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Why biofouling cannot contribute to the vertical transport of small microplastic

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Abstract
In contrast to expectations, even buoyant microplastics like polyethylene and polypropylene are found at high concentrations in deep sediment traps and deep-sea sediments. To explain the presence of such buoyant microplastic particles at great ocean depths, several vertical transport mechanisms are under discussion with biofouling as one of the most referred. Biofouling is thought to increase the density of microplastic particles to the point that they sink to the deep sea, but this has mostly been shown on large microplastic particles ≥ 1 mm. However, although microplastics are defined as particles between 1 and 5000 µm, most microplastics are < 100 µm. In the ocean plastic particles continuously fragment, converting each "large" particle into several "small" particles, and particle abundance increases drastically with decreasing size. We argue that biofouling is not a reasonable transport mechanism for small microplastic particles ≤ 100 µm, which form the majority of microplastics. Biofilm density depends on its community and composition. A biofilm matrix of extracellular polymeric substances and bacteria has a lower density than seawater, in contrast to diatoms or large organisms like mussels or barnacles. We suggest that a small microplastic particle cannot host a biofilm community consisting of the heavy organisms required to induce sinking. Furthermore, to reach the deep sea within a reasonable timespan, a microplastic particle needs to sink several meters per day. Therefore, the excess density has to not only exceed that of seawater, but also be large enough to enable rapid sinking. We thus argue that biofouling cannot be an efficient vertical transport mechanism for small microplastic. However, biofouling of small microplastic may promote the likelihood of its incorporation into sinking marine snow and increase the probability of its ingestion, allowing its transport to depth.

Keywords Microplastic, Biofouling, Biofilm, Density change, Buoyancy, Sinking velocity, Vertical transport

Introduction

Microplastics are plastic particles ranging from 1 µm to 5 mm. They can now be found in all parts of the ocean, from the polar regions [1] to the deep sea [2, 3], and are a known danger to marine ecosystems [4]. Most microplastic enters the ocean mainly through rivers [5], and once in the ocean fragment further, continuing to produce smaller particles [6]. This results in an abundance–size distribution of microplastic particles following a power law [6], meaning that in the ocean small microplastic particles are much more abundant than large microplastic particles. The vast majority of all microplastic particles in the ocean seem to be smaller than around 10 µm [7–10]. Around 80% of microplastic particles in one liter samples from three different areas, the northeastern coast of Venezuela, the Gulf Stream current at the U.S. coast, and the Pacific Arctic Ocean, had an average ESD (equivalent spherical diameter) under 6 µm [9]. A study at the

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Norwegian coast found the majority of microplastic particles (~58%) at their lowest detectable size of 11 µm [10]. In another study in New York Bight around 90% of the microplastic ranged from less than 1 µm to 3 µm ESD [8]. And in the North Atlantic the smallest measured size class of 10 µm had also the highest abundance of microplastic particles [7]. To acknowledge size differences, we will distinguish between "*large*" microplastics, particles larger than 100 µm, and "*small*" microplastics, particles smaller than 100 µm.

It was long assumed that the majority of plastics, including microplastics, can be found near the ocean surface since the most produced plastic types, polyethylene (PE) and polypropylene (PP) [11], are buoyant with a density considerably lower than that of seawater. However, the discovery of such "buoyant" microplastic particles, including small microplastic particles < 100 µm, in deep-sea sediments [3] as well as in deep sediment traps [12], which collect sinking particles, has raised the question of how these particles make their way to the sea floor.

Three main transport pathways are currently under discussion: Physical integration into marine snow aggregates, biological uptake and incorporation into food webs, or biofouling of microplastics that makes the particles heavy enough to sink [13]. Marine snow aggregates, defined as >0.5 mm in size [14], often consist of organic detritus, microorganisms, and clay minerals [14] and are responsible for a large part of organic matter transport to the deep sea [15]. Sinking marine snow can incorporate microplastic, and transport these particles from the surface ocean to depths [16]. A similar mechanism has been shown to effectively transport buoyant oil droplets to the deep sea floor [17–19]. Alternatively, microplastic particles that are ingested may be vertically transported within sinking fecal pellets or via successional consumers [20]. Lastly, the growth of a biofilm on plastic particles may increase the density of the biofouled plastic until it starts sinking [6]. The sinking velocity of a spherical particle in water can be calculated using Stokes's law

$$\omega_s = \frac{g\,(\rho_P - \rho_{sw})d^2}{18\mu}$$

which relates the sinking velocity ω_s to the gravitational acceleration (g), the density (ρ_P) and diameter (d) of the particle as well as to the density (ρ_{sw}) and dynamic viscosity (μ) of the surrounding water. Recent reviews suggest biofouling as a major transport pathway for microplastic [5, 13, 21–24] and biofouling has been provided as an explanation for the discovery of low-density microplastics like PE in marine sediments [13, 21, 23]. It has, however, also been acknowledged that biofouled microplastic may remain suspended in the water column, rather than sink to the seafloor [5, 22, 24].

The conclusion that biofouling is responsible for the sedimentation of microplastic is based on several experimental studies investigating density changes and sinking of biofouled plastic and microplastic. Some of these studies have directly investigated the sinking behavior due to biofouling on microplastic particles [25–32]. In several of these experiments, the effect of biofouling is measured as the time (days) until ≥50% of particles sink below the surface [25–28, 33] and results suggest that composite density (density of plastic plus biofilm) of many types of microplastic particles > 0.1 mm does increase due to biofouling. Sinking velocity measurements of large biofouled microplastics, 1–5 mm, demonstrate that at times, velocities of hundreds of meters per day can be reached even for buoyant plastic types [26], but in the majority of cases no or negligible sinking was observed [29–32]. And while one study reported sinking of small biofouled microplastics (70 µm) after several days [28], apparently, no sinking velocity measurements exist for small biofouled microplastics. These studies also reveal that type, size and shape of plastic all impact composite particle density. For example, changes of composite density are more rapid for films than fibers than granules [27]. And density changes occur slower, the larger a microplastic particle is [26, 27]. Thus, although published work provides evidence that biofouling can be an important transport pathway for many types of large microplastics, there is no evidence that this finding can be extrapolated to small microplastics.

In the following, we argue that it is highly unlikely that small microplastic particles sink to the deep ocean and seafloor due to biofilm formation. To provide background we first briefly explain the process of biofouling as well as key parameters driving sinking velocity, with a focus on density, and explore existing knowledge of the density of biofilms. We then provide our argument why most small microplastic will not sink due to biofouling. In short, we propose that biofilm communities and thus the composite density of a biofouled microplastic particle depends on the size of the plastic particle, and that it is unlikely that composite density of a small biofouled microplastic is high enough to cause relevant sedimentation. This means that results obtained with large microplastics should not be extrapolated to the whole size range of microplastic particles.

Background

The process of biofouling starts as soon as any particle enters the ocean [34]. Organic molecules immediately start to adsorb to the particle's surface, bacteria and phytoplankton attach to the particle, and invertebrates and macroalgae may begin colonizing. Many of these organisms create biofilms by exuding an organic matrix [35]. Such a biofilm community, if associated with a plastic particle, is frequently referred to as a plastisphere [36] and can change the physical characteristics and fate of the microplastic particle. Adding a biofilm will change the dimensions of the emerging particle, its surface properties, as well as its composite density, and thus its propensity to sink.

The sinking velocity of particles in water depends on many factors, such as shape, roundness, and surface texture, and especially for sheets, biofilm formation may result in decreased settling velocities, after a threshold, due to interaction with drag forces [32]. Nevertheless, density is a key factor in determining the propensity of a particle to sink, as well as for the argument that biofouling induces sinking.

The velocity of a spherical particle sinking through water is influenced by the density difference between the particle and surrounding water, called excess density, and by the size squared of the particle (Stokes's law). Therefore, whether a biofouled microplastic particle sinks or not, and at what speed, depends, among other factors, on its excess density in relation to its size. The excess density of a biofouled microplastic particle is determined by its composite density, which depends on the plastic type and the characteristics of the biofilm. Plastic types have various densities from below fresh water density (1000 kg m^{-3}) to above seawater density (1025 kg m^{-3}) (Table 1). The plastic types with densities above seawater density can sink without an additional biofilm, e.g. a 10 µm solid plastic sphere may reach sinking velocities between 54 and 1637 m d^{-1} depending on the plastic type (Table 1). Microplastic particles with excess densities below the surrounding water (like PE and PP) depend on the density increase from a biofilm to reach the composite excess density required for sinking.

The density of a biofilm depends, among other things, on its composition. A biofilm potentially consists of bacteria, phytoplankton, protozoans, fungi, all embedded in a matrix of extracellular polymeric substances (EPS) [35]. The different organisms as well as the exopolymer matrix of the biofilm have different densities. Diatoms known to form a biofilm on plastic have estimated densities of 1150–1180 kg m^{-3} [53], bacteria have an average density of 1100 kg m^{-3} [37, 38] and the density of fresh (bacteria free) transparent exopolymer particles (TEP), a type of EPS, was measured to range between 700 and 840 kg m^{-3} [39]. A review by Stewart in 1998 [40] listed the non-aqueous biomass density of biofilms. Six of the listed were intact mixed microbial biofilms and ranged from 24 to 141 kg m^{-3}, with a median of 83 kg m^{-3}, and an average (±std) of 78±42 kg m^{-3}. Characklis [41] found a slightly lower dry density range of 10–50 kg m^{-3}. The water content in biofilms is 70 to 98% by weight [42]. A 90% seawater content (the aqueous phase) by weight converts to a 40% water content by volume which has to be used to calculate the wet density of microbial biofilms from their dry density. Using a seawater density of 1025 kg m^{-3}, the calculated average hydrated (wet) density of the intact mixed microbial community biofilms (including bacteria) by Stewart [40] is only 457 kg m^{-3}. This density is even lower than the measured hydrated density of freshly generated TEP (without bacteria) [39]. The estimated density of microbial biofilms cultured in synthetic wastewater treatment conditions were, in comparison, slightly higher at 1001–1020 kg m^{-3} [43]. While variable, all estimates of bacterial biofilms suggest densities below that of seawater (1025 kg m^{-3}) and would not promote sinking. Only the presence of organisms heavier than bacteria will generate biofilms that can increase the density of a biofilm to above that of seawater.

Why biofouling cannot sink small microplastic particles

One main reason why microplastic is assumed to sediment due to biofouling, is an extrapolation of processes observed on large microplastic particles and plastic debris. However, if the colonizing biofilm communities depend on the size of the plastic particle, the extrapolation from mm-sized particles to small microplastic particles is questionable. While it has been acknowledged that the biofilm community differs with location, season, and the properties of the particle surface, such as hydrophobicity and roughness [44], the role of particle size has rarely been considered. Bacterial biofilm communities have been shown to differ as a function of size of the microplastic particles [45, 46]; e.g. the biofilm community

Table 1 Densities and theoretical sinking velocities for 10 µm spheres of 10 common plastic types

Plastic type	Exp. PS	PP	LDPE	HDPE	PS	Nylon 66	PMMA	PC	PET	PVC
Density [kg m^{-3}]	11–32	900–910	910–930	940–970	1040–1070	1130–1150	1170–1200	1200	1380–1390	1350–1450
Sinking velocity [m d^{-1}]	-3918–-3837	-486–-448	-448–-371	-332–-216	54–170	401–479	556–672	672	1366–1405	1251–1637

Sinking velocities are calculated after Stokes's law with the following equation:

$$\omega_s = \frac{g\,(\rho_P - \rho_{sw})d^2}{18\mu}$$

where ω_s is the sinking velocity, g is the gravitational acceleration, ρ_P is the density of the plastic, ρ_{sw} is the density of sea water, d is the diameter of the plastic sphere, and µ is the dynamic viscosity of sea water. Negative numbers mean ascension speed of particles

Exp. PS=expanded polystyrene, PP=polypropylene, LDPE=low-density polyethylene, HDPE=high-density polyethylene, PS=polystyrene, PMMA=poly(methyl methacrylate), PC=polycarbon, PET=polyethylene terephthalate, PVC=polyvinyl chloride compositions, investigated genetically, differed between particles of 50 µm vs. 3000 µm, and 10 µm vs. 120 µm. This suggests that particle size is central to the development of a specific biofilm community. It has also been demonstrated that the ratio of bacteria to diatoms on plastic debris between 1 and 10 mm varies from 15 to 0.1, suggesting great variability in the importance of diatoms in biofilms of plastic debris [47]. Biofouling communities on plastic particles ≥ 1 mm often include macroalgae or sessile invertebrates like mussels [25, 29], organisms which are likely not able to colonize small microplastic particles. The presence of mussels in the biofilm community may be required for (large) microplastic particles to sink [29]. We reason that the biofouling community that exists on small microplastic particles commonly differs from that observed on large particles, and that sinking due to biofouling is not likely for small microplastics, given that bacterial biofilms cannot increase the excess density of a microplastic particle to be above the density of seawater.

Accordingly, current modeling studies that investigate biofouling as a cause of microplastic sedimentation, do not consider the composition of a biofilm to vary with particle size, but rather use the density of diatoms to calculate biofilm thickness and composite density, independent of plastic size [48–50]. However, the smallest diatom reported in biofilms is around 3 µm [47, 51] and the cell size of the smallest known diatoms are around 2 µm. It is thus physically not possible, even for these tiny diatoms, to adhere to microplastic spheres of 1–2 µm in diameter. Even for a 100 µm PE sphere to reach a density of 1035 kg m^{-3}, a 10 µm thick diatom biofilm layer, consisting of three layers of 3 µm-sized cells, would have to cover the plastic sphere completely, as Amaral-Zettler and coauthors [53] calculated. They state that even for such a 100 µm- sized microplastic particle a biofilm that promotes sinking, "while theoretically possible, …is unlikely".

These existing modelling studies use a biofilm density of 1388 kg m^{-3} [48, 50] or 1170 kg m^{-3} [49] to predict sinking of microplastic, which we argue is an overestimate. The density of 1388 kg m^{-3} is derived from phytoplankton cell density measurements [52], and justified with the low density and relative average abundance of bacteria in comparison to diatoms observed on marine plastic debris between 1 and 10 mm [47]. The lower density of 1170 kg m^{-3} was based on measurements of diatom cultures used to form a microplastic biofilm [53]. Both density assumptions are based on the notion of a diatom-dominated biofilm coating marine plastic. We argue that these modeling calculations vastly overestimate biofilm density, in part because they ignore the contribution of the exopolymer matrix, which accounts for 50–90% of the organic carbon in a biofilm [54], and has a low density. A back of the envelope calculation illustrates that composite density would drop below seawater density, if a part of a diatom-based biofilm (1388 kg m^{-3}) is replaced with just more than 66% of TEP (840 kg m^{-3}), a proxy for the biofilm matrix. This approximation demonstrates that a biofilm solely based on diatoms might severely overestimate biofilm density in all cases, not only for microplastic particles too small to harbor many diatoms.

Interestingly, even with the assumption of a diatom-derived biofilm density, the majority of these modeled small microplastic particles do not reach the mesopelagic zone: Instead they reach either a maximum depth of 50 m [48], or only around 0.0001% of the modeled microplastics (10–100 µm) reach depths between 120 m and 2000 m depending on ocean basin [49]. This is in part because, besides the magnitude of excess density, the size of the particle, and related drag forces determine sinking velocity [32]. These model calculations thus do not support the idea that most small microplastic sink due to biofouling, but instead suggest that biofouling cannot explain the presence of microplastic in the deep ocean.

To illustrate the importance of the magnitude of excess density when addressing the question on how microplastics reach the deep ocean, we present another simple back of the envelope estimate. Assuming a diatom-based biofilm density of 1170 kg m^{-3} [49], we calculated the biofilm thickness needed to exceed seawater density (1025 kg m^{-3}) using equation #5 from Kooi and coauthors [48]:

$$\rho_{tot} = \frac{r_{pl}^3\rho_{pl} + [(r_{pl} + t_{bf})^3 - r_{pl}^3]\rho_{bf}}{(r_{pl} + t_{bf})^3} \tag{5}$$

with ρ_{tot} as the composite density of the microplastic particle with biofilm, r_{pl} the radius of the microplastic particle, ρ_{pl} the plastic density, t_{bf} the biofilm thickness, and ρ_{bf} the biofilm density.

Solving this equation for the biofilm tickness, a PE sphere with a diameter of 1 µm would need only a 0.10 µm thick biofilm to exceed seawater density. However, the sinking velocity (V_s) of a 1 µm PE sphere with a ten times thicker biofilm of 1 µm reaches only 0.047 m d^{-1}, calculated using equations #2 - #5 from the same study [48]:

$$V_s = -\left(\frac{\rho_{tot} - \rho_{sw}}{\rho_{sw}} g\, \omega_* v_{sw}\right)^{1/3} \tag{2}$$

$$\omega_* = 1.74 \times 10^{-4} D_*^2 \tag{3}$$

$$D_* = \frac{(\rho_{tot} - \rho_{sw})\ g\ D_n^3}{\rho_{sw} v_{sw}^2} \tag{4}$$

With g as the gravitational acceleration, ω_* the dimensionless settling velocity, v_{sw} the kinematic viscosity of seawater, D_* the dimensionless particle diameter, and D_n the equivalent spherical diameter (equation #5 and other parameter: see above).

It would take a biofouled particle with a sinking velocity of 0.047 m d^{-1} over 233 years to reach a depth of 4000 m. Since microplastic pollution is a recent problem, this is clearly an unreasonable result, although we presumed a dense and thick biofilm. If we assume an average sinking velocity of 10 m d^{-1}, equal to a sedimentation time of just over a year to reach the seafloor at 4000 m, our 1 µm PE sphere would need a 21 µm thick biofilm, whereas a 10 µm PE sphere would still need a biofilm 17 µm thick to reach that velocity. Whereas marine biofilms may reach a thickness of 30 µm on large surfaces, a thickness of <15 µm appears more common even on large surfaces, as biofilm thickness seems to be limited by different factors [55–57]. Obviously, these back-of-the-envelope calculations do not realistically simulate sinking of particles in the ocean. Instead they are meant to demonstrate that a small microplastic particle would need an unrealistic biofilm several times thicker than the size of the microplastic, if it were to reach the seafloor by biofouling, even assuming a relatively heavy biofilm community.

Conclusion

We argue that assuming a realistic biofilm composition and thickness for small microplastic particles, biofouling will not lead to gravitational settling substantial enough to explain the presence of these small particles in the deep ocean (>1000 m) or in deep-sea sediments. This is in contrast to large microplastic particles and plastic detritus, for which biofouling might be an important vertical transport pathway, especially in coastal waters [58]. While the biofilm of small microplastics cannot promote the vertical transport due to excess density, it can promote other possible transport paths, such as incorporation into marine snow and food chains. Biofilms are sticky and promote aggregation. In experiments, aggregation and adhesion to the experimental devices of biofouled microplastic particles has been observed [26, 27]. Michels and co-authors [59] showed that biofilm-covered microplastic particles were incorporated into aggregates at a higher rate than clean microplastic particles. Biofilm also seems to make microplastics more attractive to zooplankton, and increased ingestion of biofouled microplastics has been shown for copepods [60, 61], rotifers [62], and jellyfish [63]. Once integrated within the food web, microplastic particles may be transported downward via feces, trophic transfer or via vertical migration [64].

Vertical transport ways of small microplastics are likely to be complex. Data from deep sea sediment traps in the Atlantic looked at inorganic and organic particle fluxes as well as microplastic flux (10 to 200 µm in size) [12]. These data suggest a relationship between specific low-density microplastic types and inorganic particles flux, but not a direct relationship between microplastic particles and particulate organic carbon (POC) flux. It seems that there is no direct and simple relationship between the sedimentation of marine particles and small microplastics. Likely, a differentiation between shapes and types of microplastic is required. Fibers, films, and fragments, for example, may all sink vastly differently. Additionally, plastics and microplastics that have been exposed to UV irradiation fragment easily and produce large amounts of small microplastics [65]. Therefore, microplastic particles can also form during the vertical transport and at depth due to fragmentation of plastic debris that did sink. We propose that it is time to be more discerning when talking about vertical transport pathways and causes for high concentrations of small microplastic in the deep sea.

Abbreviations

EPS	Extracellular polymeric substances
ESD	Equivalent spherical diameter
PE	Polyethylene
PP	Polypropylene
TEP	Transparent exopolymer particles

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I.B. conceived the idea, I.B. and U.P. wrote and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

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Not applicable.

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Competing interests
The authors declare no competing interests.

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