

Effects of plastic residues and microplastics on soil ecosystems: A global meta-analysis

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Title: Effects of plastic residues and microplastics on soil ecosystems: A global
 meta-analysis

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25 ABSTRACT

26 Plastic pollution is one of the global pressing environmental problems, threatening the health of aquatic and terrestrial ecosystems. However, the influence of plastic residues and 27 microplastics (MPs) in soil ecosystems remains unclear. We conducted a global meta-analysis 28 to quantify the effect of plastic residues and MPs on indicators of global soil ecosystem 29 functioning (i.e. soil physicochemical properties, plant and soil animal health, abundance and 30 diversity of soil microorganisms). Concentrations of plastic residues and MPs were 1-2,700 31 kg ha⁻¹ and 0.01–600,000 mg kg⁻¹, respectively, based on 6,223 observations. Results show 32 33 that plastic residues and MPs can decrease soil wetting front vertical and horizontal movement, dissolved organic carbon, and total nitrogen content of soil by 14%, 10%, 9%, and 34 35 7%, respectively. Plant height and root biomass were decreased by 13% and 14% in the presence of plastic residues and MPs, while the body mass and reproduction rate of soil 36 animals decreased by 5% and 11%, respectively. However, soil enzyme activity increased by 37 7%-441% in the presence of plastic residues and MPs. For soil microorganisms, plastic 38 residues and MPs can change the abundance of several bacteria phyla and families, but the 39 effects vary between different bacteria. 40

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42 Keywords: plastic residues, microplastics, quantitative effect, soil ecosystem function,
43 meta-analysis

44

45 **1 Introduction**

Over the past 50 years, plastics have become widely used in various industries (Maity 46 and Pramanick, 2020; Yang et al., 2021; Zhang, Z. et al., 2022). Annual global plastic 47 48 production has accelerated over the past decade, reaching 368 million tons in 2020 (Plastics Europe, 2021). Much of this plastic results in pollution of the environment and has attracted 49 great attention due to its global ubiquity (Jambeck et al., 2015; Maity and Pramanick., 2020; 50 Zhang, Z. et al., 2022), and its potential to cause ecological damage in aquatic and terrestrial 51 systems (Kwak et al., 2022; Ng et al., 2018; Rochman et al., 2016). Plastics in the 52 environment can decompose into small plastic pieces with a diameter < 5 mm, defined as 53 microplastics (MPs). Particles > 5 mm are called macroplastics, and the smaller size 54 classification of 1 nm to 1µm are defined as nanoplastics (Frias and Nash, 2019; Thompson et 55 al., 2004). In the last decade, the ubiquitous presence of MPs in aquatic environments (e.g. 56 oceans, lakes, and rivers) have been reported in many studies and MPs have been shown to 57 adversely impact aquatic organisms, causing a loss of marine and freshwater ecosystem 58 functioning (Dong et al., 2021; Rochman et al., 2016; Wang et al., 2019). As 80% of plastics 59 arriving in the oceans are produced, used, and disposed of on land, the pollution of terrestrial 60 systems with plastic residues and MPs could be just as serious (Rochman, 2018). 61

Because plastic residues and MPs are long-lasting with very low biodegradability, they have accumulated rapidly in the global terrestrial environment (Jambeck et al., 2015; Zhang, Z. et al., 2022), with the abundance of plastic residues and MPs varying by up to 6 orders of magnitude between different terrestrial environments (Koutnik et al., 2021). It is estimated that around 63,000–430,000 and 44,000–300,000 tons of MPs have been generated annually

67	in European and North American farmland soils, respectively (Nizzetto et al., 2016). A survey
68	of soils in Lahar, Pakistan displayed that the abundance of MPs varied from 1,750 to 12,200
69	pieces kg ⁻¹ (Rafique et al., 2020). The concentrations of MPs in farmland soils of Yong-In,
70	Korea were about 10 to 7,630 pieces kg^{-1} (Kim et al., 2021). The abundance of MPs in
71	farmland across Ontario, Canada were observed at between 4 and 541 MPs kg ⁻¹ (Crossman et
72	al., 2020). As China is the world's biggest producer and consumer of plastic and is suffering
73	from serious plastic pollution, a substantial number of studies of plastic residues and MPs in
74	farmland soils have been carried out in China (Plastics Europe, 2021; Qi, R. et al., 2020). The
75	occurrence and distribution of plastic residues and MPs in several Chinese farmlands have
76	been investigated, showing a large spatial difference of their abundance, 0.1-411.2 kg ha ⁻¹
77	and 1.6-690,000 individual items kg ⁻¹ , respectively (Du et al., 2005; Hu, 2019; Huang et al.,
78	2020; Lv et al., 2019; Zhou et al., 2019; Liu et al., 2018). A study by Ren et al. (2021)
79	reported that agricultural much film contributed 10%-30% of total MPs in Chinese
80	agricultural soil. In addition to agricultural mulch film, the large accumulation of plastic
81	residues and MPs in farmland soils is also due to the result of other sources of inputs, such as
82	municipal waste (Liu et al., 2018; He et al., 2019), sewage sludge application(Long et al.,
83	2019), organic fertilizer and agricultural compost (Weithmann et al., 2018), atmospheric
84	deposition, flooding, littering and runoff (Ng et al., 2018; Yang et al., 2021). However,
85	comparisons between studies should be made with caution, as different studies have used
86	different extraction and detection methods for MPs.

Plastic residues, including MPs, are a threat to the soil ecosystem. Recently, several
review studies have emphasized the potential adverse effect of plastic residues, including MPs,

on the soil environment (Mbachu et al., 2021; Ng et al., 2018; Qi, R. et al., 2020; Wang et al., 89 2022; Wang, Q. et al., 2021; Zhou, J. et al., 2021). For example, Mbachu et al. (2021) and 90 Wang et al. (2022) revealed that soil MPs can affect plant health and soil fertility. Wang, Q. et 91 92 al. (2021) highlighted that MPs can cause adverse effects on the growth, lifespan, reproduction, and survival of soil fauna, via diverse toxicity mechanisms, particularly for 93 earthworms and nematodes. The effect of MPs and plastic residues on soil properties and 94 terrestrial biota depends on its chemical composition, concentration, and shape (Mbachu et al., 95 2021). Specifically, polyester (PES, 0.4%, w/w) fibers could increase the water holding 96 capacity of a loamy sand soil, but at the same time, decrease the soil microbial activity (de 97 98 Souza Machado et al., 2018). However, high-density polyethylene (HDPE) (2%, w/w) 99 fragments had no significant impact on these soil-related indicators (de Souza Machado et al., 2018). Moreover, PES fibers could increase the ratio of dry biomass between root and leaf, 100 101 while polyamide (PA) beads had an inverse impact. PA beads in the soil could increase the nitrogen content and total biomass of plant leaves, indicating that PA beads would have a 102 similar effect on plant leaves as nitrogen fertilizer in respect of nitrogen content and biomass 103 (de Souza Machado et al., 2019). Furthermore, several studies reported that plastic particles 104 with the size of 0.08–1.00 µm can penetrate the stele of rice, cucumber, wheat and lettuce, 105 leading to efficient uptake of smaller microplastic (Li et al., 2020; Li, Z. et al., 2021; Liu et al., 106 107 2022). It indicates that MPs can be transferred to the human body through the food chain, causing a potential threat to human health (Lwanga et al., 2017; Zhou, C. et al., 2021). 108 Furthermore, exposure to MPs could affect the growth and reproduction of soil animals 109 (Kwak and An, 2021), causing intestinal damage and neurotoxicity (Lei et al., 2018). In 110

addition, MPs could impact microbial activity, as they can increase the abundance of specific
microbial communities, such as dominant phyla (*a-proteobacteria* and *acidobacteria*) (Lu et
al., 2018; Liu et al., 2021).

114 To tackle the pollution caused by the wide use of conventional polymers (e.g., polyethylene, PE), the application of biodegradable plastic mulches (BDMs) has been 115 regarded as a promising solution (Kasirajan and Ngouajio, 2012). BDMs degrade at rates 116 faster than conventional PE film (Chamas et al., 2020), and their agricultural benefits are 117 comparable with conventional PE films (Yin et al., 2019). However, the widespread use of 118 BDMs has been hindered due to the high cost and poor suitability in different geographical 119 and climatic conditions (Liu et al., 2021). Moreover, there are uncertainty about short- and 120 121 long-term ecological impacts of BDMs on soil ecosystems (Liu et al., 2021; Qi, Y. et al., 2020a; Qi et al., 2018). 122

At present, the study of the impact of plastic residues and MPs on soil ecosystem 123 functioning is still in its infancy. To better understand the effect of plastic residues and MPs 124 on global soil ecosystem function (as indicated by soil physicochemical properties, plant and 125 soil animal health, and soil microorganisms), we conducted a systematic study of available 126 data. Meta-analysis is often used as a statistical method to compare and integrate the results of 127 multiple studies. It can elicit general patterns on regional and global scales (Zheng and Peng, 128 129 2001). For example, Gao et al. (2019) and Zhang et al. (2020) explored the effects of plastic mulch film and plastic residues on crop yield and water use efficiency (WUE) in China by 130 using a meta-analysis. To our knowledge, this is the first time a meta-analysis has been used 131 to systematically quantify the effect of plastic residues and MPs on global soil ecosystem 132

133 function.

134

135 2 Materials and methods

136 2.1 Literature search and data collection

We used the three literature databases, Web of Science (WOS), EI Compendex, and 137 China Knowledge Resource Integrated Database (CKRI), with the keywords "plastic residue" 138 or "plastic debris" or "macroplastic" or "microplastic" or "nanoplastic" and "soil or terrestrial" 139 to identify papers published from January 1, 2000, to January 31, 2021. These keywords 140 aimed to generate data to answer our main questions about the effects of plastic residues and 141 MPs on the global soil ecosystem. All the keywords associated with plastics were linked by 142 143 the Boolean operator "OR", and synonymous relevant to edaphic were connected with operator "AND". By searching using these keywords, we obtained 5,212 scientific papers 144 145 (WOS 3,381, CKRI 1,211, and EI 620), excluding reviews and conference articles. Details of search strings and the process of literature collection are presented in Table S1 and Figure S1 146 in Supporting Information (SI). 147

To explore the effects of plastic residues and MPs on soil ecosystems, we divided the research subjects into soil properties, plants, soil animals and soil microorganisms. In summary, these papers were chosen according to the following selection criteria: (a) the study must have compared experimental treatments against controls, with three or more replicates; (b) the experimental groups must have the addition of MPs or plastic residues solely without extra addition of heavy metals and/or plasticizers; (c) the number of replications and average value had to be presented in the article. By applying these selection criteria, we finally selected 105 valid articles for our analysis (Table S2), of which 48, 38, 35, and 23 were related to soil properties, plants, soil animals, and soil microorganisms, respectively. Then, a total of 6,223 observations were extracted for meta-analysis, of which 3,325, 1,240, 799 and 859 were related to soil properties, plants, soil animals and soil microorganisms, respectively.

159 2.2 Global meta-analysis

The suitability of using either a fixed effect or a random effect model for the meta-analysis was determined using Akechi Information Criterion (AIC). The smaller value for the AIC was observed when the random effect model was applied, meaning that the goodness-of-fit of the random effect model was better than that of the fixed effect model.

Three essential factors were extracted from the papers: the mean (M), the number of replicates (N) and standard deviation (SD) of the selected variables. If SD was not provided directly in the paper, it was calculated from the standard error (SE) (Hao and Yu, 2005). The conversion formula is as follows:

168

173

$$SD = SE \times \sqrt{N} \tag{1}$$

where *N* is the sample size and *SD* is the standard deviation of the treated or control group. A significant number (43.7 %) of articles did not provide the *SD* or *SE* values, therefore, we used the average coefficient of variation of all data to calculate the *SD* and multiplied it by the reported mean (M_r) (Zhang et al., 2020). The formula is as follows:

$$SD_i = m_r \times M_i \tag{2}$$

174
$$m_r = \frac{\sum \frac{5D_r}{M_r}}{n_r}$$
(3)

where m_r refers to the average coefficient of variation of the reported, which comes from the sum of the ratio of each known SD (*SD_r*) and mean (*M_r*), divided by the number of known 177 data (n_r) . SD_i is calculated by the data of articles that did not report the SD and is derived 178 from the sum of m_r and the mean of the literature (M_i) .

The effect value is the combined statistics in the quantitative meta-analysis, whose calculation method mainly depends on the acquisition of data from the original literature. We used a natural log-transformed response ratio (ln RR) as a metric of the effects of different sizes of MPs or plastic residues on a response variable relative to the control where plastic residue was not used.

184
$$\log RR = In(\frac{\overline{X_E}}{\overline{X_C}})$$

185 (4)

186
$$V_{\ln RR} = SD_p^2 \left(\frac{1}{N_E \overline{X_E}^2} + \frac{1}{N_C \overline{X_C}^2}\right)$$

187 (5)

188
$$SD_p = \sqrt{\frac{(N_E - 1)SD_E^2 + (N_C - 1)SD_C^2}{N_E + N_C - 2}}$$
(6)

189 where $\overline{X_i}$, SD_i , and N_i denotes the mean, standard deviation, and number of replicates, 190 respectively, and the subindices E and C refer to experimental treatments and the control 191 group, respectively. SD_p is the pooled standard deviation, and X includes varieties of different 192 indicators that affect soil ecosystems.

Our study included an assessment of publication bias using a funnel plot approach (Figure S2–5; Borenstein et al., 2021). In general, in the absence of publication bias, the scatter will be due to sampling variation only, and the plot will resemble a symmetrical inverted funnel. It should be noted that when the study size is too small (< 10, see Figure S3-Body length and Figure S4-Phyla number), the funnel plot cannot accurately reflect the bias situation (Sterne et al., 2011).

199	We also conducted a subgroup meta-analysis of the shape and chemical component of
200	plastic residues and MPs to explore the impact of different types of plastics on the indicators
201	of soil ecological environment. According to the specific surface area, plastics are classified
202	into fiber, film, and granule, where shapes of sphere and pellet were regarded as granule. The
203	plastic components in this study included polyethylene (PE), polyethylene terephthalate
204	(PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and biodegradable
205	(Bio) plastics (including polylactic acid (PLA) and polybutylene adipate terephthalate
206	(PBAT)). In addition, a random effect meta-regression analysis was carried out to assess the
207	relationship between MPs loading rate and the soil eco-environmental indicator.
208	The "metafor" package (version 3.0-2) and "forestplot" package (version 2.0.1) in R
209	(version 4.1.1) (https://www.r-project.org/) were used for the meta-analysis. We used and
210	modified the codes from Zhang et al. (2020), which are provided from the repository:
211	https://github.com/pablogalaviz/Micro-Plastics-Meta-Analysis.git.
212	
213	3 Results
214	In current study, the concentrations of plastic residues and MPs in field experiments
215	were 1–2,700 kg ha ^{-1} and 90–2,700 mg kg ^{-1} , respectively, based on 2,497 observations.

based on 3,726 observations. The detailed information of observation data is presented in the
Excel file named Raw data in SI. The results of effects of plastic residues and MPs on soil

While they were 50-2,700 kg ha⁻¹ and 0.01-600,000 mg kg⁻¹ in the laboratory experiments

219 ecosystems are based on the above concentrations.

216

220 3.1 Effects of plastic residues and MPs on soil properties

221	The response of soil to plastic residues and MPs is mainly reflected in the changes of soil
222	basic properties, i.e., carbon content, nitrogen content, phosphorus content and enzymes
223	activities (Zhang et al., 2020; Zhou et al., 2020b). As shown in Figure 1a, for soil basic
224	properties, plastic residues and MPs reduced soil pH, porosity, water content and soil water
225	movement ($p < 0.05$) based on the summary effect (represented by the red diamond in Figure
226	1a). Plastic residues and MPs decreased pH by 1% with summary effect size of 0.99 [95% CI:
227	0.99, 1] ($p < 0.05$), while according to the chemical components, PE plastic debris reduced pH
228	by 2% with response size of 0.98 [0.97, 0.99], and Bio plastic increased pH by 3% with
229	response size of 1.03 [1.01, 1.04] ($p < 0.05$). The electrical conductivity (EC) and bulk density
230	of soil were not impacted by plastic residues and MPs with the summary effect size equal to 1
231	However, according to the chemical component of plastics, Bio plastic residues and MPs
232	decreased EC by 19%, while PE plastic debris had almost no effect on EC. Though the
233	summary effect size of bulk density was equal to1, fibrous plastic debris decreased the bulk
234	density by 5%, while film plastic debris increased the bulk density by 1% ($p < 0.05$). The PE
235	plastic debris increased the bulk density by 1%, while the PET plastic debris decreased the
236	bulk density by 4% ($p < 0.05$). The summary effect sizes of wetting front horizontal
237	movement (WFHM) and wetting front vertical movement (WFVM) were 0.9 [0.88, 0.92] and
238	0.86 [0.84, 0.89] ($p < 0.05$) with the plastic residues addition of 80–1,280 kg ha ⁻¹ .

As shown in Figure 1b, the content of soil organic carbon (SOC) and dissolved organic carbon (DOC) were not significantly affected by the plastic residues (80–2,700 kg ha⁻¹) and MPs (1,000–280,000 mg kg⁻¹) with the summary effect sizes of 1.01 [0.97, 1.05] and 0.95 [0.88, 1.02] (p > 0.05), although PE plastic debris increased SOC content by 5%, and

decreased DOC by 15% (p < 0.05). All types of plastic residues promoted soil basal 243 respiration (SBR) with summary effect size of 1.38 [1.24, 1.54] (p < 0.05). Dissolved organic 244 nitrogen (DON) and total nitrogen (TN) were reduced by most types of plastic residues 245 246 $(450-2,700 \text{ kg ha}^{-1})$ and MPs $(20-280,000 \text{ mg kg}^{-1})$ with summary effect size of 0.92 [0.88, 0.96] and 0.93[0.89, 0.96] (p < 0.05), except for PP plastic debris that promoted DON by 60% 247 (p < 0.05). In contrast, nitrate nitrogen (NO₃⁻–N) in soil was increased by 12% (p < 0.05), 248 while the changes of ammonium nitrogen (NH₄⁺–N) and nitrite nitrogen (NO₂⁻–N) were not 249 significant (p > 0.05). The dissolved organophosphorus (DOP) content was increased by 41% 250 by plastic residues and MPs (p < 0.05), while the total organophosphorus (OP) and total 251 phosphorus (TP) content were decreased by 17% and 4% (p < 0.05). In general, the effects of 252 plastic residues and MPs on soil carbon, nitrogen and phosphorus varied with the forms of the 253 elements as well as chemical component and shape of plastics. However, the total contents of 254 255 these elements (i.e. SOC, TN and TP) in soils decreased in the presence of plastic residues and MPs. 256

The activity of acid phosphatase (ACP), catalase (CAT), phosphatase and urease were enhanced by plastic residues and MPs with summary effect sizes of 1.12 [1.1, 1.15], 1.07 [1.02, 1.13], 1.2 [1.13, 1.27] and 1.06 [1.03, 1.09], respectively (p < 0.05; Figure 1c). Among all plastic types, granular plastic residues and MPs show the greatest effect, increasing the activity of CAT and urease by 22% and 26% (p < 0.05). However, other enzymes (AKP, CHB and FDAse) had almost no response to plastic residues and MPs with the summary effect size of 1.02 [0.93, 1.11], 0.91 [0.75, 1.11] and 0.94 [0.87, 1.01], respectively (p > 0.05).

264 3.2 Effects of plastic residues and MPs on plants

265	The effects of plastic residues and MPs on plants are mainly reflected in plant growth
266	and the indicators of oxidative stress of plants (Pignattelli et al., 2020; Qi, R. et al., 2020). As
267	shown in Figure 2a, plastic residues and MPs significantly reduced plant height, total biomass,
268	shoot biomass and root biomass by 13%, 12%, 12% and 14%, respectively ($p < 0.0001$). All
269	types of plastic residues and MPs (film, granule, Bio, PE, PS, and PVC) inhibited plant
270	growth with the summary effect size from 0.59 [0.55, 0.63] to 1 [0.9, 1.21], and the response
271	of shoot biomass to granular plastic was greatest with the response ratio of 0.59 [0.55, 0.63].
272	In this study, the oxidative stress indicators in plant response to plastic residues and MPs
273	include antioxidant enzymes (ascorbate peroxidase (APX), CAT, peroxidase (POD),
274	superoxide dismutase (SOD)), corresponding substrates and products (ascorbic acid (AsA),
275	glutathione (GSH), hydrogen peroxide (H2O2), malonaldehyde, proline (Pro)). As shown in
276	Figure 2b, the contents of APX, CAT, AsA, H ₂ O ₂ , MDA and Pro were increased markedly by
277	plastic residues and MPs ($p < 0.0001$), and APX had the greatest response with summary
278	effect size of 1.46 [1.31, 1.64]. Among all types of plastic, the effect of PET plastic debris to
279	GSH was greatest with the response ratio of 4.74 [3.96, 5.67]. The summary effect of plastic
280	residues and MPs to GSH was not significant ($p > 0.05$), although GSH was decreased by
281	37% by PE plastic debris, and increased by 3.74 times by PET plastic debris ($p < 0.0001$)
282	according to the chemical component of plastics. However, the contents of POD and SOD
283	were not altered by most types of plastics ($p > 0.05$) with the exception of PVC plastic debris
284	that increased SOD by 27% ($p < 0.05$).

285 3.3 Effects of plastic residues and MPs on soil animals

286 The meta-analysis results show that plastic residues and MPs have different degrees of

impact on growth, behavior, feeding, reproduction, survival, energy metabolism and oxidative
stress response of soil animals (e.g. mice, earthworm, snail, nematode, springtail, Isopods, and
honey bee) as shown in Figure 3.

290 Plastic residues and MPs can inhibit animal growth. This is reflected in Figure 3a, where body length, body weight, growth rate, liver organ weight and relative liver weight of animals 291 were reduced by 7%, 5%, 19%, 8% and 6% (p < 0.05), respectively. Life span was also 292 shortened by 8% (p < 0.05) with the adding of plastic. Moreover, all types of plastics inhibited 293 animal growth to different degrees, e.g. PS plastic debris reducing the body weight, liver 294 weight, relative liver weight and life span of animals with the response ratios of 0.97 [0.95, 295 (0.99], (0.92, (0.87, 0.96), (0.94, (0.92, 0.97)) and (0.81, (0.74, 0.88)), respectively (p < 0.05). 296 297 Behaviors of soil animals was also affected by plastic residues and MPs, with body bending and head thrash frequency decreased by 9% and 19% (p < 0.0001), respectively. The response 298 299 of animal's head thrash to granular plastic was greatest with the response ratio of 0.65 [0.6, 0.7]. However, the locomotion speed of animals increased slightly, although not significantly 300 (p > 0.05). In addition, animal feeding rate was slightly reduced by plastic residues and MPs 301 (p > 0.05), although it was increased by 24% by the PE plastic residues and MPs (p < 0.05). 302

As shown in Figure 3b, plastic residues and MPs had a marked negative effects on animals reproduction and survival, reducing the reproduction rate, sperm count and vitality, the contents of succinate dehydrogenase (SDH) and testosterone and survival rate by 11%, 34%, 26%, 47%, 47% and 3%, respectively (p < 0.0001), and increasing the rate of sperm deformity by 1.37 times (p < 0.0001). The effects of all types of plastic residues and MPs on animal reproduction are similar to the summary effects, such as PS plastic debris decreasing reproduction rate, sperm count and vitality by 7%, 35% and 47%, respectively (p < 0.05). In addition, plastic residues and MPs significantly changed the energy metabolism of animals, decreasing the contents of lipids, proteins and total cholesterol (TCH) and energy available by 10%, 9%, 30% and 13%, respectively (p < 0.05), but increasing lactate dehydrogenase (LDH) by 25% (p < 0.0001).

Similar to the effects on plants, plastic residues and MPs also caused oxidative stress in 314 animals, increasing the ROS and MDA concentration by 63% and 2% with the summary 315 effect sizes of 1.78 [1.39, 2.29] and 1.02 [1.01, 1.03], respectively (p < 0.0001; Figure 3c). 316 Correspondingly, the activities of acetylcholinesterase (AChE), glutathione peroxidase 317 (GSH-Px), SOD and the content of GSH increased by 26%, 441%, 15% and 10%, 318 319 respectively (p < 0.0001), in response to the oxidative stress caused by plastic residues and MPs. However, the activities of antioxidative enzymes CAT and GST were inhibited by 5% 320 321 and 19%, respectively (p < 0.0001). The change in thiobarbituric acid reactants (TBARS) was not significant (p > 0.05). Furthermore, the effects of various plastic residues and MPs on the 322 antioxidative system of animals was similar to the summary effect, such as the granular and 323 PS plastic debris increasing the content of ROS. 324

325 3.4 Effects of plastic residues and MPs on soil microorganisms

There are fewer studies about the effects of plastic residues and MPs on soil microorganisms compared to that of soil properties and plants. The sample size of microbial meta-analysis in this study is 859, which is smaller than that for soil properties (N = 3,325) plants (N = 1,240). Published research has mainly focused on microorganisms at phylum and family level, and the abundance of different microorganisms varies greatly with the influence 331 of plastic residues and MPs.

Specifically, plastic residues and MPs significantly reduced the abundance of *Bacteroidetes, Cyanobacteria, Fimicutes*, and *Planctomycetes* by 9%, 41%, 15% and 9%, respectively, while the abundance of *Nitrospirae* increased by 33% at the phylum level (p <0.05; Figure 4a). Bio plastic residues and MPs also reduced the abundance of *Cyanobacteria* with the response ratios of 0.8 [0.67, 0.96] (p < 0.05). However, most types of plastic residues and MPs had no significant effect on bacterial phylum abundance.

In addition, at the family level, plastic residues and MPs decreased the proliferation of 338 Paenibacillaceae, Bradyrhizobiaceae, Nocardioidaceae, Sphingobacteriaceae 339 and Xanthobacteraceae with response ratios of 0.77 [0.64, 0.93], 0.59 [0.49, 0.71], 0.69 [0.57, 340 0.84], 0.69 [0.52, 0.91] and 0.8 [0.67, 0.96], while the abundance of Chitinophagaceae and 341 Comamonadaceae were promoted by 34% and 67%, respectively (p < 0.05; Figure 4b). The 342 effects of all types of plastic were similar to the summary effect sizes, such as PVC plastic 343 of Bradyrhizobiaceae, Nocardioidaceae debris decreasing the abundance 344 and Paenibacillaceae by 35%, 37% and 26% (p < 0.05). Furthermore, the effect of plastic 345 residues and MPs on microbial biomass carbon (C) and nitrogen (N) was not significant 346 compared to those without adding plastic (p > 0.05). In addition, plastic residues and MPs 347 reduced the number of observed species by 18% (p < 0.05), but had no significant effect on 348 349 other alpha diversity indexes (such as AEC, Chao1, Coverage, Shannon and Simpson) of the bacterial community with p > 0.05 (Figure 4c). 350

351

352 4 Discussion

353 4.1 Response of soil properties to plastic residues and MPs

According to the results of this first meta-analysis to determine the effects of plastic 354 residues and MPs on soil ecosystem functioning, we found that plastic residues and MPs 355 356 significantly inhibited the horizontal and vertical migration of soil water and slightly reduced soil water content by 2% (Figure 1a). Li et al. (2013) found that plastic residues would hinder 357 soil water migration and infiltration, and reduce soil moisture, which is consistent with our 358 meta-analysis result. In contrast, Hu (2020) suggested that residual film accelerates water 359 migration in both vertical and horizontal directions. These differences could be due to the 360 different quantity of plastic residues and MPs. Li et al. (2015) reported that when the loading 361 rate of plastic residues is very large (> 720 kg ha^{-1}), the movement of water through the soil 362 will be facilitated (Franklin et al., 2007), but water evapotranspiration will be hindered 363 (Figure S6-Evapotranspiration). This means that the effect of plastic residues and MPs on 364 water migration and evapotranspiration can change with the accumulation of plastic residues 365 and MPs in the farmland soil. The effect of Bio plastic on pH and EC is markedly different 366 from PE, which is probably due to their different degradation characteristics, such as the 367 differences in degradation rates and products (Qi, Y. et al., 2020b; Wang et al., 2020). 368

DOC is widely known as the source of soil microbial energy and nutrients (Kaiser and Kalbitz, 2012). Previous studies have found that plastic residues and MPs can increase soil DOC content by reducing the leaching of DOC and stimulating the enhancement of related enzyme activities in soil (Gao et al., 2021; Liu et al., 2017). However, the results of our meta-analysis show that the addition of plastic residues and MPs reduced soil DOC content by 9%. This difference may be because the soil was divided into different aggregates according to the particle size in several studies, and the DOC content is quite different in different aggregate sizes (Figure S6-DOC; Hou, 2020). Therefore, the effect of plastic residues and MPs on soil DOC dynamics needs further investigation, with a focus on different soil aggregate sizes.

The contents of TDN, DON, TDP and DOP in soil increased, indicating that plastic 379 residues and MPs promote the release of soil nutrients to soil solution and DOM accumulation 380 (Liu et al., 2017). Plastic residues and MPs can stimulate soil microbial activities, thus 381 increasing the activities of some enzymes in the soil. Soil enzymes also decompose organic 382 matter and catalyze important transitions in the C, N, and P cycles (Zhou and Staver, 2019). 383 384 Additionally, the decrease of TN, TP and DOC content in soil may provide an explanation for the inhibition of plant growth by plastic residues and MPs. These results indicate the 385 interactions caused by MPs between soil element cycling, soil enzyme activity and plant 386 growth. The interplay of these indicators should be investigated in the future study of effects 387 of plastic residue and MPs on soil ecosystems. 388

In addition, several parameters (such as water evapotranspiration, DOC, SBR, CAT) show a dose-effect relationship with MPs (Figure S6), meaning that the effect of MPs on the soil ecological environment could have a cumulative effect. In general, plastic residues and MPs could hinder soil water transport, reduce the total soil nutrient content, and increase the soil enzyme activities. These findings are helpful for the exploration of the ecological threshold of plastic residues and MPs in farmland soils.

395 4.2 Response of plants to plastic residues and MPs

396

6 Plastic residues and MPs in soil have a negative impact on plant growth (Boots et al.,

2019; de Souza Machado et al., 2019; Qi, Y. et al., 2020a; Zhang, J. et al., 2022), and these
negative effects show a dose-effect with MPs, i.e., SOD enzyme activity, MDA and biomass
decreased with the increase of MPs content (Figure S7).

400 In this meta-analysis, we found plastic residues and MPs reduced plant height and biomass by 11% and 12%, respectively (Figure 2a). Dong et al. (2015) suggested that boll 401 weight, yield and biomass of cotton decreased with increasing plastic residues content in soil. 402 Pignattelli et al. (2020) found that MPs produced acute and chronic toxicity to Lepidium 403 sativum, reducing plant height and aboveground biomass at different exposure durations (6 404 and 21 days). Similar conclusions can be found in studies on the response of maize, wheat and 405 rice to plastic residues and MPs (Qi et al., 2018; Urbina et al., 2020; Zhou, C. et al., 2021). 406 These effects could be explained that plastic residues and MPs can hinder the movement of 407 water and nutrients in soil and the activities of plant roots (Zhao et al., 2021), thus limiting the 408 absorption and utilization of water and nutrients by plants. In addition, MPs can also affect the 409 structure and metabolic process of rhizosphere microbial community, changing the root 410 growth environment and plant vital activities (Qi et al., 2018). Therefore, although the use of 411 plastic film mulching can increase crop yields (Sun et al., 2020), we recommend more 412 research addresses the potential negative effect of plastic residues and MPs on the plant 413 growth and quality. 414

Bio plastic residues and MPs inhibited plant growth, which is similar to conventional PE, PS and PVC plastic residues and MPs. However, the effects of Bio plastic residues and MPs on plant growth varied between different studies. A previous study by Qi et al. (2018) reported that starch-based Bio MPs had a stronger negative effect on plant height, leaf number and 419 biomass of wheat than low-density polyethylene MPs. In contrast, Li, B. et al. (2021) found that adding 0.1% and 0.5% Bio plastic residues increased plant height and leaf area of 420 soybean to different degrees at seedling, flowering and harvesting. The inconsistent results of 421 422 studies on the ecological effects of Bio plastic residues and MPs in soil may be attributed to different exposure duration. Bio plastics are more easily degraded and utilized by 423 microorganisms, and the plastic residues and MPs formed from Bio plastics can affect the soil 424 425 biome (such as earthworms) and soil biophysical properties (including bulk density, soil aggregates and water holding capacity), indirectly affecting the soil nutrient cycling and plant 426 growth (Lwanga et al., 2017; Zhao et al., 2021). Bio plastics may produce greater numbers of 427 428 plastic residues and MPs in the short term, while the increased rates of biodegradation may 429 result in less plastic residues and MPs in the longer term, and thus potentially have a reduced impact on plants compared to conventional plastics (Zhao et al., 2021). However, long-term 430 degradation studies are needed to further assess the effects of Bio plastic residues and MPs on 431 plants and soil processes. 432

In the current study, the activities of antioxidant enzymes in plants was generally 433 improved in the presence of plastic residues and MPs, which has been verified in previous 434 studies (Gao et al., 2021; Pignattelli et al., 2021; Pignattelli et al., 2020). In organisms, 435 antioxidant enzymes and antioxidants neutralize reactive oxygen species (ROS) to avoid 436 possible oxidative damage (Mates, 2000). In plant cells, SOD enzyme exists in the cytoplasm, 437 chloroplast, mitochondria and peroxisome and can convert O_2 .⁻ to H_2O_2 (Bowler et al., 1992). 438 Excess toxic H₂O₂ can spread rapidly through cell membranes (Foyer et al., 1997), and CAT 439 and GSH-Px can break down H₂O₂ in mitochondria, microsomes and chloroplasts (Kuźniak 440

and Skłodowska, 2001; Thounaojam et al., 2012). Therefore, we call for further research at 441 the cellular level to explore the mechanism of the effects of MPs on plant antioxidant systems. 442 APX is a key enzyme in the ascorbate-glutathione pathway and catalyzes AsA oxidation to 443 444 remove H₂O₂ (Asada, 1999; Diaz-Vivancos et al., 2006). Therefore, the enhanced activities of antioxidant enzymes and the production of corresponding antioxidant products in plants may 445 be typical responses to the exposure of plastic residues and MPs. However, there was no 446 significant decrease in GSH content in this study, which may be due to the shorter exposure 447 time used in relevant studies. Pignattelli et al. (2020) found that although the GSH content of 448 garden cress increased in the chronic (21 d) toxicological effect experiment due to MPs 449 450 stimulation, the GSH content decreased substantially in the acute (6 d) toxicological effect experiment. This may be because ROS stimulated by MPs consume the existing GSH in 451 plants, but new GSH has not yet been generated. Therefore, studies of the effects of plastic 452 residues and MPs on plant antioxidant systems should be carried out over long periods of time, 453 e.g. throughout a complete cropping season. 454

455

4.3 Response of soil animals to plastic residues and microplastics

Our current study shows that growth parameters, including body length, body weight, 456 growth rate, liver organ weight, relative liver organ weight and life span are markedly reduced 457 by most types of plastic residues and MPs (Figure 3), indicating plastic residues and MPs 458 459 inhabit the animal growth. The decreased frequency of body bending and head thrashing reveals the disturbed locomotor behaviors of animals caused by plastic residues and MPs 460 (Kim and An, 2019). Additionally, the slight increase of locomotion speed provides the 461 evidence of excitatory toxicity caused by plastic residues and MPs (Lei et al., 2018). However, 462

463 similar to the effect of plastic residues and MPs on plant GSH, the movement rate of animals may also be affected by longer-term exposure to plastic residues and MPs, but this needs to be 464 confirmed through appropriate long-term toxicology experiments. Plastic residues and MPs 465 466 had no significant effect on animal feeding rate, which is due to the large difference in the results of animal feeding studies in this meta-analysis (Figure 3a). Several studies reported 467 that the feeding rate of honey bee was reduced by MPs (Wang, K. et al., 2021), but other 468 studies showed the opposite results that MPs improve earthworm (Lumbricus terrestris) 469 feeding activities (Lwanga et al., 2016). This difference may be associated with MPs exposure 470 duration, short exposure time may increase animals' food intake, while long exposure time 471 could reduce animal appetite. Plastic residues and MPs are difficult to be digested by animals, 472 473 diluting and limiting the bioavailability of nutrients in food (Besseling et al., 2013), so animals have to intake more food to meet their physiological needs (Lwanga et al., 2016). 474 475 However, long-term exposure to MPs may cause gastrointestinal walls damage and decrease the feeding desire of animals (Song et al., 2019). A subgroup meta-analysis of plastic residues 476 and MPs exposure time was not carried out in our current study because of the difficulty of 477 integrating the exposure time of different soil animals, as well as the lack of sufficient data for 478 certain soil animals. 479

The decline of reproduction rates (i.e. juveniles number), sperm count and vitality and testosterone, as well as the increase in sperm deformity rate suggest that all types of plastic residues and MPs are harmful to animal reproduction (Figure 3), and these damages are more serious with the increasing MPs (Figure S8). Both LDH and SDH are sperm-specific enzymes involved in sperm development and energy metabolism (Chen et al., 2015; Zhu et al., 2014; 485 Zhu et al., 2019). The decrease of SDH level indicates disordered energy metabolism caused by plastic residues and MPs. However, plastic residues and MPs led to an increase in LDH 486 content, which can be explained by altered in energy metabolism pattern. Rodríguez-Seijo et 487 488 al. (2018) found that the increase of LDH may alter energy consumption to counteract the effects of oxidative stress imposed by the large addition of MPs. These results are also in 489 agreement with other studies that explored the energy metabolism and LDH levels among 490 mice and earthworm when they were exposed to different content and type of MPs (Deng et 491 al., 2017; Kwak and An, 2021). Therefore, studies on the effects of plastic residues and MPs 492 on animal reproduction should consider the response of the entire reproductive system, 493 including the changes in the number and morphology of germ cells and the level of sex 494 495 hormones, and disturbances in energy metabolism.

The contents of lipids and proteins in the animal body decreased with the presence of plastic residues and MPs, resulting in a reduction of available energy (Lu et al., 2018). The decrease of total cholesterol (TCH) content also provides evidence of lipid metabolism disorders. The possible reason is that exposure to MPs may cause an inflammatory response, which leads to lipid metabolism disorders in the liver, suppressing feeding activity (Jaeschke et al., 2002; Wright et al., 2013).

502 Similar to the response of plant antioxidant system to plastic residues and MPs, oxidative 503 stress responses in animals are also intensified by plastic residues and MPs (Chen et al., 2022; 504 Zhou et al., 2020a). Firstly, exposure to MPs increases ROS levels in animals, thereby 505 activating the cellular antioxidant defense system. As a toxic end product of lipid peroxidation, 506 the increase in MDA contents reflects the oxidative stress caused by ROS and lipid peroxidation (Yu et al., 2018). Then AChE, GSH-Px and SOD enzymes activities and GSH content increased to eliminate these oxidative damages. However, CAT and GST enzymes activities decline, which may be related to the MPs exposure duration. Chen et al. (2020) found that the decrease of CAT activity in the first 7 days of MPs exposure may be related to the inactivation of enzyme, the decline of enzyme synthesis rate or the change of enzyme subunit assembly, while the highest CAT activity at 28 days may be due to the stress response of the body to increased H_2O_2 content.

In summary, the meta-analysis results show that plastic residues and MPs have a 514 negative effect on the growth, metabolism, reproduction and survival of soil animals. Most 515 types (granule in shape, and PA, PE, PET, PS, PVC in component) of plastic residues and 516 MPs had similar effects on soil animals. Furthermore, these responses indicators show a 517 dose-effect with MPs (Figure S8). Given the damage of MPs to animals and the fact that 518 519 humans can intake MPs through ingestion and inhalation, MPs are also a potential threat to human health (Leslie et al., 2022; Senathirajah et al., 2021). Therefore, toxicity tests of MPs 520 in animals and human tissue cells could be carried out to assess the human health risks of 521 MPs. 522

523 4.4 Response of soil microorganisms to plastic residues and MPs

Many studies have shown a dose-response relationship between plastic addition and soil microorganism abundance and diversity (Figure S9). Zhang et al. (2017) found that a low amount of plastic residues could improve soil microbial activity, but the microbial biomass, microbial community abundance and soil enzyme activity decreased significantly in the soil with plastic residues amount > 450 kg ha⁻¹. Lu et al. (2018) showed that PS-based MPs can induce intestinal microflora disorders in mice. Although some family-level bacteria had significant responses to plastic addition, the study sample is too small, with < 10 observations for each bacteria family. Most types of plastics had small effect on bacterial abundance at the phylum level. Therefore, more studies are needed to assess the effects of MPs on microbial genus and species abundance levels.

Currently, the mechanism of the effect of plastic residues and MPs on soil 534 microorganisms is still unclear. Soil habitat changes caused by plastic residues and MPs are 535 thought to be a possible cause of microorganisms' change (Naveed et al., 2016; Ng et al., 536 2021; Qi, Y. et al., 2020b). Changes of soil aggregate structure, porosity, water and oxygen 537 concentration caused by plastic residues and MPs may affect microhabitats and change local 538 microbial community structure (Boots et al., 2019; Rillig and Bonkowski, 2018; Veresoglou et 539 al., 2015; Zhang et al., 2019). However, the responses of most indicators of bacterial alpha 540 541 diversity and microbial biomass to plastic residues and MPs were not significant. This also indicates that conventional plastics (PE and PVC) are not easily utilized by microorganisms 542 and do not result in changes in the microbial community structure in the short term. There 543 have been few studies on the effect of Bio plastics on microorganisms, so, further studies 544 should focus on the response of soil microbial abundance and community structure to Bio 545 plastic residues and MPs. 546

547 **4.5 Limitation**

The effect of plastic residues and MPs on global soil ecosystem functioning (as indicated by soil physicochemical properties, plants and soil animal health, and the abundance and diversity of soil microorganisms) has been quantified by using a meta-analysis in this study. 551 However, most of these quantitative results are based on laboratory or plot experiments, where very high rates of plastic debris including MPs were added to the soil in experimental 552 treatments. For example, the additive amount of plastic residues in several studies was up to 553 554 800 kg ha⁻¹ (Hu, 2020), and the MPs addition was 140,000 mg kg⁻¹ (Liu et al., 2017). In practice, the maximum concentration of plastic residues and MPs in the soil worldwide can be 555 as high as 411.1 kg ha⁻¹ and 67,500 mg kg⁻¹ (Fuller and Gautam, 2016; Scheurer and Bigalke, 556 2018), respectively. In this meta-analysis study, 36% of the 2,940 experiments that included a 557 macroplastics treatment, applied the macroplastics at a rate of > 411.1 kg ha⁻¹, while 22% of 558 the 2,405 experiments with MPs treatments, applied MPs at > 67,500 mg kg⁻¹. Therefore, 559 uncertainties exist when extrapolating the results of this meta-analysis to the typical levels of 560 plastic residues and MPs found in typical agricultural systems. In addition, studies on the 561 effect of degradable plastic residues on soil ecosystem functioning are rare, with only 51 562 observations in our meta-analysis (out of 6223). Therefore, there is still a large knowledge gap 563 in understanding the effects of degradable plastics on the soil environment. 564

565

566 **5 Conclusion**

For the first time, we quantified the effect of different shapes and components of plastic residues and MPs on indicators of global soil ecosystem functioning (i.e. soil physicochemical properties, plant and soil animal health and abundance and diversity of microorganisms) by a meta-analysis with 6,223 observations. Plastic residues and MPs changed 30 key soil physiochemical property indexes with summary effect sizes of 0.83–1.41, 13 key plant-related indexes with summary effect sizes of 0.86–1.46, 32 soil animal-related indexes with summary effect sizes of 0.53–5.41, and 33 soil microbial-related indexes with summary effect sizes of 0.59–2.66. This study demonstrates that plastic residues and MPs pose a threat to soil ecosystems by altering the physicochemical properties of soils, hindering the growth and development of plants and soil animals, and producing oxidative stress damage. However, the effects of plastic residues and MPs on the abundance and diversity of different phylum and family microorganism vary between different bacteria.

This work gives an important insight into the abundance of plastic residues and MPs in farmland soils of China and their effect on the global soil ecosystem, enhancing our understanding of the potential effects of plastic pollution on ecosystem functioning in agricultural soils. Finally, we call for more long-term positioning experiments conducted in field conditions using realistic concentrations of conventional and degradable macroplastics and/or MPs to provide a more realistic understanding of the impact of plastic debris on soil ecosystems.

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