Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

Dysregulation of gut health in zebrafish by differentially charged nanoplastics exposure: An integrated analysis of histopathology, immunology, and microbial informatics

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Table S1-S2, Figures S1-S7

WS1

The gut samples were cut to a thickness of less than 1mm by ultra-thin freezing microtome (Leica CM1520, Germany), and observed on the red-light channel (535/600nm) by laser confocal microscope (Zeiss LSM980, Germany). In addition, gut tissues were placed in a vacuum freeze-dryer to prepare dried samples. Approximately 0.1g of the lyophilized sample was digested in 1-mL of concentrated nitric acid for 2 hours, then diluted to 5mL, and finally used for the determination of MPs content in gut tissue using a fluorescence spectrophotometer (Hitachi F2500, Japan).

Gut tissues were put into 10% formalin solution, dehydrated with alcohol, and then embedded in paraffin for HE staining. Images were taken by laser scanning confocal microscope and collected from the whole visual field. Quantitative analysis was carried out by Zeiss LSM Image Browser (version 3.0) to determine the goblet cell number, mucosa thickness, and muscularis thickness of gut tissue.

WS2

Approximately 0.1-g gut tissue was ground to homogenate, and the supernatant obtained by centrifugation was used to determine diamine oxidase (DAO) activity. This homogenate was also used to determine that D-lactate content, antioxidant enzymes (SOD, CAT, POD, and GSH-px) activities, and the activities of acid phosphatase (ACP) and alkaline phosphatase (AKP). Lysozyme level was measured using the turbidity method. These biochemical indexes were detected using commercial test kits (Solarbio, China) and represented as a percentage of the control based on protein concentration measured by the Bradford method. The total RNA was extracted according to the above method.

Table S1 The composition characteristics of test nanoplastic.

Component: Name	CAS Number	Percentage
Polystyrene	9003-53-6	<0.1-10
Divinylbenzene	9003-70-7	<0.1
Water	7732-18-5	>90-99.9
Dispersant/surfactant	Proprietary	<0.5 (if any)
Preservative	Proprietary	<0.1 (if any)

Table S2 Primer sets of the OXPHOS-related genes used for real-time PCR analysis

Gene Name	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
mt-ndl	AGCCTACGCCGTACCAGTATT	GTTTCACGCCATCAGCTACTG
ndufs4	TGTAGGCTGGCAGAGGGACA	GACAGGCCGAAACAGGATGG
coxl	GGATTTGGAAACTGACTTGTG	AAGAAGAAATGAGGGTGGAAG
uqcrcl	GGATTGCATCTGAGGAGACCAAC C	GACTCCACTGCCTGTTCAAGAGC
cox5ab	GGTCACCGGAGCTTCAGGAT	TCGAGCCGAGAGGTAGAAAAAC C
GAPDH	GAAGCTTACTGGAATGGCTTTCC	GGCAGGTCAGGTCAACAACAG

Table S3 Primer sets of the immune-related genes used for real-time PCR analysis

Gene Name	Forward Primer (5’ to 3’)	Reverse Primer (5’ to 3’)
IL-6	ACACTGGCTACACTCTTC	TCCACATCCTGAACTTCG
IL-10	CATTTGTGGAGGGCTTTC	GGTTCCAAGTCATCGTTG
IL-1β	TAAGACGGCACTGAATCC	CCTGAACAGAATGAAGCAC
TNF-α	TGGTGTCTAGGAGGAAAG	GTCTTATGGAGCGTGAAG
TGF-β	TGGGCTGGCGGTGGAT	CCTCTGGGTTCAGCGTGTT
β-actin	CCATTGAGCACGGTATTG	CTGTTGGCTTTGGGATTC

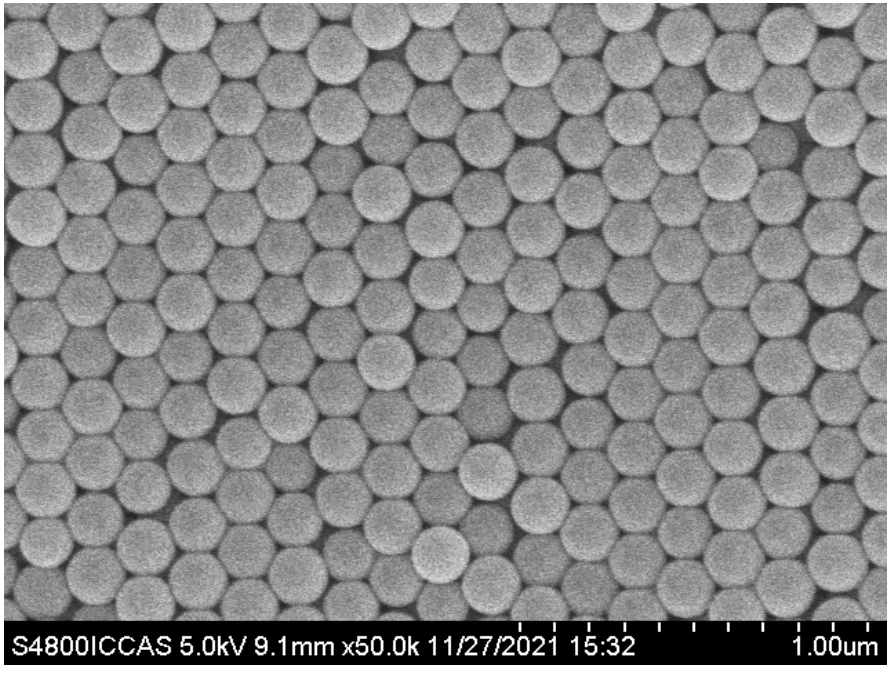

Figure S1 The SEM image of test microplastics.

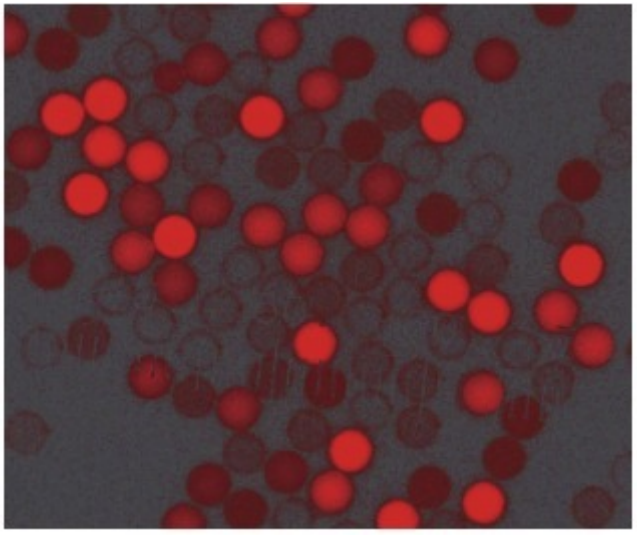

Figure S2. The red fluorescence image of test nanoplastic.

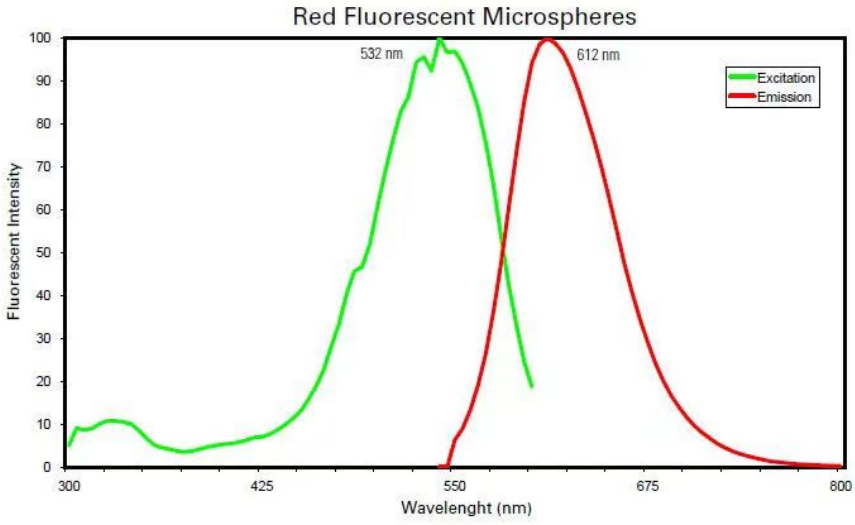

Figure S3. The fluorescent dye spectrum of test nanoplastic.

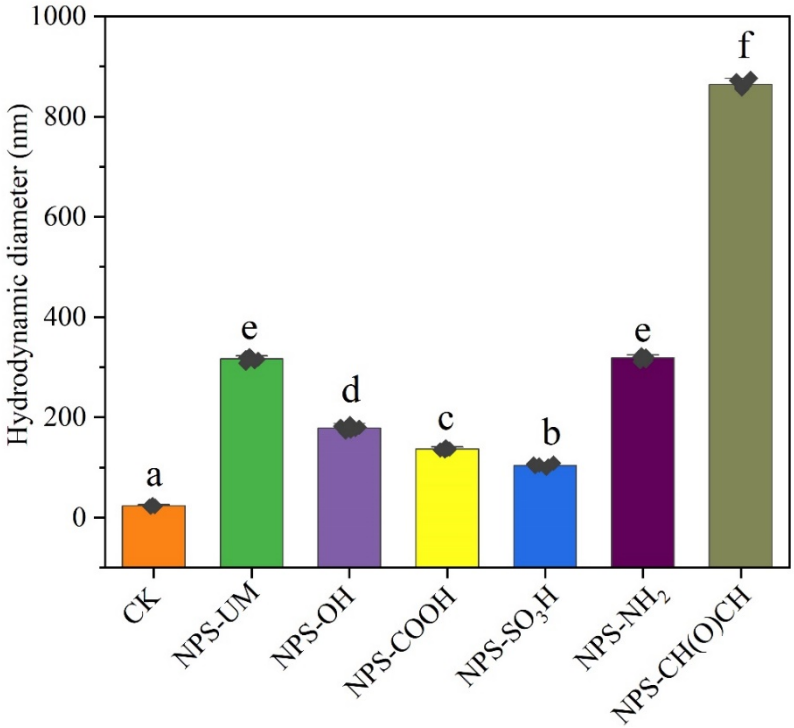

Figure S4. Hydrodynamic diameters of microplastics with different functional groups in water environment. Different lowercase letters indicated significant differences between experimental treatments (p-value < 0.05, n=6).

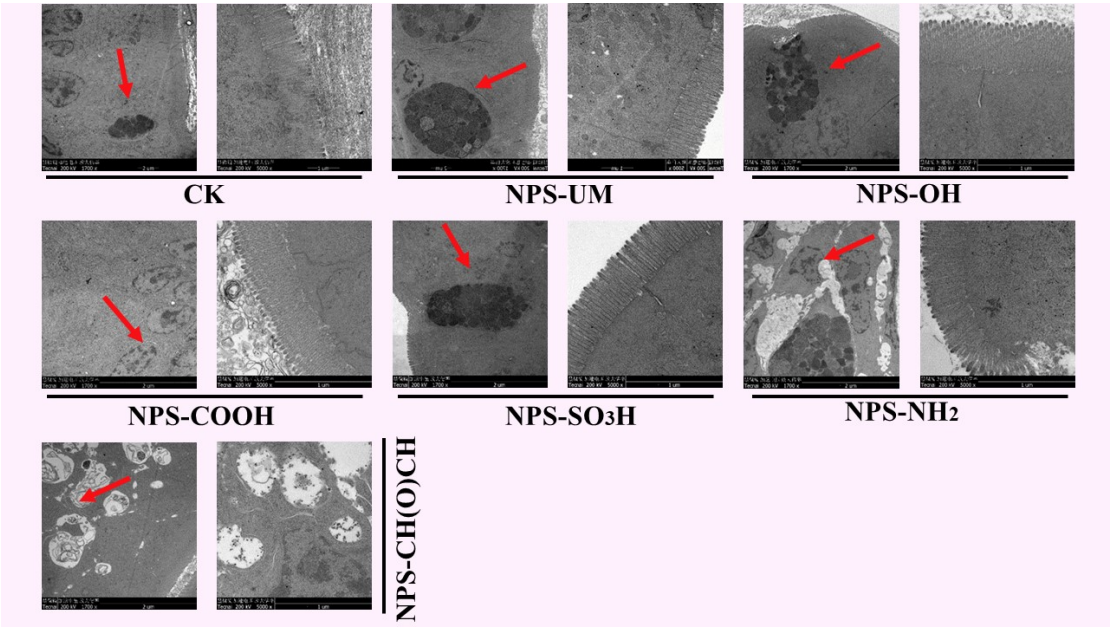

Figure S5. Representative SEM images of gut tissue (including the mast cells and intestinal villi).

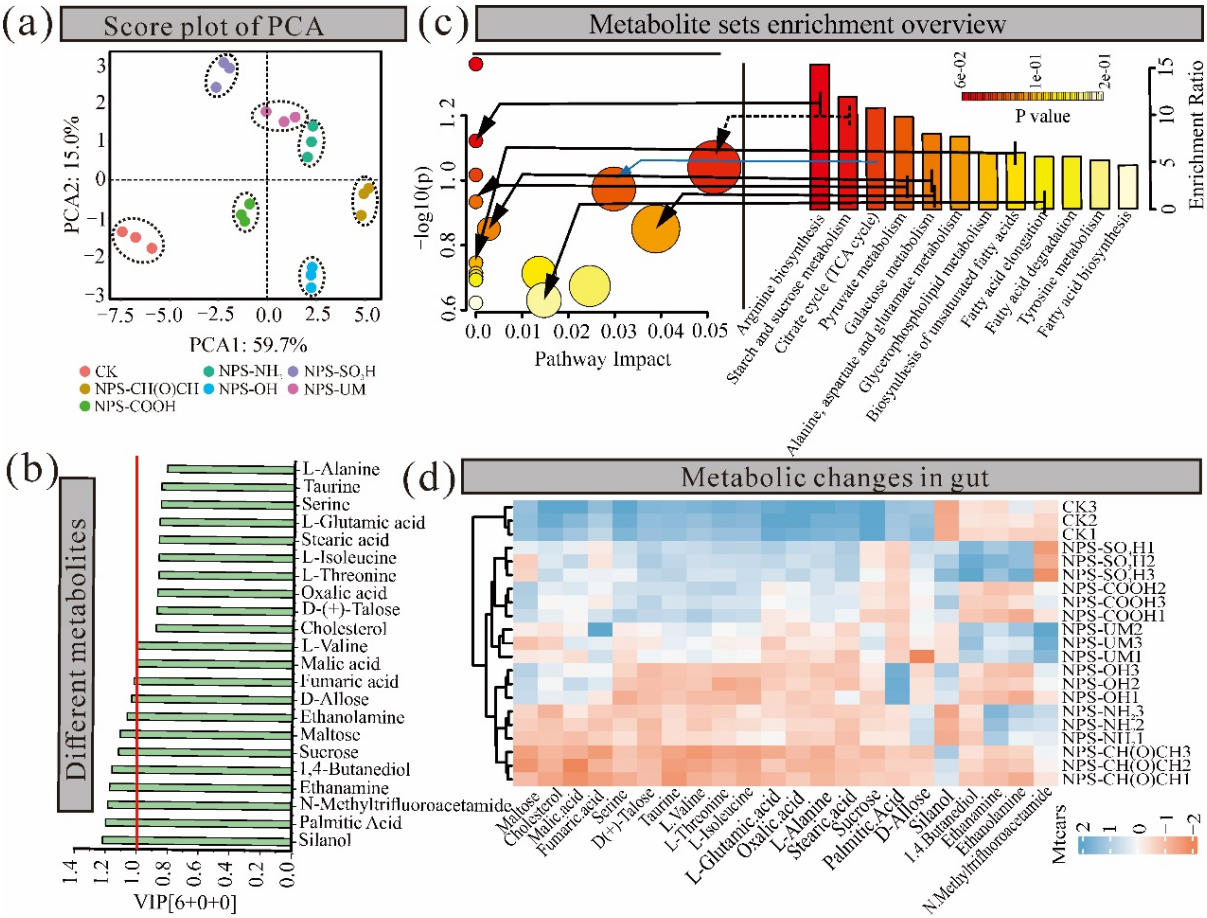

Figure S6. Metabolomic alterations in gut tissue of zebrafish after exposure to microplastics with different functional groups. (a) Principal component analysis (PCA) score plots of gut metabolites among experimental treatments. (b) OPLS-DA coefficient plot of metabolites which contribute most to the groups separations (VIP>1). (c) KEGG enrichment pathway analysis of significantly different metabolites (VIP > 1 and P<0.1). (d) Heat map of changed metabolites among experimental treatments.

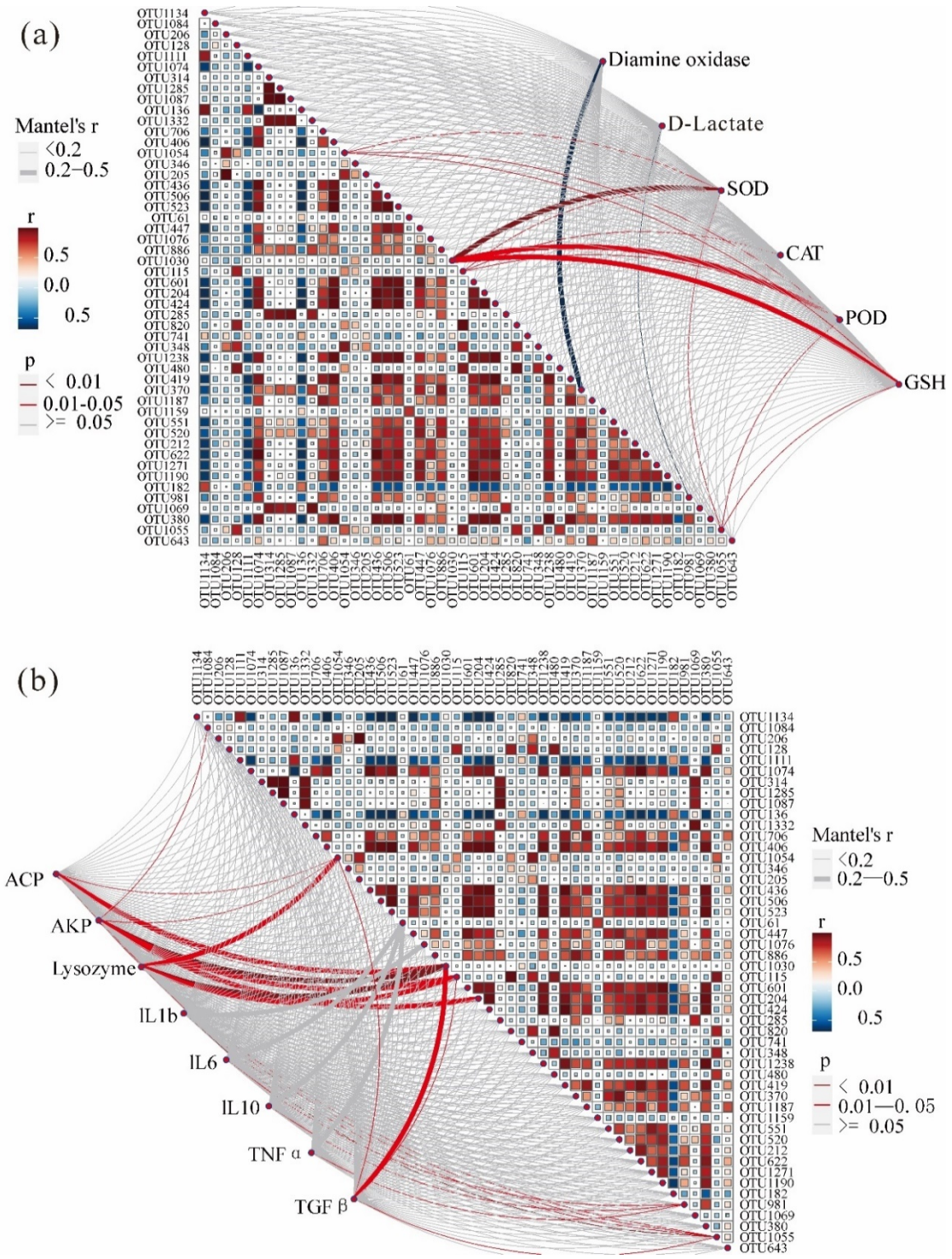

Figure S7. Correlation analysis among oxidative damage-related indexes (a), immune-related indexes (b), and core-enriched bacterial communities (top 30 most abundant OTUs) in gut of zebrafish based on the Mantel test. The red color of the line represents a significant positive correlation (p-values). The size of the line represents correlation coefficients (Mantel's r). The color gradient indicates Spearman's rank correlation coefficients, with more positive values (dark red) indicating stronger positive correlations, and more negative values (dark blue) indicating stronger negative correlations.