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# Association between microplastics and the functionalities of human gut microbiome

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ABSTRACT

As an integral part of humans, the gut microbiome plays a significant role in the physiological and pathological processes of the host, and dysbiosis of the gut microbiome is linked to various diseases. The impact of microplastics on the diversity and composition of human gut microbiome has been reported previously. However, effects of microplastics on the functionality of the gut microbiome in humans have not been well studied. In the present study, concentrations of microplastics in human blood were detected through pyrolysis-gas chromatography/mass spectrometry in 39 adults. Five types of microplastics were found in human blood, including polyvinyl chloride, polyethylene, polypropylene, polystyrene, and polyamide 66. Shotgun metagenomic sequencing was further employed to analyze the metagenomes of the human stool samples and fecal samples from mice exposed to microplastics. Associations were found between microplastics and microbial species, as well as microbial genes encoding invasion-related virulence factors, quorum sensing, autoinducer and transporter system, and microplastic biodegradation enzymes. The findings are of significance to improve the understanding of functional changes in the gut microbiome associated with microplastic exposure, as well as raising awareness regarding the health risks of microplastics in the human population.

## 1. Introduction

The human gut microbiome is a complex ecosystem that evolved along with its hosts. The gut microbiome plays a key role in the maintenance of the physiological and mental health of humans. A healthy gut flora is closely related to the overall health of the host. The gut microbiome is acquired at birth and maintains its temporal stability during adulthood (Adak and Khan, 2019). The gut microbiome varies among individuals depending on host genotype and environmental factors, such as use of antibiotics (Fassarella et al., 2021). The gut microbiota is closely linked to the human health and it has been considered as a toxicity target for environmental pollutants (Lu et al., 2019). A variety of environmental pollutants could influence the diversity and stability of the gut microbiome, such as pesticides (Gao et al., 2017b), heavy metals (Gao et al., 2017c), personal care products (Gao et al., 2017d), as well as microplastics (Ke et al., 2023; Liu et al., 2023b; Tamargo et al., 2022).

Microplastics are plastic particles with a size of less than 5 mm, including nanosized particles with size less than 1 μm (Vethaak and Legler, 2021). Microplastics have become a global environmental pollution issue, which have been detected in aquatic and terrestrial environments, as well as in food and drinking water (Mamun et al., 2023; Yang et al., 2024). The average concentration of microplastics was 191 items/L in glaciers and 55 items/L in urban stormwater (Koutnik et al., 2021). In the bay areas, the range of microplastics ranged from 0.01 to $3.62 \times 10^5$ items/$m^3$ and 0–$6.75 \times 10^5$ items/kg in the seawater and sediment, respectively (Tong et al., 2023). Up to 10.5 items/g microplastics were detected in bivalves and 20 items/individual were detected in fish (Jin et al., 2021). The mean concentration of microplastics was 0.14 and 0.13 items/g in different tissues of cow and sheep, respectively (Bahrani et al., 2024). Within 3 minutes of heating with a microwave, about 2.11 billion nanoplastics and 4.22 million microplastics could be released from some plastic food containers (Hussain et al., 2023).

Due to the omnipresence of microplastics in the environment and food, exposure of microplastics to humans is inevitable. The route of microplastic exposure includes ingestion, inhalation, and dermal

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contact (Zhao et al., 2024). It is estimated that individuals ingest 39, 000–52,000 particles annually depending on age and sex (Cox et al., 2019). The gastrointestinal tract serves as a primary accumulation site for ingested microplastics. The ubiquity and long-standing existence of microplastics have raised public health concerns. Microplastics have been detected in various human tissues and body fluids, including the placenta (Ragusa et al., 2021; Zhu et al., 2023), meconium, infant feces, breast milk (Liu et al., 2023a), and arteries (Liu et al., 2024). Microplastics have been considered a new cardiovascular risk factor as microplastics were detected in the carotid artery plaque of patients and a higher risk of death was found in patients in whom microplastics were detected than in patients in whom microplastics were not detected (Marfella et al., 2024).

We previously reported the disruption of the gut microbiota by polystyrene (PS) microplastics in a mouse model (Gao et al., 2023; Shen et al., 2024). Oral exposure to 1000 μg/L PS microplastics induced dysbiosis in mice's gut microbiota and disorder of hepatic lipid metabolism (Lu et al., 2018). Commercial PS microplastics and realistic PS microplastics showed distinct toxicity on zebrafish gut microbiota and histopathology (Guo et al., 2022). Polyethylene (PE) microplastics have also led to the dysbiosis of gut microbiota in mice (Xu et al., 2024). Polyvinyl chloride (PVC) microplastics induced dysfunction of the gut barrier function and the dysbiosis of the gut microbiota in mice (Chen et al., 2022). The disruption of the gut microbiota might be caused by the consumption of microplastics or the chemical and mechanical disturbance in the gastrointestinal tract (Fackelmann and Sommer, 2019).

In a quasi-experimental study, the gut microbiota differed in the human participants served hot food in disposable plastic tableware than those served in non-disposable plastic tableware for one month (Zhang et al., 2024). In a cross-sectional study conducted in preschool children, microplastics were inversely correlated with alpha indices, and certain probiotic taxa were affected by microplastic exposure (Ke et al., 2023). In a study that included 18 mother-infant pairs, an inverse association was found between PS microplastics and the Chao index of meconium microbiota, meanwhile, PE was inversely associated with several genera of placenta microbiota (Liu et al., 2023b). Polyethylene terephthalate (PET) microplastics have been reported to affect the human gut microbial communities during simulated digestion (Tamargo et al., 2022).

Although the impact of microplastics on the diversity and composition of the human gut microbiome has been documented in prior studies, their effects on the functional capacities of the gut microbiome remain poorly understood. To address this gap, this paper presents a pilot study aimed at elucidating the relationship between human gut microbiome functionality and microplastic exposure. Participants were recruited using convenience sampling from Health Centers of two counties in Zhejiang Province, China. Briefly, the inclusion criteria focused on adults aged 25–69 years to capture a broad yet metabolically stable demographic, avoiding confounding effects from puberty, aging, or age-related diseases. Exclusion criteria eliminated participants with systemic diseases, chronic conditions, or recent antibiotic use (within three months), ensuring the functionality of the gut microbiome was not influenced by these factors and allowing for a clearer assessment of microplastic-induced effects. The purposes of the study were to examine the associations between microplastics and the gut microbiota in adults, including the gut microbial species and genes encoding virulence factors, quorum sensing system, transporter system, and microplastic biodegradation enzymes, as well as to compare the presence and effects of microplastics in men and women. The findings from this study are helpful for the health risk assessment of microplastics in human population.

## 2. Materials and methods

### 2.1. Study subjects and sample collection

A cross-sectional study was conducted to explore microplastic exposure and its association with gut microbiome functionalities in adults. Adults aged 25–69 years old were recruited from Health Centers in two counties in Zhejiang Province, China. C county is an inland region known for its industrial activities, while T county is a coastal region with a less industrialized environment. Adults with diagnosis of systemic diseases, including cancer, kidney disease, autoimmune disease, or chronic conditions such as hypertension, diabetes, and cardiovascular disease were excluded from the study. No antibiotics or antibacterial agents were administered to the recruited subjects in the previous three months. A total of 39 adults were included in the present pilot study with characteristics shown in Table 1. All participants signed the informed consent. Whole blood samples were collected from each subject by venipuncture, stored in 4 mL glass heparinized vacutainer tubes with rubber seal (BD Biosciences, Plymouth, UK), and kept in a −80°C freezer until further analysis. For fecal sample collection, participants were provided with sterile fecal collection containers, along with clear instructions to avoid contamination with urine, water, or toilet paper. Fresh fecal samples (5–10 g) were collected directly into the container or transferred using a clean liner, sealed immediately, and labeled with the participant's ID, date, and time of collection. Samples were stored at 4 °C and delivered to the research facility within 24 hours, frozen at −80 °C for long-term preservation. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Zhejiang Provincial Center for Disease Control and Prevention (2020–040–01). The subjects were divided into a low-exposure group and high-exposure group according to the total microplastic burden. In the low-exposure group, the total microplastic burden of the subjects was lower than the median of the total microplastic burden of the population. Meanwhile, in the high-exposure group, the total microplastic burden in the subjects was higher than the median of the population.

### 2.2. Microplastic detection

Detection of microplastics in human blood samples using pyrolysis-gas chromatography/mass spectrometry has been reported (Ha et al., 2022). The detection of microplastics in this study was performed as reported previously (Guo et al., 2024; Song et al., 2024). Briefly, 400 μL blood sample from each subject was transferred to a sterile glass beaker and dried overnight at 60℃. The sample was extracted with trichloromethane, hexafluoroisopropanol, and dimethylbenzene and concentrated at 80℃. Before use, all of the extraction solvents were filtered through a polytetrafluoroethylene filter three times. The concentrate was transferred to a pyrolysis cup and evaporated to dryness and subjected to pyrosis using a PY-3030D Frontier pyrolyzer at 550℃ (Frontier Laboratories Ltd., Fukushima, Japan). Pyrolysis products were injected into a Restek Rtx-5MS column (Pennsylvania, U.S.) on Shimadzu

**Table 1**
Subject characteristics.

| Characteristics | N (%) | |
|---|---|---|
| Age (years) | 41.13 ± 12.74 | |
| | [25–40) | 25 (64.10 %) |
| | [40–55) | 8 (20.51 %) |
| | [55–70) | 7 (17.95 %) |
| Sex | | |
| | Male | 16 (41.03 %) |
| | Female | 23 (58.97 %) |
| Area | | |
| | T County | 29 (74.36 %) |
| | C County | 10 (25.64 %) |

QP2020NX GCMS (Shimadzu Corporation, Tokyo, Japan). Blank samples for the instrument were run without the injection of any samples. Blank samples for the method were run with all solvents added without blood samples. Standard samples were injected into the instrument six times consecutively to test the precision of the instrument. Microplastic standards were spiked into the blood sample to test the recovery rate. Py-GCMC data passed the quality control only if (1) the background signal was blank for blank samples; (2) RSD for standards less than 10 %; and (3) the recovery rate for the standards injected into the blood samples was 90 %-110 %. Eleven types of microplastic standards were analyzed, including PE, PET, PS, PVC, polypropylene (PP), polycarbonate (PC), polyamide 6 (PA6), polyamide 66 (PA66), polymethylmethacrylate (PMMA), poly-lactic acid (PLA), and polybutylene adipate terephthalate (PBAT). The source company and purity of each standard were provided in Table S1. The spectra of these standards were shown in Fig. S1-S42.

### 2.3. DNA extraction

Genomic DNA of gut microbiota was extracted from fecal samples using the Mag-bind Soil DNA Kit (TianGen Biotech Co., LTD, Beijing, China). Briefly, 0.25–0.5 g of fecal sample was mixed with 500 μL Buffer SA, 100 μL Buffer SC, and 0.25 g grinding beads in a 2 mL centrifuge tube, vortexed, and centrifuged at 1000 rpm for 1 minute. The supernatant (~500 μL) was transferred to a new tube, mixed with 200 μL Buffer SH, and incubated at 4 °C for 10 minutes. After centrifugation at 12,000 rpm for 3 minutes, the supernatant was mixed with 5 μL Buffer GFA and 10 μL magnetic bead suspension, vortexed for 5 minutes, and placed on a magnetic stand for separation. The beads were washed sequentially with 700 μL Protein Removal Solution RD and 700 μL Wash Solution PWD (repeated once), air-dried for 5–10 minutes and eluted with 50–100 μL Elution Buffer TB at 56 °C for 5 minutes. The eluted DNA was collected and stored for further analysis.

### 2.4. Shotgun metagenomic sequencing

Fecal DNA was extracted from 34 subjects whose stool samples were available. Fecal DNA was fragmented to around 350 bp using Covaris M220 (Covaris, LLC., Massachusetts, U.S.) followed by library preparation. Sequencing was performed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Metagenomic sequencing data analysis was performed using the online platform Majorbio Cloud Platform (www.majorbio.com). Fastp v0.20.0 was used for the quality control of the raw sequencing reads. BWA v0.7.17 was used to remove host sequences. Metagenomic assembly was performed using Megahit v1.1.2. CD-HIT v4.6.1 was used to remove the redundant or highly similar sequences. Metagenomic sequencing reads were normalized using reads per kilobase of transcript per million reads mapped (RPKM). Taxonomic profiling of the gut microbiome was performed using BLATP against the non-redundant database, which contains SwissProt, Protein Information Resource, Protein Research Foundation, Protein Data Bank and CDS data from GeneBank and RefSeq. Virulence Factor Database (http://www.mgc.ac.cn/VFs/) was used for the annotation of virulence factors. Transporter Classification Database (http://www.tcdb.org/) was used for the annotation of transporters. Quorum sensing database was constructed through searching quorum sensing genes in UniprotKB (http://www.uniprot.org/), which were classified into ten classes, including activator, autoinducer, autoinducer receptor, autoinducer producer, decomposer, effector, regulator, repressor, transporter, and unclear. Information on microplastic biodegradation enzymes was collected through a literature search.

### 2.5. Animal experiment

Male C57BL/6 mice (6–8 weeks old) were housed under controlled conditions (22 °C, 40–70 % humidity, 12 h light/dark cycle) and acclimated for 1 week before being randomly divided into two groups: a control group (n = 5) and a microplastic group (n = 5). The microplastic group received 80 mg/kg body weight/day of 5 μm PS microplastics via gavage, while the control group was gavaged with an equivalent volume of ultrapure water. The dose and diameter were chosen based on a previous study (Huang et al., 2022). The experiment lasted 14 weeks. Fecal samples were collected from each mouse in separate cages, frozen in liquid nitrogen, and stored at −80 °C. The study was conducted at Zhejiang Chinese Medical University, with approval from its Animal Management and Ethics Committee (Approval No. 20230213–07), adhering to relevant guidelines and regulations.

### 2.6. Statistical analysis

Wilcoxon rank-sum test was used to determine the significant microplastics between male and female subjects. The Chao, Ace, Sobs, Shannon, and Simpson index were used for alpha diversity analysis with the Wilcoxon rank-sum test to examine the significance between male and female subjects. For beta diversity, the Bray-Curtis and Euclidean distances were used for the analysis of microbial compositions and visualized using principal coordinate analysis (PCoA). Permutational multivariate ANOVA (PERMANOVA) was used to examine changes in microbial compositions between male and female subjects. Spearman correlation was used to analyze the correlation between microplastics and microbial species or functions.

## 3. Results

### 3.1. Correlation between microplastics and gut microbial species

PE, PVC, PS, PP, and PA66 were detected in the blood samples of adults in our study and the concentration range was 0–96.2 μg/mL, 0–62.4 μg/mL, 0–4.2 μg/mL, 0–7.4 μg/mL and 0–39.4 μg/mL, respectively. PE was the most frequently detected, followed by PVC and PS (Fig. S43A). The average concentration of PE was the highest in this population, followed by PVC and PA66 (Fig. S43B). The concentration of PA66 was higher in female subjects compared with male subjects, meanwhile, no significant difference was found between the two sexes for PS, PE, PP and PVC (Fig. 1A). Association was found between microplastics and various microbial species, including the positive correlation with unclassified species belonging to *Enterobacteriaceae* and *Escherichia coli*, as well as the negative correlation with *Faecalibacterium prausnitzii*, unclassified species belonging to genus *Faecalibacterium* (Fig. 1B). There was no significant difference between male and female in the alpha diversity and beta diversity analysis (Fig. S44). We further compared the relative abundance of the gut microbiota between the low-exposure group and high-exposure group. The subjects were divided into two groups according to the total microplastic burden of the population. Significant gut microbiota was shown in Fig. 2A. Consistent with the correlation analysis, the relative abundance of *Faecalibacterium prausnitzii* was significantly higher in the low-exposure group compared with the high-exposure group. There was no significant difference between the two groups in the alpha diversity and beta diversity analysis (Fig. 2B).

### 3.2. Correlation between microplastics and virulence factors and the quorum sensing system

A positive correlation was found between microplastics and the microbial genes encoding invasion-related virulence factors, such as Ail, invasinC, Type III secretion system (TTSS), Bsa type III secretion system (T3SS), and type 1 fimbriae. A negative correlation was found between flagella and PS, PP and PA66 (Fig. 3A). Contribution of bacterial genera to invasion-related virulence factors was shown in Fig. S45A. Unclassified genera belonging to Enterobacteriaceae family and *Escherichia* genus were the top two contributing genera (Fig. S45A). When

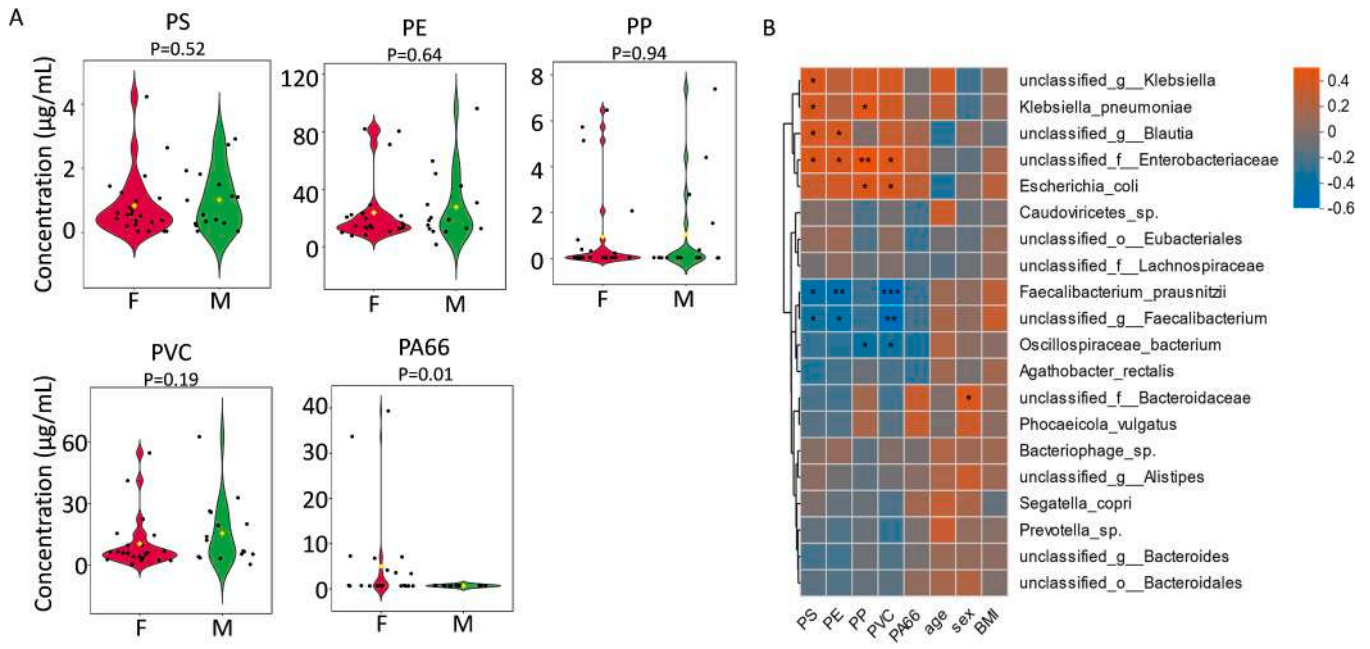


**Fig. 1.** Association between microplastics and gut microbial species. (A) Concentration of five types of microplastics in human blood. (B) Spearman correlation between microplastics and top 20 abundant gut microbial species. * : $p$-value < 0.05; * *: $p$-value < 0.01; * ** : $p$-value < 0.001.

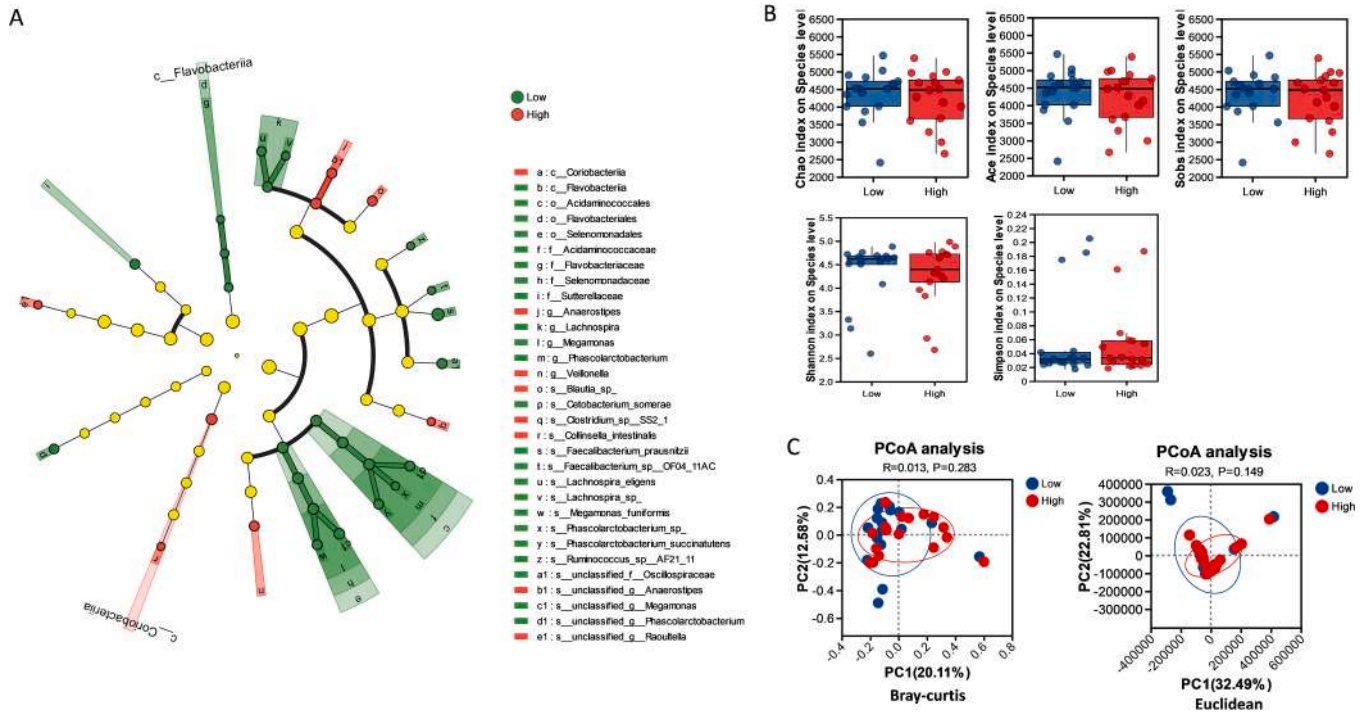


**Fig. 2.** Significant gut microbiota in low-exposure and high-exposure groups. (A) Significant gut microbiota enriched in two groups of subjects. Green: gut microbiota enriched in low-exposure group; Red: gut microbiota enriched in high-exposure group. (B) Alpha diversity analysis. (C) Beta diversity analysis.

comparing invasion-related virulence factors in two groups, we found that Flagella was significantly higher in the low-exposure group, meanwhile, LpeA was significantly higher in the high-exposure group (Fig. 3B). Next, we examined the correlation between microplastics and microbial genes in the quorum sensing system. A positive correlation was found between the effector gene and PS, PE, PP, and PVC, meanwhile negative correlation was found between microbial genes encoding autoinducers and PS, PE, and PVC, as well as between microbial genes encoding autoinducer receptor and PS (Fig. 3C). Consistent with the correlation analysis, the relative abundance of microbial genes encoding autoinducer receptor was significantly higher in the low-exposure group (Fig. 3D). We further checked the association between microplastics and microbial genes within the class of effector and autoinducer. Murein hydrolase effector LrgB was positively correlated with PS, PE, PP, and PVC (Fig. S45B). Putative poly(Beta-D-mannuronate) O-acetylase, aldose 1-epimerase, tRNA-cytidine(32) 2-sulfurtransferase, and DNA topoisomerase were negatively correlated with microplastics (Fig. 4A). Significant microbial genes between low-exposure group and high-

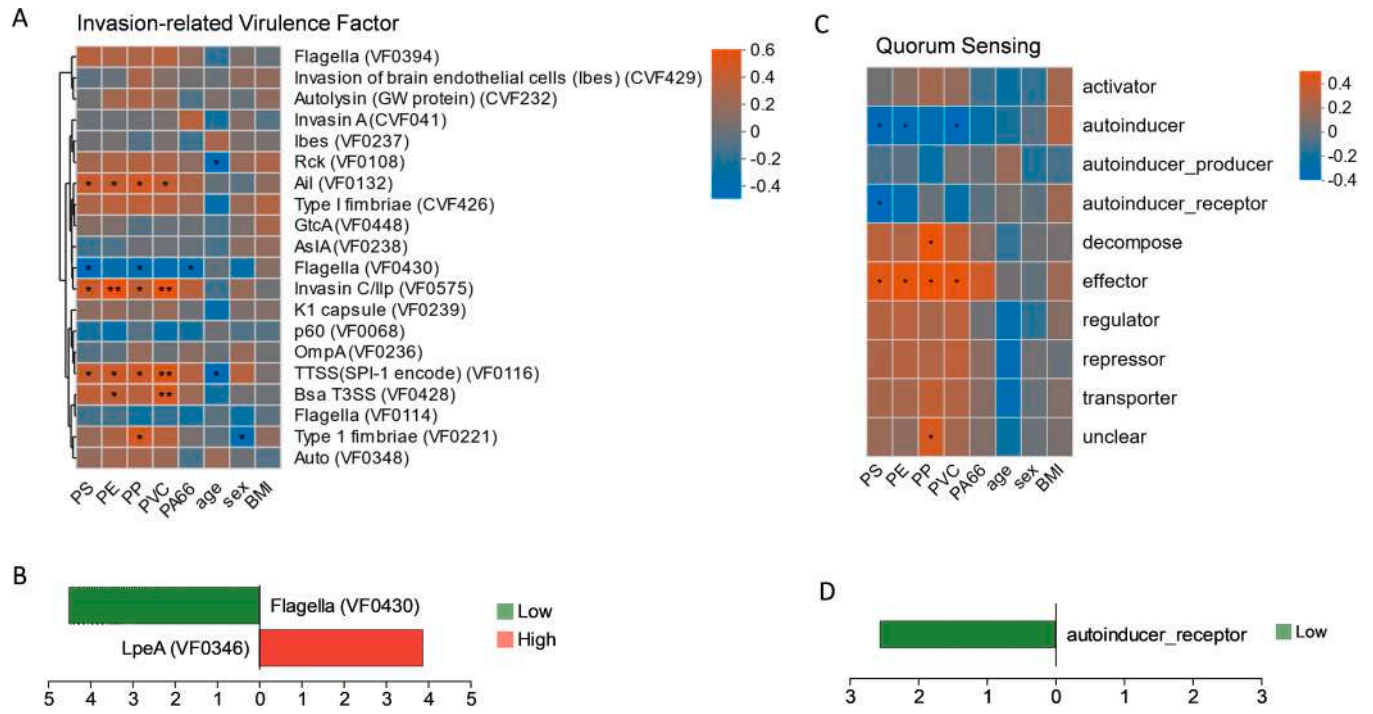


**Fig. 3.** Association between microplastics and virulence factors and quorum sensing system. (A) Spearman correlation between microplastics and top 20 abundant invasion-related virulence factors. * : *p*-value < 0.05; * *: *p*-value < 0.01. (B) Significant microbial genes encoding invasion-related virulence factors in two exposure groups. Green: enriched microbial genes in low-exposure group; Red: enriched microbial genes in high-exposure group. (C) Spearman correlation between microplastics and quorum sensing system. (D) Significant microbial class of the quorum sensing system in two exposure groups. Green: enriched in low-exposure group.

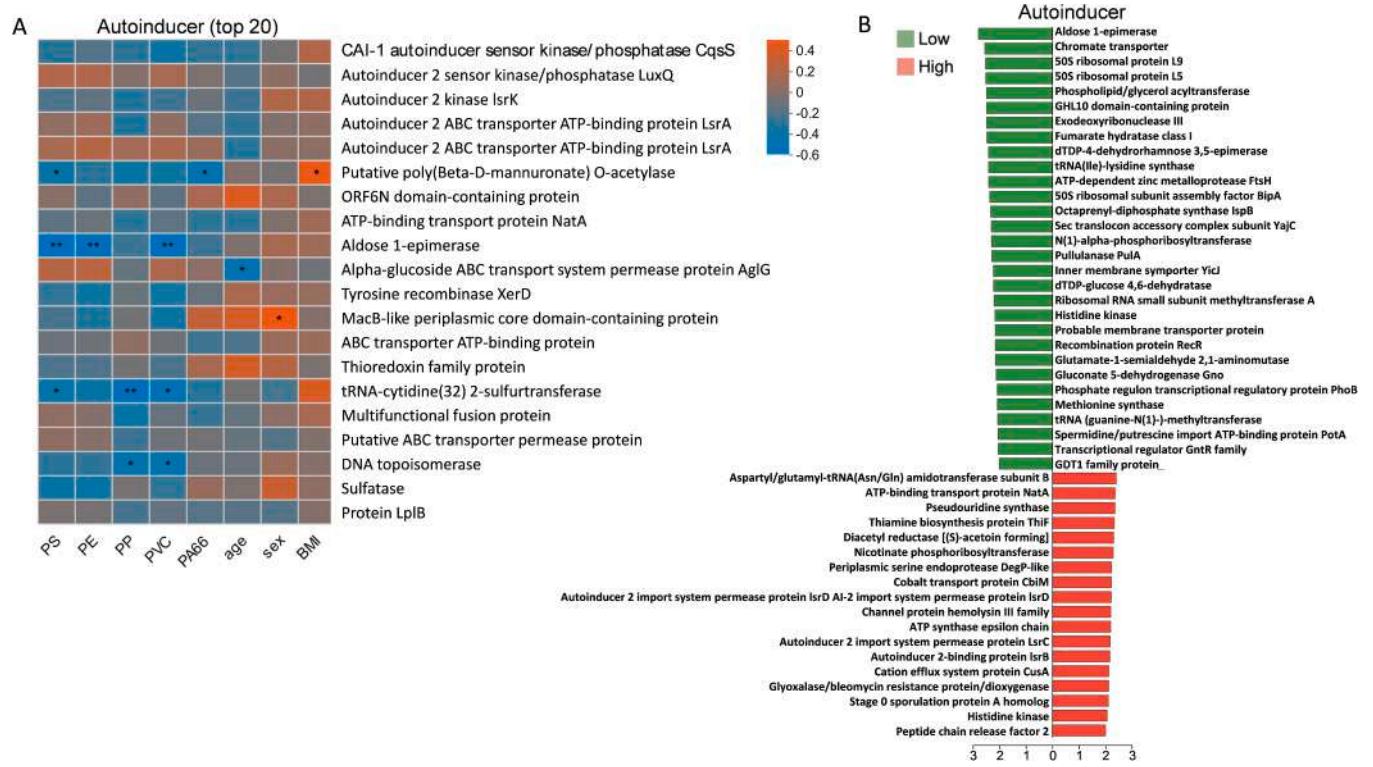


**Fig. 4.** Association between microplastics and autoinducers in the quorum sensing system. (A) Spearman correlation between microplastics and top 20 abundant autoinducer genes. * : *p*-value < 0.05; * *: *p*-value < 0.01; * ** : *p*-value < 0.001. (B) Significant microbial genes encoding autoinducers in two exposure groups; LDA value great than 2.0 was shown in the figure. Green: enriched microbial genes in low-exposure group; Red: enriched microbial genes in high-exposure group.

exposure group were shown in Fig. 4B. Consistent with the correlation analysis, the relative abundance of aldose 1-epimerase was significantly higher in the low-exposure group (Fig. 4B)

### 3.3. Correlation between microplastics and transporter system

A positive correlation was found between microbial genes encoding group translocators, electrochemical potential-driven transporters, and PP, as well as between transmembrane electron carriers and PS, PE, PP, and PVC (Fig. 5A). Specifically, microplastics were positively correlated with disulfide bond oxidoreductase, nitrate reductase, dimethyl sulfoxide reductase, trimethylamine-N-oxide reductase, thiosulfate reductase and sulfoxide reductase heme-binding flavocytochrome subunit (Fig. 5B). Significant microbial genes within the class of transmembrane electron carrier were shown in Fig. 5C. Consistent with the correlation analysis, dimethyl sulfoxide reductase DmsABC was significantly higher in the high-exposure group (Fig. 5C).

### 3.4. Correlation between microplastics and microplastic biodegradation genes

Microbial genes encoding microplastic degradation enzymes are shown in Fig. S46. These enzymes were involved in the biodegradation of PVC, PS, PE, and nylon. In addition to microplastics, enzymes involved in the biodegradation of plasticizers di(2-ethylhexyl) phthalate (DEHP) and diethyl phthalate (DP) were also detected in the human gut microbiome. The correlation between microplastics and microplastic biodegradation genes was shown in Fig. 6. A positive correlation was found between PVC, PP, PE, PS, and genes encoding microplastic biodegradation enzymes, such as acetyl-CoA acetyltransferase, 3-hydroxyacyl-CoA dehydrogenase, and catalase-peroxidase. A positive correlation was also found between PVC, PP, PE, PS and microbial genes encoding plasticizer biodegradation enzymes, such as acetaldehyde dehydrogenase.

### 3.5. Effect of PS microplastics on the functionalities of the gut microbiota in mice

We further examine the effects of microplastics on the gut microbiota in mice. PS microplastics significantly disturbed the gut microbial composition at the species level (Fig. 7). PS microplastics also disturbed the microbial genes encoding invasion-related virulence factor, autoinducer, as well as transmembrane electron carrier in mice (Fig. 8).

## 4. Discussion

Microplastics have been detected in a variety of human tissues and organs, including the liver (Horvatits et al., 2022), lungs (Jenner et al., 2022), blood (Ha et al., 2022), *etc*. In excised carotid plaque specimens collected from patients who underwent carotid endarterectomy, patients with microplastics detected showed a higher risk for a primary end-point event including a composite of myocardial infarction, stroke, or death from any cause, suggesting a possible link between microplastics in the blood vessels and the risk of cardiovascular disease (Marfella et al., 2024). A total of 15 microplastics have been detected in human feces and the concentration of fecal microplastics was correlated with the status of inflammatory bowel disease (Yan et al., 2022). More studies are needed to investigate the effects of microplastics on human health.

The effects of microplastics on the functional metagenome were revealed in adults in our study. For instance, several invasion-related virulence factors were positively correlated with microplastics in our study, including Ail, Invasin C, TTSS, Bsa T3SS and Type 1 fimbriae. Ail is a surface-associated protein with multiple roles in pathogenesis. For instance, contribution of Ail protein to plaque pathogenesis has been reported (Kolodziejek et al., 2022). Invasin C is a putative autotransporter protein, which reduced the recruitment of neutrophils in the

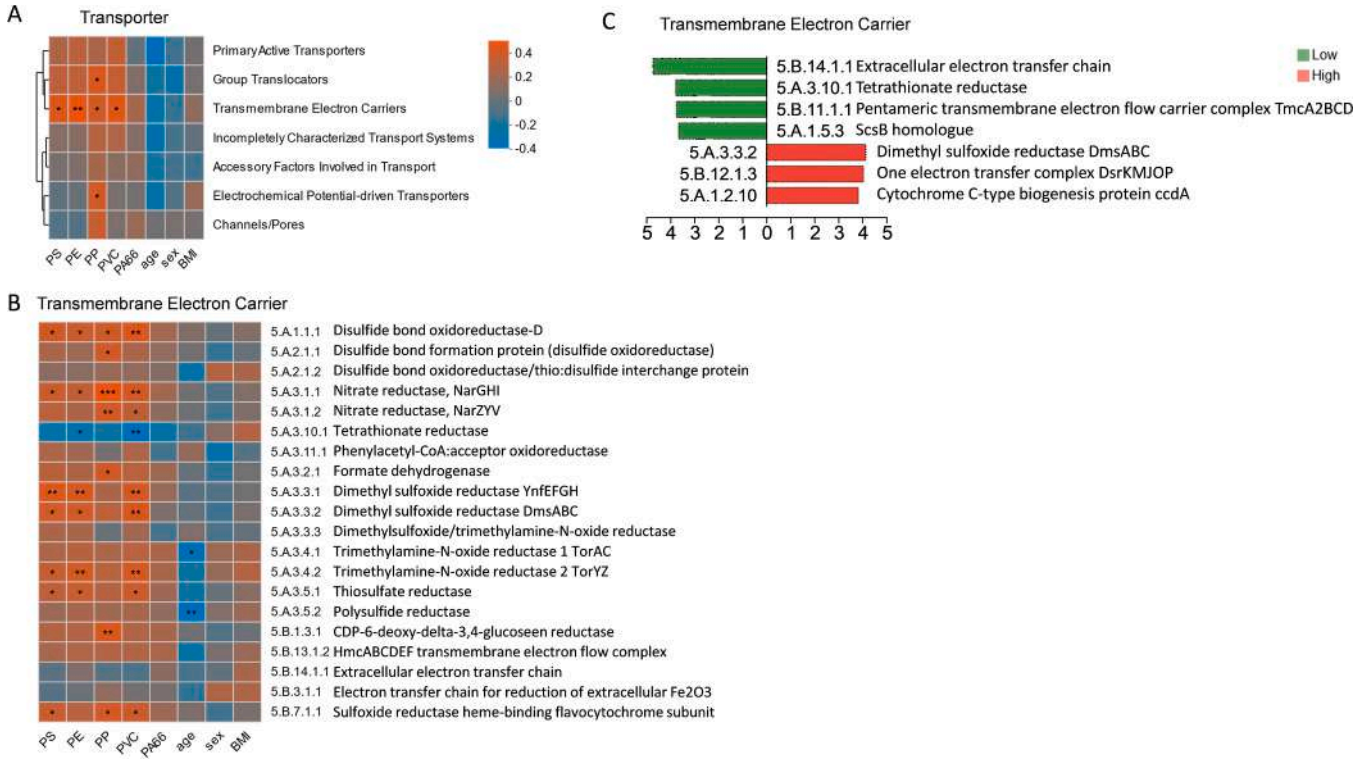


**Fig. 5.** Association between microplastics and transporter system. (A) Spearman correlation between microplastics and transporter system. (B) Spearman correlation between microplastics and microbial genes encoding transmembrane electron carrier. * : $p$-value < 0.05; * *: $p$-value < 0.01; * ** : $p$-value < 0.001. (C) Significant microbial genes encoding transmembrane electron carrier in two exposure groups. Green: enriched microbial genes in low-exposure group; Red: enriched microbial genes in high-exposure group.

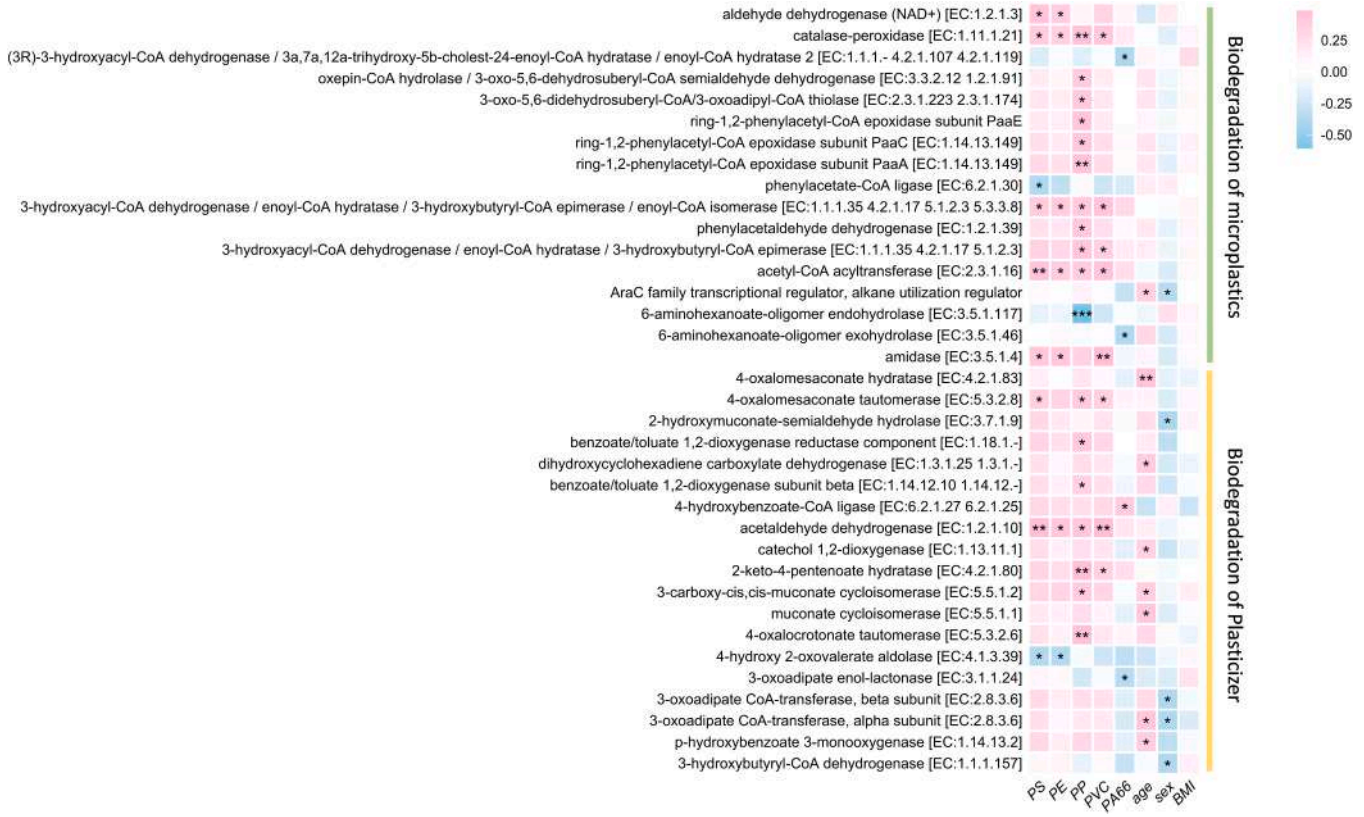


**Fig. 6.** Spearman correlation between microplastics and microbial genes encoding microplastic degradation enzymes. * : $p$-value $< 0.05$; * *: $p$-value $< 0.01$; * ** : $p$-value $< 0.001$.

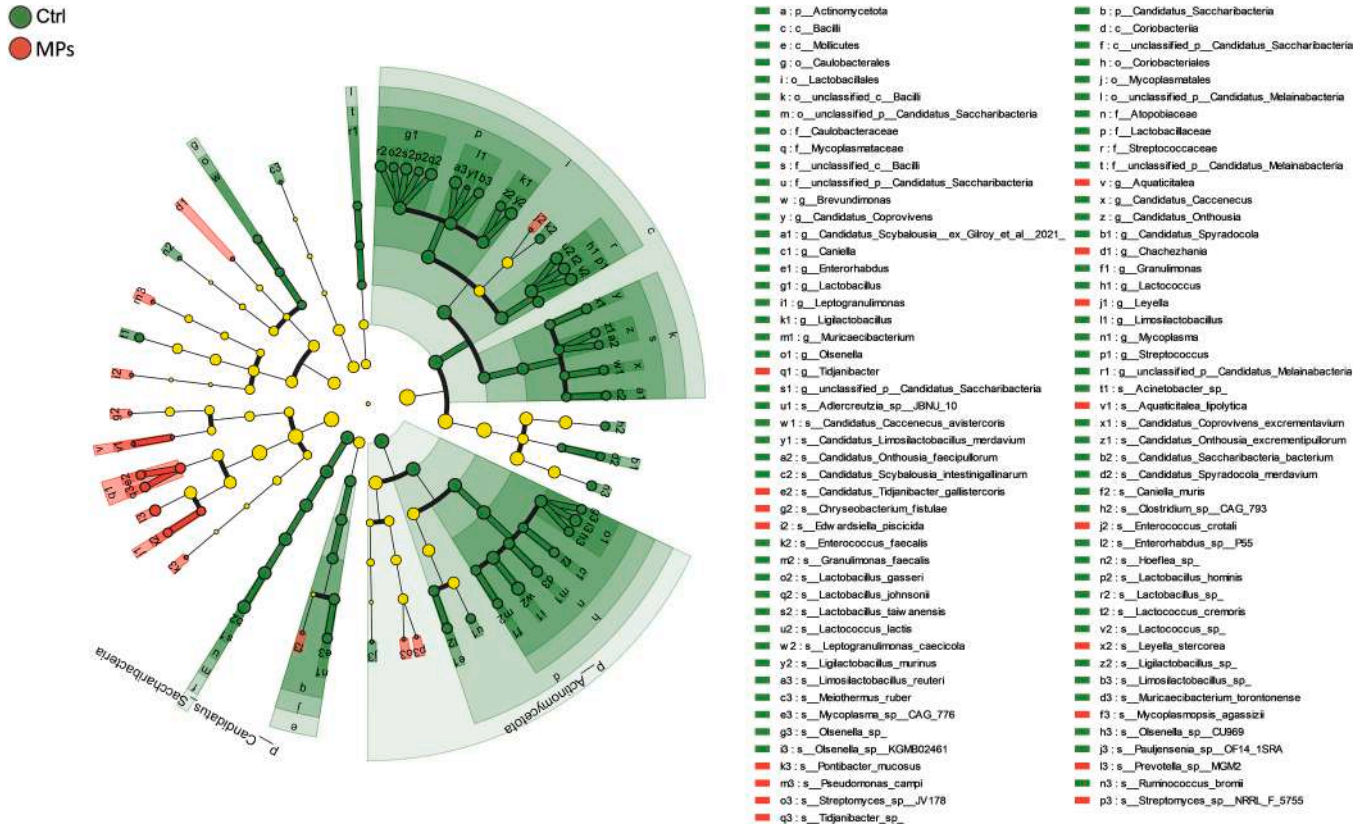


**Fig. 7.** Impact of PS microplastics on the gut microbial species in mice. Green: Significant gut microbiota enriched in the control group; Red: Significant gut microbiota enriched in the microplastics exposure group.

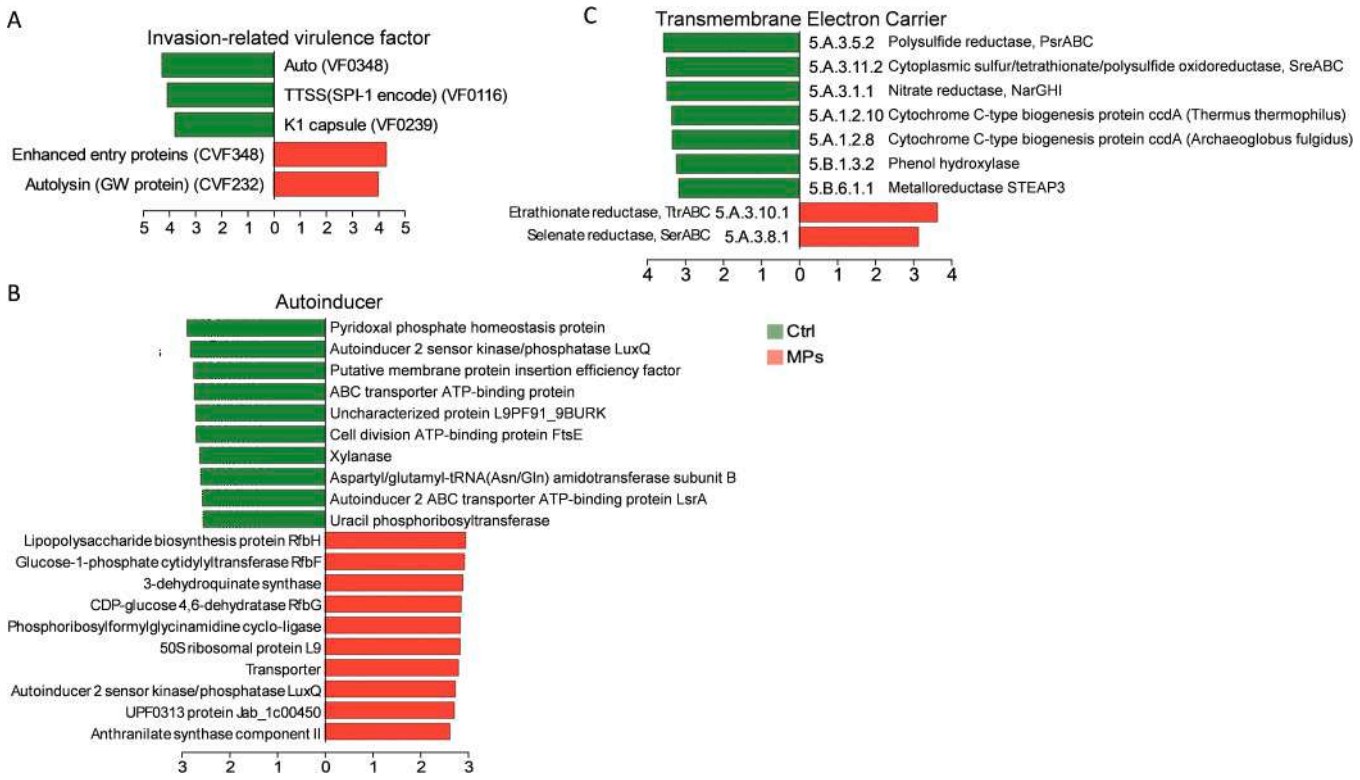


**Fig. 8.** Impact of PS microplastics on the gut metagenome of mice. (A) Significant microbial genes encoding invasion-related virulence factor. (B) Significant microbial genes encoding autoinducer. (C) Significant microbial genes encoding transmembrane electron carrier; Top 10 in each group was shown in the figure. Green: Significant microbial genes enriched in the control group; Red: Significant microbial genes enriched in the microplastics exposure group.

Peyer's patches (Pisano et al., 2012). TTSS within Salmonella pathogenicity island 1 (SPI-1) could inject effector proteins into host cells, which trigger invasion and inflammatory processes (Ehrbar et al., 2003). TTSS is associated with pathogenesis, which could secret proteins in response to various environmental conditions (Whitlock et al., 2008). T3SS could be exploited by some pathogens to deliver effector proteins into host cells, which contributes to bacterial ability to evade the immune system and induces diseases (Muangman et al., 2011). Type 1 fimbriae play a key role during interactions between *Salmonella typhimurium* and *Acanthamoeba castellanii* (Mannan et al., 2020).

Quorum sensing is a communication process between bacterial cells mediated by signaling molecules, which allow bacteria to respond to changes in the population density synchronously and regulate bacterial behaviors such as the formation of biofilm and production of secondary metabolites (Mukherjee and Bassler, 2019). This cell-to-cell communication mechanism allows the bacteria to activate the beneficial phenotypes and express various virulence genes to guarantee bacterial survival (Ruiz et al., 2022). The quorum sensing system has been considered a hidden force that drives the structure of microbial communities (Zeng et al., 2023). In our previous study, we have demonstrated that an organophosphate pesticide diazinon altered the gut microbial community through the disruption of the quorum sensing system (Gao et al., 2017a). In the natural environment, biofilms are often attached to the surface of microplastics where bacteria with quorum sensing systems are pivotal participants (Xu et al., 2023). Quorum sensing effector murein hydrolase effector LrgB was positively correlated with PS, PE, PP, and PVC in the present study. LrgB is a potential membrane-associated protein and lrgAB gene products inhibit extracellular murein hydrolase activity and promote penicillin tolerance (Groicher et al., 2000). Murein hydrolase is an enzyme family that cleaves the bacterial cell wall component specifically, which participates in the process of cell growth and division, as well as contributes to the susceptibility to antibiotics (Höltje and Tuomanen, 1991). The quorum sensing system is able to regulate the metabolic function of a variety of bacteria, including the production of extracellular hydrolases which may play an essential role in the degradation of microplastics (Jatt et al., 2015).

Microplastics might disturb the gut microbiota through the quorum sensing system. In addition, microplastics could support microbial colonization and biofilm production (Fajardo et al., 2023), which might further affect the microbial structure. The disruption of the gut microbiota by microplastics might further impact the immune system and metabolic function of the host. In mouse model, dysbiosis of the gut microbiota was also linked to the increased intestinal permeability, higher lipopolysaccharide in the serum, and increased systematic inflammation (Liang et al., 2024). PE has been reported to affect the distribution of the gut microbiota and the development of inflammation in the C57BL/6 mice model, including the increase of interleukin-1α in the serum, the decrease of the percentage of Th17 and Treg cells among CD4 cells, obvious inflammation, and elevated expression of TLR4, AP-1, and IRF5 in the intestine (Li et al., 2020). PS microplastics have been reported to induce an elevation in the number of endocrine cells, the number of cells in the lamina propria, the content of highly sulfated mucins in goblet cells, and a reduction in the volume of macrophages in healthy mice (Zolotova et al., 2023). Microplastics-induced gut dysbiosis is also linked to lipid, nucleic acid, and hormone metabolism (Sofield et al., 2024).

Correlations between microplastics and microbial genes encoding transmembrane electron carriers were found in the present study, such as disulfide bond oxidoreductase-D and nitrate reductase NarGHI. Disulfide bond oxidoreductase-D is a redox enzyme that consists of a disulfide bond in Gram-negative bacteria, whose enzymatic functions are regulated through redox switching of the disulfide bond mediated by the electron transfer (Nair et al., 2022). Disulfide bond oxidoreductase-D has been considered as a redox interaction hub, which interacts with four redox proteins in *Escherichia coli* (Stirnimann et al., 2006). Nitrate reductases reduce nitrate to nitrite, which have been classified into

periplasmic, cytoplasmic, and membrane-bound (Nar) nitrate reductases (Coelho and Romão, 2015). Nitrate reductase NarGHI oxidizes ubiquinol or menaquinol and reduces nitrate (Rothery et al., 2001).

Positive correlations between microplastics and microbial genes encoding microplastic biodegradation enzymes were found in our study, such as acetyl-CoA acetyltransferase and catalase-peroxidase. In *Galleria melonella*, a total of 47 enzymes were found to be involved in PE metabolism, including acetyl-CoA acetyltransferase (Peydaei et al., 2020). In bacterium isolated from the gut of insect larvae, several enzymes have been identified to be involved in the biodegradation of PVC, including catalase-peroxidase, which is an enolase with lyase activity (Zhang et al., 2022). Catalase-peroxidases have shown redox ability and depolymerization capability of polymers including lignin (Deangelis et al., 2013).

This study has several limitations. First, some cofounders might influence the human gut microbiota, such as eating habits, acute and chronic stimuli. Second, the sample size of the study subjects is small and the findings from this study need to be validated in an independent cohort with a larger sample size. Third, this is an observational study and only correlations were found in the human population.

## 5. Conclusion

In conclusion, PE, PVC, PP, PS, and PA66 were detected in the human blood samples. Correlations between microplastics and microbial species were found in this study, as well as microbial genes encoding invasion-related virulence factors, quorum sensing system, autoinducer, transporter system, and enzymes involved in the biodegradation of microplastics. Quorum sensing might serve as the mechanism through which microplastics disturb the gut microbiota. Functional alterations in the gut microbiota might lead to various health problems for the host. The findings from this study are of significance in improving the understanding of the health risks of microplastics on the gut microbiome in the human population.

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## CRediT authorship contribution statement

**Pengcheng Tu:** Writing – review & editing. **Zhijian Chen:** Investigation. **Shirui Zhang:** Data curation. **Lizhi Wu:** Investigation. **Zhe Mo:** Investigation. **Sunan Zhao:** Data curation. **Lixia Chen:** Data curation. **Bei Gao:** Writing – original draft, Formal analysis.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bei Gao reports financial support was provided by National Natural Science Foundation of China (grant NO. 42107459) and Key Technology R&D Program of Jiangsu Province (BE2022788).

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117497.

## Data availability

Data will be made available on request.

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