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Combined effects of degradable film fragments and micro/nanoplastics on growth of wheat seedling and rhizosphere microbes☆

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ABSTRACT

Multiple sources of microplastics (MPs) in farmland could result in the changing of microbial community and the plant growth. Most studies of MPs in agricultural system have focused on the effects of single types of MPs on growth of plants, while neglect interactions between multiple types of MPs. In this study a pot-experiment was conducted to investigate the effects of multiple types of MPs, including polystyrene beads: M1, 5 μm, M2, 70 nm and degradable mulching film (DMF) fragments on growth of wheat seedlings and associated rhizosphere microbial community. CKD (adding DMF) significantly reduced plant height and base diameter of wheat seedlings. DMF in combination with M2, significantly increased plant height and aboveground biomass, but decreased the base diameter. *Actinobacteria* was the dominant taxa in the rhizosphere bacterial community in various treatments. PCoA analysis showed that the bacterial composition in M2HD (100 mg kg^{-1} M^2 with DMF) was significantly different from that of CKD and M2LD (10 mg kg^{-1} M^2 with DMF). At the level of genera, the dominant fungi in CKD and M2LD were in the genus *Fusarium*, which is the cause of wheat fusarium blight and *Alternaria,* which results in decreased base diameter. In CK (control group) and M2HD, *Blastobotrys* exhibited the greatest abundance, which assisted wheat seedlings in resisting *Verticillium* disease. Cluster and PCoA analysis showed the fungal composition in CKD was significantly different from CK, M2LD and M2HD. These findings suggest MPs potentially have selective effects on pathogens that affect growth of plants and potentially safety of the food.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important grains globally. Growth of wheat seedlings is directly related to yields of grain. After sowing, protein in grain could maintain sprouting and growth of wheat cotyledons until appearance of the first leaves. At that stage, seedlings start to absorb nitrogen nutrients, such as NO_3^- and NH_4^+, while roots are still fragile (Zörb et al., 2018). Health of the root is vital for growth of wheat seedlings, which is also affected by characteristics in soils, such as nitrogen and carbon contents. Soil provides water and nutrients to plants, which help plants to defend the adverse environment and plays an important role in crop health and human health (Sanaullah et al., 2020).

The land was the largest carrier of wastewater irrigation and application of sludge because a large amount of MPs enter agricultural soils through wastewater and sludge application (Edo et al., 2020; Li et al., 2019). Various types of microplastics (MPs) can be derived from multiple sources. Irrigation with wastewater and application of sludge

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(Nizzetto et al., 2016) is the main sources of MPs to agricultural soils. Typical polymer types of MPs include polyethylene (PE), polypropylene (PP), polystyrene (PS) beads, polyvinyl chloride (PVC), microfibers and so on. PS accounted for 7.1% of the total world plastic production (Wu et al., 2017). PS MPs are widely used in production and daily life (Rodrigues et al., 2019), such as microbeads. They have drawn great attention among the main types of MPs and were always used in cosmetics (Cole et al., 2011), then released to agricultural systems. They are primary microplastics (Ngo et al., 2019) and intended for only one use (Kutralam-Muniasamy et al., 2020; Xanthos and Walker, 2017). Despite that microbeads account for <10% of the total microparticle count in wastewater effluent, researchers identified microbeads as one of the predominant MPs types in the treated effluent (McCormick et al., 2016; Sol et al., 2020). Although constituting just a small proportion in numbers, microbeads make up a greater proportion in mass (Hamidian et al., 2021). Even if wastewater was treated before discharging, but it still had potential hazards. Due to the structural characteristics, PS-beads has the adsorption effect on the environmental pollutants (Wang et al., 2019), posing a serious threat to the ecological environment health. Their shapes are generally round, oval or cylindrical and PS is one of the main types of microbeads (Kutralam-Muniasamy et al., 2020).

Mulching film, which is an extensively applied agricultural technique, is another source of MPs to farmland soils. Mulching film could enhance quality of the soil and benefit growth of seedlings as well as prevent growth of weeds and reduce harmful insects and diseases (Wang et al., 2009), which results in greater yields (Hou et al., 2019). In China, the use of mulching film, which results in large increases of yields, has been increasing by 30% (Espí et al., 2006). In Mexico, coverage of mulching film in top soils used to grow vegetables is 40% ~ 60% (Huerta Lwanga et al., 2016). In North America, total input of MPs to farmland could reach 44,000 ~ 300,000 t yr^{-1} (Ng et al., 2018).

As an exogenous matter, MPs can affect structures of soil ecosystems (Rillig, 2012). Soil is both a source and sink of MPs and ecological effects of MPs on soil not only affect overall quality of the soil environment, but might pose a serious threat to terrestrial biodiversity. Previous studies have shown that MPs affected soil dissolved organic matter (DOM) and its constituents (Liu et al., 2017), soil structure (de Souza Machado et al., 2019), soil microbial community (Zhang et al., 2019) and plant growth (Boots et al., 2019; de Souza Machado et al., 2019). Microbes that contribute to hydrolytic activity of MPs (Zhang et al., 2019) are present on surfaces of MPs, especially on uneven or thinner parts. MPs can enrich specific microorganisms, such as *Actinobacteria* in farmland, which could degrade polymers by synthesizing hydrolases (Muhonja et al., 2018). Results of a previous study indicated that MPs could change the microbial community in fertilized soil, and further influence emissions of greenhouse gases. Degradable mulching film might be able to be completely degraded (Brodhagen et al., 2014), so is therefore used in place of other farmland mulching films. Large degradable plastic film could also be broken into fragments by environment effects or soil animals. Results of a recent study showed that compared with high-density polyethylene (HDPE) MPs, polylactic acid (PLA) MPs significantly reduced aggregation of particle size (<63 μm) (Boots et al., 2019). PLA MPs could significantly reduce NH_4^+-N, increase the NO_3^--N and NO_2^--N, which affects soil nitrogen cycling and changes characteristics and contents of humic and fulvic acid (Chen et al., 2020).

As one of the main types of microbeads (Kutralam-Muniasamy et al., 2020), the effect of PS-beads on plants are related to sizes of particles as well as absolute and relative concentration. Results of previous studies have shown that 100 nm PS-beads significantly increased plant height at the concentration of 10 mg·kg^{-1}, while significantly inhibited that at the concentration of 100 mg·kg^{-1}; 5 μm PS-beads had no significant effect on root length and plant height at 100 mg·kg^{-1}, but significantly inhibited plant height and root length at 10 mg·kg^{-1} (Liao et al., 2019). In addition, PS beads could enter into the plant body by transpiration pull or the crack-entry at sites of lateral root emergence (Li et al., 2020). These studies provided direct evidence for effect of MPs on growth of plants.

The MPs in natural environment having different surface morphology and chemical composition tend to have larger surface areas and surface roughness, and further provide adsorption sites for soil microorganisms and enrich specific microbes (Ya et al., 2021). Besides that, MPs can also enrich microbes related to MPs degradation process (Yu et al., 2021). By enriching the specific microorganisms MPs potentially affect growth of plants. The degradation process of degradable plastics often results in lower molecular-weight-fragments under specific environmental conditions or by the action of naturally occurring microorganisms, which causes loss of some properties (Krzan et al., 2006). At the genus level, degradable plastics could enhance abundances of *Bacillius*, *Variovorax*, *Comaonadaceae*, *Bradyrizobium* and *Cellvibrio* in rhizospheres (Qi et al., 2020). Some microorganisms in the genus *Bacillius* are pathogenic.

Until now, most research has focused on effects of single types of MPs on growth of plants. However, various types of MPs in soil come from multiple sources. Therefore, it is important to evaluate risks to health and safety of multi-sources MPs on crops. In this study, two sizes of PS-beads were selected to simulate MPs from irrigation with wastewater and fertilization with sludge; and fragments of degradable mulching film (DMF), mainly composed of polylactic acid (PLA) and polybutylene adipate-co-terephthalate (PBAT), were used to simulate the mulching film residues. The study presented here focused on the combined effects of PS-beads and DMF on: (1) growth index of wheat; (2) soil DOM and its functional groups; (3) rhizosphere microbial community and (4) mechanisms of the potential microbial ecological effect.

2. Materials and methods

2.1. Microplastics

5 μm (M1) and 70 nm (M2) polystyrene beads (PS-beads) were used in the present study to simulate MPs from irrigation with municipal wastewater and application of sewage sludge to agricultural lands. PS-beads were purchased from Baseline ChromTech Research Centre (Tianjin, China, product number No.6-1-0007 and No.6-1-0500, respectively). The concentration of the emulsion solution was 25 mg ml^{-1} (PS-beads dry powder/deionized water). Sizes of PS-beads were characterized by transmission electron microscope (TEM) (JEM-100CXII, Japan). The TEM image of PS-beads used in our research was shown in Fig. S1 and their shapes were round. Degradable mulching film (DMF) fragments composed of PLA and PBAT were obtained from an agricultural products store. The film was cut into small pieces by using sharp blades and scissors, ~4.5 mm in length.

2.2. Treatments and replicates

Before use, the dialyzed emulsion PS-beads solutions at the concentration of 25 mg ml^{-1} were ultrasonicated for 10 min and diluted to final concentration of 10 or 100 mg kg^{-1} (PS-beads per kilogram of soil). Soil was collected from a reserved field without film mulching or known direct pollution and placed into experimental containers of 4 × 8 seedling trays made of PS. Trays were 54 cm in length, 28 cm in width. Each pot was 6 cm in depth, 6 × 6 cm in top and 3 × 3 cm in bottom. Each pot was filled with 60 g soil. L and H were used to represented low (10 mg·kg^{-1}) and high (100 mg·kg^{-1}) concentration of PS-beads, respectively. M1 and M2 represented 5 μm and 70 nm PS-beads, respectively. PS-beads of different concentration and particle sizes with or without DMF were mixed into the soil and then water was added (Table S1), resulting in ten treatments: 1) CK: no PS-beads adding, 2) CKD: no PS-beads adding+1% DMF, 3) M1L: 10 mg·kg^{-1} 5 μm PS-beads, 4) M1LD: 10 mg·kg^{-1} 5 μm PS-beads+1% DMF, 5) M1H: 100 mg·kg^{-1} 5 μm PS-beads, 6) M1HD: 100 mg·kg^{-1} 5 μm PS-beads+1% DMF, 7) M2L: 10 mg·kg^{-1} 70 nm PS-beads, 8) M2LD: 10 mg·kg^{-1} 70 nm PS-beads+1%

DMF, 9) M2H: 100 mg·kg^{-1} 70 nm PS-beads, 10) M2HD: 100 mg·kg^{-1} 70 nm PS-beads+1% DMF. Wheat (*Triticum aestivum* L.) used in this study was *Nongda 212*. Seeds of the same size and color were selected and sterilized with 3% H_2O_2 for 20 min, then washed for three times with distilled water. Excess water on surfaces of seeds was absorbed by filter paper before seeds were sown in pots. Once sprouted, seedlings were thinned to 4 plants in each pot. Each treatment was repeated in four replicates (Table S1). Trays were cultured at room temperature, and ventilation was maintained during the experiment.

2.3. Measurements of plant growth parameters and sample collection

Two weeks after sowing, six seedlings of each treatment were selected. The plant height and the base diameter were measured. Rhizosphere soil samples were collected after gently shaking the roots to remove the loosely adhered soil and immediately stored at −20 °C for further analysis. Then the roots were washed, and masses of above- and below-ground portions determined. After harvesting, bulk soil was sampled from pots for analyzing soil physical and chemical properties, dissolved organic matter (DOM) and the functional groups.

2.4. Analysis of soil properties

Soil DOM and related functional group characteristics were determined by use of previously reported methods (Jaffrain et al., 2007; Ren et al., 2020) (Detailed information is shown in supplementary materials). The concentration of DOM is normally expressed as the concentration of dissolved organic carbon (DOC) (Borggaard et al., 2019). The DOM solutions were analyzed by multi N/C 3100 (Analytik Jena AG, Germany) for DOC. Samples were measured by UV–Vis spectrophotometer (LAMBDA-35, PerkinElmer, USA) using a 10-mm quartz cuvette with deionized water as blank. Absorption value was recorded from 200 to 500 nm (1 nm steps). The specific UV absorbance at 210, 250, 254, 260, 265, 272, 280, 285, 300, 340, 350, 365, 400, 436, and 465 nm were selected to characterize the functional groups. Detailed information as well as the wavelengths used in this study and their corresponding organic functional groups were shown in Table S2. The soil NO_3^--N was determined by UV-spectrophotometry (TU-1810DASPC, PERSEE, Beijing, China) according to the method GB/T 32,737–2016, the extracts were tested at 220 nm and 275 nm, respectively.

2.5. DNA extraction, PCR and 16S rRNA sequencing

Microbial community genomic DNA was extracted from soils, by use of the FastDNA® Spin Kit (MP Biomedicals, Santa Ana, Ca, USA) according to manufacturer's instructions. Extracted DNA was checked on 1% agarose gel electrophoresis, and the concentration and purity were determined using NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). The V3–V4 region of the bacterial 16SrRNA genes was amplified using bacterial primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), and the ITS region of the fungal rRNA gene was amplified using the fungal-specific primer pair ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC -3′). The triplicate PCR products were extracted by 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) following the manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequencing (2 × 250) was performed on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Detailed PCR methods and bioinformatic pipeline are given in the supplemental materials.

2.6. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 24.0. One-way analysis of variance was used to determine effects of treatments on soil properties and physiological indicators of plants. Means of significant effects at $p < 0.05$ were then compared using the Duncan's multiple-range test. Operational taxonomic unit (OTUs)-level of alpha diversity indices, Chao1 (Chao, 1984), abundance-based coverage estimators (ACE) (Chao and Yang, 1993) and Shannon index (Shannon, 1948) were calculated using the OTUs table in Mothur (Schloss et al., 2009). Figures were visualized by R 3.6.1 (R Core Team, 2019) and RStudio 1.1.463 (RStudio Team, 2018). Package information and detailed data analysis method were listed in our previous studied (Ren et al., 2020).

3. Results and discussion

3.1. Effects of MPs on dissolved organic matter (DOM) and relative functional group characteristics

The trend of total dissolved carbon (TC) and dissolved organic carbon (DOC) was similar (Fig. 1). In soils without PS-beads (CK and CKD), DMF had no significant effects on soil DOC. However, in the presence of PS-beads, soil DOC was significantly ($p < 0.001$, LSD, $p < 0.05$) greater. Without DMF, M1 and M2 have no significant effect on DOC; in soil with DMF, PS-beads could significantly affect DOC. Content of dissolved inorganic carbon (DIC) was related to size and concentration of PS-beads (see Fig. 2). Without PS-beads, the presence of DMF had no significant effects on DIC. While in M1 at the greater concentration (100 mg·kg^{-1}), DIC was significantly less (M1H < CK, $p < 0.001$). In M2 at the lesser concentration (10 mg·kg^{-1}), DIC was significantly less than that in the control group (M2L < CK, $p = 0.003$; M2LD < CKD, $p = 0.018$). Therefore, PS-beads had no significant effect on DOC in soil without DMF, but PS-beads could significantly increase DOC and TC in the presence of DMF. Content of DIC in soil was related to size and concentration of PS-beads.

Without DMF, except for the significant increasing of contents of total dissolved nitrogen (TN) in M1L ($p = 0.001$, LSD, $p < 0.05$), M1H, M2L and M2H had no significant effect on TN (Fig. 2). However, in the presence of DMF, M1LD, M2LD and M2HD could significantly reduce soil TN. PS-beads had no significant effect on NO_3^--N in soil without DMF. In the presence of the lesser concentration M2 and DMF, the content of NO_3^--N in soil was significantly greater.

Without DMF, as for the $SUVA_{210}$ representing the amine substance, M1 significantly increased the value of $SUVA_{210}$ while M2 had no significant effect on that compared with CK (Fig. 3 and Table S3). Besides size effects, the values of $SUVA_{210}$ were inversely proportional to concentrations of PS-beads. As for the $SUVA_{254}$, $SUVA_{260}$, $SUVA_{272}$, $SUVA_{280}$, $SUVA_{285}$ and $SUVA_{340}$ which related to the contents of aromatics, hydrophobic C and the humification index (Table S2), they had the same changing trend related to the concentration effect, namely contents of aromatics were significantly greater in the presence of the lesser concentration of PS-beads. DMF had no significant effect on the contents of amines and aromatic substances in soil without PS-beads. PS-beads at different concentrations and of different particle sizes combined with DMF significantly decreased the contents of amines and aromatic substances. There was no significant difference of the $SUVA_{210}$, $SUVA_{254}$, $SUVA_{260}$, $SUVA_{272}$, $SUVA_{280}$, $SUVA_{285}$ or $SUVA_{340}$ values among different treatment groups of PS-beads combined with DMF, indicating the content of amine and aromatic substances was mainly related to the presence of PS-beads, but particle size and concentration had no significant effect on them in soil with DMF. PS-beads and DMF had no significant effect on A_{250}/A_{365} and A_{300}/A_{400} which represented the molecular weight of DOC and the degree of soil aggregation. A_{253}/A_{203} reflects the molecular structure, the degree of the substitution of aromatic ring and its types: low ratio indicating the substituents on the

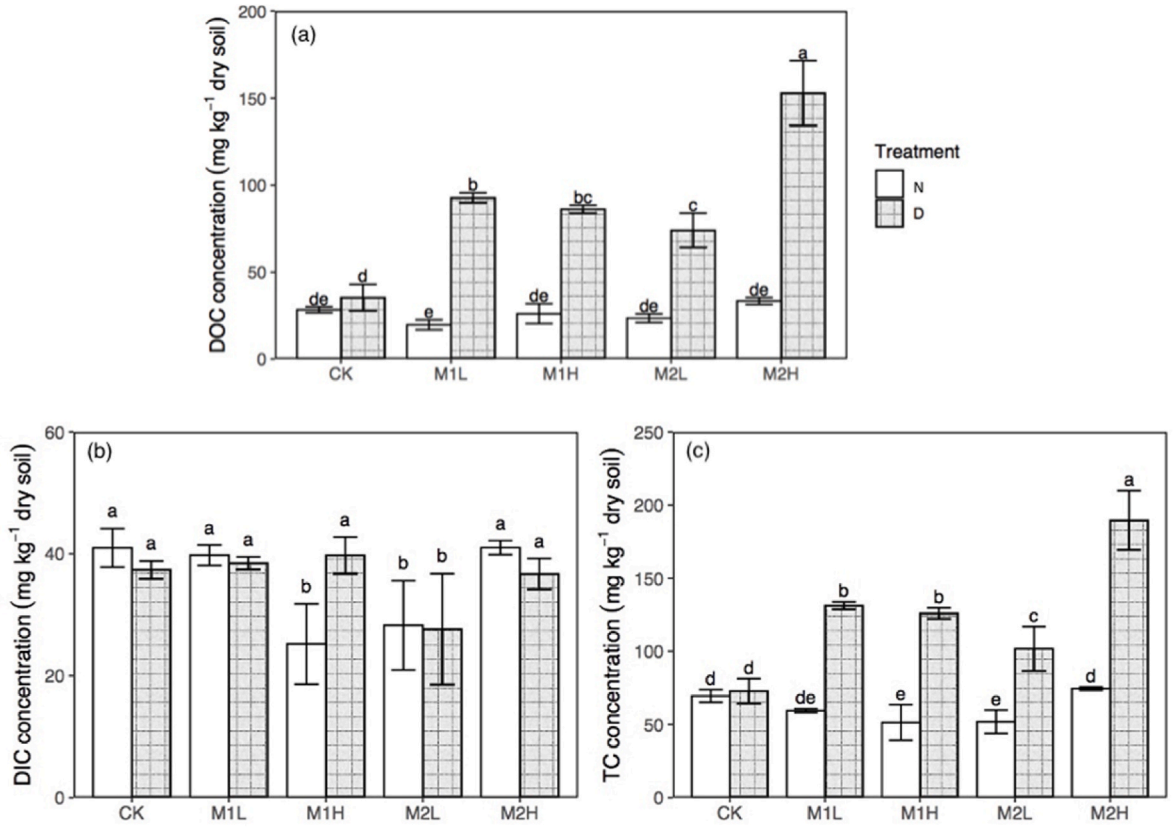

Fig. 1. DOC、DIC、TC concentration of different treatments. (a) DOC concentration; (b) DIC concentration; (c) TC concentration. Different letters mean significant differences and the same letter means no significant difference, Duncan ($p < 0.05$)); N and D indicated adding or no adding degradable film fragments in soil with or without PS; M1L-10 mg·kg^{-1} 5 μm PS , M1H-100 mg·kg^{-1} 5 μm , M2L-mg·kg^{-1} 70 nm PS , M2H-100 mg·kg^{-1} 70 nm PS.

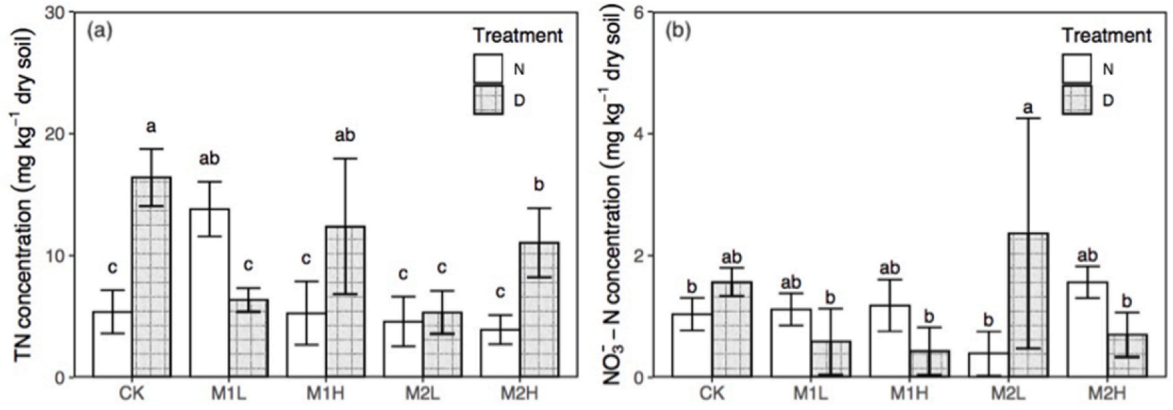

Fig. 2. TN、NO_3^--N concentration of different treatments. (a) TN concentration; (b) NO_3^--N concentration.

aromatic ring are mainly aliphatic chains; high ratio indicating the substituents are mainly carbonyl, carboxyl, hydroxyl and lipid. When there were no DMF in soil, the value of A_{253}/A_{203} in M1 treatment group was less than that of M2, which indicated that the main substituents on the aromatic ring of DOM in M1 were mainly aliphatic chains. In the M2 group, the values of A_{253}/A_{203} were higher than other groups, indicating the contents of carbonyl, carboxyl, hydroxyl and lipid in the substituents on the aromatic ring were greater than the other treatments. The value of A_{265}/A_{465} was to estimate the content of ketones (C═O) of soil DOM. M1LD had the highest A_{265}/A_{465} value which was 2.2407 indicating the potentially highest content of ketones (C═O) in the DOM of M1LD.

3.2. Effects of MPs on plant growth parameters

There was no significant difference on belowground biomass among treatments (Table 1). Except for the M2 at lesser concentration group, the presence of DMF resulted in lesser biomass of root (CKD < CK, M1LD < M1L, M1HD < M1H, M2HD < M2H). DMF also resulted in lesser aboveground biomass. Exposure to combinations of M1LD, M1HD, M2LD or M2HD resulted in greater aboveground biomass of wheat, which was even greater than that of seedlings exposed to CKD. The greatest concentrations of M2 resulted in significantly greater masses of total aboveground biomass (CKD and M2HD, $p = 0.017$; LSD, $p < 0.05$). Compared with CKD, M2HD resulted in 28% greater aboveground biomass and the ratio of total aboveground/belowground biomass

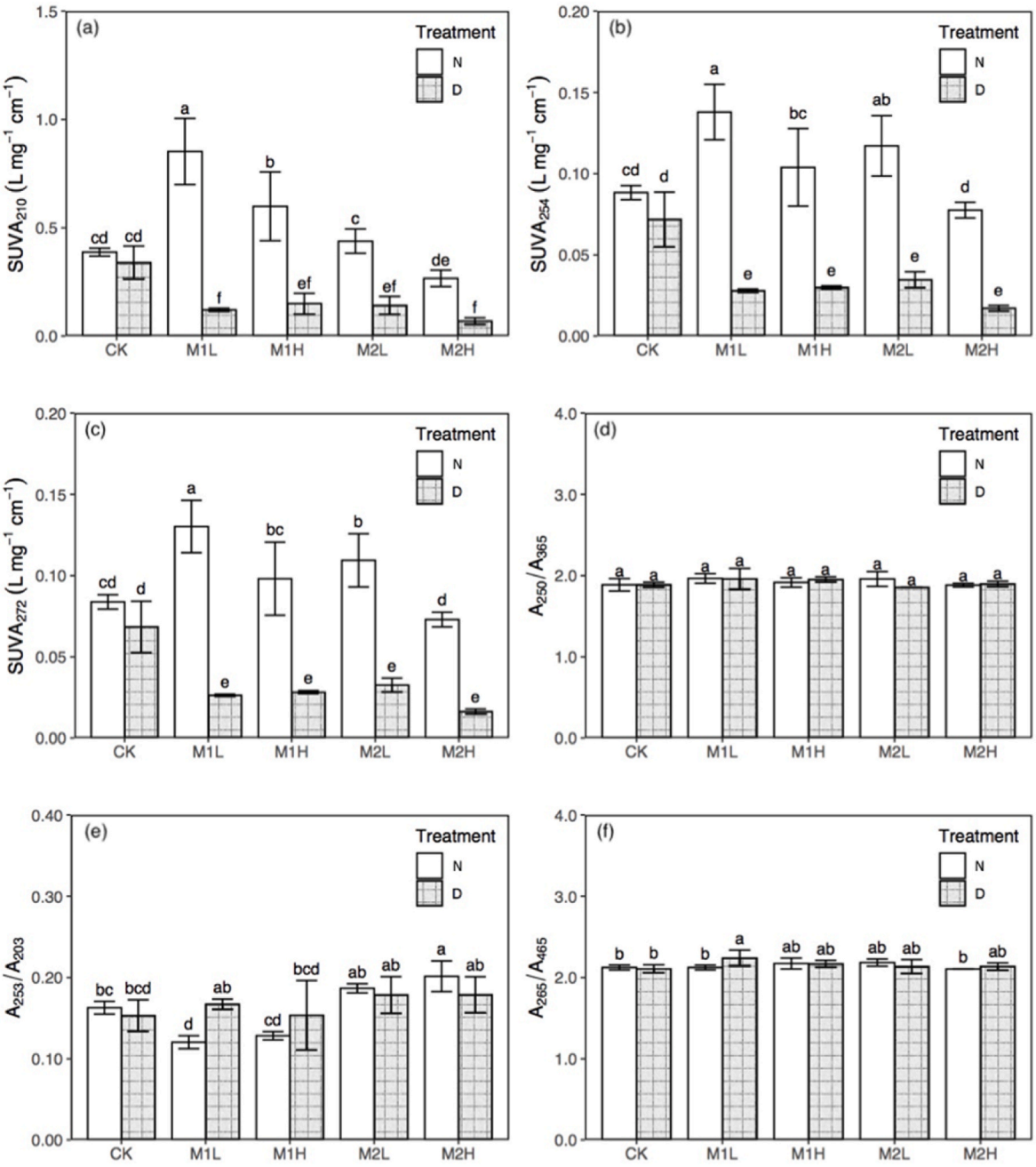

Fig. 3. Specific ultraviolet absorbance (SUVA) at different wavelengths: (a) $SUVA_{210}$, (b) $SUVA_{254}$, (c) $SUVA_{272}$, (d) A_{250}/A_{365}, (e) A_{253}/A_{203} and (f) A_{265}/A_{465} of different treatments.

increased from 1.5 of CK group to 2.03. Plant height is an important trait that influences the yield and sustainability of wheat productions as well as a critical indicator to represent the status of crop growth and nutrient absorption (Jiang et al., 2020). DMF decreased the height of wheat. In the absence of PS-beads, compared with CK, DMF decreased height of wheat by 26% (CKD < CK, $p = 0.004$, LSD, $p < 0.05$). In the presence of DMF, the height of wheat was directly proportional to concentrations of PS-beads. The plant height of wheat was significantly greater in the presence of the greatest concentration of M2 (CKD < M2HD, $p = 0.003$; LSD, $p < 0.05$). Among all treatments, CK resulted in the greatest wheat base diameter. Compared with CKD, both M1LD and M2LD significantly decreased the wheat base diameter (M1LD, $p = 0.007$; M2LD, $p = 0.006$, $p < 0.05$, LSD). With DMF in the soil, PS-beads decreased the wheat base diameter while increased the plant height indicating the PS-beads elongated the wheat seedlings (Table 1).

3.3. Effects of MPs on rhizosphere microbial community

Compared with CK, DMF (CKD) caused significantly change in ($p = 0.004$) height and base diameter of the wheat seedlings ($p = 0.028$; LSD, $p < 0.05$) and biomass was 14% less. Compared with CKD, exposure to M2LD or M2HD resulted in significantly greater aboveground biomass, but less plant height, as well as lesser mean base diameter (see Table 1). The between-subject tests showed that DMF significantly affected height ($F = 10.777$; $p = 0.003$) and basal diameter ($F = 7.344$; $p = 0.011$). Concentrations of MPs had significant effects on the plant mass ($F = 7.786$; $p = 0.009$) height ($F = 10.984$; $p = 0.002$) and basal diameter ($F = 11.485$; $p = 0.002$). Therefore, effects of CK, CKD, M2LD or M2HD on rhizosphere microbial community of wheat seedlings were studied.

Table 1
Wheat seedling biomass of belowground and aboveground as well as plant height and base diameter under the different treatments.

	Total belowground biomass (g)	Total aboveground biomass (g)	Plant height (cm)	Base diameter (mm)	Ratio of aboveground/belowground biomass
CK	0.36 ± 0.01 a	0.54 ± 0.02 abcd	16.05 ± 0.81 abc	2.05 ± 0.02 a	1.50
CKD	0.33 ± 0.11 a	0.46 ± 0.11 d	11.87 ± 3.46 d	1.78 ± 0.15 b	1.39
M1L	0.34 ± 0.07 a	0.55 ± 0.04 abcd	14.15 ± 1.09 bcd	1.45 ± 0.18 c	1.62
M1LD	0.28 ± 0.08 a	0.55 ± 0.04 abcd	14.15 ± 1.09 bcd	1.45 ± 0.18 c	1.96
M1H	0.31 ± 0.13 a	0.64 ± 0.07 a	17.08 ± 0.76 ab	1.68 ± 0.19 bc	2.06
M1HD	0.27 ± 0.04 a	0.53 ± 0.09 abcd	14.55 ± 1.86 abcd	1.63 ± 0.23 bc	1.96
M2L	0.27 ± 0.07 a	0.49 ± 0.04 bcd	14.49 ± 2.39 abcd	1.51 ± 0.15 c	1.81
M2LD	0.33 ± 0.04 a	0.48 ± 0.04 cd	13.72 ± 2.17 cd	1.44 ± 0.16 c	1.45
M2H	0.35 ± 0.10 a	0.60 ± 0.09 ab	17.48 ± 1.17 a	1.78 ± 0.16 b	1.71
M2HD	0.29 ± 0.02 a	0.59 ± 0.11 abc	16.29 ± 2.27 abc	1.54 ± 0.14 bc	2.03

Letters after the same column of numbers indicate that there was significant difference between different treatment groups under the same particle size ($p < 0.05$), the same below.

3.3.1. *Effects of MPs on diversity of microbes*

Coverage rates of sample libraries measured in this study was more than 95%, which indicated that sequencing results accurately represented samples (Figs. S2 and S3). Compared with CK, CKD, M2LD or M2HD all resulted in significantly less diversity (Shannon index) of the microbial community. The Shannon index of CKD group was least, which indicated that presence of DMF could reduce diversity of the microbial community. The Simpson index also showed that CKD, M2LD and M2HD reduced α-diversity of the soil microbial community. The trend in the ACE index was consistent with that of Chao Index. CKD and M2LD decreased both the ACE and Chao indices of bacteria, while M2HD increased them, which indicated CKD and M2LD both decreased richness of the bacterial community, while M2HD increased diversity. Compared with CK, CKD, M2LD and M2HD all decreased the ACE and Chao indices of the fungal community, and there were significant differences between M2LD and M2HD. The OTUs of bacteria and fungi were 1407–1610 and 286–422, respectively. The total number of OTUs were 1061 and 179, respectively. The number of OTUs of bacteria and fungi in M2HD was the greatest among the four treatments. Therefore, although diversity of the bacteria community in the rhizosphere was less in the presence of DMF, M2HD could increase richness of the microbial community and the number of OTUs. These results indicated that, due to the decreasing of soil bacterial evenness, the diversity of bacterial communities was significantly reduced at the high concentration of M2 and DMF.

3.3.2. *Effects of MPs on microbial community structure*

PS-beads and DMF did not change dominant bacteria at the level of phyla. *Actinobacteria* was the dominant taxa in the rhizosphere bacterial community in various treatments (Fig. 4). Compared with CK, which had an abundance of *Actinobacteria* of 50.51%, CKD and M2HD reduced abundance of *Actinobacteria* to 49.91% and 47.01%, respectively, while M2LD increased abundance of *Actinobacteria* to 57.50%. Therefore, abundance of *Actinobacteria* in soil was related to concentrations of PS-beads. In addition to *Actinobacteria*, change in abundance of *Verrucomicrobia* was also related to concentrations of PS-beads, which demonstrated the decreasing trend under lesser concentrations and greater at greater concentration in order of M2LD < CKD < M2HD). CKD, M2LD and M2HD decreased abundances of *Proteobacteria*, but the abundance was greater at great concentrations of PS-beads (M2HD > M2LD). After adding DMF, the abundance of *Patesscibacteria* increased significantly from CK (0.55%) to CKD (1.89%), M2LD (1.36%) and M2HD (7.89%). Compared with CK (5.93%), CKD increased the abundance of *Bacteroidetes* to 9.66%, while M2LD and M2HD decreased its abundance to 3.99% and 3.86%, respectively. CKD reduced abundance of *Cholroflexi* (1.44%), compared with CK (2.85%). M2LD and M2HD increased its abundance to 2.05% and 2.78%, respectively.

DMF and PS-beads did not change dominant species of fungi at the level of phyla. Ascomycota was the dominant species in the four treatments. The abundance of *Ascomycota* (98.28%) of CKD was significantly greater than that of CK (80.21%). Compared with CKD, M2LD and M2HD increased abundance of *Ascomycota* to 90.01% and 89.83%, respectively. It has been shown that some species in *Ascomycota* could secrete class I proteins, which is fairly insoluble in aqueous solution, so that it forms functional amyloid fibers that arrange into rod shapes, or class II protein, which is more soluble, and played a role in degradation of fiber or plastic (Sánchez, 2020). DMF significantly decreases abundance of *Basidiomycota* from 15.04% in CK to 0.75% in CKD, 3.19% in

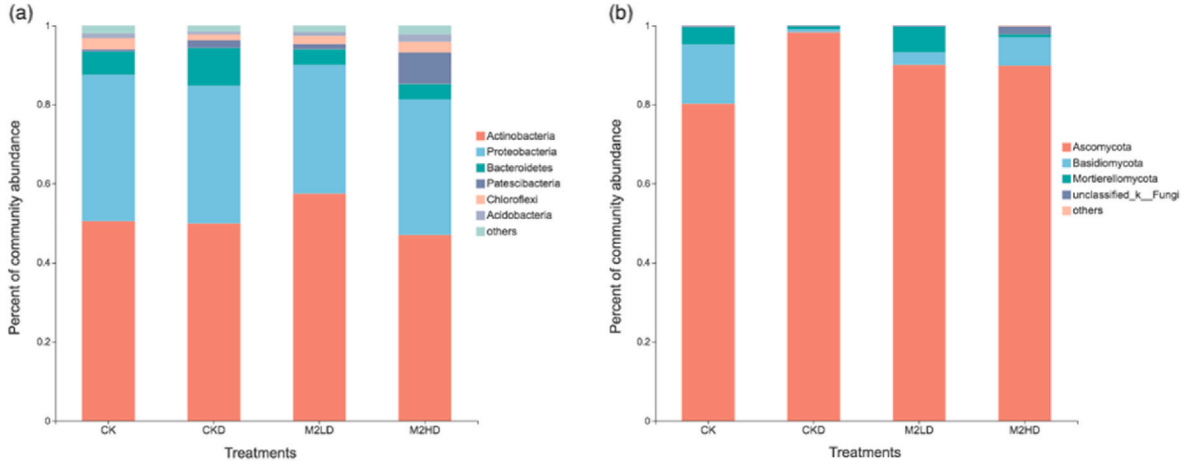

Fig. 4. Community composition at the phylum level (a) bacteria, and (b) fungi.

M2LD and 7.16% in M2HD. In soil with DMF, abundance of *Basidiomycota* increased in direct proportion to concentrations of PS-beads. In addition, CKD and M2HD significantly reduced abundance of *Mortierellomycota* from 4.34% in CK to 0.81% in CKD and 0.86% in M2HD, while M2LD increased the abundance to 6.53%.

At the genera level of bacteria, among the four treatments, *Nocardioides*, *Arthrobacter* and *Marmoricola* had greater abundance (Fig. S4). *Nocardioides* was the dominant species. CKD and M2HD decreased the abundance of *Nocardioides*, but M2LD increased it. Compared with CK, the abundance of *Arthrobacter* in CKD, M2LD and M2HD was significantly greater. Results of previous studies have shown that *Arthrobacter* can degrade some plastics (Raddadi and Fava, 2019; Yuan et al., 2020). Therefore, the presence of DMF and PS-beads resulted in greater masses of the microorganism *Arthrobacter* on roots. CKD resulted in greater abundance of *Marmoricola,* but M2LD and M2HD resulted in less. Phthalate can accumulate *Marmoricola* (Zhu et al., 2019). In this study, the abundance of *Marmoricola* was greater in CKD, which indicated that there might be phthalate additives in the mulch film. Alternatively, abundance of *Marmoricola* was less in the presence of DMF and PS-beads together in soil, which might be due to the change of microbial interspecific relationship. Compared with CK, the presence of DMF (CKD, M2LD, M2HD) decreased *Massilia*, *Sphingomonas*, *Lysobacter*, *Limnobacter*, *Promicromonospora*, *Altererythrobacter*, *Qipengyuania*, *Streptomyces*, *Microbacterium*, *g_norank_o_Microtrichales*, *Ohtaekwangia*, *g_norank_c_Gitt-GS-136* and *Kribbella*, which indicated that residues of DMF changed composition of the microbial community.

Changes in structures of bacterial communities were related to the presence of PS-beads in soil. Both M2LD and M2HD decreased the abundance of *Devosia*, *Allorhizobium*, *Brevundimonas*, *Pedobacter*, *Promicromonospora*, *Ensifer* and *Sphingobacterium*, increased the abundance of *Hydrogenophaga* and *Acidovorax*. Moreover, change in the microbial community were related to concentrations of PS-beads. Different concentrations of PS-beads caused different effects on *Cellvibrio*, *Ramlibacter*, *Rhodococcus* and *Rhizobacter*. M2HD increased abundances of *Rhodococcus* and *Rhizobacter*. M2LD caused greater abundances of *Rhodococcus* and *Rhizobacter*.

When added alone, neither DMF nor PS-beads changed the dominant fungi determined at the level of phyla, but when present together, the combined effects of DMF and PS-beads changed the dominant fungi at the level of genera (Fig. S4). Those results showed that CKD increased abundance of *Fusarium*. In CK and M2HD, *Blastobotrys* exhibited the greatest abundance and in M2LD, *Alternaria* was most abundant. Some fungal species in the genus *Fusarium* are pathogens that wheat scab (De Corato et al., 2020; Gqozo et al., 2020). *Blastobotrys* and *Alternaria* were significantly affected by PS-beads. Some species in *Blastobotrys* could resist plant disease caused by *Verticillium* (Papasotiriou et al., 2013). *Alternaria* is also a pathogen (García-Calvo et al., 2018), causing the weak growth of wheat. In addition to changes of the dominant fungi, after adding DMF and PS-beads, the combined effects also had the selective effects on specific fungi. Compared with CK, CKD, M2LD and M2HD could all significantly reduce abundances of *Guehomyces*, *Kockovaella*, *Holtermanniella*, *Thermomyces*, *Cutaneotrichosporon*, *Pseudocercospora*, *Acremonium*, *Trichoderma*, *Occultifur* and *Pyrenochaeta*. M2LD and M2HD increased abundances of *Meyerozyma*, *Pseudogymnoascus*, *Penicillium*, *Mortierella*, *Phialemoniopsis* and *Neosetophoma*. Abundances of fungi were also related to concentrations of PS-beads. M2HD increased the abundance of *Talaromyces*, while M2LD increased abundance of *Lectera*. Therefore, concentrations of PS-beads also affected the structure of the fungal community.

3.3.3. Statistical analysis on microbial community structure

Results of a cluster analysis showed that structures of the bacterial communities of M2LD and CKD were similar (Figure S4 (A)). CKD significantly changed the bacterial community compared with CK. This result was consistent with results of a PCoA analysis, based on Bray-Curtis distance (Fig. 5 (A)). PCoA results showed that the confidence ellipses of M2LD and CKD overlapped, which indicated that compositions of the microbial communities in M2LD and CKD were similar, based on classification at the OTUs level. Results of the Adonis test (permutation test by 999) also showed that there were significant differences in structures of bacterial communities among treatments. To further investigate the significance of these changes on genera, a Kruskal-Wallis test was conducted based on the 50 most abundant bacteria (Figure S5 (A)). The results showed that the DMF increase abundances of *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Devosia*, *Pedobacter* and *Sphingobacterium*. M2LD caused significantly greater abundance of *Noviherbaspirillum*, while M2HD significantly decreased *Noviherbaspirillum*, which indicated that *Noviherbaspirillum* was affected in a concentration-dependent way by PS-beads in soil that included DMF. In the presence of DMF, PS-beads also affected *norank_f_norank_o_Saccharimonadales*, *Cellvibrio*, *Ramlibacter*, *Hydrogenophaga*, *Limnobacter* and *Bradyrhizobium* in soil in a dose-dependent way.

The cluster analysis results of Figure S4 (B) showed that the structure of the fungal communities in M2HD and M2LD were similar, and that in CKD was significantly different from the others. PCoA results (Fig. 5 (B)) show that, the first axis separates CK, M2HD and M2LD from CKD. The confidence ellipses of CK, M2HD and M2LD coincided, indicating the compositions of fungal communities were similar for these three treatments, but the composition of CKD community was quite different from

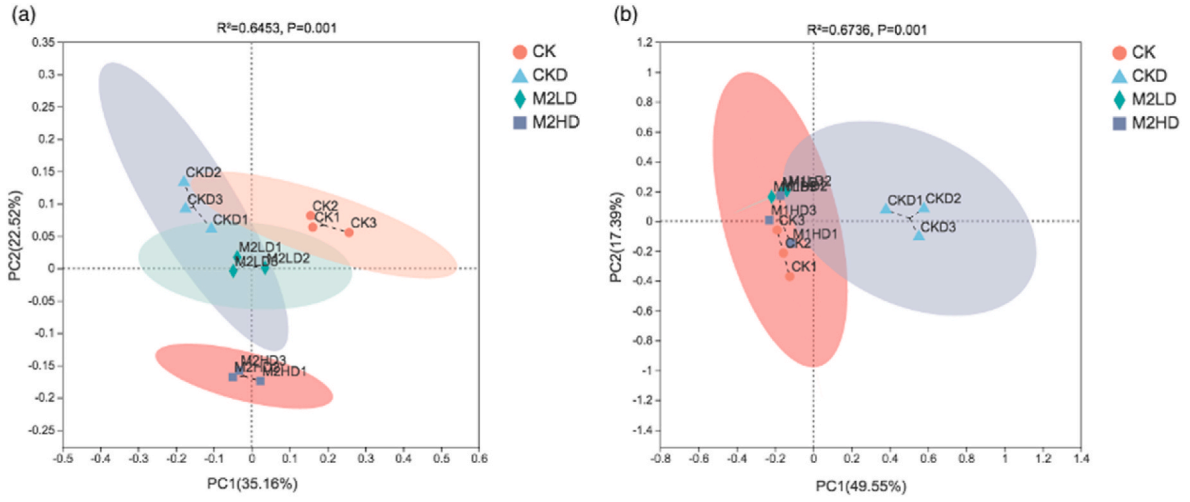

Fig. 5. Principal coordinate analysis (PCoA) based on bray-curtis distance (OTU level): (a) bacteria, and (b) fungi. (Adonis test and permutation test by 999).

those of the other three treatments. The results of an Adonis test also showed that there were significant differences in structures of microbial communities among treatments. The fungi, *Fusarium*, *Blastobotrys*, *Alternaria* were affected differentially (Kruskal-Wallis of fungi) among treatments (Fig. S5B). DMF caused significantly greater abundance of *Fusarium*. Therefore, DMF and PS-beads could increase abundance of pathogens to wheat seedlings, which could pose a threats to agricultural production. M2LD and M2HD resulted in significantly greater abundance of *Meyerozyma*, a potential probiotic (de Melo Pereira et al., 2018).

In conclusion, the combined effect of different sources of MPs had the selective effect on specific microorganisms, thus potentially affecting the function and metabolism of microbial community. This could in further affect the soil physical and chemical environment and soil organisms.

3.4. Potential relationship among plant growth, environmental factors and microbial communities

The decreased height of wheat seedlings might be due to DMF hindering absorption and metabolism of nitrogen in the rhizosphere, which potentially affects adsorption of nutrients by seedlings. Without DMF, the effect of PS-beads on height of wheat seedlings is concentration-dependent, which might be related to effects of particle size and concentration on soil DIC. Different particle sizes and specific surface areas of PS-beads could result in different adsorption of PS-beads on DIC. The changing of wheat basal diameter caused by PS-beads was concentration-dependent. Plant height and basal diameter were related to the DMF and concentrations of PS-beads. Masses of wheat seedling was related to concentrations of PS-beads. In soil without DMF, small concentrations of PS-beads decreased total masses of wheat seedlings, while greater concentration increased values. In soil with DMF, both lesser and greater concentrations increased total masses with the greater concentration PS-beads causing significant effects. Results of the study reported here are consistent with results of previous where a concentration of 1% film fragments significantly inhibited growth of wheat (Qi et al., 2018).

Changes in soil DOC might further affect the structure, function and metabolism of microbial communities, which can, in turn, affect microbial metabolic function (Rédei, 2008). Results of KEGG analyses illustrated that MPs could change metabolic functions of microbial communities, thus further affecting cycling of nutrients in soil and ultimately growth of plant (Fig. S6). At level 1, compared with CK, CKD, M2LD and M2HD all significantly increased “Environmental Information Processing”, “Genetic Information Processing” and “Metabolism” ($p = 0.005$, $p = 0.021$, $p = 0.012$, LSD, $p < 0.05$). With DMF (CKD, M2LD, M2HD), the “Cellular Processes”, “Environmental Information Processing”, “Genetic Information Processing”, “Metabolism” and “Biological System” of bacterial communities were significantly correlated with concentrations of PS-beads. Compared with CKD and M2LD, M2HD significantly inhibited all five of these functions. At level 2, the comparison of KEGG indicated that various treatments affected pathways differently (Fig. S6B). Compared with CK, CKD significantly promoted “Carbohydrate metabolism” ($p = 0.007$), “Amino acid metabolism” ($p = 0.026$), “Global and overview maps” ($p = 0.007$), “Metabolism of cofactors and vitamins” ($p = 0.007$), “Membrane transport” ($p = 0.009$) and “Nucleic acid metabolism” ($p = 0.004$). The main functions of level 2 were also related to concentrations of PS-beads. In CKD and M2LD, bacterial diversity was less, but basic metabolic function was greater, which was potentially due to the selective effect on the microbes owing to specific metabolism functions.

Change in the bacterial community might also change cooperation or competition among bacteria. With DMF (CKD), the total below-ground and above-ground biomass, plant height and basal diameter of wheat seedlings were less than those of CK, which might be due to occurrence of *Fusarium*, which is the dominant genus in the rhizosphere. Mean basal diameter of wheat was significantly less in M2LD, which was the least of the four treatments. This might be due to greater abundance of *Alternaria* in M2LD. However, the effect of M2HD on below-ground biomass and mean basal diameter of wheat might be due to absorption at the greatest concentration of nano-PS condition. As shown in previous studies, in wheat, M2 could be transported from the root to the stem and leaf (Lian et al., 2020). The lesser under-ground biomass and mean basal diameter might be due to toxic effects of M2.

4. Conclusions

This research presented here focused on effects of multiple combinations of MPs on soil DOM, survival and growth of seedlings of wheat and their associated rhizosphere microbes. Combined effect of DMF and PS-beads resulted in lesser α-diversity of soil microbes and had selective effects on specific microbes, which could influence growth of wheat seedlings. Though at the genus level, DMF and PS-beads did not change the dominant bacteria, they did have significant effects on the dominant fungi. The structures of the bacterial communities of M2LD and CKD were similar. The dominant fungus in CKD *Fusarium*, could cause Wheat Scab, subsequently decreasing the wheat growth index. The dominant group in the M2LD *Alternaria* was also a pathogen to wheat, weakening growth. CK and M2HD had the greatest abundance of *Blastobotrys*, which could help wheat seedlings to resist Verticillium disease. Cluster analysis and PCoA analysis showed that combined effects of various MPs from various sources could change the microbial community composition, indicating the combined effects of MPs could adversely affect crops.

Author contribution statement

Xinwei Ren conducted the studies and prepared the original draft of the manuscript, Jingchun Tang designed the experiment and prepared parts of the manuscript and revised the manuscript, Lan Wang did data analyses, Hongwen Sun and John Giesy wrote the final draft and edited the manuscript for English grammar and syntax.

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118516o.

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