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Occurrence and characteristics of microplastics in soils from greenhouse and open-field cultivation using plastic mulch film

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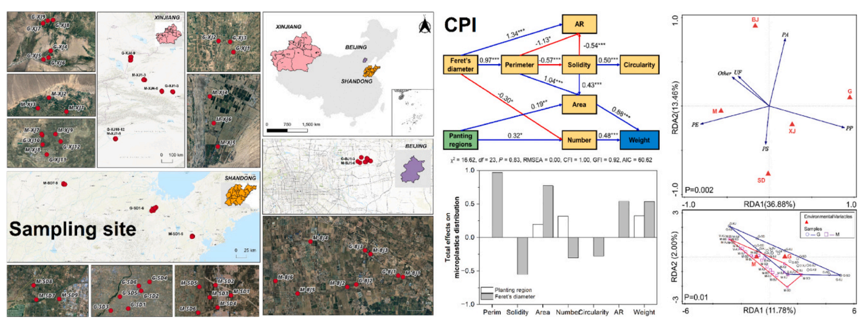
HIGHLIGHTS

- Microplastics (MPs) were investigated in typical agricultural soils in China.
- The average abundance of MPs ranged from 1.82×10^4 kg^{-1} to 4.83×10^4 kg^{-1} in soils.
- MPs were mainly 50–250 μm, with the amount decreasing as particle size increased.
- Polyethylene and polypropylene were the two dominant MPs in the soil.
- MP amounts were significantly responsive to regions, what MP types to mulching modes.

GRAPHICAL ABSTRACT



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ABSTRACT

There is a major knowledge gap concerning the extent of microplastic pollution in agronomic regions of China, which represent a plastic use hotspot. In order to clarify the amendment of agronomic region and plastic film mulching mode to microplastics distribution, the characteristics of microplastics distributed in agricultural soils from three typical regions (Beijing (BJ), Shandong (SD), and Xinjiang (XJ)) with two plastic film mulching modes (greenhouse (G) and conventional field-based film mulching (M)) in China were investigated. Microplastics weight and their response to planting regions were also evaluated in this study. The result showed that the average abundance of microplastics in soils from BJ, SD, and XJ was 1.83×10^4 items kg^{-1}, 4.02×10^4 items kg^{-1}, and 3.39×10^4 items kg^{-1}, and the estimated weight of microplastics per kg of dry soils was 3.12 mg kg^{-1}, 5.63 mg kg^{-1}, and 7.99 mg kg^{-1}, respectively. Microplastics in farmland were mainly of small particle size (50 to 250 μm), with their abundance decreasing with increasing particle size. Among the microplastics detected, polyethylene and polypropylene were the two dominant types present, accounting for 50.0% and 19.7%, respectively. The standard total effect of planting regions on microplastic number and weight was 31.8% and 32.3%, and plastic film mulching modes (G vs. M) could explain 34.4% of the total variation of microplastic compositions with a contribution rate of 65.6% in this study. This research provides key data for an assessment of the environmental risk of microplastics and supports the development of guidelines for the sustainable use of

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agricultural plastic film. Further, it is necessary to quantify and assess the contribution of other different plastic sources to microplastics in soil. Big data technologies or isotope tracer techniques may be promising approaches.

1. Introduction

Microplastic, as an emerging pollutant of global concern, is almost ubiquitous within the atmosphere, water, and terrestrial biosphere (Baho et al., 2021; Hurley et al., 2018; Li et al., 2023). Research related to microplastics has been a rapidly evolving domain; however, previous research mainly focused on microplastic pollution in marine and other aquatic ecosystems (Alimi et al., 2018; Qi et al., 2020). There is increasing evidence that microplastic pollution may pose a major threat to the functioning and sustainability of terrestrial ecosystems (Huang et al., 2020a; Li et al., 2020; Zhou et al., 2021b). Detailly, these microplastics incorporated into soil can increase contact angle and saturated hydraulic conductivity, decrease bulk density and water holding capacity (Yu et al., 2023), and alter bacterial community structure (Li et al., 2023; Zhou et al., 2021a). Microplastics can also be readily absorbed and ingested by keystone organisms, which regulate critical functions within terrestrial ecosystems (Zhou et al., 2021a; Zhou et al., 2020). They can be transported through food webs and into the human food chain (Li et al., 2020; Okeke et al., 2022), where their effects on human health remain uncertain but remain of concern (Kannan and Vimalkumar, 2021; Udovicki et al., 2022). Although investigations on microplastic pollution in terrestrial ecosystems have increased recently, most of them have focused on the environmental hazards of microplastics. Very little work has been done regarding the abundance and composition of microplastics in typical planting regions. There is a major knowledge gap concerning the extent of microplastic pollution in agronomic regions of China, which represent a plastic use hotspot.

Microplastics enter the terrestrial environment via numerous sources, such as agricultural plastic films (Huang et al., 2020b; Yang et al., 2021b), municipal-derived composts and waste disposal (Sharma et al., 2017), sewage sludge (Corradini et al., 2019), atmospheric deposition (Allen et al., 2019), animal manures/film-coated fertilizer (Weithmann et al., 2018; Yang et al., 2021a; Zhang et al., 2022), and road run-off (Blasing and Amelung, 2018). Of these, agricultural plastic film has been recognized as a dominant source of microplastics entering agricultural soil (Huang et al., 2020b; Khalid et al., 2023). In our previous study, we found that microplastic (particle sizes <5 mm) derived from four types of buried plastic mulching film persisted in agricultural soil for up to 2 years (Qi et al., 2021). Various factors, e.g., plastic components, mulching age, film types, tillage, and UV irradiation, caused plastic residues to generate microplastics that accumulated in agricultural soils (Liu et al., 2023; Revell et al., 2021). However, there is a knowledge gap about the macroscopically important effect of planting region and plastic mulching mode on the occurrence and distribution of microplastics. Not yet, evidence could directly reveal the response of microplastic distribution on agronomic regions and plastic mulching modes.

Suburbs of Beijing (BJ), intensive agricultural areas of Shandong (SD), and long-term planting regions of Xinjiang (XJ) represent three typical agronomic regions in China in which the application of agricultural plastic film is commonplace, quantification and assessment of microplastic pollution in the actual soil environment are in breach. We hypothesize that the abundance and composition of microplastics in farmland significantly depend on planting regions and mulching modes. To test this hypothesis, we detected the abundance, size, morphology, and compositions of microplastics present in soils from these distinct planting regions and under two modes of plastic use, namely greenhouse (G) and field mulching (M) conditions, aiming to reveal the total effects of planting regions and mulching modes on microplastic distribution in the typical agronomic regions. We used this information to assess the sources and possible implication of microplastic contamination in farmland. The effects of agricultural activities in different regions on the abundance and morphological characteristics of soil microplastics are also discussed and evaluated, providing data to support further research on pollution by legacy plastic in farmland.

2. Materials and methods

2.1. Test sites and sample collection

The experimental sites involved in the study were located in Beijing (BJ, 40°05′N, 116°54′E, including the experimental center for the Institute of Agricultural Environment and Sustainable Development, Shunyi, Chinese Academy of Agricultural Sciences), Shandong Province (SD, 36°26′N~37°20′N, 116°29′E~120°03′E, including the intensive agricultural areas in Qingdao, Dezhou, and Shouguang city), and Xinjiang Uygur Autonomous Region (hereinafter referred to as Xinjiang, XJ, 40°35′N~46°72′N, 81°15′E~87°19′E, including the agricultural planting areas with long-term mulching in Changji city, Tacheng District, Alar, Kuitun, and Kanas). In detail, the site of Beijing has a temperate monsoon climate with an annual average temperature of 10–12 °C and a mean annual precipitation of 644 mm. The region surrounding Shandong has a warm temperate monsoon climate with an annual average temperature of 11–14 °C and a mean annual precipitation of 650–750 mm. The region surrounding Xinjiang has a temperate continental climate with a larger difference in annual average temperature and a mean annual precipitation of 150 mm.

A total of 45 sampling sites covered with plastic film for >10 years were selected in this study (Fig. 1), including soil samples under two mulching modes: (i) greenhouse cultivation with plastic film mulching used to cover the soil (G), and (ii) conventional film mulching used to cover the soil in open fields (M). Considering the plastic film mulching mode, geographic location, crop types, sown area, yield, and so on, at least three sampling sites of a mulching mode were collected at each planting region. The information on sampling sites was detailed in Table 1S.

All soil samples were collected from June to October 2019. For each soil sample, 15 replicate points were randomly selected within each field or greenhouse. Soil was collected from each point with a stainless-steel corer (3 cm in diameter) from the 0–10 cm soil layer. Each soil sample was thoroughly mixed and quartered, then packed into an aluminum box before being brought back to the laboratory. Subsequently, the soil samples were spread out flat on kraft paper in a clean room, air-dried to a constant weight at room temperature, and then stored in an aluminum box for subsequent separation and identification of microplastics.

2.2. Protocol for isolation of microplastics in agricultural soil

In order to increase the accuracy of image recognition during the detection process, microplastics in soil were allowed to be hierarchically detected by particle size classification (Jia et al., 2022). The soil sample was mixed evenly and then successively sieved through the 5 mm and 2 mm stainless steel filter meshes. At this stage, visible plant residues, stones, and other inert material were removed. Microplastics (2000–5000 μm) left on the 2 mm stainless steel filter mesh were selected and photographed to record their number and morphological characteristics.

Considering the limitations of instrument resolution and the influence of statistical software noise, we did not perform statistical analysis for microplastics in the size range of 0–50 μm. For the detection of microplastic ranging from 50 to 2000 μm, techniques using fluorescent staining (Shruti et al., 2022) combined with total reflection Fourier-transform infrared (FTIR) methods were applied. The microplastic isolation protocol allowed the quick and efficient isolation and characterization of the main plastic types present within soil. In order to ensure the effectiveness of separating microplastics from soil, different densities of suspension were used to suspense microplastics step by step in this study (Nuelle et al., 2014; Hurley et al., 2018). The developing details of the isolation protocol were specified in the supplement materials (3 Method section). The major operational steps included: a) preparation of the soil sample; b) density separation; c) removal of impurities; d) microscopic imaging; e) chemical identification; and f) statistical analysis. Initially, 10 g of soil passed through a 2 mm filter mesh was weighed into a clean glass beaker for subsequent isolation protocols. Each soil sample was replicated three times.

For density separation, a saturated NaCl solution (1.18 g cm^{-3}) was employed to extract microplastics from the soil. Briefly, 200 ml of NaCl solution was added to the soil sample, and the mixture was stirred at 400 rev min^{-1} for 20 min before being left to settle for 24 h. The supernatant containing the microplastic particles was transferred into a clean beaker. The abovementioned procedures were repeated three times to completely extract plastic from the soil samples. Following a 1-hour period of settling, the supernatants were filtered through a stainless filter with a 48-μm pore size to recover the particles for subsequent removal of impurities.

The particles that had been recovered from the stainless filter were transferred into a small glass bottle, to which a 10% NaOH (*w/v*) solution was added and mixed evenly. After this, the bottle was placed in a water bath (50 °C) for 6 h in order to decompose the organic matter present in the extracts while causing no obvious damage to the microplastics. After the removal of impurities, the particles were rinsed using deionized water and transferred to a clean beaker (about 200 ml of solution containing microplastic).

A Nile red solution (240 μg ml^{-1}) was then added to the plastic solution at a ratio of 1:2000 (Nile red: water), and the particles were left to stain for 30 min. Then a 0.2-μm glass fiber filter membrane (diameter: 60 mm) was settled on a vacuum filtration device (inner diameter: 45 mm), and the suspension was filtered through. More than one filter membrane was used for the same sample, so all the microplastics were evenly dispersed on the membrane instead of overlapping each other. To prevent the fluorescence effect of Nile red on the filter membrane, the membrane and vacuum filter device were repeatedly rinsed using deionized water at least three times. Each filter membrane-attached microplastic was then stored in an individual glass culture dish for subsequent observations and assays.

Photographs of the plastic particles were taken with a fluorescence microscope (Leica M165 FC, Germany) within 24 h of recovery from soil. Five scopes evenly distributed across each filter membrane were selected and photographed under both white light and fluorescence (Ex 450–490 nm, Em 500–550 nm). The locations were also marked on the photo for subsequent micro-Fourier Transform Interferometer (μ-FTIR) analysis to identify the composition of the microplastics using attenuated total reflectance (ATR) mode. FTIR, undisturbed by fluorescent signals, was used to identify microplastics based on specific regions in the polymeric functional group (Baruah et al., 2021; Turner and Holmes, 2011). Within the marked locations on each glass fiber filter membrane, suspected plastic particles with distinguishable shapes were found via the microscope attached to a Fourier infrared spectrometer. About 15

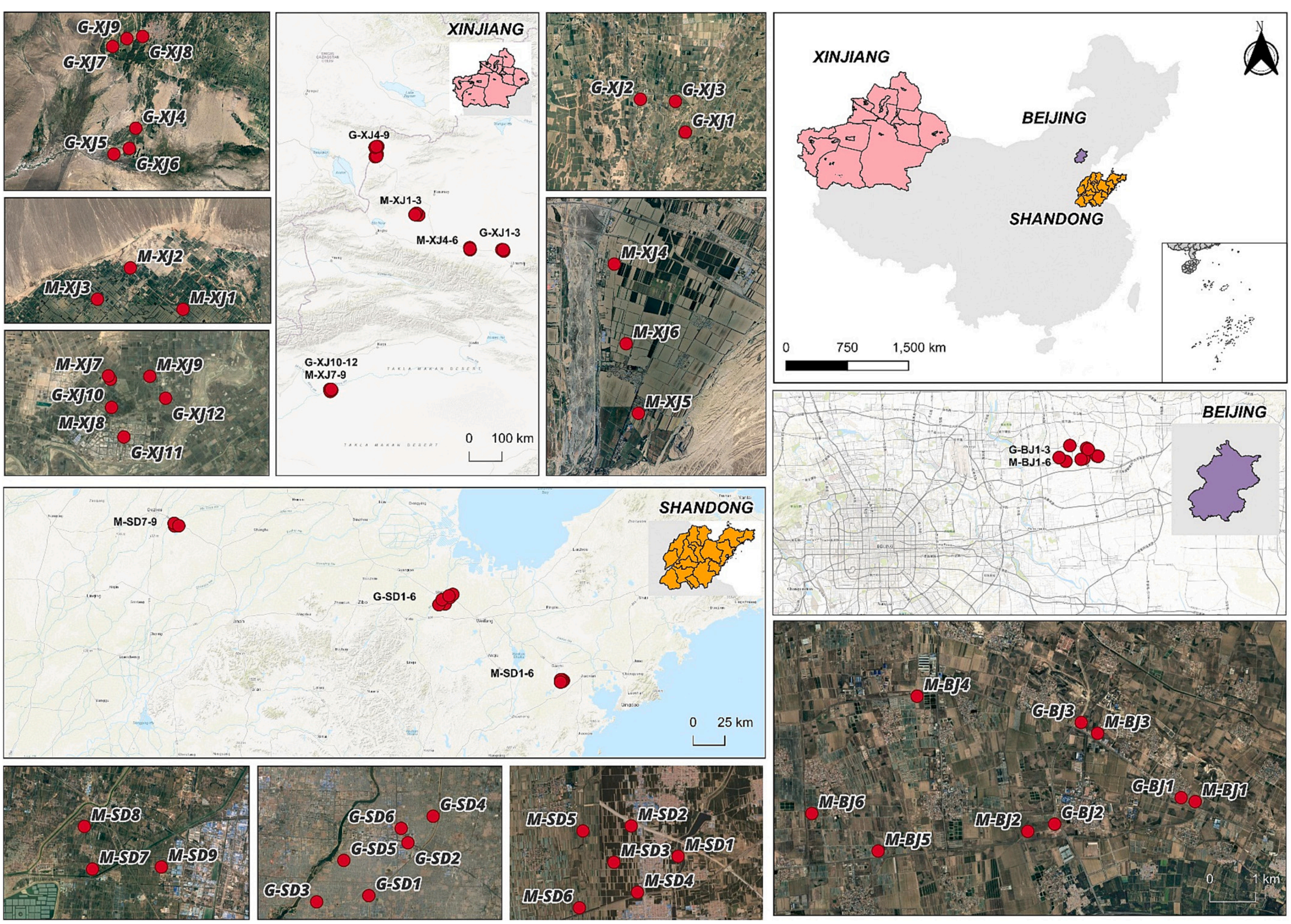

Fig. 1. Mapping of sampling sites under greenhouse (G) and field-based mulching (M) modes in Beijing, Shandong, and Xinjiang Province.

individual suspected particles for one soil sample (a total of 719 particles) were identified, whose aim was to correct the data to ensure its accuracy. The μ-FTIR spectrometer (Bruker, Germany) spectra were recorded from wavenumber ranging from 400 to 4000 cm^{-1} and 64 scans averaged at a resolution of 4 cm^{-1} in this study.

In order to reduce contamination from exogenous microplastics (e.g., in the air and from clothing), sample preparation, extraction, and detection were carried out in a clean room, and plastic products were avoided. Cotton test clothes and latex gloves were worn during the experiment. All the glassware and metalware in the experiment were pre-cleaned with deionized water and stored with tin foil when not in use.

2.3. Data analysis

Fiji ImageJ software was used to automatically count and analyze the morphological characteristics of microplastics in each fluorescence photo. In the process of using ImageJ software (Ferreira and Rasband, 2012), the image was first adjusted to an 8-bit gray scale, and the contrast and threshold of the image were adjusted to ensure that all particle fragments were included as far as possible (all particle sizes larger than 50 μm were included in the calculation).

The calculation formula of microplastic content *N* on each filter is as follows:

$$N = n \times \frac{S1}{S2 \times 5} \quad (1)$$

where *n* is the content of microplastics in each fluorescence photograph; S_1 is the total area of the filter membrane, d = 45 mm, $S_1 = \pi(d/2)^2 = 1589.6$ mm^2; and S_2 is the fluorescence photograph area ($S_2 = 4.35 \times 3.27 = 14.22$ mm^2). The constant 5 is the number of fluorescence photographs selected on each filter membrane.

The abundance of microplastics in soil *N* is expressed as the number of microplastics per kilogram of dry soil in unit of items kg^{-1}. The weight of microplastics in soils was calculated by the following formula:

$$W = A \times h \times \rho \times N \quad (2)$$

The weight of microplastics in soils *W* is expressed as mg microplastic per kilogram of dry soil (mg kg^{-1}), where *A* is the area of each microplastic detected in this study; *h* is the thickness of microplastic (set at 0.010 mm); and ρ is the density of microplastic detected in this study (set at 1.00 g cm^{-3}). The abundance of microplastics with particle sizes of 2000–5000 μm was the result of visual detection plus fluorescence identification.

Excel 2016 and IBM SPSS Statistics 25 (IBM Inc., Chicago, USA) were used to statistically analyze the data, and Duncan's method was used to test for significant differences using $P < 0.05$ as the cutoff for statistical significance. Origin 2021 mapping was used to analyze and fit the abundance and morphological characteristics of microplastics in farmland soil in different mulched areas. A structural equation model was applied to evaluate the total effect of microplastic distribution as amended by planting region and mulching modes. Redundancy analysis (RDA) was used to analyze the relationship between components of microplastic and planting regions as well as mulching modes with Canoco version 5.0.

3. Results

3.1. Abundance of microplastics in three typical agronomic regions

The abundance of microplastics in agricultural soils showed a distinct pattern dependent upon the mulching mode and geographical region we collected (Fig. 2). Overall, the abundance of microplastics in soil under greenhouse cropping was slightly greater (3.60×10^4 items kg^{-1}) than under conventional field-based mulching (3.01×10^4 items

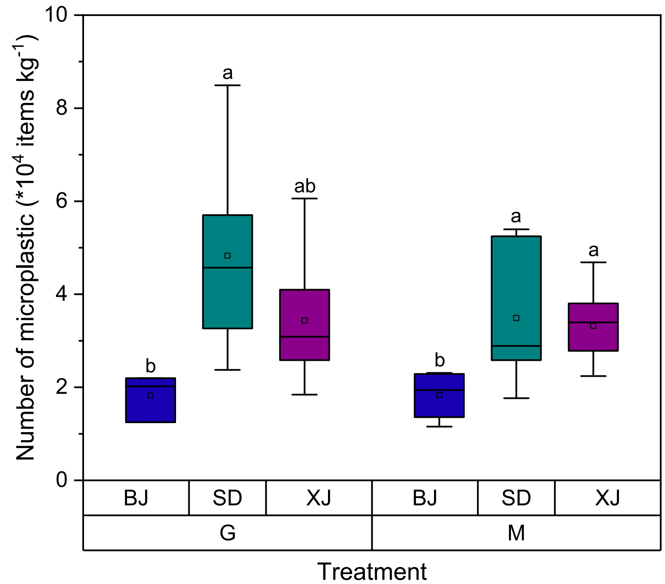

Fig. 2. Number of microplastics in soils under greenhouse (G) and field-based mulching (M) modes in Beijing, Shandong, and Xinjiang Province. BJ, SD, and XJ are shorthand for the sampling regions of Beijing, Shandong, and Xinjiang Province, respectively; G and M represent greenhouse and conventional film mulching modes, respectively; the different letters on the columns indicate that the number of microplastics in soil samples of different regions under the same mulching mode is significantly different ($P < 0.05$).

kg^{-1}), however, this proved to be non-significant (Table 2S, $P > 0.05$). The abundance of microplastics in Beijing suburban soils under both greenhouse and field mulching was 1.82×10^4 items kg^{-1} and 1.83×10^4 items kg^{-1}, respectively. In SD sites, the abundance of microplastic in intensive agricultural areas was 4.83×10^4 items kg^{-1} and 3.49×10^4 items kg^{-1} under greenhouse and field-based mulching, respectively. In XJ, microplastic abundance in the long-term planting area was 3.43×10^4 items kg^{-1} and 3.32×10^4 items kg^{-1} under greenhouse and field-based mulching, respectively. For three typical regions, the average abundance of microplastics in soils from Shandong (4.02×10^4 items kg^{-1}) and Xinjiang province (3.39×10^4 items kg^{-1}) was significantly ($P < 0.05$) higher than that from Beijing (1.83×10^4 items kg^{-1}).

A two-way ANOVA analysis (Table 2S in Supplemental Materials) showed that different mulching modes had no distinct effect on the abundance of microplastics in agricultural soil ($P > 0.05$), nevertheless planting regions (BJ, SD, and XJ) had a significant effect on microplastic abundance ($P < 0.05$). The interaction between mulching mode and regional location had no significant effect ($P > 0.05$).

3.2. Morphological characteristics of microplastics

The morphology of microplastics detected in farmland soil varied among the three mulching modes (Fig. 3); however, there were no significant differences in their area, perimeter, Feret's diameter, or length-width ratio ($P > 0.05$). In detail, the minimum area of microplastics detected was 286.3 μm^2, and the maximum was 141.6×10^4 μm^2. The perimeter values ranged from 118.4 μm to 1.95×10^4 μm, while the Feret's diameter ranged from 50.0 μm to 3345.6 μm. It should be noted that we only characterized microplastics with a size larger than 50 μm in this study. In addition, the length-width ratio ranged from 1.01 to 87.1, and the proportion of microplastics with circularity and solidity ranged from 0.5 to 1 accounted for 57.8% and 92.7%, respectively (Fig. 1S). The result indicated that most of the detected microplastics were close to round in shape, and that the majority were relatively regular in shape.

According to particle size, the detected microplastic in this study were divided into 6 grades: Class1 (50–100 μm), Class2 (100–250 μm),

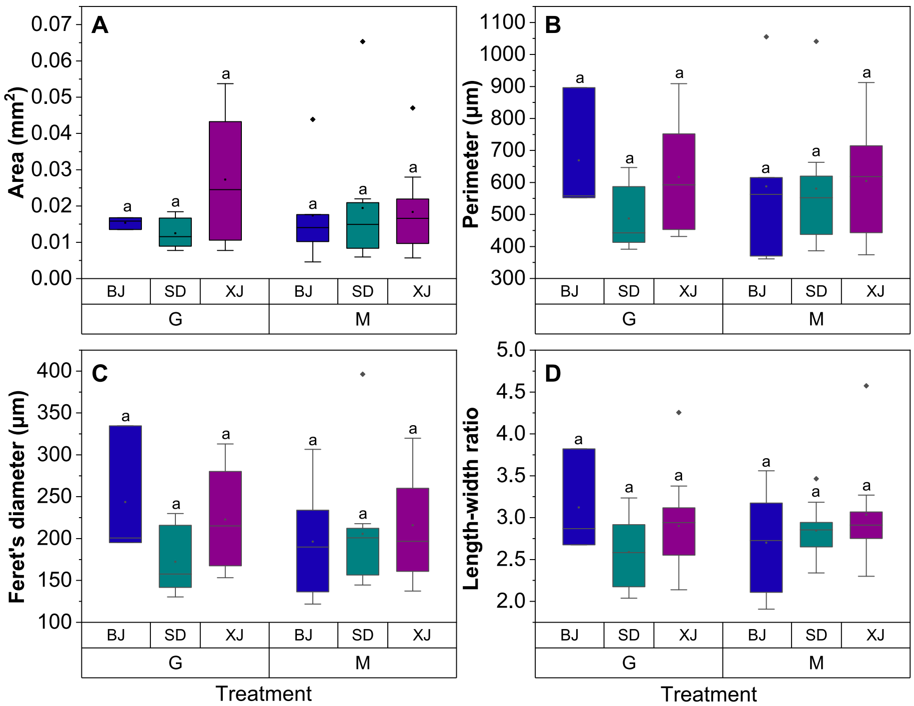

Fig. 3. Area, Perimeter, Feret's diameter and Length-width ratio of microplastic in soils under G and M modes. BJ, SD, and XJ are shorthand for the sampling regions of Beijing, Shandong, and Xinjiang, respectively; G and M represent greenhouse and conventional open field-based film mulching modes, respectively.

Class3 (250–500 μm), Class4 (500–1000 μm), Class5 (1000–2000 μm), Class6 (2000–5000 μm). The distribution of microplastics with different grades of particle size is shown in Fig. 4A-C. Microplastics with the small size at Class1 (50–100 μm) and Class2 (100–250 μm) accounted for about 80% of the total, and the microplastic abundance all decreased with the increasing particle size in the three planting regions (Fig. 4A-C). The proportions of six grades of microplastics in G mode were 49.4%, 31.8%, 9.7%, 5.4%, 2.9%, and 0.7%, respectively; in M mode, they were

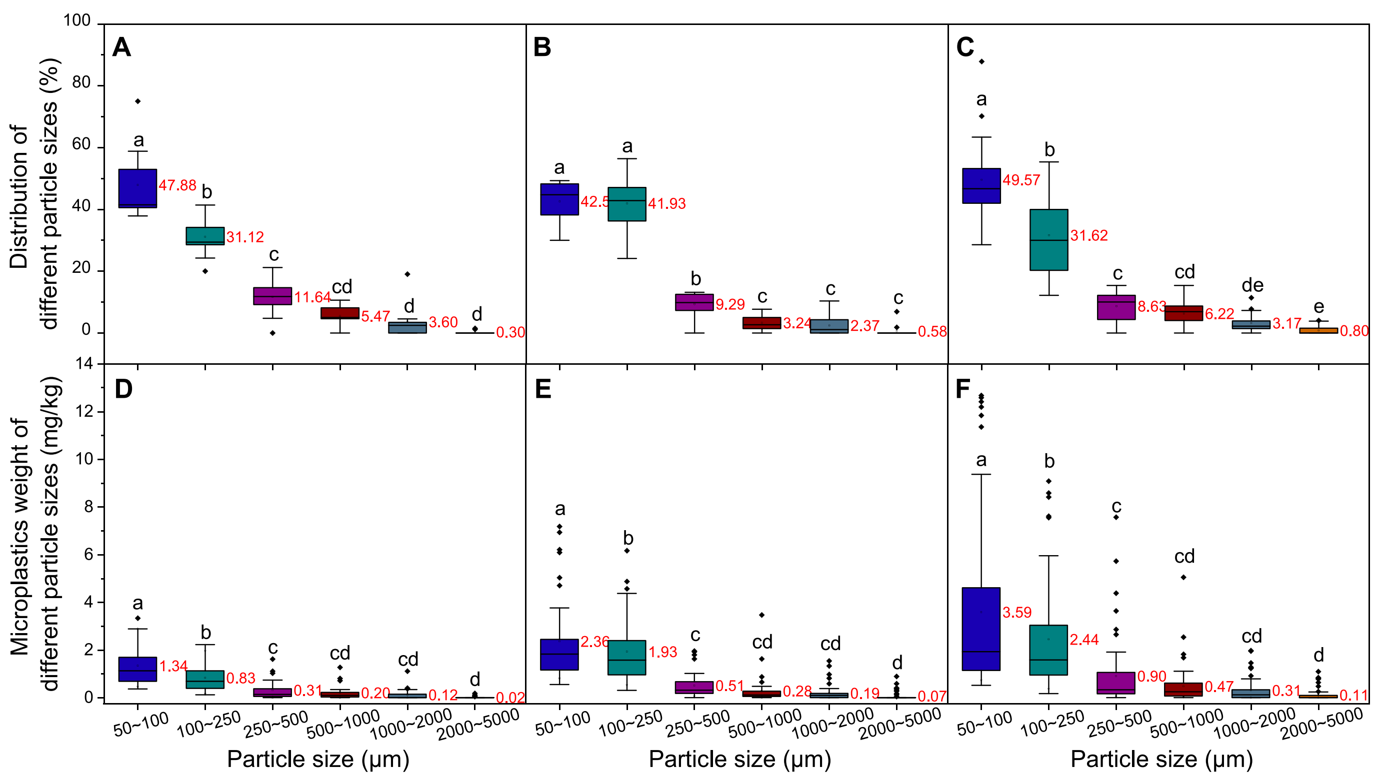

Fig. 4. Distribution of microplastic size (A-C) and weight (D—F) in soils in three planting regions (BJ, SD, and XJ). (A-C) Proportion of microplastic at six size grades in Beijing, Shandong, and Xinjiang Province, respectively. (D—F) Weight of microplastics at six size grades in Beijing, Shandong, and Xinjiang Province, respectively. The number next to each box in the figure means the average value.

44.7%, 37.7%, 9.2%, 4.8%, 3.1%, and 0.5%, respectively. Both in the two mulching modes (G and M), the microplastic abundance also decreased with increasing particle size (Fig. 2S). There was an order-of-magnitude difference between the abundance of microplastics with particle sizes of 50–2000 μm and > 2000 μm in the same soil sample.

A two-way ANOVA analysis showed that the two contrasting mulching modes had no significant effect on the distribution of microplastic particle size (Table 2S, $P > 0.05$). While the distribution of microplastics with a particle size ranging from 100 μm to 250 μm and 500 μm to 1000 μm was significantly different ($P < 0.05$) in the different planting regions (BJ, SD, and XJ). The proportion of microplastics with a particle size ranging from 100 μm to 250 μm in SD (41.9%) was significantly higher than that in BJ (31.1%) and XJ (31.6%). The trend of microplastic distribution in the size range 500–1000 μm in different planting regions was XJ > BJ > SD, and XJ was significantly higher than SD ($P < 0.05$). The interaction of mulching mode and planting region had significant effects on the distribution of microplastics with particle sizes of 50–100 μm and 100–250 μm ($P < 0.05$).

In order to better assess the risk of microplastics, their weight was estimated in this study based on the number and morphological characteristics (i.e., area, circularity, and solidity) of microplastics detected above. The reason for this was that the weight concentration of microplastics was often used in toxicological experiments. Microplastic weight all decreased with increasing particle size in the three agronomic regions (BJ, SD, and XJ), with each having a similar trend with the proportion of microplastic size (Fig. 4D-F). The average weight of microplastics on a per kg dry soil basis in the three regions was 3.12 mg kg^{-1}, 5.63 mg kg^{-1}, and 7.99 mg kg^{-1}, respectively, increasing in the order BJ < SD < XJ. Further, the microplastic weight in XJ was significantly higher than that in BJ. Nevertheless, a two-way ANOVA showed that planting region and mulching mode, as well as their interaction, had no significant effect on microplastic weight (Table 2S, $P > 0.05$).

3.3. Microplastics components detected by μ-FTIR with ATR mode

In total, 210 unquestionable microplastics from all the detected particles were selected and characterized using μ-FTIR to analyze their composition. In summary, six plastic types were found among the samples (Fig. 5A-C), namely polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (PA), urea-formaldehyde resins (UF), and other (Polyoxymethylene(Polyformaldehyde), POM), with the numbers of 105, 41, 3, 29, 31, and 1, respectively (Fig. 5A). Therefore, PE and PP represented the two dominant plastic types, accounting for 50.0% and 19.5% of all the particles analyzed, followed by UF and PA, accounting for 14.8% and 13.8%, respectively.

Regardless of the differences among regions (Fig. 5B), the dominant type of microplastic in G mode was PP, accounting for 37.0%, and PE in M mode, accounting for 63.4%. PP and PS in G mode were significantly ($P < 0.05$) higher, while PE and POM were significantly ($P < 0.05$) lower than those in M mode, respectively. There was no significant difference in the proportion of PA and UF microplastics between the two mulching modes ($P > 0.05$). Regardless of the two mulching modes' differences (Fig. 5C), PE was the dominant plastic type in Beijing, Shandong, and Xinjiang, accounting for 53.5%, 47.9%, and 48.2%, respectively, and that in Beijing was significantly higher than that in Shandong and Xinjiang ($P < 0.05$). However, there was no significant difference in PE microplastic proportion in Shandong and Xinjiang ($P > 0.05$). The proportions of PP and PS in the three planting regions were distinctly different, following the order SD > XJ > BJ. Similar to PE, PA in Beijing was 21.2% ($P < 0.05$), significantly higher than those in Shandong (10.7%) and Xinjiang (11.6%). UF in Beijing and Xinjiang were 16.2% and 16.2%, respectively, significantly higher than those in Shandong (12.1%). Contrary to PP and PS, POM was significantly different, following the order BJ > XJ > SD ($P < 0.05$).

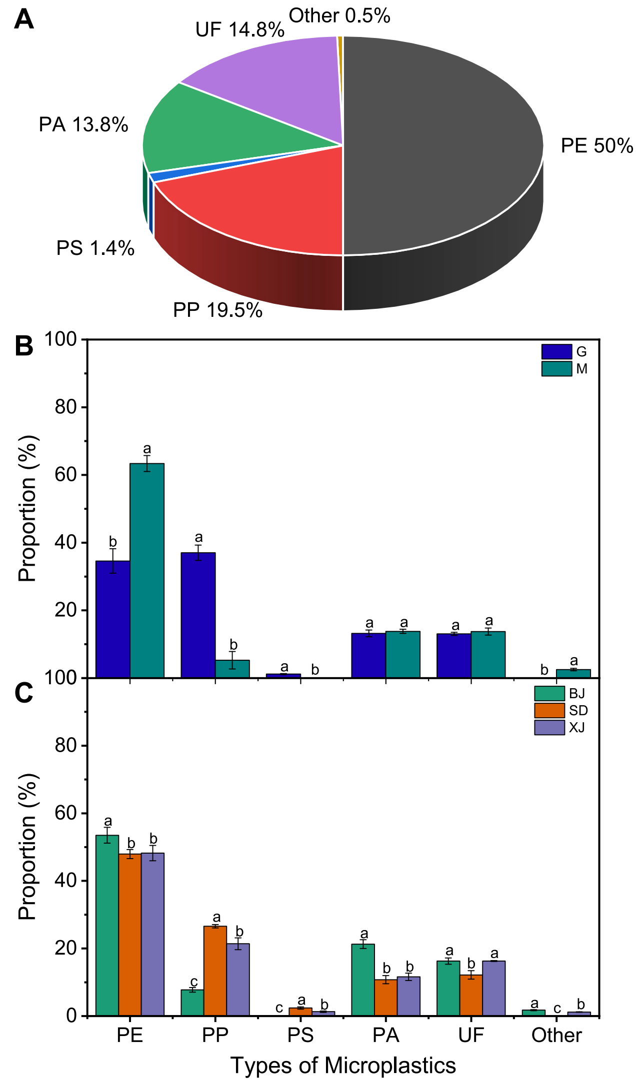

Fig. 5. Distribution of microplastic types in two mulching modes (G and M) and three planting regions in China (BJ, SD, and XJ). (A) Proportion of microplastic type determined by Fourier Transform Infrared Spectroscopy (FTIR); (B) Proportion of microplastic types in G and M modes, respectively. (C) Proportion of microplastic types in BJ, SD, and XJ planting regions. Therein, G and M represent greenhouse and conventional open field-based film mulching modes, respectively; BJ, SD, and XJ are shorthand for the planting regions of Beijing, Shandong, and Xinjiang, respectively; the different letters on the columns indicate significant differences ($P < 0.05$). PE indicates polyethylene, PP indicates polypropylene, PS indicates polystyrene, PA indicates polyamide, UF indicates urea-formaldehyde resins, and POM indicates Polyoxymethylene (Polyformaldehyde).

3.4. Effect of plastic mulching modes and planting regions on microplastic distribution

A structural equation model and RDA analysis were used to evaluate the response of microplastic distribution (i.e., abundance and morphological characteristics) and components on plastic mulching modes and planting regions. Overall, the abundance of microplastic in agricultural soils was significantly dependent on planting region, while the microplastic component was significantly determined by mulching mode (Fig. 6A-D). Detailly, the planting region directedly affected the number and area of microplastic with an estimate coefficient of 0.32 and 0.19, respectively, and then indirectly affected the microplastic weight with an estimate coefficient of 0.48 and 0.88, respectively (Fig. 6A). The standard total effects of planting regions on number, area, and weight

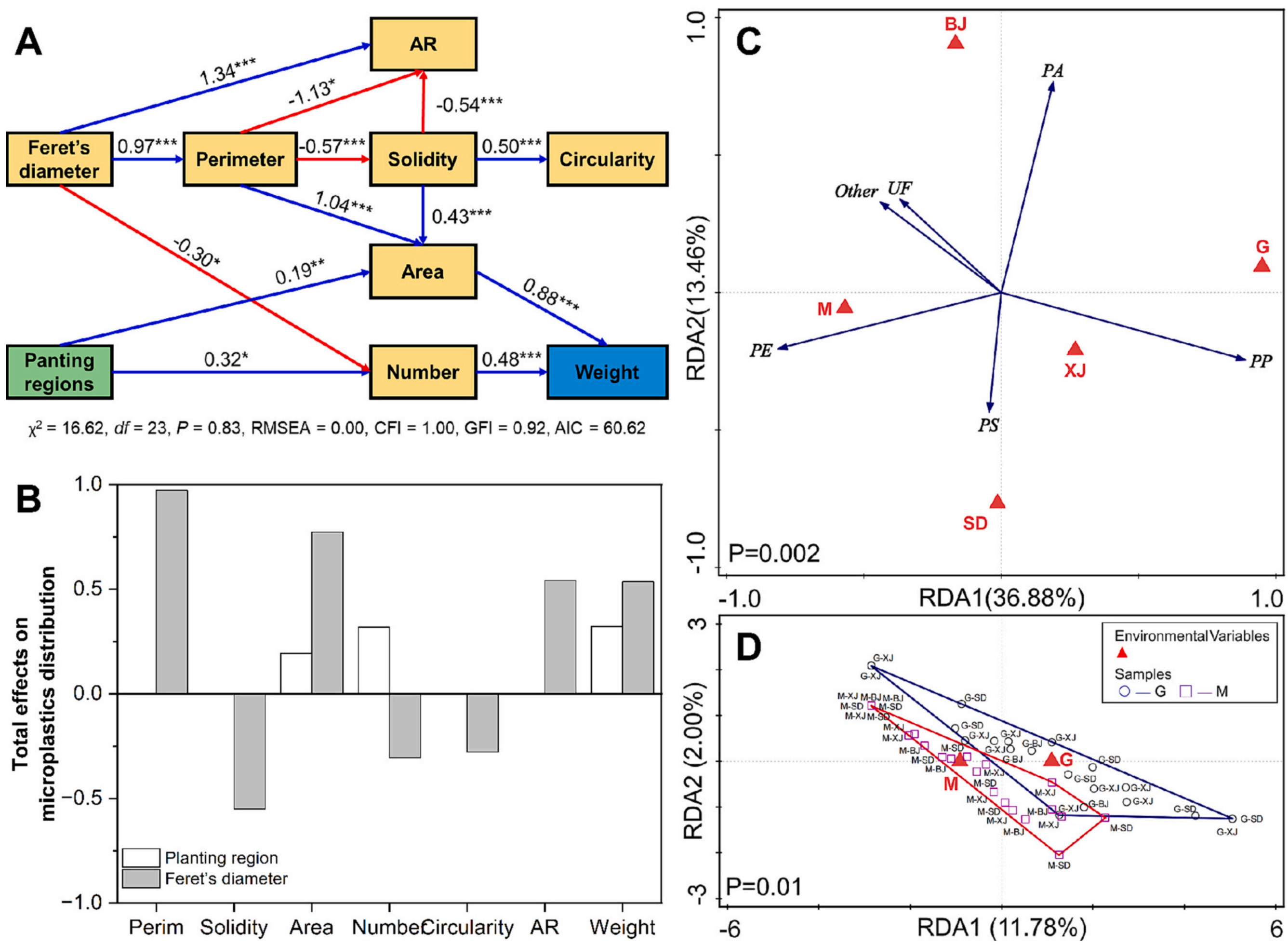

Fig. 6. Effect of plastic mulching modes (G and M) and planting regions (BJ, SD, and XJ) on the distribution of microplastics in this study. (A) A structural equation model showing the responses of microplastic abundance and morphological characteristics on plastic mulching modes and planting regions; AR indicates the length-width ratio of microplastics detected. The blue and red arrows indicate positive and negative path coefficients, respectively. The *p* values based on 1000 bootstrapping for path coefficients are indicated by * when $P < 0.05$, ** when $P < 0.01$, and *** when $P < 0.001$. (B) Standard total effect of planting regions and Feret's diameter on microplastic abundance and morphological characteristics; (C—D) RDA analysis of the microplastic types constrained by mulching modes and planting regions.

were 31.8%, 19.4%, and 32.3% (Fig. 6B, $P < 0.05$). Notably, the regression result showed that Feret's diameter was another important factor that dominated the weight of microplastics (Fig. 6A-B), which was not negligible in the detection of microplastics.

RDA showed that the explanatory variables, including plastic mulching modes and planting regions, accounted for 52.4% of the total variation of microplastic compositions, and the dominant types of microplastic, PP and PE, were significantly positively associated with G and M modes, respectively (Fig. 6C, $P < 0.05$). Further Interactive-forward-selection analysis showed that mulching modes (G and M) significantly affect the microplastic composition and could explain 34.4% of their total variation with a contribution rate of 65.6% (Table 3S in Supplemental Material). The microplastic types of samples under the two mulching modes were relatively clustered together in their respective groups (Fig. 6D, explanatory variables accounted for 13.78% of the total variation in this mode, $P < 0.05$), while there was no cluster in three typical regions. This was also confirmed by the result of the two-way ANOVA mentioned above.

4. Discussion

4.1. Distribution of microplastics in farmland

Microplastics were found in all 45 soil samples studied here. The spatial distribution of microplastics showed distinct patterns in our agricultural soils caused by geographical location and modes of mulching. Plastic films used to construct greenhouses combined with the use of conventional plastic mulching film (G mode) represent a common agronomic practice in China (Yu et al., 2021). It is a dominant factor resulting in the accumulation of microplastic in soils under this farming practice being significantly higher than under conventional open field-based plastic film mulching (M mode) in SD and XJ. Another notable phenomenon in Beijing was that microplastic abundance in M mode was higher than that in G mode. We attributed this finding to the samples collected in these urbanized farmland areas being nearer to roads (Fig. 1), leading to microplastic contamination associated with road traffic (e.g., more POM microplastics occurred). Which could be confirmed by the fact that microplastics were detected in road dust worldwide due to plastic litter fragmentation and vehicle tire abrasion (Blasing and Amelung, 2018; Myszka et al., 2023). Statistically, it has been shown that microplastic abundance was highest in intensive farming regions, followed by suburban farmland, and lowest in less intensive agricultural soil (Yang and He, 2021). In this research, microplastic abundance increased in the intensive farming regions of Shandong province, followed by the long-term agricultural region of Xinjiang, with the last being the suburban regions of Beijing. The farmland in Xinjiang province has been mulched with agricultural film for a long time, to the extent that excessive plastic film residues have visibly accumulated in these soils (Hu et al., 2021). This was the reason why the result had a discrepant trend from the abovementioned. Additionally, microplastic weight was in the order of BJ < SD < XJ, which was different from the trend of microplastic number (BJ < XJ < SD), because the microplastics in Xinjiang are relatively large on the whole (Figs. 3A and 4A-B).

In our study, the abundance of microplastic in agricultural soils from the largest vegetable production base was 4.83×10^4 items kg^{-1} (G-SD), significantly higher than that reported by Yu et al. (2021), where the abundance ranged from 310 to 5698 items kg^{-1}. One thing is certain, different separation methods for extracting microplastic from soil severely affect the abundance and spatial distributions, which cause the distinct results. Different statistical techniques (e.g., differentiated units expressed microplastic abundance) may also bring difficulties when comparing results from different studies, resulting in problems with statistical analysis of disparate datasets (Jia et al., 2022). For example, the abundance of microplastics in soil determined by density suspension combined with the heating method ranged from 5.80×10^2 to 1.19×10^4 pieces kg^{-1} in long-term mulched farmland in Gansu and northern Shaanxi (Cheng et al., 2020). The concentration of MPs in agricultural soil in Shaanxi Province ranged from 1.43×10^3 to 3.41×10^3 items kg^{-1} detected by the density fraction method (Ding et al., 2020). While the number of microplastics obtained from the soils using continuous air flotation followed by density separation was 571 pieces kg^{-1} and 263 pieces kg^{-1} in mulching and non-mulching soils on the coastal plain of Hangzhou Bay, respectively (Zhou et al., 2019). In the case of a low-density suspension for extracting microplastics, it will also inevitably underestimate the abundance of microplastics with a high density.

We found that microplastics recovered in the three planting regions mainly possessed small particle sizes within the range of 50–100 μm and 100–250 μm. Further, their abundance gradually decreased with increasing particle size, which was consistent with the previous study (Cheng et al., 2020). Microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea were also dominated by small particle sizes, with ca. 60% of the soil microplastics having a size range of <1 mm (Zhou et al., 2018). The microplastics with a size larger than 2000 μm, including those manually selected and identified by fluorescence microscopy, were analyzed in this study. There was an order-of-magnitude difference between the abundance of all microplastics with particle sizes of 50–2000 μm and >2000 μm in the same soil sample, which was consistent with the result by Jia et al. (2022), who reported microplastics with smaller sizes (10–500 μm) accounted for 96.5%–99.9% in agricultural soil in Xinjiang. Lately, microplastics in 1–1000 μm size range were defined as a new classification by Hartmann et al. (2019), which may be more likely to be transmitted or absorption/desorption toxic pollutants (i.e., heavy metal) (Khalid et al., 2021), or even absorbed by organisms (Li et al., 2020), posing an aggravated threat to the agroecosystems (Chen et al., 2023). Therefore, more attention should be paid to the toxicity of microplastics with small sizes (including nanoplastics) in the future.

4.2. Sources of microplastics in farmland

The different shapes and compositions of microplastics were expected to be closely related to their sources, such as greenhouse plastic covers, mulch film, packaging bags, manure or film-coating fertilizer, etc. In our study, the microplastic components identified by μ-FTIR included PE, PP, PS, PA, UF, and POM. Of these, PE and PP represented the two dominant microplastic types, which was consistent with the results of Liu et al. (2018). PE microplastics widely exist in agronomic regions, maybe due to their predominance within mulching film, packaging bags, irrigation pipes, farm implements, etc. On the whole, the abundance of PE microplastics in soil was related to farmland mulched with PE plastic film for a long time, this was consistent with the result of Hu et al., who indicated that microplastics in Xinjiang cotton fields were mainly PE components (Hu et al., 2021). PP may be derived from greenhouse film, agricultural drain pipe, strapping rope, farm implements, and beverage bottles (Ding et al., 2020). PP microplastic in G mode possessed a larger proportion than that in M mode, owing to PP being the major component of greenhouse plastic film. PA is mainly used as nylon, industrial cloth, medical appliances, knitwear, fishing nets, fertilizer, and pesticide bags, which are widely used in agricultural production. As the main component of coated-fertilizer, UF is widely used in farmland soil, representing a major source of microplastic in agricultural soils. PS is often used in the production of lamp-chimneys, electrical devices, packaging, etc., which are generally present in greenhouses. Therefore, this may be the reason why more PS microplastic accumulated in G mode than in M mode. POM was mainly used in parts of apparatus (such as wear-resisting parts, instrument boards, machine tools, gears, and other automotive parts); more vehicles and large machinery occurred in the suburbs of Beijing and long-term planting regions of Xinjiang, which is the reason for the higher abundance of POM microplastics. This further indicated that the slightly higher microplastic abundance under M mode in Beijing may be related to road traffic. Intensive agricultural areas of Shandong, especially under G mode, almost had no POM microplastics in the soils.

In this study, microplastics were mainly white or transparent film with a granular appearance, alongside microplastic fragments of black color and pink fiber particles derived from shed plastic film and conventional film or plastic packaging. The fiber-shaped microplastic may originate from the ropes or cloth strips used to tie vegetables to poles or as a net to allow vines to grow. The wide variety, wide application, and non-degradation of plastics resulted in large amounts of plastic residues (including microplastics and nanoplastics) accumulating in the soil, causing “white pollution” to the environment. Therefore, it is urgent to trace the source of plastic pollution, rationalize the use of plastic, and recycle of plastic waste.

4.3. Effects of plastic mulching mode and planting region on microplastics

The distinct distribution and characteristics of microplastic were related to planting regions, plastic mulching modes, soil physical and chemical properties, and other environmental factors (such as temperature and wind velocity) to a certain extent (Liu et al., 2023; Revell et al., 2021). Microplastic abundance and morphological characteristics were significantly dependent on planting regions, while microplastic components were dominated by plastic mulching modes. Planting regions significantly affected the abundance of microplastics, and the direct and indirect effects of planting regions on the weight of microplastics reached 32.30%. This could explain the different levels of plastic contamination exemplified in previous studies. Huang et al. (2020b) found a significant linear correlation between the amounts of mulch film use and the film residues in soils in the different regions of China, indicating that the application of agricultural plastic mulch films serves as one of the main sources of film-like MPs with a dominant component of PE in agricultural soils. However, plastic mulching modes had no significant effect on microplastic abundance in farmland; the microplastic pollution could not be simply attributed to the application of plastic mulch film. In our study, plastic mulching modes significantly affected the compositions of microplastics, combined with the contribution of plastic types, this conclusion can provide a reference for covering different mulch films.

Otherwise, land utilization type was one of the factors affecting the distribution of soil microplastics (Chen et al., 2022). Agricultural activity, like tillage, fertilization, irrigation, and recycling of plastic mulch film, also greatly affects the distribution of microplastics (Huang et al., 2020b; Zhang et al., 2022). The distribution of microplastic size in the mulched farmland soil in Northwest China was also linearly and negatively correlated with soil depth (Hu et al., 2021). However, issues with this study included a lack of consideration of microplastic distribution in different soil layers. The contribution of other environmental factors and anthropogenic activities to the microplastics distributed in soil needs to be further evaluated.

4.4. Perspectives for future soil microplastic research

(1) Establishment of a microplastic database and assessment of pollution levels. While microplastic contamination in terrestrial ecosystems is being increasingly investigated by researchers, there is a large knowledge gap on the extent to which agricultural soils are affected. Globally, agricultural plastic film represents a dominant source of microplastics in farmland, especially in China. It is necessary to establish a database on the distribution of microplastics in farmland soil, so as to grade and assess the level of microplastic pollution in farmland soil in China.

(2) **Tracing and controlling microplastics.** Besides the physical cracking and bio-degradation of plastics (secondary microplastics), microplastics also directly originate from a range of other activities, i.e., primary microplastics, which are specifically produced within a small size for a variety of applications, like cosmetic products or household cleaners. Tracing the source of microplastics will provide a better understanding of the contributors to microplastic pollution and be beneficial to controlling microplastic pollution at the source.

(3) **Microplastics and the carbon cycle.** As we know, biodegradable plastic can be utilized by soil microorganisms as a source of carbon, but there is a large knowledge gap in the relationship between microplastic-related carbon mineralization and greenhouse gas emissions. Further, one of the key debates is whether the advantage (e.g., carbon sequestration) of microplastics as carbon sources utilized by soil microorganisms outweighs the contribution of (micro)plastic degradation to carbon emissions. Therefore, revealing the contribution of plastics, even microplastics, to carbon sequestration or greenhouse gas emissions could provide data support for assessing the degradation effects of plastic production in the environment.

5. Conclusion

In order to reveal the contribution of planting regions and mulching modes to microplastic distribution, the characteristics of microplastics were investigated in greenhouse and open-field cultivation using plastic mulching film from three typical regions in China. Microplastics were readily detected in all 45 soil samples examined in this study. Their abundance, morphology, and composition showed distinct characteristics for each of the three typical mulched agricultural areas of China. Generally, the abundance of microplastics was highest in the Xinjiang long-term planting area, followed by the Shandong intensive planting area, and lowest in the Beijing suburbs. The microplastic particles smaller than 250 μm accounted for 80% of the total recovered, suggesting that smaller-sized microplastics play a dominant role in the three planting regions. The distribution of microplastic size and microplastic weight decreased with increasing particle size. PE and PP were the dominant microplastic types. Otherwise, the distribution of microplastic abundance was significantly responsive to agronomic region, while microplastic composition was significantly dependent on plastic film mulching mode (G vs. M). Microplastic sizes and types were not negligible in the assessment of microplastic pollution. This information provides key data for assessing the environmental risk of microplastics and preventing plastic pollution from agricultural plastic film. In the future, big data technologies or isotope tracer techniques will be considered to quantify and assess the contribution of more different plastic sources to microplastics in soil.

CRediT authorship contribution statement

All the persons who have made substantial contributions to the work reported in the manuscript, including those who contributed to the preliminary work are named in the acknowledgements section of the manuscript. The authors' major contributions are listed below:

Ruimin Qi: Conceptualization, Methodology, Validation, Investigation, Data curation, Formal analysis, Writing- Original draft preparation

Yuanyuan Tang: Resources

Davey L. **Jones:** Conceptualization, Writing- Reviewing and Editing

Wenqing He: Resources

Changrong Yan: Conceptualization, Resources, Supervision, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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