BMC Microbiology

RESEARCH

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The altitudinal patterns of global human gut microbial diversity

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Abstract

Background The human gut microbiota is closely associated with human health, influencing not only overall well-being but also the incidence and treatment outcomes of diseases. Altitudinal gradients are considered to impact gut microbial community characteristics through factors such as environmental temperature, humidity, and lifestyle. While previous studies have reported altitudinal variations in human gut microbiota in specific regions, a comprehensive exploration of these patterns at a global scale is still lacking. In this study, we analyzed 16S rRNA amplicon sequencing data from healthy human gut microbiota, spanning altitudes from 3 m to 3850 m, obtained from multiple open-access databases. The analysis focused on elucidating the altitudinal patterns of microbial diversity, community composition, and functional profiles.

Results After screening, a total of 6702 sequences from 15 countries were obtained. The diversity of human gut microbiota decreased with increasing altitude (R = -0.047, P < 0.001), but no consistent results were acquired among continents. The relative abundances of the genera *Faecalibacterium* and *Blautia* decreased with rising altitude (R = -0.131 and R = -0.135, respectively, P < 0.001 for both), while the relative abundance of the genus *Prevotella* increased with altitude (R = 0.336, P < 0.001). However, taxa such as Bacilliota, *Bacteroides*, and *Bifidobacterium* exhibit no consistent trends across different continents. The abundance of genes associated with the metabolism of terpenoids and polyketides, lipid metabolism, neurodegenerative diseases, and aging increased with altitude (R = 0.146, 0.037, 0.366, and 0.317, respectively; lipid metabolism P = 0.003, others P < 0.001). Conversely, the abundance of genes related to the immune system and carbohydrate metabolism decreased with increasing altitude (R = -0.166 and R = -0.219, respectively; P < 0.001 for both).

Conclusion Altitude significantly influences diversity, composition, and functional attributes of the human gut microbiota.

Keywords Global, Gut microbiota, Altitude, Distribution patterns

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Introduction

The relationship between gut microbiota and human health is profoundly intricate [1], as the microbiota participates in multiple processes such as digestion, nutrient absorption, and energy metabolism [2, 3]. Investigating the composition, functions, and interactions of gut microbiota with human health is essential for understanding disease mechanisms and developing innovative therapeutic strategies [4]. Altitude, defined as the height of a location relative to sea level, is considered a major factor influencing gut microbial communities and their functions [5]. As altitude increases, temperature and oxygen decrease, and industrialization typically reduces [6]. Hypoxia can trigger inflammation and other diseases, acting as a driver of gut microbiota dysbiosis [7]. Moreover, industrialization affects local air quality, with exposure to pollutants inducing significant changes in gut microbiota composition and increasing the risk of metabolic diseases [8, 9]. Additionally, people living in regions with varying levels of industrialization tend to have distinct dietary habits, potentially leading to changes in gut microbiota composition [10]. Therefore, understanding the changes in gut microbiota taxa and functions along altitudinal gradients is crucial for elucidating altitude-associated disease patterns and offering insights to disease treatment.

The trends in human gut microbial diversity with altitude remain inconclusive. Most studies suggest that gut microbial diversity decreases with increasing altitude. For example, Das B. et al. analyzed gut microbiota from populations in Ballabhgarh (low altitude) and Leh (high altitude) and found that species-level diversity was significantly lower at high altitudes [11]. Similarly, Zeng B. et al. studied populations across multiple altitudes and reported a significant decline in the Shannon index for high-altitude groups compared to low-altitude groups [12]. However, some researchers conducted studies on the gut microbiota of males from three different regions in China and reported no significant changes in gut microbiota diversity across different altitudes [13]. In contrast, Lan D et al. investigated six locations at varying altitudes and found that individuals living in high-altitude regions exhibited high bacterial diversity [14].

Regarding microbial composition, numerous studies have demonstrated significant differences along altitudinal gradients. Comparative analyses of fecal microbiota from Tibetan and Han populations living at low and high altitudes revealed a higher relative abundance of Bacilliota and lower relative abundance of Bacteroidetes in high-altitude populations. Dietary surveys attributed these differences primarily to high-fat diets [15, 16]. Furthermore, the increase or decrease in the Bacilliota/Bacteroidetes ratio is considered indicative of dysbiosis [15], with an elevated ratio being associated with obesity [17]. Additionally, Bo Zeng et al. found that genera such as *Bacteroides* and *Faecalibacterium* exhibited altitude-dependent variations [18].

From a functional perspective, Li Kang et al. discovered that the abundance of carbohydrate-active enzyme modules was higher in low-altitude populations, with significant differences in the abundance of metabolic pathways and modules across altitudes [19]. In contrast, Bhabatosh Das et al. observed higher abundances of processes such as carbohydrate and lipid metabolism, membrane transport, signal transduction, and xenobiotic metabolism in low-altitude populations, while high-altitude populations exhibited elevated levels of terpenoid and zeatin biosynthesis pathways [11]. These conflicting results indicate that the functional shifts in gut microbiota across altitudes require further investigation.

Previous studies have typically focused on a limited number of altitudinal locations, lacking a global perspective [20]. Consequently, discrepancies in findings regarding gut microbiota diversity, composition, and functions along altitudinal gradients may arise from variations in study site selection.

This study collected amplicon sequencing data from a global altitude range of 3 m to 3,700 m through database searches and literature reviews. By analyzing the altitudinal distribution patterns of gut microbiota both globally and within individual continents, this study aims to determine whether consistent global patterns exist in gut microbiota diversity, taxonomy, and functions. The findings of this study will provide a scientific foundation for microbiome-based diagnostics and personalized medicine.

Methods

Data collection and selection

We utilized the following search query to retrieve metadata from the NCBI (National Center for Biotechnology Information) Sequence Read Archive (SRA), restricting the results to DNA data up to July 2, 2024: (gut[All Fields] OR enteric[All Fields] OR intestinal[All Fields]) AND (microbiota[All Fields] OR microorganisms[All Fields] OR microbe[All Fields]) AND (Homo sapiens[All Fields] OR human[All Fields]). Additionally, equivalent searches were conducted in the European EMBL-EBI database and the GMrepo database.

This search yielded 611,970 16S rRNA gene sequencing samples. Initial filtering was performed based on the following criteria: amplicon sequencing data of 16S rRNA genes generated using Illumina sequencing platforms, and samples with clearly documented latitude and longitude. Since gut microbiota in infants and young children differs significantly from that of adults [21], we selected samples from healthy humans aged over three years without experimental intervention. After filtering, 8,332

datasets remained. The raw data were downloaded from the database, and we utilized sratoolkit version 2.9.6 to convert the 16 S rRNA gene SRA files into FASTQ format. Additionally, we organized the primer information and sample details, including geographic coordinates, country, age, and other relevant conditions, based on the information provided in the database.

GeoTIFF files containing altitude data at a 2.5-minute resolution were downloaded from the WorldClim 2.1 database. Using QGIS software, latitude and longitude information from the datasets was matched to the altitude data from the GeoTIFF files to obtain altitude information representing the host's environmental conditions.

Microbial analyses

High-throughput data were analyzed using the Easy-Amplicon pipeline on a server [22]. This pipeline facilitated the merging, trimming, and quality control of the sequencing data. Following denoising with the UNOISE 3 algorithm, sequences were clustered at 100% identity. Subsequently, chimeric sequences were identified and removed by comparing the sequences against the SILVA_16S_V123 database, where a sequence was classified as chimeric if it had no similar sequences in the reference data but was similar to 2–3 other sequences. The vsearch program was employed to generate a feature table of operational taxonomic units (Amplicon Sequence Variants, ASVs). Taxonomic annotation of the ASVs was performed using the sintax algorithm from USEARCH, referencing the SILVA_16S_V123 database, which is specifically tailored for bacterial species. Non-bacterial components, such as mitochondrial and chloroplast sequences, were removed from the ASVs, resulting in a detailed taxonomic information Table [22].

Data analysis

Alpha diversity analysis

The Chao1 and Shannon indices were calculated using the "vegan" package in R (v4.3.1). Linear regression models were applied to analyze the correlation between Chao1 and Shannon indices and altitude. To investigate differences and similarities in gut microbiota among populations, we stratified the data by continent. Given Japan's geographical isolation as an island nation, it was analyzed as an independent group alongside Eurasia, the Americas, Africa, and Oceania.

Community composition analysis

We conducted correlation analyses between elevation and gut microbiota taxa for both global and continental subgroups. A linear regression model was used to assess the overall association between the core taxa with relatively high abundance and altitude. Additionally, the Bacillota/Bacteroidota ratio was calculated, and its relationship with altitude was evaluated via linear regression at both continental and global levels. A Composition Stacked Chart was performed on the TuTU analysis platform (http://www.cloudtutu.com).

To examine microbial community divergence across altitudinal gradients, samples were partitioned into four altitude intervals:0–1000 m, 1000–2000 m, 2000–3000 m, and 3000–4000 m. We conducted beta diversity analysis using all the data. β-diversity differences were quantified using Bray-Curtis dissimilarity matrices, followed by principal coordinate analysis (PCoA) to visualize compositional variations across continents and altitude groups. Permutational multivariate analysis of variance (PERMANOVA, 999 permutations) with the adonis function in the R "vegan" package tested the significance of community differentiation [23, 24]. Pairwise adonis tests with Bonferroni correction further identified specific group differences. Linear discriminant analysis (LDA) was applied to identify taxa with significantly divergent abundances (LDA score > 2.0) among populations.

Functional gene abundance analysis

PICRUSt2 integrates existing open-source tools to predict the genomic content of environmental samples based on 16 S rRNA gene sequences. It places ASVs within a reference tree, which serves as the foundation for functional predictions. This reference tree comprises 20,000 complete 16S rRNA genes from bacterial and archaeal genomes in the Integrated Microbial Genomes database [25]. To identify differentially abundant functional processes, we employed the PICRUSt2 method to conduct functional predictions based on the taxonomic profiles. We utilized linear discriminant analysis (LDA) combined with effect size measurements to assess the impact of each differentially abundant taxonomic unit on the abundance of significantly different metabolic genes in the KEGG metabolic pathways (including secondary pathways related to terpenoid and polyketide metabolism, lipid metabolism, and carbohydrate metabolism), organismal system genes (such as those related to the immune system and aging) and disease genes (specifically pertaining to neurodegenerative diseases) [26]. Furthermore, we employed linear regression models to investigate whether there were significant differences in functional gene abundance at both continental and global levels in relation to altitude.

Results

Sequencing, quality control, and annotation statistics

We calculated the total number of sequences for each sample. For samples with a total sequence count of 0 or 1, we deemed their data quality unreliable and not reflective of the true microbial community structure; thus, these samples were excluded from subsequent analyses.

Ultimately, we obtained 275,477,930 high-quality amplicon sequences from 6,702 fecal samples. After clustering the sequences based on 100% sequence similarity, we identified a total of 27,561 amplicon sequence variants (ASVs).

The 6,702 human samples collected in this study spanned an altitude gradient from 3 m to 3,863 m (Fig. 1). Among these, there were 4,073 samples from Asia (3 m – 3,863 m); 699 samples from Europe (11–756 m); 1,255 samples from North America (9 m – 1,459 m); 359 samples from South America (26 m – 3,039 m); 170 samples from Africa (661 m – 1,068 m); and 146 samples from Oceania (17 m). Additionally, there were 3,403 samples from Japan (3–257 m). We utilized relative abundance to ensure comparability across different samples.

Global differences in gut microbial diversity

Alpha diversity of gut microbial communities

Across the continents, the Chao1 index of gut microbiota in African, American, and Eurasian populations decreased with increasing altitude (R values of -0.390, -0.550, and – 0.400, respectively; all $p < 0.001$). In contrast, the Chao 1 index of gut microbiota in the Japanese population increased with altitude ($R = 0.320$, $p < 0.001$) (Fig. 2a). The Shannon index significantly decreased with increasing altitude in the American, Eurasian, and Japanese populations (R values of -0.310, -0.220, and – 0.053, respectively; $p = 0.002$ for Japan, and $p < 0.001$ for the other continents); however, the Shannon index in Africa exhibited a significant positive correlation with altitude ($R = 0.770$, $p < 0.001$) (Fig. 2b).

At the overall level, the Chao 1 index of human gut microbiota decreased with increasing altitude (R = -0.047, $p < 0.001$) (Supplementary Figure S1a). Similarly, the Shannon index of human gut microbiota also showed a decreasing trend with altitude (R = -0.053, $p < 0.001$) (Supplementary Figure S1b).

Beta diversity of gut microbial communities

In the permutation tests conducted across different elevation gradient groups, pairwise comparisons (pairwise.adonis with Bonferroni correction) revealed that the most significant inter-group difference was observed between the 0–1000 m and 1000–2000 m elevation groups ($F = 65.472$, $R^2 = 0.010$, adjusted $P = 0.006$), indicating a substantial difference in community composition between these two elevation gradients. Additionally, a significant difference was also noted between the 0–1000 m and 3000–4000 m groups ($F = 39.859$, $R^2 = 0.006$, adjusted $P = 0.006$) (Supplementary Table 3). These results suggest that elevation gradient has a significant impact on community composition, demonstrating strong explanatory power (Fig. 3a).Furthermore, due to geographical influences, there are notable differences in gut microbiota

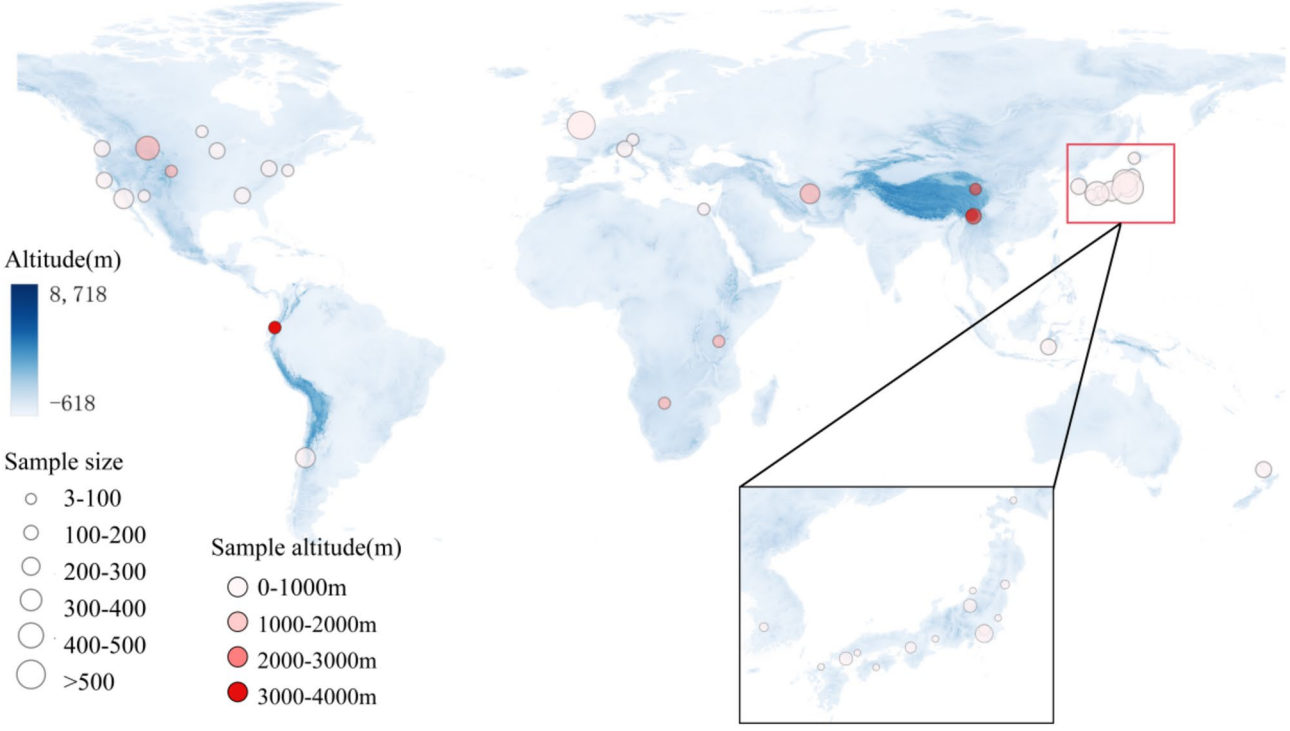

Fig. 1 Geographical distribution of samples. The varying shades of blue in the background represent different altitudes, with darker blue indicating higher altitudes. The size of the red circles corresponds to the sample quantity, and the shade of the circles reflects the altitude, with darker circles representing higher altitudes.)

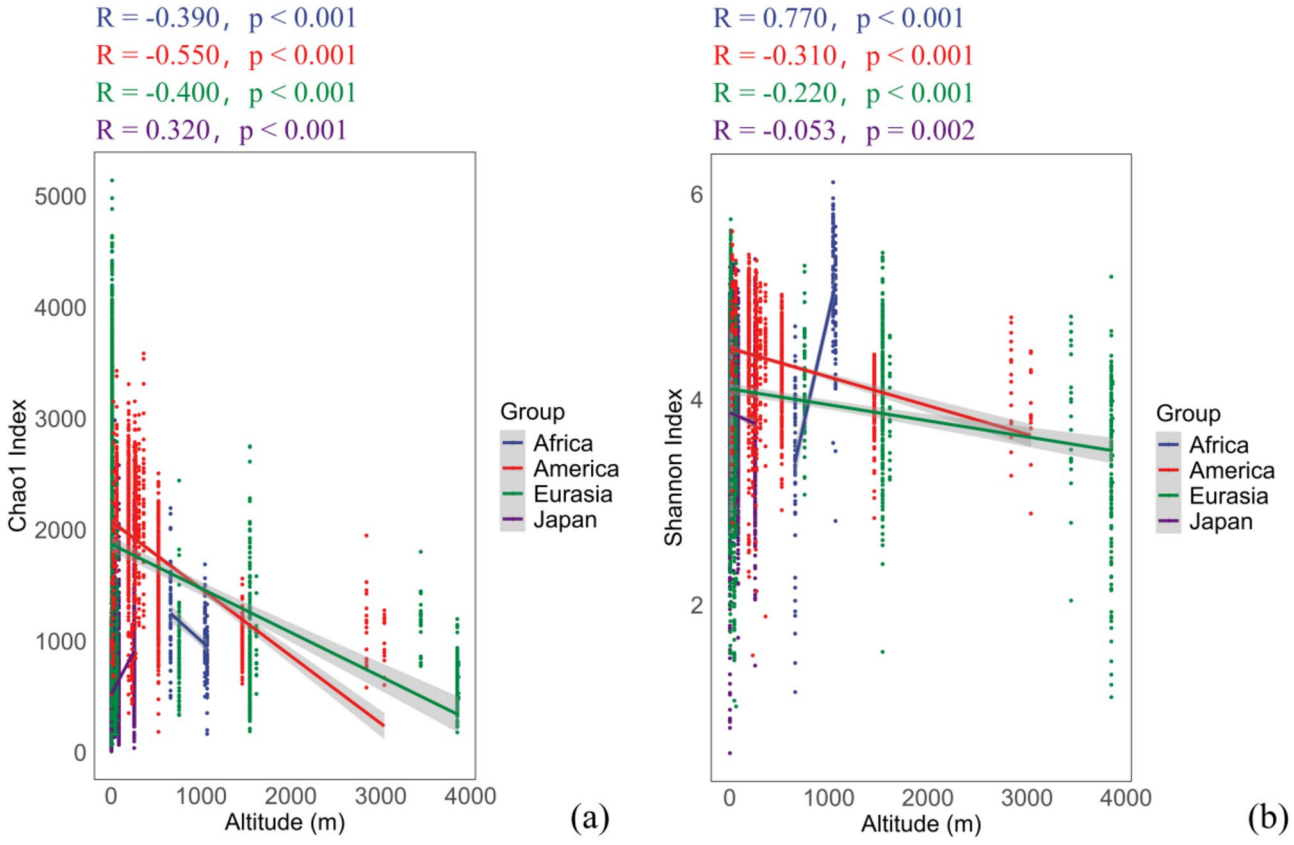

Fig. 2 The correlation between alpha diversity indices and altitude among different continental populations: **(a)** Chao 1 index; **(b)** Shannon index

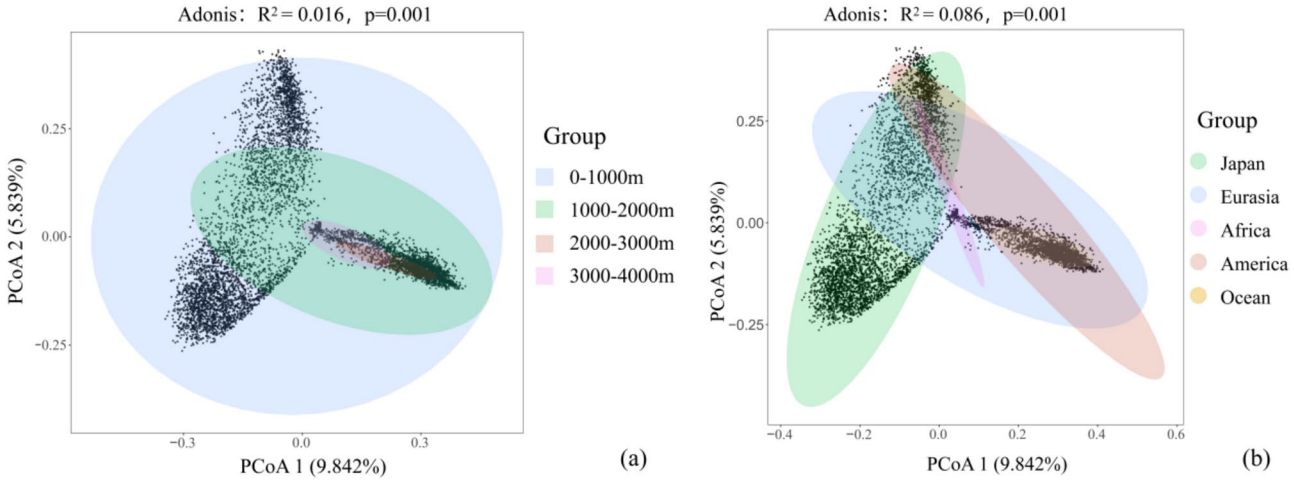

Fig. 3 Principal Coordinate Analysis (PCOA) of gut microbiota composition: **(a)** PCOA analysis of gut microbiota composition across different altitude gradient populations; **(b)** PCOA analysis of gut microbiota composition across different continental populations

among different continents, with the most significant differences observed between Japan and both the Americas and the Eurasian continent (F values of 427.300 and 212.827, R^2 values of 0.079 and 0.043, respectively, with adjusted P values of 0.010 for both comparisons) (Fig. 3b) (Supplementary Table 3).

The results of the permutation test for Africa indicated a statistically significant difference between the 0–1000 m and 1000–2000 m groups (F = 25.403, R^2=0.131, adjusted P = 0.001) (Supplementary Figure S2a) (Supplementary Table 3). In the Eurasian continent, the permutation test results showed significant differences between the 0–1000 m and 1000–2000 m groups, as well as between

the 3000–4000 m group and both the 0–1000 m and 1000–2000 m groups (F values of 47.991, 31.798, and 35.649, with R^2 values of 0.038, 0.031, and 0.074, respectively, and adjusted P values of 0.003 for all comparisons) (Supplementary Figure S2b) (Supplementary Table 3). In the Americas, the permutation test results indicated that, except for the comparison between the 2000–3000 m and 3000–4000 m groups, which did not show a significant difference (adjusted $P = 1$), all other inter-group comparisons exhibited significant differences in community composition. Notably, the difference between the 0–1000 m and 1000–2000 m groups was the most pronounced ($F = 91.417$, $R^2 = 0.055$, adjusted $P = 0.006$) (Supplementary Figure S2c) (Supplementary Table 3).

Global gut Microbiome composition across different altitudes

To characterize the gut microbiome profiles within the samples, a total of 30 phyla and 618 genera were detected. At the phylum level, the most abundant were Bacilliota (49.38%), Bacteroidetes (37.87%), Actinobacteria (5.32%), and Proteobacteria (4.78%). At the genus level, the most prevalent were *Bacteroides* (25.81%), *Faecalibacterium* (6.78%), *Prevotella* (5.84%), *Blautia*(4.85%) and *Bifidobacterium* (3.63%) (Fig. 4a and b). The dominant bacterial

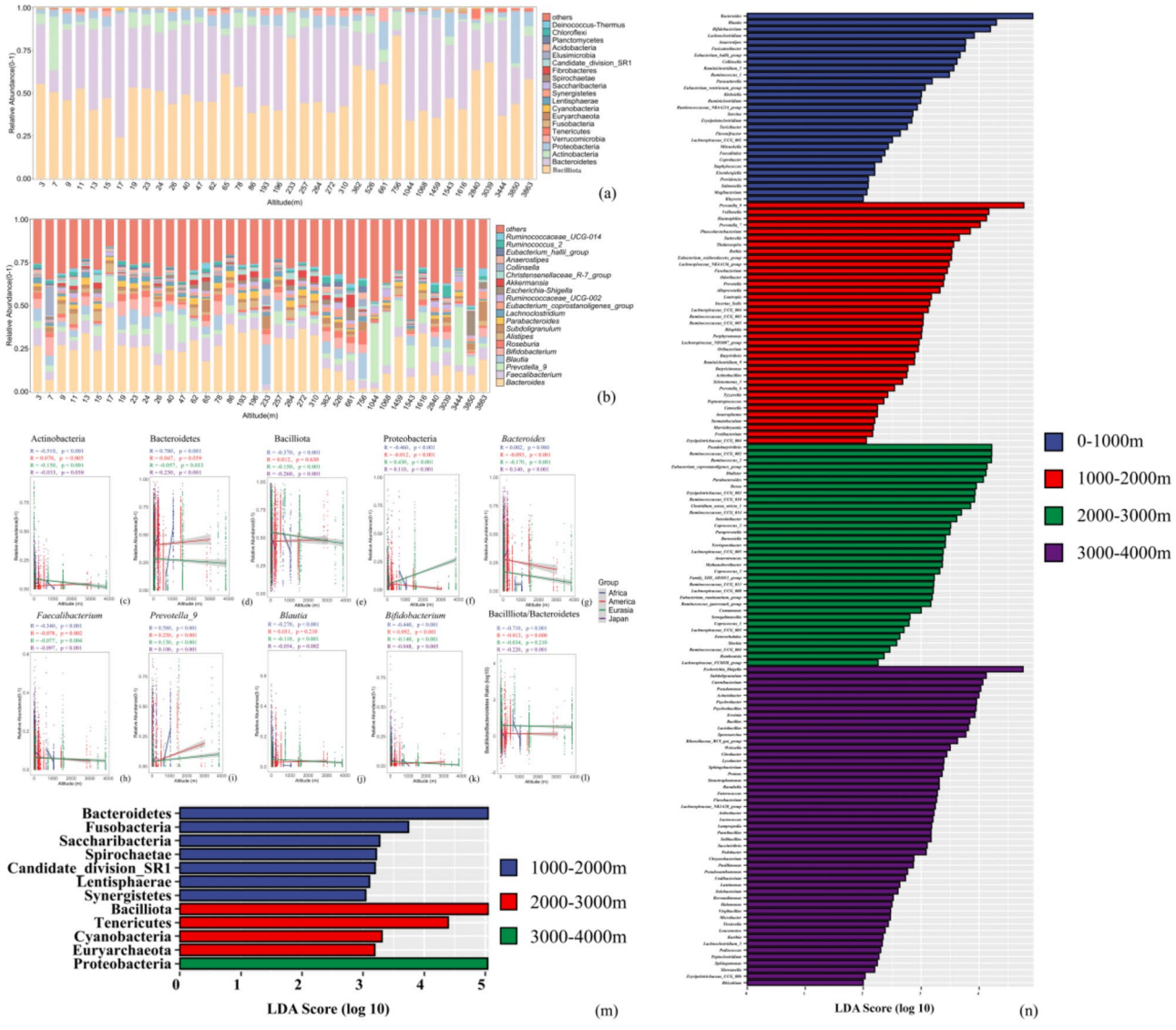

Fig. 4 **(a)** Major phylum-level composition of the human gut microbiota; **(b)** Major genus-level composition of the human gut microbiota. The stacked bar charts display the relative abundance (%) of the top 20 bacterial phyla (from bottom to top) across different populations. Other low-abundance phyla are grouped together and labeled as "Others."; **(c-k)** Correlation analysis of core phyla and genera relative abundances with altitude at the continental level; **(l)** Correlation analysis of the Bacilliota/Bacteroidetes Ratio with altitude at the continental level; **(m-n)** Differential analysis of gut microbiota across different altitude gradients using the LEfSe software (combining linear discriminant analysis [LDA] with effect size measurements). **(m)** At the phylum level; **(n)** At the genus level

taxa across continents did not show significant differences compared to the overall composition (Supplementary Figures S3a, S3b). We conducted correlation analyses between these relative abundances of core phyla and genera and altitude.

When analyzing the relationship between relative abundance at the phylum level and altitude on a continental scale, results indicated that the relative abundance of Actinobacteria significantly decreased with increasing altitude in African and Eurasian populations (R values of -0.510 and − 0.150, $P < 0.001$), while it increased with altitude in American populations ($R = 0.070$, $P = 0.005$). In the Japanese population, there was no significant relationship between Actinobacteria and altitude ($P = 0.058$) (Fig. 4c). For Bacteroidetes, the relative abundance increased with altitude in African and Japanese populations (R values of 0.780 and 0.230, $P < 0.001$), while it decreased in Eurasian populations (R=-0.057, $P = 0.033$). No significant relationship was observed in American populations ($P = 0.059$) (Fig. 4d). Additionally, the relative abundance of Bacilliota significantly decreased with increasing altitude in African, Eurasian, and Japanese populations (R values of -0.370, -0.150, and − 0.260, $P < 0.001$), while no significant change was observed in American populations ($P = 0.630$) (Fig. 4e). At the overall level, we found that the relative abundances of Actinobacteria, Bacteroidetes, and Bacilliota all decreased with increasing altitude (R values of -0.071, -0.066, and − 0.072, $P < 0.001$) (Supplementary Figures S3c, S3d, S3e). Notably, continent-level analysis indicated that the relative abundance of Proteobacteria decreased with altitude in African and American populations (R values of -0.460 and − 0.012, $P < 0.001$), while it increased in Eurasian and Japanese populations (R values of 0.430 and 0.110, $P < 0.001$) (Fig. 4f). Overall, the relative abundance of Proteobacteria significantly increased with altitude ($R = 0.336$, $P < 0.001$) (Supplementary Figure S3f).

When analyzing the correlation between relative abundance at the genus level and altitude on a continental scale, we found that the relative abundance of *Bacteroides* significantly decreased with increasing altitude in American and Eurasian populations (R values of -0.093 and − 0.170, $P < 0.001$), while it increased in the Japanese population ($R = 0.140$, $P < 0.001$). No significant differences were observed in African populations ($P = 0.980$) (Fig. 4g). The relative abundance of *Faecalibacterium* decreased with increasing altitude across all continental populations (R values of -0.340, -0.078, -0.077, and − 0.098; P values of 0.002 for America, 0.004 for Eurasia, and $P < 0.001$ for the others) (Fig. 4h). The relative abundance of *Blautia* significantly decreased with altitude in African, Eurasian, and Japanese populations (R values of -0.270, -0.110, and − 0.054; P values of 0.002 for Japan and $P < 0.001$ for the others), while no significant differences were observed in American populations ($P = 0.210$) (Fig. 4j). The relative abundance of *Bifidobacterium* decreased with increasing altitude (R=-0.106, $P < 0.001$), and this trend was consistent across African, Eurasian, and Japanese populations (R values of -0.440, -0.140, and − 0.048; P values of 0.005 for Japan and $P < 0.001$ for the others), while the abundance of *Bifidobacterium* in American populations exhibited an opposite trend ($R = 0.092$, $P < 0.001$) (Fig. 4k). At the overall level, we found that the relative abundances of *Bacteroides*, *Faecalibacterium*, *Blautia*, and *Bifidobacterium* all decreased with increasing altitude (R values of -0.200, -0.131, -0.135, and − 0.106, $P < 0.001$) (Supplementary Figures S3g, S3h, S3i, S3k). Furthermore, at the continental level, *Prevotella* exhibited a positive correlation with altitude across all populations (R values of 0.580, 0.230, 0.130, and 0.100; $P < 0.001$) (Fig. 4i). At the overall level, the relative abundance of *Prevotella* significantly increased with altitude ($R = 0.131$, $P < 0.001$) (Supplementary Figure S3i).

We conducted a continental analysis of the Bacilliota/Bacteroidetes ratio and found that in the African and Japanese populations, the ratio decreased with increasing altitude (R values of -0.710 and − 0.220, respectively, both with $P < 0.001$). However, no significant relationship was observed between the ratio and altitude in the Eurasian and American populations (P values of 0.600 and 0.210, respectively) (Fig. 4l). At the overall level, the results for the Bacillota/Bacteroidetes ratio indicated an increase with rising altitude ($R = 0.047$, $P < 0.001$) (Figure S3l).

Based on the LEfSe analysis of phylum and genus abundances, we found that *Bacteroides*, *Blautia*, and *Bifidobacterium* were significantly abundant in the 0–1000 m altitude group, while Bacteroidetes and *Prevotella* were significant in the 1000–2000 m altitude group, and Proteobacteria was significant in the 2000–3000 m altitude group (Fig. 4m and n). These taxa also exhibited different significant differences across continents (Supplementary Figures S3m, S3n).

Functional predictions of gut microbiota at different altitudes

Based on the LEfSe analysis of the secondary pathway abundance predicted by PICRUSt, we found that the abundance of carbohydrate metabolism was significantly higher in populations at altitudes of 0–1000 m. In contrast, populations at altitudes of 3000–4000 m exhibited significant abundance in lipid metabolism, terpenoid and polyketide metabolism, neurodegenerative diseases, and aging-related genes. Notably, immune system genes showed significant abundance in the 2000–3000 m altitude group (Fig. 5a). Furthermore, these genes exhibited distinct significant differences across different continents, attracting considerable attention from researchers (Supplementary Figure S4a). Consequently, we conducted an in-depth analysis of these genes.

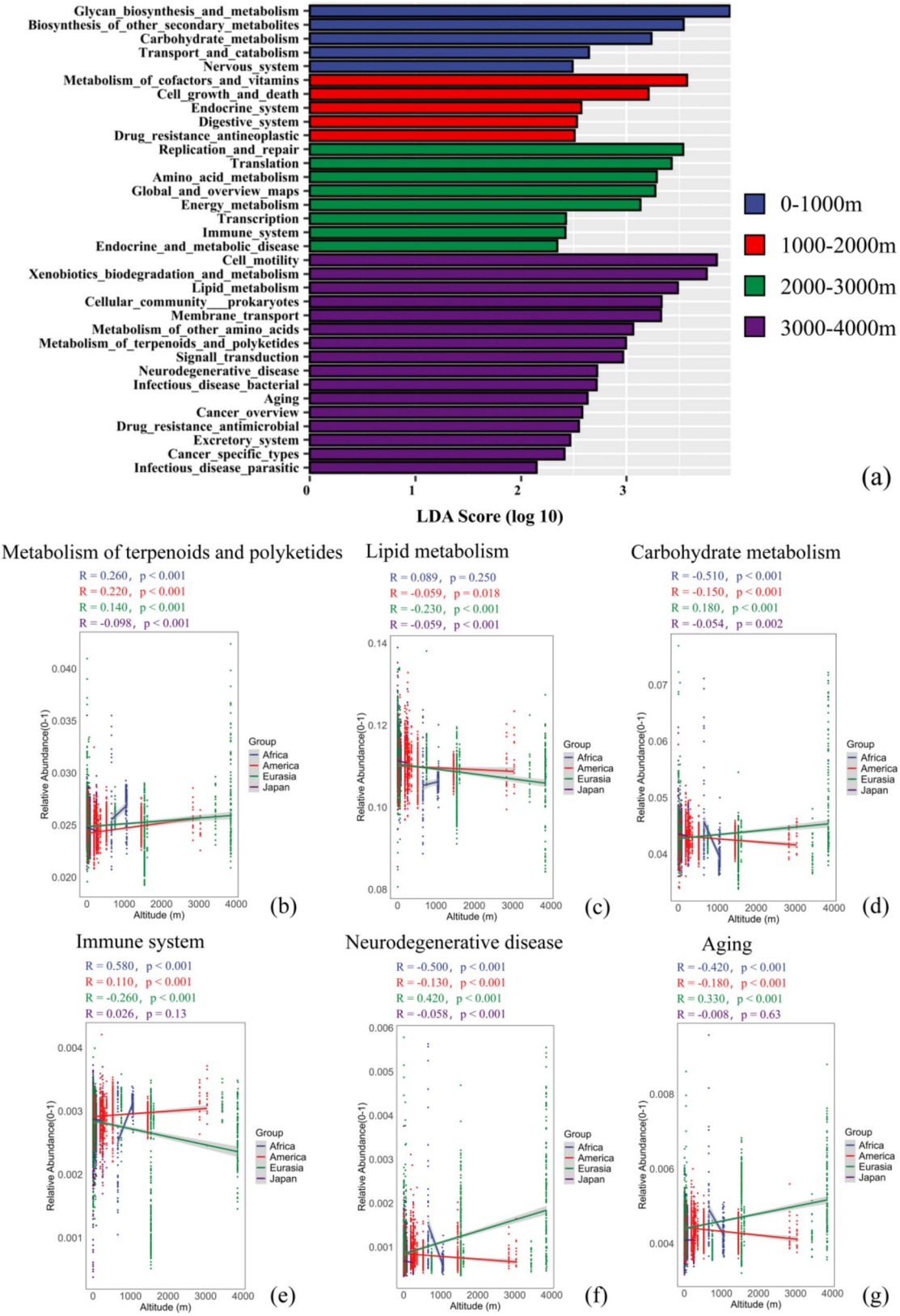

Fig. 5 **(a)** The analysis of differences in gut microbiota among populations across various altitude gradients focused on the predicted functional abundances of secondary metabolic pathways and was conducted using the LefSe software(LDA). **(b-g)** Correlation between the abundance of different functional genes and altitude at the continental level

We found that among metabolism-related genes, at the continental level, the abundance of metabolic genes for terpenoids and polyketides in African, American, and Eurasian populations increased with altitude (R values of 0.260, 0.220, and 0.140, respectively; $P < 0.001$). Conversely, in the Japanese population, the abundance of these metabolic genes decreased with increasing altitude (R = -0.098, $P < 0.001$) (Fig. 5b). The abundance of lipid metabolism genes in African, American, and Japanese populations decreased with altitude (R values of -0.059, -0.230, and – 0.059, respectively; $P < 0.001$ for all), while no significant relationship was found between lipid metabolism gene abundance and altitude in non-human populations ($P = 0.250$) (Fig. 5c). At the overall level, the gene abundance for terpenoids and polyketides, as well as lipid metabolism, increased with altitude (R values of 0.146 and 0.037, respectively; P values of < 0.001 and = 0.003) (Supplementary Figures S4b, S4c). The abundance of carbohydrate metabolism genes in African, American, and Japanese populations decreased with altitude (R values of -0.510, -0.150, and – 0.054, respectively; $P = 0.002$ for Japan, and $P < 0.001$ for the other continents), while in the Eurasian population, the abundance of carbohydrate metabolism genes increased with altitude ($R = 0.180$, $P < 0.001$) (Fig. 5d). At the overall level, the abundance of carbohydrate metabolism decreased with increasing altitude (R = -0.219, $P < 0.001$) (Supplementary Figure S4d).

In terms of organism system-related genes, the abundance of immune system genes in African and American populations increased with altitude (R values of 0.580 and 0.110, respectively; $P < 0.001$ for both), while in the Eurasian population, the abundance of immune system genes decreased with altitude (R = -0.260, $P < 0.001$). In the Japanese population, there was no significant relationship between the abundance of polysaccharide synthesis and metabolism genes and altitude ($P = 0.130$) (Fig. 5e). At the overall level, immune system gene abundance decreased with increasing altitude (R = -0.166, $P < 0.001$) (Supplementary Figure S4e).

Regarding disease-related genes, we observed that the abundance of neurodegenerative disease genes in African, American, and Japanese populations decreased with altitude (R values of -0.500, -0.130, and – 0.058, respectively; $P < 0.001$ for all), while in the Eurasian population, the abundance of neurodegenerative disease genes increased with altitude ($R = 0.420$, $P < 0.001$) (Fig. 5f). At the overall level, the abundance of neurodegenerative disease genes increased with altitude ($R = 0.366$, $P < 0.001$) (Supplementary Figure S4f).

In terms of aging-related genes, we found that the abundance of aging genes in African and American populations decreased with altitude (R values of -0.420 and – 0.180, respectively; $P < 0.001$ for both), while in the Eurasian population, the abundance of aging genes increased with altitude ($R = 0.330$, $P < 0.001$). In the Japanese population, there was no significant relationship between aging gene abundance and altitude ($P = 0.63$) (Fig. 5g). At the overall level, the abundance of aging genes increased with altitude ($R = 0.317$, $P < 0.001$) (Supplementary Figure S4g).

Discussion

Human gut microbial diversity along altitudes

In our study, we observed that human gut microbial diversity decreases with increasing altitude. At the continental level, some results from Japan and Africa exhibited opposing trends, which may be attributed to the fact that the data from Japan predominantly represent low-altitude samples. As an island nation, Japan has distinct dietary and lifestyle habits, while the African sample size was relatively small, with limited altitude coverage. The finding is consistent with the studies conducted by Das B and Zeng B et al. [11, 12]. However, it contrasts with the results of Li L and Lan D et al., who studied Han and Tibetan populations living at 3100 m and Han populations at 450 m, finding no statistically significant differences among different altitude groups [15]. Lan et al. investigated six locations across the Tibetan Plateau (altitudes ranging from 2800 to 4500 m) and concluded that individuals at higher altitudes exhibit greater bacterial diversity [14]. This discrepancy is likely due to the limitations in sampling ranges, as different sampling locations can yield varying results. This further supports our hypothesis that the altitude range and regional characteristics can influence the level of human gut microbial diversity.

Composition of human gut microbiota at different altitudes

The overall composition at the phylum and genus levels is consistent with previous studies.

LEfSe results indicate that there are differences in the composition of gut microbiota at the phylum level among populations at different elevation gradients. We found that the relative abundance of *Prevotella* increased with altitude, while the relative abundances of *Blautia* and *Faecalibacterium* decreased with altitude, exhibiting a consistent trend across continents, wich is consistent with the findings of Kang Li et al. [20]. This phenomenon may be attributed to the negative correlation between *Prevotella* and energy intake, as populations at high altitudes tend to have higher energy intake compared to those at lower altitudes [27]. Furthermore, *Prevotella* is known to produce short-chain fatty acids (SCFAs) [28, 29], which not only provide energy [30] but also help lower blood pressure through receptor mechanisms [31], thus aiding in the adaptation to the energy demands and

pulmonary hypertension associated with high-altitude environments [32]. Some studies suggest that *Blautia* increases in abundance under hypoxic conditions at high altitudes [33], which contradicts our results. This discrepancy may arise from differences in geographical locations and dietary habits of the study populations. Regarding the relationship between *Faecalibacterium* and altitude, the results of Bhabatosh Das et al. are consistent with ours, suggesting that *Faecalibacterium* may encode anti-inflammatory functions [11] to adapt to hypoxic environments at high altitudes. Conversely, Daoliang Lan et al. reported findings contrary to ours, positing that the increase in facultative anaerobes at high altitudes [14] could explain this contradiction. The differing altitude ranges selected by researchers likely contribute to these discrepancies; Bhabatosh Das et al. compared populations at 228 m and 3500 m, while Daoliang Lan et al. studied six altitude ranges between 2800 and 4500 m. This highlights the influence of selected research ranges and lifestyle differences on gut microbiota composition [34].

In our research, regarding the relative abundance of major phyla and the ratio of Bacilliota to Bacteroidetes, we did not find heterogeneous conclusions across global and continental scales with respect to altitude, suggesting that from a large geographic perspective, altitude is not a stable factor for taxonomic selection [35–37].

Functional predictions of human gut microbiota at different altitudes

LEfSe results indicate that high-altitude populations have a greater abundance of significant genes in their gut microbiota compared to low-altitude populations.

In terms of metabolism-related genes, we found that the abundance of genes involved in terpenoid and polyketide metabolism increased with altitude, exhibiting a consistent trend across continents. Terpenoids are known for their anti-inflammatory mechanisms, which can protect cells under hypoxic conditions at high altitudes, thereby contributing to the higher proportion of terpenoid-related genes in the gut microbiota of high-altitude populations [38, 39]. We also observed that the abundance of lipid metabolism genes increased with altitude, with similar trends across continents. Conversely, the abundance of carbohydrate metabolism genes decreased with increasing altitude, showing no consistent trend across continents. This may be due to differences in dietary habits among populations on different continents, a finding supported by previous researchers [19]. Populations living at lower altitudes often consume more carbohydrate-rich foods, such as grains and vegetables, while those at higher altitudes typically face colder temperatures and hypoxia, necessitating a diet higher in fats and proteins for energy storage. Consequently, individuals at lower altitudes may rely more on carbohydrate metabolism for quick energy, while high-altitude populations may depend more on fats or other energy sources. This may lead to selective pressure favoring lipid metabolism pathways over carbohydrate metabolism pathways, thereby increasing the proportion of related genes [40]. This also explains the lower diversity observed in high-altitude populations, likely due to the more varied diet of low-altitude individuals, who consume more carbohydrates and fiber-rich foods. Some researchers have also found higher abundances of carbohydrate and lipid metabolism in low-altitude groups [11], which may be attributed to lifestyle differences among high-altitude populations.

Our study also revealed that the abundance of neurodegenerative disease genes increased with altitude. This is likely due to the hypoxic environment at high altitudes, which can trigger neurological disorders, including Alzheimer's disease, Parkinson's disease, and other age-related neurodegenerative diseases [41]. We also examined immune system genes and aging-related genes, which were generally negatively and positively correlated with altitude, respectively, but did not exhibit consistent trends across continents. This may be due to the influence of multiple factors on these genes, with altitude not being the primary determinant.

Limitations and future prospects

Our research still exhibits significant geographical bias, such as the lack of data from Oceania and the disproportionately high representation of Japan as an island nation, which demonstrates exceptional uniqueness. The distribution of high-altitude data is also unbalanced, with a notable absence of high-altitude data from North America and Africa. Additionally, this study employed 16S rRNA sequencing, a technique with limited sequencing range that may lead to some inaccurate assessments of diversity; future research should incorporate more metagenomic studies. The study also utilized PICRUSt2 analysis, and the inferred genetic content may differ somewhat from the actual genomes derived from the 16 S rRNA sequences. Furthermore, we only excluded samples from infants under three years of age and made judgments based on the uploaded data without rigorously examining information regarding gender, age, and migration status. The impact of altitude on the gut microbiota of global populations and the mechanisms underlying these differences require further investigation through more comprehensive, large-scale studies.

Conclusion

Using global gut microbial data, our analysis reveals significant differences in both diversity and function of gut microbiota across human populations at varying

altitudes. On a global scale, as well as in the Eurasian and American continents where altitude variations are substantial, diversity levels decrease with increasing elevation. However, at the taxonomic level, we observed virtually no heterogeneous altitude-associated patterns except *Faecalibacterium*, *Blautia* and *Prevotella* in relative abundance across global and continental groupings, suggesting that altitude does not exert consistent selective pressure on taxonomic units. Functional analysis demonstrated that genes related to terpenoid, polyketide, and lipid metabolism increase in abundance with higher altitudes, while carbohydrate metabolism genes are more prevalent in low-altitude populations, indicating that selection may occur at the functional rather than taxonomic level. These findings provide valuable insights for the development of microbiome-based diagnostics and personalized healthcare approaches tailored to populations residing at different altitudes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03974-w.

Supplementary Material 1: Table S1 The information of data using this study.

Supplementary Material 2: Table S2 Figure illustrating the relationship between gut microbiota and altitude across different continents, focusing on diversity, species composition, and functional aspects.

Supplementary Material 3: Table S3 Results of the permutation test.

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Acknowledgements
We thank Run-Hua Hu and Xin-Yuan Zhong for their contributions in data collection.

Author contributions
L.P. was responsible for writing the main manuscript text.L.P. and F.Q. was involved in data collection, analysis, and figures preparation. K.T. contributed to manuscript revision and provided funding support. W.X. offered research ideas and finalized the manuscript text. All authors reviewed the manuscript.

Funding
This work was supported by National Natural Science Foundation of China (32360274) and Dali University Undergraduate Research Fund Project.

Data availability
All data used in this study are publically available. The datasets analysed during the current study are available in the NCBI Sequence Read Archive (SRA) database, [Accession Number: PRJDB10526,PRJDB10528, PRJDB10529, PRJDB10530, PRJDB4360, PRJDB7891, PRJDB9429, PRJEB23227, PRJEB26012, PRJEB27306, PRJEB38465, PRJEB4335, PRJEB6705, PRJNA1048169, PRJNA255266, PRJNA395034, PRJNA429968, PRJNA450340, PRJNA533120, PRJNA547591, PRJNA578008, PRJNA644097, PRJNA744878, PRJNA774503, PRJNA787573, PRJNA814893, PRJNA940351].Source data are provided in the Table S1.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

Received: 9 January 2025 / Accepted: 17 April 2025
Published online: 05 May 2025

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