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Complete List of Authors:	Rillig, Matthias; Freie Universitat Berlin Institut fur Biologie Machado, Anderson Abel; Freie Universitat Berlin Institut fur Biologie Lehmann, Anika; Freie Universitat Berlin Institut fur Biologie Klümper, Uli; University of Exeter
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Environmental context

Microplastic particles are increasingly recognized as a human-caused pollutant in soil with potential consequences for soil microorganisms. Microplastic may also have evolutionary consequences for soil microbes, because these particles may alter conditions in the soil and hence selection pressures. Including this evolutionary perspective may lead to new questions and novel insights into responses of soil microbes to this anthropogenic stressor.

Evolutionary implications of microplastics ~~in soils~~for soil biota

Matthias C. Rillig [A, B*], Anderson Abel de Souza Machado [A, B], Anika Lehmann [A, B] , Uli Klümper [C, D]

[A] *Freie Universität Berlin, Institut für Biologie, Altensteinstr. 6, D-14195 Berlin, Germany*
[B] *Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), D-14195 Berlin, Germany*
[C] *ESI & CEC, Biosciences, University of Exeter, Penryn Campus, Cornwall, United Kingdom*
[D] *European Centre for Environment and Human Health, University of Exeter, Truro, United Kingdom*

*Corresponding author
Email: matthias.rillig@fu-berlin.de

Abstract

Microplastic pollution is increasingly considered as a factor of global change: in addition to aquatic ecosystems, this persistent contaminant is also found in terrestrial systems and soils. Microplastic has been chiefly examined in soils in terms of presence and potential effects on soil biota. Given the persistence and widespread distribution of microplastic, it is also important to consider potential evolutionary implications of microplastic presence in soil; we here offer such a perspective for soil microbiota. We discuss the range of selection pressures likely to act upon soil microbes, highlight approaches for the study of evolutionary responses to microplastic, and point out obstacles to overcome. Pondering evolutionary consequences of microplastic in soils can yield new insights into the effects of this group of pollutants, including establishing 'true' baselines in soil ecology, and understanding future responses of soil microbial populations and communities.

Keyword: Ecotoxicology *(if allowed, further keywords: microplastic, soil, microbiota, evolution, selection pressures)*

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Introduction

Microplastics are emerging as a factor of global change. These particles, generally defined as plastic < 5mm (or 1mm), have been found in a range of environments, including freshwater ecosystems (Li et al. 2018a), the oceans, arctic sea ice (Peeken et al. 2018), and also in terrestrial ecosystems and the soil (Rillig 2012; Horton et al. 2017; Machado et al. 2018a). Current studies in soils focus on documenting the extent of pollution (e.g., Scheurer & Bigalke 2018), with data from soil lagging far behind our knowledge about oceans, where research has started a decade earlier (Thompson et al. 2004). Research has also started to document potential effects of microplastic particles on individual soil biota, for example earthworms (Huerta-Lwanga et al. 2017). Such studies are primarily aimed at understanding potential ecological consequences of this novel group of contaminants.

However, given the widespread - and likely long-term - presence of microplastic in the environment, it is also important to start considering evolutionary consequences. These have so far not been discussed, except perhaps in the context of the discovery of plastic-degrading microbes (Yoshida et al. 2016).

Here we discuss various aspects of selection pressures likely to act upon soil microbes (Fig. 1); we introduce approaches for the study of evolutionary responses, and highlight general obstacles to overcome. We argue that introducing an evolutionary perspective would introduce highly relevant questions to the study of these persistent contaminants in soil.

Selection pressures

Microplastic particles may affect a range of soil properties, which would present soil biota with certain selection pressures (Fig. 1). This will lead to a shift in genotypes within populations, either by selection among already existing lines, or among lines based on *de novo* mutations; that is evolution. The question therefore becomes: how might microplastics affect the environment in soil, and which organismal traits would become important as targets of selection?

The most obvious factor would be microplastic as a novel resource, i.e. a source of nutrients and carbon. In fact, microplastic may be a significant anthropogenic component of soil organic carbon already (Rillig 2018). Plastics are often made to be inert and they typically decompose very slowly; for all intents and purposes of the human time horizon they may be regarded as persistent. However, microbiota (bacteria and fungi) genotypes with an ability to utilize the carbon or other elements contained in microplastic may have a selective advantage, and such genotypes would be expected to increase in relative abundance within the population. The same is true for any other additives chemically or physically bound to the plastic polymer (e.g. plasticizers), which may be contained in microplastic particles, even though such effects may be relatively more short-lived.

Furthermore, microplastics display an elevated ability to absorb chemical substances, such as antibiotics, heavy metals and other xenobiotics (Brennecke et al. 2016; Hirai et al. 2011; Li et al. 2018b). For example, polyamides display a particularly high adsorption capacity for antibiotics containing a carbonyl group like tetracycline or ciprofloxacin, since strong hydrogen bonds between this carbonyl group and the microplastics amide group as a proton donor can be established (Li et al. 2018b). However, the sorption ability differs greatly between diverse plastic materials, sorbed substances and environmental conditions (Li et al. 2018b).

Still, through, ~~for example,~~ increased antibiotic or heavy metal concentrations, microplastics and their surroundings can constitute microniches in the soil environment with highly selective conditions. In combination with potentially providing a ~~potentially elevated~~ novel nutrient ~~availability~~ source ~~they~~ microplastics can consequently serve as so called "hot-spots" of horizontal gene transfer (HGT) and microbial evolution. While in water environments the additional surface introduced through microplastics is the major factor in enhancing plasmid transfer, plastic particles still favored microbial interactions to a larger extent than natural aggregates (Arias-Andres et al. 2018). Moreover, the presence of microplastics can positively alter the retention time of other introduced stressors in the soil environment and thus lead to longer lasting periods of exposure and subsequent evolution to these conditions (Sun et al. 2018).

Microplastics also have the potential to change the soil physical environment. The soil physical environment is governed by soil aggregation, a process to which many soil biota contribute (Lehmann et al. 2017). Soil aggregates are relatively stable entities whose interiors contain microhabitats with often drastically different conditions to those on aggregate surfaces. Such temporarily stable structures have recently been conceptualized as massively concurrent evolutionary incubators for microbes (Rillig et al. 2017a), meaning that evolutionary processes and trajectories within aggregates are different compared to those in a non-structured soil. Following this concept, any changes in soil aggregation, that is processes affecting rates of formation, stabilization or disintegration of aggregates, could also be expected to have consequences for microbial evolution. Microplastic, probably especially linear fibers, could have effects on these processes. A change in soil aggregation and, corresponding to these, pore distributions, could have multiple evolutionary consequences within communities that are currently difficult to predict in terms of traits and directions. In fact, changes in soil structure and pore spaces may even lead to local extinction because of microhabitat loss (Veresoglou et al. 2015). Recently, effects of microfibers on soil aggregation were demonstrated experimentally (Machado et al. 2018b), together with accompanying changes in bulk density and water holding capacity.

Many soil microbes interact strongly with hosts, including soil animals. Soil animals, in turn, may also interact with microplastics: earthworms have been shown to ingest polystyrene beads (Rillig et al. 2017b; Huerta Lwanga et al. 2016, 2017), and some studies have shown deleterious effects on earthworms (Huerta Lwanga et al. 2016). From earthworm guts, microbes specialized in degrading microplastic compounds have been isolated (Huerta Lwanga et al. 2018), which could be part of a newly evolved complex host-symbiont interaction in response to microplastic pollution in soils. Similarly, other soil animals may also consume these particles (e.g. Collembola; Zhu et al. 2018), with alteration in their associated microbiota. As such, we expect cascading effects of microplastic on microbiota evolution via effects on hosts.

When microplastics break down further to even smaller particles, such particles may enter the nanosize range (< 0.1 micrometer). Such nanoplastic particles may have very different properties, for example they may be able to traverse biological membranes and thus acquire toxic properties (Machado et al. 2018). Genotypes better resisting such effects would be expected to increase in abundance. These changes in community structure can further alter the complex interplay of microbial processes in the soil environment. For example, in an anaerobic digestion system the exposure to polystyrene nanoparticles caused an inhibition in community wide productivity linked with significant changes in microbial community structure (Fu et al. 2018), likely also observable in soil microbial communities.

Approaches for the study of evolutionary responses to microplastic

Several approaches are available for the study of evolutionary responses of soil biota to microplastic: experimental evolution in the lab, resurrection ecology, and observational studies using gradients.

Experimental evolution studies have a long tradition in microbial biology (e.g. Lenski et al. 1991; Buckling et al. 2000). Such studies use serial transfers in the laboratory to study effects of a certain evolutionary driver. One could test using such systems if traits predicted to be favored by the presence of microplastic increase in abundance through time. In addition, monitoring abundance of certain genes may be promising. Through its horizontal mobility across bacterial species and linkage to genes conferring diverse resistance phenotypes the relative abundance of the class 1 integron-integrase gene *intI1* is widely considered as a proxy to measure the level of and the selective pressure associated with anthropogenic pollution (Gillings et al. 2014). In environmental studies it might pose extremely difficult to disentangle the influence of microplastics on *intI1* abundance from that of other potentially stronger selective agents such as antibiotic or heavy metal residues or human associated microbial pollution (Amos et al. 2015). However, in controlled experiments microplastics have already shown to increase the persistence of *intI1* from treated wastewater when entering a freshwater microbial community (Eckert et al. 2018). Consequently, *intI1* could provide a promising target to quantitatively measure the selective pressures imposed on soil microbial communities through the addition of microplastic particles in experimental evolution experiments.

Another promising approach may be resurrection ecology (Franks et al. 2018). This is an approach where extant populations are compared with historical populations, which can be reanimated ('resurrected') from historical samples. In our case, this would entail the use of soil archives, for example from agricultural experiment stations, that include samples collected prior to the widespread use of plastics. Populations extracted from such historical samples could be compared to extant populations from the same soil, with the caveat that other factors influencing the evolution of the target organisms may have changed concurrently.

Observational studies along established gradients of contamination, which share this basic limitation with resurrection studies, can also be used to learn about evolutionary responses of populations to the presence of microplastic. Here, correlations can be used to test for the link between predicted favored traits and their relative abundance in populations along a microplastic contamination gradient.

<u>Obstacles to overcome</u>

The single most challenging aspect of studying microplastic is likely its diversity: microplastic comes in a bewildering range and combination of chemical forms, sizes, surface properties, shapes and modifications (e.g. additives). Therefore, this is very much not like studying specific contaminants, but this work encompasses a whole group of substances, additives and sizes with likely very different effects. For example, effects of beads, films and fibers on soil and soil microbes might be quite different. This imposes significant challenges on the external validity of any study, since by necessity these will be limited to few plastic types for logistical reasons.

For the understanding of evolutionary dynamics of microplastic pollution in soil, it is important to realize that this is a gradually changing factor: microplastic arrives via various processes at the soil surface, and it then accumulates gradually in the soil, because of limited rates of decomposition. This means that, in any given soil, soil biota are not abruptly exposed to high concentrations of microplastic particles, which tends to be the current practice in experimental approaches aimed at elucidating ecological or physiological effects. Thus, it may also be useful to gradually expose soils and their biota to microplastic in experiments; evolutionary dynamics in response to gradual vs. abrupt changes in the environment are expected to differ significantly.

We here focus on soil microbes, because they are eminently tractable experimentally. However, soil biota are enigmatically diverse and contain entire food webs. It is thus risky to focus on only particular groups of biota, since microplastic may modify trophic interactions, thus exerting differential top-down effects. Such effects would potentially be extremely important to gauge evolutionary responses; however, it is a real challenge to capture the entirety of soil biodiversity.

Finally, technical challenges remain, chiefly in respect to adequately quantifying types and amounts of microplastics in the soil matrix. These are certainly not unique to studies with an evolutionary focus, but will also limit such studies, for example as far as observational studies are concerned, and in terms of establishing true baseline levels of contamination in experiments.

Concluding remarks

Pondering evolutionary consequences of microplastic in soils can lead to new questions (Table 1) and yield new insights into the effects of this group of pollutants. On the one hand, by studying selection pressures experienced by a range of soil biota we learn about the ways soil biota may adapt in future soils. Importantly, this can also include interactions with other factors of global change. On the other hand, when we now measure soil biota traits or process rates, we may actually already be unknowingly capturing such responses: this therefore becomes an issue of understanding ‘true’ baselines in soil biology.

Much of what we discuss here may also be applicable to aquatic systems; however, there the provision of a surface will likely be a dominant factor (Arias-Andres et al. 2018), with the possibility of novel interactions in the particle eco-corona, including plasmid exchange.

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Conflicts of interest

The authors declare no conflicts of interest.

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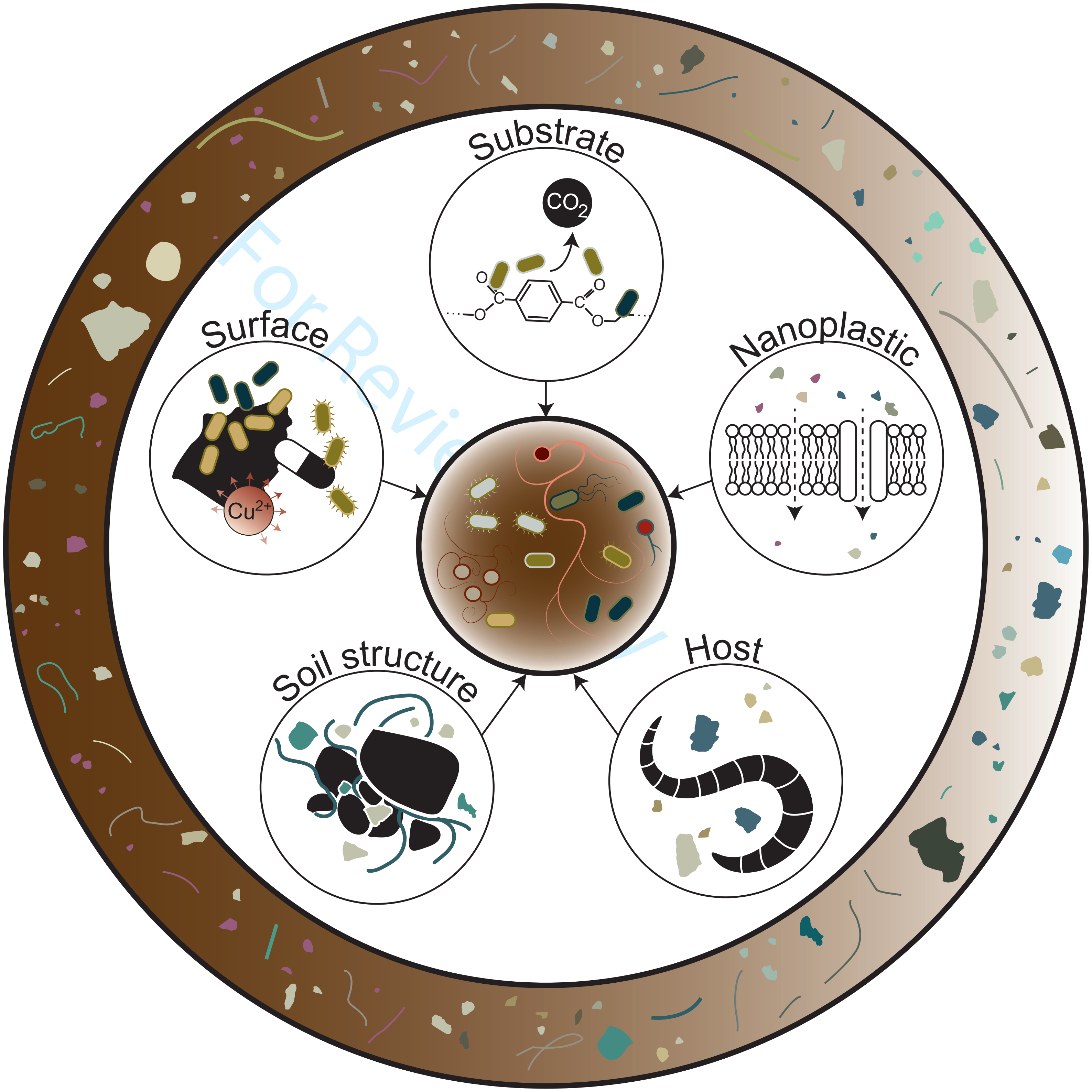
Table 1 Examples of questions on evolutionary consequences of microplastic contamination in soils.

Question	Explanation/ background
Has the presence of microplastic in soil already affected evolutionary trajectories of soil microbiota? For example, has microplastic created new niches for soil microbes?	Persistence of microplastic in soil, and the finding that microplastic appears to be ubiquitous in soil samples even from relatively non-human influenced ecosystems (Scheurer & Bigalke 2018)
Can evolutionary changes to microplastic within populations buffer against or exacerbate changes in microbial community composition? How do these changes interact with phenotypic plasticity?	Eco-evolutionary dynamics
Does microplastic lead to local extinctions of microbial populations?	Changes in soil physical structure (as a consequence of possible effects on soil aggregation) can lead to local exclusion of biota, for example soil animals, which may host specific microbes (Veresoglou et al. 2015; Zhu et al. 2018)
How does microplastic (and microplastic type) interact with other evolutionary drivers affecting soil microbial populations?	Global change is inherently a multifactorial phenomenon; also within cities or on agricultural fields there are multiple evolutionary drivers that co-occur with microplastic contamination

Figure legends:

Figure 1. Drivers of potential evolutionary effects of microplastics on soil microbes. The outer ring depicts microplastic particles of various properties (including size, shape, chemistry). Microbial communities (in the center) experience various effects triggered by microplastic particles. Typical impacts with evolutionary consequences include potential changes in soil structure, alteration of host availability or function (host microbiome), nanoplastic toxic effects, plastic particles representing a resource, and providing novel surfaces (with various chemicals attached, including heavy metals and antibiotics).


Substrate
CO_2
Surface
Cu^{2+}
Nanoplastic
Soil structure
Host