Ecotoxicological effects of microplastics in soil

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Letter to the Editor

Ecotoxicological effects of microplastics in soil: Comments on the paper by Zhu et al. (2018) 'Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition.' Soil Biology & Biochemistry 116, 302-310☆

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The increased use and emission of plastics and their degradation in the environment has led to an increased environmental load in soils of so-called microplastics, plastic particles with sizes smaller than 5 mm (Duis and Coors, 2016; Rillig, 2012; Rillig et al., 2017). In the last decade, awareness is increasing that these microplastics may pose a risk to human and environmental health. The extent of such risk, however, still remains unclear due to a lack of studies on the effects of microplastics (e.g., Rillig et al., 2017). As a result, research has been initiated to assess the emission, fate and effects of microplastics in the environment.

The recent study by Zhu et al. (2018), in this journal, presented data on the effects of PVC microplastics on the springtail *Folsomia candida* and its microbiome after 28 and 56 days of exposure in a natural soil. Although this study adds novel data on the microbiome of the gut of springtails in comparison to that of soil, we wish to raise some issues in relation to its content.

1. The study used only one exposure level, which makes it very hard to conclude that the effects observed really can be related to microplastic exposure. Only by performing a full dose-response study it is possible to unequivocally determine whether a chemical does affect organisms or processes and at what dose or concentration level such effects occur. This rule was not applied by Zhu et al. (2018).
2. Variation of the reproduction of *F. candida* can be considerable, triggering Van der Hoeven (1998) to conclude that for an unequivocal assessment of so-called No Observed Effect Concentrations in ecotoxicity tests a high number of replicates would be needed in treatments and even more in the controls. When comparing treatments in an experimental design with only two treatments (here the control and the microplastic treatment), three replicates therefore is not sufficient to confirm the differences between the treatments. The effects reported by Zhu et al. (2018) therefore cannot unequivocally be attributed to the microplastic treatment.
3. It also is essential to confirm the validity of the test. For that reason, standardized toxicity test guidelines, like the OECD guideline 232 on springtail reproduction toxicity testing (OECD, 2009), do require reporting control performance in terms of survival, number of juveniles produced and variation of juvenile reproduction in the replicate control jars. Zhu et al. (2018) do report high control survival but they do not confirm validity of their tests regarding springtail reproduction in the controls.
4. Zhu et al. (2018) reported a significant reduction in growth and reproduction of the springtails at the microplastic concentration of 1 g/kg dry soil. Where it concerns the first, this statement is not in line with Fig. 6A, which shows an increased biomass of the springtails in the microplastic treatment. This contradiction impairs the credibility of the results on decreased growth. The caption of Fig. 6 does not agree with the contents of the figure; it seems figure panels A and B have been mixed up. In any case, given that the biomass was truly affected, effects on springtail biomass and reproduction in the microplastic treatment were only 16.8% and 28.8%, respectively compared to the non-treated control. Although such a difference indeed may be statistically significant, it seems unlikely that it is significant from a biological point of view. First, springtail tests tend to show fairly high biological variation, for which reason the OECD guideline 232 (OECD, 2009) recommends the use of at least four replicate jars per treatment and control (see also our second concern). Second, whether an effect is significant from an ecotoxicological point of view, meaning that it can be attributed to the tested chemical (in this case: microplastics), can only be concluded once it would be confirmed by a dose-related increasing trend (see our first concern).
5. In case of growth, it has to be noted the effect reported (16.8% reduction) was based on a difference in springtail body weights after 56 days. Considering the life cycle of *Folsomia candida* (Fountain and Hopkin, 2005), it is expected that the 7–9 day old animals introduced at the start of the test would have reached adulthood and produced their first clutch of eggs after approx. 14 days. Considering

☆ This letter is to be interpreted as the opinions of the authors, and is not necessarily endorsed by any member of the editorial board of the journal Soil Biology & Biochemistry. It is published in the interests of open discussion of such matters.
Karl Ritz, Editor-in-Chief.

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an egg incubation time of 7–10 days, this means juveniles from these first clutch of eggs emerged after 21–24 days. Since *F. candida* shows asymptotic growth (see e.g., Crommentuijn et al., 1993), this would mean that these juveniles after 56 days, so approx. 32–35 days after hatching, would already have reached a size similar to the animals introduced at the start of the test. It remains unclear how the authors were able to distinguish the animals introduced at the start of the test from the first generation juveniles. If they were able to do so, we have doubts as to the health of the springtails. Since the animals were not fed (Zhu et al., 2018), a lack of food could also have been the reason for a very low growth. But in that case, the lack of food should also have had a strong impact on reproduction. Since information on the number of juveniles produced is lacking from the paper, no conclusions can be draw on this.

6. Zhu et al. (2018) did mention a method for determining microplastic content of the springtails but they had to conclude the springtails did not take up any microplastics. They did however, not confirm the presence and (homogeneity of the) distribution of the microplastics in soil. It therefore is not even certain whether the springtails were exposed to the microplastics, while it also is possible they managed to avoid exposure.
7. In their discussion, Zhu et al. (2018) strongly suggest that the microplastic treatment may have affected the springtails indirectly by changing the physicochemical properties of the soil. Such claim, however, is not supported by any data.
8. It has been shown in aquatic environments – both in water and in sediment – that microbes can colonize the surfaces of plastic particles and that microbial communities growing on microplastics can be very different from those in the surrounding environments (Rummel et al., 2017). This phenomenon is likely to affect the composition of food and eventually also the composition of the microbial community in the gut of microbial feeding animals, such as springtails. The results presented by Zhu et al. (2018) suggest a difference in the microbial community composition in soil and springtails. Since it is known that *Folsomia candida* prefers to feed on yeasts and fungi, such a difference is not surprising. Actually, the authors discuss that some microbial families were "enhanced by passing soil through collembolan gut", although no direct evidence was shown that collembolans would have ingested soil particles instead of feeding on microbial biomass. What is more surprising is that the community composition of the control springtail guts seems to resemble that of the soil spiked with microplastics, while that of the microplastic exposed springtails seems to resemble more the community in the control soil (Fig. 1). This aspect would deserve a more in-depth discussion and probably needs further investigation.

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