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Microplastic and soil protists: a call for research

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Abstract

Microplastic is an emerging contaminant of concern in soils globally, probably gradually increasing in soil due to slow degradation. Few studies on microplastic effects on soil biota are available, and no study in a microplastic contamination context has specifically addressed soil protists. Soil protists, a phylogenetically and functionally diverse group of eukaryotic, unicellular soil organisms, are major consumers of bacteria in soils and are potentially important vehicles for the delivery of microplastics into the soil food chain. Here we build a case for focusing research on soil protists by drawing on data from previous, older studies of phagocytosis in protist taxa, which have long made use of polystyrene latex beads (microspheres). Various soil-borne taxa, including ciliates, flagellates and amoebae take up microplastic beads in the size range of a few micrometers. This included filter feeders as well as amoebae which engulf their prey. Discrimination in microplastic particle uptake depended on species, physiological state as well as particle size. Based on the results of the studies we review here, there is now a need to study microplastic effects in a pollution ecology context: this means considering a broad range of particle types under realistic conditions in the soil, and exploring longer-term effects on soil protist communities and functions.

Keywords

protist; amoebae; flagellates; ciliates; plastic; soil; soil food web

Introduction

Microplastic, plastic particles generally defined as < 5 mm or <1 mm, have been studied relatively intensely in aquatic environments, and are recognized there as potentially deleterious for a range of biota (e.g. Cole et al. 2011). More recently, microplastics have also been considered for terrestrial ecosystems and the soil (Rillig 2012), where such particles may also affect several biota, biodiversity and ecosystem processes (Horton et al. 2017,

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Machado et al. 2018). While evidence is gradually accumulating that microplastics may indeed be quite widespread in soils (Zubris et al. 2005; Fuller and Gautam 2016; Zhang et al. 2018; Scheurer and Bigalke 2018), effects on soil biota are not well known. Earthworms are likely affected (e.g., Huerta Lwanga et al. 2016), and collembola (Maaß et al. 2017) and earthworms (Rillig et al. 2017) have been shown to move the particles. However, not much is currently known for other soil biota groups, and this includes the ecologically important group of soil protists.

Protists are a phylogenetically and functionally diverse group of eukaryotic, unicellular soil organisms (Geisen et al. 2018). As major consumers of bacteria in soils (Trap et al. 2016), protists occupy an important position at the base of soil food webs (Geisen and Bonkowski 2017), and are potentially important vehicles for the delivery of microplastics into the soil food chain.

Studies using protists from aquatic environments strongly suggest that these organisms take up microplastic particles. For example, Christaki et al. (2003) showed that marine ciliates took up microplastic (including 1.0 µm size), and that bead clearance rates (a measure of uptake) differed by a factor of five as a function of species, culture phase and microplastic particle size.

Even though there are these data from marine ciliates, there is not yet a *bona fide* study on microplastic effects on soil protists in a pollution ecology context. However, it so happens that phagocytosis research has long made use of the study of latex bead uptake (Desjardins and Griffiths 2003). These data can now be used to learn about potential consequences of microplastic uptake by these organisms. Such polystyrene latex beads have been employed to study phagocytosis already several decades ago (Roberts and Quastel 1963, Mueller et al. 1965). We here review findings of these previous studies, and use these results to build a case for the study of microplastic pollution effects on protists in soils.

Microplastic uptake into soil protists

An important component of any potential, direct environmental effect of microplastic on soil protists is the demonstration of particles associating with cells and being taken up. Microplastic uptake might be particularly important for protist filter feeders, as found in many ciliates and some flagellate taxa (Fenchel 1986; Boenigk and Arndt 2002). For example, Fenchel (1980 a,b) studied uptake of latex beads (several sizes from 0.09 to 5.7 µm) in fourteen species of ciliates, mostly isolated from freshwater environments, but also including two species of soil inhabiting ciliates (*Colpoda* spp.). The two species extracted from soil (as well as all others) took up the latex microbeads and displayed different preferences for particle size; the author explained this by differences in the mouth structure. Ciliates had uptake rates comparable to metazoa and could concentrate beads out of a dilute suspension. Pace and Bailiff (1987) found strong evidence that *Cyclidium* sp., a taxon of small and common ciliates in soils and freshwater systems, could not discriminate between uptake of latex microspheres and bacteria.

For heterotrophic flagellates, the uptake of a range of latex beads (0.5, 0.75, 1.0, and 2.0 μm) was measured in a species from a lake environment, with a strong uptake preference for 1.0- μm sized beads (Hahn and Höfle 1999). Pace and Bailiff (1987) found that flagellates from aquatic environments appeared to discriminate against the latex beads, having lower uptake. Hekman et al. (1992) studied a mycophagous soil flagellate. Even though bacteria did not support its growth, the flagellate ingested bacteria-sized 1-2 μm latex beads, and also 5-7 μm but not 15-17 μm size beads (even though it did ingest and use for growth fungal spores larger than this). Boenigk et al. (2001) found a possible explanation for the discrepancies between studies. When Boenigk et al. (2001) offered beads and bacteria simultaneously, both were ingested by three interception-feeding flagellate species, but the chrysomonads *Spumella* and *Ochromonas* egested the beads within 2-3 minutes, while the bicosoecid *Cafeteria* stored bacteria and beads for half an hour in its food vacuoles. Vacuole passage was significantly longer for starved flagellates. Boenigk et al. (2001) concluded that "selective digestion" rather than ingestion is the main mechanism responsible for discrimination of bacteria by these flagellates, and may explain the uptake of inert particles such as polystyrene microbeads (Holen and Boraas, 1991). The discrimination of prey may be particularly important for small interception and raptorial feeders in flagellates, which consume bacteria one by one and often have comparably long handling times for prey (Boenigk and Arndt 2000, 2002). For example, Landry et al. (1992) measured a discrimination of 1:20 in the uptake of dead vs. living bacteria for the marine chrysomonad flagellate *Paraphysomonas*. Taken together, these studies show that the uptake and incorporation rates of microplastics are species specific and further depend on the nutritional status of the flagellates.

Amoeba can engulf whole bacterial colonies with their pseudopodia, and therefore may not be as specific in food uptake as flagellates. *Acanthamoeba* occur ubiquitously and in high numbers in soils (Geisen et al. 2014, Fiore-Donno et al. 2016). Despite feeding studies with antibiotic producing and non-toxic mutants of pseudomonads confirmed high prey discrimination compared to bacterivorous nematodes (Jousset et al. 2009), Weisman and Korn (1967) and Korn and Weisman (1967), using light microscopy and electron microscopy, respectively, showed that latex beads (polystyrene and polyvinyltoluene) were taken up by *Acanthamoeba*. They documented that larger latex beads (1.305, 1.90, and 2.68 μm) were taken up individually into the cell, whereas smaller beads (0.557, 0.264, 0.126, and 0.088 μm) were taken up in groups. Upon encystment, the amoebae expelled previously ingested latex beads by exocytosis, resulting in latex bead-free cysts (Stewart and Weisman 1972), suggesting discrimination of food after ingestion, as discussed for flagellates above. Avery et al. (1995) also studied uptake of latex beads by *Acanthamoeba castellanii*. Using confocal laser scanning microscopy (CLSM, and sodium azide as phagocytic inhibitor), the authors could clearly distinguish between surface attachment and internalization of 1.0- μm fluorescently labeled latex beads. Even though study conditions were tightly controlled and homogeneous, there was variability in uptake of beads; cell volume was an important parameter, such that larger trophozoites phagocytosed comparatively more latex beads than younger cells. Uptake also increased linearly with bead concentration, up to a point ($1.5 \times 10^8 \text{ mL}^{-1}$) beyond which uptake appeared to be saturated (even though surface-attachment continued to increase). Elloway et al. (2006) however showed by CLSM and flow cytometry

that individual *Acanthamoeba* trophozoites could contain ‘almost uncountable numbers’ of fluorescein isothiocyanate (FITC) labeled microspheres, suggesting individual variation within species.

We can take several points from studies such as this: (i) Even though protists are well known to discriminate between different types of bacteria (e.g., Bottone et al. 1994) or toxin and non-toxin producing bacterial mutants (Jousset et al. 2009), they do take up microplastic beads; (ii) uptake varies depending on cell size, degree of starvation and culture age, meaning that even under completely homogeneous conditions there will be population-level variability in uptake and any effects; and (iii) uptake and surface-attachment are highly dependent on microbead concentration, which likely means that low availability will translate into low uptake in the soil, and, conversely, that risk of uptake rises with microbead concentration.

Taken together, this clearly means that amoebae, ciliates and flagellates from soil can take up microplastic (latex) beads in the micrometer size range under the experimental settings used. Studies generally conclude that the trigger for uptake is of a mechanical nature, and that often particle uptake rates are so high that discrimination may play only a minor role.

Research needs

Studies of bead uptake were typically conducted under tightly controlled conditions, because the goal of such studies was not environmental relevance, but rather the mechanistic study of the process of phagocytosis. As a consequence of such study goals, researchers chose time scales typically in the range of minutes or a few hours (e.g. Avery et al. 1995), and the beads were considered inert, i.e. physiological effects on the protists were not typically examined (even though one study noted that at high particle concentrations, the beads tended to clog up the mouth part of ciliates; Fenchel 1980a). Given the commercial availability of latex microbeads (often fluorescently labeled), researchers used just this type of plastic (mostly polystyrene), and not a range of different plastic materials and shapes. Taken together, this means a number of critical research needs exist to further elucidate the interaction of microplastics with soil protists; no recent studies have addressed these research questions (Table 1).

Besides taking particles up by phagocytosis, microplastic has the potential to affect soil protists indirectly. In terms of biotic effects, this can occur by either affecting prey items (bacteria and fungi, as well as other protists), and by affecting consumers. Neither effects on soil bacteria or fungi, nor those on potential consumers of protists are currently known, making it difficult to speculate on the potential importance of such effects. Other indirect effects include any potential consequences of microplastic incorporation on soil structure and other physical properties of soil.

Conclusions

Utilizing the literature on phagocytosis, which occasionally used latex beads as inert markers, we conclude that soil protists (amoebae, ciliates and flagellates) are highly likely to take up microplastic particles in the range of a few micrometers and smaller. Larger

microplastics have not been experimentally tested, but it is unlikely that particles closer to the mm-range can be processed by the majority of soil protists. Given the lack of obvious discrimination against such artificial particles from such earlier studies, there is a need to study microplastic effects now from the perspective of pollution ecology: this means testing a broader range of particle types, using realistic conditions in the soil, and examining longer-term effects on soil protist communities and functions, including effects on biogeochemical cycles.

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Capsule

Based on past studies with latex beads for phagocytosis research, we build a case that soil protists take up microplastics; the topic is now ripe for study in pollution ecology.

Table 1
Research priorities for studies on microplastic effects on soil protists, ranked by importance.

Research priority (ranked)	Specific questions
1. Test for uptake and effects in a particle-rich environment (soil or soil-analog) rather than in liquid culture	Do protists take up microplastic particles also in the presence of soil surfaces? Are microplastic particles available for uptake?
2. Study physiological effects of microplastic uptake on protists	Can microplastic or its constituents (additives) be toxic for protists?
3. Examine different microplastic types (reflecting differences in surface, chemistry, charge, eco-corona, and shape)	If properties diverge from those of latex (polystyrene) beads, will protists discriminate against them? What are differences between beads (spherical shape) and microplastic fibers? Does the accumulation of environmental materials including organisms, i.e. the eco-corona of microplastic particles, affect uptake and effects?
4. Conduct studies on longer-term (i.e. a growth season) effects on protists and their communities	Can microplastic differentially affect populations of protists, with consequences for community composition (which can be assessed by high-throughput sequencing)? Are these effects mostly direct or indirect in nature?
5. Assess functional consequences of any microplastic effects	Does microplastic affect communities of protists to a point that there are changes in ecosystem processes (e.g. nutrient cycling)?