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Article

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Individualized and temporally stable gut microbiota across training and peak-performance phases in elite Polish athletes: a case-series pilot study

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Keywords: gut microbiota; elite athletes; longitudinal; 16S rRNA; individuality; temporal stability; training periodization; PICRUSt2

Abstract

Introduction: The gut microbiota of elite athletes has been characterized largely through cross-sectional, athlete-versus-control comparisons, leaving open whether microbiota features are stable or fluctuate within individuals across a competitive season. We performed a repeated-measures, within-athlete longitudinal characterization of 11 elite Polish track-and-field athletes (national-team members; endurance and strength/speed-power disciplines), collecting repeated fecal samples aligned to athlete-specific training-period blocks (preparatory, competition, and peak-performance) rather than to fixed calendar dates.

Methods: Gut microbiota composition was profiled by 16S rRNA (V3–V4) sequencing, yielding 1,438 ASVs, with inferred functional potential from PICRUSt2. Because the design targets within-athlete dynamics, analyses used linear mixed-effects models with athlete as a random effect.

Results: Alpha diversity was dominated by stable, athlete-specific signatures: between-athlete variation accounted for over 40% of the total variance (intraclass correlation = 0.43), and Shannon and Simpson indices did not differ significantly across training periods, sport types, or their interaction. Beta-diversity analyses reinforced this individuality: athlete identity

explained 59% of the variation in community composition (PERMANOVA on Bray-Curtis distances, $R^2 = 0.585$, $P = 0.0001$), within-athlete dissimilarities were consistently lower than between-athlete dissimilarities, and neither training period nor any transition category (direction, or entering/leaving peak performance) was significantly associated with community structure or turnover. Against this stable background, exploratory analyses identified a nominal decrease in Shannon diversity on leaving the peak-performance phase and candidate genus- and pathway-level shifts that differed by sport type but did not survive multiple-testing correction.

Conclusions: Collectively, the elite-athlete gut microbiota appears strongly individualized and globally resilient across a periodized season, with any phase-related responses emerging as selective, athlete-specific changes rather than community-wide restructuring. Larger longitudinal studies using shotgun metagenomics, metabolomics, and more detailed performance and recovery measures are warranted.

Introduction

The gut microbiota is increasingly recognized as a major regulator of host physiology, with effects extending beyond the intestinal lumen to energy metabolism, immune and inflammatory signaling, epithelial barrier integrity, nutrient handling, and systemic homeostasis across the life course. Regular exercise likewise challenges systemic homeostasis and elicits coordinated multiorgan responses involving immunity, metabolism, and the microbiome-gut-brain axis, making the intestinal microbiota a biologically plausible mediator of training adaptation, recovery, and health in athletes [1,2]. This concept is particularly relevant in elite sport, where high training loads, repeated physiological stress, and tightly controlled nutritional strategies may jointly shape the gut ecosystem. Although diet remains one of the strongest determinants of microbial composition, exercise type, intensity, and duration may also modulate microbial communities and their metabolic potential through altered hormone release, substrate availability, gastrointestinal transit, and redistribution of blood flow away from the gastrointestinal tract during exercise [3–6].

Many studies indicate that athletes harbor gut microbiota profiles that differ from those of less active or sedentary individuals, and that these differences may be even more evident at the functional and metabolic level than at the taxonomic level [3,7,8]. In professional athletes, these alterations have been linked to enrichment of pathways related to amino acid biosynthesis, carbohydrate metabolism, and microbially derived short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, supporting the idea that exercise-associated microbiome remodeling may have consequences for both performance and health (Barton et al., 2018). A comprehensive metagenomic meta-analysis similarly showed that an athletic lifestyle is associated with enrichment of SCFA-producing taxa and with high-impact microbial biosynthetic functions related to vitamin B12, amino acid derivatives, and other metabolites potentially relevant to cardiometabolic health [9]. These observations support the concept that the athlete microbiome should be considered not only in compositional terms, but also in relation to its broader metabolic potential [3,9]. In this context, pathway-level analyses may complement taxonomic profiling by providing insight into the metabolic processes that accompany exercise-associated microbiome remodeling, particularly in longitudinal designs.

Despite these shared features, increasing evidence indicates that the athlete microbiome is not uniform across athletic populations and may vary according to sport-specific demands. In elite Irish athletes, both the composition and functional capacity of the gut microbiome, as well as microbial and host-derived metabolite profiles, differed across sports-classification groups despite the absence of significant dietary differences, suggesting that training type or load may contribute independently to microbiome variation [10]. Similarly, a large multi-cohort study across aerobics, wrestling, and rowing demonstrated sport-

specialized gut microbial configurations, with distinct microbial subgroups associated with inflammation, diet, anthropometric characteristics, anaerobic measurements, and sex-dependent effects of sport type and exercise intensity [11]. Comparisons between endurance– and strength-oriented athletes further suggest that genus– and species-level microbial differences may be present even when alpha- and beta-diversity do not differ significantly, and that these patterns are shaped by the interaction between training mode and sport-specific dietary practices [10,12]. Thus, athlete microbiota profiles likely emerge from the interaction between exercise modality and sport-specific nutritional practices rather than from exercise exposure alone.

Potentially beneficial training-associated adaptations coexist with substantial gastrointestinal stress, particularly during prolonged or intense endurance exercise. Such exercise can reduce splanchnic blood flow, increase intestinal hypoxia and oxidative stress, disrupt epithelial tight junctions, elevate intestinal permeability, and promote the translocation of bacterial products into the circulation. These responses are accompanied by gastrointestinal symptoms, inflammatory activation, and impaired nutrient handling during the post-exercise period, and may therefore affect both recovery and performance [13]. Li et al. [11] further showed that athlete microbial subgroups were associated with inflammatory markers and that these relationships differed by sport type and sex, supporting the view that microbiota-based signatures may reflect not only training adaptation but also susceptibility to exercise-associated inflammatory stress.

Recent matched-subgroup work in German elite athletes further supports the presence of athlete-associated gut microbiome signatures, including differences in community structure and predicted metabolic potential related to amino acid metabolism, glycolysis, fatty acid β-oxidation, and quinone biosynthesis [14]. However, this evidence remains primarily cross-sectional and based on athlete-control comparisons, leaving unresolved whether such microbiome features remain stable or fluctuate within elite athletes across training and peak-performance phases.

Longitudinal studies in elite athletes remain limited, but the studies available suggest that global community metrics may remain relatively stable even when specific taxa change over time. However, most longitudinal studies to date have focused on single disciplines or isolated changes in training load, and relatively few have examined microbiota dynamics in relation to explicit transitions into and out of peak-performance phases within the same athletes. In highly trained middle-distance runners, alterations in training volume did not significantly affect alpha diversity or global microbial composition, although several lower-taxonomic changes were detected and persisted after taper [15]. Akazawa et al. [16] showed that periodization was associated with shifts in genera such as *Prevotella*, *Bifidobacterium*, *Parabacteroides*, *Alistipes*, *Bacteroides*, and *Blautia*, and that some of these changes were

associated with aerobic capacity and tended to relate to anaerobic power. Similarly, in elite volleyball athletes, alpha diversity remained stable despite marked inter-individual variation, whereas selective temporal taxonomic changes were observed across training, competition, and recovery [17]. More recent work in highly trained rowers linked high versus low training load to differences in stool frequency, SCFA concentrations, Bacteroidia abundance, and the Bacillota/Bacteroidota ratio, together with a tendency toward lower Shannon diversity during higher training load [18]. In summary, these studies indicate that athlete microbiota may show relative global stability against a strong background of subject-specific microbial signatures, while still undergoing biologically meaningful restructuring in response to training load, periodization, and recovery. This approach is particularly relevant when the dominant signal is the inter-individual microbiota structure, whereas differences between sports and phase-related effects are expected to be more moderate and potentially detectable mainly at lower taxonomic or pathway levels.

Mechanistic studies also support a potential direct contribution of specific microbes to exercise performance. [19] showed that exercise increased the abundance of *Veillonella* in marathon runners and demonstrated that *Veillonella atypica* enhanced treadmill run time in mice through conversion of exercise-derived lactate into propionate. These data reinforce the idea that the microbiome may be relevant not only as a biomarker of training status, but also as a contributor to exercise metabolism and performance capacity.

Published findings are still heterogeneous, and inconsistencies between studies likely reflect differences in sport discipline, cohort composition, sex, diet, enterotype structure, training history, and sampling design. In addition, relatively few studies have examined elite athletes longitudinally across clearly defined training phases, and even fewer have linked temporal microbiota dynamics to performance-relevant periods within the same individuals. These gaps are particularly relevant in track and field, where athletes competing in sprint, hurdle, race walking, marathon, and other long-distance disciplines are grouped within one broad sport but are exposed to markedly different metabolic demands, training structures, and competition schedules. A longitudinal analysis spanning multiple phases of preparation and competition may therefore help disentangle the extent to which gut microbiota variation reflects stable athlete-specific signatures, sport specialization, or more selective changes associated with transitions into and out of peak-performance.

Therefore, using a repeated-measures, within-athlete longitudinal design in a rare and difficult-to-expand elite cohort, the aims of the present study were:

(i) to characterize the temporal stability and individuality of gut microbiota diversity and composition across preparatory, competition, and peak-performance phases in elite Polish athletes; and

(ii) as exploratory, hypothesis-generating analyses, to assess whether microbiota dynamics differ according to sport type and according to transitions between phases, particularly transitions into and out of the peak-performance period; and (iii) to verify the feasibility of the study protocol - the scheme of collecting repeated fecal samples aligned to individual training-period blocks - as a basis for designing a study in a larger cohort of athletes.

Materials and methods

Study design

Athletes

The study included 11 elite Polish elite and highly trained/elite athletes (Tier 4 or 5 according to [20] (Table 1), all members of the Polish national team, including 3 women and 8 men. Inclusion criteria were membership of the national athletics team resulting in their periodic health evaluation (PHE) at the National Centre for Sports Medicine in Warsaw.

Table 1. Athletes' characteristics.

Athlete	Sex	Age, years	Weight, kg	Height, cm	BMI, kg/m^2	Sport type	Sport discipline	Tier
1	F	24	60.7	174	19.98	Strength	400m sprint	5
2	F	21	56.5	168	20.02	Strength	400m sprint	4
3	F	21	54.9	170	20.55	Strength	110m hurdles	5
4	M	26	79.6	193	21.37	Strength	400m sprint	5
5	M	24	120.5	191	33.03	Strength	Discus	4
6	M	25	112.0	193	30.07	Strength	Discus	4
7	M	18	69.1	182	20.86	Endurance	Middle distance	4
8	M	21	66.2	178	20.89	Endurance	Middle distance	4
9	M	33	73.1	185	21.36	Endurance	Marathon	4
10	M	37	73.0	179	22.78	Endurance	Race walking	4
11	M	37	71.0	174	23.45	Endurance	Race walking	4

Athletes 6 and 9 were excluded from all downstream analyses (fewer than three longitudinal samples and incomplete dietary records, respectively); analyses are therefore based on the remaining nine athletes.

These screenings are mandatory for members of national teams, which ensured access to a representative population of professional athletes. Participation in the study was voluntary. Exclusion criteria were absence of consent to participate and lack of organisational capacity to complete the protocol. Based on the type of sports competition athletes were assigned to two sport-type groups: Endurance (predominantly aerobic, high dynamic and low static component) and Strength (predominantly anaerobic, low dynamic and high static component).

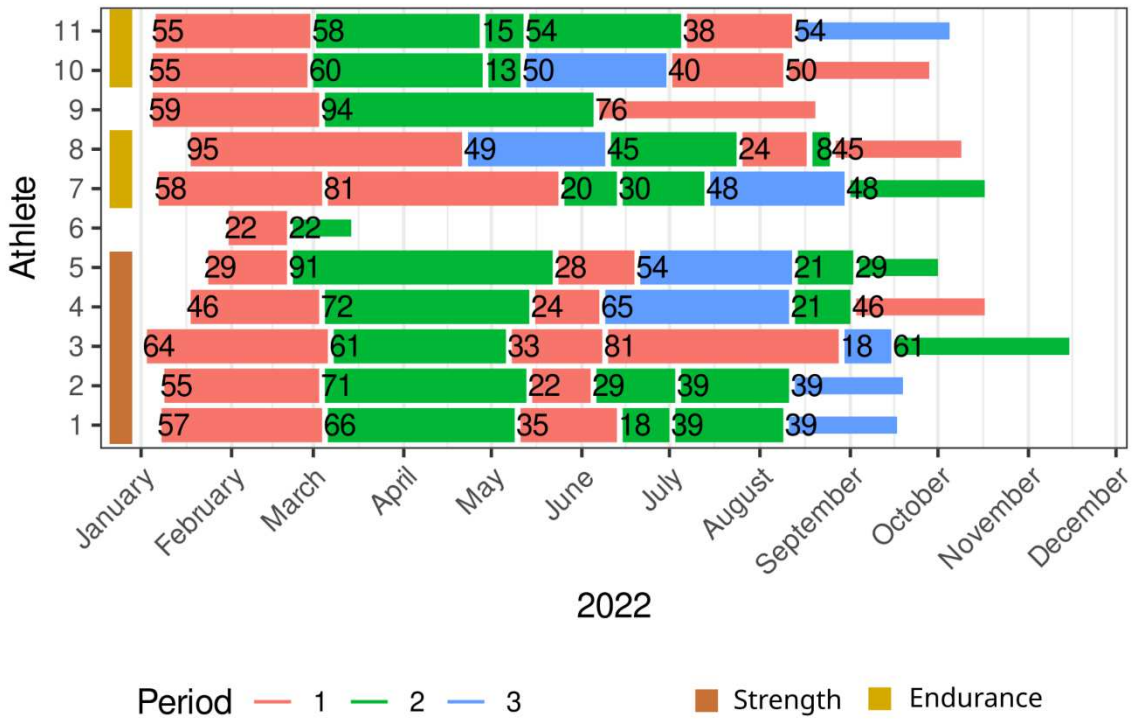
The study protocol was reviewed by the Bioethics Committee of the Pomeranian Medical University in Szczecin, which determined that formal ethics approval was not required

(decision no. KB.006.75.2024). The study was conducted in accordance with the Declaration of Helsinki, and all participants provided written informed consent before enrolment.

Sampling schedule and training-period classification

Biological material was collected longitudinally across the athletic season. Sampling was planned to capture three major training and competition contexts: Period 1, corresponding to the preparatory period; Period 2, corresponding to the competition period; and Period 3, corresponding to the competition period associated with the athlete's best starting condition or peak performance. Because the timing and duration of these phases differed between athletes according to individual training plans, event specialization, and competition schedules, samples were aligned to athlete-specific training-period blocks rather than to fixed calendar dates. The designation of these phases, and the assignment of each sample to a phase, was made by the coaching staff responsible for the athlete. The longitudinal structure of these training-period blocks across athletes is shown in Figure 1.

Figure 1. Temporal distribution of preparatory, competitive, and peak-performance phases across athletes.



Each horizontal segment represents a continuous training-period block (Period) within an individual athlete. The end of each block was set to the start date of the next block. For the final block of each athlete (thinner segment), duration was estimated by adding the athlete-specific median sampling interval to the last observed sampling date, accounting for uneven sampling frequency. Segment color denotes training-period type: Period 1, preparatory period; Period 2, competition period; and Period 3, competition period associated with best starting condition/peak performance. The end of each block was defined by the start date of the subsequent block. For the final block of each athlete, shown as a

thinner/dashed segment, duration was estimated by adding the athlete-specific median sampling interval to the last observed sampling date to account for uneven sampling frequency. Numbers displayed within segments indicate block duration, with values for the final block representing estimated duration.

Dietary assessment

Dietary data were collected using a 3-day prospective food record. The records were completed over three consecutive days in the weeks preceding each fecal sample collection for gut microbiota analysis. In the 3-day food records, participants noted every meal and beverage consumed, including the time of intake and the amount eaten. Before starting the recordings, all athletes received written and verbal instructions on how to describe foods and estimate portion sizes as precisely as possible. When portion sizes were reported using household measures, the written records were supplemented with meal photographs sent by the athletes. These photographs helped to refine portion size estimates and to verify the amounts of foods actually consumed, following the principles of the Remote Food Photography Method described by Martin et al. [21]. In cases of uncertainty regarding portion size, dietary records were verified by using an album of photographs of food products and dishes, which contained three suggested portion sizes.

All completed food records were reviewed by a dietitian, and any unclear or missing entries were discussed with the participants. After this verification step, the dietary data were entered into the Dieta 5 software (National Food and Nutrition Institute, Warsaw, Poland), which is based on Polish food composition tables [22], to calculate daily energy and nutrient intake. The analyzed dietary data included mean daily intakes of macronutrients, such as total carbohydrates and sugars, protein, and fat. In addition, the analysis covered selected dietary components, including dietary fibre, fatty acids, and cholesterol, as well as micronutrients such as sodium, potassium, folate, and vitamins from the B group, vitamin D, and vitamin C, in order to describe the overall diet composition of the athletes. The obtained values were then compared with dietary reference values for the Polish population [22] and, where applicable, with nutritional recommendations for athletes [23]. Because no statistically significant differences were found between repeated measurements taken within the same phase of the training season, the dietary data were averaged. For further analyses, results were grouped according to the phase of the training cycle: preparatory period (Period 1, P1), competition period (Period 2, P2), and peak performance period (Period 3, P3).

Microbiota composition

DNA extraction and 16S rRNA sequencing

DNA was extracted using the QIAmp PowerFecal Pro DNA kit (Qiagen), following the manufacturer's instructions, with additional mechanical disruption by bead-beating homogenization on the PowerLyzer 24 device (Qiagen), and the V3–V4 hypervariable regions of the 16S rRNA gene were amplified using PCR. The resulting amplicons were sequenced on an Illumina platform (Illumina, San Diego, USA) with a 2×250 bp paired-end protocol following the standard in-house procedure.

Raw sequences processing, taxonomic assignment and data processing

An initial quality check was performed using FastQC (v0.12.1) and summarized with MultiQC (v1.23). After this step, the sequencing data was processed through the LotuS2 (v2.32) pipeline [24]. Quality filtering and dereplication were carried out using the sdm module tailored for the MiSeq Illumina platform, with a slightly reduced minimum sequence length. Within LotuS2, sequence clustering into ASVs (Amplicon Sequence Variants) was achieved using the DADA2 algorithm [25]. To remove chimeric sequences, both reference-based and *de novo* detection methods were applied using the RDP reference database (rdp_gold.fa) with UCHIME3. By default, ASVs were aligned against the phiX genome using Minimap2 [26], and any sequences showing significant similarity were excluded. Reads that matched the human reference genome (Homo_sapiens.GRCh38.dna.toplevel.fa; release 2025-07-07) were also filtered out to eliminate host contamination.

The dataset was further refined using the LULU algorithm [27]**,** which curated sequence clusters. The remaining ASVs were aligned using Lambda v3.0.0 [28] against the SILVA 138.1 SSU [29] and KSGP 3.1 [30] databases to assign taxonomy through LotuS2's default Least Common Ancestor (LCA) method. ASVs that were assigned to categories such as Eukaryota, Archaea, mitochondria, and chloroplasts were included in the dataset. Unassigned taxonomic levels were assigned to the last known taxonomic level and sequentially numbered. In order to minimize the inflation of rare species in the community analysis, we removed ASVs that were not present more than two times in at least 10% of the samples. The filtered ASV matrix was used as input to PICRUSt2 [31] with default settings to infer KEGG Ortholog (KO) abundances and MetaCyc pathway profiles. To minimize over-interpretation from distant phylogenetic placement, we applied an NSTI quality filter and excluded ASVs with NSTI >0.15 a priori; among retained ASVs, the cohort-level NSTI was 0.06 ± 0.02 (mean ± SD), supporting close reference genomes and acceptable prediction reliability. Alpha-diversity - vegan package (version 2.7-2) 'diversity' function - Shannon and Simpson indices on ASV table (non rarefied).

Statistical analysis

To assess whether gut microbial alpha diversity differed across training periods, we fitted linear mixed-effects models with athlete identity as a random intercept to account for repeated sampling within individuals. Community-level (beta-diversity) differences were tested by permutational multivariate analysis of variance (PERMANOVA; adonis2, vegan) on ASV-level Bray-Curtis and Aitchison distance matrices with 9,999 permutations. Aitchison distances were computed as Euclidean distances on centered log-ratio values calculated across features within each sample. Because samples were collected repeatedly from the same athletes, permutations were restricted to respect the longitudinal design (permute package): the within-athlete factor (training period) was tested by permuting samples only within athletes, whereas the between-athlete factor (sport type) was tested by permuting whole athletes and never samples within them. Athlete identity was tested using unrestricted permutation, which is valid under the null hypothesis that samples are exchangeable across individuals, and the corresponding R^2 was used to quantify the contribution of individuality to community variation. As a complementary test, the period effect was also assessed by partial distance-based redundancy analysis conditioning on athlete identity. Homogeneity of multivariate dispersion across periods was examined using betadisper function. Because sport type is constant within an athlete and only nine athletes were analyzed, the between-athlete test has limited resolution (126 distinct athlete-to-sport assignments; minimum attainable $P = 0.008$). An equivalent restricted-permutation approach has recently been applied to longitudinal athlete microbiome data, in which residuals were shuffled only within each athlete for within-subject terms while the between-subject athlete effect was tested under free permutation, on the grounds that its exchangeable unit is the whole subject [32].

We next examined within-athlete changes in Shannon and Simpson diversity between consecutive samples. For each athlete, the change in diversity was calculated as the difference between successive time points (later period - earlier period). Two complementary transition frameworks were evaluated. First, transitions were categorized according to the direction of change between training periods (“Transition direction”), distinguishing forward transitions (movement to a later period), backward transitions (movement to an earlier period), and transitions occurring within the same period. We also evaluated transitions associated with entering (Entering P3) or leaving this phase (Leaving P3) - peak athletic performance - “Peak Transition”.

Transition driver analysis

Genus- and pathway-level centered log-ratio values, used for the heatmaps and for the transition-driver analyses, were likewise computed across features within each sample. Transition driver analysis was used to identify taxa/pathways showing the strongest changes

across peak-related transition categories. The analysis was performed separately for each sport group. First, subject-level median change values were calculated for each taxon/pathway within each Peak Transition category (Entering P3, Leaving P3, or Other; only one Entering P3 or Leaving P3 was present in each athlete, a subject-level aggregation occurred only across transitions in the Other category). These subject-level medians were then aggregated across subjects to obtain, for each taxon/pathway, the median value within each transition category. To summarize transition-related differences, effect estimates were calculated from contrasts between category medians, including Entering P3 vs Leaving P3 and Other vs non-Other transitions. Overall differences among the three transition categories were assessed using the Kruskal-Wallis rank-sum test. Multiple-testing correction was performed using the Benjamini-Hochberg method, and adjusted P values were transformed as -log10 (adjusted P). Each taxon/pathway was further assigned to a dominant transition class, defined as the category with the highest median value across the three transition groups: Entering P3, if the Entering P3 median exceeded both the Leaving P3 and Other medians, Leaving P3, if the Leaving P3 median exceeded both the Entering P3 and Other medians, and Other otherwise. For ranking and visualization, a driver score was computed as the product of the absolute effect size for the Entering P3 vs Leaving P3 contrast and the -log10(adjusted P) value. The top-ranking features were displayed as transition driver plots.

Results

Cohort characteristics and longitudinal sampling structure

The study included 11 elite Polish track-and-field athletes, comprising 3 women and 8 men. The mean age of the cohort was 26.1 ± 6.6 years, with a mean body mass of 76.1 ± 21.3 kg, height of 180.6 ± 9.0 cm, and BMI of 23.1 ± 4.3 kg/m². Based on event characteristics, 6 athletes were classified into the strength/speed-power group and 5 into the endurance group. The strength/speed-power group included 400 m sprinters, a hurdle athlete, and discus throwers, whereas the endurance group included middle-distance runners, a marathon runner, and race walkers. The sport-type grouping was not sex-balanced, as all female athletes were included in the strength/speed-power group and all endurance athletes were male. This event-level heterogeneity was retained intentionally, as the study aimed to evaluate whether longitudinal microbiota dynamics differed between athletes exposed to distinct physiological and training demands within the same broad sport.

Dietary intake across training-period phases

Dietary data suitable for analysis were available for 9 of 11 athletes (athletes 6 and 9 were excluded from this analysis because of incomplete dietary records). Mean daily energy intake was 2461 ± 588 kcal during the preparatory period, 2393 ± 350 kcal during the

competition period, and 2337 ± 380 kcal during the best-start/peak-performance period. Macronutrient distribution was broadly comparable across phases, with protein contributing approximately 20% of total energy intake, fat 28–30%, and carbohydrates 50–53%. Mean carbohydrate intake ranged from 322 to 338 g/day across phases, protein intake from 115 to 119 g/day, and fat intake from 73 to 84 g/day. Dietary fiber intake was 31.4 ± 8.8 g/day during the preparatory period, 30.5 ± 9.1 g/day during the competition period, and 35.3 ± 8.1 g/day during the best-start/peak-performance period. Individual dietary intake values and selected micronutrient intakes are presented in Supplementary Table S1.

Gut microbiota diversity and composition across training periods

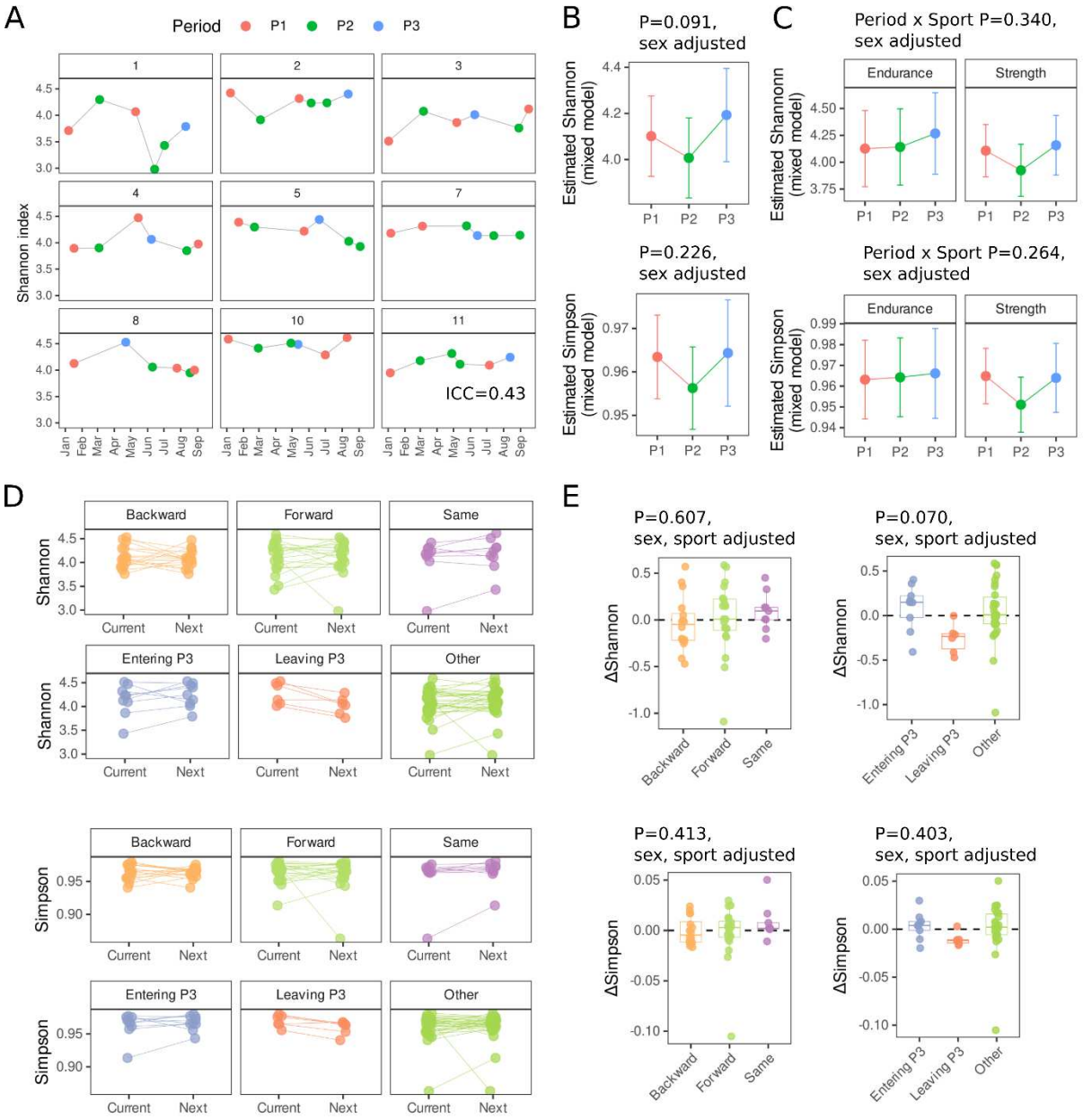
There were 1438 ASVs (the ASV abundance and prevalence are presented in Supplementary Figure 1). First we investigated whether period affects both within-sample alpha diversity. All microbiome analyses were restricted to the nine athletes with at least three longitudinal samples (54 samples; athletes 6 and 9 excluded), comprising five strength/speed-power and four endurance athletes; as in the full cohort, sport type remained completely confounded with sex, as all women belonged to the strength/speed-power group. Figure 2A presents individual Shannon-diversity trajectories over time for each athlete. Variance components indicated substantial between-athlete variability (0.040) relative to within-athlete residual variance (0.054), corresponding to an intraclass correlation, ICC = 0.43. Despite a high degree of within-athlete variability, it did not align with a training period. Shannon (Figure 2B, top) and Simpson (Figure 2B, bottom) diversity indices did not differ significantly between periods (likelihood ratio test, P=0.091, P=0.226, respectively). Compared with Period 1, diversity showed a non-significant decrease during Period 2 (Shannon: $\beta = -0.094$, standard error (SE) = 0.068, P = 0.171, Simpson: $\beta = -0.007$, SE = 0.005, P = 0.137) and a slight increase during Period 3 (Shannon: $\beta = 0.092$, SE = 0.089, P = 0.309, Simpson: $\beta = 0.0009$, SE = 0.006, P = 0.885, model-based estimates, Figure 2B).

Strength athletes tended to show lower Shannon diversity than endurance athletes (Shannon: $\beta = -0.117$, SE = 0.179, Simpson: $\beta = -0.005$, SE = 0.009), although this difference was not statistically significant (Wald t-test: P = 0.539 and P = 0.607 for Shannon and Simpson, respectively; likelihood-ratio test for the sport main effect: P = 0.433 and P = 0.512). Likewise, we found no significant interaction between training period and sport discipline (Shannon: LRT P = 0.340, Simpson: LRT P = 0.264), suggesting that period-related diversity changes were broadly similar across sport types (Figure 2C).

We next examined within-athlete changes in Shannon and Simpson diversity between consecutive time points (see Methods). Among 45 changes between training periods, 16 changes were backward transitions, 20 forward transitions, while 9 were the same period changes. Nine transitions were toward the peak performance period (Entering P3) and 6 were

from the P3 to other periods (Leaving P3, Figure 2D, Supplementary Table S2). No significant differences in ΔShannon were observed between transition directions. Compared with “same” transitions (reference), forward transitions were not associated with significant diversity changes (β = -0.085 ± 0.130, P = 0.516), and backward transitions also showed no significant effect (β = -0.133 ± 0.135, P = 0.331, Figure 2E, top/left). Because Period 3 corresponded to the phase of peak athletic performance, we next specifically evaluated transitions associated with entering or leaving this phase. Compared to “other”, entering the peak-performance phase was not associated with a significant change in Shannon diversity (β = 0.053 ± 0.117, P=0.652). Overall, peak-related transition category was not significantly associated with ΔShannon (likelihood-ratio test, P = 0.070). In an exploratory, uncorrected contrast based on only six “Leaving P3” transitions, leaving Period 3 showed a nominal decrease in Shannon diversity relative to other transitions (β = −0.298 ± 0.138, P = 0.036; Figure 2E**,** top/right). No significant changes were observed in the Simpson metric (Figure 2E, bottom). Because this contrast was uncorrected for multiple testing and rested on few transitions, it should be regarded as hypothesis-generating rather than as evidence of a robust post-peak effect, and would require confirmation in larger cohorts.

Figure 2. Gut microbiome α-diversity across the training season in elite athletes.



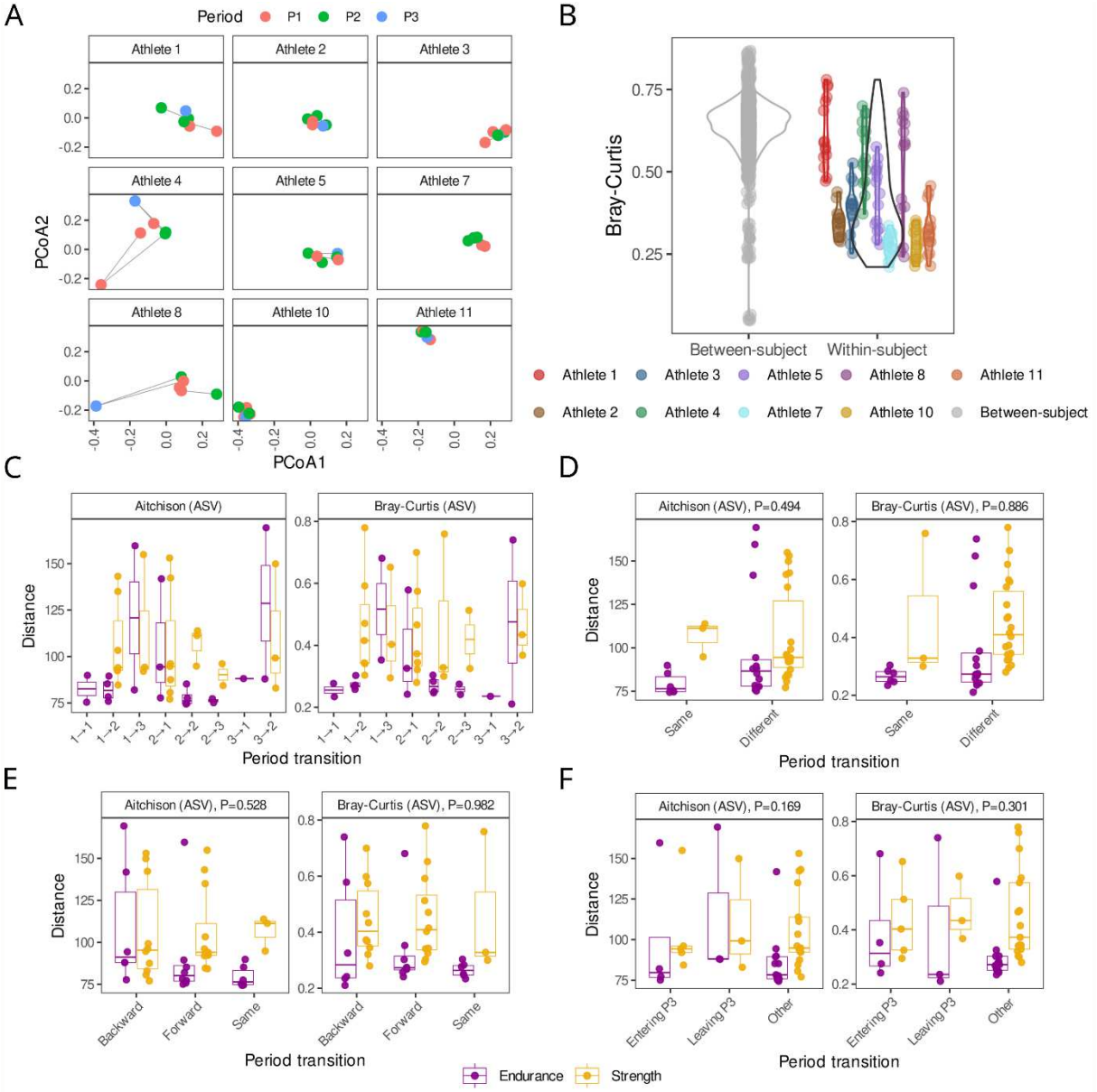
Shannon and Simpson indices were computed on non-rarefied ASV counts (vegan). Analyses include 54 samples from 9 athletes; two athletes with fewer than three longitudinal samples (subjects 6 and 9) were excluded. Samples are grouped into three training periods: preparatory (P1), competitive (P2) and peak-performance (P3), with n = 22, 23 and 9 samples, respectively. (A) Individual longitudinal trajectories of the Shannon index for each athlete (one panel per subject), plotted against sampling date. Points are colored by training period (P1–P3) and grey lines connect consecutive samples within an athlete, illustrating the marked between-subject differences and within-subject fluctuation in α-diversity over the season. (B) Period-level effect on α-diversity estimated from linear mixed-effects models (index ~ Sex + Period + (1 | SubjectID)), shown for Shannon (top) and Simpson (bottom). Points are estimated marginal means and error bars are 95% confidence intervals. Diversity did not differ significantly across periods (likelihood-ratio test vs. the Sex-only model: Shannon P = 0.091, Simpson P = 0.226); (C) The same period contrast stratified by sport discipline (Endurance vs. Strength), from models including a Period × Sport interaction (index ~ Sex + Period × Sport1 + (1 | SubjectID)), for Shannon and Simpson. Points and error bars are estimated marginal means ± 95% CI. Neither the sport main effect (Shannon P = 0.433, Simpson P = 0.512) nor the Period × Sport interaction (Shannon P = 0.340, Simpson P = 0.264) was significant. (D) Within-athlete, paired sample-to-sample transitions in α-diversity (Shannon and Simpson). Each pair of consecutive samples from the same athlete is drawn

as a "Current → Next" line, faceted both by relation to the peak-performance period (Entering P3 / Leaving P3 / Other) and by direction of movement between periods (Forward / Backward / Same), highlighting how diversity shifts around the peak-performance period. (E) Distribution of the per-transition change in diversity (Δ = index_next − index_current) for Shannon and Simpson, shown across two complementary classifications of each transition: by relation to the peak-performance period (Entering P3 / Leaving P3 / Other) and by direction of movement between periods (Forward / Backward / Same). Boxplots show the median and interquartile range (outliers hidden) with individual transitions overlaid as points; the dashed line marks no change (Δ = 0). Changes did not differ significantly for either classification (LRT in Δindex ~ Sex + Sport1 + group + (1 | SubjectID): by peak transition, ΔShannon P = 0.070, ΔSimpson P = 0.403; by transition direction, ΔShannon P = 0.607, ΔSimpson P = 0.413).

Figure 3A presents PCoA ordination of individual athlete samples based on ASV-level Bray-Curtis beta diversity. Athletes 1, 4, and 8 showed the most pronounced shifts in microbiome composition across training periods, whereas the remaining athletes displayed relatively limited changes. Accordingly, within-individual dissimilarities were generally lower than between-individual dissimilarities (Figure 3B), with the exception of these three athletes. Consistent with this, PERMANOVA attributed the largest share of community variation to athlete identity (Bray-Curtis: $R^2 = 0.585$; Aitchison: $R^2 = 0.546$; both P = 0.0001, unrestricted permutation), with a mean within-athlete Bray-Curtis dissimilarity of 0.41 compared with 0.64 between athletes. In contrast, training period explained approximately 2% of the variation and was not significant when permutations were restricted within athletes (Bray-Curtis: $R^2 = 0.020$, P = 0.540; Aitchison: $R^2 = 0.025$, P = 0.177), a result confirmed by partial distance-based redundancy analysis conditioning on athlete identity (P = 0.821 and P = 0.366, respectively). Sport type was likewise non-significant when whole athletes were permuted (Bray-Curtis: $R^2 = 0.071$, P = 0.498; Aitchison: $R^2 = 0.068$, P = 0.406), and multivariate dispersion did not differ between training periods (P = 0.354 and P = 0.520). To assess whether microbiome turnover differed across training period changes, within-athlete dissimilarities were further examined by transition category.

Within-athlete beta-diversity distances were analyzed using linear mixed-effects models with subjects included as a random effect. No significant differences were observed for Bray-Curtis or Aitchison distances when transitions were classified as “Same” versus “Different” (Bray-Curtis: P = 0.886; Aitchison: P = 0.494, Figure 3D), by transition direction (Bray-Curtis: P = 0.982; Aitchison: P = 0.528, Figure 3E, Supplementary Table S3), or by peak-related transition category (Bray-Curtis: P = 0.301; Aitchison: P = 0.169, Figure 3F, Supplementary Table S3).

Figure 3. Longitudinal β-diversity of the gut microbiome across the training season.

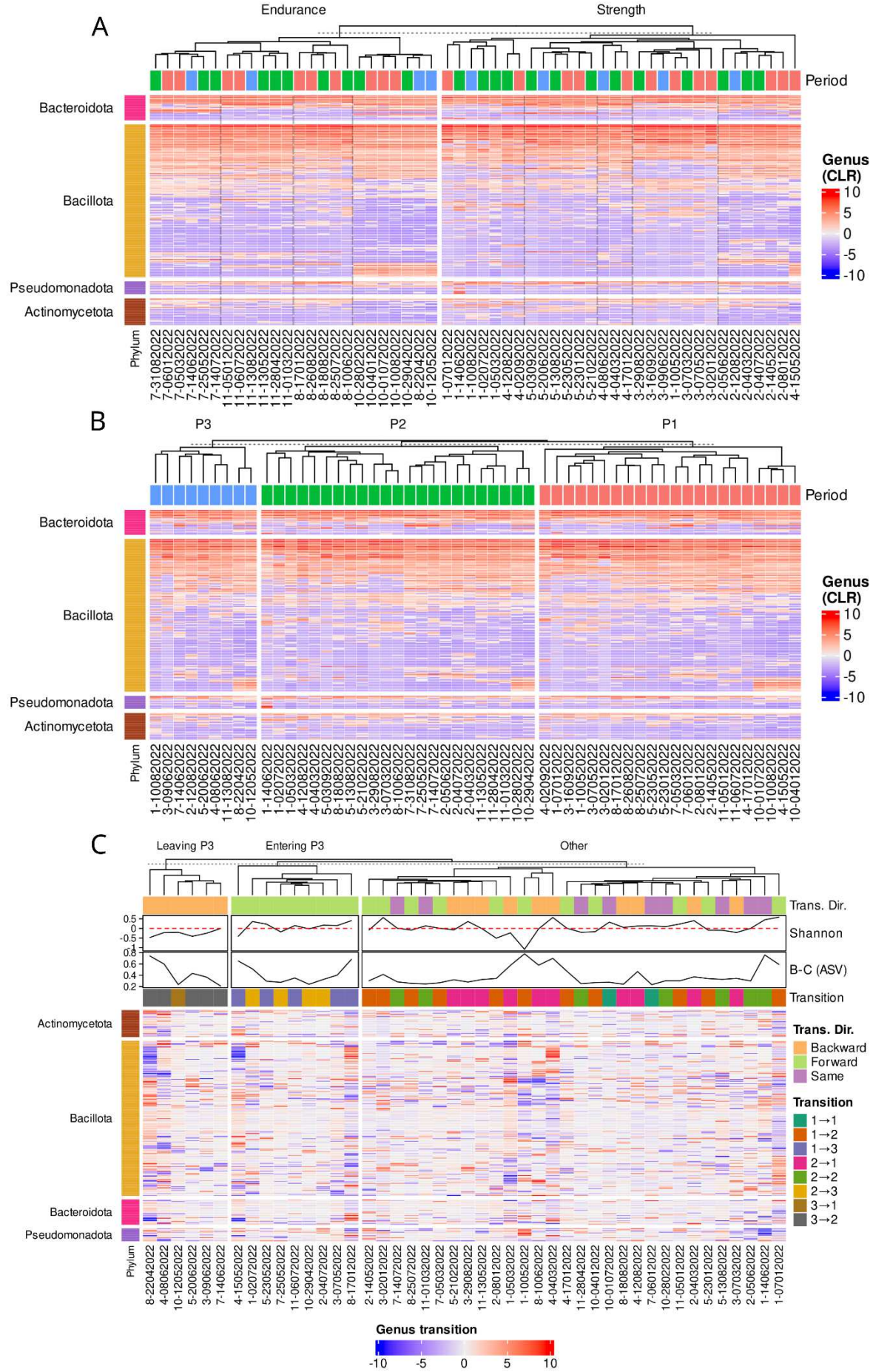


Analyses are based on 54 samples from 9 athletes (subjects 6 and 9, with fewer than three samples, excluded), using a prevalence-filtered ASV table. Two complementary dissimilarity metrics are used throughout: Bray-Curtis on relative abundances and Aitchison distance (Euclidean distance on centred log-ratio-transformed abundances). Training periods are preparatory (P1), competitive (P2) and peak-performance (P3); "i→j" denotes a transition between consecutive samples from period i to period j. (A) Principal coordinates analysis (PCoA; classical multidimensional scaling) of ASV-level Bray-Curtis distances, shown per athlete (one panel per subject). Points are individual samples coloured by training period, connected in sampling order to trace each athlete's longitudinal microbiome trajectory. Within-athlete movement is small relative to the separation between athletes, indicating strong individual specificity. (B) Violin plots of ASV-level Bray-Curtis dissimilarities for between-individual sample pairs versus within-individual pairs, the latter shown both pooled and separately for each athlete (colours). Within-individual dissimilarities are consistently lower than between-individual dissimilarities, quantifying individual microbiome stability. (C) ASV-level Aitchison and Bray-Curtis distances between consecutive within-athlete samples (boxplots: median and interquartile range, outliers hidden, individual transitions overlaid as points), grouped by the full period-transition category and coloured by sport type (Endurance, Strength). Panels (D–F) show the same distances after collapsing transitions into coarser categories: (D) "Same" (no period change; 1→1, 2→2) versus "Transition" (all changes); (E)

"Backward" (2→1, 3→1, 3→2), "Forward" (1→2, 1→3, 2→3) and "Same" (1→1, 2→2); and (F) "Entering P3" (1→3, 2→3), "Leaving P3" (3→1, 3→2) and "Other" (1→1, 2→2, 1→2, 2→1). In D–F, p-values shown in the facet strip labels are from likelihood-ratio tests comparing a linear mixed model with the grouping term against an intercept-only model, each with a per-athlete random intercept (distance ~ group + (1 | SubjectID)); none of the transition classifications was significantly associated with either distance metric.

Consistent with the beta-diversity analyses, genus-level CLR-transformed heatmaps (Figure 4) showed that microbiota structure was driven predominantly by athlete-specific clustering rather than by training period or transition category. The patterns described in this paragraph are based on inspection of the clustered heatmaps and were not tested statistically. In all heatmaps, only genera belonging to the four dominant phyla - Bacillota, Bacteroidota, Actinomycetota, and Pseudomonadota - were displayed, with rows split accordingly by phylum. In the overall heatmap (Figure 4A), columns were split by sport type (endurance vs strength), and although samples were still clustered mainly by athlete, modest differences between sport types were apparent, particularly among genera within Bacillota. With only minor exceptions for athletes 1, 4, and 8, this pattern also suggested relative stability of within-individual genus-level composition over time. When training periods were visualized separately (Figure 4B), genus-level profiles remained heterogeneous within each period and did not show a consistent period-specific pattern. Likewise, the transition-based heatmap (Figure 4C), annotated by transition direction, transition type, Shannon diversity, and within-subject Bray-Curtis distance, did not reveal a distinct genus-level pattern for Entering P3, Leaving P3, or Other transitions.

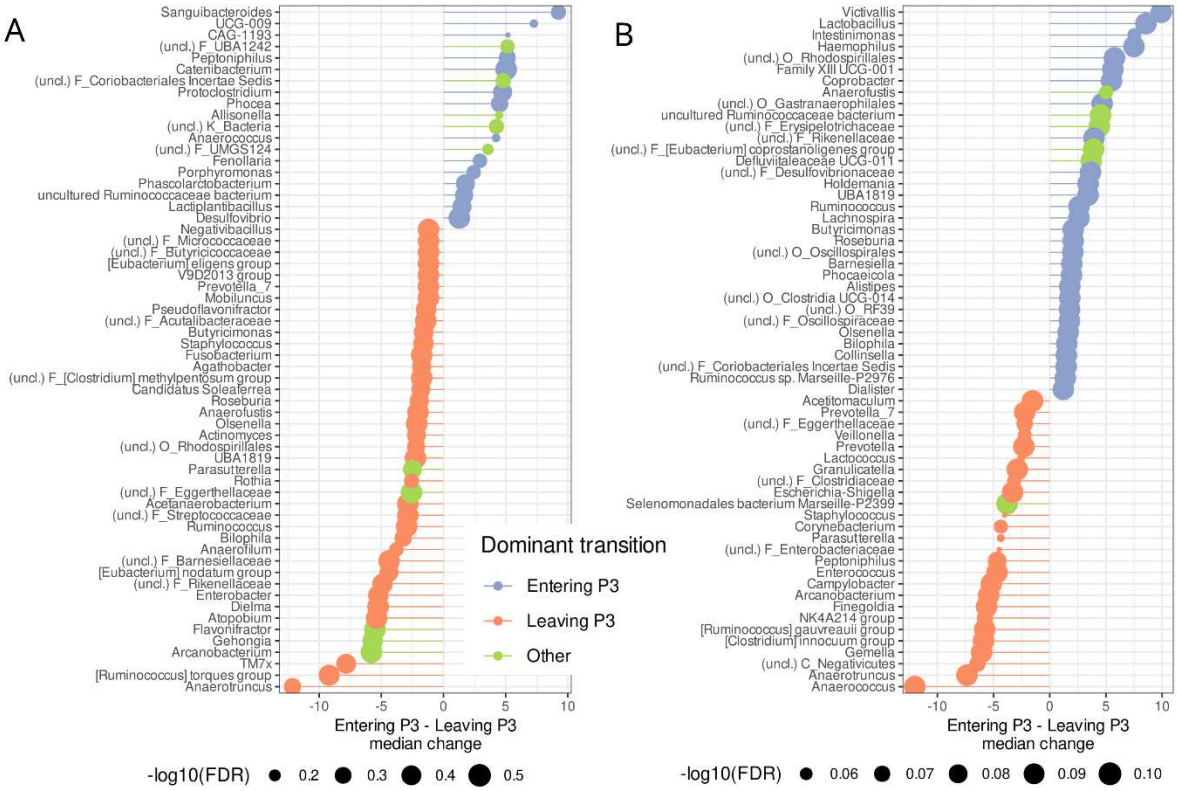
Figure 4. Genus-level CLR-transformed heatmaps across training periods and transition categories.


A
Endurance
Strength
Period
Bacteroidota
Bacillota
Pseudomonadota
Actinomycetota
Phylum
Genus (CLR)
10
5
0
-5
-10
B
P3
P2
P1
Period
Bacteroidota
Bacillota
Pseudomonadota
Actinomycetota
Phylum
Genus (CLR)
10
5
0
-5
-10
C
Leaving P3
Entering P3
Other
Trans. Dir.
Shannon
B-C (ASV)
Transition
Actinomycetota
Bacillota
Bacteroidota
Pseudomonadota
Phylum
Trans. Dir.
Backward
Forward
Same
Transition
1→1
1→2
1→3
2→1
2→2
2→3
3→1
3→2
Genus transition
-10
-5
0
5
10

CLR-transformed genus-level heatmaps of gut microbiota composition across athletes, training periods, and transitions after 5% prevalence filtering. Only genera assigned to the four dominant phyla - Bacillota, Bacteroidota, Actinomycetota, and Pseudomonadota - were included, with rows split by phylum in all panels. (A) - Overall genus-level heatmap with columns annotated by training period and split by sport type (endurance and strength). Samples clustered predominantly by athlete, with athlete-associated clusters separated by vertical dashed gray lines. The clusters for athletes 5, 7, 8, and 11 contained only samples from the corresponding athlete, whereas four clusters each included one or two samples from a different athlete: one athlete 4 sample within the athlete 2 cluster, one athlete 1 sample within the athlete 3 cluster, one athlete 8 sample within the athlete 10 cluster, and two athlete 4 samples within the athlete 1 cluster. Column names represent individual athlete samples. (B) - Genus-level heatmap shown separately for each training period (P1, P2, P3) using column splitting. Genus-level profiles remained heterogeneous within each period and did not show a consistent period-specific pattern across athletes. Column names represent individual athlete samples. (C) - Transition-based heatmap (a difference in CLR-transformed genus abundance) including Leaving P3, Entering P3, and Other transitions, with column annotations for transition direction, transition type, Shannon diversity, and within-subject Bray-Curtis distance. No distinct genus-level pattern was observed for Entering P3 or Leaving P3 transitions. Column names represent the first time point used to calculate genus-level transitions and Bray-Curtis distance. P1 - pre-season, P2 - in-season , P3 - in-season peak-performance period

Given the absence of clear global transition-related patterns in the beta-diversity and heatmap analyses, we next used transition driver plots to assess whether individual genera nevertheless contributed to specific transition dynamics. Genus-level transition driver analysis suggested distinct descriptive patterns in endurance and strength athletes (Figure 5**,** Supplementary Tables S4-S5). In endurance athletes (Figure 5A**,** Supplementary Table S4), Entering P3 was represented by genera such as *Sanguibacteroides*, *UCG-009*, *Peptoniphilus*, and *Anaerococcus*, whereas *Anaerotruncus*, the *[Ruminococcus] torques* group, and TM7x were more strongly associated with Leaving P3. In strength athletes (Figure 5B**,** Supplementary Table S5), the distribution across transition categories was more balanced, with *Victivallis*, *Lactobacillus*, *Haemophilus*, and *Intestinimonas* among the main Entering P3 genera, and *Anaerococcus*, *Anaerotruncus*, *Gemella*, and the *[Ruminococcus] gauvreauii* group among the main Leaving P3 genera. Several genera, including *Anaerococcus*, *Peptoniphilus*, *Roseburia*, and *Ruminococcus*, differed in dominant transition category between sport types, suggesting possible sport-type-dependent genus-level transition patterns.

Figure 5. Genus transition drivers in endurance and strength athletes.



Lollipop plots of the top 60 genus ranked by driver score, defined as the product of the absolute Entering P3 − Leaving P3 median difference and the -log10 adjusted P value (FDR). Analyses were performed separately for endurance (A) and strength (B) athletes. Genera were assigned to a dominant transition class according to the Peak Transition category (Entering P3, Leaving P3, or Other) with the highest median value across subjects. In each panel, segment length and point position represent the Entering P3 − Leaving P3 median difference, point size corresponds to -log10 (FDR), and color indicates dominant transition class. Size scales are panel-specific.

A similar approach was then applied to MetaCyc pathways by assigning each pathway to the transition category with the highest median value. In both endurance and strength athletes (Figure 6**,** Supplementary Tables S6-S7), dominant-class assignments were not strongly skewed toward either peak-related transition category. Because these assignments rest on contrasts between category medians that are frequently close to zero, their relative frequencies were unstable and are not interpreted further; only the most extreme-ranking features are described below. In endurance athletes (Figure 6A), pathways dominantly associated with Entering P3 were mainly related to amino-acid, fatty-acid, and energy metabolism, including L-tyrosine degradation I (TYRFUMCAT-PWY), petroselinate biosynthesis (PWY-5367), ergothioneine biosynthesis I (PWY-7255), and fatty-acid degradation pathways (PWY-561, PWY66-391, FAO-PWY). In strength athletes (Figure 6B), Entering P3 was more often represented by pathways involved in amino-acid degradation, carbohydrate metabolism, and central energy metabolism, including L-histidine degradation (PWY-5028), L-lysine degradation (PWY-6328), sucrose degradation (PWY66-373), and glycolysis/Entner–Doudoroff (GLYCOLYSIS-E-D). Notably, some pathways differed in dominant transition category between sport types, including L-tyrosine degradation I and

oleate β-oxidation, which were classified as Entering P3 in endurance but Leaving P3 in strength (TYRFUMCAT-PWY, PWY0-1337). Overall, these findings should be interpreted as descriptive, as no genus or pathway remained significant after multiple-testing correction, although more nominally significant associations were observed for pathway transitions than for genus-level transitions (Supplementary Tables S4–S7).

Discussion

In this longitudinal study of elite Polish athletes, we examined fecal microbiota diversity, composition, and inferred functional profiles across preparatory, competition, and peak-performance phases. The principal finding was that gut microbiota structure was dominated by strong athlete-specific signatures rather than by uniform changes across training periods. Alpha diversity did not differ significantly between periods, sport-type groups, or period-by-sport interactions, and within-athlete beta-diversity distances were not significantly associated with transition direction or peak-performance transition category. Nevertheless, an exploratory, uncorrected analysis suggested a nominal decrease in Shannon diversity on leaving the peak-performance period, and descriptive genus- and pathway-level transition analyses suggested that selected taxa and inferred metabolic pathways may differ between sport groups around peak-performance transitions. These findings indicate that the gut microbiota of elite athletes may remain globally stable across the training season while still showing selective, individualized changes at lower taxonomic and inferred pathway levels.

Two features of the present design affect how these findings should be read. First, the cohort comprised current members of a national team competing at the highest domestic level, a population that is small by definition and cannot be enlarged at will, the number of athletes therefore reflects the size of the accessible population rather than a sampling decision. Second, samples were aligned to each athlete's own training-period blocks rather than to fixed calendar dates, and those phases were designated by the coaching staff responsible for each athlete. Phase comparisons therefore reflect the periodization actually planned and recognised in practice for each individual, under ordinary training, travel and competition conditions. Together these features favour repeated observation of each athlete over the number of athletes available for comparison: contrasts between athletes or between sport groups are weakly powered, whereas the interpretations that follow rest principally on the measurements repeated within each athlete.

Inter-individual differences dominated the structure of this dataset: samples clustered mainly by athlete in both the ordination and the genus-level heatmaps (Figures 3A, 4A), with only a few athletes showing more pronounced longitudinal shifts (athletes 1, 4, 8). Athlete identity accounted for 58.5% of the variation in ASV-level Bray-Curtis composition (PERMANOVA, P = 0.0001) and 54.6% of the variation in Aitchison distance, while the subject intraclass correlation for Shannon diversity was 0.43. Comparable partitions have been reported in non-athlete cohorts: in a one-year study of 75 disease-free adults from the Swedish general population, only 23% of total compositional variance was attributable to intra-individual variation [33], and a two-year study of 15 healthy adults reported intra-individual variability of 40% against inter-individual variability of 75% [34]. Our estimates are therefore of the same order, although differences in cohort size, sampling interval and sequencing approach preclude a direct comparison. Such stability may extend to the strain level: approximately 60% of the roughly 200 strains carried by an adult are retained over a five-year sampling period, and extrapolation of the observed power-law decay suggests that most strains persist for decades [35]. The physiological basis of this individuality is beginning to be defined: in 61 adults sampled on nine consecutive days, whole-gut and segmental transit times together with luminal pH accounted for part of the inter-individual variation in gut microbiome composition, whereas day-to-day intra-individual variation tracked stool moisture and faecal pH [36].

Two aspects of these estimates deserve comment. First, in the present cohort the proportion of variance attributable to athlete identity was considerably larger for whole-community composition (58.5%) than for Shannon diversity (intraclass correlation 0.43), although these two quantities derive from different estimators and the comparison is qualitative. This is expected, because diversity indices differ in how repeatable they are: in a general-population cohort of 20 adults profiled daily for six weeks, intraclass correlations computed on relative-abundance data using the same variance-components definition applied here were 0.85 for richness, 0.64 for the Shannon index and 0.46 for evenness [37]. Whole-community composition is therefore a more stable personal property than any single diversity index, and the Shannon index - which reflects evenness as well as richness - falls between the two. Second, the magnitude of an intraclass correlation depends on the interval over which samples are collected. Our value of 0.43 was estimated across an entire competitive season, whereas the 0.64 above derives from daily sampling over six weeks, and repeatability declines as the interval lengthens because progressively more within-individual variation is captured. An ICC of 0.43 therefore represents moderate individuality at the scale of a full season, and is better understood as microbiome repeatability over that season than as a reliability coefficient for a single measurement.

Athletic status itself explains remarkably little by comparison. In a cross-study analysis of 855 individuals (656 athletes, 199 non-athletes), athlete versus non-athlete classification

explained 1.63% of the variance in community structure, whereas geographic region explained 19.99%, and taxonomic associations with athletic status were reported to remain inconsistent across studies [38]. Because that analysis pools cohorts differing in sequencing and bioinformatic processing, region cannot be separated from study of origin, and these percentages are not directly comparable with within-cohort estimates such as ours. The ordering nonetheless points to athletic status as a minor axis of variation relative to the individual.

Within athlete cohorts specifically, our findings align closely with recent longitudinal work. In 62 fit recreational runners sampled at four timepoints by shotgun metagenomics, individual identity explained a large share of species-level variation (PERMANOVA $R^2 = 0.74$, $P = 0.001$), beta diversity did not change over time, and neither fitness level nor habitual training volume was associated with composition or predicted function [39]. A compositional reanalysis of an elite volleyball cohort likewise identified athlete identity as the largest single source of compositional variance, accounting for 51.31% at family level and 50.75% at genus level, with no taxon-performance association surviving correction [40], although in that study the individual effect dominated at family and genus level but not at phylum level. One difference from the present study is worth noting: they detected statistically significant phase effects ($P \leq 0.0004$) alongside the dominant individual effect, whereas we did not. Their phase contrast incorporated a designated rest period, a sharper physiological separation than the preparatory, competition and peak-performance blocks sampled here, all of which involved active training.

The individuality documented here is consistent with the concept that adult gut microbiota profiles are highly individualized and relatively stable over time, even when exposed to physiological or lifestyle perturbations [41]. Furber et al. [42] reported that greater gut microbial stability during acute dietary periodization was associated with better endurance performance in highly trained runners, supporting the possibility that stability itself may represent a relevant ecological feature of the athlete gut microbiome rather than merely the absence of change. Comparable stability of diversity indices across training phases has been reported in middle-distance runners and elite volleyball athletes, in which alpha diversity changed little while selective taxonomic shifts were identified across training or competition phases [15,17]. Together with the findings of Lenz et al. [14], this supports the interpretation that alpha diversity should not be treated as a simple marker of 'better' athletic adaptation. Within-athlete dissimilarities were generally lower than between-athlete dissimilarities, and no consistent beta-diversity shifts were detected across transition categories, a pattern also reported in previous athlete cohorts, where global community structure remained stable even when lower-taxonomic changes were detectable [16]. Athletes 1, 4 and 8 accounted for most of the longitudinal movement, and the same three athletes contributed the samples that

clustered outside their own athlete-associated group in the genus-level heatmap (Figure 4A). Temporal variability may therefore be a personalized trait in its own right, with individuals differing systematically in how much their communities fluctuate and more diverse communities tending to be more compositionally stable [43].

The absence of significant period, sport-type or transition effects on global community metrics is consistent with a rapidly accumulating set of independent observations. In 11 national-level taekwondo athletes sampled before and after an eight-week pre-competition training block, Shannon diversity was unchanged ($P = 0.278$) and training phase explained 2.8% of community variation (PERMANOVA $P = 0.647$) [44]. In 13 competitive weightlifters sampled across preparation, competition and transition phases, diversity indices and phylum- and genus-level profiles remained stable with shifts in individual genera tracking energy intake rather than phase [45]. Comparison of taekwondo and diving athletes revealed no difference in either alpha or beta diversity between disciplines, although taxon-level differences and 12 differentially abundant predicted metabolic pathways were reported [46], and a three-month longitudinal study combining full-length 16S profiling with shotgun sequencing found no differences in alpha diversity between sport groups or over time, with no gene or pathway remaining significant after multiple-testing correction [47]. The convergence of these results with ours indicates that the limited responsiveness of global community metrics to training phase is a reproducible feature of the athlete gut microbiome.

This interpretation should not be overstated, because contrary observations exist. In 17 Japanese male handball players sampled at three timepoints spanning an off-season, a significant within-athlete change in the Shannon index was observed ($P = 0.022$), whereas no such change occurred in non-athletic controls - the athletes' alpha diversity exceeded that of controls during both athletic-season timepoints but not during the off-season [48]. Notably, the Chao1 index followed the same trend without reaching significance ($P = 0.091$), so that observation rests on a single index. A season-long case report in a world-class mountain trail runner described increasing diversity across the competitive season, with the highest values at peak performance [49]. Both studies contrast states that are more widely separated in training load than the preparatory, competition and peak-performance blocks sampled here, and the off-season in particular represents a degree of detraining that our design did not capture. Whether detectable microbiome change requires a larger or more prolonged perturbation than routine within-season periodization provides is an open question that our data cannot resolve.

Although alpha diversity was broadly stable across periods, an exploratory, uncorrected transition-based contrast pointed to a nominal decrease in Shannon diversity when athletes moved out of the peak-performance period; this contrast was not supported by the overall test and rested on few transitions, so we treat it as hypothesis-generating. Were it

to be confirmed, the absence of a corresponding change in Simpson diversity would suggest an effect on richness or less abundant taxa rather than on dominant community members. Evidence bearing on such post-peak effects is mixed. Craven et al. [15] similarly found no significant changes in Shannon, Chao1, or inverse Simpson indices after high-volume training in highly trained middle-distance runners. In contrast, exercise-microbiome studies summarized by Dziewiecka et al. [50] indicate that diversity responses may depend on the type, intensity, and timing of the exercise stimulus. In physique athletes followed across a 23-week post-competition period, most taxonomic changes accrued during contest preparation returned toward baseline after weight regain [51], a pattern more consistent with post-competition reversion than with lasting ecological disruption.

Sport type was not associated with alpha diversity (likelihood-ratio test, $P = 0.433$) or with beta diversity (PERMANOVA, $P = 0.498$). Differences between endurance and strength/speed-power athletes were nevertheless apparent on inspection of the genus-level heatmap, and the highest-ranking features in the transition-driver analyses, which were computed separately within each sport group, differed between groups. Neither observation constitutes a formal comparison of the two groups at genus or pathway level, and no feature survived correction for multiple testing; both are therefore descriptive. They are consistent with previous work showing that microbiota differences between athlete groups may be detectable at genus, species, metabolomic, or functional levels even when alpha- and beta-diversity metrics are not significantly different [17,52], and with reports of sport-specialized microbial configuration across athlete cohorts [10,11]. In the present cohort, however, all female athletes belonged to the strength/speed-power group, so any sport-type contrast is inseparable from sex - a caution reinforced by a longitudinal cohort in which sex rather than sport group was the strongest determinant of beta diversity [47].

The genus- and pathway-level transition-driver analyses were exploratory: they were computed separately within each sport group, they rest on dominant-class assignments whose relative frequencies were unstable, and no feature survived correction for multiple testing. The genus-level analysis suggested that selected genera may contribute differently to Entering P3 and Leaving P3 transitions in endurance and strength/speed-power athletes, with the highest-ranking features in each group listed in the Results. Mechanistic work supports the general rationale for examining individual taxa: Scheiman et al. [19] showed that exercise-responsive gut bacteria can influence endurance-related performance through lactate-to-propionate metabolism, illustrating that performance-relevant microbial effects may depend on specialized metabolic functions rather than on broad diversity changes alone.

Top-ranked pathways in the two sport-type groups differed in which peak-related transition they were most strongly associated with, and several of these categories are among the athlete-associated functional signatures reported by Lenz et al. [14]. Barton et al. [3] and

Fontana et al. [9] both emphasized that athlete-associated microbiome differences may be particularly evident at the functional or metabolic level. Because these pathways were inferred from 16S rRNA data using PICRUSt2, however, they identify candidates for validation by shotgun metagenomic, metatranscriptomic or metabolomic methods rather than evidence of functional remodelling.

The dietary data provide important context for interpreting these microbiota findings. Mean energy intake and macronutrient distribution were broadly comparable across training-period phases, with protein, fat, carbohydrate, and fiber intakes showing no major phase-dependent shifts at the cohort level. At the same time, dietary data were available for only 9 athletes, and individual-level dietary variation may still have contributed to athlete-specific microbiota patterns. Jang et al. and Furber et al. [12,42] showed that sport-specific dietary practices and short-term dietary periodization can influence gut microbial structure and performance-related outcomes in athletes. The comparable macronutrient distribution observed across phases here therefore makes it unlikely that the stability we report reflects a systematic phase-dependent shift in cohort-level intake.

These findings have practical consequences for the design of athlete microbiome studies. If an athlete's gut community is a stable personal signature that routine periodization does not reorganize, then a single cross-sectional sample cannot distinguish that signature from a transient, phase-dependent state. Increasing the number of observations per athlete may therefore be more informative than increasing the number of athletes sampled once. Recent methodological work in this field has reached similar conclusions, recommending within-subject longitudinal designs, documentation of training load, and short-term dietary recording as minimum requirements, and identifying 16S rRNA amplicon profiling as an appropriate tool for repeated monitoring across a training season [32], alongside broader calls for standardized collection and analysis practices in sport microbiome research [53].

Limitations

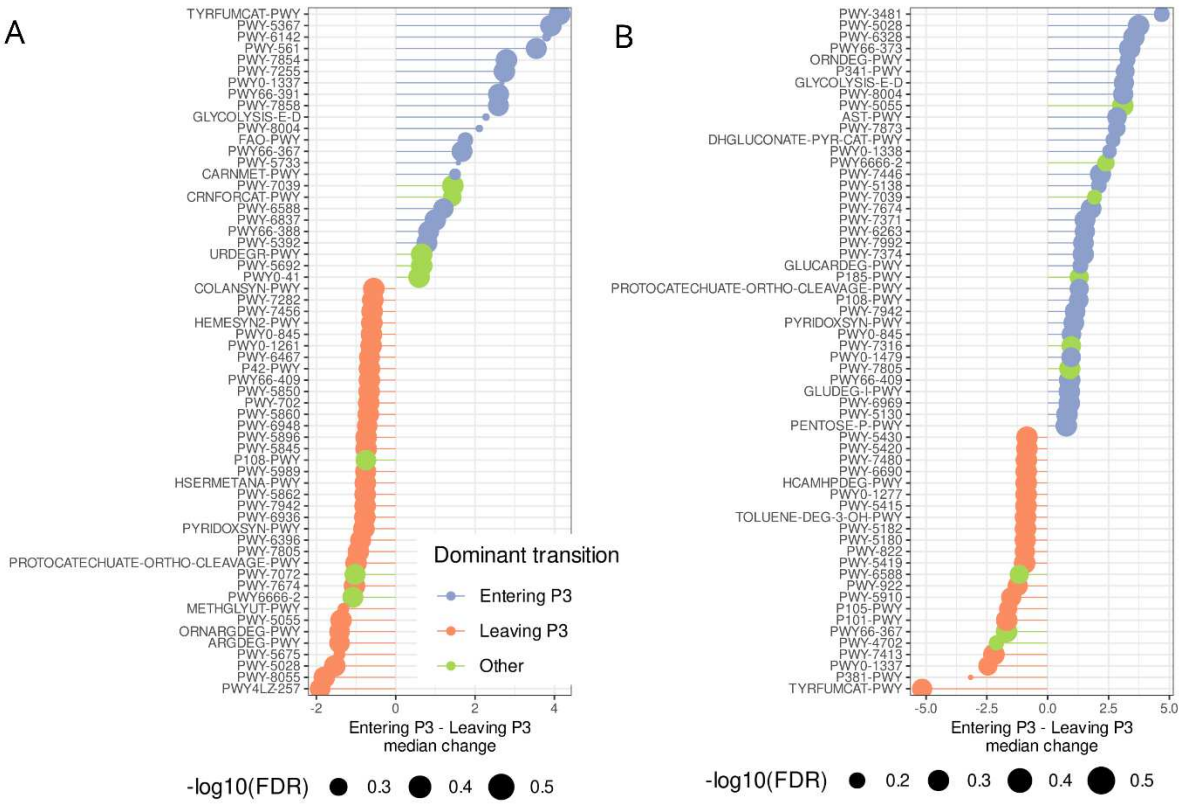
Several limitations should also be acknowledged. Because the study was designed as a longitudinal within-athlete analysis, it cannot determine whether the observed microbiota profiles differ from sedentary or recreationally active individuals. The cohort was necessarily small, reflecting the limited pool of national-team athletes. Accordingly, the analyses of sport type, sex, and transition subgroups - which rest on few athletes and, for some transitions, few events - should be interpreted as exploratory and hypothesis-generating rather than as adequately powered tests of independent effects. Because all female athletes belonged to the strength/speed-power group and all endurance athletes were male, sport-type and sex effects are additionally confounded and cannot be disentangled in this cohort. In addition, dietary data

were incomplete for two athletes and were summarized by training phase, which may not fully capture short-term dietary variation around sampling. The functional analyses were based on PICRUSt2 inference from 16S rRNA amplicon data rather than direct metabolomic measurement. The number of Entering P3 and Leaving P3 transitions was small, which limits conclusions about the reproducibility of the genus- and pathway-level patterns. A further and important limitation is the absence of any comparison between the microbiota data and performance parameters, which could not be carried out for organizational reasons.

Conclusions

In conclusion, the gut microbiota of elite Polish track-and-field athletes was strongly individualized and temporally stable across training and competition phases. Athlete identity accounted for the largest share of variation in community composition ($R^2 = 0.585$), and roughly 40% of the variance in Shannon diversity (intraclass correlation 0.43), whereas training period explained approximately 2% and was not statistically significant. Exploratory, uncorrected analyses identified a nominal decrease in Shannon diversity after leaving the peak-performance phase and candidate genus- and pathway-level features that differed by sport type, none of which survived correction for multiple testing. These findings support a model in which the athlete gut microbiota is characterized by strong individual stability, with more subtle and potentially sport-type-dependent changes detectable at lower taxonomic and inferred functional levels. Larger longitudinal studies using shotgun metagenomics, metabolomics, and more detailed performance and recovery measures are needed to validate these observations and determine whether specific microbiota features contribute to training adaptation, gastrointestinal resilience, or competitive performance. Analyzing the relationship between the gut microbiota and athletic performance is feasible, but only where comprehensive, individualized monitoring of athletes and multi-omic analyses can be implemented.

Figure 6. Pathway transition drivers in endurance and strength athletes.



Lollipop plots of the top 60 MetaCyc pathways ranked by driver score, defined as the product of the absolute Entering P3 − Leaving P3 median difference and the -log10 adjusted P value (FDR). Analyses were performed separately for endurance (A) and strength (B) athletes. Pathways were assigned to a dominant transition class according to the Peak Transition category (Entering P3, Leaving P3, or Other) with the highest median value across subjects. In each panel, segment length and point position represent the Entering P3 − Leaving P3 median difference, point size corresponds to -log10 (FDR), and color indicates dominant transition class. Size scales are panel-specific.

Conflict of Interest

I. Ł. is a probiotic company shareholder. K. S.-Ż., M. K. , D. C-L., A.-W., K.-P. - receive remuneration from a probiotic company

Author Contributions

J.K. – Writing – original draft, Writing – review and editing, Conceptualization, Investigation, Data curation, Supervision, Project administration, Validation, Funding acquisition
M. K. - Writing – original draft, Writing – review and editing, Data curation, Methodology, Formal Analysis, Visualization
D. C.-L. - Writing – original draft, Writing – review and editing, Software, Data curation, Methodology, Formal analysis
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Data availability statements

The raw 16S rRNA gene sequencing data generated during the current study are available in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1535945: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1535945/.

References

1. Cerdá, B. *et al.* Gut Microbiota Modification: Another Piece in the Puzzle of the Benefits of Physical Exercise in Health? *Front Physiol* **7**, 51 (2016).
2. Codella, R., Luzi, L. & Terruzzi, I. Exercise has the guts: How physical activity may positively modulate gut microbiota in chronic and immune-based diseases. *Dig Liver Dis* **50**, 331–341 (2018).
3. Barton, W. *et al.* The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. *Gut* **67**, 625–633 (2018).
4. Bressa, C. *et al.* Differences in gut microbiota profile between women with active lifestyle and sedentary women. *PLOS ONE* **12**, e0171352 (2017).
5. Clark, A. & Mach, N. Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes. *J Int Soc Sports Nutr* **13**, 43 (2016).
6. Suryani, D. *et al.* Type, Intensity, and Duration of Exercise as Regulator of Gut Microbiome Profile. *Curr Sports Med Rep* **21**, 84–91 (2022).
7. Cronin, O., Molloy, M. G. & Shanahan, F. Exercise, fitness, and the gut. *Curr Opin Gastroenterol* **32**, 67–73 (2016).
8. Estaki, M. *et al.* Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. *Microbiome* **4**, 42 (2016).

9. Fontana, F. *et al.* The human gut microbiome of athletes: metagenomic and metabolic insights. *Microbiome* **11**, 27 (2023).
10. O'Donovan, C. M. *et al.* Distinct microbiome composition and metabolome exists across subgroups of elite Irish athletes. *J Sci Med Sport* **23**, 63–68 (2020).
11. Li, Y. *et al.* Gut microbiota and inflammation patterns for specialized athletes: a multi-cohort study across different types of sports. *mSystems* **8**, e00259-23 (2023).
12. Jang, L.-G. *et al.* The combination of sport and sport-specific diet is associated with characteristics of gut microbiota: an observational study. *J Int Soc Sports Nutr* **16**, 21 (2019).
13. Sumi, D. & Suzuki, Y. Gastrointestinal function and microbiota in endurance athletes. *Front. Physiol.* **16**, (2025).
14. Lenz, C. *et al.* Signatures in the gut microbiome of German elite athletes: insights from a matched-subgroup analysis. *mSystems* **0**, e00489-26 (2026).
15. Craven, J. *et al.* The influence of exercise training volume alterations on the gut microbiome in highly-trained middle-distance runners. *European Journal of Sport Science* **22**, 1222–1230 (2022).
16. Akazawa, N. *et al.* Gut microbiota alternation with training periodization and physical fitness in Japanese elite athletes. *Front Sports Act Living* **5**, 1219345 (2023).
17. Carlone, J. *et al.* Dynamic stability of gut microbiota in elite volleyball athletes: microbial adaptations during training, competition and recovery. *Front. Sports Act. Living* **7**, (2025).
18. Charlesson, B. *et al.* Training load influences gut microbiome of highly trained rowing athletes. *J Int Soc Sports Nutr* **22**, 2507952 (2025).
19. Scheiman, J. *et al.* Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. *Nat Med* **25**, 1104–1109 (2019).
20. McKay, A. K. A. *et al.* Defining Training and Performance Caliber: A Participant Classification Framework. *Int J Sports Physiol Perform* **17**, 317–331 (2022).

21. Martin, C. K. *et al.* A novel method to remotely measure food intake of free-living individuals in real time: the remote food photography method. *Br J Nutr* **101**, 446–456 (2009).
22. *Normy żywienia dla populacji Polski i ich zastosowanie*. (Warsaw, 2020).
23. Thomas, D. T., Erdman, K. A. & Burke, L. M. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance. *J Acad Nutr Diet* **116**, 501–528 (2016).
24. Özkurt, E. *et al.* LotuS2: an ultrafast and highly accurate tool for amplicon sequencing analysis. *Microbiome* **10**, 176 (2022).
25. Callahan, B. J. *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**, 581–583 (2016).
26. Li, H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **34**, 3094–3100 (2018).
27. Frøslev, T. G. *et al.* Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nat Commun* **8**, 1188 (2017).
28. Hauswedell, H., Singer, J. & Reinert, K. Lambda: the local aligner for massive biological data. *Bioinformatics* **30**, i349-355 (2014).
29. Yilmaz, P. *et al.* The SILVA and 'All-species Living Tree Project (LTP)' taxonomic frameworks. *Nucleic Acids Res* **42**, D643-648 (2014).
30. Grant, A. *et al.* KSGP 3.1: improved taxonomic annotation of Archaea communities using LotuS2, the genome taxonomy database and RNAseq data. *ISME Commun* **5**, ycaf094 (2025).
31. Douglas, G. M. *et al.* PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology* **38**, 685–688 (2020).
32. Carlone, J., Ribeiro, Á. C. da S., Parisi, A., Giampaoli, S. & Fasano, A. Profiling the Athletes' Gut Microbiome: A Critical Methodological Perspective on 16S Metabarcoding and Shotgun Metagenomics. *Biology (Basel)* **15**, 600 (2026).

33. Olsson, L. M. *et al.* Dynamics of the normal gut microbiota: A longitudinal one-year population study in Sweden. *Cell Host Microbe* **30**, 726-739.e3 (2022).
34. Senina, A. *et al.* Two-Year Study on the Intra-Individual Dynamics of Gut Microbiota and Short-Chain Fatty Acids Profiles in Healthy Adults. *Microorganisms* **12**, 1712 (2024).
35. Faith, J. J. *et al.* The long-term stability of the human gut microbiota. *Science* **341**, 1237439 (2013).
36. Procházková, N. *et al.* Gut physiology and environment explain variations in human gut microbiome composition and metabolism. *Nat Microbiol* **9**, 3210–3225 (2024).
37. Vandeputte, D. *et al.* Temporal variability in quantitative human gut microbiome profiles and implications for clinical research. *Nat Commun* **12**, 6740 (2021).
38. Lewis, G. S. *et al.* The athlete microbiome project: integrating deep learning to reveal microbial associations of physical fitness. *Physiol Genomics* **58**, 199–211 (2026).
39. O' Donovan, C. M. *et al.* Temporal stability and lack of variance in microbiome composition and functionality in fit recreational athletes. *Sci Rep* **15**, 5619 (2025).
40. Carlone, J. *et al.* Athlete or Season? Gut Microbiota Variance in Elite Volleyball Players: A Compositional Performance-Based Reanalysis. *Sports (Basel)* **14**, 292 (2026).
41. O'Brien, M. T., O'Sullivan, O., Claesson, M. J. & Cotter, P. D. The Athlete Gut Microbiome and its Relevance to Health and Performance: A Review. *Sports Med* **52**, 119–128 (2022).
42. Furber, M. J. W. *et al.* Gut Microbial Stability is Associated with Greater Endurance Performance in Athletes Undertaking Dietary Periodization. *mSystems* **7**, e0012922 (2022).
43. Flores, G. E. *et al.* Temporal variability is a personalized feature of the human microbiome. *Genome Biol* **15**, 531 (2014).
44. Xie, L., Chen, X., Wan, X. & Li, B. Gut microbiota and pre-competitive anxiety in high-performance taekwondo athletes during pre-competition training: an exploratory pilot study. *Front. Microbiol.* **17**, (2026).

45. Kuo, C.-Y., Lo, Y.-C., Chen, W.-L. & Hsu, Y.-J. Phase-Specific Alterations in Gut Microbiota and Their Associations with Energy Intake and Nutritional Clustering in Competitive Weightlifters. *Nutrients* **17**, 3199 (2025).
46. Chen, X., Xie, L. & Li, B. Impact of sport disciplines on gut microbiota in elite athletes: evidence from Taekwondo and diving athletes. *Front Microbiol* **17**, 1770499 (2026).
47. Biazzo, M. *et al.* Hybrid-sport participation is associated with gut microbiota composition: an exploratory longitudinal study. *Front. Sports Act. Living* **8**, (2026).
48. Toda, K. *et al.* An Exploratory Study on Seasonal Variation in the Gut Microbiota of Athletes: Insights from Japanese Handball Players. *Microorganisms* **12**, 781 (2024).
49. Álvarez-Herms, J., Burtscher, M., González-Benito, A., Corbi, F. & Odriozola-Martínez, A. The Gut Microbiota Characterization of a World-Class Mountain Trail Runner During a Complete Competition Season: A Case Report. *J Athl Train* **60**, 252–258 (2025).
50. Dziewiecka, H. *et al.* Physical activity induced alterations of gut microbiota in humans: a systematic review. *BMC Sports Sci Med Rehabil* **14**, 122 (2022).
51. Driuchina, A. *et al.* Unveiling the impact of competition weight loss on gut microbiota: alterations in diversity, composition, and predicted metabolic functions. *Journal of the International Society of Sports Nutrition* **22**, 2474561 (2025).
52. Pérez-Castillo, Í. M. *et al.* The athlete gut microbiota: state of the art and practical guidance. *Benef Microbes* **15**, 97–126 (2024).
53. Mancin, L. *et al.* Standardization of gut microbiome analysis in sports. *Cell Rep Med* **5**, 101759 (2024).

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