

**Influences of different sizes and contents  
of microplastics on soil macroaggregate  
formation and properties**

Master of Philosophy

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# Abstract

Microplastics (MPs) in the soil environment have received particular attention in recent years due to their potential widespread ecological and human health impacts. Given that well-structured soil aggregates can contribute to the abilities of a soil to store carbon, resist erosion and support plant growth, evaluating the impacts of MPs on soil aggregates is important. A major knowledge gap is whether the effects of MPs on aggregates formation and properties depend on MP particle size.

This thesis aimed to investigate the effects of different sizes and concentrations of microplastics (MPs) on soil aggregate formation and properties. The study used polyethylene MPs of three different sizes (<35, <125, and <500  $\mu\text{m}$ ) mixed with soils and subjected to wet-dry cycles to incorporate the MPs into the aggregates.

The findings revealed that smaller MPs (<35  $\mu\text{m}$ ) had a more significant impact on aggregate formation, leading to a decrease in the mean weight diameter of newly-formed macroaggregates. Increasing concentrations of the smaller MPs also resulted in a significant reduction in the mean weight diameter of newly-formed macroaggregates. Additionally, the wettability of aggregates decreased with increasing MP concentrations and increased with increasing MP sizes. However, the water stability of aggregates showed a non-monotonic response to the concentrations and sizes of MPs. Furthermore, the study developed practical methods for characterizing the particle density of pristine MPs and detecting their concentrations in the laboratory and proved that ethanol could help laser diffraction method more precisely measure the particle size distribution of MPs. Overall, this study provides evidence that the influence of pristine MPs on soil aggregates increases with decreasing size and higher concentrations. Further research is needed to understand the breakdown process of MPs and the impacts of aged MPs on aggregate properties.

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# 1 Introduction

Microplastics (MPs) are defined as plastic particles smaller than  $< 5$  mm. Over the past few decades, human activity has released far more plastic into the environment than has naturally degraded, and due to the fragmentation and mobility of plastics, net MP accumulations have occurred in major ecosystems worldwide (Chamas et al., 2020; Petersen and Hubbart, 2021; Yu et al., 2020). Soil is now regarded as a primary sink of MPs as it annually receives considerable plastics from various sources, such as sewage sludge, landfill leakage, urban-rainfall washes, atmospheric deposition, and agricultural mulching (Blasing and Amelung, 2018; Tian et al., 2022). Although the majority of MPs are recalcitrant in the soil environment (Chamas et al., 2020), they can leach harmful additives, adsorb pollutants, fragment, and become incorporated into soil structure (Hodson et al., 2017; Rillig et al., 2017a; Zhu et al., 2022; Zimmermann et al., 2021). Thus, the presence of MPs likely disrupts the delicate balance of soil ecosystems and has far-reaching consequences for soil environment. To date, research have shown that MPs can have significant negative impacts on flora (Boots et al., 2019; de Souza Machado et al., 2019), fauna (Huerta Lwanga et al., 2016), microbial structures and functions (Hou et al., 2021; Zhu et al., 2022), soil physics (de Souza Machado et al., 2018) and soil chemistry (Boots et al., 2019; Lozano et al., 2021). Given the important roles aggregates play in soil health and ecosystem functioning, MPs-bearing aggregates are currently receiving special concern.

The formation of soil aggregates from primary soil particles is driven by both biotic (e.g., secretion of binding agents) and abiotic (e.g., wet-dry cycles) processes (Six et al., 2000). Well-structured soil aggregates can reduce soil erosion (Barthès and Roose, 2002), build continuous network of pore spaces that facilitate water infiltration, air circulation, and root growth (Horn and Smucker, 2005; Zhang et al., 2020), and serve as vital habitats for soil organisms by offering water, nutrients, and protected spaces (Bach et al., 2018; Hou et al., 2021). It is worth noting that the performance of the above services and functions are primarily determined by aggregate properties. For instance, soil aggregates, especially water-stable aggregates (WSAs), are resistant to break up during water flow, thus enabling aggregates to persist as functional units (Rabot et al., 2018). By regulating the intake, diffusion, and retention of pore water and nutrients from the interspaces among aggregates, wettability can alter the water stability of aggregates as well as the living conditions of organisms that inhabit aggregates (Eynard et al., 2004a; Lipiec et al., 2009). Additionally, reduced particle density

may lower the bulk density of aggregates, with a resulting reduction in the strength of aggregates (Zhang, 1994). If soil aggregate properties are altered, the services that they provide may loss, which underlines the necessity for evaluating the influences of MPs on aggregate properties.

Through literature review, this study defines three major knowledge gaps. Firstly, few research has explored the effects of MPs on wettability and particle density of aggregates. Secondly, little is known about the influences of different sized MPs on aggregate formation and properties. Thirdly, due to the incorporation of MPs into aggregates, the vertical transport of MPs through undisturbed soil by water may differ from that through disturbed soil, or columns of sand or glass beads. To further study the impacts of aggregates on MP mobility in soil, it is crucial to first comprehend the influences of MPs on soil aggregate properties.

Therefore, the experiments reported here aimed to determine the impacts of different sizes (<35, <125, <500  $\mu\text{m}$ ) and concentrations (0, 0.1, 1, 10%) of polyethene MPs on aggregate formation, wettability, water stability, particle density and MP concentration. This MPhil thesis consists of 5 chapters, including one main laboratory experiments. We hypothesized that higher MP exposure concentrations increase the MP concentration incorporated into aggregates, while decreasing the aggregate formation, wettability, water stability, particle density, and that smaller MP sizes increase the impacts of MPs on aggregate formation, wettability, water stability. The contents of each chapter, excluding the introduction, are described briefly as follows.

**Chapter 2** is a literature review contextualizing this thesis. This chapter summarizes the details of some environmental factors and how they relate to the mobility of MPs in the soil systems. This literature review also places this thesis in the context of the influences of MPs on soil aggregate formation and properties, outlines the knowledge gaps that this thesis attempted to address, and raises the corresponding hypotheses waiting to be tested.

**Chapter 3:** agricultural and woodland soils of different acidity, organic matter, effective CEC, total extractable Mn, Al, Fe and SAR were collected, air dried, sieved to <250  $\mu\text{m}$ , and evenly mixed with 0, 0.1, 1, and 10% of pristine polyethene powders <35  $\mu\text{m}$ , <125  $\mu\text{m}$ , <500  $\mu\text{m}$ . A 75-day wet-dry cycles at 30°C in lab was operated to achieve the incorporation of MPs into newly-formed aggregates. Then the microplastic-containing aggregates were collected to determine the impacts of different sizes and concentrations of MPs on the properties of newly-formed macroaggregates (> 250  $\mu\text{m}$ ). This chapter also introduces the

development of some improved and/or new methods for determining the aggregate properties, such as size distribution, wettability, water stability, particle density, percentage of MPs, along with some improved techniques for analysing the particle density and size distribution of tested polyethylene microplastics.

**Chapter 4** consists the results of MPs properties, the results of soil properties affecting aggregate formation and stability, and the influences of different sizes and concentrations of MPs on the properties of newly-formed soil macroaggregates ( $> 250 \mu\text{m}$ ).

**Chapter 5** discusses the feasibility of using ethanol pycnometer method to measure the particle density of MPs as well as the repeatability and applicability of this method compared with other existing methods; the error analysis of using laser granulometer to measure the particle size distribution of MPs and how 35% ethanol, as a new dispersant, could help laser granulometer increase the accuracy and repeatability when measuring particle size distribution of MPs; the effectiveness of determining the percentage of MPs in aggregates based on changes in particle density and the potential improvements for this method. This section also discusses the differences in the formation and properties of newly-formed macroaggregates, due to the addition of different sizes and concentrations of MPs, and considers the mechanisms by which MPs exert influences.

## 2 Literature review

### 2.1 Influence of soil physical properties on the mobility of MPs in soils

Much of the literature relevant to the movement of microplastics (MPs) in soils used other coarse porous media (i.e., sand, gravel, and glass sphere) rather than real soils, which allowed researchers to study soil physical properties that affect MPs mobility under well-constrained conditions (e.g., specific particle types, grain sizes, and porosities).

Texture can influence pore structure to impact the transport of MPs by water flow (Table 2-1). Assumed that the mineral particles have been well compacted, when the diameter of particles making up the porous media decreases, MPs that are moving through the pore media tend to be intercepted by solid particles since aperture gap reduced (Hou et al., 2020; Porubcan and Xu, 2011; Yao et al., 1971). For instance, studies of plastic migration based on coarse porous media showed reduced matrix sizes significantly declined the maximum migration distance of MPs, with reducing porosity significantly decreasing migration depth of MPs in

glass beads ( $P < 0.001$ ) (Hou et al., 2020; Waldschlager and Schuttrumpf, 2020; Wang et al., 2021).

It is noteworthy that pore structure cannot solely explain the variation in the migration of plastics in natural soils. For example, a positive correlation between soil porosity and the retention rate of NPs in columns filled with different natural soils was discovered in Wu et al.' (2020) study of natural soils. In this case, higher percentage of clay-sized particles (3.56-25.39%) in soil samples, which would have provided more deposition sites for MPs and enhanced electrostatic attraction between MPs and soil particles, was likely responsible for the reduced mobility at a higher porosity (Wu et al., 2020). Besides, as water flowed through the soil, clay-sized particles may have been carried to the end of the column where soil pores were likely to be plugged (Aksu et al., 2015; Norgaard et al., 2018). Clay minerals, especially montmorillonite and kaolinite could also have swelled due to hydration (Aksu et al., 2015), subsequently blocking the soil pores thereby reducing the mobility of MPs in soils.

Considering all this evidence, it seems that when a mixture of different grain sizes is present, as in soil, the mobility of MPs is much more complicated, which may be due to narrowing aperture gaps, increased deposition sites, fine migration, and clay mineral swelling.



**Table 2-1. Studies that have investigated the influences of the physical properties of porous media on the mobility of micro-nanoplastic**

Microplastics Properties		Physical Properties of Porous Media			Methods	Hydrologic Condition	Main Findings	References
Type	Shape	Grain sizes	Density	Porosity				
Polystyrene, High Density Polyethene, Polypropylene	Particle (75–425 µm) Microbead (45-53 µm)	Sand (365 µm on average) Gravel (2000-8000 µm)	NA	NA	Irrigated sand/gravel columns with liquid containing microplastics	Irrigation (unsaturated)	① Microplastics were found throughout the gravel column (0-80 cm) , whereas for sand column, microplastics only migrated downward by 10 cm from the surface.	Wang et al., (2021)
Polyethene, terephthalate, Polyvinyl chloride, Styrene butadiene rubber, Polyamide, Copolyimide, Polystyrene, Polyethene, Polypropylene	Fragment (0.6-1.5 mm) Pellet (2.29-3.91 mm) Fibre (1.08-2.15 mm) Sphere (1.00-5.00 mm) Cubic (0.75-1.00 mm)	Glass spheres (1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 11.0 mm)	NA	0.322-0.365	Simulated rainfall experiments were carried out in columns (30 cm in depth) filled with different sizes of glass spheres and with microplastics on the surface	Simulated rainfall (unsaturated)	① Maximum migration depth of the microplastics increased with decreasing diameter of the microplastic (d <sub>MP</sub> ) particles and with increasing diameter of the glass spheres(d <sub>GS</sub> ); ② The average maximum migration depth of microplastics was 13.14, 4.63, and 1.18 cm when d <sub>MP</sub> /d <sub>GS</sub> <0.11, 0.11<d <sub>MP</sub> /d <sub>GS</sub> <0.32, 0.32<d <sub>MP</sub> /d <sub>GS</sub> , respectively.	Waldschlag er and Schuttrumpf (2020)
Polyethene	Graininess (40-48 µm)	Quartz sand (1-2 mm, 2-4 mm)	1.54 g/cm <sup>3</sup> , 1.50 g/cm <sup>3</sup>	0.42 0.43	Upward migration experiments were carried out in columns (20 cm in depth) filled with different sizes of sands.	Pumping water (saturated)	① For porous media with larger diameter, higher percentage of microplastics broke through sand column after injection of 5 pore volumes of microplastics suspensions and ultrapure water, respectively.	Hou et al., (2020)
Polystyrene	Spherical (100 nm)	Desert soil (Clay: 3.56%) Black soil (Clay: 11.90%) Red soil (Clay: 25.39%)	NA	Desert soil (0.3) Black soil (0.42) Red soil (0.45)	Upward migration experiments were carried out in columns (10 cm in depth) filled with different soils.	Pumping water (saturated)	① Soils with higher percentage of clay had greater capacity to retain nanoplastics in soil, with maximum of 96.8%, 87.5%, 0% of polystyrene nanoparticles eventually penetrating columns filled with desert soil, black soil, and red soil, respectively.	Wu et al., (2020)

## **2.2 Influence of soil chemical properties on the mobility of MPs in soil**

MPs, due to their small size, large surface area, surface charges and functional groups, presumably interact with other soil chemical components that affect the stability of MP particles in soil pore water and their mobility in soils. To date, what we know about the influences of soil chemical properties on the mobility of MPs/ NPs in soil are largely based upon column experiments that investigated the influences of pH, ionic strength, organic matters, and the concentration of various metal oxides. Although much of the work that gave insight into chemical controls on the mobility of MPs had been derived from work using saturated artificial porous media comprising either well-defined glass spheres or quartz sand with different chemistry, these findings were wanted to be verified with investigations conducted in the real soils (Table 2-2).

### **2.2.1 Influence of pH on the mobility of MPs in soil**

The majority of studies relevant to the impacts of soil chemistry on MP mobility included pH. However, only a small percentage of them considered pH in any detail (Table 2-2). Increasing pH can influence the surface charge of soil particles and MPs, contributing to the enhanced particle-to-particle electrostatic repulsions, and thus increasing the MPs mobility. For example, Wu et al. (2020) detected a significant increase in effluent recovery of MPs when the soil pH increased from 4.97 to 9.75 at the same ionic strength (1 mM NaCl), due mainly to the enhanced negative surface charge of soils and NPs (soils:  $-5.1 \pm 0.1$  mV to  $-28.6 \pm 0.8$  mV; MPs:  $-25.7 \pm 0.9$  mV to  $-42.7 \pm 1.2$  mV). Additionally, since the pH increased and finally exceeded the point of zero charges of soil iron oxides (pH: 7.5-9.0) (Parks and Bruyn, 1962) and that of aluminium oxides (pH: 7.18) (Schwarz et al., 1984), the reversion from electrostatic attraction to electrostatic repulsion between Fe/Al oxides and NPs appeared and presumably accounted for the increased NPs mobility. However, in these experiments, pH was not systematically varied for a single soil. As different soils were compared, other properties which differed between the soils may have also impacted MP mobility. To further verify the influence of pH on MPs/NPs mobility, Wu et al. (2020) and Cai et al. (2019) artificially increased the pH of solution in a system comprising saturated quartz sand. Likewise, as the zeta potential of MPs/NPs and quartz sand became more negatively charged at higher pH, the mobility of both MPs and NPs in quartz sand was promoted, which was in accordance with the calculation of DLVO theory. In summary, these studies suggested an increase in MP mobility with pH, but relatively few studies in soils suggested that further investigations are warranted.

### 2.2.2 Influence of ionic strength on the mobility of MPs in soil

Much of the current literature has compared MP mobility with different ionic strengths of metal cations in pore water. A consensus among published studies is that the increased concentration of metal cations in the higher ionic strength solutions rendered MPs less mobile (Chu et al., 2019; Keller et al., 2020; Tong et al., 2020; Wu et al., 2020; Zhao et al., 2021). This is mainly because the negative charge neutralization effect of electrolytes can reduce particle-to-particle repulsions and progressively increase the size of MP aggregations. Wu et al. (2020) have established that when the ionic strength of soil solution increased from 1 to 5 mM  $\text{CaCl}_2$ , the zeta potential of the MPs in desert soil, black soil, and red soil decreased from about -18.3 to -15.3 mV, -16.7 to -13.4 mV, and -15.2 to -11.6 mV, respectively, with the observations of decreasing repulsive forces between particles, ultimately, an abrupt drop in the effluent recovery of NPs. Furthermore, results from other experiments using artificial porous media indicated that the deposition of MPs/NPs caused by increasing ionic strength was reversible. When the ionic strength reduced again, a fraction of the retained MPs/NPs was found to detach from the quartz sand mainly because of the growing electrostatic repulsion (Hou et al., 2020; Tong et al., 2020). While for real soils, this hypothesis is warranted to be well tested due to the complex composition of the soil.

As carrying more charges, multivalent electrolytes usually showed a stronger negative charge neutralization effect on MP than monovalent electrolytes under the same ionic strength. This corresponds with the classical Schulze-Hardy rule that the critical coagulation concentrations (CCC) of colloidal particles decrease with increasing ionic valence (Lu et al., 2018; Trefalt et al., 2020). For example, the CCC of MP in divalent electrolytes, such as  $\text{CaCl}_2$  (2.95 mM) and  $\text{BaCl}_2$  (3.20 mM) solutions, were significantly lower than those in monovalent electrolytes, such as  $\text{NaCl}$  (14.9 mM),  $\text{NaNO}_3$  (13.7 mM) and  $\text{KNO}_3$  (14.8 mM) solutions at pH 6 (Li et al., 2018b). Thus, compared with the electric double layer (EDL) repulsions among particles in monovalent electrolytes solution, though ionic strength is the same, the EDL repulsions decreased in divalent electrolytes solution. Such a decrease in EDL repulsions would both increase the aggregation size among MPs and the attraction of matrix particles to MPs, thereby inhibiting MP mobility more strongly. Wu et al. (2020) verified that under the same ionic strength, the negative zeta potential of MP in  $\text{CaCl}_2$  solution was smaller than that in  $\text{NaCl}$  solution, thus  $\text{CaCl}_2$  caused much more MP retention in natural soils. These findings were also consistent with the results from investigations comparing the influence of  $\text{CaCl}_2$  and  $\text{KCl}$  on

MP mobility in sand (Zhao et al., 2021). Together, above studies indicated that increasing ionic strength of electrolytes, especially multivalent electrolytes, can inhibit MP mobility. In the future, more research is needed on MP mobility in real soils with different electrolytes and concentrations in solutions.

### **2.2.3 Influence of oxyhydroxides on the mobility of MPs in soil**

Aluminium and iron oxyhydroxides as ubiquitous soils minerals typically have points of zero charges (PZC) of  $> 7$  and are usually positively charged (Kosmulski, 2009; Li et al., 2019; Regelink et al., 2014; Wu et al., 2020). In contrast, unmodified MPs/NPs are generally negatively charged in soils (Li et al., 2021b; Wu et al., 2020). Given the opposite electrical charges between aluminium/ iron oxyhydroxides and MPs/NPs, these oxyhydroxides are favour to inhibit MP mobility due to their involvement in the aggregation of MPs and interactions with matrix particles (Chowdhury et al., 2011). For instance, Li et al. (2019) reported that the addition of iron oxides (goethite, hematite) resulted in larger aggregations of MPs/NPs relative to those without iron oxide addition. Consequently, the effluent recovery of  $0.2 \mu\text{m}$  NPs dropped from 73% to approximate 27%, while for  $2 \mu\text{m}$  MPs, this figure reduced from 70.7% to approximate 2% (Li et al., 2019). Also, Wu et al. (2020) argued that the attachment rate of PSNPs was correlated with soil Fe/Al oxides content, partly due to the increased electrostatic attraction between NPs and the soil matrix under acidic conditions (pH: 4.97-6.57). However, above findings were based upon the electrostatic attractions between positively charged aluminium/iron oxyhydroxides and negatively charged unmodified NPs/MPs under acidic conditions (pH $<7$ ). Considering alkaline conditions can reverse the surface charge of the aluminium/iron oxyhydroxides and enhance the negative charge of MP as discussed above, an increase in MPs mobility with aluminium/ iron oxyhydroxides are likely to occur, needing further investigations.

### **2.2.4 Influence of dissoluble organic matters on the mobility of MPs in soil**

Dissoluble organic matters, such as humic acid, fulvic acid, naphthalene, influenced MP stability and aggregate size through changing particle-to-particle repulsion, therefore altering MP mobility (Dong et al., 2020; Hou et al., 2020; Li et al., 2018b). As the zeta potentials of most MPs are originally negative in pore water, negatively charged dissoluble organic compounds enhanced mobility of MPs by increasing electrostatic repulsions. For instance, in an unsaturated soil leaching tested by Yan et al. (2020), the addition of humic acid enhanced electrostatic dispersion of MPs by increasing the negative zeta potential of MPs from

-20mV to -34 mV, leading to an increase in maximal downward distance of raw MPs from 3-4 cm to 9-10 cm (Li et al., 2018b; Yan et al., 2020). Investigations on nonpolar naphthalene reported that the mobility of NP was higher when it solely existed in the pore-water than that when in the presence of naphthalene, primarily because nonpolar naphthalene may shield parts of the negative surface charge of PSNP and weaken the repulsion between particles (Hu et al., 2020). However, our observation to the behaviour of hydrophobic polyethylene MPs in non-electrolyte solution showed the addition of ethanol significantly reduced the surface tension of MPs and decreased the aggregation of MPs in water by reducing hydrophobic attraction force between MPs, which may favour to increasing the mobility of MPs.

Environmental MPs may serve as vectors for multiple organic matters. On the other hand, the adsorption of these organic compounds could influence MP mobility as well. Dong et al. (2020) reported that NPs with a large adsorbed amount of negative bovine serum albumin (BSA) could induce negatively charged corona around MP thus generating steric repulsion. In contrast, NPs with a small adsorbed amount of the positively charge lysozyme increase the aggregate sizes of NPs via connecting MPs with protein “bridges” (Dong et al., 2020).

Collectively, it seems that the natures (e.g., size, charge, hydrophobic/hydrophilic) of dilutable organic matter and the degree of adsorption on MP may be curial for surface interactions controlling MP mobility (Dong et al., 2020; Hou et al., 2020; Shaniv et al., 2021). Note that when evaluating the impacts of organic matter on the mobility of MP in real soil, much of research only considers the concentration of a single soil organic matter (SOM). In the future, more research comparing the influences of different fractions of SOM and their interactions is recommended.

**Table 2-2. Influence of soil chemical properties on MPs/NPs mobility**

Microplastics type	Soil Chemical Properties				Porous media	Methods	Hydraulic Condition	Main Findings	References
	pH	ionic strength	organic matter	metal oxidates					
polystyrene	7	Na+ (0.1-20 mM)	NA	NA	glass sphere	upward migration experiments in columns with a depth of 10 cm	pumping water (saturated)	① The retention rate of MPs increased with an increase in ionic strength.	Chu et al., (2020)
polystyrene	8.0 ± 0.2	seawater background solution	bovine serum albumin (BSA), lysozyme (LSZ)	NA	sand	upward migration experiments in columns with a depth of 10 cm	pumping water (saturated)	① The BSA corona drove the dispersion of NPs (200 and 500 nm) due dominantly to the induced colloidal steric hindrance; ② The LSZ corona triggered the aggregation of 200 nm NPs via electrostatic adsorption and bridging effect, thereby inhibiting the transport of 200 nm NPs.	Dong et al., (2020)
polystyrene	NA	NA (0.5-50 mM)	naphthalene	NA	sand	upward migration experiments in columns with a depth of 10 cm	pumping water (saturated)	① The mobility of NPs in the sand column was lower when NPs copresented with naphthalene in the pore-water than when naphthalene was absent; ② The retention rate of MPs increased with an increase in ionic strength.	Hu et al., (2020)
polystyrene	6.5-7.2	K+ (1-100 mM) Ca2+ (1-100 mM)	tetracycline	NA	glass bead	upward migration experiments in columns with a depth of 10 cm	pumping water (saturated)	① Ionic strength of K+ negligibly affected the transport of MPs; ② Increasing ionic strength of Ca2+ significantly reduced the transport of MPs; ③ Tetracycline slightly inhibited MPs mobility in K+ solutions but facilitated it in Ca2+ solutions.	Zhao et al., (2021)
polystyrene	7.0-7.1	raw groundwater (12 mM), filtered groundwater (11 mM), artificial groundwater (Ca2+:3.66 mM, Na+: 1.00 mM)	25-50 g/mL	NA	aquifer sand	upward migration experiments in columns with a depth of 6 cm	pumping water (saturated)	① Larger solid organic matter promotes NP stability and mobility, while smaller soluble organic matter has opposite effect; ② Calcium ions assist in hetero-aggregation between likely-charged NP and OM.	Song et al., (2019)
carboxylate- and amine-modified polystyrene latex (CMPs and AMPs)	6	Na+ (5-25 mM)	bovine serum albumin (BSA), trypsin	NA	aquifer sand	upward migration experiments in columns with a depth of 10 cm	pumping water (saturated)	① BSA increased the transport of both CMPs and AMPs; ② trypsin decreased the transport of CMPs but increased the transport of AMPs; ③ the capacity of CMPs mobility at low ionic strength was higher, while the alteration of AMPs mobility is negligible at both ionic strengths.	Rong et al., (2019)
polyacrylonitrile, ethylene terephthalate	NA	high, low	unknown organic matter	NA	glass sphere	downward migration experiments in columns with a depth of 25 cm	flow was applied from a height of 6 cm	① 50% of microplastics cotransported with organic matter eventually passed through column; ② the retention and aggregation of MPs increased with higher ionic strength.	Keller et al., (2020)
polypropylene	5	NA	Humic acid	NA	Loamy sand soil	downward migration experiments in columns with a depth of 15 cm	artificial rainfall at 1 mL min-1	① Humic acid accelerated the migration of MPs from soil surface to deeper layer.	Yan et al., (2020)
polyethylene	NA	0, 10, 50 mM	fluvic acid	NA	quartz sand	downward migration experiments in columns with a depth of 20 cm	pumping water (saturated)	① Increasing ionic strength reduced the repulsive force between MPs and sand, subsequently retaining more MPs in sand; ② Higher concentration of fluvic acid increased repulsive energy between MPs and sand, which facilitated the migration of MPs.	Hou et al., (2020)
polystyrene	7.0-8.5	Ca2+ (0.45-2.5mM)	Humic acid	Fe2O3 Al2O3	Loamy sand soil, sand	upward migration experiments in columns with a depth of 20 cm	pumping water (saturated)	① Calcium-driven aggregation may increase NPs retention in saturated soil; ② Humic acid may affect NPs mobility by reducing its surface charge (~ +20 to ~ -15.5 mv).	Shaniv et al., (2021)

**Table 2-2. (continued)**

Microplastics type	Soil Chemical Properties				Porous media	Methods	Hydraulic Condition	Main Findings	References
	pH	ionic strength	organic matter	metal oxides					
polystyrene	9.75, 6.57, 4.97	Na <sup>+</sup> (1-20 mM) Ca <sup>2+</sup> (1-5 mM)	0.05%, 2.57%, 0.12%	Fe oxides Al oxides	desert soil black soil red soil	upward migration experiments in columns with a depth of 10 cm	pumping water (saturated)	① The retention of NPs was positively correlated with Fe/Al oxides contents; ② Increasing ionic strength, especially Ca <sup>2+</sup> greatly inhibited the transport of NPs in natural soils; ③ The retention rate of MPs decreased, mainly due to the transition from favourable condition to unfavourable condition between MPs and soil minerals, with increasing pH.	Wu et al., (2020)
carboxylate-modified polystyrene latex	6	Na <sup>+</sup> (5, 25 mM)	biochar Fe <sub>3</sub> O <sub>4</sub> -biochar	Fe <sub>3</sub> O <sub>4</sub> -biochar	quartz sand	upward migration experiments in columns with a depth of 10 cm	pumping water (saturated)	① The addition of biochar and Fe <sub>3</sub> O <sub>4</sub> -biochar in quartz sand decreases the transport and increases the retention of plastic particles in porous media, ② with Fe <sub>3</sub> O <sub>4</sub> -biochar having higher inhibitory effect; ③ Small amounts of the retained NPs detached from the sand, because of decreasing ionic strength.	Tong et al., (2020)
Carboxylate-modified polystyrene latex	6	Na <sup>+</sup> (5, 25 mM)	NA	Goethite Hematite	quartz sand	upward migration experiments in columns with a depth of 10 cm	pumping water (saturated)	① Increasing ionic strength caused lower breakthrough curves and corresponding higher retained profiles of MPs; ② Both Goethite and Hematite tended to reduce the transport of MPs and increase retention of MPs in quart sand.	Li et al., (2019)
polystyrene	5, 7	Na <sup>+</sup> (0.1-10 mM)	NA	nTiO <sub>2</sub>	quartz sand	upward migration experiments in columns with a depth of 10 cm	pumping water (saturated)	① Lower breakthrough curves and corresponding the higher retained profiles of MPs with nTiO <sub>2</sub> in suspensions relative to those without nTiO <sub>2</sub> were obtained, because the size of MP with TiO <sub>2</sub> increased; ② The retention rate of MPs increased, mainly due to decreased repulsive energy between MPs and sand, with increasing ionic strength.	Cai et al., (2019)

### 2.3 The influences of MP physical properties on the mobility of MP in soils

The evidence from column experiments and field investigations provided insight into the MP physical properties (i.e., shape, size, and density) and the relevant mechanisms controlling MP mobility (Table 2-3).

Shape seems to influence the intensity of MP entanglement with soil particles when MPs are being moved by water flow thus changing MP mobility (Waldschlager and Schuttrumpf, 2020). Regarding MP particles with granular shapes, round MPs such as spheres, pellets, beads tend to be more mobile in soil than angular MPs such as cubic, cylinder, bar, fragment because the surface protrusions are more likely to be caught in the throats of pores (Knappenberger et al., 2015; Lamy et al., 2013; Pradel et al., 2020; Waldschlager and Schuttrumpf, 2020). Also, the decreased MP mobility could be attributed to the orientation-dependent interaction energies, since side-on orientation increased the secondary-minimum depth of DLVO energy profile between MPs and medium particles under unfavourable attachment conditions (in the absence of MPs-medium particle surface electrostatic repulsion) (Knappenberger et al., 2015; Pradel et al., 2020). Both the observations from a field-controlled study by Crossman et al. (2020) and an indoor simulated experiment by Keller et al. (2020) revealed that the application of waste treatment sludge (biosolid) resulted in a higher leaching risk of granular MPs to surrounding soils than the risk of MP fibres mainly because liner shape resulted in entanglement within the soil happening more frequently (de Souza Machado et al., 2018). Despite showing downward migration of MP fibres was less than that of granular MPs, a lack of standard methods to compare the size of MPs with different shapes seemed to prevent these studies from distinguishing the impacts of MP shapes or sizes. In contrast, Waldschlager and Schuttrumpf (2020) used the cubic root of the product of length, width and height as the equivalent particle diameter of MP ( $d_{MP}$ ) when comparing the mobility of different MPs, providing more convincing evidence that MP fibres had less mobility than MP spheres with similar  $d_{MP}$ .

Existing evidence (Table 2-3) showed that the maximum migration depth and effluent recovery of granular MPs decreased with MPs' average diameter under unfavourable attachment conditions (Keller et al., 2020; Pradel et al., 2020; Waldschlager and Schuttrumpf, 2020). Thinner fibre diameter and shorter fibre length contributed to the deeper migration depth of MP fibres (Waldschlager and Schuttrumpf, 2020). This is mainly because straining, which reduces MP movement by water flow, is more likely to occur for larger and longer MPs when



pore morphology is the same (Pradel et al., 2020; Shaniv et al., 2021; Waldschlager and Schuttrumpf, 2020). Generally, the ratio of MP to matrix particle diameters is recognized as a predictor variable for the extent of MP straining because this parameter can reflect the relative size of MP to pore, with a larger ratio leading to a decrease in MP mobility (Bradford et al., 2002; Porubcan and Xu, 2011; Torkzaban et al., 2008). For instance, Waldschlager and Schuttrumpf (2020) found that hardly any MP infiltration occurred when that ratio  $> 0.32$ . Despite the principle role that the actual diameter of a single MP plays in straining, MP aggregation induced by chemical factors (e.g., pH, ionic strength, surface charge) can increase the size of MPs, thus further promoting MPs straining (Crist et al., 2005; Dong et al., 2018). In addition to straining, MP sizes may also impact other factors influencing MP mobility. Knappenberger et al. (2014) revealed when the diameter of MP exceeded the water film thickness on the surface of unsaturated matrix particles, MP could be trapped within the water films due to capillary force, which reduced MP mobility and is often referred to as film straining. Moreover, smaller angular MPs, which might be preferentially ingested by surface feeding earthworms (*Lumbricus terrestris*), can accelerate the transport of granular MP via ingestion-egestion pathway (Huerta Lwanga et al., 2016; Rillig et al., 2017b). In summary, microplastics that break down into smaller fragments in the soil are presumably more mobile.

Density affected MP mobility via changing the balance of gravity and buoyancy when soil pore is filled with water (Chrysikopoulos and Syngouna, 2014; Li et al., 2021a; O'Connor et al., 2019). Investigations in aquatic environments (e.g., ocean, river, lake) reported that if the specific gravity of MP  $< 1.0$ , the MPs will float at the surface with hardly deposition occurring (Chen et al., 2019; Chubarenko et al., 2016; Nizzetto et al., 2016). Comparable studies based on soils, which mainly considered physical effects, demonstrated that the downward migration of MP increased with density for both primary and secondary granular MPs. For instance, O'Connor et al. (2019) found after twelve 83 mm infiltration events, small pristine PP (diameter: 29  $\mu\text{m}$ , density: 0.833  $\text{g}/\text{cm}^3$ ) still stayed at surface layer, while larger pristine PE (diameter: 349  $\mu\text{m}$ ) with a higher density (0.893  $\text{g}/\text{cm}^3$ ) migrated much deeper. Similarly, Yan et al. (2020) reported that the vertical migration of naturally-aged MPs with and without cleaning in soil accorded with the observations in aquatic environments, suggesting that an increase in the density of naturally-aged MP resulting from hetero-aggregation with other soil colloids could favour MP mobility. In comparison, density did not impact the vertical movement of plastic fibre, potentially because other hydrodynamic forces, such as positive frontal pressure and

shear force, dominate over buoyance and gravity under the high flow rate (Shen et al., 2014; Waldschlager and Schuttrumpf, 2020).

**Table 2-3. Influence of MPs physical properties on MPs mobility**

MPs Physical Properties				Porous Mediums	Methods	Hydraulic Conditions	Main Findings	References
type	shape	size	density					
polystyrene	graininess	50 ~ 190 nm	1.05 g/cm <sup>3</sup>	Loamy sand soil, sand	upward migration experiments in columns with a depth of 20 cm	pumping water (saturated)	① Maximum elution value of 90% from the soil was found for the 50 nm PS-NPs, in contrast a maximum value of 48% was for 110 nm PS-NPs; ② the breakthrough curve for 190 nm PS-NPs demonstrated a maximum elution value of 60%.	Shaniv et al. (2021)
polypropylene, polyethylene	granular, pellet, sphere	21 to 535 µm	0.833-0.898 g/cm <sup>3</sup>	sand	downward migration experiments in columns with a depth of 25 cm	wet-dry cycles (unsaturated)	① Microplastics undergo accelerated vertical migration in sand soil due to small size; ② the larger sized PE particles i.e., PE-course (349 µm), facewash (181 µm) and body scrub (535 µm) all reached maximum penetration depths of ~3.5 cm within the columns, while the PP MPs (29µm) migrated to a maximum depth of only ~1.5 cm due to lower density; ③ for owning higher density, course PE reached further penetration than fine PE.	O'Connor et al. (2019)
Polyethylene terephthalate, Polyvinyl chloride, Styrene butadiene rubber, Polyamide, Copolyimide, Polystyrene, Polyethylene, Polypropylene	Fragment, Pellet, Fiber, Sphere, Cubic	0.6-1.5 mm, 2.29-3.91 mm, 1.08-2.15 mm, 1.00-5.00 mm, 0.75-1.00 mm	0.870-1.368 g/cm <sup>3</sup>	glass sphere	downward migration experiments in columns with a depth of 50 cm	Artificial rainfall (unsaturated)	① the infiltration depth of the microplastic particles increased with decreasing diameter of the microplastic particles (dMP); ② in case of fibres, particularly the fibre diameter influences the depth of infiltration, with thinner fibre diameters leading to deeper infiltration depths; ③ Fragments (e.g., tire abrasion) infiltrated less deeply than spherical particles, probably due to the entanglement of the angular particles in the pores.	Waldschlager and Schuttrumpf (2020)
polyacrylonitrile covered by a polystyrene shell, ethylene terephthalate	particles, fiber	187 ± 22 nm, 510 ± 12 µm	NA	glass sphere	upward migration experiments in columns with a depth of 10 cm	irrigating water (unsaturated)	① a very high retention of microplastic fibres both within the sludge bed and in the porous media in contrast to particle MP.	Keller et al. (2020)
Polystyrene, High Density Polyethylene, Polypropylene	particle, microbead	75–425 µm, 45-53 µm	0.90-1.05 g/cm <sup>3</sup>	sand, gravel	upward migration experiments in columns with a depth of 80 cm	irrigating water (unsaturated)	① Microbeads had higher permeability because the size of microbeads was 45–53 µm which was much smaller than the size of the other MPs (75–425 µm).	Wang et al. (2021)
Polystyrene, Polyvinyl chloride, Car tyre, Polyethylene terephthalate	beads, fragments, fibers	Na	Na	farmed soils	applied biosolids containing MPs to farmed land and then continuously monitored soil MPs concentrations	rainfall, irrigation	① following biosolids applications, two fields demonstrated significant increases in soil MP concentrations, with preferential retention of MP fibres over fragments observed.	Crossman et al. (2020)
carboxylate-modified polystyrene	Spherical barrel	1 µm	Na	sand	downward migration experiments in columns with a depth of 15 cm	pumping water (unsaturated to saturated)	① MPs were retained and released from a secondary energy minimum with more angular-shaped MPs being retained and released under varying ionic strength.	(Knappenberger et al., 2015)

## 2.4 Bioturbation and its impacts on mobility of microplastics in soils

The position of microplastics in soils can be directly or indirectly altered by the activities of soil biota. In previous studies, different activities of terrestrial fauna and flora have been found to impact the mobility of plastic particles in soils (Table 2-4)

The existing literature on soil invertebrates is extensive and focuses particularly on microplastics transported by earthworms. Burrowing invertebrates can vertically migrate microplastics into deeper soil via the ingestion-egestion pathway. Huerta Lwanga et al. (2016) found that microplastics in surface soil can be ingested by the surface feeding earthworm *Lumbricus terrestris* and then concentrated in their egested casts due to the partial digestion of organic matters. In some cases, microplastics (polyethene bag fragments) may have the same mobility as other soil particles during ingestion-egestion transportation, because earthworms (*Lumbricus terrestris*) neither preferentially ingested nor avoided microplastics when eating soil and did not retain microplastics in their gut after complete defecation (Hodson et al., 2017). Other researchers who have looked at the size-selective movement of microplastics by earthworms, have highlighted that smaller microplastic particles migrated downward to a greater extent (Huerta Lwanga et al., 2016, 2017a; Huerta Lwanga et al., 2017b; Rillig et al., 2017b; Yu et al., 2019), which may be explained by both the preferential intake of smaller particles and the grinding of larger plastics into smaller sizes during transit through the gut (Huerta Lwanga et al., 2016).

Earthworms and other soil invertebrates can also transport plastic particles without ingesting them, whereby plastic particles can be translocated further. Zhang et al. (2018) observed that earthworms purposely drag polyethene mulches (1.5 cm×1.5 cm) into burrows up to 20 cm, while not necessarily ingesting them, proving that earthworms had a potential to transport macroplastics (>5 mm). (Vinkovic et al., 2021). Accidental adhesion also plays a role in the movement of very small MPs (<1 mm). Rillig et al. (2017b) found that polyethene beads (710–850 µm) adhered to the earthworm skin and thus can be transferred elsewhere simply by the movement of the earthworm. Collembolans (*Folsomia candida*, *Proisotoma minuta*) and mites (*Hypoaspis aculeifer*) can also transport MPs via attaching MPs on their cuticle (Maass et al., 2017; Zhu et al., 2018), though their transport sizes (< 250 µm) were smaller than that of earthworms (*Lumbricus terrestris*) because of their small size. Moreover, interspecies relationship may affect the efficiency of adhesion transportation. For example, tropic predator (mite)–prey (collembolans) relationship accelerated the movement of both mites and

collembolans. Consequently, the number of MPs (80-250 $\mu$ m) horizontally dispersed via adhering to soil microarthropods increased by 40% (Zhu et al., 2018).

Earthworm burrows provide preferential flow paths for water which may increase the risk of microplastics leaching into shallow groundwater (Huerta Lwanga et al., 2016, 2017a; Rillig et al., 2017b). Subsequent studies have confirmed this. Yu et al. (2019) reported HDPE particles up to 1 mm penetrated 50 cm depth of sandy soil and even leached into drainage due to earthworm burrows. Similarly, Wang et al. (2021) detected the appearance of multiple microplastics (PS particles, HDPE particles, and microbeads) in the leachate of an 80 cm-depth sand column. Lastly, thanks to preferential flow, microplastics deposited in the topsoil and earthworms' burrows rather than that in soil matrix are more likely to migrate downward (Yu et al., 2019).

Studies on flora activities that affect the movement of microplastics in soil are remarkable scarce. However, a seminal investigation by Li et al. (2020) revealed cracks, where lateral roots grow out from the endodermis and cortex of the primary root, offer 0.2 and 2.0  $\mu$ m PS beads entryways into wheat (*Triticum aestivum*) and lettuce (*Lactuca sativa*) root xylem. On entering roots, these microplastics were subsequently transferred to upper edible organs including shoot-tips by the driving force of transpiration. Furthermore, Li et al. (2021a) compared the different roles of fine roots and thick roots in the process of translocating microplastics. The fine ryegrass roots seemed to fix microplastics in a dense root network. In comparison, MPs less dense than water rose through water occupying macropores created by coarse corn roots. Together, these studies indicate that roots of crop plants tend to move microplastics upward or immobilize them in soil via absorption, fixation and affecting soil hydraulic properties.

**Table 2-4. Microplastics transported by terrestrial fauna and flora**

Microplastics Properties			Porous Mediums	Methodologies	Organisms	Main Findings	References
type	shape	size					
Low Density Polyethene	Graininess	<150 µm.	Sandy soil	Plant litter containing microplastics was placed on the surface of mesocosms.	Earthworm ( <i>Lumbricus terrestris</i> )	① Microplastic was concentrated in cast; ② size-selective egestion by the earthworms.	Huerta Lwanga et al. (2016)
High Density Polyethene	Fragment	0.92±1.09 mm <sup>2</sup>	Woodland soil	Earthworms were exposed in mesocosms of moist soil containing microplastics.	Earthworm ( <i>Lumbricus terrestris</i> )	① Earthworms neither preferentially ingested nor avoided the microplastics when eating soil; ② earthworms didn't leave microplastics in gut.	Hodson et al., (2017)
Low Density Polyethene	Graininess	<150 µm	Sandy soil	Plant litter containing microplastics was placed on the surface of mesocosms.	Earthworm ( <i>Lumbricus terrestris</i> )	① Size-selective downward transport of microplastics by earthworms; ② microplastics can be pushed into the earthworm burrows with plant litter.	Huerta Lwanga et al., (2017a)
Polyethene	Sphere	710-2800 µm	Albic Luvisol	Microplastics with four size ranges were added to the surface of soil column.	Earthworm ( <i>Lumbricus terrestris</i> )	① The smallest microplastics (710–850 µm) was found the most in the bottom (7-10.5 cm); ② polyethene beads adhered to the earthworm skin.	Rillig et al., (2017)
Low Density Polyethene	Graininess	<1 mm	Sandy soil	Leaching experiments were carried out after 14 days of earthworm's incubation in soil columns.	Earthworm ( <i>Lumbricus terrestris</i> )	① Earthworm constructed pathways (burrows) for microplastics to be transported into groundwater.	Yu et al., (2019)
Urea-formaldehyde	Graininess	<200 mm	Plaster of Paris, activated charcoal	Microplastics was placed on the center of specimen cup filled with thin artificial soil.	Eollembolans ( <i>Folsomia candida</i> , <i>Proisotoma minuta</i> )	① Collembolans horizontally dispersed microplastics more than 4 cm via attaching to cuticle.	Maass et al., (2017)
Polyethene	Mulch	1.5 cm×1.5 cm	Silt loam	Plastic mulches were placed on the mesocosms filled with soil.	Earthworm ( <i>Lumbricus terrestris</i> )	① Earthworms purposely dragged mulches into their burrows up to 20 cm.	Zhang et al., (2018)
Polystyrene, High Density Polyethene, Polypropylene	Particle, Bead	particle (75–425 µm), bead (45-53 µm)	Sand	leaching experiments were carried out after 20 days of incubation in sand columns with a depth of 80 cm.	Earthworm ( <i>Lumbricus terrestris</i> )	① Microplastics were found in the excreta of earthworms in the bottom layer and in the effluent as well.	Wang et al. (2021)
Polyvinyl Chloride	Particle	80-250 µm	Plaster of Paris, activated charcoal	Microplastics was placed on the center of specimen dishes filled with thin artificial soil.	collembolan ( <i>Folsomia candida</i> ), mite ( <i>Hypoaspis aculeifer</i> )	① Soil microarthropods translocated microplastic particles up to 9 cm; ② microarthropod predator (predatory mite)-prey (collembolan) relationships can promote the horizontal dispersion of microplastics.	Zhu et al. (2018)
Polystyrene	Bead	0.2µm, 2 µm	Sand, sandy soil	Crop plants grew in a sand matrix or sandy soil with different contents of microbeads.	Wheat ( <i>Triticum aestivum</i> ), lettuce ( <i>Lactuca sativa</i> )	① Polystyrene beads permeated the stele of roots using the crack-entry mode at sites of lateral root emergence; ② higher transpiration rates enhanced the uptake of plastic particles.	Li et al. (2020)
Polypropylene	Film, bead	<1 mm	Cinnamon soil	Crop plants grew in soil column with microplastics placed on surface (E1) and mixed well with soil (E2)	Crop, Soybean, Ryegrass	① significant positive correlation between microplastic numbers and tertiary roots of ryegrass were observed, because microplastics were more easily fixed by fine roots; ② thick corn roots might create water-filling spaces that provide buoyancy for upward migration of microplastics	Li et al. (2021)

## 2.5 The influences of environmental processes on MP mobility in soils

MPs released into the environment are subject to various environmental processes (e.g., ageing, formation of biofilm, ploughing, embedding in soil aggregates), exerting enormous influences on MP mobility, as such processes change the physiochemical properties of MPs and surrounding soils and even directly serve as driving forces (Table 2-5).

MPs that are present in the environment for a lengthy period of time are susceptible to exposure to ultraviolet (UV) radiation, thermal degradation, biodegradation, weathering and oxidation, which can change the MP properties and are often referred to as ageing (Jahnke et al., 2017). Ageing can markedly modify the original surface chemistry, such as charge, functional groups, and hydrophobicity, which are vital properties controlling MP mobility (Table 2-6). Liu et al. (2019) found that the exposure of polystyrene nanoplastics to both UV irradiation and ozone induced the formation of oxygen-containing functional groups (e.g., epoxy/hydroxyl, carbonyl, and carboxyl groups). These groups not only increased the negative charge of NPs, but more importantly, enhanced hydrophilicity leading to a significant increase in hydrophilic repulsion between particles (Liu et al., 2019). Consequently, the aged NPs became more mobile than the pristine NPs during downward migration. Similarly, Crist et al. (2005) verified that more hydrophilic carbonylated MPs were eluted in the leachate than unmodified hydrophobic MPs. However, the influence of other ageing factors (i.e., high temperature, biodegradation) still needs to be explored. Continuous exposure to ageing factors would generate more properties changes on MP surface, thus causing more MP movement (Liu et al., 2019; Ren et al., 2021). For example, when UV irradiation increased from 6 to 12 h, an abrupt increase in carboxyl functional groups on the MP surface from 0.75 to 4.01 wt % was obtained, with the peak of breakthrough curve further growing from about 0.6 to 0.9 (C/Co) at 10 mM NaCl (Liu et al., 2019). Furthermore, UV-persulfate ageing established a synergetic effect on promoting MP mobility, compared with the impact of UV and persulfate ageing alone, suggesting that the cumulative effects of various ageing processes can increase the risk of MP to surrounding soils (Ren et al., 2021). Although the influences of ageing-induced physical changes, especially reduced MP sizes and increasing MP surface roughness, were not discussed in published studies (Liu et al., 2019; Ren et al., 2021), it can be inferred that ageing processes can favour MP mobility by reducing size. In contrast, increasing surface roughness may make MP more easily trapped in the throats of the soil pores.

Biofilms, a community of microorganisms (largely bacteria) accumulated in the matrix of self-secreted extracellular polymeric substances (EPS), are ubiquitous on the surface of porous media particles and MPs. The published literature contains conflicting claims about the change in surface properties of biofilm-coated matrix particles and how these changes affect MP and other colloids mobility. Much of the research agrees that biofilm formation on matrix particles can inhibit MP mobility through narrowing flow path because biofilm is considered impermeable to water flow (Chen et al., 2021; Cunningham et al., 2002; Kim et al., 2010; Rittmann, 1993; Wang et al., 2021). He et al. (2020) compared the pore structure of a biofilm-coated sand column with that of a bare sand column using computed X-ray microtomography imaging. They found that biofilm significantly increased the retention of MP induced by straining, through substantially reducing porosity and flow path confluent points (He et al., 2020). Similarly, the observations from other microparticle movement experiments (e.g., ZnO, Ag, TiO<sub>2</sub>) corroborate this point of view (Kurlanda-Witek et al., 2015; Li et al., 2013; Xiao and Wiesner, 2013). In addition, biofilm formation could largely decrease soil permeability by as much as three orders of magnitudes, an association with a substantial reduction of shear force provided by water flow, whereby MP mobility may reduce (Cunningham et al., 2002; Dennis and Threr, 1998; Kim et al., 2010). Much of the research found that classical DLVO theory cannot adequately explain the change of MP and other colloids mobility in porous media. This is mainly due to the uneven distribution of biofilm on porous media particles (Kim et al., 2010; Li et al., 2013; Xiao and Wiesner, 2013) as well as the roles of other non-DLVO interactions (e.g., steric repulsion, hydrophobic attraction, hydrophilic repulsion, etc). In order to calculate the energy interaction considering surface roughness impact, He et al. (2020) used modified DLVO theory. They found that the interaction energy between MP and bare sand was more repulsive than that between MP and biofilm-coated sand, a result contrary to the calculation of classical DLVO theory, suggesting that increasing roughness inhibited MP mobility. In contrast, some emphasized the critical role that steric repulsion plays in facilitating colloids mobility. Biomacromolecule chains (e.g., cell polysaccharides and proteins) on bacterial surface could induce steric hindrance between MPs and matrix particles beyond the effective range of classical DLVO interactions, with direct potential consequence that physical contact between particles can be prevented (Camesano and E, 2000; Xiao and Wiesner, 2013). Others regard that the relative abundance of hydrophobic fraction (e.g., proteins) compared to hydrophilic fraction (e.g., polysaccharides) determine that biofilm would make matrix particles more attractive to hydrophobic microparticles (e.g., pristine MPs) by hydrophobic attraction



(Habimana et al., 2011; Xiao and Wiesner, 2013). In summary, these findings indicate that MP movement in biofilm-coated soil matrix could be influenced by various complex physicochemical factors, which should be carefully considered and verified in future studies.

Biofilm can grow on MPs exposure to environment (Chinaglia et al., 2018; Ren et al., 2015; Wu et al., 2019). Thus far, possible consequences of biofilm formation on MP mobility in soils are virtually unknown. However, evidence is accumulating that the biofilm has the capacity to influence MPs mobility through modifying the properties of MPs. As discussed in the last paragraph, hetero aggregation with other soil colloids facilitated the mobility of ageing MPs less dense than water by increasing overall density (Yan et al., 2020). Regarding biofilm-coated MPs, the overall density of a particle depended on MP density, biofilm density and the volume fraction of biofilm (Besseling et al., 2017; Rummel et al., 2017). Thus, it is logical that biofilm, as a fraction denser than water, may also increase the downward movement of float MPs in water-filled soil pores (Chen et al., 2019; Kooi et al., 2017; Miao et al., 2021). Furthermore, biofilm also introduces increasing particle size and surface roughness to MPs, likely resulting in MPs being more easily caught in the throats of soil pores (Knappenberger et al., 2015; Waldschlager and Schuttrumpf, 2020). In addition to the impacts of physical factors, the biological matrix covering MPs contains a mass of extracellular polymeric substances, such as proteins, lipids, extracellular DNA, and polysaccharides. When these biopolymers are present on the surface, MP chemistry, such as surface charge, hydrophobicity, or hydrophilicity, will inevitably change thus affecting soil-MP interactions. For example, comprehensive investigations of proteins' influences on MPs mobility conducted by Dong et al. (2020) have shown that proteins (e.g., BSA, LSE) adsorbed on MPs can either disperse or aggregate MPs in matrix solutions by electrostatic interactions, steric repulsion, polymer bridging, thus changing MP mobility, which mainly depended on the nature of proteins as well as the degree of adsorption. However, it is still unclear what effects the real biofilm and its compositions have on MP mobility because few studies focus on the relationship between biofilm and MPs mobility in soils compared to biofilm-coated MPs mobility in aquatic environments.

Ploughing was seen as an effective driving force that can quickly bring residual plastics staying at soil surface to the subsurface deep into the depth of ploughing, through squeezing, embedding, and mixing soil containing plastics (Rillig et al., 2017a). Studies concerning the influence of ploughing emphasized the maximum depth of different tillage practices because such depth to soil surface was regarded as the region where plastics can be distributed by

ploughing (Rillig et al., 2017a). But in fact, the roles of some other factors relevant to ploughing might be neglected previously. For instance, plastics incorporated into soil blocks, especially plastics with thin and flat shapes such as mulching film, are readily stretched and tore during machinery ploughing. One can be expected that these plastics can thus break into smaller debris with a higher mobility. Ploughing also creates abundant soil pores that can serve as pathways for the movement of MPs by water flow. Furthermore, soil cover produced by ploughing may shield the MPs from UV radiation, high temperature, and ozone above soil surface. As a result, MPs buried in soil would be less influenced by above abiotic ageing factors but more likely to be affected by biodegradation, suggesting a change in the contributions of different ageing factors to MP mobility.

The spaces between aggregates form a range of pore sizes with preferential water flow typically occurring through macropores, leading to the formation of preferential water flow (Kodešová et al., 2012; Regelink et al., 2015; Rillig et al., 2017a). Therefore, it is reasonable to assume that the vertical movement of solid particles transported by that water in structured soils containing aggregates could be more complex than those in the simplified soil matrixes without structure. Field evidence has shown that up to 72% of MPs in farmland soil are incorporated into aggregates (Zhang and Liu, 2018). Since soil aggregates, especially water-stable aggregates (WSAs) are resistant to breaking up during infiltration, undisturbed soils in fields containing aggregates likely retain more MPs than disturbed soil without aggregates did in laboratories, which has been overlooked in previous simulated experiments. In addition, as soil aggregates are not permanently stable but constantly forming and breaking up, MPs trapped in soil aggregates may be continuously re-released and intermittently move (Rillig et al., 2017a). Hence, the formation and duration of soil aggregates containing MPs are vital for estimating MP mobility in undisturbed soil. Recently, researchers successively cultivated soil aggregates embedding MPs in laboratories, revealing that the quantity and the stability of soil aggregates are also affected by the shape and content of microplastics (de Souza Machado et al., 2018; Liang et al., 2019; Zhang and Zhang, 2020). Among different MPs, WSAs% only significantly decreased with increasing polyester concentrations ( $p < 0.01$ ), suggesting a potential weak immobilization of soil aggregates to plastic fibres (de Souza Machado et al., 2018). Additionally, properties including the life cycle of soil aggregates, the proportion of MPs entering soil aggregates, and the water stability of soil aggregates will be necessary to evaluate when exploring the impacts of soil aggregates on MP mobility. More importantly,

corresponding migration experiments should be conducted in soil containing aggregates cultivated in laboratories and ultimately in the field.

**Table 2-5. the influences of the environmental processes on MP mobility in soils**

MP types	Environmental processes	Changes in MP properties	Changes in soil properties	Porous Medias	MP mobility	References
polystyrene	Ultraviolet (UV) ageing ozone ageing	① both UV ageing and O <sub>3</sub> ageing formed epoxy/hydroxyl, carbonyl, and carboxyl groups on the surface of MP, which subsequently ② increased surface negative charge and the hydrophilicity. ④ reduced size; ⑤ increased surface roughness.	NA	Lufa soil (loamy sand)	① aged PSNPs exhibited higher mobility in saturated soil largely because of their higher surface hydrophilicity; ② whereas enhanced negative charge contributed less to increased MP mobility.	Liu et al., (2019)
polystyrene	Ultraviolet (UV) ageing, persulfate ageing, UV-persulfate ageing	① both UV ageing and persulfate ageing increased oxygen-containing functional groups and ② surface roughness of MP; ③ aged MPs with increasing surface functional groups tended to have more negative zeta potential ( $R^2=0.958$ ); ④ the UV-persulfate ageing showed synergetic effect on promoting MP mobility, compared to the effect of UV and PS alone.	NA	clay loam sandy soil	① UV-persulfate MPs had the highest breakthrough percentage of 89.2% which was approximately 1.68 times of UV MPs (53%), 2.57 times of PS MPs (34.7%), and 2.92 times of pristine MPs (30.5%) in sandy soil. ② similar observations were reported in clay loam.	Ren et al., (2021)
polyethene polyethene terephthalate	biofilm growth on MPs and matrix particles	① biofilm increase the density of floating MP; ② increased MP size	① reduced the aperture gap; ② enhanced the adhesion	gravel	① both the formation of biofilm on MPs and on matrix particles attributed to reduced MP mobility.	Chen et al., (2021)
Polystyrene	The absorption of proteins on NPs	① NPs with a large adsorbed amount of negative bovine serum albumin (BSA) could induced negatively charged corona around MP thus generating steric repulsion; ② NPs with a small adsorbed amount of the positively charge lysozyme increase the aggregate sizes of NPs via connecting MPs with protein “bridges”	NA	sand		
polystyrene	biofilm growth on matrix particles	NA	① narrowed the path of water flow; ② negatively increased the surface charge; ③ increased surface roughness	sand	① Although biofilm coating increased the interaction energy between MPs and sand, the presence of biofilm ① narrowed the flow path in the inlet of the column and ② increased the surface roughness of porous media, which led to a decrease in MP mobility.	He et al., (2021)
polystyrene, polyethene, polypropylene	biofilm growth on matrix particles	NA	The biofilm could ① enhance the water retention, ② lead to higher humidity.	sand	① the growth of biofilm slightly enhanced the filtering capacity of sand, leading to a tiny decrease in MPs mobility.	Wang et al., (2021)

## **2.6 The effects of MPs on soil aggregate formation and properties: the major knowledge gaps and hypotheses**

Aggregate formation and properties play important roles in ecosystem services and functioning (Barthès and Roose, 2002; Eynard et al., 2004a; Horn and Smucker, 2005; Shakesby et al., 2000; Zhang, 1994). Field investigations have shown that MPs in soils are primarily present in aggregates (Rehm et al., 2021; Zhang and Liu, 2018). Due to the significant difference between the properties of MPs and other soil components (e.g., chemical composition, bioavailability, hydrophobicity, particle density) (de Souza Machado et al., 2019; Lehmann et al., 2021; Pathak and Navneet, 2017; Qi et al., 2020), this could have a significant impact on aggregate formation and properties. Recently, MPs have been reported to reduce the sizes and water stability of aggregates, most likely by affecting microbial structures and activities, introducing fracture points, and reducing cohesion (Boots et al., 2019; de Souza Machado et al., 2018; Lehmann et al., 2019; Lehmann et al., 2021). Additionally, since MPs usually have much higher hydrophobicity and lower particle density than most soil fractions (Hao et al., 2008; Li et al., 2018a; Pathak and Navneet, 2017), their incorporation may cause reductions in aggregate wettability and particle density, thus affecting the ecological functions of aggregates. Despite this, minimal research has explored the effects of MPs on these critical aspects of aggregates.

While accumulating in soils, plastics can fragment into smaller debris due to UV radiation, high temperature, biodegradation, and mechanical forces of tillage (Lee et al., 1991; Tian et al., 2022). The resulting increased concentrations and reduced sizes of MPs can pose long-term challenges to aggregated soil structure. It is likely that some MP features (e.g., hydrophobic surface area, additive leakage) can become more notable as MP sizes decrease, hence magnifying the effects of MPs on aggregates. Thus far, researcher have discovered that impacts of MPs on soil aggregates depend on MP concentrations, shapes, and polymer-types (Boots et al., 2019; de Souza Machado et al., 2019; Lehmann et al., 2021; Lozano et al., 2021). Nevertheless, little is known about the effects of different sized MPs on aggregates formation and properties.

Therefore, two scientific questions were considered in this thesis:

1. Whether MPs have size-dependent effects on aggregate formation and properties?
2. Whether MPs have concentration-dependent effects on aggregate formation and properties?

Five hypotheses were raised in this thesis:

- i. MPs can be embedded in newly-formed soil aggregates which larger than the size of MPs particle. When soil is exposed to higher concentration of MPs, the MP concentration in newly-formed soil aggregates increases.
- ii. Higher MP exposure concentration in soil may reduce the formation of newly-formed soil aggregates. Reduced MP sizes increase the impacts of MPs on aggregate formation.
- iii. Pristine polyethene particles, as an extremely hydrophobic material relative to most soil fractions, can significantly enhance the hydrophobicity of aggregates. Increased MP concentration can further decrease aggregate wettability. Smaller MPs can increase this impact because of the larger surface-to-mass ratio (i.e., larger hydrophobic surface area).
- iv. Higher concentration of MPs likely reduces the water stability of aggregates. Smaller MP size may increase this impact.
- v. Higher MP concentration incorporated into aggregate may lower the overall particle density of MPs-containing aggregates, since the particle density of polyethene MPs are much less dense than that of soil mineral particles. However, MP sizes will not significantly change this property.

# 3 Methods and Materials

## 3.1 Microplastic properties analyses

Three size fractions of pristine polyethene powders were sold by Goonvean Fibres Ltd in Devon, the UK as being < 35, < 125 and < 500  $\mu\text{m}$  in size. And we referred to them as MP35, MP125, MP500. Their chemical components were identified as polyethene by FTIR (Bruker Optics, Karlsruhe, Germany) (Figure 3-1).

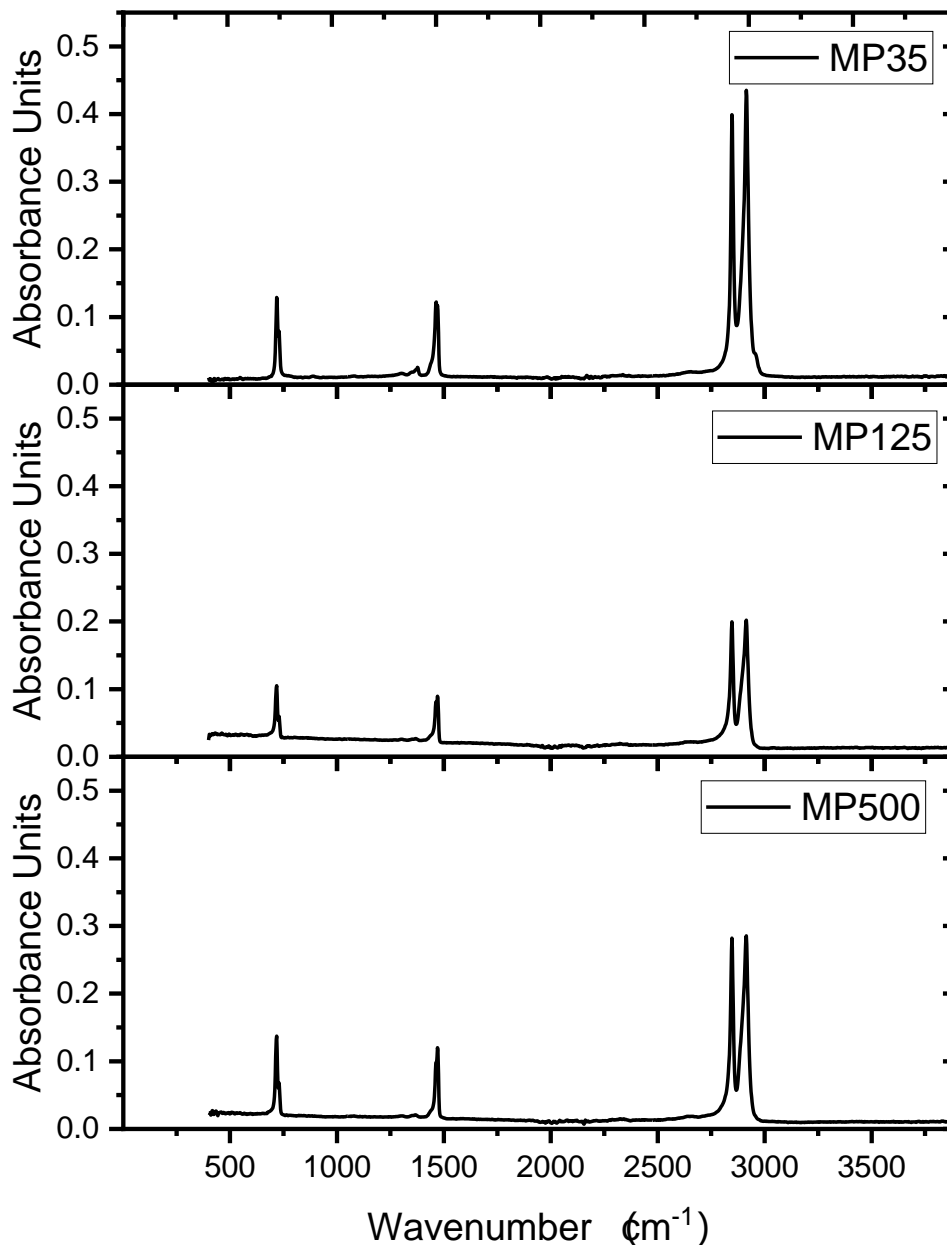


Figure 3-1. The FTIR spectrum of MP35, MP125, and MP500. The characteristic PE absorbance bands were all located at  $718\text{ cm}^{-1}$ ,  $1470\text{ cm}^{-1}$ ,  $2847\text{ cm}^{-1}$ ,  $2914\text{ cm}^{-1}$ , which were the same as the results of D'Amelia et al. (2016).

Those pristine polyethene microplastics are strongly hydrophobic and less dense than water. Due to the problems of floating, aggregation, and forming MPs-coated air bubbles on water surface that this creates (Figure 3-1. A-C), it is challenging to use analytical methods that rely on water as a dispersant for measuring MPs' physical properties. However, polyethene MPs were able to suspend and/or sink, and cannot be dissolved in > 35% ethanol absolute (Figure 3-1. D-F). Thus, the substituting water for ethanol offer opportunities to update traditional analytical techniques for identifying MPs' physical properties.

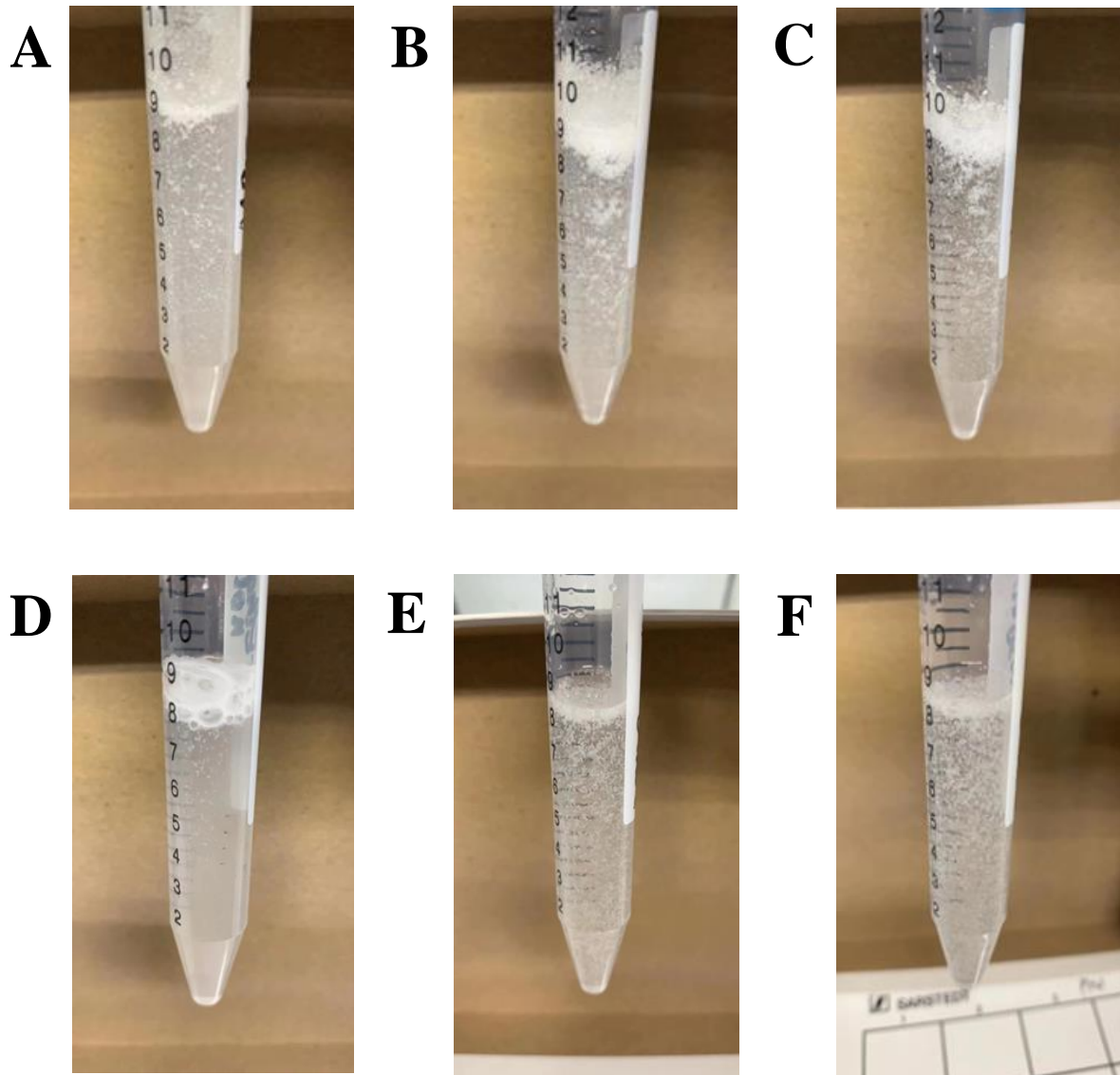


Figure 3-1. Status of MP35 (A), MP125 (B), MP500 (C) in deionized water and MP35 (D), MP125 (E), MP500 (F) in 35% ethanol absolute after shaking.



### 3.1.1 Analytical method for particle density analyses

A traditional particle density determination method using a pycnometer was improved by replacing deionized water with 99.8% ethanol absolute (VWR Chemicals BDH Co., Ltd, Leicestershire, United Kingdom) in this study. Briefly, 5 mL pycnometers (Pomex Glassware Co., Ltd, Beijing, China) both empty and containing deionized water of known density ( $0.998 \text{ g/cm}^3$ ) were weighed (accurate to four decimal places) on an analytical balance at  $20.0 \text{ }^\circ\text{C}$  to determine their inner volumes (Equation 1). Using the known volume of pycnometers, the density of 99.8% ethanol absolute ( $0.789 \text{ g/cm}^3$ ) that acted as a dispersant at  $20 \text{ }^\circ\text{C}$  could be obtained using pycnometers (Equation 2).

$$V_p = \frac{M_{pw} - M_p}{\rho_w (20 \text{ }^\circ\text{C})} \quad (1)$$

Where  $V_p$  is the volume of pycnometer ( $\text{cm}^3$ );  $M_{pw}$  is the mass of pycnometer fully filled with deionized water (g);  $M_p$  is the mass of empty pycnometer (g);  $\rho_w (20 \text{ }^\circ\text{C})$  is the density of deionized water at  $20^\circ\text{C}$ .

$$\rho_e (20 \text{ }^\circ\text{C}) = \frac{M_{pe} - M_p}{V_p} \quad (2)$$

Where  $\rho_e(20^\circ\text{C})$  is the density of ethanol ( $\text{g/cm}^3$ ) at  $20^\circ\text{C}$ ;  $M_{pe}$  is total mass of pycnometer fully filled with ethanol (g);  $M_p$  is the mass of empty pycnometer (g);  $V_p$  is the volume of pycnometer.

The mass of ethanol solution replaced by MPs in the pycnometer was then measured in order to calculate the volume of MPs, which is equal to excluded ethanol's volume, and to determine the precise particle density of MPs. Specifically, the precise mass of empty pycnometer and a pycnometer containing  $\sim 1.0 \text{ g}$  of MPs were weighed and recorded. And then using a syringe, 99.8% ethanol was gently added to the pycnometer containing MPs until liquid level is closing to the bottleneck. After that opening was immediately sealed with cling film to

reduce the possible change in solution density due to evaporation. A 5 min sonication was then applied to exclude air among MPs. All samples were left overnight to ensure that MPs sank to the bottom and would not interfere with the subsequent installation of the glass stopper (Figure 3.2). Ethanol should be continued to inject until the criterion that the MPs and ethanol could fully fill the pycnometer after the installation of stopper was met. Finally, the mass of pycnometer filled with ethanol and tested MPs was precisely measured to calculate the particle density of MPs according to equation (3). Each size fraction of MPs had 5 replicates, giving 15 measurements in total.



Figure 3-2. Status of MPs after sonication and then being left in the pycnometer containing 99.8% ethanol overnight. Note that ethanol filled out the gap among the MPs in the bottom and that no MPs particles were residue in the supernate and bottleneck.

$$\rho_{mp} = \frac{\rho_e(20^\circ\text{C})(M_{pmp} - M_p)}{(M_{pmp} - M_p) + (M_{pe} - M_{pmp})} \quad (3)$$

Where  $\rho_{mp}$  is the particle density of microplastic ( $\text{g}/\text{cm}^3$ );  $\rho_e(20^\circ\text{C})$  is the density of ethanol ( $\text{g}/\text{cm}^3$ ) at  $20^\circ\text{C}$ ;  $M_p$  is the mass of empty pycnometer (g);  $M_{pmp}$  is the total mass of pycnometer and MPs (g);  $M_{pe}$  is total mass of pycnometer fully filled with ethanol (g);  $M_{pmp}$  is the mass of pycnometer fully filled with MPs and ethanol (g).

### **3.1.2 Analytical method for particle size distribution (PSD) analyses**

A Mastersizer 2000 (Malvern Panalytical Ltd., Malvern, UK) was used to measure the particle size distribution of the MPs. A 35% ethanol solution was shown to be a reliable dispersant in practice as a replacement for water, because in addition to having a density (0.94 g/cm<sup>3</sup>) similar to tested polyethene powders (0.93~0.95 g/cm<sup>3</sup>) and a refractive index (1.34) approaching to deionized water (1.33), 35% ethanol also significantly lowered MPs' surface tension and dispersant density. Higher ethanol concentrations were not used, because of the risk involved in operating flammable liquid and the increase in dispersant refractive index. To meet the demands of PSD tests, the manual model was chosen. The pump speed on the laser granulometer was firstly set to 1500 rpm for measuring the background of dispersant. To better disperse MPs, pump speed was then increased to 3000 rpm and a sonicator was activated when MPs were added to the instrument. When obscuration reached 4.5%, we stopped adding MPs, reduced the pump speed to 1500 rpm, closed the sonicator and then wait for 30s for restoring the background before measuring. Water was used to analyze the same samples as contrasts and standard sand was also used to discover differences induced by dispersant change. Each sample had 3 continuous tests in each dispersant, giving 24 measurements in total. The sizes of single MP35 (n=30) were also measured under an optical microscope to validate the results of laser granulometer measurements.

### **3.2 Soil collection and basic properties analyses**

Each 100 kg soil sample without obvious plastic pollution was collected from the top 10 cm of local deciduous woodland (SE 62084 50860) and arable field (SE 62635 49583) in York, UK, after removal of any litter layer (Figure 3-3). All the samples were fully air-dried, crushed and sieved to < 2 mm to remove any obvious stone, roots, faunas. Subsequently, this partially disaggregated soil was sieved to < 250 µm for removal of any macroaggregate, and the fraction passing this sieve was used for the experiment. Prior to incubation of aggregates, the following properties affecting soil aggregates formation for tested soils were measured: soil texture by feel ([Guide to Texture by Feel | NRCS Soils - USDA](#)); soil pH at a soil /water ratio of 2:5 (w/v) by a pH meter (Jackson et al., 1986); soil organic matter using loss on ignition method at 350 °C; soil effective cation exchange capacity, concentration of exchangeable Na, sodium pyrophosphate, sodium dithionite and ammonium oxalate extractable Fe, Al, Mn using ICP-OES (Carter and Gregorich, 2007).



Figure 3-3. Images of air-dried woodland soil (left) and agricultural soil (right).

### **3.3 Incubation of microplastic-bearing aggregates**

Wetting-drying cycles were used to generate aggregates in the soil. A full factorial pot experiment was designed with three different levels of size (35, 125, 500  $\mu\text{m}$ ) and four different levels of concentration (0, 0.1, 1, 10%) of polyethene particles in two types of soil. The maximum mass fraction of MPs was set as 10% corresponding to the published field investigations up to 7% (Fuller and Gautam, 2016). Each treatment had 5 replicates, giving 120 pots in total. Although we had a control of 0% MP associated with each size fraction, for statistical analysis these controls were combined, giving 15 control replicates per soil. The MPs were thoroughly mixed with 300 g of the  $< 250 \mu\text{m}$  air-dried soil in PVC pots. Before wet-drying cycles, water holding capacities were measured from 30.0 g of soil containing 0, 0.1, 1, 10% each size of MPs. Using a watering can, deionized water was gently sprayed to the sample surface so that the soil was not disturbed. All the samples were initially maintained at a moisture content of 90% water holding capacity for 10 days in a controlled temperature room at 30 °C. Following wetting, all soil samples were naturally dried until soil moisture content decreased to 40% of the water holding capacity, at which point these soil samples were rewetted to 90% of the water holding capacity by gently spraying water on the surface. According to the evaporation rate of each sample, 4~9 times of wet-dry cycles were repeated during a 75-day incubation (Figure 3-4).



Figure 3-4. Soil aggregate incubation by (left) running wetting-drying cycles in a CT room at 30°C. And the mixture of soil and MPs after 75-day incubation (right).

### **3.4 Soil aggregate collection and properties analyses**

#### **3.4.1 Soil aggregate separation and collection**

All the soil samples were fully air dried at 30°C and then removed from pots. The size distribution of the newly-formed soil aggregates was determined by gently, manually sieving ~100 g of slightly crushed soil through a set of stack sieves (mesh opening of 4000  $\mu\text{m}$ , 2000  $\mu\text{m}$ , 1000  $\mu\text{m}$ , 250  $\mu\text{m}$ ); sieves were shaken for 50 times within 20 s. The mass of each sieved fraction was measured. After that, collected aggregates were carefully stored in glass sample bottles for further analyses (Figure 3-5).

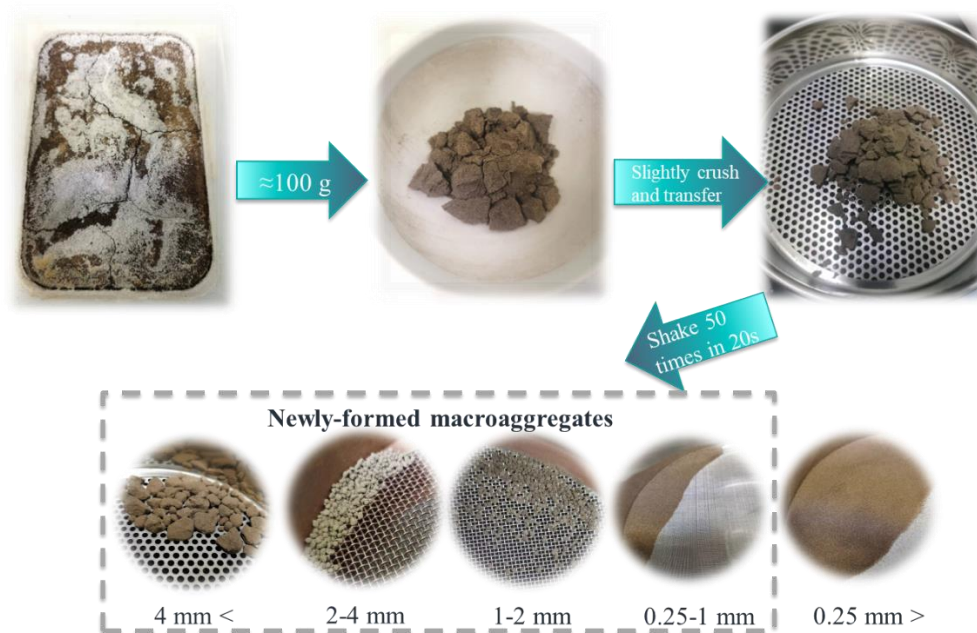


Figure 3-5. Schematic diagram of the procedures for soil aggregate separation and collection.

### 3.4.2 water stability of soil aggregates

The water stability of aggregates was measured by making 2 measurements of the continuous size distribution ( $<2000 \mu\text{m}$ ) of aggregates before and after sonication treatment in a laser granulometer (Rawlins et al., 2015). Before adding samples, aggregates were left on double-layer filter paper (No. 42 Whatman, GE Life Sciences, Little Chalfont, the UK) that had been previously pre-wetted with 1 mL of deionized water for 30 min to simulate the moist content in the field. Those pre-wetted aggregates were then gently knocked down into the aqueous vessel. After adding sample, 30 seconds were reserved for allowing aggregates to disperse and circulate within the laser granulometer.

The mean weight diameter (MWD) of the aggregate was continually analyzed three times and averaged (Equation 4). This number was equivalent to the MWD of water stable aggregates, because the mild disruptive force due to water flow was insufficient to break down the majority of water stable aggregate within several minutes. After the first measurement, a sonicator ran for 5 min. To make sure that roughly the same amount of energy was inputted in each analysis, the powers used to heat the water for each test were calculated by measuring the change in water temperature before and after sonication. The resulting strong disruptive force made the rest of water stable aggregates further disaggregate into finer particles whose MWD

were also measured three times and averaged. Lastly, DR value could be calculated by subtracting the averaged MWD2 from MWD1 to evaluate aggregate water stability (Equation 5). About 250 mg of 1000-2000  $\mu\text{m}$  soil aggregate per treatment was used to test the water stability, because when too much sample had been added, light obscuration after sonication exceeded 20% which can reduce the accuracy of MWD measurement.

$$MWD = \sum_{i=1}^n \bar{x}_i w_i \quad (4)$$

Where  $\bar{x}_i$  is the mean diameter of each size fraction ( $\mu\text{m}$ ), and  $w_i$  is the volume proportion of the sample corresponding to that size fraction.

$$DR = MWD1 - MWD2 \quad (5)$$

Where DR is disaggregation reduction, with a larger DR value indicating aggregates are more stable in water, MWD1 is the mean weight diameter of water stable aggregate in the first PSD measurement, and MWD2 is the mean weight diameter of sample after sonication in the second PSD measurement.

### 3.4.3 Wettability of soil aggregates

A water drop penetration time (WDPT) method, based on that by Blanco-Canqui et al. (2007) was developed for aggregate wettability in this study. An optical tensiometer equipped with a micro syringe and a high-speed camera was used to place 1  $\mu\text{L}$  of deionized water on top of a discrete aggregate with a mass of  $\sim 0.5$  g and a diameter  $> 4000$   $\mu\text{m}$  and record the time necessary for the drop to completely penetrate the aggregate at the frequency of 60 frames per second (Figure 3-6). The longer time for a drop to penetrate the sample means less wettability (hydrophilicity) of aggregate. Each treatment had 5 replicates with one measurement for each sample, giving 120 measurements in total.



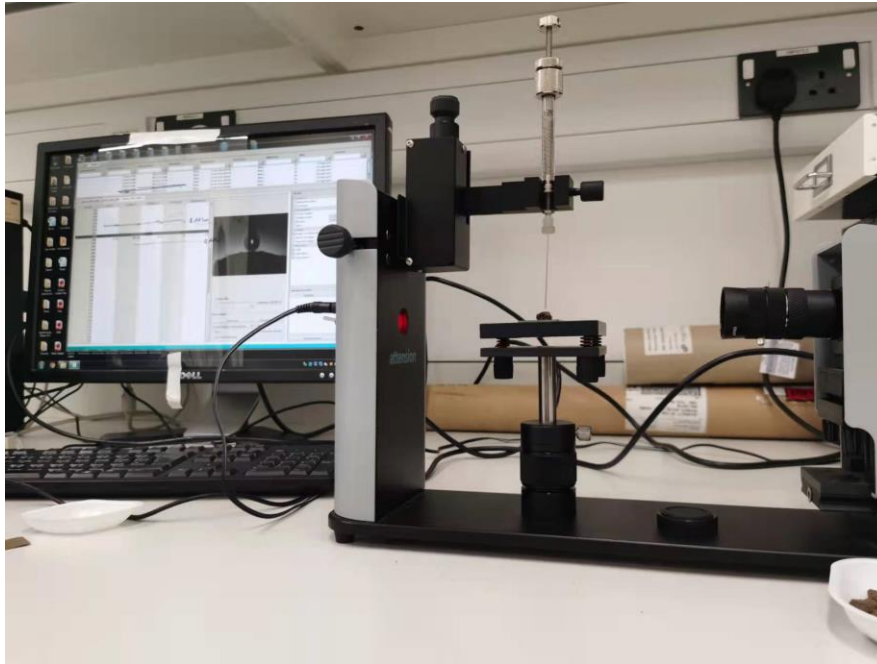


Figure 3-6. Measurement of water drop penetration time based on an optical tensiometer. On the top of the aggregate, a micro syringe was for placing a water drop. On the right side of the aggregate, a high-speed camera is observing the penetration of the drop. The penetration time can be computed by reading the times of images which recorded water drop just contact to aggregate and was fully absorbed.

#### **3.4.4 Particle density of soil aggregate and the estimation of %MP based on particle density**

The method for analysing the particle density of MPs in Section 2.1.1 was also used to measure aggregates containing MPs. Each treatment had 5 replicates, giving 120 samples in total. Presuming that the particle densities of microplastics and soils did not change after incubation and that the total volume of the particles making up the aggregates equalled the volume of MPs particles plus the volume of soil particles, the particle density of aggregates with a series of different soil/MP proportions could be calculated on basis of measured data from tested soils and MPs according to Equation (6) below. Calculated and measured values of  $\rho_a$  were compared and the solver function in Excel was used to determine values of  $\theta$  that minimized the relative error (Equation 7).



$$\rho_a \text{ (g/cm}^3\text{)} = \frac{\rho_s \rho_{mp}}{\theta \rho_s + (1-\theta) \rho_{mp}} \quad (6)$$

Where  $\rho_a$  is the particle density of aggregate (g/cm<sup>3</sup>),  $\rho_s$  is the particle density of soil (g/cm<sup>3</sup>),  $\rho_{mp}$  is the particle density of MPs (g/cm<sup>3</sup>).  $\theta$  is the concentration of MPs in aggregate.

$$\delta = \frac{\rho_{ac} - \rho_{am}}{\rho_{am}} \times 100\% = 0 \quad (7)$$

Where  $\delta$  is the relative error of calculated and measured values of  $\rho_a$  (g/cm<sup>3</sup>),  $\rho_{ac}$  is the calculated particle density of aggregate (g/cm<sup>3</sup>),  $\rho_{am}$  is the measured particle density of aggregate (g/cm<sup>3</sup>).

# 4 Results

## 4.1 Properties of tested MPs

### 4.1.1 Particle density of MPs

Particle density of MPs, ranging between  $0.934 \pm 0.004$  and  $0.952 \pm 0.003$  g/cm<sup>3</sup> (n=5), displayed significant difference among different sizes (F=38.817, P<0.001) (Table 4-1). The particle density of MP125 was larger compared to MP35 and MP500 (P<0.05).

Table 4-1. Mean particle density of tested samples ( $\pm$  SEM, n = 5).

Name	Type	Particle Density (g/cm <sup>3</sup> )
MP35	PE	$0.934 \pm 0.004^a$
MP125	PE	$0.952 \pm 0.003^b$
MP500	PE	$0.936 \pm 0.003^a$

Superscript letters indicate significant differences  $\alpha < 0.05$  between the treatments

#### 4.1.2 Size distribution of MPs

MPs dispersed in water floated on the surface, formed aggregates and became coated in air bubbles in the laser granulometer (Figure 4-1. A). The ethanol additions prevented those behaviours (Figure 4-1. B). The change of the dispersant did not affect the PSD results of standard sand, but significantly impacted the PSD of MP35 (Table 4-2). Since aggregated MPs dominated the volume in measurement, results of MP35 tested with water largely exceeded the expected size (35  $\mu\text{m}$ ). In comparison, the PSD curves of MPs tested with ethanol tended to ranges with a smaller size. The most remarkable was the redistribution of MP35, suggesting a net loss of aggregated MPs and a net gain of finer MPs (Figure 4-2. B). The size fractions of MPs tested with ethanol generally had smaller sizes and standard deviations (Figure 4-2, Table 4-2). And the observation of MP35 under an optical microscope, whose results were  $14.0 \pm 3.4$   $\mu\text{m}$  (minimum=8.6  $\mu\text{m}$ , median=13.6  $\mu\text{m}$ , maximum=23.0  $\mu\text{m}$ , n=30), was closer to PSD of MP35 tested with ethanol (Table 4-2). Altogether, we verified that ethanol increased the accuracy and repeatability of laser granulometer-based technique for measuring PSD of MPs (Table 4-3).

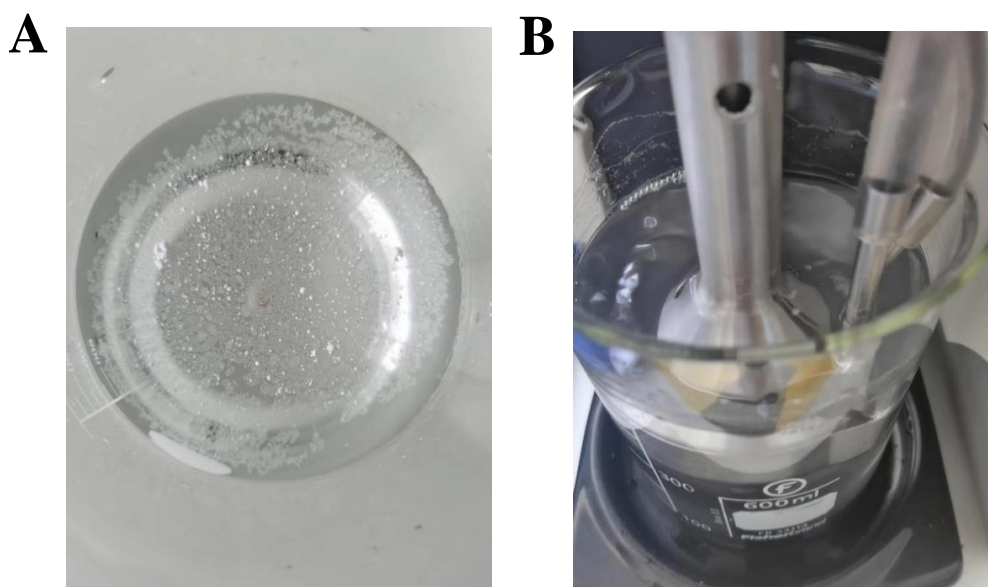


Figure 4-1. Status of MP35 dispersed in water after measurement(A) and in 35% ethanol during measurement (B).

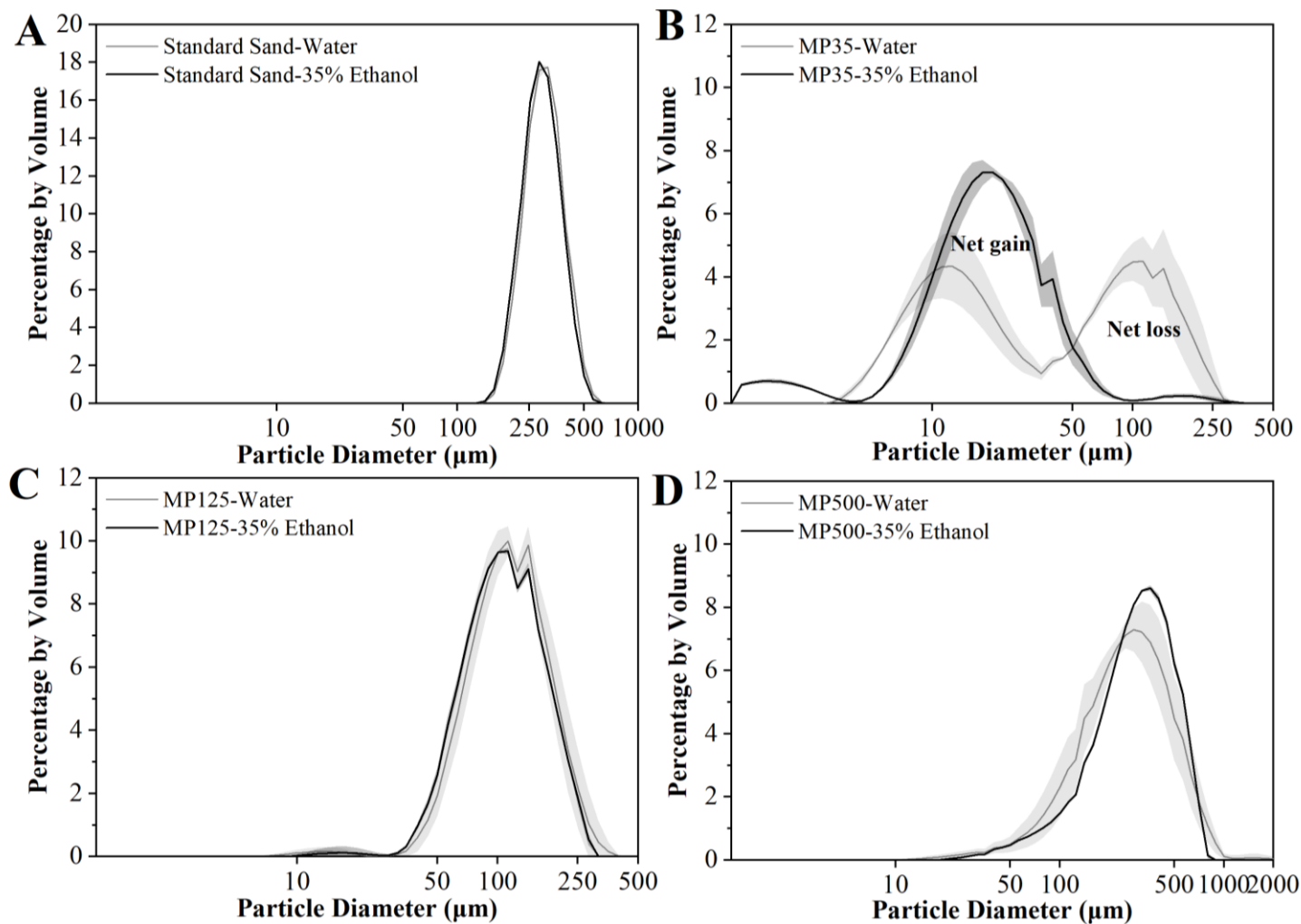


Figure 4-2. Size distribution (log<sub>10</sub> scale) curves (mean ± SEM, n=3) of samples tested with water and 35% ethanol as a dispersant: (A) Standard sand, (B) MP35, (C) MP125, (D) MP500. Light (water) and dark (ethanol) grey belts above and beneath curves are the range of error bar. Areas where the change of dispersant led to a net loss of coarse material which accumulated at finer sizes (shown as net gain) were highlighted in (B).

Table 4-2. Measurement data for particle size distribution of standard sand and tested MPs.

Name	Type	Dispersant	Particle Size Distribution		
			D10 (µm)	D50 (µm)	D90 (µm)
MP35	PE	water	7.3±0.1 <sup>a</sup>	39.7±19.6 <sup>a</sup>	<b>141.1±26.3<sup>b</sup></b>
MP35	PE	35% ethanol	6.8±0.4 <sup>a</sup>	17.5±1.6 <sup>a</sup>	<b>38.0±4.1<sup>a</sup></b>
MP125	PE	water	59.6±6.2 <sup>a</sup>	103.0±4.8 <sup>a</sup>	185.5±22.4 <sup>a</sup>
MP125	PE	35% ethanol	55.5±1.0 <sup>a</sup>	100.4±0.5 <sup>a</sup>	177.4±1.8 <sup>a</sup>
MP500	PE	water	100.9±22.7 <sup>a</sup>	244.7±39.8 <sup>a</sup>	500.2±47.1 <sup>a</sup>
MP500	PE	35% ethanol	115.0±1.6 <sup>a</sup>	281.5±3.1 <sup>a</sup>	505.8±2.4 <sup>a</sup>
Standard Sand	Sand	water	199.4±1.3 <sup>a</sup>	274.2±1.0 <sup>a</sup>	374.3±2.3 <sup>a</sup>
Standard Sand	Sand	35% ethanol	199.8±1.0 <sup>a</sup>	273.9±1.3 <sup>a</sup>	375.0±1.6 <sup>a</sup>

Data are the results of laser granulometer (Mean ± SEM, n=3).

D10 is the maximum diameter of 10% of the particles, D50 is the maximum diameter of 50% of the particles, D90 is the maximum diameter of 90% of the particles.

Superscript letters indicate significant differences  $\alpha < 0.05$  between the treatments.

Table 4-3. Measurement data for particle size distribution of standard sand and tested MPs.

Name	Type	Dispersant	Coefficient of Variation in Particle Size Distribution (%)		
			D10	D50	D90
MP35	PE	water	0.93	49.29	18.64
MP35	PE	35% ethanol	5.74	9.00	10.77
MP125	PE	water	10.41	4.68	12.07
MP125	PE	35% ethanol	1.87	0.47	1.04
MP500	PE	water	22.53	16.27	9.42
MP500	PE	35% ethanol	1.35	1.12	0.47
Standard Sand	Sand	water	0.65	0.35	0.62
Standard Sand	Sand	35% ethanol	0.51	0.47	0.43

Data are the results of laser granulometer (n=3).

D10 is the maximum diameter of 10% of the particles, D50 is the maximum diameter of 50% of the particles, D90 is the maximum diameter of 90% of the particles.

## **4.2 Properties of soil samples**

Physicochemical properties that affect aggregates were significantly different between soils (Table 4-4). Features such as higher acidity, organic matter, effective CEC, total extractable Mn, Al, Fe and lower SAR were thought to lead to increases in soil aggregation and aggregate stability (Amézketa, 1999; Regelink et al., 2015; Totsche et al., 2017). Therefore, higher acidity, organic matter, effective CEC, total extractable Mn may help aggregation in woodland soil, while richer extractable Al, Fe and lower SAR were likely to improve aggregation in agricultural soil. Except for the treatments of MP125 addition in woodland soil, increasing concentrations of MPs significantly enhanced soil water holding capacity, and with only 10% MPs treatments obviously boosting this parameter ( $P < 0.05$ ) (Table 4-5).

Table 4-4. The physicochemical properties affecting soil aggregates formation and properties for tested soils ( $\pm$ SEM, n = 5).

Properties	Soil types	
	Woodland soil (n=5)	Agricultural soil (n=5)
Soil texture	Silty clay loam	Clay loam
Acidity (pH)	6.40 $\pm$ 0.02 <sup>a</sup>	6.65 $\pm$ 0.02 <sup>b</sup>
Sodium adsorption ratio (SAR)	0.054 $\pm$ 0.00 <sup>a</sup>	0.043 $\pm$ 0.00 <sup>b</sup>
<b>Organic matter wt. %</b>	<b>6.92<math>\pm</math>0.06<sup>a</sup></b>	<b>3.99<math>\pm</math>0.02<sup>b</sup></b>
Effective CEC (cmol (+)/kg)	20.23 $\pm$ 0.28 <sup>a</sup>	12.20 $\pm$ 0.21 <sup>b</sup>
Pyrophosphate extractable Al (mg/kg)	880 $\pm$ 14 <sup>a</sup>	1083 $\pm$ 20 <sup>b</sup>
Pyrophosphate extractable Fe (mg/kg)	1591 $\pm$ 10 <sup>a</sup>	2448 $\pm$ 62 <sup>b</sup>
Pyrophosphate extractable Mn (mg/kg)	165 $\pm$ 3 <sup>a</sup>	68 $\pm$ 4 <sup>b</sup>
Dithionite extractable Al (mg/kg)	1073 $\pm$ 21 <sup>a</sup>	1348 $\pm$ 18 <sup>b</sup>
Dithionite extractable Fe (mg/kg)	3952 $\pm$ 69 <sup>a</sup>	4477 $\pm$ 60 <sup>b</sup>
Dithionite extractable Mn (mg/kg)	295 $\pm$ 6 <sup>a</sup>	177 $\pm$ 4 <sup>b</sup>
Oxalate extractable Al (mg/kg)	138 $\pm$ 2 <sup>a</sup>	159 $\pm$ 1 <sup>b</sup>
Oxalate extractable Fe (mg/kg)	2186 $\pm$ 44 <sup>a</sup>	1612 $\pm$ 14 <sup>b</sup>
Oxalate extractable Mn (mg/kg)	64 $\pm$ 1 <sup>a</sup>	37 $\pm$ 1 <sup>b</sup>

Superscript letters indicating significant differences at  $\alpha < 0.05$  between the soils.

Table 4-5. Soil water holding capacity under different MPs addition treatments.

Soil	MP Size ( $\mu\text{m}$ )	MP Content (%)	Water Holding Capacity (%)
Woodland soil	35	0	61.33 $\pm$ 0.01 <sup>a</sup>
		0.1	60.48 $\pm$ 0.01 <sup>a</sup>
		1	60.62 $\pm$ 0.01 <sup>a</sup>
		10	<b>67.81<math>\pm</math>0.03<sup>b</sup></b>
	125	0	61.33 $\pm$ 0.01 <sup>a</sup>
		0.1	59.61 $\pm$ 0.01 <sup>a</sup>
		1	60.18 $\pm$ 0.01 <sup>a</sup>
		10	61.11 $\pm$ 0.01 <sup>a</sup>
	500	0	61.33 $\pm$ 0.01 <sup>a</sup>
		0.1	62.5 $\pm$ 0.01 <sup>a</sup>
		1	61.43 $\pm$ 0.01 <sup>a</sup>
		10	<b>65.89<math>\pm</math>0.04<sup>b</sup></b>
Agricultural soil	35	0	45.7 $\pm$ 0.01 <sup>ab</sup>
		0.1	46.6 $\pm$ 0.01 <sup>b</sup>
		1	44.6 $\pm$ 0.01 <sup>a</sup>
		10	<b>51.97<math>\pm</math>0.03<sup>c</sup></b>
	125	0	45.7 $\pm$ 0.01 <sup>b</sup>
		0.1	45.65 $\pm$ 0.01 <sup>b</sup>
		1	44.52 $\pm$ 0.01 <sup>a</sup>
		10	<b>50.05<math>\pm</math>0.01<sup>c</sup></b>
	500	0	45.7 $\pm$ 0.01 <sup>a</sup>
		0.1	46.56 $\pm$ 0.01 <sup>a</sup>
		1	46.16 $\pm$ 0.01 <sup>a</sup>
		10	<b>52.76<math>\pm</math>0.04<sup>b</sup></b>

Data are means ( $\pm$ SEM, n=15 for control groups, n = 5 for treatments).

Superscript letters indicating significant differences  $\alpha < 0.05$  between the treatments.



## **4.3 Properties of newly-formed soil aggregates**

### **4.3.1 Influences of various MPs on soil structure and aggregate formation**

After 75 days of wet-drying cycles, all MPs-addition treatments produced newly-formed soil macroaggregate ( $> 250 \mu\text{m}$ ) containing MPs, of which  $> 4 \text{ mm}$  and  $0.25\text{--}1 \text{ mm}$  fractions made up the majority. MPs affected the soil structure, however, the impacts varied depending on soil type, MP size, and content (Figure 4-3. A-F). The result of three ways Anova analysis showed woodland soil formed higher concentration of macroaggregates ( $> 0.25\mu\text{m}$ ) than agriculture soil ( $P<0.01$ ). There was no significant change in each fraction of macroaggregates when MP concentrations increased from 0% to 1% ( $P>0.05$ ) (Figure 4-3. A-F). As the concentration of MP further increased to 10%, MP35 appeared to result in the formation of fewer 2 - 4 mm and  $> 4 \text{ mm}$  macroaggregates and more 0.25 - 1 mm and 1 - 2 mm macroaggregates (Figure 4-3. A, D). For instance, the addition of MP35 to woodland soil (WS) induced a reduction by a factor of c. 2 in  $> 4 \text{ mm}$  fraction and a 1-fold increase in 0.25-1 mm fraction compared to the control ( $P<0.05$ ). And the addition of MP35 to agricultural soil (AS) caused a 0.7-fold reduction in the percentage of  $>4 \text{ mm}$  and a 1.25-fold increase in 0.25-1 mm fraction relative to the control ( $P<0.05$ ). In contrast, MP125 had no significant effect on aggregate formation ( $P>0.05$ ) (Figure 4-3. B, E). The influences of MP500 on soil structure were primarily limited to  $<1 \text{ mm}$  fractions ( $P<0.05$ ) (Figure 4-3. C, F). When the concentration of MP500 rose, the proportion of aggregate between 0.25-1 mm increased in AS ( $P<0.05$ ), and the overall percentage of macroaggregates ( $>250 \mu\text{m}$ ) in both soils were impacted ( $P<0.05$ ), but no discernible trend emerged.

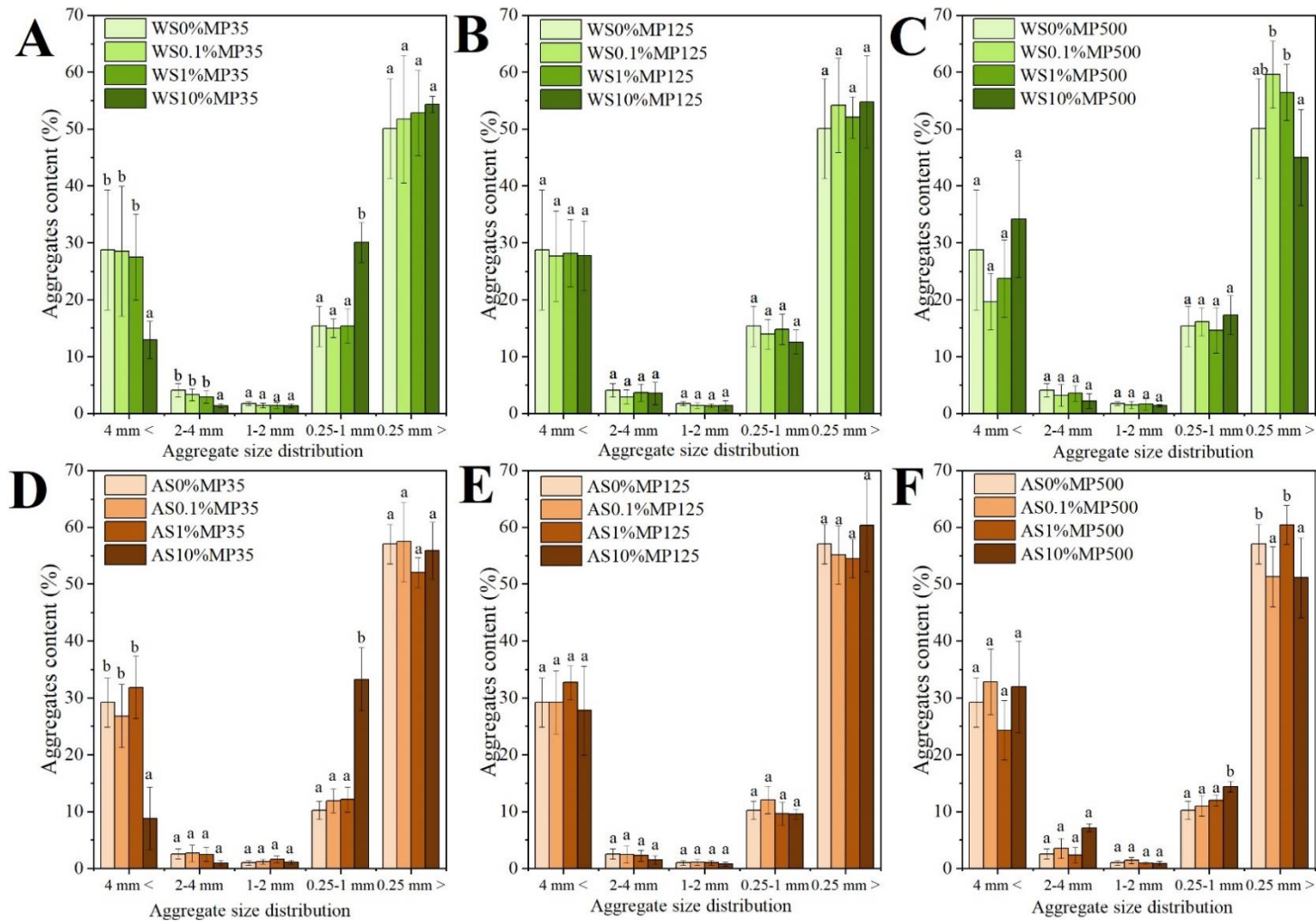


Figure 4-3. Structure of woodland soil under MP35 (A), MP125 (B), MP500 (C) addition treatments and agricultural soil under MP35 (D), MP125 (E), MP500 (F) addition treatments after 75 days of wet-drying cycles. Data are means ( $\pm$ SEM,  $n=5$ ). Statistical results are included (one-way ANOVA). Different letters indicate significant differences at  $\alpha < 0.05$ .

#### 4.3.2 Influences of various MPs on soil aggregate wettability

The water drop penetration time (WDPT) of newly-formed aggregates (> 4 mm), ranging between  $0.03\pm 0.01$ s and  $9.02\pm 3.85$ s ( $n=5$ ), showed significant difference with MP size ( $F=46.229$ ,  $P<0.001$ ) and concentration ( $F=45.706$ ,  $P<0.001$ ). The interaction between MP size and content ( $F=45.261$ ,  $P<0.001$ ) also significantly influenced aggregate wettability (Table 4-6). Compared to other sizes of MPs, increasing content of MP35 dramatically slowed water from penetrating aggregates (> 4 mm), with WDPT climbing from  $0.05\pm 0.02$ s to  $9.02\pm 3.85$ s ( $n=5$ ) for WS and from  $0.03\pm 0.01$ s to  $8.16\pm 4.14$ s ( $n=5$ ) for AS ( $P<0.001$ ) (Figure 4-4. A, D). Indeed, 1% MP35 have had profound influence as there is a significant ~2 folds increase in WDPT between the control and 1% MP35 treatment ( $P<0.05$ , if only comparing 0% ~ 1% MP35 addition treatments). Such rises in WDPT indicated that the addition of MP35 could significantly reduce aggregate wettability ( $P<0.001$ ). Despite the considerable difference in the MP35 treatments, we only found a modest, though still significant, increase from  $0.03\pm 0.01$ s to  $0.05\pm 0.01$ s ( $P<0.05$ ) in the WDPT of AS aggregate containing MP125 (Figure 4-4. E), when the MPs level increased from 0 to 10% in the other treatments.

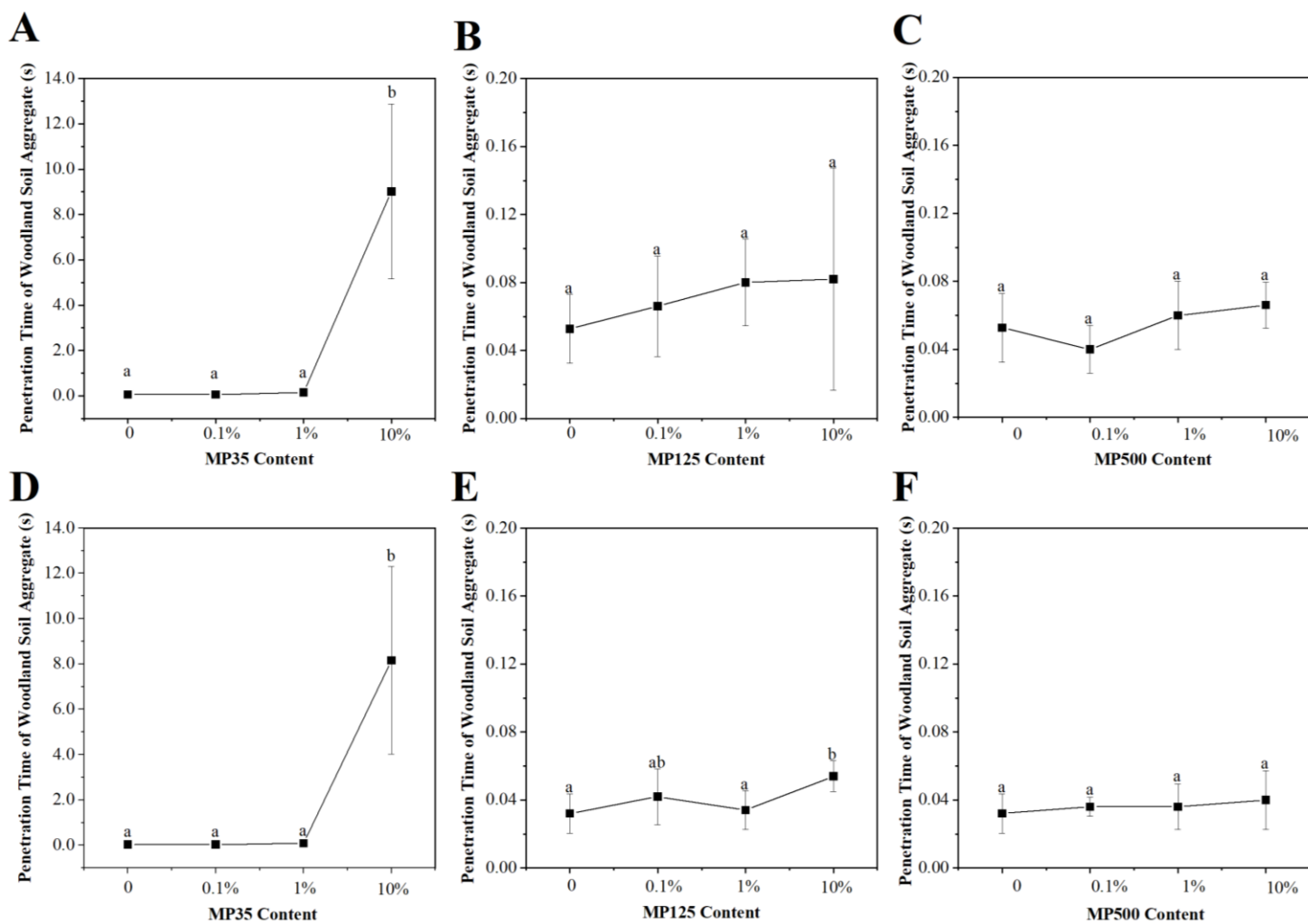


Figure 4-4. Water penetration time of woodland soil aggregates (> 4 mm) under MP35 (A), MP125 (B), MP500 (C) addition treatments and agricultural soil aggregates under MP35 (D), MP125 (E), MP500 (F) addition treatments. Data are means ( $\pm$ SEM, n=5). Partial error bars in (A) and (D) are too narrow to find. Statistical results are included (one-way ANOVA). Different letters indicate significant differences at  $\alpha < 0.05$ .

Table 4-6. Summary output from three-way ANOVA analysis of the variance of water penetration time.

<b>Factors</b>	<b>Degrees of freedom</b>	<b>Sum of Sq.</b>	<b>Mean Sq.</b>	<b>F value</b>	<b>Sig.</b>
Soil	1	0.276	0.276	0.207	0.65
<b>MP size</b>	2	123.14	61.57	46.229	<b>&lt;0.001</b>
<b>MP content</b>	3	182.618	60.873	45.706	<b>&lt;0.001</b>
Soil×MP size	2	0.317	0.158	0.119	0.888
Soil×MP content	3	0.44	0.147	0.11	0.954
<b>MP size×MP content</b>	6	361.68	60.28	45.261	<b>&lt;0.001</b>
Soil×MP size×MP content	6	0.843	0.14	0.105	0.996
Residual	96	127.857	1.332		

### 4.3.3 Influences of various MPs on soil aggregate water stability

For all the treatments, the application of sonication removed most of the aggregate structure because the MWD of the disaggregated material was far smaller than that of the WSA. Water stability of newly-formed aggregate (1-2 mm), ranging between  $52.13 \pm 15.41 \mu\text{m}$  and  $198.65 \pm 3.85 \mu\text{m}$  ( $n=5$ ), had different responses under varying soil ( $F= 117.118$ ,  $P<0.001$ ), MP size ( $F= 15.291$ ,  $P<0.001$ ), MP content ( $F= 12.827$ ,  $P<0.001$ ). And the interactions among above factors also significantly influenced water stability of aggregates (Table 4-7). WS aggregates were more stable than AS soil aggregates, since WS aggregates had  $\sim 1.60$  to  $\sim 2.02$  times mean DR relative to AS aggregates when the added MPs were the same size ( $P<0.001$ ) (Figure 4-5. A-F). In terms of MPs addition, WS aggregate was sensitive to MP35 and MP125, with significant decreases observed for 1% MP35 and 0.1-10% MP125 compared to control ( $P<0.05$ ), while 10% MP35 increased DR value of AS aggregate ( $P<0.05$ ) (Figure 4-5. A-B, D). Considering all MPs sizes in both soils, when addition of MPs increased from 0% to 1%, the average DR value continually reduced from  $117.54 \pm 55.30 \mu\text{m}$  to  $89.54 \pm 32.18 \mu\text{m}$  ( $P<0.05$ ), then significantly increased to  $130.01 \pm 62.00 \mu\text{m}$  ( $P<0.05$ ) from 1% to 10% though still insignificant compared to control ( $P>0.05$ ). Such variance suggested that aggregates appeared to become less and then the same water stable with increasing %MP in soils. As the size of MPs increased from  $\sim 35 \mu\text{m}$  to  $\sim 500 \mu\text{m}$ , the mean DR value of aggregates including controls reduced from  $124.07 \pm 60.68 \mu\text{m}$  (MP35) to  $90.22 \pm 37.48 \mu\text{m}$  (MP125) ( $P<0.05$ ) then rose to  $112.70 \pm 46.15 \mu\text{m}$  (MP500) ( $P<0.05$ ), indicating MP125 had a greater influence on reducing aggregate water stability.

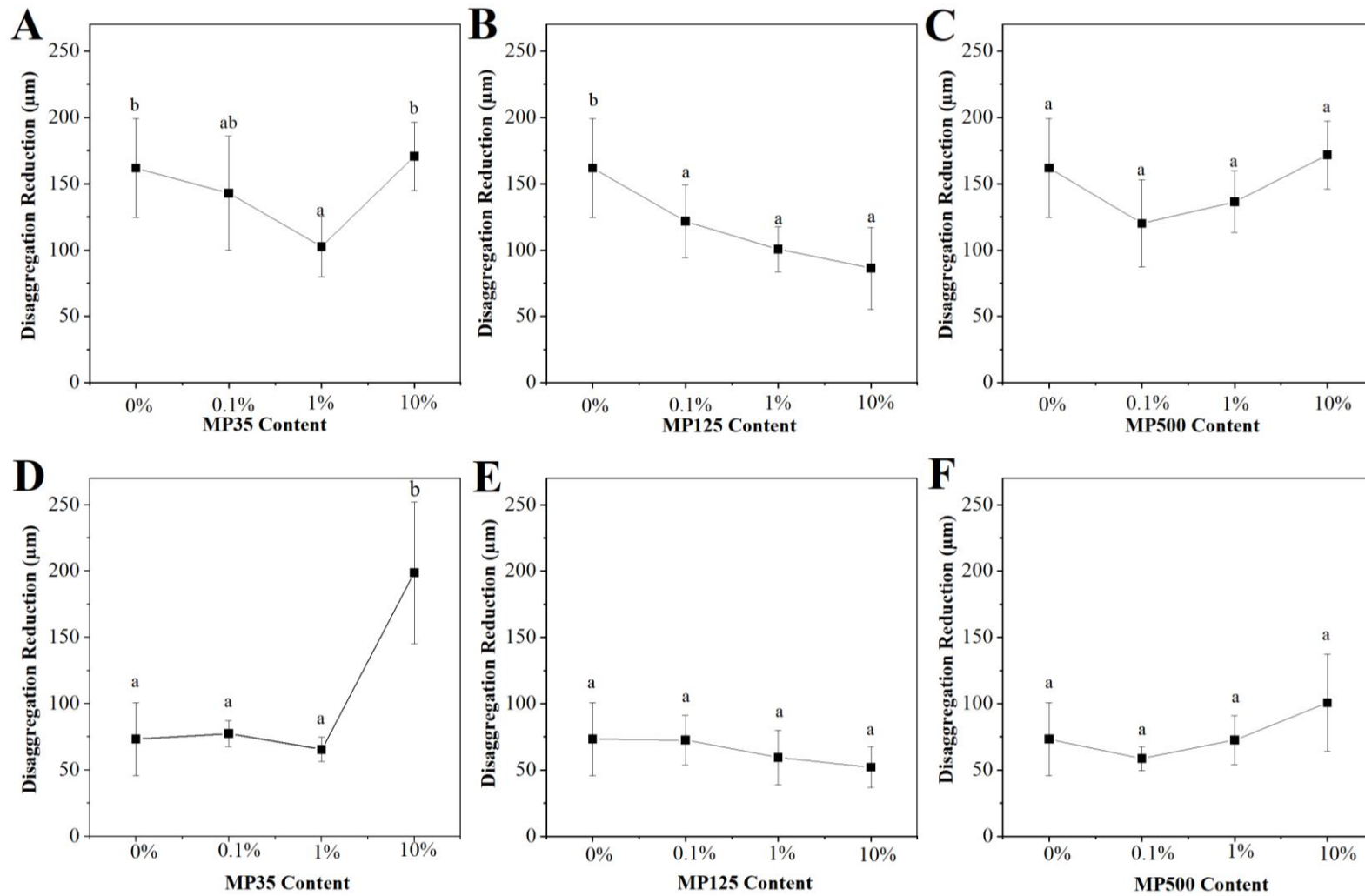


Figure 4-5. The water stability of woodland soil aggregates (> 4 mm) incubated under MP35 (A), MP125 (B), MP500 (C) addition treatments and agricultural soil aggregates (> 4 mm) under MP35 (D), MP125 (E), MP500 (F) addition treatment. Data are means ( $\pm$ SEM,  $n=5$ ). Statistical results are included (one-way ANOVA). Different letters indicate significant differences at  $\alpha < 0.05$ .

Table 4-7. Summary output from three-way ANOVA analysis of the variance of disaggregation reduction value.

Factors	Degrees of freedom	Sum of Sq.	Mean Sq.	F value	Sig.
<b>Soil</b>	1	90873.902	90873.902	117.118	<b>&lt;0.001</b>
<b>MP size</b>	2	23730.081	11865.040	15.292	<b>&lt;0.001</b>
<b>MP content</b>	3	29858.524	9952.841	12.827	<b>&lt;0.001</b>
Soil×MP size	2	2191.699	1095.849	1.412	0.249
<b>Soil×MP content</b>	3	15338.276	5112.759	6.589	<b>&lt;0.001</b>
<b>MP size×MP content</b>	6	49215.889	8202.648	10.572	<b>&lt;0.001</b>
<b>Soil×MP size×MP content</b>	6	19472.852	3245.475	4.183	<b>&lt;0.001</b>
Residual	96	74488.336	775.920		



#### **4.3.4 Influences of various MPs on particle density of soil aggregates and the estimation of %MP.**

The particle density of newly-formed aggregates (> 4 mm), ranging between  $2.162 \pm 0.034$  and  $2.604 \pm 0.032$  g/cm<sup>3</sup> (n=5), was significantly different between MP-addition treatments. Soil (F=151.460, P<0.001), MP content (F=640.812, P<0.001) and the interaction between soil and MP content (F=3.111, P<0.05) explained a large portion of the variation in aggregate particle density (Figure 4-8 and Figure 4-9). Compared with AS aggregates including controls, WS aggregates with the same MP-addition treatment had lower particle density (P<0.05). When the concentration of MPs increased from 0 to 0.1%, most particle densities slightly decreased but no significant difference could be detected relative to controls. As the concentration of MPs further increased, the particle density of agricultural soil aggregates under 1% MP35 and MP500 treatments, and all soil aggregates under 10% MP treatments significantly dropped compared to their controls (P<0.05). Despite the obvious difference induced by MP content, the impact of MP size was insignificant (P>0.05). Predicting %MP in aggregates and addition of MPs were well liner correlated, with adjusted R<sup>2</sup> ranging between 0.998 and 1.000 (P<0.001). The %MP determined by our method ranged -0.19 ~ 0.19% with 0.1% MPs addition, 0.92 ~ 1.21% with 1% MPs addition, and 8.79 ~ 10.14 % under 10% MPs addition. If measured by the relative error between two parameters, the predicting results at 1% and 10% levels, in comparison to the level at 0.1%, were closer to the addition of %MP in soil.

Table 4-8. Summary output from the analysis of particle density of macroaggregates (> 4 mm) and the estimation of %MP in aggregates.

<b>Treatments</b>	<b>Calculated particle density (g/cm<sup>3</sup>)</b>	<b>Measured particle density (g/cm<sup>3</sup>)</b>	<b>Relative error in particle density (%)</b>	<b>Addition of MPs (%)</b>	<b>Percentage of MPs (%)</b>
WS0%MP35	2.509	2.509±0.045 <sup>b</sup>	0.00	<b>0.00</b>	<b>0.00</b>
WS0.1%MP35	2.505	2.506±0.021 <sup>b</sup>	0.04	<b>0.10</b>	<b>0.08</b>
WS1%MP35	2.468	2.462±0.032 <sup>b</sup>	-0.24	<b>1.00</b>	<b>1.14</b>
WS10%MP35	2.148	2.180±0.036 <sup>a</sup>	1.50	<b>10.00</b>	<b>8.96</b>
WS0%MP125	2.509	2.509±0.045 <sup>b</sup>	0.00	<b>0.00</b>	<b>0.00</b>
WS0.1%MP125	2.505	2.502±0.052 <sup>b</sup>	-0.15	<b>0.10</b>	<b>0.19</b>
WS1%MP125	2.469	2.461±0.039 <sup>b</sup>	-0.33	<b>1.00</b>	<b>1.21</b>
WS10%MP125	2.156	2.194±0.044 <sup>a</sup>	1.71	<b>10.00</b>	<b>8.79</b>
WS0%MP500	2.509	2.509±0.045 <sup>bc</sup>	0.00	<b>0.00</b>	<b>0.00</b>
WS0.1%MP500	2.505	2.518±0.036 <sup>c</sup>	0.49	<b>0.10</b>	<b>-0.19</b>
WS1%MP500	2.468	2.466±0.017 <sup>b</sup>	-0.09	<b>1.00</b>	<b>1.06</b>
WS10%MP500	2.148	2.162±0.034 <sup>a</sup>	0.63	<b>10.00</b>	<b>9.56</b>
AS0%MP35	2.604	2.604±0.032 <sup>c</sup>	0.00	<b>0.00</b>	<b>0.00</b>
AS0.1%MP35	2.600	2.603±0.030 <sup>c</sup>	0.13	<b>0.10</b>	<b>0.02</b>
AS1%MP35	2.559	2.557±0.038 <sup>b</sup>	-0.07	<b>1.00</b>	<b>1.04</b>
AS10%MP35	2.209	2.235±0.026 <sup>a</sup>	1.15	<b>10.00</b>	<b>9.24</b>
AS0%MP125	2.604	2.604±0.032 <sup>b</sup>	0.00	<b>0.00</b>	<b>0.00</b>
AS0.1%MP125	2.600	2.600±0.047 <sup>b</sup>	0.00	<b>0.10</b>	<b>0.10</b>
AS1%MP125	2.560	2.562±0.019 <sup>b</sup>	0.08	<b>1.00</b>	<b>0.95</b>
AS10%MP125	2.219	2.215±0.026 <sup>a</sup>	-0.20	<b>10.00</b>	<b>10.14</b>
AS0%MP500	2.604	2.604±0.032 <sup>c</sup>	0.00	<b>0.00</b>	<b>0.00</b>
AS0.1%MP500	2.600	2.598±0.034 <sup>bc</sup>	-0.06	<b>0.10</b>	<b>0.13</b>
AS1%MP500	2.559	2.562±0.019 <sup>b</sup>	0.14	<b>1.00</b>	<b>0.92</b>
AS10%MP500	2.210	2.228±0.043 <sup>a</sup>	0.82	<b>10.00</b>	<b>9.46</b>

Data are means (±SEM, n=15 for control groups, n = 5 for treatments).

Superscript letters indicating significant differences  $\alpha < 0.05$  between the treatments.

Table 4-9. Summary output from three-way ANOVA analysis of the variance of particle density value.

<b>Factors</b>	<b>Degrees of freedom</b>	<b>Sum of Sq.</b>	<b>Mean Sq.</b>	<b>F value</b>	<b>Sig.</b>
<b>Soil</b>	1	0.206	0.206	151.460	< <b>0.001</b>
MP size	2	0.001	0.000	0.186	0.831
<b>MP content</b>	3	2.619	0.873	640.812	< <b>0.001</b>
Soil×MP size	2	0.001	0.000	0.214	0.808
<b>Soil×MP content</b>	3	0.013	0.004	3.111	<b>0.030</b>
MP size×MP content	6	0.004	0.001	0.498	0.809
Soil×MP size×MP content	6	0.003	0.001	0.401	0.877
Residual	96	0.131	0.001		

# 5 Discussions

## 5.1 Properties of MPs

### 5.1.1 The particle density of MPs measured by ethanol pycnometer method

In the present study, manufacturer did not provide the density data. So, modified liquid pycnometer method based on 99.8% ethanol solution was used to measure the particle density of different sizes of polyethene MPs. The particle density of our tested MPs ( $0.934 \pm 0.004$  -  $0.952 \pm 0.003$  g/cm<sup>3</sup>) were compared with of other granular polyethene particles, such as  $0.950 \pm 0.0002$  (n=7) and  $0.951 \pm 0.0001$  g/cm<sup>3</sup> (n=10) tested by Senfter et al. (2022) using automatic gas pycnometer with helium gas flow,  $0.936$  g/cm<sup>3</sup> by Waldschlager and Schuttrumpf (2020), and  $0.8$ - $0.9$  g/cm<sup>3</sup> (n=1) by Li et al. (2018a) using density gradient solutions method. The variation in different size fractions maybe due to the extra voids produced during the compounding and processing of plastic. In addition, variation among studies may be also explained by the slight difference in the composition of raw materials and the systematic error between different methodologies. In general, our results were in the usual range of polyethene density, but still needs to be compared with other precise methods such as the automatic gas pycnometer method.

The prerequisites for using liquid pycnometer methods include being able to submerged and fully wetted by dispersant. Ethanol was applied in our study, because it has a lower density ( $0.789$  g/cm<sup>3</sup>) than most plastics ( $0.8$  –  $1.6$  g/cm<sup>3</sup>) and is effective in reducing surface tension that makes hydrophobic MPs aggregate, and eliminating generation of MPs-coated air bubbles on dispersant surface (Li et al., 2018a; Pabortsava and Lampitt, 2020). This method is probably applicable to other plastic types, since the preliminary results showed that polyethene terephthalate and polypropylene cut from disposable water bottles, as well as Poly (butylene adipate-co-butylene terephthalate) from disposable spoon could be sunk and fully wetted in 99.8% ethanol. This method was demonstrated to be applicable to pristine MPs from factory and soil samples containing MPs, and it is expected to work with other environmental samples containing MPs.

Some advantages of this method include being precise enough (accurate to three decimal places); environmental friendliness and low cost (each test only requires the use of 10 mL of ethanol and some deionized water as reagents); high efficiency (with sufficient pycnometers, an operator can perform at least 20 tests per day); good repeatability (coefficient

of variation only up to 0.44% (n=5)); simplicity (without additional gas supply system). On the other hand, some limitations to this method are noteworthy, for instance, the challenge of consistently keeping the lab temperature at 20 °C to maintain the dispersant density. However, a 20 °C water bath could be used to achieve the same purpose. The density of ethanol may change during storage due to denaturation and volatilization, which could introduce inaccuracy into the outcome. To minimize this error, samples should be sealed by cling film and stored in a dark place following ethanol procession.

In comparison to the ethanol pycnometer method, the density gradient solutions method can only roughly estimate the range of particle density of tested MPs and relies on a series of density gradient solutions made of multiple chemicals. The automatic gas pycnometer method, which can analyse the particle density of MPs more quickly and accurately while also excluding the impacts of MPs hydrophobicity, has a coefficient of variation as low as 0.01%. However, the high cost of the apparatus and the necessity of the additional gas supply system for operation limit the scope of its use. Taken together, the method's repeatability and precision were sufficient to produce high-quality quantitative data. The ethanol pycnometer approach can complement other methods for determining the particle density of MPs, particularly in laboratories without automatic gas pycnometers and additional gas supply systems.

### **5.1.2 The particle size distribution of MPs based on laser granulometer**

The forces obviously interfering the determination of the particle size distribution of MPs during the laser granulometer test include hydrophobic attraction, electrostatic attraction, and buoyancy. The hydrophobic attraction between particles immersed in water is caused by the AB free energy of cohesion among the water molecules which surround particles in water (van Oss, 2008). Pristine polyethene MPs, having hydrophobic surface, are easily influenced by this force in the water and tend to aggregate to reduce its total areas contact with water. Electrostatic charges on highly insulating materials, such as tested MPs, can be produced during processes and transportation due to triboelectrification (Mort, 2003), and induced electrostatic attraction which strengthened the aggregation of MPs in water. Additionally, given that air can be embedded in MP aggregates and polyethene MPs are less dense than water, buoyancy can offset the gravitational pull and the hydrodynamic forces of the circulating water, leading partial MPs to float rather than constantly circulating through the apparatus and being analysed (Rawlins et al., 2013).

The accuracy and repeatability of PSD measurement by laser granulometer depend on the status of MPs that circulate through apparatus. Before the measurement of PSD, sonication was off and the pump speed lowered to 1500 rpm for restoring measuring background. With less energy being inputted, dispersive MPs particles restarted to aggregate and float on dispersant surface during measurement. Therefore, three PSD curves successively measured from the same sample were influenced by the reducing amount of detectable and increasing size of MPs particles, with obvious standard deviations being observed in results tested with water (light grey belts in Figure 4-2, B). Among all measurements, the results of MP35 largely exceeded the expected values and had the largest coefficient of variation. This was probably because MP35, which was finer, lighter and had larger specific surface area than other MPs, was easier to be affected by hydrophobic attraction in water and electrostatic attraction, thus producing a larger degree of aggregation in test.

Ethanol prevented MPs from floating and aggregating in the laser granulometer by reducing dispersant density and surface tension as described in Section 2.1. Therefore, the status of MPs in ethanol changed less over time and three PSD curves tested with ethanol were more consistent, with smaller error ranges (dark grey belts) being observed (Figure 4-2). Standard sand was always well dispersed in water and ethanol, and did not float during measurement, hence the PSD curves of sand tested with water and ethanol were highly coincident and only with narrow error ranges. This result suggests the differences in properties between water and ethanol are insufficient to visibly alter results of PSD in a way other than allowing sample to disperse and sink.

Despite the fact that a microscope can precisely measure the size of a single MP, measuring the PSD of the entire MPs sample by eye is reduplicative and easy to ignore some tiny particles. In contrast, laser granulometer is more suitable for the latter work. The evidence from this study suggests that ethanol could help laser granulometer overcome the drawbacks of measuring the PSD of MPs with water. This improved method could release the researcher from the duplication of labour in measuring MPs sizes using microscopes and allows them to quickly obtain high-quality quantitative data on the PSD of MPs from laser granulometer.

## **5.2 Effects of different sizes and contents of MPs on aggregate properties**

### **5.2.1 Effects of MPs on soil structure and the formation of soil macroaggregates**

Mineralogy and soil organic matter are important determinants for aggregation (Regelink et al., 2015; Totsche et al., 2017). In this study, WS had higher values for organic matter, CEC, extractable Mn than AS, whereas AS has a higher concentration of extractable Fe and Al and lower SAR. Although a variety of factors impact on aggregation, organic matter content is a key one and because WS had a higher OM content than AS, more aggregation occurred ( $P < 0.01$ ).

Previous studies have noticed that the influence of MPs on soil aggregate formation depends on MPs types, shapes, contents (de Souza Machado et al., 2018; Lehmann et al., 2021; Zhang et al., 2019). MPs are different in terms of their chemical composition, utilization rate for soil biotas, adhesivity, hydrophobicity, absorbability, particle size distribution, thus likely resulting in different soil aggregation (Boots et al., 2019; de Souza Machado et al., 2019; Liang et al., 2019). In terms of underlying mechanisms behind the influences of MPs, existing research mainly attempted to illustrate from the following two aspects: firstly, effects of MPs on soil aggregation require the presence of soil biota. MPs could induce changes in microenvironmental conditions, in turn the responses from microbial community structure may influence aggregation (Lehmann et al., 2019). In addition to changing living conditions of microorganisms, such as pH, gas and water permeability of soil, MPs have a chance in altering microbiome and their activity by releasing the monomers or additives which are useful for or hazardous to microorganisms. A recent study by Zhu et al. (2022) has showed that plasticizer-containing PVC at 0.5% (w/w) and plasticizer (phthalate) both altered nitrogen cycling and shaped soil microbiota into a microbial system with more nitrogen-fixing microorganisms. Conversely, PVC without plasticizer had no effect, indicating additives could be a main driver of changes in soil microorganisms. Soil aggregation could be indirectly affected by changes in microorganisms. Research by Liang et al. (2019) showed the addition of 0.4% w/w polyacrylic fibres to soils inhibit partial fungi activity, as measured by the fluorescein diacetate (FDA) hydrolytic activity, with resulting decreased WSA% in partial treatments. Similarly, de Souza Machado et al. (2018) reported that WSA% and FDA synchronously decreases with polyester and polyacrylic fibrous; Secondly, MPs as foreign substances lead to reduced tightness of the binds between soil particles. For example, when Liang et al. (2021) observed decreasing percentage of WSA with rising fibrous MPs, they speculated that the more fracture points produced by increased incorporation of fibrous MPs into soil aggregates play a key role. Similarly, Boots et al. (2019) suspected that reducing aggregate cohesion could be caused by incorporation of granular HDPE MPs, with resulting fewer macro-aggregates ( $> 2$  mm). However, above

explanations about mechanical strength are not verified so far. Moreover, previous studies were not designed for determining the impacts of various sizes of MPs on aggregation under control variable, therefore the effects of various sizes of MPs on soil aggregation are still poorly known.

To our knowledge, a negative relation between the size of MPs and the size of newly-formed macroaggregates has not been showed in the literature before. We deduce that MPs sizes could affect soil aggregation in the following aspects: firstly, if assuming that the shape of the microplastics is strictly consistent, surface area of MP35 are ~4 times that of MP125 and ~16 times that of MP500. Larger surface-to-mass ratio made 10% MP35 have considerable surface area interacting with soil particles. Therefore, it perhaps raises the risk in the leaching of additives hazardous to the soil microbes involved in the aggregation and thus likely inhibiting some activities important to aggregation, for example secretion of binding agents (e.g., polysaccharides, mycelia); Secondly, larger surface-to-mass ratio also rendered extreme water repellence of aggregates in 10% MP35 treatments (Figure 4-4, A, D). That might reduce the concentration of water-soluble binding agents in the aggregate and therefore weaken aggregate strength when the aggregates dry out. Meanwhile, under water deficit stress, the moisture required by soil microbials to secrete binding agents (e.g., polysaccharides, mycelia) might not be well satisfied and thus aggregation at 10% MP35 were weaker than other treatments (Blankinship et al., 2011; Goebel et al., 2005; Holland et al., 2013); Thirdly, despite the same MP contents, reducing size could lead to a higher number of MPs debris in aggregates, thus likely increasing fracture points; Lastly, the spatial distribution of MPs is related to their sizes even if the same MPs content and may influence their engagements in different size fractions of aggregates. We hereby raise a concept model to explain this mechanism (Figure 5-1). Briefly stated, smaller MPs, acting as building units, tend to directly engage in the formation of smaller new aggregates, which serve as the foundation for larger aggregates. Meanwhile, larger surface-to-mass ratios enhance the negative influences of smaller MPs. As those MPs surfaces are not only embedded in aggregates but also densely spread on aggregate surface, formation of small aggregates and further aggregation from small aggregates to larger macroaggregates could be more intensively interfered. In contrast, larger MPs are less likely to have an impact on the formation of new microaggregates, as we can observe in figure of black frame that the uncontaminated area between MPs are wider (Figure 5-1). Additionally, due to much smaller surface-to-mass ratios, those large MPs might not intensively inhibit aggregation in uncontaminated area and are possible to passively incorporate into macroaggregates as a result of the aggregation of surrounding microaggregates and small macroaggregates.



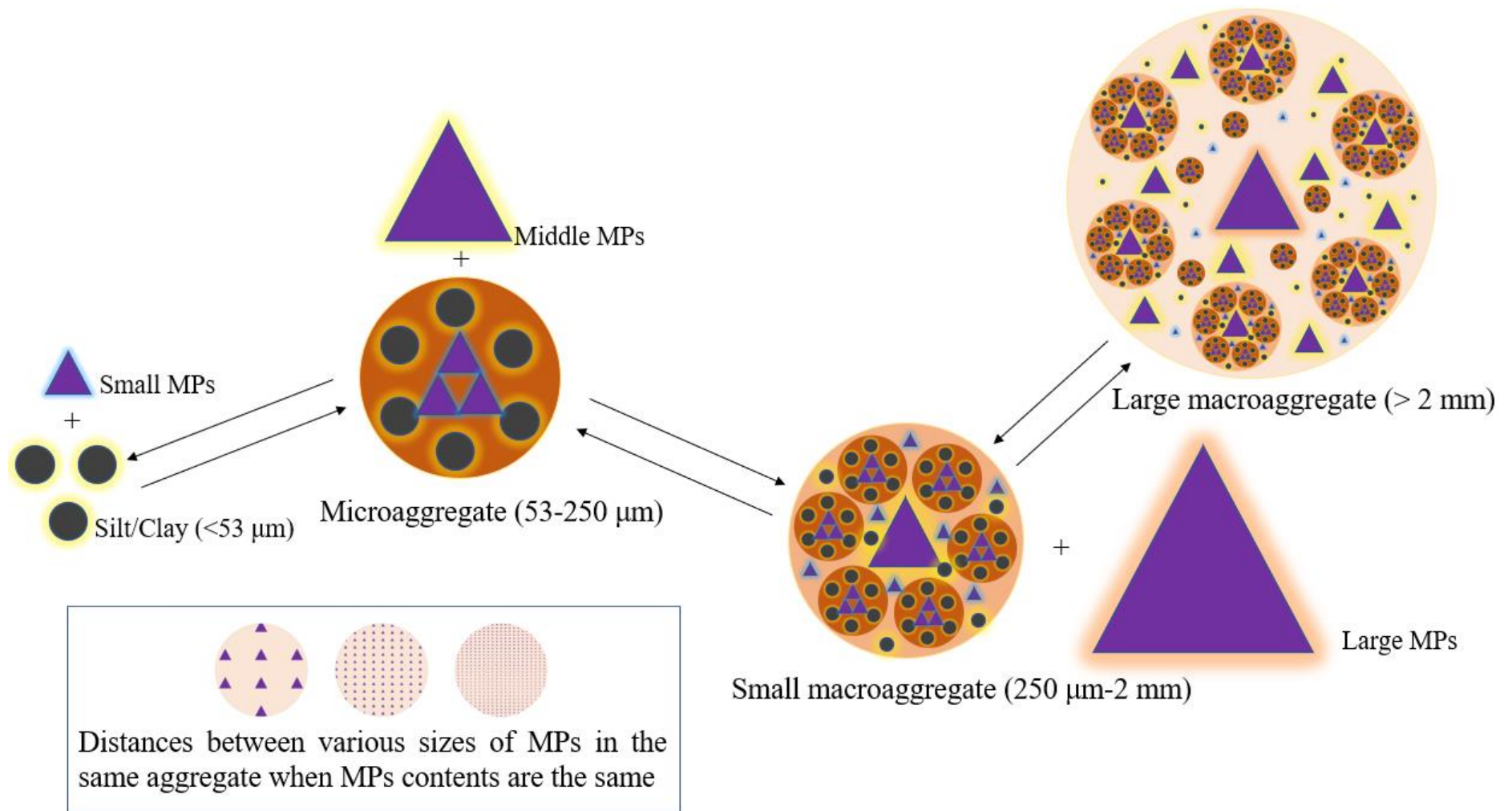


Figure 5-1. A concept model for the engagement of different sized granular MPs in formation of new soil aggregates.

On basis of findings from this indoor study, we deduced that serious degradation of soil structure could happen if soil contaminated by granular polythene MPs meets the both criterions that the majority of MPs are <35 mm and that the MPs content is close to 10%, whereas granular MPs (>125µm) are unlikely to change soil structure even at an extreme MPs loading. However, this deduction also needs to be verified by the results from more field investigations, because the conditions in field (e.g., soil texture, bioturbation, plant growth, humidity) are much more complicated than laboratory. In addition, 75-days incubation in this study is a short term compared to recalcitrant nature of polythene MPs. Hence, we recommended conducting a multiple-years mesocosm study for observing the long-term impacts of MPs on the distribution of aggregates.

### **5.2.2 Effects of various MPs on newly-formed soil aggregate wettability**

Aggregate wettability is the property of the aggregate to become wet, and can be measured by the rate of water intake (Eynard et al., 2004b). As wettability often positively correlates with water stability of aggregates (Blanco-Canqui et al., 2007; Chenu et al., 2000) and controls the allocation of surface and subsurface runoff in rainfall, it can be assumed as an important index for evaluating mobility of MPs in soils aggregates. (Rehm et al., 2021). Additionally, aggregate wettability plays an important role in soil processes like gas, water, and nutrient fluxes, the accessibility and storage of nutrients, and define the living conditions for microbes including their decomposing or precipitation capacities (Lipiec et al., 2009).

Aggregate wettability is mainly controlled by the characteristics of soil pore and the spatial distribution of hydrophilic and hydrophobic surface on pore wall (Eynard et al., 2004b). The wettability of AS aggregates was larger than WS aggregates. This could be mainly explained by the SOM concentration of AS is lower, because SOM is considered as the main source of hydrophobicity (Goebel et al., 2005). MPs powders, characterized by varied sizes and strong hydrophobicity, have the potential to affect aggregate wettability by altering aggregate pore and introducing extra hydrophobic surface. However, data on effect of MPs on aggregate wettability is rare.

Our experimental results showed aggregate wettability decreased with MPs contents and increased with MPs sizes. Aggregate wettability reduced with MPs contents because aggregates, including external surface and internal pores, are built up from a higher proportion of hydrophobic units. In order to interpret the impacts of different MPs sizes on aggregate

wettability, we hereby raise a concept model (Figure 5-2). Firstly, smaller MPs are with larger surface-to-mass ratios, could thus enhance the hydrophobicity by boosting hydrophobic area under same MP content. For instance, if assuming that the shapes of different sizes of plastics are strictly the same, the surface to mass ratio of MP35 could be ~4 times that of MP125 and ~16 times that of MP500; Secondly, the spatial distribution of larger MPs is more heterogeneous (loose). As a result, the diameter of hydrophilic throats (uncontaminated area) between hydrophobic MPs increases with MP sizes under same MPs content. For aggregates containing larger MPs, water could still rapidly through soil via wide hydrophilic throats even at 10% MP loading; Lastly, MP500 are coarser than used soils (< 250  $\mu\text{m}$ ). So, the incorporation of large MPs into macroaggregates make aggregate texture coarser and might produce additional macropores on MP-soil interface, offsetting partial effect of enlarged hydrophobic area due to increasing MPs content. This theory is supported by the research of Qi et al. (2020), who found when concentration of LDPE powders (< 1000  $\mu\text{m}$ ) increased from 0 – 2%, WDPT of sandy soil significantly increased by ~60% then decreased to the control level, indicating the formation of new macropores that facilitate unsaturated infiltration.

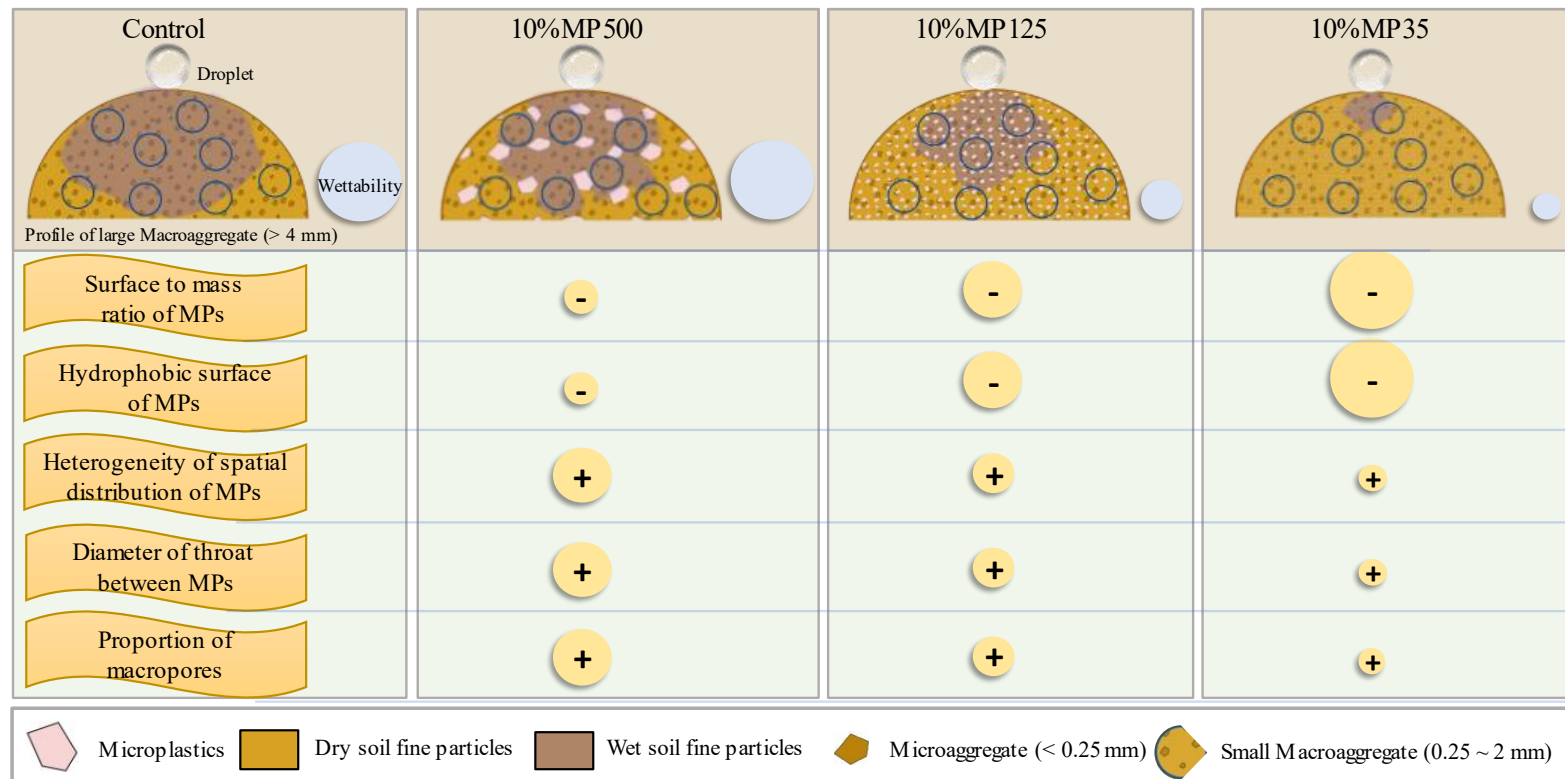


Figure 5-2. A concept model for the impacts of properties related to MPs size on aggregate wettability. When the size reduces from MP500 (< 500  $\mu\text{m}$ ) to MP125 (< 125  $\mu\text{m}$ ) then to MP35 (< 35  $\mu\text{m}$ ), MPs have a higher surface to mass ratio, with resulting enlarged hydrophobic surface; The heterogeneity of the spatial distribution of MPs reduced, meaning MPs tend to distribute in the whole aggregate (like MP35) rather than concentrating in a few sites (like MP500) and MPs get closer to each other (like MP35); Wetting front have to across narrower hydrophilic throats (uncontaminated area) between hydrophobic MPs. The affinity for soil to water decrease more rapidly in throat because MPs are closer to each other, thus slowing down wetting front; Aggregate texture became finer, so the potential for the formation of additional macropores MP-soil interface reduced. Consequently, the wettability of aggregate reduces with MPs sizes.

Some increases in water repellence (difficulty in wettability), like the influence of 10% MP125, can prevent water rapidly entering into dry aggregates, and theoretically enhance water stability by slowing the disintegration and slaking of aggregates (AchimEllies et al., 1995). So, it may imply reduction in soil erosion. In contrast, if water repellence goes too far, like influences of 1% and 10% MP35, any reduction in erosion will be offset by increasing splash erosion, surface runoff, and rill formation, and consequently cause lateral dispersion of MPs pollution (Rehm et al., 2021; Shakesby et al., 2000). Therefore, the MPs (< 35  $\mu\text{m}$ ) pollution in agroecosystems should receive specific attention, since a small increase in this component of the MPs loading is possible to trigger a mutation of aggregate wettability, then cause complicated consequences.

Understanding the long-term effects of MPs on aggregate wettability is essential to assessing the impacts of MPs in the soil environment. Numerous studies have reported that natural aging processes (e.g., ultraviolet (UV) radiation, biodegradation, and oxidation) render MPs more hydrophilic due to the formation of surface functional groups (Liu et al., 2019; Ren et al., 2021; Yan et al., 2020). If MPs get covered in biofilm, the hydrophobicity of MPs will also decrease (He et al., 2023). In our experiments, aging processes were very likely to happened during soil incubation, but their variety, intensity, and duration might be very limited compared to outdoor environment. Given that soil microbial community were preserved, it is possible that biofilm have built on our MPs samples. However, after 75 days of incubation, none of the influences of above factors proved sufficient to completely offset the extra water repellence introduced by MPs. Considering it is still unclear whether the wettability of these aggregates can be restored by further aging processes and formation of biofilm, we thereby recommend continuous observation of the long-term effects of aged MPs (< 35  $\mu\text{m}$ ) on aggregate wettability.

### **5.2.3 Effects of various MPs on newly-formed soil aggregate water stability**

The SOM% of the AS (6.92%) was much higher than that of WS (2.99%). This may in part explain the greater DR value of WS ( $P < 0.001$ ), as SOM can enhance aggregate stability (Goebel et al., 2005; Regelink et al., 2015). Previously published studies on the effect of MPs found types, shapes, and contents of MPs significantly affected aggregate water stability, and most of research presented that aggregate stability reduced with contents of fibrous MPs (e.g., polyacrylic, polyester) ( $P < 0.05$ ) (de Souza Machado et al., 2019; de Souza Machado et al.,

2018; Lehmann et al., 2021; Liang et al., 2019). The underlying mechanisms behind the detrimental influences likely include the production of fracture points by MPs inside aggregates (Liang et al., 2021), inhibiting activity of soil microbial (Liang et al., 2019), reducing cohesion of particles in aggregate (Boots et al., 2019). In contrast, on basis of finding in **Section 5.2.2**, incorporation of MPs could enhance aggregate hydrophobicity and likely improve water stability. Unfortunately, the effects of different size of MPs on aggregate stability are currently poorly understood, because previous research did not further investigate the influences of various sizes of MPs on aggregate water stability under variable control.

In this study, diverse impacts of different sizes and contents of MPs on water stability of aggregates (1-2 mm) were observed. To our knowledge, these are the first findings on MPs size-induced effects on water stability of aggregates. Water stability of aggregates were not significantly affected by the content of MP500 ( $P > 0.05$ ), as found for other studies on PE powders (~500  $\mu\text{m}$ ) (de Souza Machado et al., 2019; de Souza Machado et al., 2018; Qi et al., 2020). This was likely a result of those MPs being too large, which prevented them from significantly strengthening those impacts that could destabilise aggregate, or reducing wettability of either soil to increase stability (Figure 4-4, C, E). In contrast, water stability is significantly reduced by treatments of 0.1-10% MP125 and 1% MP35 in WS ( $P < 0.05$ ). A similar result reported by Boots et al. (2019) showed sandy clay loam soil in treatment of 0.1% granular HDPE MPs with an average size of 102.6  $\mu\text{m}$  had 26% lower MWD of water stable aggregates relative to the control, and the authors speculated cohesion may reduce and thus destabilizing aggregates. Meanwhile, we also observed that dry aggregates containing MP35 and MP125 were more fragile. The evidence presented thus far supports the idea that aggregate containing smaller sizes of MPs are more vulnerable. On the other hand, smaller MPs size boosts water repellence of aggregate. If contents of small MPs continuously increase, the rapidly rising water repellence could offset the increases in detrimental effects of MPs and even enhanced aggregate stability in some cases, likely explaining why treatment of MP35 in WS regained water stability after a decline and treatment of MP35 in AS sharply increased in water stability (Figure 4-4, A, D; Figure 4-5, A, D). The differences in respond of water stability to MPs between AS and WS were likely due to different soil texture, because soil mineralogy and clay content play important roles in the formation and stability of aggregates (Regelink et al., 2015; Totsche et al., 2017).

In order to better clarify the potential impacts of factors related to MP sizes on aggregate water stability, we hereby raise a concept model on basis of current knowledge, experimental results, and deductions (Figure 5-3).

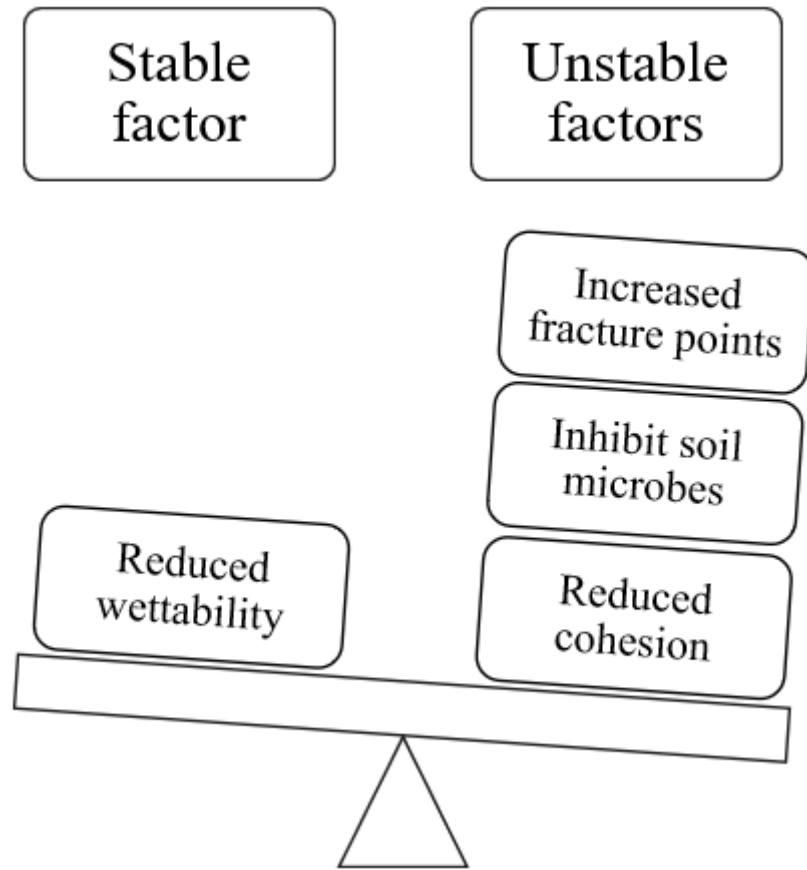


Figure 5-3. A concept model for the underlying mechanisms behind influences of MPs on aggregate water stability. Arrows are impacts of MPs. When MP contents are the same, reducing size could lead to a higher number of MPs debris in aggregates, thus likely increasing fracture points; increase MPs surface area and thus perhaps more intensively inhibiting activity of soil microbial; and might further reduce cohesion of aggregates. As a result, aggregate should have become less water stable with reducing MP size. However, wettability reduced with MP sizes and thus offsetting those detrimental effects, and even improve aggregate water stability in some cases. As same, increasing content of MPs could enhance those negative or positive effects as well. However, the degree of enhancement for each effect varies at different MP sizes, thus finally leading to diverse water stability.

Taken together, this study has uncovered a size dependency of MPs effects on water stability of aggregate. These findings could be very useful for understanding the long-term challenges from plastic pollutions in agroecosystems, because accumulated polyethene plastic

debris in arable field will eventually fragment into MPs or even NPs (nanoplastics) (Qi et al., 2020), thus being prone to undermine soil health by incorporating into aggregates (Zhang and Zhang, 2020). In the future, we need to investigate other potential factors (e.g., microbial activity, cohesion of aggregate) altering aggregate stability and their relationships with different sized MPs, in order to further explore the reasons for the distinct changes in aggregate water stability.

#### **5.2.4 Effects of various MPs on newly-formed soil aggregate particle density and rough estimation of %MPs in aggregates on basis of change in particle density**

The density of minerals commonly found in soils varies from 2.60 to 2.75 g/cm<sup>3</sup> (Hao et al., 2008). However, if large amount of ‘light’ components such as natural organic matter (1.3-1.5 g/cm<sup>3</sup>), MPs (0.8-1.6 g/cm<sup>3</sup>) are present in a soil, the particle density of the soil may be less than 2.60 g/cm<sup>3</sup>. In this study, the particle density of AS and WS are both below 2.60 cm<sup>3</sup>. This could be mainly explained by the high SOM contents. Since polythene (0.92-0.97 g/cm<sup>3</sup>) is less dense than natural SOM (1.3-1.5 g/cm<sup>3</sup>), the particle density of aggregates is perhaps more sensitive to the polythene MPs%. In the real world, the widespread usage of mulching with polythene film results in the residual of a rising amount of plastic waste in the agroecosystem (Qi et al., 2020). These ‘light’ component may reduce the particle density of topsoil. Our experimental results confirmed that as the MPs% increased from 0-10%, the particle density of aggregates in AS and WS both sharply declined.

Based on the considerable difference in particle density between uncontaminated soil and MPs, this study is the first attempt to determine the MPs% in soil aggregates using known soil and MPs particle density and the particle density of MPs-containing aggregate measured by pycnometer. Increasing concentration of MPs reduced the particle density of aggregates ( $P < 0.05$ ), whereas MPs size did not influence the particle density of aggregate ( $P > 0.05$ ) (Table 4-7). The MPs% predicted by changes in aggregate particle density only linearly responds to addition of MPs in soil, with adjusted  $R^2$  ranging between 0.998 and 1.000 ( $P < 0.001$ ), indicating that the MPs added to the soil were incorporated into the aggregates (> 4 mm) in a uniform way. This method has some advantages compared to the traditional digestion-separation-filtration-weighing processes, such as environmental friendliness and low cost (each test only requires the use of 10 mL of ethanol as reagent); high efficiency (each person can test 20 samples per day); simplicity (no necessity to separate the MPs from sample); accurate when



measuring 1-10% level of MPs because added MPs were incorporated uniformly into the aggregates at a level broadly equivalent to that at which the MPs were added to the bulk soil. Thus, this method might be useful when it comes to checking MPs% and a lot more efficient than traditional density separation & weighing.

On the other hand, due to the limited accuracy and the precision of the current quantitative measurement, there was no significantly different particle density in the treatments of 0.1% and partial 1% MPs addition compared to the control. These deviations probably affect the accuracy in predicting tiny concentration of MPs. Besides, the changes in the particle density of soil components (excluding MPs) are perhaps responsible for introducing deviations to the results. For example, the degradation of soil 'light' components, in particularly a reduction in organic matter, could slightly increase the particle density of soil, with resulting underestimation of tiny concentration of MPs. Lastly, this method only works for experiments where researcher have control samples (i.e., uncontaminated soil and MPs). As a result, it is predictable in practice that applying this approach to real soil polluted by MPs in field will meet some challenges, in particular, determining the particle densities of MPs in soil and particle density of components without MPs in soil. Nonetheless, the variation of soil particle density in the same plot can also reflect different degree of MP pollution to a certain extent.

In follow-up studies, we hope to verify the results of the MPs% in aggregates by other methods (e.g., density separation & weighing, thermal analysis). More precise methods such as automated gas pycnometer should be applied for measuring the particle density of aggregates under 0.1% and 1% MPs addition treatments to improve the accuracy of raw data required for the calculation of MPs%. Furthermore, we hope to tested the particle density of wider types of MPs and soils and the soils polluted by MPs in experiments, and measure the MPs% in the mixed samples, so as to verify the reliability, precision, accuracy and applicability of this methods. Via being improved by above measures, this method is perhaps quite useful in following fields: knowing the distribution of MPs% in different sized aggregates, long-term monitoring of changes in MPs% in soil.

## 6 Conclusions

This study investigated whether pristine polyethene MPs have size- and concentration-dependent effects on the formation of newly-formed soil macroaggregates and their properties (i.e., wettability, water stability, particle density, MP concentration) in WS and AS. Meanwhile, some efficient tools for analysing particle density and particle size distribution of hydrophobic MPs and an innovative method for determining MPs concentration in aggregate samples were developed. Based on the results and discussions, the following main conclusions can be drawn.

1. 99.8% ethanol solution as a substitute dispersant for water could help traditional liquid pycnometer methods achieve precise determination of the particle density of strong hydrophobic MPs less dense than water, because 99.8% ethanol, whose density ( $0.789 \text{ g/cm}^3$ ) is lower than polyethene MPs ( $0.92\text{-}0.97 \text{ g/cm}^3$ ), can prevent MPs floating, and is effective in reducing surface tension that makes hydrophobic MPs aggregate.
2. The particle size distribution of polyethene MPs tested with 35% ethanol generally had smaller sizes and standard deviations compared with the results tested with water, because 35% ethanol largely reduced the random floating and aggregation of tested MPs in dispersant during measurements, particularly for smaller MPs that have greater surface-to-mass ratios (i.e., hydrophobic surface area). 35% ethanol solution, as a substitute dispersant for water, has been shown to improve the accuracy and precision of determining the particle size distribution of MPs using a laser granulometer.
3. The concentrations of MPs incorporated into aggregates ( $>4 \text{ mm}$ ) were broadly equivalent to that at which the MPs were added to the soil. Therefore, **we cannot reject the hypothesis (i) that higher MP exposure concentrations in soil can encourage more MPs to incorporate into aggregates.** It is worth noting that for smaller aggregates, especially microaggregates, large MPs might be more difficult to incorporate compared with smaller MPs. Therefore, the MP concentration in different sized aggregates might not be same.
4. Aggregates produced in soil containing 10 wt% of  $<35 \text{ }\mu\text{m}$  MPs had a significantly lower MWD than controls; other treatments showed no significant difference to the

control. More additives leaching, reduced water availability, and change in spatial distribution of MP within aggregate may result in these impacts. Therefore, **we cannot reject the hypothesis (ii) that higher concentrations of MPs can reduce aggregate formation and smaller MP sizes can increase this impact.**

5. Increased MP concentration significantly reduced the wettability of MPs-containing aggregates (>4 mm), because aggregates are built up from a higher proportion of hydrophobic units (MPs). The degree of reduction in wettability increased with MP sizes. Surface-to-mass ratio (i.e., hydrophobic surface area) and MP-induced soil porosities likely result in this size-dependent effect. Hence, **we cannot reject the hypothesis (iii), i.e., higher concentrations of MPs can reduce aggregate wettability and smaller MP sizes can increase this impact.**
6. The water stability of aggregates (1-2 mm) containing MPs was significantly impacted by both MP sizes and concentrations. Despite this, water stability nonmonotonically responded to either MP sizes or concentrations. The results of some treatments were inconsistent with the hypothesis (iv) that higher concentrations of MPs can reduce aggregate water stability and smaller MP sizes can increase this impact. This inconsistency is likely because increased MP concentrations and reduced MP size can make aggregate more hydrophobic, thus offsetting partial detrimental impacts of MPs and even enhancing aggregate water stability. This also highlights the complexity of the effects of MPs on aggregates. Given that, **we reject the hypothesis (iv).**
7. As a 'light' component in soil, increased MP concentration in aggregates significantly lowered particle density of newly-formed macroaggregate (>4 mm), whereas MP sizes had no effect. **We are not able to reject the hypothesis (v)** as it is consistent with observed results. The determination of MP concentration in aggregates on basis of change in particle density could be useful when it comes to verifying MP concentration in experiments and a lot more efficient than traditional methods. However, for environmental samples, this method still has some limitations.

Collectively, the size- and concentration-dependent effects of MPs on aggregate formation and properties were uncovered by this study. This knowledge can aid in

understanding the long-term impacts of plastic debris on terrestrial ecosystems in the context that plastics are constantly accumulating and fragmenting in soil. Additionally, it can help researchers better study how aggregates influence the mobility of MPs at different sizes and concentrations in soil. Given the more obvious impacts of MP35, the contamination of MPs (<35  $\mu\text{m}$ ) should be more strictly controlled in real world to protect soil aggregated structure. It must also be noted that research on the specific impacts of different sized MPs on soil health is still limited, and more studies are needed to fully understand the potential risks and impacts. Equally important is the monitoring of long-term impacts of aged MPs on aggregates since it can be anticipated that these impacts will vary as MP properties (e.g., sizes, wettability, chemical components) will change over time in the field. Based on the findings of this research, soil column experiments will be designed to investigate whether the incorporation of MPs into aggregates can mitigate their mobility in soil. Additionally, a field study will examine the effects of aged MPs on soil aggregate properties, including their seasonal variations.

## Author's declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for a degree or other qualification at this University or elsewhere. All sources are acknowledged as references.

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