

Responses of soil enzyme and microbial community under co-loading of different microplastics and sulfamethoxazole

Huang Juan Chen Hsuan Cao Meifang Ma Yixuan Qian Xiuwen

(School of Civil Engineering, Southeast University, Nanjing 211189, China)

Abstract: Polyamide/polyethylene (PA/PE) microplastics were injected into soil containing sulfamethoxazole (SMX) to investigate their combined effects on SMX removal, soil enzyme activity, and microbial communities. The results show that both PA and PE transiently increase SMX removal and inhibit the stimulation of microbial species diversity by SMX. The effect of PE is more significant. Meanwhile, PE combined with SMX increases the relative abundances of *Actinobacteria* and *Pseudomonas*, while PA combined with SMX decreases the relative abundances of *Nocardioide*s and *Streptomyces*. In addition, PA/PE combined with SMX can increase dehydrogenase, urease, ammonia monooxygenase, and nitrate reductase activities in the soil while inhibiting the activity of laccase. Compared with PA combined with SMX, the activities of dehydrogenase, urease, ammonia monooxygenase, and laccase of PE combined with SMX increase by 9.82% , 10.41% , 8.07% , and 5.47% , while the activities of nitrate reductase and neutral phosphatase decrease by 1.47% and 6.78% .

Key words: microplastics; sulfamethoxazole; combined effect; soil enzyme; microbial community

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Microplastics (MPs) and antibiotics have received growing attention in recent years as emerging contaminants for their possible threats to the environment^[1]. The amount of plastic waste released into the terrestrial environment each year is 4-23 times the volume of marine inputs^[2]. In soil media, MPs can be detected in agricultural mulch use^[3], biosolid addition^[4], wastewater irrigation^[5], atmospheric deposition^[6], or surface runoff^[7]. Polyamide (PA) and polypropylene (PP) are the main types of microplastic pollutants in soil^[8]. Polyethylene (PE) was the first material to be used for manufacturing disposable plastic bags^[9], and its residues account for 10% of plastic films^[10]. Because of the persistence and

resistance of MPs, they can easily accumulate in terrestrial ecosystems, thus affecting nutrient cycling^[11], energy flow^[12] and stability of ecological functions^[13] in the soil. Sulfonamide antibiotics (SAs) are one of the most commonly used types of antibiotics known to be the largest in the world in terms of production scale and usage^[14]. Sulfamethoxazole (SMX) is the most representative one of these antibiotics. Studies have shown that the highest concentration of SAs was detected in soil, followed by that in water^[15]. Additionally, antibiotics are characterized by difficult degradation^[16], accumulating after entering the environment and causing far-reaching effects on the operation and functions of soil ecosystems^[17].

MPs and SMX coexist in most soil environments. The possible combined effects of the two on soil ecology have now made them the topics of urgent focus for research and improvement. The adsorption of MPs and alteration of soil microbial community structure can directly affect the biodegradation^[18] and migration^[19] behaviors of antibiotics in the soil. The combined effects of antibiotics and microplastics also have a profound impact on soil microorganisms^[20]. However, most of the studies considered only the effect of MPs in combination with antibiotics on antibiotic removal and nitrogen cycle-related enzyme activities in soil. Systematic comparative studies on the effect of different MPs in combination with SMX on soil ecology were lacking.

Thus, this study aims to explore the combined effects of SMX and different MPs on SMX removal from soils, with or without PA or PE injection, and various impacts on soil enzyme activities and microbial communities with different MPs.

1 Materials and Methods

1.1 Soils and chemicals

Test soil was taken from unused land without PE, PA, and SMX exposure history at the Jiulonghu Campus of Southeast University (Nanjing, China). In addition, there are no potential sources of contamination from either substance in the surrounding area. To minimize sampling variance, five soil samples were manually collected in the surface layer (0-20 cm) with a shovel and mixed thoroughly. Collected soil samples were air-dried and sieved through a 2-mm sieve to remove gravel, debris, and plant

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Biography: Huang Juan (1980—), female, doctor, professor, 101010942@seu.edu.cn.

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rhizomes. Various soil samples were stored in a refrigerator at 4 °C as backup. The potential of hydrogen in the soil sample was determined to be 6.70, with an organic matter content of 14.5 g/kg, effective nitrogen content of 0.1507 g/kg, effective phosphorus content of 0.0436 g/kg, and effective potassium content of 0.1244 g/kg.

SMX, 98% purity, and acetonitrile (chromatographic grade) were purchased from Aladdin Biochemical Technology (Shanghai) Company. PA powder (without additives) was purchased from Sheng Hao Plastic Company. Other reagents (analytical grade) were provided by McLean Biochemical Technology Company. SMX solution with a concentration of 100 mg/L was prepared with ultra-pure water and stored in a refrigerator at 4 °C.

1.2 Experimental design

According to Ref. [21], the average abundance of MPs of Chinese farmland was 4536.6 items/kg. PA and PE were the most abundant polymers in the arable land^[22]. Considering the large-scale production and emissions of plastic products in the future and the dose selection of microplastics in the relevant literature^[23–24], the exposure dose of PA and PE in this study was set at 1% of the dry weight of the soil. Studies have shown that the highest concentration of sulfamethazine (SMZ) in Chinese soil is 0.001% of that of soil dry weight^[25]. Because of the accumulation of SMX in soil and amplification of the concentration in the same type of research^[26], the exposure level of SMX in this study was set at 0.01% of that of soil dry weight.

Four groups of experimental equipment were set up in this study. Among them, the CK group is the control group treated only with ultra-pure water; the SMX10 group refers to the treatment group with the 0.01% soil mass fraction SMX alone; the PA-SMX10 group refers to the treatment group with the 0.01% soil mass fraction SMX combined with 1% soil mass fraction PA. PE-SMX10 refers to the treatment group exposed by a concentration of 0.01% soil mass fraction of SMX combined with a concentration of 1% soil mass fraction of PE. The selected microplastic particle size was 75–80 µm. Three replications were performed for each treatment group. Each device set contained 2 kg of soil and was covered by a tin foil with 12 small holes at the top. The sides and bottom of the devices were covered with black tape. Before the start of the formal experiment, the soil moisture content was ensured to be at 60% of the maximum water holding capacity and preincubated at 25 °C for 14 d. Formal experiments were begun with one period of SMX application, each period of continuous incubation for 15 d, and the device was operated continuously for three periods.

1.3 SMX removal rate measurement

1 g of soil samples were taken on the 1st, 3rd, 7th, 11th, and 15th days of each phase of equipment operation

for quantitative monitoring of SMX, the CaCl₂ extractable state content of SMX. The extraction method was based on Technical Report No. 117 of the European Center for Ecotoxicology and Research on Chemicals (ECETOC). Specific steps were as follows: 5 mL of 0.01 mol/L CaCl₂ solution was added to soil samples, and extraction was conducted at a temperature of 25 °C for 8 h with an oscillation frequency of 200 revolutions per minute. Centrifugation was carried out, and the supernatant was collected. The extraction was repeated thrice, and the supernatants were combined. The supernatant combination was filtered through a 0.22 µm filter membrane and transferred to a liquid phase brown injection bottle. The supernatant was filtered by a 0.22 µm filter membrane and transferred to a liquid phase brown injection bottle for quantitative detection of SMX concentration by ultra-high performance liquid chromatography combined with ultra-violet detector (Waters, UPLC-UV). The inner diameter of the Waters BEH C18 column was 2.1 mm; the length was 100 mm; and the diameter of the stationary phase particles filled in the column was 1.7 µm. The detection temperature was 30 °C; each sample size was 10 µL; the flow rate was 0.25 mL/min; the detection wavelength was 270 nm; the mobile phase solution volume ratio of 0.01 mol/L H₃PO₄ was 80%, and that of acetonitrile was 20%.

1.4 Measurement of soil enzyme activity

Soil enzyme activities in the present study were spectrophotometrically measured. Urease (UR) activity was measured using the method described by Fang et al^[27]. Laccase (LA) activity was determined using the method described by Bourbonnais et al^[28]. Dehydrogenase (DHA) was assessed by measuring the reduction reaction of 2,3,5-triphenyltetrazolium chloride^[29]. The activity of ammonia monooxygenase (AMO) was expressed by measuring the amount of nitrous produced using the method of Zheng et al^[30]. The nitrate reductase (NAR) activity was expressed by determining the nitrate nitrogen content consumed using the method of Chen et al^[31]. Neutral phosphatase (PST) activity was determined by measuring the absorbance at 405 nm^[27].

Soil from four groups of installations, CK, SMX10, PA-SMX10, and PE-SMX10, was sampled. The above six enzyme activities were then measured on the 1st, 3rd, 7th, 11th, and 15th days of each period for three consecutive periods. The average enzyme activity measurements for the CK group without any added contaminants were set at 100%, and three parallel groups were then set up. The relative activity of soil enzymes in each pollutant exposure group was calculated as a percentage of the measured value in each pollutant exposure group compared to the CK group.

1.5 Microbial community composition determination

After three periods of device operation, the soil in four

device groups, CK, SMX10, PA-SMX10, and PE-SMX10, was sampled and sent to Megger Bio-technology Ltd. in Guangzhou, China, for 16S rRNA high-throughput sequencing. Soil DNA was extracted according to the instructions of MOBIO PowerSoil® DNA Kit, and then the V4-V5 region of microbial 16S rRNA was amplified using the Illumina Nova 6000 platform with primers with barcode and PremixTaq. The V4-V5 region of microbial 16S rRNA was amplified according to the standard procedure of NEBNEXT® UltraTM II DNA Library Prep Kit. A sequencing library was established according to the standard process of NEBNEXT® UltraTM II DNA Library Prep Kit. After data filtering, splicing, and sequence quality filtering, Usearch software was used to cluster the OTUs at a 97% similarity level using the UPARSE method and the longest sequence in each OTU was selected as the representative sequence for subsequent data analysis.

1.6 Statistical analysis

All data in this study were processed and analyzed by using Excel and SPSS 22.0. One-way ANOVA and LSD were used to test the significance of soil enzyme activities. A significant difference was considered when *p* was less than 0.05.

2 Results and Discussion

2.1 Impact of various MPs on the removal rate of SMX

Fig. 1 shows the variations of SMX concentration and removal for each pollutant exposure group. The SMX removal of each pollutant group showed a decreasing trend with the increase in the number of SMX injections. Compared to the SMX10 group, the PA-SMX10 group showed an increase in the average removal of SMX by 4.44% and 2.71% during the periods I and II, respectively. It is hypothesized that SMX in the soil was adsorbed by PA at the beginning of the experiment, thus increasing the SMX removal rate. It is also possible that microorganism growth was stimulated by exogenous pollutants, thereby increasing the abundance of organic matter-degrading bacteria. With the increase in SMX exposure time and the secondary dosing of SMX, PA can reach adsorption saturation, and the phenomenon of SMX desorption can appear^[32]. These changes can lead to a gradual increase in SMX concentration in the soil. Note that the average removal of SMX in the PE-SMX10 group was 0.71% lower in the period I than that in the SMX10 group, while it was 8.83% higher in the period II. The influence trend is different from that of dosing PA. This can be due to the fact that PE has a weaker adsorption capacity than PA^[33], resulting in less SMX adsorption at the beginning of the experiment. As the exposure time of SMX increased, PE adsorbed more SMX from the soil, thus contributing to the removal rate of SMX. By comparing the PA-SMX and PE-SMX10 groups, it is found that different

MPs had different effects on SMX removal. Moreover, the average SMX removal of the PA-SMX10 and PE-SMX10 groups in the period III were 3.65% and 1.82%, respectively, lower than that in the SMX10 group. This can be due to the desorption of SMX by PA and the aging of MPs^[34]. The results show that PE has a slight advantage over PA during SMX removal.

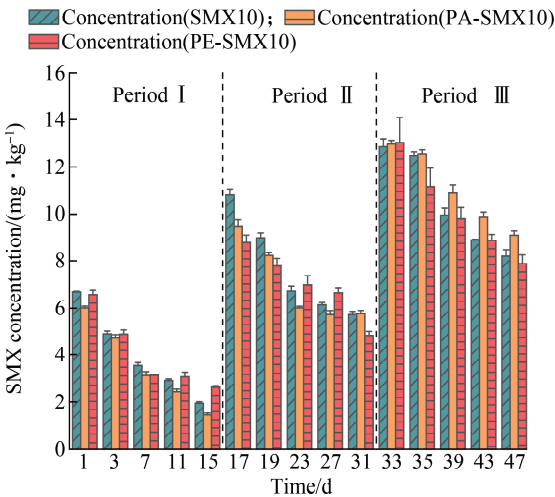


Fig. 1 SMX removal and concentration changes for each pollutant exposure group

2.2 Soil-enzyme activity under co-loading of MP and SMX

Responses of key soil enzymes can reflect the toxicity stresses of MPs and SMX. Fig. 2 demonstrates the effect of different MPs combined with SMX on DHA, UR, AMO and NAR activities in soil, and * in the figure indicates a statistically significant difference (*p* < 0.05). Fig. 2 (a) demonstrates variations of DHA activity in each pollutant group, reflecting the effect of different pollutants on the ability of soil microorganisms to remove organic pollutants^[35]. All pollutant exposure groups show significant and persistent inhibition of DHA (*p* < 0.05). The SMX10 group shows the strongest effect in the period III. A decrease in the relative activity of DHA by 48.61% was observed in relation to the CK group, which is consistent with previous studies^[36]. During the period I, DHA inhibition was lower in all combined exposure groups than in the SMX10 group. However, in contrast to the PE-SMX10 group, the inhibition rate of DHA activity was higher in the PA-SMX10 group than in the SMX10 and PE-SMX10 groups during the periods II and III. Similar to the change in DHA activity, understanding the change in the activity of UR in each pollutant group (see Fig. 2(b)) helps understand the effects of different pollutants on the ability of soil to supply nitrogen^[37]. The SMX10 and PA-SMX10 groups exhibited significant inhibition of urease activity (*p* < 0.05) in contrast to the PE-SMX10 group, which showed a weak effect on urease activity. During the periods I and II, the inhibition of

urease activity was lower in the combined exposure group than in the SMX group. However, the PA-SMX10 group showed a higher inhibition compared to the SMX10 group during the period III, which was opposite to that of the PE-SMX10 group. Similarly, the variations in AMO activity in each pollution group (see Fig. 2(c)) reflect the impact on the soil nitrification process^[38]. The SMX10 group showed inhibition of AMO activity during the periods I and II and promotion in the period III. This differed from the trend of influences in the combined exposure group. All contamination groups showed significant inhibition of AMO activity in the period I ($p < 0.05$). However, in the period II, the co-exposure group exhibits a promoting effect on AMO, in contrast to the SMX10 group. During the period III, all the pollutant groups exhibit a promoting effect on AMO activity. The PA-SMX10 group has a higher rate of effect on AMO activity than the PE-SMX10 group in the period II and lower

than the PE-SMX10 group in the period III. As an enzyme involved in the denitrification process^[39], the variations of NAR activity in each pollutant group can be observed in Fig. 2(d). NAR activity in the SMX10 group indicates an inhibition-promotion trend, and the combined exposure group shows a continuous promotion of NAR activity. During the period I, the co-exposure groups exhibited a promotion of AMO activity, which was in contrast to the SMX10 group. During the periods II and III, all pollutant groups show promotion of NAR activity. However, the PA-SMX10 group shows lower promotion than the CK group in period III, which is different from the PE-SMX10 group.

Analyzing the changes of the four enzyme activities mentioned above, at the beginning of the experiment, MPs can weaken the inhibitory effect of SMX on soil enzyme activities by adsorbing SMX in the soil. Nevertheless, as SMX was dosed several times, PA underwent a

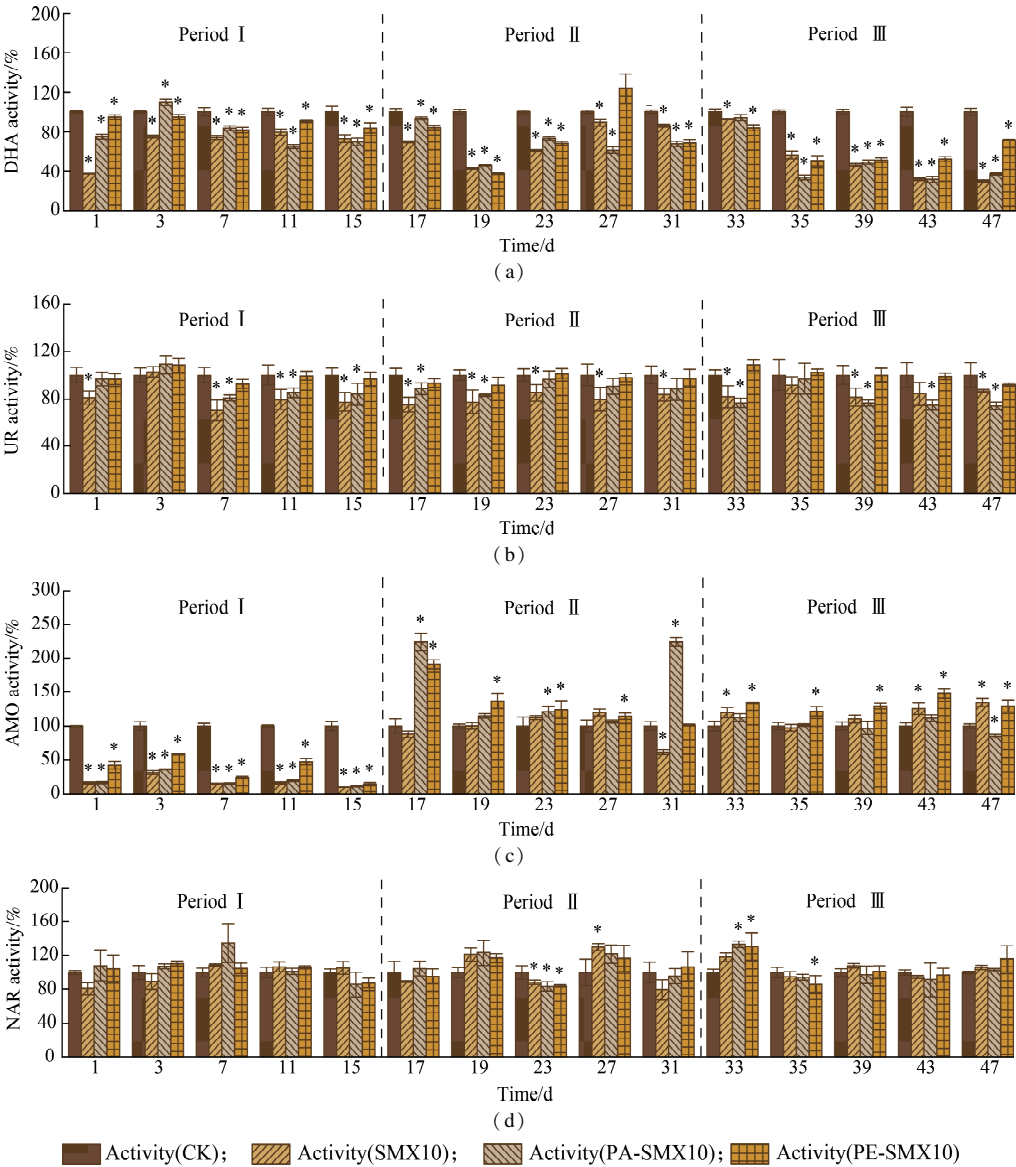


Fig. 2 Changes in different enzyme activities and absolute impact rates of each pollutant exposure group. (a) DHA; (b) UR; (c) AMO; (d) NAR

desorption-adsorption cyclic process because of adsorption saturation, which altered the SMX concentration in the soil and thus had a fluctuating effect on soil enzyme activities. In contrast, PE can undergo aging and thus adsorb additional SMX, thus reducing the SMX concentration in the soil and weakening the inhibition rate on soil enzyme activity^[40]. The variations of SMX concentration in Fig. 1 can support this speculation. The whole experiment shows that compared with PA combined with SMX exposure, PE combined with SMX exposure increased DHA, UR, and AMO by 9.82%, 10.41%, and 8.07%, respectively, and decreased NAR activity by 1.47%. The experiment also revealed that PE can easily alleviate SMX inhibition on soil compared to PA.

Fig. 3 demonstrates the effect of different MPs combined with SMX on PST and LA activities in soil. * in the figure indicates a statistically significant difference ($p < 0.05$). PST is often used as an important indicator of the ability of soil microorganisms to transform organic phosphorus decomposition and the level of bio-effective phosphorus^[41]. As shown in Fig. 3 (a), all pollutant groups showed an increase in PST activity in all three periods, with a significant difference in PST activity in the period I compared to the CK group ($p < 0.05$). Co-loading of PE and SMX resulted in higher PST activity than that in the SMX10 group, while relative activity in the PA-SMX10 group was even lower than SMX10 by 3.29%-3.36%. In the present study, PE is more prone to reduce SMX hazards on PST compared to PA. The effects of different pollutants on the ability of microorganisms to degrade organic pollutants were explored by

analyzing the changes in LA activity^[42] in each pollution group (see Fig. 3(b)). The LA activities of both the SMX10 and PE-SMX10 groups showed a trend of promotion-inhibition-promotion, while only the inhibition-promotion trend was observed in the case of the PA-SMX10 group. Compared to the SMX10 group, the PE-SMX10 group exhibited a higher effect on LA activity during the periods I and II, while the effect was lower in the period III. In contrast, the PA-SMX10 group exhibited an inhibitory effect on LA activity in periods the I and II, whereas it showed a weak promoting effect during the period III.

The effects of different pollutants on the activities of PST and LA differed from those of the four enzymes mentioned above. During the period I, PA adsorbed SMX from the soil, thus inhibiting the promotion of PST and LA activities by SMX^[43]. In contrast, PE may have enhanced the ecological effects of SMX when it was present alone^[44]. It indicated that the different effects of MPs on phosphatase can be related to the type of MPs, soil type, and coexisting pollutants. As SMX was dosed multiple times, PA desorbed and increased the SMX concentration in the soil, creating a difference in the changes of the two enzyme activities. During the periods II and III, the increase in SMX concentration inhibited its promoting effect on PST activity. In contrast, LA activity may have been increased by microorganisms viewing SMX as a carbon source, stimulated by increased SMX concentration^[45]. In addition, PE can undergo aging with time, increasing the adsorption capacity for SMX, thereby differentially affecting the two enzyme activities.

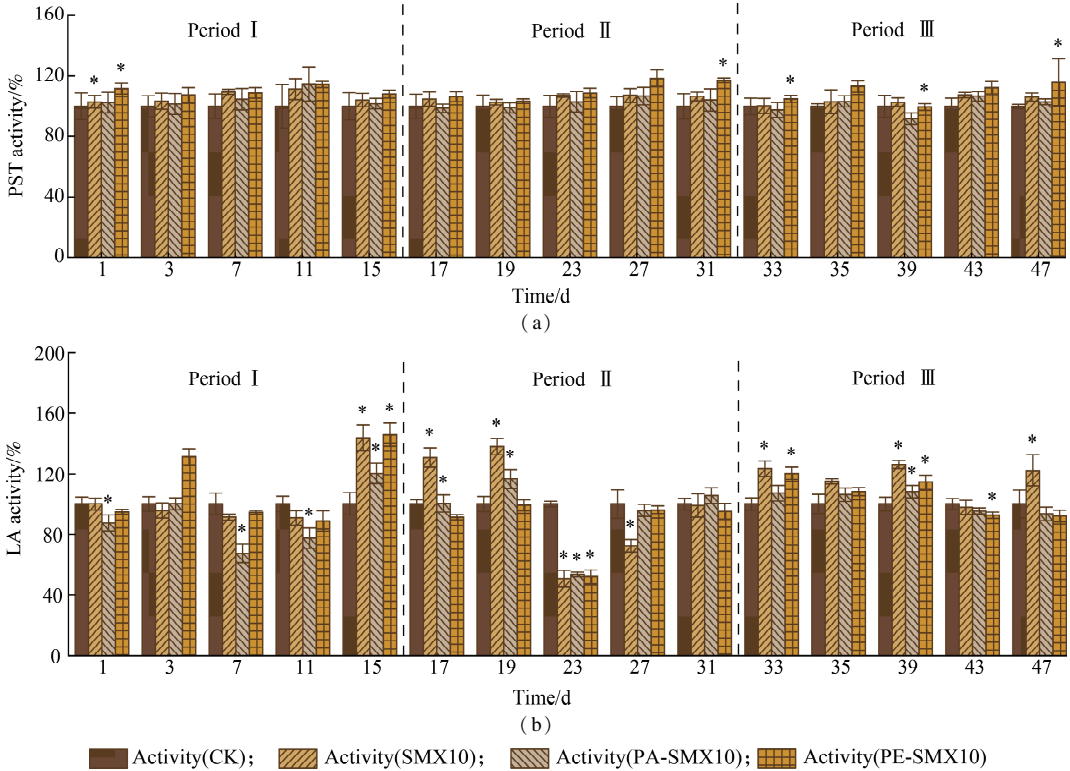


Fig. 3 Changes in different enzyme activities and absolute impact rates for each pollutant exposure group. (a) PST; (b) LA

In the case of PST, PE adsorption of more SMX resulted in a smaller facilitated decrease in enzyme activity than in the SMX10 group. Nevertheless, the higher inhibition of LA activity in the period II can be due to the lack of growth factors for the microorganisms because of SMX adsorption. During the period III, PE adsorption was saturated and microbial activity was revitalized because of the increase in SMX concentration in the soil. The variation of SMX concentration in Fig. 1 can support this speculation. From the whole experiment, compared with PA combined with SMX exposure, PE combined with SMX exposure increased LA by 5.47%, while PST activity decreased by 6.78%. These results further indicate that the effects of different MPs and SMX combinations on soil enzymes were different.

2.3 Microbial diversity under co-loading of MP and SMX

2.3.1 Differences in community structure and analysis of functional microorganisms

As shown in Table 1, the Chao1 indices of SMX10, PA-SMX10 and PE-SMX10 were higher than that of the CK group, suggesting that SMX can elevate soil bacterial community richness, regardless of coexistence with MPs. However, compared to the SMX10 group, lower Chao1 was discovered in co-exposed groups, which implied that MPs curtailed the augmented species richness effect prompted by SMX, particularly in the PE group. On comparing the PA-SMX10 group and PE-SMX10 group, it was found that the difference in the Chao1 index between the two was small. This indicates that the effect of different microplastic types combined with SMX exposure on species richness was not significant. Besides, both Simpson and Shannon indices in the PA-SMX10 group were higher than those in PE-SMX10, which indicates that PA addition is more likely to raise microbial diversity in soil with SMX exposure.

Table 1 Statistics on the number of sample sequences, number of OTUs and alpha diversity index

Sample ID	Sequence No.	OTUs No.	Index		
			Chao 1	Simpson	Shannon
CK	65 666	2 281	2 281.4	0.042 5	5.15
SMX10	58 164	2 687	2 687.2	0.036 3	5.39
PA-SMX10	58 927	2 586	2 586.2	0.038 2	5.35
PE-SMX10	55 281	2 571	2 571.2	0.041 2	5.26

2.3.2 Analysis of microbial community

Relative abundance above 0.01% was selected and analyzed for abundance of dominant microorganisms at the phylum level (see Fig. 4(a)). In view of bacteria at the phylum level, *Actinobacteria* (36.57%-58.76%) was the dominant phylum with the highest relative abundance, which was involved in the process of decomposition and denitrification of organic matter^[46]. In SMX ex-

posure and co-loading groups, it was 18.82%-22.19% lower than that in CK, suggesting that *Actinomycetes* responded sensitively to SMX and MPs. Moreover, the PA-SMX10 group exhibited an adverse effect on the *Actinobacteria* phylum, while the PE-SMX10 group demonstrated a stimulatory and promotive effect on the same phylum. It implied that variations in microplastic types contribute to divergent outcomes regarding the effects of combined SMX exposure on the *Actinobacteria*. In contrast, the abundance of *Proteobacteria*, *Bacteroidetes*, and *Acidobacteria* in each pollutant group was elevated compared to the CK group. The relative abundance of *Proteobacteria* increased by 19.18%-20.25% from 16.87% in the CK group by the exposure of SMX alone and by the combined exposure of MPs and SMX. Analyzing the differences in *Bacteroidetes* showed that the relative abundance of *Bacteroidetes* in the SMX10, PA-SMX10, and PE-SMX10 groups increased to 12.14%, 13.01%, and 12.12%, in comparison with the CK group. Compared with the results of the individual and combined exposure groups, PA slightly enhanced the promotion effect of SMX, while PE showed no effect. For *Acidobacteria*, the relative abundance ranged from 9.50% to 10.56% in the contaminant group, all higher than that in the CK group (6.32%). Studies have shown that the *Proteobacteria* phylum has the ability to transform sulfonamide antibiotics^[47], and the *Acidobacter* phylum has the ability to degrade plastic polymers. *Bacteroides* and *Acidobacteria* both have the potential to degrade organic pollutants in the environment^[48]. The increase in the relative abundance of *Ascomycetes*, *Anaplasma*, and *Acidobacteria* indicated that soil microorganisms responded to MPs and SMX pollution threats. In addition, the relative abundance of *Thaumarchaeota* in the SMX10, PA-SMX10, and PE-SMX10 was higher than that in the CK group. It was reported that the MPs and SMX enhanced the ammonia oxidation process within the soil^[49], which is consistent with the increased activity of AMO in period III. The relative abundance of the phylum *Nitrospiraea* also increased, with *Nitrospira* being the dominant nitrifying bacterium in the soil as a genus of this bacterial phylum.

Further analysis of microbial community composition was performed at the class level. As shown in Fig. 4(b), the largest proportion was in *Actinobacteria* (33.71%-52.36%), aligned with that of phylum *Actinobacteria* (see Fig. 4(a)). It was followed by *Anabaena* (2.86%-12.96%), γ -*Ascomycetes* (8.56%-12.36%), *Bacillariophyta* (6.65%-9.23%) and α -*Ascomycetes* (6.16%-7.69%). Note that *Anaerolineae* have a denitrification function^[50]. Its abundance percentage was 1.21%-1.50% in the pollutant exposure group, which was higher than the control group (0.72%). It suggests that the denitrification process within the soil can be enhanced, consistent

with the weak increase in NAR activity that occurred in the previous section.

The 30 genera with the highest relative abundance were selected to form a heat map (see Fig. 5). Except for the undefined and uncultured genus, *Bacillus* (6.08%-8.46%) was the genus with the highest relative abundance. It was shown that *Bacillus*, *Pseudomonas*, and *Flavobacterium* are involved in phosphorus solubilization and nitrogen fixation^[51-52]. Compared with group CK, the relative abundance of *Bacillus*, *Pseudomonas*, and *Flavobacterium* in the groups SMX10, PA-SMX10, and PE-SMX10 increased by 34.20%-39.20% ,

42.69%-164.03% , and 152.15% -186.66% , respectively. Meanwhile, the relative abundance of these bacteria in the PE-SMX10 and PA-SMX10 groups was higher than that in the SMX10 group, with the highest abundance observed in the PE-SMX10 group. The results showed that both SMX alone and SMX combined with MPs were beneficial to soil nitrogen and phosphorus cycling, and PE combined with SMX showed a higher promoting effect. The relative abundance of *Bacillus* in the PA-SMX10 group was 3.73% higher than that in the PE-SMX10 group. It indicated that different MPs combined with SMX had different effects on the microorgan-

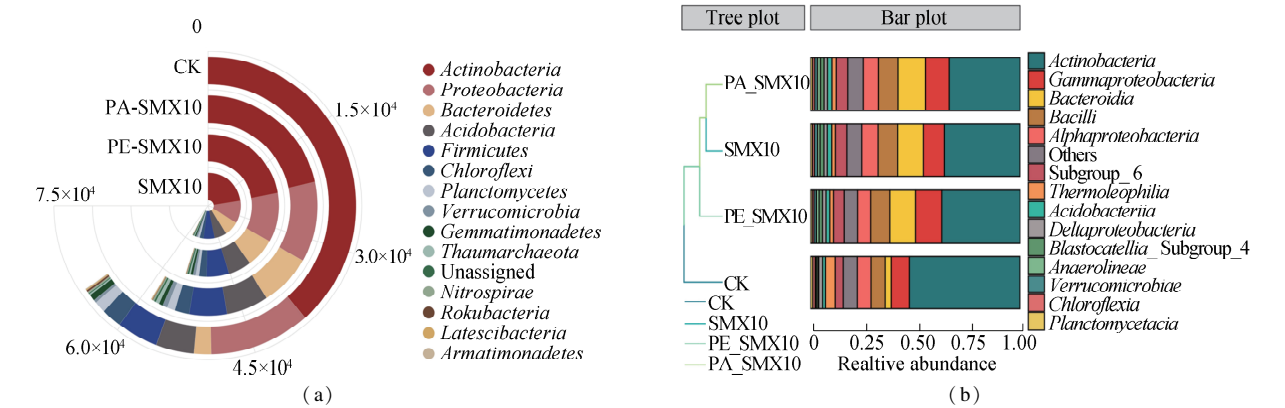


Fig. 4 Microbial community composition. (a) Phylum level; (b) Class level

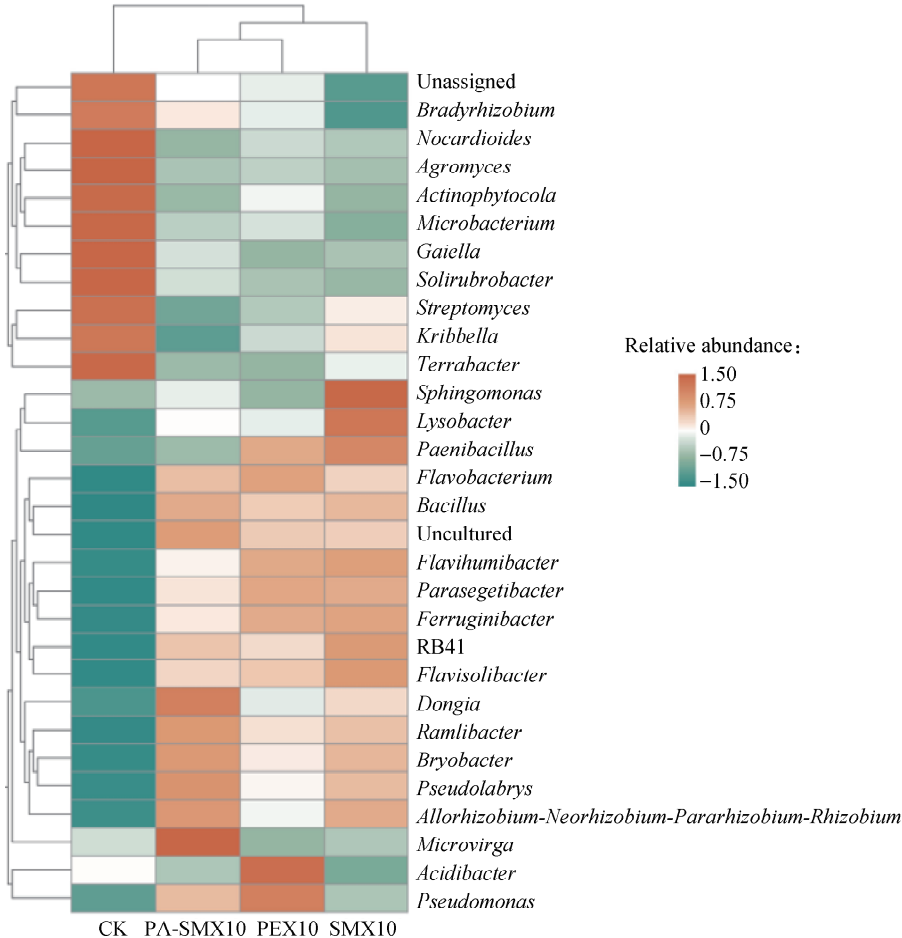


Fig. 5 Relative abundance of the top 30 genera

isms, which are involved in soil nitrogen and phosphorus conversion. Moreover, it was shown that *Pseudomonas* is involved in the degradation of SMX and MPs^[53]. Compared with the SMX10 group, the *Pseudomonas* in the PA-SMX10 and PE-SMX10 groups increased by 51.65% and 85.03%, respectively, indicating that the MP and SMX association promoted the growth and enrichment of degrading bacteria. Simultaneously, the abundance of *Pseudomonas* in the PE-SMX10 group was higher than that in the PA-SMX group, indicating that the combination of PE and SMX had a more obvious promoting effect on degrading bacteria. Studies have shown that *Nocardioides* and *Streptomyces* are the dominant genera of soil carbon sequestering microorganisms^[54-55], and their relative activities in SMX10, PA-SMX10 and PE-SMX10 groups decreased by 43.17%-53.83% and 28.12%-52.42%, compared with CK groups. The results indicated that SMX alone exposure and SMX combined exposure with MPs can adversely affect the retention and stability of soil organic carbon. The relative abundances of *Nocardioides* and *Streptomyces* in the PE-SMX10 group (2.84% and 4.05%) were higher than those in the PA-SMX10 group (2.29% and 3.36%). It shows that the inhibition effect of PA combined with SMX is stronger. The relative abundance of *Nocardioides* in the PE-SMX10 group was 8.86% lower than that in the SMX10 group. It was shown that PE injection can reduce the inhibitory effect of SMX on *Nocardioides*.

3 Conclusions

- 1) MPs may ephemerally enhance the removal of SMX through adsorption. With multiple SMX dosing, PA can undergo a cyclic process of adsorption-desorption because of adsorption saturation. PE has a slight advantage over PA for the removal of SMX.
- 2) MPs can inhibit the toxic effects of SMX on DHA, UR, AMO and NAR and the promotion of effects of PST and LA by adsorbing SMX in soil. The results show that the combined effect of PE and SMX on soil enzymes is better than that of PA.
- 3) The addition of MPs inhibited the stimulatory promotion of soil microbial diversity and abundance by SMX exposure alone. PE was more inhibitory than PA. In addition, SMX exposure alone and combined exposure of MPs and SMX stimulated the growth and enrichment of organic-degrading bacteria, ammonia-oxidizing archaea, and soil dominant nitrifying bacteria.

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不同微塑料和磺胺甲噁唑共同负载下土壤酶和微生物群落响应

黄娟 陈璿 曹美芳 马溢轩 钱秀雯

(东南大学土木工程学院,南京 211189)

摘要:分别将聚酰胺(PA)和聚乙烯(PE)微塑料投加至含有磺胺甲噁唑(SMX)的土壤中,研究其对SMX去除、土壤酶活性和微生物群落的影响.结果表明,PA和PE均能短暂提高SMX的去除率,抑制SMX对微生物物种多样性的刺激,且PE的影响更为显著.PE联合SMX可提高放线菌和假单胞菌的相对丰度,PA联合SMX则可降低类诺卡式菌和链霉菌的相对丰度.PA/PE联合SMX诱使土壤中脱氢酶、脲酶、氨单加氧酶、硝酸还原酶活性提高,同时抑制了漆酶活性.与PA联合SMX相比,PE联合SMX组的脱氢酶、脲酶、氨单加氧酶和漆酶活性分别提高9.82%、10.41%、8.07%和5.47%,硝酸还原酶和中性磷酸酶的活性则分别降低1.47%和6.78%.

关键词:微塑料;磺胺甲噁唑;综合效应;土壤酶;微生物群落

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