Effect of Microplastics on Gut Bacterial Community of the Earthworm *Pheretima guillelmi*

Qiongqiong Shang [a], Mingxia Tan and Jie Chi
School of Environmental Science and Engineering, Tianjin University, Tianjin, 300350, PR, China

Keywords: Earthworm, Microplastics, Intestinal.

Abstract: Earthworm is an important part of the soil ecosystem. Study on the responses of earthworm gut bacteria to microplastics (MPs) is still lacking. In this work, the effects on non-biodegradable polyethylene (PE) and biodegradable poly (butylene adipate-co-terephthalate) (PBAT) MPs on bacterial community of earthworm gut were investigated. The results showed that the number of operational Taxonomic Units (OTUs) and α diversity indexes in treatments with MPs were higher than those in treatment without MPs. The number of OTUs was in the order of treatments with aged-PE MPs > treatments with unaged-PE MPs > treatments with PBAT MPs. The number of OTUs in treatments with lower MPs concentration was higher than that in treatments with higher MPs concentration. Addition of MPs increased the relative abundances of genera *Ensifer*, *Bacteroides* and *Bacillus*, but decreased the relative abundances of genera *Salmonella*, *Escherichia-Shigella* and *Paracoccus*. Therefore, MPs significantly impact the microbial community of earthworm gut, which is related to types of MPs.

1 INTRODUCTION

Soil animals are widely distributed all over the world and play a key role in soil health and biodiversity (BARDGETT 2014). Among them, earthworms are the largest invertebrates in the soil, which can enhance soil structure and fertility (BERTRAND 2015). Most of the ecological functions of earthworms are connected to their internal microbial communities, which are very sensitive to external environmental interference (ZHANG 2022). However, our research on the intestinal microflora of soil animals has just begun, and the understanding of the composition diversity and ecological functions of the earthworms gut bacteria is still lacking, and further research is required.

Microplastics (MPs) are widely present in the soil environment, and absorb organic pollutants and metal pollutants in the soil, even have a certain impact on animals and microorganisms in soils. At the same time, exposure to MPs disturbs the growth of earthworms (HUERTA 2016), thereby affecting their intestinal microbial community. However, the concentration and properties of MPs are different, and the effects on earthworms are also distinct.

Therefore, in this research, two types of MPs, non-biodegradable polyethylene (PE) and biodegradable poly (butylene adipate-co-terephthalate) (PBAT), were selected and earthworms *(Pheretima guillelmi*) were the target species to study the effects of different types and different amounts of MPs particles on the structure of the earthworm gut bacterial community.

2 MATERIALS AND METHODS

2.1 Preparation and Characterization of Materials

Polyethylene (PE) and poly (butylene adipate-co-terephthalate) (PBAT)with particle size ranges of 104-178 μm are purchased from Dongguan Plastic Technology Co. Ltd. The MPs were washed with methanol for 48 hours (changing methanol every 24 hours), and then dried in a fume hood at room temperature. Aged PE (APE) was prepared by 18% (v/w) H_2O_2 and UV exposure (HUFFER 2018). The functional groups of MPs were determined by FTIR

[a] https://orcid.org/0000-0002-1939-4009

Shang, Q., Tan, M. and Chi, J.
Effect of Microplastics on Gut Bacterial Community of the Earthworm Pheretima guillelmi.
DOI: 10.5220/0011199300003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 260-264
ISBN: 978-989-758-595-1

spectrometer (Varian Excalibur HE 3100). The carbonyl index (CI) is often used as an indicator of the presence of carbonyl groups (SONG 2017). The calculation of CI of three types of MPs was determined based on the absorbance at 1720, 2850 cm^{-1}: CI=Abs (1720 cm^{-1}) / Abs (2850 cm^{-1}). The crystallinity of MPs was determined by X-ray diffraction (XRD) of Bruker company.

The soil was collected from the Beiyang Park Campus, Tianjin University, China. After air-dried, the soil samples were sieved through a 2-mm mesh. The basic physicochemical properties of the soil samples were as follows: pH 8.08, total organic content 1.50%, and composition of 6.09% sand, 68.33% silt and 25.58% clay.

The earthworm *Pheretima guillelmi* was selected for this work. The earthworms were first incubated in soil for 7~14 days to adapt to the laboratory conditions, maintaining soil moisture content at 70%~85% during culture. Before use, healthy earthworms were selected and placed on hydrated filter paper at 20 ±3 °C for 24 h to clear their gut contents.

2.2 Experiments Methods

There seven treatments were set up, including soils without MPs (CK), soil +0.2% (w/w) MPs (i.e.PE,APE and PBAT) and soil +2% (w/w) MPs (i.e.PE,APE and PBAT). Portions of 200 g soil with or without MPs were added into a series of 500 mL glass beakers (distilled water was added into the beakers to keep soil moisture content of 85%). Each treatment was replicated 3 times. Five healthy earthworms (have obvious reproductive ring, sexual maturity) were washed with distilled water, weighed and added into the beakers, which covered gauze to prevent the earthworm escaping. These beakers were placed in an intelligent illumination incubator (3200 Lux) with 12 h light/12 h dark at 20 ±3 °C for 28 days. The earthworms were removed from soil on day 28, washed with distilled water and placed in alcohol. After being inactivated, the earthworm was dissected and the gut was removed to measure the microbial community structure (MA 2017).

3 RESULTS AND DISCUSSIONS

3.1 Characterization of Microplastics

The FTIR fingerprints appear to be quite different for MPs derived (Fig. 1). Compared to the spectra of PE and APE, the APE has an obvious peak in the carbonyl region (1870~1540cm^{-1}). The CI increased from 0.04 (PE) to 0.27 (APE). It is indicating that the polarity of the PE increased after aging. In addition, the peak of PBAT spectra in the carbonyl region is more obvious, and the CI of PBAT is 0.92, and the polarity is the strongest.

According to the degree of crystallinity, MPs can be divided into crystalline state, semi-crystalline state and amorphous state (amorphous). Amorphous MPs also include glassy state and rubber state (GUO 2012). The X-ray diffractograms of PE/APE/PBAT composites are shown in Fig.2. The crystallinity of MPs varies. As semi-crystalline plastics, PE has obvious crystallization peaks, APE also shows obvious crystallization peaks. Through jade data processing, PE crystallinity is 56.85%, while APE is 61.67%. Compared with PE, the crystallinity of APE increased slightly. PBAT has no obvious crystallization peak and is a rubber polymer.

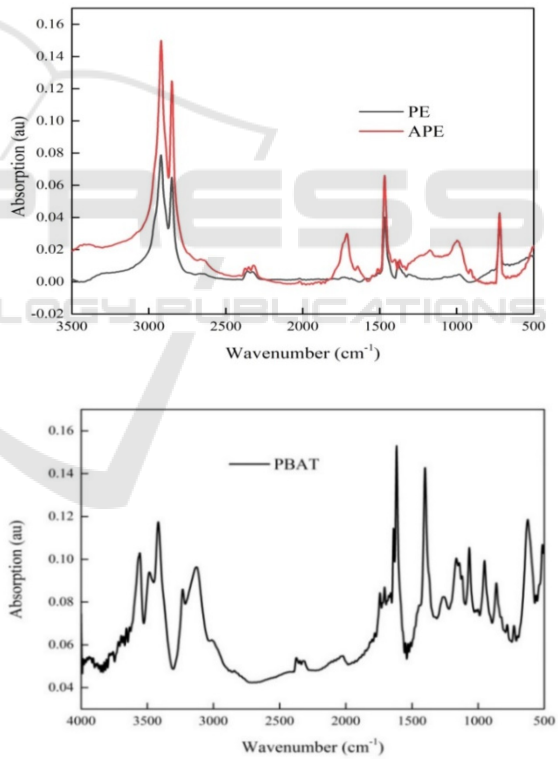

Figure1: FTIR spectra of microplastics.

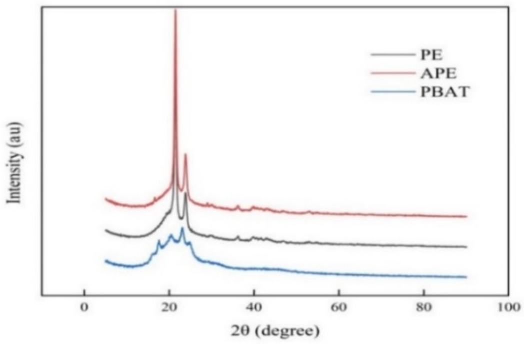

Figure 2: XRD of microplastics.

3.2 Effects of Microplastics on Earthwormsgut Bacteria

At the end of the experiment, 16SrDNA sequences ofthe samples of earthworms guts have been tested, and the results of OTUs analysis were as shown in Table 1. The results show that, compared with the CK, the number of OTUs in treatments with MPs significantly increased. The number of OTUs of the low concentration treatments were higher than that of the high at the same type of MPs. The variation trend of α diversity index (Shannon and Simpson) of the earthworm gut bacterial community was essentially consistent with the number of OTUs.

Figure 3 shows the top 10 phyla in the relative abundances of the earthworm gut bacteria. It can be seen that the relative abundances of *Verrucomicrobiota* and *Firmicutes* in the earthworm gut were the highest for all cultivation. Compared with CK, the relative abundances of *Firmicutes*, *Crenarchaeota*, *Bacteroidota*, *Actinobacteriota* and *Planctomycetes* increased significantly in those treatments with MPs with the highest increase of *Firmicutes* by 3.28~23.78%, while the relative abundances of *Proteobacteria* significantly reduced by 53.70%~69.10%. And the relative abundances of *Bacteroidota* increased when the concentration of MPs increased.

Table 1: The number of OTUs and α diversity index of earthworm gut bacteria.

Group	OTUs	Shannon	Simpson
CK	357	2.135	0.588
PE-0.2	1774	7.297	0.979
PE-2	1163	6.768	0.982
APE-0.2	945	4.045	0.829
APE-2	778	5.952	0.958
PBAT-0.2	764	5.534	0.939
PBAT-2	752	7.034	0.973

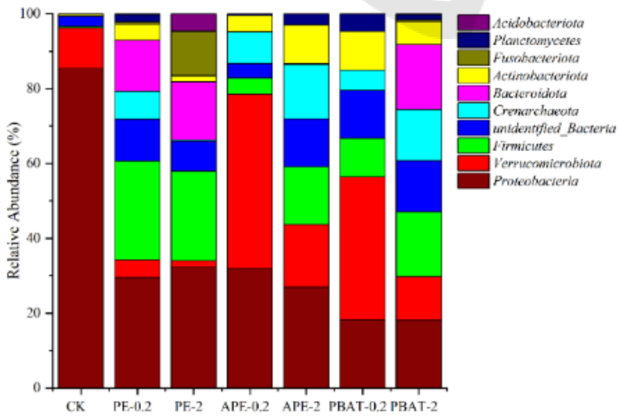

Figure 3: Thebacterial community composition of earthworm gut is shown as relative abundance (%) at the phylum levels.

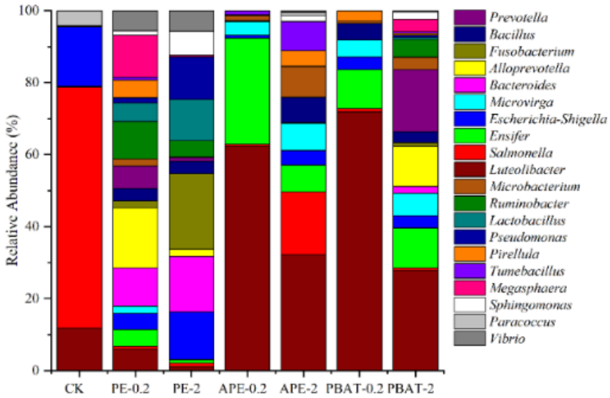

Figure 4: The bacterial community composition of earthworm gut is shown as relative abundance (%) at the genera levels.

Figure 4 lists the top 20 bacterial genera in the relative abundance of the earthworm gut bacteria, belonging to 6 phyla, namely *Verrucomicrobia*, *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, *Bacteroidetes* and *Firmicutes*. Additionally, compared with CK, the relative abundance of *Ensifer*, *Bacteroides*, and *Bacillus* in the earthworm gut increased significantly after added MPs, while the relative abundances of *Salmonella*, *Escherichia-Shigella* and *Paracoccus* decreased significantly.

Many studies have reported that earthworms produce more intestinal secretions when they ingest materials without rich fresh organic matter. Therefore, MPs addedto the soil may stimulate earthworms to produce more intestinal mucus. Simultaneously, the bacteria in the gut of earthworms is stimulated, which has a significant effect on the consumption of pollutants (HUERTA 2016). In this study, the number of OTUs and α diversity indexes of the earthworm gut bacteria increased significantly after adding MPs to the soil where earthworms live. Addition of MPs increased the relative abundances of genera *Ensifer*, but decreased the relative abundances of genera *Salmonella*. Earthworms change the growing environment of their own gut microbial communities by swallowing soil containing MPs.

4 CONCLUSIONS

In this work, the changes of earthworms gut bacteria were observed for a 28-day experiment in the MPs added soil. The results showed that the microbial community structure of earthworms gut was significantly affected by non-biodegradable polyethylene (PE) and biodegradable poly (butylene adipate-co-terephthalate) (PBAT) MPs. The number of operational Taxonomic Units (OTUs) and α diversity indexes in treatments with MPs were higher than those in treatment without MPs. The number of OTUs was in the order of treatments with aged-PE MPs > treatments with unaged-PE MPs > treatments with PBAT MPs. The number of OTUs in treatments with lower MPs concentration was higher than that in treatments with higher MPs concentration. Addition of MPs increased the relative abundances of genera Ensifer, Bacteroides and Bacillus, but decreased the relative abundances of genera Salmonella, Escherichia-Shigella and Paracoccus. Therefore, MPs significantly impact the microbial community of earthworm gut, which is related to types of MPs.

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