Supporting Information for

Distinct effects of nano-polystyrene, erythromycin and their mixtures on composition and metabolic profile of intestinal microbiota in *Nereis succinea*

Dali Wang[a], Yuheng Zhong[a], Qi Ding[a], Xiaofeng Han[a], Kai Chen[b], Bo Pan[c], Jing You[*a]

[a] Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Guangdong Key Laboratory of Environmental Pollution and Health, Center for Environmental Microplastics Studies, School of Environment, Jinan University, Guangzhou 511443, China

[b] Institute of Photonics Technology, Jinan University, Guangzhou 511443, China

[c] Faculty of Environmental Science & Engineering, Kunming University of Science & Technology, Kunming 650500, China

* Corresponding author. E-mail: youjing@jnu.edu.cn

Chemical analysis of erythromycin

The concentrations of erythromycin in water were measured using salting-out assisted liquid-liquid extraction followed by HPLC-MS/MS analysis. In detail, 2 mL of water samples from the exposure beakers were extracted using 3 mL of acetonitrile after adding 20 μg/L of roxithromycin-d_7 as the surrogate standard, and NaCl was added to the solution to reach a final concentration of 12.5%. After a 5-min vortex, the upper layer of acetonitrile was collected. The operations were repeated three times, and the acetonitrile extracts were combined and concentrated to nearly 1 mL under a gentle flow of nitrogen. Then sulfamethoxazole-d_4 (20 μg/L) was added as the internal standard and the final volume was adjusted to 1 mL. The solution was filtered with a 0.22-μm filter before instrumental analysis.

Erythromycin was quantified using a QTRAP 5500 HPLC-MS/MS (AB SCIEX, USA) and a Waters ACQUITY-UPLC®-HSS-C18 column (2.1 × 100 mm, 1.8 μm) connected with an ACQUITY-UPLC®-HSS-C18 VanGuardTM C18 pre-column (2.1 × 5 mm, 1.8 μm) was used for chemical separation. Column temperature was maintained at 40 °C. The mobile phase consisted of 0.1% formic acid aqueous solution (A) and acetonitrile (B), with a flow rate of 0.3 mL/min. The procedures of gradient elution were as follows: 0–0.5 min, 10% B; 1.0 min, 35% B; 5.0 min, 70% B; 5.0–5.2 min, 10% B; 5.2–7.0 min, 10% B. Injection volume was 2 μL. The quantification was achieved using multiple-reaction monitoring (MRM) mode and positive electrospray ionization (ESI) mode. Temperature of drying gas was set at 550 °C. Ion source gas was set at 55 psi, and nitrogen was used as atomized gas. Pressure of curtain and collision gases was at 40 and 7 psi, respectively. Positive ESI voltages were set at 5500 V. Entrance potential and collision cell exit potential were at 10.0 and 16.0

V, respectively. Instrument parameters of the compounds are shown in Table S1.

Quality control samples

Samples including a solvent blank, a matrix blank, and a matrix spike as well as its duplicate were analyzed simultaneously with the exposure water samples. Surrogates were added to each sample before extraction to check the performance of sample preparation processes. The instrument was examined by analyzing a calibration standard every ten samples, and variation of each analyte was less than 20%. The recoveries of target contaminants in the matrix spikes were in the range of 91.9 ± 7.4%. The recoveries of the surrogates in all samples were 91.4 ± 20.2%.

Table S1 Instrument parameters for analyzing erythromycin.

Compound	Retention time (min)	Ionization mode	Transition mass (m/z)	Declustering Potential (V)	Collision energy (eV)
Erythromycin	4.41	ESI+	734.5→158.0	50	37
Sulfamethoxazole-d_4 (internal standard)	4.64	ESI+	258.0→160.0	90	20
Roxithromycin-d_7 (surrogate standard)	5.17	ESI+	844.5→686.5	30	31

Table S2 Substrates in the wells of the Biolog EcoPlate. The A5-H9 wells contained the same substrates as in the A1-H4 wells and each 96-well plate had three replicates.

A1	**A2**	**A3**	**A4**
Water (control)	ß-Methyl-D-Glucoside	D-Galactonic Acid γ-Lactone	L-Arginine
B1	**B2**	**B3**	**B4**
Pyruvic Acid Methyl Ester	D-Xylose	D-Galacturonic Acid	L-Asparagine
C1	**C2**	**C3**	**C4**
Tween 40	I-Erythritol	2-Hydroxy Benzoic Acid	L-Phenylalanine
D1	**D2**	**D3**	**D4**
Tween 80	D-Mannitol	4-Hydroxy Benzoic Acid	L-Serine
E1	**E2**	**E3**	**E4**
a-Cyclodextrin	N-Acetyl-D-Glucosamine	y-Hydroxybutyric Acid	L-Threonine
F1	**F2**	**F3**	**F4**
Glycogen	D-Glucosaminic Acid	Itaconic Acid	Glycyl-L-Glutamic Acid
G1	**G2**	**G3**	**G4**
D-Cellobiose	Glucose-1-Phosphate	a-Ketobutyric Acid	Phenylethyl-amine
H1	H2	**H3**	**H4**
a-D-Lactose	D,L-a-Glycerol	D-Malic Acid	Putrescine

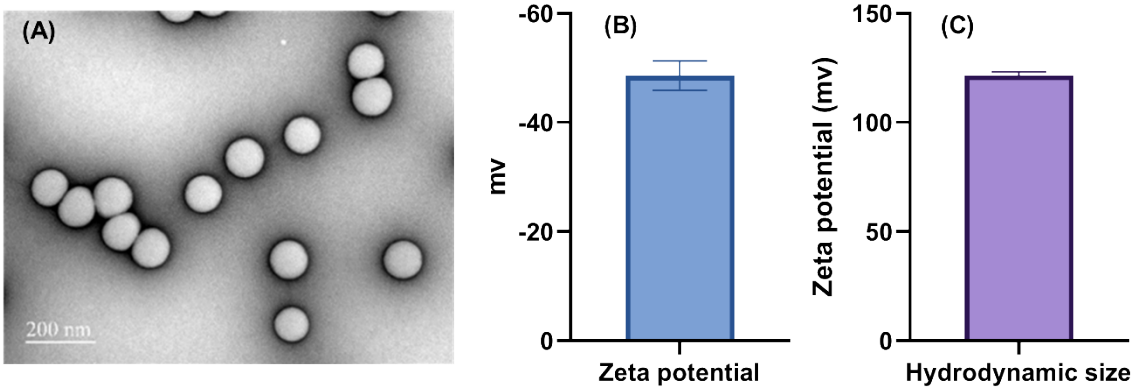

Fig. S1 (A) Transmission electron microscopy image, (B) zeta potential, and (C) hydrodynamic size of the polystyrene nanoplastics used in the present study.

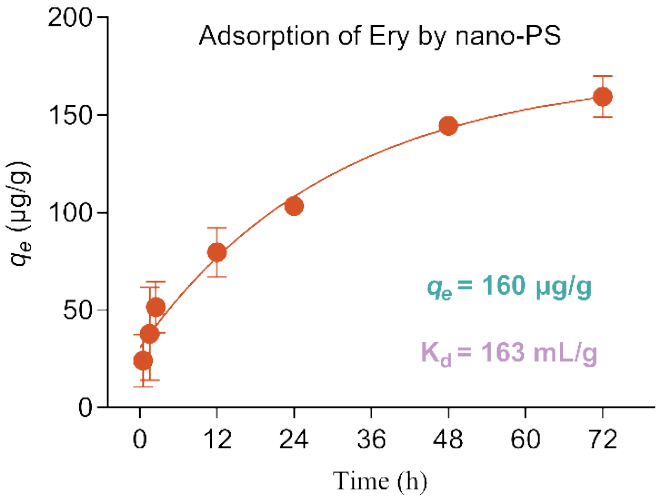

Fig. S2 Adsorption of erythromycin (Ery) by nano-PS. The parameter q_e is the maximum adsorption capacity at adsorption equilibrium, K_d is the distribution coefficient of Ery between PS and water ($K_d=C_{NPs}/C_{water}$). The adsorption experiment was carried out in a mixture solution of 100 mg/L of PS NPs and 1000 μg/L of erythromycin. Detailed information about the adsorption experiment can be found in our previous research (Ning et al. 2022).

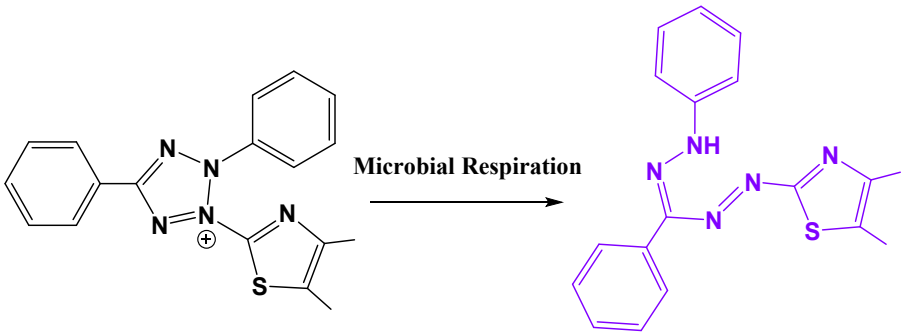

Fig. S3 Reduction of triphenyl tetrazolium to formazan during the respiration of the microbes in the Biolog EcoPlates.

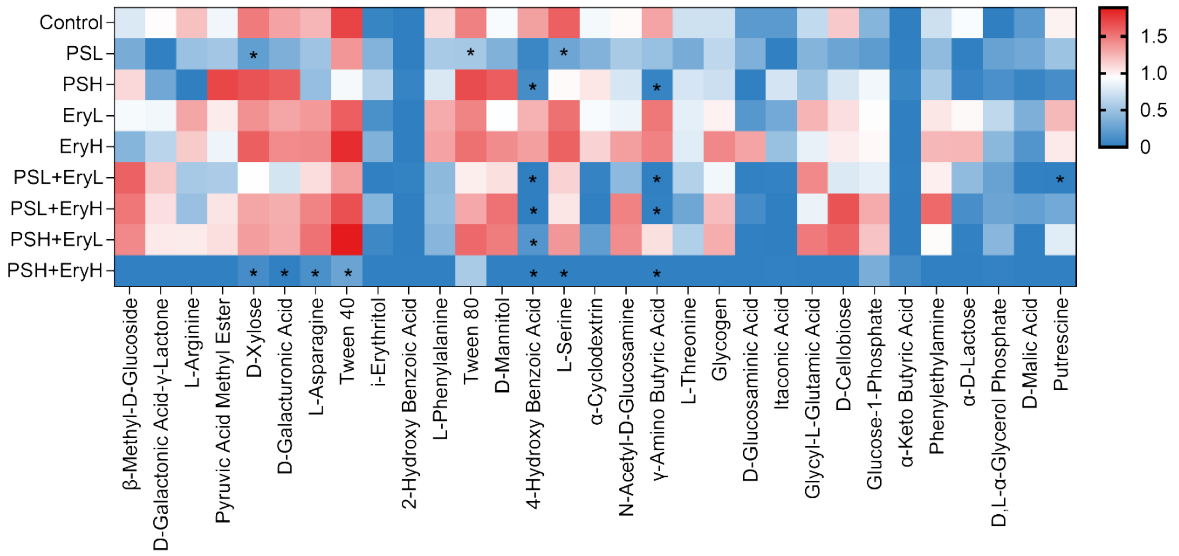

Fig. S4 Carbon utilization patterns of cultivable microbiota in intestines of *N. succineas* under different exposure conditions. The asterisks (*) indicate the significant difference ($p < 0.05$) of the carbon utilization between the treated group and the control. PS: polystyrene nanoplastics; Ery: erythromycin; L: low; H: high.

References

Ning Q, Wang D, An J, Ding Q, Huang Z, Zou Y, Wu F, You J. 2022. Combined effects of nanosized polystyrene and erythromycin on bacterial growth and resistance mutations in *Escherichia coli*. Journal of Hazardous Materials 422, 126858.