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Association of Accelerometer-Determined Physical Activity and Sedentary Behavior With the Gut Microbiome in Middle-Aged Women: A Compositional Data Approach

Inmaculada Pérez-Prieto[1,2] | Jairo H. Migueles[3] | Nerea M. Molina[1,2] | Alberto Sola-Leyva[4,5,6] | Eduardo Salas-Espejo[1] | Riikka K. Arffman[7] | Marjukka Nurkkala[8,9] | Maisa Niemelä[10] | Kreete Lüll[11] | Elin Org[11] | Stephen Franks[12] | Juha S. Tapanainen[13,14] | Andres Salumets[4,5,6,15] | Terhi T. Piltonen[7] | Francisco B. Ortega[3,16,17] | Signe Altmäe[1,2,4,5]

[1]Department of Biochemistry and Molecular Biology I, Faculty of Sciences, University of Granada, Granada, Spain | [2]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain | [3]Department of Physical Education and Sports, Faculty of Sport Sciences, Sport and Health University Research Institute (iMUDS), University of Granada, Granada, Spain | [4]Division of Obstetrics and Gynecology, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Huddinge, Stockholm, Sweden | [5]Department of Gynaecology and Reproductive Medicine, Karolinska University Hospital, Huddinge, Stockholm, Sweden | [6]Competence Centre on Health Technologies, Tartu, Estonia | [7]Department of Obstetrics and Gynecology, Research Unit of Clinical Medicine, Medical Research Center, Oulu University Hospital, University of Oulu, Oulu, Finland | [8]Department of Sports and Exercise Medicine, Oulu Deaconess Institute Foundation sr, Oulu, Finland | [9]Research Unit of Population Health, Faculty of Medicine, University of Oulu, Oulu, Finland | [10]Research Unit of Health Sciences and Technology, Medical Faculty, University of Oulu, Oulu, Finland | [11]Institute of Genomics, Estonian Genome Centre, University of Tartu, Tartu, Estonia | [12]Faculty of Medicine, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK | [13]Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland | [14]Department of Obstetrics and Gynaecology, HFR – Cantonal Hospital of Fribourg and University of Fribourg, Fribourg, Switzerland | [15]Department of Obstetrics and Gynaecology, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia | [16]CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Granada, Spain | [17]Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland

Correspondence: Inmaculada Pérez-Prieto (inmapprieto@ugr.es) | Signe Altmäe (signealtmae@ugr.es)

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Keywords: accelerometry | exercise | gut microbiota | moderate-to-vigorous physical activity | short-chain fatty acids

ABSTRACT

The beneficial effects of physical activity (PA) on gut microbiome have been reported, nevertheless the findings are inconsistent, with the main limitation of subjective methods for assessing PA. It is well accepted that using an objective assessment of PA reduces the measurement error and also allows objective assessment of sedentary behavior (SB). We aimed to study the associations between accelerometer-assessed behaviors (i.e., SB, light-intensity physical activity [LPA] and moderate-to-vigorous physical activity [MVPA]) with the gut microbiome using compositional data analysis, a novel approach that enables to study these behaviors accounting for their inter-dependency. This cross-sectional study included 289 women from the Northern Finland

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Birth Cohort 1966. Physical activity was measured during 14 days by wrist-worn accelerometers. Analyses based on the combined effect of MVPA and SB, and compositional data analyses in association with the gut microbiome data were performed. The microbial alpha- and beta-diversity were not significantly different between the MVPA-SB groups, and no differentially abundant microorganisms were detected. Compositional data analysis did not show any significant associations between any movement behavior (relative to the others) on microbial alpha-diversity. Butyrate-producing bacteria such as *Agathobacter* and *Lachnospiraceae* CAG56 were significantly more abundant when reallocating time from LPA or SB to MVPA ($\gamma = 0.609$ and 0.113, both p-values = 0.007). While PA and SB were not associated with microbial diversity, we found associations of these behaviors with specific gut bacteria, suggesting that PA of at least moderate intensity (i.e., MVPA) could increase the abundance of short-chain fatty acid–producing microbes.

1 | Introduction

Strong scientific evidence supports the notion that regular physical activity (PA) exerts beneficial effects on different health conditions, such as cardiovascular, insulin resistance, and physical fitness, among others [1]. In the last decades, epidemiological studies have detected a decrease in the levels of PA worldwide, with one-third of adults not meeting the minimum WHO recommendations [2]. Further, there is gender inequality also in inactivity, with a recent study reporting a 7.5% higher inactivity prevalence in women than in men worldwide [3]. This increase of physical inactivity in women and in the general population has been defined as a "pandemic" and prevails as the fourth leading cause of death worldwide with 3.9–5.3 million annual premature deaths [4]. In this context, scientific efforts are directed towards investigating the molecular mechanisms underlying the health benefits of PA and the detrimental consequences of sedentary behavior (SB), with a new focus on the microbiota (the collection of all microbes) that symbiotically coexists within and on the body. Pioneering studies of microbiome (i.e., the study of the microbial genomes) are associating regular PA with a healthy gut microbiome [5].

Physical activity seems to be a cornerstone in promoting an optimal ecosystem for a healthy diverse gut microbiome, which in turn, may benefit overall host health [5]. This is especially evident in athletes, although various types of exercise have been shown to influence gut microbial diversity differently [6]. This variability in response to exercise hampers our understanding of the effects of PA on the gut microbial communities. Furthermore, the interaction between PA, SB and the gut microbiome in physically inactive individuals is scarcely investigated [7].

Most studies have independently investigated the associations of SB and PA of different intensities (light intensity PA [LPA] and moderate-to-vigorous PA [MVPA]), without considering the interplay between these behaviors [8]. Since the hours of the day are limited to 24 or to waking hours, an increase in the time spent in one behavior necessarily comes with a reduction in the time spent in other behaviors that day. This closure effect and interdependency of the variables should be mathematically modeled, but has not been done in the past. In this context, compositional data analysis (CoDA) emerges as a shift from a univariate to a 24-h time-use paradigm that analyzes how the daily time spent in different codependent movement behaviors (i.e., MVPA, LPA, and SB) synergistically impacts our health (as dedicating more time in one of these behaviors means a reduction of time in the others) [9]. This new analytic tool provides a more advanced approach to the time spent on each activity during the day and whether a single ideal combination of these movements actually exists in the context of a study outcome [1, 10]. Thus, there is a need for studies on bigger sample size and homogenous study groups with rigorous PA data (objectively measured PA using accelerometry). Furthermore, the association of a movement behavior (i.e., MVPA, LPA, or SB, relative to the others) with microbiome diversity and composition, and whether there is an ideal combination of PA of different intensities associated with a healthy gut microbiome remain unexplored.

In the present study, we aimed to determine the joint associations between the accelerometer-measured MVPA, LPA, and SB with microbiome diversity and composition in a subset of women from a wide population-based birth cohort study from Northern Finland, using CoDA. To the best of our knowledge, this is the first study investigating the associations of combinations of daily time spent in PA of different intensities and SB using CoDA in relation to the gut microbiome.

2 | Material and Methods

2.1 | Study Population

This cross-sectional study analyzed a subset of women of the Northern Finland Birth Cohort 1966 (NFBC1966) [11, 12], a longitudinal population-based cohort study which includes all expected births in the year 1966 in the two northernmost provinces of Finland [13]. During the follow-up, anthropometric measures, clinical examinations, health and personal information, and blood and fecal samples were collected. Our study population consisted of a total of 303 women, where 102 women had been diagnosed with polycystic ovary syndrome (PCOS) and 201 were age- and body mass index (BMI)-matched controls with no PCOS at 46 years of age [14]. Women with hormonal contraceptive or antibiotic, antimycotic, letrozole, or tamoxifen treatment within the last 3 months preceding sample collection were excluded. Since a previous study in this subset did not find any statistically significant differences in the gut microbiome profile of women with PCOS and controls [14], we included the whole population in a single group, considering diagnosis as a covariate for statistical analyses. The study has been approved by the ethical committee of Northern Ostrobothnia hospital district.

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All participants of the NFBC1966 provided informed consent for the data and samples to be used for scientific purposes.

2.2 | Accelerometer-Assessed Physical Activity

Accelerometry has been widely demonstrated to be a more valid and comparable method to obtain a more precise estimation of each PA and SB component, compared to self-reported questionnaires. Accelerometers are wearable monitors that allow to objectively and continuously monitor the accelerations (movements) of the body segment in which the devices are attached to during a relatively long period of time (usually ~7 days). Participants were asked to wear a wrist-worn accelerometer (Polar Active, Polar Electro Oy, Kempele, Finland) during 24 h/day for at least 14 days on the nondominant hand. Polar Active is a waterproof activity monitor that records metabolic equivalents of task (MET) values every 30 s based on daily PA, and using clinical data (height, weight, sex, and age) as predefined inputs. This dispositive has been demonstrated to correlate ($R^2 = 0.74$) with the double-labeled water technique when measuring energy expenditure during exercise [15]. Accelerometer readings were blinded to the wearer (and investigator) during the data collection period, and participants were asked to mail them back after the clinical examinations ended. Briefly, these devices categorize PA according to five levels—very light: 1–2 METs; light: 2–3.5 METs; moderate: 3.5–5 METs; vigorous: 5–8 METs; and very vigorous ≥8 METs [16]. Daily duration spent in each PA category was calculated in min/day for all participants. Polar Active data were filtered for minutes at intensities of 1 MET or higher, so that sleep and non-wear time were not included in the analyses. As the standard definition of SB encompasses activities of low-energy expenditure (<1.5 METs) including sitting, reclining, or laying while awake, we considered the activities of very light intensity (1–2 METs) as equivalent to SB. PA level ≥3.5 was classified as MVPA. The first accelerometer-monitored day was not considered in the analyses. Women with data from 4 or more valid days (defined as wear time at least 600 min/day) were included in the analyses. Wear time was calculated as a sum of all five activity levels.

2.3 | Self-Reported Physical Activity

Leisure-time PA was self-reported with questions on the frequency and duration of brisk PA (causing at least some sweating and breathlessness) and light PA (causing no sweating or breathlessness) during leisure time. PA frequency had six response options: (1) once a month or less often, (2) two to three times a month, (3) once a week, (4) two to three times a week, (5) four to six times a week, and (6) daily. PA duration had the following response options: (1) not at all, (2) less than 20 min, (3) 20–39 min, (4) 40–59 min, (5) 1–1.5 h, and (6) more than 1.5 h [16]. Weekly averages of MET-min scores of light and brisk PA were calculated by multiplying the PA volume (duration*frequency) by its intensity (light PA, 3 METs; brisk PA, 5 METs) [16]. Daily sitting time (ST) was self-reported with the amount of sitting in five domains (at work, at home watching TV or video, at home in front of computer, in a vehicle, and in another place). Average daily ST (min/day) was calculated as a sum of durations of these sedentary behaviors [16].

2.4 | Sample Collection and DNA Extraction

The fecal samples were collected at home by the study participants. It was recommended that the fecal sample should be delivered in a cooler on the day of collection. When it was not possible, the sample was stored for 1–2 days in a freezer at −20°C until delivery. After delivery, the fecal samples were initially stored at −20°C and then at −70°C until analyses were performed.

For microbial DNA isolation, the fecal samples were first homogenized in a Stomacher-400 blender. DNA was extracted using QIAamp Stool Mini Kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's instructions, except for the incubation step where samples were mixed with lysis buffer and incubated at 95°C instead of 70°C in order to ensure the lysis of both Gram-negative and Gram-positive bacteria. The extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, DE, USA). DNA yield was determined by measuring the absorbance ratios spectrophotometrically, adjusting A260/280 nm for protein and A260/230 nm for salt and phenol contamination.

2.5 | 16S rRNA Gene Sequencing

Gut microbiome was profiled by amplifying the hypervariable V3–V4 regions of the 16s rRNA gene using the forward 5′-CCTACGGGNGGCWGCA-3′ and reverse 5′-GACTACHVGGGTATCTAATCC-3′ primers pair and sequencing on a MiSeq Illumina instrument. All PCR reactions were carried out in a 25-μL final reaction volume containing 12.5 μL 2X KAPA HiFi Hotstart ready mix (KAPA Biosystems, Woburn, MA, USA), 5 μL of each primer (1 μM), and 2.5 μL of extracted DNA (10 ng) under the following cycling conditions: initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s, with a final extension step at 72°C for 5 min. PCR clean-up was done with AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA). Next, a PCR to index the amplicons was performed using the Nextera XT Index Kit (Illumina, San Diego, CA, USA) with conditions: 95°C for 3 min; 8 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, with a final extension of 5 min at 72°C, and hold at 4°C. The pooled PCR products were purified with AMPure XP beads (Beckman Coulter) before quantification. The final library was paired-end sequenced (2 × 300 bp) using a MiSeq Reagent Kit v.3 on the Illumina MiSeq sequencing platform (Illumina).

2.6 | Microbiome Analysis

Raw sequences were demultiplexed with Illumina bcl2fastq2 Conversion Software v2.20 and imported to Qiime2 software v.2022.11 with a PairedEndFastqManifestPhred33 input format. DADA2 was used for the denoising step. Low-quality regions were trimmed considering a quality score below 25 to create high quality forward and reverse reads, using the "q2-dada2" function with the following parameters: trunc_len_f=288, trunc_len_r v=241, trim_left_f=16, and trim_left_r=0. Taxonomy assignment of amplicon sequence variants (ASVs)

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was performed using the "classify-sklearn" function against the SILVA 16S v132_99 database, along with a similarity threshold of 99%.

2.7 | Statistics

Descriptive characteristics of the study participants were reported as mean and standard deviation (SD) or geometric mean and covariance, as appropriate. As fiber intake is well-known to influence the gut microbiome, a fiber score based on the weekly frequency consumption of fresh/boiled vegetables, fruits and berries, and grain-contained products, as well as daily fiber intake from bread was calculated (Table S2). Six women did not provide any information about the fiber consumption. We estimated these values using multiple imputation method in SPSS v.28.0.1.0. BMI, accelerometer wear time, PCOS diagnosis, and fiber score were considered confounders in our statistical analyses.

All statistical analyses were performed in R (v.4.2.1) under RStudio (v.2022.07). Statistical significance was set to 0.05 (i.e., p-value or q-value <0.05 for analyses using Benjamini–Hochberg false discovery rate (FDR) for multiple correction). To analyze the combined effect of MVPA and SB, we categorized participants into four groups according to the median of the time spent (min/day) in each behavior: Low MVPA-High SB, Low MVPA-Low SB, High MVPA-High SB, and High MVPA-Low SB. CoDA captures a more real approach of the movement composition of the day, quantifying the effect of increasing a specific behavior while reducing the others on a continuous scale. Hence, multiple regression models over compositional data were constructed to investigate the associations of increasing time spent in one single behavior (while proportionally reducing the others) with microbiome outcomes (i.e., Shannon diversity index and richness and relative abundances of bacteria >0.1%). For this purpose, one time-use composition was defined and included SB, LPA, and MVPA. Isometric log ratios were calculated in sequential binary partition and included as explanatory variables as previously reported [17]. Compositional models were adjusted by BMI, PCOS diagnosis, fiber score, and accelerometer wear time. The strength and direction of each association were indicated by gamma (γ) coefficients. Prediction analyses for specific time reallocations were additionally performed minute-by-minute up to 30 min/day for each behavior. An additional model including SB, LPA, and moderate and vigorous PA as separated components was performed to corroborate the previous results.

Microbiome diversity analyses were conducted and visualized using phyloseq, vegan, microviz, and ggplot2 packages. Microbial taxa were aggregated to phylum and genus level in further analysis. Within-sample microbiome diversity (i.e., alpha-diversity) was estimated by Shannon diversity index and richness (i.e., number of microbial taxa), using the "diversity" and "specnumber" functions from the vegan package, respectively. Between-sample microbiome dissimilarity (beta-diversity) was visualized using principal coordinate analysis (PCoA), based on the Bray–Curtis distance. For alpha-diversity comparisons, post hoc analyses following analysis of covariance (ANCOVA) were conducted for significance testing among MVPA-SB groups with the function "emmeans_test" from the rstatix package, controlling for BMI, PCOS diagnosis, fiber score, and accelerometer wear time as potential covariates. For beta-diversity significance testing, permutational multivariate analysis of variance (PERMANOVA) was permuted using the "adonis2" function from vegan package. Differential abundance analysis was performed in those bacterial taxa with a relative abundance >0.1% using the metagenomeSeq package. MetagenomeSeq models microbiome data with a zero-inflated distribution, providing statistically significant differentially abundant taxa between MVPA-SB groups (q-value <0.05 after FDR correction). Furthermore, sensitivity analyses were conducted by excluding women with PCOS to corroborate the previous analyses.

3 | Results

Of the initial study population of 303 women, a total of 289 presented valid objectively measured PA data. All these participants wore the devices for at least 4 days and provided valid accelerometer data whose mean daily wear time (SD) was 971.5 min (57.0). Descriptive characteristics and accelerometer data of study participants are summarized in Tables 1 and S1.

3.1 | Microbiome Composition Across the Study Population

After 16S rRNA gene sequencing, 72 742 ASVs were detected, with a total of 20 phyla and 523 genera. The most dominant phyla were *Firmicutes* (53.8%) and *Bacteroidetes* (32.6%), followed by other sub-dominant phyla such as *Proteobacteria* (6.7%), *Actinobacteria* (3.2%), and *Verrucomicrobia* (2.0%), representing >98% of the gut microbiome. At genus taxonomic level, 103 taxa were present in a relative abundance over 0.1%, where *Bacteroides* (18.4%), *Alistipes* (7.0%), *Faecalibacterium* (4.5%), *Blautia* (2.4%), *Ruminococcaceae* UCG-002 (2.4%), and *Roseburia* (2.2%) were the most abundant bacteria in the gut.

TABLE 1 | Descriptive characteristics of the study participants.

Characteristics	$N = 289$ women
BMI, mean ± SD	27.8 ± 5.4
Accelerometer valid days, mean ± SD	13.6 ± 1.3
Accelerometer wear time, mean ± SD (min/day)	971.5 ± 57.0
Moderate-to-vigorous PA, geometric mean (min/day)	54.0
Light PA, geometric mean (min/day)	289.0
Sedentary behavior, geometric mean (min/day)	608.0

Note: Data presented as mean ± SD, or geometric means.
Abbreviations: BMI, body mass index; PA, physical activity; SD, standard deviation.

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3.2 | Microbiome Analysis in Groups of MVPA and SB

As a first step, we analyzed the combined effect of MVPA and SB on the gut microbiome profile. Therefore, we established four study groups based on the median of the time spent in each behavior (median [q1; q3] of MVPA: 57.6 [39.7; 73.5]; SB: 616.8 [561.0; 673.3]): Low MVPA-High SB ($n=95$), Low MVPA-Low SB ($n=49$), High MVPA-High SB ($n=50$), and High MVPA-Low SB ($n=95$). Self-reported time spent in sedentary time and PA (METs-min/week) was in agreement with the MVPA/SB groups derived from the accelerometer data (Table S1). Notably, 46% of the study participants reported exercising >150 min of MVPA per week, following the WHO guidelines [18].

Alpha-diversity evaluated by Shannon diversity index and richness indicated no significant differences between the groups (Figure 1A,B; pairwise comparisons following ANCOVA: all p-values >0.05). All models included BMI, PCOS diagnosis, accelerometer wear time, and fiber score as potential confounders. Beta-diversity analysis based on Bray–Curtis distances did not detect any significant dissimilarity between the microbial populations of each group (Figure 1C; pairwise PERMANOVA test accounting for covariates, all $R^2 \leq 0.01$, all p-values >0.05).

Next, we performed a differential abundance analysis using metagenomeSeq to detect specific taxa that could be differentially abundant in the gut microbiome of MVPA-SB groups (Tables S4–S9). *Bifidobacterium* and a member of *Ruminococcaceae* family

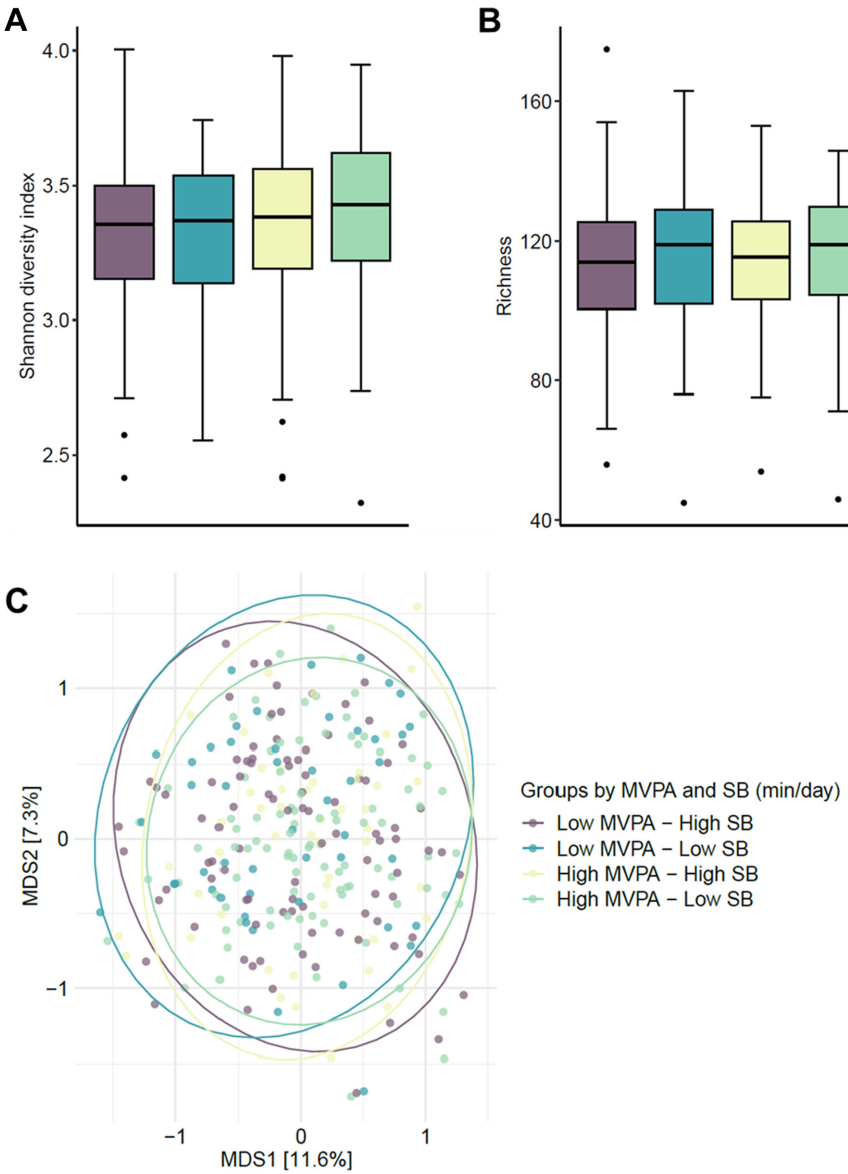

FIGURE 1 | Microbial diversity measures in the MVPA-SB groups. Panels A and B represent alpha-diversity analysis (i.e., Shannon diversity index and richness). Four study groups were stablished based on the median of each behavior (MVPA: 57.6 min/day; SB: 616.8 min/day): Low MVPA-High SB ($n=95$), Low MVPA-Low SB ($n=49$), High MVPA-High SB ($n=50$), and High MVPA-Low SB ($n=95$). Pairwise comparisons indicate no significant differences between groups (post hoc analysis following ANCOVA: all p-values >0.05). Panel C represents beta-diversity analysis: principal coordinate analysis (PCoA) of Bray–Curtis distances. There is no significant dissimilarity between groups (pairwise PERMANOVA: all $R^2 \leq 0.01$, all p-values >0.05). MVPA, moderate-to-vigorous physical activity; SB, sedentary behavior.

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were less abundant in groups with high SB compared to low SB groups, while *Eubacterium xylanophilum*, *Lachnospiraceae* CAG56, and *Ruminococcus* 1 were increased (Tables S4 and S7). Interestingly, when we compared the most opposite groups (i.e., Low MVPA-High SB vs. High MVPA-Low SB), *Coprobacter* and a member of *Muribaculaceae* family were increased in women with high MVPA and low SB (Table S6). However, differences between the abundance of these bacteria did not remain statistically significant after FDR correction (all *p*-values >0.05).

3.3 | Compositional Data Analysis

Movement composition behavior of the women included in this study was visualized in a ternary plot illustrated in Figure 2. The geometric mean for each behavior was 54 min/day of MVPA, 289 min/day of LPA, and 608 min/day of SB. Covariance matrices for the daily time-use in movement behaviors are indicated in Table S3.

We examined the dose–response curves relating to the effect of increasing one behavior while proportionally reducing others on microbial diversity (Figure 3). MVPA, LPA, or SB (relative to the remaining behaviors) were not associated with any alpha-diversity metric (all *p*-values >0.05). Next, we investigated the associations between compositional data and the relative abundance of bacteria present in a relative abundance >0.1% (Figure 4). At phylum level, LPA (relative to the other behaviors) was negatively associated with *Tenericutes* ($\gamma = -0.844$, $p = 0.035$). For example, increasing 30 min per day of LPA was associated with a 0.09% decrease in its relative abundance (expected change [CI]: −0.089 [−0.195; 0.017]).

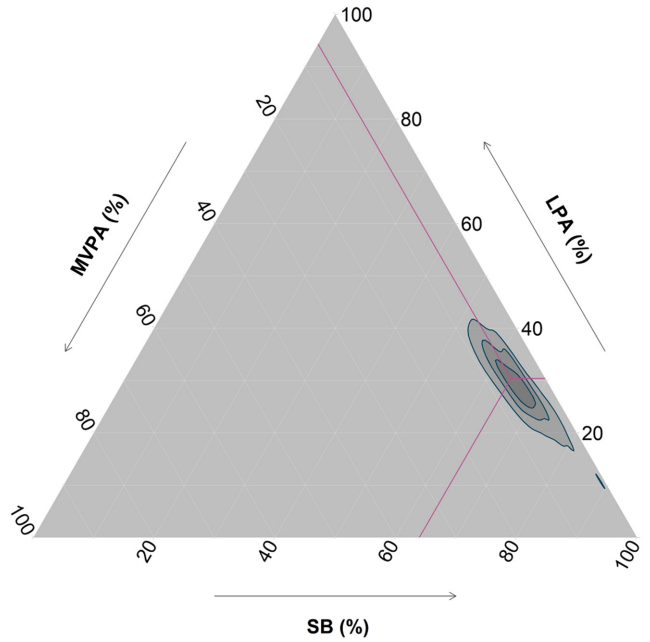

FIGURE 2 | Ternary plot for the daily time-use in the movement behaviors in the study participants ($n = 289$). The crosshair marks represent the geometric mean of the behaviors (i.e., MVPA: 54 min/day, LPA: 289 min/day, SB: 608 min/day). Concentric rings represent the 25%, 50%, and 75% confidence regions. LPA, light physical activity; MVPA, moderate-to-vigorous physical activity; SB, sedentary behavior.

At genus level, 10 bacteria showed significant associations with accelerometer-assessed behaviors (Figure 4). Reallocating time proportionally from LPA or SB to MVPA was positively associated with the relative abundance of *Agathobacter* ($\gamma = 0.609$, $p = 0.007$), *Lachnospiraceae* CAG56 ($\gamma = 0.113$, $p = 0.007$), and an unidentified bacterium from *Muribaculaceae* family ($\gamma = 0.473$, $p = 0.015$). For example, increasing 30 min per day of MVPA was associated with a 0.2% (expected change [CI]: 0.164 [0.048; 0.280]), 0.03% (expected change [CI]: 0.030 [0.009; 0.052]), and 0.1% (expected change [CI]: 0.127 [0.027; 0.227]) increase in relative abundance of *Agathobacter*, *Lachnospiraceae* CAG56, and *Muribaculaceae*'s bacterium, respectively. However, MVPA (relative to the other movement behaviors) was negatively associated with *Asteroleplasma* ($\gamma = -1.329$, $p = 0.041$). Reallocating 30 min per day to MVPA at expenses of the other behaviors was associated with a 0.4% decrease in the relative abundance of this genus (expected change [CI]: −0.379 [−0.713; −0.045]).

Next, LPA was positively associated with *Asteroleplasma* ($\gamma = 3.387$, $p = 0.001$), while negatively associated with *Agathobacter* ($\gamma = -0.892$, $p = 0.014$), *Lachnospiraceae* CAG56 ($\gamma = -0.157$, $p = 0.020$), the bacterium from *Muribaculaceae* family ($\gamma = -0.662$, $p = 0.035$), *Eubacterium xylanophilum* ($\gamma = -0.409$, $p = 0.009$) and two members belonged to the *Clostridiales vadin* BB60 group ($\gamma = -0.546$, $p = 0.026$ and $\gamma = -0.565$, $p = 0.020$). For example, an increment of 30 min per day in LPA was associated with a 0.4% increase in *Asteroleplasma* (expected change [CI]: 0.407 [0.132; 0.683]), and decrease in the relative abundance of *Agathobacter* (expected change [CI]: −0.142 [−0.238; −0.046]), *Lachnospiraceae* CAG56 (expected change [CI]: −0.026 [−0.044; −0.008]), *Muribaculaceae* (expected change [CI]: −0.108 [−0.191; −0.026]), *Eubacterium xylanophilum* (expected change [CI]: −0.052 [−0.093; −0.011]), and *Clostridiales vadin* BB60 group's bacteria (expected changes [CI]: −0.071 [−0.136; −0.007] and −0.058 [−0.123; 0.006]).

Finally, SB (relative to LPA and MVPA) was positively associated with higher abundances of two bacteria from *Clostridiales vadin* BB60 group ($\gamma = 0.415$, $p = 0.037$) and *Izimaplasmatales* order ($\gamma = 0.636$, $p = 0.028$), while demonstrating an inverse association with *Asteroleplasma* ($\gamma = -2.058$, $p = 0.016$), *Eubacterum ventriosum* ($\gamma = -0.134$, $p = 0.033$), and *Intestinibacter* ($\gamma = -0.151$, $p = 0.041$). Prediction analysis from reallocating 30 min per day from LPA or MVPA to SB reported slightly higher abundances of *Clostridiales vadin* BB60 group (0.005 [−0.038; 0.047]) and *Izimaplasmatales* (0.031 [−0.030; 0.094]), while 0.05% and 0.01% decreases in *Asteroleplasma* (−0.047 [−0.135; 0.229]), *Eubacterum ventriosum* (−0.014 [−0.027; −0.001]), and *Intestinibacter* (−0.014 [−0.030; 0.002]).

The results presented above are confirmed by an additional data analysis in which we included four components: SB, LPA, moderate PA, and vigorous PA (Figures S1 and S2).

3.4 | Sensitivity Analysis

A sensitivity analysis excluding those women who have been diagnosed with PCOS was performed to corroborate the obtained results. A total of 190 control women had valid accelerometry data and were included. The results are in line

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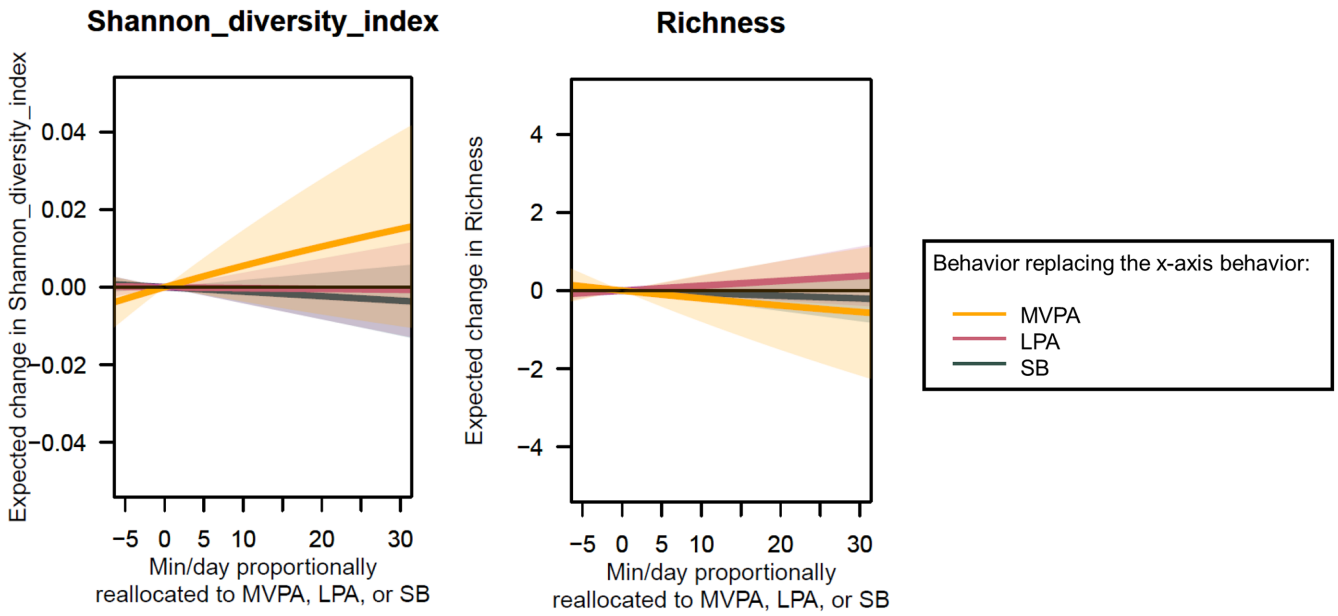

FIGURE 3 | Joint associations of the movement behavior (i.e., MVPA, LPA, and SB) composition with alpha-diversity metrics, that is, Shannon diversity index and richness, both $p > 0.05$. The models are adjusted by BMI, PCOS diagnosis, accelerometer wear time, and fiber score. Each line represents time in a behavior while proportionally reducing the others. Shaded areas represent the 95% confidence intervals. BMI, body mass index; LPA, light physical activity; MVPA, moderate-to-vigorous physical activity; PCOS, polycystic ovary syndrome; SB, sedentary behavior. Study sample size was 289 participants.

with those of the whole sample analyses, with no statistical differences between the MVPA-SB groups detected in the microbial alpha- (pairwise comparisons following ANCOVA: all p-values >0.05) and beta-diversities (pairwise PERMANOVA test, all $R^2 \leq 0.03$; all p-values >0.05), after adjustment by BMI, fiber score, and accelerometer wear time (Figure S3). Additionally, we did not detect any significant differentially abundant taxon after correcting by multiple comparisons (Tables S10–S15).

A ternary plot illustrating the movement composition behavior of the participants included in the sensitivity analysis is presented in Figure S4. Concerning sensitivity analyses on compositional data, the previous findings were confirmed with no significant associations between accelerometer-assessed time of MVPA, LPA, or SB and alpha-diversity metrics (p-values >0.05, Figure S5). Results from analyzing the association of movement behaviors with relative abundances of specific bacteria although, in agreement, were attenuated. Specifically, *Agathobacter*, *E. ventriosum*, *E. xylanophylum*, *Intestinibacter*, *Lachnospiraceae* CAG56, and the unidentified member of *Izimaplasmatales* order showed similar expected-change curves compared to the main analysis, but the associations did not reach the statistical significance. Additionally, SB time (relative the other behaviors) was negatively associated with the abundance of *Bifidobacterium* (Figure S6).

4 | Discussion

To our knowledge, this is the first study investigating the joint associations of MVPA, LPA, or SB (relative to the other behaviors) with the gut microbiome outcomes. CoDA represents a more real approach of the time spent in each behavior during the entire day, as increasing time in one activity means commensurately reducing time in other behaviors. Our findings support that PA of different intensities and SB are associated with the abundance of specific gut bacteria, although not with overall microbial diversity.

In our first analysis, we characterized and compared the gut microbiome of women with different MVPA and SB levels. A greater alpha-diversity has been related to an overall gut stability and health, while a low diversity has been linked to several diseases such as obesity, diabetes, bowel diseases, or colon cancer, among others [19]. We did not detect any significant association of MVPA and SB with the gut microbiome diversity in the middle-aged women. A previous study also failed to find any significant differences in the alpha- or beta-diversities when comparing the gut microbiome profile of 40 active and sedentary women categorized according to WHO recommendations (i.e., active were those who performed at least 3 h of PA per week, while sedentary were those who did not practice at least 3 days of PA per week for 30 min at a moderate intensity) [20]. However, they found significant correlations between the accelerometer assessed sedentary parameters (i.e., sedentary time and breaks) and alpha-diversity metrics (Shannon diversity and Simpson indexes) [20]. Similarly, numerous studies did not find any associations between the alpha-diversity and PA levels of individuals of different gender, age, and health conditions [21–24], while other studies described an increased gut microbiome diversity among participants with higher PA levels [25, 26].

Self-reported questionnaires generally rely on subjective information, lack of detail when assessing behaviors (as they do not usually differentiate PA of different intensities, e.g., LPA and MVPA), and do not allow to perform analyses on a continuous scale. Few studies have analyzed the association between the

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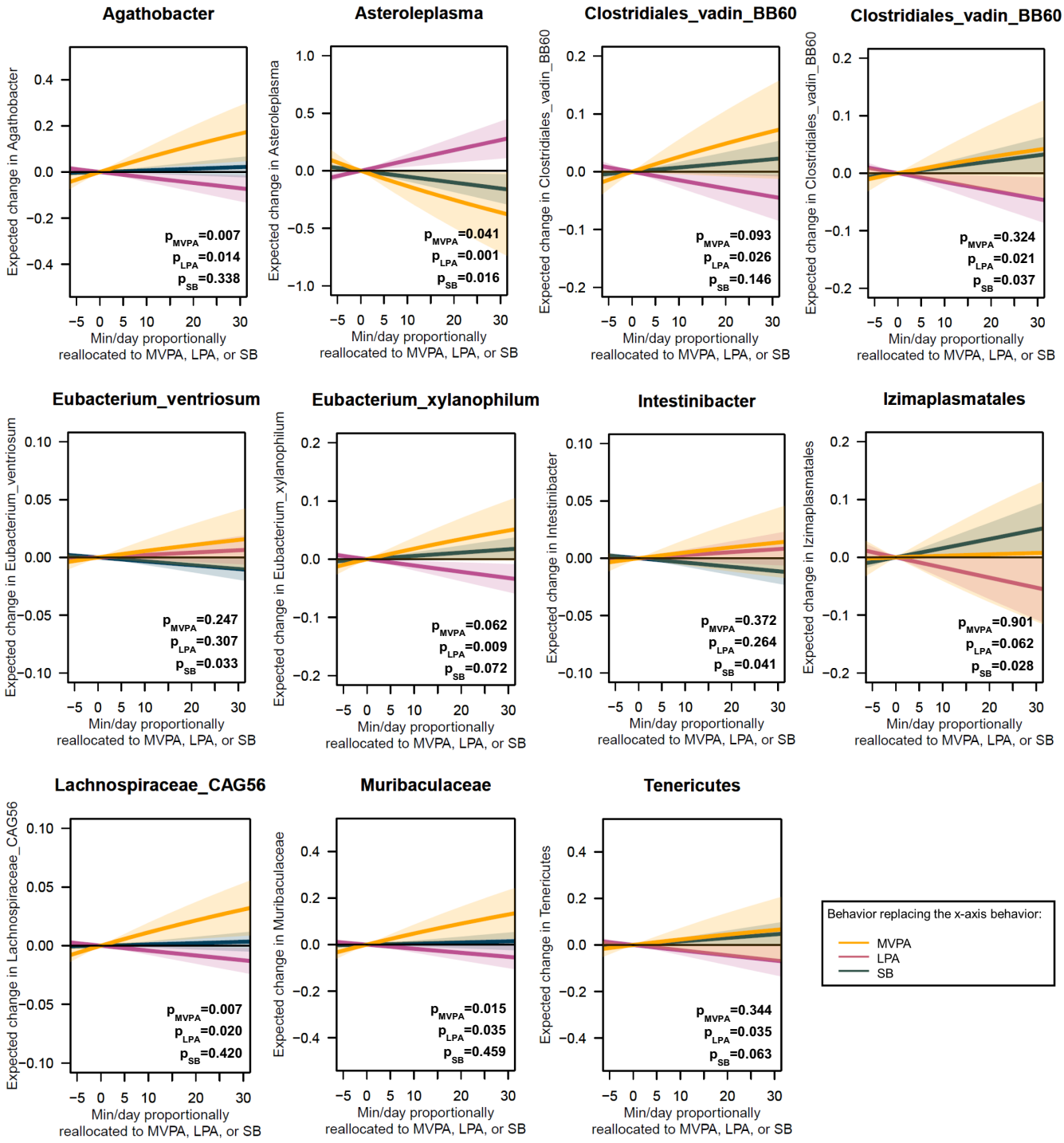

FIGURE 4 | Joint associations of the movement behavior (i.e., MVPA, LPA, and SB) composition with bacteria with a relative abundance >0.1%. Only statistically significant models ($p < 0.05$) are shown. The models are adjusted by BMI, PCOS diagnosis, accelerometer wear time, and fiber score. Each line represents time in a behavior while proportionally reducing the others. Shaded areas represent the 95% confidence intervals. Study sample size was 289 participants. BMI, body mass index; LPA, light physical activity; MVPA, moderate-to-vigorous physical activity; PCOS, polycystic ovary syndrome; SB, sedentary behavior.

accelerometer assessed-PA and the gut microbiome [22, 27, 28]. Carter et al. [27] recorded PA patterns over a 10-day period by a hip-worn triaxial accelerometer in 37 breast cancer survivors. They quantified MVPA (min/day) to perform multiple regression analysis and examined the associations between MVPA and alpha-diversity. Their results showed that cardiorespiratory fitness (a genetic component modifiable by PA), while not MVPA, correlated positively with gut microbiome diversity [27]. These results are in accordance with those obtained by Zhong et al. [28], where they analyzed whether different accelerometer movement behaviors (i.e., SB, LPA, and MVPA) were associated with microbiome outcomes in 100 older female and male participants. Multiple regression analysis did not detect any relationship between physical behavior and alpha-diversity; however,

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associations were identified for MVPA and beta-diversity and the relative abundance of specific gut bacteria. In our study, where we analyzed 303 women at the same age and considered BMI matching together with fiber score and accelerometer wear time as confounders, no gut microbial diversity indices associated with PA and SB.

In our cohort, we integrated SB, LPA, and MVPA as intrinsically codependent behaviors by using CoDA. Traditionally, researchers have analyzed movement behaviors as isolated components which leads to an unreal approach of the time distribution during a 24-h day [8]. To our knowledge, this is the first CoDA study investigating the inter-relationships between physical behaviors and the gut microbiome. We did not find evidence of a significant association of MVPA, LPA or SB with any alpha-diversity metrics (Shannon diversity index and richness), which is in line with previous studies. It has been argued whether the type, dose and duration of PA is determining to produce considerable influence on the gut microbiome [7]. Therefore, there is a need for clarifying how much and what type of PA would be sufficient to increase microbial diversity in the gut [20, 29]. Specially, several studies including participants from sports which demand high-intensity level of exercise and usually require considerable fitness and dietary requirements, generally report a more diverse gut microbiome in athletes compared to nonathletes [30, 31]. In this context, a recent meta-analysis of the metagenomics sequencing data of 207 fecal samples aimed to compare the gut microbiome between athletes and individuals with reduced PA, where athletes showed a significantly more diverse microbiome compared to nonathletes [29]. Otherwise, the relationship between physical fitness and the gut microbiome remains largely unexplored in physically inactive participants. Recent evidence suggests that exercise at moderate intensity exerts improvements in cardiorespiratory fitness and body composition that could be associated with substantial changes in the gut microbial diversity and composition [32]. Future studies in inactive population are needed to better understand the mechanistic interaction between PA, physical fitness, and the gut microbiome.

Our differential abundance analysis detected statistically significant bacteria associated with MVPA, LPA, and SB. Particularly, *Agathobacter* and *Lachnospiraceae* CAG56 (both belong to *Lachnospiraceae* family) were positively associated with MVPA (relative to the other behaviors). *Lachnospiraceae* family members are among the main producers of short-chain fatty acids (SCFAs), particularly acetate and butyrate, in the gut [33]. SCFAs are metabolic products originating from dietary nondigestible carbohydrates and have been linked to human physiology, being substrates for energy metabolism and important signaling molecules implicated in the gut–microbiome axis, the regulation of the immune response, and the skeletal muscle lipid metabolism [34]. In fact, a decrease in the relative abundance of *Lachnospiraceae* has negative health consequences due to the loss of relevant beneficial functions performed by members of this family, such as colonization resistance (i.e., capacity to limit the growth of potential microbial pathogens) or butyrate-conducted pleiotropic beneficial effects for the host metabolism and immune regulation [33]. In line with our study results, numerous human observational [21, 25, 28] and experimental [35–39] studies have associated higher abundances of SCFAs-producing gut bacteria from *Lachnospiraceae* family, such as *Roseburia*, *Coprococcus*, *Lachnospira*, and *Blautia*, among others, with higher PA levels or PA interventions ranging from 2 to 12 weeks. For example, Whisner et al. [21] pointed to *Lachnospiraceae* and *Lachnospira* as important microbial markers for college students with greater MVPA. Similarly, Zhong et al. [28] reported four unclassified bacteria from *Lachnospiraceae* family to be positively associated with accelerometer-assessed MVPA in older adults.

Asteroleplasma was the only microbe associated with the three PA components, being positively associated with LPA while negatively with MVPA and SB. Several studies have linked *Asteroleplasma* to chronic conditions, such as type-2 diabetes and tumor metastasis [40], although its biological role in human physiology has not been elucidated. Neither are the functional roles of *Clostridiales vadin* BB60 group and *Izimaplasmatales* in human health well-known, which we found to increase when reallocating time from MVPA or LPA to SB. Interestingly, the butyrate producers, *Eubacterium ventriosum* and *Eubacterium xylanophilum* showed a negative association with SB and LPA, respectively. *E. ventriosum* has been described to be important for the gut health and proposed as a biomarker of low risk of colorectal cancer [41].

4.1 | Limitations and Strengths

The impact of PA on the gut microbiome independently from host and lifestyle factors such as gender, age, or diet, among others, is still unclear as the current knowledge relys on heterogeneous studies [7]. The strength of this study is to include a homogenous female population of representative sample size. A major strength is the use of accelerometers which enable to objectively register PA of different intensities and SB. Notably, PA behaviors were codependently analyzed (on a continuous scale) using CoDA by considering different time reallocations. CoDA mirrors more precisely "real life" of study participants, in contrast with the traditional univariate approach that analyzes movement behaviors as independent domains.

A limitation in our CoDA was that we did not have information on sleep time and, therefore, it was not included in the analyses. Therefore, we were not able to analyze the entire 24-h day, but just the waking hours of the day. Additionally, it is important to note that the cross-sectional design restricts CoDA to establishing associations between body movements and the gut microbiome, rather than inferring causality. While accelerometry is the best and most reliable measuring method, wrist-worn accelerometers have several limitations. For example, they do not capture all types of PA precisely, with an overestimation of upper body movements while underestimating others activities such as cycling [16]. Regarding microbiome analysis, fecal samples were analyzed by marker gene sequencing which reliably identifies bacterial genera, but fails in identifying bacteria on species level. Hence, studies using whole-metagenome sequencing that enables microbiome detection on species level are required to provide more detailed knowledge of the relationship between PA, SB, and microbiome.

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5 | Perspective

Previous microbiome studies have analyzed PA and SB without accounting for their inter-dependency. Further, results from different studies are hardly comparable due to disparity of the participants (i.e., age, gender, and health status), wide use of self-reported PA questionnaires, lack of standardized criteria to define which PA level categorizes the study population as sufficiently active, and relevant covariates missing, especially diet. Therefore, studies of bigger sample size, on different ethnicities, with rigorous PA measurements (objectively obtained by accelerometry) and dietary well-controlled studies are warranted to unravel the real influence of PA on the gut microbial ecosystem. The current study provides a novel insight through the application of a compositional data approach in the PA-microbiome field. Our findings using CoDA do not support a significant association of PA with gut microbial diversity. On the other hand, our data indicate that more time in MVPA (relative to LPA and SB) was associated with an increase in the relative abundance of beneficial SCFAs-producing bacteria. Further research in other cohorts that analyzes compositional data including sleep time is warranted to precisely analyze the entire 24-h day. Also, longitudinal studies with a dietary control are needed to unravel the complex interaction between PA, physical fitness, and the gut microbiome.

Author Contributions

R.K.A., S.F., J.S.T., A.S., F.B.O., and S.A. conceived and designed the study; I.P.-P., J.H.M., and F.B.O. conducted the compositional data analyses; I.P.-P., N.M.M., A.S.-L., E.S.-E., K.L., and E.O. performed the microbiome analyses; R.K.A., M.Nu., M.Ni., and T.T.P. participated in data generation; I.P.-P., J.H.M., E.S.-E., M.Nu., E.O., S.F., J.S.T., A.S., T.T.P., F.B.O., and S.A. interpreted the results; I.P.-P., J.H.M., F.B.O., and S.A. drafted the manuscript. All authors reviewed the manuscript draft for important intellectual content. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Individual-level 16S RNA sequencing data are submitted in the Sequence Read Archive (SRA) under the reference PRJNA669650 (https://trace.ncbi.nlm.nih.gov/Traces/sra/?study=SRP287519; https://www.ncbi.nlm.nih.gov/bioproject/669650). NFBC data are available from the University of Oulu, Infrastructure for Population Studies. Permission to use the data can be applied for research purposes via electronic material request portal. In the use of data, we follow the EU general data protection regulation (679/2016) and Finnish Data Protection Act. The use of personal data is based on cohort participant's written informed consent at his/her latest follow-up study, which may cause limitations to its use. Please, contact NFBC project center (nfbcprojectcenter@oulu.fi) and visit the cohort website (www.oulu.fi/nfbc) for more information.

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