



# Microplastics in soil-plant system: effects of nano/microplastics on plant photosynthesis, rhizosphere microbes and soil properties in soil with different residues

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## Abstract

**Aims** To investigate the effects of polystyrene microplastics (PS-beads) on the soil properties, photosynthesis of Flowering Chinese cabbage, the rhizosphere microbial community and their potential correlation in soil with different residues.

**Methods** The influences of PS-beads (PS-MPs, M1, 5 µm; PS-NPs, M2, 70 nm) on the plant photosynthesis and growth parameters, soil dissolved organic matter (DOM) and the characteristic functional groups, the microbial community and metabolism prediction were studied by a pot-experiment in soil without residues (N), with biochar (B), degradable mulching film (DMF) fragments (D), or biochar and DMF (BD).

**Key results** Chlorophyll a was more susceptible to the exogenous substances than Chlorophyll b. In soil with different residues, PS-beads of different sizes could change different components, structures and functional groups in aromatic rings of DOM, might further change the microbial community and metabolism. M2

decreased TDN and  $\text{NO}_3^-$  and increased the weight of the plant in group D. M2 increased the weight of the plant in group N. M2 decreased the net photosynthetic rate in group B. The different sizes of PS-beads affected the different parameters of plant growth and potentially changed the plant growth and photosynthetic parameters through altering the microbial metabolism and the correlation among microbes. The potential mechanisms of PS-beads changing the plant growth were different in soil with different residues.

**Conclusions** Our results evidenced the PS-beads potentially changed the plant growth and photosynthesis by changing the microbial metabolism and the correlation among microbes.

**Keywords** Degradable mulching film fragments · Biochar · Microplastics · Photosynthesis · Rhizosphere microbes · PICRUST 2

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## Introduction

Soil is the survival foundation for organisms. Research on soil pollution is one of the key issues in biogeochemical cycle of terrestrial ecosystem (Chae and An, 2018). The total global plastic production has increased from 2 million tons in 1950s (Shen et al., 2020) to 348 and 359 million tons in 2017 and 2018, respectively (PlasticsEurope, 2019). Featured by low cost and durability, plastics are widely used in the industry and our life, leading to the discharge of plastic waste at an alarming rate (Geyer et al., 2017). Most of the plastic waste ends up in the landfill and is difficult to be degraded, resulting in gradually accumulating in the environment (Wright and Kelly, 2017). Under the effects of UV, high temperature and soil abrasion, or by the process “ingestion-excretion” of soil fauna, the larger plastics break into smaller pieces, namely microplastics (MPs) (Barnes et al., 2009; Rillig, 2012). MPs was first proposed by Thompson (Thompson, 2004), referring to the plastic of the particle size <5 mm (Arthur et al., 2008) or 1 mm (Claessens et al., 2011). MPs are easy to be swallowed by organisms, and accumulated in the food chain (Rillig, 2012). Moreover, MPs could adsorb pollutants or microorganisms on the surface (Law and Thompson, 2014), posing severely negative effects on the ecosystem (Scheurer and Bigalke, 2018).

One major source of MPs in soil is the mulching film such as polyethylene (PE) film featured by the long-life cycle while only used for one growing season. The residues are hard to be recovered and dealt with, therefore accumulated in the field. Besides film fragments, MPs could also source from landfill (He et al., 2019), soil conditioner using (Zubris and Richards, 2005), sludge application (Corradini et al., 2019; Li et al., 2018), wastewater irrigation (Gündoğdu et al., 2018), compost and organic fertilizer application (Weithmann et al., 2018). Polystyrene (PS) accounts for 7.1% of the world's total plastic production (Wu et al., 2017) and exists in products for daily use (Mahon et al., 2017), such as plastic beads in cosmetics (Cole et al., 2011), resulting in wide spreading in wastewater and sewage sludge (Ngo et al., 2019). The concentration of MPs in sewage sludge from wastewater irrigation could reach 15,385 particles  $\text{kg}^{-1}$  and might be brought into farmland (Nizzetto et al., 2016).

Our previous research illustrated that PE-MPs could influence the soil dissolved organic carbon (DOC)

composition and the microbial community (Ren et al., 2020). This might further threaten the rhizosphere microbes and the physiological characteristics of plants. Recent study showed that MPs could significantly affect plant properties and functions by changing soil structure and water content (de Souza Machado et al., 2019). Moreover, MPs in the soil also affected the competition of different plant species (Kleunen et al., 2019), indicating that MPs might potentially threaten the biodiversity in terrestrial environments. The negative effect of MPs was also related to its sorption feature (Koelmans et al., 2016) because the synergistic effect with pollutants could enhance the toxicity of MPs or pollutants. Several studies has shown that the synergistic effect of MPs and pollutants such as dibutyl phthalate or As (III) could aggravate the damage of pollutants to the plants, negatively affecting the growth parameters, photosynthesis and the chlorophyll contents of the plants (Dong et al., 2020; Gao et al., 2019; Liu et al., 2019). These results provide evidence for the combined effect of MPs and pollutants with more toxic effects on plant, directly.

Owing to the possibility of replacing PE film in agriculture (Chek et al., 2017; Sander, 2019), degradable mulching film (DMF) have been widely concerned by researchers (Bandopadhyay et al., 2018; Brodhagen et al., 2014; Steinmetz et al., 2016) though there was doubt about degradable film (Brodhagen et al., 2014). DMF could significantly inhibit the growth of wheat, affect the bacterial community and the volatiles in rhizosphere (Qi et al., 2020). Polylactic acid (PLA) MPs inhibited seed germination, significantly reduced seedling height (Boots et al., 2019). Besides DMF, researchers also found that the PS-beads showed size and concentration effects on the growth of different plants and the diameter might be the key determinants for micro/nanoplastics to penetrate the tissues of the plants. As for the 100 nm PS-beads, except for increasing the wheat biomass and the metabolism (Lian et al., 2020), it also could be accumulated in *V. faba*'s roots and block cell connections or cell wall pores, affecting the nutrients transportation (Jiang et al., 2019). Li et al. (2019) indicated that 0.2  $\mu\text{m}$  PS-beads could be enriched in the roots of lettuce and migrated from the roots to the aboveground parts, accumulated and distributed in the stems and leaves. Liao et al. (Liao et al., 2019) showed 10  $\text{mg kg}^{-1}$  PS-beads (100 nm and 5  $\mu\text{m}$ ) could reduce the root length, 100 nm increased the seedling height while 5  $\mu\text{m}$  decreased that. These results directly illustrated the accumulation and transportation

of MPs in soil-plant system, indicating the potential possibility of MPs entering into the food chain (Jassby et al., 2019). Li et al. (2020) further indicated that the mechanism of submicrometre- (0.2  $\mu\text{m}$ ) and micrometre-sized (2  $\mu\text{m}$ ) plastic particles penetrating the plants was related to the crack-entry pathway of the lateral root emergence sites and features of the polymeric particles leading to the efficient uptake of submicrometre plastic.

Biochar application in farmland could potentially improve the soil quality and plant productivity. Due to its highly aromatic and porous features, biochar could increase soil pH, soil carbon content and nutrient retention (Yi et al., 2020). At present, most studies paid attention to the effects of the single MPs source. While the farmland soil is a complex mixture of exogenous substances.

Microplastics (MPs) are emergent pollutant in soil-plant system. Biochar and degradable mulching film (DMF) fragments were common residues in farmland. While the response of the soil-plant system to the combined effects of MPs and different residues is still unclear. Therefore, this study was carried out to illustrate how plant-microbe-soil system response to exogenous materials to contribute to a more comprehensive understanding of the mechanisms. In our study, two particle sizes PS-beads were selected to simulate the MPs from sludge application and wastewater irrigation; the degradable mulching film (DMF), mainly composed of PLA and Polybutylene adipate-co-terephthalate (PBAT), was used to simulate the mulch residues; sawdust biochar was selected, so as to simulate the multimedia soil. The plant used in this study was the Flowering Chinese cabbage. Our research tried to make clear the combined effect of biochar and MPs on the plant growth, photosynthetic activity and microbial community in rhizosphere of the plant.

## Materials and methods

### Soil and experiment site

The clay soil used in this study was collected from a reserved field without any known direct pollution, Houge Village, Beichen District, Tianjin, China. Before use, stones and plant residues were removed. After soil samples were air dried at room temperature, the dried soil was sieved through a 2 mm steel sieve and mixed

thoroughly for the subsequent microcosm experiment. The pot experiment was carried out in the greenhouse at College of Environmental Science and Engineering, Nankai University, Tianjin, China.

### Microplastics and biochar

Two types of polystyrene microplastics (PS-beads) were used in the present study. PS-beads (70 nm, PS-NPs and 5  $\mu\text{m}$ , PS-MPs) was purchased from Tianjin Baseline ChromTech Research Centre (Tianjin, China, product number No.6-1-0007 and No.6-1-0500, respectively). The degradable mulching film was obtained from an agricultural products store, composed of PLA and PBAT. The film was cut into small pieces by using sharp blades and scissors and the length and width were  $\sim 4.5$  mm (Qi et al., 2018). The biochar used in this study was poplar woodchips biochar heated at 500  $^{\circ}\text{C}$  in a muffle furnace for 3 h. The preparation of PS-beads solution and biochar as well as the characterization of PS-beads and biochar were listed in the supplementary material Text S1.

### Treatments and replicates

Twelve treatments were divided into four groups: biochar (B) and degradable mulching film fragments (DMF) were added to each group, respectively (see Table 1). CK: soil without PS-beads; M1: soil with PS-MPs-5  $\mu\text{m}$ ; M2: soil with PS-NPs-70 nm. The concentration of PS-beads was 10  $\text{mg}\cdot\text{kg}^{-1}$  soil. The information on selecting the concentration of PS-beads and residues in the study was described in the Supplementary material (Text S2 and Table S1).

Polypropylene (PP) pots were used in the experiment, with upper diameter 10 cm, lower diameter 7.6 cm, height 10 cm. PS-beads, and DMF and biochar were added to soil samples according to the amount listed in the Table 1. The required amount of materials was spiked in 400 g tested soil for each pot, evenly mixed, then placed in PP pot and balanced for one week after adding water. The prepared pots with soil were placed in the greenhouse. The temperature and the humidity were the same as them in pre-experiment (see Text S2), with day/night photoperiod of 16/8 h. The pots were sterilized before use.

Flowering Chinese cabbage (*Brassica rapa* syn. *Campestris* L. ssp. *Chinensis* var. *utilis* Tsen et Lee) was used as the tested plant. The seeds of the same size

**Table 1** Treatments with their corresponding group names

Group setting	CK	M1	M2
N group - No adding	CKN	M1N	M2N
B group - 1% biochar adding	CKB	M1B	M2B
D group - 1% DMF adding	CKD	M1D	M2D
BD group - 1% biochar and 1% DMF	CKBD	M1BD	M2BD

and color were selected (the germination rate was tested by pre-experiment). After sterilization with 3% H<sub>2</sub>O<sub>2</sub> for 20 mins, the seeds were washed with distilled water for three times, and the water on the surface of the seeds was absorbed by filter paper. The seeds were sowed in the pots, and the seedlings were thinned to 5 plants in each pot at the stage of two cotyledons and one carpel. Each treatment was carried out in four replicates.

### Sample collection

Twenty-one days after sowing, the photosynthetic gas exchange parameters and the content of chlorophylls were determined (see section 2.3), the biomass and other physiological indexes of cabbage leaves were measured (see section 2.4). Rhizosphere soil samples were collected after gently shaking the roots to remove the loosely adhered soil and stored at -20 °C for further analysis. After harvesting, bulk soil was sampled from pots for analyzing soil physical and chemical properties, dissolved organic matter (DOM), and functional groups (see Table S5).

### Analysis of soil properties

Soil DOM and related functional group characteristics were determined according to the method described in the previous studies (Jaffrain et al., 2007; Ren et al., 2020). The extracts were analyzed by multi N/C 3100 (Analytik Jena AG, Germany) for DOM. The functional groups of the subsamples were measured by UV-Vis spectrophotometer (LAMBDA-35, PerkinElmer, USA). UV-Vis absorption from 200 to 500 nm (1 nm steps) was measured in a 10-mm quartz cuvette with deionized water as blank. The specific UV absorbance at 210, 250, 254, 260, 265, 272, 280, 285, 300, 340, 350, 365, 400, 436, and 465 nm were measured for all samples. Detailed information, the wavelengths used in this study and their corresponding organic functional groups were shown in Table S2. The soil NO<sub>3</sub><sup>-</sup>-N was determined by

UV-spectrophotometry (TU-1810DASPC, PERSEE, Beijing, China) according to the method GB/T 32737–2016. Detailed method for extracting DOM, NO<sub>3</sub><sup>-</sup>-N determination and equation for calculating NO<sub>3</sub><sup>-</sup>-N were shown in supplementary material Text S3, Eq. (4).

### Photosynthetic activity parameters and chlorophyll content

Leaf photosynthetic gas exchange parameters were measured using LI-6800 Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, NE, USA). The net photosynthetic rate (P<sub>n</sub>, μmol m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (G<sub>s</sub>, mol m<sup>-2</sup> s<sup>-1</sup>), intercellular carbon dioxide concentration (C<sub>i</sub>, μmol mol<sup>-1</sup>) and transpiration rate (Tr, mmol m<sup>-2</sup> s<sup>-1</sup>) were measured under an ambient light source (190 μmol m<sup>-2</sup> s<sup>-1</sup>) in greenhouse. The instantaneous water use efficiency (WUE) and the limiting value of stomata (L<sub>s</sub>) were also used to illustrate the photosynthetic parameters of plants. The chlorophyll a, chlorophyll b and the total chlorophyll were analyzed according to the method of Ren et al. (2012) using an UV-Vis spectrophotometer (TU-1810DASPC, PERSEE, Beijing, China). The detailed information of LI-6800 settings and method of chlorophyll determinations, the equations for calculating WUE, L<sub>s</sub>, chlorophyll a and b, as well as total chlorophyll were listed in the supplementary material Text S4, Eqs. (5), (6), (7), (8), (9).

### Measurements of plant growth parameters

Three seedlings from each group were washed with distilled water and dried. After the seedlings being weighed, the seedlings were placed in the oven for 15 mins at 105 °C, followed by 80 °C until constant weight was obtained and weighed again (dry weight). The water content of the seedlings was calculated according to the following Eqs. (1):

$$\text{Water content (\%)} = \frac{(\text{Fresh weight} - \text{dry weight}) \times 100\%}{\text{Fresh weight}} \quad (1)$$

### DNA extraction, PCR and 16S rRNA sequencing

Microbial community genomic DNA was extracted from soil samples using the FastDNA® Spin Kit (MP

Biomedicals, Santa Ana, Ca, USA) according to manufacturer's instructions. The 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 primers used in this study were listed in Table 2. 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). The detailed PCR method and bioinformatic analysis were listed in the supplementary material Text S5 and Text S6.

#### Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 24.0. One-way ANOVA was used to determine the effect of different treatments on soil properties and physiological indicators of plants. The means of significant effects at  $p < 0.05$  were then compared using the Duncan's multiple-range test. Operational taxonomic unit (OTU)-level alpha diversity indices, Chao1 (Chao, 1984), abundance-based coverage estimators (ACE) (Chao and Yang, 1993) and Shannon (Shannon, 1948) were calculated using the OTU table in Mothur. 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). Network analysis was based on spearman's rank correlation calculating by RStudio and visualized by Gephi 0.9.2.

## Results

Effects of PS-beads on dissolved organic matter (DOM) and relative functional group characteristics in soil with different residues

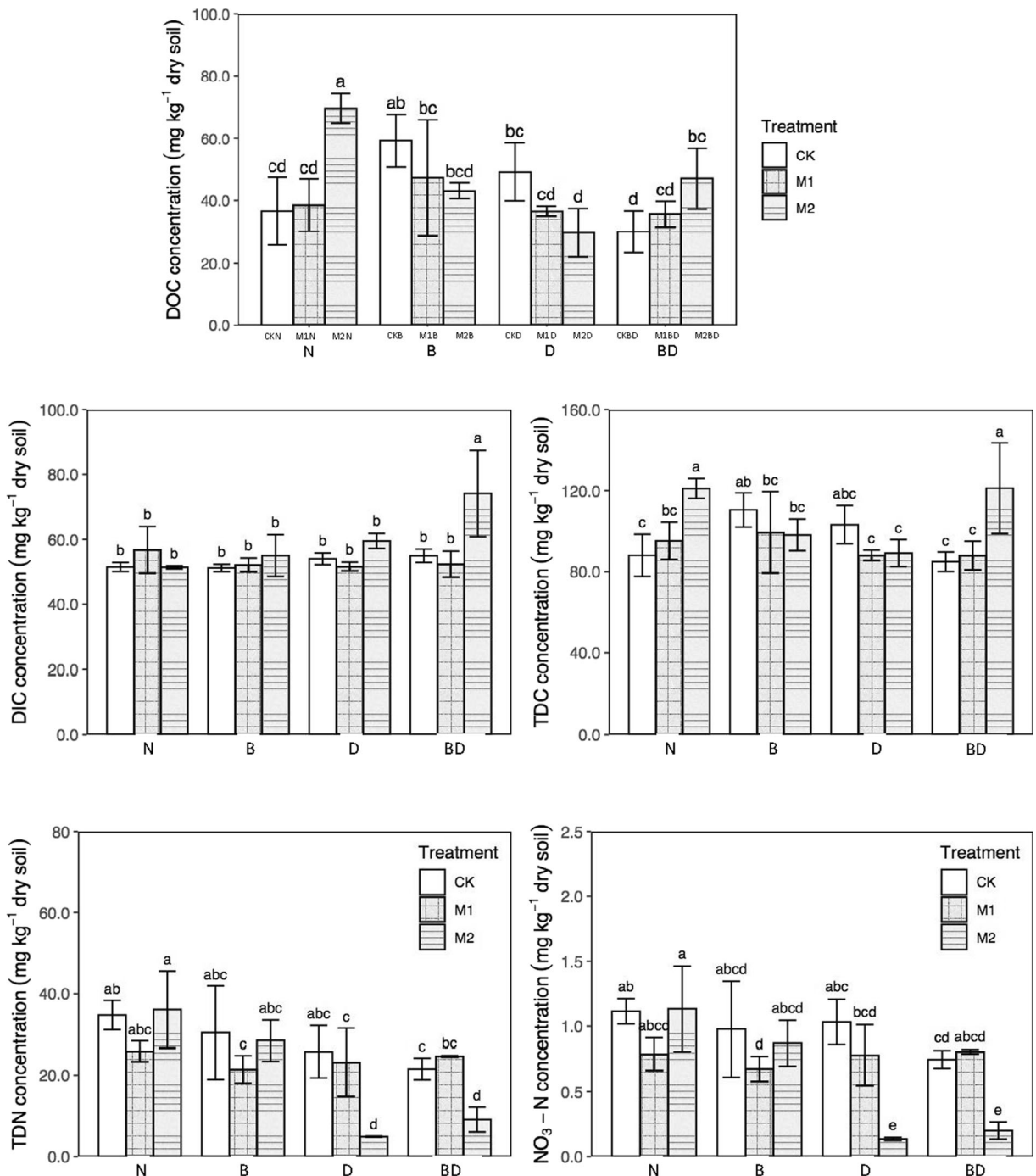
The effects of different particle sizes of PS-beads on the total dissolved carbon (TDC), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total dissolved nitrogen (TDN) and  $\text{NO}_3^-$ -N in soil with different residues were shown in Fig. 1. The contents of DOC and TDC had the similar changing trend. In group N, B, D and BD, PS-MPs (M1) had no significant influence on soil DOC, DIC, TDC, TDN and  $\text{NO}_3^-$ -N. In group N and BD, PS-NPs (M2) significantly increased the content of TDC compared with CK (N,  $p = 0.001$ ; BD,  $p = 0.000$ , LSD,  $p < 0.05$ ). The increasing of TDC in group N was attributed to the increasing of DOC ( $p = 0.000$ , LSD,  $p < 0.05$ ) while in group BD was owing to the significantly raising in both DOC ( $p = 0.027$ , LSD,  $p < 0.05$ ) and DIC ( $p = 0.000$ , LSD,  $p < 0.05$ ). In group B and D, M2 significantly decreased the DOC compared with CK (B,  $p = 0.035$ ; D,  $p = 0.013$ , LSD,  $p < 0.05$ ). As for the dissolved nitrogen, PS-beads showed size effect on TDN and  $\text{NO}_3^-$ -N in group N, D and BD (M1 and M2: TDN, N,  $p = 0.041$ , D,  $p = 0.001$ , BD,  $p = 0.003$ ;  $\text{NO}_3^-$ -N, N,  $p = 0.032$ , D,  $p = 0.000$ , BD,  $\text{NO}_3^-$ -N,  $p = 0.000$ , LSD,  $p < 0.05$ ). In D and BD group, M2 significantly decreased the contents of TDN and  $\text{NO}_3^-$ -N.

For further get to know the variation of DOM components, the relative functional group characteristics of DOM were detected. The key wavelengths selected in this study and their corresponding organic functional groups were shown in Table S2 and the results were shown in Fig. 2 and Table S5.  $\text{SUVA}_{210}$  is used to characterize amines in the DOC.  $\text{SUVA}_{254}$ ,  $\text{SUVA}_{260}$ ,  $\text{SUVA}_{272}$ ,  $\text{SUVA}_{280}$ ,  $\text{SUVA}_{285}$  and  $\text{SUVA}_{340}$  represented the aromaticity and humification index, they had the similar changing trends in treatments.  $A_{250}/$

**Table 2** Primers used in PCR amplification

	Primers	Sequences	Amplification length
Bacterial	338F 806R	5'-ACTCCTACGGGAGGCAGCA-3' 5'-GGACTACHVGGGTWTCTAAT-3'	468 bp
Fungi	ITS1F ITS2R	5'-CTTGGTCATTTAGAGGAAGTAA-3' 5'-GCTGCGTTCTTCATCGATGC-3'	300 bp



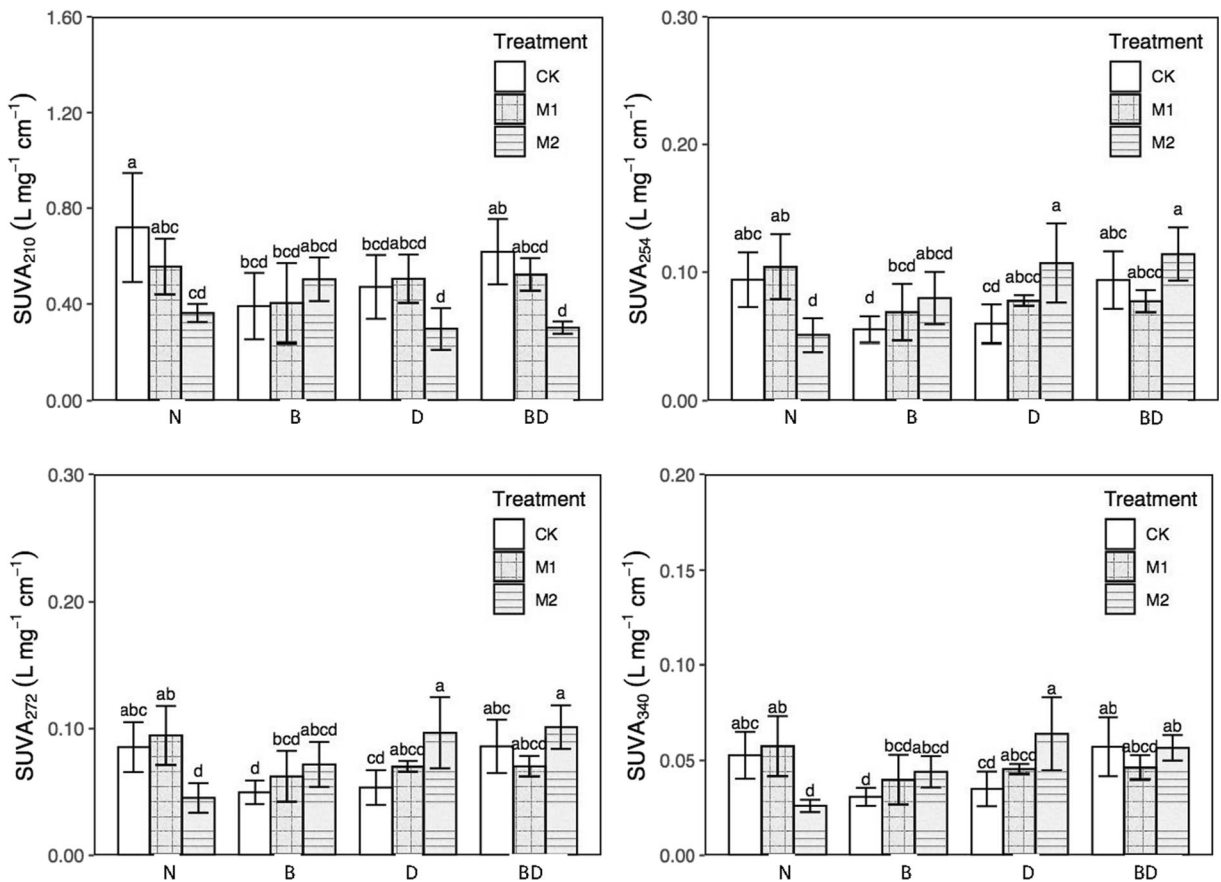


**Fig. 1** Dissolved carbon (DOC, DIC, TC) and dissolved nitrogen (TDN, NO<sub>3</sub><sup>-</sup>-N) concentration of different treatments. Different letters mean significant differences and the same letter means no significant difference, Duncan ( $p < 0.05$ ). CK, M1, M2

represented soil without PS, soil with 5  $\mu$ m PS and 70 nm PS, respectively; N-soil without biochar or degradable mulching film fragments (DMF), B-soil with biochar, D-soil with DMF. BD-soil with biochar and DMF. The same below

$A_{365}$  and  $A_{300}/A_{400}$  reflect the degree of humification and molecular weight of organic matter. The  $A_{253}/A_{203}$

represents the relative abundance functional group. In group N, M2 significantly reduced the SUVA<sub>210</sub>



**Fig. 2** Specific ultraviolet absorbance (SUVA) at different wavelengths: SUVA<sub>210</sub>, SUVA<sub>254</sub>, SUVA<sub>272</sub> and SUVA<sub>340</sub> of different treatments

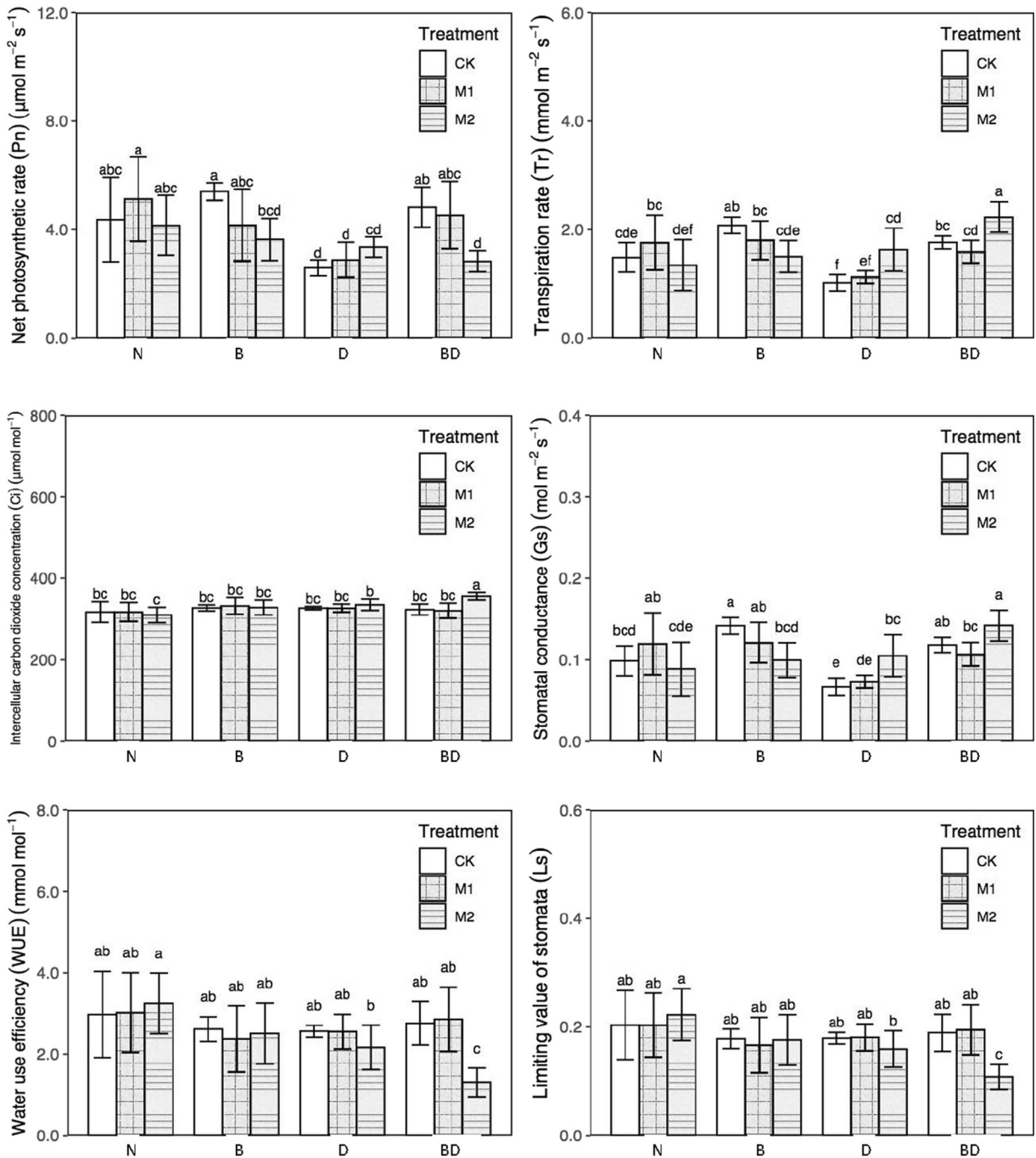
compared with CK ( $p = 0.002$ , LSD,  $p < 0.05$ ) and significantly decreased the aromatization and humification degree compared with CK and M1. The  $A_{253}/A_{203}$  in M1 was higher, indicating the higher content of carbonyl, carboxyl, hydroxyl and ester in the aromatic ring while the higher content of aliphatic chain in CK and M2 (Table S2, Table S5). In group D, M2 significantly reduced SUVA<sub>210</sub> ( $p = 0.049$ , LSD,  $p < 0.05$ ) while significantly increased the aromatic substances ( $p = 0.006$ , LSD,  $p < 0.05$ ) compared with CK as well as the  $A_{253}/A_{203}$ , namely the content of carbonyl, carboxyl, hydroxyl and ester in the aromatic ring. In group BD, M2 significantly reduced SUVA<sub>210</sub> compared with CK ( $p = 0.004$ , LSD,  $p < 0.05$ ) while significantly increased the  $A_{250}/A_{365}$  and  $A_{300}/A_{400}$  indicating the lower molecular weight of DOC and degree of soil aggregation compared with CK. M2 also significantly

increased the content of carbonyl, carboxyl, hydroxyl and ester in the aromatic ring, as well as the  $A_{265}/A_{465}$ , indicating more C=O structures or main color functional groups in DOC after adding M2.

The results of correlation analysis among the particle sizes of PS-beads, DOM and its corresponding functional groups were shown in Fig. S4. In group N (Fig. S4 (A)), TDN and  $\text{NO}_3^-$ -N had the negative correlation with the particle size of PS-beads, M1 decreased the contents of TDN and  $\text{NO}_3^-$ -N while M2 increased them. While  $A_{253}/A_{203}$  positively correlated with the particle size of PS-beads: M1 increased the content of carbonyl, carboxyl, hydroxyl and ester in the aromatic rings while M2 had the higher content of aliphatic chain in the aromatic rings. In group B, the DOC negatively correlated with SUVA<sub>210</sub> and SUVA<sub>254</sub> representing the amine substance and aromatic matter, respectively. While in group D, DOC had negative correlation with SUVA<sub>254</sub> and  $A_{253}/A_{203}$ , in

BD, DOC negatively correlated with  $SUVA_{210}$  and  $A_{250}/A_{365}$ . Therefore, in soil with different residues, PS-beads had different influence on the DOC through changing the

different components as well as the structures and functional groups in aromatic rings, with M2 having the more obvious influence.



**Fig. 3** Influence of different treatments on the photosynthetic parameters of Flower Chinese cabbage. Different letters mean significant differences and the same letter means no significant difference, Duncan ( $p < 0.05$ )



### Effects of PS-beads on chlorophyll content and photosynthetic parameters in soil with different residues

The contents of chlorophyll were listed in Table S6. The result showed that compared with chlorophyll b (Chl b), chlorophyll a (Chl a) was more easily affected by the different additives. M1 and M2 could promote the content of Chl a in group N, D and BD while decreased that in B. Total chlorophyll (total Chl) had the highest value under the co-effect of DMF and M2.

Figure 3 showed the results of the photosynthetic parameters of Flower Chinese Cabbage. PS-beads had no significant effect on net photosynthetic rate (Pn) in group N and D. In group B, M1 and M2 significantly decreased the value of Pn compared with CK (M1,  $p = 0.033$ ; M2,  $p = 0.003$ , LSD,  $p < 0.05$ ). In group BD, M2 significantly declined the Pn compared with CK and M1 (CK,  $p = 0.001$ ; M1,  $p = 0.004$ , LSD,  $p < 0.05$ ). M2 significantly decreased the Tr and Gs in group B compared with CK (Tr,  $p = 0.002$ ; Gs,  $p = 0.001$ , LSD,  $p < 0.05$ ) while increased them in group D (Tr,  $p = 0.001$ ; Gs,  $p = 0.003$ , LSD,  $p < 0.05$ ). As for the intercellular carbon dioxide concentration (Ci), limiting value of stomata (Ls) and water use efficient (WUE), compared with CK and M1, M2 significantly increased the Ci (CK,  $p = 0.001$ ; M1,  $p = 0.000$ , LSD,  $p < 0.05$ ) while decreased the values of Ls and WUE (Ls, CK,  $p = 0.001$ , M1,  $p = 0.001$ ; WUE, CK,  $p = 0.000$ , M1,  $p = 0.000$ , LSD,  $p < 0.05$ ).

### Effects of PS-beads on plant growth parameters in soil with different residues

The fresh weight, dry weight and water content of plant were listed in Table S7. In group N, M2 significantly increased the fresh and dry weight compared with CK ( $p = 0.033$ ;  $p = 0.020$ , LSD,  $p < 0.05$ ) and M1 ( $p = 0.045$ ;  $p = 0.024$ , LSD,  $p < 0.05$ ) while there was no significant difference in plant water content among different treatments. In group D, M1 significantly increased fresh weight by 112.83% and 132.54% as well as raised the dry weight by 129.35% and 122.60% compared with CK and M2, respectively. Meanwhile, the water content of the plant in M1 was lower than that in CK, indicating that biochar in soil with M1 could promote the accumulation of the dry matter of the plant. In group BD, M2 significantly declined the fresh weight compared with that of CK ( $p = 0.010$ , LSD,  $p < 0.05$ ).

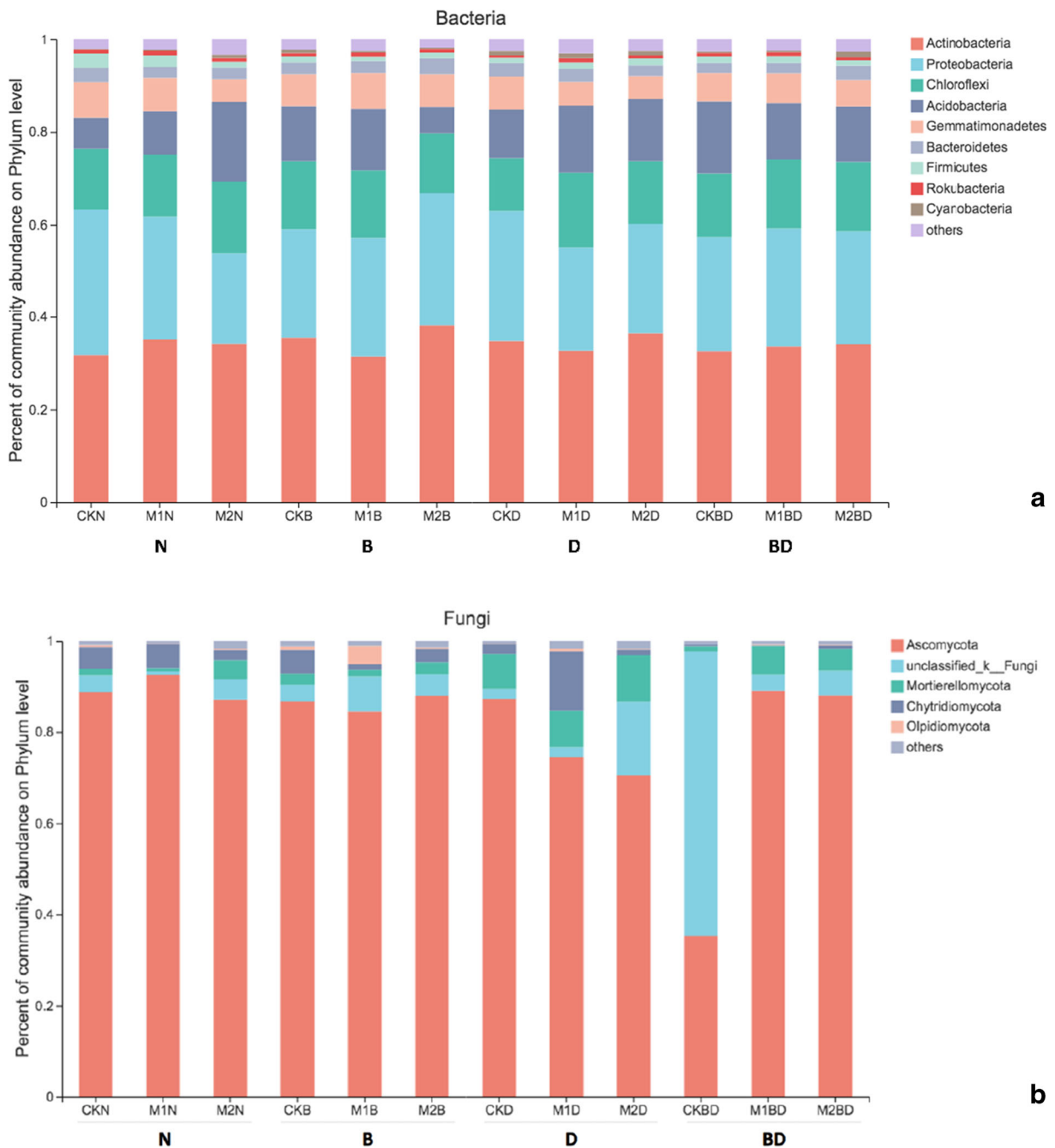
Fig. S5 showed the correlation among the plant growth parameters and chlorophyll content. The particle size of PS-beads significantly negatively correlated with Chl a in group B, positively correlated with the dry weight of the plant in group D and negatively correlated with water content in group BD. Therefore, in soil with different residues, different sizes of PS-beads affected different plant growth parameters.

### Effects of PS-beads on the diversity of rhizosphere microbial community in soil with different residues

The shared OTUs of different treatments were shown in Venn diagram (Fig. S6, S7, S8). The total OTUs of bacteria and fungi ranged 1800–2007 and 317–484, respectively. For the treatments CK, M1 and M2, the shared OTUs of bacteria and fungi were 1160, 1188 and 1158 as well as 159, 173 and 187, respectively. The shared OTUs of bacteria and fungi were 1238, 1264, 1360 and 1288 as well as 172, 222, 247 and 78, respectively. The diversity indexes were listed in Table S8. In group N, compared with CK and M1, M2 increased the OTUs number, Shannon index as well as the ACE and Chao1 of bacteria and fungi, indicating the increased diversity and richness of microbes. Compared with CK, M1 decreased the richness of bacterial community (ACE, Chao1) while increased the richness of fungal community (ACE, Chao1). In group B, M1 increased the OTUs number of bacteria and fungi compared with CK, meanwhile raised the diversity (Shannon) and richness (Chao1) of bacterial community as well as the richness (ACE and Chao1) of fungi. M2 decreased the OTUs number, the diversity and the richness of bacteria and fungi. In group D, M1 increased the OTUs number and Shannon index of bacteria and fungi, improving the richness of bacteria while declining that of fungi. M2 decreased the OTUs number and the richness of bacteria and fungi. In group BD, M2 increased the OTUs and the richness of bacteria. Both M1 and M2 could increase the OTUs, diversity and richness of fungal community.

### Effects of PS-beads on the rhizosphere microbial community structure in soil with different residues

The microbial community structure of bacteria and fungi of different treatments were shown in Fig. 4. At the phylum level, the main bacterial communities included *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Gemmatimonadales*, *Acidobacteria*, *Firmicutes*,



**Fig. 4** Community composition at the phylum level: (A) bacteria, and (B) fungi

*Bacteroidetes*, *Rokubacteria* and *Cyanobacteria*. *Actinobacteria* was the dominant phylum in all treatments. In group N, different sizes of PS-beads had the similar effect on the main phyla. While PS-beads of different particle sizes showed different effects on the main phylum with different residues in the soil. In group B and D, PS-beads showed size effect on the abundance

of *Actinobacteria*. In group N and BD, PS-beads decreased the abundance of *Actinobacteria*. In N and D, PS-beads decreased the abundance of *Proteobacteria*, *Gemmatimonadetes* and *Bacteroidetes*, while increased that in group B. In group N, D and BD, PS-beads increased the abundance of *Chloroflexi*, which was decreased in group B. PS-beads increased the

abundance of *Acidobacteria* in group N and D while decreased that in BD. PS-beads decreased the abundance of *Firmicutes* in group N, B and BD while increased that in group D. With DMF in the soil, PS-beads of different particle sizes showed similar effects on main bacterial community except for *Actinobacteria* while showed size effects in group B. The main fungal community at phylum level included *Ascomycota*, *Mortierellomycota*, *Chytridiomycota*, *Basidiomycota*, *Olpidiomycota* and *Unclassified\_Fungi*. *Ascomycota* was the dominant species in all treatments except CKBD. In group N and B, PS-beads of different particle sizes showed opposite effects on the main phyla. In group D, PS-beads decreased the abundance of *Ascomycota*, increased the abundance of *Mortierellomycota*, *Basidiomycota* and *Olpidiomycota*. In group BD, PS-beads increased the abundance of *Ascomycota*, *Mortierellomycota* and *Chytridiomycota*.

In group D, the change of bacterial community based on genus level was mainly related to the existence of PS-beads and the particle size (Fig. S9). Cluster analysis based on genus level showed that in group D, the addition of M1 and M2 resulted in the similar bacterial community structure while M2 and CK had the similar fungal community structure. In group B, M2 had the similar microbial community compared with CK.

#### KEGG pathway analysis

The changing of microbial community structure will further influence the microbial function. PICRUSt 2 was used to predict the microbial function by using the surrogate test sequences with the comprehensive reference database information. Six major categories were involved in the present study which were metabolism, genetic information processing, environmental information processing, cellular processes, organic systems and human diseases. The results fell into 46 subfunctions, among which, “carbohydrate metabolism”, “global and overview maps”, “amino acid metabolism”, “energy metabolism” and “metabolism of cofactors and vitamins” had the higher abundance (Fig. S10). At level 2, in group N, PS-beads increased the abundance of “carbohydrate metabolism”, “global and overview maps”, “amino acid metabolism” and “metabolism of cofactors and vitamins”, and M2 also accelerated the “energy metabolism”. In group D, PS-beads promoted “carbohydrate metabolism”, “global and overview map” and “energy metabolism”, M1 raised “metabolism of cofactors and vitamins” and “amino acid metabolism”,

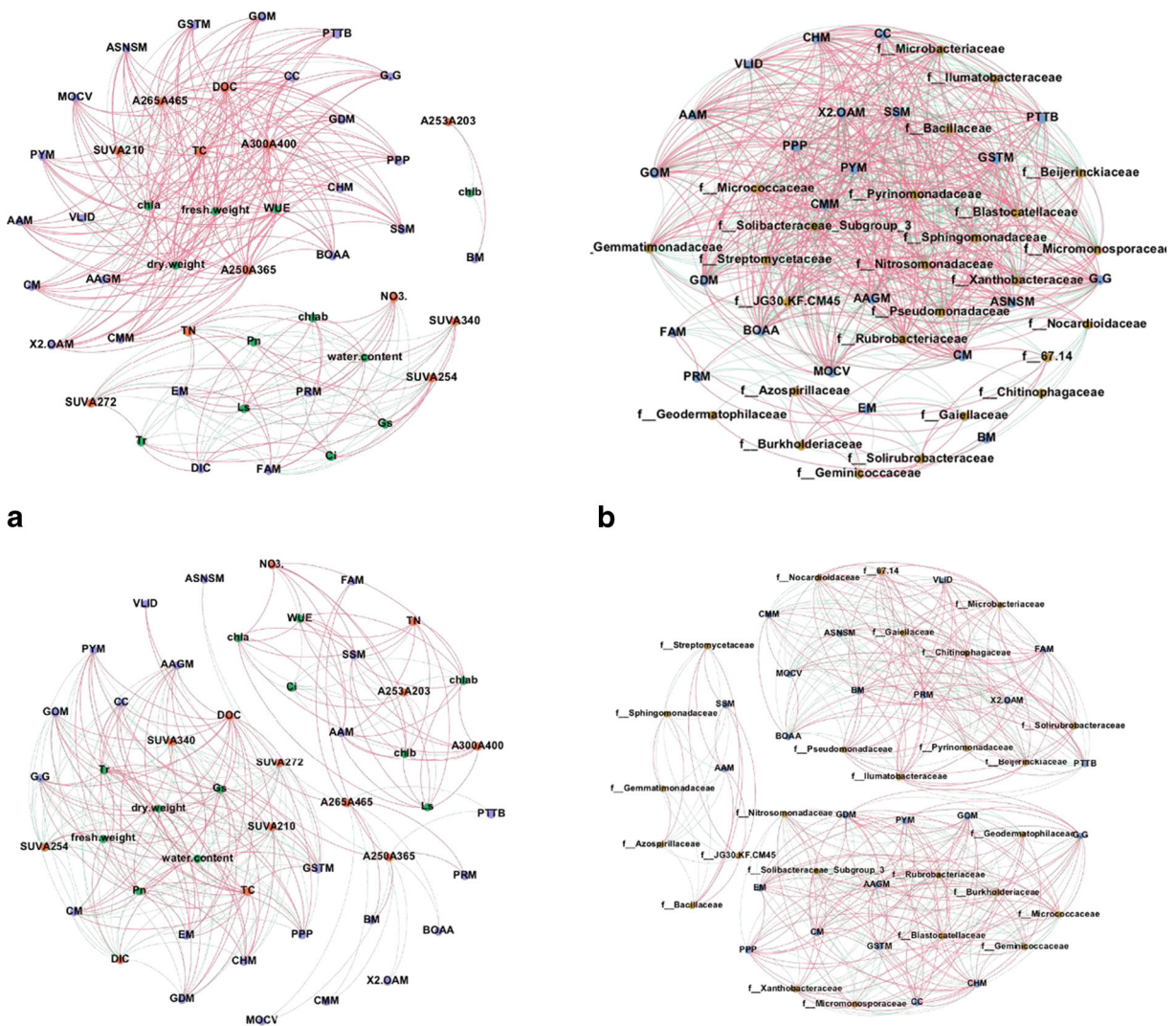
respectively. Therefore, PS-beads could promote microbial metabolism in group N and D.

#### Discussion

The rhizosphere microbes play a vital role in the root-soil-microbes system (Zhang et al., 2017), soil carbon turnover, root stimulation and other activities (He et al., 2020). The rhizosphere microbes and the plants are interdependent to each other (Berendsen et al., 2012; Mueller and Sachs, 2015), having close interactions (Uzoh and Babalola, 2018). The microbes provide the nutrients for plants by metabolism, participate in the interaction of root-soil, affecting the growth and health of plants and potentially influence the terrestrial material circulation. The changes of soil DOM and its composition interact with the metabolism of microbes. Amino acid is mainly decomposed into  $\alpha$ -ketoacids, amines and carbon dioxide through deamination, transamination, combined deamination or decarboxylation in process of “amino acid metabolism” (Yang et al., 2020), related to alanine, aspartic acid and other carbohydrate metabolism. PS-beads improving the abundance of “amino acid metabolism” (Neis et al., 2015), which may potentially affect soil carbon and nitrogen cycle (Huang et al., 2019). Therefore, the variations of microbial metabolism directly affect the soil nutrient which may further influence the physiological and biochemical characteristics of plants. However, in group B and BD, except for M1 (PS-MPs) promoted “metabolism of cofactors and vitamins” and M2 (PS-NPs) promoted “amino acid metabolism” in group B as well as M2 raised “carbohydrate metabolism” and “amino acid metabolism” in group BD, PS-beads inhibited several major metabolic processes. Therefore, the effect of PS-beads on microbial metabolism was influenced by the presence of biochar in soil.

For further illustrating the correlation among plant growth parameters, photosynthetic parameters, environmental factors and microbial metabolism as well as the correlation between bacterial community and microbial metabolism, network analysis was carried out (Fig. 5). The abbreviations and their corresponding parameters were listed in the supplementary material Table S9.

In group N, bacterial community and the KEGG pathway based on level 2 (Fig. 5 (B)) owned the more complicated network than that of B, D and BD (Fig. 5 (D)(F)(H)). M2 significantly increased the fresh and dry weight of the plant which was similar to the previous study that PS-beads at nano scale could raise the wheat



**Fig. 5** Network analysis: (A)(C)(E)(G)-correlation among soil DOM, plant growth and photosynthetic parameters and KEGG pathway at level 2; (B)(D)(F)(H)-correlation among bacterial community and microbial function. A connection represents for a strong (Spearman's  $|\rho| > 0.6$ ) and significant ( $p$  value  $< 0.01$ )

biomass (Lian et al., 2020). This might be due to PS-beads could enter into the plant via a crack-entry mode (Li et al., 2020) and therefore raised the plant biomass. Besides that, M2 significantly increased the soil DOC and rhizosphere microbial metabolism. PICRUST2 prediction at level 2 showed that M2 raised the functions of Carbohydrate metabolism, Amino acid metabolism, Global and overview maps, Metabolism of cofactors and vitamins which positively correlated with DOC

content (Fig. 5(A)). This could attribute to the accumulation of biomass by the enhancement of microbial metabolism, increasing the available nutrients absorbed by the roots. The DOC functional group analysis showed that the contents of amines, aromatics and macromolecules in DOC decreased as well as the DOC molecular weight declined which might attribute to the increasing of metabolism. M2 decreased the Pn and the stomatal conductance (Gs) lower than that of CK and



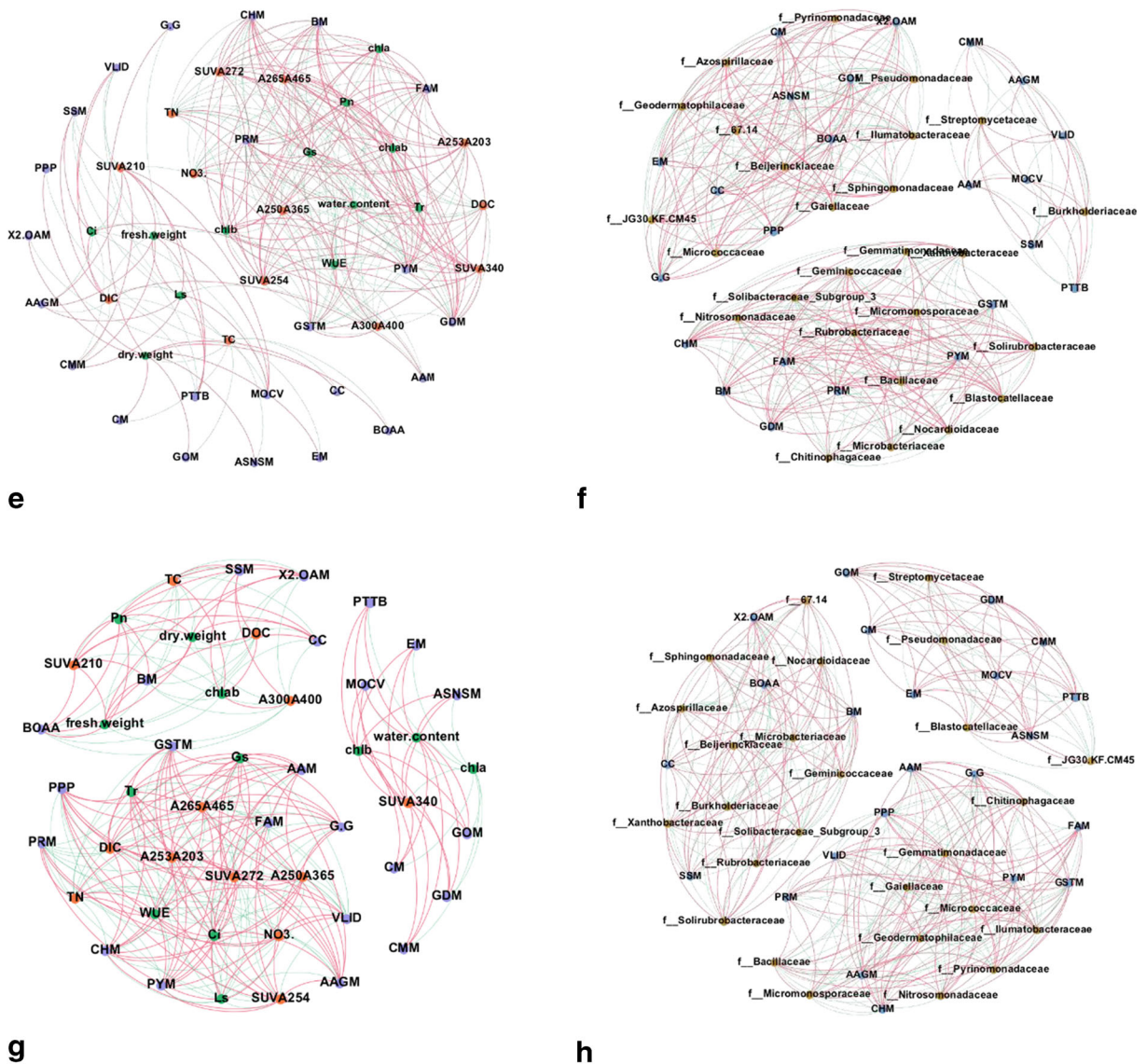


Fig. 5 continued.

M1, the limiting value of stomata (Ls) increased and the value of transpiration rate (Tr) decreased resulting in the water accumulation. Furthermore, the diversity and evenness of the community could also indirectly reflect the community function.

In group B, the fresh and dry weight had no significant variation after adding PS-beads. However, M2 significantly increased the water content of the plant which might be due to the decrease of Gs and Tr. Pn and Gs decreased with the decrease of PS particle size. The total Chlorophyll (total Chl) and Chl a/Chl b decreased, which might be the reason

for the decreasing of Pn positively correlating with the dry weight. In addition, the changing of correlation among microbes and the main microbial functions could change the microbial metabolism (Fig. 5(D)). The dry weight was positively correlated with the main microbial metabolism, such as Carbohydrate metabolism, Global and overview map as well as Energy metabolism (Fig. 5(C)). Therefore, PS-beads decreased the photosynthetic pigment, which might potentially affect Pn and in further influence the dry weight accumulation. Gs and Tr decreased and the water content increased.



In group D, Pn was positively correlated with Gs, Chl a and Chl b and total Chl. With the decrease of PS-beads particle size, the chlorophyll content increased. M1 had the highest Chl a/Chl b, indicating that the M1 treatment had a strong light harvesting ability (Esteban et al., 2015). Fresh weight and Ls, water content and water use efficient (WUE) had the positive correlation. The dry weight was positively correlated with Global and overview map and Energy metabolism in microbial metabolism (Fig. 5 (E)). Dry weight had a significant positive correlation with Glycolysis/Gluconeogenesis, Biosynthesis of amino acids and Carbon metabolism (Fig. 5, Fig. S8). M1 had the higher bacterial and fungal OTUs number, richness, evenness and diversity, potentially having the positive effect on the plant biomass.

In group BD, the fresh and dry weight had the significant positive correlation with Pn. M2 increased the Gs and Tr, decreased WUE and Pn as well as dry and fresh weight. At level 3, the dry and fresh weight were related to amino acid synthesis (Fig. 5(G)).

## Conclusion

This study was carried out for exploring the effects of different sizes of PS-beads on the soil properties, plant photosynthetic parameters and microbes as well as the potential correlation among them in soil with different residues. The results showed that the particle size of MPs did affect the soil properties, rhizosphere microbial community composition, plant photosynthesis and growth. The results showed that in soil with different residues, PS-beads of different sizes could change the different components, structures and functional groups in aromatic rings of DOM, which might further change the microbial community and metabolism. The different sizes of PS-beads affected different plant growth parameters and potentially changed the plant growth parameters and photosynthetic parameters by changing the microbial metabolism through the influence the correlation among microbes. In soil with different residues, the potential mechanisms of PS-beads changing the plant growth were different. Due to the multi-source of MPs in soil meanwhile more and more background MPs values researches being carried on, a better understanding of the different residues and different sizes of residues related to the

environmental concentration should be taken into consideration on soil-plant system is needed.

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