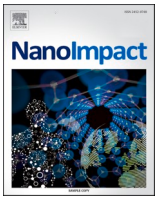
NanoImpact 36 (2024) 100531

Contents lists available at ScienceDirect

NanoImpact

journal homepage: www.elsevier.com/locate/nanoimpact



Gut-lung microbiota dynamics in mice exposed to Nanoplastics

Nur Kaluç [a], Sara Bertorello [b], Oğuz Kaan Tombul [c], Simone Baldi [b], Giulia Nannini [b], Gianluca Bartolucci [d], Elena Niccolai [b,*], Amedeo Amedei [b,e,f,*]

[a] Department of Medical Biology, University of Health Sciences, Istanbul, Turkey
[b] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
[c] Experimental Animal Application and Research Center, Maltepe University, İstanbul, Turkey
[d] Department of Neurosciences, Psychology, Drug Research and Child Health Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Florence, Italy
[e] Laboratorio Congiunto MIA-LAB (Microbiome-Immunity Axis research for a Circular Health), University of Florence, Italy
[f] Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Florence, Italy

ARTICLE INFO

Editor: Dr. Bernd Nowack

Keywords:
Nanoplastics
Microbiota
Gut-lung axis
Polyethylene terephthalate
Lung microbiota
Health

ABSTRACT

Concern has grown over potential health effects of micro- and nanoplastics (M/NPs) exposure. There is significant interest in understanding their impact on animal and human microbiota due to its crucial role in preserving health, as research in this area is rapidly advancing. We conducted a sub-chronic exposure study involving 12 male mice, divided into two groups: a control group ($n = 6$) and a PET-NPs exposure group (n = 6). PET-NPs, administered by oral gavage at a dose of 0.5 mg/day in 0.1 ml/mice, were given daily for 28 days. Microbiota analyses were performed on lung, colon, oral cavity, and stool samples using 16S rRNA sequencing. Additionally, fecal short and medium-chain fatty acids were analyzed by GC/MS. No significant changes were observed in the fecal and oral microbiome of the treated mice, nor in the fecal fatty acid levels. However, there were prominent alterations in the colon, characterized by increased abundance of Gram-negative bacteria belonging to *Veillonella* and *Prevotella* genera, and of amino acid metabolism pathways, coupled with a decrease in *Lactobacillus*. PET-NPs ingestion caused unexpected alterations in the lung microbiome with an increase in the *Pseudomonas* and changes in microbial energy metabolism and nitrogen utilization. This study provides insights into the differential impact of PET-NPs exposure on various microbiome niches.

1. Introduction

Plastic pollution is an emerging global concern over decades. While the pollution generated by macroplastics have received more attention, the emergence of micro (MPs) and nanoplastics (NPs) have considerably complicated this worldwide problem. Plastic materials widely used in daily life can produce micro- and nanoplastics, and these particles have potential risks to both environmental and human health (Gigault et al., 2018; Wright and Kelly, 2017). These materials usually include common plastics such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET). MPs are primarily generated from land sources (~80 %) and also from marine sources (~20 %) (Barboza et al., 2019) and have the ability to travel long distances due to their properties such as lightweight, durability, buoyancy, shape, and color. Terrestrial ecosystems are considered the main sources and transport pathways of MPs into the marine environment (Horton et al., 2017).

Humans are exposed to M/NPs through ingestion, inhalation, and dermal contact. Studies estimate that humans ingest up to 4.7×10^3 microplastics annually from tap water and up to 7.3×10^4 particles from table salt (Zhang et al., 2020). Additionally, MPs inhalation is significant, with indoor exposure ranging from 1.9×10^3 to 1.0×10^5 particles annually, and outdoor exposure potentially reaching up to 3.0×10^7 particles (Zhang et al., 2020). However, it is also important to consider variations in daily respiration rates among different demographic groups, such as men and women, adults and children, as well as the fact that some amount of these nanoparticles will also be exhaled. Therefore, there remains a significant gap between current estimates and the actual amount of MNP inhaled by humans (Feng et al., 2023). Regarding intake through skin contact, it is possible for them to enter through wounds,

* Corresponding authors at: Department of Experimental and Clinical Medicine, University of Florence, Italy.
E-mail addresses: nur.kaluc@sbu.edu.tr (N. Kaluç), sara.bertorello@unifi.it (S. Bertorello), oguzkaan.tombul@maltepe.edu.tr (O.K. Tombul), simone.baldi@unifi.it (S. Baldi), giulia.nannini@unifi.it (G. Nannini), gianluca.bartolucci@unifi.it (G. Bartolucci), elena.niccolai@unifi.it (E. Niccolai), amedeo.amedei@unifi.it (A. Amedei).

https://doi.org/10.1016/j.impact.2024.100531
Received 25 July 2024; Received in revised form 20 September 2024; Accepted 21 October 2024
Available online 22 October 2024

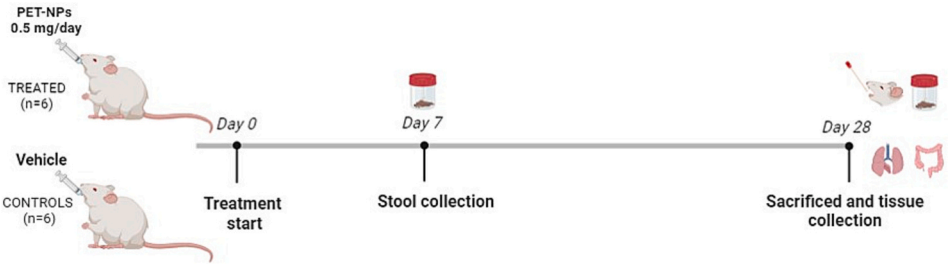


Fig. 1. Study design. The diagram illustrates the experimental setup and timeline for the study.

sweat glands, and mostly through hair follicles (Schneider et al., 2009). Regardless of exposure routes, nanoplastics can distribute and accumulate in body tissues (Zhao et al., 2023). Furthermore, it has been shown that oral exposure of polyethylene and polystyrene MPs induce gut microbiome (GM) dysbiosis in mice (Jin et al., 2019; Li et al., 2020; Lu et al., 2018). Although potential toxicity of nanoplastics on the human health has drew the focus of researchers, the majority of the studies have primarily examined on the toxicity of microplastics or conducted in vitro assessments of several commercially available nanoplastics (Domenech et al., 2021; Liu et al., 2023; Yang et al., 2021; Yin et al., 2021). This highlights a critical gap in understanding the in vivo effects of nanoplastics, especially PET-NPs, and their broader impacting for human health.

PET is the preferred material for packaging food and beverages, primarily owing to its exceptional material features, which encompass its resistance to breakage, low weight, and cost-efficiency. Unfortunately, a recent study showed that PET-bottled waters contain an average of 118 microplastics per liter and 84 % of these particles may originate from their bottle (Schymanski et al., 2018). While the intake of PET microplastics have been estimated as 166 mg/day (Tamargo et al., 2022); the daily ingestion of PET-NPs for human is still not known. Furthermore, studies that reflect the potential effects of PET-NPs on the human health are very limited (Alzaben et al., 2023; Ji et al., 2020; Zhang et al., 2022b).

Current research primarily focuses on the impact of M/NPs on GM (Harusato et al., 2023; Lin et al., 2023), but it is crucial to investigate their effect on other physiological microbiome niches. Microbial communities are essential for maintaining symbiosis with the host, playing a critical role in homeostasis and immune regulation. So, the microbiome dysbiosis can lead to functional disturbances in the body and contribute to various diseases, including cardiovascular and respiratory diseases, autoimmune disorders, and cancers (Hou et al., 2022). The GM, recognized as paramount for human health, consists of diverse bacteria engaged in food fermentation, pathogen protection, immune stimulation, and vitamin production (Hillman et al., 2017). This microbial diversity includes the oral cavity lungs, vagina, and skin in addition to the gut. The oral microbiome, acknowledged as the second-largest microbial community (Lozupone et al., 2012), displays diversity across multiple habitats, undergoing rapid changes, influenced by different factors such as pH variations and bacterial interactions (Segata et al., 2012). The lungs, contrary to prior beliefs, also host a core microbiome shaped by microbial immigration, elimination, and reproduction rates (Dickson and Huffnagle, 2015). Within various microbial niches, there is a reciprocal exchange of microorganisms, their metabolites, and immunomodulatory signals, as observed in established gut-organ axis, such as gut-lung (Haldar et al., 2023; Saxami et al., 2023). Consequently, intestinal dysbiosis may have implications for respiratory, immune, neurological, hepatic, and renal health. Understanding the dynamics of these diverse microbiomes is crucial for well and finely unraveling their profound impact on human health and disease.

Despite advances in understanding M/NPs' effects on GM, significant knowledge gaps remain regarding PET-NPs' impact on microbial communities across different body sites. Recent studies documented a lack of information on how PET-NPs induce microbial dysbiosis, particularly in the nasal and lung microbiome (LM) (Zha et al., 2023). Furthermore, the long-term effects of PET-NPs, mechanisms underlying microbiome disruptions, and the comparative risks posed by varying particle sizes have not been thoroughly explored (Zhang et al., 2023b).

In this study, we hypothesize that ingestion of PET-NPs leads to alterations in microbiome profiles not only within the gut but also in other niches such as the oral cavity and lungs. Specifically, we aim to assess how sub-chronic exposure to PET-NPs affects microbiome diversity, composition, and functional profiles across multiple niches in a murine model. By investigating the effects of PET-NPs of defined sizes and dosages, we seeked to fill critical knowledge gaps regarding the distant-site impact of PET-NPs on microbiome health, providing new insights into the environmental and health risks made by nanoplastics.

2. Materials and methods

2.1. Animals and ethics statement

A total of 12 five-week-old male mice (*Mus musculus*) were obtained from Maltepe University Experimental Animals Research and Application Center (Istanbul, TURKEY). Experimental protocol was approved by Maltepe University Laboratory Animal Experiments Ethics Committee (protocol number: 2022.09.03, date: 29.09.2022) and was made in accordance with Turkish Law 6343/2 and with the Helsinki Declaration of Animal Rights. Animal care and experiments were carried out in the standard plastic cages with stainless steel at 25 ± 4 °C temperature, 50 ± 5 % relative humidity and 12 h light and 12 h dark cycle and fed standard laboratory chow and water ad libitum. 12 five-week-old mice were randomly selected and divided into two groups: control group (n = 6), and 0.5 mg/day PET-NPs exposure groups (n = 6) (Fig. 1). PET-NPs were administered by oral gavage in a fixed volume of 0.1 ml/mice daily for 28 days. 5 mg/ml of PET-NPs solutions were freshly prepared in physiologic saline for 0.5 mg/day group, respectively. Before oral gavage, both solutions were sonicated, and only physiologic saline was administrated to control group. Oral swab samples and 24-h stool samples were collected from mice individually at 7th (Week one, W1) and 28th days (Week 4, W4) of exposure and stored at −80 °C. After 28 days of exposure, all mice were anesthetized by intraperitoneal injection of ketamine (10 mg/kg) and xylazine (90 mg/kg) and sacrificed by collecting whole blood from heart. Colon and lung tissues were collected and frozen at −80 °C. The anesthetics were used solely for euthanasia and administered under identical conditions to both control and treated groups. Given that Gerb et al. found no significant GM changes from daily ketamine administration (100 mg/kg) over 10 days (Gerb et al., 2021), we do not expect our single, lower-dose administration to affect the gut microbiota. While prolonged exposure could impact microbial communities, a one-time dose prior to euthanasia is unlikely to do so.

The exposure concentration of 0.5 mg/day PET-NPs was selected based on doses used in previous studies investigating the effects of other M/NPs in animal models with similar age, exposure methods, and durations (Deng et al., 2017; Jeong et al., 2022; Nie et al., 2021). At study starting, no in vivo studies on PET-NPs were available, and no

Table 1
DLS analysis data of PET-NPs in physiological saline.

	Mean
Z- Average (nm)	91.11
Polydispersity Index (PI)	0.2803
Conductivity (mS/cm)	0.3328
Zeta Potential (mV)	−29.1

established daily exposure limits existed. Upon comparison with LC50 values from subsequent PET-NP research, the chosen concentration was confirmed to be appropriate for our experiments (Lin et al., 2023).

2.2. Polyethylene terephthalate Nanoplastics (PET-NPs)

The used PET-NPs were previously produced and fine characterized in our previous report (Kaluç et al., 2024) (**Fig. S1**). Transmission electron microscopy (TEM) confirmed that the nanoplastics had an actual size of 56 nm. Additionally, Fourier-transform infrared spectroscopy (FT-IR) analysis showed the characteristic absorption bands of PET, confirming that the chemical structure of the polymer remained intact despite the reduction in particle size.

Since the PET-NPs were to be administered via oral gavage in physiological saline, hydrodynamic diameter (*Z*-average), zeta potential, polydispersity index (PDI) and conductivity in physiological saline was also analyzed by Dynamic Light Scattering (DLS) and given in Table 1.

2.3. Microbiome characterization

Genomic DNA from stool and oral swab was extracted using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany), while the DNA from lung and colon fresh tissue with DNeasy Blood and Tissue kit from frozen (−80 °C) samples. The quality and quantity of extracted DNA were assessed with NanoDrop ND-1000 (Thermo Fisher Scientific, Waltham, MA, United States) and the Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA, United States) and then frozen at −20 °C until sequencing. Extracted DNA samples were sent to IGA Technology Services (Udine, Italy) where amplicons of the variable V3-V4 region of the bacterial 16S rRNA gene, delimited through primers 341F and 805R, were sequenced in paired-end on the Illumina MiSeq platform, according to the Illumina 16S Metagenomic Sequencing Library Preparation protocol (Pagliai et al., 2020).

The resulting raw data as FASTQ files were processed following the software pipeline MICCA (MICrobial Community Analysis). Demultiplexed sequence reads were processed using QIIME2 2022.11. The Cutadapt tool was used to discard the sequences without primers. DADA2 was used to trim low-quality nucleotides from both forward and reverse reads (−-p-trunc-len-f 228 –p-trunc-len-r 214) and to perform paired-end filtering, merging, and removing chimeras. Possible host sequences were identified by aligning the possible amplicon sequence variants (ASV) to GRCm39 (murine reference genome) and deleted using Bowtie2 2.4.4. The remaining reads were imported into QIIME2 and the taxonomic assignments were performed through the Scikit-learn multinomial Bayes classifier re-trained on the Silva database (release 138) V3-V4 iper-variable region.

Every ASV whose average relative abundance between the genera was less than the cutoff of 0.005 % was removed to minimize the presence of any contaminants (Bokulich et al., 2013). Furthermore, the Chloroplast genus was removed because considered a contaminant (Lusk, 2014).

2.4. Short- and medium-chain fatty acids analysis

The qualitative and quantitative evaluation of fecal short (SCFA) and medium chain fatty acids (MCFA) was performed using an Agilent GC–MS system composed of an HP 5971 single quadrupole mass spectrometer, an HP 5890 gas chromatograph, and an HP 7673 autosampler, as previously described (Niccolai et al., 2019). Briefly, just before the analysis, stool samples were thawed and mixed with a 0.25 mM sodium bicarbonate solution (1,1 *w/v*) in a 1.5 ml centrifuge tube. Then, the resulting suspensions were sonicated for 5 min, centrifuged at 5.000 rpm for 10 min and then the supernatants were collected. The SCFA and MCFA were finally extracted as follows: an aliquot of 100 µl of sample solution (corresponding to 0.1 mg of stool sample) was added to 50 µL of internal standards mixture, 1 ml of tert-butyl methyl ether and 50 µl of HCl 6 M + 0.5 M NaCl solution in a 1.5 ml centrifuge tube. Subsequently, each tube was shaken in a vortex apparatus for 2 min, centrifuged at 10.000 rpm for 5 min, and lastly, the solvent layer was transferred to an autosampler vial and processed three times.

2.5. Statistical analyses

The statistical analyses of bacteria communities were performed in R 4.2.2 using the following packages: phyloseq 1.42.0, vegan 2.6–4, DEseq2 1.38.3, ggplot2 3.2.4, ggpubr 0.6.0 and other packages satisfying their dependencies. Saturation analysis was performed and satisfied for each sample using the rarecurve function. The observed richness and Shannon indices were used to perform alpha diversity analyses. The Pielou's evenness index was calculated using the formula E = S/log (R), where S is the Shannon index while R is the observed ASV richness in the sample.

Differences in all indices between grouped samples were tested using paired and not paired Wilcoxon tests and *p*-value less than 0.05 were considered statistically significant. The permutational ANOVA (PERMANOVA) with 9999 permutations was applied to beta diversity distance matrices (performed using Hellinger distance on Hellinger transformed genera abundances) to test the significance between samples' clusters observed following coordinate analysis (PCoA). At the different taxonomic ranks, the differential abundances (DA) have been computed through the DESeq2 algorithm on raw count data. The DA with an associated *P*-value (adjusted through Benjamini–Hochberg method) less than 0.05 has been considered significant. Moreover, every DA with a grand mean count <100 has been discarded from the displayed results to avoid the most likely noisy ones. Potentially expressed Metacyc pathways have been predicted through PICRUST2 v2.4.2 with EPA-ng algorithm and then significant differences among groups have been explored using LEFSE (LDA Effect Size) analysis**.** Finally**,** GraphPad Prism (v.5) was used for statistical analysis of fecal SCFA and MCFA levels before and after treatments.

3. Results

3.1. Resilience of Oral and fecal microbiome composition under PET-NPs exposure

To evaluate the impact of PET-NPs ingestion on the fecal microbiome, we analyzed stool samples from control and treated mice at W1 and W4. Alpha and beta diversity analyses indicated no significant differences in microbial structure or composition between treated and control mice at both time points (**Fig. S2**). The fecal microbiome of exposed mice at W1 consisted mainly of five phyla: Bacteroidota (49.3 %), Firmicutes (41.7 %), Campilobacterota (5.5 %), Verrucomicrobiota (1.3 %), and Proteobacteria (0.5 %), similar to control mice. The top genera in exposed mice included *Bacteroides* and *Odoribacter*, mirroring control mice (**Fig. S3**).

At W4, both groups continued to show comparable microbiome compositions, dominated by Firmicutes (47.6 %) and Bacteroidota (45.5 %). Univariate analysis did not reveal significant differences in microbial taxa abundance between treated and control mice (**Fig. S3**).

Similarly, oral microbiome analysis at W4 revealed no significant differences between the treated and control groups. Both groups

Table 2
SCFA and MCFA analysis. Percentage of fecal SCFA and MCFA (median, (IQR) in treated and untreated mice, at week 1 and week 4. *P* values were calculated using Mann-Whitney test. *P* value<0.05 are considered statistically significant.
Percentage of fecal SCFA and MCFA (median, (IQR); %).

Analyte	W1 untreated	W1 treated	*P* value	W4 untreated	W4 treated	*P* value
Acetic	72.418 (3.023)	73.280 (5.415)	0.937	73.323 (1.059)	71.361 (2.799)	0.132
Propionic	16.312 (3.342)	13.150 (2.305)	1	14.710 (2.330)	13.107 (2.986)	0.240
Butyric	9.073 (1.594)	9.495 (6.619)	1	9.581 (2.833)	12.422 (5.688)	0.132
isoButyric	0.545 (0.057)	0.563 (0.209)	0.818	0.503 (0.044)	0.588 (0.080)	0.064
isoValeric	0.306 (0.025)	0.305 (0.117)	0.393	0.257 (0.029)	0.295 (0.111)	0.179
2-MethylButyric	0.232 (0.027)	0.264 (0.112)	0.484	0.197 (0.050)	0.237 (0.074)	0.148
Valeric	1.032 (0.343)	1.040 (0.418)	0.818	0.884 (0.176)	0.953 (0.142)	0.240
IsoHexanoic	0.004 (0.002)	0.005 (0.005)	1	0.005 (0.006)	0.008 (0.011)	0.376
Hexanoic	0.0300 (0.011)	0.025 (0.011)	0.520	0.041 (0.013)	0.052 (0.010)	0.297
Heptanoic	0.009 (0.006)	0.006 (0.001)	0.157	0.007 (0.003)	0.007 (0.002)	0.682
Octanoic	0.005 (0.002)	0.002 (0.003)	0.154	0.006 (0.003)	0.006 (0.002)	0.566
Nonanoic	0.004 (0.003)	0.003 (0.001)	0.287	0.003 (0.002)	0.004 (0.001)	0.677
Decanoic	0.005 (0.004)	0.004 (0.001)	0.357	0.004 (0.002)	0.005 (0.001)	0.866
Dodecanoic	0.010 (0.007)	0.007 (0.001)	0.214	0.008 (0.004)	0.008 (0.002)	0.935

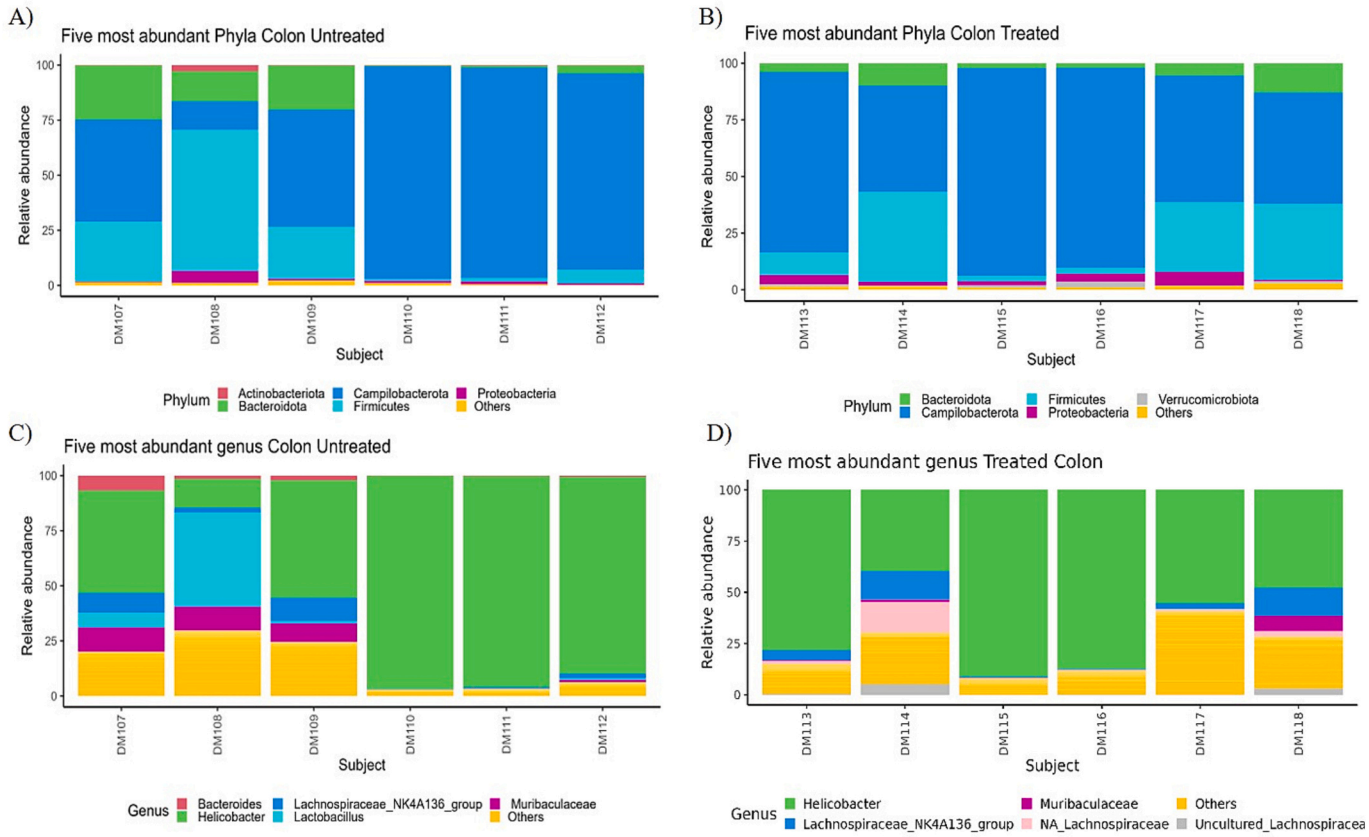


Fig. 2. Most represented Phyla and Genera in colon samples. Bar plot showing the relative abundance of the five most represented Phyla (A, B) and Genera (C, D) at week four documented in colon samples of untreated mice and treated mice.

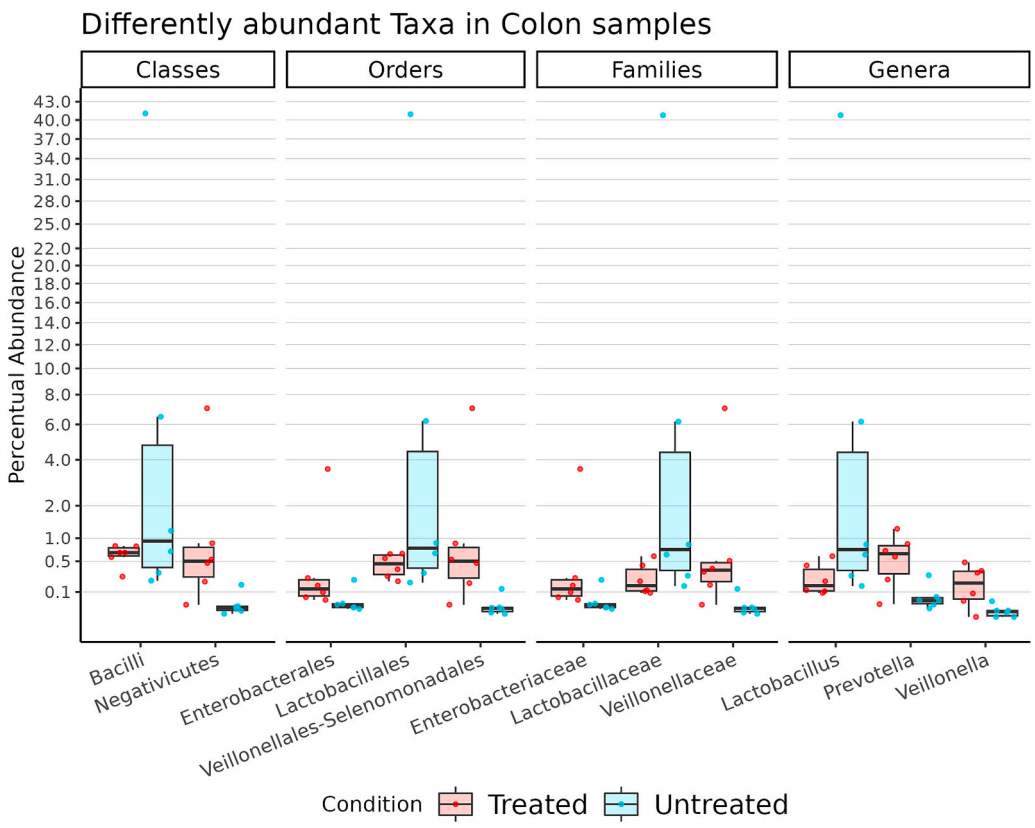


Fig. 3. Results of multivariate analysis of colon samples. Box plot showing the significant differentially abundant taxa among untreated and treated mice after four weeks of treatment at Class, Order, Family, and Genus levels.

exhibited a similar microbial composition, with Proteobacteria and Firmicutes as the dominant phyla. In control mice, Proteobacteria accounted for 54 %, followed by Firmicutes at 40 %, while in treated mice, Proteobacteria made up 55.6 % and Firmicutes 36.9 %. The most abundant genera were consistent across groups, with *Muribacter* and *Streptococcus* being the predominant genera in both treated and control mice (**Fig. S4**). Alpha and beta diversity analyses further confirmed that the microbial diversity and overall composition in the oral microbiome remained unaffected by PET-NPs exposure (**Fig. S5**).

3.2. Fecal short- and medium-chain fatty acids analysis

The analysis of fecal SCFA and MCFA showed no significant differences in their abundance between treated and untreated mice at both W1 and W4 (**Table 2**).

3.3. Fluctuations in relative taxon abundance in Colon and Lung microbiome following PET-NP exposure

We assessed the impact of PET-NPs exposure on the gut and lung microbiome by analyzing microbial communities in the colon mucosa and lungs of mice. While alpha and beta diversity analyses showed no significant structural changes between treated and control mice (**Fig. S6**), variations in relative taxon abundance were noted across both microbial niches.

In the colon mucosa, control mice were dominated by Campilobacterota (63.5 %) and Firmicutes (23.6 %), while treated mice exhibited an increase in Campilobacterota (66.6 %) and Proteobacteria (3 %) (Fig. 2). Univariate analysis revealed that control mice had a significant enrichment of Bacilli (padj = 0.002), Lactobacillales (padj = 0.0056), Lactobacillaceae (padj = 0.0004), and *Lactobacillus* (padj = 0.0008). In contrast, treated mice showed higher levels of Negativicutes (padj = 9.64E-008), Veillonellales-Selenomonadales (padj = 1.73E-006), Enterobacterales (padj = 0.0294), Veillonellaceae (padj = 1.08E-005), *Veillonella* (padj = 0.0258), and *Prevotella* (padj = 0.040) (Fig. 3).

In the lungs, the most abundant phyla in control mice were Firmicutes (45.2 %) and Bacteroidota (29.2 %), whereas treated mice showed increased Proteobacteria (25.8 %) and Verrucomicrobiota (9.3 %) (Fig. 4 **A,B**). Treated mice also exhibited a higher abundance of *Escherichia-Shigella* (9.5 %) and *Akkermansia* (9.3 %), compared to control mice, where *Bacteroides* and *Burkholderia-Caballeronia-Paraburkholderia* were more prominent (Fig. 4 **C,D**).

Finally, the DeSeq analysis identified significant differences in the microbial communities between treated and control mice. Specifically, the order Pseudomonales was significantly enriched in treated mice (padj = 0.0016), as well as the family Pseudomonaceae (padj = 0.0014). Within this family, the genus *Pseudomonas* was notably more abundant in the treated group (padj = 0.0058) (Fig. 5). These findings highlight a specific increase in Pseudomonas-related taxa, suggesting a potential selective pressure exerted by nanoplastic particles on certain microbial populations. This enrichment was consistent across multiple taxonomic levels, suggesting that PET-NPs may influence microbial dynamics by promoting specific taxa like Pseudomonas, which are known for their resilience in various environmental contexts.

3.4. Functional profiles of microbiome in lung and colon tissue

Given the significant alterations observed in the microbiome of lung and colon tissue, we employed PICRUST2 analysis to estimate differences in the bacterial functional profiles between treated and untreated mice.

In the colon, we identified 10 pathways as significantly different among treated and untreated mice (Fig. 6A). The treated mice showed higher activities in superpathway of sulfate assimilation and cysteine

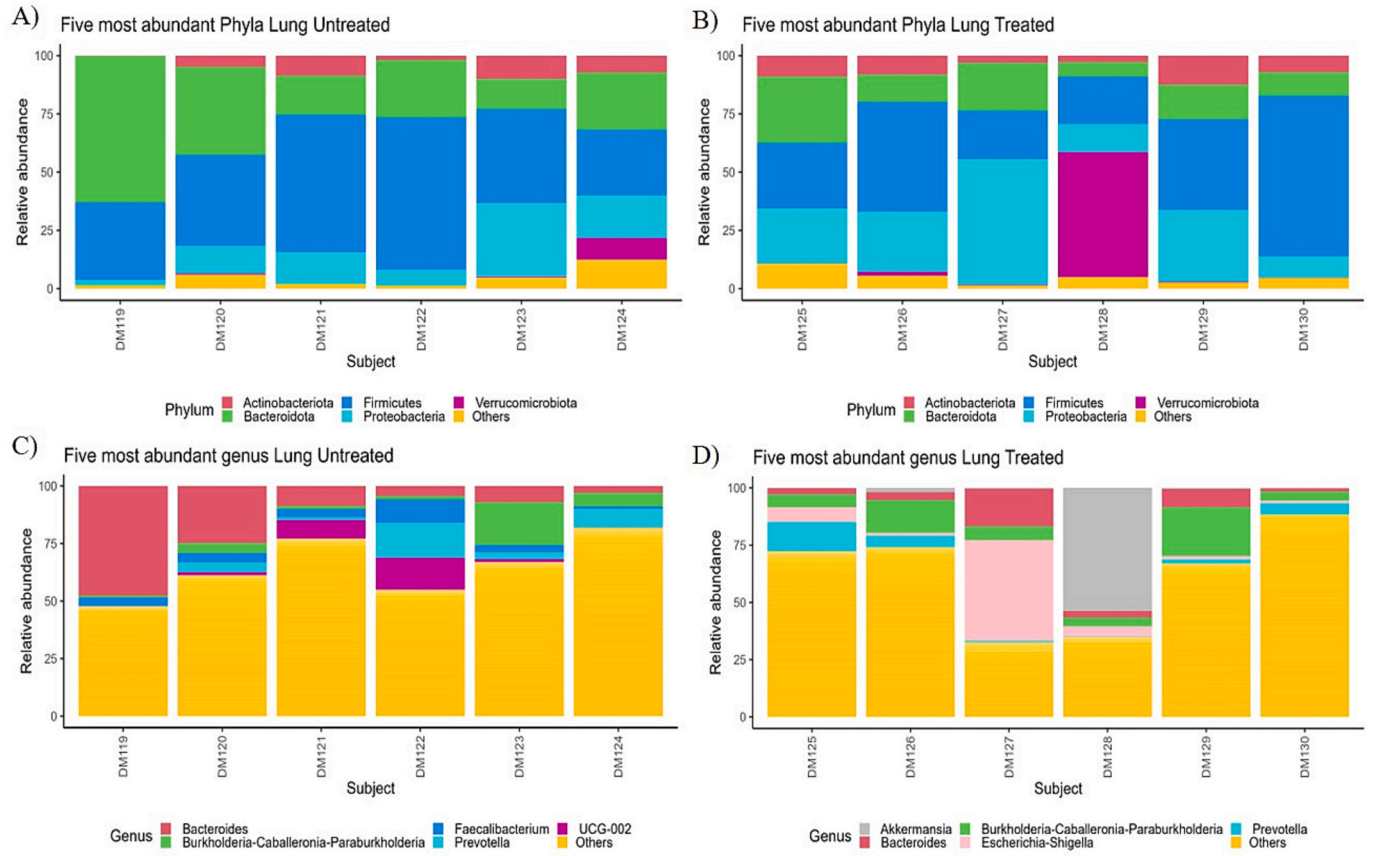


Fig. 4. Most represented Phyla and Genera in lung samples. Bar plot showing the relative abundance of the five most represented Phyla (A, B) and Genera (C, D) at week four documented in lung samples of untreated mice and treated mice.

biosynthesis, L-methionine biosynthesis (by sulfhydrylation), menaquinol-8 biosynthesis I, menaquinol-7,11,12,13 biosynthesis and (Kdo)2-lipid A biosynthesis compared to control. Conversely, in untreated mice the superpathways of pyruvate fermentation to acetate and lactate II and with dTDP-L-rhamnose biosynthesis were highly expressed.

In the lung, we found 13 differentially abundant pathways (Fig. 6B). In detail, treated mice showed e significant enrichment of the superpathway of L-alanine biosynthesis, allantoin degradation to glyoxylate III, D-galactarate degradation I, D-glucarate and D-galactarate degradation, L-arginine degradation II (AST pathway), 1,4-dihydroxy-2-naphthoate biosynthesis I, purine nucleobases degradation I (anaerobic) and superpathway of phylloquinol biosynthesis. On the other hand, in the lung of untreated the superpathway of L-aspartate and L-asparagine biosynthesis, pentose phosphate pathway (non-oxidative branch), gondoate biosynthesis (anaerobic), peptidoglycan biosynthesis III (mycobacteria), and UDP-*N*-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing) are enhanced.

4. Discussion

In recent years, there has been an increasing focus on the potential adverse impacts of exposure to micro and nanoplastics on health. Studies have demonstrated that M/NPs can induce oxidative stress, inflammation, toxicity, and potentially disrupt key biological processes, including those linked to the microbiome, which plays a pivotal role in maintaining overall well-being (Giri et al., 2024; Prata et al., 2020; Qiao et al., 2019; Zhang et al., 2022a). As such, investigating the dynamic interplay between M/NPs and this essential microbial ecosystem has become a growing area of research. To contribute to this field, our study aimed to assess the effects of sub-chronic PET-NPs exposure on various microbiome niches in a murine model. Male mice were exposed to PET-NPs for 28 days, followed by a comprehensive evaluation of oral, lung, colon mucosa, and gut fecal microbiome. While the exposure not significantly affecting oral and fecal microbiome, notable alterations were observed in microbiome composition and function of the lung and colon mucosa.

Recent investigations have explored the effects of various M/NPs on the fecal microbiome of mice, employing diverse protocols, exposure durations, and particle characteristics. For instance, Lin et al. studied the health effects of single exposure to PET-NPs, observing that the smallest particles (<250 nm) caused adverse effects on gut health, including loss, cyst formation, intestinal obstruction, organ damage, and mortality, coupled with significant alterations in the fecal microbiome composition (Lin et al., 2023). In contrast, larger PET-derived NPs (> 400 nm) and MPs (<3.5 μm) did not have marked effects on microbiome composition (Harusato et al., 2023; Lin et al., 2023). However, independent studies by other research groups revealed that exposure to 5 μm polystyrene microplastics (PS-MPs) led to significant changes in the fecal microbiome of mice, including an increase in *Prevotella* and a decrease in *Lactobacillus* species (Wen et al., 2022; Zhang et al., 2023a).

Moreover, Chen and colleagues have conducted an experiment in which mice were exposed to PVC-MPs at a dose of 100 mg/kg for 60 days. They revealed a prominent increase in the abundance of *Prevotella* in the colon mucosa of treated mice compared to the control group (Chen et al., 2022); and our study is consistent with these findings, as sub-chronic exposure to PET-NPs (approximately 100 nm) resulted in alterations in the colon mucosa microbiome, characterized by an increase in the abundance of *Veillonella* and *Prevotella* genera, and a decrease in *Lactobacillus*.

These observations raise questions about the implications of mucosal microbiome changes following PET-NPs exposure for the host's health

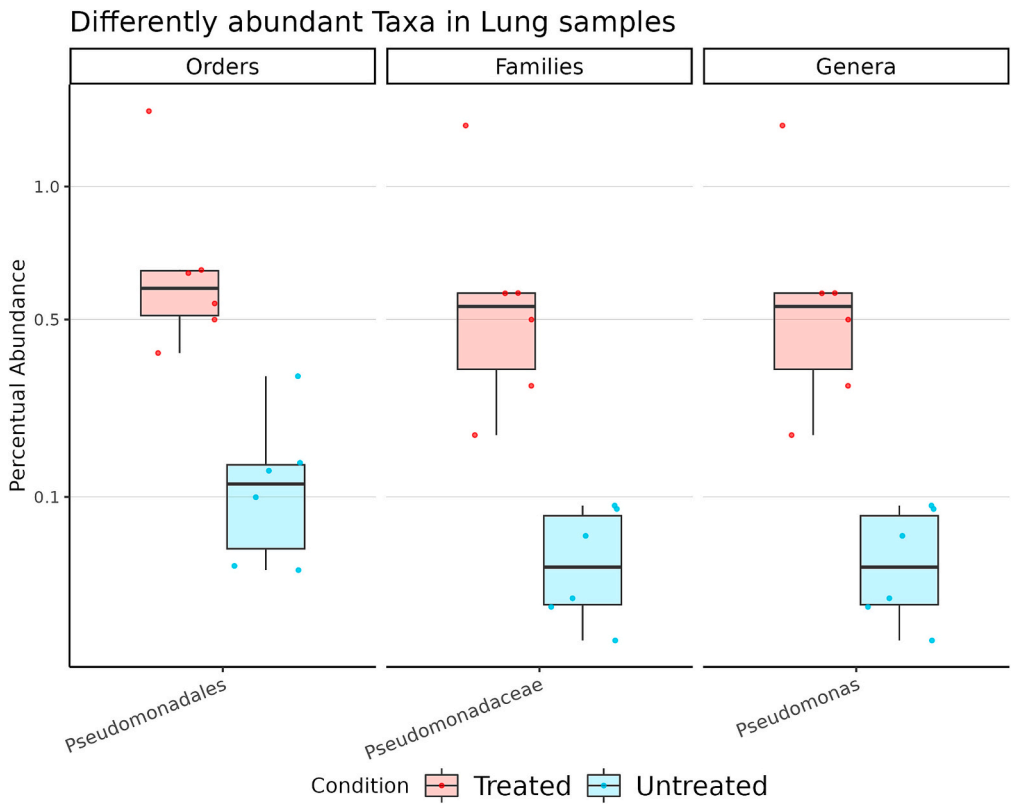


Fig. 5. Results of multivariate analysis in lung samples. Box plot showing the significant differentially abundant taxa among untreated (control) and treated mice after four weeks of treatment at Order, Family, and Genus levels.

and metabolism. It is remarkable that *Prevotella*, a gram-negative bacterium, has been associated with several human diseases, including periodontitis (Davison et al., 2021), rheumatoid arthritis (Alpizar-Rodriguez et al., 2019), vaginal (Gosmann et al., 2017) and intestinal dysbiosis (Wedenoja et al., 2022). Notably, additional studies have established a connection between intestinal *Prevotella* levels and sperm viability in male infertility patients (Ning et al., 2020), suggesting a potential interplay between the composition of the gut microbiome and male reproductive health. Finally, recent data have also shed light on the immunomodulatory role of *Prevotella* spp. in the intestine, including how specific species may exacerbate mucosal inflammation (Iljazovic et al., 2021). Moreover, the concurrent decrease in the well-known intestinal probiotic *Lactobacillus* (Fang et al., 2018; Fernández et al., 2018) could contribute further to localized inflammation (Gosmann et al., 2017).

Similar to *Prevotella,* the significant rise in *Veillonella,* on colon of treated mice, presents relevant queries regarding its potential implications for intestinal health and inflammation. Recent research has lessened how the oral bacterium *Veillonella parvula*, elevated in the intestines of patients with inflammatory bowel disease, undergoes metabolic adaptations to colonize the gut (Rojas-Tapias et al., 2022). This is particularly relevant since patients with ulcerative colitis have exhibited a decrease in short-chain fatty acids producers, like *Lactobacillus*, and an expansion of bacteria typically found in the oral mucosa, including *Hemophilus* and *Veillonella* (Basha et al., 2023). Additionally, the work of Zhan et al. has revealed that an overabundance of *Veillonella parvula* has the potential to trigger intestinal inflammation by activating macrophages through the LPS-TLR4 pathway in mice (Zhan et al., 2022). Collectively considering these findings, it is conceivable that the surge in *Veillonella* within the colon mucosa of PET-NPs treated mice could be a response to the altered gut environment induced by microplastic exposure. This observation prompts further investigation to elucidate the fine mechanisms involved and the potential implications of *Veillonella's* presence in the colon mucosa.

In addition, our PICRUST analysis showed notable differences in functional profiles between the control and treated groups in the colon tissues. The significant increase in activities related to sulfate assimilation, cysteine biosynthesis, L-methionine biosynthesis (by sulfhydrylation), menaquinol biosynthesis (specifically menaquinol-8 and menaquinol-7,11,12,13), and (Kdo)2-lipid A biosynthesis in treated mice suggests a potential modulation of sulfur metabolism, amino acid biosynthesis, and lipid A formation. In line with our findings Sun et al. found that the absorption of PE-MPs in mice led an increase in the amino acid metabolism pathway within the microflora, achieved by modifying the composition of the intestinal microflora (Sun et al., 2021).

These microbiome changes may reflect adaptive responses to PET-NPs exposure. GM dysbiosis has been associated with an increased risk of inflammatory-related disorders, as alteration in microbial composition influence host immunity, potentially triggering pro-inflammatory responses (Di Vincenzo et al., 2024; Hou et al., 2022; Shan et al., 2022). In summary, exposure to plastic M/NPs, regardless of their source or size, may disrupt the gut microbiome, and through the disruption of microbial homeostasis, contribute to inflammatory processes.

Furthermore, our findings surprisingly reveal that sub-chronic ingestion of PET-NPs has a discernible effect on the composition of the lung microbiome. To date, limited information exists regarding the influence of M/NPs on the lung microbiome. A recent investigation by Zha et al. explored the intranasal administration of 5 μm polystyrene MPs and 99 nm polystyrene NPs. The authors found that airborne M/NPs could modify the nasal microbiome in mice, with MPs exerting a more pronounced influence on the lung microbiome than NPs (Zha et al., 2023).

In our study, PET-NPs exposure resulted in a remarked increase in the *Pseudomonas* genus. This discovery is particularly intriguing given the association of elevated *Pseudomonas* species in the lung microbiome

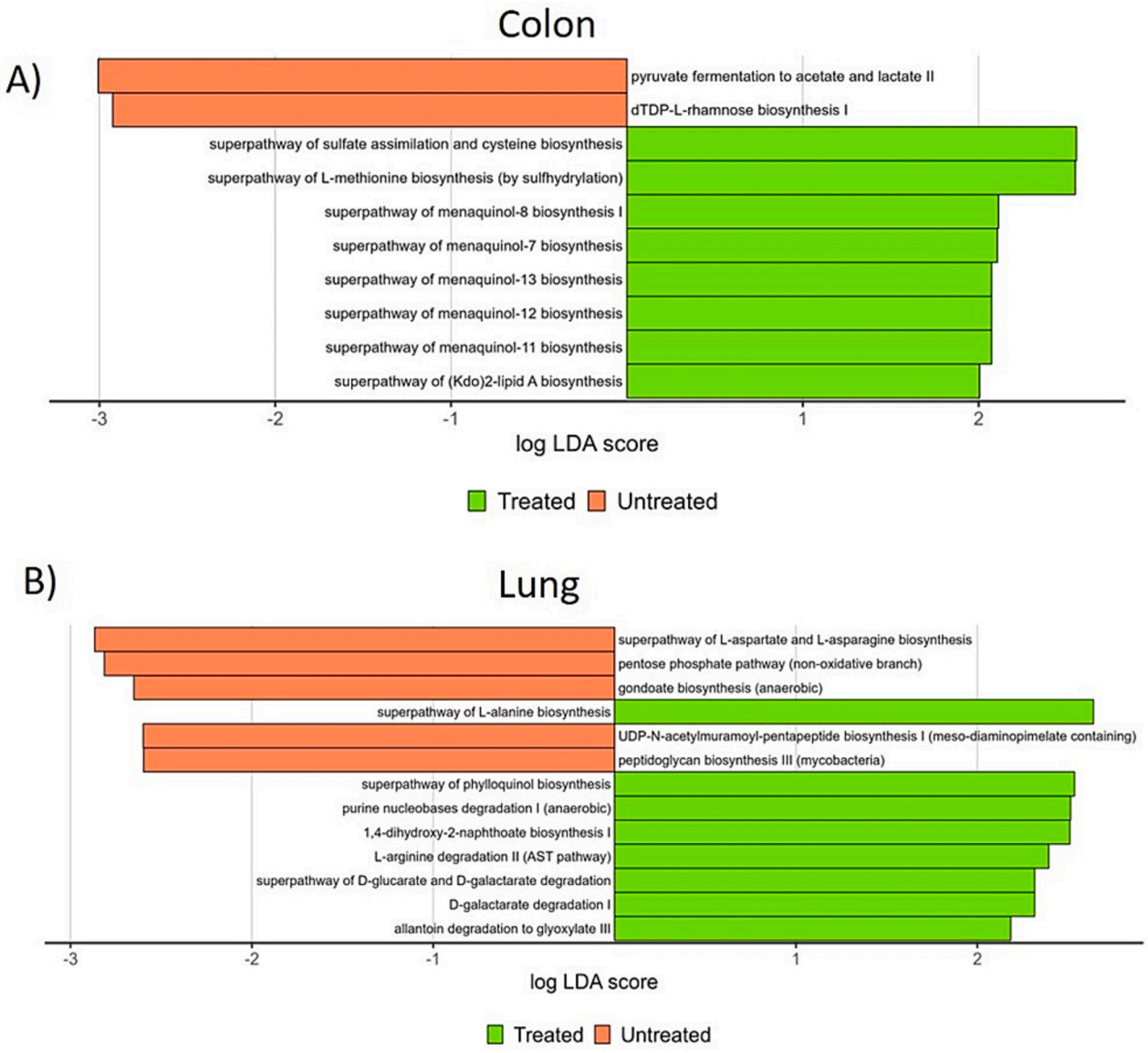


Fig. 6. PICRUST2 analysis results. Computed LDA scores of the significantly different functions in treated and untreated groups in A) colon and B) lung samples. Negative LDA scores (orange) are enriched in the Untreated group while positive LDA scores (green) are enriched in the Treated group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with various respiratory infections and lung diseases (Gaibani et al., 2021; Wang et al., 2019). Notably, the *Pseudomonas* genus includes potential respiratory pathogens, such as *P. aeruginosa*, which could potentially dominate the commensal microbiome in the airways, leading to a reduction in microbial diversity within the lungs. In the context of adult cystic fibrosis patients, this decrease of microbial diversity has been correlated with decreased lung function and an increased frequency of pulmonary exacerbations (Boutin et al., 2015; Coburn et al., 2015).

Finally, in the lung, we observed a significant alteration of microbial functional profiles in response to PET-NPs exposure; treated mice exhibited a significant enrichment of pathways involved in the biosynthesis of amino acids, such as L-alanine biosynthesis, and the degradation of purine nucleobases. The enhanced activities in allantoin degradation to glyoxylate III, D-galactarate degradation I, D-glucarate and D-galactarate degradation, and L-arginine degradation II (AST pathway) suggest potential shifts in energy metabolism and nitrogen utilization.

The alterations observed in colon and lung microbiome in response to PET-NPs exposure in mice suggest potential cross-implications of the gut-lung axis. In the context of nanoparticle exposure, a hypothesis could involve the direct passage of nanoparticles from the gut to the lungs. It is known that nanoparticles can cross biological barriers, and their ingestion might lead to systemic distribution, affecting distant organs such as the lungs (Jani et al., 2011; Raftis and Miller, 2019). This mechanism could trigger immune responses and disrupt the local microbiome environment in both gut and lungs (Stock et al., 2019).

However, considering recent research by Xuan e al., which explores the gut-lung axis in the context of nano/microplastics, an intriguing connection emerges (Xuan et al., 2023). Xuan's study demonstrates that M/NPs exposure induces lung and intestinal mucosal damage, along with gut microbiota dysbiosis and altered lactate levels in the lungs. The gut microbiota's influence on lactate, a key metabolite, is implicated in exacerbating lung damage through the HIF1a/PTBP1 pathway. This research suggests that changes in the gut microbiota composition may impact lung health, offering a compelling perspective on how PET-NPs exposure could potentially affect both the gut and lung microbiome through intricate systemic interactions.

These findings provide a basis for exploring the multifaceted dynamics of the gut-lung axis in the context of nanoparticle exposure, raising questions about the specific mechanisms at play and their potential contribution to observed alterations in microbiome profiles. Further investigations into these complex interactions will contribute to a more comprehensive understanding of the impact of PET-NPs on both gut and lung health.

While our study sheds light on the impact of sub-chronic PET-NPs exposure on the microbiome of the lung and colon mucosa, emphasizing the critical interplay between gut and lung health, it essential to acknowledge certain limitations. One lies in the inability to measure circulating levels of metabolites (i.e. circulating SCFA, bile acids) or mediators (i.e. cytokines) that could potentially elucidate the gut-lung connection. While our investigation provides valuable insights into microbial composition alterations, the lack of data on specific metabolites or signalling molecules hinders a comprehensive understanding of the functional consequences of sub-chronic PET-NPs exposure. Another limitation is the use of exclusively male mouse models and the absence of investigation of female models.

So, future research endeavours should consider incorporating advanced analytical techniques to measure circulating metabolites, allowing for a more nuanced exploration of the mechanistic pathways underlying the observed changes in microbiome across different niches and their potential implications for respiratory health. Additionally, this study was conducted utilizing an in vivo mouse model and reflects sub-chronic exposure conditions, do not designed to assess effects that have a long period. Since information regarding the daily intake of PET-NPs are not known, PET-NP dose that used in the study were determined based on previous studies conducted with PS-NPs or MPs in the mouse model of the same age group and exposure periods.

5. Conclusions

In conclusion, our study provides novel insights into the complex interplay between nanoplastics and the microbiome, particularly in the context of the gut-lung axis. While sub-chronic exposure to PET-NPs did not induce significant changes in oral and fecal microbiome, the alterations observed in the colon and lung microbiome underscore the potential systemic impact of nanoplastic exposure. The increase in *Veillonella* and *Prevotella* genera in the colon, coupled with a decrease in *Lactobacillus*, suggests a disturbance in the mucosal microbial balance. Furthermore, the notable increase in the *Pseudomonas* genus in the lung microbiome raises concerns about respiratory health. These findings contribute to the evolving understanding of nanoplastic interactions with diverse microbiome niches and highlight the need for further research to unravel the functional implications and mechanistic pathways involved.

Ethical approval

Experimental protocol was approved by Maltepe University Laboratory Animal Experiments Ethics Committee (protocol number: 2022.09.03, date: 29.09.2022) and was made in accordance with Turkish Law 6343/2 and with the Helsinki Declaration of Animal Rights.

CRediT authorship contribution statement

Nur Kaluç: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Sara Bertorello:** Writing – original draft, Visualization, Formal analysis, Data curation. **Oğuz Kaan Tombul:** Formal analysis. **Simone Baldi:** Investigation. **Giulia Nannini:** Investigation. **Gianluca Bartolucci:** Investigation. **Elena Niccolai:** Writing – review & editing, Visualization, Methodology, Data curation, Conceptualization. **Amedeo Amedei:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was supported by COST Action Plastics monitoRIng detectiOn RemedIaTion recovery—PRIORITY, CA20101, supported by COST (European Cooperation in Science and Technology).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.impact.2024.100531.

Data availability

The 16S rRNA sequence data have been deposited in the NCBI Gene Expression Omnibus (GEO) repository (XXX).

References

Alpizar-Rodriguez, D., Lesker, T.R., Gronow, A., Gilbert, B., Raemy, E., Lamacchia, C., et al., 2019. Prevotella copri in individuals at risk for rheumatoid arthritis. Ann. Rheum. Dis. 78, 590–593.
Alzaben, M., Burve, R., Loeschner, K., Møller, P., Roursgaard, M., 2023. Nanoplastics from ground polyethylene terephthalate food containers: genotoxicity in human lung epithelial A549 cells. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 892, 503705.
Barboza, L.G.A., Cózar, A., Gimenez, B.C.G., Barros, T.L., Kershaw, P.J., Guilhermino, L., 2019. Chapter 17 - macroplastics pollution in the marine environment. In: Sheppard, C. (Ed.), World Seas: An Environmental Evaluation (Second Edition). Academic Press, pp. 305–328.
Basha, O.M., Hafez, R.A., Salem, S.M., Anis, R.H., Hanafy, A.S., 2023. Impact of gut microbiome alteration in ulcerative colitis patients on disease severity and outcome. Clin. Exp. Med. 23, 1763–1772.
Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., et al., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59.
Boutin, S., Graeber, S.Y., Weitnauer, M., Panitz, J., Stahl, M., Clausznitzer, D., et al., 2015. Comparison of microbiomes from different niches of upper and lower airways in children and adolescents with cystic fibrosis. PLoS One 10, e0116029.
Chen, X., Zhuang, J., Chen, Q., Xu, L., Yue, X., Qiao, D., 2022. Polyvinyl chloride microplastics induced gut barrier dysfunction, microbiota dysbiosis and metabolism disorder in adult mice. Ecotoxicol. Environ. Saf. 241, 113809.
Coburn, B., Wang, P.W., Diaz Caballero, J., Clark, S.T., Brahma, V., Donaldson, S., et al., 2015. Lung microbiota across age and disease stage in cystic fibrosis. Sci. Rep. 5, 10241.
Davison, E., Johnston, W., Piela, K., Rosier, B.T., Paterson, M., Mira, A., et al., 2021. The subgingival plaque microbiome, systemic antibodies against Bacteria and Citrullinated proteins following periodontal therapy. Pathogens 10.
Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci. Rep. 7, 46687.
Di Vincenzo, F., Del Gaudio, A., Petito, V., Lopetuso, L.R., Scaldaferri, F., 2024. Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. Intern. Emerg. Med. 19, 275–293.
Dickson, R.P., Huffnagle, G.B., 2015. The lung microbiome: new principles for respiratory bacteriology in health and disease. PLoS Pathog. 11, e1004923.
Domenech, J., de Britto, M., Velázquez, A., Pastor, S., Hernández, A., Marcos, R., et al., 2021. Long-Term Effects of Polystyrene Nanoplastics in Human Intestinal Caco-2 Cells. 11, 1442.
Fang, F., Xu, J., Li, Q., Xia, X., Du, G., 2018. Characterization of a Lactobacillus brevis strain with potential oral probiotic properties. BMC Microbiol. 18, 221.
Feng, Y., Tu, C., Li, R., Wu, D., Yang, J., Xia, Y., et al., 2023. A systematic review of the impacts of exposure to micro- and nano-plastics on human tissue accumulation and health. Eco-Environment & Health 2, 195–207.
Fernández, S., Fraga, M., Silveyra, E., Trombert, A.N., Rabaza, A., Pla, M., et al., 2018. Probiotic properties of native Lactobacillus spp. strains for dairy calves. Benefic. Microbes 9, 613–624.
Gaibani, P., Viciani, E., Bartoletti, M., Lewis, R.E., Tonetti, T., Lombardo, D., et al., 2021. The lower respiratory tract microbiome of critically ill patients with COVID-19. Sci. Rep. 11, 10103.
Gerb, S.A., Dashek, R.J., Ericsson, A.C., Griffin, R., Franklin, C.L., 2021. The effects of ketamine on the gut microbiome on CD1 mice. Comp. Med. 71, 295–301.
Gigault, J., Halle, A.T., Baudrimont, M., Pascal, P.Y., Gauffre, F., Phi, T.L., et al., 2018. Current opinion: what is a nanoplastic? Environ. Pollut. 235, 1030–1034.

Giri, S., Lamichhane, G., Khadka, D., Devkota, H.P., 2024. Microplastics contamination in food products: occurrence, analytical techniques and potential impacts on human health. Current Research in Biotechnology 7, 100190.
Gosmann, C., Anahtar, M.N., Handley, S.A., Farcasanu, M., Abu-Ali, G., Bowman, B.A., et al., 2017. Lactobacillus-deficient Cervicovaginal bacterial communities are associated with increased HIV Acquisition in Young South African Women. Immunity 46, 29–37.
Haldar, S., Jadhav, S.R., Gulati, V., Beale, D.J., Balkrishna, A., Varshney, A., et al., 2023. Unravelling the gut-lung axis: insights into microbiome interactions and traditional Indian Medicine's perspective on optimal health. FEMS Microbiol. Ecol. 99.
Harusato, A., Seo, W., Abo, H., Nakanishi, Y., Nishikawa, H., Itoh, Y., 2023. Impact of particulate microplastics generated from polyethylene terephthalate on gut pathology and immune microenvironments. iScience 26, 106474.
Hillman, E.T., Lu, H., Yao, T., Nakatsu, C.H., 2017. Microbial ecology along the gastrointestinal tract. Microbes Environ. 32, 300–313.
Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127–141.
Hou, K., Wu, Z.-X., Chen, X.-Y., Wang, J.-Q., Zhang, D., Xiao, C., et al., 2022. Microbiota in health and diseases. Signal Transduct. Target. Ther. 7, 135.
Iljazovic, A., Roy, U., Gálvez, E.J.C., Lesker, T.R., Zhao, B., Gronow, A., et al., 2021. Perturbation of the gut microbiome by Prevotella spp. enhances host susceptibility to mucosal inflammation. Mucosal Immunol. 14, 113–124.
Jani, P., Halbert, G.W., Langridge, J., Florence, A.T., 2011. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J. Pharm. Pharmacol. 42, 821–826.
Jeong, B., Baek, J.Y., Koo, J., Park, S., Ryu, Y.K., Kim, K.S., et al., 2022. Maternal exposure to polystyrene nanoplastics causes brain abnormalities in progeny. J. Hazard. Mater. 426, 127815.
Ji, Y., Wang, C., Wang, Y., Fu, L., Man, M., Chen, L., 2020. Realistic polyethylene terephthalate nanoplastics and the size- and surface coating-dependent toxicological impacts on zebrafish embryos. Environ. Sci. Nano 7, 2313–2324.
Jin, Y., Lu, L., Tu, W., Luo, T., Fu, Z., 2019. Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. Sci. Total Environ. 649, 308–317.
Kaluç, N., Çötelli, E.L., Tuncay, S., Thomas, P.B., 2024. Polyethylene terephthalate nanoplastics cause oxidative stress induced cell death in Saccharomyces cerevisiae. J. Environ. Sci. Health A 59, 180–188.
Li, B., Ding, Y., Cheng, X., Sheng, D., Xu, Z., Rong, Q., et al., 2020. Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. Chemosphere 244, 125492.
Lin, X., Xie, H., Zhang, Y., Tian, X., Cui, L., Shi, N., et al., 2023. The toxicity of nano polyethylene terephthalate to mice: intestinal obstruction, growth retardant, gut microbiota dysbiosis and lipid metabolism disorders. Food Chem. Toxicol. 172, 113585.
Liu, W., Zhang, B., Yao, Q., Feng, X., Shen, T., Guo, P., et al., 2023. Toxicological effects of micro/nano-plastics on mouse/rat models: a systematic review and meta-analysis. Front. Public Health 11, 1103289.
Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K., Knight, R., 2012. Diversity, stability and resilience of the human gut microbiota. Nature 489, 220–230.
Lu, L., Wan, Z., Luo, T., Fu, Z., Jin, Y., 2018. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Sci. Total Environ. 631-632, 449–458.
Lusk, R.W., 2014. Diverse and widespread contamination evident in the unmapped depths of high throughput sequencing data. PLoS One 9, e110808.
Niccolai, E., Baldi, S., Ricci, F., Russo, E., Nannini, G., Menicatti, M., et al., 2019. Evaluation and comparison of short chain fatty acids composition in gut diseases. World J. Gastroenterol. 25, 5543–5558.
Nie, J.H., Shen, Y., Roshdy, M., Cheng, X., Wang, G., Yang, X., 2021. Polystyrene nanoplastics exposure caused defective neural tube morphogenesis through caveolae-mediated endocytosis and faulty apoptosis. Nanotoxicology 15, 885–904.
Ning, D., Xin, Z., Xue Di, Z., Jun, J., Shan Shan, L., Yun Ping, M., et al., 2020. Impairment of spermatogenesis and sperm motility by the high-fat diet-induced dysbiosis of gut microbes. Gut 69, 1608.
Pagliai, G., Russo, E., Niccolai, E., Dinu, M., Di Pilato, V., Magrini, A., et al., 2020. Influence of a 3-month low-calorie Mediterranean diet compared to the vegetarian diet on human gut microbiota and SCFA: the CARDIVEG study. Eur. J. Nutr. 59, 2011–2024.
Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2020. Environmental exposure to microplastics: an overview on possible human health effects. Sci. Total Environ. 702, 134455.
Qiao, R., Sheng, C., Lu, Y., Zhang, Y., Ren, H., Lemos, B., 2019. Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. Sci. Total Environ. 662, 246–253.
Raftis, J.B., Miller, M.R., 2019. Nanoparticle translocation and multi-organ toxicity: a particularly small problem. Nano Today 26, 8–12.
Rojas-Tapias, D.F., Brown, E.M., Temple, E.R., Onyekaba, M.A., Mohamed, A.M.T., Duncan, K., et al., 2022. Inflammation-associated nitrate facilitates ectopic colonization of oral bacterium Veillonella parvula in the intestine. Nat. Microbiol. 7, 1673–1685.
Saxami, G., Kerezoudi, E.N., Eliopoulos, C., Arapoglou, D., Kyriacou, A., 2023. The Gut–Organ Axis within the Human Body: Gut Dysbiosis and the Role of Prebiotics, 13, p. 2023.
Schneider, M., Stracke, F., Hansen, S., Schaefer, U.F., 2009. Nanoparticles and their interactions with the dermal barrier. Dermato-Endocrinology 1, 197–206.
Schymanski, D., Goldbeck, C., Humpf, H.U., Fürst, P., 2018. Analysis of microplastics in water by micro-Raman spectroscopy: release of plastic particles from different packaging into mineral water. Water Res. 129, 154–162.
Segata, N., Haake, S.K., Mannon, P., Lemon, K.P., Waldron, L., Gevers, D., et al., 2012. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genome Biol. 13, R42.
Shan, Y., Lee, M., Chang, E.B., 2022. The Gut Microbiome and Inflammatory Bowel Diseases. 73, 455–468.
Stock, V., Böhmert, L., Lisicki, E., Block, R., Cara-Carmona, J., Pack, L.K., et al., 2019. Uptake and effects of orally ingested polystyrene microplastic particles in vitro and in vivo. Arch. Toxicol. 93, 1817–1833.
Sun, H., Chen, N., Yang, X., Xia, Y., Wu, D., 2021. Effects induced by polyethylene microplastics oral exposure on colon mucin release, inflammation, gut microflora composition and metabolism in mice. Ecotoxicol. Environ. Saf. 220, 112340.
Tamargo, A., Molinero, N., Reinosa, J.J., Alcolea-Rodriguez, V., Portela, R., Bañares, M. A., et al., 2022. PET microplastics affect human gut microbiota communities during simulated gastrointestinal digestion, first evidence of plausible polymer biodegradation during human digestion. Sci. Rep. 12, 528.
Wang, W., Gao, H., Jin, S., Li, R., Na, G., 2019. The ecotoxicological effects of microplastics on aquatic food web, from primary producer to human: a review. Ecotoxicol. Environ. Saf. 173, 110–117.
Wedenoja, S., Saarikivi, A., Mälkönen, J., Leskinen, S., Lehto, M., Adeshara, K., et al., 2022. Fecal microbiota in congenital chloride diarrhea and inflammatory bowel disease. PLoS One 17, e0269561.
Wen, S., Zhao, Y., Liu, S., Yuan, H., You, T., Xu, H., 2022. Microplastics-perturbed gut microbiota triggered the testicular disorder in male mice: via fecal microbiota transplantation. Environ. Pollut. 309, 119789.
Wright, S.L., Kelly, F.J., 2017. Plastic and human health: a Micro issue? Environ. Sci. Technol. 51, 6634–6647.
Xuan, L., Xu, Z., Luo, J., Wang, Y., Yan, Y., Qu, C., et al., 2023. Lactate exacerbates lung damage induced by nanomicroplastic through the gut microbiota-HIF1a/PTBP1 pathway. Exp. Mol. Med. 55, 2596–2607.
Yang, S., Cheng, Y., Chen, Z., Liu, T., Yin, L., Pu, Y., et al., 2021. In vitro evaluation of nanoplastics using human lung epithelial cells, microarray analysis and co-culture model. Ecotoxicol. Environ. Saf. 226, 112837.
Yin, K., Wang, Y., Zhao, H., Wang, D., Guo, M., Mu, M., et al., 2021. A comparative review of microplastics and nanoplastics: toxicity hazards on digestive, reproductive and nervous system. Sci. Total Environ. 774, 145758.
Zha, H., Xia, J., Li, S., Lv, J., Zhuge, A., Tang, R., et al., 2023. Airborne polystyrene microplastics and nanoplastics induce nasal and lung microbial dysbiosis in mice. Chemosphere 310, 136764.
Zhan, Z., Liu, W., Pan, L., Bao, Y., Yan, Z., Hong, L., 2022. Overabundance of Veillonella parvula promotes intestinal inflammation by activating macrophages via LPS-TLR4 pathway. Cell Death Dis. 8, 251.
Zhang, Q., Xu, E.G., Li, J., Chen, Q., Ma, L., Zeng, E.Y., et al., 2020. A review of microplastics in table salt, drinking water, and air: direct human exposure. Environ. Sci. Technol. 54, 3740–3751.
Zhang, Q., He, Y., Cheng, R., Li, Q., Qian, Z., Lin, X., 2022a. Recent advances in toxicological research and potential health impact of microplastics and nanoplastics in vivo. Environ. Sci. Pollut. Res. 29, 40415–40448.
Zhang, Y., Wang, S., Olga, V., Xue, Y., Lv, S., Diao, X., et al., 2022b. The potential effects of microplastic pollution on human digestive tract cells. Chemosphere 291, 132714.
Zhang, Y., Hou, B., Liu, T., Wu, Y., Wang, Z., 2023a. Probiotics improve polystyrene microplastics-induced male reproductive toxicity in mice by alleviating inflammatory response. Ecotoxicol. Environ. Saf. 263, 115248.
Zhang, Z., Xu, M., Wang, L., Gu, W., Li, X., Han, Z., et al., 2023b. Continuous oral exposure to micro- and nanoplastics induced gut microbiota dysbiosis, intestinal barrier and immune dysfunction in adult mice. Environ. Int. 182, 108353.
Zhao, X., Sun, J., Zhou, L., Teng, M., Zhao, L., Li, Y., et al., 2023. Defining the size ranges of polystyrene nanoplastics according to their ability to cross biological barriers. Environ. Sci. Nano 10, 2634–2645.