







Effect of aerobic exercise and particulate matter exposure duration on the diversity of gut microbiota

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ABSTRACT

Inhalation of ambient particulate matter (PM) can disrupt the gut microbiome, while exercise independently influences the gut microbiome by promoting beneficial bacteria. In this study, we analyzed changes in gut microbial diversity and composition in response to combined interventions of PM exposure and aerobic exercise, extending up to 12 weeks. This investigation was conducted using mice, categorized into five groups: control group (Con), exercise group (EXE), exercise group followed by 3-day exposure to PM (EXE + 3-day PM), particulate matter exposure (PM), and PM exposure with concurrent treadmill exercise (PME). Notably, the PM group exhibited markedly lower alpha diversity and richness compared to the Con group and our analysis of beta diversity revealed significant variations among the intervention groups. Members of the *Lachnospiraceae* family showed significant enhancement in the exercise intervention groups (EXE and PME) compared to the Con and PM groups. The biomarker *Lactobacillus*, *Coriobacteraceae*, and *Anaerofustis* were enriched in the EXE group, while *Desulfovibrionaceae*, *Mucispirillum schaedleri*, *Lactococcus* and *Anaeroplasm* were highly enriched in the PM group. Differential abundance analysis revealed that *Paraprevotella*, *Bacteroides*, and *Blautia* were less abundant in the 12-week PM exposure group than in the 3-day PM exposure group. Moreover, both the 3-day and 12-week PM exposure groups exhibited a reduced relative abundance of *Bacteroides uniformis*, *SMB53*, and *Staphylococcus* compared to non-PM exposure groups. These findings will help delineate the possible roles and associations of altered microbiota resulting from the studied interventions, paving the way for future mechanistic research.

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1. Introduction

Particulate matter (PM) is a complex mixture of tiny solid particles and liquid droplets that enter the atmosphere through various means, including vehicle emissions, industrial and agricultural activities, construction, and natural sources such as dust and pollen. PM consists of a blend of organic chemicals, metals, acids, and other particles, making it one of the most significant contributors to air pollution (Anderson et al. 2012). This poses serious concerns for both environmental and public health (Arias-Pérez et al. 2020). As per the 2019 World Health Organization (WHO) report, a staggering 99% of the global population lived in areas where air quality

fell short of WHO standards (Organization 2023). PM particles come in various sizes, classified by their aerodynamic diameter as PM 10 (coarse particles, < 10µm), PM 2.5 (fine particles, < 2.5µm), and PM 0.1 (ultrafine particles, < 0.1µm). Coarse and fine particles often originate from different sources and exhibit various chemical compositions. Exposure to elevated levels of PM can result in damage to the lungs and various other organs in the body (Schraufnagel et al. 2019). Notably, PM 2.5, a major air pollutant, caused approximately 4 million deaths globally in 2019 (Collaborators and Årnlöv 2020), and was associated with an increased risk of neurodegeneration. Among PM 2.5 components, black carbon and

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sulfate displayed the strongest associations with dementia and Alzheimer's disease (Shi et al. 2023). PM 10, which is generally found in higher proportions than PM 2.5, primarily stems from forest fires, waste combustion, and industrial and agricultural waste (Tian et al. 2021). While PM's adverse effects on the respiratory (Télez-Rojo et al. 2000; Leikauf et al. 2020; Misiukiewicz-Stepien and Paplinska-Goryca 2021; Albano et al. 2022; Lee et al. 2023) and cardiovascular systems (Hamanaka and Mutlu 2018; Tschofen et al. 2019; Basith et al. 2022; Ji 2022) have been extensively studied, recent evidence has revealed its detrimental impacts on the gastrointestinal (GI) tract (Vignal et al. 2017; Pambianchi et al. 2022), including the modulation of the gut microbiome. In addition to direct deposition in the respiratory system, PM can be indirectly deposited in the oropharynx through mucociliary clearance and via the swallowing of mucus and saliva (Mutlu et al. 2018; Xie et al. 2022).

The gut microbiome encompasses the total genetic material of all microbes in the gastrointestinal tract. Its genetic diversity and biochemical capacity can surpass the functional capability of the human genome (Qin et al. 2010). The gut microbiota forms a complex ecosystem of microorganisms, and its stability and resilience are crucial ecological features for human health (Fassarella et al. 2021). The gut ecosystem influences various essential physiological functions, including digestion, nutrient absorption, immune system development, and synthesis of certain vitamins and neurotransmitters, among other critical functions (Hillman et al. 2017; Lee et al. 2023; Yun and Hyun 2023). Nonetheless, the diversity and composition of the gut microbiome can be influenced by diet, genetics, age, and environmental exposures (Hasan and Yang 2019; Su and Liu 2021), which also includes PM exposure and its association with the GI diseases. In relation to diet, the physical characteristics of the dietary components such as substrate form, dietary fiber, and particle size are important determinants in the colonization of gut bacteria. The attachment of gut bacteria to substrates is preliminary for their utilization. Bacteria have variable attachment abilities to the same dietary fiber or particles (Zhang 2022). In the gut, PM can serve as a substrate for microbial growth, thereby altering the composition of gut microbiota. Moreover, the inflammatory response triggered by PM in the lungs can have downstream effects on gut microbiota, promoting the growth of specific microorganisms. The indirect consequences of PM exposure, including systemic inflammation and oxidative stress, can impact gut permeability, mucus production, and immune function. These effects can, in turn influence the gut microbial diversity (Salim et al. 2014; Gupta et al. 2022).

Physical activity and exercise have the potential to enrich the gut microbiota and enhance the Bacteroidetes/Firmicutes ratio. This in turn, may contribute to weight reduction, a lowered risk of obesity-related health issues, and fewer GI complications. Furthermore, exercise can stimulate the proliferation of beneficial bacteria by influencing mucosal immunity and improving barrier functions. This can reduce the incidence of metabolic diseases and promote the growth of short-chain fatty acid (SCFA)-producing bacteria, thereby safeguarding against GI disorders and certain cancers (Monda et al. 2017). Exercise can independently impact the composition and functional capacity of gut microbiota (Mailing et al. 2019). The connection between the gut microbiome and exercise carries substantial implications for athletes, and potential mechanisms are discussed here (Wegierska et al. 2022). Recent research conducted on overweight women unveiled the potential of aerobic exercise to induce metabolic shifts that provide substrates for beneficial gut microbiota, such as *Akkermansia* (Hintikka et al. 2023). Additionally, a recent systematic review concluded that moderate-to high-intensity exercise (30–90 min) performed at least three times per week for more than 8 weeks can effectively alter the gut microbiota in both clinical and healthy individuals (Boyatar et al. 2023).

The combined effect of PM and exercise has been studied. A study examining all-cause mortality rates in older adults found that moderate physical activity was linked with delayed mortality; however, vigorous physical activity was associated with accelerated mortality in older adults when exposed to high levels of PM 10 (Park et al. 2023). Moreover, the combined effect of reduced physical activity and PM 2.5 exposure can accelerate the onset and progression of type 2 diabetes (T2D) and mood disorders (Luo et al. 2023). A recent Chinese cohort study revealed that prolonged higher ambient exposure to PM 2.5 can significantly attenuate the effects of increased physical activity-induced cardiovascular benefits. Additionally, farmers exposed to 54 $\mu\text{g}/\text{m}^3$ or higher concentrations of PM 2.5 along with increased active farming and commuting were at a higher risk of developing cerebrovascular disease (Sun et al. 2023).

Several studies have elaborated on the role of physical exercise and exposure to PM in combination with the gut microbiota or on the combination of the effects of PM exposure and physical activity in the context of the risk of developing diseases. However, the combined effects of aerobic exercise and exposure to PM on the alteration of gut microbiota and the protective effects of exercise in relation to PM exposure and gut microbiota outcomes are limited. We aimed to determine the changes in the composition and diversity

of the gut microbiota over a period of 12 weeks with treadmill exercise and PM exposure (simultaneous, non-simultaneous) interventions in a mouse model.

2. Materials and methods

2.1. Animal care

Four-week-old female C57BL/6J mice were randomly separated into five groups: Con, control group ($n=5$); PM, particulate matter exposure ($n=5$); PME, PM exposure with concurrent treadmill exercise ($n=5$); EXE, the treadmill exercise group ($n=5$); EXE + 3-day PM, 5 exercise group followed by 3-day exposure to PM ($n=5$). The mice were housed in sterile cages at a controlled temperature (22 ± 2 °C), relative humidity ($50 \pm 10\%$), and standard light–dark cycle (12:12), with free access to chow food (fat 4%; rodent NIH-31 open formula auto diet; Zeigler Feed) and water. Mice were supplied by Koatech (South Korea). Authorization for performing animal experiments was granted by the Inha University Ethics Committee (approval number: INHA 220203-811).

2.2. Treadmill exercise and PM exposure

The treadmill exercise protocol spanned 12 weeks, and PM exposure was performed for either 3 or 12 weeks. – A custom-made PM treadmill chamber (Korea patent registration No. 10-2529955 and 10-2529956) consisting of air circulation, PM supply, mouse treadmill, and a central control unit (So et al. 2021) was utilized for PM exposure and treadmill exercise (Figure 1). The PM used in this study was ISO 12103-1 (Powder Technology Inc., U.S.), and the concentration was maintained above

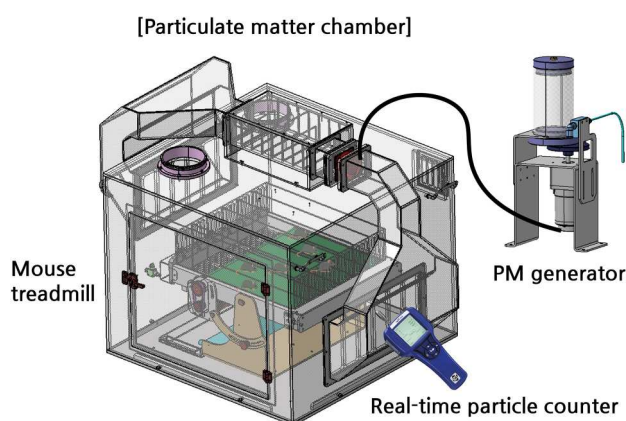


Figure 1. A schematic of the PM Chamber. The PM generator supplies fine dust to the chamber, and the concentration of fine dust in the chamber is being monitored by a real-time particle counter. The PM chamber is equipped with a mouse treadmill with adjustable exercise intensity and duration.

$100 \mu\text{g}/\text{m}^3$ (So et al. 2021). The PM concentration in the chamber was maintained by continuous monitoring and measurement using an AeroTrak 9306-V2 particle counter (TSI Inc., MN, U.S.). The EXE, EXE + 3-day PM, and PME groups underwent 1 h of treadmill running per day at an intensity of 20 m/min with a 5-degree uphill incline, following 1 week of acclimatization to treadmill exercise.

2.3. Fecal collection and DNA extraction

Feces were collected after 12 weeks of intervention. The animals were restrained in sterile containers to collect fecal pellets, which were snap-frozen and stored at -80 °C, until further processing. DNA was extracted from ~ 100 mg of thawed fecal material and homogenized in a Fast-Prep 24 (MP Biomedicals, Irvine, CA, USA) using the SPINeasy DNA Kit for Feces (MP Biomedicals). The quality of the extracted DNA was analyzed by electrophoresis using a 1% agarose gel and visualizing it using a ChemiDoc imaging system (Bio-Rad, Hercules, CA, USA). Further, quality metrics were analyzed using a SpectraMax iD3 spectrophotometer (Molecular Devices, San Jose, CA, USA), and DNA was quantified using Qubit 4 (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. 16S rDNA sequencing and library preparation

The bacterial 16S rRNA gene was amplified by a two-step PCR amplification protocol. Briefly, in the first PCR run, the V4 region was amplified using 515F and 806R primers (F-primer: 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG CCA GCM GCC GCG GTA A-3' and R-primer: 5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3') and 2X KAPA Hifi HotStart ReadyMix (KAPA Biosystems, UK). The second PCR resulted in the attachment of dual indices and sequencing adapters, and the Nextera XT Index Kit v2 (Illumina, San Diego, CA, USA) was used to generate libraries. AMPure XP beads (Beckman Coulter, USA) were used for PCR product clean-up. PCR thermal cycling conditions were as described in our previous study (Yun et al. 2022). The purified libraries were pooled in equimolar concentrations, followed by paired-end sequencing on an iSeq 100 Illumina platform (150 cycles) at a targeted depth of 1.2 Gb.

2.5. Bioinformatics analysis

Raw reads were imported into QIIME 2 v2022.02.01 (Bolyen et al. 2019) using the paired-end format and

inspected for quality control by generating quality plots in FastQC. Primers were trimmed using Cutadapt v4.0 (Martin 2011). The joining of paired-end reads yielded insufficient merged reads; therefore, further analysis was carried out with forward reads. Denoising of reads was performed using DADA2 to extract the abundances and representative sequences. The evolutionary relationship was depicted by a SATé-enabled phylogenetic placement (SEPP) (Janssen et al. 2018) -based phylogenetic tree via the fragment-insertion method against the Greengenes 13.8 reference database (McDonald et al. 2012). The Naive Bayes classifier (Pedregosa et al. 2011) was trained using the Greengenes database to extract V4 region sequences to assess the bacterial taxonomy of representative reads. The samples were rarified to a minimum library size (27450). The QIIME 2 output files were exported and modified to match the input criteria for use with MicrobiomeAnalyst (Chong et al. 2020) for data visualization and further analysis. Alpha diversity was estimated using the observed features and ACE, Shannon, and Fisher metrics at the feature level using filtered reads. Comparisons of intervention groups were performed using the Kruskal–Wallis post-hoc test with false discovery rate (FDR)-corrected p -values. Beta diversity was analyzed at the feature level using the Bray–Curtis metric and confirmed via analysis of similarities (ANOSIM). The composition of gut microbial samples was analyzed at the phylum, family, genus, and species levels to highlight the biomarker taxa, and statistical significance was calculated using two-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. Differentially abundant taxa were examined using the linear discriminant analysis (LDA) effect size (LEfSe) algorithm. A multiple linear regression model was employed for the association of PM exposure duration with microbial taxa after adjusting for covariates. The significance threshold was set at $p < 0.05$.

3. Results

The bacterial structure and composition of the mouse gut microbiota were characterized via 16S rRNA gene sequencing analysis of samples exposed to PM and exercise interventions.

3.1. Bacterial diversity and community structure

Alpha diversity was measured using metrics of richness by considering the unique features (observed) and low-abundance features (ACE), whereas the diversity within samples was estimated using the Shannon and Fisher metrics, which account for both richness and

evenness. Figure 2 shows that the richness of samples in the EXE + 3-day PM, PM, and PME intervention groups was significantly decreased compared to that in the Con group ($p = 0.02$) when estimated using the observed feature and ACE metrics. However, alpha diversity was higher in the PME group than in the Con and EXE groups ($p = 0.03$), as estimated using the Shannon metric. The diversity derived from the Fisher metric was similar to the richness estimates, where the Con group showed greater diversity than the EXE + 3-day PM, PM, and PME groups ($p = 0.02$) (Figure 2).

Microbial communities were compared using the Bray–Curtis dissimilarity distance method. The feature-level taxonomic variations among the intervention groups showed significant separation along the axes, where axis 1 accounted for 22.6% of the total variance, as revealed via ANOSIM (Figure 3). Each group clustered significantly differently than the other groups, explaining the dissimilarity between them.

3.2. Gut bacterial abundance profiling

Nine bacterial phyla, including two dominant inhabitant phyla (Bacteroidetes and Firmicutes), were identified in the mouse gut (Figure 4). The relative proportion of Bacteroidetes significantly decreased by 1.3-fold in all the intervention groups, including EXE, PM, and PME groups ($p < 0.0001$), and by 1.1-fold in the EXE + 3 d PM group ($p = 0.03$), compared to that in the Con group. Alternatively, the abundance of Bacteroidetes was relatively higher in the EXE + 3 d PM group than in the EXE ($p = 0.008$), PM ($p = 0.004$), and PME ($p = 0.0009$) groups (Figure 4). The compositional variation in Bacteroidetes was compensated by the change in the relative abundance of Firmicutes. The relative proportion of Firmicutes was higher in all intervention groups (EXE, PM, and PME; $p < 0.0001$, EXE + 3-day PM; $p = 0.008$) than in the Con group. However, the EXE + 3-day PM group had a lower percentage of Firmicutes than the EXE ($p = 0.0004$), PM ($p = 0.006$), and PME ($p = 0.0001$) groups (Figure 4). No differences were observed in the phylum-level taxonomy among the remaining phyla.

Taxonomic abundance at the family level was also analyzed. *Lachnospiraceae* family (Firmicutes) abundance was significantly higher in the EXE group than in the Con group ($p = 0.003$; Figure 4). Moreover, the members of this family also expanded in the PME group compared to that in the PM ($p = 0.04$) and Con ($p < 0.0001$) groups. However, EXE + 3-day PM had significantly lower proportions of *Lachnospiraceae* than did EXE ($p < 0.0001$), PM ($p = 0.01$), and PME ($p < 0.0001$). No differences were observed between the

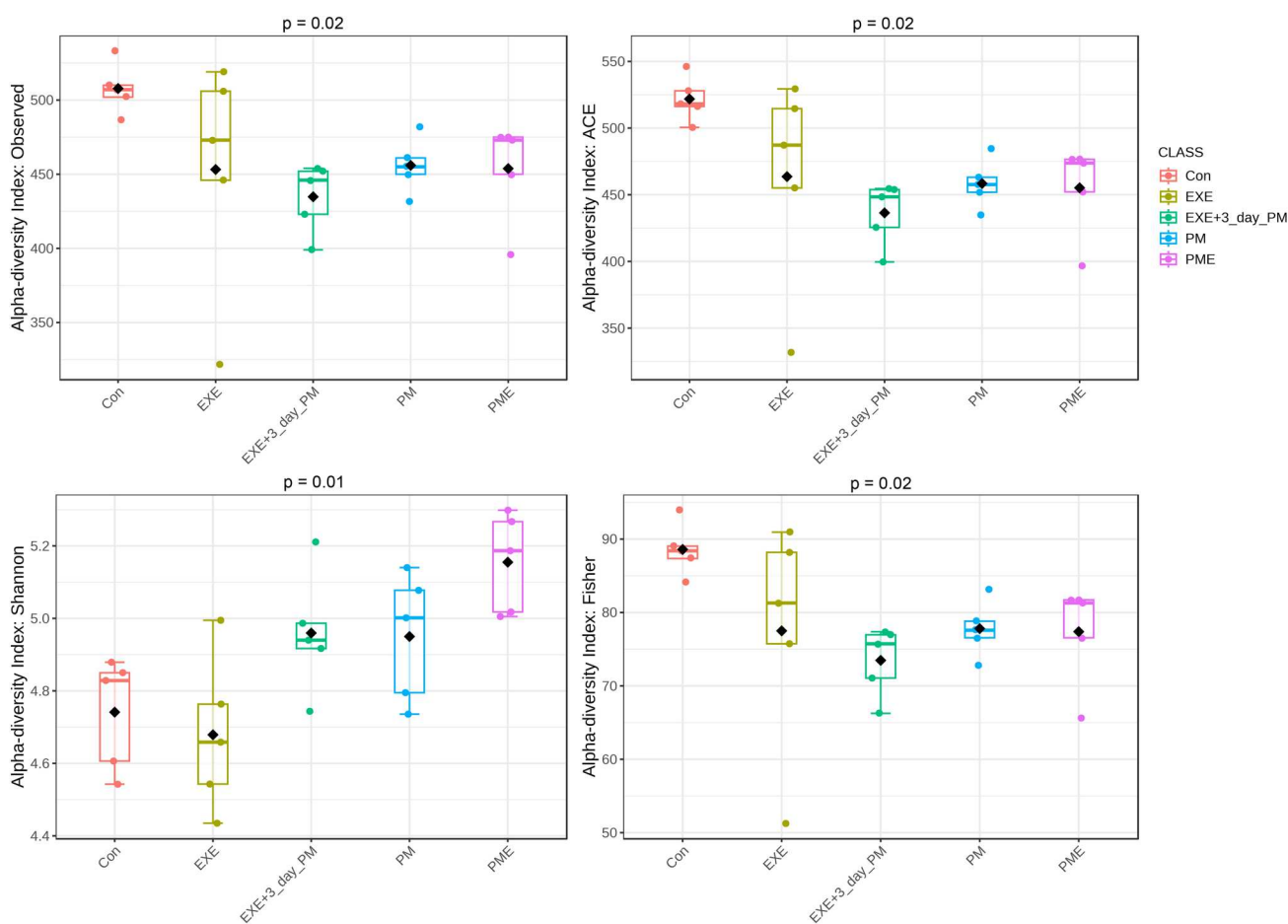


Figure 2. Alpha-diversity boxplots illustrating the feature richness (observed features and ACE metrics) and diversity (Shannon and Fisher metrics) using filtered data. Post-hoc pairwise comparisons were made using the Kruskal–Wallis test. p -values were adjusted based on the Benjamini–Hochberg procedure or false discovery rate (FDR) correction.

Con, PM, and EXE + 3-day PM groups. *Lactobacillaceae* family (Firmicutes) abundance was not significantly different among the Con, EXE, and PM intervention groups. However, the relative abundance of *Lactobacillaceae* was higher in EXE + 3-day PM than in Con ($p = 0.004$). Similarly, compared to that in the Con group, the PME group showed an increased abundance of this family ($p = 0.04$; Figure 4).

Paraprevotellaceae family (Bacteroidetes) members were the most abundant in the Con group compared to that in the remaining groups—EXE ($p = 0.01$), EXE + 3-day PM ($p = 0.001$), PM, and PME ($p < 0.0001$). Its proportion was significantly lower in the PME group than in the EXE ($p < 0.0001$) and EXE + 3-day PM ($p = 0.0004$) groups. *Prevotellaceae* family (Bacteroidetes) members showed a similar trend to *Paraprevotellaceae*, where EXE ($p = 0.01$), EXE + 3-day PM ($p = 0.009$), and PM and PME ($p = 0.001$) showed shrinkage of the taxa compared to that in Con. Furthermore, members of the *Rikenellaceae* family (Bacteroidetes) were less

abundant in PME than in Con ($p = 0.002$) and EXE ($p = 0.04$). The predominant family of mouse gut microbiota, *S24_7*, now called *Muribaculaceae*, belongs to the phylum Bacteroidetes. Its abundance was decreased in all experimental groups—EXE ($p = 0.001$), EXE + 3-day PM, PM, and PME ($p < 0.0001$)—compared to that in the Con group. Moreover, the relative proportion of *S24_7* was lower in the PM group than in the EXE group ($p = 0.001$; Figure 4).

3.2.1. Genus or species level composition

Approximately 40% of bacterial taxa remained unclassified at the genus or species level. *Akkermansia muciniphila* abundance was not significantly different between the groups, except for Con and EXE + 3-day PM ($p = 0.01$), and Con and PME ($p < 0.0001$), although the proportion of *A. muciniphila* was higher in the Con group (Figure 5). *Lactobacillus salivarius* was more abundant in the EXE ($p = 0.0004$) and PM ($p = 0.01$) groups than in the Con group. However, its proportion was lower in

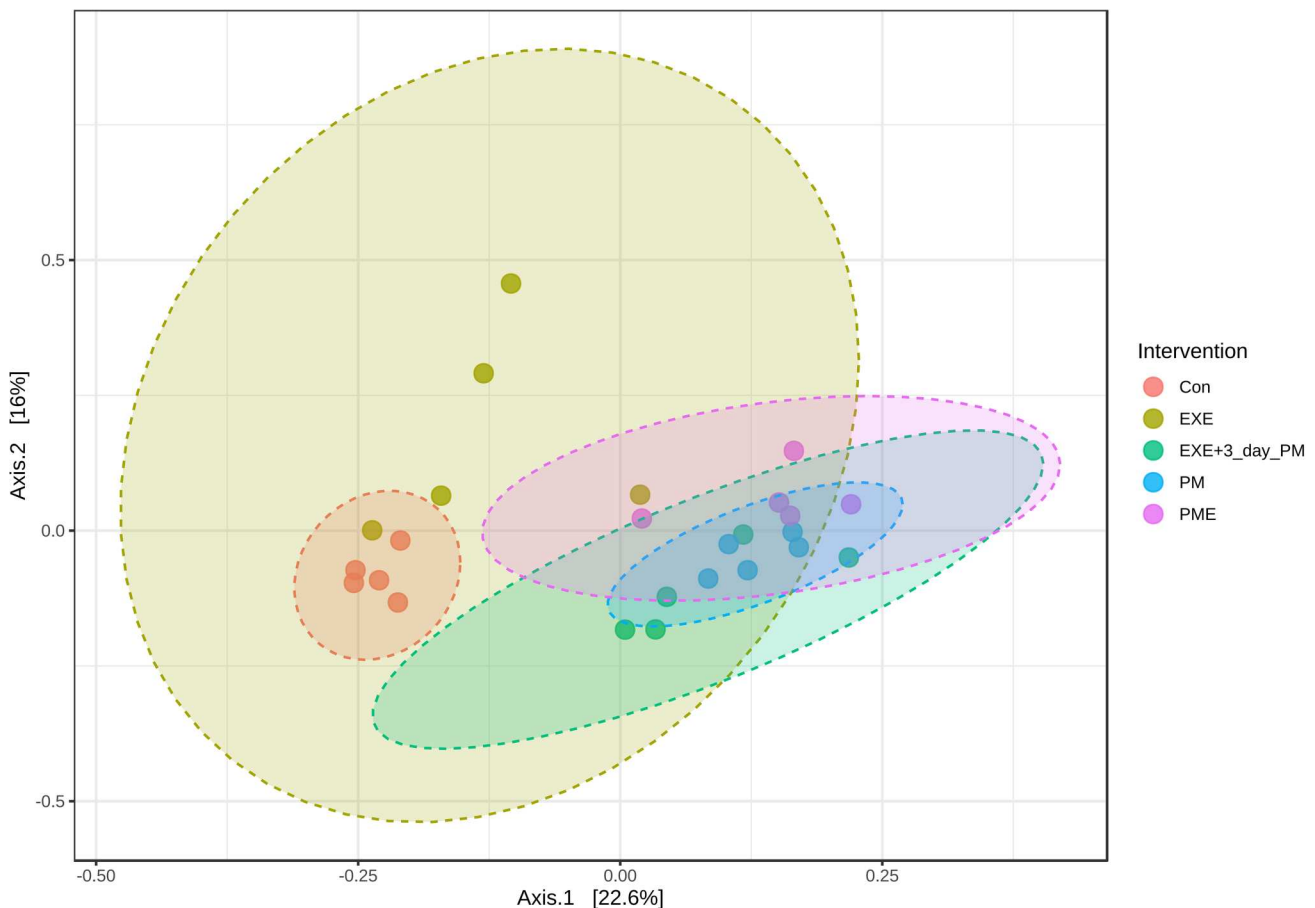


Figure 3. Principal coordinate analysis (PCoA) of the intervention groups using the Bray–Curtis distance metric based on the feature level. Individual samples are represented by the data points. The values along the axes show the percentage of total variance explained by each axis. Statistical significance was determined using analysis of similarities (ANOSIM) ($R: 0.71696$; $p < 0.001$).

the EXE + 3-day PM group than in the EXE ($p = 0.0002$) and PM ($p = 0.008$) groups. Another *Lactobacillus* sp. was significantly expanded in the EXE group compared to that in all other intervention groups ($p < 0.0001$). By contrast, the EXE + 3-day PM group had the lowest abundance among all groups: Con ($p = 0.03$), PM ($p = 0.02$), and PME ($p < 0.0001$). *Bacteroides* abundance was higher in the PM ($p = 0.009$) and EXE + 3-day PM ($p < 0.0001$) groups than in the EXE group. Moreover, EXE + 3-day PM had a higher relative proportion of *Bacteroides* than did the Con ($p = 0.005$) and PME ($p = 0.001$) groups. *Oscillospira* proportions were similar in the PM, PME, and EXE + 3-day PM groups. In addition, its proportion was similar in the control and EXE groups. However, the PM, PME, and EXE + 3-day PM intervention groups had a higher proportion of *Oscillospira* than did the Con ($p < 0.007$) and EXE ($p < 0.0003$) groups (Figure 5). *Paraprevotella* abundance was higher in the Con group than in all the other groups ($p < 0.0001$). However, its relative abundance was lowest in the PME group ($p < 0.0001$). Notably, the EXE + 3-day PM group had a higher percentage of *Paraprevotella* than did the

PM group ($p = 0.02$), similar to the EXE group. *Prevotella* was found to have a greater relative abundance in the Con group than in the other intervention groups ($P < 0.0001$; Figure 5).

3.2.2. Biomarker bacterial taxa

The statistically differential bacterial taxa abundance was analyzed by performing LEfSe, demonstrating 26 bacterial taxa that significantly surpassed the LDA score of > 2 (Figure 6). *Paraprevotella*, *A. muciniphila*, *Corynebacterium*, *RF32*, *Parabacteroides*, *Butyricimonas*, *Turibacter*, *Facklamia*, *Aerococcaceae*, *SMB53*, *Bacillaceae*, and *Sporosarcina* were enriched in the control group. *Lactobacillus*, *Coriobacteraceae*, and *Anaerofustis* were enriched in the EXE group. The EXE + 3-day PM group showed enrichment for *Bacteroides*, *Bacteroidales*, *Clostridiaceae*, and *Dorea*. *Desulfovibrionaceae*, *Mucispirillum schaedleri*, *Lactococcus*, and *Anaeroplasma* were highly enriched in the PM group, whereas *Rikenellaceae*, *Ruminococcus flavefaciens*, and *AF12* were enriched in the PME group (Figure 6).

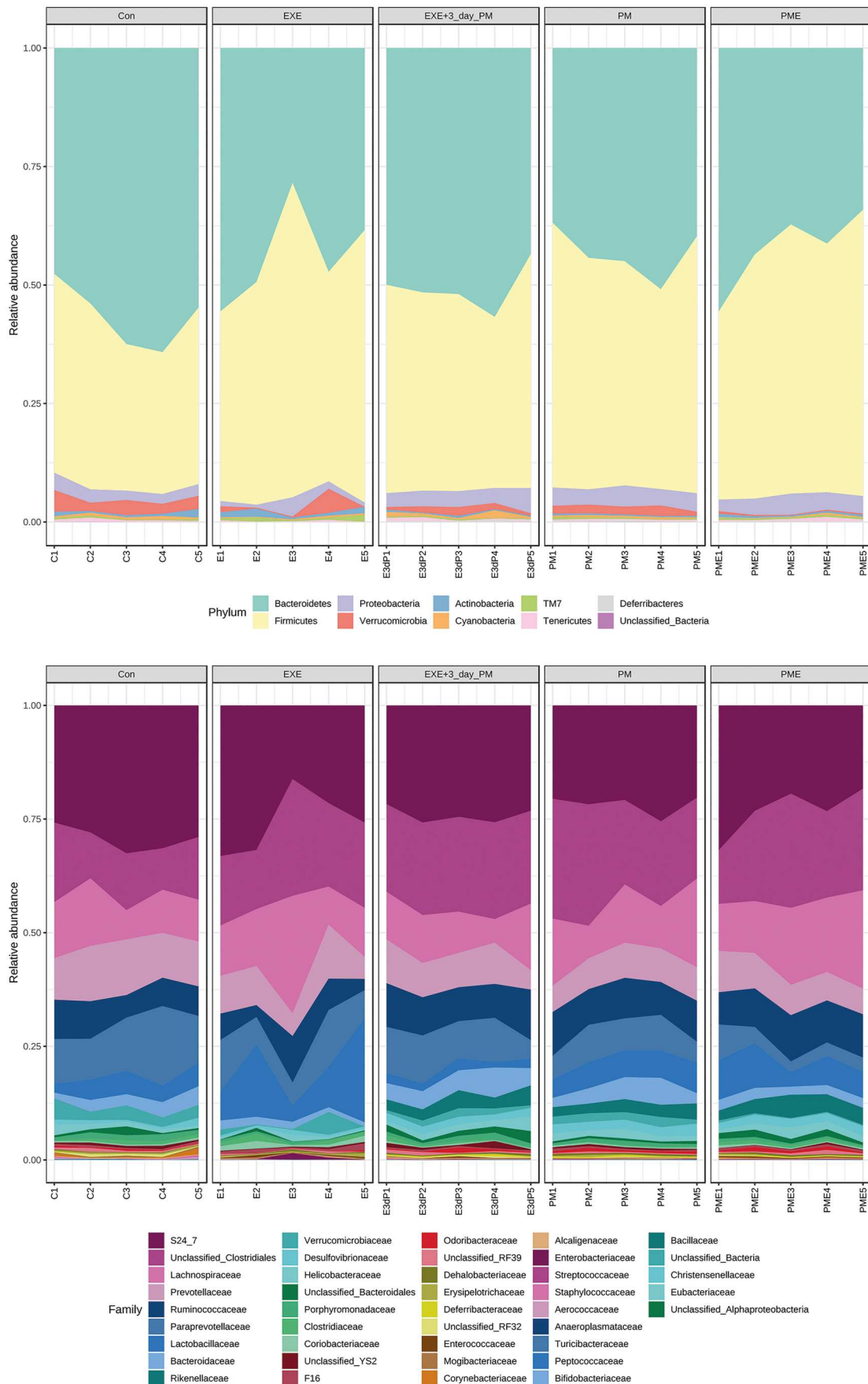


Figure 4. Relative taxonomic abundance profiling of mouse gut microbiota across the experimental groups. The graph illustrates phylum level taxonomic profiling (top) and family level relative abundance (bottom). Statistical significances were calculated using two-way ANOVA and Tukey's multiple comparison tests.

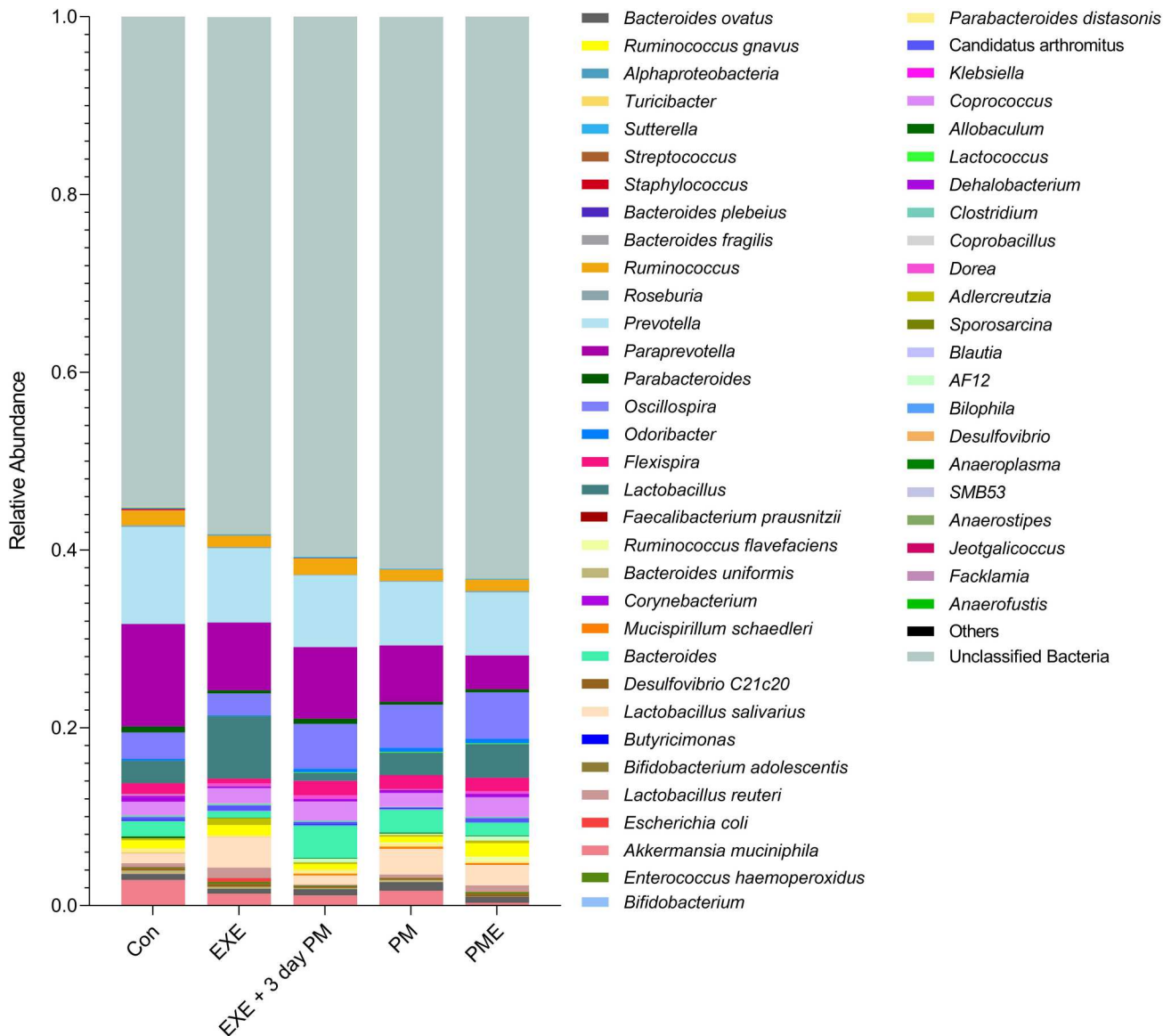


Figure 5. Percent relative abundance of mice gut microbiota at the genus or species level. Statistical significance was calculated using two-way ANOVA and Tukey's multiple comparison tests ($p < 0.05$).

The bacterial taxa significantly associated with PM exposure duration are presented in Figure 6. A multiple linear regression model was used to determine the association between gut bacteria and PM exposure duration (12 weeks, 3 days, non-PM). The confounding variable exercise was controlled to identify significant bacterial taxa. *Paraprevotella* was significantly reduced in the 12-week PM group, whereas *Bacteroides* abundance was significantly increased in the 3-day PM ($p = 0.02$) and 12-week PM groups ($p = 0.01$) compared with that in the non-PM group. Moreover, the 12-week PM group had a lower abundance of *Paraprevotella* ($p = 0.04$) and *Bacteroides* ($p = 0.01$) than that in the 3-day PM group (Figure 7). *Bacteroides uniformis* ($p = 0.03$), *SMB53* ($p = 0.02$), and *Staphylococcus* ($p = 0.04$)

decreased in the PM exposure groups (3-day and 12-week) compared with that in the non-PM exposure groups. *Lactobacillus reuteri* ($p = 0.02$), *L. salivarius* ($p = 0.03$), *Lactobacillus* sp. ($p = 0.004$), and *Ruminococcus gnavus* ($p = 0.03$) were present in lower proportions in the 3-day PM exposure group than that in the non-PM exposure group (Figure 7). *Flexispira* ($p = 0.04$) and *Lactococcus* were significantly more abundant in the PM exposure groups (3-day and 12-week PM) than that in the control non-PM exposure group. *Blautia* was higher in the 3-day PM exposure group than that in the 12-week PM exposure group ($p = 0.03$), and the 12-week PM exposure group had a lower abundance than that in the non-PM group ($p = 0.03$). *Butyricimonas* abundance was associated with PM exposure, whereas the

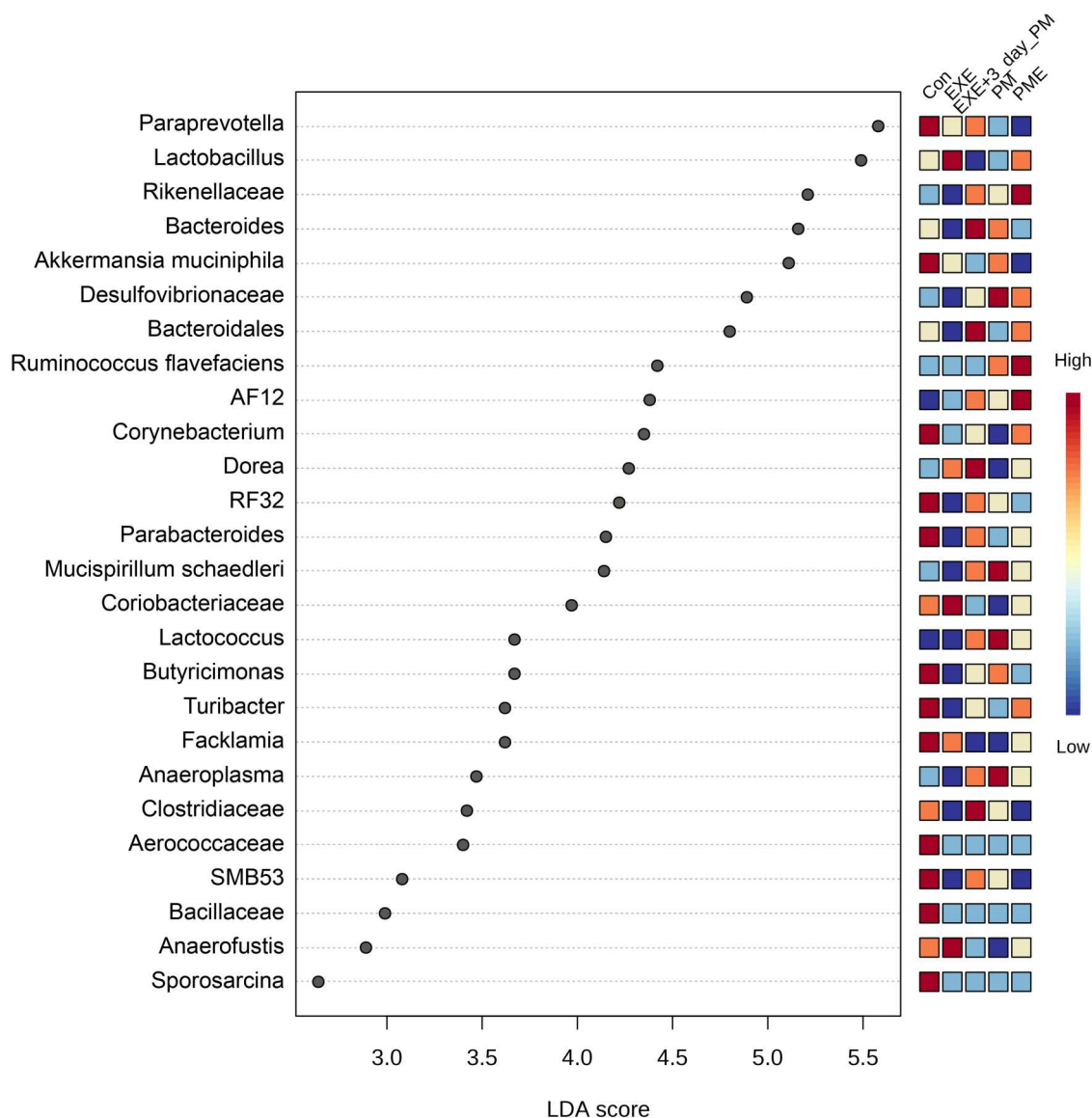


Figure 6. Differentially abundant taxa across intervention groups estimated using the LefSe algorithm. The bacterial taxa illustrated an LDA > 2 and a significance threshold of 0.05.

12-week PM exposure showed reduced proportions compared with that in the 3-day PM exposure ($p = 0.03$). *Parabacteroides distasonis* was significantly reduced after 12-week PM exposure compared with that in the 3-day PM exposure group ($p = 0.04$) (Figure 7).

4. Discussion

This study aimed to identify alterations in the gut microbiota composition and diversity during a 12-week period of treadmill exercise and PM exposure. Physical activity and cardiorespiratory fitness are positively linked to increased alpha diversity in fecal bacteria (Ortiz-Alvarez et al. 2020). Moderate endurance exercise can contribute

to human health by reducing inflammation and improving body composition as well as gut microbial diversity. Elite athletes possess high gut microbial diversity and are enriched in amino acid biosynthesis and carbohydrate and fiber metabolism-related bacterial species, consequently producing key SCFAs (Clauss et al. 2021; Imdad et al. 2022). Exercise intervention did not influence the alpha and beta diversities of the human gut microbiota but had an impact on SCFA producers (Motiani et al. 2020; Bycura et al. 2021; Moitinho-Silva et al. 2021). Our findings did not reveal significant differences in alpha diversity between the control and exercise intervention groups. However, the beta-diversity (Bray–Curtis) of the gut microbiota significantly differed between the Con and EXE groups, as evidenced

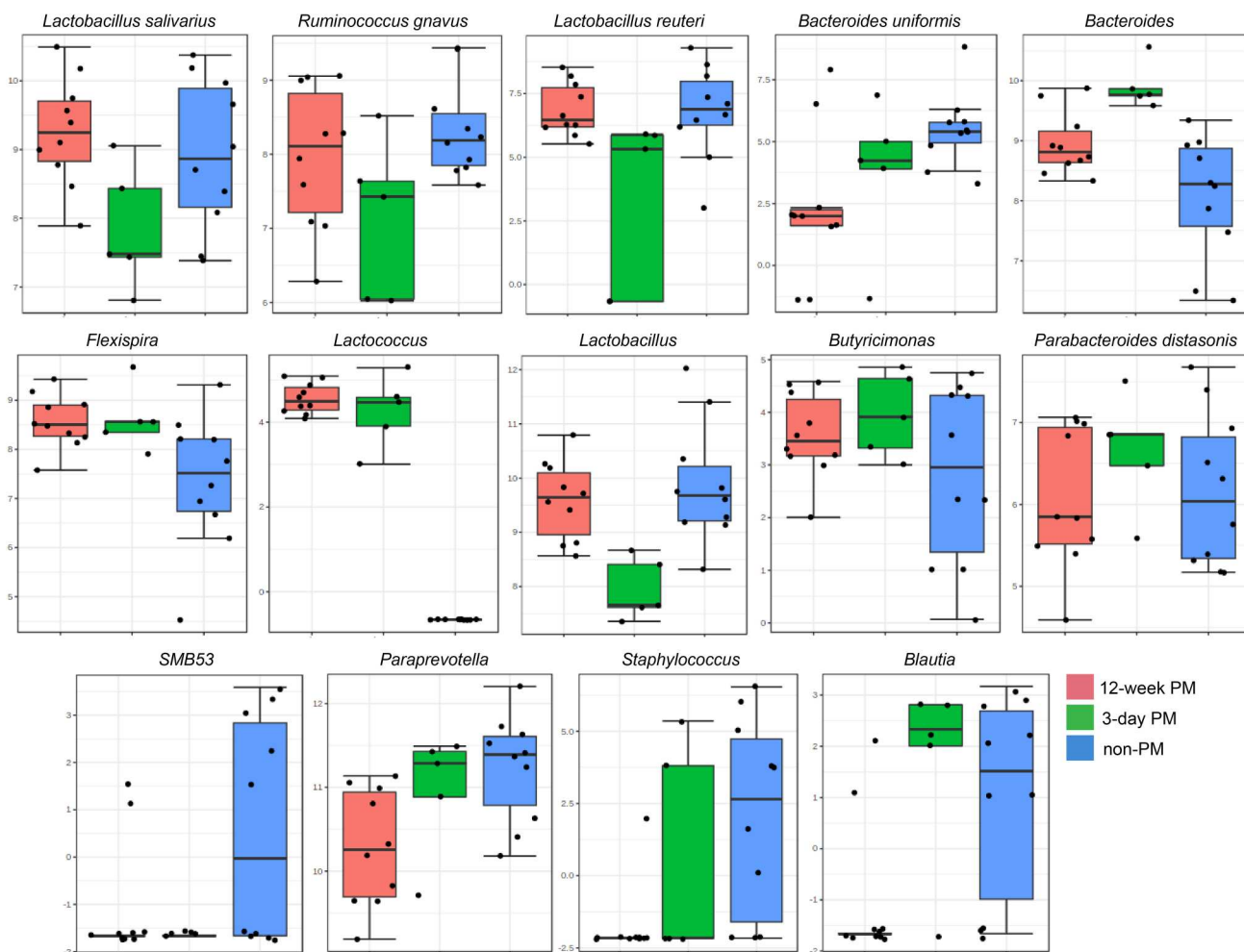


Figure 7. Association of gut microbial species with PM exposure duration following adjustment of exercise covariate using multiple linear regression model. The log-transformed counts of adjusted significantly altered bacterial genera/species are displayed on the Y-axis, with a p -value cut-off of 0.05 (FDR-corrected).

by separate clustering (Figure 2). PM, a study variable that can modulate the gut microbial diversity. Its exposure can cause gut microbial dysbiosis (Ran et al. 2021) and promote cardiovascular disease (CVD) (Chen et al. 2019). A recent study reported an alteration of microbial profiles in the small intestine by exposure to diesel exhaust particles, which can lead to systemic inflammation and early CVD biomarkers (Phillippi et al. 2022). Our findings revealed that PM exposure decreased the alpha diversity (Fisher) and richness (observed features and ACE) of the gut microbiota in mice, irrespective of exposure duration (3 days or 12 weeks) and exercise intervention (Figure 2). Beta-diversity analysis of the gut microbiota indicated that the intervention groups were significantly dissimilar from each other, as estimated using the Bray–Curtis metric (Figure 3).

Numerous investigations have identified adverse links between exposure to air pollution and indices

related to the diversity, evenness, and richness of the intestinal microbiome. PM exposure can elevate colonic immune cell infiltration, triggering heightened inflammatory responses through intestinal tissue damage, and concurrently inducing alterations in the gut microbial community through the initiation of oxidative stress. Bacteroidetes to Firmicutes ratio and the richness of the gut microflora were significantly reduced after PM 2.5 exposure, according to a recent study using mice (Dai et al. 2022). Across various studies, exposure to air pollution exhibited negative association with taxa from *Acidobacteria*, *Cyanobacteria*, *Deferribacteres*, *Fibrobacteres*, *Gemmatimonodota*, *Lentisphaerae*, *Spirochaetes*, *Synergistetes* *Thermodesulfobacteriota*, and *Verrucomicrobia*, while showing positive associations with taxa from *Bdellovibrionales*, *Deltaproteobacteria*, *Elusimicrobiota*, and *Fusobacteria*. Similarly, our results revealed a reduction in the abundance of Bacteroidetes in the PM exposure groups and increased

abundance of Firmicutes compared with that in the Con group (Figure 4). A recent cohort study from China corroborates our data, confirming the association of reduced Bacteroidetes abundance with PM 2.5 constituents revealed by weighted correlation analysis. The authors demonstrated that long-term PM 2.5 exposure, specifically exposure to black carbon, is associated with modulation in gut microbiota; therefore, this should be monitored to mitigate its adverse effects (Li et al. 2023) including inflammation, oxidative stress, and gut damage and permeability, on gut microbiota (Van Pee et al. 2023).

The *Lachnospiraceae* family was highly abundant in the exercise (EXE) group, compared to the case for the Con group, and in the PME group, compared to the case for the PM alone group (Figure 4). A recent report demonstrated that aerobic exercise (3 months) together with a Mediterranean diet (low glycemic index) led to an increased abundance of species from *Lachnospiraceae* family and some other species in subjects with nonalcoholic fatty liver disease (NAFLD), in comparison to the diet and exercise alone groups (Calabrese et al. 2022). The present study highlights the role of aerobic exercise with a stronger covariate diet (Yun et al. 2022) in the expansion of *Lachnospiraceae*. However, our data were gathered from healthy mice; therefore, exercise alone with a normal chow diet showed the same effects even in the presence of PM exposure. The bacterial families *Lachnospiraceae* and *Ruminococcaceae* (from the phylum Firmicutes) are known for their butyrate-producing bacteria, the abundances of which are significantly reduced in patients with T2D, according to clinical evidence (James et al. 2022). A recent study demonstrated that voluntary and endurance exercise capacity is driven by members of *Lachnospiraceae* family, and these intestinal microbes appear to act via the gut–brain axis (Thaiss 2023). Intense physical activity stimulates a dopamine release surge in the brain striatum in the presence of the microbiome (Friend et al. 2017). This can be explained by the crosstalk between the microbiome and the brain through microbiome-derived molecules in systemic circulation or via direct neurotransmitter-based connections between the gut and the brain (Agirman et al. 2021).

Bacteroides, a genus from the phylum Bacteroidetes, was found to be less abundant in male C57BL/6 mice after 14 weeks of aerobic exercise compared to the case in the control mice (Yang et al. 2021). In agreement with a previous study, our analysis showed a reduction in the abundance of *Bacteroides* in the EXE group compared to that in the Con group. Moreover, the effects of exercise were sustained in the PME group, which showed a lower abundance of members from

Bacteroides than the PM group (Figures 5 and 6). Cigarette smoking has been associated with increased abundances of members from Proteobacteria and *Bacteroides* spp. in the gut; however, smoking cessation has been shown to decrease its abundance (Biedermann et al. 2014). Moreover, an increased abundance of members from *Bacteroides* is independently associated with higher levels of lipopolysaccharides (LPSs) and lower insulin sensitivity in T2D patients than those in non-T2D controls; this might be linked to gut barrier function (Wang et al. 2020). A study showed that strength training exercise for four weeks was associated with a higher abundance of members from *Dorea* and other bacterial spp. than that in healthy controls (Chen et al. 2021). The genus *Dorea*, classified within the family *Lachnospiraceae*, possesses the capacity to ferment glucose and other sugars, resulting in the production of ethanol, formic acid, and acetic acid. The functional role of this genus can exhibit either pro-inflammatory or anti-inflammatory characteristics, contingent upon the specific composition of the surrounding gut bacteria (Shahi et al. 2017). Furthermore, *Lactobacilli* protect the intestinal barrier from infections by promoting mucus production and synthesizing barrier-related proteins. They release antimicrobial substances such as short-chain fatty acids (SCFAs), bacteriocins, and hydrogen peroxide to inhibit the growth or eliminate pathogens. Moreover, *Lactobacilli* modulate the host's immune response to pathogens (Dempsey and Corr 2022). Our data showed that *Lactobacillus salivarius*, another species from the genus *Lactobacillus*, and members from the genus *Dorea* were enriched in the EXE group compared to the case for the Con group; further, 12 weeks of PM exposure in combination with exercise (PME) was associated with a higher abundance of members from *Dorea* and *Lactobacillus* sp. than PM exposure alone (Figures 5 and 6).

The abundance of members from the genus *Butyricimonas* was lower in the EXE and PME groups than in the Con and PM groups, respectively (Figure 6), showing an association of *Butyricimonas* with PM exposure (Figure 7). A study reported that Microplastics (MPs) can adsorb and transport toxic phthalate esters (PAEs) into the mouse gut, which can increase intestinal permeability and induce inflammation, compared to the case for exposure to MPs and phthalate esters alone. The same study revealed the relative abundance of members from *Butyricimonas* in the mouse gut after exposure to MPs and phthalate-contaminated MPs (Deng et al. 2020), corroborating our data. Members from the *Butyricimonas* produce butyrate, a short-chain fatty acid with anti-inflammatory and anti-oxidative properties (Fu et al. 2019). We hypothesized that the

abundance of *Butyricimonas* after exposure to either PM or MPs was able to cope with increasing stress. PM is known to induce the expression of biomarkers of oxidative stress. A metabolomics analysis revealed that higher exposure to PM (2.5 μm) led to a significant elevation in the levels of stress hormones, such as cortisol, cortisone, epinephrine, and norepinephrine (Li et al. 2017), which may indirectly contribute to oxidative stress.

4.1. Conclusions

Alpha and beta diversity are influenced by the interventions compared to the case for the control group (no interventions). However, exercise did not yield significant alpha diversity variations in the gut microbiota. Interestingly, members from *Lachnospiraceae* were associated with exercise intervention in the absence and presence of PM exposure. The biomarker taxa *Lactobacillus*, *Coriobacteriaceae*, and *Anaerofustis* were enriched in the EXE group, while *Desulfovibrionaceae*, *Mucispirillum schaedleri*, *Lactococcus*, and *Anaeroplasmata* were highly enriched in the PM group. The PM exposure (3-day and 12-week) groups showed a reduced relative abundance of *Bacteroides uniformis*, *SMB53*, and *Staphylococcus* compared to the non-PM exposure groups. The gut microbiota can interact with the host system, particularly by means of microbiota-derived metabolites. However, many of these interactions and the mechanisms underlying these interactions remain unknown. Filling this knowledge gap can advance our understanding of the etiological properties of gut microbial taxa with regard to certain diseases associated with PM exposure. Furthermore, our findings underscore the beneficial effects of exercise, which not only enhances the athletic abilities of the body but also boosts physiological functions for overall human health.

Disclosure statement

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