Effects of microplastics on chemodiversity of dissolved organic matter: A comparison with different types of polymers

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1. Supporting information of materials and methods

1.1. DNA Extraction

Microbial communities were analyzed by Illumina high-throughput sequencing. Total genomic DNA was extracted using E.Z.N.A.®Soil DNA Extraction Kit (Omega Bio-Tek, US) according to the manufacturer's instructions. The extracted DNA was evaluated by 1% agarose gel electrophoresis, and the quality and concentration of the extracts were determined with a spectrophotometer (NanoDrop, ND2000, Thermo Scientific, Wilmington, DE, USA).

1.2. PCR amplification and sequencing

The 16S rRNA gene in bacteria was amplified using forward and reverse primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), respectively. The ITS1 gene in fungi was amplified using forward and reverse primers ITS5F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS1R (5′-GCTGCGTTCTTCATCGATGC-3′), respectively. The PCRs were performed using the following protocol: 3 min of predenaturation at 95 °C, 30 s denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. Each sample has 3 replicates. The PCR products from the same sample were mixed, then were purified using 2% agarose gel and the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Purified amplicons were sequenced in a paired end format using the Illumina MiSeq platform by Majorbio BioPharm Technology Co. Ltd (Shanghai, China).

1.3. High-throughput sequencing data analysis

Raw sequences yielded from Illumina sequencing were processed using Quantitative Insights Into Microbial Ecology (QIIME, version 1.9.1). Briefly, paired reads were assembled and demultiplexed, and any sequences with a quality score < 20 or with truncated reads shorter than 50 bp were removed from the data set. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using Uparse 7.0, and chimeric sequences were identified and removed using Uchime. The obtained OTUs were aligned to the SILVA and UNITE reference databases to determine their taxonomic classification level (Threshold: 0.8–1) (kingdom, phylum, class, order, family, genus, species); the non-bacterial and non-fungal reads were further removed. OTU abundance information was normalized via a standard of the sample with the least sequences number.

All data analysis was conducted on the Meiji Biocloud platform (https://cloud.majorbio.com). Alpha diversity index, such as Simpson, Shannon, Chao, and Ace indices, was calculated using Mothur software (http://www.mothur.org/wiki/Calculators).

2. Figure legends of the supplementary information

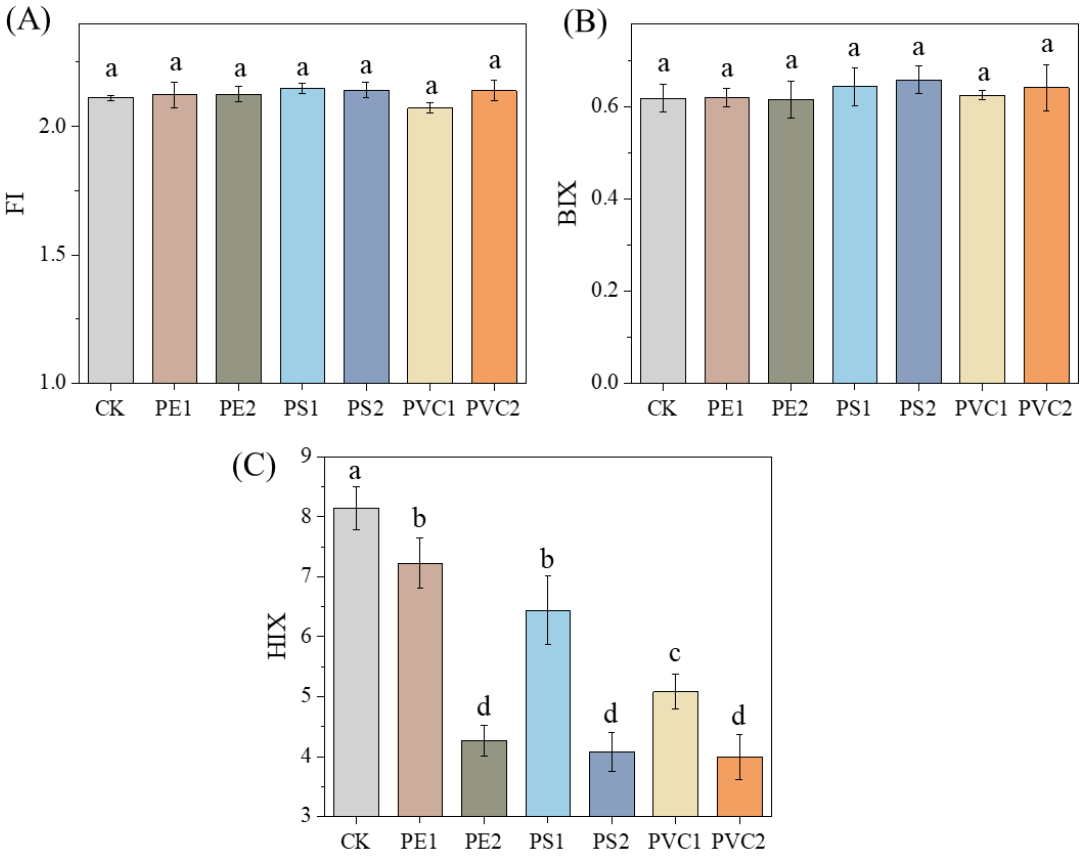
Fig. S1. 3D fluorescence spectroscopy characteristic parameters of Soil DOM. Different lowercase letters above bars represent significant differences among treatments at $p < 0.05$ level.

Fig. S2. Parallel factor analysis identified six independent fluorescence components of soil DOM.

Fig. S3. The number of unique molecules in each treatment.

Fig. S4. Van Krevelen diagrams for (a) PE1, (b) PE2, (c) PS1, (f) PS2, (e) PVC1, (f) PVC2, and (g) CK treatments.

Fig. S5. Van Krevelen diagrams for the core compounds common to all treatments.



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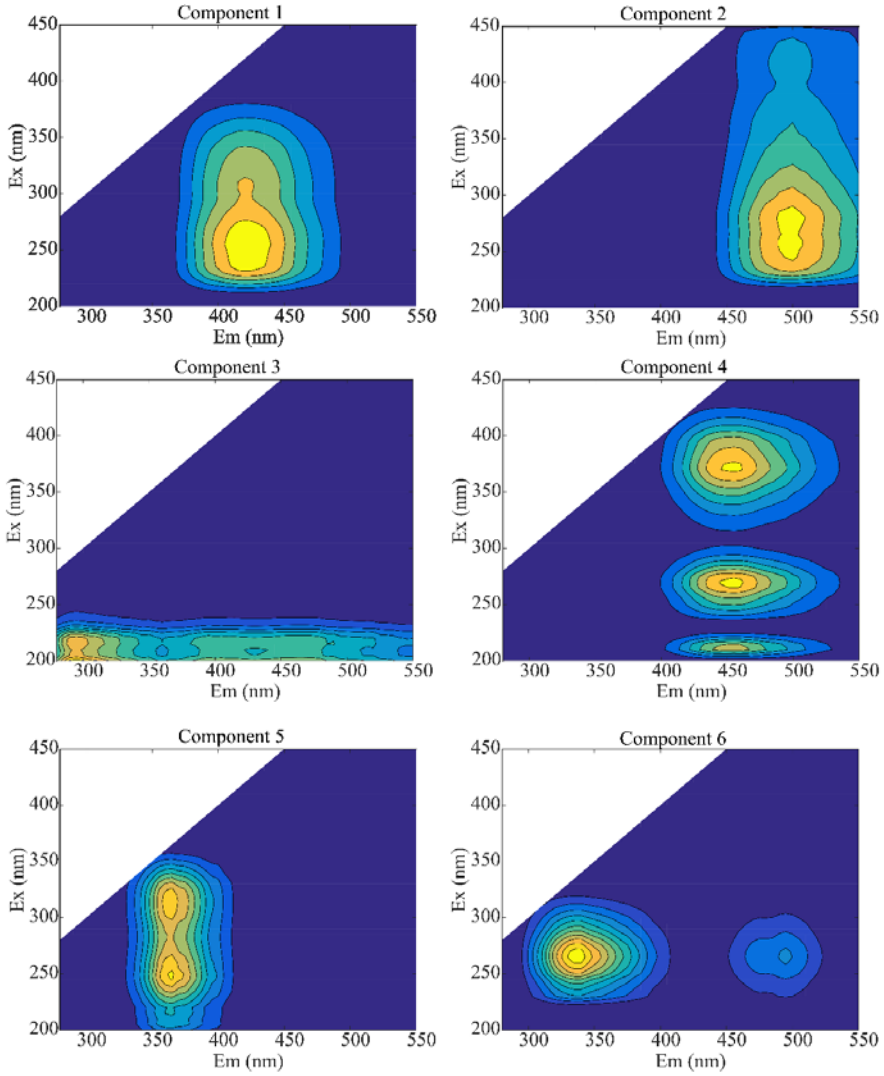
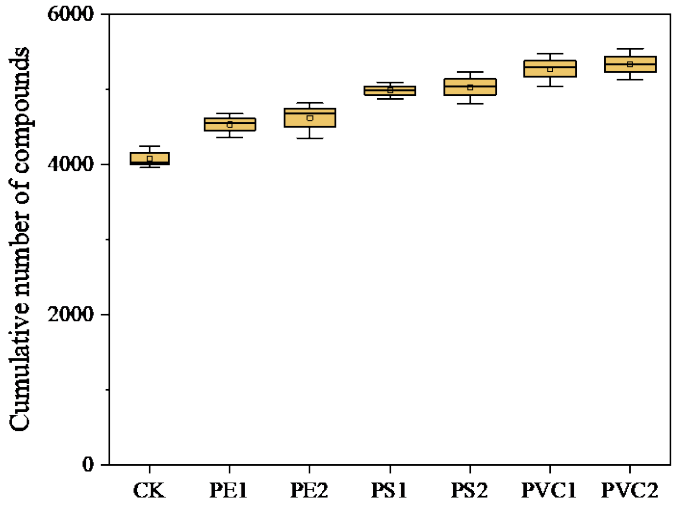


Fig. S2. Parallel factor analysis identified six independent fluorescence components of soil DOM.



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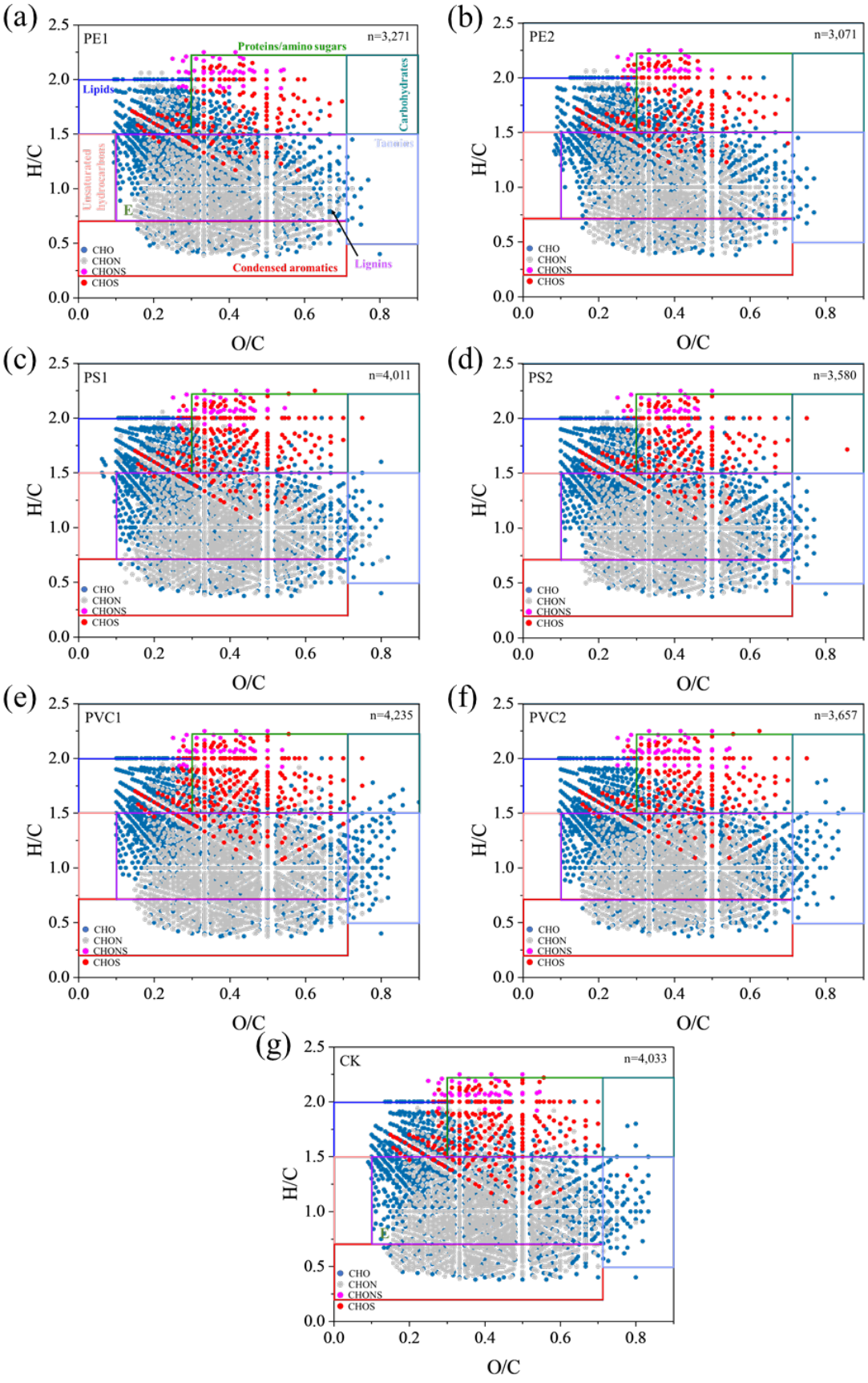

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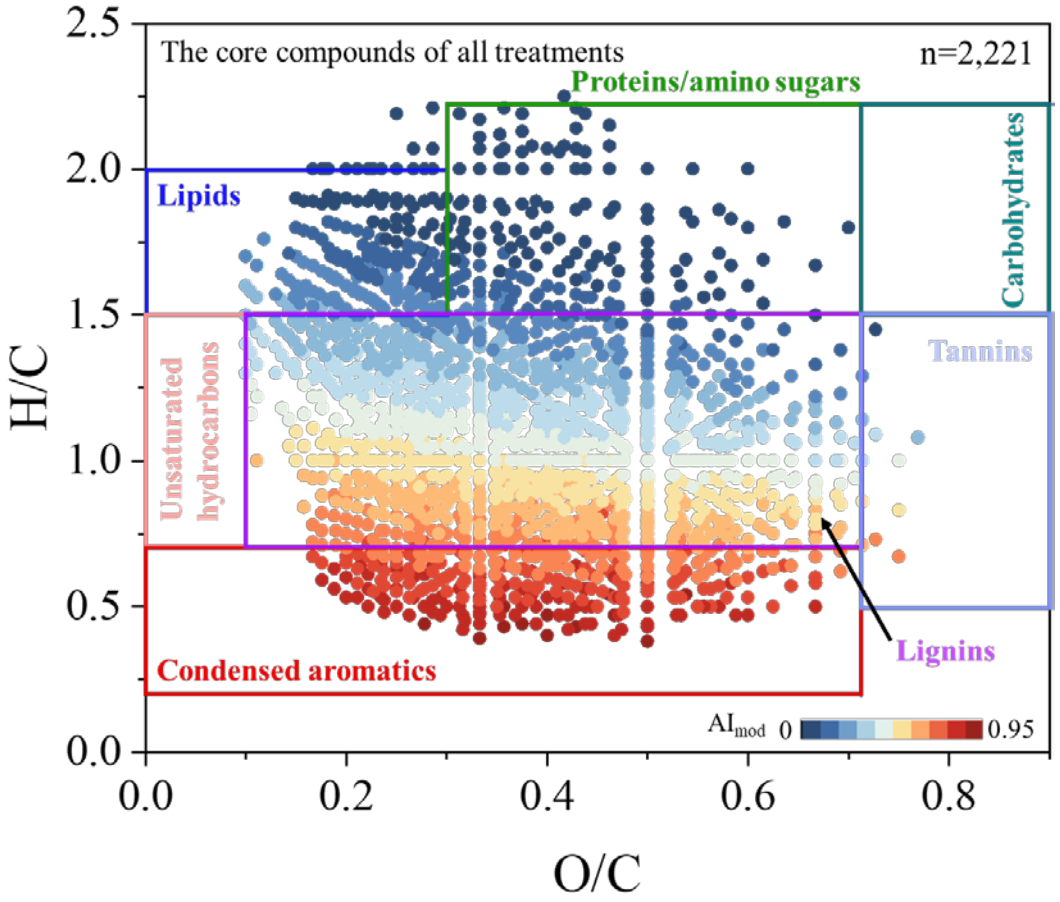

Fig. S5. Van Krevelen diagrams for the core compounds common to all treatments.

3. Table legends of the supplementary information

Table S1 Changes of soil properties induced by different MPs.

Table S2 Description of characteristic parameters of UV–Vis absorption and fluorescence spectra.

Table S3 Magnitude-weighted Parameters and Diversity Estimators of DOM Compounds indifferent treatments.

Table S1 Changes of soil properties induced by different MPs.

Soil properties		CK	PE1	PE2	PS1	PS2	PVC1	PVC2
pH		8.02±0.12c	8.54±0.18a	8.28±0.14b	8.45±0.13ab	8.32±0.10ab	8.32±0.08ab	8.41±0.20ab
EC (mS m^{-1})		10.3±0.9a	13.2±2.1a	10.2±1.4a	14.2±1.7a	15.3±1.3a	13.0±2.3a	23.3±4.1b
TN (g kg^{-1})		0.12±0.02a	0.10±0.01ab	0.09±0.01b	0.11±0.02ab	0.09±0.01b	0.11±0.02ab	0.12±0.01a
SOM (g kg^{-1})		1.8±0.16d	3.8±0.32c	7.6±0.62a	6.0±0.42b	6.2±0.22b	1.8±0.12d	1.8±0.13d
DOC (mg kg^{-1})		159.1±11.5a	129.4±10.3c	128.4±6.3c	111.8±8.2cd	96.7±7.8d	141.6±6.7b	110.9±9.6cd
CAT (μmol H_2O_2 g^{-1} $24h^{-1}$)		66.5±4.3c	84.4±6.0b	84.4±5.7b	80.7±5.6c	83.8±6.7b	71.6±5.3d	92.1±7.1a
URE (mg NH_3–N g^{-1} $24h^{-1}$)		108.6±10.4d	116.9±8.5cd	117.4±9.5cd	190.5±13.5a	114.3±7.6cd	181.8±14.4b	123.0±11.3c
ALP (mmol pheno g^{-1} $24h^{-1}$)		6.46±0.53e	7.59±0.62bc	8.36±0.54a	6.66±0.47d	7.44±0.55c	7.88±0.62b	6.84±0.48d
Bacteria	Simpson	0.0032±0.0001a	0.0033±0.0002a	0.0038±0.0003a	0.0040±0.0003a	0.0033±0.0002a	0.0046±0.0003a	0.0636±0.0003b
	Shannon	7.07±0.25a	6.87±0.12ab	6.91±0.20a	7.01±0.27a	7.09±0.22a	6.47±0.02b	4.76±0.13c
	Chao	3896±281a	3800±266a	3734±197a	3916±218a	3950±226a	3558±282a	2396±161b
	Ace	3958±283a	3756±212a	3894±223a	3955±256a	4142±303a	3477±251a	2856±221b
Fungal	Simpson	0.0795±0.005a	0.0651±0.004a	0.1335±0.009b	0.0571±0.003a	0.0618±0.004a	0.2082±0.014c	0.365±0.028d
	Shannon	4.04±0.28a	3.89±0.23ab	3.48±0.19b	4.21±0.29a	4.07±0.29a	2.41±0.17c	1.89±0.11d
	Chao	643±38a	600±35a	627±41a	642±45a	623±46a	392±28b	364±24b
	Ace	657±49a	598±34a	635±46a	638±47a	610±39a	385±24b	368±27b

Note: EC, electrical conductivity; TN, total nitrogen; SOM, soil organic matter; DOC, dissolved organic carbon; CAT, catalase; URE, urease; ALP, alkaline phosphatase. Values are described as mean ± SD (n = 3). Different lowercase letters indicate significant differences at $p < 0.05$ level among treatments.

Table S2 Description of characteristic parameters of UV–Vis absorption and fluorescence spectra.

Spectroscopic parameters	Calculation method	Description
$SUVA_{254}$	$SUVA_{254}$ = α(254)/DOC; a(254) is the absorption coefficient at 254 nm UV wavelength.	Indicates the aromaticity of DOM and shows a positively correlation with the aromaticity of DOM (Wu et al., 2020).
$SUVA_{260}$	$SUVA_{260}$ = α(260)/DOC; a(260) is the absorption coefficient at 260 nm UV wavelength.	Indicates the content of hydrophobic DOM containing aromatic carbon groups and a positive correlation with the hydrophobic component of DOM (Wu et al., 2020).
E2/E3	The absorbance at 250 nm divides by the absorbance at 365 nm.	Indicates the degree of DOM humification, and shows a negative correlation with molecular weight of DOM (Wu et al., 2020).
E3/E4	The absorbance at 300 nm divides by the absorbance at 400 nm.	Indicates DOM source. E3/E4 > 3.5, indicating that DOM is mainly fulvic acid; E3/E4 < 3.5 is humic acid (Wu et al., 2020).
Fluorescence Index（FI）	The ratio of the fluorescence intensity at Em is 470 nm to that at 520 nm when Ex is 370 nm	Represents DOM source. FI > 1.9 represents DOM mainly from microbial activities; FI < 1.4 represents DOM mainly from terrestrial plants and SOM (Liu et al., 2022).
Biological index (BIX)	The ratio of Em intensity at 380 nm to the maximum Em between 420 and 435 nm when Ex is 310 nm.	Indicates the contribution of newly produced autochthonous DOM content. BIX > 1.0 means that DOM is mainly from an autogenous source and newly produced, and BIX of 0.6–0.7 means that authigenic components of DOM are less (Liu et al., 2022).
Humification index (HIX)	The regional area of 435 nm < Em < 480 nm divides by that of 300 nm < Em < 345 nm when Ex is 254 nm.	Indicates humification levels of DOM. HIX > 10 indicates a high humification level of DOM, and HIX < 4 indicates a low humification level of DOM (Liu et al., 2022).

Table S3 Magnitude-weighted Parameters and Diversity Estimators of DOM Compounds indifferent treatments.

Treatments	Average formula	$(MW)_W$	$(DBE)_W$	$(O/C)_W$	$(H/C)_W$	$(DBE/C)_W$	$(DBE/O)_W$	$(DBE-O)_W$	$(AI_{mod})_W$	$(NOSC)_W$	MLB_L (%)
CK	$C_{17.43}H_{20.49}N_{0.46}O_{6.47}S_{0.17}$	344.87	8.41	0.38	1.24	0.48	1.30	1.94	0.36	-0.32	27.5
PE1	$C_{17.80}H_{25.16}N_{0.33}O_{5.58}S_{0.21}$	339.26	6.38	0.32	1.48	0.36	1.14	0.80	0.25	-0.70	41.9
PE2	$C_{17.75}H_{27.20}N_{0.24}O_{5.66}S_{0.24}$	341.84	5.27	0.33	1.60	0.30	0.93	-0.39	0.19	-0.82	46.0
PS1	$C_{17.47}H_{24.07}N_{0.35}O_{5.16}S_{0.15}$	325.95	6.61	0.30	1.44	0.38	1.28	1.45	0.27	-0.70	37.3
PS2	$C_{17.45}H_{23.29}N_{0.35}O_{5.50}S_{0.16}$	330.77	6.98	0.32	1.40	0.40	1.27	1.48	0.29	-0.61	33.0
PVC1	$C_{17.46}H_{22.74}N_{0.41}O_{6.09}S_{0.16}$	340.50	7.29	0.36	1.37	0.42	1.20	1.20	0.30	-0.50	27.3
PVC2	$C_{17.43}H_{22.34}N_{0.39}O_{5.93}S_{0.16}$	337.12	7.46	0.35	1.35	0.43	1.26	1.53	0.31	-0.50	28.2

$(MW)_W$: intensity weighted molecular weight (Da); $(DBE)_W$: intensity weighed averaged double bond equivalent; $(O/C)_W$: intensity weighted averaged oxygen-to-carbon ratios; $(H/C)_W$: intensity weighted averaged hydrogen-to-carbon ratios; $(DBE/C)_W$: intensity weighted averaged DBE-to-carbon ratios; $(DBE/O)_W$: intensity weighted averaged DBE-to-oxygen ratios; $(DBE-O)_W$: intensity weighted averaged DBE with numbers of oxygen atoms subtracted; $(AI_{mod})_W$: intensity weighted averaged modified aromatic index; $(NOSC)_W$: intensity weighted averaged nominal oxidation state of carbon; MLB_L: the percentage of labile compounds above the molecular lability boundary.

References

Liu, Y., Hu, W., Huang, Q., Qin, J., Zheng, Y., Wang, J., Li, X., Wang, Q., Guo, G., Hu, S., 2022. Plastic mulch debris in rhizosphere: Interactions with soil-microbe-plant systems. Sci. Total Environ. 807(Pt 2), 151435.

Wu, D., Ren, C., Jiang, L., Li, Q., Zhang, W., Wu, C., 2020. Characteristic of dissolved organic matter polar fractions with variable sources by spectrum technologies: Chemical properties and interaction with phenoxy herbicide. Sci. Total Environ. 724, 138262.