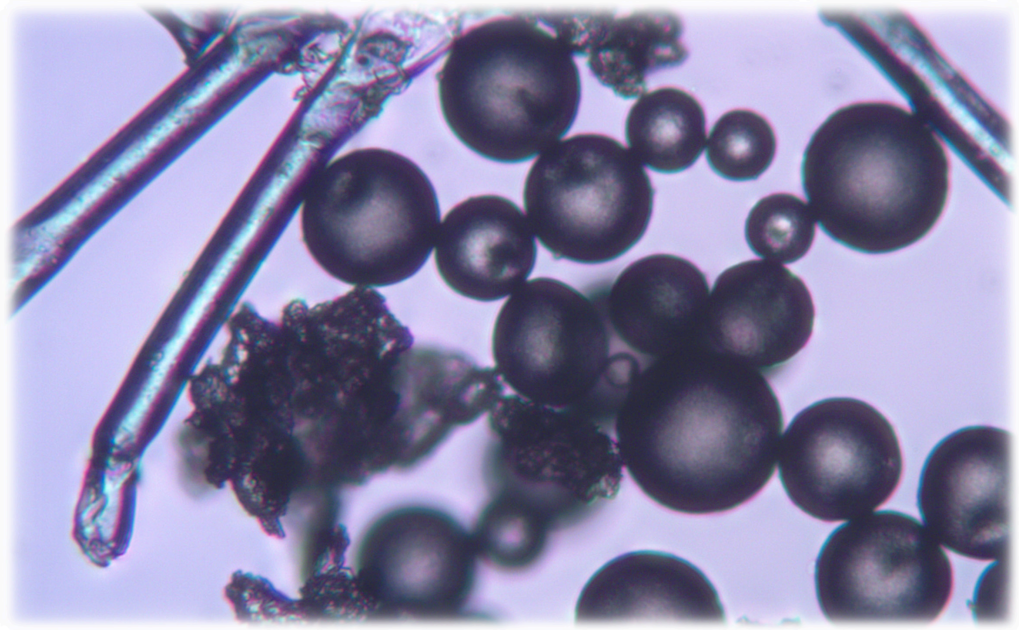
Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2024:63
Faculty of Natural Resources and Agricultural Science

Microplastics in freshwater ecosystems

Effects and drivers

Ze Hui Kong



Microplastics in freshwater ecosystems

Effects and drivers

Ze Hui Kong
Faculty of Natural Resources and Agricultural Science
Department of Aquatic Sciences and Assessment
Uppsala

SLU
SWEDISH UNIVERSITY
OF AGRICULTURAL
SCIENCES

DOCTORAL THESIS
Uppsala 2024

Acta Universitatis Agriculturae Sueciae
2024:63

Cover: Microplastic fibres, fragments and spheres (photo: Ze Hui Kong)

ISSN 1652-6880
ISBN (print version) 978-91-8046-354-6
ISBN (electronic version) 978-91-8046-390-4
https://doi.org/10.54612/a.lm7pgi91kf

Swedish University of Agricultural Sciences, Department of Aquatic Sciences and Assessment, Uppsala, Sweden

Print: SLU Grafisk service, Uppsala 2024

Microplastics in freshwater ecosystems: effects and drivers

Abstract

Microplastics (MPs) are recognised as a pervasive contaminant of freshwater environments and their key resource processing chains. However, the impacts of MPs on freshwater ecosystems remain unclear. In this thesis, I assessed the effects of MP exposure on freshwater microorganisms, macroinvertebrates, and ecosystem functions through a series of laboratory and field experiments. I further aimed to identify key drivers of MP effects, including particle properties and potential interactions with natural sources of variation and anthropogenic stress. MP effects were detected in all my experiments, most often altering microbial growth and activity, which then propagated to affect macroinvertebrate growth or ecosystem functions. These responses were often facilitated by MP properties associated with higher surface area availability, including high concentrations and irregular fragment shapes. MP effects on ecosystems were often smaller compared to natural sources of variation, including macroinvertebrate presence and resource quality. These factors also frequently modified MP effects, such as macroinvertebrate presence, which dampened some MP effects on microorganisms and ecosystem functions. Additionally, MPs had some complex interactions with another global anthropogenic stressor, warming, especially on ecosystem metabolism, although their impacts on other response variables were largely independent of one another. Furthermore, MP leachates stimulated cellulose decomposition in my field study. While I detected many MP effects occurring at concentrations that exceed those generally recorded in the environment, other MP effects also occurred at realistic concentrations, particularly those on microorganisms and macroinvertebrates. Overall, the findings of this thesis highlight the potential for MPs to affect real-world freshwater ecosystems, including as temperatures rise due to global warming. However, MP effects on microbial communities and ecosystem functioning may be less extreme than other common sources of environmental variation.

Keywords: microplastics, macroinvertebrates, microbial community, ecosystem functioning, multiple stressors

Mikroplaster i sötvattensekosystem: effekter och drivkrafterna

Sammanfattning

Mikroplaster (MP) är en genomgripande förorening av sötvatten miljöer och dess ekosytem processeser. Trots detta är MP påverkan på sötvattensekosystem fortfarande oklara. I denna avhandlingen undersökte jag effekter av MP på sötvattensmikroorganismer, bottenfauna och ekosystemfunktioner genom laboratorie- och fältexperiment, och de nyckelfaktorer som driver MP effekter, inklusive partikelegenskaper och potentiella interaktioner med naturliga källor till variation i miljön och antopogena stressorer. Effekter från MP var upptäckta i alla experiment och framförallt involverade förändringar i mikrobiell tillväxt och aktivitet, som i sin tur ibland påverkade tillväxt hos bottenfauna eller ekosystemfunktioner. Dessa effekter oftast drevs av MP-faktorer förknippade med ökad ytarea, inklusive höga MP koncentrationer och fragmenterade partikelformer. Dock var MP effekter på ekosystem ofta mindre jämfört med naturliga variationskällor, t. ex. tätheten av bottenfauna och resurskvaliteten hos organiskt material. Dessa faktorer även ändrade MP påverkan i ekosystemet, t.ex. förekomsten av bottenfauna minskade vissa MP-effekter på mikroorganismer och ekosystemfunktioner. Andra viktiga resultat inkludera några komplexa interaktioner emellan MP och stress från uppvärmning på ekosystemmetabolism i mikrocosms, och en stimulerade effekt av urlakade kemikalier från MP på cellulosanedbrytningshastighet i en fältexperiment. Många MP-effekter inträffade vid realistiska MP koncentrationer, framförallt på mikroorganismer och bottenfauna, medan andra inträffade endast vid väldig höga koncentrationer. Denna avhandling belyser potentialen för MP att påverka sötvattensekosystem, dock kan några MP-effekter på vara mindre än andra vanliga källor till miljövariationer.

Nyckelord: mikroplaster, makroinvertebrater, mikrobsamhällen, ekosystemfunktion, multipel stressorer

淡水里的微塑料: 效应与驱动因素

摘要

微塑料已被辨为淡水环境及其关键加工链的普遍污染物。依然，它们在淡水环境内的效应依然不清。在此论文内，我使用实验室里的模拟实验与野外实地实验，评估了微塑料对淡水微生物、大型无脊椎动物与生态系统功能对微塑料的效应。我也确认了推动微塑料效应的关键因素，包括颗粒特征以及与其他自然和人造压力源的潜在互相作用。微塑料效应在所有实验里均检测到：其效应通常归因于改变微生物生长与活动，从而影响到了大型无脊椎动物或生态系统的功能。这些反应是由表面积较高的微塑料颗粒特征促进，包括高颗粒浓度及其粗糙碎片形状。此外，微塑料对生态环境带来的效应经常小于大自然的胁迫与压力。这些因素也经常改变微塑料导致的效应，包括大型无脊椎动物的存在以及资源质量。我也展现了微塑料与另外一种广泛的人造压力—全球变暖—有一些夫扎的相互作用，列如产生了对生态系统代谢的影响。尽管如此，微塑料与变暖大部分的效应都被检测为相对独立。此外，从微塑料中浸出的渗滤液增加了实地实验内的纤维素分解率。许多在此论文里被确认的微塑料效应都发生于比真实环境中的微塑料浓度高。然而此论文的实验也证明了存有微塑料在实际浓度下的效应，尤其是对微生物群落与大型无脊椎动物的效应。此论文里的结果强调了微塑料对现实淡水生态系统的潜在影响，尤其是世界未来因人造全球暖化而继续暖和。即使，微塑料对微生物群落和生态系统功能的效应或许比大自然的胁迫与压力相比之下未必太极端。

关键词：微塑料，大型无脊椎动物，微生物群落，生态系统功能，多重压力

Dedication

Grandma

“Life in plastic, it’s fantastic”(?)

Aqua, Barbie Girl

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Kong, Z.H., Burdon, F.J., Truchy, A., Bundschuh, M., Futter, M.N., Hurley, R. and McKie, B.G. (2023). Comparing effects of microplastic exposure, FPOM resource quality, and consumer density on the response of a freshwater particle feeder and associated ecosystem processes. Aquatic Sciences, 85(3), p.70. https://doi.org/10.1007/s00027-023-00964-w.

II. Kong, Z.H., Tong, L., Burdon, F.J., Truchy, A., Futter, M.N., Bundschuh, M., Bertilsson, S. and McKie, B.G. Microplastics in freshwaters: Comparing effects of particle properties and an invertebrate consumer on microbial communities and ecosystem functions (in review (revisions) with Ecotoxicology and Environmental Safety).

III. Kong, Z.H., Burdon, F.J., Truchy, A., Bundschuh, M., Futter, M.N. and McKie, B.G. Contrasting the impacts of warming and realistic microplastic exposure on brown and green resource-use pathways in a model freshwater ecosystem. (manuscript).

IV. Kong, Z.H., Stangl, M., Oester, R., Rehnstam, S., Futter, M.N., Bundschuh, M. and McKie, B.G. Polypropylene medical mask waste: effects of macroplastic and microplastic exposure on freshwater leaf and cotton decomposition in the field. (manuscript).

All published papers are published open access.

The contribution of Ze Hui Kong to the papers included in this thesis was as follows:

- I. Contributed to the study design, conducted the experiment, and performed laboratory work and data analysis for all data. Wrote the original manuscript.
- II. Contributed to the study design, conducted the experiment and performed all laboratory work related to macroinvertebrates. Performed flow cytometry and enzymatic activity assays with lab assistance (Renes, Sophia). Performed DNA extractions, PCR amplification of 16s RNA and illumina sequencing with co-author (Tong, Liu). Performed data analysis for all data collected except for microbial community structure. Wrote the original manuscript.
- III. Conceived initial study idea, contributed to the study design and conducted the experiment. Sample collection with assistance from supervisor (Mckie, Brendan G.). Performed the majority of laboratory work, with lab assistance (Widenfalk, Siri) for macroinvertebrate sample preparation. Stable isotope analysis was done at UC Davis. Performed data analysis for all data and wrote the original manuscript.
- IV. Conceived initial study idea and contributed to the study design and some fieldwork. Prepared samples for ergosterol analysis by co-author (Oester, Rebecca). Performed data analysis for all data. Preparation of the manuscript from original draft written by co-author (Stangl, Martina).

List of tables

List of figures

Abbreviations

MP	Microplastic
FPOM	Fine particulate organic matter
CPOM	Coarse particulate organic matter
PE	Polyethylene
PP	Polypropylene
PET	Polyethylene terephthalate
PS	Polystyrene
DO	Dissolved oxygen
ER	Ecosystem respiration
NEM	Net ecosystem metabolism
GPP	Gross primary production
LMM	Linear mixed model
ANOVA	Analysis of variance

1. Introduction

The ever increasing production of cheap, disposable plastic goods has opened a Pandora's Box, unleashing an endless wave of plastic that has contaminated the planet since the late 1950s (Ostle et al., 2019). The earliest reports of plastic pollution generally consisted of marine plastic debris with particle diameters (⌀) of 100-500 µm (Carpenter and Smith, 1972; Carpenter et al., 1972). At this early stage, there were no explicit distinctions made between the sizes of different plastic waste particles. This practice was to change in the coming decades, where studies began organising collected plastic debris by different size classes (Ryan and Moloney, 1990; Thompson et al., 2004), ultimately resulting in the broadly accepted definition of microplastics (MPs) proposed by Frias and Nash (2019):

> "Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water."

Historically, the focus of plastic (including MP) pollution has firmly been on the marine environment, driven by the detection of large-scale accumulation of plastic waste such as the great Pacific garbage patch (Moore et al., 2002). It was only in the 2010s that research attention on plastic pollution, specifically MP pollution, in freshwater environments began gathering pace (Eriksen et al., 2013; Imhof et al., 2013; Free et al., 2014). The findings of these studies revealed the extent of global MP pollution in freshwaters, with MP occurrence recorded from all sampled habitats (Li, Busquets and Campos, 2020; Cera et al., 2022). In view of this, it is unsurprising that MP contamination has been reported in tap water (Kosuth, Mason and Wattenberg, 2018), commercial drinks (Liebezeit and Liebezeit, 2014), dietary salt (Karami et al., 2017), human digestive systems (Schwabl et al., 2019) and body tissues (Ragusa et al., 2021).

The public consciousness is thus confronted with the reality of pervasive and inexorable MP contamination everywhere, not just in outdoor environments but also within our bodies. This signified a drastic reduction of psychological distance between the public and plastic pollution, and became a driving force behind rising public awareness and interest in the issue of MP pollution (Garcia-Vazquez and Garcia-Ael, 2021). Consequently, MP regulations were introduced in response to public concerns, such as the measures adopted under the European Union regulation on the registration, evaluation, authorisation and restriction of chemicals (REACH) to restrict the direct sale of MP products and limit the MP content in products (European Commission, 2023). However, such actions are motivated primarily by the *precautionary principle*, in an instance of policy preceding science, where the vast scale of MP pollution necessitates action despite scientific uncertainty on the impacts of MP exposure on organisms and ecosystems (Backhaus and Wagner, 2019; McKie et al., 2023).

Early research efforts to quantify MP impacts retooled existing toxicological and ecotoxicological protocols and methods, typically designed to investigate soluble chemical contaminants (Abdolahpur Monikh et al., 2023). These studies provided relatively swift insights into the MP effects on a selection of taxa typically used in toxicological assays, such as *Daphnia magna* zooplankton and zebrafish (Jemec et al., 2016; Lu et al., 2016). However, MPs differ from many chemical contaminants in being insoluble and characterised by wide variation in e.g. particle density and shape, and thus require additional considerations on exposure conditions and effect pathways during testing (Blettler et al., 2018; De Ruijter et al., 2020). Indeed, there are major research questions that remain unanswered after the early ecotoxicological assessments. First, it is unclear how environments and ecosystems that are most exposed to MP pollution are affected. Second, there is a mismatch in the MPs used in laboratory tests and those detected in real-world settings (Rozman and Kalčíková, 2022), resulting in a knowledge gap on the effects of MPs with properties commonly found in freshwater environments. Third, it is uncertain how MP effects on organisms translate to higher ecological levels, particularly in more complex ecosystems with interactions between multiple taxa. More generally, there is a pressing need to better align experiments on the impacts of MP exposure with

Table 1. Key concepts and definitions referred to within this thesis.

Anthropogenic stressor	"A variable that, as a result of human activity, exceeds its range of normal variation and affects (whether negatively or positively) individual taxa, community composition, or ecosystem functioning relative to a reference condition." (Piggott, Townsend and Matthaei, 2015)
Effect drivers	A variable or factor that influences or is the cause of effects in a system or process.
Ecosystem functioning	"The joint effects of all processes that sustain an ecosystem" (Reiss et al., 2009)
Food dilution effect	The reduction of nutrient concentration with a resource pool due to the addition of a nutrient-poor or -void material. Alternatively known as dietary dilution (Lee, Raubenheimer and Simpson, 2004)
Metabolic theory of ecology	The theory states that community structuring and ecosystem functioning is dictated by organism processes and metabolic rates, and thus can be predicted through factors that affect metabolic rates, including temperature and body size (Brown et al., 2004).
Microbial conditioning	The "successional changes" of biochemistry and microbial community growth on substrates (Cummins, 1974).
Precautionary Principle	An approach to address uncertain risks in environmental policy, where action can be motivated by the potential of a serious or irreversible threat of an issue, despite scientific uncertainty (Sand, 2000).
Stressor	Factors or variables which shift the environment beyond the optimal condition range of organisms, leading to changes in organism survival, growth and other processes
Stressor Interactions	Interactions between multiple stressors can influence their overall impacts on organisms and ecosystems, where their combined effects can be equal to (*additive*), less than (*antagonistic*) or more than (*synergistic*) the sum of individual stressor effects (Jackson et al., 2016).

environmentally relevant conditions and MP types, to inform priority setting in policy and management, allowing policymakers to scale back dependence on the precautionary principle and base action on scientific knowledge.

1.1 Context: Particle rich freshwater ecosystems

Even in the absence of MPs, freshwater ecosystems are typically characterised by high and pervasive occurrence of particulate organic matter

(POM), including detritus, faecal particles and organic colloids, that are utilised as resources by various organisms. The POM within freshwater ecosystems is largely generated through two trophic pathways associated with key basal resources, allochthonous detritus and autochthonous primary production, which drive the *brown* and *green* trophic pathways respectively (Figure 1). The brown pathway is largely based on microbial and macroinvertebrate breakdown of allochthonous terrestrial detritus, which contributes significantly to the carbon and nutrient pool in many types of freshwater environments (Cummins and Klug, 1979; Pace et al., 2004). The green pathway is driven by production and consumption of autotrophic phytoplankton, periphyton and, to a lesser extent, macrophytes (Vadeboncoeur and Steinman, 2002; Vis et al., 2007). Additionally, the dynamics of the brown pathway are captured in the *detrital processing chain* concept (Heard, 1994), which begins with the introduction of plant detritus, also termed course particulate organic matter (CPOM; ⌀>1000 µm), into freshwaters. This plant material is rapidly colonised by bacteria and fungi that act as key decomposers, in a process referred to as *conditioning* (Cummins and Klug, 1979; Findlay et al., 2002; Anesio, Abreu and Biddanda, 2003; Romaní et al., 2006). The colonisation of microorganisms also facilitate the consumption of leaf litter by shredder invertebrates by forming biofilms on detritus and enhancing detrital nutritional content (Cummins and Klug, 1979; Wright and Covich, 2005).

A by-product of detrital decomposition activities is the production of fine particulate organic matter (FPOM; ⌀ 0.45-1000 µm), which includes detritivore faecal pellets and microbial aggregates (Cummins and Klug, 1979; Shepard and Minshall, 1984). Fractions of FPOM may also be derived through the green pathway, with the dislodgement of algal biofilm fragments (Hart, 1985; Scrimgeour et al., 1991), formation of algae aggregates (Hamilton, Sippel and Bunn, 2005) and faecal pellets from the activities of grazer invertebrates (Heard and Buchanan, 2004). FPOM forms the primary resource base of a dominant feeding guild within freshwater ecosystems, consisting of collector organisms that gather and/or filter particles from benthic deposits and the water column (Wallace and Webster, 1996). This manifests as the tight linkage described by the *river continuum concept*, (Vannote et al., 1980) between downstream collectors capturing and exploiting the inefficiencies and conditioning of resource processing by upstream organisms via FPOM.

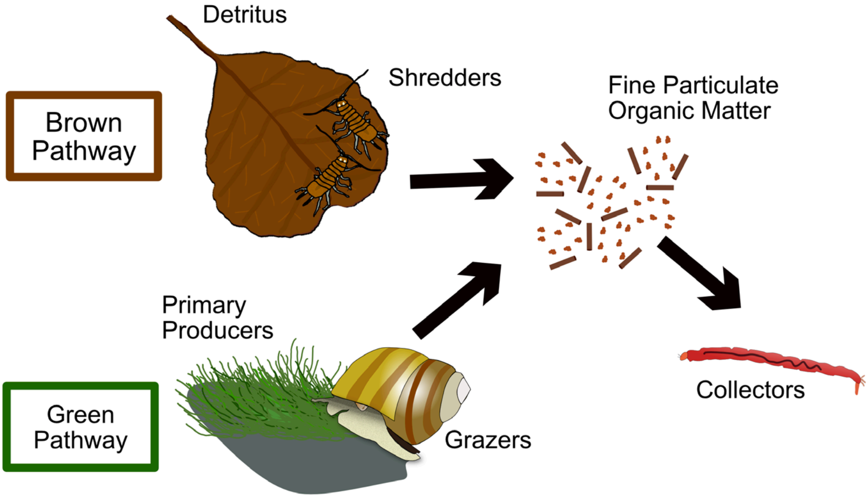


Figure 1. The two main energy use pathways present in freshwater ecosystems. Basal resources (detritus and primary producers) are broken down and consumed by consumers (shredders and grazers), which produce fine particulate organic matter that is subsequently consumed by collector organisms, including both gatherers and filter-feeders.

Today, the contents of particle pools within freshwater ecosystems extend beyond naturally-occurring particles, encompassing particles generated from anthropogenic sources, including MPs. The entry of MPs into freshwaters may occur through sewage effluent (Lasee et al., 2017; Park et al., 2020), terrestrial runoff (Wang et al., 2022) and atmospheric deposition (Evangeliou et al., 2020; Stanton et al., 2020), or be directly generated in-situ from the degradation of macroplastic waste. Reflecting their sources, MPs are classed as either *primary* or *secondary*. Primary MPs are directly produced for domestic and industrial uses, such as exfoliating or abrasive agents (Zitko and Hanlon, 1991). Secondary MPs are generated from the breakdown of larger macro-plastics or primary MPs (referred here as the plastic breakdown chain), driven primarily through environmental abiotic photo-, thermal-, hydrolytic and mechanical degradation (Andrady, 2015; Zhang et al., 2021), and limited contributions from microbial biodegradation (Gewert, Plassmann and Macleod, 2015; Khoironi, Anggoro, and Sudarno, 2019). Given that the vast majority of the approximately 4900 million metric tonnes of plastic ever produced have been discarded and are slowly degrading throughout the planet (Geyer, Jambeck and Law, 2017), it comes as no surprise that the majority of MPs found in the environment are

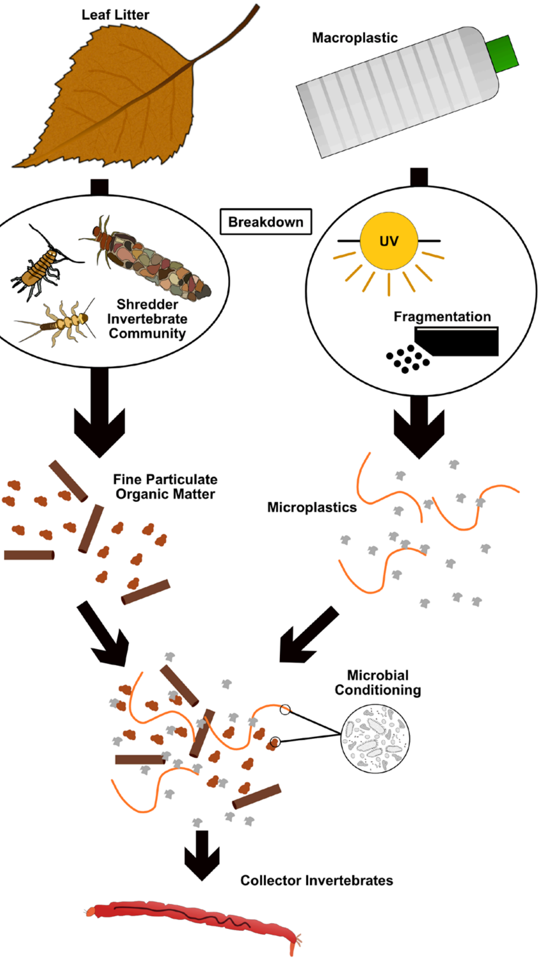


Figure 2. The breakdown of leaf litter and macroplastics by various biotic and abiotic processes generates numerous small particles, i.e. fine particulate organic matter (⌀ 1-1000 µm) and microplastics (⌀ 1-5000 µm) respectively. Subsequently, fine particulate organic matter and microplastics are likely to co-occur in benthic deposits and consumed by collector macroinvertebrates, including both gatherers and filter-feeders.

secondary (Burns and Boxall, 2018; An et al., 2020). Both primary and secondary MPs can be found in a wide variety of polymer compositions, shapes, colours, and with different chemical additives, echoing the diverse range of applications that plastics are used for (Rochman et al., 2019).

The plastic breakdown chain parallels that of the detrital processing chain (Figure 2), where initial materials are degraded into numerous small particles, which subsequently disperse into the aquatic environment. The particles produced from both chains, i.e. MPs and FPOM, have overlapping size ranges and material densities (EPA, 1992; Thomas et al., 2001). Thus, the fates of FPOM and MP particles in freshwater environments intersect, with both types of particles depositing in the same areas (Alimi et al., 2018; Vincent and Hoellein, 2021). Furthermore, both particle types are able to serve as substrates for the growth of microbial biofilms, which modifies their densities and depositional behaviours, and improves their palatability for consumers (Cummins and Klug, 1979; Joyce, Warren and Wotton, 2007). These properties predispose FPOM-reliant feeding guilds to MP ingestion, especially the gatherer and filter-feeding guilds of collector invertebrate consumers (Akindele, Ehlers and Koop, 2020; Bertoli et al., 2022).

Despite these similarities, key distinctions remain between naturally occurring FPOM and MPs. Biological breakdown of plastics, driven by microbial taxa that produce plastic-degrading enzymes (Amobonye et al., 2021), is a slow process. Additionally, MPs may contain chemical additives that leach out into the environment and potentially affect organisms (Rochman et al., 2019). The combination of overlapping and distinct properties of MPs, relative to FPOM, makes them a contaminant that can easily integrate into naturally particle-rich freshwater habitats, yet also have the potential to affect organisms and processes that are adapted to utilise FPOM resources.

1.2 Consequence: Microplastic effects on organisms

Our current understanding of MP impacts is primarily built upon laboratory single-species studies (Ockenden et al., 2021), which have afforded us insight into the impacts and mechanisms behind MP exposure effects. For microorganisms, MP effects primarily relate to their potential to act as a growth substrate for microbial biofilms. Compared to natural particles such as FPOM, the various polymers of MPs form novel substrates in the freshwater environment, and host surficial biofilms with distinct microbial assemblages and metabolic pathways (Arias-Andres, Rojas-Jimenez and Grossart, 2019; Miao et al., 2019). In particular, biofilms on MPs have been shown to harbour higher fractions of plastic-degrading microbial taxa (Gambarini et al., 2021). The accumulation of biofilms on MP surfaces can in turn change the properties of MP particles, e.g. altering buoyancy

(Semcesen and Wells, 2021) or increasing sorption of pollutants (Wang, Guo and Xue, 2021), and thus altering the bioavailability of pollutants and thus toxicity (Wang et al., 2020). Besides acting as a growth substrate, MPs may also release chemical leachates containing plastic additives such as toxic plasticisers and dyes, which can inhibit microorganism growth (Tetu et al., 2019; Capolupo et al., 2020). However, MPs may also release dissolved organic carbon (Romera-Castillo et al., 2018) or trace chemicals within leachates (Chae, Hong and An, 2020) that can stimulate microbial growth, such as that of microalgae (Canniff and Hoang, 2018; Chae, Kim and An, 2019). Through the alterations in microbial community composition, MP presence has been shown to change microbial-driven nitrogen cycling process rates, such as nitrification and denitrification (Seeley et al., 2020).

Owing to their larger size, macroorganisms interact with MPs differently from microorganisms. For example, macroorganisms can ingest MPs, with studies documenting this across a variety of freshwater organism groups, including zooplankton (Scherer et al., 2017; Canniff and Hoang, 2018) and invertebrates (Au et al., 2015; Ward et al., 2019). The ingestion of MPs by macroorganisms has been identified as a major determinant of MP impacts on individuals (Wagner et al., 2014; Horton, Svendsen, et al., 2017; Backhaus and Wagner, 2019), through mechanisms including false satiation, gut blockages, toxic effects from chemical leachates and internal damage (Ma et al., 2020). Consequently, MP exposure and ingestion has been recorded to cause increased mortality (Ogonowski et al., 2016; Tian et al., 2020), reduced assimilation efficiency (Blarer and Burkhardt-Holm, 2016), and reduced growth and reproduction (Au et al., 2015; Redondo-Hasselerharm et al., 2018) in macroorganisms. These MP effects on macroorganisms have also been shown to propagate, leading to knock-on effects on ecosystem processes. For example, the negative effects of MP exposure on macroinvertebrate survival and activity were shown to reduce macroinvertebrate-driven leaf litter decomposition (López-Rojo et al., 2020) and sediment nitrogen removal (Huang et al., 2021).

However, there is a notable of variation in MP effects on macroorganisms and ecosystem functions across taxa and studies, as reviewed in Foley et al. (2018). MPs were frequently found to have no effects on macroorganisms (Imhof and Laforsch, 2016; Foley et al., 2018; Redondo-Hasselerharm et al., 2018; Weber et al., 2018; Aljaibachi et al., 2020) or on ecosystem functions such as decomposition (Silva et al., 2022; Marchant et al., 2023). This variability between studies suggests that there are aspects of MP exposure that might not be easily generalizable, and highlights the knowledge gaps surrounding the influence of MP particle properties and conditioning on its effects on biota and associated ecosystem processes.

1.3 Relevance: Microplastic particle properties and conditions

The environmental relevance of results has been touted as a limitation of laboratory MP research (Connors, Dyer and Belanger, 2017), reflecting discrepancies between the types, concentrations and conditions of MP particles commonly found in the environment and those used in laboratory testing. In the environment, the majority of MPs recorded globally are fragments and fibres (≥ 29 and 45 % respectively; Burns and Boxall, 2018). Additionally, most MPs in the environment are comprised of polyethylene (PE), polyethylene terephthalate (PET), polyacrylamide (PA), polypropylene (PP) and polystyrene (PS) polymers (Burns and Boxall, 2018). The dominance of these MP shapes and polymers reflect common plastic products, including disposable plastic packaging and synthetic fabrics which breakdown and release MPs into the environment (Xu et al., 2020). In contrast to the profile of MPs commonly found in the environment, laboratory studies tend to use commercially purchased MP spheres (Rozman and Kalčíková, 2022) and MPs comprised of PS and PE polymers (Burns and Boxall, 2018). Spherical MPs make up a relatively small fraction (≤ 10 %) of all MPs detected globally (Burns and Boxall, 2018), although local sources can create hotspots of such MP types (Castañeda et al., 2014; Peng et al., 2018). Furthermore, the concentrations of MPs used in laboratory studies were generally higher than concentrations detected in real-world freshwater environments (Lenz, Enders and Nielsen, 2016; de Sá et al., 2018).

The disconnect between MPs found in the environment and those used in the laboratory is of concern as MP particle properties are important factors in influencing their fate in the environment, and thus the potential risk for organisms. For example, MPs generated from dense polymers are likely to settle into the benthos quickly, to be colonised by benthic microbial communities and encountered by benthic macroinvertebrates. Obviously, exceedingly high concentrations of MPs also increase the likelihood of detecting MP impacts on organisms due to the increased potential to interact with and ingest MP particles. Consequently, studies that use MPs and exposure conditions uncommon in the real world may only be relevant for specific locations and yield results that are inappropriate to extrapolate to the general state of MP pollution in the environment.

The original properties of MPs are further altered in freshwater environments through various abiotic and biotic processes. In the environment, MPs are subject to solar and UV radiation, which can increases the roughness of particle surfaces and the adsorption capacity of MPs for heavy metals (Lin et al., 2020). MPs are also rapidly colonised by microbes which form biofilms (Sooriyakumar et al., 2022), with this process referred to as the *biofouling* of MP particles. The presence of biofilms result in changes to the overall density of MP particles, and thus their settling rates (Miao et al., 2021; Semcesen and Wells, 2021). Additionally, the biofouling of particles also lead to the preferential ingestion of MPs by macroorganisms (Vroom et al., 2017; Polhill et al., 2022), potentially through changes in the chemical signature of biofouled plastics that induce foraging responses from odour-guided foragers (Savoca et al., 2017). Evidently, the biofouling of MPs is important in influencing their fate and effects in the environment. However, this aspect of MP exposure is less frequently incorporated into laboratory study designs, which tend to opt for the direct addition of virgin MPs (Rummel et al., 2017).

Thus, identifying key MP particle properties that drive encounters and impacts on organisms and ecosystems may provide us a more practical avenue to derive insight into the effects of MPs in the environment.

1.4 Complexity: Complex ecosystems and multiple stressors

The ecosystem processes underpinning the green and brown trophic pathways in freshwaters, including decomposition, primary productivity, CO_2 sequestration and nutrient cycling (De Groot, Wilson and Boumans, 2002), are key components of wider ecosystem functioning (Truchy et al., 2015). Correspondingly, responses of individual components in these trophic pathways to stressors, whether natural or anthropogenic, have the potential to reverberate throughout food webs and ecosystems. This may occur through stressors altering interactions between taxa, or resource quantity/quality (e.g. Dawoud et al., 2017). For example, changes in microbial communities and associated microbial functions may lead to direct effects on ecosystem processes (Miao et al., 2019; Chen et al., 2020), and indirect effects on consumer growth through changes in resource quality of microbial aggregates (Wei, Liao and Wang, 2016).

The strength of indirect effects is further dependent on the relative importance of top-down vs. bottom-up biotic processes in food webs, e.g. predator suppression of prey (Carpenter, Kitchell and Hodgson, 1985) vs. primary production limitation of consumer populations (McQueen, Post and Mills, 1986). These interactions between taxa and trophic levels, together with the number of trophic levels (vertical trophic diversity) and taxa within each level (horizontal trophic diversity) collectively make up food web complexity (Gessner et al., 2010). More complex food webs may be able to resist change and maintain processes through stability and resilience conferred by functional redundancy and diversity in biodiverse communities (Frainer and McKie, 2015), referred to as the *insurance hypothesis* (Naeem and Li, 1997; Yachi and Loreau, 1999). These emergent properties of complex communities and ecosystems demonstrate the challenge of extrapolating responses from simple ecological models, such as those frequently used in testing stressor responses, and highlight the importance of integrating multiple ecological levels in investigative studies on stressor impacts (Petchey et al., 2004; Woodward, 2009).

Even in undisturbed ecosystems, environmental variation imposes multiple naturally occurring sources of stress on organisms, including fluctuations in the nutritional value of resources, presence of competitors and extreme temperatures. Against the backdrop of these naturally occurring stressors, freshwater ecosystems are further beset by a wide range of anthropogenic stressors, often simultaneously, including plastic pollution and anthropogenic global warming (Dudgeon et al., 2006; Reid et al., 2019). The occurrence of these stressors lead to altered conditions for organisms, which may respond by prioritising energy allocation to physiological processes that mitigate stressor effects, potentially to the detriment of other processes (Kooijman, 2010). Furthermore, co-occurring stressors may interact, resulting in various outcomes, with non-additive interactions being the most challenging for management as they present scenarios where managing just one stressor may not result in the intended outcome if other stressors remain unaddressed (Côté, Darling and Brown, 2016; Johnson et al., 2017). Anthropogenic stressors may also interact with natural stressors, resulting in unexpected outcomes when natural stressors are not taken into account (Holmstrup et al., 2010). The mechanisms behind these interactions sometimes reflect direct abiotic interactions between the stressors themselves. For example, increased temperatures associated with anthropogenic global warming are likely to increase photo- and thermal-degradation of plastics (Andrady, Hamid and Torikai, 2003), leading to higher MP generation from macroplastics. However, stressor interactions are more often mediated through the effects and feedbacks of biota and ecosystem functioning (Piggott, Townsend and Matthaei, 2015a; Dawoud et al., 2017). For example, organism traits that protect against a stressor may alter its tolerance towards other co-occurring stressors, either reducing it (*negative co-tolerance*) it due to physiological trade-offs or increasing it (*positive co-tolerance*) it due to traits that protect against multiple stressors (Vinebrooke et al., 2004).

At this point, it is worth pointing out that the biotic responses I have described are most relevant when stressor effects on mortality are limited, such that co-occurring stressors form complex networks of interacting sub-lethal effects in the environment. In such scenarios, considerations towards how stressors co-occur, whether temporally (Jackson, Pawar and Woodward, 2021) or across ecological scales (Simmons et al., 2021), can help improve predictions of the impacts of multiple stressors on ecosystems. In this respect, MP contamination overlaps with anthropogenic global warming in temporal and ecological scales, with both being long-term potentiating stressors that can act on basal food web resources, with the potential for effects to reverberate through the rest of the ecosystem. MPs are guaranteed to be a mainstay stressor for the foreseeable future due to the persistence and slow degradation of plastic polymers, and the challenges in removing MPs from the environment (Eerkes-Medrano, Thompson and Aldridge, 2015; Pan et al., 2022). Similarly, global temperatures are predicted to continue rising throughout the foreseeable future, potentially reaching up to 4 °C of warming by the end of the 21st century (Lee et al., 2021). Warming fundamentally accelerates biochemical kinetics, which increases metabolic rates and costs, as described by the *metabolic theory of ecology* (Brown et al., 2004). Thus, warming may lead to increased ingestion rates to fulfil metabolic demand, and may thus increase the inadvertent consumption of MPs and expose organisms to potential negative effects associated with that (Hasan et al., 2023). However, warming does not simply drive a continuous increase in process rates, as the biological machinery of organisms, e.g. enzymes, are adapted for specific thermal niches (Clarke and Fraser, 2004). Deviations from the thermal optimum reduce the efficiency or degrade enzymatic reactions, altering organism process rates and negative physiological effects (McKie, Cranston and Pearson, 2004; Miller and Stillman, 2012), with potential implications for the overall outcomes of co-occurrence with MPs. These scenarios point towards the broad range of possible outcomes associated with the co-occurrence of MP pollution with other, globally pervasive, stressors.

The complexities of natural ecosystems and multiple co-occurring stressors highlight the challenges in assessing the potential risks of new (or newly recognised) stressors on the environment. In particular, it is a challenge to balance complexity and practicality to ensure studies remain environmentally relevant. Previous laboratory studies focused on individual taxa (Rozman and Kalčíková, 2022), which while informative, is difficult to extrapolate onto the complex inter-species relationships and resulting ecosystem processes present in real-world environments (Baho, Bundschuh and Futter, 2021; Ockenden et al., 2021). Similarly, research on the impacts of individual stressors are challenging to generalize onto freshwater ecosystems that are subjected to complex combinations of stressors in both space and time (Folt et al., 1999; Piggott, Townsend and Matthaei, 2015b). To this end, this thesis incorporates environmentally relevant MP types and concentrations within an ecological context perceived to be at high risk of MP pollution, investigating the impacts of MP contamination throughout the ecological levels of freshwater ecosystem elements associated with detrital processing, and the influence of MP particle properties and co-occurring stressors on these effects.

2. Aim of thesis and specific objectives

This thesis aimed at assessing the impacts of MP exposure on freshwater ecosystems, through elements of the resource processing chains, and using a range of environmentally relevant MP types in both laboratory and field studies, in which impacts of MPs are assessed simultaneously with other key abiotic and biotic environmental drivers.

The research in this thesis had the following objectives:

- To assess the effects of MP exposure on freshwater microorganisms, macroinvertebrates and associated ecosystem functions (**paper I- IV**).
- To determine key MP particle factors that drive effects in freshwater ecosystems (**paper II** & **IV**).
- To quantify the magnitude of MP effects relative to those of other natural sources of variation including competition and resource quality (**paper I** and **II**) and other anthropogenic stressors including warming (**paper III**) and macro-plastic exposure (**paper IV**).
- To assess interactions between MPs and these additional biotic drivers and stressors, and evaluate outcomes of co-exposure with these factors for the effects of MPs (**paper I-III**).
- To assess the extent to which MP impacts on microorganisms propagate to higher trophic levels and ecosystem functions (**paper I-IV**).
- To assess the effects and feedbacks between macroinvertebrate presence and MPs on freshwater ecosystems (**paper I-III**).

3. Materials and methods

3.1 Study systems

As discussed in the introduction, ecologically simplistic laboratory experiments lack the ecological scale and relevance of field studies, and in isolation, are difficult to extrapolate to far more complex real-world environments (Carpenter, 1996). However, practicality cannot be discounted, with laboratory experiments being indispensable for MP research due to challenges with limiting external MP contamination and avoiding the accidental release of MPs into the environment. This emphasizes the importance of developing complementary experiments across multiple scales, to generate knowledge capable of informing management and policy decisions (Benton et al., 2007). Therefore, I aimed to investigate the responses of the at-risk detrital processing chain towards MP exposure, particularly of organisms and processes associated with FPOM, through interconnected laboratory and field experiments. In addition, I aimed to identify key drivers of MP effects by utilising different MP types and co-occurring sources of stress.

Over a linked series of experiments, I increased elements of vertical or horizontal trophic and environmental complexity (Figure 3). This begins with **paper I**, where I compared the effects of exposure to a single realistic concentration of MP against naturally occurring sources of stress (n = 60). The same microcosm set-ups where used in **paper II**, which compared the influence of a range of MP polymers, shapes and concentrations on its effects on ecosystems (n = 240). These two experiments culminated in my final laboratory study in **paper III**, where I used a mixture of fragment and fibre MPs, deviating from the single MP type treatments used in my two earlier

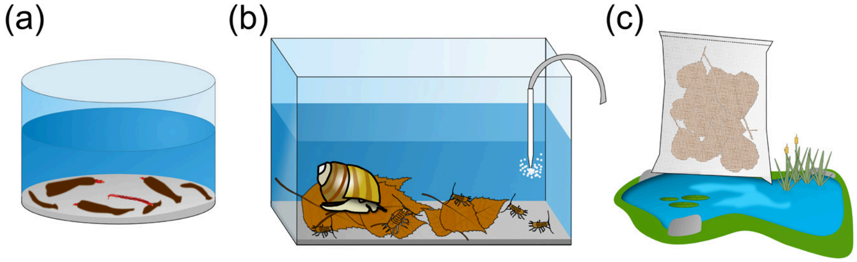


Figure 3. Experimental systems used in this thesis: (a) Microcosms with chironomid larvae and FPOM resource. (b) Aerated microcosms with isopods, snails and chironomid larvae. (c) Leaf litter decomposition assays in a field site.

laboratory experiments (n = 54). Furthermore, **paper III** used an expanded biological model system of the detrital processing chain that increased horizontal trophic complexity, and was maintained for a longer study period, crossing generations for some of the macroinvertebrate study organisms. Following these laboratory microcosm experiments, I took my research out into the field with **paper IV**, aiming to test the responses of microbial communities and processes towards a common MP type in the more complex real-world environment (n = 150).

The following section provides a general overview of the experimental systems and methods used for this thesis. For more details, please refer to **papers I-IV**.

3.1.1 Laboratory microcosms (**paper I-III**)

Experiments reported in **paper I-III** were conducted using laboratory microcosms in climate-controlled facilities at SLU Uppsala, set to specified temperatures and with a 16:8 light-dark cycle. Microcosms in **paper I** and **II** consisted of glass crystallisation dishes (300 mL) containing larvae of the non-biting midge *Chironomus riparius* (Diptera) as a particle feeder, and a layer of FPOM resource (Figure 3a). **Paper III** microcosms consisted of aerated glass aquaria containing birch leaf litter and three macroinvertebrate species representing three feeding groups: a detritivore (isopod *Asellus aquaticus*), grazer (snail *Viviparus viviparus*) and particle feeder (*C. riparius*) (Figure 3b). For **paper I-III**, water was collected from a local stream (Hågaån, Uppsala), which served as our primary colonization source for introducing microbial communities (bacteria, fungi, etc.) into the microcosms. Stream water was filtered through a 25 µm sieve to remove potential MP contamination prior to addition to the microcosms. To recreate the particle-rich environments that are susceptible to MP pollution, I added both inorganic sand (Fontainebleau Sand, VWR) and organic FPOM into microcosms. One of two types of FPOM were used in the resource quality treatments of **paper I**: (i) naturally-occurring FPOM collected from Fibyån stream (Uppsala) or (ii) FPOM prepared using a ratio of 1:2:2 of TetraPhyll fish food (Tetra, Germany), ground alder leaves and birch leaves. FPOM in **paper II** comprised of the prepared FPOM used in **paper I**. I did not directly add FPOM into microcosms for **paper III**, instead relying on grazer and detritivores macroinvertebrates to generate faecal particles from the ingestion of algal biofilms and intact leaf litter respectively.

Three MP shape types of various polymers were used across the three microcosm experiments. Spherical MPs were commercially purchased (Cospheric). I produced all fragment MPs by grinding liquid nitrogen frozen plastic pellets with a ball mill. PP fibre was produced by cutting synthetic fabrics. MPs PET fibre MPs were generated at the Norwegian Institute of Water Research (NIVA) in Oslo by washing microfiber blankets and collecting the loose MP fibres from the effluent (Schell et al., 2022). In this thesis, all MP concentrations are expressed as the number of MP particles per kilogram of sediment (p/kg). All experiments included an environmentally relevant sediment concentration of MPs (1,000 p/kg) that is referred to as the *realistic* concentration, and some experiments also including a concentration significantly higher than most recorded concentrations (50,000 p/kg for **paper II**, 100,000 p/kg for **paper III** and 23,000 p/kg for **paper IV**) referred to as the *high* concentration. These concentrations were determined through compilation of published sediment MP concentrations in freshwater environment worldwide (**paper II**). To better replicate the condition in which MPs are likely encountered in the environment, microcosms were set up with MPs and left to condition in stream water for approximately a week (**paper I & II**) or a month (**paper III**) prior to introduction of macroinvertebrate consumers to promote biofilm growth on particle and other microcosm surfaces. In **paper III**, MPs were also photodegraded prior to the experiment through UV irradiation in a climate chamber, to better mimic exposure to sunlight in freshwater environments.

In **paper I**, I tested the effects of PE MP sphere (45-53 µm) exposure at a realistic environmental concentration (1,000 p/kg) on non-biting midge *C. riparius* larvae and ecosystem respiration, at varying food qualities (low quality field collected FPOM vs high quality prepared FPOM) and larvae densities (0, 10 and 20) over 21 days. Results from **paper I** were used to inform the design of **paper II**, which tested the influence of MP particle concentration (0, 1,000, 50,000 p/kg), shape (fragment, fibre, sphere) and polymer (PE, PET, PP, PS) on MP responses from the microbial community, chironomid larvae and ecosystem functions (ecosystem respiration and chlorophyll-a concentration) over 20 days. Microcosms for both **paper I** and **II** were incubated at 20 °C, matching the culturing conditions for the chironomid larvae used. In **paper III**, I tested the effects of exposure to different concentrations (0, 1,000, 100,000 p/kg) of a 1:1 mix of PET fragment and fibre MPs and temperatures (16 ,19, 22 °C) on the microbial communities, macroinvertebrates and ecosystem functions (decomposition, ecosystem respiration, chlorophyll-a concentration) over 121 days. All organisms used in **paper III** were acclimated to 16 °C in the laboratory for a month before the experiment.

3.1.2 Field study (**paper IV**)

I conducted litter decomposition assays (Figure 3c) in the naturally formed Kunskapsparken pond, located on campus of the Swedish University of Agricultural Sciences, Uppsala. I compared the effects of exposure to commercially available synthetic medical mask derived macro- (60 mm) and microplastics (3 mm), and associated leachates (virgin vs. pre-leached), with wood shavings on microbial-driven leaf litter decomposition. Dried alder leaves were enclosed in cotton bags (0.5 mm mesh) which were submerged and incubated in the pond for 2, 7, 14, 21 or 34 days. For treatment bags, 0.2 g of macroplastics or MPs, or wood shavings were added, equating to approximately 23,000 p/kg of microplastics, 57 p/kg of macroplastics and 5,000 p/kg of wood shavings.

3.2 Measurements

3.2.1 Microbial community and processes (**paper II-IV**)

Water column microbial abundance was assessed through analytical flow cytometry (CytoFLEX, Beckman Coulter); where samples were stained with SYBR Green® and filtered through a 20 µm sieve prior to analysis (**paper II & III**). Microbial exoenzyme activity, specifically of β-glucosidase and chitinase, was assessed using methylumbelliferone (MUF)-linked substrates according to Renes et al. (2020) (**paper II**). Briefly, water samples from microcosms were incubated with MUF-linked substrates at saturating conditions, after which the reaction was terminated with glycine buffer and the fluorescent signal measured at λex/em = 355/460 nm (Hidex Sense microplate reader). Microbial community composition was assessed through 16s rRNA amplicon sequencing with the Illumina Miseq platform, with amplicons aligned and annotated using the DADA2 pipeline (**paper II**). In **paper IV**, I measured lead litter fungal biomass based on ergosterol content of leaf disks (Nylund and Wallander, 1992).

3.2.2 Macroinvertebrate consumers (**paper I-III**)

For **paper I**, **II** and **III**, macroinvertebrates were collected at the end of the experiment and survivorship assessed. Growth was assessed through quantification of dry biomass and body length, with the exception of snail growth that was assessed through change in wet mass. Body condition of chironomids was assessed by measuring body lipid content (**paper II**; as in Reznick, 1983). Stable isotopes and C:N stoichiometry of invertebrates and FPOM resources were also tested in **paper III**, to detect possible shifts in dietary sources and resource quality (McKie et al., 2023).

3.2.3 Ecosystem functions (**paper I-IV**)

Three ecosystem functions were assessed in this thesis: First, respiration was assessed using a modified dark-light bottle method (Johnson, Tank and Dodds, 2009) as detailed in (Kong et al., 2023; **paper I**), where microcosms or vessels were filled with oxygen-saturated water, sealed and incubated in the dark. Respiration was determined to be the difference in dissolved oxygen (DO) before and after incubation, measured suing Firesting O2 probes (Pyroscience). This was used to assess total ecosystem respiration (**paper I & II**) and leaf litterbag respiration (**paper IV**). For **paper III**, the microcosm systems were not sealed for respiration measurements. Rather, DO concentration was measured continuously during a period that included the transition from light and dark periods, with DO change during the light period used as an estimate of net ecosystem metabolism (NEM; Johnson, Tank and Dodds, 2009) and the DO change during the dark period used as an estimate of ecosystem respiration (ER). Gross primary production (GPP) was calculated as the sum of NEM and ER. Second, chlorophyll-a concentration was measured as a proxy for algal biomass (Huot et al., 2007), using fluorescence analysis for the water column at λex/em = 444/680 nm (Hidex Sense microplate reader; **paper II & III**) and a BenthoTorch (bbe Moldaenke) for the benthic biofilms (Kahlert and McKie, 2014; **paper III**). Third, decomposition was assessed based on loss of leaf litter mass that were deployed in experimental microcosms (**paper III**) or in cotton litterbags deployed in the field (**paper IV**). The field study (**paper IV**), included an additional measure of cellulose decomposition by measuring the tensile strength loss of strips cut from cotton litterbags used for the litter decomposition assays, in a similar manner to the use of cotton strips as a decomposition assay (Tiegs et al., 2013).

3.3 Data Analyses

The main statistical method used in this thesis was linear mixed model (LMM) analysis, and the significance of factors manipulated in the experiments tested using an analysis of variance (ANOVA) approach, including fitting of one or more random effects accounting for background variation associated with spatial or temporal blocking design elements of the experiments. In **paper II** and **IV**, a nested fixed effect design was used to assess affects associated with our particle characteristic treatments, which were nested within plastic presence. For post-hoc analysis, **paper I** and **III** used t-tests, but due to the nested analysis of **paper II** and **IV**, log response ratios were used instead to explore differences among levels of main or nested fixed effects identified as significant in LMMs.

For the microbial community multivariate analysis in **paper II**, non-metric multi-dimensional scaling (NMDS) and permutational multivariate analyses of variance (PERMANOVA) were used to visualise community dissimilarities and identify dominant factors driving community structure.

Data were split into groups based on the dominant non-MP factors and linear discriminant analysis (LDA) used to highlight differences in microbial relative abundances driven by MP presence within these groups.

All statistical analyses were conducted using R v4.3.1 (R Core Team, 2023) through RStudio v3.62. R packages used were *lme4* to construct LMMs (Bates et al., 2015) and *lmerTest* to calculate p-values for ANOVAs of LMMs (Kuznetsova, Brockhoff and Christensen, 2017). All graphs were made with *ggplot2* (Valero-Mora, 2010).

4. Results and discussion

4.1 Effects of exposure: Key microplastic properties

4.1.1 Microorganisms

Across **paper I–III**, I detected several effects of MPs on microbial abundance, enzyme activity and community composition, with effect sizes ranging from 1.2 to 42.6 %. Most of these effects can be related to MP properties associated with (i) increased available surface area of fine particles in the system, namely high MP concentrations and presence of MP fragments (**paper II & III**), and/or (ii) the role of MPs as a novel substrate (**paper II**). For example, I found that water microbial abundance was increased when exposed to the high concentrations of MPs, but only in the presence of macroinvertebrates (Figure 4). The increased surface area associated with the high concentration of MP particles can promote biofilm formation rates (Taylor et al., 1998; Hossain et al., 2019), which is further facilitated by macroinvertebrate activity that cycles nutrients and suspends settled MPs and other particulates into the water column (Hölker et al., 2015; Malli et al., 2022). Additionally, exposure to fragment MPs, which have the highest surface-area-to-volume ratio of all the MP shapes I investigated, was associated with a small increase (1.2 %) in β-glucosidase activity, a key enzyme involved in carbon breakdown, that contributes towards leaf litter decomposition (**paper II**).

I detected that exposure to the realistic concentration of MPs resulted in decreased water microbial community richness (**paper II**). This was further reflected by changes in the relative abundances of microbial taxa associated

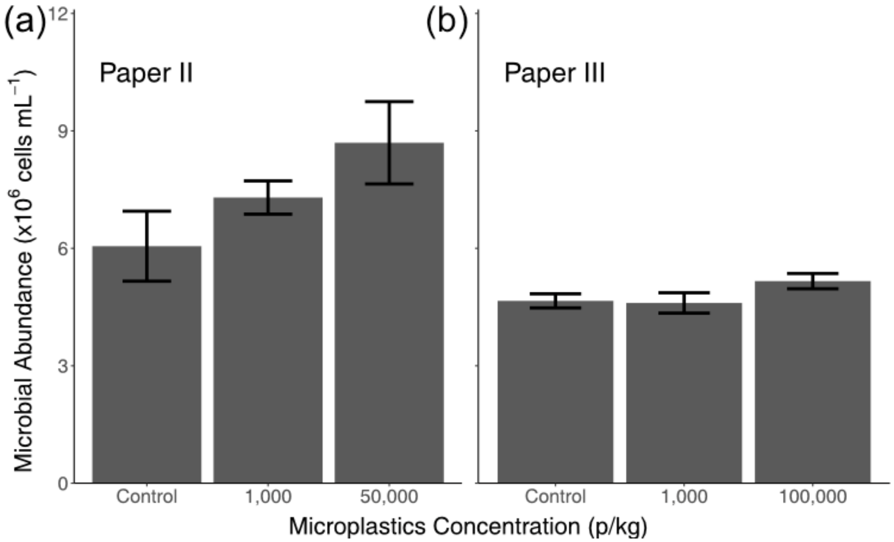


Figure 4. Mean (± se) of water column microbial abundance when exposed to different concentrations of microplastics in (a) **paper II** and (b) **III**. Data for **paper II** was pooled across all the different MP shapes and polymers used, and **paper III** used a mixture of PET microplastic fibre and fragments. Data presented here was from sampling on the 21st day for **paper II** and 110th day for **paper III**.

with plastic degradation in the presence of MPs (**paper II**), including the genera *Pseudomonas* and *Flavobacterium* which include species known to degrade the polymers used in my study (Gambarini et al., 2021). Thus, MP particles may provide a novel niche supporting microbial taxa with genes encoding for plastic-degrading enzymes, and thus able to derive energy from plastic polymer breakdown (Quero and Luna, 2017; Gambarini et al., 2021).

4.1.2 Macroinvertebrates

MP exposure was associated with both decreases and increases in macroinvertebrate growth and development. I observed reduced chironomid larvae body length when exposed to spherical PE MPs (Figure 5a) and reduced *V. viviparus* snail growth when exposed to PET fibre and fragment MPs (Figure 5b). MP exposure has been previously found to lead to reduced growth in macroinvertebrates (Redondo-Hasselerharm et al., 2018; Ziajahromi et al., 2018), potentially due to food dilution from the contamination of resources with nutritionally-void MPs (Foley et al., 2018). The food dilution effect refers to the presence of a nutritionally poor or void

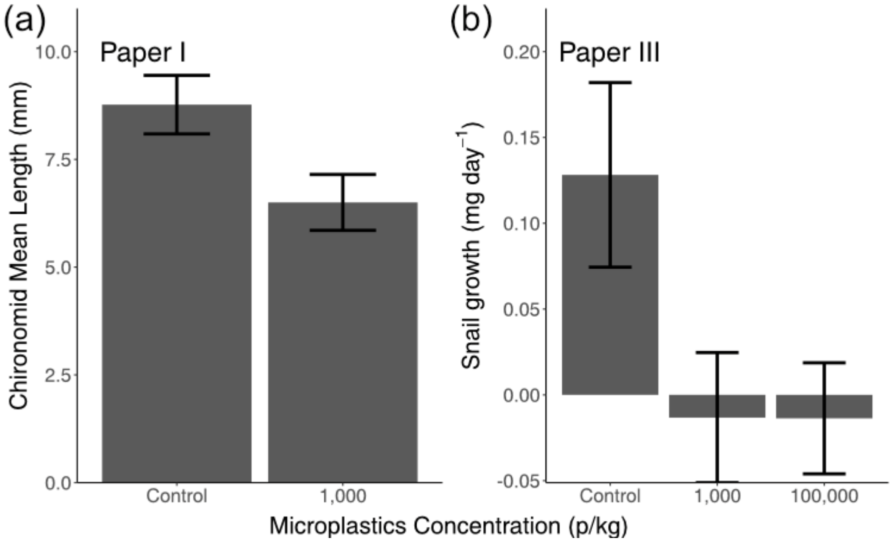


Figure 5. Mean (± se) of (a) chironomid mean body length when exposed to the low concentration of PE microplastic spheres (**paper I**), and (b) snail growth when exposed to different concentrations of a mixture of PET microplastic fibre and fragments (**paper III**). **Paper I**'s study design only included one MP concentration treatment. **Paper III** used a mixture of PET microplastic fibre and fragments.

material within dietary resource pools that cannot be easily filtered out (Lee, Raubenheimer and Simpson, 2004), leading to an overall reduction in nutritional value of resources. Furthermore, reduced organism growth in response to MP exposure may suggest that organisms are allocating energy to processes other than somatic growth (Kooijman, 2010). For example, MP exposure may cause oxidative stress in organisms, resulting in the upregulation of the antioxidant system (Trestrail, Nugegoda and Shimeta, 2020).

As opposed to the negative effects of MP exposure, I also observed increased chironomid larvae biomass when exposed to MP fragments and increased chironomid lipid content when exposed to a high concentration of MPs (Figure 6a). These MP responses from chironomids contrast with the reduced chironomid growth observed in **paper I**, and may be related to differences in MP size ranges used, where **paper I** (⌀ 45-53 µm) used a narrower range of MPs compared to **paper II** (⌀ 25-63 µm). Additionally, isopod populations demonstrated increased development rates when exposed to increasing MP concentrations, resulting in higher final abundances of medium-sized relative to small-sized individuals (Figure 6b). As discussed

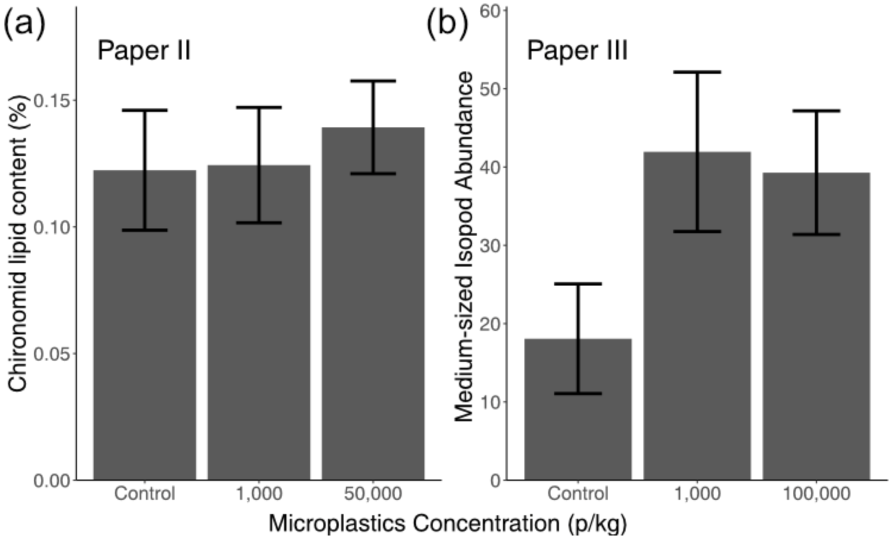


Figure 6. Mean (± se) of (a) chironomid body lipid content when exposed to different concentrations of microplastics. Data for **paper II** was pooled across all the different MP shapes and polymers used. (b) Mean ± se of medium-sized isopod relative abundance when exposed to different concentrations of a mixture of PET microplastic fibre and fragments (**paper III**).

in the previous section, MP presence can facilitate increased microbial abundance and enzyme activity within both benthic and pelagic habitats, through the growth of biofilms on particle surfaces. Consequently, the increased microbial biomass present may be consumed by shredders and particle consumers, which rely on surficial biofilms for a major fraction of their diet as compared to the comparatively nutrition-poor detrital substrates (Cummins and Klug, 1979; Findlay et al., 2002).

My results demonstrate some taxa-specific effects of MP exposure on macroinvertebrates, such as the contrasting responses of *V. viviparus* snails and *A. aquaticus* isopods raised in the same aquariums in **paper III** (Figure 5b & Figure 6b). *V. viviparus* snails may be more susceptible to the effects of MP exposure due to their ability to filter feed and graze benthic biofilms, potentially increasing the MP load that is ingested by snails relative to isopods. The ingestion of MPs may lead to increased metabolic costs related to particle expulsion, and may also affect snail respiration efficiency due to their filter feeding mechanism, which captures food particles on the surface of internal gills before transporting them to the mouth (Höckelmann and Pusch, 2000). I further detected altered elemental ratios of snails in response to MP exposure, with C:N ratios increasing with MP concentrations at 16 °C. I hypothesised that this finding may be due to changes in snail nutrient assimilation efficiency, which is enhanced for carbon-rich carbohydrates when metabolic demand increases (Croll and Watts, 2004; Carreira et al., 2016, 2020). More research is required to assess this hypothesis, but if confirmed then this indicates that effects of MP exposure on the dietary intake of *V. viviparus* snails may have consequences for organism growth.

Conversely, I found that the C:N ratio of first cohort isopods (initial isopods added into microcosms) decreased when exposed to the high MP concentration (**paper III**), which I hypothesise might signify reduced carbon-rich lipid accumulation in favour of faster development (Lau, Goedkoop and Vrede, 2013). *A. aquaticus* isopods can tolerate high proportions of highly refractory organic carbon in their diet, which is often further mixed with large quantities of inorganic sediment (Moore, 1975). The need to process these materials might afford *A. aquaticus* a greater capacity to limit negative physical effects of ingesting highly refractory MPs whilst utilising the surficial biofilms thereupon, resulting in higher growth and altered body composition. Together, my results highlight the potential for different taxa to respond differently to MP effects, which may be influenced by feeding modes and natural dietary tolerances for refractory materials.

4.1.3 Ecosystem Functions

In systems without macroinvertebrates, ecosystem respiration was reduced in the presence of a realistic concentration of PE spheres (Figure 7a), which I hypothesise may be due to changes in microbial community composition and functioning related to MP exposure (Yang et al., 2020; Huang et al., 2021). Additionally, I observed increased ecosystem respiration in the presence of a realistic concentration of PET fragments and a high concentration of PP fragments or fibres in systems without macroinvertebrates (presented as part of overall pooled data, Figure 7b). I hypothesise that is due to increased available surface area provided by MP particles, promoting microbial activity and respiration. Water chlorophyll-a content, as a proxy for primary production, was reduced when exposed to the high concentration of PP MPs in macroinvertebrate-free microcosms (**paper II**). This response may be due to the formation of hetero-aggregates between algae cells and PP MPs, leading to sedimentation and thus lower concentration of algae cells in the water column, which is a process that has

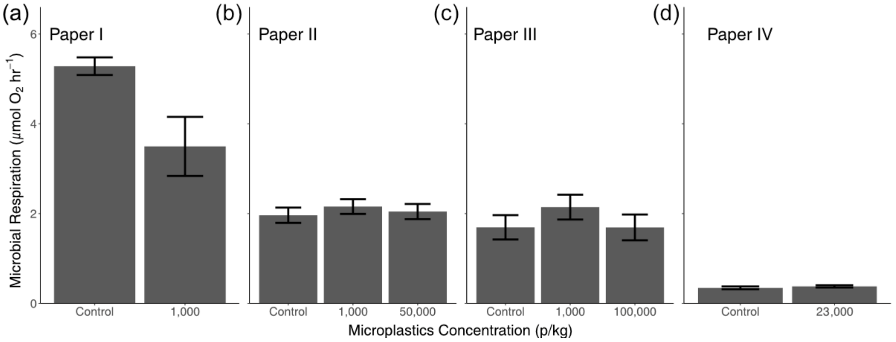


Figure 7. Mean (± se) of microbially-mediated ecosystem respiration when exposed to different concentrations of microplastics for (a) **paper I**, (b) **II**, (c) **III** and (d) **IV**. All data, except for **paper III**, was for treatments in the absence of chironomids. **Paper I**'s study design only one concentration of PE microspheres, and the data presented here was only for microcosms under the high quality resource treatment. Data for **paper II** was pooled across all the different MP shapes and polymers used. **Paper III** used a mixture of PET microplastic fibre and fragments, and included macroinvertebrate presence as part of the experiment design. **Paper IV** used a high concentration of PP MP fragments.

been observed in a previous study (Lagarde et al., 2016). In the field, I found that the presence of unleached MPs results in increased microbial cellulose decomposition but not leaf litter decomposition. I hypothesize that MP leachates stimulate activity of microbes growing on simpler organic substrates (i.e. cellulose), but not of the more complex, fungal dominated communities growing on leaf litter (**paper IV**). This stimulating effect may be driven by the presence of bioavailable nutrients in plastic leachates, which can contain bioavailable dissolved organic matter or other limiting nutrients (Romera-Castillo et al., 2018, 2022; Sheridan et al., 2022).

In the presence of macroinvertebrates, there were no detectable effects of MPs on ecosystem respiration or water chlorophyll-a concentration in **paper I** or **II**. Additionally, microbial-driven ecosystem respiration in **paper III**, which was in the presence of macroinvertebrates, was not affected by MP exposure (Figure 7c). However, in **paper III** also, I observed an increase in benthic algae biomass in the presence of the realistic concentration of a mixture of fragment and fibre PET MPs, which was primarily attributable to an increase in green algae concentration, and a subsequent decrease at the high concentration (Figure 9a). While MPs have been shown to have negative effects on microalgae growth (Liu et al., 2020) and lipid composition (Guschina, Hayes and Ormerod, 2020), it has also been shown that phytoplankton can adapt to MP exposure and undergo enhanced growth

(Mao et al., 2018). MP presence has also been shown to increase the growth of microalgae *Raphidocelis subcapitata*, potentially serving as a growth substrate (Canniff and Hoang, 2018). Thus, the observed effects of a realistic concentration of MPs on benthic algae biomass may be driven by the increased surface area on the benthos provided by the settling of the dense PET MPs (1.34 g cm^{-3}) used in **paper III**. At the high concentration, the inhibition of benthic algae biomass may be driven by a shading effect from increased abundance of pelagic microbes and MP particles that have been resuspended into the water column (Vadeboncoeur, Lodge and Carpenter, 2001; Zhao et al., 2019).

4.2 Environmental and anthropogenic sources of stress: Contrast with microplastic exposure

4.2.1 Natural sources of variation

In this thesis, the natural sources of variation tested (resource quality, presence of natural refractory particles and consumer presence or density) produced the expected responses from organisms and ecosystem functions. I found that the provision of a low quality FPOM resource reduced chironomid growth (Figure 8a) and ecosystem respiration (**paper I**). The quality of FPOM in the environment can vary due to differences in composition and microbial conditioning, with potential consequences for consumers, e.g., FPOM that contains high fractions of refractory material, such as lignin and cellulose (Ward and Aumen, 1986), is a relatively poor resource for macroinvertebrates (Callisto and Graça, 2013). Refractory particles can also affect microorganism growth, as shown in **paper IV**, where exposure to refractory wood shavings led to negative fungal biomass accrual in leaf litter. The microbial breakdown of wood is slow due to its high lignin and cellulose content, and thus wood may act as a physical barrier to microbial growth (Vargo, Neely and M. Kirkwood, 1998; Canhoto and Graça, 1999). Additionally, wood may release compounds that are naturally produced by plants as a defence mechanism against insect herbivores (Salminen and Karonen, 2011) and microbial infection (Scalbert, 1991), such as tannins (Anttila et al., 2013). Thus, I hypothesise that the observed decreased fungal growth and leaf litter decomposition may be caused by the barrier effect of wood and the leaching of antimicrobial compounds (**paper IV**). This effect

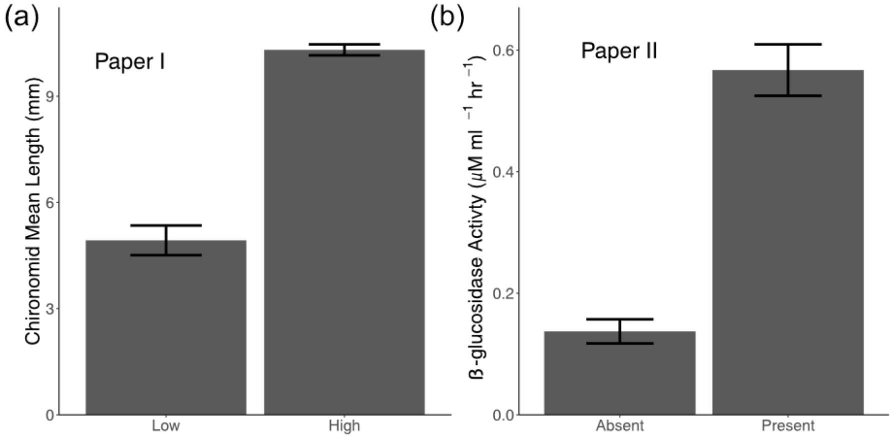


Figure 8. Mean (± se) of (a) chironomid mean body length when provided different food qualities (**paper I**) and (b) microbial β-glucosidase activity in the absence and presence of chironomids (**paper II**).

may also have knock-on effects for shredder invertebrate growth, due to their dependence on fungi as a major dietary resource (Danger et al., 2012).

In **paper II**, I found that chironomid presence increased β-glucosidase enzyme activity (Figure 8b), microbial abundance, and microbial-driven ecosystem respiration, and decreased water microbial taxa richness. The presence of chironomids increases nutrient availability and nutrient cycling in freshwaters, through the release of soluble nutrients (e.g., carbon, nitrogen and phosphorus) in their excretions (Fukuhara and Sakamoto, 1987) and bioturbating activity within their dwelling tubes (Svensson, 1997). Thus, chironomids drive an increase in nutrient availability that microorganisms can utilise for growth and processes. Chironomids also actively graze on microbial biofilms in and around their dwelling tubes (Mason and Bryant, 1975; Johnson, Boström and van de Bund, 1989), further altering microbial communities and their functions (Yeager, Foreman and Sinsabaugh, 2001). Additionally, I found that higher chironomid density reduced chironomid growth (**paper I**), likely due to an increase in the degree of intraspecific competition. *C. riparius* larvae have been previously shown to respond to reduced space and resources, as might be caused by high competition, by slowing down development and growth (Hooper et al., 2003).

4.2.2 Anthropogenic stressors

Beyond natural sources of variation, I also compared the effects of MP exposure against other anthropogenic stressors, warming (simulating anthropogenic global warming scenarios) and macroplastic exposure. In **paper III**, warming was shown to accelerate isopod development, specifically at 3 °C of warming above 16 °C, with 16 °C being the temperature at which test organisms were acclimated prior to the experiment. This corresponds with the predictions of the metabolic theory of ecology, where increased metabolic activity at warmer temperatures drive increased organism development rates (Brown et al., 2004). Additionally, warming enhances microbial decomposition activity (Boyero et al., 2011), increasing the bioavailability of nutrients bound in refractory substrates. However, I found that isopod development was reduced at 6 °C of warming (**paper III**), possibly indicating that the temperature optima of isopods had been exceeded. When exposed to temperatures outside the temperature optima range, organisms may suffer negative effects such as reduced growth due to high metabolic demand, protein deformation and other negative physiological effects (Ward and Stanford, 1982; Mckie, 2004). For *A. aquaticus* specifically, previous research has shown that newly hatched individuals have a temperature optima between 14.5 and 18.8 °C, after which growth rate decreases (Roshchin and Mazelev, 1979). Furthermore, I found that warming over the same temperature range led to a U-shaped response for total benthic algae biomass (Fig 8a). This response from benthic algae might reflect a temperature-driven increase in algae consumption by isopods at 3 °C of warming, with algae being a known part of *A. aquaticus* diets (Moore, 1975). At 6°C of warming, the increase in benthic algae biomass (relative to 3 °C of warming) may be driven by lower isopod feeding pressure due to temperatures surpassing their temperature optima.

MPs are only one size-class of plastic waste, with other plastic size-classes, including macroplastics, abundant in the freshwater environment (Battulga, Kawahigashi and Oyuntsetseg, 2019; Blettler et al., 2019), and likely to interact with and impact freshwater organisms and ecosystem functioning. In **paper IV**, I found that macroplastic exposure decreased fungal biomass accrual on leaf litter in the field. Macroplastics may act as physical barriers to fungal conidia attachment on leaves or limiting fungal hyphae expansion and growth (Aleklett et al., 2021). Additionally, the plastics used in **paper IV** were found to contain slip agents, which can further reduce the biofouling rate on plastic surfaces by reducing friction (Getachew et al., 2016).

4.2.3 Comparing effect sizes

Generally, I found that the effects of naturally-occurring sources of stress on microorganisms, macroinvertebrates and ecosystem functions often had larger effect sizes (±11-473 %) compared with MP exposure (±1-89 %). The most striking comparison was the effect of high resource quality on chironomid growth and microbial-driven ecosystem respiration, which were approximately one order of magnitude higher than MP effects on these responses (Table 2). Additionally, microbial community composition was more strongly driven by chironomid presence, habitat (sediment vs. water) and time than MP exposure (**paper II**), although MPs did elevate the relative abundances of certain microbial taxa. Furthermore, the effect of wood exposure on leaf litter fungal biomass accrual was also almost tenfold higher than the effect of MP exposure (Table 2). I hypothesise that the stronger effect of wood may relate to the leaching of natural antimicrobial compounds, while the disposable facemasks, which the MPs were derived from, generally do not contain antimicrobial compounds as they are mainly used as a physical barrier against aerosol transmission (Rengasamy et al., 2017). The effects of wood on fungal biomass observed here is also shown to occur in the presence of kaolin clay particles, which led to a decreasing trend in fungal biomass and reduced fungal sporulation rates (Sanpera-Calbet, Chauvet and Richardson, 2012). Together, these results highlight that while MP particles have effects on fungi communities that are different from natural refractory particles, the effects of natural particles are not necessarily benign and may even have negative effects on fungal growth.

For anthropogenic stressors, the effect sizes (±8-97 %) of the degrees of warming I manipulated, 3–6 °C, generally had a comparable range to those of MP exposure (±11-142 %; Table 2). In **paper III**, MP exposure increased isopod development (Figure 6b), potentially due to higher food quality and availability through increased biofilm growth facilitated by the additional surface area from the high concentration of MP particles (Cummins, 1974; Huang et al., 2021). Warming had similar outcomes as with MP exposure on isopod development, but only up to 3 °C of warming above 16 °C, beyond which the thermal optima of isopods may be exceeded (**paper III**). Additionally, the U-shape response of benthic algae biomass to warming

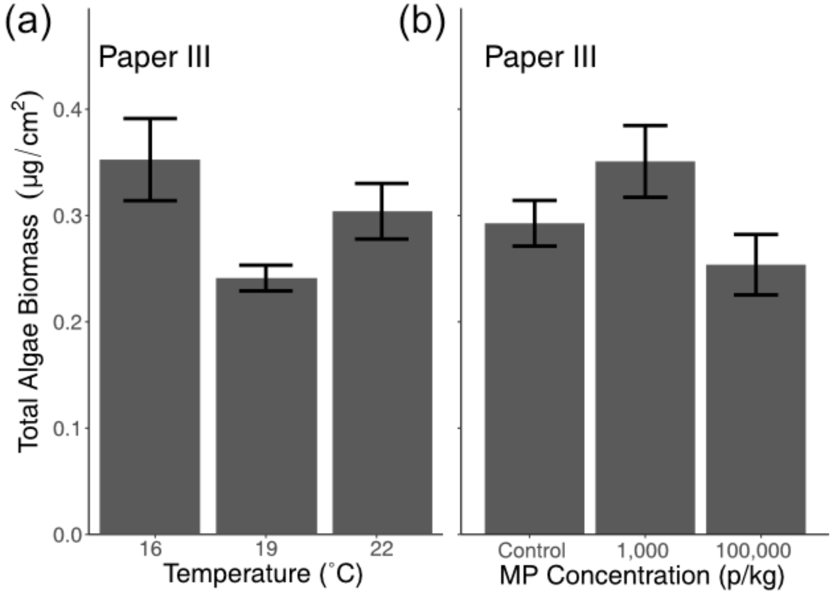


Figure 9. Mean (± se) of total algae biomass when exposed to different (a) temperatures and (b) concentrations of a mixture of PET microplastic fibre and fragments in **paper III**.

(Figure 8a) is mirrored by the hump-shaped response from benthic algae biomass (Figure 9b). As discussed in the previous section, warming may alter benthic algae biomass through changes in isopod consumption of algae. The effects of MPs on benthic algae biomass may instead be driven by the increased dominance of pelagic relative to benthic microbes, as indicated by the observed increase in water microbial abundance in **paper III**. An increase in pelagic microbial abundance increases shading of the benthos and nutrient competition with benthic microbes, likely resulting in reduced benthic algae biomass.

The other anthropogenic stressor I tested, macroplastic exposure, decreased fungal biomass accrual with effect sizes that were approximately two-fold higher than that of MP exposure (Table 2). Macroplastics form a continuous barrier compared to the dispersed MP particles, and may thus have a stronger barrier effect on fungal growth.

In this thesis, I assessed two anthropogenic stressors, but freshwater ecosystems are exposed to a much wider range of anthropogenic stressors, with different magnitude of effects relative to MPs. Previous research has demonstrated that other anthropogenic stressors, including the insecticide

Table 2. Comparison between effect sizes represented by percentage change relative to controls of non-MP sources of stress and MP treatments. Only measures that responded to both non-MP sources of stress and MP exposure were included, and the higher degree of change between the two comparisons bolded. All responses reported here were statistically significant ($p<0.050$).

Response	**Change (%)**	
	Other stressors	MPs
Natural sources of variation		
Paper I - High quality resource		
Microbial respiration	**335**	-26.2
Chironomid length	**245**	-26.7
Paper II - Chironomid presence		
Microbial abundance	**65.1**	42.6^{+chiro}
Water microbial richness	**-16.8**	-9.3
β-glucosidase enzyme activity	**313**	-1.2
Ecosystem respiration	**34.8**	12.6
Water chlorophyll-a concentration	**228**	44.8
Paper IV – Wood shavings		
Fungal biomass accrual	**-119**	-18.8
Anthropogenic Stressors		
Paper III - Warming		
Medium-size isopod abundance	96.9	**142**
1st cohort isopod C:N ratio	-8.6	**-10.9**
Benthic algae biomass	**-31.5**	-13.4
Paper IV - Macroplastics		
Fungal biomass accrual	**-53.5**	-18.8

$^{+chiro}$ effect only observed in the presence of chironomids

dimethoate (Horton et al., 2018) and inorganic copper (Weber et al., 2021), have larger effects sizes on invertebrate survival, reproduction and mobility, relative to MP exposure. Thus, more research is needed to assess the relative severity of MP exposure compared with a broader range of anthropogenic stressors.

4.2.4 Interaction between MPs and other stressors

The natural sources of variation tested, chironomid presence or density, often modified the effects of MP exposure. The effect of MP exposure on

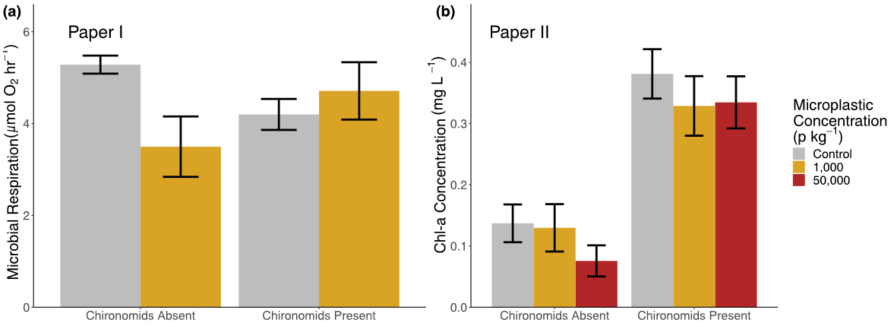


Figure 10. Mean (± se) of (a) microbial respiration under the influence of chironomid presence and PE microplastic presence (**paper I**). Mean (± se) of (b) water chlorophyll-a concentrations under the influence of chironomid presence and exposure to different concentration of PP microplastics (**paper II**). **Paper I**'s study design only one concentration of PE microspheres. Data for **paper II** was pooled across all the different MP shapes and polymers used.

ecosystem respiration was only detected in the absence of chironomids (Figure 10a), which suggests that chironomids and their activities, which release nutrients from the benthos, dampen the subtler effects of MP exposure on ecosystem functioning. Additionally, I found that chironomid presence reverses the effects of MP exposure on microbial abundance (**paper II**). In the absence of chironomids, exposure to PP MPs reduced microbial abundance, but in the presence of chironomids, exposure to the high concentration of MPs increased microbial abundance (Figure 10**b**). These chironomid-mediated responses of microorganisms and ecosystem functions are likely driven by increased nutrient availability in the water column due to the bioturbating activities of chironomids that drive nutrient cycling and the resuspension of benthic particles, including microbial cells and MPs (Figure 11; Hölker et al., 2015; Malli et al., 2022). Additionally, the effects of MPs on chironomid growth were only observed at the low chironomid density (**paper I**). I hypothesise that low chironomid density results in lower rates of nutrient cycling and faecal particle production, which may exacerbate the negative effects of MP exposure despite the lower level of intraspecific competition. Taken together, these results highlight the importance of nutrient availability and microbial conditioning for the detection of MP exposure effects.

For anthropogenic stressors, I observed interactions between MP exposure and temperature (simulating anthropogenic global warming

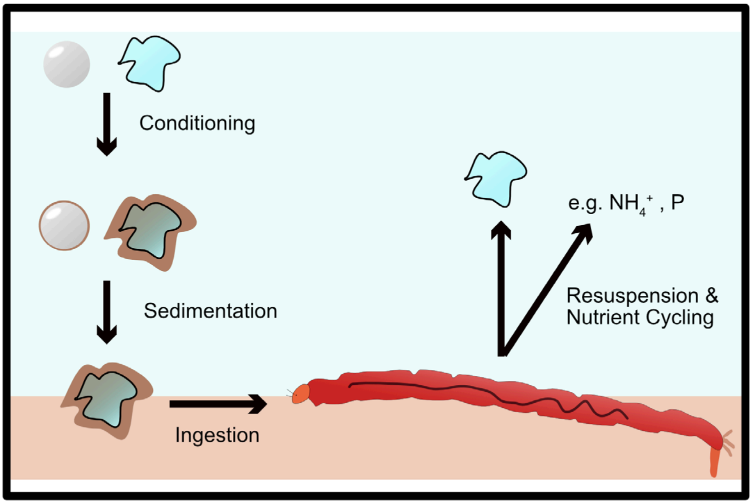


Figure 11. Conceptual figure illustrating a potential pathway of microplastic sedimentation and resuspension. Microplastics with higher surface-area-to-volume ratios (e.g. irregular fragments compared to uniform spheres) are likely to have higher microbial biofilm growth and volume during conditioning, which increases the sedimentation rate of particles. Subsequent encounters and ingestion of particles by benthic macroinvertebrates such as the particle-feeding *Chironomus riparius* non-biting midge larvae would strip particles of biofilm. Consequently, larvae helps resuspend microplastics back into the water column after egestion, alongside driving nutrient cycling in freshwater ecosystems.

scenarios) on the responses of ecosystem GPP:ER and snail C:N ratios (**paper III**). Generally, GPP:ER ratios had a U-shaped pattern over time, indicating a temporary shift towards heterotrophy before bouncing back towards the initial levels of autotrophy. I found that MP exposure resulted in a more sustained shift towards heterotrophy, particularly at the realistic concentration at 16 °C, and the high concentration at 3 and 6 °C of warming above 16 °C. MPs may favour a shift towards heterotrophy by supporting elevated fractions of heterotrophic microbes. This is supported by the observation of increased pelagic microbial abundance at the high MP concentration with no corresponding increase in water chlorophyll-a concentration. MP particles have also been previously recorded to support elevated activity of heterotrophic microbes (Arias-Andres et al., 2018).

Additionally, MP exposure appears to alter the response of snail C:N ratios to warming. In the absence of warming, snail C:N ratios increase with warming, potentially indicating increased carbohydrate intake to fulfil metabolic demand (Croll and Watts, 2004; Carreira et al., 2016), and increased lipid accumulation due to a stress-induced shift away from lipid metabolism (Díaz-Morales et al., 2023). The presence of MPs at high concentrations appears to reverse this response, where C:N ratios are highest at cooler temperatures but decreases with warming. Results from **paper III** highlight the potential for complex outcomes from co-exposure to MPs and warming, but the consequences of these interactions for organism growth and other aspects of ecosystem functioning remain unclear.

While I have discussed various instances where the effects of MPs are often overshadowed or modified by naturally occurring sources of stress and other anthropogenic stressors, I also observed effects on some organisms and ecosystem functions that were exclusive to MP exposure. In **paper III**, I found that MP exposure alone had an effect on microbial abundance and snail growth, independent of warming. These results point towards key properties of MP particles and associated mechanisms of action that are able to affect certain biota differently from other stressors. This may e.g. include the role of MPs as a buoyant, refractory, and novel growth substrate, which can continually migrate between the benthos and water column as particle density changes due to biofilm growth (Fig 8; Li et al., 2023). Overall, the natural sources of variation tested here appear to have larger effects on ecosystems relative to MPs exposure, while the effect sizes of warming were comparable to MP exposure. Even so, I highlight the potential for MP exposure to have distinct responses compared to natural refractory particles and anthropogenic warming.

4.3 Ecological complexity and magnitude of microplastic effects on microbial communities and ecosystem functions

While I did not explicitly test for the effects of ecological complexity in my studies, the experiment designs allow for certain comparisons of trophic diversity and environmental complexity (Figure 12). For *comparison 1*, the chironomid-free microcosms in **paper I** and **II** are paired with **paper IV** to compare microbial responses to MP exposure, and the propagation of effects to ecosystem functions, across systems varying in *environmental complexity* (laboratory vs field). **Paper IV**, in which I excluded macroinvertebrates, was

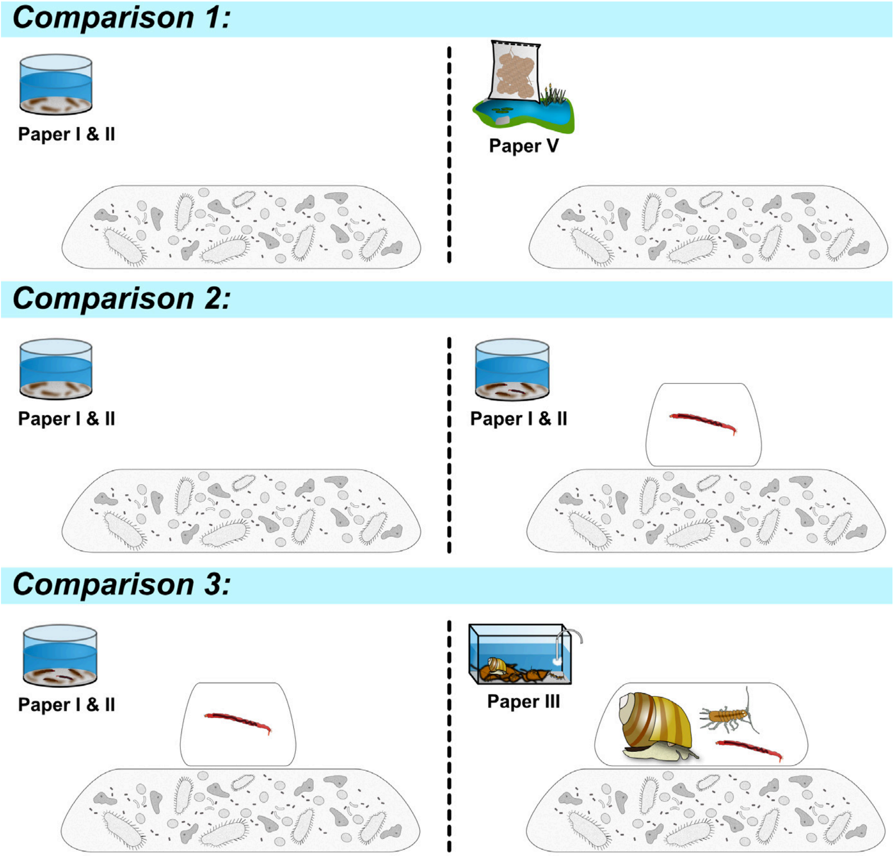


Figure 12. The comparisons of environmental complexity and trophic diversity across different experiments made in section 4.3. *Comparison 1* relates to the differences in environmental complexity, which refers to the level of control achievable within experimental systems, where simple single-species microcosm studies have lower complexity relative to multi-species microcosms or field systems. *Comparison 2* related to differences in vertical trophic diversity, which refers to the changes in the number of trophic levels. *Comparison 3* relates to differences in horizontal trophic diversity, which refers to changes in the number of taxa or functional groups within trophic levels.

conducted in the field. For *comparison 2*, the chironomid-free microcosms are compared with the chironomid-present microcosms in **paper I** and **II** to provide a direct, albeit simplistic, comparison of how increased *vertical trophic diversity* (i.e. more trophic levels; Jabiol et al., 2013) alters MP exposure effects on microbial communities, and if the propagation of effects from microbial communities to ecosystem functions is maintained. The presence of chironomids form a trophic level above microbial communities through their ingestion of microbial biofilms. For *comparison 3*, the chironomid-present microcosms in **paper I** and **II** were paired with **paper III** to compare responses of microorganisms and macroinvertebrates across increased *horizontal trophic diversity* (i.e. greater diversity of feeding groups; Jabiol et al., 2013). Due to the diversity of responses measured across studies, only relevant responses that have either direct or comparable surrogates between studies are mentioned in this section.

4.3.1 Comparison 1: Lab and field microbial responses

In the field, microbial communities are exposed to a wider range of environmental variation, including in temperature and hydrological regimes, and a more complex sediment matrix. In comparison, laboratory experiments subject microbial communities to stable controlled environments and may lead to responses that are less relevant in real-world environments outside the laboratory (Calisi and Bentley, 2009). Throughout my laboratory experiments, MP exposure often affected microbial-driven ecosystem respiration (Figure 7a-b), but this effect was no longer observed when microbial communities were incubated in the field (Figure 7d). Furthermore, a subset of MPs in the laboratory study of **paper II** and the MPs used in the field study of **paper IV** were made from the same material (PP medical facemasks), with exposure to these MPs in **paper II** resulting in increased microbial-mediated ecosystem respiration in the laboratory only. As discussed in the previous section, natural sources of variation, specifically food quality and consumer presence/density, often had stronger effects than MP exposure and further regulated the effects of MPs on organisms and ecosystem functions. Therefore, these environmental variables, or others that were uncontrolled for, are likely more important drivers of microbial respiration in the field than the presence of MPs. Nonetheless, this may not be true for all ecosystem functions as I observed MP effects on another ecosystem function, cellulose decomposition, in the field (**paper IV**).

4.3.2 Comparison 2: Chironomid presence in microcosms

The presence of chironomids altered the effect of MP exposure, especially at high concentrations, on microbial abundance. I hypothesise that this is driven by increased nutrient cycling from chironomid activity, which facilitates increased microbial biofilm growth on the surfaces of MP particles. Previous research has also detected increased biomass of sediment biofilm in the presence of MPs (Huang et al., 2021). In turn, higher microbial conditioning and biofilm growth is likely to facilitate increased feeding and growth of consumers, as seen in effects of microbial growth on leaf-consuming detritivores (Jabiol et al., 2013). In **paper II**, the high concentration of MPs, and the resulting elevated microbial abundance, was associated with higher lipid content in chironomid larvae. Higher lipid content is an indicator that chironomid larvae are ingesting better quality resources, which allows for more fat stores (Wei, Liao and Wang, 2016). The accumulation of both lipids and glycogen in chironomids occurs in preparation for development stages that have high energy requirements, such as pupation (Hamburger, Lindegaard and Dall, 1996). Thus, the responses of microorganisms to MP exposure appear to affect macroinvertebrate growth, through increased biofilm growth on food particles, and thus higher quality resources for consumers. Additionally, there was some evidence for direct effects of MP exposure on chironomids, which also had reduced growth when exposed to MPs in **paper I**. This response may be driven by the food dilution effect of MPs in the FPOM resources of chironomids (Redondo-Hasselerharm et al., 2018) or the increased metabolic costs associated with excreting MP particles (Foley et al., 2018).

The presence of chironomid larvae appears to dampen the effects of MP exposure on ecosystem functions. In the absence of chironomids, MP exposure altered water chlorophyll-a concentrations and microbial-driven ecosystem respiration, demonstrating the propagation of MP effects from the microbial community to ecosystem functions. In the presence of chironomids, I no longer detect any MP responses from ecosystem functions. As discussed above, chironomids drive nutrient cycling in freshwater ecosystems and these stronger effects of chironomid presence may dampen the propagation of MP effects from the microbial community to ecosystem functions.

4.3.3 Comparison 3: Horizontal trophic diversity

In the previous section, I hypothesised that macroinvertebrate-driven nutrient cycling facilitates increased microbial abundance in the presence of a high concentration of MPs. The ecosystem processes that contribute to nutrient cycling, such as decomposition and egestion, may be enhanced by increased biodiversity (Naeem et al., 1994; Tilman, Isbell and Cowles, 2014), particularly with gains in taxa that complement one another in resource use

(Fargione et al., 2007). Furthermore, the body mass of consumers is a dominant factor driving egestion rates (Allgeier et al., 2015; Vanni and McIntyre, 2016), and thus nutrient cycling. In **paper III**, I included additional macroinvertebrate functional feeding groups, isopod shredders and snail grazers, into the microcosms, in addition to the chironomid collectors used in **paper II**. These additional taxa are significantly bigger in terms of body mass, with the average body mass (±SE) of isopod and snails being 1.7 (±0.2) and 110 (±5) mg respectively, relative to 0.49 (±0.01) mg for chironomids. Thus, increasing the horizontal diversity of macroinvertebrates, particularly larger taxa with different niches and functional feeding groups, may increase the process rates conferred by macroinvertebrates and result in a higher magnitude of MP-driven increase in microbial abundance.

The magnitude of microbial abundance increase was higher in the simpler (**paper II**) than in the more complex trophic web (**paper III**). I found that MPs were associated with a greater increase (relative to the controls) in microbial abundance in **paper II** (42.6 %) compared to **paper III** (10.8 %). While the microcosms were smaller in volume for **paper II**, their ratio of total macroinvertebrate biomass to water volume was in fact lower (0.03) relative to the microcosms in **paper III** (0.04), indicating that the differences in microbial abundance increase is unlikely to be due to the size and water volume of microcosms.

These results indicate that species interactions in a community with a higher diversity of functional groups may suppress microbial productivity. Alternatively, differences in the starting conditions of the microcosms between the two experiments might have been important in influencing microbial productivity. Overall, these results highlight the need for more research on how trophic complexity regulates MP effects on ecosystems.

Through the three comparisons in this section, I draw some insights into the potential responses of natural freshwater ecosystems to MP exposure. *Comparison 1* demonstrated the laboratory-to-field attenuation of MP effects on ecosystem respiration, highlighting how MP effects may be dependent by environmental conditions that are not often seen in real-world environments, such as the complete absence of macroinvertebrate consumers, or stable thermal regimes. *Comparison 2* showed that the MP responses of microbial-driven ecosystem respiration and chlorophyll-a concentration appear to be dampened by chironomid presence, despite MP responses of the chironomid larvae themselves. *Comparison 3* highlighted the potential for increased horizontal trophic diversity modify the interactions between macroinvertebrates and MP particles, and the resulting outcomes on microorganisms. Together, these findings suggest that the effects of MPs in the environment are likely to be smaller than the effects found in laboratory studies. However, some MP effects on basal microbial communities and their functioning were still apparent in the more complex systems that I studied, including increased microbial abundance and cellulose decomposition. The potential for such changes to propagate and have knock-on effects on higher trophic levels and ecosystem functioning in real-world environments requires more investigation.

5. Conclusion and future considerations

5.1 Conclusion

There is an ongoing debate on the significance of the risk MPs pose to real-world environments (as demonstrated by Backhaus and Wagner, 2019). On one end, the opinion is that MPs *per se* do not constitute a significant risk in the environment due to the low concentrations recorded in real-world environments, and the limited scientific evidence demonstrating MP effects at these recorded concentrations (Burton, 2017). On the other end, the stance is that there is a high probability of negative consequences in the environment from MP pollution due to accumulative exposure over time and across broad spatial scales, and thus preventative action is warranted despite limited scientific evidence of MP effects in the environment (Rochman et al., 2015; Guerranti et al., 2019). Ultimately, arguments from both sides of the discussion are rooted in the lack of scientific evidence surrounding the effects of MPs on real-world ecosystems, which hampers scientific evidence based action on MPs.

Through this thesis, I aimed to improve our understanding of the effects of MP exposure on biota associated with the freshwater processing chain, and its potential implications for freshwater ecosystems as a whole. Furthermore, I aimed to investigate the drivers of MP effects, including particle properties and other factors, and contrast the effects of MPs against these other stressors. The main conclusions of this thesis are summarised as follows:

I. MPs impacted all ecological levels assessed, altering microbial community structure and growth, macroinvertebrate growth, development and body condition, ecosystem respiration and chlorophyll-a concentration.

II. MP effects were frequently driven by particle properties supporting increased microbial growth, including high particle concentrations and irregular fragment shapes that provide larger surface areas for biofilm growth, and leachate compounds that stimulate microbial activity.

III. Natural sources of variation and stress (resource quality, macroinvertebrate presence/density, presence of refractory organic material such as wood) often had higher effect sizes than MPs and often further regulated the effects of MP exposure.

IV. The anthropogenic stressors I studied (warming simulating anthropogenic global warming scenarios and macroplastic pollution) showed variable effects relative to MP exposure. Interactions between warming and MPs were few, but have the potential to result in complex ecosystem-level effects.

V. MP effects generally propagated from microbial communities, leading to effects on macroinvertebrates or ecosystem functions.

VI. The presence of macroinvertebrates appears to dampen the effects of MP exposure on ecosystem functions, regardless of MP effects on macroinvertebrates themselves.

Overall, the findings in my thesis suggest that many of the effects of MP exposure seen in laboratory studies may be less relevant in the real-world environment. However, I still detected MP effects associated with MP types and environmental conditions that correspond more closely to real-world environments, including realistic concentrations of environmentally common MP types, macroinvertebrate presence and most saliently, in field conditions. Thus, MPs have the potential to affect real-world freshwater ecosystems, albeit likely to a lesser extent than suggested by laboratory studies.

5.2 Future considerations and final thoughts

Microplastics are small refractory plastic particles, barely discernible to the human eye and easily transportable hydrodynamic and atmospheric currents, contaminating every environment known to man. The almost insurmountable challenge posed by MPs in global clean-up efforts is compounded by the continuing production of vast volumes of plastic waste, adding to that already accumulated in the environment. MPs have been detected in every tested environment, with average concentrations of 10 particles per m^3 of water or 1,000 particles per kg of sediment in freshwaters globally. These recorded environmental MP concentrations are low relative to the threshold concentrations where toxic effects are typically detected in freshwater organisms (Burns and Boxall, 2018). In my thesis, I find that MP effects were frequently associated with MP freshwater sediment concentrations one or two orders of magnitude higher than the global average. However, MPs are likely to increase over time as the existing pool of macroplastic waste in the environment continues to breakdown. Additionally, the detection and characterisation of MPs remain an ongoing challenge, as particles at the low end of the MP size range (⌀ < 50 µm) are particularly difficult to identify and characterise due to methodological limitations (Horton, Walton, et al., 2017; Caputo et al., 2021). Overall, the scale of MP pollution in the environment may be currently underestimated (Conkle, Báez Del Valle and Turner, 2018; Weber and Kerpen, 2023) and is unlikely to reduce in magnitude over time, unless decisive action is taken.

The insights derived from this thesis contribute to the ever-growing body of research surrounding the issue of widespread MP pollution. The frequency of MP effects on microbial communities are particularly interesting and warrant further research, as microbial communities form the base of the world's ecosystems and drive a major fraction of ecosystem functioning. Particularly, the changes in leaf litter fungal biomass observed in **paper IV** may reflect changes in fungal communities, and further investigation into this may better characterise the mechanism of MP effects on fungal communities. Additionally, I hypothesised that many of the effects detected throughout the experiments are likely to be driven by changes in microbial biofilm volume or growth. Thus, future research targeting the biofilms on MPs or on surfaces exposed to MPs, may help further inform the mechanisms behind some of my findings. Furthermore, future work may consider including additional trophic levels into their experiments, as my results suggest that MP effects have the potential to propagate across trophic levels. The findings in this thesis also motivates further research to investigate the interactions of MP exposure with other anthropogenic stressors, which would allow us to better understand the potential ramifications of MP pollution in environments that are affected by multiple anthropogenic stressors.

As a final note, MPs have gained significant attention in recent years, likely driven by a continuous stream of studies reporting the presence of MP particles in food, drink and human tissue. However, MPs are but one aspect of the continuum of plastic production and degradation. The extraction of fossil fuels provide the petrochemical raw materials that are used in the mass production of plastic products, which are subsequently discarded into the environment, existing as macroplastic pollutants, and then degrading over time into MPs and nanoplastics. There exist the risk of environmental pollution at every step of this process, and the effects of macroplastic exposure shown in this thesis highlight how MP pollution is one of many environmental issues that originate from the process of plastic production and disposal.

References

Abdolahpur Monikh, F., Baun, A., Hartmann, N.B., Kortet, R., Akkanen, J., Lee, J.-S., Shi, H., Lahive, E., Uurasjärvi, E., Tufenkji, N., Altmann, K., Wiesner, Y., Grossart, H.-P., Peijnenburg, W. and Kukkonen, J.V.K. (2023) 'Exposure protocol for ecotoxicity testing of microplastics and nanoplastics', *Nature Protocols*, 18(11), pp. 3534–3564. Available at: https://doi.org/10.1038/s41596-023-00886-9.

Akindele, E.O., Ehlers, S.M. and Koop, J.H.E. (2020) 'Freshwater insects of different feeding guilds ingest microplastics in two Gulf of Guinea tributaries in Nigeria', *Environmental Science and Pollution Research*, 27(26), pp. 33373–33379. Available at: https://doi.org/10.1007/s11356-020-08763-8.

Aleklett, K., Ohlsson, P., Bengtsson, M. and Hammer, E.C. (2021) 'Fungal foraging behaviour and hyphal space exploration in micro-structured Soil Chips', *The ISME Journal*, 15(6), pp. 1782–1793. Available at: https://doi.org/10.1038/s41396-020-00886-7.

Aljaibachi, R., Laird, W.B., Stevens, F. and Callaghan, A. (2020) 'Impacts of polystyrene microplastics on Daphnia magna: A laboratory and a mesocosm study', *Science of the Total Environment*, 705, p. 135800. Available at: https://doi.org/10.1016/j.scitotenv.2019.135800.

Amobonye, A., Bhagwat, P., Singh, S. and Pillai, S. (2021) 'Plastic biodegradation: Frontline microbes and their enzymes', *Science of The Total Environment*, 759, p. 143536. Available at: https://doi.org/10.1016/j.scitotenv.2020.143536.

An, L., Liu, Q., Deng, Y., Wu, W., Gao, Y. and Ling, W. (2020) 'Sources of Microplastic in the Environment', in D. He and Y. Luo (eds) Microplastics in Terrestrial Environments: *Emerging Contaminants and Major Challenges*. Cham: Springer International Publishing, pp. 143–159. Available at: https://doi.org/10.1007/698_2020_449.

Andrady, A.L. (2015) 'Persistence of Plastic Litter in the Oceans', in M. Bergmann, L. Gutow, and M. Klages (eds) *Marine Anthropogenic Litter*. Cham: Springer International Publishing, pp. 57–72. Available at: https://doi.org/10.1007/978-3-319-16510-3_3.

Andrady, A.L., Hamid, H.S. and Torikai, A. (2003) 'Effects of climate change and UV-B on materials', *Photochemical & Photobiological Sciences*, 2(1), pp. 68–72. Available at: https://doi.org/10.1039/b211085g.

Anttila, A.-K., Pirttilä, A.M., Häggman, H., Harju, A., Venäläinen, M., Haapala, A., Holmbom, B. and Julkunen-Tiitto, R. (2013) 'Condensed conifer tannins as antifungal agents in liquid culture', Holzforschung, 67(7), pp. 825–832. Available at: https://doi.org/10.1515/hf-2012-0154.

Arias-Andres, M., Kettner, M.T., Miki, T. and Grossart, H.P. (2018) 'Microplastics: New substrates for heterotrophic activity contribute to altering organic matter cycles in aquatic ecosystems', *Science of the Total Environment*, 635, pp. 1152–1159. Available at: https://doi.org/10.1016/j.scitotenv.2018.04.199.

Arias-Andres, M., Rojas-Jimenez, K. and Grossart, H.-P. (2019) 'Collateral effects of microplastic pollution on aquatic microorganisms: An ecological perspective', *TrAC Trends in Analytical Chemistry*, 112, pp. 234–240. Available at: https://doi.org/10.1016/j.trac.2018.11.041.

Au, S.Y., Bruce, T.F., Bridges, W.C. and Klaine, S.J. (2015) 'Responses of Hyalella azteca to acute and chronic microplastic exposures', *Environmental Toxicology and Chemistry*, 34(11), pp. 2564–2572. Available at: https://doi.org/10.1002/etc.3093.

Backhaus, T. and Wagner, M. (2019) 'Microplastics in the Environment: Much Ado about Nothing? A Debate', *Global Challenges*, 1900022, p. 1900022. Available at: https://doi.org/10.1002/gch2.201900022.

Baho, D.L., Bundschuh, M. and Futter, M.N. (2021) 'Microplastics in terrestrial ecosystems: Moving beyond the state of the art to minimize the risk of ecological surprise', *Global Change Biology*, 27(17), pp. 3969–3986. Available at: https://doi.org/10.1111/gcb.15724.

Bates, D., Mächler, M., Bolker, B.M. and Walker, S.C. (2015) 'Fitting linear mixed-effects models using lme4', *Journal of Statistical Software*, 67(1). Available at: https://doi.org/10.18637/jss.v067.i01.

Battulga, B., Kawahigashi, M. and Oyuntsetseg, B. (2019) 'Distribution and composition of plastic debris along the river shore in the Selenga River basin in Mongolia', *Environmental Science and Pollution Research*, 26(14), pp. 14059–14072. Available at: https://doi.org/10.1007/s11356-019-04632-1.

Benton, T.G., Solan, M., Travis, J.M.J. and Sait, S.M. (2007) 'Microcosm experiments can inform global ecological problems', *Trends in Ecology & Evolution*, 22(10), pp. 516–521. Available at: https://doi.org/10.1016/j.tree.2007.08.003.

Bertoli, M., Pastorino, P., Lesa, D., Renzi, M., Anselmi, S., Prearo, M. and Pizzul, E. (2022) 'Microplastics accumulation in functional feeding guilds and functional habit groups of freshwater macrobenthic invertebrates: Novel insights in a riverine ecosystem', *Science of The Total Environment*, 804, p. 150207. Available at: https://doi.org/10.1016/j.scitotenv.2021.150207.

Blarer, P. and Burkhardt-Holm, P. (2016) 'Microplastics affect assimilation efficiency in the freshwater amphipod Gammarus fossarum', *Environmental Science and Pollution Research*, 23(23), pp. 23522–23532. Available at: https://doi.org/10.1007/s11356-016-7584-2.

Blettler, M.C.M., Abrial, E., Khan, F.R., Sivri, N. and Espinola, L.A. (2018) 'Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps', *Water Research*, 143, pp. 416–424. Available at: https://doi.org/10.1016/j.watres.2018.06.015.

Blettler, M.C.M., Garello, N., Ginon, L., Abrial, E., Espinola, L.A. and Wantzen, K.M. (2019) 'Massive plastic pollution in a mega-river of a developing country: Sediment deposition and ingestion by fish (Prochilodus lineatus)', *Environmental Pollution*, 255, p. 113348. Available at: https://doi.org/10.1016/j.envpol.2019.113348.

Boyero, L., Pearson, R.G., Gessner, M.O., Barmuta, L.A., Ferreira, V., Graça, M.A.S., Dudgeon, D., Boulton, A.J., Callisto, M., Chauvet, E., Helson, J.E., Bruder, A., Albariño, R.J., Yule, C.M., Arunachalam, M., Davies, J.N., Figueroa, R., Flecker, A.S., Ramírez, A., Death, R.G., Iwata, T., Mathooko, J.M., Mathuriau, C., Gonçalves, J.F., Moretti, M.S., Jinggut, T., Lamothe, S., M'Erimba, C., Ratnarajah, L., Schindler, M.H., Castela, J., Buria, L.M., Cornejo, A., Villanueva, V.D. and West, D.C. (2011) 'A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration', *Ecology Letters*, 14(3), pp. 289–294. Available at: https://doi.org/10.1111/j.1461-0248.2010.01578.x.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. and West, G.B. (2004) 'Toward a metabolic theory of ecology', *Ecology*, pp. 1771–1789. Available at: https://doi.org/10.1890/03-9000.

Burns, E.E. and Boxall, A.B.A. (2018) 'Microplastics in the aquatic environment: Evidence for or against adverse impacts and major knowledge gaps', *Environmental Toxicology and Chemistry*, 37(11), pp. 2776–2796. Available at: https://doi.org/10.1002/etc.4268.

Burton, G.A.Jr. (2017) 'Stressor Exposures Determine Risk: So, Why Do Fellow Scientists Continue To Focus on Superficial Microplastics Risk?', *Environmental Science & Technology*, 51(23), pp. 13515–13516. Available at: https://doi.org/10.1021/acs.est.7b05463.

Calisi, R.M. and Bentley, G.E. (2009) 'Lab and field experiments: Are they the same animal?', *Hormones and Behavior*, 56(1), pp. 1–10. Available at: https://doi.org/10.1016/j.yhbeh.2009.02.010.

Callisto, M. and Graça, M.A.S. (2013) 'The quality and availability of fine particulate organic matter for collector species in headwater streams', *International Review of Hydrobiology*, 98(3), pp. 132–140. Available at: https://doi.org/10.1002/iroh.201301524.

Canhoto, C. and Graça, M.A.S. (1999) ‘Leaf Barriers to Fungal Colonization and Shredders (Tipula lateralis) Consumption of Decomposing Eucalyptus globulus’, *Microbial Ecology*, 37(3), pp. 163–172. Available at: https://doi.org/10.1007/s002489900140.

Canniff, P.M. and Hoang, T.C. (2018) ‘Microplastic ingestion by Daphnia magna and its enhancement on algal growth’, *Science of the Total Environment*, 633, pp. 500–507. Available at: https://doi.org/10.1016/j.scitotenv.2018.03.176.

Capolupo, M., Sørensen, L., Jayasena, K.D.R., Booth, A.M. and Fabbri, E. (2020) ‘Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms’, *Water Research*, 169, p. 115270. Available at: https://doi.org/10.1016/j.watres.2019.115270.

Caputo, F., Vogel, R., Savage, J., Vella, G., Law, A., Della Camera, G., Hannon, G., Peacock, B., Mehn, D., Ponti, J., Geiss, O., Aubert, D., Prina-Mello, A. and Calzolai, L. (2021) ‘Measuring particle size distribution and mass concentration of nanoplastics and microplastics: addressing some analytical challenges in the sub-micron size range’, *Journal of Colloid and Interface Science*, 588, pp. 401–417. Available at: https://doi.org/10.1016/j.jcis.2020.12.039.

Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P. and Peck, B.B. (1972) ‘Polystyrene spherules in coastal waters’, *Science*, 178(4062), pp. 749–750. Available at: https://doi.org/10.1126/science.178.4062.749.

Carpenter, E.J. and Smith, K.L. (1972) ‘Plastics on the Sargasso Sea Surface’, *Science*, 175(4027), pp. 1240–1241. Available at: https://doi.org/10.1126/science.175.4027.1240.

Carpenter, S.R. (1996) ‘Microcosm Experiments have Limited Relevance for Community and Ecosystem Ecology’, *Ecology*, 77(3), pp. 677–680. Available at: https://doi.org/10.2307/2265490.

Carpenter, S.R., Kitchell, J.F. and Hodgson, J.R. (1985) ‘Cascading Trophic Interactions and Lake Productivity’, *BioScience*, 35(10), pp. 634–639. Available at: https://doi.org/10.2307/1309989.

Carreira, B.M., Segurado, P., Laurila, A. and Rebelo, R. (2020) ‘Heat waves trigger swift changes in the diet and life-history of a freshwater snail’, *Hydrobiologia*, 847(4), pp. 999–1011. Available at: https://doi.org/10.1007/s10750-019-04155-3.

Carreira, B.M., Segurado, P., Orizaola, G., Gonçalves, N., Pinto, V., Laurila, A. and Rebelo, R. (2016) ‘Warm vegetarians? Heat waves and diet shifts in tadpoles’, *Ecology*, 97(11), pp. 2964–2974. Available at: https://doi.org/10.1002/ecy.1541.

Castañeda, R.A., Avlijas, S., Simard, M.A. and Ricciardi, A. (2014) ‘Microplastic pollution in St. Lawrence River sediments’, *Canadian Journal of Fisheries* *and Aquatic Sciences*, 71(12), pp. 1767–1771. Available at: https://doi.org/10.1139/cjfas-2014-0281.

Cera, A., Gallitelli, L., Cesarini, G. and Scalici, M. (2022) 'Occurrence of Microplastics in Freshwater', in M.Z. Hashmi (ed.) *Microplastic Pollution: Environmental Occurrence and Treatment Technologies*. Cham: Springer International Publishing (Emerging Contaminants and Associated Treatment Technologies), pp. 201–226. Available at: https://doi.org/10.1007/978-3-030-89220-3_10.

Chae, Y., Hong, S.H. and An, Y.-J. (2020) 'Photosynthesis enhancement in four marine microalgal species exposed to expanded polystyrene leachate', *Ecotoxicology and Environmental Safety*, 189, p. 109936. Available at: https://doi.org/10.1016/j.ecoenv.2019.109936.

Chae, Y., Kim, D. and An, Y.J. (2019) 'Effects of micro-sized polyethylene spheres on the marine microalga Dunaliella salina: Focusing on the algal cell to plastic particle size ratio', *Aquatic Toxicology*, 216, p. 105296. Available at: https://doi.org/10.1016/j.aquatox.2019.105296.

Chen, Xianchuan, Chen, Xiaofei, Zhao, Y., Zhou, H., Xiong, X. and Wu, C. (2020) 'Effects of microplastic biofilms on nutrient cycling in simulated freshwater systems', *Science of the Total Environment*, 719, p. 137276. Available at: https://doi.org/10.1016/j.scitotenv.2020.137276.

Clarke, A. and Fraser, K.P.P. (2004) 'Why Does Metabolism Scale with Temperature?', Functional Ecology, 18(2), pp. 243–251.

Conkle, J.L., Báez Del Valle, C.D. and Turner, J.W. (2018) 'Are We Underestimating Microplastic Contamination in Aquatic Environments?', *Environmental Management*, 61(1), pp. 1–8. Available at: https://doi.org/10.1007/s00267-017-0947-8.

Connors, K.A., Dyer, S.D. and Belanger, S.E. (2017) 'Advancing the quality of environmental microplastic research', *Environmental Toxicology and Chemistry*, 36(7), pp. 1697–1703. Available at: https://doi.org/10.1002/etc.3829.

Côté, I.M., Darling, E.S. and Brown, C.J. (2016) 'Interactions among ecosystem stressors and their importance in conservation', *Proceedings of the Royal Society B: Biological Sciences*, 283(1824), p. 20152592. Available at: https://doi.org/10.1098/rspb.2015.2592.

Croll, S.L. and Watts, S.A. (2004) 'The Effect of Temperature on Feed Consumption and Nutrient Absorption in Procambarus clarkii and Procambarus zonangulus', *Journal of the World Aquaculture Society*, 35(4), pp. 478–488. Available at: https://doi.org/10.1111/j.1749-7345.2004.tb00113.x.

Cummins, K.W. (1974) 'Structure and Function of Stream Ecosystems', *BioScience*, 24(11), pp. 631–641. Available at: https://doi.org/10.2307/1296676.

Cummins, K.W. and Klug, M.J. (1979) 'Feeding Ecology of Stream Invertebrates', *Annual Review of Ecology and Systematics*, 10(1), pp. 147–172. Available at: https://doi.org/10.1146/annurev.es.10.110179.001051.

Danger, M., Cornut, J., Elger, A. and Chauvet, E. (2012) 'Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa', *Freshwater Biology*, 57(5), pp. 1017–1030. Available at: https://doi.org/10.1111/j.1365-2427.2012.02762.x.

Dawoud, M., Bundschuh, M., Goedkoop, W. and McKie, B.G. (2017) 'Interactive effects of an insecticide and a fungicide on different organism groups and ecosystem functioning in a stream detrital food web', *Aquatic Toxicology*, 186, pp. 215–221. Available at: https://doi.org/10.1016/j.aquatox.2017.03.008.

De Groot, R.S., Wilson, M.A. and Boumans, R.M.J. (2002) 'A typology for the classification, description and valuation of ecosystem functions, goods and services', *Ecological Economics*, 41(3), pp. 393–408. Available at: https://doi.org/10.1016/S0921-8009(02)00089-7.

De Ruijter, V.N., Redondo-Hasselerharm, P.E., Gouin, T. and Koelmans, A.A. (2020) 'Quality Criteria for Microplastic Effect Studies in the Context of Risk Assessment: A Critical Review', *Environmental Science and Technology*, 54(19), pp. 11692–11705. Available at: https://doi.org/10.1021/acs.est.0c03057.

Díaz-Morales, D.M., Bommarito, C., Knol, J., Grabner, D.S., Noè, S., Rilov, G., Wahl, M., Guy-Haim, T. and Sures, B. (2023) 'Parasitism enhances gastropod feeding on invasive and native algae while altering essential energy reserves for organismal homeostasis upon warming', *Science of The Total Environment*, 863, p. 160727. Available at: https://doi.org/10.1016/j.scitotenv.2022.160727.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J. and Sullivan, C.A. (2006) 'Freshwater biodiversity: Importance, threats, status and conservation challenges', *Biological Reviews of the Cambridge Philosophical Society*, 81(2), pp. 163–182. Available at: https://doi.org/10.1017/S1464793105006950.

Eerkes-Medrano, D., Thompson, R.C. and Aldridge, D.C. (2015) 'Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs', *Water Research*, 75, pp. 63–82. Available at: https://doi.org/10.1016/j.watres.2015.02.012.

EPA (1992) 'Plastic Pellets in the Aquatic Environment: Sources and Recommendations', United States Environmental Protection Agency, p. 56.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H. and Amato, S. (2013) 'Microplastic pollution in the surface waters of the

Laurentian Great Lakes', *Marine Pollution Bulletin*, 77(1–2), pp. 177–182. Available at: https://doi.org/10.1016/j.marpolbul.2013.10.007.

European Commission (2023) 'Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards synthetic polymer microparticles'.

Evangeliou, N., Grythe, H., Klimont, Z., Heyes, C., Eckhardt, S., Lopez-Aparicio, S. and Stohl, A. (2020) 'Atmospheric transport is a major pathway of microplastics to remote regions', *Nature Communications*, 11(1). Available at: https://doi.org/10.1038/s41467-020-17201-9.

Fargione, J., Tilman, D., Dybzinski, R., Lambers, J.H.R., Clark, C., Harpole, W.S., Knops, J.M.H., Reich, P.B. and Loreau, M. (2007) 'From selection to complementarity: shifts in the causes of biodiversity–productivity relationships in a long-term biodiversity experiment', *Proceedings of the Royal Society B: Biological Sciences*, 274(1611), pp. 871–876. Available at: https://doi.org/10.1098/rspb.2006.0351.

Findlay, S., Tank, J., Dye, S., Valett, H Maurice, Mulholland, P.J., McDowell, W.H., Johnson, S.L., Hamilton, S.K., Edmonds, J., Dodds, W.K. and Bowden, W.B. (2002) 'A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams', *Microbial Ecology*, 43(1), pp. 55–66. Available at: https://doi.org/10.1007/s00248-001-1020-x.

Foley, C.J., Feiner, Z.S., Malinich, T.D. and Höök, T.O. (2018) 'A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates', *Science of the Total Environment*, 631–632, pp. 550–559. Available at: https://doi.org/10.1016/j.scitotenv.2018.03.046.

Folt, C.L.;, Chen, C.Y.;, Moore, M.V.; and Burnaford, J. (1999) 'Synergism and antagonism among multiple stressors', *Limnology and Oceanography*, 44(3), pp. 864–877. Available at: https://doi.org/10.1002/ece3.1465.

Frainer, A. and McKie, B.G. (2015) 'Chapter Seven - Shifts in the Diversity and Composition of Consumer Traits Constrain the Effects of Land Use on Stream Ecosystem Functioning', in S. Pawar, G. Woodward, and A.I. Dell (eds) *Advances in Ecological Research*. Academic Press (Trait-Based Ecology - From Structure to Function), pp. 169–200. Available at: https://doi.org/10.1016/bs.aecr.2015.03.002.

Frias, J.P.G.L. and Nash, R. (2019) 'Microplastics: Finding a consensus on the definition', *Marine Pollution Bulletin*, 138, pp. 145–147. Available at: https://doi.org/10.1016/J.MARPOLBUL.2018.11.022.

Fukuhara, H. and Sakamoto, M. (1987) 'Enhancement of Inorganic Nitrogen and Phosphate Release from Lake Sediment by Tubificid Worms and Chironomid Larvae', *Oikos*, 48(3), p. 312. Available at: https://doi.org/10.2307/3565519.

Gambarini, V., Pantos, O., Kingsbury, J.M., Weaver, L., Handley, K.M. and Lear, G. (2021) 'Phylogenetic Distribution of Plastic-Degrading Microorganisms', *Am Soc Microbiol.* Edited by A.D. Kent, 6(1), pp. e01112-20. Available at: https://doi.org/10.1128/mSystems.01112-20.

Garcia-Vazquez, E. and Garcia-Ael, C. (2021) 'The invisible enemy. Public knowledge of microplastics is needed to face the current microplastics crisis', *Sustainable Production and Consumption*, 28, pp. 1076–1089. Available at: https://doi.org/10.1016/j.spc.2021.07.032.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. and Hättenschwiler, S. (2010) 'Diversity meets decomposition', *Trends in Ecology and Evolution*, 25(6), pp. 372–380. Available at: https://doi.org/10.1016/j.tree.2010.01.010.

Getachew, P., Getachew, M., Joo, J., Choi, Y.S., Hwang, D.S. and Hong, Y.-K. (2016) 'The slip agents oleamide and erucamide reduce biofouling by marine benthic organisms (diatoms, biofilms and abalones)', *Toxicology and Environmental Health Sciences*, 8(5), pp. 341–348. Available at: https://doi.org/10.1007/s13530-016-0295-8.

Gewert, B., Plassmann, M.M. and Macleod, M. (2015) 'Pathways for degradation of plastic polymers floating in the marine environment', *Environmental Sciences: Processes and Impacts*, 17(9), pp. 1513–1521. Available at: https://doi.org/10.1039/c5em00207a.

Geyer, R., Jambeck, J.R. and Law, K.L. (2017) 'Production, use, and fate of all plastics ever made', *Science Advances*, 3(7). Available at: https://doi.org/10.1126/sciadv.1700782.

Guerranti, C., Martellini, T., Perra, G., Scopetani, C. and Cincinelli, A. (2019) 'Microplastics in cosmetics: Environmental issues and needs for global bans', *Environmental Toxicology and Pharmacology*, 68, pp. 75–79. Available at: https://doi.org/10.1016/j.etap.2019.03.007.

Guschina, I.A., Hayes, A.J. and Ormerod, S.J. (2020) 'Polystyrene microplastics decrease accumulation of essential fatty acids in common freshwater algae', *Environmental Pollution*, 263, p. 114425. Available at: https://doi.org/10.1016/j.envpol.2020.114425.

Hale, R.C., Seeley, M.E., La Guardia, M.J., Mai, L. and Zeng, E.Y. (2020) 'A Global Perspective on Microplastics', *Journal of Geophysical Research: Oceans*, 125(1), p. e2018JC014719. Available at: https://doi.org/10.1029/2018JC014719.

Hamburger, K., Lindegaard, C. and Dall, P.C. (1996) 'The role of glycogen during the ontogenesis of Chironomus anthracinus (Chironomidae, Diptera)', *Hydrobiologia*, 318(1), pp. 51–59. Available at: https://doi.org/10.1007/BF00014131.

Hamilton, S.K., Sippel, S.J. and Bunn, S.E. (2005) 'Separation of algae from detritus for stable isotope or ecological stoichiometry studies using density fractionation in colloidal silica', *Limnology and Oceanography: Methods*, 3(3), pp. 149–157. Available at: https://doi.org/10.4319/lom.2005.3.149.

Hart, D.D. (1985) 'Grazing Insects Mediate Algal Interactions in a Stream Benthic Community', *Oikos*, 44(1), p. 40. Available at: https://doi.org/10.2307/3544041.

Hasan, J., Siddik, M.A., Ghosh, A.K., Mesbah, S.B., Sadat, M.A. and Shahjahan, M. (2023) 'Increase in temperature increases ingestion and toxicity of polyamide microplastics in Nile tilapia', *Chemosphere*, 327, p. 138502. Available at: https://doi.org/10.1016/j.chemosphere.2023.138502.

Heard, S.B. (1994) 'Processing Chain Ecology: Resource Condition and Interspecific Interactions', *The Journal of Animal Ecology*, 63(2), p. 451. Available at: https://doi.org/10.2307/5562.

Heard, S.B. and Buchanan, C.K. (2004) 'Grazer-collector facilitation hypothesis supported by laboratory but not field experiments', *Canadian Journal of Fisheries and Aquatic Sciences*, 61(6), pp. 887–897. Available at: https://doi.org/10.1139/F04-022.

Höckelmann, C. and Pusch, M. (2000) 'The respiration and filter-feeding rates of the snail Viviparus viviparus (Gastropoda) under simulated'.

Hoellein, T.J., Shogren, A.J., Tank, J.L., Risteca, P. and Kelly, J.J. (2019) 'Microplastic deposition velocity in streams follows patterns for naturally occurring allochthonous particles', *Scientific Reports*, 9(1), pp. 1–11. Available at: https://doi.org/10.1038/s41598-019-40126-3.

Hölker, F., Vanni, M.J., Kuiper, J.J., Meile, C., Grossart, H.P., Stief, P., Adrian, R., Lorke, A., Dellwig, O., Brand, A., Hupfer, M., Mooij, W.M., Nützmann, G. and Lewandowski, J. (2015) 'Tube-dwelling invertebrates: Tiny ecosystem engineers have large effects in lake ecosystems', *Ecological Monographs*, 85(3), pp. 333–351. Available at: https://doi.org/10.1890/14-1160.1.

Holmstrup, M., Bindesbøl, A.M., Oostingh, G.J., Duschl, A., Scheil, V., Köhler, H.R., Loureiro, S., Soares, A.M.V.M., Ferreira, A.L.G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P.E., Bayley, M., Svendsen, C. and Spurgeon, D.J. (2010) 'Interactions between effects of environmental chemicals and natural stressors: A review', *Science of The Total Environment*, 408(18), pp. 3746–3762. Available at: https://doi.org/10.1016/J.SCITOTENV.2009.10.067.

Hooper, H.L., Sibly, R.M., Hutchinson, T.H. and Maund, S.J. (2003) 'The influence of larval density, food availability and habitat longevity on the life history and population growth rate of the midge Chironomus riparius', *Oikos*, 102(3), pp. 515–524. Available at: https://doi.org/10.1034/j.1600-0706.2003.12536.x.

Horton, A.A., Vijver, M.G., Lahive, E., Spurgeon, D.J., Svendsen, C., Heutink, R., van Bodegom, P.M. and Baas, J. (2018) 'Acute toxicity of organic pesticides to Daphnia magna is unchanged by co-exposure to polystyrene microplastics', *Ecotoxicology and Environmental Safety*, 166, pp. 26–34. Available at: https://doi.org/10.1016/j.ecoenv.2018.09.052.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E. and Svendsen, C. (2017) 'Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities', *Science of the Total Environment*, 586, pp. 127–141. Available at: https://doi.org/10.1016/j.scitotenv.2017.01.190.

Hossain, M.R., Jiang, M., Wei, Q.H. and Leff, L.G. (2019) 'Microplastic surface properties affect bacterial colonization in freshwater', *Journal of Basic Microbiology*, 59(1), pp. 54–61. Available at: https://doi.org/10.1002/jobm.201800174.

Huang, Y., Li, W., Gao, J., Wang, F., Yang, W., Han, L., Lin, D., Min, B., Zhi, Y., Grieger, K. and Yao, J. (2021) 'Effect of microplastics on ecosystem functioning: Microbial nitrogen removal mediated by benthic invertebrates', *Science of the Total Environment*, 754, p. 142133. Available at: https://doi.org/10.1016/j.scitotenv.2020.142133.

Huot, Y., Babin, M., Bruyant, F., Grob, C., Twardowski, M.S. and Claustre, H. (2007) 'Does chlorophyll a provide the best index of phytoplankton biomass for primary productivity studies?', *Biogeosciences Discussions*, 4(2), pp. 707–745.

Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R. and Laforsch, C. (2013) 'Contamination of beach sediments of a subalpine lake with microplastic particles', *Current Biology*, 23(19), pp. R867–R868. Available at: https://doi.org/10.1016/j.cub.2013.09.001.

Imhof, H.K. and Laforsch, C. (2016) 'Hazardous or not – Are adult and juvenile individuals of Potamopyrgus antipodarum affected by non-buoyant microplastic particles?', *Environmental Pollution*, 218, pp. 383–391. Available at: https://doi.org/10.1016/j.envpol.2016.07.017.

Jabiol, J., Mckie, B.G., Bruder, A., Bernadet, C., Gessner, M.O. and Chauvet, E. (2013) 'Trophic complexity enhances ecosystem functioning in an aquatic detritus-based model system', *Journal of Animal Ecology*, 82(5), pp. 1042–1051. Available at: https://doi.org/10.1111/1365-2656.12079.

Jackson, M.C., Loewen, C.J.G., Vinebrooke, R.D. and Chimimba, C.T. (2016) 'Net effects of multiple stressors in freshwater ecosystems: A meta-analysis', *Global Change Biology*, 22(1), pp. 180–189. Available at: https://doi.org/10.1111/gcb.13028.

Jackson, M.C., Pawar, S. and Woodward, G. (2021) 'The Temporal Dynamics of Multiple Stressor Effects: From Individuals to Ecosystems', *Trends in Ecology & Evolution*, 36(5), pp. 402–410. Available at: https://doi.org/10.1016/j.tree.2021.01.005.

Jemec, A., Horvat, P., Kunej, U., Bele, M. and Kržan, A. (2016) 'Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna', *Environmental Pollution*, 219, pp. 201–209. Available at: https://doi.org/10.1016/j.envpol.2016.10.037.

Johnson, L.T., Tank, J.L. and Dodds, W.K. (2009) 'The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions', *Canadian Journal of Fisheries and Aquatic Sciences*, 66(7), pp. 1081–1094. Available at: https://doi.org/10.1139/F09-065.

Johnson, R.K., Angeler, D.G., Hallstan, S., Sandin, L. and McKie, B.G. (2017) 'Decomposing multiple pressure effects on invertebrate assemblages of boreal streams', *Ecological Indicators*, 77, pp. 293–303. Available at: https://doi.org/10.1016/j.ecolind.2017.02.020.

Johnson, R.K., Boström, B. and van de Bund, W. (1989) 'Interactions between Chironomus plumosus (L.) and the microbial community in surficial sediments of a shallow, eutrophic lake', *Limnology and Oceanography*, 34(6), pp. 992–1003. Available at: https://doi.org/10.4319/lo.1989.34.6.0992.

Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M.C., Rivett, D.W., Salles, J.F., van der Heijden, M.G.A., Youssef, N.H., Zhang, X., Wei, Z. and Hol, W.H.G. (2017) 'Where less may be more: how the rare biosphere pulls ecosystems strings', *The ISME Journal*, 11(4), pp. 853–862. Available at: https://doi.org/10.1038/ismej.2016.174.

Joyce, P., Warren, L.L. and Wotton, R.S. (2007) 'Faecal pellets in streams: Their binding, breakdown and utilization', Freshwater Biology, 52(10), pp. 1868–1880. Available at: https://doi.org/10.1111/j.1365-2427.2007.01828.x.

Kahlert, M. and McKie, B.G. (2014) 'Comparing new and conventional methods to estimate benthic algal biomass and composition in freshwaters', *Environmental Sciences: Processes and Impacts*, 16(11), pp. 2627–2634. Available at: https://doi.org/10.1039/c4em00326h.

Karami, A., Golieskardi, A., Keong Choo, C., Larat, V., Galloway, T.S. and Salamatinia, B. (2017) 'The presence of microplastics in commercial salts from different countries', *Scientific Reports*, 7(1), p. 46173. Available at: https://doi.org/10.1038/srep46173.

Khoironi, A., Anggoro, S., and Sudarno (2019) 'Evaluation of the Interaction Among Microalgae Spirulina sp, Plastics Polyethylene Terephthalate and Polypropylene in Freshwater Environment', *Journal of Ecological Engineering*, 20(6), pp. 161–173. Available at: https://doi.org/10.12911/22998993/108637.

Kong, Z.H., Burdon, F.J., Truchy, A., Bundschuh, M., Futter, M.N., Hurley, R. and McKie, B.G. (2023) 'Comparing effects of microplastic exposure, FPOM resource quality, and consumer density on the response of a freshwater particle feeder and associated ecosystem processes', *Aquatic Sciences*, 85(3), p. 70. Available at: https://doi.org/10.1007/s00027-023-00964-w.

Kooijman, S.A.L.M. (2010) 'Dynamic energy budget theory for metabolic organisation'. Cambridge university press.

Kosuth, M., Mason, S.A. and Wattenberg, E.V. (2018) 'Anthropogenic contamination of tap water, beer, and sea salt', *PLOS ONE*, 13(4), p. e0194970. Available at: https://doi.org/10.1371/journal.pone.0194970.

Kuznetsova, A., Brockhoff, P.B. and Christensen, R.H.B. (2017) 'lmerTest Package: Tests in Linear Mixed Effects Models', *Journal of Statistical Software*, 82(13), pp. 1–26. Available at: https://doi.org/10.18637/JSS.V082.I13.

Lasee, S., Mauricio, J., Thompson, W.A., Karnjanapiboonwong, A., Kasumba, J., Subbiah, S., Morse, A.N. and Anderson, T.A. (2017) 'Microplastics in a freshwater environment receiving treated wastewater effluent', *Integrated Environmental Assessment and Management*, 13(3), pp. 528–532. Available at: https://doi.org/10.1002/ieam.1915.

Lau, D.C.P., Goedkoop, W. and Vrede, T. (2013) 'Cross-ecosystem differences in lipid composition and growth limitation of a benthic generalist consumer', *Limnology and Oceanography*, 58(4), pp. 1149–1164. Available at: https://doi.org/10.4319/lo.2013.58.4.1149.

Lee, J.-Y., Marotzke, J., Bala, G., Cao, L., Corti, S., Dunne, J.P., Engelbrecht, F., Fischer, E., Fyfe, J.C., Jones, C., Maycock, A., Mutemi, J., Ndiaye, O., Panickal, S. and Zhou:, T. (2021) 'Future global climate: scenario-based projections and near-term information, Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change'. Available at: https://doi.org/10.1017/9781009157896.006.553.

Lenz, R., Enders, K. and Nielsen, T.G. (2016) 'Microplastic exposure studies should be environmentally realistic', *Proceedings of the National Academy of Sciences of the United States of America*, 113(29), pp. E4121–E4122. Available at: https://doi.org/10.1073/pnas.1606615113.

Li, C., Busquets, R. and Campos, L.C. (2020) 'Assessment of microplastics in freshwater systems: A review', *Science of the Total Environment*, 707, p. 135578. Available at: https://doi.org/10.1016/j.scitotenv.2019.135578.

Li, J., Shan, E., Zhao, J., Teng, J. and Wang, Q. (2023) 'The factors influencing the vertical transport of microplastics in marine environment: A review', *Science of The Total Environment*, 870, p. 161893. Available at: https://doi.org/10.1016/j.scitotenv.2023.161893.

Liebezeit, G. and Liebezeit, E. (2014) 'Synthetic particles as contaminants in German beers', *Food Additives & Contaminants: Part A*, 31(9), pp. 1574–1578. Available at: https://doi.org/10.1080/19440049.2014.945099.

Lin, J., Yan, D., Fu, J., Chen, Y. and Ou, H. (2020) 'Ultraviolet-C and vacuum ultraviolet inducing surface degradation of microplastics', *Water Research*, 186, p. 116360.

Liu, G., Jiang, R., You, J., Muir, D.C.G. and Zeng, E.Y. (2020) 'Microplastic Impacts on Microalgae Growth: Effects of Size and Humic Acid', *Environmental Science & Technology*, 54(3), pp. 1782–1789. Available at: https://doi.org/10.1021/acs.est.9b06187.

López-Rojo, N., Pérez, J., Alonso, A., Correa-Araneda, F. and Boyero, L. (2020) 'Microplastics have lethal and sublethal effects on stream invertebrates and affect stream ecosystem functioning', *Environmental Pollution*, 259, p. 113898. Available at: https://doi.org/10.1016/j.envpol.2019.113898.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L. and Ren, H. (2016) 'Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (Danio rerio) and Toxic Effects in Liver', *Environmental Science and Technology*, 50(7), pp. 4054–4060. Available at: https://doi.org/10.1021/acs.est.6b00183.

Luo, H., Liu, C., He, D., Sun, J., Li, J. and Pan, X. (2022) 'Effects of aging on environmental behavior of plastic additives: Migration, leaching, and ecotoxicity', *Science of The Total Environment*, 849, p. 157951. Available at: https://doi.org/10.1016/j.scitotenv.2022.157951.

Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S. and Xing, B. (2020) 'Microplastics in aquatic environments: Toxicity to trigger ecological consequences', *Environmental Pollution*, 261, p. 114089. Available at: https://doi.org/10.1016/j.envpol.2020.114089.

Malli, A., Corella-Puertas, E., Hajjar, C. and Boulay, A.-M. (2022) 'Transport mechanisms and fate of microplastics in estuarine compartments: A review', *Marine Pollution Bulletin*, 177, p. 113553. Available at: https://doi.org/10.1016/j.marpolbul.2022.113553.

Mao, Y., Ai, H., Chen, Y., Zhang, Z., Zeng, P., Kang, L., Li, W., Gu, W., He, Q. and Li, H. (2018) 'Phytoplankton response to polystyrene microplastics: Perspective from an entire growth period', *Chemosphere*, 208, pp. 59–68. Available at: https://doi.org/10.1016/j.chemosphere.2018.05.170.

Marchant, D.J., Martínez Rodríguez, A., Francelle, P., Jones, J.I. and Kratina, P. (2023) 'Contrasting the effects of microplastic types, concentrations and nutrient enrichment on freshwater communities and ecosystem functioning', *Ecotoxicology and Environmental Safety*, 255, p. 114834. Available at: https://doi.org/10.1016/j.ecoenv.2023.114834.

Mason, C.F. and Bryant, R. j. (1975) 'Periphyton production and grazing by chironomids in Alderfen Broad, Norfolk', *Freshwater Biology*, 5(3), pp. 271–277. Available at: https://doi.org/10.1111/j.1365-2427.1975.tb00140.x.

Mckie, B.G. (2004) 'Disturbance and investment: Developmental responses of tropical lotic midges to repeated tube destruction in the juvenile stages', *Ecological Entomology*, 29(4), pp. 457–466. Available at: https://doi.org/10.1111/j.0307-6946.2004.00622.x.

McKie, B.G., Cranston, P.S. and Pearson, R.G. (2004) ‘Gondwanan mesotherms and cosmopolitan eurytherms: effects of temperature on the development and survival of Australian Chironomidae (Diptera) from tropical and temperate populations’, *Marine and Freshwater Research*, 55(8), pp. 759–768. Available at: https://doi.org/10.1071/MF04023.

McKie, B.G., Taylor, A., Nilsson, T., Frainer, A. and Goedkoop, W. (2023) ‘Ecological effects of mosquito control with Bti: evidence for shifts in the trophic structure of soil- and ground-based food webs’, *Aquatic Sciences*, 85(2), p. 47. Available at: https://doi.org/10.1007/s00027-023-00944-0.

McKie, B.G., Woodward, G., Hladyz, S., Nistorescu, M., Preda, E., Popescu, C., Giller, P.S. and Malmqvist, B. (2008) ‘Ecosystem functioning in stream assemblages from different regions: Contrasting responses to variation in detritivore richness, evenness and density’, *Journal of Animal Ecology*, 77(3), pp. 495–504. Available at: https://doi.org/10.1111/j.1365-2656.2008.01357.x.

McQueen, D.J., Post, J.R. and Mills, E.L. (1986) ‘Trophic relationships in freshwater pelagic ecosystems.’, *Canadian Journal of Fisheries and Aquatic Sciences*, 43(8), pp. 1571–1581. Available at: https://doi.org/10.1139/f86-195.

Miao, L., Gao, Y., Adyel, T.M., Huo, Z., Liu, Z., Wu, J. and Hou, J. (2021) ‘Effects of biofilm colonization on the sinking of microplastics in three freshwater environments’, *Journal of Hazardous Materials*, 413, p. 125370. Available at: https://doi.org/10.1016/j.jhazmat.2021.125370.

Miao, L., Wang, P., Hou, J., Yao, Y., Liu, Z., Liu, S. and Li, T. (2019) ‘Distinct community structure and microbial functions of biofilms colonizing microplastics’, *Science of the Total Environment*, 650, pp. 2395–2402. Available at: https://doi.org/10.1016/j.scitotenv.2018.09.378.

Miller, N.A. and Stillman, J.H., 2012. ‘Physiological optima and critical limits’. *Nature Education Knowledge*, *3*(10), p.1. Available at: https://www.nature.com/scitable/knowledge/library/physiological-optima-and-critical-limits-45749376/ (Accessed: 20 March 2024).

Moore, C.J., Moore, S.L., Weisberg, S.B., Lattin, G.L. and Zellers, A.F. (2002) ‘A comparison of neustonic plastic and zooplankton abundance in southern California’s coastal waters’, *Marine Pollution Bulletin*, 44(10), pp. 1035–1038. Available at: https://doi.org/10.1016/S0025-326X(02)00150-9.

Moore, J.W. (1975) ‘The Role of Algae in the Diet of Asellus aquaticus L. and Gammarus pulex L.’, *Journal of Animal Ecology*, 44(3), pp. 719–730. Available at: https://doi.org/10.2307/3714.

Naeem, S. and Li, S. (1997) ‘Biodiversity enhances ecosystem reliability’, *Nature*, 390(6659), pp. 507–509. Available at: https://doi.org/10.1038/37348.

Naeem, S., Thompson, L.J., Lawler, S.P., Lawton, J.H. and Woodfin, R.M. (1994) ‘Declining biodiversity can alter the performance of ecosystems’, *Nature*, 368(6473), pp. 734–737. Available at: https://doi.org/10.1038/368734a0.

Nylund, J.-E. and Wallander, H. (1992) ‘5 Ergosterol Analysis as a Means of Quantifying Mycorrhizal Biomass’, in J.R. Norris, D.J. Read, and A.K. Varma (eds) *Methods in Microbiology*. Academic Press, pp. 77–88. Available at: https://doi.org/10.1016/S0580-9517(08)70088-6.

Ockenden, A., Tremblay, L.A., Dikareva, N. and Simon, K.S. (2021) ‘Towards more ecologically relevant investigations of the impacts of microplastic pollution in freshwater ecosystems’, *Science of the Total Environment*, 792, p. 148507. Available at: https://doi.org/10.1016/j.scitotenv.2021.148507.

Ogonowski, M., Schür, C., Jarsén, Å. and Gorokhova, E. (2016) ‘The effects of natural and anthropogenic microparticles on individual fitness in daphnia magna’, *PLoS ONE*. Edited by A. Mukherjee, 11(5), p. e0155063. Available at: https://doi.org/10.1371/journal.pone.0155063.

Ostle, C., Thompson, R.C., Broughton, D., Gregory, L., Wootton, M. and Johns, D.G. (2019) ‘The rise in ocean plastics evidenced from a 60-year time series’, *Nature Communications*, 10(1), p. 1622. Available at: https://doi.org/10.1038/s41467-019-09506-1.

Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., Van De Bogert, M.C., Bade, D.L., Kritzberg, E.S. and Bastviken, D. (2004) ‘Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs’, *Nature*, 427(6971), pp. 240–243. Available at: https://doi.org/10.1038/nature02227.

Pan, Y., Gao, S.-H., Ge, C., Gao, Q., Huang, S., Kang, Y., Luo, G., Zhang, Z., Fan, L., Zhu, Y. and Wang, A.-J. (2022) ‘Removing microplastics from aquatic environments: A critical review’, *Environmental Science and Ecotechnology*, 13, p. 100222. Available at: https://doi.org/10.1016/j.ese.2022.100222.

Park, T.-J., Lee, S.-H., Lee, M.-S., Lee, J.-K., Park, J.-H. and Zoh, K.-D. (2020) ‘Distributions of Microplastics in Surface Water, Fish, and Sediment in the Vicinity of a Sewage Treatment Plant’, *Water*, 12(12), p. 3333. Available at: https://doi.org/10.3390/w12123333.

Peng, G., Xu, P., Zhu, B., Bai, M. and Li, D. (2018) ‘Microplastics in freshwater river sediments in Shanghai, China: A case study of risk assessment in mega-cities’, *Environmental Pollution*, 234, pp. 448–456. Available at: https://doi.org/10.1016/j.envpol.2017.11.034.

Petchey, O.L., Downing, A.L., Mittelbach, G.G., Persson, L., Steiner, C.F., Warren, P.H. and Woodward, G. (2004) ‘Species loss and the structure and functioning of multitrophic aquatic systems’, *Oikos*, 104(3), pp. 467–478. Available at: https://doi.org/10.1111/j.0030-1299.2004.13257.x.

Peter, H., Beier, S., Bertilsson, S., Lindström, E.S., Langenheder, S. and Tranvik, L.J. (2011) ‘Function-specific response to depletion of microbial diversity’, *The ISME journal*, 5(2), pp. 351–361. Available at: https://doi.org/10.1038/ismej.2010.119.

Piggott, J.J., Townsend, C.R. and Matthaei, C.D. (2015a) ‘Climate warming and agricultural stressors interact to determine stream macroinvertebrate community dynamics’, *Global Change Biology*, 21(5), pp. 1887–1906. Available at: https://doi.org/10.1111/gcb.12861.

Piggott, J.J., Townsend, C.R. and Matthaei, C.D. (2015b) ‘Reconceptualizing synergism and antagonism among multiple stressors’, *Ecology and Evolution*, 5(7), pp. 1538–1547. Available at: https://doi.org/10.1002/ece3.1465.

Polhill, L., de Bruijn, R., Amaral-Zettler, L., Praetorius, A. and van Wezel, A. (2022) ‘Daphnia magna’s Favorite Snack: Biofouled Plastics’, *Environmental Toxicology and Chemistry*, 41(8), pp. 1977–1981. Available at: https://doi.org/10.1002/etc.5393.

Quero, G.M. and Luna, G.M. (2017) ‘Surfing and dining on the “plastisphere”: Microbial life on plastic marine debris’, *Advances in Oceanography and Limnology*, 8(2). Available at: https://doi.org/10.4081/aiol.2017.7211.

R Core Team (2023) ‘R: A Language and Environment for Statistical Computing’. Vienna, Austria: R Foundation for Statistical Computing. Available at: https://www.R-project.org/.

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D’Amore, E., Rinaldo, D., Matta, M. and Giorgini, E. (2021) ‘Plasticenta: First evidence of microplastics in human placenta’, *Environment International*, 146, p. 106274. Available at: https://doi.org/10.1016/j.envint.2020.106274.

Redondo-Hasselerharm, P.E., Falahudin, D., Peeters, E.T.H.M. and Koelmans, A.A. (2018) ‘Microplastic Effect Thresholds for Freshwater Benthic Macroinvertebrates’, *Environmental Science and Technology*, 52(4), pp. 2278–2286. Available at: https://doi.org/10.1021/acs.est.7b05367.

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D. and Cooke, S.J. (2019) ‘Emerging threats and persistent conservation challenges for freshwater biodiversity’, *Biological Reviews*, 94(3), pp. 849–873. Available at: https://doi.org/10.1111/brv.12480.

Renes, S.E., Sjöstedt, J., Fetzer, I. and Langenheder, S. (2020) ‘Disturbance history can increase functional stability in the face of both repeated disturbances of the same type and novel disturbances’, *Scientific Reports*, 10(1), pp. 1–13. Available at: https://doi.org/10.1038/s41598-020-68104-0.

Rengasamy, S., Shaffer, R., Williams, B. and Smit, S. (2017) ‘A comparison of facemask and respirator filtration test methods’, *Journal of Occupational and Environmental Hygiene*, 14(2), pp. 92–103. Available at: https://doi.org/10.1080/15459624.2016.1225157.

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

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., Frond, H.D., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A. and Hung, C. (2019) ‘Rethinking microplastics as a diverse contaminant suite’, *Environmental Toxicology and Chemistry*, 38(4), pp. 703–711. Available at: https://doi.org/10.1002/etc.4371.

Rochman, C.M., Kross, S.M., Armstrong, J.B., Bogan, M.T., Darling, E.S., Green, S.J., Smyth, A.R. and Veríssimo, D. (2015) ‘Scientific Evidence Supports a Ban on Microbeads’, *Environmental Science & Technology*, 49(18), pp. 10759–10761. Available at: https://doi.org/10.1021/acs.est.5b03909.

Romera-Castillo, C., Mallenco-Fornies, R., Saá-Yánez, M. and Álvarez-Salgado, X.A. (2022) ‘Leaching and bioavailability of dissolved organic matter from petrol-based and biodegradable plastics’, *Marine Environmental Research*, 176, p. 105607. Available at: https://doi.org/10.1016/j.marenvres.2022.105607.

Romera-Castillo, C., Pinto, M., Langer, T.M., Álvarez-Salgado, X.A. and Herndl, G.J. (2018) ‘Dissolved organic carbon leaching from plastics stimulates microbial activity in the ocean’, *Nature Communications*, 9(1), pp. 1–7. Available at: https://doi.org/10.1038/s41467-018-03798-5.

Roshchin, V. and Mazelev, K. (1979) ‘The influence of constant temperature on the embryonic growth of Asellus aquaticus L.(Crustacea)[Translation from: Vestsi Akademii Navuk Belorusskoi SSR, Seriya Biyal. 1979 (1) 128-130]’.

Rozman, U. and Kalčíková, G. (2022) ‘Seeking for a perfect (non-spherical) microplastic particle – The most comprehensive review on microplastic laboratory research’, *Journal of Hazardous Materials*, 424, p. 127529. Available at: https://doi.org/10.1016/j.jhazmat.2021.127529.

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D. and Schmitt-Jansen, M. (2017) ‘Impacts of Biofilm Formation on the Fate and Potential Effects of Microplastic in the Aquatic Environment’, *Environmental Science & Technology Letters*, 4(7), pp. 258–267. Available at: https://doi.org/10.1021/acs.estlett.7b00164.

Ryan, P. and Moloney, C.L. (1990) ‘Plastic and other artefacts on South African beaches: temporal trends in abundance and composition’, South African *Journal of Science*, 86, pp. 450–452.

de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L. and Futter, M.N. (2018) ‘Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future?’, *Science of the Total*

Environment, 645, pp. 1029–1039. Available at: https://doi.org/10.1016/j.scitotenv.2018.07.207.

Salminen, J.-P. and Karonen, M. (2011) ‘Chemical ecology of tannins and other phenolics: we need a change in approach’, *Functional Ecology*, 25(2), pp. 325–338. Available at: https://doi.org/10.1111/j.1365-2435.2010.01826.x.

Sanpera-Calbet, I., Chauvet, E. and Richardson, J.S. (2012) ‘Fine sediment on leaves: shredder removal of sediment does not enhance fungal colonisation’, *Aquatic Sciences*, 74(3), pp. 527–538. Available at: https://doi.org/10.1007/s00027-011-0245-0.

Savoca, M.S., Tyson, C.W., McGill, M. and Slager, C.J. (2017) ‘Odours from marine plastic debris induce food search behaviours in a forage fish’, *Proceedings of the Royal Society B: Biological Sciences*, 284(1860), p. 20171000. Available at: https://doi.org/10.1098/rspb.2017.1000.

Scalbert, A. (1991) ‘Antimicrobial properties of tannins’, *Phytochemistry*, 30(12), pp. 3875–3883. Available at: https://doi.org/10.1016/0031-9422(91)83426-L.

Schell, T., Martinez-Perez, S., Dafouz, R., Hurley, R., Vighi, M. and Rico, A. (2022) ‘Effects of Polyester Fibers and Car Tire Particles on Freshwater Invertebrates’, *Environmental Toxicology and Chemistry*, 41(6), pp. 1555–1567. Available at: https://doi.org/10.1002/etc.5337.

Scherer, C., Brennholt, N., Reifferscheid, G. and Wagner, M. (2017) ‘Feeding type and development drive the ingestion of microplastics by freshwater invertebrates’, *Scientific Reports*, 7(1), pp. 1–9. Available at: https://doi.org/10.1038/s41598-017-17191-7.

Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T. and Liebmann, B. (2019) ‘Detection of Various Microplastics in Human Stool’, *Annals of Internal Medicine*, 171(7), pp. 453–457. Available at: https://doi.org/10.7326/M19-0618.

Scrimgeour, G.J., Culp, J.M., Bothwell, M.L., Wrona, F.J. and McKee, M.H. (1991) ‘Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly-diatom systems’, *Oecologia*, 85(3), pp. 343–348. Available at: https://doi.org/10.1007/BF00320609.

Seeley, M.E., Song, B., Passie, R. and Hale, R.C. (2020) ‘Microplastics affect sedimentary microbial communities and nitrogen cycling’, *Nature Communications*, 11(1), pp. 1–10. Available at: https://doi.org/10.1038/s41467-020-16235-3.

Semcesen, P.O. and Wells, M.G. (2021) ‘Biofilm growth on buoyant microplastics leads to changes in settling rates: Implications for microplastic retention in the Great Lakes’, *Marine Pollution Bulletin*, 170, p. 112573. Available at: https://doi.org/10.1016/j.marpolbul.2021.112573.

Shepard, R.B. and Minshall, G.W. (1984) 'Role of Benthic Insect Feces in a Rocky Mountain Stream: Fecal Production and Support of Consumer Growth', *Ecology*, pp. 119–127.

Sheridan, E.A., Fonvielle, J.A., Cottingham, S., Zhang, Y., Dittmar, T., Aldridge, D.C. and Tanentzap, A.J. (2022) 'Plastic pollution fosters more microbial growth in lakes than natural organic matter', *Nature Communications*, 13(1), p. 4175. Available at: https://doi.org/10.1038/s41467-022-31691-9.

Silva, C.J.M., Machado, A.L., Campos, D., Rodrigues, A.C.M., Patrício Silva, A.L., Soares, A.M.V.M. and Pestana, J.L.T. (2022) 'Microplastics in freshwater sediments: Effects on benthic invertebrate communities and ecosystem functioning assessed in artificial streams', *Science of the Total Environment*, 804(4), pp. 467–488. Available at: https://doi.org/10.1016/J.SCITOTENV.2021.150118.

Simmons, B.I., Blyth, P.S.A., Blanchard, J.L., Clegg, T., Delmas, E., Garnier, A., Griffiths, C.A., Jacob, U., Pennekamp, F., Petchey, O.L., Poisot, T., Webb, T.J. and Beckerman, A.P. (2021) 'Refocusing multiple stressor research around the targets and scales of ecological impacts', *Nature Ecology & Evolution*, 5(11), pp. 1478–1489. Available at: https://doi.org/10.1038/s41559-021-01547-4.

Sooriyakumar, P., Bolan, N., Kumar, M., Singh, L., Yu, Y., Li, Y., Weralupitiya, C., Vithanage, M., Ramanayaka, S., Sarkar, B., Wang, F., Gleeson, D.B., Zhang, D., Kirkham, M.B., Rinklebe, J. and M Siddique, K.H. (2022) 'Biofilm formation and its implications on the properties and fate of microplastics in aquatic environments: A review', *Journal of Hazardous Materials Advances*, 6, p. 100077. Available at: https://doi.org/10.1016/j.hazadv.2022.100077.

Stanton, T., Johnson, M., Nathanail, P., MacNaughtan, W. and Gomes, R.L. (2020) 'Freshwater microplastic concentrations vary through both space and time', *Environmental Pollution*, 263, p. 114481. Available at: https://doi.org/10.1016/j.envpol.2020.114481.

Svensson, J.M. (1997) 'Influence of Chironomus plumosus larvae on ammonium flux and denitrification (measured by the acetylene blockage- and the isotope pairing-technique) in eutrophic lake sediment', *Hydrobiologia*, 346(1–3), pp. 157–168. Available at: https://doi.org/10.1023/a:1002974201570.

Taylor, R.L., Verran, J., Lees, G.C. and Ward, A.J.P. (1998) 'The influence of substratum topography on bacterial adhesion to polymethyl methacrylate', *Journal of Materials Science: Materials in Medicine*, 9(1), pp. 17–22. Available at: https://doi.org/10.1023/A:1008874326324.

Teste, F.P., Karst, J., Jones, M.D., Simard, S.W. and Durall, D.M. (2006) 'Methods to control ectomycorrhizal colonization: effectiveness of chemical and physical barriers', *Mycorrhiza*, 17(1), pp. 51–65. Available at: https://doi.org/10.1007/s00572-006-0083-4.

Tetu, S.G., Sarker, I., Schrameyer, V., Pickford, R., Elbourne, L.D.H., Moore, L.R. and Paulsen, I.T. (2019) 'Plastic leachates impair growth and oxygen production in Prochlorococcus, the ocean's most abundant photosynthetic bacteria', *Communications Biology*, 2(1), pp. 1–9. Available at: https://doi.org/10.1038/s42003-019-0410-x.

Thomas, S.A., Newbold, J.D., Monaghan, M.T., Minshall, G.W., Georgian, T. and Cushing, C.E. (2001) 'The influence of particle size on seston deposition in streams', *Limnology and Oceanography*, 46(6), pp. 1415–1424. Available at: https://doi.org/10.4319/lo.2001.46.6.1415.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D. and Russell, A.E. (2004) 'Lost at Sea: Where Is All the Plastic?', *Science*, 304(5672), pp. 838–838. Available at: https://doi.org/10.1126/science.1094559.

Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., Gilbreath, A., Sutton, R., Scholz, N.L., Davis, J.W., Dodd, M.C., Simpson, A., McIntyre, J.K. and Kolodziej, E.P. (2020) 'A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon', *Science*, p. eabd6951. Available at: https://doi.org/10.1126/science.abd6951.

Tiegs, S.D., Clapcott, J.E., Griffiths, N.A. and Boulton, A.J. (2013) 'A standardized cotton-strip assay for measuring organic-matter decomposition in streams', *Ecological Indicators*, 32, pp. 131–139. Available at: https://doi.org/10.1016/j.ecolind.2013.03.013.

Tilman, D., Isbell, F. and Cowles, J.M. (2014) 'Biodiversity and Ecosystem Functioning', *Annual Review of Ecology, Evolution, and Systematics*, 45(Volume 45, 2014), pp. 471–493. Available at: https://doi.org/10.1146/annurev-ecolsys-120213-091917.

Trestrail, C., Nugegoda, D. and Shimeta, J. (2020) 'Invertebrate responses to microplastic ingestion: Reviewing the role of the antioxidant system', *Science of The Total Environment*, 734, p. 138559. Available at: https://doi.org/10.1016/j.scitotenv.2020.138559.

Truchy, A., Angeler, D.G., Sponseller, R.A., Johnson, R.K. and McKie, B.G. (2015) 'Linking biodiversity, ecosystem functioning and services, and ecological resilience: Towards an integrative framework for improved management', *Advances in Ecological Research*, pp. 55–96. Available at: https://doi.org/10.1016/bs.aecr.2015.09.004.

Vadeboncoeur, Y., Lodge, D.M. and Carpenter, S.R. (2001) 'Whole-Lake Fertilization Effects on Distribution of Primary Production Between

Benthic and Pelagic Habitats', *Ecology*, 82(4), pp. 1065–1077. Available at: https://doi.org/10.1890/0012-9658(2001)082[1065:WLFEOD]2.0.CO;2.

Vadeboncoeur, Y. and Steinman, A.D. (2002) 'Periphyton Function in Lake Ecosystems', *The Scientific World Journal*, 2, pp. 1449–1468. Available at: https://doi.org/10.1100/tsw.2002.294.

Valero-Mora, P.M. (2010) 'ggplot2: Elegant Graphics for Data Analysis', Journal of Statistical Software, 35(Book Review 1), p. 212. Available at: https://doi.org/10.18637/jss.v035.b01.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R. and Cushing, C.E. (1980) 'The river continuum concept.', *Canadian Journal of Fisheries and Aquatic Sciences*, 37(1), pp. 130–137. Available at: https://doi.org/10.1139/f80-017.

Vincent, A.E.S. and Hoellein, T.J. (2021) 'Distribution and transport of microplastic and fine particulate organic matter in urban streams', *Ecological Applications*, 31(8), p. e02429. Available at: https://doi.org/10.1002/eap.2429.

Vinebrooke, R.D., Cottingham, K.L., Norberg, J., Marten Scheffer, Dodson, S.I., Maberly, S.C. and Sommer, U. (2004) 'Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance', *Oikos*, 104(3), pp. 451–457. Available at: https://doi.org/10.1111/j.0030-1299.2004.13255.x.

Vis, C., Hudon, C., Carignan, R. and Gagnon, P. (2007) 'Spatial Analysis of Production by Macrophytes, Phytoplankton and Epiphyton in a Large River System under Different Water-Level Conditions', *Ecosystems*, 10(2), pp. 293–310. Available at: https://doi.org/10.1007/s10021-007-9021-3.

Vroom, R.J.E., Koelmans, A.A., Besseling, E. and Halsband, C. (2017) 'Aging of microplastics promotes their ingestion by marine zooplankton', *Environmental Pollution*, 231, pp. 987–996. Available at: https://doi.org/10.1016/j.envpol.2017.08.088.

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M. and Reifferscheid, G. (2014) 'Microplastics in freshwater ecosystems: what we know and what we need to know', Environmental Sciences Europe, 26(1), pp. 1–9. Available at: https://doi.org/10.1186/s12302-014-0012-7.

Wallace, J.B. and Webster, J.R. (1996) 'The role of macroinvertebrates in stream ecosystem function', *Annual review of entomology*. Vol. 41, 41, pp. 115–139. Available at: https://doi.org/10.1146/annurev.ento.41.1.115.

Wang, J., Bucci, K., Helm, P.A., Hoellein, T., Hoffman, M.J., Rooney, R. and Rochman, C.M. (2022) 'Runoff and discharge pathways of microplastics into freshwater ecosystems: A systematic review and meta-analysis', *FACETS*, 7, pp. 1473–1492. Available at: https://doi.org/10.1139/facets-2022-0140.

Wang, J., Guo, X. and Xue, J. (2021) ‘Biofilm-Developed Microplastics As Vectors of Pollutants in Aquatic Environments’, *Environmental Science & Technology*, 55(19), pp. 12780–12790. Available at: https://doi.org/10.1021/acs.est.1c04466.

Wang, T., Wang, L., Chen, Q., Kalogerakis, N., Ji, R. and Ma, Y. (2020) ‘Interactions between microplastics and organic pollutants: Effects on toxicity, bioaccumulation, degradation, and transport’, *Science of The Total Environment*, 748, p. 142427. Available at: https://doi.org/10.1016/j.scitotenv.2020.142427.

Ward, G.M. and Aumen, N.G. (1986) ‘Woody Debris as a Source of Fine Particulate Organic Matter in Coniferous Forest Stream Ecosystems’, *Canadian Journal of Fisheries and Aquatic Sciences*, 43(8), pp. 1635–1642. Available at: https://doi.org/10.1139/f86-202.

Ward, J.E., Zhao, S., Holohan, B.A., Mladinich, K.M., Griffin, T.W., Wozniak, J. and Shumway, S.E. (2019) ‘Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (Mytilus edulis) and Eastern Oyster (Crassostrea virginica): Implications for Using Bivalves as Bioindicators of Microplastic Pollution’, *Environmental Science and Technology*, 53(15), pp. 8776–8784. Available at: https://doi.org/10.1021/acs.est.9b02073.

Ward, J.V. and Stanford, J.A. (1982) ‘Thermal Responses in the Evolutionary Ecology of Aquatic Insects’, *Annual Review of Entomology*, 27(Volume 27, 1982), pp. 97–117. Available at: https://doi.org/10.1146/annurev.en.27.010182.000525.

Weber, A., von Randow, M., Voigt, A.-L., von der Au, M., Fischer, E., Meermann, B. and Wagner, M. (2021) ‘Ingestion and toxicity of microplastics in the freshwater gastropod Lymnaea stagnalis: No microplastic-induced effects alone or in combination with copper’, *Chemosphere*, 263, p. 128040. Available at: https://doi.org/10.1016/j.chemosphere.2020.128040.

Weber, A., Scherer, C., Brennholt, N., Reifferscheid, G. and Wagner, M. (2018) ‘PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate Gammarus pulex’, *Environmental Pollution*, 234, pp. 181–189. Available at: https://doi.org/10.1016/j.envpol.2017.11.014.

Weber, F. and Kerpen, J. (2023) ‘Underestimating microplastics? Quantification of the recovery rate of microplastic particles including sampling, sample preparation, subsampling, and detection using µ-Ramanspectroscopy’, *Analytical and Bioanalytical Chemistry*, 415(15), pp. 2963–2973. Available at: https://doi.org/10.1007/s00216-022-04447-z.

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

Woodward, G. (2009) ‘Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle’, *Freshwater Biology*, 54(10), pp. 2171–2187. Available at: https://doi.org/10.1111/j.1365-2427.2008.02081.x.

Wright, M.S. and Covich, A.P. (2005) ‘Relative importance of bacteria and fungi in a tropical headwater stream: Leaf decomposition and invertebrate feeding preference’, *Microbial Ecology*, 49(4), pp. 536–546. Available at: https://doi.org/10.1007/s00248-004-0052-4.

Xu, C., Zhang, B., Gu, C., Shen, C., Yin, S., Aamir, M. and Li, F. (2020) ‘Are we underestimating the sources of microplastic pollution in terrestrial environment?’, *Journal of Hazardous Materials*, 400, p. 123228. Available at: https://doi.org/10.1016/j.jhazmat.2020.123228.

Yachi, S. and Loreau, M. (1999) ‘Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis’, *Proceedings of the National Academy of Sciences of the United States of America*, 96(4), pp. 1463–1468. Available at: https://doi.org/10.1073/pnas.96.4.1463.

Yang, Y., Liu, W., Zhang, Z., Grossart, H.-P. and Gadd, G.M. (2020) ‘Microplastics provide new microbial niches in aquatic environments’, *Applied Microbiology and Biotechnology*, 104(15), pp. 6501–6511. Available at: https://doi.org/10.1007/s00253-020-10704-x.

Yeager, P.E., Foreman, C.L. and Sinsabaugh, R.L. (2001) ‘Microbial community structure and function in response to larval chironomid feeding pressure in a microcosm experiment’, *Hydrobiologia*, 448(1949), pp. 71–81. Available at: https://doi.org/10.1023/A:1017504114692.

Zhang, K., Hamidian, A.H., Tubić, A., Zhang, Y., Fang, J.K.H., Wu, C. and Lam, P.K.S. (2021) ‘Understanding plastic degradation and microplastic formation in the environment: A review’, *Environmental Pollution*, 274, p. 116554. Available at: https://doi.org/10.1016/j.envpol.2021.116554.

Zhao, T., Tan, L., Huang, W. and Wang, J. (2019) ‘The interactions between micro polyvinyl chloride (mPVC) and marine dinoflagellate Karenia mikimotoi: The inhibition of growth, chlorophyll and photosynthetic efficiency’, *Environmental Pollution*, 247, pp. 883–889. Available at: https://doi.org/10.1016/j.envpol.2019.01.114.

Ziajahromi, S., Kumar, A., Neale, P.A. and Leusch, F.D.L. (2018) ‘Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates’, *Environmental Pollution*, 236, pp. 425–431. Available at: https://doi.org/10.1016/j.envpol.2018.01.094.

Zitko, V. and Hanlon, M. (1991) 'Another source of pollution by plastics: Skin cleaners with plastic scrubbers', *Marine Pollution Bulletin*, 22(1), pp. 41–42. Available at: https://doi.org/10.1016/0025-326X(91)90444-W.

Popular science summary

Plastic is undoubtedly the material of choice for the mass production of cheap and disposable everyday products. Unfortunately, improper disposal of these plastic goods has led to rampant pollution. Over time, plastic debris fragment through environmental forces, resulting in the generation of microplastics (MPs), small plastic particles with diameters between 0.5 millimetres (mm) to 1 micrometre (µm). MPs are also directly manufactured for synthetic fabric or as abrasives in creams, which are eventually released into the environment. In freshwater environments, MPs may contaminate deposits of natural particles, thus potentially impacting organisms and processes associated with these natural particles, and the overall functioning of ecosystems.

My thesis aimed to investigate the effects of MPs on freshwater microbial communities, macroinvertebrates and ecosystem processes, including detritus decomposition, algal productivity and oxygen consumption. Additionally, I aimed to identify the drivers of these effects, including particle properties and natural or anthropogenic sources of stress. In the first experiment, I assessed the effects of spherical polyethylene (PE) MPs on non-biting midge *Chironomus riparius* larvae (referred to as chironomids), and associated ecosystem processes, under different environmental conditions. I found that exposure to a realistic concentrations of MPs reduced chironomid growth and microbial-driven ecosystem respiration, though effects were generally less pronounced and further modified by resource quality and chironomid population density.

In the second experiment, I aimed to identify MP properties – such as particle polymer, shape and concentration - that drive MP effects on the experimental system used in experiment one under conditions that led to MP effects. I found that MP effects were generally associated with higher surface area availability of MPs, such as high particle concentrations and fragment shapes. MP effects also tended to propagate from microbial communities to chironomids or ecosystem processes. However, chironomid presence often dampened the effect of MP exposure on ecosystem processes.

In the third experiment, I assembled a more complex food web than the previous two experiments, which included leaf eating *Asellus aquaticus* isopods, biofilm eating *Viviparus viviparus* snails and particle eating chironomids (the same species used in the previous experiments). I assessed the effects and interactions of different concentrations of common polyethylene terephthalate (PET) MPs and range of temperatures simulating global warming. MPs had effects on microbial communities that propagated onwards onto macroinvertebrates. MP effects were also evident despite up to 6 °C of warming, with some evidence that co-exposure to MPs and warming results in complex outcomes for ecosystem functioning.

The first three experiments I conducted were all in laboratory conditions, where the environment is controlled and manipulated, which may yield results that do not correspond to impacts in the real-world environment. Thus, I took my experiments out into the field, assessing and comparing the responses of leaf litter decomposition to exposure to polypropylene (PP) medical mask derived MP and macroplastic particles, and wood shavings as a proxy for natural organic materials that do not decompose easily. MPs reduced fungal biomass, a key decomposer of leaf litter. The leachates of MPs, which contain various plastic additives, stimulated the decomposition of cellulose, which is a major component of leaf litter. Additionally, macroplastics and wood shavings were often shown to have stronger effects on decomposition rates relative to MPs.

MP pollution has firmly grasped public attention in recent years, with MPs detected in even the most remote corners of the world, in our food and in our bodies. The insights from this thesis contribute to our knowledge on the impacts of widespread MP pollution. The frequency of MP effects on microbial communities, coupled with the potential for effects to spread to other parts of the ecosystem, warrant further research, particularly as microorganisms form the base of the world’s ecosystems and thus drive a major part of ecosystem functioning.

Populärvetenskaplig sammanfattning

Plast är utan tvekan förstahandsmaterialet för massproduktion av billiga engångsprodukter. Men tyvärr är plast också kopplat till den välkända världsomfattande plastföroreningen. Allt detta skräp har med tiden, och naturliga processer, brutits ner till mikroplaster (MP), små plastpartiklar som mäter mellan 1 mikrometer (µm) och 0.5 millimeter (mm) i diameter. Mikroplaster tillverkas dessutom som startmaterial för produktion av syntetiskt tyg och slipmedel i krämer, vilka också i slutändan släpps ut i miljön. MP kan i sötvatten förorena ansamlingar av naturligt partiklar och kan potentiellt påverka organismer och processer associerade med dessa naturliga partiklar, och ekosystemsets övergripande funktion. Målet med min avhandling var att undersöka effekterna av MP i mikrobsamhällen, på makroinvertebrater och ekosystemprocesser (inkluderar detritusnedbrytning, algproduktivitet och syreförbrukning), i sötvatten. Jag har också haft som mål att identifiera drivkrafter i miljön, vilket har inkluderat karaktärisering av partiklar och naturliga eller mänskliga miljöstressorer. I det första experimentet utvärderade jag effekterna av mikrosfärer, gjorda av polyeten (PE), på larver från ett släkte av fjädermyggor *Chironomus riparius* (refereras till här som chironomider), och ekosystemfunktionerna, under olika miljöförhållanden. Jag upptäckte att realistiska koncentrationer av MP sänkte tillväxten av chironomider och den mikrobdrivna ekosystemsrespirationen. Effekten var dock generellt mindre än, och även reglerad av, kvaliteten på resurser och chironomidernas populationsstorlek. I det andra experimentet valde jag att identifiera MP-faktorer, som typ av polymer, form och koncentration. Dessa undersöktes under förhållanden som i det första experimentet påvisat effekter av MP. Jag upptäckte att MP effekterna ofta var kopplade till hög yt-area av MP, vilket inkluderade höga MP-koncentrationer och ojämna plastformer. Effekterna av MP kunde också spridas från mikrobsamhällerna till chironomider eller påverka ekosystemsprocesserna. Närvaro av chironomider skulle dock kunna minska effekten från MP på ekosystemsprossesserna. I det tredje experimentet satte jag ihop en mer komplex näringsväv an de två föregående experimenten. Detta experiment inkluderade lövätande isopoder *Asellus aquaticus*, biofilmsätande sniglar *Viviparus viviparus* och partikelätande chironomidlarver (samma art som första och andra experiment). Jag utvärderade effekterna och interaktionerna för flera olika koncentrationer av vanliga polyetenterephtalat (PET) MP med temperaturer som simulerade global uppvärmning. MP påverkade mikrobsamhällerna, och effekterna spreds till makroinvertebraterna. Efekterna av MP var också påtagliga trots upp till 6 °C uppvärmning, med vissa bevis för att samexponering av MP och uppvärmning ledde till komplexa resultat för ekosystemets funktion. De första tre experimentet var alla utförda i ett laboratorium där miljön var helt kontrollerad och manipulerad, vilket kan resultera i respons som inte stämmer överens med verkligheten. Därför tog jag mitt experiment ut i fält med syftet att utvärdera effekterna på bladnedbrytning från exponering av MP och makroplaster från kirurgansiktsmasker i polypropen (PP) och jämföra det med burspån som simulerade naturliga svårnedbrytbara material. MP minskade svampbiomassan, som är en nyckelspelare i bladnedbrytning. Kemikalier från MP-lakvatten, vilket inehöll plasttillsatser, stimulerade nedbrytningen av cellulosa, vilket är en viktig komponent i blad. Utöver detta så hade makroplaster och burspån ofta en högre påverkan på nedbrytningshastighet än vad MP hade. Den världsomfattande MP-föroreningen har fått mycket uppmärksamhet i samhället på senare år, med fokus på MP i mat, vatten och människor. Insikterna från min avhandling bidrar med ny kunskap om effekterna av utbredd MP förorening. Frekvensen av MP-effekter på sötvattensmikrobsamhällen, och potentialen för effekter att sprida till andra delar av ekosystemet, motiverar mer förskning då mikroorganismer utgör basen för världens ekosystem och därmed driver en stor del av ekosystemets funktion.

科普总结

塑料毫无可疑的是生产大量便宜与一次性产品的主要材料。但不幸的是，处置不当的塑料产品使得全球环境受到了广泛的塑料污染。随着时间的推移，这些塑料废物因环境力量分解成微塑料。微塑料是直径为 0.5 毫米（mm）和 1 微米 (µm) 之间的小塑料颗粒或碎片。此外，微塑料因可以被直接用来合成纤织物或作为霜中的研磨剂， 而最终被释放到环境中。在淡水环境中，微塑料能污染天然颗粒沉积物。于是，微塑料有潜在的能影响跟天然微颗粒相关的生物与流程，以及生态系统的总体功能。

我的论文目标是调查微塑料在淡水微生物、大型无脊椎动物与生态系统功（包括碎屑分解，藻类生产力与耗氧量）能对微塑料的效应。此论文也旨在识别造成这些影响的原因，包括微塑料颗粒特征与天然或人造压力。在我第一个实验里，我评估了聚乙烯（PE）微塑料球在伸展摇蚊（*Chironomus riparius*）幼虫及相关生态系统功能中，且在不同环境条件下的效应。我发现实际浓度的微塑料减少了伸展摇蚊幼虫的生长且影响到了微生物驱动的生态系统的耗氧量。 然而，微塑料效应通常都比资源质量与幼虫种群密度所带来的影响小，但也时常受这些因素的调节。

在第二个实验里，我的宗旨就是识别能驱动微塑料的效应因素，包括颗粒聚合物、形状和浓度。这实验利用了第一个实验里模拟导致微塑料效应的系统条件。我发现微塑料效应通常和具有较大的表面积有关，例如高颗粒浓度要和粗糙碎片形状。微塑料效应也倾向于从微生物群落开始，接着传播到幼虫或生态系统功能。然而，幼虫的存在也抵消了微塑料在生态系统功能上的影响。

在第三个实验，我建了个比前两个实验较复杂的食物网，其中包括食叶等足类动物（*Asellus aquaticus*）、食生物膜的蜗牛（*Viviparus viviparus*）和食自然颗粒的伸展摇蚊幼虫 （*Chironomus riparius*）。我在这模型系统调查不同聚对苯二甲酸（PET）微塑料浓度和变暖（模

拟全球变暖）的效应以及之间的相互作用。微塑料在微生物群落的影响能传播到幼虫。尽管升温高达 6 ° C，微塑料的效应仍然很明显。然而，同时暴露于微塑料与变暖也产生了一些对生态系统功能的复杂效应。

我前三个实验都是在实验室受控和操纵环境内实施。然而，这种受控条件可能会产生与现实环境中的影响不相符的结果。因此，我在野外实地进行了再一项实验，旨在评估和比较聚丙烯（PP）医用口罩衍生的微塑料和大塑料颗粒以及木屑（作为不易分解的天然材料的代表）对落叶分解的影响。我发现微塑料降低了真菌的生物量。真菌实际上是个落叶层的主要分解者。微塑料的渗滤液含有各种塑料添加剂，而刺激了纤维素分解。纤维素是落叶的其中一个主要组成物。此外，与微塑料相比，大塑料和木屑通常对分解速率有更强的影响。

近年来，微塑料污染引起了公众的广泛关注，都因再世界最偏远的角落、在我们食物和我们身体内都发现了它。此论文内的简介贡献于我们对广泛微塑料污染产生的影响的了解。世界生态系统的基础都被微生物构成，并对生态系统功能有重大的驱动。因此，微塑料对微生物群落的效应，加上这些影响传播到生态系统其他部分的潜能，都应激发了对该领域进一步研究。

Ringkasan Sains Umum

Plastik merupakan bahan pilihan untuk produk yang murah dan sekali pakai. Sayangnya, pelupusan produk plastik yang tidak terkawal telah menyebabkan pencemaran plastik yang meluas. Secara beransur-ansur, sampah plastik pecah melalui proses semula jadi dan menghasilkan mikroplastik (MP), zarah plastik kecil dengan diameter antara 0.5 milimeter (mm) hingga 1 mikrometer (µm). MP juga dihasilkan secara langsung untuk pembuatan fabrik sintetik atau sebagai pelelas, yang akhirnya dilepaskan ke alam sekitar. Dalam alam air tawar, MP mungkin mencemari sedimen zarah semula jadi, dan berpotensi menjejaskan organisma dan proses yang berkaitan dengan zarah semula jaid, dan keseluruhan fungsi ekosistem.

Tesis saya bertujuan untuk menyiasat kesan MP terhadap komuniti mikrob, makroinvertebrata dan proses ekosistem air tawar, termasuk penguraian detritus, produktiviti alga dan penggunaan oksigen. Saya juga bertujuan mengenalpasti pendorong kesan MP, termasuk ciri zarah dan sumber tekanan semula jadi atau antropogenik. Dalam eksperimen pertama, saya menilai kesan MP sfera polietilena (PE) terhadap larva *Chironomus riparius* (dirujuk sebagai chironomid), dan fungsi ekosistem yang berkaitan, dengan keadaan persekitaran yang berbeza. Saya mendapati bahawa MP mengurangkan pertumbuhan chironomid dan respirasi ekosistem oleh mikrob. Kesan-kesan MP kerap lebih kecil berbanding dengan kesan keadaan persekitaran dan juga diubahsuai oleh kualiti sumber dan kepadatan populasi chironomid.

Dalam eksperimen kedua, saya menyasarkan untuk mengenalpasti faktor MP - seperti polimer, bentuk dan kepekatan zarah - yang memacu kesan MP pada sistem eksperimen yang digunakan sebelum ini di bawah keadaan persekitaran yang terhasil kesan MP. Secara umumnya, saya mendapati bahawa kesan MP berkaitan dengan kawasan permukaan zarah yang lebih besar, disebabkan kepekatan zarah melebihi tahap persekitaran dan bentuk serpihan. Kesan MP juga sering bersebar daripada komuniti mikrob kepada chironomid atau proses ekosistem. Tetapi, kehadiran chironomid sering mengurangkan kesan MP pada proses ekosistem.

Dalam eksperimen ketiga, saya menggunakan rankaian makanan yang lebih komplex berbanding dengan dua eksperimen sebelum ini, dengan tambahan pemakan daun isopod *Asellus aquaticus*, pemakan biofilem siput *Viviparus viviparus* dan pemakan zarah larva chironomid (sama spesis seperti eksperimen sebelum ino). Saya menilai kesan dan interaksi kepekatan MP polietilena tereftalat (PET) dan suhu simulasi pemanasan global. MP mempunyai kesan pada komuniti mikrob yang sebar ke makroinvertebrata. Walaupun tahap suhu ditinggikan kepada pemanasan 6 °C, kesan MP tetap jelas, dengan beberapa bukti bahawa pendedahan bersama MP dan pemanasan menghasilkan kesan yang kompleks terhadap fungsi ekosistem.

Ketiga-tiga eksperimen pertama semuanya dijalankan dalam persekitaran dalam makmal, di mana keadaan telah dikawal dan dimanipulasi. Keadaan semacam ini mungkin menghasilkan keputusan yang tidak sepadan dengan kesan MP di persekitaran dunia sebenar. Oleh itu, saya menjalankan eksperimen di luar makmal, menilai dan membandingkan tindak balas penguraian daun apabila didedah kepada zarah MP dan makroplastik yang diperolehi daripada topeng perubatan polipropilena (PP), dan serpihan kayu sebagai bahan rujukan untuk bahan semula jadi yang tidak mudah terurai. Pendedahan MP mengurangkan biojisim kulat, yang merupakan salah satu pengurai utama daun. Bahan larut lesap MP, yang mengandungi pelbahai bahan temabahan plastik, meningkatkan pernguaraian selulosa, yang merupakan komponene utama daun. Selain itu, makroplastik dan serpihan kayu sering mempunyai kesan yang lebih kuat pada kadar penguraian berbanding dengan MP.

Pencemaran MP telah menarik perhatian umum sejak beberapa tahun kebelakangan ini, di mana MP ditemui di alam dunia yang antara paling terpencil, dalam makanan kita dan badan manusia. Hasil tesis ini menyumbang kepada pengetahuan kita mengenai kesan pencemaran MP yang meluas. Kekerapan kesan MP pada komuniti mikrob, bersama dengan potensi kesan merebak ke bahagian ekosistem yang lain, mewajarkan penyelidikan lanjut kerana mikroorganisma membentuk asas ekosistem dunia dan juga memacu sebahagian besar fungsi ekosistem.

Acknowledgements

Naturvårdsverket and swedish tax payers: *for the opportunity*

My supervisors: *without whom this would not be possible*
Brendan, with all your advice and experience, I have been really lucky to have had you as a PhD supervisor. **Frank**, for the pieces of wisdom over the years. **Mirco**, for all your advice and direction in writing and experiment design, and the rapid turnover of any drafts. **Martyn**, for your perspective, thoughts and ideas in my work. **Amélie,** for the help and advice over the years. **Rachel**, for the study visit to NIVA, I learnt a lot about microplastics that has stuck with me ever since.

FUME: *for enriching my work*
Stefan, for the advice and direction provided for my experiments and writing. **Prune**, for all your guidance in the lab. **Tong**, for working with me in the lab and office. **Javi & Lauren,** for the great company and help in the lab and beyond**. Maliheh & Anna,** for helping me with questions I had with lab work and general advice. **Klas,** for the all the bits of advice. **Xavi,** for the crash course in flow cytometry and general help in the lab. **Moritz,** for helping me understand bioinformatics just a little bit better. **Jennifer,** for the fungi knowledge of which you have plenty.

My help during the PhD: *who made my life easier*
Sophia Renes, for all the hours and effort for the lab work for paper II. **Martina,** for submerging yourself in the pond for paper IV**. Siri,** for grinding and weighing samples (a lot of them) for paper III.

PhD students, past and present: w*ithout whom my days would be longer*
Carlotta, for all the good times, and making my start in Sweden a lot easier. **Alberto (Italian),** for the good company over the years. **Valentina,** for all the conversations about plants and shrimp. **Svante**, for all the support and great times over the years, things are definitely much more interesting with you around. **Yao,** for all the fun times over the years. **Oscar**, for the great positive company and conversations. **Daniel,** no slide tackling indoors big man. **Joel,** for the great company during our trip to Brisbane. **Sanne, Björn, Paul, Malhun, Khadija, Maxi, Patrick, Harold, Winnie, Tohn, and others,** for friendly conversations, coffee and fun times. **Jasmina**, for your efforts in helping me settle in during the early months of the pandemic. **Sara, Emma, Jenny,** for the warm reception to the department years ago.

The IVM department: *for broadening my knowledge*
Bio-group: Richard. Willem, **Stina, Ulf, Danny, Fernando (C.), James, Simon, Peter, Micke,** for the help and advice over the years, and sharing your work with us all.
Benthic Fauna Lab (**Magda-Lena & Karin**), for the help with taxa knowledge and working the microscopes.
Marcus (K.), for all the help in the lab and ordering 40 kilos of sand for me.
Alina, Alberto (Spanish), for all the conversations and advice.
Lars, for always keeping in mind our welfare.
Rest of the dept. for sharing your expertise and being a friendly work environment.

Invertebrates I worked with: *without whom this work would not exist*
Chironomids, keep emerging. **Isopods,** you guys were fun. **Snails,** for all the stress, especially that one snail who pretended to be dead a lot.

SLU Phytotron (Ayano & Fredric): for the help with setting up and maintaining the temperature controlled cabinets.

SUPSI: Andreas, Camilla, Federica, for taking the time to show me around and discuss our work.

Imperial: Guy, Michelle, Bruno, Emma *where my journey with freshwater ecology started.*

Friends: *highly recommended*

Catherine, for ending up in Uppsala also (somehow) and being a great friend for almost a decade now. **Si Cong** & **Jenny,** for all the dinners (and for coming over for dinner!). **Boz & Grace,** for all the fun times in Uppsala. **Arief**, for all the good times in Uppsala and letting me indulge in being Malaysian. **LAH,** for being Mississippian (fun word).

Milan, it has been to my eternal fortune to have you as a friend. **Elan**, for being a great friend despite us moving to different countries. **Harry**, for keeping in me in mind often despite the difficulties in scheduling.

Qing Xi, for wanting to play badminton with me. **Li Hao,** for training together with me.

Gupta FC, for all the football that we lost and those that we won.

Kai Liang, for keeping in touch and visiting me in Europe. **Boon Siang, Sze Yan, Tytus, Naren**, for meeting up with me whenever I go home.

Family: *Without whom I would be lesser*

Mom, it is pretty obvious how I ended up here, considering all the time I spend around aquariums growing up. **Papa** 谢谢你们帮我走到这里来。

HK Uncle, for the help in getting me to Europe in the first place. **Ze Bing, Ze Chen, Ze Jie**, I hope that things will work out the way you want them to.

Grandma 婆婆，谢谢你永远关心我。

Sammi, I must have quite difficult to deal with at times over the years, especially towards the end of my PhD when I started screaming in my sleep. It has not been the easiest but I appreciate you sticking by me all this time. You the best.

Aquatic Sciences (2023) 85:70
https://doi.org/10.1007/s00027-023-00964-w

Aquatic Sciences

RESEARCH ARTICLE

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

Ze Hui Kong[1] · **Francis J. Burdon**[1,2] · **Amélie Truchy**[1,3] · **Mirco Bundschuh**[1,4] · **Martyn N. Futter**[1] · **Rachel Hurley**[5] · **Brendan G. McKie**[1]

Received: 11 May 2022 / Accepted: 27 March 2023

Abstract

Fine particulate organic matter (FPOM) is an important basal resource in stream ecosystems for deposit- and filter-feeding macroinvertebrates (collectively 'particle feeders'). Microplastics (MP) share many characteristics with FPOM (e.g. size range, surface area to volume ratios) and are potentially consumed by particle feeders. Accordingly, MP contamination of natural FPOM pools might affect particle feeder growth and survival, particularly when background FPOM resource quality is low, or intraspecific competition is high. We conducted a microcosm experiment to evaluate how a realistic (1400 particles/kg sediment) polyethylene MP ($\phi = 45$–53 µm) concentration interacts with FPOM ($\phi = 63$–250 µm) resource quality (low versus high nutrient content) and consumer density (10 versus 20 individuals per microcosm) to affect growth and survival of larval *Chironomus riparius* (Diptera: Chironomidae), a model particle feeder. We additionally quantified community respiration, based on three hour measurements of oxygen consumption in the microcosms at the end of the experiment. MP exposure reduced larval body lengths by 26.7%, but only under the low consumer density treatment. MPs reduced community respiration by 26.2%, but only in the absence of chironomids, indicating an impact on microbial respiration. In comparison, low resource quality and high consumer density were associated with 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 the 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.

Keywords Fine particulate organic matter · Microplastics · *Chironomus riparius* · Consumer density · Resource quality · Community respiration

Introduction

Stream and river networks produce and transport tonnes of fine particulate organic matter (FPOM) [particle $\phi > 45$ µm, < 1000 µm (Hutchens et al. 2017)] every day (Malmqvist et al. 2001). FPOM particles include faecal particles (Shepard and Minshall 1984), other particulate detritus (Cummins and Klug 1979) and organic colloid flocculates (Wotton 2007). FPOM is a major component of nutrient and energy cycling in streams. FPOM provides a substrate for microbial biofilm formation, and constitutes a primary resource for deposit and filter feeding invertebrates, collectively termed 'particle feeders' (Fisher 1977; Malmqvist et al. 2001). However, stocks of FPOM in streams and rivers

✉ Ze Hui Kong
ze.hui.kong@slu.se

[1] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden

[2] Te Aka Mātuatua - School of Science, University of Waikato, Hamilton, New Zealand

[3] UR RiverLy, Centre Lyon-Grenoble Auvergne-Rhône-Alpes, French National Institute for Agriculture, Food, and Environment (INRAE), Lyon, France

[4] iES Landau, Institute for Environmental Sciences, RPTU Kaiserslautern-Landau, Landau, Germany

[5] Norwegian Institute for Water Research (NIVA), Oslo, Norway

are increasingly contaminated by pollution from microplastic (MP) particles (Alimi et al. 2018), with a size range of 1–5000 µm (Frias and Nash 2019). Vast quantities of plastic waste enter aquatic ecosystems via terrestrial runoff (e.g. storm water from urban and industrial areas where plastic use is high), wastewater or wind (Zeng 2018). Some of this waste comprises primary MPs, directly released into the environment as spheres, irregular shapes (Gregory 1996) or fibres (Henry et al. 2019). In contrast, secondary MPs are generated from larger plastic waste through biotic-, e.g. microbial or animal activity (Immerschitt and Martens 2020), and abiotic degradation processes, e.g. UV photo-degradation (Barnes et al. 2009). FPOM and MP particles have overlapping size ranges (Webster and Meyer 1997; Thomas et al. 2001; Frias and Nash 2019) and densities (EPA 1992; Thomas et al. 2001), contain bioavailable organic carbon (Romera-Castillo et al. 2018) and host surficial biofilms (Zettler et al. 2013; Hossain et al. 2019; Chen et al. 2020). Consequently, contamination of lotic FPOM pools with MPs has potential to affect both the microbes and invertebrate particle feeders that regulate the cycling of FPOM in freshwater catchments.

Key particle feeding organisms in lotic ecosystems include larval Chironomidae (Diptera), especially the non-predacious species from the subfamilies Orthocladinae and Chironominae. These chironomids not only consume benthic FPOM, but also incorporate FPOM into construction of silken tubes within which they live (Mckie 2004; Goedkoop et al. 2007; Hölker et al. 2015). The deposit feeding and tube construction activities of chironomids in turn alter microhabitat structures and sediment microbial communities (Yeager et al. 2001). Larval chironomids contribute to bioturbation as individuals pump water through their tubes (Svensson 1997). Bioturbation and the potential for associated increases in bacterial production (van de Bund et al. 1994) together enhance the community respiration of microbes associated with detritus and sediments (Nogaro et al. 2008; Baranov et al. 2016) and support denitrification (Svensson 1998). These microbe-consumer interactions may be disrupted by MPs, directly affecting the survival and activity of chironomids and/or microorganisms (Huang et al. 2021). MPs might also be incorporated into chironomid tubes, as observed previously for Trichoptera larvae (Ehlers et al. 2019), potentially altering the functioning of these structures (Ehlers et al. 2020).

MPs have been shown to cause a range of effects on benthic particle-feeding organisms in aquatic ecosystems (de Sá et al. 2018). For example, Stanković et al. (2020) found that MP exposure slowed development time and increased final body size in the chironomid *Chironomus riparius*. Other recorded effects include reduced feeding of the marine lugworm *Arenicola marina* (Besseling et al. 2013) and reduced growth rates in the amphipod *Hyalella azteca* (Au et al. 2015). Such effects are likely to be at least partly attributable to a 'food dilution effect' (Ogonowski et al. 2018). 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 (Ogonowski et al. 2016; Welden and Cowie 2016). Besides that, MP exposure can also affect metabolism and other physiological functions (Kratina et al. 2019), which over time might accumulate to impact consumer growth and survival, and ecosystem functioning (Prinz and Korez 2020). Despite the potential vulnerability of freshwater FPOM-based food webs to MP exposure, only one previous study has specifically investigated the impacts of MPs on ecosystem processes associated with FPOM particle feeders. Huang et al. (2021) focussed on processes of nitrogen removal mediated by microbes and *Chironomus riparius*. They found that although MP exposure promoted growth and activity of denitrifying microbes, the role *C. riparius* in mediating nitrogen removal was reduced, possibly due to a negative impact on its bioturbating activities.

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) (McNamara and Buchanan 2005; Ieromina et al. 2014). The nutrient quality of FPOM is notably low in comparison with other aquatic resources (e.g. algae) (Callisto and Graça 2013; Bundschuh and McKie 2016), but is normally enhanced through the growth of surficial biofilms on particles (Cummins and Klug 1979; Joyce et al. 2007). In turn, surficial biofilm growth is strongly regulated by substrate characteristics [e.g. refractory carbon (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 might have particularly strong potential to impact particle feeders (Miao et al. 2019a). Consumer density is a further key factor regulating resource availability. As consumer density increases, resource limitation is intensified as a consequence of competition (McKie et al. 2008). This may lead to negative density dependent effects on individual behaviour, growth and survival (Hooper et al. 2003), and on associated ecosystem processes (Klemmer et al. 2012). Both intra- and interspecific competition (i.e. competition within and between species, respectively) increases the vulnerability of organisms to additional stressors, e.g. exposure to pesticides, nutrients and low pH (McKie et al. 2009; Op de Beeck et al. 2018). 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.

Here, we conducted a laboratory microcosm experiment to compare the effects of FPOM resource quality (low or high quality) and consumer density (0, 10 or 20 chironomid individuals per microcosm) with those of microplastic exposure on the growth and survival of *C. riparius* as a model particle feeder, and also on community respiration (i.e. bulk respiration of all organisms in the microcosms, including microbes and chironomids) as an ecosystem process. Our microplastic consisted of polyethylene (PE) particles, among the most dominant polymer types detected in environmental samples (Shim et al. 2018; Koelmans et al. 2019). The PE was in the form of primary MP bead particles, widely used in personal care products and thus readily entering freshwater habitats via wastewater. FPOM and spherical PE MPs were conditioned in stream water to grow surficial biofilm before the addition of chironomids. We expected that the survival of chironomid larvae would be reduced when reared at high density, reflecting the negative density-dependent effects of intraspecific-competition, and on the lower quality FPOM resource. We further hypothesised that these impacts would be strongest in microcosms contaminated by MPs due to the additional stress imposed by contamination of the consumers' FPOM food resource (i.e. in line with a food dilution effect). Finally, we hypothesised that community respiration would be greatest at the highest chironomid density and on the high quality FPOM resource, but would be reduced by the presence of MPs due to negative effects of MP exposure on chironomid biomass accrual and/or disruptions in the activity of microbial biofilms.

Methods

Experimental design

The study was conducted in a controlled environment room set at 20 ± 3 °C on a 16:8 h light–dark cycle. Glass crystallisation dishes (300 mL) were used as microcosms, each containing 80 g of sterile sand (Fontainebleau Sand, VWR). The experiment employed a full factorial design with three variables: FPOM resource quality (two levels: low quality, field-derived FPOM and high quality, laboratory-produced FPOM), chironomid consumer density (three levels: absence, 10 individuals or 20 individuals per microcosm) and MP presence (two levels: presence or absence of MPs). Each treatment was replicated five times leading to 60 microcosms across five randomised blocks.

Treatments

FPOM resource quality – two FPOM resource types were used in the experiment: the first was low quality, naturally occuring FPOM sourced from a forest stream, and the second consisted of a high quality heterogeneous lab mixture. The low quality FPOM resource was collected from depositional habitats in Fibyån Stream (59.884 N, 17.354 E), which flows through the Fiby Old Forest Nature Reserve (Ålandsdal, Sweden). We collected FPOM by carefully siphoning the top layer of the sediment using a 50 mL syringe. The collected material was filtered through nested sieves (250 µm and 63 µm) to remove any large debris and invertebrates. The FPOM material retained in the 63 µm sieve was then dried in an oven at 60 °C for 48 h and stored in glass jars. The high quality FPOM was prepared by combining ground Tetra Phyll fish food (Tetra, Germany) together with ground alder and birch leaves in a ratio of 1:1:2. The leaves were collected freshly abscised during autumn 2017 from one small alder and one small birch forest stand near Uppsala, Sweden that were not subject to anthropogenic fertilization. The high quality FPOM was passed through the same sieve set as used for the low quality FPOM, to ensure a comparable size distribution of particles. The carbon and nitrogen content of these FPOM resources were analysed at the UC Davis Stable Isotope Facility with an PDZ-Europe ANCA-GSL elemental analyser (SERCON Ltd, Cheshire, UK) interfaced to a continuous flow isotope ratio mass spectrometer (UC Davis 2020). The high and low quality FPOM resources had an average C:N ratio of 16.2 ± 0.0 and 47.5 ± 0.4, respectively. It is possible that the source materials used in both the low and high quality FPOM treatments were contaminated with some level of MP pollution. However, given we collected alder and birch leaf litter and natural FPOM from single locations, and used a single commercial preparation of fish food, we did not anticipate substantial variation in the level of such contamination, and expected that any such contamination was evenly distributed among our replicates.

Consumer density – the density of chironomids was varied by adding either 0, 10 or 20 newly hatched larvae (< 24 h old) into each microcosm dish. Five chironomid egg masses were obtained from a laboratory culture maintained at the department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, and hatched in a petri dish with the same water and temperature as the source culture (20 °C). Hatched larvae were gently mixed to facilitate random allocation of individuals from the five clutches among the microcosms. Once added to the microcosms, chironomid individuals settled on the bottom substrate and constructed tubes predominantly composed of the FPOM resource provided. In a pilot study, a consumer density of 10 allowed all individuals to construct robust, well-spaced tubes incorporating abundant FPOM particles (personal observation, Supplementary Information, Fig. S1a). The doubling of density in the 20 individual treatments was thus presumed to increase competition for FPOM, and resulted in less well-spaced tubes incorporating lower quantities of FPOM per tube (personal observation; Supplementary Information, Fig. S1b). The densities used in this experiment (10 and 20 corresponding to 1411 and 2822 individuals m^{-2}, respectively) are within the range of densities (157 and 3384 individuals m^{-2}, mean 954 individuals m^{-2}) observed previously for northern European lakes (Mousavi 2002).

Microplastics – clear virgin polyethylene microspheres (Cospheric, 45–53 µm) were used in the MP treatment, at a concentration equivalent to 1400 MPs per $kg_{sediment}$ (0.669 mg per $kg_{sediment}$) or 747 MPs per litre of water (0.357 mg per L_{water}). The concentration used here is within the range of globally observed environmental sediment MP concentrations in some urban sites (Yang et al. 2021). We prepared a MP stock suspension of 0.01 g per L of de-ionised water. Unintended MP inputs were minimised by using glassware in the laboratory, with all containers pre-rinsed with 95% ethanol. Personnel always wore cotton clothing in the laboratory, and leaf litter was collected by hand and stored in cardboard boxes. When producing FPOM, we used a grinder with a stainless steel burr. Additionally, the stream water used in the microcosms were filtered through a stainless steel 25 µm sieve to remove any MPs above that size before the MP treatment was applied.

Addition of FPOM and MPs to the microcosms

Suspensions of FPOM resource (low or high quality) were prepared and added into each microcosm separately with 0.11 g of FPOM resource in 50 mL of de-ionised water. This amount of high quality FPOM resource was found in a pilot test to be sufficient for chironomids at a consumer density of 10 to reach pupation within 15 d. For microcosms exposed to MPs, we pipetted 5.35 mL of the MP stock solution into each MP treatment microcosm to achieve the desired concentration. The stock was sonicated for 5 s before each application to help evenly distribute particles. We confirmed realisation of the target concentration and effectiveness of sonication by visual enumeration of MP particles in stock samples under a light microscope (Supplementary Information, Fig. S2). All microcosms were then filled with 150 mL filtered stream water. The FPOM resource and MPs were conditioned in the microcosms for 7 d (after McKie et al. 2008) before chironomids were added. During this period, all microcosms were refilled every two days with approximately 25 mL filtered stream water to supplement the initial microbial colonisation, and to maintain both water depth (2 cm) and water nutrient concentrations close to that of the source stream. After the conditioning period, chironomids were added, with de-ionised water used for subsequent water level maintenance to minimise addition of dissolved minerals and colonisation by additional microbes. These experimental conditions were maintained for 14 days before the final measurements were made.

Data collection

At the end of the experiment, community respiration (i.e. bulk respiration of all organisms in a microcosm) was quantified in sets of eight microcosms (henceforth referred to as ‘assay batch’) using the dark–light bottle method of Johnson et al. (2009), as modified by Truchy et al. (2020). Briefly, each microcosm was filled with oxygen-saturated de-ionised water and the dissolved oxygen (DO) concentration measured using calibrated optical sensors (Firesting O_2 Meter, PyroScience, Aachen, Germany). Where possible, microcosms were taken from the same experimental block for each assay batch. Four crystallisation dishes containing only de-ionised water were also included to measure background O_2 fluctuations in the microcosm dishes (i.e. ‘blanks’ with only de-ionised water and sterile sand). All dishes were then sealed with parafilm, with care taken to avoid trapped air bubbles, and incubated in the dark. After three hours, the microcosm dishes were carefully unsealed and DO concentrations measured again. Community respiration (CR) is calculated as the difference between the initial and final DO concentration, corrected for the volume of crystallisation dishes and incubation time (Supplementary Information, Eq. S1). This was further adjusted for background DO fluctuations measured in the blanks.

Following respiration measurements, chironomids were collected and survivorship assessed. The collected chironomids were left to empty their guts overnight in de-ionised water prior to quantification of biomass. After 24 h, the chironomids were sedated with carbonated water and photographed under 10 × magnification. The photographs were analysed using ImageJ (V1.53a, National Institutes of Health, Bethesda, USA) (Schneider et al. 2012) to obtain the individual body lengths of chironomids. The chironomids from each microcosm were then dried at 60 °C in an oven for 24 h before total biomass was weighed to the closest 0.001 mg on a microbalance. Per capita biomass was calculated for each microcosm by dividing total biomass with the number of survivors.

Data analysis

Data were analysed using linear mixed models (LMM) with consumer density, FPOM quality and MP treatments fitted as fully crossed fixed effects. Random effects varied according to the response variable. Experimental block was fitted as a random effect for mortality and per capita biomass, while body length data was analysed using microcosm identity nested within experimental blocks as a random effect. For analysis of respiration data, assay batch was fitted as a random effect to account for background variation among batches. Across all microcosm respiration rates, more random variation was explained by assay batches than the original experimental blocks (33.3% versus 16.2%, respectively). The primary test of significance was derived from the LMMs, but we additionally included significance tests based on Tukey's post-hoc test in our figures, to aid in assessment of differences among groups. One replicate was excluded from all analyses due to an error in the initial allocation of the number of chironomid larvae to that microcosm. There were also three replicates with extremely low survivorship ($\leq 15\%$, one with and two without MPs). However, exclusion of these replicates did not change the outcomes of our statistical tests (no change in which terms were significant), and so were retained in our analyses. All data analysis was carried out using RStudio V3.62 (R Core Team 2019). R packages used were *ggplot2* for graphical plotting (Wickham 2009), *lme4* to construct LMMs (Bates et al. 2015), *lmerTest* to derive p-values for the LMMs (Kuznetsova et al. 2017) and *emmeans* to perform Tukey tests on the models (Lenth 2019).

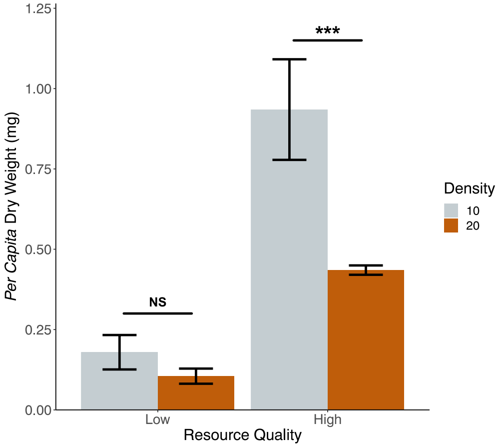


Fig. 1 Effects of FPOM resource quality and consumer densities on mean [±1 standard error (SE)] per capita dry weight of chironomid larvae. Mixed model significance tests: density ($p<0.001$), resource quality ($p<0.001$), density × resource quality ($p<0.010$). Tukey's post-hoc tests plotted on the graph. *NS* non-significant; $^{**}p<0.010$, $^{***}p<0.001$

Results

Chironomid survivorship, biomass and body length

Mean percentage survivorship was 66.7 ± 4.1%. Neither the three-way interaction between resource quality, consumer density and MP exposure (ANOVA, $F_{1,27}=3.25$, $p<0.100$) (Supplementary Information, Fig. S3), nor any other interactions or main effects (all remaining $F\leq 1.1$, $p>0.100$) (Supplementary Information, Table S1) were significant.

Chironomid mean per capita dry weight (g) was 473% higher for larvae fed the high (0.672 ± 0.093) compared with the low (0.142 ± 0.030 mg) quality FPOM resource ($F_{1,27}=52.2$, $p<0.001$). Chironomid mean per capita dry weight was also 50% lower for larvae kept at the high (0.27 ± 0.04 mg) compared with low density (0.54 ± 0.12 mg) treatment ($F_{1,27}=15.0$, $p<0.001$), pooling across the resource quality treatments. Additionally, there was a significant interaction between FPOM resource quality and consumer density ($F_{1,27}=8.38$, $p<0.01$), with the negative effect of increased density on per capita dry weight more pronounced for individuals reared on the high quality FPOM resource (Fig. 1).

The individual body lengths of chironomid larvae were 245% higher when fed the high quality FPOM (10.3 ± 0.1 mm) compared with the low quality FPOM (4.2 ± 0.08 mm) resource ($F_{1,15}=236$, $p<0.001$). Additionally, there was a significant interaction between consumer density and MP presence (ANOVA, $F_{1,15}=6.30$, $p<0.05$). Individual body length was significantly lower for chironomids exposed to MPs under the low, but not high, density treatment (Fig. 2).

Community respiration

Community respiration was significantly affected by FPOM resource quality (ANOVA, $F_{1,27}=175$, $p<0.001$). Respiration was 374% higher in microcosms with the high (4.41 ± 0.27 μmol O_2 hr^{-1}) compared with low (1.18 ± 0.09 μmol O_2 hr^{-1}) quality FPOM resource. There was also a significant interaction between consumer density and MP presence (ANOVA, $F_{2,24}=4.46$, $p<0.050$), with respiration reduced only when MPs were present in the chironomid-free microcosms (Fig. 3). This interaction was further explored by analysing data for microcosms without chironomids alone, in which respirations is primarily attributable to microbial organisms. In these microcosms, respiration was 335% greater in replicates with the high (4.39 ± 0.44 μmol O_2 hr^{-1}) compared with low (1.31 ± 0.16 μmol O_2 hr−1) quality FPOM (ANOVA, $F_{1,11}=75.6$, $p<0.001$). MP presence had a significantly negative effect on community respiration (ANOVA, $F_{1,12}=6.91$, $p<0.050$; Fig. 4). A further interaction between FPOM resource and MP presence was non-significant (ANOVA, $F_{1,15}=3.86$, $p>0.050$), with a trend for lower community respiration in the presence of MPs when

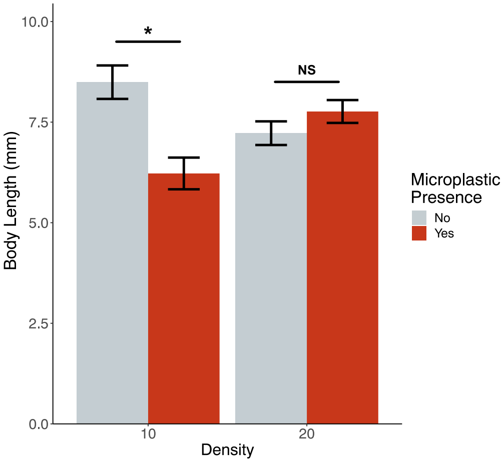


Fig. 2 Effects of consumer densities and microplastic exposure on mean (± 1 SE) chironomid body length. Mixed model significance tests: density (p < 0.100), MP (p < 0.100), resource quality (p < 0.001), density x MP (p < 0.050). Tukey's post-hoc tests plotted on the graph. *NS* non-significant; ***p < 0.001; *p < 0.050

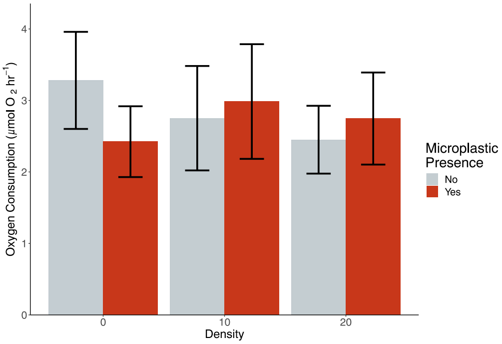


Fig. 3 Effects of consumer density and microplastic exposure on mean (±1 SE) community respiration of microcosms. Mixed model significance tests: density (p > 0.050), MP (p > 0.050), density × MP (p < 0.050). No Tukey's post-hoc test significance

provided with the high quality, but not the low quality, FPOM. In contrast, community respiration of microcosms with chironomids was significantly affected by FPOM resource quality only (ANOVA, $F_{1,\,32} = 111$, $p < 0.001$) (Supplementary Information, Fig. S4), with rates 380% higher in microcosms with the high (4.22 ± 0.35 µmol O_2 hr^{-1}) compared with low (1.11 ± 0.11 µmol O_2 hr−1) quality FPOM resource.

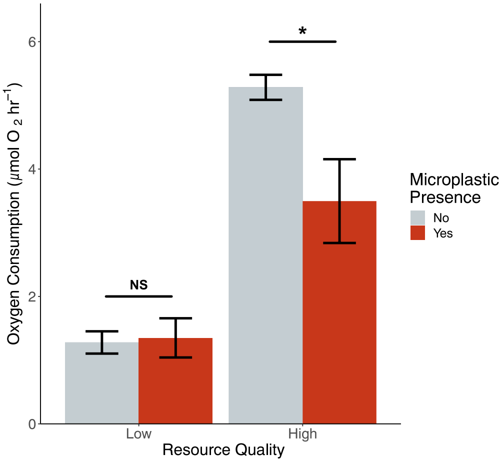


Fig. 4 Effects of FPOM resource quality and microplastic exposure on community respiration (± 1 SE) of microcosms without chironomids. Mixed model significance tests: resource quality (p < 0.001), MP (p < 0.050), resource quality × MP (p > 0.050). Tukey's post-hoc tests plotted on the graph. *NS* non-significant; *p < 0.050

Discussion

In a microcosm experiment, we evaluated the effects of a realistic substrate concentration of polyethylene MP particles on the survival and growth of a model particle feeding chironomid and on community respiration, relative to those of additional environmental drivers. FPOM resource quality and consumer density were clearly the predominant drivers of both organism growth and community respiration. Nevertheless, there was evidence for contingent impacts of MP exposure on chironomid body length and community respiration. MP exposure reduced chironomid body length under the high, but not low, FPOM resource quality treatment. MP exposure also reduced community respiration, but only in the microcosms without chironomids present, indicating an impact on microbially-mediated community respiration. The effect sizes of these MP impacts were limited relative to the much stronger effects of consumer density and resource quality, similar to previous studies which demonstrated lower levels of impact from MPs relative to other stressors, such as temperature (Hiltunen et al. 2021) and pesticides (Horton et al. 2018).

Individual and population responses

We did not observe a systematic effect of either resource quality, consumer density or MP presence on survivorship of chironomids. High mortality in some individual replicates was also not clearly associated with any of the experimental treatments or blocks, and is thus difficult to explain, but seems most likely to reflect random variation in the initial survivorship of the newly hatched larvae, which was not easily quantified owing to their extremely small size. Neither resource quality, consumer density nor MP exposure were predicted a priori to induce a strong acute mortality response. However, we did observe a systematic effect of resource quality on chironomid growth, which we hypothesise could compromise survivorship over a longer period than assessed here. Growth was very limited for larvae reared on low quality FPOM, with most individuals failing to develop beyond the second instar. This suggests the low quality FPOM resource was very limited in availability of key nutrients required for supporting growth and development, which are likely to affect survivorship in the longer term, and in particular the success of pupal metamorphosis (Wesner et al. 2020). Accordingly, a longer study period, including rearing the larvae to adulthood, might be necessary for detection of potential survivorship differences associated with the low resource quality treatment.

The limited development of larvae grown on the low quality FPOM resource was manifested in reduced individual body length and per capita biomass, relative to the high quality FPOM treatment. Notably, the C:N ratio of our low quality, field collected, FPOM (47.5) was higher than any previously reported value for naturally occurring FPOM in heterotrophic steam ecosystems (e.g. C:N ~ 10–35) (Cross et al. 2003; Callisto and Graça 2013; Yoshimura et al. 2008). In contrast, the C:N ratio of our high quality FPOM resource (16.2) is characteristic of the highest quality plant detritus in stream ecosystems, such as the nitrogen rich litter of *Alnus* (17.5–19.2) (García-Palacios et al. 2016), and was supplemented with additional nutrients from the commercially available fish food included in the mixture. The field-collected FPOM in our study was dried to facilitate mass determination, but was then reconditioned in stream water to re-establish surficial biofilms, which is an important factor in enriching the nutrient quality of particles in situ (Cummins and Klug 1979). We might not have achieved levels of conditioning in the laboratory comparable to what is typically seen in situ, which would then have further limited nutrient quality of the field collected FPOM. Nevertheless, these results indicate that a diet consisting of naturally occurring FPOM on its own has extremely limited capacity for supporting consumer growth. This is important in contextualizing potential MP impacts on heterotrophic freshwater ecosystems, given that naturally occurring FPOM pools are not themselves necessarily highly nutritious. For example, the potential for microplastic contamination of organic sediments to be associated with significant 'food dilution effects' on consumers is likely to be limited when the organic matter is itself extremely low quality (Ogonowski et al. 2018), as observed here.

Contrary to our hypothesis, there was no evidence that exposure of chironomid larvae to the greater potential stress associated with the low food quality or high consumer density increased the likelihood of negative impacts of MP contaminationthe opposite for per-capita growth. Rather, we observed the opposite for per-capita growth: MP exposure negatively impacted individual body lengths of chironomids reared at low consumer density. MP exposure has been associated with reduced growth in macroinvertebrates (Au et al. 2015; Redondo-Hasselerharm et al. 2018), likely due to dilution of dietary intake with MP particles characterised by minimal nutritional value, and possibly the increased metabolic costs to excrete these particles (Foley et al. 2018). Reduced growth of prey organisms due to MP exposure may lead to knock-on effects on predators as smaller prey organisms become more common. Our findings suggest that negative effects of MPs on growth are more likely to affect chironomids in lower density aggregations. We hypothesise that this surprising result might reflect the production of higher volumes of faecal particles in the higher density chironomid aggregations. Invertebrate faecal particles are colonised by bacteria which thrive in invertebrate digestive tracts, and contribute additional exudates and bacterial cell biomass compared with FPOM prior to gut passage, and might be primed for further conditioning upon egestion (Ward and Cummins 1979; Wotton and Malmqvist 2001). These rapidly conditioning and tightly packed particles might in turn reduce negative effects of MP exposure by increasing the pool of more nutritious FPOM. Alternatively, the lower level of competition at low consumer densities might have allowed individual larvae to spend more time in resource acquisition compared with inter-specific interactions, increasing their exposure to potential negative effects of MPs contaminating their food resource. Further research is required to assess these alternative explanations.

Community respiration and chironomids

Unexpectedly, there was no significant difference in community respiration between microcosms with and without chironomids, and consumer density did not affect respiration rates. These findings indicate that, overall, community respiration was dominated by microorganisms (Fisher and Likens 1973), without any evidence that chironomid bioturbation increased net respiration rates. Instead, community respiration was overwhelmingly driven by FPOM resource quality, with respiration rates substantially greater in microcosms with the high quality FPOM resource. The very low respiration rates observed on the low quality FPOM are in line with those observed for consumer growth, highlighting the limited capacity of the natural FPOM resource to support metabolic activity compared with the more nutrient rich, high quality resource.

MP presence reduced community respiration, but only in microcosms without chironomids. This suggests that MP exposure reduces the respiration of microbes associated with the organic sediment and other substrates in our microcosms, but that this effect was obscured when chironomids were present. There was also a trend for a stronger negative effect ($p = 0.068$) of MP presence on microbially-mediated community respiration (in the absence of chironomids) in microcosms with the high quality FPOM resource, but more research is required to confirm this result. MPs have been shown to harbour distinct and often less diverse microbial assemblages compared with naturally occurring substrates (McCormick et al. 2014; Kettner et al. 2017; Miao et al. 2019b; Li et al. 2020). Changes in microbial community composition and diversity have been linked with altered ecosystem functioning in detrital food webs (Gardeström et al. 2016), and there is some evidence linking shifts in microbial communities associated with MP and nanoplastic exposure to changes in ecosystem functioning (Arias-Andres et al. 2018; Huang et al. 2021; Seena et al. 2022). It is similarly possible that the change in microbial respiration observed here reflects a change in microbial composition, activity and/or biomass.

Implications and conclusions: microplastic impacts in freshwater particle-processing chains

Our study is most relevant for benthic food webs in heterotrophic streams and rivers with nutrient-poor pools of FPOM and high levels of mineral sediment (as in our experimental microcosms). In these habitats, the overall resource quality of the particle pool is unlikely to be substantially impacted by the addition of MP particles. This contrasts with many previous studies of MP impacts on freshwater organisms, which have focussed on filter feeding pelagic organisms consuming algal and bacterial cells, and where ambient concentrations of mineral sediments and low quality FPOM are much lower than those our benthic deposit feeders are exposed to (Canniff and Hoang 2018; Aljaibachi et al. 2020). Our study focussed on one type of polymer (albeit one of the most abundant in most freshwater habitats) and presented only as spheres, which is relatively uncommon in the environment (Burns and Boxall 2018), and it is possible the impacts of the different shape and polymer combinations could differ from those observed here. Nevertheless, our results highlight the risk for MPs to affect consumer life histories and microbial processes in benthic habitats. In particular, the interaction observed between MP exposure and consumer density demonstrates the potential for MPs to interact with other environmental drivers, highlighting the need for a more thorough investigation of potential interactions between MP exposure and additional stressors.

In the Anthropocene, MP pollution is just one pressing environmental issue amongst the broad array of anthropogenic pressures currently impacting ecosystems. Up to now, the lack of research focussed on ecosystem-level impacts of MPs in freshwaters limits the possibility for scientific knowledge to inform priority setting in monitoring, policy and management of MP pollution, relative to other anthropogenic pressures. Our findings suggest that MPs, applied at an environmentally realistic concentration might have some impacts on the growth and survival of consumers and on microbial respiration in benthic stream FPOM-based food webs. Although the magnitude of these impacts were limited relative to the much stronger effects of resource quality and consumer density, we emphasise that we always used MP concentrations that spanned the range of currently observed real-world concentrations. It is thus possible that some of these effects will strengthen with projected increased MP concentrations in the future, in line with the amount of plastic debris already accumulated in freshwater habitats, which has yet to degrade to MP size. Furthermore, our evidence for an effect of MPs on microbial respiration in particular point to risks for knock-on effects on other organism groups and ecosystem properties, given the key role of microorganisms as not only a resource but also in mediating fundamental ecosystem processes, including nutrient and carbon cycling.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00027-023-00964-w.

Acknowledgements We thank Willem Goedkoop for access to the *Chironomus riparius* culture used in this study and for comments on the manuscript.

Funding Open access funding provided by Swedish University of Agricultural Sciences. This research was funded by grant NV-03728-17 (to BGM, MB, MNF and RH), from the Swedish Environmental Protection Agency (Naturvårdsverket).

Data availability Data collected and analysed are available from the authors upon reasonable request.

Declarations

Conflict of interest All authors declare no conflicts of interests.

Ethical approval No ethical approval was required for this study as experimental work was conducted with an unregulated invertebrate species (*Chironomus riparius*).

References

Alimi OS, Farner Budarz J, Hernandez LM, Tufenkji N (2018) Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. Environ Sci Technol 52:1704–1724

Aljaibachi R, Laird WB, Stevens F, Callaghan A (2020) Impacts of polystyrene microplastics on *Daphnia magna*: a laboratory and a mesocosm study. Sci Total Environ 705:135800. https://doi.org/10.1016/j.scitotenv.2019.135800

Arias-Andres M, Kettner MT, Miki T, Grossart HP (2018) Microplastics: new substrates for heterotrophic activity contribute to altering organic matter cycles in aquatic ecosystems. Sci Total Environ 635:1152–1159. https://doi.org/10.1016/j.scitotenv.2018.04.199

Au SY, Bruce TF, Bridges WC, Klaine SJ (2015) Responses of *Hyalella azteca* to acute and chronic microplastic exposures. Environ Toxicol Chem 34:2564–2572. https://doi.org/10.1002/etc.3093

Baranov V, Lewandowski J, Romeijn P, Singer G, Krause S (2016) Effects of bioirrigation of non-biting midges (Diptera: Chironomidae) on lake sediment respiration. Sci Rep 6:1–10. https://doi.org/10.1038/srep27329

Barnes DKA, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc B Biol Sci 364:1985–1998. https://doi.org/10.1098/rstb.2008.0205

Bates D, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. J Stat Softw. https://doi.org/10.18637/jss.v067.i01

Besseling E, Wegner A, Foekema EM, Van Den Heuvel-Greve MJ, Koelmans AA (2013) Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ Sci Technol 47:593–600. https://doi.org/10.1021/es302763x

Bundschuh M, McKie BG (2016) An ecological and ecotoxicological perspective on fine particulate organic matter in streams. In: Freshwater Biology. Blackwell Publishing Ltd, pp 2063–2074

Burns EE, Boxall ABA (2018) Microplastics in the aquatic environment: evidence for or against adverse impacts and major knowledge gaps. Environ Toxicol Chem 37:2776–2796

Callisto M, Graça MAS (2013) The quality and availability of fine particulate organic matter for collector species in headwater streams. Int Rev Hydrobiol 98:132–140. https://doi.org/10.1002/iroh.201301524

Canniff PM, Hoang TC (2018) Microplastic ingestion by *Daphnia magna* and its enhancement on algal growth. Sci Total Environ 633:500–507. https://doi.org/10.1016/j.scitotenv.2018.03.176

Chen X, Chen X, Zhao Y, Zhou H, Xiong X, Wu C (2020) Effects of microplastic biofilms on nutrient cycling in simulated freshwater systems. Sci Total Environ 719:137276. https://doi.org/10.1016/j.scitotenv.2020.137276

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

UC Davis (2020) Carbon and nitrogen in solids | stable isotope facility. https://stableisotopefacility.ucdavis.edu/carbon-and-nitrogen-solids Accessed 6 May 2022

de Sá LC, Oliveira M, Ribeiro F, Rocha TL, Futter MN (2018) Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? Sci Total Environ 645:1029–1039. https://doi.org/10.1016/j.scitotenv.2018.07.207

Ehlers SM, Manz W, Koop JHE (2019) Microplastics of different characteristics are incorporated into the larval cases of the freshwater caddisfly Lepidostoma basale. Aquat Biol 28:67–77. https://doi.org/10.3354/ab00711

Ehlers SM, Al Najjar T, Taupp T, Koop JHE (2020) PVC and PET microplastics in caddisfly (*Lepidostoma basale*) cases reduce case stability. Environ Sci Pollut Res 27:22380–22389. https://doi.org/10.1007/s11356-020-08790-5

EPA (1992) Plastic pellets in the aquatic environment: sources and recommendations. United States Environmental Protection Agency EPA842-B-92-010: 11-17

Fisher SG (1977) Organic matter processing by a stream-segment ecosystem: Fort River, Massachusetts, U.S.A. Int Rev der gesamten Hydrobiol und Hydrogr 62:701–727. https://doi.org/10.1002/iroh.1977.3510620601

Fisher SG, Likens GE (1973) Energy Flow in Bear Brook, New Hampshire : an integrative approach to stream ecosystem metabolism. Ecol Monogr 43:421–439

Foley CJ, Feiner ZS, Malinich TD, Höök TO (2018) A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci Total Environ 631–632:550–559

Frias JPGL, Nash R (2019) Microplastics: finding a consensus on the definition. Mar Pollut Bull 138:145–147. https://doi.org/10.1016/J.MARPOLBUL.2018.11.022

García-Palacios P, Mckie BG, Handa IT, Frainer A, Hättenschwiler S (2016) The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and across biomes. Funct Ecol 30:819–829. https://doi.org/10.1111/1365-2435.12589

Gardeström J, Ermold M, Goedkoop W, McKie BG (2016) Disturbance history influences stressor impacts: effects of a fungicide and nutrients on microbial diversity and litter decomposition. In: Freshwater Biology. John Wiley & Sons, Ltd, p 2171–2184

Goedkoop W, Demandt M, Ahlgren G (2007) Interactions between food quantity and quality (long-chain polyunsaturated fatty acid concentrations) effects on growth and development of *Chironomus riparius*. Can J Fish Aquat Sci 64:425–436. https://doi.org/10.1139/F07-016

Gregory MR (1996) Plastic scrubbers' in hand cleansers: a further (and minor) source for marine pollution identified. Mar Pollut Bull 32:867–871. https://doi.org/10.1016/S0025-326X(96)00047-1

Henry B, Laitala K, Klepp IG (2019) Microfibres from apparel and home textiles: prospects for including microplastics in environmental sustainability assessment. Sci Total Environ 652:483–494. https://doi.org/10.1016/J.SCITOTENV.2018.10.166

Hiltunen M, Vehniäinen ER, Kukkonen JVK (2021) Interacting effects of simulated eutrophication, temperature increase, and microplastic exposure on *Daphnia*. Environ Res 192:110304. https://doi.org/10.1016/j.envres.2020.110304

Hölker F, Vanni MJ, Kuiper JJ, Meile C, Grossart HP, Stief P, Adrian R, Lorke A, Dellwig O, Brand A, Hupfer M, Mooij WM, Nützmann G, Lewandowski J (2015) Tube-dwelling invertebrates: tiny ecosystem engineers have large effects in lake ecosystems. Ecol Monogr 85:333–351

Hooper HL, Sibly RM, Hutchinson TH, Maund SJ (2003) The influence of larval density, food availability and habitat longevity on the life history and population growth rate of the midge *Chironomus riparius*. Oikos 102:515–524. https://doi.org/10.1034/j.1600-0706.2003.12536.x

Horton AA, Vijver MG, Lahive E, Spurgeon DJ, Svendsen C, Heutink R, van Bodegom PM, Baas J (2018) Acute toxicity of organic pesticides to *Daphnia magna* is unchanged by co-exposure to polystyrene microplastics. Ecotoxicol Environ Saf 166:26–34. https://doi.org/10.1016/j.ecoenv.2018.09.052

Hossain MR, Jiang M, Wei QH, Leff LG (2019) Microplastic surface properties affect bacterial colonization in freshwater. J Basic Microbiol 59:54–61. https://doi.org/10.1002/jobm.201800174

Huang Y, Li W, Gao J, Wang F, Yang W, Han L, Lin D, Min B, Zhi Y, Grieger K, Yao J (2021) Effect of microplastics on ecosystem functioning: microbial nitrogen removal mediated by benthic invertebrates. Sci Total Environ 754:142133. https://doi.org/10.1016/j.scitotenv.2020.142133

Hutchens JJ, Wallace JB, Grubaugh JW (2017) Transport and storage of fine particulate organic matter. In: Methods in stream ecology, 3rd edn. Academic Press, p 37–53

Ieromina O, Peijnenburg WJGM, de Snoo G, Müller J, Knepper TP, Vijver MG (2014) Impact of imidacloprid on *Daphnia magna* under different food quality regimes. Environ Toxicol Chem 33:621–631. https://doi.org/10.1002/etc.2472

Immerschitt I, Martens A (2020) Ejection, ingestion and fragmentation of mesoplastic fibres to microplastics by *Anax imperator* larvae (Odonata: Aeshnidae). Odonatologica 49:57–66. https://doi.org/10.5281/zenodo.3823329

Johnson LT, Tank JL, Dodds WK (2009) The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. Can J Fish Aquat Sci 66:1081–1094. https://doi.org/10.1139/F09-065

Joyce P, Warren LL, Wotton RS (2007) Faecal pellets in streams: their binding, breakdown and utilization. Freshw Biol 52:1868–1880. https://doi.org/10.1111/j.1365-2427.2007.01828.x

Kettner MT, Rojas-Jimenez K, Oberbeckmann S, Labrenz M, Grossart HP (2017) Microplastics alter composition of fungal communities in aquatic ecosystems. Environ Microbiol 19:4447–4459. https://doi.org/10.1111/1462-2920.13891

Klemmer AJ, Wissinger SA, Greig HS, Ostrofsky ML (2012) Nonlinear effects of consumer density on multiple ecosystem processes. J Anim Ecol 81:770–780. https://doi.org/10.1111/j.1365-2656.2012.01966.x

Koelmans AA, Mohamed Nor NH, Hermsen E, Kooi M, Mintenig SM, De France J (2019) Microplastics in freshwaters and drinking water: critical review and assessment of data quality. Water Res 155:410–422. https://doi.org/10.1016/j.watres.2019.02.054

Kratina P, Watts TJ, Green DS, Kordas RL, O'Gorman EJ (2019) Interactive effects of warming and microplastics on metabolism but not feeding rates of a key freshwater detritivore. Environ Pollut 255:113259. https://doi.org/10.1016/j.envpol.2019.113259

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

Lenth R (2019) Emmeans: estimated marginal means. R Packag. version 1.4.2. https://cran.r-project.org/package=emmeans.Accessed on 15 March 2022

Li C, Gan Y, Dong J, Fang J, Chen H, Quan Q, Liu J (2020) Impact of microplastics on microbial community in sediments of the Huangjinxia Reservoir—water source of a water diversion project in western China. Chemosphere 253:126740. https://doi.org/10.1016/j.chemosphere.2020.126740

Malmqvist B, Wotton RS, Zhang Y (2001) Suspension feeders transform massive amounts of seston in large northern rivers. Oikos 92:35–43. https://doi.org/10.1034/j.1600-0706.2001.920105.x

McCormick A, Hoellein TJ, Mason SA, Schluep J, Kelly JJ (2014) Microplastic is an abundant and distinct microbial habitat in an urban river. Environ Sci Technol 48:11863–11871. https://doi.org/10.1021/es503610r

Mckie BG (2004) Disturbance and investment: developmental responses of tropical lotic midges to repeated tube destruction in the juvenile stages. Ecol Entomol 29:457–466. https://doi.org/10.1111/j.0307-6946.2004.00622.x

McKie BG, Woodward G, Hladyz S, Nistorescu M, Preda E, Popescu C, Giller PS, Malmqvist B (2008) Ecosystem functioning in stream assemblages from different regions: contrasting responses to variation in detritivore richness, evenness and density. J Anim Ecol 77:495–504. https://doi.org/10.1111/j.1365-2656.2008.01357.x

McKie BG, Schindler M, Gessner MO, Malmqvist B (2009) Placing biodiversity and ecosystem functioning in context: Environmental perturbations and the effects of species richness in a stream field experiment. Oecologia 160:757–770. https://doi.org/10.1007/s00442-009-1336-7

McNamara JM, Buchanan KL (2005) Stress, resource allocation, and mortality. Behav Ecol 16:1008–1017. https://doi.org/10.1093/beheco/ari087

Miao L, Hou J, You G, Liu Z, Liu S, Li T, Mo Y, Guo S, Qu H (2019a) Acute effects of nanoplastics and microplastics on periphytic biofilms depending on particle size, concentration and surface modification. Environ Pollut 255:113300. https://doi.org/10.1016/j.envpol.2019.113300

Miao L, Wang P, Hou J, Yao Y, Liu Z, Liu S, Li T (2019b) Distinct community structure and microbial functions of biofilms colonizing microplastics. Sci Total Environ 650:2395–2402. https://doi.org/10.1016/j.scitotenv.2018.09.378

Mousavi SK (2002) Boreal chironomid communities and their relations to environmental factors - the impact of lake depth, size and acidity. Boreal Environ Res 7:63–75

Nogaro G, Mermillod-Blondin F, Montuelle B, Boisson JC, Gibert J (2008) Chironomid larvae stimulate biogeochemical and microbial processes in a riverbed covered with fine sediment. Aquat Sci 70:156–168. https://doi.org/10.1007/s00027-007-7032-y

Ogonowski M, Schür C, Jarsén Å, Gorokhova E (2016) The effects of natural and anthropogenic microparticles on individual fitness in daphnia magna. PLoS One 11:e0155063. https://doi.org/10.1371/journal.pone.0155063

Ogonowski M, Gerdes Z, Gorokhova E (2018) What we know and what we think we know about microplastic effects – a critical perspective. Curr Opin Environ Sci Heal 1:41–46. https://doi.org/10.1016/j.coesh.2017.09.001

Op de Beeck L, Verheyen J, Stoks R (2018) Competition magnifies the impact of a pesticide in a warming world by reducing heat tolerance and increasing autotomy. Environ Pollut 233:226–234. https://doi.org/10.1016/j.envpol.2017.10.071

Prinz N, Korez Š (2020) Understanding how microplastics affect marine biota on the cellular level is important for assessing ecosystem function: a review. In: YOUMARES 9 - The oceans: our research, our future. Springer International Publishing, p 101–120

R Core Team (2019) A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R--project.org. Accessed on 15 March 2022

Redondo-Hasselerharm PE, Falahudin D, Peeters ETHM, Koelmans AA (2018) Microplastic effect thresholds for freshwater benthic macroinvertebrates. Environ Sci Technol 52:2278–2286. https://doi.org/10.1021/acs.est.7b05367

Romera-Castillo C, Pinto M, Langer TM, Álvarez-Salgado XA, Herndl GJ (2018) Dissolved organic carbon leaching from plastics stimulates microbial activity in the ocean. Nat Commun 9:1–7. https://doi.org/10.1038/s41467-018-03798-5

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675

Seena S, Gutiérrez IB, Barros J, Nunes C, Marques JC, Kumar S, Gonçalves AMM (2022) Impacts of low concentrations of nanoplastics on leaf litter decomposition and food quality for detrsitivores in streams. J Hazard Mater 429:128320. https://doi.org/10.1016/j.jhazmat.2022.128320

Shepard RB, Minshall GW (1984) Role of benthic insect feces in a rocky mountain stream: fecal production and support of consumer growth. Ecography 7(2):119–127

Shim WJ, Hong SH, Eo S (2018) Marine microplastics: abundance, distribution, and composition. In: Microplastic contamination in aquatic environments: an emerging matter of environmental urgency. Elsevier, p 1–26

Stanković J, Milošević D, Savić-Zdraković D, Yalçın G, Yildiz D, Beklioğlu M, Jovanović B (2020) Exposure to a microplastic mixture is altering the life traits and is causing deformities in the non-biting midge *Chironomus riparius* Meigen (1804). Environ Pollut 262:114248. https://doi.org/10.1016/j.envpol.2020.114248

Svensson JM (1997) Influence of *Chironomus plumosus* larvae on ammonium flux and denitrification (measured by the acetylene blockage- and the isotope pairing-technique) in eutrophic lake sediment. Hydrobiologia 346:157–168. https://doi.org/10.1023/a:1002974201570

Svensson JM (1998) Emission of N_2O, nitrification and denitrification in a eutrophic lake sediment bioturbated by *Chironomus plumosus*. Aquat Microb Ecol 14:289–299. https://doi.org/10.3354/ame014289

Thomas SA, Newbold JD, Monaghan MT, Minshall GW, Georgian T, Cushing CE (2001) The influence of particle size on seston deposition in streams. Limnol Oceanogr 46:1415–1424. https://doi.org/10.4319/lo.2001.46.6.1415

Truchy A, Sarremejane R, Muotka T, Mykrä H, Angeler DG, Lehosmaa K, Huusko A, Johnson RK, Sponseller RA, McKie BG (2020) Habitat patchiness, ecological connectivity and the uneven recovery of boreal stream ecosystems from an experimental drought. Glob Chang Biol 26:3455–3472. https://doi.org/10.1111/gcb.15063

van de Bund WJ, Goedkoop W, Johnson RK (1994) Effects of deposit-feeder activity on bacterial production and abundance in profundal lake sediment. J North Am Benthol Soc 13:532–539. https://doi.org/10.2307/1467849

Ward GM, Cummins KW (1979) Effects of food quality on growth of a stream detritivore, *Paratendipes albimanus* (Meigen) (Diptera: Chironomidae). Ecology 60:57–64. https://doi.org/10.2307/1936468

Webster JR, Meyer JL (1997) Organic matter budgets for streams: a synthesis. J North Am Benthol Soc 16:141–161. https://doi.org/10.2307/1468247

Welden NAC, Cowie PR (2016) Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. Environ Pollut 218:895–900. https://doi.org/10.1016/j.envpol.2016.08.020

Wesner J, Kraus JM, Henry B, Kerby J (2020) Metamorphosis and the impact of contaminants on ecological subsidies. Contaminants and ecological subsidies. Springer, Cham, pp 111–125

Wickham H (2009) ggplot2. Springer, New York

Wotton RS (2007) Do benthic biologists pay enough attention to aggregates formed in the water column of streams and rivers? J North Am Benthol Soc 26:1–11

Wotton RS, Malmqvist B (2001) Feces in aquatic ecosystems. Bioscience 51:537. https://doi.org/10.1641/0006-3568(2001)051[0537:fiae]2.0.co;2

Yang L, Zhang Y, Kang S, Wang Z, Wu C (2021) Microplastics in freshwater sediment: a review on methods, occurrence, and sources. Sci Total Environ 754:141948

Yeager PE, Foreman CL, Sinsabaugh RL (2001) Microbial community structure and function in response to larval chironomid feeding pressure in a microcosm experiment. Hydrobiologia 448:71–81. https://doi.org/10.1023/A:1017504114692

Zeng EY (2018) Microplastic contamination in aquatic environments: an emerging matter of environmental urgency. Elsevier

Zettler ER, Mincer TJ, Amaral-Zettler LA (2013) Life in the “plastisphere”: microbial communities on plastic marine debris. Environ Sci Technol 47:7137–7146. https://doi.org/10.1021/es401288x

Supplementary Information

Figure S1 – Comparison of two microcosms with the same high quality FPOM resource and no microplastic addition but with different consumer densities. Generally, chironomids in microcosms with a lower consumer density of 10 (a) constructed more robust and well-spaced tubes compared to those in microcosms with 20 individuals (b). The white rectangles (5 mm graph paper) were added during photography for scale.

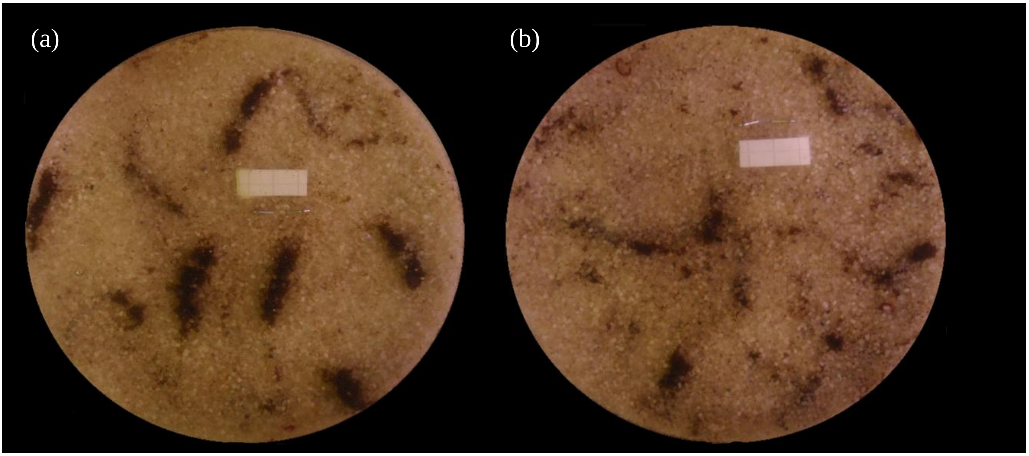
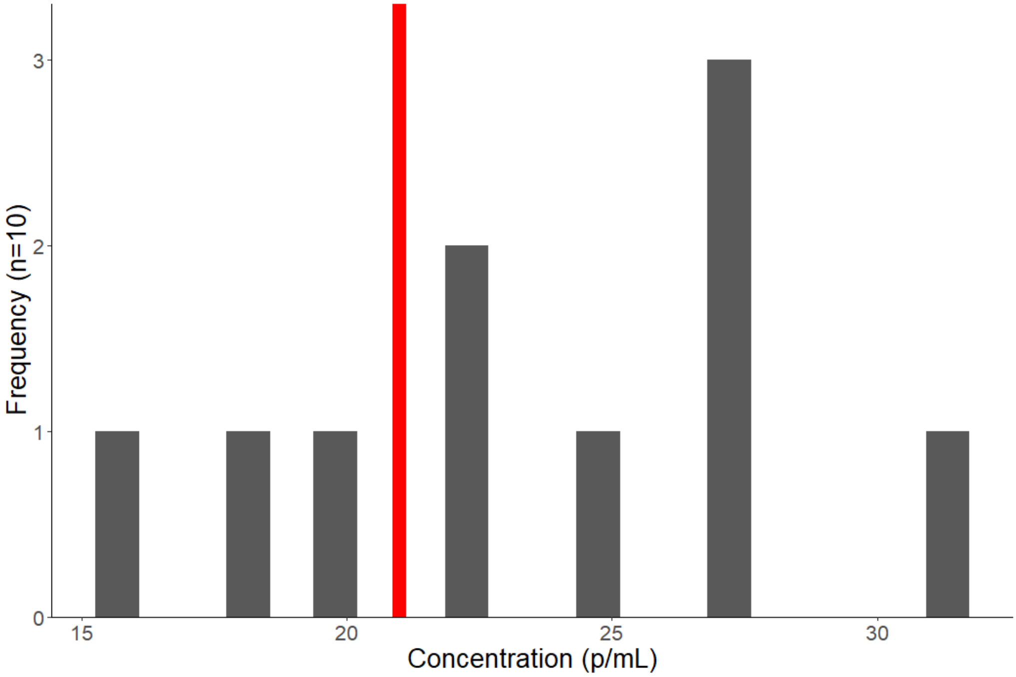


Figure S2 – Verification of MP stock concentration: Concentration of MP stock (particles per mL) calculated from the number of MP beads counted under a microscope. The red vertical line indicates the target concentration (20.9 particles/mL).



Equation S1:

$$CR = \left[\left(DO_{m_0} - DO_{m_t}\right) - \left(DO_{b_0} - DO_{b_t}\right)\right] \times \frac{V_{H2O}}{t} \qquad (1)$$

Where DO_{m_0} is the DO concentration in the microcosm at the start of the incubation; DO_{m_t} is the DO concentration in the microcosm at the end of the incubation period t; DO_{b_0} and DO_{b_t} are the DO concentrations in the blanks at the start of the incubation and t, respectively; and V_{H2O} is the volume of water in the crystallisation dish.

Table S1 – ANOVA table of linear mixed models for measured responses. Models were simplified by removing non-significant effects. One replicate was excluded from all analyses due to an error in the initial allocation of larvae.

Fixed Effects	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F Value**	**Pr(>F)**
(1) Survivorship (log(x+1))						
Food	0.003	0.003	1	27.2	0.00	0.334
Density	0.282	0.282	1	27.2	0.21	0.674
MP	1.456	1.456	1	27.2	1.11	0.882
Food*Density	0.385	0.385	1	27.2	0.29	0.156
Food*MP	0.064	0.064	1	27.2	0.05	0.175
Density*MP	0.039	0.039	1	27.2	0.04	0.973
Food*Density*MP	4.278	4.278	1	27.2	3.25	*0.096*
(2) *Per capita* Dry Weight						
Food	2.935	2.935	1	27.3	52.22	**<0.001**
Density	0.842	0.842	1	27.3	14.99	**<0.001**
MP	0.009	0.009	1	27.3	0.15	0.700
Food*Density	0.471	0.471	1	27.3	8.38	**0.007**
Food*MP	0.103	0.103	1	27.3	1.83	0.188
Density*MP	0.024	0.024	1	27.3	0.43	0.517
Food*Density*MP	0.101	0.101	1	27.3	1.81	0.190
(3) Individual length						
Food	363.800	363.800	1	14.7	236.41	**<0.001**
Density	5.710	5.710	1	14.7	3.71	*0.074*
MP	6.420	6.420	1	14.7	4.17	*0.059*
Food*Density	4.950	4.950	1	14.7	3.21	*0.094*
Food*MP	0.010	0.010	1	14.7	0.00	0.936
Density*MP	9.700	9.700	1	14.7	6.30	**0.024**
Food*Density*MP	0.030	0.030	1	14.7	0.02	0.891
(4) Microcosm Respiration						
Total						
Food	128.186	128.186	1	26.7	175.14	**<0.001**
Density	0.277	0.138	2	25.9	0.19	0.828
MP	0.001	0.001	1	25.1	0.22	0.964
Food*Density	0.488	0.244	2	27.6	0.33	0.718
Food*MP	0.613	0.613	1	26.5	0.84	0.365
Density*MP	6.525	3.263	2	24.2	4.46	**0.018**
Food*Density*MP	1.227	0.614	2	28.8	0.84	0.439
Chironomid-only						
Food	93.842	93.842	1	26.7	100.17	**<0.001**
Density	0.337	0.337	1	26.0	0.36	0.554
MP	2.038	2.038	1	25.1	2.18	0.153
Food*Density	0.242	0.242	1	27.6	0.26	0.615
Food*MP	0.000	0.000	1	26.5	0.00	0.687
Density*MP	0.042	0.042	1	24.2	0.05	0.834

Food*Density*MP	0.224	0.224	1	28.7	0.24	0.629
Microbial-only						
Food	41.682	41.682	1	11.0	75.61	**<0.001**
MP	3.809	3.809	1	12.3	6.91	**0.022**
Food*MP	2.129	2.129	1	15.1	3.86	*0.068*

Table S2 – Comparison of C:N ratios of FPOM used within this study and the source organic material used in the high quality FPOM.

FPOM	**Mean C:N ratio**
Low Quality from Fybiån Stream, Sweden	47.5
High Quality	16.2
Leaf litter	
Alder	26.1
Birch	44.9
Fish Food	
Tetra Phyll Flakes	5.97

Figure S3 – Survival of chironomid larvae. Error bars represent standard error.

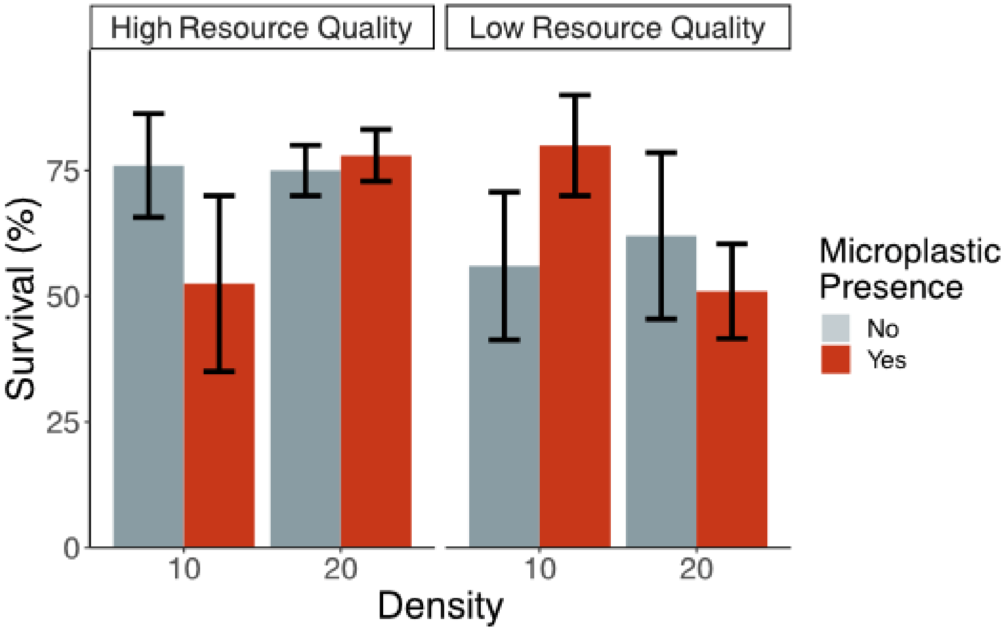
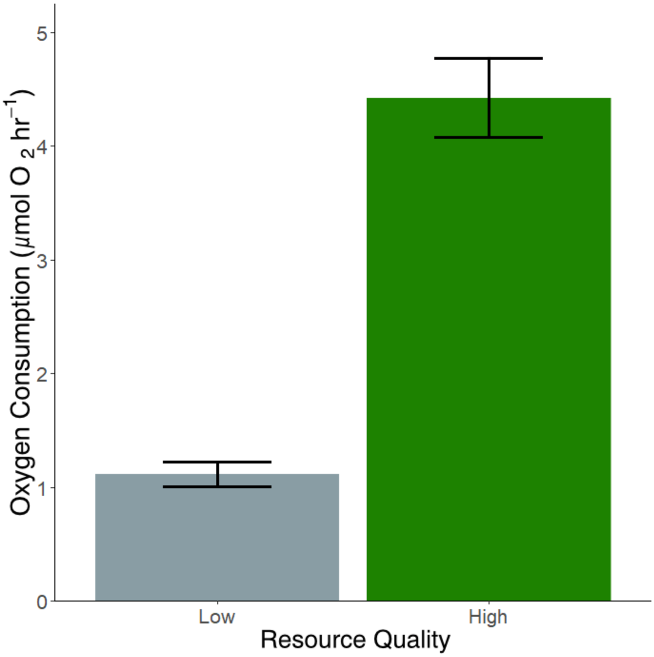


Figure S4 –Respiration of communities with chironomid larvae was only significantly affected by FPOM resource quality. Error bars represent standard error.



ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

DOCTORAL THESIS NO. 2024:63

Microplastics are a widespread contaminant with the potential to interact with freshwater biota and their associated resource processing chains. This thesis aims to assess the effects of microplastics, and the drivers of these effects on freshwater microorganisms, macroinvertebrates and ecosystem functions, through a series of laboratory and field experiments. The findings of this thesis highlight the potential for microplastics to affect real-world freshwater environments, although these effects may be less pronounced than other common sources of environmental variation.

Ze Hui Kong received his doctoral education at the Department of Aquatic Sciences and Assessment at the Swedish University of Agricultural Sciences. He holds an M.Sc. in Ecological Applications and a B.Sc. in Biology, both from Imperial College London, UK.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8046-354-6
ISBN (electronic version) 978-91-8046-390-4