Abstract: The transport of eDNA is one of the key environmental behaviors for its spreading and dispersal. Microplastics (MPs) are widely present in the soil environment and directly affect the environmental behavior of co-existing soil pollutants. However, the effect of MPs on eDNA transport and its mechanism remain unclear. In this study, we systematically investigated the effect of MPs types and functional groups on eDNA transport. The results showed that different kinds of MPs promoted eDNA transport, but there was no significant difference between these two MPs types. MPs with two different functional groups inhibited eDNA transport, and the transport rate of eDNA decreased by 8.9% and 7.0%, respectively. PS-NH3 inhibited eDNA transport by reducing electrostatic repulsion, enhancing electrostatic adsorption, and reducing porosity of porous media, enhancing the interaction between eDNA and MPs. In the presence of kaolin, the inhibition effect of MPs on eDNA transport increased with the proportion of kaolin increased. The positive charge of kaolin enhanced the electrostatic adsorption between MPs and eDNA, and inhibited the transport of eDNA. This study revealed the transport rule of eDNA in the presence of MPs, and provided a theoretical basis for a comprehensive assessment of the environmental and ecological risks of coexistence of MPs and eDNA.

1. Introduction

Extracellular DNA (eDNA) is intracellular DNA released by cell lysis or actively secreted by the cell. eDNA is present in various environmental media (soil, sediments, marine)\(^1\). Soil provides a good ecological environment for the enrichment of eDNA. eDNA exists widely in soil environment, the highest concentration of eDNA can reach 200 μg g\(^{-1}\). eDNA can persist in the soil for a long time without being degraded. Some eDNA-carrying antibiotic resistance genes (ARGs) can be internalized by microbial sensory cells and integrated into their genomes through natural transformation. The natural transformation of ARGs promotes the spread of antibiotic resistance, and its risks to human health and ecology have aroused widespread concern\(^2\). Co-existing soil contaminants affect the environmental behavior of eDNA through adsorption of eDNA. For example, montmorillonite prevents the degradation of eDNA by adsorption of eDNA. The interaction between co-existing soil pollutants and eDNA directly determines the spreading and dispersal of eDNA in the soil.

Microplastics enter the soil through agricultural mulching, organic fertilizer application, wastewater irrigation, etc. and accumulate in the soil in different shapes and types\(^3\). The pollution of MPs in soil is already very serious, and the concentration of MPs in soil can reach up to 7% of the dry weight of the soil\(^4\). MPs have large specific surface area, porous and hydrophobicity, and can adsorb coexisting pollutants in the soil through pore filling and hydrophobic interactions\(^5\). The effect of MPs on the environmental behavior of co-existing soil pollutants is controlled by MPs characteristics, such as types, surface, and functional groups. For example, there are significant differences in the effects of MPs with different functional groups on E.coli transport. Most studies have mainly focused on the effects of MPs on the adsorption and transport of organic matter, heavy metals, and few studies have focused on the effects of MPs on eDNA transport.

In this study, transport experiments were conducted using two types and two functional groups of MPs to evaluate the effect of MPs on eDNA transport. The effects of MPs on eDNA transport mediated by soil minerals were investigated by mixing MPs with kaolin. The effects of different types and surface charges of MPs as well as kaolin-mediated MPs on eDNA transport were explained by the transport rate and the breakthrough curve (BTC). The results of this study fill the gap in the current research on the transport of eDNA.

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2. Materials and methods

2.1 Plasmids and microplastics

Calf thymus DNA was used as the test eDNA, purchased from Sangong Bioengineering. The background solution of eDNA is 10 mM Tris-HCl (pH = 7) with an initial concentration of 25 mg L\(^{-1}\). Polystyrene (PS) and polyvinyl chloride (PVC) were purchased from Ruixiang Plasticizing as test MPs, and 177 μm PVC and PS were obtained by sieving through a sieve mesh. MPs with different surface functional groups were purchased from the Tianjin Besler Chromatography Technology Development Center. The particle sizes of the quartz sands used in the transport experiments ranged from 595-841 μm. The quartz sands were soaked in concentrated NaOH, concentrated HCl, and 30% H\(_2\)O\(_2\) sequentially for at least 24 h. The quartz sands were thoroughly washed with deionized water after each soaking until the pH = 7. The cleaned quartz sands were dried at 105 °C overnight, and then baked in a muffle furnace at 850 °C for 8 h.

2.2 Standard curve and stability of eDNA

The standard curve for eDNA was set to 0, 5, 10, 15, 20, and 25 mg L\(^{-1}\), the standard curve was prepared as a serial dilution. The stability of the eDNA within 120 h was determined before the transport experiment. eDNA was stored in a 20 mL liquid phase vial and placed under natural light, and samples were taken at 2, 4, 8, 12, 24, 72, and 120 h, respectively. The absorbance of eDNA was measured with a UV spectrophotometer (UV-9000S, Lambda35, USA) after sampling, and the concentration of eDNA was calculated according to the absorbance.

2.3 Transport experiment

The transport column used in this study was a Plexiglas cylinder. 200 mesh filters are placed inside the top and bottom cover of the transport column to prevent small porous media from blocking the inlet and outlet water holes or flowing into the silicone tube. The bottom end of the transport column is connected with a silicone tube, and the top end is connected with a peristaltic pump (BT100F-1). The background solution is pumped into the transport column by a peristaltic pump in a bottom-to-top direction. The effluent was collected with an automatic distillate collector (EBS-20) at a rate of once 5 min.

The transport experiments were mainly divided into three stages. Stage I: 10 Pore Volume (PV) Tris-HCl was passed into the packed column, this process was mainly for the exclusion of gas in the column and to reduce the effect of preferential flow on eDNA transport. Stage II: 3 PV eDNA solution was passed into the transport column from the bottom up at the same speed. During the pumping process of eDNA, the eDNA is magnetically stirred to prevent it from settling or condensing, so as to ensure the uniformity of solution concentration during the transport process. Stage III: Another 3 PV Tris-HCl was passed into the packed column at the same rate to elute the eDNA until the concentration of the substance to be measured was barely detectable in the effluent. The effluent was collected using an automatic fraction collector, and the effluent was collected every 5 min during the experiment. Quantification of eDNA concentration using a UV spectrophotometer. The eDNA solution was collected once at both the beginning and end of the experiment and its concentration was measured as the initial feed concentration of eDNA. The BTC was plotted for the relative concentration, C/C\(_0\), and time.

2.4 Statistical analysis

Experimental data were analyzed and plotted by Excel 2019. ANOVA and Duncan's multiple comparisons (P < 0.05, indicated by lowercase letters) were used for significant difference analysis by SPSS 20.0.

3. Results and discussion

3.1 Standard curve and stability of eDNA

A linear standard curve of the eDNA solution was obtained by diluting eDNA at a concentration of 25 mg L\(^{-1}\) (Figure 1). The stability of eDNA was determined and found to be stable for 120 h in 10 mM Tris-HCl (pH = 7) background solution without degradation and sedimentation. eDNA can be used for subsequent transport experiments.

![Figure 1. Standard curve (a) and stability of eDNA (b).](image-url)
3.2. Effect of different types of MPs on eDNA transport

The effects of PVC and PS on eDNA transport were studied to reveal the effects of different types of MPs on eDNA transport. The effects of PVC and PS on the transport rate and BTC of eDNA are shown in Figure 2. PVC and PS significantly promoted the transport speed of eDNA, but there was no significant difference in the promotion effect of the two MPs. The transport rates of eDNA in CK, PS and PVC treatment groups were 96.7%, 99.6% and 99.5%, respectively. The BTC of eDNA reached its peak at 1.44 PV, 1.25 PV and 1.25 PV, and the end of the plateau phase at 3.66 PV, 4.13 PV and 4.56 PV. In the MPs treatment group, the transport rate and transport speed of eDNA increased significantly, indicating that MPs promoted the transport of eDNA.

MPs promoted the transport of eDNA by occupying the deposition site of eDNA on the surface of porous media. At the same time, MPs promoted the transport of eDNA by increasing the porosity of porous media and reducing the trapping effect of porous media on eDNA. Compared with porous media, the surface of MPs has more negative charge, which enhanced the electrostatic repulsion of eDNA and promoted the transport of eDNA[6].

![Figure 2. Effect of different types of MPs on eDNA transport breakthrough curve (a) and transport rate (b).](image)

3.3. Effect of MPs with different functional groups on eDNA transport

Electrostatic interactions are the key mechanism for the interaction between MPs and eDNA. The effects of MPs with different functional groups on eDNA transport rate and BTC are shown in Figure 3. PS-NH$_2$ and PS-COOH significantly reduced the transport rate of eDNA and delayed the time for BTC to reach its peak. The transport rates of DNA in CK, PS-NH$_2$, and PS-COOH were 96.7%, 87.8%, and 89.7%, respectively and the time to reach the peak of BTC was 1.88 PV, 2.08 PV, and 2.08 PV. The decrease of eDNA transport and the delay of BTC peak time indicated that PS-NH$_2$ and PS-COOH significantly inhibit eDNA transport. The inhibitory effect of PS-NH$_2$ on transport was more remarkable than that of PS-COOH.

The PS-NH$_2$ inhibited eDNA transport by enhancing the electrostatic adsorption between MPs and eDNA. The PS-COOH aggregates together to form MPs agglomerates with larger particle sizes, providing more adsorption sites for eDNA, promoting the adsorption of eDNA, and inhibiting the transport of eDNA. PS-COOH aggregations reduced the porosity of porous media, promoted the retention of eDNA, and inhibited the transport of eDNA[7].

![Figure 3. Effect of MPs with different functional groups on eDNA transport breakthrough curve(a) and transport rate (b).](image)

3.4. Effect of MPs on eDNA transport mediated by kaolin

Soil kaolin changes the transport of eDNA by adsorbing MPs and eDNA. By exploring the effect of MPs on eDNA transport in the presence of kaolin, we can reveal how MPs affect soil mineral-mediated eDNA transport. The kaolin and MPs were mixed well and filled into the
transport column, and the flushed kaolinite is continuously pumped back into the transport column using a peristaltic pump until there is no kaolin in the effluent solution. The effect of MPs on eDNA transport in the presence of kaolin is shown in Figure 4. The inhibitory effect of MPs on eDNA transport was enhanced with the increase of kaolin proportion. The transport rates of eDNA were 94.8%, 81.4%, 72% and 70% in the treatment groups with kaolin proportion of 0%, 1%, 4% and 8%, respectively. The peak time of BTC is delayed with the increase of kaolin proportion.

The positively charged kaolin was prone to heterogeneous aggregation with MPs to form large particle size aggregates, and large particle size aggregates provided more adsorption sites for eDNA. At the same time, the positively charged kaolin reduced the electrostatic repulsion of MPs to eDNA, enhanced the electrostatic attraction, and inhibited the transport of eDNA. By adsorption on porous media, kaolin reduced the porosity of porous media, weakened the retention effect of porous media on eDNA, and inhibited the transport of eDNA.

4. Conclusions
Based on the above experimental results and analysis, the following conclusions were drawn in this study. PVC and PS facilitated eDNA transport by enhancing electrostatic repulsion, increasing porosity of porous media, and occupying eDNA deposition sites. There was no significant difference between PVC and PS in promoting eDNA transport. PS-NH$_2$ and PS-COOH significantly inhibited eDNA transport. PS-NH$_2$ with positive charge enhanced electrostatic attraction between MPs and eDNA and inhibited eDNA transport. The negatively charged PS-COOH inhibited the transport of eDNA by forming aggregates and provide more adsorption sites for eDNA. Kaolin and MPs formed larger aggregates, which provided more adsorption sites for eDNA. The positive charge on the kaolin surface enhanced the electrostatic adsorption between MPs and eDNA, thus inhibiting the transport of eDNA.

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References


