Supporting information of

Metabolic response of lettuce (*Lactuca sativa* L.) to foliar exposed polystyrene nanoplastics and microplastics

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Text S1 Determination of SOD and CAT activities and MDA concentration

After grinding in liquid nitrogen, fresh leaves were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8) and centrifugated (4 °C, 10,000×g) for 10 min. Next, the supernatant was collected for determining SOD, CAT, and MDA. These three indexes were measured according to the instructions of assay kits provided by Keming Biotechnology Co., Ltd. (Suzhou, China).

SOD can inhibit the photochemical reduction of nitroblue tetrazolium (NBT). One unit of SOD activity (U) was defined as the amount of enzyme that caused a 50% decrease in the SOD-inhibited NBT reduction. Specifically, an aliquot of sample supernatant (V_t) was added into tubes (referred as sample tube) which containing reaction mixture, i.e., phosphate buffer, methionine, ethylenediaminetetraacetic acid, NBT, and xanthine. In control and blank tubes, Milli-Q water, instead of supernatant, was added into the reaction mixture. Next, the blank tubes were stored in dark for 15 min whereas other tubes were irradiated under light for 15 min. Finally, the absorbance of all solutions was measured at 560 nm using a spectrophotometer. The SOD activity was calculated according to the equation as follows:

$$SOD\ activity\ (U\ g^{-1}) = \frac{(A_{CK} - A_E) \times V}{0.5 \times A_{CK} \times W \times V_t} \quad (1)$$

Where A_{CK} and A_E are the absorbance of control and sample solution at 560 nm, respectively; V is the total volume of sample solution (mL); W is the fresh weight of lettuce leave sample (g); V_t is the supernatant volume used to determination (mL).

CAT activity was determined by following the consumption of H_2O_2 at 240 nm. One unit of CAT activity (U) was defined as the amount of enzyme catalyzing the decomposition of 1 nmol of H_2O_2 per min. Specifically, 10 μL of supernatant was mixed thoroughly with 190 μL reaction mixture, which containing H_2O_2 and phosphate buffer. Afterwards, the absorbance of solutions was measured

at 240 nm at 0 (A_1) and 1 min (A_2). The CAT activity was calculated according to the equation as follows:

$$CAT\ activity\ (nmol\ min^{-1}\ g^{-1}) = \frac{(A_1 - A_2) \times 918}{W} \quad (2)$$

Where W is the fresh weight of lettuce leave sample (g).

Thiobarbital acid (TBA) colorimetric method was applied to measure the MDA content. MDA can react with TBA to produce a red product with a maximum absorption peak at 532 nm. To measure MDA content, an aliquot of sample supernatant was added into the reaction mixture consisted of trichloroacetic acid and thiobarbituric acid. The obtained mixture was then reacted for 30 min in a boiling water bath (90 °C). After cooling, the mixture was centrifuged at 10,000×g for 10 min at 25 °C. Afterwards, the absorbance of supernatants was measured at 532 nm (A_{532}) and 600 (A_{600}) nm. The MDA content was calculated according to the equation as follows:

$$MDA\ content\ (nmol\ g^{-1}) = \frac{\left[6.432 \times (A_{532} - A_{600}) - 0.559 \times A_{450}\right] \times V_t}{V_s \times W} \quad (3)$$

Where A_{450} is the absorbance of supernatants at 450 nm; V_t is the total volume of sample solution (mL); V_s is the supernatant volume used to determination (mL); W is the fresh weight of lettuce leave sample (g).

Text S2

Determination of soluble protein, soluble sugar, ascorbic acid, and nitrate in lettuce leaves

Soluble protein: The fresh lettuce leaves were rapidly frozen with liquid nitrogen. After grinding, 0.2 g of fresh lettuce leaves were extracted with 2 mL of extraction solution. The obtain extracts were centrifuged (8000 g, 4 ℃) for 10 min. Next, the supernatant was collected to determine the soluble protein content according to Coomassie brilliant blue method.

Soluble sugar: 0.1 g of fresh lettuce leaves were homogenized in 1 mL of Milli-Q water. The obtained homogenate was bathed in water at 95 ℃ for 10 min, cooled, and centrifuged (8000 g, 25 ℃) for 10 min. Next, the supernatant was collected to determine the content of soluble sugar according to anthrone colorimetric method.

Ascorbic acid: 0.1 g of fresh lettuce leaves were homogenized in 1 mL of ice-cold extraction solution. Next, the obtained homogenate was centrifuged (8000 g, 4 ℃) for 20 min. The supernatant was collected to determine the content of ascorbic acid according to 2, 6-dichlorophenol sodium indigophenol titration method

Nitrate: 0.1 g of fresh lettuce leaves were homogenized in 1 mL of Milli-Q water. The obtained homogenate was shake in a constant temperature oscillation chamber at 90 ℃ for 30 min, cooled, and centrifuged (12000 g, 25 ℃) for 15min. Next, the supernatant was collected to determine the content of nitrate nitrogen according to salicylic acid method. The nitrate content was calculated by multiplying nitrate nitrogen by 4.4.

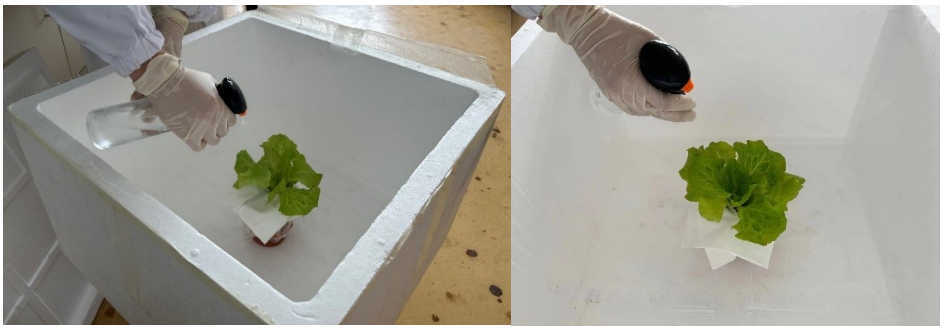

Figure S1 Images of lettuce spraying detail

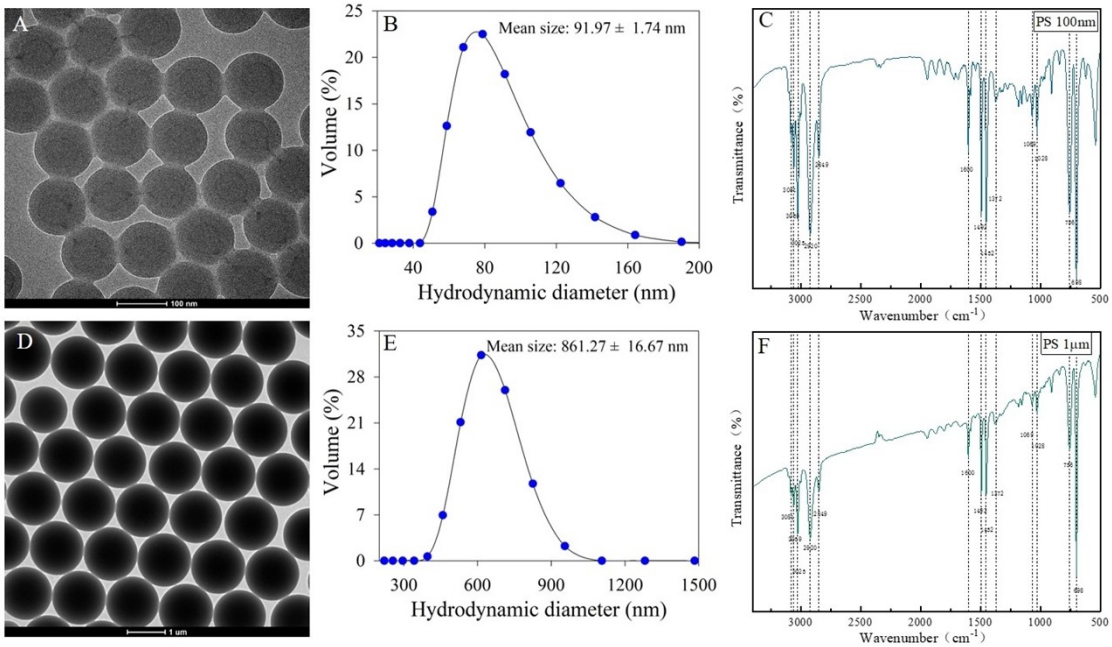

Figure S2 Transmission electron microscopy images (A and D) and dynamic light scattering particle size distribution (B and E), as well as Fourier transform infrared spectrograms (C and F) of PS NPs (A-C) and PS MPs (D-F).

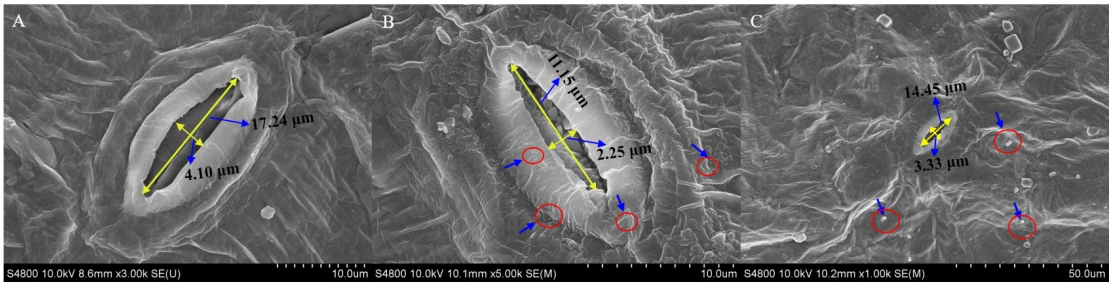

Figure S3 Scanning electron microscope images of lettuce leaf surface from CK (A), NP-HC (B), and MP-HC treatments (C).

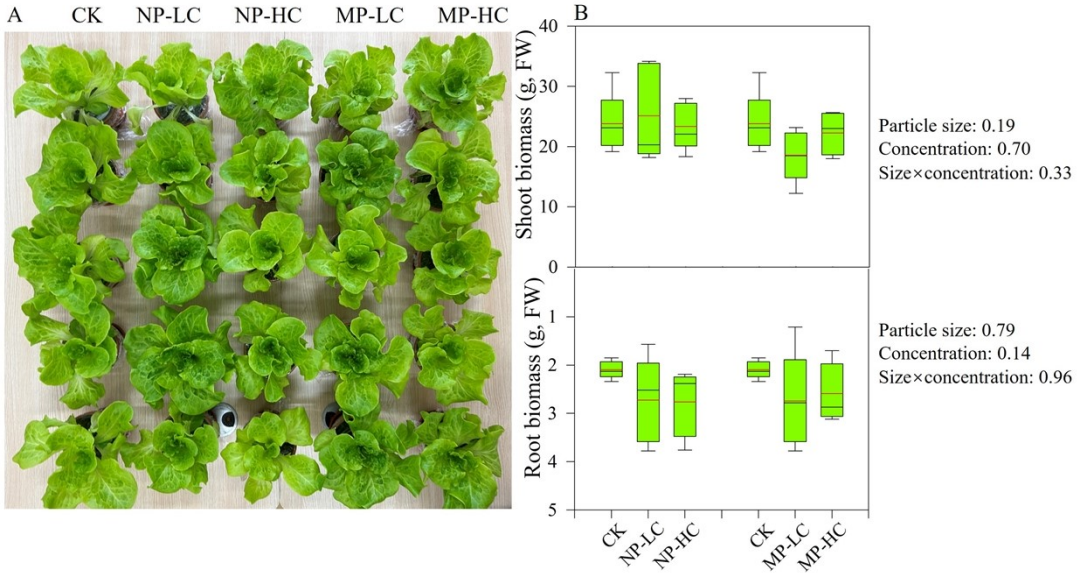

Figure S4 Lettuce morphology after PS exposure (A) and the effects of PS on the fresh biomass of lettuce leaves and roots (B). The lettuce was foliar exposed to Milli-Q water (control, CK), 100 nm PS at a low concentration of 3.5 mg L^{-1} (NP-LC) and a high concentration of 35.0 mg L^{-1} (NP-HC), 1 μm PS at a low concentration of 3.5 mg L^{-1} (MP-LC) and a high concentration of 35.0 mg L^{-1} (MP-HC). The red and black lines in the box plot represent the mean and median values, respecitively. The effects of PS particle size, concentration, and their interations are analyzed by two-way analysis of variance and the F values are presented in the test. Data are presented as mean ± SD (n = 5).

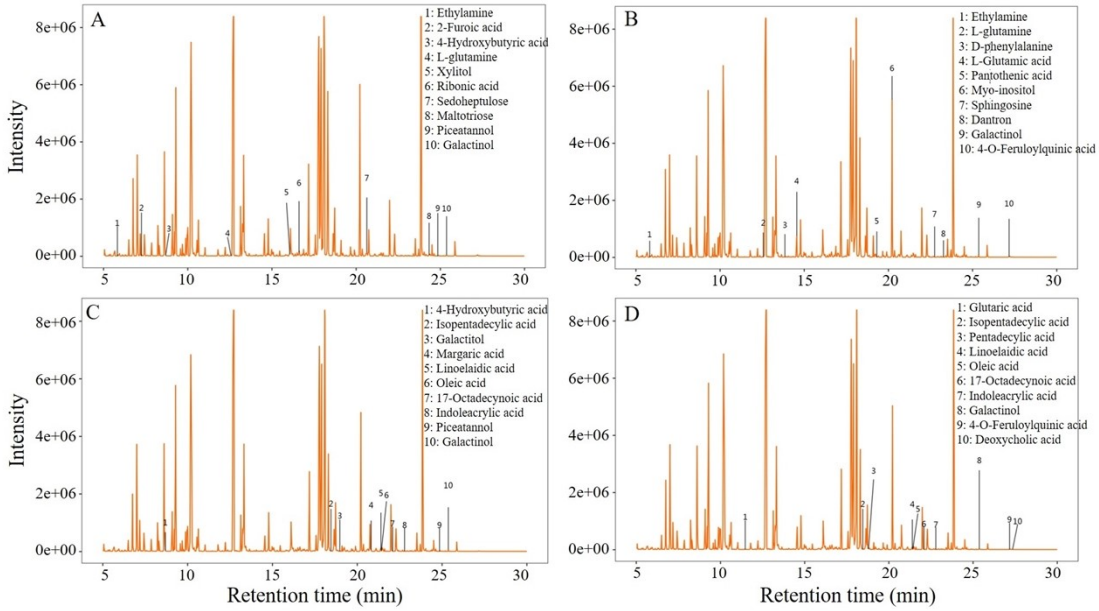

Figure S5 The total ion chromatogram of metabolites in lettuce leaves in NP-LC (A), NP-HC (B), MP-LC (C), and MP-HC (D) treatments. The number represents the top 10 metabolites which were selected based on VIP values (VIP >1). The lettuce was foliar exposed to 100 nm PS at a low concentration of 3.5 mg L^{-1} (NP-LC) and a high concentration of 35.0 mg L^{-1} (NP-HC), 1 μm PS at a low concentration of 3.5 mg L^{-1} (MP-LC) and a high concentration of 35.0 mg L^{-1} (MP-HC).

Table S1 The data related to the calculation of capture rate.

Replicates	Total spraying volume (mL)	The volume wasted in the plastic box (mL)	Capture rate (%)	Average of capture rate (%)	SD of capture rate (%)
1	6.10	3.28	46.29	41.57	3.61
2	3.80	2.37	37.51		
3	6.70	3.96	40.92		

Table S2 The properties of artificial soil used in this study. Data are given as mean ± SD (n = 3).

pH	OM (g kg^{-1})	WHC (%)	CEC (cmol kg^{-1})
5.40 ± 0.02	44.96 ± 3.15	46.83 ± 5.36	15.94 ± 0.76

Table S3 Effects of PS NPs and PS MPs foliar exposure on the concentration of chlorophyll a, chlorophyll b, total chlorophyll, and nitrate, as well as catalase activity in lettuce leaves. Data are presented as mean ± SD (n = 5).

Particle size	Concentration	Chlorophyll a (mg kg^{-1})	Chlorophyll b (mg kg^{-1})	Total chlorophyll (mg kg^{-1})	Catalase activity (nmol min^{-1} g^{-1})	Nitrate (mg kg^{-1})
Nanoplastics	CK	0.55±0.09a	0.26±0.04a	0.80±0.11a	18.39±2.05a	1712.31±109.27a
	LC	0.54±0.05a	0.22±0.01a	0.76±0.06a	25.36±3.53b	1856.80±56.30a
	HC	0.41±0.03b	0.18±0.01b	0.60±0.05b	93.76±2.36c	1189.70±129.63b
Microplastics	CK	0.55±0.09a	0.26±0.04a	0.80±0.11a	18.39±2.05a	1712.31±109.27a
	LC	0.60±0.03a	0.25±0.01a	0.85±0.03a	14.16±0.55b	1630.84±89.26a
	HC	0.31±0.06b	0.15±0.0.1b	0.46±0.0.6b	18.81±2.12a	1279.40±95.99b
Particle size		1.81	0.51	1.80	1183.84***	1.43
Concentration		38.30***	50.44***	54.75***	884.12***	78.97***
Size × concentration		6.09**	7.15**	8.29**	782.12***	6.55**

Note: LC and HC mean PS exposure at a low concentration of 3.5 mg L^{-1} and a high concentration of 35.0 mg L^{-1}, respectively. Different lowercase letters represent significant differences among treatments with the same particle size as revealed by two-way ANOVA followed by Bonferroni paired comparison. The results of two-way ANOVA are shown in the text. ** and *** mean significant effects at $p < 0.01$ and $p < 0.001$, respectively.

Table S4 Effects of PS NPs and PS MPs foliar exposure on superoxide dismutase (SOD) acivity and the concentration of malonaldehyde (MDA), soluble protein, soluble sugar, and ascorbic acid in lettuce leaves. Data are presented as mean ± SD (n = 5).

Concentration	SOD activity ($U\ g^{-1}$)	MDA concentration ($nmol\ g^{-1}$)	Soluble protein ($mg\ g^{-1}$)	Soluble sugar ($mg\ g^{-1}$)	Ascorbic acid ($mg\ kg^{-1}$)
CK	324.50±24.41a	4.64±0.66a	13.38±1.55a	8.47±1.05a	92.05±11.84a
LC	270.31±34.32b	6.14±0.59b	16.55±1.51b	8.64±1.04a	101.31±15.74a
HC	255.00±23.52b	7.43±1.06c	18.56±2.36c	11.39±2.20b	96.00±9.16a
Particle size	2.41	1.75	1.64	4.10	4.07
Concentration	18.29***	31.75***	23.00***	14.51***	1.73
Size × concentration	1.16	1.32	2.72	3.10	2.95

Note: LC and HC mean PS exposure at a low concentration of 3.5 mg L^{-1} and a high concentration of 35.0 mg L^{-1}, respectively. The data presented in the table are the mean and SD values of all CK, LC treatments (including NP-LC and MP-LC treatments), and HC treatments (including NP-LC and MP-LC treatments), respectively. Different lowercase letters represent significant differences among treatments by two-way ANOVA followed by Bonferroni paired comparison. The results of two-way ANOVA are shown in the text. *** means significant effects at $p < 0.001$.

Table S5 The detailed information of metabolites with significant difference selected by PLS-DA (VIP > 1) and t-test ($p < 0.05$) in lettuce leaves upon exposure to PS NPs.

NP-LC vs CK			NP-HC vs CK		
Metabolites	**VIP**	***p*-value**	**Metabolites**	**VIP**	***p*-value**
P-Hydroxyphenylacetic Acid	1.24	0.0073	Galactinol	2.94	0.0002
3-Hydroxyphenylacetic Acid	1.32	0.0029	Pantothenic Acid	2.37	0.0000
Myo-Inositol	1.44	0.0002	4-O-Feruloylquinic Acid	2.35	0.0052
Quinic Acid	1.21	0.0017	Myo-Inositol	2.34	0.0000
Ethylamine	2.38	0.0006	Ethylamine	2.27	0.0071
Sphingosine	1.70	0.0002	Sphingosine	2.23	0.0050
L-Glutamine	2.38	0.0000	L-Glutamine	2.12	0.0048
D-Phenylalanine	1.69	0.0002	D-Phenylalanine	2.02	0.0126
Dl-Pyroglutamic Acid	1.26	0.0010	L-Glutamic Acid	2.02	0.0016
Beta-Alanine	1.22	0.0045	Dantron	1.93	0.0003
L-Glutamic Acid	1.20	0.0064	3-Hydroxybenzoic Acid	1.91	0.0000
L-Alanine	1.11	0.0017	Deoxycholic Acid	1.88	0.0142
Dantron	1.27	0.0059	Galactitol	1.81	0.0142
Meso-Tartrate	1.12	0.0010	Galactose 1-Phosphate	1.76	0.0137
Maltotriose	1.92	0.0000	Xylitol	1.72	0.0053
Xylitol	1.92	0.0001	D-Threonic Acid	1.72	0.0006
Ribonic Acid	1.91	0.0087	Maltotriose	1.70	0.0043
Galactose 1-Phosphate	1.87	0.0025	N-Acetylmannosamine	1.61	0.0003
Isomaltose	1.80	0.0036	Beta-Lactose	1.59	0.0168
Beta-Lactose	1.74	0.0003	Melibiose	1.56	0.0025
L-Fucitol	1.73	0.0126	D-(+)-Mannose	1.54	0.0001
Dl-Glyceric Acid	1.69	0.0000	D-Tagatose	1.53	0.0003
D-Arabitol	1.67	0.0001	Dl-Glyceric Acid	1.48	0.0001
D-Threonic Acid	1.65	0.0001	D-Arabitol	1.43	0.0014
N-Acetylmannosamine	1.65	0.0001	1,5-Anhydrosorbitol	1.43	0.0003
Glucose	1.63	0.0000	L-Fucitol	1.39	0.0441
2-Ketoglucose	1.59	0.0419	Glutaric Acid	1.38	0.0001
D-Glucose	1.43	0.0000	4-Hydroxybutyric Acid	1.38	0.0412
D-Lyxose	1.41	0.0002	17-Octadecynoic Acid	1.38	0.0194
1-Deoxy-D-Glucitol	1.36	0.0026	Margaric Acid	1.36	0.0406
D-Xylose	1.35	0.0006	Oleic Acid	1.36	0.0077
D-(+)-Mannose	1.23	0.0043	Isopentadecylic Acid	1.35	0.0107
D-Tagatose	1.16	0.0008	Pentadecylic Acid	1.34	0.0198
Fructose	1.15	0.0000	Caproic Acid	1.34	0.0019
L-Lyxulose	1.14	0.0022	Myristic Acid	1.30	0.0003
Erythritol	1.07	0.0030	Lactitol	1.28	0.0163
Sucrose	1.07	0.0009	Leucrose	1.27	0.0334
1,5-Anhydrosorbitol	1.04	0.0247	2-Furoic Acid	1.26	0.0002
Formamide	1.15	0.0107	Erythrono-1,4-Lactone	1.25	0.0090
D-Xylono-1,5-Lactone	1.62	0.0000	Dehydroascorbic Acid	1.23	0.0411

Succinic Acid	1.19	0.0006	Indoleacrylic Acid	1.23	0.0072
Pgf2alpha	1.40	0.0002	Linoelaidic Acid	1.22	0.0006
4-Hydroxybutyric Acid	2.53	0.0000	6-Hydroxyhexanoic Acid	1.21	0.0155
Daminozide	1.26	0.0063	Glycerol 1-Myristate	1.20	0.0266
D-(-)-Citramalic Acid	1.07	0.0066	Methylphosphate	1.19	0.0149
Lactitol	1.39	0.0041	Nicotinic Acid	1.19	0.0017
2-Furoic Acid	1.90	0.0043	Uracil	1.19	0.0001
Dehydroascorbic Acid	1.45	0.0001	Thymine	1.18	0.0002
Erythrono-1,4-Lactone	1.15	0.0026	2-Hydroxyglutaric Acid	1.18	0.0344
Oxoglutaric Acid	1.24	0.0027	Isocitric Acid	1.17	0.0079
Linoelaidic Acid	1.35	0.0480	Triacetin	1.17	0.0005
6-Hydroxyhexanoic Acid	1.89	0.0003	Piceatannol	1.16	0.0017
Glycerol 1-Myristate	1.43	0.0402	Ethyl-Alpha-D-Glucopyranoside	1.14	0.0117
Tyramine	1.05	0.0046	2-Ketobutyric Acid	1.13	0.0058
2-Hydroxyglutaric Acid	1.25	0.0011	Beta-Mannosylglycerate	1.12	0.0019
Triacetin	1.21	0.0011	Sedoheptulose	1.11	0.0020
Galactinol	3.17	0.0004	5-Methyluridine	1.11	0.0015
Piceatannol	2.10	0.0066	Tartaric Acid, Ethyl Ester	1.10	0.0068
Sedoheptulose	1.97	0.0000	Mannobiose	1.09	0.0135
Tartaric Acid, Ethyl Ester	1.89	0.0091	Conduritol-Beta-Epoxide	1.09	0.0261
D-Fucitol	1.72	0.0000	3-(3-Hydroxyphenyl)Propanoic Acid	1.08	0.0027
Beta-Mannosylglycerate	1.67	0.0235	2-Hydroxycyclohexane-1-Carboxylic Acid	1.06	0.0176
5-Methyluridine	1.54	0.0423	5-Methylimidazolidine-2,4-Dione	1.05	0.0170
Ethyl-Alpha-D-Glucopyranoside	1.47	0.0005	5,5-Dimethylhydantoin	1.04	0.0022
Conduritol-Beta-Epoxide	1.39	0.0003	1-C-Octylhexopyranose	1.04	0.0153
1-C-Octylhexopyranose	1.18	0.0029	2,3,4-Tri-O-Methyl-D-Galactopyranose	1.04	0.0075
D-Fructose	1.18	0.0000	D-Fucitol	1.02	0.0352
2,3,4,5-Tetrahydroxypentanoic Acid	1.14	0.0027	Furfuryl Alcohol	1.01	0.0082
Levoglucosan	1.09	0.0369	5-Methylpyrrolidin-2-One	1.01	0.0095

Table S6 The detailed information of metabolites with significant difference selected by PLS-DA (VIP > 1) and t-test ($p < 0.05$) in lettuce leaves upon exposure to PS MPs.

MP-LC vs CK			MP-HC vs CK		
Metabolites	**VIP**	***p* value**	**Metabolites**	**VIP**	***p* value**
1,2,4,5-Cyclohexanetetrol	1.76	0.0439	4-O-Feruloylquinic Acid	2.13	0.0011
4-O-Feruloylquinic Acid	1.73	0.0036	1,2,4,5-Cyclohexanetetrol	1.98	0.0228
4-Methoxycyclohexanol	1.72	0.0121	4-Methoxycyclohexanol	1.72	0.0121
Shikimic Acid	1.32	0.0104	Shikimic Acid	1.33	0.0032
Ethylamine	2.06	0.0002	Sphingosine	1.18	0.0011
Sphingosine	1.41	0.0002	Beta-Alanine	1.11	0.0027
Cadaverine	1.19	0.0059	1,3-Benzenediol	1.05	0.0398
Beta-Alanine	1.55	0.0001	Hydroxyhydroquinone	1.01	0.0004
L-Serine	1.09	0.0026	3-Hydroxybenzoic Acid	1.38	0.0026
Hydroxyhydroquinone	1.05	0.0003	Hydroxypropionic Acid	1.16	0.0000
3-Hydroxybenzoic Acid	1.34	0.0032	Deoxycholic Acid	2.25	0.0021
Hydroxypropionic Acid	1.24	0.0001	Galactose	1.63	0.0106
Deoxycholic Acid	1.83	0.0053	D-Gulose	1.58	0.0135
Galactitol	2.11	0.0000	Maltotriose	1.45	0.0002
Galactose 1-Phosphate	1.38	0.0129	L-(-)-Sorbose	1.44	0.0135
D-Threonic Acid	1.38	0.0001	Galactose 1-Phosphate	1.33	0.0001
Xylitol	1.34	0.0003	Glucose	1.27	0.0008
N-Acetylmannosamine	1.33	0.0001	Beta-Lactose	1.27	0.0011
Dl-Glyceric Acid	1.12	0.0002	Xylitol	1.27	0.0010
L-Fucitol	1.11	0.0051	D-Threonic Acid	1.24	0.0001
Glucose	1.02	0.0095	1-Deoxy-D-Glucitol	1.16	0.0066
Galactaric Acid	1.02	0.0056	Methyl-Beta-Galactopyranoside	1.13	0.0253
Glutaric Acid	2.07	0.0013	N-Acetylmannosamine	1.12	0.0005
Pgf2alpha	1.07	0.0036	L-Fucitol	1.09	0.0042
17-Octadecynoic Acid	2.38	0.0044	D-Glucose	1.02	0.0029
Oleic Acid	2.37	0.0069	Glutaric Acid	2.53	0.0002
Isopentadecylic Acid	2.31	0.0061	Isopentadecylic Acid	2.95	0.0033
4-Hydroxybutyric Acid	2.12	0.0000	Oleic Acid	2.86	0.0044
Margaric Acid	2.11	0.0050	17-Octadecynoic Acid	2.37	0.0044
Pentadecylic Acid	2.05	0.0065	Pentadecylic Acid	2.34	0.0041
Caproic Acid	1.43	0.0015	Margaric Acid	2.10	0.0050
Myristic Acid	1.34	0.0071	4-Hydroxybutyric Acid	1.68	0.0042
Heptylic Acid	1.21	0.0070	Itaconic Acid	1.62	0.0274
Stearic Acid	1.18	0.0064	Caproic Acid	1.54	0.0008
Palmitic Acid	1.10	0.0097	Myristic Acid	1.42	0.0049
Leucrose	1.22	0.0115	Heptylic Acid	1.33	0.0031
Lactitol	1.03	0.0094	Stearic Acid	1.32	0.0028
2-Furoic Acid	1.62	0.0043	Palmitic Acid	1.23	0.0047

Erythrono-1,4-Lactone	1.06	0.0010	Caprylic Acid	1.09	0.0016
Indoleacrylic Acid	2.22	0.0052	Lactitol	1.16	0.0023
Linoelaidic Acid	2.20	0.0084	Cetyl Alcohol	1.02	0.0120
6-Hydroxyhexanoic Acid	1.20	0.0001	2-Furoic Acid	1.61	0.0043
Nicotinic Acid	1.58	0.0044	3-Pyridinol	1.10	0.0120
Thymine	1.33	0.0032	Indoleacrylic Acid	2.21	0.0052
Uracil	1.30	0.0002	Linoelaidic Acid	2.48	0.0059
2-Hydroxyglutaric Acid	1.01	0.0030	Nicotinic Acid	1.70	0.0027
Galactinol	2.75	0.0001	Thymine	1.56	0.0010
Piceatannol	2.12	0.0000	Uracil	1.32	0.0002
Ethyl-Alpha-D-Glucopyranoside	1.76	0.0000	Ginkgolide A	1.26	0.0002
Beta-Mannosylglycerate	1.67	0.0038	Galactinol	2.16	0.0119
3-(3-Hydroxyphenyl)Propanoic Acid	1.56	0.0016	Talose	1.85	0.0104
5,5-Dimethylhydantoin	1.50	0.0035	Ethyl-Alpha-D-Glucopyranoside	1.77	0.0000
5-Methylimidazolidine-2,4-Dione	1.44	0.0016	3-(3-Hydroxyphenyl)Propanoic Acid	1.76	0.0006
1-C-Octylhexopyranose	1.35	0.0002	5,5-Dimethylhydantoin	1.69	0.0016
Mannobiose	1.34	0.0076	5-Methylimidazolidine-2,4-Dione	1.53	0.0010
Conduritol-Beta-Epoxide	1.21	0.0003	Furfuryl Alcohol	1.36	0.0022
Furfuryl Alcohol	1.20	0.0046	Beta-Mannosylglycerate	1.33	0.0184
2-Mercaptophenol	1.20	0.0009	Piceatannol	1.32	0.0462
Pterostilbene	1.13	0.0018	2-Mercaptophenol	1.25	0.0006
Levorphanol	1.10	0.0031	5-Methylpyrrolidin-2-One	1.25	0.0011
Sedoheptulose	1.09	0.0009	Mannobiose	1.07	0.0437