Environment International 161 (2022) 107096

Contents lists available at ScienceDirect

Environment International

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

Seasonal variations and feedback from microplastics and cadmium on soil organisms in agricultural fields

Xiaofeng Jiang [a], Yang Yang [a], Qian Wang [a], Na Liu [b], Mei Li [a,*]

[a] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023, China
[b] Norwegian Research Center NORCE, Nygårdsgaten 112, 5008 Bergen, Norway

ARTICLE INFO

Handling Editor: Da Chen

Keywords:
Plastic film mulching
Cadmium
Seasonal variations
Polyethylene microplastics
Multiomics techniques

ABSTRACT

Plastic film mulching is an important agricultural technology that plays a critical role in increasing crop yield and maintaining soil moisture. However, long-term coverage and untimely recovery lead to a large amount of plastic residues in soils. This decomposes into smaller plastics over time, which can reduce sowing quality, destroy the soil structure, and have adverse effects on soil organisms. In this study, the seasonal variations and correlations of microplastics and cadmium (Cd) in Wuxi farmland soils of Taihu Lake, China, were investigated in the spring and winter. The microplastics were mainly in the form of films, fibers, and debris and were mainly transparent and black in color. Microplastic abundance reached 890 particles/kg soil, with the majority of microplastics (>72.5%) being 0–500 μm. Polyethylene microplastics were the main polymers, accounting for >54.65%. In addition, the abundance of soil microplastics in the winter was significantly correlated with Cd, indicating that microplastics and heavy metals present a risk of coexposure to soil organisms. Furthermore, the response of *in situ* earthworms to microplastic–Cd pollution revealed that microplastics can be used as a vector to transfer heavy metals in the soil environment and may accumulate in the bodies of soil organisms. Multiomics techniques demonstrated bacterial community structure dysbiosis and metabolic changes of *in situ* earthworms under microplastic heavy metal-contaminated soils. The abundance of microplastics in earthworm casts and intestines was higher than that in the soil samples. These results reveal the potential risks from microplastics entering the soil environment and heavy metal pollution in soil ecosystems.

1. Introduction

Plastic products are widely produced and used in daily life. According to a report from the European Plastics Association, global plastic production reached 348 million tons in 2017 (PlasticsEurope, 2018). However, due to the untimely recycling of plastic products, an increasing amount of plastic waste is discharged into the environment. These plastics can degrade into microplastic particles of <5 mm via light, physical, or chemical actions. Microplastic pollution in marine, freshwater and estuarine ecosystems has recently attracted widespread attention (Horton et al., 2017a; Li et al., 2018; Han et al., 2020; Zhang et al., 2021).

Soil environments have received much less attention, even though the microplastics released into soil environments are 4–23 times higher than those in aquatic environments (Horton et al., 2017b). The sources of microplastics in soil vary and include sewage irrigation, tire wear, atmospheric deposition, organic fertilizer application, sludge utilization, and agricultural plastic film residue (Baensch-Baltruschat et al., 2021; Corradini et al., 2021; Yang et al., 2021). In the agricultural soils of China, plastic film residue is the main source of microplastic pollution, owing to its intensive application and improper disposal (Horton et al., 2017b; Chen et al., 2020; Y. Huang et al., 2020b; Li et al., 2020). However, to collect the mulching film from farmlands is time and labor-consuming, thus many of them are left in the agricultural soil with intentionally or unintentionally (Yang et al., 2021). Plastic mulching is an important agricultural technology that can improve crop yield and retain soil moisture (Y. Huang et al., 2020b; Yang et al., 2021). China is the largest user of plastic film mulching in the world. From 1991 to 2017, approximately 3–14.7 $\times$ 10^5 tons of plastic film was applied for crop cultivation (Xue et al., 2017). Although the use of mulching film can increase crop yield by 20–50%, large amounts of residues after crop harvest accumulate in the soil and eventually decompose into microplastics (Shi et al., 2017; Haixin et al., 2022). Residual microplastics can adhere to the skin of organisms and inhibit their mobility (Kim and An,

* Corresponding author at: 163 Xianlin Ave., Nanjing University, Nanjing 210023, China.
E-mail address: meili@nju.edu.cn (M. Li).

https://doi.org/10.1016/j.envint.2022.107096
Received 12 October 2021; Received in revised form 6 January 2022; Accepted 12 January 2022
Available online 21 January 2022

2019). In addition, microplastic particles might be accidentally ingested by soil organisms and transferred through the food chain (Rillig et al., 2017; Song et al., 2019; Guo et al., 2020). The ingestion of microplastics can cause false satiation, both mechanical and physiological damages of organisms, which subsequently leads to potential adverse effects, i.e., feeding disruption, growth decrease, reproductive reduction, energy depletion, intestinal damage, metabolic disturbances and even death (Zhu et al., 2018; Selonen et al., 2020; Y. Huang et al., 2020b). Previous studies indicate that these resultant changes are significantly correlated with the size and shape of microplastics (Lei et al., 2018; Guo et al., 2020; Kim et al., 2020). Furthermore, due to the small size, strong hydrophobicity, and large specific surface area of microplastics, they can absorb toxic pollutants in the soil, such as heavy metals (Vedolin et al., 2018; Zhou et al., 2019). Microplastics could enhance the accumulation of these pollutants in soil organisms and change the bioavailability of the pollutants; thus, they may aggravate the risk to ecosystems (Bradney et al., 2019; Y. Zhou et al., 2020a). In addition, heavy metals adsorbed on microplastics could travel with microplastics and potentially be released into the surrounding environment (Song et al., 2019; M. Li et al., 2021b). Therefore, microplastics and heavy metals can pose a risk of synergistic pollution to the environment, resulting in potentially adverse impacts on soil organisms.

Taihu Lake, located in one of the most industrialized areas, is the third largest freshwater lake in China, and the main source of drinking water for the city of Wuxi, Jiangsu Province (Zhang et al., 2021). The soil properties in nearby regions are closely related to the food security and human health of the whole Taihu Lake basin. The abundance of microplastics in Taihu Lake has reached 0.01–6.8 $\times$ 10^6 items/km^2 in plankton net samples and 11.0–234.6 items/kg dry weight in sediments, indicating serious microplastic pollution in this basin, which could directly affect the safety of drinking water for Wuxi residents (Su et al., 2016). However, the potential microplastic contamination of farmland due to long-term plastic mulching and the toxicological effects on soil organisms have not been well investigated. Furthermore, the abundance and distribution of residual microplastics in farmlands will be influenced by anthropogenic activity and crop cultivation in different seasons (Huang et al., 2021; Haixin et al., 2022). Therefore, it is necessary to understand the abundance and distribution of agricultural mulching microplastics in the soil environment and to study the potential ecological effects of soil microplastics and heavy metal pollution, especially with respect to the toxicological effects on soil organisms.

In this study, the aims were to (1) investigate the seasonal variations in microplastic abundance during spring and winter and the correlation with heavy metals in the soil and (2) explore the response of *in situ* earthworms to synergistic microplastic–heavy metal pollution. These results provide a comprehensive evaluation of the potential risk of microplastics and heavy metals to soil ecosystems.

2. Materials and methods

2.1. Experiment 1: Seasonal variations and correlation analysis of microplastics and Cd

2.1.1. Soil sampling

In April 2019 (spring) and December 2019 (winter), 10 topsoil (0–20 cm) samples were collected from agricultural farmlands in the Wuxi area of Taihu Lake, China (Fig. S1). These samples, from fruit and vegetable farmlands, represent typical agricultural planting patterns that incorporate spring planting with plastic mulching and harvesting in the autumn or winter. The locations, pH, and organic matter of the sampling sites were determined according to our previously published work (Liu et al., 2020) (Table S1). Detailed procedures for measuring the Cd content in soils are provided in the supplementary materials (Text S1). At each site, three duplicate topsoil samples were collected. After removing visible debris and stones, approximately 1 kg of soil per sample was collected using a stainless-steel soil sampler, stored in sterile Ziplock bags, and placed on ice from each sampling site. All soil samples were dried in an oven at 55 °C for 48 h. The soils were then sieved with a 2 mm mesh and stored in aluminum foil bags for further analysis.

2.1.2. Microplastic extraction and digestion

The extraction of microplastics was carried out via density separation, according to Liu et al. (2018) with some modifications. Briefly, a 50 g soil sample was added to a 250 mL conical beaker and mixed with 200 mL saturated NaCl solution. Subsequently, the mixtures were fully stirred for 2 min, oscillated at 180 rpm for 1 h, and then left to settle for 24 h to collect the supernatant. The above settlement procedure was conducted three times to float microplastics. The supernatant was filtered through a 20 μm nylon net filter (Merck Millipore) to obtain the final supernatant. Next, the supernatant was digested with 30% H_2O_2 for 72 h at 50 °C to thoroughly digest the organic matter. The digested solution was then filtered through a 20 μm nylon net filter with a vacuum pump. Finally, the filters were stored in glass Petri dishes and dried at room temperature for microplastic identification and assays. Each test was performed in triplicate (60 filters in total in spring and winter samples).

2.1.3. Observation and identification of microplastics

The number of microplastics on the filters was counted under a stereomicroscope (SteREO Discover V8, Carl Zeiss, Germany). The shape (e.g., fiber, fragment, film) and color (blue, black, white, transparent, brown, green, red) of the microplastic particles were recorded. Accordingly, the microplastics were classified into 12 categories based on their size: <50 μm, 50–100 μm, 100–200 μm, 200–300 μm, 300–400 μm, 400–500 μm, 500–600 μm, 600–700 μm, 700–800 μm, 800–900 μm, 900–1000 μm, and >1 < 5 mm. According to the previous stereomicroscopy results, three random 1 $\times$ 1 cm^2 areas in the middle of each filter were selected with tweezers and further identified by micro-Fourier transformed infrared spectroscopy (μ-FTIR) (Nicolet iN10 MX, Thermo, USA). The spectral range was set at 675–4000 cm^{-1}, and the acquisition time was 3 s with a scan 32 times. To determine the polymer composition of the microplastics, the results were compared with Hummel Polymer and Additives and Polymer Laminate Films (Thermo Fisher Scientific, USA). When the matching degree was ≥70%, the result was considered credible.

2.1.4. Quality assurance and control

To avoid and reduce interference from anthropogenic or external plastics, the stainless-steel sampler was rinsed with ultrapure water prior to use at each location to prevent cross pollution. During the microplastic separation, digestion, and identification processes, all the glass instruments were rinsed 3–5 times with ultrapure water and then dried before the experiments. During the experiments, researchers were required to wear cotton laboratory coats and gloves. In addition, microplastic separation and digestion procedures were conducted in an enclosed environment, and the flasks were covered with aluminum foil bags to reduce atmospheric plastic and operator pollution.

2.1.5. Statistical analysis

The means and standard deviations (SD) for all treatments were calculated using SPSS 23.0 software (IBM, Armonk, NY, USA). Significant differences between the treatments and the control were determined by one-way analysis of variance (ANOVA, $p < 0.05$), followed by Tukey's post-hoc test. Origin 9.0 was used for drawing the figures.

2.2. Experiment 2: Response of in situ earthworms to soil microplastic–Cd pollution

Experiment 2 was performed to investigate the response of *in situ* earthworms to soil microplastic–heavy metal pollution. In September 2020, ten of the previous sampling sites were selected to harvest earthworms and soil. However, earthworms (*Amynthas corticis*, see the

detailed information in Text S2) were only found at three sampling sites, namely, F2, F3, and F6. Therefore, soil samples and 10 earthworms from sites F2, F3, and F6 were stored and carried to the laboratory according to our previous procedures (Liu et al., 2020). The abundances of microplastics in the soils (referred to as samples SF2, SF3, and SF6), earthworm intestines/guts (samples GF2, GF3, and GF6), and casts (samples CF2, CF3, and CF6) were measured according to the procedure outlined in "Experiment 1". Determination of the Cd content in earthworms is described in Text S3.

2.2.1. DNA extraction, PCR, and 16S rRNA sequencing

DNA from the earthworm intestine content or soil (0.5 g) was extracted using the FastDNA Spin Kit (MP Biomedicals, CA) following the manufacturer's directions. The content and purity of the extracted DNA were measured via spectrophotometric analysis (Nanodrop ND-1000, Thermo Fisher) and checked by agarose gel electrophoresis (1%, w/v). Finally, the intestine and soil DNA were stored at –20 °C until further analysis. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified with the forward primer 341F (ACTCCTACGGGAGGCAGCAG) and the reverse primer 806R (GGACTACHVGGGTWTCTAAT). All PCRs were carried out in 30 μL reactions containing 15 μL Phusion®High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM forward and reverse primers, and approximately 10 ng template DNA. Thermal cycling consisted of an initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s, with a final elongation at 72 °C for 5 min. PCR products were mixed in equidensity ratios. The pooled PCR products were then purified with an AxyPrepDNA Gel Extraction Kit (AXYGEN). Sequencing of the 16S rRNA gene was conducted using the Illumina MiSeq platform at Applied Protein Technology Co., Ltd. (Shanghai, China). High-throughput sequencing data were analyzed with Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) (Caporaso et al., 2010). Sequences with ≥97% similarity were assigned to the same operational taxonomic unit (OTU). After obtaining the representative sequence for each OTU, the RDP classifier was used to annotate the taxonomic information. The Shannon, Simpson, ACE, Chao1, observed species, and Good's coverage indices were used to describe the alpha diversity. Beta diversity, the difference in microbial communities between the two groups, was assessed using principal component analysis (PCA).

Metabonomics analysis was performed by Applied Protein Technology Co., Ltd. (Shanghai, China) using both untargeted and targeted metabonomics methods.

2.2.2. LC–MS-based untargeted metabolomics

Briefly, 80 mg of intestinal tissues from F2, F3, and F6 were used in triplicate to extract metabolites. First, the samples were thawed at 4 °C. Next, 200 μL ultrapure water and 800 μL of cold methanol/acetonitrile (1:1, v/v) were added to the samples and mixed to remove the protein. The mixture was centrifuged for 20 min (14000 rcf, 4 °C). The supernatant was dried in a vacuum centrifuge and then stored at –80 °C. Prior to LC–MS analysis, the samples were separated by ultrahigh-performance liquid chromatography (UHPLC, 1290 Infinity LC, Agilent Technologies, Santa Clara, CA, USA). Mobile phase A consisted of 25 mM ammonia, 25 mM ammonium acetate, and ultrapure water. Mobile phase B was acetonitrile. The flow rate was 0.3 mL/min, the column temperature was 25 °C, and the injection volume was 2 μL. During the analysis process, samples were maintained at 4 °C in the automatic sampler. The detailed elution program can be seen in Table S2. After UHPLC separation, electrospray ionization (ESI) was used for detection in both positive and negative ion modes. The conditions were as follows: ion spray voltage floating (ISVF) ± 5.5 KV; declustering potential (DP) ± 60 V; collision energy 35 ± 15 eV; heater temperature 300 °C. Mass spectrometry analysis and metabolite identification were performed using an Agilent 6550 iFunnel Q-TOF spectrometer (Agilent Technologies) and a Triple TOF 5600 mass spectrometer (SCIEX, Framingham, MA, USA), respectively. The conditions were as follows: TOF MS scan *m/z* range 60–1000 Da; product ion scan *m/z* range 25–1000 Da; TOF MS scan accumulation time 0.20 s/spectra; product ion scan accumulation time 0.05 s/spectra. Information-dependent acquisition (IDA) was performed with high sensitivity. Quality control samples were analyzed at the beginning of the batch and then every six samples to evaluate the stability of the experimental data. The raw data were converted to the mzXML format using Proteowizard 3.0 (USA) and then imported into XCMS software for further analysis, including retention time correction, peak alignment, and picking. Following Pareto-scaling preprocessing, the data were subjected to multivariate analysis with partial least square discriminant analysis (PLS-DA) and univariate statistical analysis, including fold change (FC) analysis and t tests. The mean metabolite concentration in each group was used to calculate the FC. Differentially expressed metabolites were selected according to the following screening criteria: variable importance in projection VIP > 1.5 and $p < 0.05$.

2.2.3. Multiple reaction monitoring of targeted metabolomics

Targeted metabolomics was performed using the multiple reaction monitoring (MRM) technique. The sample pretreatment and LC–MS analysis are the same as for the untargeted metabonomics. After ultra-HPLC separation, mass spectrometry was performed on an AB 5500 QqQ spectrometer (AB SCIEX) using the following parameters in positive and negative electrospray ionization mode: sheath gas temperature 350 °C; dry gas temperature 350 °C; sheath gas flow 11 L/min; dry gas flow 10 L/min; capillary voltage 4000 V (positive mode) or −3500 V (negative mode); nozzle voltage 500 V; and nebulizer pressure 30 psi. Using MRM mode, the dwell time of each MRM ion pair was 3 ms, and the total cycle time was 1.263 s. The raw data were processed using the MRM Analyzer (R), and the peak area, data acquisition, and data analysis of each metabolite were obtained according to a previously published method (Cai et al., 2015).

3. Results

3.1. Seasonal variations of microplastics and Cd in spring and winter

To investigate the seasonal variations and the correlations of microplastics and heavy metals during the spring and winter seasons, the content of heavy metals was first detected in the spring, the results of which are presented in Table S3. Among these heavy metals, only the Cd concentration exceeded the risk screening values (0.30 mg/kg) for soil contamination of agricultural land, according to the Chinese soil environmental quality guidelines (Chinese soil environmental quality, 2018). Hence, the Cd content was further detected in the winter soils (see Tables S3 and S4). The results show that the Cd content decreased slightly during the winter compared with the spring, which may be related to the biogeochemical cycle of Cd itself, as well as the meteorological conditions, environmental factors, and the use of pesticides and fertilizers (Hu et al., 2020). Therefore, Cd is the main pollutant in agricultural fields of Taihu Lake, Wuxi area.

Microplastics were found at all sampling sites in both the spring and winter. The microplastic abundances were 56.67–180.33 particles/kg soil for the spring samples (Fig. 1A) and 206.15–890.49 particles/kg soil for the winter samples (Fig. 1C). In addition, the size of the microplastics was categorized as 0–500 μm, 500–1000 μm, and >1 < 5 mm, representing 72.45%, 17.63% and 9.92%, respectively, in the spring samples (Fig. 1B) and 88.87%, 9.28% and 1.85%, respectively, in the winter samples (Fig. 1D).

3.2. Shape, size distribution, and color of microplastics in farmland soils

The shape, size distribution, and color of the microplastics at each sampling site during the spring and winter were determined by stereomicroscopy (Fig. S2). The microplastic shapes were mainly in the form of

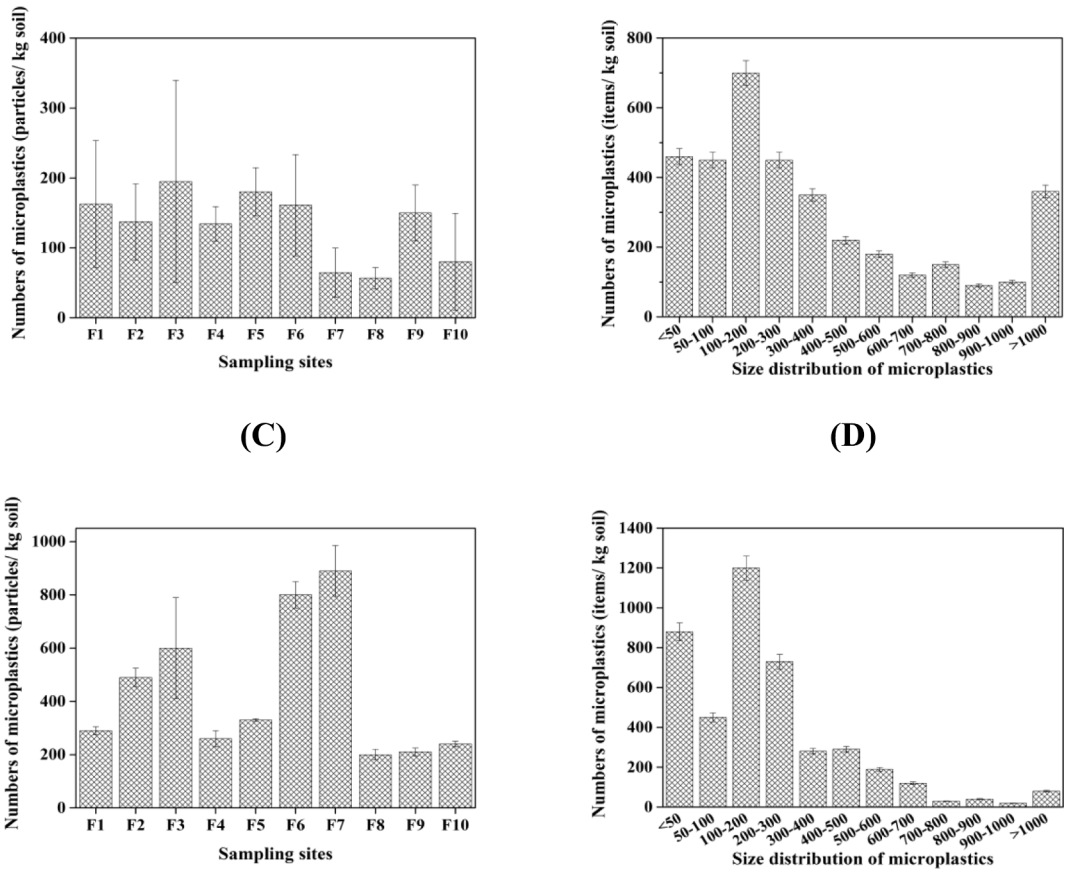

Fig. 1. The distribution and size of microplastics at the sampling sites during the spring (A, B) and winter (C, D). Error bars represent the mean ± standard deviation ($n = 3$).

fibers, films, fragments, foams, and pellets (Fig. S3). For the criteria for classifying the shapes of MPs, see Text S4. The colors were black, transparent, red, blue, brown, and green, and the size ranged from <50 μm to >1 mm. Furthermore, the overall color and shape of the microplastics at the sampling sites during the spring and winter were determined (Fig. 2). In the spring, the top three microplastic colors were transparent (58%), green (17%), and black (11%), while fragments were the dominant shape, accounting for 46% of all microplastics, followed by films (26%) and fibers (23%). In the winter, the top three microplastic colors were transparent (44%), green (22%), and black (22%). However, the overall shape of microplastics was slightly different from that of the spring samples, with fibers being the dominant shape,

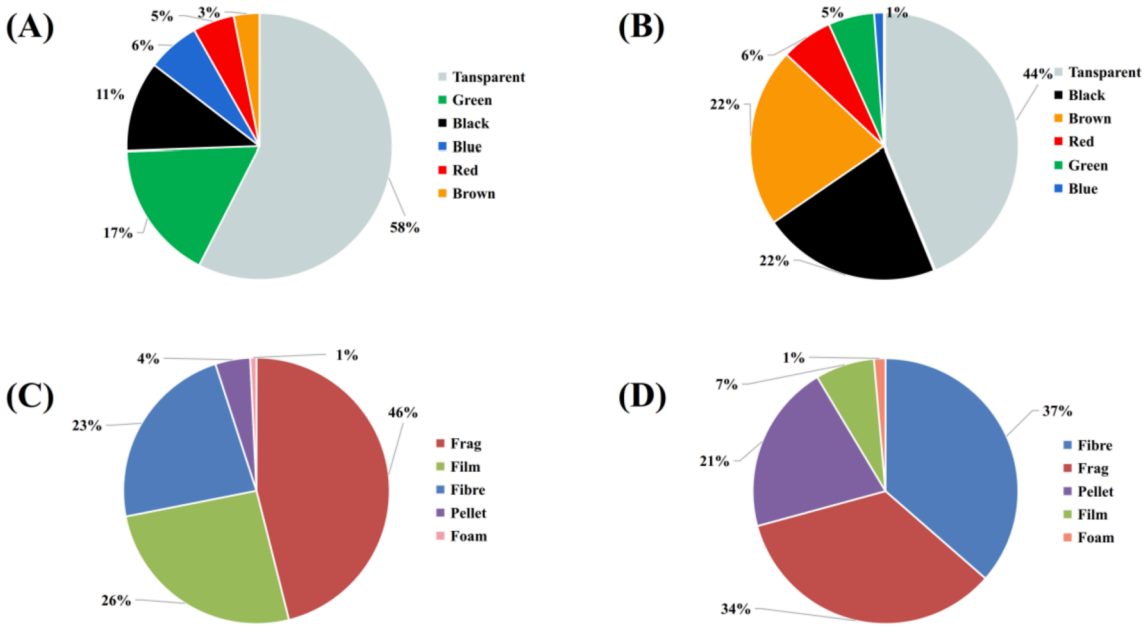

Fig. 2. Overall distribution of microplastic colors and shapes during the spring (A, C) and winter (B, D) at the sampling sites.

accounting for 37% of all microplastics, followed by fragments (34%) and pellets (21%).

3.3. Microplastic polymer types

The polymer composition of the microplastics was further identified by μ-FTIR (Table 1). Eight microplastic polymers were identified in the spring samples, and ten polymers were identified in the winter. Polyethylene microplastics were the most abundant polymer, accounting for 67.26% in the spring and 54.65% in the winter samples.

3.4. Correlation analysis of soil microplastics and Cd

The linear regression of microplastic abundance and Cd content is shown in Fig. 3A and 3B. There was no significant relationship between the abundance of microplastics and the content of Cd during the spring at the sampling sites ($p > 0.05$). However, in the winter samples, the Cd content linearly increased with increasing microplastic abundance ($p < 0.05$).

Furthermore, to investigate the response of *in situ* earthworms to soil microplastic-heavy metal pollution, 10 of the previous sampling sites were selected to harvest earthworms.

3.5. The shape, colors, and size distribution of microplastics from sampling sites, earthworm intestines, and casts

The shapes of the microplastics from the sampling sites, earthworm intestines, and casts were mainly composed of fibers, films, fragments, and pellets (Fig. S4A). Photographs of typical microplastics are shown in Fig. S5. The percentages of microplastic shapes in the F2, F3, and F6 fields show high variation. Briefly, the dominant microplastic shapes were fragments (38%), fibers (83%), and fragments (80%) in earthworm casts, intestines, and sampling soils (Fig. S6B, S6D, and S6F), respectively. Five colors of microplastics were found, namely, black, yellow, green, red, and transparent (Fig. S4B). The most common microplastic colors were black (28%) and transparent (28%) in earthworm casts, black (33%) and transparent (33%) in intestines, and transparent (60%) in soils (Fig. S6A, S6C, and S6E). In addition, all sizes of microplastics, from 0 to 900 μm, were detected (Fig. S4C). Among these, the 0–200 μm size accounted for the largest proportion in earthworm casts and intestines, indicating that small microplastics are more likely to be ingested and enter the intestinal tissue of earthworms (Huerta Lwanga et al., 2016).

3.6. Determination of microplastics and Cd in earthworm intestines, casts, and sampling sites

The abundance of microplastics in SF2, SF3, and SF6 soils was 1.21 ± 0.08, 1.03 ± 0.06, 1.75 ± 0.12 items/g, respectively; in GF2, GF3, and GF6 intestines it was 2.30 ± 0.12, 2.65 ± 0.15, and 2.41 ± 0.10 items/g, respectively; in CF2, CF3, and CF6 casts it was 9.63 ± 0.50, 13.04 ± 0.80, and 47.19 ± 3.00 items/g, respectively (Fig. 4A). Additionally, in F2, F3, and F6, significant differences were found between groups, with F6 exhibiting the highest abundance of microplastics. Similar results were also found in earthworm casts. It is worth noting that the abundance of microplastics in earthworm casts and intestines was higher than that in the soil environment, suggesting that microplastics may accumulate in the bodies of soil organisms and cause potential health risks.

Furthermore, the Cd contents in earthworm casts, intestines, and soils from sites F2, F3, and F6 were detected (Fig. 4B). The results show that the Cd contents were 0.20 ± 0.01, 0.22 ± 0.02, and 0.24 ± 0.02 mg/kg in CF2, CF3, and CF6 casts, respectively; 0.28 ± 0.04, 0.34 ± 0.02, and 0.46 ± 0.02 mg/kg in SF2, SF3, and SF6 soils, respectively; and 1.42 ± 0.08, 1.52 ± 0.01, and 2.32 ± 0.10 mg/kg in GF2, GF3, and GF6 intestines, respectively. In addition, the soil at F6 had the highest Cd content. Furthermore, the Cd content in earthworm intestines was higher than that in both the sampling sites and casts.

Table 1
Percentage of different polymer microplastics in sampling soils.

Polymer types	Spring	Winter
Acrylates	7.08	10.99
Polyamide	2.21	6.16
Polyethylene (PE)	67.26	54.65
Polyethylene Terephthalate (PET)	0.44	7.62
Polypropylene (PP)	13.72	3.02
Polystyrene (PS)	1.33	2.09
Polyurethane (PU)	6.64	9.19
Polyvinylchloride (PVC)	1.33	3.14
Polycarbonate (PC)		1.80
Polymethylmethacrylate (PMMA)		1.34

3.7. Differences in bacterial communities between in situ soil and earthworm intestine

High-throughput sequencing was used to characterize the changes in soil and intestinal bacterial communities. The shared OTUs in the soils and intestines are shown using a Venn diagram (Fig. 5A and B). The total OTUs in the soils and intestines ranged from 4358 to 4600 and 1967–2067, respectively. Alpha diversity indices for the soil and intestine communities are included in Table S5. There was no significant difference between the sampled soils and earthworm intestines; however, all the alpha diversity indices in earthworm intestines were significantly lower than those in the soil ($p < 0.05$).

The differences in microbiota patterns can be directly attributed to the changes in the relative abundance of dominant intestinal bacteria (Fig. 5C). The *in situ* soil and earthworm intestines had a relatively similar community composition, with Proteobacteria as the predominant phylum, accounting for 41%, 36%, and 38% of the relative abundance in SF2, SF3, and SF6 soils, respectively, and 37%, 55%, and 52% in GF2, GF3, and GF6 intestines, respectively. There were no differences in the relative abundance of other phyla in soils. However, in the earthworm intestines, with the increase in microplastic abundance in SF2, SF3, and SF6 *in situ* soils, the relative abundance of Bacteroidetes increased (15%, 13%, and 29%, respectively), while Firmicutes decreased (40%, 21%, and 8.4%, respectively). Specifically, the ratio of Bacteroidetes to Firmicutes in the GF2, GF3, and GF6 intestines increased by 0.38, 0.63, and 3.46, indicating that microbial community structure dysbiosis was exacerbated. According to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database functional analysis, the relative abundance and functional composition of the community structure were mainly amino acid metabolism, membrane transport, and carbohydrate metabolism in the soil and earthworm intestines (Fig. 5D). However, the relative abundance of functional composition was not lucid different between the *in situ* soil and earthworm intestines. In addition, the PCA results showed that the bacterial communities in soils and earthworm intestines have different structures (Fig. S7).

3.8. Characteristics of metabolite changes in in situ earthworms

To more directly and accurately reflect the physiological responses of earthworms to contaminated soils, metabolite changes in earthworm intestines were further explored via LC–MS-based untargeted metabonomics and MRM-based targeted metabonomics. LC–MS-based untargeted metabolomics identified 485 metabolites in earthworm intestines. Among these metabolites, the largest proportion were organic acids and their derivatives (16.2%); nucleosides, nucleotides, and their analogs (9.9%); and oxygen-containing organic compounds (8.5%) (Fig. S8). Furthermore, PLS-DA was used to compare metabolite changes in earthworms induced by the contaminated soils at their respective sites (F2, F3, and F6). However, there was no clear distinction among the

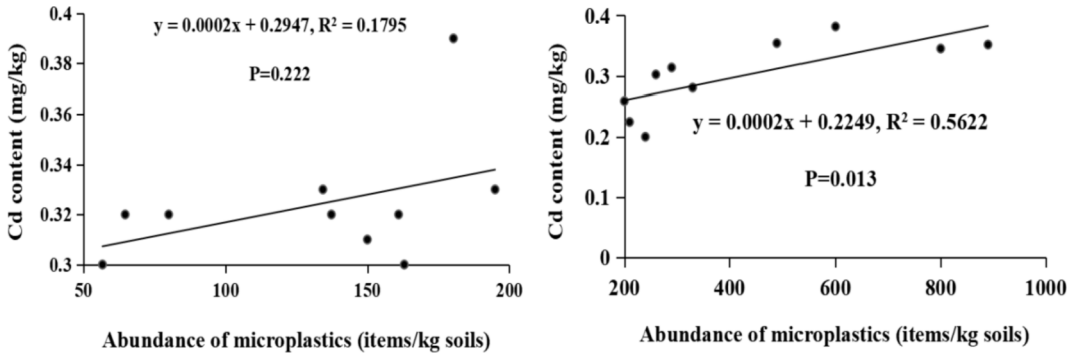

Fig. 3. Correlation between microplastic abundance and Cd content in the spring (A) and winter (B) at the sampling sites.

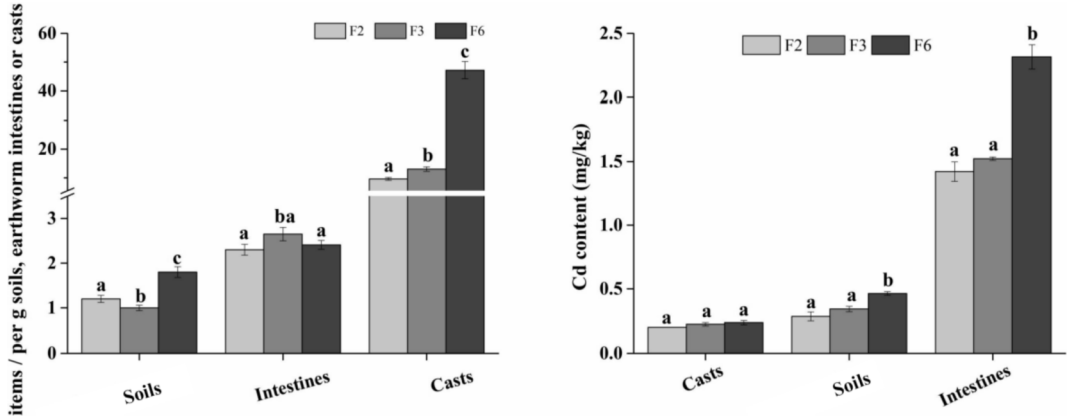

Fig. 4. The abundance of microplastics (A) and Cd content (B) in soils, earthworm casts, and intestines.

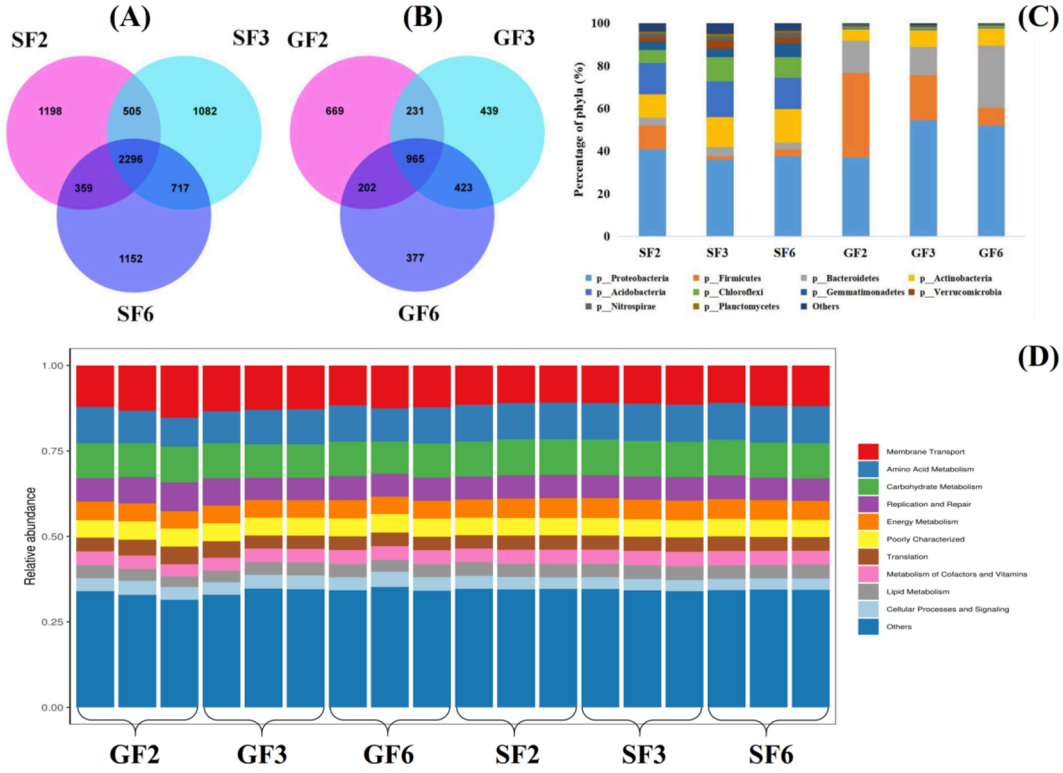

Fig. 5. Venn diagram of operational taxonomic units (OTUs) in soils (A) and earthworm intestines (B). Relative abundances at the phylum level for *in situ* soils and earthworm intestines (C). Relative abundance and functional composition of the community structure (D).

different earthworm intestines (Fig. S9).

Using the KEGG database, it was determined that the main pathways affected by contaminated soils were metabolic pathways, propionic acid metabolism, pyruvate metabolism, and niacin and nicotinamide metabolism (Fig. 6A), which are related to changes in organic acid and amino acid metabolism. However, the absolute content of differential metabolites could not be quantified. Therefore, MRM-based targeted metabolomics was used to absolutely quantify the content of differential metabolites in the earthworm intestines. Two metabolites, namely, L-phenylalanine (Fig. 6B) and succinic acid (Fig. 6C), were identified as having significant differences between *in situ* earthworms from sites F2, F3, and F6.

4. Discussion

4.1. Seasonal variations and correlation analysis of microplastics and Cd

In the present study, the abundance of microplastics in winter samples was much higher than that in spring, which could be explained by three factors. First, agricultural activities after spring sowing, such as irrigation and mechanical operations during harvest, might introduce new sources of microplastics to the soils (Haixin et al., 2022). Second,

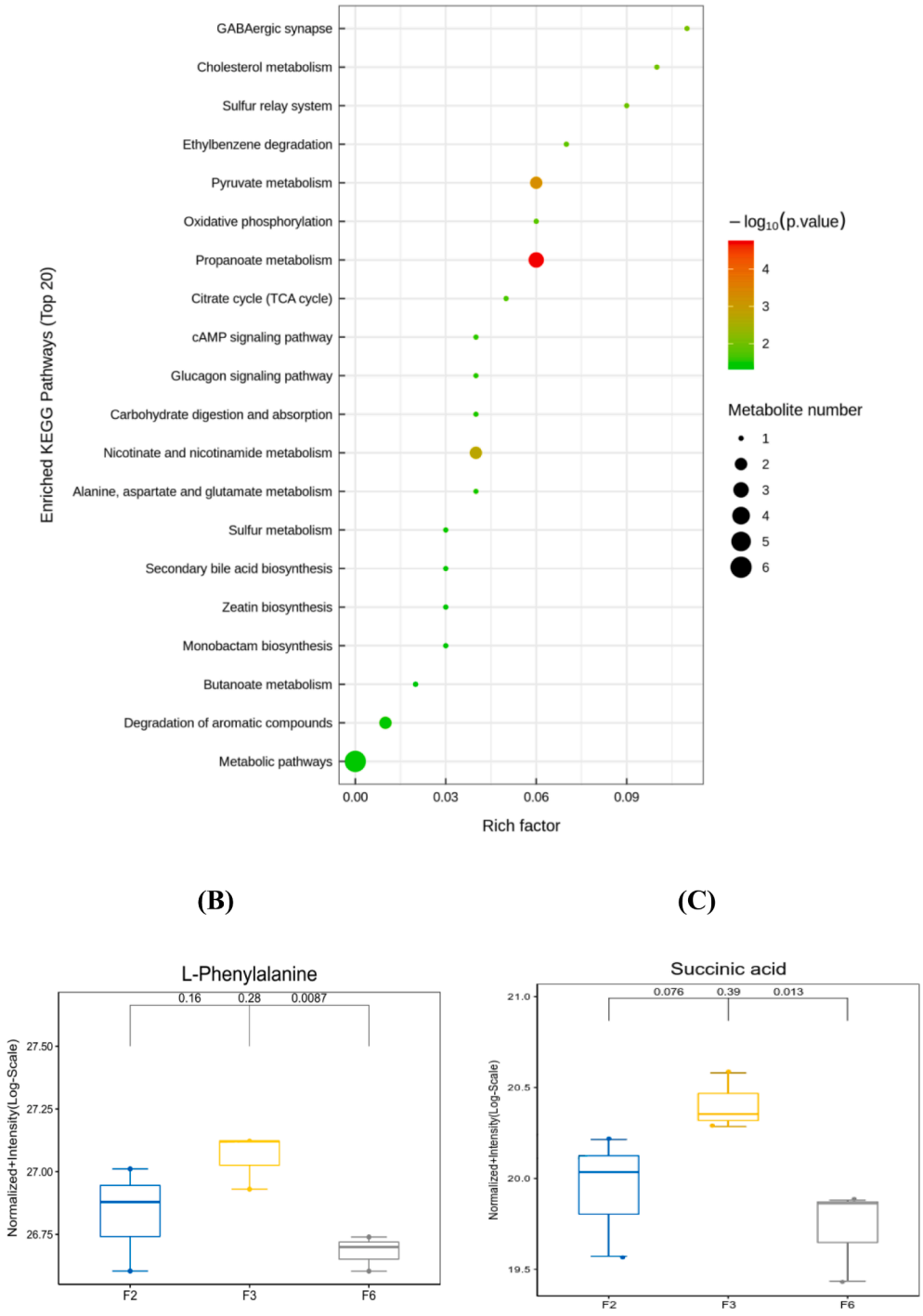

Fig. 6. Bubble plot of significantly affected KEGG pathways in earthworm intestines (A). Changes in metabolites from earthworm intestines at different sampling sites. (B: L-Phenylalanine, C: Succinic acid).

agricultural film residues could accelerate aging and degradation under ultraviolet radiation and high temperature (Rillig et al., 2017; Shi et al., 2017). Third, precipitation was an important factor in atmospheric MP deposition and introduced it into terrestrial soils (Bi et al., 2020; Y. Zhang et al., 2020b).

Previous studies have also shown seasonal variations in microplastics at the same site, which are mainly related to sampling methodology, agricultural activities, and meteorological conditions during different seasons (Rodrigues et al., 2018). In the spring and winter, the shapes, colors, and sizes of soil microplastics were relatively consistent with each sampling site, while the overall distribution varied, mainly due to the increase in pellets and the decrease in fragments. The pellets are linked to personal care products including cleaning products and cosmetics (Xu et al., 2020). Thus, this phenomenon may be related to the use of cosmetics and facial cleansers, which will be discharged into wastewater treatment and potentially used for sludge utilization and increase the MP abundance of pellets in farmland (Horton et al., 2017b; Gao et al., 2020). Therefore, in addition to agricultural films and fragments, human activities may also be a source of agricultural microplastic pollution (Liu et al., 2018).

According to previous studies, various types of microplastics, including polypropylene (PP), polyethylene (PE), polyvinylchloride (PVC), and polystyrene (PS), have been detected in agricultural soils (W. Wang et al., 2020b; Yang et al., 2021), indicating that soil microplastic pollution may be closely related to the source of mulch film plastic in agricultural farmland. Mulching, an important source of soil microplastics, can be used to protect fragile fruits and vegetables from moisture loss and frost (Chen et al., 2020). Plastic films are widely used in different agricultural applications, such as greenhouses, sheds, and mulch (Horodytska et al., 2018). Furthermore, the residual macroscopic and microscopic characteristics of polyethylene and biodegradable mulch film have certain adverse effects on soil microbial composition, soil structure, crop nutrition supply and reproductive growth (De Souza Machado et al., 2018; Qi et al., 2018; Huang et al., 2019). In summary, PF is the main source of microplastic pollution in Taihu Lake farmland soil. Reports have demonstrated that microplastics can adsorb heavy metals from soils (Vedolin et al., 2018; F. Wang et al., 2019a) and that the concentration of heavy metals on the surface of microplastics is higher than that in the surrounding environment (Lee et al., 2014). In our study, the Cd content increased with increasing microplastic abundances in the winter samples, indicating that microplastics and Cd may cause synergistic pollution to the environment and thus have potentially adverse effects on organisms (S. Zhang et al., 2020a). A previous study also proved that coexposure to microplastics and Cd can inhibit the growth rate and increase the mortality rate of *E. fetida* (Y. Zhou et al., 2020a). In addition, according to statistics from the national general survey of soil contamination in China, approximately one-fifth of the total cultivated area in China has been polluted by heavy metals, such as Cd (F. Huang et al., 2020a; Ogunkunle et al., 2020). This may result in biomagnification and toxic effects to soil animals and plants through enrichment, thereby causing great harm to the environment and human health through the food chain (Guo et al., 2020; Yang et al., 2021). Therefore, in addition to the individual ecotoxicity of microplastics, the combined ecological risk induced by microplastics and heavy metals should not be neglected.

4.2. The feedback of soil organisms to microplastics and cadmium pollution in agricultural fields

Studies have shown that microplastics may affect soil physicochemical properties and functions, as well as soil microcosms, with the migration of microplastics. In addition, microplastic particles can adsorb heavy metals, organic pollutants, etc., and when ingested by soil organisms, they can be transferred to higher nutrients and accumulate, which can adversely affect soil organisms (Vedolin et al., 2018; F. Huang et al., 2020a). Huerta Lwanga et al. (2016) identified that small microplastics are more likely to be ingested and enter the intestinal tissue of earthworms, and aging microplastics may exacerbate intestinal damage (abrasion and clogging) due to the greater specific surface area and stronger adsorption. This will lead directly to weight loss, alteration of soil microbial diversity, biofilm formation, and even death (Rillig et al., 2019; J. Wang et al., 2019b).

In this study, Cd enrichment in earthworm intestines was higher at SF6, which had high microplastic abundance. Previous results showed that microplastics can adsorb and transport heavy metal contaminants (Hodson et al., 2017) and that metals accumulated on the surface of microplastics are highly likely to be released due to the action of intestinal surfactants into intestinal tissues (Y. Zhou et al., 2020a), which is also consistent with the present findings (Fig. 4). Thus, the toxic effects of microplastics on soil animals are of high concern. The lack of knowledge about the behavior and effects of these microplastics will have potentially serious consequences on soil ecosystem health and biodiversity, posing a major threat to the agroenvironment (Li et al., 2018; J. Wang et al., 2019b).

Earthworms are widely distributed in soil, and their intestinal flora can accumulate or degrade pollutants through biodegradation, adsorption transformation, and bioaccumulation without secondary pollution, which is conducive to the stability of soil ecosystems and sustainable development of the environment (Biswas et al., 2018; Cao et al., 2018; Huerta Lwanga et al., 2018). Earthworm intestines are rich in endophytic bacteria, which not only play an important regulatory role in their metabolism and functional stability but also facilitate the decomposition of organic matter and the transformation of nutrient elements in the soil (Hu et al., 2018). However, the presence of excessive contaminants can also negatively affect the immune system and the diversity of the intestinal bacterial community. Therefore, the species and abundance of earthworm gut bacterial communities change accordingly to multiple factors, such as species and the external environment. Huerta Lwanga et al. (2018) isolated Firmicutes and Actinobacteria from the intestine of the earthworm *Lumbricus terrestris* (Oligochaeta, Lumbricidae) and incubated them in low-density polyethylene (LDPE) at a dose of 1% (w/w). The degradation products were mainly n-octadecane, eicosane, docosane, and eicosane. Therefore, earthworm intestinal bacteria could also degrade the chemical structure of microplastics in the environment. However, when the microplastic content is too high, it can inhibit the earthworm's biological functions and change the composition of the intestinal bacterial community. In this study, the increase in Firmicutes and Actinobacteria may be a feedback effect of intestinal microorganisms on exogenous substances.

Although the current results can clarify which functions are differentially altered by microorganisms in soil and earthworm gut microbes in heavy metal–microplastic contaminated soils, the specific biological processes of these functions are not clear. Therefore, the important pathways were analyzed using metabolomics to further evaluate the feedback of earthworms to heavy metal–microplastic pollution. Amino acids are the main nutrients involved in protein synthesis, energy supply, and the formation of mediators, either directly (as mediators) or through their metabolism. Amino acids also act as precursors of biologically active molecules, such as neurotransmitters, second messengers, and cytokines. Thus, dysregulation of amino acid metabolism may lead to the development of various pathological symptoms in living organisms (Ding et al., 2018; Dirckx et al., 2019). Carbohydrates are the main components of the living cell structure and the main energy suppliers, and they have important functions in regulating cellular activities (Lankadurai et al., 2015). Carbohydrate metabolism mainly includes processes such as glycolysis and the tricarboxylic acid cycle (TCA), which have important roles in the energy transfer process of organisms. In addition, the increase in intermediates in the TCA cycle is the cause of energy metabolism disorders (Horton et al., 1996).

The content of phenylalanine varied between F2, F3, and F6, with the lowest content in the F6 group. According to previous studies, phenylalanine can be converted into a substrate of the TCA cycle, which

can be used as an energy supply for earthworms suffering from external stress that depletes energy (Lankadurai et al., 2015; Dani et al., 2019). In addition, the F6 group had the lowest content in succinate metabolism. KEGG metabolic pathways showed that succinate was involved in pathways including the TCA cycle; oxidative phosphorylation; alanine, aspartate, and glutamate metabolism; lysate metabolism; tyrosine degradation; phenylalanine metabolism; pyruvate metabolism; butyrate metabolism; niacin and nicotinamide metabolism; carbon metabolism; and GABAergic synapses. The TCA cycle is an important intermediate of metabolites, so the decrease in succinic acid indicates that the TCA cycle and carbohydrate metabolism were inhibited and that the organisms could not produce more energy to offset the side effects caused by insufficient external energy (Lankadurai et al., 2015; Dani et al., 2019). The changes in these metabolites can be used as potential biomarkers for the toxic effects to earthworms caused by microplastic exposure.

Based on the above results, although there was no significant difference between the abundance of microplastics and the effects of metabolites, it can be suggested that the higher the abundance of microplastics, the greater the impact on earthworm metabolites. Therefore, microplastic pollution, especially the synergistic effect of microplastic and heavy metal pollution in farmland soils, should be focused on alleviating the threats to soil ecological safety.

4.3. Environmental implications and future perspective for soil microplastic pollution

The effectiveness of plastic film mulching and greenhouse covering in improving crop quality and yield means that they have been widely and intensively applied in agricultural production (Gao et al., 2019; Sander, 2019; Y. Huang et al., 2020b; Yang et al., 2021). However, the low recovery rate of film mulching residues is an important reason for the increase in microplastics in agricultural soils (Astner et al., 2019; Qi et al., 2020). The United Nations Environment Program (UNEP) listed microplastic pollution as one of the top ten environmental problems in 2014 (UNEP, 2014) and called on researchers to conduct more research on the impact of microplastic pollution on the soil environment (UNEP, 2018). In addition, the combined pollution of soil microplastics and heavy metals in the soil has become a practical problem in soil environments. Soil organisms may unintentionally ingest MPs, in which the metals adsorbed by MPs are released in their body (Y. Zhou et al., 2020a; M. Li et al., 2021a). Residual microplastics will affect soil physical and chemical properties, which will change plant nutrient absorption, thus affecting plant growth and leading to ecotoxicity and genotoxicity (Jiang et al., 2019; Y. Zhou et al., 2020b). Plants can also take up Cd by the adsorption of heavy metals on root surfaces (F. Wang et al., 2020a). Due to technical limitations, it is difficult to detect nanosized microplastics, and the concentration of nanoplastics in the soil environment is largely underestimated (Xu et al., 2020). Furthermore, there is a process of continuous degradation from microplastics to nanoplastics owing to external conditions such as UV radiation or high temperature and wind erosion (Ekvall et al. 2019). Under these circumstances, it is necessary to conduct more studies on nanoplastics to assess the potential toxicity to higher plant and soil organisms. Additionally, the use of multiomics technology to clarify the interaction between soil animals, plants and soil microorganisms can improve the understanding of the long-term effects of soil pollution on farmland ecosystems. This is of profound significance for further understanding the soil environmental geochemical cycle, controlling the migration and transformation of soil pollutants, and food safety evaluations.

5. Conclusion

The seasonal variations of microplastics in agricultural fields from Taihu Lake, Wuxi, China, were investigated, and the responses of *in situ* earthworms to soil microplastic–Cd pollution were revealed. These findings show that the abundance of microplastics was different during the spring and winter at the sampling sites, while the size distribution of microplastics was similar. Polyethylene microplastics were the main polymers, illustrating that the microplastics in the agricultural soil predominantly originated from plastic film mulch. In addition, the abundance of soil microplastics in the winter was significantly linked to the Cd content ($p < 0.05$), indicating that microplastics and heavy metals had a coexposure risk to soil organisms. Moreover, the response of *in situ* earthworms to soil microplastic–Cd pollution revealed that microplastics can act as vectors to transfer heavy metals in the soil environment to organisms, subsequently becoming enriched. In addition, the results from 16S rRNA sequencing and metabonomics illustrated that microplastic–heavy metal contaminated soils can lead to bacterial community structure dysbiosis and disruption of energy depletion of *in situ* earthworms. The abundance of microplastics in earthworm casts and intestines was higher than that in the soil environment, suggesting that microplastics may accumulate in the body of soil organisms, causing potential health risks. These findings provide a comprehensive evaluation of the potential risk of coexisting soil microplastics and heavy metals in the soil ecosystem and play an important role in maintaining soil ecological security.

CRediT authorship contribution statement

Xiaofeng Jiang: Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Yang Yang:** Methodology, Formal analysis. **Qian Wang:** Investigation. **Na Liu:** Writing – review & editing. **Mei Li:** Conceptualization, Validation, Supervision, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by National Natural Science Foundation of China (No. 41773115, 41571468), Science and Technology Support Program of Jiangsu Province (No. BE2016736) and Nanjing University Innovation and Creative Program for PhD candidate (No. CXCY19-61). Xiaofeng Jiang and Yang Yang contributed equally to this work (co-first author).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107096.

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