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1	Occurrence and characteristics of microplastics in soils from
2	greenhouse and open-field cultivation using plastic mulch film
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15	Abstract
16	There is a major knowledge gap concerning the extent of microplastic pollution in agronomic

17 regions of China, which represent a plastic use hotspot. In order to clarify the amendment of

- 18 agronomic region and plastic film mulching mode to microplastics distribution, the characteristics
- 19 of microplastics distributed in agricultural soils from three typical regions (Beijing (BJ), Shandong
- 20 (SD), and Xinjiang (XJ)) with two plastic film mulching modes (greenhouse (G) and conventional

21	field-based film mulching (M)) in China were investigated. Microplastics weight and their response
22	to planting regions were also evaluated in this study. The result showed that the average abundance
23	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
24	3.39×10^4 items kg ⁻¹ , and the estimated weight of microplastics per kg of dry soils was 3.12 mg kg ⁻¹
25	¹ , 5.63 mg kg ⁻¹ , and 7.99 mg kg ⁻¹ , respectively. Microplastics in farmland were mainly of small
26	particle size (50 to 250 μ m), with their abundance decreasing with increasing particle size. Among
27	the microplastics detected, polyethylene and polypropylene were the two dominant types present,
28	accounting for 50.0% and 19.7%, respectively. The standard total effect of planting regions on
29	microplastic number and weight was 31.8% and 32.3%, and plastic film mulching modes (G vs. M)
30	could explain 34.4% of the total variation of microplastic compositions with a contribution rate of
31	65.6% in this study. This research provides key data for an assessment of the environmental risk of
32	microplastics and supports the development of guidelines for the sustainable use of agricultural
33	plastic film. Further, it is necessary to quantify and assess the contribution of other different plastic
34	sources to microplastics in soil. Big data technologies or isotope tracer techniques may be promising
35	approaches.

Keywords: Microplastics, Spatial distribution, Morphology and composition, Plastic mulch film,
Planting regions.

38 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

42	mainly focused on microplastic pollution in marine and other aquatic ecosystems (Alimi et al., 2018;
43	Qi et al., 2020). There is increasing evidence that microplastic pollution may pose a major threat to
44	the functioning and sustainability of terrestrial ecosystems (Huang et al., 2020a; Li et al., 2020;
45	Zhou et al., 2021b). Detailly, these microplastics incorporated into soil can increase contact angle
46	and saturated hydraulic conductivity, decrease bulk density and water holding capacity (Yu et al.,
47	2023), and alter bacterial community structure (Li et al., 2023; Zhou et al., 2021a). Microplastics
48	can also be readily absorbed and ingested by keystone organisms, which regulate critical functions
49	within terrestrial ecosystems (Zhou et al., 2021a; Zhou et al., 2020). They can be transported through
50	food webs and into the human food chain (Li et al., 2020; Okeke et al., 2022), where their effects
51	on human health remain uncertain but remain of concern (Kannan and Vimalkumar, 2021; Udovicki
52	et al., 2022). Although investigations on microplastic pollution in terrestrial ecosystems have
53	increased recently, most of them have focused on the environmental hazards of microplastics. Very
54	little work has been done regarding the abundance and composition of microplastics in typical
55	planting region. There is a major knowledge gap concerning the extent of microplastic pollution in
56	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</p> 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.

71 Suburbs of Beijing (BJ), intensive agricultural areas of Shandong (SD), and long-term planting 72 regions of Xinjiang (XJ) represent three typical agronomic regions in China in which the application 73 of agricultural plastic film is commonplace, quantification and assessment of microplastic pollution 74 in the actual soil environment are in breach. We hypothesize that the abundance and composition of 75 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 76 77 in soils from these distinct planting regions and under two modes of plastic use, namely greenhouse 78 (G) and field mulching (M) conditions, aiming to reveal the total effects of planting regions and 79 mulching modes on microplastic distribution in the typical agronomic regions. We used this 80 information to assess the sources and possible implication of microplastic contamination in 81 farmland. The effects of agricultural activities in different regions on the abundance and 82 morphological characteristics of soil microplastics are also discussed and evaluated, providing data 83 to support further research on pollution by legacy plastic in farmland.

84 2 Materials and Methods

85 2.1 Test sites and sample collection

86 The experimental sites involved in the study were located in Beijing (BJ, 40°05'N, 116°54'E, 87 including the experimental center for the Institute of Agricultural Environment and Sustainable 88 Development, Shunyi, Chinese Academy of Agricultural Sciences), Shandong Province (SD, 89 36°26'N~37°20'N, 116°29'E~120°03'E, including the intensive agricultural areas in Qingdao, 90 Dezhou, and Shouguang city), and Xinjiang Uygur Autonomous Region (hereinafter referred to as 91 Xinjiang, XJ, 40°35'N~46°72'N, 81°15'E~87°19'E, including the agricultural planting areas with 92 long-term mulching in Changji city, Tacheng District, Alar, Kuitun, and Kanas). In detail, the site 93 of Beijing has a temperate monsoon climate with an annual average temperature of 10-12°C and a 94 mean annual precipitation of 644 mm. The region surrounding Shandong has a warm temperate 95 monsoon climate with an annual average temperature of 11-14°C and a mean annual precipitation 96 of 650-750 mm. The region surrounding Xinjiang has a temperate continental climate with a larger 97 difference in annual average temperature and a mean annual precipitation of 150 mm.

A total of 45 sampling sites covered with plastic film for more than 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.

104 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.

111 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 μ m) left on the 2 mm stainless steel filter mesh were selected and photographed to record their number and morphological characteristics.

118 Considering the limitations of instrument resolution and the influence of statistical software 119 noise, we did not perform statistical analysis for microplastics in the size range of 0-50 µm. For the 120 detection of microplastic ranging from 50-2000 µm, techniques using fluorescent staining (Shruti et 121 al., 2022) combined with total reflection Fourier-transform infrared (FTIR) methods were applied. 122 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 123 124 microplastics from soil, different densities of suspension were used to suspense microplastics step 125 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 minutes before being left to settle for 24 hours. 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 hours 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 minutes. 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

148 sample, so all the microplastics were evenly dispersed on the membrane instead of overlapping each 149 other. To prevent the fluorescence effect of Nile red on the filter membrane, the membrane and 150 vacuum filter device were repeatedly rinsed using deionized water at least three times. Each filter 151 membrane-attached microplastic was then stored in an individual glass culture dish for subsequent 152 observations and assays.

153 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 154 155 membrane were selected and photographed under both white light and fluorescence (Ex 450-490 156 nm, Em 500-550 nm). The locations were also marked on the photo for subsequent micro-Fourier 157 Transform Interferometer (μ -FTIR) analysis to identify the composition of the microplastics using 158 attenuated total reflectance (ATR) mode. FTIR, undisturbed by fluorescent signals, was used to 159 identify microplastics based on specific regions in the polymeric functional group (Baruah et al., 160 2021; Turner and Holmes, 2011). Within the marked locations on each glass fiber filter membrane, 161 suspected plastic particles with distinguishable shapes were found via the microscope attached to a 162 Fourier infrared spectrometer. About 15 individual suspected particles for one soil sample (a total 163 of 719 particles) were identified, whose aim was to correct the data to ensure its accuracy. The μ -164 FTIR spectrometer (Bruker, Germany) spectra were recorded from wavenumber ranging from 400-165 4000 cm⁻¹ and 64 scans averaged at a resolution of 4 cm⁻¹ in this study. 166 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 167

- 168 products were avoided. Cotton test clothes and latex gloves were worn during the experiment. All
- 169 the glassware and metalware in the experiment were pre-cleaned with deionized water and stored

170 with tin foil when not in use.

171 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 et al., 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).

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

178
$$N = n \times \frac{S1}{S2 \times 5}$$
 (Eqn. 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, $S_1 = \pi (d/2)^2 = 1589.6 \text{ mm}^2$; and S_2 is the fluorescence photograph area $(S_2 = 4.35 \times 3.27 = 14.22 \text{ mm}^2)$. The constant 5 is the number of fluorescence photographs selected on each filter membrane.

183 The abundance of microplastics in soil N is expressed as the number of microplastics per 184 kilogram of dry soil in unit of items kg⁻¹. The weight of microplastics in soils was calculated by the 185 following formula:

186 $W = A \times h \times \rho \times N$ (Eqn. 2)

187 The weight of microplastics in soils W is expressed as mg microplastic per kilogram of dry soil 188 (mg kg⁻¹), where A is the area of each microplastic detected in this study; h is the thickness of 189 microplastic (set at 0.010 mm); and ρ is the density of microplastic detected in this study (set at 1.00 190 g cm⁻³). The abundance of microplastics with particle sizes of 2000-5000 µm was the result of visual 191 detection plus fluorescence identification.

192 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 193 194 the cutoff for statistical significance. Origin 2021 mapping was used to analyze and fit the 195 abundance and morphological characteristics of microplastics in farmland soil in different mulched 196 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 197 analyze the relationship between components of microplastic and planting regions as well as 198 199 mulching modes with Canoco version 5.0.

200 3 Results

201 3.1 Abundance of microplastics in three typical agronomic regions

202	The abundance of microplastics in agricultural soils showed a distinct pattern dependent upon
203	the mulching mode and geographical region we collected (Fig. 2). Overall, the abundance of
204	microplastics in soil under greenhouse cropping was slightly greater $(3.60 \times 10^4 \text{ items } \text{kg}^{-1})$ than
205	under conventional field-based mulching $(3.01 \times 10^4 \text{ items kg}^{-1})$, however, this proved to be non-
206	significant (Table 2S, $P > 0.05$). The abundance of microplastics in Beijing suburban soils under
207	both greenhouse and field mulching was 1.82×10^4 items kg ⁻¹ and 1.83×10^4 items kg ⁻¹ , respectively.
208	In SD sites, the abundance of microplastic in intensive agricultural areas was 4.83×10^4 items kg ⁻¹
209	and 3.49×10^4 items kg ⁻¹ under greenhouse and field-based mulching, respectively. In XJ,
210	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).

220 3.2 Morphological characteristics of microplastics

221 The morphology of microplastics detected in farmland soil varied among the three mulching 222 modes (Fig. 3); however, there were no significant differences in their area, perimeter, Feret's 223 diameter, or length-width ratio (P > 0.05). In detail, the minimum area of microplastics detected 224 was 286.3 μ m², and the maximum was 141.6 × 10⁴ μ m². The perimeter values ranged from 118.4 μ m to $1.95 \times 10^4 \mu$ m, while the Feret's diameter ranged from 50.0 μ m to 3345.6 μ m. It should be 225 226 noted that we only characterized microplastics with a size larger than 50 µm in this study. In addition, 227 the length-width ratio ranged from 1.01 to 87.1, and the proportion of microplastics with circularity 228 and solidity ranged from 0.5 to 1 accounted for 57.8% and 92.7%, respectively (Fig. 1S). The result 229 indicated that most of the detected microplastics were close to round in shape, and that the majority 230 were relatively regular in shape.



232	Class1 (50-100 µm), Class2 (100-250 µm), Class3 (250-500 µm), Class4 (500-1000 µm), Class5
233	(1000-2000 μ m), Class6 (2000-5000 μ m). The distribution of microplastics with different grades of
234	particle size is shown in Fig. 4A-C. Microplastics with the small size at Class1 (50-100 μ m) and
235	Class2 (100-250 μ m) accounted for about 80% of the total, and the microplastic abundance all
236	decreased with the increasing particle size in the three planting regions (Fig 4A-C). The proportions
237	of six grades of microplastics in G mode were 49.4%, 31.8%, 9.7%, 5.4%, 2.9%, and 0.7%,
238	respectively; in M mode, they were 44.7%, 37.7%, 9.2%, 4.8%, 3.1%, and 0.5%, respectively. Both
239	in the two mulching modes (G and M), the microplastic abundance also decreased with increasing
240	particle size (Fig. 2S). There was an order-of-magnitude difference between the abundance of
241	microplastics with particle sizes of 50-2000 μ m and > 2000 μ m in the same soil sample.
242	A two-way ANOVA analysis showed that the two contrasting mulching modes had no
243	significant effect on the distribution of microplastic particle size (Table 2S, $P > 0.05$). While the
244	distribution of microplastics with a particle size ranging from 100 μ m to 250 μ m and 500 μ m to
245	1000 μ m was significantly different ($P < 0.05$) in the different planting regions (BJ, SD, and XJ).
246	The proportion of microplastics with a particle size ranging from 100 μ m to 250 μ m in SD (41.9%)

248 distribution in the size range 500-1000 μm in different planting regions was XJ >BJ >SD, and XJ

was significantly higher than that in BJ (31.1%) and XJ (31.6%). The trend of microplastic

247

- 249 was significantly higher than SD (P < 0.05). The interaction of mulching mode and planting region 250 had significant effects on the distribution of microplastics with particle sizes of 50-100 µm and 100-251 250 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

254 detected above. The reason for this was that the weight concentration of microplastics was often 255 used in toxicological experiments. Microplastic weight all decreased with increasing particle size in 256 the three agronomic regions (BJ, SD, and XJ), with each having a similar trend with the proportion 257 of microplastic size (Fig 4D-F). The average weight of microplastics on a per kg dry soil basis in 258 the three regions was 3.12 mg kg⁻¹, 5.63 mg kg⁻¹, and 7.99 mg kg⁻¹, respectively, increasing in the 259 order BJ < SD < XJ. Further, the microplastic weight in XJ was significantly higher than that in BJ. 260 Nevertheless, a two-way ANOVA showed that planting region and mulching mode, as well as their 261 interaction, had no significant effect on microplastic weight (Table 2S, P > 0.05).

262 3.3 Microplastics components detected by μ -FTIR with ATR mode

263 In total, 210 unquestionable microplastics from all the detected particles were selected and 264 characterized using µ-FTIR to analyze their composition. In summary, six plastic types were found 265 amongst the samples (Fig. 5A-C), namely polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (PA), urea-formaldehyde resins (UF), and other (Polyoxymethylene(Polyformaldehyde), 266 267 POM), with the numbers of 105, 41, 3, 29, 31, and 1, respectively (Fig. 5A). Therefore, PE and PP 268 represented the two dominant plastic types, accounting for 50.0% and 19.5% of all the particles 269 analyzed, followed by UF and PA, accounting for 14.8% and 13.8%, respectively. 270 Regardless of the differences among regions (Fig. 5B), the dominant type of microplastic in G 271 mode was PP, accounting for 37.0%, and PE in M mode, accounting for 63.4%. PP and PS in G 272 mode were significantly (P < 0.05) higher, while PE and POM were significantly (P < 0.05) lower 273 than those in M mode, respectively. There was no significant difference in the proportion of PA and

274 UF microplastics between the two mulching modes (P > 0.05). Regardless of the two mulching

275	modes' differences (Fig. 5C), PE was the dominant plastic type in Beijing, Shandong, and Xinjiang,
276	accounting for 53.5%, 47.9%, and 48.2%, respectively, and that in Beijing was significantly higher
277	than that in Shandong and Xinjiang ($P < 0.05$). However, there was no significant difference in PE
278	microplastic proportion in Shandong and Xinjiang ($P > 0.05$). The proportions of PP and PS in the
279	three planting regions were distinctly different, following the order $SD > XJ > BJ$. Similar to PE,
280	PA in Beijing was 21.2% ($P < 0.05$), significantly higher than those in Shandong (10.7%) and
281	Xinjiang (11.6%). UF in Beijing and Xinjiang were 16.2% and 16.2%, respectively, significantly
282	higher than those in Shandong (12.1%). Contrary to PP and PS, POM was significantly different,
283	following the order $BJ > XJ > SD$ ($P < 0.05$).

284 3.4 Effect of plastic mulching modes and planting regions on microplastic distribution

285 A structural equation model and RDA analysis were used to evaluate the response of 286 microplastic distribution (i.e., abundance and morphological characteristics) and components on 287 plastic mulching modes and planting regions. Overall, the abundance of microplastic in agricultural 288 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 289 290 affected the number and area of microplastic with an estimate coefficient of 0.32 and 0.19, 291 respectively, and then indirectly affected the microplastic weight with an estimate coefficient of 0.48 292 and 0.88, respectively (Fig. 6A). The standard total effects of planting regions on number, area, and 293 weight were 31.8%, 19.4%, and 32.3% (Fig. 6B, P < 0.05). Notably, the regression result showed 294 that Feret's diameter was another important factor that dominated the weight of microplastics (Fig. 295 6A-B), which was not negligible in the detection of microplastics.

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

306 4 Discussion

307 4.1 Distribution of microplastics in farmland

308	Microplastics were found in all 45 soil samples studied here. The spatial distribution of
309	microplastics showed distinct patterns in our agricultural soils caused by geographical location and
310	modes of mulching. Plastic films used to construct greenhouses combined with the use of
311	conventional plastic mulching film (G mode) represent a common agronomic practice in China (Yu
312	et al., 2021). It is a dominant factor resulting in the accumulation of microplastic in soils under this
313	farming practice being significantly higher than under conventional open field-based plastic film
314	mulching (M mode) in SD and XJ. Another notable phenomenon in Beijing was that microplastic
315	abundance in M mode was higher than that in G mode. We attributed this finding to the samples

316 collected in these urbanized farmland areas being nearer to roads (Fig. 1), leading to microplastic 317 contamination associated with road traffic (e.g., more POM microplastics occurred). Which could 318 be confirmed by the fact that microplastics were detected in road dust worldwide due to plastic litter 319 fragmentation and vehicle tire abrasion (Blasing and Amelung, 2018; Myszka et al., 2023). 320 Statistically, it has been shown that microplastic abundance was highest in intensive farming regions, 321 followed by suburban farmland, and lowest in less intensive agricultural soil (Yang and He, 2021). 322 In this research, microplastic abundance increased in the intensive farming regions of Shandong 323 province, followed by the long-term agricultural region of Xinjiang, with the last being the suburban 324 regions of Beijing. The farmland in Xinjiang province has been mulched with agricultural film for 325 a long time, to the extent that excessive plastic film residues have visibly accumulated in these soils 326 (Hu et al., 2021). This was the reason why the result had a discrepant trend from the abovementioned. 327 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 328 329 large on the whole (Fig. 3A and Fig. 4A-B). 330 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

338	with the heating method ranged from 5.80×10^2 to 1.19×10^4 pieces kg ⁻¹ in long-term mulched
339	farmland in Gansu and northern Shaanxi (Cheng et al., 2020). The concentration of MPs in
340	agricultural soil in Shaanxi Province ranged from 1.43×10^3 to 3.41×10^3 items kg ⁻¹ detected by the
341	density fraction method (Ding et al., 2020). While the number of microplastics obtained from the
342	soils using continuous air flotation followed by density separation was 571 pieces kg ⁻¹ and 263
343	pieces kg ⁻¹ in mulching and non-mulching soils on the coastal plain of Hangzhou Bay, respectively
344	(Zhou et al., 2019). In the case of a low-density suspension for extracting microplastics, it will also
345	inevitably underestimate the abundance of microplastics with a high density.
346	We found that microplastics recovered in the three planting regions mainly possessed small
347	particle sizes within the range of 50-100 µm and 100-250 µm. Further, their abundance gradually
348	decreased with increasing particle size, which was consistent with the previous study (Cheng et al.,
349	2020). Microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea were also
350	dominated by small particle sizes, with ca. 60% of the soil microplastics having a size range of < 1
351	mm (Zhou et al., 2018). The microplastics with a size larger than 2000 μ m, including those manually
352	selected and identified by fluorescence microscopy, were analyzed in this study. There was an order-
353	of-magnitude difference between the abundance of all microplastics with particle sizes of 50-2000
354	μ m and > 2000 μ m in the same soil sample, which was consistent with the result by Jia et al. (2022),
355	who reported microplastics with smaller sizes (10-500 μ m) accounted for 96.5%–99.9% in
356	agricultural soil in Xinjiang. Lately, microplastics in 1-1000 µm size range were defined as a new
357	classification by Hartmann et al. (2019), which may be more likely to be transmitted or
358	absorption/desorption toxic pollutants (i.e., heavy metal) (Khalid et al., 2021), or even absorbed by
359	organisms (Li et al., 2020), posing an aggravated threat to the agroecosystems (Chen et al., 2023).

360 Therefore, more attention should be paid to the toxicity of microplastics with small sizes (including361 nanoplastics) in the future.

362 4.2 Sources of microplastics in farmland

363 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-364 coating fertilizer, etc. In our study, the microplastic components identified by µ-FTIR included PE, 365 366 PP, PS, PA, UF, and POM. Of these, PE and PP represented the two dominant microplastic types, 367 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, 368 369 irrigation pipes, farm implements, etc. On the whole, the abundance of PE microplastics in soil was 370 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 371 372 (Hu et al., 2021). PP may be derived from greenhouse film, agricultural drain pipe, strapping rope, 373 farm implements, and beverage bottles (Ding et al., 2020). PP microplastic in G mode possessed a 374 larger proportion than that in M mode, owing to PP being the major component of greenhouse plastic 375 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 376 377 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 378 379 devices, packaging, etc., which are generally present in greenhouses. Therefore, this may be the 380 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 longterm 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.

387 In this study, microplastics were mainly white or transparent film with a granular appearance, 388 alongside microplastic fragments of black color and pink fiber particles derived from shed plastic 389 film and conventional film or plastic packaging. The fiber-shaped microplastic may originate from 390 the ropes or cloth strips used to tie vegetables to poles or as a net to allow vines to grow. The wide 391 variety, wide application, and non-degradation of plastics resulted in large amounts of plastic 392 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, 393 rationalize the use of plastic, and recycle of plastic waste. 394

395 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 402 403 levels of plastic contamination exemplified in previous studies. Huang et al. (2020b) found a 404 significant linear correlation between the amounts of mulch film use and the film residues in soils 405 in the different regions of China, indicating that the application of agricultural plastic mulch films 406 serves as one of the main sources of film-like MPs with a dominant component of PE in agricultural 407 soils. However, plastic mulching modes had no significant effect on microplastic abundance in 408 farmland; the microplastic pollution could not be simply attributed to the application of plastic 409 mulch film. In our study, plastic mulching modes significantly affected the compositions of 410 microplastics, combined with the contribution of plastic types, this conclusion can provide a 411 reference for covering different mulch films.

412 Otherwise, land utilization type was one of the factors affecting the distribution of soil 413 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., 414 415 2020b; Zhang et al., 2022). The distribution of microplastic size in the mulched farmland soil in 416 Northwest China was also linearly and negatively correlated with soil depth (Hu et al., 2021). 417 However, issues with this study included a lack of consideration of microplastic distribution in 418 different soil layers. The contribution of other environmental factors and anthropogenic activities to 419 the microplastics distributed in soil needs to be further evaluated.

420 4.4 Perspectives for future soil microplastic research

421 (1) Establishment of a microplastic database and assessment of pollution levels. While
 422 microplastic contamination in terrestrial ecosystems is being increasingly investigated by

423 researchers, there is a large knowledge gap on the extent to which agricultural soils are affected. 424 Globally, agricultural plastic film represents a dominant source of microplastics in farmland, 425 especially in China. It is necessary to establish a database on the distribution of microplastics in 426 farmland soil, so as to grade and assess the level of microplastic pollution in farmland soil in China. 427 (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 428 activities, i.e., primary microplastics, which are specifically produced within a small size for a 429 430 variety of applications, like cosmetic products or household cleaners. Tracing the source of 431 microplastics will provide a better understanding of the contributors to microplastic pollution and 432 be beneficial to controlling microplastic pollution at the source.

433 (3) Microplastics and the carbon cycle. As we know, biodegradable plastic can be utilized by 434 soil microorganisms as a source of carbon, but there is a large knowledge gap in the relationship 435 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 436 437 sources utilized by soil microorganisms outweighs the contribution of (micro)plastic degradation to 438 carbon emissions. Therefore, revealing the contribution of plastics, even microplastics, to carbon 439 sequestration or greenhouse gas emissions could provide data support for assessing the degradation 440 effects of plastic production in the environment.

441 5 Conclusion

In order to reveal the contribution of planting regions and mulching modes to microplasticdistribution, the characteristics of microplastics were investigated in greenhouse and open-field

444 cultivation using plastic mulching film from three typical regions in China. Microplastics were 445 readily detected in all 45 soil samples examined in this study. Their abundance, morphology, and 446 composition showed distinct characteristics for each of the three typical mulched agricultural areas 447 of China. Generally, the abundance of microplastics was highest in the Xinjiang long-term planting 448 area, followed by the Shandong intensive planting area, and lowest in the Beijing suburbs. The 449 microplastic particles smaller than 250 µm accounted for 80% of the total recovered, suggesting that 450 smaller-sized microplastics play a dominant role in the three planting regions. The distribution of 451 microplastic size and microplastic weight decreased with increasing particle size. PE and PP were 452 the dominant microplastic types. Otherwise, the distribution of microplastic abundance was significantly responsive to agronomic region, while microplastic composition was significantly 453 454 dependent on plastic film mulching mode (G vs. M). Microplastic sizes and types were not 455 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 456 457 plastic film. In the future, big data technologies or isotope tracer techniques will be considered to 458 quantify and assess the contribution of more different plastic sources to microplastics in soil.

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466 References

- 467 Alimi, O.S., Farner Budarz, J., Hernandez, L.M., Tufenkji, N., 2018. Microplastics and Nanoplastics in
- 468 Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. Environ.
 469 Sci. Technol. 52, 1704-1724.
- 470 Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Durántez Jiménez, P., Simonneau, A., Binet, S., Galop,
- 471 D., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment.
- 472 Nat. Geosci. 12, 339-344.
- 473 Baho, D.L., Bundschuh, M., Futter, M.N., 2021. Microplastics in terrestrial ecosystems: moving beyond
- the state of the art to minimize the risk of ecological surprise. Glob. Chang. Biol. 27, 3969-3986.
- 475 Baruah, A., Sharma, A., Sharma, S., Nagraik, R., 2021. An insight into different microplastic detection
- 476 methods. Int. J. Environ. Sci. Te. 19, 5721-5730.
- 477 Blasing, M., Amelung, W., 2018. Plastics in soil: Analytical methods and possible sources. Sci. Total.
- 478 Environ. 612, 422-435.
- 479 Chen, F., Aqeel, M., Khalid, N., Nazir, A., Irshad, M.K., Akbar, M.U., Alzuaibr, F.M., Ma, J., Noman, A.,
- 480 2023. Interactive effects of polystyrene microplastics and Pb on growth and phytochemicals in
 481 mung bean (Vigna radiata L.). J. Hazard. Mater. 449.
- 482 Chen, L., Yu, L., Li, Y., Han, B., Zhang, J., Tao, S., Liu, W., 2022. Spatial Distributions, Compositional
- 483 Profiles, Potential Sources, and Intfluencing Factors of Microplastics in Soils from Different
- 484 Agricultural Farmlands in China: A National Perspective. Environ. Sci. Technol. 56, 16964-16974.
- 485 Cheng, W.I., Fan, T.I., Wang, S.y., Li, S.z., Zhang, J.j., Zhao, G., Wang, L., Dang, Y., 2020. Quantity and

- 486 distribution of microplastics in film mulching farmland soil of Northwest China. J. Agro-Environ.
- 487 Sci. (in Chinese). 39, 8.
- 488 Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., Geissen, V., 2019. Evidence of
- 489 microplastic accumulation in agricultural soils from sewage sludge disposal. Sci. Total. Environ.
- 490 671, 411-420.
- 491 Ding, L., Zhang, S., Wang, X., Yang, X., Zhang, C., Qi, Y., Guo, X., 2020. The occurrence and
 492 distribution characteristics of microplastics in the agricultural soils of Shaanxi Province, in north-
- 493 western China. Sci. Total. Environ. 720, 137525.
- 494 Ferreira, T., Rasband, W., 2012. Image J user-guide.
- 495 Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S.,
- 496 Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., Wagner,
- 497 M., 2019. Are We Speaking the Same Language? Recommendations for a Definition and

498 Categorization Framework for Plastic Debris. Environ. Sci. Technol. 53, 1039-1047.

- 499 Hu, C., Lu, B., Guo, W., Tang, X., Wang, X., Xue, Y., Wang, L., He, X., 2021. Distribution of
- 500 microplastics in mulched soil in Xinjiang, China. Int. J. Agri. Biol. Eng. 14, 196-204.
- Huang, C., Ge, Y., Yue, S., Zhao, L., Qiao, Y., 2020a. Microplastics aggravate the joint toxicity to
 earthworm Eisenia fetida with cadmium by altering its availability. Sci. Total. Environ. 753, 142042.
- 503 Huang, Y., Liu, Q., Jia, W., Yan, C., Wang, J., 2020b. Agricultural plastic mulching as a source of
- 504 microplastics in the terrestrial environment. Environ. Pollut. 260, 114096.
- 505 Hurley, R.R., Lusher, A.L., Olsen, M., Nizzetto, L., 2018. Validation of a Method for Extracting
- 506 Microplastics from Complex, Organic-Rich, Environmental Matrices. Environ. Sci. Technol. 52,
- 507 7409-7417.

- 508 Jia, W., Karapetrova, A., Zhang, M., Xu, L., Li, K., Huang, M., Wang, J., Huang, Y., 2022. Automated
- identification and quantification of invisible microplastics in agricultural soils. Sci. Total. Environ.
 844, 156853.
- 511 Kannan, K., Vimalkumar, K., 2021. A Review of Human Exposure to Microplastics and Insights Into
- 512 Microplastics as Obesogens. Front. Endocrinol. (Lausanne) 12, 724989.
- 513 Khalid, N., Aqeel, M., Noman, A., Fatima Rizvi, Z., 2023. Impact of plastic mulching as a major source
- of microplastics in agroecosystems. J. Hazard. Mater. 445.
- 515 Khalid, N., Aqeel, M., Noman, A., Khan, S.M., Akhter, N., 2021. Interactions and effects of microplastics
- 516 with heavy metals in aquatic and terrestrial environments. Environ. Pollut. 290.
- 517 Li, K., Jia, W., Xu, L., Zhang, M., Huang, Y., 2023. The plastisphere of biodegradable and conventional
- microplastics from residues exhibit distinct microbial structure, network and function in plasticmulching farmland. J. Hazard. Mater. 442.
- 520 Li, L., Luo, Y., Li, R., Zhou, Q., Peijnenburg, W.J.G.M., Yin, N., Yang, J., Tu, C., Zhang, Y., 2020.
- 521 Effective uptake of submicrometre plastics by crop plants via a crack-entry mode. Nature Sustain.
 522 3, 929-937.
- 523 Liu, M., Lu, S., Song, Y., Lei, L., Hu, J., Lv, W., Zhou, W., Cao, C., Shi, H., Yang, X., He, D., 2018.
- 524 Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China. Environ.
- 525 Pollut. 242, 855-862.
- 526 Liu, Y., Rillig, M.C., Liu, Q., Huang, J., Khan, M.A., Li, X., Liu, Q., Wang, Q., Su, X., Lin, L., Bai, Y.,
- 527 Guo, G., Huang, Y., Ok, Y.S., Hu, S., Wang, J., Ni, H., Huang, Q., 2023. Factors affecting the
- 528 distribution of microplastics in soils of China. Front. Environ. Sci. Eng. 17.
- 529 Myszka, R., Enfrin, M., Giustozzi, F., 2023. Microplastics in road dust: A practical guide for

- identification and characterisation. Chemosphere 315, 137757.
- 531 Nuelle, M.T., Dekiff, J.H., Remy, D., Fries, E., 2014. A new analytical approach for monitoring
- 532 microplastics in marine sediments. Environ. Pollut. 184, 161-169.
- 533 Okeke, E.S., Okoye, C.O., Atakpa, E.O., Ita, R.E., Nyaruaba, R., Mgbechidinma, C.L., Akan, O.D., 2022.
- 534 Microplastics in agroecosystems-impacts on ecosystem functions and food chain. Resour. Conserv.
- 535 Recy. 177.
- 536 Qi, R., Jones, D.L., Li, Z., Liu, Q., Yan, C., 2020. Behavior of microplastics and plastic film residues in
- the soil environment: A critical review. Sci. Total. Environ. 703, 134722.
- 538 Qi, R., Jones, D.L., Liu, Q., Liu, Q., Li, Z., Yan, C., 2021. Field test on the biodegradation of
- 539 poly(butylene adipate-co-terephthalate) based mulch films in soil. Polym. Test. 93.
- 540 Revell, L.E., Kuma, P., Le Ru, E.C., Somerville, W.R.C., Gaw, S., 2021. Direct radiative effects of
- 541 airborne microplastics. Nature 598, 462-467.
- 542 Sharma, B., Sarkar, A., Singh, P., Singh, R.P., 2017. Agricultural utilization of biosolids: A review on
- 543 potential effects on soil and plant grown. Waste Manag. 64, 117-132.
- 544 Shruti, V.C., Perez-Guevara, F., Roy, P.D., Kutralam-Muniasamy, G., 2022. Analyzing microplastics with
- 545 Nile Red: Emerging trends, challenges, and prospects. J. Hazard. Mater. 423, 127171.
- 546 Turner, A., Holmes, L., 2011. Occurrence, distribution and characteristics of beached plastic production
- 547 pellets on the island of Malta (central Mediterranean). Mar. Pollut. Bull. 62, 377-381.
- 548 Udovicki, B., Andjelkovic, M., Cirkovic-Velickovic, T., Rajkovic, A., 2022. Microplastics in food:
- scoping review on health effects, occurrence, and human exposure. Int. J. Food Contamin. 9.
- 550 Weithmann, N., Möller, J.N., Löder, M.G.J., Piehl, S., Laforsch, C., Freitag, R., 2018. Organic fertilizer
- as a vehicle for the entry of microplastic into the environment. Sci. Adv. 4, 8.

- 552 Yang, J., Li, R., Zhou, Q., Li, L., Li, Y., Tu, C., Zhao, X., Xiong, K., Christie, P., Luo, Y., 2021a.
- Abundance and morphology of microplastics in an agricultural soil following long-term repeated
 application of pig manure. Environ. Pollut. 272, 116028.
- 555 Yang, Y., He, W.Q., 2021. Research status and progress of farmland soil microplastic pollution. Envir.
- 556 Eng. (in Chinese).
- Yang, Y., Li, Z., Yan, C., Chadwick, D., Jones, D.L., Liu, E., Liu, Q., Bai, R., He, W., 2021b. Kinetics of
 microplastic generation from different types of mulch films in agricultural soil. Sci. Total. Environ.
- 559814, 152572.
- 560 Yu, L., Zhang, J., Liu, Y., Chen, L., Tao, S., Liu, W., 2021. Distribution characteristics of microplastics
- in agricultural soils from the largest vegetable production base in China. Sci. Total. Environ. 756,143860.
- 563 Yu, Y., Battu, A.K., Varga, T., Denny, A.C., Zahid, T.M., Chowdhury, I., Flury, M., 2023. Minimal
- 564 Impacts of Microplastics on Soil Physical Properties under Environmentally Relevant
 565 Concentrations. Environ. Sci. Technol. 57, 5296-5304.
- 566 Zhang, J., Wang, X., Xue, W., Xu, L., Ding, W., Zhao, M., Liu, S., Zou, G., Chen, Y., 2022. Microplastics
- 567 pollution in soil increases dramatically with long-term application of organic composts in a wheat–
- 568 maize rotation. J. Clean. Prod. 356.
- 569 Zhou, B., Wang, J., Zhang, H., Shi, H., Fei, Y., Huang, S., Tong, Y., Wen, D., Luo, Y., Barcelo, D., 2019.
- 570 Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, east China: Multiple
- 571 sources other than plastic mulching film. J. Hazard. Mater. 121814.
- 572 Zhou, J., Gui, H., Banfield, C.C., Wen, Y., Zang, H.D., Dippold, M.A., Charlton, A., Jones, D.L., 2021a.
- 573 The microplastisphere: Biodegradable microplastics addition alters soil microbial community

- 574 structure and function. Soil Biol. Biochem. 156.
- 575 Zhou, J., Wen, Y., Marshall, M.R., Zhao, J., Gui, H., Yang, Y., Zeng, Z., Jones, D.L., Zang, H., 2021b.
- 576 Microplastics as an emerging threat to plant and soil health in agroecosystems. Sci. Total. Environ.
- 577 787.
- 578 Zhou, Q., Zhang, H., Fu, C., Zhou, Y., Dai, Z., Li, Y., Tu, C., Luo, Y., 2018. The distribution and
- 579 morphology of microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea.
 580 Geoderma 322, 201-208.
- 581 Zhou, Z., Wang, C., Luo, Y., 2020. Meta-analysis of the impacts of global change factors on soil microbial
- 582 diversity and functionality. Nat. Commun. 11, 3072.