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Effects of polystyrene microplastics on uptake and toxicity of phenanthrene in soybean

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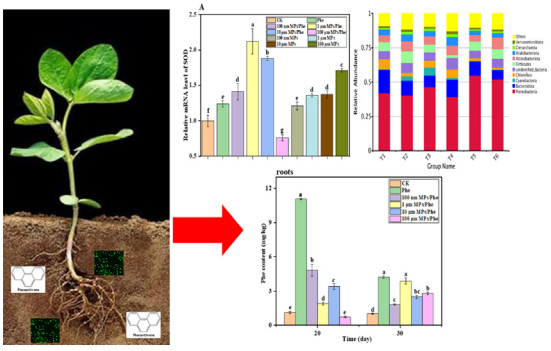
HIGHLIGHTS

- Microplastics (MPs) decreased the uptake of phenanthrene in soybean.
- MPs and phenanthrene co-exposure caused higher toxicity to soybean tissue.
- Micron-size MPs and phenanthrene exhibited higher genotoxicity to soybean.
- Micron-size MPs inhibited relative abundance of *Proteobacteria* in rhizosphere soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MPs) can influence the availability of contaminants in the soil and have adverse effects on plants. Up to now, the effects of MPs on the uptake of organic pollutants by leguminous plants are still unclear. In this study, we explored the impacts and mechanisms of polystyrene MPs of different sizes on the uptake of phenanthrene (Phe) by soybean seedlings. The results showed that MPs decreased the uptake of Phe in soybean roots and leaves. Micron-size MPs showed a higher inhibition of Phe uptake in roots than nano-size MPs (4.83 mg/kg) at the beginning with concentrations of 1.89 mg/kg, 3.40 mg/kg, and 0.72 mg/kg in groups 1 μm, 10 μm, and 100 μm MPs/Phe, respectively. The combined toxicity of micron-size MPs and Phe to soybean plants was higher than that of nano-size MPs and Phe, and 100 μm MPs and Phe co-contaminant show the highest toxicity to soybean. The activities of antioxidative enzymes and their gene expression showed that micron-size MPs induced higher genotoxic and oxidative damage to soybean roots than nano-size MPs, which decreased the activity of roots, thus leading to the lower uptake of Phe by soybean roots and leaves. This study highlights that the combined exposure to MPs and Phe causes harmful effects on soybean plants and MPs inhibit the uptake of organic pollutants by higher plants.

1. Introduction

Microplastics (MPs) are a diverse group of high-molecular polymer particles (<5 mm) (Wright et al., 2013), which are widely distributed in marine and terrestrial ecosystems (Collard et al., 2019; Lenaker et al., 2019). It has been estimated that about 63,000–430,000 and 44,000–300,000 tons of MPs enter farmland soils per year with the application of sewage sludge in Europe and North America, respectively (Nizzetto et al., 2016). They may also enter the farmland by the fragmentation of agricultural films, sewage irrigation and application of organic fertilizer (Ng et al., 2018).

So far, a few works have studied effects of MPs on plants. They could be adsorbed on the root surface of plants (Urbina et al., 2020), and

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reduce the water conductivity in roots, which hinder the transpiration, nutrient uptake, and growth of plants. Polyethylene (PE) MPs were also reported to impact the germination of seed by blocking the pores of seed capsule (Bosker et al., 2019). The size of MPs is a critical factor in eco-toxicological risk assessments for plants. Growth limitations and biomass decrement are observed in wheat after exposure to micron-size MPs (Qi et al., 2018). Nano-size MPs can be taken up by plants and have adverse impacts on their seed germination and growth. They can penetrate cell wall, enter the cell and cause adverse effects to root-tip cells (Jiang et al., 2019). Bandmann et al. revealed that tobacco cells could incorporate 20–40 nm MPs in the process of cell culture (Bandmann et al., 2012). In a previous study, nano-size MPs exhibited size-dependent growth inhibition rates, but 5 μm MPs had little effect on the growth of *Chlorella pyrenoidosa* (Li et al., 2020). Smaller MPs significantly affect fertility and growth of algae and photosynthesis is also disturbed (Chen et al., 2020). In addition, plants can take up and translocate nano-size MPs (Dong et al., 2021; Huang et al., 2021; Khalid et al., 2020). MPs in the hundred-micrometer range have no significant toxic effects to diatom (Guo et al., 2020), whereas nano-size MPs can accumulate in roots and induce higher genotoxic and oxidative damage to broad bean than micron-size MPs (Jiang et al., 2019).

Higher plants are widely used to detect and evaluate the toxicity of pollutants, such as heavy metals, nanomaterials and MPs (Dong et al., 2021; Ge et al., 2018; Urbina et al., 2020). Soybean is an important cash crop and the fifth largest crop in agricultural production in the world (Yusefi-Tanha et al., 2020). Studies on potential toxic effects and mechanisms of MPs have not been well explored in cash crops. Due to the large surface area and functional groups, MPs can adsorb organic pollutants and impact their translocation in aquatic plants (Kalcikova et al., 2017). Until now, effects of MPs with different sizes on the toxicity of organic contaminants to cash crops are relatively scarce.

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic pollutants and have become a worldwide environmental problem. PAHs can be taken up by plants and cause toxicity to plants (Zhan et al., 2015). The accumulation of PAHs in plants decreases the chlorophyll content, plant height, fresh weight and induces oxidative stress (Meng and Chi, 2015; Shen et al., 2017). A previous study found that MPs decreased the toxicity of phenanthrene (Phe) to diatom (Guo et al., 2020). In addition, Tang et al. reported that (Tang et al., 2020) the toxicity of PAHs was generally aggravated by nano-size MPs and mitigated by micron-size MPs. However, effects of MPs on the uptake and accumulation of PAHs by higher plants and mechanisms have not been clarified. Their combined toxicity on higher plants has also rarely been studied at the gene expression level.

Higher plants are the primary basis of many food chains, and the accumulation of PAHs in higher plants may have adverse impacts on the production, quality and safety of foods. The aims of this study were to study the uptake and toxicity of Phe in different parts of soybean in the presence of polystyrene (PS) MPs with different sizes, and to reveal their individual and combined genotoxicity. Our study will contribute to a comprehensive understanding toward the toxic effects of MPs on higher plants.

2. Materials and methods

2.1. Materials

Fluorescent PS MPs (100 nm, 1 μm, 10 μm and 100 μm) were purchased from Tianjin BaseLine ChromTech Research Centre, Tianjin, China. The functional groups, elemental composition and morphology of MPs were measured by Fourier transform infrared spectroscopy (FT-IR, Nicolet NEXUS-670, Thermo Fisher, USA), transmission electron microscope (TEM, JEM-2100F, JEOL, Japan) and scanning electronic microscopy, (SEM, SU-8010, Hitachi, Japan). Phe (purity 97.0%) was purchased from Macklin Industrial Corporation, Shanghai, China). Soybean seeds (*Glycine* max L. *Merrill*) were obtained from Jilin Province Seed Company, Changchun, China. ELISA kits and root activity Kits were purchased from Shanghai MLBIO Biotechnology Co. Ltd. and Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China), respectively.

2.2. Exposure of soybean to MPs

The soil was collected from farmland in Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China. The physical and chemical properties of the soil are shown in Table S1. Phe was dissolved with acetone and added into the soil to achieve 1 mg/kg. After that, the soil was placed in the fume cupboard for 48 h to evaporate the solvent. Then, the soil was amended with fluorescent MPs to achieve 10 mg/kg. Ten groups were set up: T1, Phe + 100 nm MPs; T2, Phe + 1 μm MPs; T3, Phe + 10 μm MPs; T4, Phe + 100 μm MPs; T5, Phe alone; T6, no Phe or MPs (CK); T7, 100 nm MPs; T8, 1 μm MPs; T9, 10 μm MPs; T10, 100 μm MPs.

Eight soybean seeds were planted in a 5000 ml box with 5000 g of spiked soil and placed in a greenhouse at 25 °C and 50% humidity for 30 days after sprouting. The soybean plants were sampled on day 10, 20, 30, and cut into root, stem and leaf. The samples were lyophilized and stored in a refrigerator (−20 °C) until further analysis.

2.3. Sample analysis

One gram soybean plant samples (root, stem and leaf; dry weight) were placed in 10 ml of HNO_3 at 70 °C for 5 h until the samples were digested completely. After that, the samples were added with 1% HNO_3 solution to 10 ml and the concentrations of MPs were determined by a fluorescence spectrophotometer (RF-5301PC, Shimadzu, Japan) at excitation and emission wavelengths of 570 nm and 526 nm, respectively. The concentrations of Phe in root, stem and leaf of soybean were measured by a gas chromatography–mass spectrometry, (GC–MS, Trace 1300 ISQ, Thermo Fisher, USA). The details are shown in the Supplementary Material.

The soybean plant samples were weighed and disrupted with homogenizer in phosphate buffer saline (PBS) solution (pH = 7.4). The mixture was put into a tube and centrifuged at 3000 r/min, 4 °C for 20 min. The supernatant was collected to measure physiological indexes, including peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and Reactive Oxygen Species (ROS) by ELISA kits. The root activity was determined by the Kit.

2.4. Analysis of gene expression and high-throughput sequencing

Soybean roots were ground in an ice bath with a glass homogenizer. Total RNA of soybean roots were extracted with 1 ml TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The absorbance was determined by an ELIASA (Synergy H1, Biotek, USA). cDNA was synthesized by the testing kit (FSQ-101; TOYOBO, Shanghai, China) and then subjected to a quantitative polymerase chain reaction (q-PCR, Mx3000p, Agilent, USA). Fluorescence Quantitative Detection Kits (TIANGEN, Beijing, China) were employed for q-PCR analysis. High-throughput 16S rRNA sequencing was performed to detect the microbial community in rhizosphere soil. Details of q-PCR reaction system, gene primers (Table S2), 16S rRNA gene amplification, high-throughput sequencing, and bioinformatic analysis are shown in the Supplementary Material. The relative expression levels were measured by the $2^{-\Delta\Delta Ct}$ method.

2.5. Statistical analysis

All the samples were analyzed in triplicate. Data were presented as the mean ± standard deviation (SD) and analyzed by SPSS 22.0 software. The analysis of variance (ANOVA) and LSD test were performed to examine the differences among groups. The differences were considered significant at $p < 0.05$. Origin 8.0 software was used to draw the figures.

3. Results

3.1. Occurrence of Phe in soybean tissues

The characteristics of the PS MPs are shown in the Supplementary Material (Fig. S1). MPs in soybean plants were not detected. As shown in Fig. 1A, Phe in soybean roots in groups T5, T1 and T3 (Phe alone, 100 nm, and 10 μm MPs/Phe) showed the same trend, which decreased at day 30 compared with day 20. In groups M2 and M4 (1 μm and 100 μm MPs/Phe), concentrations of Phe in soybean roots increased significantly on day 30. The highest level of Phe was found in T5 compared with other groups on day 20, indicating that MPs may restrain the uptake of Phe in soybean roots. As shown in Fig. 1b, Phe in soybean stems in groups T5, T2 and T3 (Phe alone, 1 μm, and 10 μm MPs/Phe) exhibited the same trend, and the highest concentrations of Phe were found on day 20. In groups T1 and T4 (100 nm and 100 μm MPs/Phe), the concentrations of Phe in soybean stems increased significantly with exposure time. The concentrations of Phe in soybean leaves are shown in Fig. 1c. Phe in leaves in groups T5 and T1 (Phe alone, 100 nm MPs/Phe) decreased significantly on day 30. In groups T2, T3 and T4, the concentrations of Phe in soybean leaves increased significantly at the end of exposure time. Generally, MPs decreased the accumulation of Phe in soybean roots and leaves, and increased the accumulation of Phe in soybean stems. The occurrence of Phe in stems and leaves revealed its acropetal translocation in soybean. The distribution of Phe in soybean plants followed the descending order roots > stems > leaves, and the uptake of Phe by soybean plants can be attributed to root uptake and acropetal translocation.

3.2. Oxidative stress of Phe and MPs to soybean plants

Biomarkers were measured to evaluate the toxic effects of contaminants to soybean plants (Figs. 2, S2, S3). The contents of ROS and MDA (Fig. 2A and B) in roots increased significantly ($p < 0.05$) in all the treatment groups, and the highest content was found in group T4. CAT activity in roots showed ascending trends with exposure time in all treatment groups except T4, where CAT activity decreased slightly after 20 days and then increased significantly (Fig. 2C). Similarly, SOD (Fig. 2D) and POD (Fig. 2E) activity also increased with exposure time in all treatment groups except T4 and T5, respectively. The activity of SOD in group T4 increased significantly at the beginning and then decreased. POD activity in group T5 decreased significantly with exposure time.

The contents of ROS, MDA and CAT (Fig. S2A, S2B and S2C) in stems increased significantly ($p < 0.05$) in groups T1-T6 with exposure time. The activities of SOD and POD increased significantly in groups T1-T6 with exposure time except group T3, where they decreased slightly at the beginning and then increased significantly. The biomarkers in leaves increased significantly, and showed higher values in group T5 compared with other groups (Fig. S3). However, the biomarkers in MPs alone exposure groups had no obvious change in soybean stems and leaves except CAT and SOD in leaves, where they showed an ascending trend.

3.3. Effects of MPs and Phe on root activity and gene expression

The activity of soybean roots is shown in Fig. 3. In general, root activity increased with exposure time, and was significantly affected by individual or combined MPs and Phe treatment. The activity of soybean

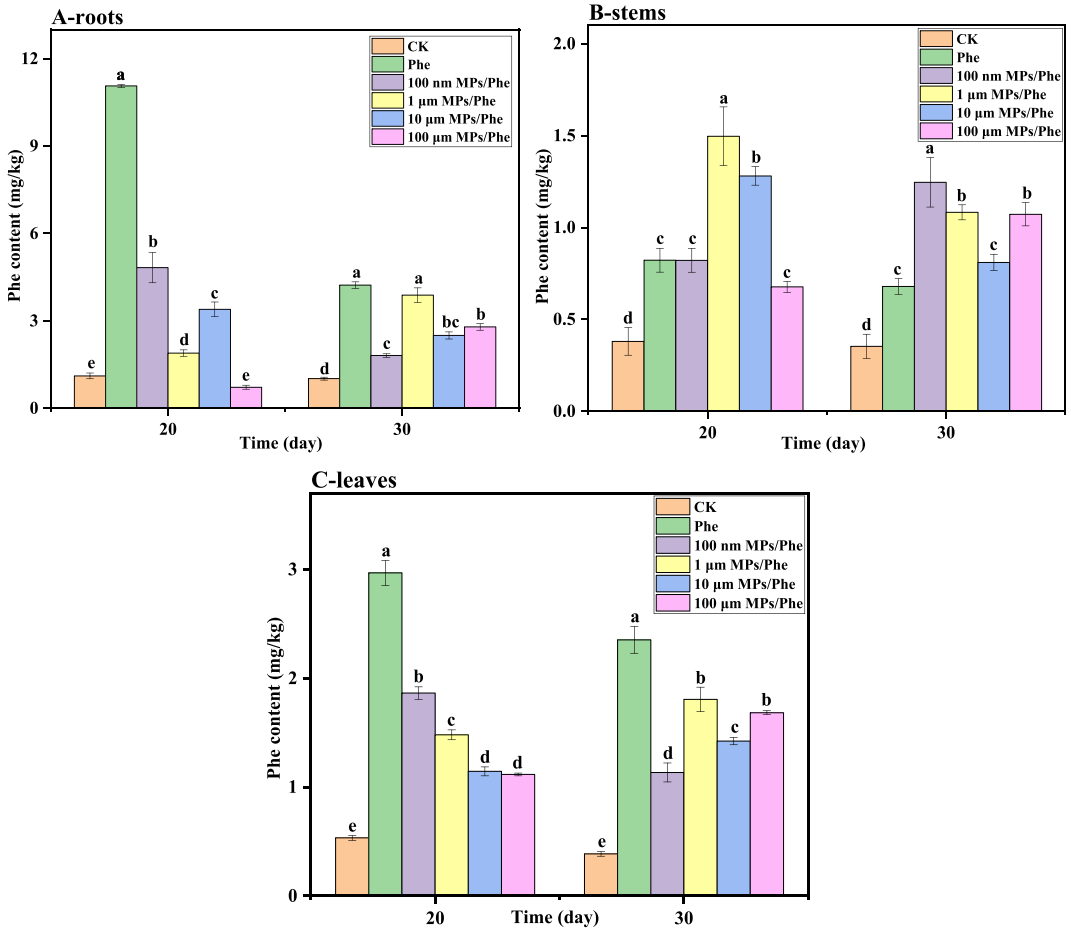


Fig. 1. The concentration of phenanthrene in different parts of soybeans, (A) roots, (B) stems, (C) leaves. Different letters indicate significant differences in same day ($p < 0.05$).

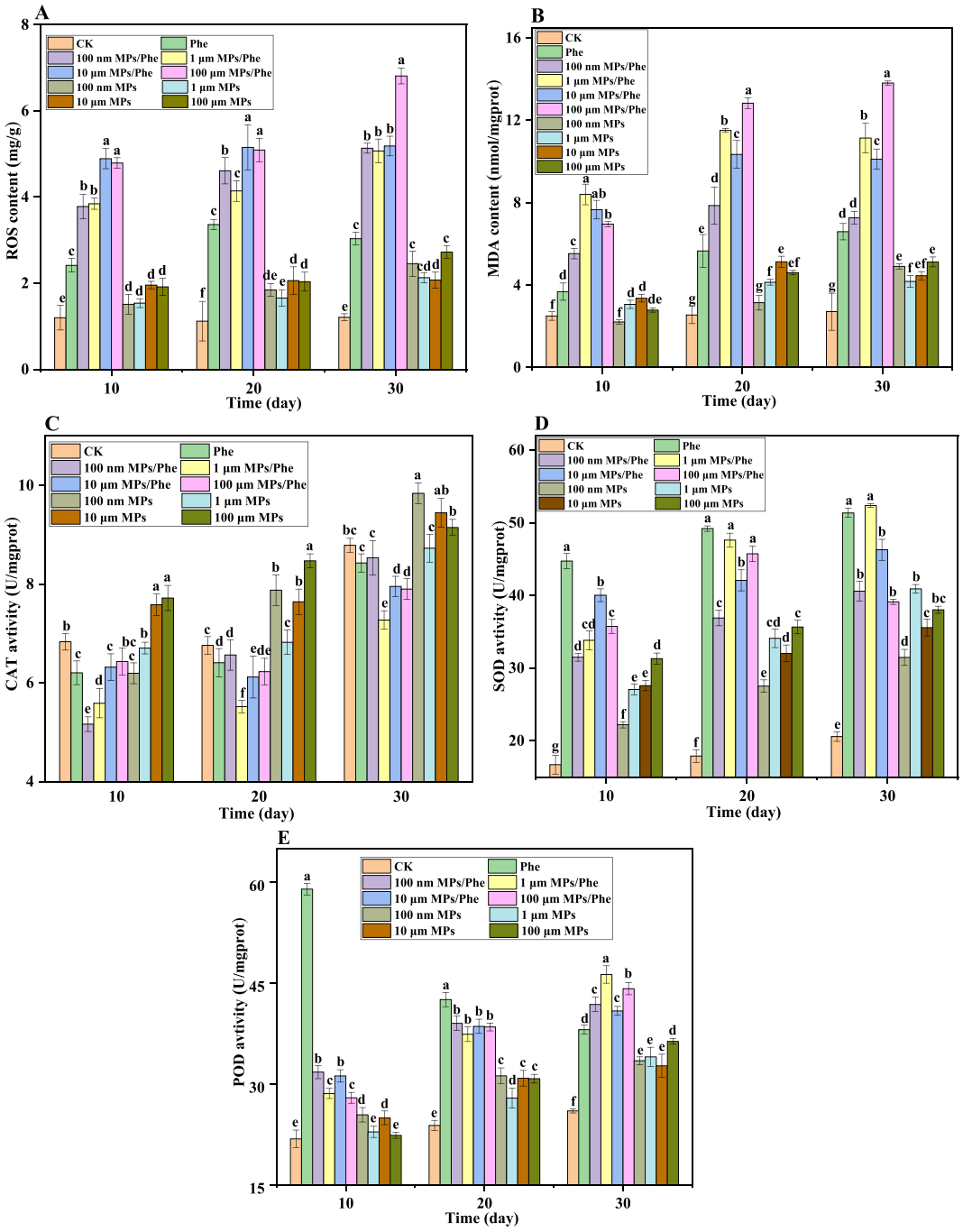


Fig. 2. Antioxidant enzyme activities in soybean roots. (A) ROS, (B) MDA contents, and (C) CAT, (D) SOD, (E) POD activity. Different letters indicate significant differences between treatments in the same day.

roots was lower in all treatment groups compared with CK, with the lowest activity in group T4.

The relative expression levels of three antioxidant enzyme genes are shown in Fig. 4. The relative expression levels of SOD (Fig. 4A) gene in all treatments were significantly higher than that in CK group ($p < 0.05$) except group T4 (0.76), with the highest expression in group T2 (1.88). The relative expression of CAT gene (Fig. 4B) was inhibited in groups T1-T5 (0.88, 0.81, 0.45, 0.64, and 0.65), and significantly lower than that in CK group. In contrast, the relative expression of CAT gene was stimulated after exposure to MPs individual treatments. The relative expression of POD gene (Fig. 4C) was also stimulated after exposure to pollutants. The relative expression levels in co-exposure groups were higher than other groups.

3.4. Effects of MPs and Phe on rhizosphere soil microbial community

The results of 16S rRNA genes in rhizosphere soil characterized by high-throughput sequencing are shown in Figs. 5, 6, S4 and S5. Venn and flower diagrams show that 2558 OTUs were shared in rhizosphere soil from all the groups, and 2969 OTUs were shared in rhizosphere soil from co-exposure groups (Fig. 5). The numbers of unshared OTUs in rhizosphere soils of co-exposure groups were lower than the CK group. The relative abundance of *Proteobacteria* decreased significantly in rhizosphere soils from co-exposure groups (groups T1-T4), whereas the relative abundance of *cyanobacteria*, and *chloroflexi* increased significantly (Fig. 6A).

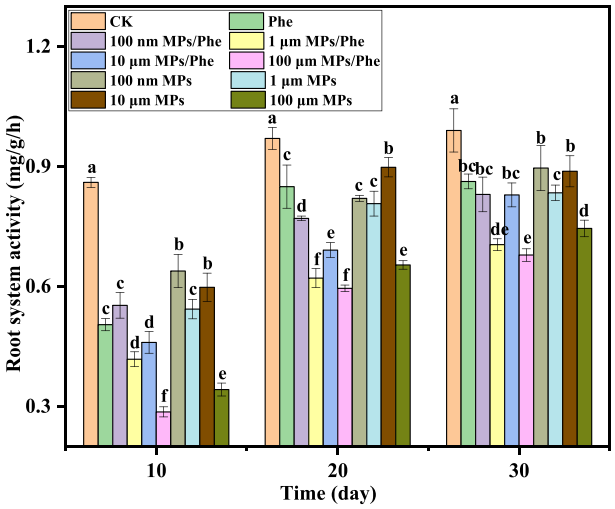


Fig. 3. The activity of soybean roots. Different letters indicate significant differences between treatments in the same day.

The structure and abundance of the rhizosphere soil microbiotas of soybean plants were affected by the co-exposure of Phe and MPs with different sizes. At the phylum level, *Proteobacteria*, *Bacteroidota*, *Actinobacteriota* and *Firmicutes* were the most abundant phyla in rhizosphere soil microbiotas, accounting for 90% of all sequence reads. The abundance of *Proteobacteria* decreased dramatically after co-exposure to Phe and MPs compared with CK and showed the lowest abundance in group T4 (100 μm MPs/Phe). The abundance of *Cyanobacteria* increased significantly in groups T2, T3 and T4 compared with other groups.

The results of PCoA plots based on the Bray-Curtis distance (Fig. S4) indicated that the rhizosphere soil community structures among different groups were significantly separated ($P < 0.01$). The PCoA primary (PC1) and secondary axis (PC2) displayed the separation of the rhizosphere soil, explaining 35.6% and 16.7% inter sample variation. After co-exposed to contaminants, the Alpha-diversity in groups T1-T4 increased significantly in soybean rhizosphere soils compared with other groups (Fig. 6B), which can be confirmed by the rarefaction curves of OTUs (Fig. S5).

4. Discussion

4.1. Effects of MPs on Phe uptake and translocation in soybean plants

Nano-size (40 nm) and micron-size (1 μm) PS-MPs can accumulate at root surface of wheat, especially at the root tip (Taylor et al., 2020). Micron-size MPs affect the root growth by mechanical blocking (Kalcikova et al., 2017). In this study, MPs inhibited the uptake of Phe by soybean roots from soil, which can be attributed to the adsorption of MPs on the soybean roots and blocking the connections between cells or cell wall pores for transport of contaminants (Jiang et al., 2019).

The uptake of Phe by roots was significantly different in treatment groups due to the changes in root activity. In contaminated soils, Phe is adsorbed on the root surface upon activation in rhizosphere, and transported to the other parts through apoplastic and symplastic uptake

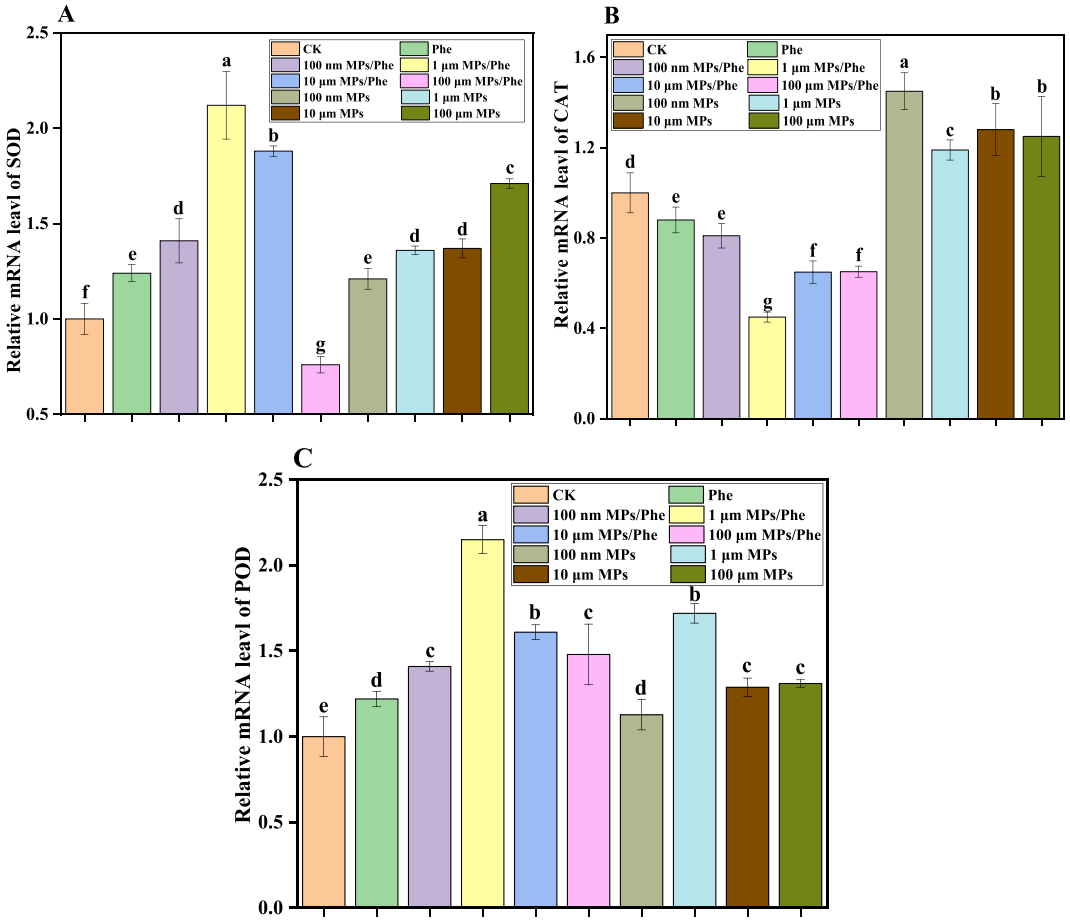


Fig. 4. Relative mRNA levels of (A) SOD, (B) CAT and (C) POD in soybean roots on day 30. Different letters indicate significant differences between treatments ($p < 0.05$).

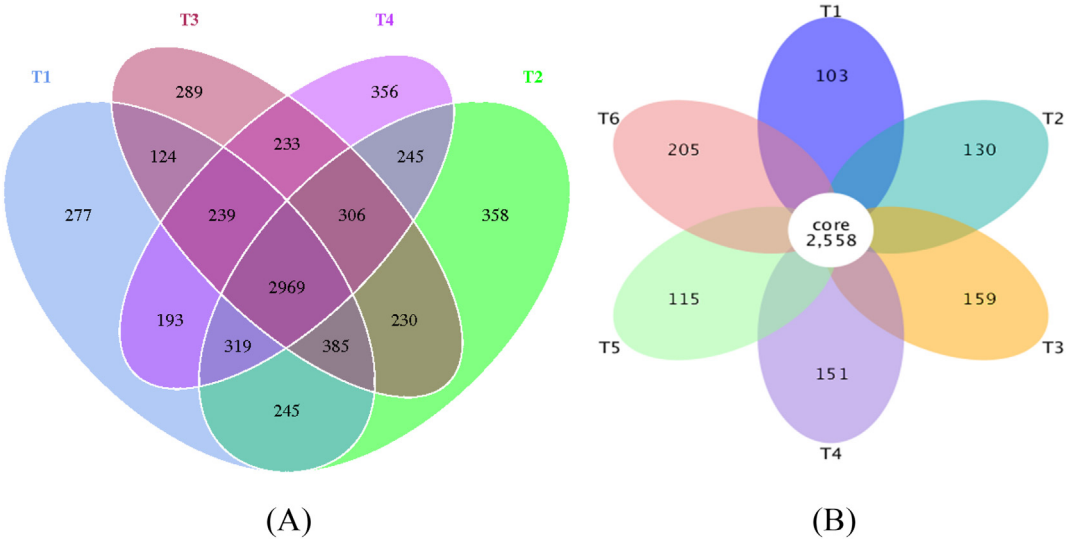


Fig. 5. Venn and flower diagrams showing the number of bacterial OTUs shared in the rhizosphere soils among different treatments. (A) The number of shared OTUs was counted between samples for each of the MPs and phenanthrene co-exposure groups. (B) The number of shared OTUs was observed between the CK and treatment groups.

(Redjala et al., 2010). Due to their insolubility, PS-MPs can be adsorbed onto the surface of soybean roots (Dong et al., 2020). In soils, MPs were adsorbed on the surface of soybean roots, and they may compete with Phe for adsorption sites and affect the absorption of Phe by influencing the root activity and transpiration (Li et al., 2021a; Li et al., 2021b).

MPs can reduce the photosynthetic rate of plants by changing the response of guard cells, resulting in partial closure of stomata and decrease in stomatal conductance (Dong et al., 2020). The decrease of root activity would decrease the transpiration rate, thus reducing the ability to absorb water. Phe could induce the thylakoid membrane damage, destroy chlorophyll structure and reduce activity of photosynthesis-related enzymes (Guo et al., 2020), thus affecting plant growth and elimination of contaminants.

4.2. Toxicity to soybean plants by Phe and MPs

MPs inhibited the accumulation of Phe in soybean roots and leaves. Meanwhile, the presence of MPs amplified the negative effects caused by Phe. ROS would be generated in plant tissue when the plant is exposed to pollutants. In this study, the activities of SOD, POD and CAT were inhibited under Phe stress, leading to the accumulation of ROS and damage of cell membrane. The contaminants could change the tertiary structure of enzyme proteins and result in the decrease of antioxidant enzyme activity (Jobby et al., 2016). In addition, the expression of relative gene was inhibited, indicating that Phe caused the damage to enzyme genes and further led to the decrease of antioxidant enzyme activity. The mechanism of oxidative stress induced by MPs is different from Phe. MPs are not able to enter plant cells, but they may cause mechanical damage to plant roots (Minibayeva et al., 2015) and increase the activity of antioxidant enzymes. ROS contents and SOD and CAT activities in treatment groups were higher than CK group, suggesting that soybean plants responded to excess ROS by increasing enzyme activity. However, overload of ROS may exceed the tolerance of plant cells, causing the cell damage and inhibiting the expression of genes. The increase of MDA revealed that MPs and Phe induced the membrane lipid peroxidation in soybean tissues.

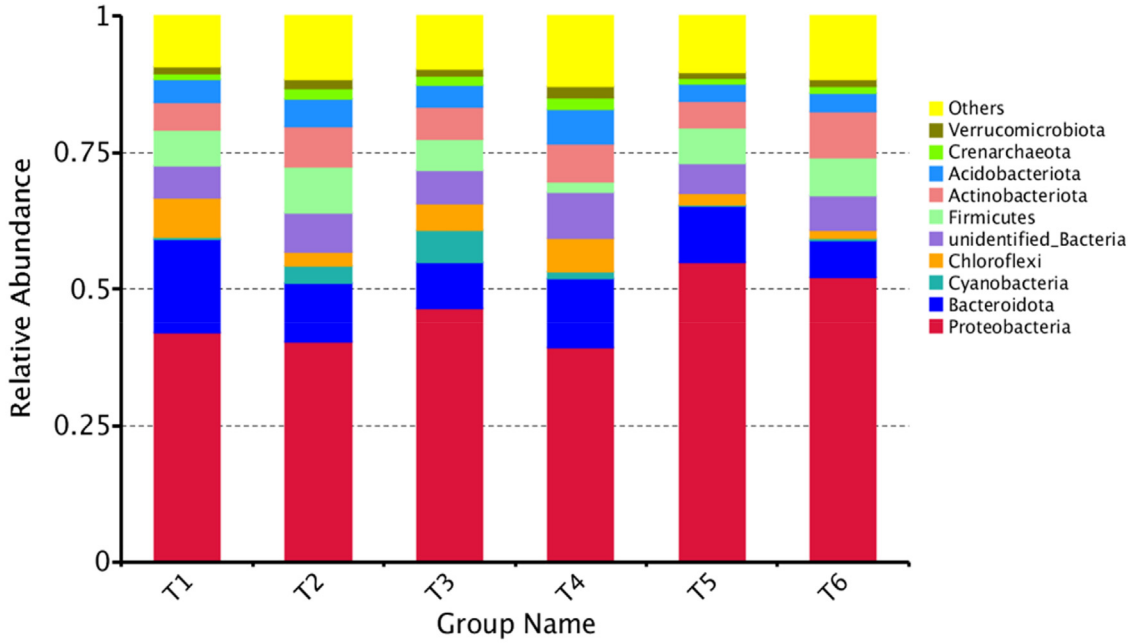
MPs increased the accumulation of Phe in soybean stems at the end of experiment. MPs and Phe co-exposure treatment damaged the soybean plant cells and micron-size MPs (100 μm) caused the highest toxicity to cells, which is consistent with the higher accumulation of di-butyl phthalate in lettuce by micron-size MPs (Gao et al., 2021). In this study, we further demonstrated that gene injury in soybean roots was related to the MP sizes, consistent with the results of the oxidative stress assays (ROS, MDA, CAT, SOD and POD enzyme). Gene injury could be related to oxidative stress induced by ROS (Jiang et al., 2019). Therefore, damages in soybean tissues partially contributed to the accumulation of Phe by soybean stems.

The leguminous plant root cell wall pores have an average radius of 7–20 nm (Hylmö, 1958), MPs accumulate at the root surface of soybean and barely enter the plant due to their larger size. Therefore, the uptake of nutrients and water through plant root tips were blocked and the activity of roots was inhibited (Jiang et al., 2019). Due to the inhibition of MPs on root activity, the ability of taking up Phe was restricted, therefore, the concentrations of Phe in soybean roots and leaves were reduced. A previous study has also found that MPs can induce the accumulation of As in rice by decreasing the root activity (Dong et al., 2020). These results revealed that the size of MPs is an important factor in determining the uptake and distribution of Phe in crops.

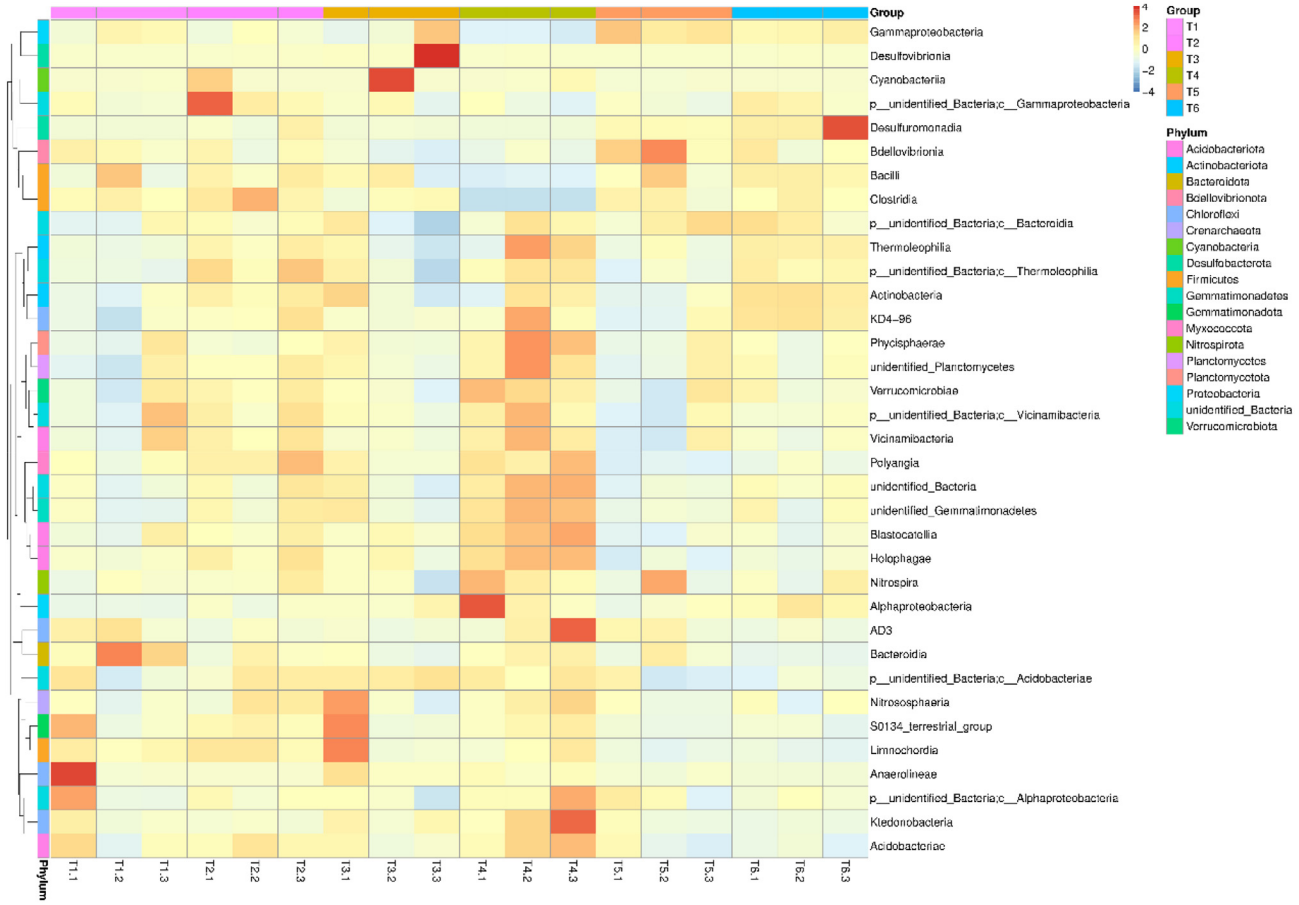
MPs in soils enhanced the toxicity of Phe to soybean plants, and the micron-size MPs led to more oxidative damage and affected the gene expression of soybean roots. The relative gene expression levels of SOD, POD, CAT and contents of MDA and ROS indicated the chronic toxicity of micron-size MPs to soybean roots and leaves, which may explain the decrease of Phe in roots and leaves in groups T2-T4. To further explore its mechanism, microbial community structures in soybean rhizosphere soil were measured by high-throughput 16S rRNA sequencing analysis.

4.3. Co-contaminant effects on soybean rhizosphere soil microbiota

The microbiota community composition and structure of soybean rhizosphere soils were significantly changed after co-exposure of MPs and Phe. MPs can affect soybean root activity, and exposure to MPs may alter root exudates and thus lead to changes in the rhizosphere soil microflora. Previous studies have also revealed that MPs impacted rhizosphere soil microbiota via changing soil physical properties and nutrient conditions in agricultural soils (Huang et al., 2019; Ren et al., 2020; Wang et al., 2020). *Bacteroidetes* are considered to be specialized in degrading complex organic matter in biosphere, which are widely distributed in various ecosystems (Wolińska et al., 2017). *Proteobacteria* prefer to colonize in nutrient-rich and low bulk density soils. Recently, researchers found that PE MPs increased the rates of soil water evaporation and decreased the soil moisture contents (Wan et al., 2019), which could change microbial community in soil. In this study, rhizosphere soil



(A)



(B)

Fig. 6. Relative abundance of bacterial at phylum (A) and clustering map of species abundance (B) in the rhizosphere soil among different treatments. Categories with relative abundance <1% were clustered into "Other".

diversity decreased significantly after exposure to MPs and Phe compared with the CK, which is supported by the large number of OTUs in the rhizosphere soil (Fig. 5). Similar results were also found by other researchers (Wang et al., 2020). Some rhizosphere microorganisms could secrete biological surfactants, which promote the uptake of organic pollutants by plants (Zang et al., 2020). It is worth noting that the relative abundance of *Proteobacteria* decreased significantly in rhizosphere soils in co-exposure groups, which contains these microbes. Specifically, the relative abundance of *Proteobacteria* was lower in groups T1-T4, which corresponds with the lower concentrations of Phe in groups T1-T4.

5. Conclusions

MPs decreased the uptake of Phe in soybean roots and leaves by damaging the roots. Micron-size MPs inhibited the soybean root activity and decreased the relative abundance of microbial in rhizosphere soil, leading to reduced uptake of Phe in soybean roots. The co-exposure of micron-size MPs and Phe showed a higher genotoxicity compared with nano-size MPs and Phe. MPs enhanced the toxic effects of Phe at cellular and molecular levels, and modulated kinetics and toxicity of Phe in soybean plants. This study reveals the size effects of MPs on uptake of organic pollutants by higher plants and provides insights into their combined toxicity to plants. The co-exposure of MPs and Phe should attract attention for the safety in production of grain.

CRediT authorship contribution statement

Guanghui Xu: Investigation, Methodology, Writing – original draft. **Yang Liu:** Formal analysis. **Yong Yu:** Supervision, Funding acquisition, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147016.

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