Soil Biology and Biochemistry 116 (2018) 302–310

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition

Dong Zhu[a,b], Qing-Lin Chen[a,b], Xin-Li An[a,b], Xiao-Ru Yang[a], Peter Christie[c], Xin Ke[d], Long-Hua Wu[c], Yong-Guan Zhu[a,e,*]

[a] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen 361021, China
[b] University of the Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, China
[c] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[d] Institute of Plant Physiology and Ecology, Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China
[e] State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

ARTICLE INFO

Keywords:
Core microbiota
Gut bacteria
Plastics
Microarthropods
Isotopic fractionation

ABSTRACT

Effects of microplastics on aquatic organisms have been widely studied in recent years but effects on soil biota, and especially on the gut microbiota of soil animals, remain poorly understood. An experiment was therefore conducted using the common soil collembolan *Folsomia candida* exposed to microplastics for 56 days to investigate the effects of plastics on gut microbiota, growth, reproduction and isotopic turnover of collembolans in the soil ecosystem. A diverse microbial community was observed in the collembolan gut, consisting of (at phylum level) Actinobacteria (~44%), Bacteroidetes (~30%), Proteobacteria (~12%) and Firmicutes (~11%). Distinctly different bacterial communities and lower microbial diversity were found in the collembolan gut compared with the surrounding soil. We also found that exposure to microplastics significantly enhanced bacterial diversity and altered the microbiota in the collembolan gut. Moreover, collembolan growth and reproduction were significantly inhibited (by 16.8 and 28.8%, respectively) and higher $\delta^{15}N$ and $\delta^{13}C$ values were observed in the tissues after exposure to microplastics. These results indicate that exposure to microplastics may impact non-target species via changes in their microbiota leading to alteration of isotopic and elemental incorporation, growth and reproduction. The collembolan gut microbial data acquired fill a gap in our knowledge of the ecotoxicity of microplastics.

1. Introduction

Polymers are widely used in our daily life (Thompson et al., 2009; Wright and Kelly, 2017) and > 280 million tonnes of plastics are consumed annually (Duis and Coors, 2016). Plastic wastes have become major urban wastes (Zhao et al., 2015) because their degradation is very slow (Al-Salem et al., 2009; Wang et al., 2015). Polymers enter the environment and can then disintegrate with concomitant formation of small plastic particles (Rillig, 2012; Huerta Lwanga et al., 2016b). When the particle size is < 5 mm the material is defined as microplastics (Wright et al., 2013). Microplastic particles are accumulating in the seas and on land due to their durability and this has become a global problem of growing concern (Wright and Kelly, 2017). The effects of microplastics on marine organisms have been intensively studied (Lenz et al., 2016). Numerous studies so far have demonstrated that microplastics can harm aquatic organisms physically and also increase the accumulation of chemical pollutants in the tissues of organisms and disturb their metabolism (Auta et al., 2017; Sussarellu et al., 2017). However, the consequences of microplastics for soil organisms remain largely unknown (Maaβ et al., 2017). So far, only a few studies have investigated the effects of microplastics in soils and terrestrial systems (Huerta Lwanga et al., 2016a,b; Rodriguez-Seijo et al., 2017; Stamiatiadis and Dindal, 1983).

Microplastics can enter the soil environment by a variety of ways including the application of sewage sludge or the residues of plastic mulching films and evidence for the accumulation of microplasticss in soils is increasing (Huerta Lwanga et al., 2016b; Karen and Anja, 2016; Mahon et al., 2017). For instance, approximately 700 plastic particles per kg soil were found in European agricultural land (Barnes et al., 2009; Briassoulis et al., 2010). In north China a large number of agricultural sites are covered with plastic film to retain soil moisture and most of this material is discarded in soils in an unregulated manner (Huerta Lwanga et al., 2016a). It has been reported that soils in many (sub)tropical countries contain large amounts of plastic waste (Huerta

* Corresponding author. Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen 361021, China.
E-mail address: ygzhu@iue.ac.cn (Y.-G. Zhu).

http://dx.doi.org/10.1016/j.soilbio.2017.10.027
Received 16 August 2017; Received in revised form 21 October 2017; Accepted 25 October 2017

Lwanga et al., 2016b). There is therefore an urgent need to evaluate the environmental risk of microplastics in soil ecosystems for the rational management of plastic wastes. Huerta Lwanga et al. (2016a) studied the exposure of earthworms to litter spiked with microplastics and observed that the plastic particles significantly lowered their survival and growth rates. Associated histopathological damage and atrophy or detachment of the gut epithelium have been confirmed in earthworms (Rodriguez-Seijo et al., 2017). Several recent studies have also shown that earthworms and collembolans in soils can transport microplastics through their activities (Huerta Lwanga et al., 2016a; Maaβ et al., 2017; Rillig et al., 2017). However, current knowledge of the effects of microplastics on the soil fauna remains inadequate for future ecological risk assessments of microplastics in soils.

Soil collembolans, or springtails, are micro-arthropods comprising a key group of soil mesofauna (Zhu et al., 2016; D'Annibale et al., 2017). They are widespread globally and can occur in high abundance in surface soils (e.g. 1000-100, 000 individuals m^{-2}) (Potapov et al., 2016). They play crucial functions by feeding on soil detritus, litter and microbiota to promote organic decomposition and nutrient cycling in soil ecosystems (Potapov et al., 2016). Furthermore, they are direct ecological vectors for soil pollutants (Chelinho et al., 2017). *Folsomia candida* is a model collembolan that has been frequently used in soil eco-toxicological and genomic studies (Agamennone et al., 2015; Princz et al., 2017). Rillig (2012) has suggested that collembolans may ingest microplastics and one recent study indicates that collembolans can transport microplastics (Maaβ et al., 2017). However, the effects of microplastics on collembolans remain largely unknown and this greatly restricts our understanding of the ecotoxicity of microplastics in soils.

Gut microbiota make important contributions to host health, metabolism and immunity (Agamennone et al., 2015; Berg et al., 2016). For example, gut microbiota play a key role in the absorption of nutrients by many arthropods (Engel et al., 2012). In addition, pollutants may alter the composition of animal gut microbiota but the pattern of shift in gut microbiota is different in different species (Brule et al., 2015; Pass et al., 2015; Raymann et al., 2017). Thus, more studies on responses of gut microbiota to environmental pollutants are needed. Histopathological damage in the earthworm gut were observed in an exposure study of soils spiked with microplastics (Rodriguez-Seijo et al., 2017). Previous studies on aquatic organisms also reveal that microplastics may scratch gut tissues and be retained in the gut (Grigorakis et al., 2017; Vendel et al., 2017). However, the effects of microplastic exposure on the gut microbiota of collembolans have never been investigated. Moreover, information about the composition of collembolan gut microbiota is also lacking. Studying the gut microbial community of *F. candida* will contribute to the identification of the core microbial community in the gut of collembolans and an understanding of the effects of the microbiota on animal health.

The nitrogen and carbon isotope ($\delta^{15}N$ and $\delta^{13}C$) composition of animal tissues are useful indicators of the trophic positions and feeding habits of animals (Ek et al., 2016; Zhu et al., 2016). Recently there has been increasing evidence that environmental pollutants may alter stable isotope contents of animal tissues by interfering with growth rate and metabolic turnover (Ek et al., 2015, 2016; Zhu et al., 2016) but different pollutants have different effects in this regard. The $\delta^{15}N$ and $\delta^{13}C$ values of daphnids exposed to lindane were significantly elevated compared to controls (Ek et al., 2015). In line with this, using collembolans fed cadmium-contaminated yeast, Zhu et al. (2016) found significantly higher $\delta^{15}N$ values in collembolans fed Cd-contaminated yeast than in controls, suggesting a slower rate of nitrogen turnover under the influence of Cd. Against that, Banas et al. (2009) observed that exposure to DDT did not change the $\delta^{15}N$ value of fish, and the $\delta^{15}N$ value of exposed snails generally decreased (Ek et al., 2016). In addition, the characteristics of microplastics are markedly different from those of other chemical pollutants (Costa et al., 2016). This suggests that further studies are needed to explore the effects of microplastics on the stable isotope values of organisms.

The aims of the present study were to identify the core microbial community of the collembolan gut, to compare the microbial communities in soil and in the collembolan gut, to evaluate the effect of microplastic exposure on the microbial community of the collembolan gut using 16S RNA gene high-throughput sequencing, and to explore the changes in the stable isotope composition of exposed collembolan tissues, with concomitant growth inhibition and metabolic imbalance. These results will contribute to enhancing our understanding of the ecological risk from microplastics in soil ecosystems and the inherent relationships between soil and collembolan gut microbial communities.

2. Materials and methods

2.1. Exposure treatment

The parthenogenetic collembolan *Folsomia candida* used in the present study was originally sourced from Aarhus University in Denmark and has been cultured for more than six months in our laboratory. Referring to the standardized methods of the Organization for Economic Co-operation and Development (Zhu et al., 2016), we arranged a suitable breeding environment for *F. candida* in Petri dishes with a layer of moist plaster of Paris/activated charcoal mixture (ratio 8:1 w/w) and obtained age-synchronized *F. candida* individuals. Before microplastic exposure, 7-9-day-old collembolan juveniles were transferred into the test soil (clay loam, WHC 46.8%, pH($CaCl_2$) 4.76, CEC 13.86 cmol kg^{-1}, OM content 24.6 g kg^{-1}, total N content 3.8 g kg^{-1}) from Ningbo, east China, which was not contaminated with microplastics, and pre-incubated for one week at 20 ± 2 °C and 75% relative humidity (RH) with a 16:8 h dark/light photoperiod (800 lux) to acclimate the animals to the new cultivation environment. Throughout the pre-incubation process the mortality of the animals was < 1% and we therefore used these collembolans to start the microplastic exposure experiment. Field conditions were mimicked by withholding food during pre-incubation and exposure experiments. Soil moisture content was maintained by adding distilled water twice weekly.

Commercial polyvinyl chloride (PVC) particles (K-value 72-71) purchased from Aladdin Industrial Corporation (Shanghai, China) were selected as model microplastics to conduct exposure experiments because PVC is one of the commonest polymers in use worldwide. Most of the test PVC particles were between 80 and 250 μm in diameter (Fig. S1) and their viscosity number was 135-127 mL g^{-1}. Uncontaminated PVC particles were obtained by removing any surface absorbed solvent-soluble plastic monomers and other allogenic materials using octane and pentane. The cleaned PVC particles were dried at 50 °C and stored at 4 °C before use.

Two experiments were conducted to test the effect of MP exposure on (1) growth, reproduction and isotope composition and (2) the gut microbiota of the collembolans. In experiment 1, eight 14-16-day-old pre-incubated collembolans were transferred into glass cylinders (inner diameter 5.3 cm, 6.5 cm high) containing 30 g moist soil. The experimental treatments consisted of a control (0 g microplastics kg^{-1} dry soil) and microplastic exposure (1 g microplastics kg^{-1} dry soil). Each exposure treatment was separately repeated five times and the whole exposure test lasted for 56 days. On days 0, 28 and 56, collembolan samples were collected to determine body weight (to reflect body size), elemental composition and isotope values. Moreover, reproduction was counted after 28 days of exposure. In experiment 2, 60 acclimated 14-16-day-old collembolans were exposed to 0 g kg^{-1} (control) or 1 g microplastics kg^{-1} with three replicates. The exposure experiment was conducted in the same cylinders each containing 65 g moist soil. After 56 days of exposure all collembolans were collected for analysis of gut microbiota. The microplastic exposure concentration was chosen on the basis of concentrations found in contaminated soils and the results of studies on the effects of microplastics on earthworms (Huerta Lwanga et al., 2016b).