Institute of Biochemistry and Biology

Microbial Gene Exchange on Microplastic Particles

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in partial fulfillment for the award of the degree

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by

María de Jesús Arias Andrés

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in der Wissenschaftsdisziplin "Ecology"

eingereicht an der

Mathematisch-Naturwissenschaftlichen Fakultät

der Universität Potsdam

Von

María de Jesús Arias Andrés

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Table of contents

Preface

This scientific work was conducted mainly at the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Dept. Experimental Limnology, Alte Fischerhuette 2, D-16775 Stechlin, Germany. This work was carried out from August 2014 until May 2018. Research was conducted independently with the funding from the Leibniz Association (Germany) and partially with funds from COST-European Cooperation in Science and Technology, COST Action ES1403. The Ph.D. of María de Jesús Arias Andrés was supported by a scholarship from Universidad Nacional (Costa Rica).

List of Publications

This thesis is a publication-based dissertation. Three publications constitute the central part of the thesis and are integrated as chapters, each with individual figures and tables numbering, supplementary data and individual reference list. María de Jesús Arias Andrés's share of work is explained for each. The name used for publications is Maria Arias-Andres.

Chapter I

Eckert, E.M., Di Cesare, A., Kettner, M.T., Arias-Andres, M., Fontaneto, D., Grossart, H.-P., and Corno, G. (2018) Microplastics increase impact of treated wastewater on freshwater microbial community. Environ. Pollut. **234**: 495–502. doi:10.1016/j.envpol.2017.11.070.

Maria Arias-Andres participated together with the other co-authors in designing the experiment, performing the analyses and writing the manuscript.

Chapter II

Arias-Andres, M., Klümper, U., Rojas-Jimenez, K., and Grossart, H.-P. (2018) Microplastic pollution increases gene exchange in aquatic ecosystems. Environ. Pollut. **237**: 253–261. doi: 10.1016/j.envpol.2018.02.058

Maria Arias-Andres designed major parts of the experiment with help from HPG and UK, performed most of the analysis with the help of KRJ, and wrote major parts of the manuscript with inputs from all co-authors.

Chapter III

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

Maria Arias-Andres participated in designing the experiment together with TK and HGP, performed the analysis with TK and TM and wrote major parts of the manuscripts with input from all co-authors.

Summary

Plastic pollution is ubiquitous on the planet since several millions of tons of plastic waste enter aquatic ecosystems each year. Furthermore, the amount of plastic produced is expected to increase exponentially shortly. The heterogeneity of materials, additives and physical characteristics of plastics are typical of these emerging contaminants and affect their environmental fate in marine and freshwaters. Consequently, plastics can be found in the water column, sediments or littoral habitats of all aquatic ecosystems. Most of this plastic debris will fragment as a product of physical, chemical and biological forces, producing particles of small size. These particles (< 5mm) are known as "microplastics" (MP). Given their high surface-to-volume ratio, MP stimulate biofouling and the formation of biofilms in aquatic systems.

As a result of their unique structure and composition, the microbial communities in MP biofilms are referred to as the "Plastisphere." While there is increasing data regarding the distinctive composition and structure of the microbial communities that form part of the plastisphere, scarce information exists regarding the activity of microorganisms in MP biofilms. This surface-attached lifestyle is often associated with the increase in horizontal gene transfer (HGT) among bacteria. Therefore, this type of microbial activity represents a relevant function worth to be analyzed in MP biofilms. The horizontal exchange of mobile genetic elements (MGEs) is an essential feature of bacteria. It accounts for the rapid evolution of these prokaryotes and their adaptation to a wide variety of environments. The process of HGT is also crucial for spreading antibiotic resistance and for the evolution of pathogens, as many MGEs are known to contain antibiotic resistance genes (ARGs) and genetic determinants of pathogenicity.

In general, the research presented in this Ph.D. thesis focuses on the analysis of HGT and heterotrophic activity in MP biofilms in aquatic ecosystems. The primary objective was to analyze the potential of gene exchange between MP bacterial communities vs. that of the surrounding water, including bacteria from natural aggregates. Moreover, the thesis addressed the potential of MP biofilms for the proliferation of biohazardous bacteria and MGEs from wastewater treatment plants (WWTPs) and associated with antibiotic resistance. Finally, it seeks to prove if the physiological profile of MP biofilms under different limnological conditions is divergent from that of the water communities. Accordingly, the thesis is composed of three independent studies published in peer-reviewed journals. The two laboratory studies were performed using both model and environmental microbial communities. In the field experiment, natural communities from freshwater ecosystems were examined.

In **Chapter I**, the inflow of treated wastewater into a temperate lake was simulated with a concentration gradient of MP particles. The effects of MP on the microbial community structure and the occurrence of integrase 1 (*int 1*) were followed. The *int 1* is a marker

associated with mobile genetic elements and known as a proxy for anthropogenic effects on the spread of antimicrobial resistance genes. During the experiment, the abundance of *int1* increased in the plastisphere with increasing MP particle concentration, but not in the surrounding water. In addition, the microbial community on MP was more similar to the original wastewater community with increasing microplastic concentrations. Our results show that microplastic particles indeed promote persistence of standard indicators of microbial anthropogenic pollution in natural waters.

In **Chapter II**, the experiments aimed to compare the permissiveness of aquatic bacteria towards model antibiotic resistance plasmid pKJK5, between communities that form biofilms on MP vs. those that are free-living. The frequency of plasmid transfer in bacteria associated with MP was higher when compared to bacteria that are free-living or in natural aggregates. Moreover, comparison increased gene exchange occurred in a broad range of phylogenetically-diverse bacteria. The results indicate a different activity of HGT in MP biofilms, which could affect the ecology of aquatic microbial communities on a global scale and the spread of antibiotic resistance.

Finally, in **Chapter III**, physiological measurements were performed to assess whether microorganisms on MP had a different functional diversity from those in water. General heterotrophic activity such as oxygen consumption was compared in microcosm assays with and without MP, while diversity and richness of heterotrophic activities were calculated by using Biolog® EcoPlates. Three lakes with different nutrient statuses presented differences in MP-associated biomass build up. Functional diversity profiles of MP biofilms in all lakes differed from those of the communities in the surrounding water, but only in the oligo-mesotrophic lake MP biofilms had a higher functional richness compared to the ambient water. The results support that MP surfaces act as new niches for aquatic microorganisms and can affect global carbon dynamics of pelagic environments.

Overall, the experimental works presented in **Chapters I** and **II** support a scenario where MP pollution affects HGT dynamics among aquatic bacteria. Among the consequences of this alteration is an increase in the mobilization and transfer efficiency of ARGs. Moreover, it supposes that changes in HGT can affect the evolution of bacteria and the processing of organic matter, leading to different catabolic profiles such as demonstrated in **Chapter III**. The results are discussed in the context of the fate and magnitude of plastic pollution and the importance of HGT for bacterial evolution and the microbial loop, i.e., at the base of aquatic food webs. The thesis supports a relevant role of MP biofilm communities for the changes observed in the aquatic microbiome as a product of intense human intervention.

Zusammenfassung

Die Plastikverschmutzung ist auf dem Planeten allgegenwärtig, da jährlich mehrere Millionen Tonnen Plastikabfall in die aquatische Ökosystemen gelangen. Darüber hinaus wird erwartet, dass die Menge an produziertem Plastik in naher Zukunft exponentiell ansteigen wird. Die Heterogenität der Kunststoffmaterialien, ihrer Additive und physikalischen Eigenschaften ist typisch für diese neu auftretenden Schadstoffe und beeinflusst deren Umweltverhalten in Meeres- und Süßwasser. Als Folge kann Plastik in der Wassersäule, den Sedimenten oder Küstenlebensräumen aller aquatischen Ökosysteme gefunden werden. Die meisten dieser Plastikabfälle fragmentieren durch das Zusammenspiel physikalischer, chemischer und biologischer Kräfte, wodurch kleine Partikel erzeugt werden. Diese Partikel (<5mm) sind auch bekannt als "Mikroplastik" (MP). Aufgrund ihres hohen Oberflächen-Volumen-Verhältnisses stimuliert MP das Biofouling und somit die Bildung von Biofilmen in aquatischen Systemen.

Aufgrund ihrer einzigartigen Struktur und Zusammensetzung werden die mikrobiellen Gemeinschaften in MP-Biofilmen als "Plastisphäre" bezeichnet. Während es immer mehr Daten über die spezifische Zusammensetzung und Struktur der mikrobiellen Gemeinschaften – die Teil dieser Plastisphäre sind – gibt, existieren hingegen nur wenige Informationen über die Aktivität von Mikroorganismen in MP-Biofilmen. Dieser Lebensstil des Anheftens und Besiedelns von Oberflächen ist oft mit der Zunahme von horizontalem Gentransfer (HGT) unter Bakterien verknüpft. Diese Art der mikrobiellen Aktivität stellt eine besonders relevante Funktion dar und sollte daher in MP-Biofilmen analysiert werden. Der horizontale Austausch von mobilen genetischen Elementen (MGEs) ist ein wesentliches Merkmal von Bakterien. Er ist verantwortlich für die schnelle Evolution dieser Prokaryoten und ihre Anpassungsfähigkeit an verschiedenste Umweltbedingungen. Der Prozess des HGT ist zudem entscheidend für die Verbreitung von Antibiotikaresistenzen sowie für die Entwicklung von Pathogenen, da viele MGEs bekanntermaßen Antibiotikaresistenzgene (ARGs) und genetische Determinanten für Pathogenität enthalten.

Im Allgemeinen konzentriert sich die Forschung in der vorliegenden Dissertation auf die Analyse des HGT und der heterotrophen Aktivität in MP-Biofilmen in aquatischen Ökosystemen. Das Hauptziel besteht darin, das Potenzial des Genaustausches zwischen MP-Bakteriengemeinschaften und dem des umgebenden Wassers, einschließlich der Bakterien in natürlichen Aggregaten, zu analysieren. Darüber hinaus befasst sich diese Doktorarbeit mit dem Potenzial von MP-Biofilmen zur Ausbreitung biologisch gefährlicher Bakterien und MGEs, die aus Kläranlagen stammen und mit Antibiotikaresistenzen assoziiert sind. Schließlich soll bei verschiedenen limnologischen Bedingungen überprüft werden, ob das jeweilige physiologische Profil von MP-Biofilmen von dem der Wassergemeinschaften abweicht. Dementsprechend besteht die Arbeit aus drei unabhängigen Studien, die in

Fachzeitschriften veröffentlicht wurden. In den beiden Laborstudien wurden sowohl mikrobielle Modell- als auch Umwelt-Gemeinschaften betrachtet. Im Freilandexperiment wurden schließlich die natürlichen Gemeinschaften aus Süßwasserökosystemen untersucht.

In Kapitel I wurde der Zufluss von geklärtem Abwasser mit einem Konzentrationsgradienten von MP-Partikeln in einen See der gemäßigten Klimazone simuliert. Dabei wurden die Effekte von MP auf die mikrobielle Gemeinschaftsstruktur und das Auftreten von Integrase 1 (int 1) verfolgt. Int 1 ist ein Marker, der mit mobilen genetischen Elementen assoziiert ist und zur Abschätzung anthropogener Einflüsse auf die Ausbreitung antimikrobieller Resistenzgene verwendet ist. Während des Experiments erhöhte sich das Vorkommen von Int1 in der Plastisphäre mit zunehmender MP-Partikelkonzentration, jedoch nicht im umgebenden Wasser. Darüber hinaus ähnelte die mikrobielle Gemeinschaft auf MP zunehmend der ursprünglichen Abwassergemeinschaft mit steigender Mikroplastikkonzentration. Unsere Ergebnisse zeigen, dass Mikroplastikpartikel tatsächlich die Persistenz von Standardindikatoren mikrobieller anthropogener Verschmutzung in natürlichen Gewässern fördern.

In Kapitel II wurde die Permissivität von aquatischen Bakterien gegen das Modell-Plasmid für Antibiotikaresistenz pKJK5 zwischen Gemeinschaften, die Biofilme auf MP bilden, gegenüber denen, die frei leben, verglichen. Die Häufigkeit des Plasmidtransfers unter den MP-assoziierten Bakterien war höher als unter Bakterien, die frei oder in natürlichen Aggregaten leben. Der verstärkte Genaustausch trat darüber hinaus bei einem breiten Spektrum phylogenetisch diverser Bakterien auf. Die Ergebnisse deuten auf eine unterschiedliche Aktivität von HGT in MP-Biofilmen hin, welche die Ökologie aquatischer mikrobieller Gemeinschaften auf globaler Ebene sowie die Verbreitung von Antibiotikaresistenzen beeinflussen könnten.

Schließlich wurden in Kapitel III physiologische Messungen durchgeführt, um festzustellen, ob Mikroorganismen auf MP eine andere funktionelle Diversität aufwiesen als jene im Wasser. Die generelle heterotrophe Aktivität, wie der Sauerstoffverbrauch, wurde in Mikrokosmentests mit und ohne MP verglichen, während die Diversität und Vielfalt heterotropher Aktivitäten mit Hilfe von Biolog® EcoPlates berechnet wurden. Drei Seen mit unterschiedlichen Nährstoffbedingungen wiesen Unterschiede in der Ausprägung der MP-assoziierten Biomasse auf. In allen Seen unterschieden sich die funktionellen Diversitätsprofile der MP-Biofilme von denen der Gemeinschaften im umgebenden Wasser, aber nur die MP-Biofilme des oligo-mesotrophen Sees hatten eine höhere funktionelle Vielfalt im Verglichen zum Umgebungswasser. Die Ergebnisse verdeutlichen, dass MP-Oberflächen als neue Nischen für aquatische Mikroorganismen fungieren und die globale Kohlenstoffdynamik im Pelagial beeinflussen können.

Insgesamt unterstützen die in den Kapiteln I und II vorgestellten experimentellen Studien ein Szenario, in dem die Umweltverschmutzung durch MP die HGT-Dynamik zwischen aquatischen Bakterien beeinflusst. Zu den Folgen dieser Veränderung gehört eine Erhöhung

der Mobilisierungs- und Übertragungseffizienz von ARGs. Darüber hinaus wird vermutet, dass eine Beeinflussung des HGT die Evolution von Bakterien und die Umsetzung von organischem Material verändern könnte, was zu verschiedenen katabolischen Profilen führt, wie in Kapitel III gezeigt. Die Ergebnisse werden in Zusammenhang mit dem Ausmaß der Plastikverschmutzung sowie der Bedeutung von HGT für die bakterielle Entwicklung und „mikrobielle Schleife“, d. h. an der Basis der aquatischen Nahrungsnetze, diskutiert. Diese Doktorarbeit veranschaulicht die Bedeutung von MP-Biofilmgemeinschaften für die beobachteten Veränderungen des aquatischen Mikrobioms als eine Folge der intensiven anthropogenen Eingriffe.

List of abbreviations

MP: microplastic(s)

HGT: horizontal gene transfer

MGEs: mobile genetic elements

ARGs: antibiotic resistant genes

WWTPs: wastewater treatment plants

FL: free-living bacteria

PA: particle-associated bacteria

FCM: flow cytometry

FACS: fluorescence-activated cell sorting

Introduction

Plastic pollution has recently caught the attention of both the scientific community and the public opinion. Increasing amounts of manufactured plastic polymers end up accumulating on landfills or the natural terrestrial and aquatic environments (Geyer *et al.*, 2017). This waste is easily fragmented and weathered down to particles of less than a few millimeters denominated "microplastics" (MP), whose complete mineralization could take up to centuries (Keswani *et al.*, 2016). These particles are easily transported to freshwater ecosystems and finally to the oceans. In natural environments, MP surfaces represent a new habitat for biofilm formation, where diverse microbial assemblages develop, often referred to as the "plastisphere" (Zettler *et al.*, 2013). However, very few studies have addressed microbial activities of MP biofilms and their repercussions for ecosystem functioning and alteration of water quality.

Our knowledge of microbial biofilms tells us they are essential for both biodiversity and functioning of aquatic ecosystems (Battin *et al.*, 2016). The proximity of prokaryotic and eukaryotic cells together with a polymer matrix to protect them from many environmental stressors (e.g., physical abrasion, UV radiation) (Demeter *et al.*, 2016), provides conditions to maximize interactions between different groups colonizing on MP. The result is a diverse network of metabolic co-operation that transforms organic matter and shape water biogeochemistry, with many ecological implications for the entire aquatic food web. The metabolic diversity in biofilms is also a product of horizontal gene transfer (HGT) among different species, whereby mobile genetic elements (MGEs) are shared integrating, e.g., different degradation pathways (Flemming *et al.*, 2016). The increased proximity of cells on biofilms enhances HGT, an essential driver in the evolution of organismic, particularly of bacterial communities on Earth (Madsen *et al.*, 2012). It is also a very relevant process for the spread of antibiotic resistance genes (ARGs) in the world, a long-recognized threat by the World Health Organization (Balcázar *et al.*, 2015).

This dissertation starts with an introduction to the magnitude of current plastic and MP pollution in the environment and highlights of current knowledge of the microbial communities that make up the plastisphere. Recent knowledge of HGT in biofilm lifestyles is presented to contextualize the relevance of studies on MP biofilms in aquatic ecosystems. Finally, a brief overview of the main techniques used in this work is presented in the context of the primary research hypothesis.

Microplastic pollution and the "Plastisphere"

Plastic is a generic term for materials made out from different polymers, thereby having different physical and chemical properties such as density (Cole *et al.*, 2011). The manufacture of plastic materials in a year represents the consumption of around 8% of the world's oil production, and it was estimated in more than 332 million metric tons (MT) in 2015 (P.C.H. Hollman *et al.*, 2013; PlasticsEurope, 2015). Among the polymers most used are low and high-density polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET) (Ivar do Sul and Costa, 2014). Plastic materials can be easily molded and manufactured, they have a low cost, and display high resistance to different water, chemical, temperature and light conditions (da Costa *et al.*, 2017). These characteristics, and others that are given by different types of additives, enhance their performance and encourage the widespread use of plastics.

An approximate amount of 6300 MT of plastic waste produced by the end of 2015 ended up in aquatic ecosystems, and it is expected that plastic pollution may double by 2050 (Geyer et al., 2017). Physical, chemical, and biological degradation of plastic waste in the environment result in constant inputs of plastic particles, which are not so readily mineralized (Cole et al., 2011; Law and Thompson, 2014). Microplastics (MP) is the term generally used for those particles of less than 5 mm, either product of plastic fragmentation or from the industrial origin (Keswani *et al.*, 2016). Floating plastic debris found in all subtropical gyres is mainly composed of < 1cm diameter particles (Cózar et al., 2014), with estimations of 15-51 trillion plastic particles in the oceans (van Sebille *et al.*, 2015). Nevertheless, MP are also ubiquitous in all forms of freshwaters, whereby substantial amounts stem from inputs of wastewater treatment plants (WWTPs) (Wagner and Lambert, 2018; Leslie *et al.*, 2017; Mintenig *et al.*, 2017).

Although MP pollution can be formed by fragmentation of large plastic litter, it also originates from fibers that pass the WWTPs (Law and Thompson, 2014). The most commonly found MP in the environment include PE, PP, low-density PE (LDPE), PVC (Bakir *et al.*, 2012) and PET (Oberbeckmann *et al.*, 2014). Further, The analysis of persistent organic pollutants (POPs) in marine MP shows that they can concentrate some of these substances such as polychlorinated biphenyls (PCBs) by several orders of magnitude in respect to the surrounding water (Andrady, 2011; Hirai *et al.*, 2011). All these particles have therefore different composition with respect to other types of particulate material in aquatic ecosystems and provide an increased surface-to-volume ratio that makes them attractive for microbial attachment.

Microplastics interact with microorganisms, the base of aquatic food webs. The specific term, the "plastisphere," was coined to refer to all the assemblages in MP particles (Zettler *et al.*, 2013). Several observations in samples of water and sediments found that the microbial colonization of MP particles presents a different structure and compositions to the surrounding habitats in marine and freshwaters, converging towards specific groups

(Harrison *et al.*, 2014; McCormick *et al.*, 2016). After this, there has been increasing calls to research MP-associated microorganisms, and they have been identified as a significant threat to aquatic biota (Kalogerakis *et al.*, 2014; McCormick *et al.*, 2014).

From a microbial perspective, the presence of microbial pathogens as hitchhikers in MP biofilms is a cause for concern (Kirstein *et al.*, 2016). Also, experiments with zooplankton taxa have demonstrated the passage of MP particles with their associated biofilms in the planktonic food web (Setälä *et al.*, 2014). On the other hand, the recently found potential differences in metabolism of MP-associated bacteria (Bryant *et al.*, 2016) could translate into alterations of the biogeochemical cycles and the evolution of bacteria in aquatic systems. One of the reasons this can happen is via changes in the genetic exchange between microorganisms that form part of the MP biofilm communities.

Horizontal gene transfer (HGT) in aquatic ecosystems and MP biofilms

There are different mechanisms for HGT, meaning the sharing of genetic material between organisms of different species or not a parent-offspring relationship (Soucy *et al.*, 2015). Among these, conjugation requires direct intercellular exchange while transduction and transformation are a virus-mediated transport and environmental uptake of DNA, respectively (Drudge and Warren, 2012). All of these mechanisms have in common that they give microorganisms the ability to adapt to changing conditions (McDaniel *et al.*, 2012). Also, the incorporation of those changes to their genome causes the evolution of microbial life (Linz *et al.*, 2007).

The attention towards transfer dynamics of MGEs was triggered by their use in genetic engineering to provide applications useful for human life, and because of its role in the problem of antibiotic resistance in pathogenic bacteria (Aminov, 2011). However, with the advent of the "omics" era, there is a reassessment of the definitions of MGEs and their role in ecosystems (Aminov, 2011). Conjugation by plasmids, double-stranded circular or linear DNA molecules capable of autonomous replication (Carattoli *et al.*, 2014), and the ubiquity of integrons, genetic platforms that acquire exogenous genes in the form of mobile gene cassettes (Gillings, 2017), are two types of MGEs studied in this Ph.D. thesis.

The presence of transferable plasmids and conjugation events has been shown to positively affect biofilm formation (Broszat and Grohmann, 2014). Thereby, plasmid-encoded factors are believed to influence biofilm development (Madsen *et al.*, 2012). Biofilms are aggregations of microorganisms that adhere to a solid surface in a matrix composed of extracellular biopolymers (Dickschat, 2010). They may adhere to an inert or biotic surface or exist as free-floating communities. The reasons why biofilm lifestyles offer suitable characteristics for HGT include the high density that allows for contact between cells, and matrix concentration of chemical compounds that induce gene transfer (Aminov, 2011; Sezonov *et al.*, 2007).

Experimental data provide evidence of enhanced conjugative transfer in biofilms vs. free-living bacteria: more transconjugants can be found after mating on a filter compared with mating in liquid culture (Broszat and Grohmann, 2014). HGT via bacterial conjugation is also a major force driving the recent antibiotic resistance spread (Reisner *et al.*, 2012). Nevertheless, there is less detailed information on the extent of HGT in the spatially structured populations in biofilms (Broszat and Grohmann, 2014) and in particular on pelagic particles including MP in aquatic systems.

Differences in diversity between free-living and particle-associated microorganisms have been documented in both limnic and marine environments (Bižić-Ionescu *et al.*, 2015; Rösel *et al.*, 2012). In the particle associated lifestyle, some features are more common such as chemotaxis, as well as the pressure effects of grazing (Tang *et al.*, 2006). Indeed, MP biofilms in marine systems have shown enrichment in genes associated with chemotaxis, frequent cell-to-cell interactions, and nitrogen fixation (Bryant *et al.*, 2016). The concentration of nutrients in particles of organic matter in water (e.g., oceans) can be several orders of magnitude higher than in the surrounding waters and serves as an anchor point for bacteria. Hence, MP particles are an ideal hotspot for gene exchange, since they provide an environment rich in carbon sources and ideal for bacteria to meet and exchange information (Stewart, 2013).

Objectives and study approach

In this Ph.D. thesis gene exchange was analyzed among bacterial communities in MP biofilms and complemented with the study of microbial composition and heterotrophic activities in the plastisphere. These topics were addressed in three scientific publications with the following research questions:

1. **Chapter I**: Does MP provide an ecological niche for wastewater-derived bacteria and the spread of ARGs by HGT?
2. **Chapter II**:
    a. Does MP surface increase HGT frequency in aquatic ecosystems?
    b. Does bacteria in MP biofilms from aquatic ecosystems are more permissive to plasmid transfer than bacteria in the surrounding water?
3. **Chapter III**: Are MP new niches for enhanced heterotrophic activities in aquatic ecosystems?

The thesis aim is to provide evidence that MP represents a new habitat for bacteria, which alters the rates of horizontal gene exchange and heterotrophic activities in freshwater ecosystems. The study comprises bacteria from natural aggregates and the free-living community. The functional features analyzed include **a)** the spread of the integron class 1, a marker of anthropogenic pollution associated with mobile genetic elements, and known as a

proxy for the spread of antimicrobial resistance genes; **b)** the transfer frequency of a self-conjugative plasmid encoding resistance to a given antibiotic substance; and **c)** the functional diversity profile based on the use of different carbon sources by microbial communities on microplastics.

Traditional experiment-based quantification of HGT has limitations such as growth conditions of bacterial species in a model system, scaling and limited capacity to single cell analysis. Detection limits are around one event every 10^9-10^{10} bacteria exposed (Nielsen *et al.*, 2014). Over the last decade, advances in reporter gene technology have provided new insights into the extent and spatial frequencies of HGT *in vitro* and in natural environments (Reisner *et al.*, 2012). Accordingly, a set of different tools like fluorescent-based flow cytometry (FCM) and cell sorting (FACS), next-generation sequencing, qPCR, and metabolic fingerprint methods were used in this thesis. Notably, the use of FCM equipped with FACS and qPCR were chosen because they are suitable alternatives to cultivation-based methods for routine measurement of plasmid persistence and sorting of single bacterial cells, thereby opening avenues for high-throughput analyses (Loftie-Eaton *et al.*, 2014).