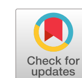
Science of the Total Environment 650 (2019) 2395–2402

Contents lists available at ScienceDirect

Science of the Total Environment

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

Distinct community structure and microbial functions of biofilms colonizing microplastics



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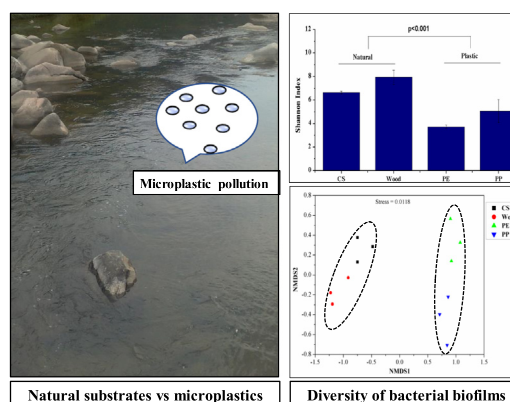
HIGHLIGHTS

- Alpha diversity of biofilms was lower on microplastic than on natural substrates.
- Community structure and composition varied between biofilms on different substrates.
- Metabolic pathways were altered in biofilms colonizing microplastic.
- Microplastic is a new microbial niche affecting microbial structure and function.
- This alteration in biofilms may have an ecological impact on aquatic ecosystems.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 August 2018
Received in revised form 27 September 2018
Accepted 29 September 2018
Available online 01 October 2018

Editor: Jay Gan

Keywords:
Microplastic
Biofilm
Species sorting
Metabolic pathways
Substrate type

ABSTRACT

Microplastics are frequently detected in freshwater environments, serving as a new factitious substrate for colonization of biofilm-forming microorganisms. Distinct microbial assemblages between microplastics and surrounding waters have been well documented; however, there is insufficient knowledge regarding biofilm colonization of plastic and non-plastic substrates, despite the fact that microbial communities generally aggregate on natural solid surfaces. In this study, the effects of substrate type on microbial communities were evaluated by incubation of biofilms on microplastic substrates (polyethylene and polypropylene) and natural substrates (cobblestone and wood) for 21 days under controlled conditions. Results from high-throughput sequencing of 16S rRNA revealed that the alpha diversity (richness, evenness, and diversity) was lower in the microplastic-associated communities than in those on the natural substrates, indicating substrate-type-coupled species sorting. Distinct community structure and biofilm composition were observed between these two substrate types. Significantly higher abundances of *Pirellulaceae*, *Phycisphaerales*, *Cyclobacteriaceae*, and *Roseococcus* were observed on the microplastic substrates compared with the natural substrates. Simultaneously, the functional profiles (KEGG) predicted by Tax4Fun showed that the pathways of amino acid metabolism and metabolism of cofactors and vitamins were increased in biofilms on the microplastic substrates. The findings illustrate that microplastic acts as a distinct microbial habitat (compared with natural substrates) that could not only change the community structure but also affect microbial functions, potentially impacting the ecological functions of microbial communities in aquatic ecosystems.

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Abbreviations: ANOVA, analysis of variance; CS, cobblestone; KEGG, Kyoto Encyclopedia of Gene and Genomes; MP, microplastics; NMDS, non-metric multidimensional scaling; OTUs, operational taxonomic units; PE, linear low-density polyethylene; PP, polypropylene; STAMP, Statistical Analysis of Metagenomic Profiles.

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https://doi.org/10.1016/j.scitotenv.2018.09.378
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1. Introduction

Global plastic production has increased rapidly in the past 60 years, reaching 322 million tons in 2015 with an upward trend (PlasticsEurope, 2016). Large amounts of plastic debris are continuously released into the environment directly or indirectly (Dris et al., 2015; Ivleva et al., 2017) and then fragment into smaller particles collectively termed microplastics (MP; particles with diameter < 5 mm) by biological, photo-, and/or mechanical degradation (Shim et al., 2017). MP have been identified as an emerging environmental threat to aquatic ecosystems because of their negative effects on a range of aquatic organisms from phytoplankton to zooplankton, fish, and cetaceans (Cole et al., 2011; Connors et al., 2017; Eerkes-Medrano et al., 2015; Ogonowski et al., 2018; Sharma and Chatterjee, 2017). MP are widespread in marine habitats, and numerous studies have been performed to investigate their temporal and spatial distribution, fate, and ecological impacts in marine environments (Harrison et al., 2011; Ivleva et al., 2017; Oberbeckmann et al., 2015). Recent investigations revealed that MP were also discovered in various freshwater environments, such as rivers, lakes, and reservoirs (Di and Wang, 2018; Klein et al., 2015; McCormick et al., 2014; Su et al., 2016). Thus, the fate and potential impact of MP in freshwater systems should be further investigated.

When released in aquatic habitats, buoyant MP may float in the water for months and finally accumulate in benthic environments (Besseling et al., 2017; Eerkes-Medrano et al., 2015). During their long-distance transport by water flow and winds, MP can serve as factitious surfaces for planktonic microorganism colonization and assemblage formation (De Tender et al., 2015; Harrison et al., 2014; Rummel et al., 2017). Studies have reported that rapid formation of microbial biofilms was observed on MP surfaces within 1–2 weeks in aquatic environments, and the taxonomic composition of biofilms on plastic particles was distinct from the microbial assemblages of the surrounding water (De Tender et al., 2017; McCormick et al., 2014; McCormick et al., 2016). These MP are therefore suggested as a specific niche for microbial life, known as the "plastisphere" (Zettler et al., 2013). Moreover, MP were hypothesized as vectors for transport of pathogens and harmful algae species in natural ecosystems (Arias-Andres et al., 2018a; Koelmans et al., 2016; Viršek et al., 2017). For example, Eckert et al. (2018) demonstrated that increasing quantities of MP promote the survival of wastewater treatment plant (WWTP)-derived bacteria in fresh water. Therefore, the introduction of MP colonized by specific assemblages is likely to alter the microbial communities and genetic exchange in natural water and consequently affect the ecological function of the microbial communities.

Generally, in natural water, microbial communities exist in the form of biofilm attached to natural solid surfaces (such as rock and wood) with an assortment of colonies and cellular and extracellular polymers (Flemming et al., 2016). Biofilms have been demonstrated to be of great significance to microbial function and ecological processes in fresh water (Battin et al., 2016). The formation and growth of biofilms are significantly affected by environmental conditions, among which the types and properties of solid surfaces are directly associated with early biofilm formation (Cardinale et al., 2002). As MP serve as a new surface for biofilm colonization, it is essential to compare the microbial communities developing on plastic and natural substrates inoculated with the same source communities. Nonetheless, most current research is focused on the comparison of MP-associated and aquatic communities (Chae and An, 2017; De Tender et al., 2015; De Tender et al., 2017; Jiang et al., 2018), and investigations of biofilm formation on plastic and non-plastic surfaces are scarce (Ogonowski et al., 2018). More importantly, the specific assemblages colonizing MP might reveal distinct microbial functions compared with those of assemblages on natural substrates, resulting in notable ecological consequences.

Thus, in this study, we hypothesized that compared with natural substrates, the introduction of MP as a new substrate may result in a shift of the community structure of biofilms formed on these substrates and then change the functional diversity of biofilm communities, which consequently might result in unpredictable influences on the freshwater ecosystems. To test this hypothesis, an indoor biofilm culture experiment was performed using two types of substrates—natural (cobblestone and wood) and MP (polyethylene and polypropylene)—with a bacterioplankton community from Xuanwu Lake (Nanjing, China) as the inoculum. The microbial richness, composition, and structure of biofilm communities were compared between the natural and MP substrates. Moreover, comparative analysis of the predicted functional diversity was performed to investigate the ecological impacts of MP on biofilm in aquatic ecosystems.

2. Materials and methods

2.1. Microplastics and natural substrates

To evaluate the potential impacts of substrate type (natural and MP) on aquatic biofilm communities, four different substrates (two of each type) were selected for microbial colonization. Linear low-density polyethylene particles (PE; diameter 3–4 mm, density 0.92 g cm^{-3}) and polypropylene particles (PP; diameter 3–4 mm, density 0.91 g cm^{-3}) were purchased from Aladdin Biochemical Technology Co. LTD (Shanghai, China) and served as MP substrates. PP and PE were used because they represent the most abundant plastics detected in the aquatic environments (Cózar et al., 2014), and to compare with previous studies (Zettler et al., 2013), in which bacterial communities colonizing PP and PE were studied. Cobblestone (CS; diameter 3–4 cm) and short-cut wood (length 5 cm; width 2 cm; depth 1 cm), which are ubiquitous in freshwater environments, served as the natural reference substrates.

The source community was retrieved from fresh water collected from Xuanwu Lake, Nanjing, East China. Fifty liters of water was collected in sterile jars, transported to the lab on ice, and then filtered with a 10-μm sieve to remove the large particles and small organisms. The water quality parameters were also determined (pH = 7.7; total nitrogen = 2.3 mg L^{-1}; total phosphorous = 0.13 mg L^{-1}; ammonia = 0.62 mg L^{-1}; and nitrate = 0.85 mg L^{-1}).

2.2. Biofilm incubation

Bacterial biofilms were incubated in 12 experimental tanks (length 50 cm; width 50 cm; depth 30 cm), with three tanks per substrate type. To provide comparable surface areas for colonization, tanks were loaded with 200 particles of microplastics (PE and PP), and 10 pieces of natural substances (CS and Wood) (see Table S1). The tanks were situated in a greenhouse that was exposed to natural light, and the roofs were covered with black cloth to block approximately 50% of the incoming solar radiation. Evaporation loss was replenished daily by adding dechlorinated tap water. In order to facilitate the development of biofilms, 100 mL Woods Hole culture medium (Table S2) was pre-added into experimental tanks (described below) to provide normal levels of nutrition (Sun et al., 2018). The tanks were stirred manually four times per day for 5–6 min. After 21 days of incubation, the substrate material was washed three times with sterile water, and the biofilms were collected and used in further experiments.

2.3. DNA extraction, amplification, and sequencing

Approximately 0.5 g biofilm was collected, from which genomic DNA was extracted using the E.Z.N.A.® Tissue DNA kit (Omega Biotek, Norcross, GA, USA). DNA integrity and purity were monitored on 1% agarose gels. DNA concentration and purity were measured simultaneously using the NanoDrop One (Thermo Fisher Scientific, Waltham, MA, USA). 16S rRNA genes of the V4 region were amplified using the 515F and 806R primers for bacterial communities. Then, the PCR products were detected by 1% agarose gel electrophoresis and purified. Sequencing libraries were generated using NEBNext® Ultra™ DNA

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