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Microplastics increase impact of treated wastewater on freshwater microbial community☆

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ABSTRACT

Plastic pollution is a major global concern with several million microplastic particles entering every day freshwater ecosystems via wastewater discharge. Microplastic particles stimulate biofilm formation (plastisphere) throughout the water column and have the potential to affect microbial community structure if they accumulate in pelagic waters, especially enhancing the proliferation of biohazardous bacteria. To test this scenario, we simulated the inflow of treated wastewater into a temperate lake using a continuous culture system with a gradient of concentration of microplastic particles. We followed the effect of microplastics on the microbial community structure and on the occurrence of integrase 1 (*int*1), a marker associated with mobile genetic elements known as a proxy for anthropogenic effects on the spread of antimicrobial resistance genes. The abundance of *int*1 increased in the plastisphere with increasing microplastic particle concentration, but not in the water surrounding the microplastic particles. Likewise, the microbial community on microplastic was more similar to the original wastewater community with increasing microplastic concentrations. Our results show that microplastic particles indeed promote persistence of typical indicators of microbial anthropogenic pollution in natural waters, and substantiate that their removal from treated wastewater should be prioritised.

1. Introduction

Global production of plastic dramatically and constantly increased in the past 60 years reaching 322 million of tons in 2015 with rising tendencies (PlasticsEurope, 2015). Substantial parts of this huge amount of plastic escape dumping at landfill sites, recycling, or waste treatment and thus enters the environment, where it accumulates, particularly in aquatic habitats (Eriksen et al., 2013; Law, 2017). In the environment, plastic remains almost unchanged for a long time and its complete mineralization has been estimated to require centuries (Barnes and Milner, 2005; Krueger et al., 2015). The term *microplastic* has been coined to describe manufactured microbeads (primary microplastic) or fragments of < 5 mm in diameter that are formed during plastic degradation (secondary microplastic) and their total number floating in the oceans has been estimated to range between 15 and 51 trillion particles in 2014 (Van Sebille et al., 2015). Plastic-derived hazards are well described for numerous aquatic organisms ranging from zooplankton to mammals (Cole et al., 2011; Gall and Thompson, 2015; Li et al., 2016). Although identified as an emerging environmental threat for the oceans, little is known about microplastic in freshwater ecosystems and its ecological consequence (Eerkes-Medrano et al., 2015; Wagner et al., 2014). In particular, wastewater treatment plants (WWTP) effluents represent an important point source for microplastic particles for freshwater environments (Leslie et al., 2017; Mintenig et al., 2017). Although WWTPs remove between 83 and 95% of all microplastic particles (Dris et al., 2015), there is still a substantial quantity; e.g. around 9×10^3 pieces of microplastic m^{-3} were found in the effluent of a German WWTP. Based on the annual effluents of the twelve tested WWTPs, a total discharge of up to

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4×10^9 microplastic particles and fibres per WWTP can be expected to be released into the environment (Mintenig et al., 2017).

One feature of microplastic particles is that they constitute new submerged surfaces for bacterial and eukaryotic colonization, dispersal, nutrient cycling, and biofilm formation (Kettner et al., 2017; Mincer et al., 2016; Oberbeckmann et al., 2015). The fact that microplastic particles host specific assemblages differing from the open waters led to formulate the term *plastisphere* (Zettler et al., 2013). Microplastic particles have been hypothesized to even act as a vector for opportunistic microbial colonisers that otherwise might not be able to proliferate in the surrounding waters (Keswani et al., 2016). For example, the potential pathogen *Vibrio parahaemolyticus* was found on floating microplastic particles (Kirstein et al., 2016). Within the biofilm, such bacteria can be protected from grazing pressure and competition for nutrients is reduced (Corno et al., 2014; Costerton et al., 1999). Another point of concern is that the close vicinity of cells growing in biofilms might increase Horizontal Gene Transfer (HGT) between different bacteria and may thus favour the transfer of pathogenicity and antibiotic resistance in the environment (Costerton et al., 1999).

The here proposed experiment is based on the notion that wastewater effluents contain specific microbial communities, which can include potential human pathogens (Cai and Zhang, 2013; Wéry et al., 2008) and antibiotic resistance genes (ARGs (Di Cesare et al., 2016a),). If microplastic and potential pathogens are released concomitantly, microplastic particles might provide an ecological niche for WWTP-derived pathogens. Moreover, the presumed enhanced HGT in biofilms might facilitate the spread of ARGs (Suzuki et al., 2017). Therefore, we aimed to evaluate the role of microplastic particles in the accumulation of class 1 integrons, which are gene cassettes capture elements (Hall and Collis, 1995) associated with mobile genetic elements involved in the spread of ARGs in the environment (Ma et al., 2017; Stalder et al., 2014). We set up a continuous culture experiment in chemostats with increasing numbers of microplastic particles incubated in different vessels. We used a microbial community from an equimolar mix of waters from a large oligotrophic lake (Lake Maggiore) and from the effluent of the largest municipal WWTP that directly discharges into the lake (Fig. 1). Our experiment mimicked the direct outlet of WWTPs to a receiving aquatic ecosystem such as a lake or a river, where both natural and WWTP waters mix. Since particles and bacterial inoculum were added at the same time, both communities had equal chances of colonizing the microplastic particles.

2. Material and methods

2.1. Experimental set-up

Continuous cultures in chemostats were set up to mimic conditions where water from a WWTP effluent enters into a freshwater system. Therefore, for the inoculum, on September 23rd, 2015, 10 L of lake water were sampled from the shore of Lake Maggiore (WGS84 coordinates: 45.924647° N, 8.545711° E), and concomitantly water was sampled from the municipal WWTP effluent of Verbania (Italy). Both waters were subsequently filtered through 126 μm and 10 μm plankton nets to remove large grazers and particles, but keep the bacterial communities and the smaller eukaryotic predators. Cell numbers were determined immediately by flow cytometry and the waters were mixed to achieve a balanced bacterial community half in cell numbers each from the WWTP effluent and from Lake Maggiore. The starting community consisted of 2.57×10^6 bacterial cells mL^{-1}. Each chemostat vessel was filled with 750 mL of the inoculum solution, including the mixed bacterial communities of the lake and WWTP.

Autoclaved water from the same lake, without any additional bacterial community, was used as a medium during the experiment: 60 L of surface lake water was sampled from the same station as sampled for the inoculum, at the shore of Lake Maggiore (on September 21st, 2015), and pre-filtered over glass microfiber filters (grade GF/C). The medium water was aliquoted into three bottles (18 L), each of them supplemented with chitin from the stock solution (see below), autoclaved, and each bottle used to feed a triplet of running chemostat vessels (Fig. 1).

Chitin was chosen as a supplementary carbon source since this refractory substrate represents one of the most prevalent autochthonous biopolymers in natural aquatic ecosystems (Corno et al., 2015; Köllner et al., 2012). Since medium water was pre-filtered, natural sources of biopolymers, e.g. chitinous body parts of dead zooplankton, were potentially removed and were thus hereby replaced. A final concentration of approximately 4 mg L^{-1} dissolved

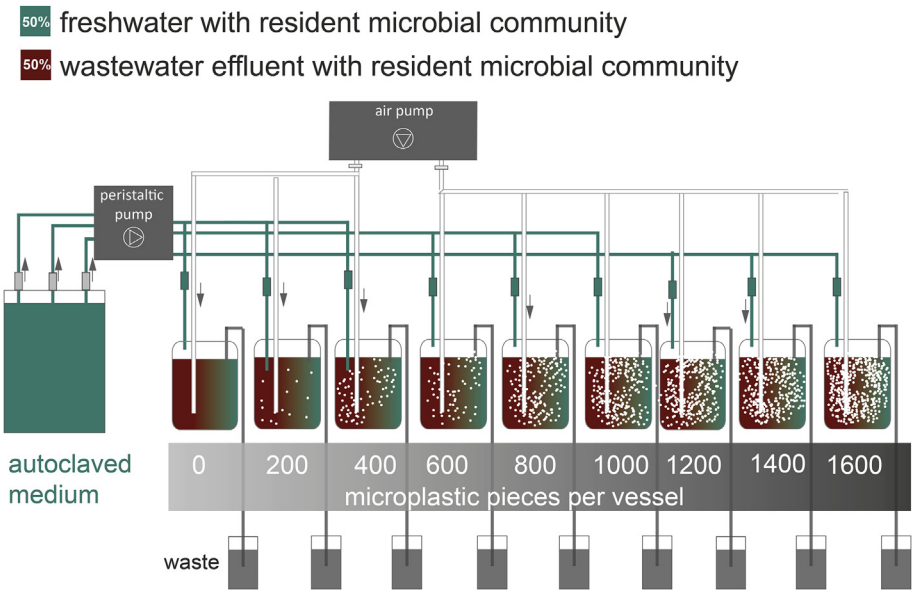

Fig. 1. Schematic representation of the chemostat set-up.