Response of soil dissolved organic matter to microplastic addition in Chinese loess soil

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HIGHLIGHTS

- Microplastic addition stimulated soil activity of fluorescein diacetate hydrolase (FDAse) in soil.
- The lower level of microplastic addition had a negligible effect on the nutrient contents in DOM solution at day 30.
- The higher level of microplastic addition significantly increased the nutrient contents in DOM solution.

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ABSTRACT

Plastic debris is accumulating in agricultural land due to the increased use of plastic mulches, which is causing serious environmental problems, especially for biochemical and physical properties of the soil. Dissolved organic matter (DOM) plays a central role in driving soil biogeochemistry, but little information is available on the effects of plastic residues, especially microplastic, on soil DOM. We conducted a soil-incubation experiment in a climate-controlled chamber with three levels of microplastic added to loess soil collected from the Loess Plateau in China: 0% (control, CK), 7% (M1) and 28% (M2) (w/w). We analysed the soil contents of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), NH4\(^+\), NO3\(^-\), dissolved organic phosphorus (DOP), and PO4\(^3-\) and the activities of fluorescein diacetate hydrolase (FDAse) and phenol oxidase. The higher level of microplastic addition significantly increased the nutrient contents of the DOM solution. The lower level of addition had no significant effect on the DOM solution during the first seven days, but the rate of DOM decomposition decreased in M1 between days 7 and 30, which increased the nutrient contents. The microplastic facilitated the accumulation of high-molecular-weight humic-like material between days 7 and 30. The DOM solutions were mainly comprised of high-molecular-weight humic-like material in CK and M1 and of high-molecular-weight humic-like material and tyrosine-like material in M2. The Microplastic stimulated the activities of both enzymes. Microplastic addition thus stimulated enzymatic activity, activated pools of organic C, N, and P, and was beneficial for the accumulation of dissolved organic C, N and P.

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1. Introduction

The increasing use of plastic has increased the amount of plastic debris in the environment, especially in the oceans. More than 240 million tonnes of plastic are used every year (Thompson et al., 2009), contributing to 11.7–25% of the municipal waste in Europe and the USA. Only 1–2% of this plastic is recycled (Shent et al., 1999;...
Plastic debris is accumulating in the environment because of its durability and the limitation of recycling technology (Barnes et al., 2009; Rillig, 2012). Microplastic, particles <5 mm in size (Claessens et al., 2011), have been studied in the marine environment and on shorelines, but few studies have focused on the microplastic in soil and terrestrial systems (Rillig, 2012; do Sul and Costa, 2014; Van Cauwenbergh et al., 2015). Microplastic can enter the soil either as primary microplastic from industrial abrasives and cosmetic products by sludge application (Cole et al., 2011) or secondary microplastic from the environmental degradation of plastic mulch. Agricultural sites and landfills in Europe contain 1000–4000 microplastic particles per kg of sludge dry mass (Zubris and Richards, 2005; Barnes et al., 2009). The use of plastic films as agricultural mulches increased in China, especially in northern regions, nearly four-fold from 1991 to 2011, from 0.32 to 1.25 million tonnes which has contributed to the accumulation of microplastic in farmland (Yearbook, 2012; Wang et al., 2013; Liu et al., 2014). Microplastic can be ingested by fauna due to their small size and can thus accumulate in the food chain (Besselink et al., 2013; Huerta Lwanga et al., 2016b, 2017). Some studies have identified interactions between microplastic and the chemical pollutants they absorb on their surfaces (Ivar do Sul and Costa, 2014). Microplastic can also change the physical properties of soil and can accumulate in soil, reaching levels that can affect soil function and biodiversity (Rillig, 2012).

Dissolved organic matter (DOM) plays an important role in numerous physical, chemical, and biological processes in soil (Kalbitz et al., 2000), including the cycling of soil organic carbon (C) and the transport of nutrients such as nitrogen and phosphorus (Kalbitz et al., 2003b). DOM represents <0.25% of the total soil organic matter but facilitates the solubility and mobility of metals and organic compounds (Kalbitz et al., 1997; Temminghoff et al., 1997) and thus increases the transport of pollutants (Kalbitz et al., 2000). DOM is a complex mixture of various labile and recalcitrant organic substances (Michel et al., 2006) and is a substrate and the most important C source for microorganisms (Marschner and Kalbitz, 2003; DeForest et al., 2004a, 2004b). Soil dissolved organic C (DOC) is a more sensitive indicator of changes in soil quality changes than total organic C (TOC), because short-term changes in TOC are not easily detected (Purakayastha et al., 2008; Gong et al., 2009; Li et al., 2016).

The accumulation of microplastic in soil can affect microbial activity, attributed to absorbed harmful contaminants (Rillig, 2012), earthworm activity (Huerta Lwanga et al., 2016b, 2017) and soil physical properties, such as soil porosity and aggregate structure (Ladd et al., 1993; Rillig, 2012; Zhang et al., 2015). The activities of soil enzymes represent microbial activities and the availability of substrates for microorganism uptake. Soil enzymes with high catalytic capacities are produced by soil microorganisms and are the main medium controlling the cycling of soil nutrients such as C, N, and P (Dick et al., 1994; Allison and Jastrow, 2006; Trasar-Cepeda et al., 2008). Phenol oxidase (PO) is involved in the degradation of phenolic compounds which are recalcitrant DOMs and decreases the DOM biodegradability (Marschner and Kalbitz, 2003). PO activity is negatively correlated with soil humification (Li et al., 2015). Fluorescein diacetate hydrolase (FDAse) can represent overall microbial metabolic activity and is an effective indicator of short-term changes of soil quality (Muscolo et al., 2014, 2015).

So far the effect of microplastic on soil fertility and microbial activity were still not clear, although researches demonstrated that plastic-film residues can decrease soil porosity, air circulation, microbial biomass and microbial activity and can probably affect soil fertility (Moreno and Moreno, 2008; Kasirajan and Ngouajio, 2012). A detailed study of the effects of microplastic on the dynamics of soil DOM and enzymatic activities is needed for minimizing environmental risks and determining the sustainability of farming practices. We used three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy, a rapid, sensitive, selective, and reagent-free technique for fingerprinting organic matter in terrestrial ecosystems, to study the composition of soil DOM (Chen et al., 2003; Matilainen et al., 2011).

Our specific aims were to: (1) determine the effect of microplastic on the quantities and composition of soil DOM, (2) determine the effect of microplastic on the dynamics of soil enzymatic activity, and (3) clarify the relationships between soil DOM and enzymatic activity.

2. Materials and methods

2.1. Experimental design

This experiment was conducted in the climate-controlled chamber (AGC-Doo3N, Hangzhou, China) at the Institute of Soil and Water Conservation, Chinese Academy of Sciences, Yangling, China. The soil used in this experiment was collected in Ansai county on the Loess Plateau, and soil properties can be seen in Table 1. The soil is mainly composed of Cultivated loessial soils (Calcic cambisols, FAO) developed on wind-deposited loess parental material and is characterised by the parental material, the absence of bedding, a loose silky texture, macroporosity, and wetness-induced collapsibility. Two hundred grams were incubated in PVC pots 10 cm in diameter and 9 cm in height. Our experiment contained three treatments: 1) CK, no microplastic added to the soil; 2) M1, 14 g of microplastic added to the soil (7% w/w); 3) M2, 56 g microplastic added to the soil (28% w/w). The microplastic used in this experiment is made of polypropylene (Yongling-TECH company, Beijing, China). The density of Microplastic is 0.91 g/cm³, and its bending strength is 200 kg/cm². The particle size of microplastic is below 180 μm. The soils were slightly compacted using a small manual soil compactor. The soil compactor was set to fall 10 times by gravity at the pot height to guarantee the same compaction for all samples. The microplastic contents were chosen based on the research of Huerta Lwanga et al. (2016a) but simulating the hotspots of plastic debris in the field. Soil moisture was maintained at 60% of field capacity throughout the experiment. Each treatment had three replicates. The pots were incubated in the light at 28 °C (relative humidity of 80%, 300 μ (photons) m⁻² s⁻¹). The soil was sampled from each pot after 0, 1, 3, 7, 14, and 30 days, so the experiment had a total of 54 pots: 3 treatments × 3 replicates × 6 days. The soil samples were passed through a 2 mm sieve, and then one subsample was stored at −80 °C for analysing soil enzymatic activities, and another subsample was stored at 4 °C for measuring DOM chemical properties.

2.2. Analysis of soil DOM concentration and composition

DOM was extracted by adding 120 mL of distilled water to 40 g subsamples of homogenised soil (1:3 soil:water, w/w) as described by Kalbitz et al. (2003a, 2013b). All extracts were centrifuged at 4000 rpm for 10 min, and the supernatants were filtered through pre-rinsed 0.45 μm cellulose-acetate membranes (Schleicher & Schuell). The filtered solutions were stored frozen until analysis. Total dissolved N (TDN), DOC, NH₄⁺, NO₃⁻, total dissolved P (TDP), and PO₄³⁻ contents were measured in all samples using standard soil test procedures of the Chinese Ecosystem Research Network (CERN Editorial Committee, 1996). DOC contents were determined using a TOC analyser (liquid TOC II, Elementar, Germany). TDN contents were determined using alkaline persulfate digestion-UV spectrophotometric method (Doyle et al., 2004). TDP contents were measured using the ammonium molybdate
spectrophotometric method (Galhardo and Masini, 2000). PO₄³⁻ contents were determined using phospho-molybdenum blue method (Jarvie et al., 2002). NH₄⁺ content was measured by an AA3 continuous flow autoanalyzer (AutoAnalyzer3–aa3, Bran + Luebbe, Germany). NO₃⁻ content was determined by ultraviolet colorimetry with an ultraviolet spectrophotometer (UV2300, Shanghai, China). Dissolved organic N (DON) and dissolved organic P (DOP) contents were calculated as TDN- (NH₄⁺ + NO₃⁻) and TDP- PO₄³⁻, respectively. UV–Vis absorption from 200 to 500 nm (1 nm steps) was measured in a 10-mm quartz cuvette, with Milli-Q-water used as a blank. The specific UV absorbance at 254, 280 and 365 nm were measured for all samples. EEM spectrograms of the subsamples were measured using an F-4600 fluorescence spectrometer (HITACHI, Japan). The voltage of the photomultiplier tubes was set at 700 V, and the slits for both excitation and emission were 5 nm at a scanning speed of 1200 nm min⁻¹. EEM were recorded in a range of excitation wavelengths of 200–450 nm and in a range of emission wavelengths of 250–450 nm. Before measurement, the wavelength-dependent light intensity of the light source and the light sensitivity of the detector were corrected. Excitation and emission intensities for instrument-specific biases were corrected based on correction factors supplied by manufacturer. Each EEM was corrected for inner filtering effects by multiplying it by a correction matrix, which was calculated for each wavelength pair from the sample absorbance by assuming excitation (Ex) and emission (Em) pathlengths of 5 mm in a 10 mm cuvette (Ohno, 2002). The EEM spectrograms were combined into a three-dimensional data array: 54 samples × 51 excitations × 41 emissions. The Raman scatter effects were removed from the data set before analysis by subtracting the spectrogram for Milli-Q-water from the sample spectrogram. Rayleigh scatter effects were removed from the dataset by excluding emission measurements made at wavelengths less than or equal to the excitation wavelength +20 nm.

The EEM contours of four components identified by the PARAFAC analysis are shown in Fig. 5. Component 1 had two protein-like fluorescence peaks centered at excitation/emission (Ex/Em) wavelength pairs of 215/280 and 260/280 nm. This component was associated with tyrosine-like substances which indicated a more degraded peptide material (Coble et al., 1998; Parlanti et al., 2000). The Ex/Em wavelength pairs of component 2 were centered at 225/ 450 nm, and this component was identified as a UVC humic-like components associated with high-molecular-weight and aromatic humus (Stedmon and Markager, 2005; Murphy et al., 2006). Component 3 had a humic-like fluorescence peak centered at the Ex/Em wavelength pairs at 235/420 nm. This component was associated with high-molecular-weight humus (Stedmon et al., 2003; Murphy et al., 2006). The Ex/Em wavelength pairs of component 4 were centered at 220/440 nm. This component originated from a UVA humic-like substance associated with fluorescence resembling that of fulvic acid (Cory and McKnight, 2005; Stedmon and Markager, 2005).

The activities of two enzymes were measured: PO and FDase. FDase activity was measured by the method adapted from (Green et al., 2006; Daou et al., 2016). One gramme of soil was added to 9 mL of 0.1 M phosphate buffer, pH 7, and the suspension was shaken for 30 min. After shaking, 180 μL of the sample suspension were dispensed into wells of a black 96-well microplate, 20 μL of 20 mM fluorescein diacetate (FDA) were dispensed to columns to serve as an assay, and 20 μL of phosphate buffer were dispensed into columns to serve as controls. Reference standard wells received 20 μL of substrate plus 180 μL phosphate buffer. Each assay microplate also contained one column of phosphate buffer blanks for measuring background fluorescence in the substrate. Phosphate buffer blank wells received 200 μL phosphate buffer. The microplates were covered and incubated in the dark at 30 °C for 1 h. The FDase activities were expressed in units of mg Kg⁻¹ h⁻¹.

PO activity was determined spectrophotometrically in clear 96-well microplates using L-3, 4-dihydroxyphenylalanine (l-DOPA) as a substrate as described by Deforest (2009). One gramme dry mass of fresh soil was mixed with 125 mL of 50 mM acetate buffer (pH 5.0) made by mixing sodium acetate trihydrate with distilled water. The samples were shaken for 30 min. After shaking, 50 μL of the soil suspensions were placed in microplate wells containing 150 μL of buffer that served as controls or containing 150 μL of 25 mM l-DOPA for sample analysis. Reference standard wells received 150 μL of substrate plus 50 μL acetate buffer. Each assay microplate also contained one column of phosphate buffer blanks for measuring background fluorescence in the substrate. Phosphate buffer blank wells received 200 μL acetate buffer. The microplates were covered and incubated in the dark for 24 h. PO activity was quantified by measuring absorbance at 450 nm and expressed in units of μmol h⁻¹ g⁻¹.

2.3. Data analysis

One-way analysis of variance was used to determine the effects of microplastic addition and time on chemical properties of soil DOC and enzyme activities. The means of significant effects at $P < 0.05$ were then compared using the Duncan’s multiple-range test. All statistical analyses were performed using SPSS 21.0. The EEM data were analysed using MatLab 2010a (MathWorks Inc., USA). Parallel factor analysis (PARAFAC) modelling of the fluorescence EEMs was conducted with MATLAB using the DOMFluor toolbox (Stedmon and Bro, 2008) following the procedures described by (Stedmon and Bro, 2008). Figures were drawn using SigmaPlot 10.0 software.

3. Results

3.1. Impact of microplastic addition on the dynamics of soil WEOM

The microplastic significantly affected DOC contents (Fig. 1). DOC content was higher in M2 and significantly lower in M1 than CK on days 0 and 1. DOC content increased significantly in M1 and M2 during the first three days after microplastic addition, decreased between days 3 and 7, and then remained stable until the end of the experiment. DOC content was similar in CK and M1 but was significantly higher in M2 between days 3 and 30. At day 30, DOC content in M2 increased by 35% relative to CK.

TDP contents ranged from 21.4 to 42.1 mg kg⁻¹. TDP content was significantly lower in M1 than CK and M2 on days 0, 1 (Fig. 2) but was significantly higher in M1 and M2 than CK between days 7 and 30, and was highest in M2. DON contents ranged from 3.4 to

### Table 1

<table>
<thead>
<tr>
<th>Properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size distribution:</td>
<td></td>
</tr>
<tr>
<td>&lt;0.002 mm (clay) (%)</td>
<td>18.39</td>
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<tr>
<td>0.002–0.02 mm (%)</td>
<td>24.95</td>
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<tr>
<td>0.02–2 mm (%)</td>
<td>55.85</td>
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<tr>
<td>&gt;2 (%)</td>
<td>3.81</td>
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<tr>
<td>pH</td>
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</tr>
<tr>
<td>Total organic carbon (g Kg⁻¹)</td>
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<tr>
<td>Total nitrogen (g Kg⁻¹)</td>
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</tr>
<tr>
<td>Total phosphorus (g Kg⁻¹)</td>
<td>0.55</td>
</tr>
<tr>
<td>Nitrate nitrogen (mg Kg⁻¹)</td>
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</tr>
<tr>
<td>Ammonium nitrogen (mg Kg⁻¹)</td>
<td>3.37</td>
</tr>
<tr>
<td>Available phosphorus (mg Kg⁻¹)</td>
<td>3.04</td>
</tr>
</tbody>
</table>
24.1 mg kg\(^{-1}\). Microplastic addition significantly increased DON content, especially between days 14 and 30. Microplastic addition, however, significantly decreased NO\(_3^-\) content during the first three days, but NO\(_3^-\) content did not differ significantly among the treatments between days 7 and 30. Microplastic addition had no significant effect on NH\(_4^+\) content. TDN, DOC, and NO\(_3^-\) contents decreased quickly in CK between days 1 and 7, but Microplastic addition notably slowed the decrease.

TDP and DOP contents ranged from 0.2 to 1.68 mg kg\(^{-1}\) and from 0.05 to 1.57 mg kg\(^{-1}\), respectively. Microplastic addition significantly increased TDP and DOP contents, especially by day 30, and the contents were highest in M2 (Fig. 1). TDP and DOP contents tended to increase in M1 and M2 relative to CK. In contrast, PO\(_4^{3-}\) contents decreased quickly in M1 and M2 between days 14 and 30 and were similar in all treatments by 30 day. PO\(_4^{3-}\) content was highest in M2 between days 0 and 15 but did not notably between CK and M1.
3.2. Impact of microplastic addition on the dynamics of soil FDAse and PO activities

FDAse activities ranged from 5.7 to 24.2 mg kg\(^{-1}\) h\(^{-1}\). FDAse activity decreased quickly in CK and M1 in the first three days and increased between days 3–7. FDAse activity decreased in M2 within the first day, increased between days 1 and 3, and then decreased between days 3 and 7 (Fig. 3). FDAse activity in CK and M2 remained stable between days 7 and 30, but it in M1 decreased between days 7 and 30. Microplastic addition significantly increased FDAse activity, which was highest in M2. PO activities ranged from 5.2 to 9.8 mg kg\(^{-1}\) h\(^{-1}\). PO activity decreased in M1 and M2 in the first three days, subsequently increased between days 3 and 7, and then decreased between days 7 and 30. PO activity varied oppositely in CK.

3.3. Changes in the UV/Vis absorption spectrum of DOM

Specific UV absorbance (SUVA) at 254 nm is associated with natural organic matter content in natural waters (Najm et al., 1994). SUVA 280 was introduced to represent total aromaticity (Sarathy and Mohseni, 2007), and SUVA 365 has been demonstrated to increase with molecular size (Pehruvori and Pihlaja, 2004). SUVA 254, SUVA 280 and SUVA 365 tended to vary similarly over time after microplastic addition (Fig. 4). SUVA 254, SUVA 280 and SUVA 365 increased in M2 during the first seven days and then sharply decreased between days 7 and 14, which indicated the formation of high-molecular-weight aromatic compounds during the first seven days and their decomposition between days 7 and 14. SUVA 254, SUVA 280 and SUVA 365 increased in M2 during the first seven days and then sharply decreased between days 7 and 14, which indicated the formation of high-molecular-weight aromatic compounds during the first seven days and their decomposition between days 7 and 14. SUVA 254, SUVA 280 and SUVA 365 remained stable in M2 between days 14 and 30. SUVA 254, SUVA 280, and SUVA 365 sharply increased in CK during the first day, decreased between days 1 and 3, then increased until day 14, and finally decreased until the end of the experiment. Similarly, SUVA 254, SUVA 280, and SUVA 365 immediately increased in M1 during the first day, decreased between days 1 and 3, increased until day 14, and finally decreased until the end of the experiment. SUVA 254, SUVA 280, and SUVA 365 were highest in M2 and lowest in M1, indicating that a
moderate level of Microplastic inhibited, and that a high level of Microplastic facilitated the formation of high-molecular-weight aromatic compounds.

Clear differences in EEM appearance at the start and end of the incubations were apparent (Fig. 6). Component 1 accumulated in CK between days 0 and 7 and subsequently decomposed until the end of the experiment. Component 1 increased in M1 during the first 14 days and subsequently decomposed until the end of the experiment. Component 1 increased in M2 during the first three days, decreased until day 7, and then increased between days 7 and 30 (Fig. 7). Component 1 content was higher in CK than M1 and M2 between days 1 and 14. However, component 1 content was significant higher in M2 than CK and M1 at day 30 while it in CK was as low as M1. Component 2 varied similarly over time in CK, M1 and M2 after Microplastic addition. It accumulated rapidly during the first day after microplastic addition, decreased until day 7, then increased between days 7 and 14, and finally decomposed until the end of the experiment. The component 2 content was significantly higher in M2 than CK and M1 throughout the experiment, especially at days 1 and 30. However, component 2 content in M1 was as low as CK at day 1, 14 and 30. Component 3 also increased quickly in M1 and M2 during the first day after Microplastic addition, decomposed sharply between days 1 and 7, then accumulated quickly until day 14, and finally remained stable until the end of the experiment. In contrast, component 3 decomposed immediately in CK until day 7 and then remained constant until the end of the experiment. Component 3 was higher in CK than M1 and M2 at day 0 but was higher in M1 and M2 than CK between days 14 and 30. Component 3 was higher in M2 than M1 between days 1 and 3. Component 4 immediately increased in CK and M2 during the first day after microplastic addition, decreased until day 7, then increased between days 7 and 14, and finally decomposed until the end of the experiment. The component 4 content was significantly higher in M2 than CK and M1 throughout the experiment, especially at days 1 and 30. However, component 4 content in M1 was as low as CK at day 1, 14 and 30.
day, decreased in M2 until day 3 and in CK until day 7, then accumulated until day 14, and finally decomposed until day 30. Component 4 accumulated in M1 between days 0 and 7 and decomposed sharply between days 7 and 30. Component 4 throughout the experiment was lowest in CK. Component 4 was higher in M2 than CK and M1 at days 0, 1, 14 and 30. However, component 4 content in M1 was as low as CK at day 0 and 30.

4. Discussion

4.1. Effects of microplastic addition on soil DOM quantity

The soil microbial community plays a vital role in nutrient cycling, including mineralization, biodegradation (Firestone et al., 2002; Murugan et al., 2014). Plastic residues in soil decrease soil saturated hydraulic conductivity (Wang et al., 2015) and influence soil microbial communities (Jiang et al., 2014). Soil microbial carbon, nitrogen, soil fluorescein diacetate hydrolysis, soil dehydrogenase and Simpson indices declined by about $28.9\text{--}73.5\%$, $38.2\text{--}76.2\%$, $1.6\text{--}30.7\%$, $14.9\text{--}59.0\%$, $1.8\text{--}18.7\%$, respectively (Wang et al., 2016) after the addition of plastic residues. However, these results only concern about the effect of the addition of larger particle size debris ($20 \text{ mm } \times 20 \text{ mm}$ pieces) instead of microplastics on soil microbial activities. Our study initially presented the effects of microplastic addition on soil enzyme activities. Microplastic addition significantly promoted FDAse between day 7 and 30, and

![Fig. 6. Representative fluorescence emission—excitation matrices (EEMs) at the start and end of 30-day incubations with or without microplastic addition. Panels show EEMs from CK (A,B), M1 (C,D), and M2 (E,F). Panel columns represent EEM at the start of the incubation experiment, left, and at the end of the incubation, right.](image)
and lignin (Keuskamp et al., 2015). PO activity increased after degradation of recalcitrant (phenolic) compounds such as tannin pounds, thus increasing dissolved organic matter. FDAse activity decomposed into easily dissolved low-molecular-weight compounds in soil microplastic addition, especially between days 7 and 14, leading to more poorly dissolved high-molecular-weight compounds in soil decomposed into easily dissolved low-molecular-weight compounds, thus increasing dissolved organic matter. FDAse activity can represent general metabolic activity and is a good indicator of the intensity of soil life and microbial activity (Perucci, 1992). Microplastic addition significantly stimulated the activity of FDAse, resulting in the enhancement of microbial hydrolytic activity on soil organic matter or DOM, which would lead to the accumulation of DOM (Fig. 3). An increase in DOM hydrolysis would not alter the apparent DOC content, because dissolved compounds remain soluble after hydrolysis (Gogo et al., 2014).

Fig. 7. Changes of the Fmax values of the four components identified by PARAFAC after microplastic addition. Error bars are standard deviations. Capital letters and Lowercase letters designate significant differences between sampling time and treatments, separately (P < 0.05).

increased PO activities between day 7 and 14, and these beneficial effects were positively correlated with the content of microplastic (Fig. 3). Microplastic can sorb harmful contaminants from the soil solution and alter soil physical properties, such as increasing porosity and changing aggregate structure, so microbial activity can be incorporated into the dynamic structure of aggregates (Ladd et al., 1993; Rillig, 2012; Zhang et al., 2015; Huerta Lwanga et al., 2016b, 2017). Previous studies have shown that porosity, specific surface area, and aggregate structure are positively correlated with soil microbial activities (Girvan et al., 2003; Arthur et al., 2012; Naveed et al., 2016). An increase in porosity may increase the flow of air in soil, which would enrich aerobic microorganisms (Rubol et al., 2013).

TDN, DON, TDP, and DOP contents in the DOM solution were significantly higher in M2 than M1 and CK, indicating that the high level of microplastic addition facilitated the release of soil nutrients into the soil solution and the accumulation of DOM. TDN, DON, TDP, and DOP contents were also higher in M1 than CK between days 14 and 30 but did not differ significantly between CK and M1 during the first seven days, so that the beneficial effect of low content microplastic addition on accumulation of DOM is a slow process. DOM dynamics depend on the imbalance between production and in situ mineralization (Schimel and Weintraub, 2003; Gogo et al., 2014). In our experiment, no external organic matter was added to the soil, so the soil microorganisms likely played an important role in DOM dynamics. The activities of soil enzymes can be indicators of microbial activity and the availability of substrates for microbial uptake. Soil enzymes also decompose organic matter and catalyse important transformations in C, N, and P cycles (Wallenstein and Burns, 2011). PO activity is involved in the degradation of recalcitrant (phenolic) compounds such as tannin and lignin (Keuskamp et al., 2015). PO activity increased after microplastic addition, especially between days 7 and 14, leading to more poorly dissolved high-molecular-weight compounds in soil decomposed into easily dissolved low-molecular-weight compounds, thus increasing dissolved organic matter. FDAse activity can represent general metabolic activity and is a good indicator of the intensity of soil life and microbial activity (Perucci, 1992). Microplastic addition significantly stimulated the activity of FDAse, resulting in the enhancement of microbial hydrolytic activity on soil organic matter or DOM, which would lead to the accumulation of DOM (Fig. 3). An increase in DOM hydrolysis would not alter the apparent DOC content, because dissolved compounds remain soluble after hydrolysis (Gogo et al., 2014).

The beneficial effect of microplastic addition on DOM nutrient availability tended to increase over time after microplastic addition. Some of the changes in concentrations of DOM observed during the first few days of the incubation might be partly related to the sample disturbance during mixing. As the influence of sample disturbance on DOM nutrient availability decreases over time, the results of the later sampling are more reliable. Similarly, the beneficial effect of microplastic addition on microbial activity also increased over time, further promoting the cycling of soil C, N and P, and increasing the bioavailability of soil C, N and P. DOC and DON are concentrated in different fractions of DOM (hydrophobic and hydrophilic, respectively) (Kaiser and Zech, 2000; Petrone et al., 2009). The hydrophobic acid fraction is considered to be less degradable than hydrophilic neutral fraction (Jandl and Sollins, 1997; Kalbitz et al., 2003a). The labile fractions of DOM, such as carbohydrates, are preferentially degraded by microorganisms, so refractory DOM primarily accumulates during biodegradation (Ogawa et al., 2001; Kalbitz et al., 2003a, 2003b). In this study, microplastic addition had a more beneficial effect on DOC accumulation than DON within the first 3 days, as the DON contents in M1 and M2 were the same as CK at day 3. The lowerer decline rates of DON and TDN contents in M1 and M2 between days 3 and 14 relative to CK contributed to the accumulation of DON and TDN. The main DOM components were likely recalcitrant materials such as high-molecular-weight humic-like material and fulvic acid, indicating more recalcitrant material in M1 and M2 at day 7 relative to CK (Fig. 7). PO, which can degrade insoluble high-molecular-weight compounds I, such as Lignin, likely played a crucial role in DOM
mineralization between days 7 and 30 (Yamashita and Tanoue, 2003, 2004; Fellman et al., 2008). The higher PO activities in M1 and M2 relative to CK between days 7 and 14 contributed to the decomposition of insoluble high-molecular-weight materials, thus promoting the accumulation of DON.

Our results suggested that microplastic addition immediately contributed to the suppression of the accumulation of NO3\textsuperscript{–}, but this effect was transient. Although Microplastic increases soil porosity, specific surface area, and oxygen content which can enhance the nitrification process, the low content of NH4\textsuperscript{+} in soil is the limit for the nitrification process. Microplastic addition stimulated soil microbial activity which urged microbes to fiercely compete for limited NH4\textsuperscript{+} in soil, therefore limiting the nitrification process. The reduction of NO3\textsuperscript{–} content after microplastic addition was not mainly on account of microbial degradation because microbes preferentially utilized the DON component of the DOM solution as N source relative to NO3\textsuperscript{–} and NH4\textsuperscript{+} (Ghani et al., 2013). Microplastic addition increased microbial activities and favored DOC accumulation, whereas increased C-substrates exists to enable heterotrophic microbial growth (NH4\textsuperscript{+} immobilization) to dominate over autotrophic growth (NH4\textsuperscript{+} oxidation) (Jones et al., 2004), thus inhibiting the NO3\textsuperscript{–} accumulation. The NH4\textsuperscript{+} and inorganic P contents in the DOM solution were very low relative to the NO3\textsuperscript{–} content. Microplastic addition had a negligible effect on NH4\textsuperscript{+} content in DOM solution. The high level of microplastic addition could potentially increase inorganic P contents. However, Microplastic addition had no significant effect on long-term inorganic P contents. The dynamics of inorganic N and P depend on the balance between mineralization of organic matter and in situ immobilization by chemoautotrophic bacteria. FDAse activity was much higher at day 0 than days 14 and 30, indicating a slower rate of soil microbial mineralization between days 14–30, which would decrease the contents of inorganic N and P.

4.2. Effects of microplastic addition on soil DOM quality

High level of microplastic addition facilitated the formation of high-molecular-weight aromatic compounds in the DOM solution during the first seven days. However, SUVA 254, SUVA 280, and SUVA 365 subsequently decreased sharply in M2 between days 7 and 14, suggesting that the high-molecular-weight aromatic compounds in the DOM solution were decomposed into smaller compounds, as supported by the EEM spectroscopic characteristics of DOM (Fig. 7). High level of microplastic addition tended to facilitate the long-term accumulation of high-molecular-weight aromatic compounds relative to CK, while lower level of microplastic addition initially promoted the degradation of high-molecular-weight aromatic compounds, but finally had a negligible effect on high-molecular-weight aromatic compounds contents and the molecular size of humic substance.

A large proportion of the DOM in soil is humic acid (HA) and fulvic acid (FA) (Thurman, 1985). HA, with a molecular weight >1200 Da, is more readily degraded than FA, with a molecular weight of 500–1200 Da, because of its highly aromatic structure (Kisand et al., 2008; Rocker et al., 2012). Component 2, representing high-molecular-weight and aromatic humic materials, were significantly higher in M2 than CK throughout the experiment, especially at days 1 and 30, but there were no significant differences of component 2 between CK and M1 throughout the experiment, suggesting that high level of microplastic addition promoted the accumulation of humic substances while low level of microplastic addition had a negligible effect on humic substances. However, component 3, representing high-molecular-weight humic material, accumulated rapidly between days 7 and 30 in M1 and M2 relative to CK. This accumulation can be attributed to the different variations of PO activities among CK, M1, and M2. PO activities between days 7 and 30 decreased in M1 and M2, but increased in CK, which slowed the decomposition of humic-like material after microplastic addition, thus facilitating the accumulation of humic-like material. HA-rich materials are useful amendments for soil remediation because they improve soil structure, stability, water-holding capacity, nutrient availability, and sorption of metal ions (Schnitzer, 2000; Clemente and Bernal, 2006; Hayes and Malcom, 2001a, 2001b). Microplastic addition promoted the accumulation of high-molecular-weight humic-like material which improved the quality of soil. FA is the most mobile fraction and a major component of DOM in the environment (Stevenson, 1994; Maie et al., 2004). The content of FA in soil poses a large environmental risk, because it affects the transport and bioavailability of environmental contaminants, including heavy metals (Chirenje et al., 2002; Plaza et al., 2005), polycyclic aromatic hydrocarbons (PAHs) (Perminova et al., 2001; Gunasekara and Xing, 2003), and other chemicals (Stevenson, 1994) as carrying agents and complexing media. Component 4, representing FA, was significantly higher in M2 than CK throughout the experiment, especially at days 1 and 30, and was significantly higher in M1 than CK between days 1 and 14, but were the same between CK and M1 at days 1 and 30, implying that the beneficial effect of microplastic addition on fulvic acid content is positively correlated with the content of microplastic. The increase in FA-like material after microplastic addition therefore indicates a higher risk of environmental pollution (Fig. 7).

Microbial metabolites such as peptides generally accumulate when carbohydrate content is low so that other compounds such as lignin-derived moieties and lipids can serve as energy and C sources for microorganisms. Tyrosine-like fluorescence indicates more highly degraded peptides, and tryptophan-like fluorescence may indicate the presence of intact proteins or less degraded peptides (Mayer et al., 1999; Yamashita and Tanoue, 2003, 2004). Previous study showed that protein-like component is a useful predictor of biodegradable DOC compared to other fluorescent indicators (Fellman et al., 2008). In this study, microplastic addition initially decreased the tyrosine-like material in the DOM solution, which may have been due to the higher microbial activity in M1 and M2, contributing to enhanced the decomposition of tyrosine-like material. The tyrosine-like material, however, decreased in CK and M1 between days 7 and 30 but increased sharply in M2 between days 14 and 30, implying that the low level of microplastic addition had no significant influence on the long-term content of tyrosine-like material and that the beneficial effect of high level of microplastic addition on the accumulation of tyrosine-like material in the DOM solution could persist for a long time. Finally, the DOM in CK and M1 was mainly composed of high-molecular-weight humic material by the end of the experiment, indicating that this material was less biodegradable. However, the DOM solution in M2 was composed of high content tyrosine-like material, high-molecular-weight humic material and fulvic acid, which was related to the high microbial enzyme activity in M2.

5. Conclusions

The occurrence of microplastic in soil affected soil DOM and the activities of soil enzymes. Microplastic addition stimulated soil activity of fluorescein diacetate hydrolase (FDAse) in soil. The lower level of microplastic addition had a negligible effect on the contents of organic carbon, inorganic nitrogen, total phosphorus, high-molecular-weight humic-like material and fulvic acid in DOM solution at day 30 after microplastic added. The higher level of addition significantly increased the nutrient contents of the DOM, including those of DOC, DON, NO3\textsuperscript{–}, DOP, and PO4\textsuperscript{3–} and high-
molecular-weight humic-like material and fulvic acid. These imply that the accumulation of microplastic probably stimulate soil enzymatic activity which is useful for organic C, N, and P dissolved in soil and increasing the available source for plant. Thus, further study needs to be considered on the interaction between microplastic accumulation and plant growth, as well as the risk assessment of particle uptake by plant.

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