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Oral microbiota diversity and compositional shifts in relation to sleep quality: a cross-sectional study in Beijing adults

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Abstract

Background

Accumulating evidence indicates that the oral microbiome may modulate sleep homeostasis via the oral–gut–brain axis, yet population-based epidemiological data from Asian cohorts remain scarce. This study aimed to quantify the associations of oral microbial diversity and community composition with subjective sleep quality, and identify taxonomic biomarkers linked to sleep disturbances among urban Chinese adults.

Methods

A cross-sectional survey was conducted recruiting 73 adults aged 20–60 years in Beijing between April and July 2024. Salivary microbiota was profiled via 16S rRNA V3–V4 high-throughput sequencing. Sleep quality was evaluated using the validated Chinese version of the Pittsburgh Sleep Quality Index (PSQI). Participants were stratified into a high sleep quality group (HSQ, PSQI ≤ 5, n = 30) and a low sleep quality group (LSQ, PSQI > 5, n = 43). Alpha diversity (Shannon index) and beta diversity (Bray–Curtis, unweighted UniFrac distances) were compared between groups, while differential taxa were screened using linear discriminant analysis effect size (LEfSe) with a linear discriminant analysis (LDA) threshold of > 2.0.

Results

The LSQ group exhibited significantly higher Shannon alpha diversity than the HSQ group (Tukey's test, P = 0.048). PCoA combined with PERMANOVA revealed no significant intergroup divergence in overall oral microbial community structure (Bray–Curtis: P = 0.356, R^2 = 0.0371; unweighted UniFrac: P = 0.414, R^2 = 0.0353). Six bacterial taxa displayed significantly different relative abundances between groups (all P < 0.05). *Actinobaculum timonae* and *Stomatobaculum longum* were enriched in LSQ individuals, whereas *Bifidobacterium adolescentis*, *Bacillaceae*, *Fusobacterium canifelinum* and *Haemophilus influenzae* were overrepresented in the HSQ group. LEfSe analysis identified 22 discriminatory taxa: LSQ subjects were enriched in *Bacteroidota*-derived lineages (*Prevotellaceae, Porphyromonadaceae*), while HSQ subjects harbored elevated *Deinococcota* and *Thermus*-related taxa.

Conclusions

Distinct oral microbial taxonomic signatures differentiate adults with good versus poor sleep quality in this Beijing cohort, supporting an intimate link between oral microbiota and sleep status. Longitudinal and interventional trials are required to clarify causal relationships and develop microbiome-targeted strategies for sleep improvement.

Background

Sleep is a physiological state characterized by reduced physical and mental activity that is essential for maintaining mental and physical wellness(1). Sleep disturbance is a critical public health issue that affects both mental and physical health(2). Epidiological studies have shown that approximately one-third of adults experience at least one insomnia symptom, and 10% meet formal clinical diagnostic criteria for insomnia disorder(3, 4). Beyond conventional risk factors including chronic stress, excessive caffeine intake and prolonged screen exposure, emerging evidence suggests that alterations in the human microbiota are closely linked to the pathogenesis of insomnia(5).

Most existing research has predominantly examined the gut microbiome in relation to sleep quality; the oral cavity remains comparatively understudied. The oral cavity harbors over 600 bacterial taxa and shares anatomical continuity with the upper airway; ooral dysbiosis readily triggers chronic low-grade systemic inflammation(6–8). Notably, oral dysbiosis has been associated with periodontitis, type 2 diabetes, and an expanding list of neurological disorders(9, 10). Emerging evidence further suggests that oral microbial communities exhibit circadian rhythmicity and may modulate sleep–wake regulation through the oral–gut–brain bidirectional signaling axis(7).

Sleep quality is a critical determinant of overall health, with lower sleep quality(LSQ) linked to cardiovascular diseases, metabolic disorders, and cognitive decline(11). Globally, sleep disturbances affect approximately 20–30% of adults, posing significant economic and social burdens(4). In China, rapid urbanization and irregular daily routines have exacerbated sleep problems, particularly in metropolitan areas such as Beijing. Concurrently, the human microbiota, especially the oral microbiome, has gained attention for its role in systemic health through immune modulation and inflammatory pathways.

To date, large-scale population data elucidating the oral microbiota-sleep correlation remain limited. Two small US studies reported associations between α-diversity and self-reported sleep duration, however, objective sleep monitoring metrics and East Asian populations were absent from these analyses(12). Accordingly, we performed this cross-sectional Beijing Oral–Sleep Survey to test the hypothesis that reduced oral microbial diversity and taxonomic compositional shifts correlate with deteriorated subjective sleep quality.

Methods

1. Cohort and study design

We conducted a population-based cross-sectional study between April and July 2024 in Beijing, China. Participants were recruited from health center of the ninth medical center of PLA general hospital. A total of 73 participants were included in this study, comprising 30 with HSQ and 43 with LSQ. The study flow chart was depicted in Fig. 1. This study aimed to quantify the associations of oral microbial diversity and community composition with subjective sleep quality, and identify taxonomic biomarkers

linked to sleep disturbances among urban Chinese adults. The study protocol was approved by the Institutional Review Board of the ninth medical center of PLA general hospital (Approval Date: November 12, 2024), and All participants provided written informed consent prior to enrollment, and all study procedures complied with the Declaration of Helsinki.

2. Participant Recruitment

Inclusion criteria included: (1) age 20–60 years; (2) permanent residence in Beijing for a minimum of 5 consecutive years; (3) ability to complete questionnaires in Mandarin.

Exclusion criteria included: (1) antibiotic use within the preceding month; (2) diagnosed sleep disorders; (3) severe periodontal disease; (4) pregnancy or lactation; (5) severe systemic diseases; (6) regular oral intake of hypnotic drugs; (7) acute infections, malignant tumors, acute and chronic liver diseases, and autoimmune diseases; and (8) individuals who refused or were unable to complete the informed consent procedure.

3. Oral Microbiome Sampling

Saliva samples were collected by trained nurses or physicians following unified standardized protocols. Participants were instructed to refrain from eating, chewing gum, or smoking for 30 minutes prior to specimen collection. After rinsing their mouths with purified water, participants relaxed and massaged their cheeks to allow saliva to flow naturally into the collection device. A required volume of 3.5–4.0 ml was obtained, free of sputum. Samples were transported to the laboratory under cold-chain logistics and stored at − 80°C until further processing.

4. 16S rRNA Sequencing

DNA extraction was performed using the DNeasy PowerSoil Pro Kit (Qiagen, Germany) according to the manufacturer's instructions. The V3–V4 hypervariable regions of the 16S rRNA gene were amplified using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). Sequencing was performed on the Illumina NovaSeq 6000 platform (Illumina, USA) with 2×250 bp paired-end sequencing layout.

5. Laboratory Measurements

Blood samples were drawn from all participants between 7:00 and 9:00 am after 8 to 10 hours of overnight fasting. Blood samples were centrifuged within 30 to 45 minutes of collection. Weight, height, and blood pressure were measured according to standard protocols by the same trained staff. Serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) were quantified via enzymatic colorimetric assays. Thyroid-stimulating hormone (TSH), free triiodothyronine (FT3) and free

thyroxine (FT4) were detected using electrochemiluminescence immunoassays. All biochemical tests were completed on the sampling day, and sample analysis was only conducted when internal quality control indicators met acceptable standards.

6. Sleep Quality Assessment

Subjective sleep quality was evaluated using the Chinese validated version of the PSQI(13), a 19-item self-reported questionnaire measuring sleep disturbances over the past month. The PSQI generates seven subdomain scores and a global total score ranging from 0 to 21; higher scores correspond to worse sleep quality. Participants with a global PSQI score > 5 were classified into the LSQ group, while those with scores ≤ 5 were assigned to the HSQ group.

7. Statistical Analysis

Microbiome Data Processing: Alpha diversity was quantified using the Shannon index, and intergroup disparities were tested via the Wilcoxon rank-sum test. Beta diversity was visualized through principal coordinate analysis (PCoA) based on Bray–Curtis and unweighted UniFrac distances at the operational taxonomic unit (OTU) level. PERMANOVA was applied to test statistical differences in overall community composition. LEfSe was adopted to screen differentially abundant taxa with an LDA threshold of > 2.0.

Statistical Methods: Continuous variables were expressed as mean ± standard deviation or median (interquartile range), while categorical variables were summarized as counts and percentages. Independent t-tests were adopted for intergroup comparisons of continuous clinical indicators. The false discovery rate (FDR) correction was applied to adjust P values for multiple testing where appropriate. All statistical analyses were performed using SPSS 26.0 (IBM, Armonk, NY), with a two-tailed significance threshold set at $P < 0.05$.

Results

1.Baseline Clinical Characteristics

A comparison of baseline clinical characters between the he HSQ and LSQ groups is presented in Table 1. A total of 30 participants were enrolled in the HSQ group and 43 in the LSQ group. No significant intergroup differences were observed in age, BMI, lipid profiles, blood pressure, thyroid hormones (TSH, FT3 and FT4), fasting blood glucose (FBG) and uric acid (UA) (all $P > 0.05$).

Table 1 Comparison of clinical characters between HSQ and LSQ groups ($x \pm s$)

Group	HSQ	LSQ	t	*P*
n	30	43		
Age (y)	36.5±8.2	37.1±7.3	-0.58	0.56
BMI(kg/m2)	22.87±3.4	23.26±2.6	0.76	0.45
TCHO(mmol/L)	4.72±0.66	4.86±0.86	0.79	0.43
TG(mmol/L)	1.05±0.38	1.03±0.36	-0.17	0.86
LDL-C	2.73±0.64	2.87±0.75	0.99	0.32
SBP(mmHg)	118.4±12.2	118.2±13.4	-0.11	0.92
DBP(mmHg)	72.4±7.7	73.1±7.9	0.52	0.6
TSH(mIU/ml)	2.27±1.21	2.19±1.14	-0.35	0.73
FT3（pmol/L）	3.14±0.48	3.25±0.92	1.26	0.14
FT4（pmol/L）	10.07±1.50	9.78±1.71	-0.9	0.37
FBG（mmol/L）	4.85±0.31	4.98±0.42	1.38	0.17
UA(umol/L)	330.9±88.3	349.9±81.3	1.32	0.19

Abbreviations: BMI: Body Mass Index, TCHO: Total Cholesterol, TG: Triglycerides, LDL-C: Low-Density Lipoprotein Cholesterol, SBP/DBP: Systolic/Diastolic Blood Pressure, TSH: Thyroid-Stimulating Hormone, FT3/FT4: Free Triiodothyronine / Free Thyroxine, FBG: Fasting Blood Glucose,UA: Uric Acid

2. Alpha diversity analysis

Four complementary alpha diversity metrics were compared between the HSQ and LSQ groups (Figure 2). The Shannon index and Pielou's evenness were significantly higher in the LSQ group (Tukey's post-hoc test, P = 0.048 and P = 0.043, respectively). The LSQ group exhibited a median Shannon index of approximately 6.0 with a compact interquartile range, whereas the HSQ group showed a lower median (~5.6) with broader data dispersion and a distinct low-value outlier (Shannon ≈ 1.8). In contrast, observed species richness and Faith's phylogenetic diversity showed no intergroup statistical differences (P = 0.17, P = 0.926). These results indicated that sleep quality mainly regulates microbial community evenness and overall diversity, rather than taxonomic richness or phylogenetic evolutionary divergence.

3. Beta Diversity Analysis

PCoA combined with PERMANOVA was performed to evaluate intergroup divergence in overall oral microbial community composition (Figure 3). Two ordination plots were generated based on Bray–Curtis distance (Figure 3A) and unweighted UniFrac distance (Figure 3B). The first two PCoA axes explained 24.83% (13.47% + 11.36%) and 23.52% (15.11% + 8.41%) of total community variance, respectively. Visual inspection revealed extensive overlap of sample scatter points and 95% confidence ellipses between the two groups. Consistent with visual patterns, PERMANOVA confirmed non-significant overall community separation (Bray–Curtis: P = 0.356,

R^2 = 0.0371; unweighted UniFrac: P = 0.414, R^2 = 0.0353).

4. Differential abundance analysis of bacterial taxa

Six bacterial species exhibited significantly different relative abundances between HSQ and LSQ groups (all P < 0.05, Figure 4). *Actinobaculum timonae* (P = 0.0141) and *Stomatobaculum longum* (P = 0.049) were markedly enriched in LSQ participants. *A. timonae* had a median relative abundance of ~0.03% with multiple high-value outliers reaching 0.14%, while *S. longum* showed a median abundance of ~0.25% with wider distribution.

Four taxa were overrepresented in the HSQ group: *Bifidobacterium adolescentis* (P = 0.037), unclassified *Bacillaceae* (P = 0.037), *Fusobacterium canifelinum* (P = 0.0147) and *Haemophilus influenzae* (P = 0.00894). *B. adolescentis* and *Bacillaceae* were nearly undetectable in LSQ saliva samples, with sporadic high-abundance outliers only observed in HSQ. *H. influenzae* presented the most prominent intergroup difference, with a median relative abundance of ~0.015% and extreme outliers up to 0.17% in HSQ, while most LSQ samples contained undetectable levels of this species. Although both groups exhibited broad distribution ranges, *F. canifelinum* displayed elevated median abundance in the HSQ cohort.

5. LEfSe Analysis of Differentially Abundant Taxa

LEfSe analysis with an LDA threshold > 2.0 identified 22 taxa with significantly divergent abundances across multi-taxonomic levels (Figure 5). Taxa affiliated with phylum Bacteroidota were enriched in LSQ individuals, including class *Bacteroidia*, order *Bacteroidales*, families *Prevotellaceae* and *Porphyromonadaceae*, as well as genera *Prevotella*, *Porphyromonas* and *Leptotrichia*. At the species level, *Porphyromonas pasteri* served as a characteristic LSQ biomarker, indicating that poor sleep facilitates the colonization of oral anaerobic pathobionts.

In contrast, the HSQ group showed specific enrichment of phylum *Deinococcota* and its subordinate lineages (class *Deinococci*, order *Deinococcales*), alongside genus *Thermus* (including *T. scotoductus*) and family *Thermaceae*. Species-level biomarkers for HSQ included *Rothia dentocariosa*. Although commensal bacteria existed in both cohorts, their species-level composition differed substantially.

Collectively, LSQ oral microbiota was dominated by *Bacteroidota*-derived anaerobes, whereas HSQ saliva harbored stress-resistant *Deinococcota* and *Thermus*-related taxa, demonstrating that sleep quality drives targeted enrichment of functionally distinct bacterial lineages.

Linear discriminant analysis (LDA) scores showing taxa significantly enriched in the LSQ (green) and HSQ(orange) groups. Only taxa with an LDA score > 2.0 are displayed. Taxonomic levels are denoted by prefixes: p_, phylum; c_, class; o_, order; f_, family; g_, genus; s_, species.

6. Spearman Correlation of Top 20 Microbial Taxa at Phylum and Genus Levels

We evaluated inter-taxa associations of the top 20 abundant microbiota via Spearman correlation heatmaps at phylum and genus levels (Figure X), with hierarchical clustering grouping taxa with identical correlation profiles. Red cells signified positive covariation, whereas blue cells represented negative correlation. At the phylum level, rare phyla like *Gemmatimonadota* formed a strongly positively correlated cluster, and core phyla (*Bacteroidota, Actinomycetota*) showed moderate negative correlation. At the genus level, *Thermus* displayed widespread negative associations with dominant genera including *Prevotella* and *Porphyromonas*, while a group of fermentative genera *(Prevotella, Nanosynbacter, Lancefieldella*) exhibited robust positive co-occurrence. The microbiota exhibited clear co-occurrence and competitive co-exclusion relationships among core and subdominant taxa, Fig 6.

7. Functional Profiling of Microbial KEGG Orthologs and Validation of Core Carbon Metabolic Genes

A row z-score normalized heatmap visualized normalized abundance of the top 50 dominant KEGG Orthology (KO) genes between HSQ and LSQ groups (Figure 7 left), exhibiting obvious group-biased functional clustering patterns. Four core carbon-metabolism KO orthologs (K00253, K00158, K00009, K00034) were further quantified via scatter-box plots to verify intergroup functional divergence (Figure 7 right). These orthologs encode key enzymes regulating the tricarboxylic acid (TCA) cycle, glycolysis and lactic acid fermentation pathways. Despite subtle visual disparities in average raw read counts, FDR multiple testing correction confirmed no statistically significant intergroup differences for all four genes ($P>0.05$, unified $q= 0.699$).

Discussion

1. Elevated Alpha Diversity Is a Distinct Microbial Signature of Poor Sleep Quality

This study demonstrates a significant association between oral microbiome composition and sleep quality in a Beijing population. Specifically, we observed higher Shannon diversity in the LSQ group compared to the HSQ group, along with distinct taxonomic signatures between the two groups. These findings align with emerging research on the oral-gut-brain axis, suggesting that oral dysbiosis may influence sleep through immune and neural pathways.

While the observation of elevated α-diversity in individuals with poor sleep may appear counterintuitive to the prevailing ecological paradigm that greater microbial diversity confers health benefits, this pattern is consistent with the concept of dysbiotic expansion, wherein pathological conditions facilitate the proliferation of opportunistic and pathobiontic taxa normally suppressed within a healthy ecosystem(8, 9). Comparable phenomena have been well documented in periodontitis and inflammatory bowel disease, wherein disease-associated increases in richness reflect ecological degradation rather than functional resilience(14). In the specific context of sleep disturbance, nocturnal mouth breathing, diminished salivary flow, and altered immune surveillance may generate microenvironments conducive to the expansion of anaerobic and pro-inflammatory taxa, thereby inflating diversity metrics while concomitantly compromising community stability.

These findings appear to contrast with those of the NIH-AARP cohort study by Dalton et al.(12) who reported that shorter self-reported sleep duration was associated with reduced oral microbiome diversity. Several methodological and biological factors may account for this apparent discrepancy. First, Dalton et al. operationalized sleep as a continuous duration variable, whereas we employed the PSQI-defined sleep quality construct, which captures multidimensional sleep disturbances (including sleep latency, efficiency, and daytime dysfunction) beyond mere sleep duration. Second, ethnic and dietary differences between US and Chinese populations may exert differential modulatory effects on the microbiome–sleep relationship. Third, our relatively modest sample size (n=73) may have increased susceptibility to outlier-driven diversity estimates, as evidenced by the greater dispersion and low-value outliers observed within the HSQ group.

2. Sleep Quality Fine-Tunes Taxonomic Proportions Without Rewriting Overall Community Architecture

Despite significant differences in α-diversity and specific taxonomic abundances, β-diversity analyses revealed no statistically significant separation between HSQ and LSQ groups. This suggests that sleep quality may act as a fine-tuning force on the oral microbiome—altering the relative proportions of constituent members without dismantling the overall community architecture. Such a pattern is biologically plausible: the oral cavity is a highly selective environment with strong colonization resistance, and profound community restructuring may require more severe or chronic perturbations than subclinical sleep disturbances(6, 12). Nevertheless, the small effect sizes ($R^2 < 4\%$) indicate that sleep quality explains only a modest fraction of microbiome variance, underscoring the dominant roles of diet, oral hygiene, and host genetics in shaping oral microbial ecology.

3. LSQ Enrichment of Pro-Inflammatory Anaerobic Taxa and HSQ Enrichment of Sleep-Beneficial Commensals

The LEfSe analysis revealed a striking enrichment of *Bacteroidota*-associated lineages—specifically *Prevotellaceae*, *Porphyromonadaceae*, *Prevotella*, *Porphyromonas*, and *Leptotrichia*—in the LSQ group. These taxa are archetypal anaerobes commonly associated with periodontal disease, oral inflammation, and systemic endotoxemia.Their enrichment in poor sleepers may reflect several interconnected mechanisms. First, sleep disturbances are frequently accompanied by mouth breathing and reduced

nocturnal salivary flow, which lowers oral oxygen tension and creates favorable niches for anaerobic proliferation. Second, chronic sleep restriction activates the hypothalamic-pituitary-adrenal (HPA) axis and elevates circulating cortisol, which can impair local immune responses and reduce the production of antimicrobial peptides in saliva, thereby permitting pathobiont expansion(5, 15). Third, these anaerobic taxa are potent producers of lipopolysaccharide (LPS) and other pro-inflammatory metabolites that can translocate across the oral mucosa or be swallowed into the gastrointestinal tract, triggering systemic low-grade inflammation that further disrupts sleep–wake regulation via the oral-gut-brain axis(5, 6).

The differential abundance analysis corroborated this anaerobic signature: *Actinobaculum timonae* and *Stomatobaculum longum* were significantly enriched in the LSQ group. Both species are associated with anaerobic, protein-rich environments and have been implicated in opportunistic infections and inflammatory conditions. Their presence may serve as sentinel markers of oral ecosystem dysbiosis driven by sleep-related physiological changes.

Conversely, the HSQ group exhibited enrichment of taxa with potentially protective or stabilizing roles. *Bifidobacterium adolescentis*, though traditionally considered a gut commensal, has been detected in the oral cavity and is recognized for its ability to produce γ-aminobutyric acid (GABA), a primary inhibitory neurotransmitter critical for sleep initiation and maintenance(16). Preclinical and clinical studies indicate that *B.adolescentis* can attenuate neuroinflammation, modulate HPA axis activity, and improve intestinal barrier function—mechanisms that may indirectly support sleep homeostasis through the gut-brain axis(5, 16, 17), Murakami et al.(17) recently demonstrated that oral supplementation with B. adolescentis significantly increased total sleep time, REM sleep, and wakefulness in adults with sleep complaints, providing direct clinical evidence for its sleep-promoting properties. Its enrichment in good sleepers suggests that beneficial oral taxa may constitute a microbial reservoir contributing to systemic neurochemical balance.

The HSQ group was also characterized by enrichment of the phylum *Deinococcota* and its descendant lineages, including *Thermus* and *Rothia dentocariosa*. Members of *Deinococcota* are renowned for their extreme resistance to oxidative stress, radiation, and desiccation, conferred by robust enzymatic and non-enzymatic antioxidant defense systems dominated by manganese complexes and catalases(18). In the oral cavity, these properties may confer resilience against environmental fluctuations and host-derived oxidative bursts, potentially stabilizing the microbial community under conditions of metabolic and immunological homeostasis. *Rothia dentocariosa*, a facultative anaerobe with both cariogenic and probiotic properties depending on ecological context, may also contribute to community stability through competitive exclusion of more pathogenic taxa. The co-enrichment of these stress-resistant and potentially beneficial taxa in good sleepers supports the hypothesis that sleep quality fosters a robust, resilient oral microbiome.

The significant enrichment of *Haemophilus influenzae* in the HSQ group is noteworthy. While *H.influenzae* is a recognized respiratory pathogen, non-encapsulated strains commonly colonize the nasopharynx and oral cavity as commensals without causing disease. In the context of this study, its presence may reflect

a healthy, uncompromised upper airway–oral continuum, whereas sleep disturbances—particularly those associated with nocturnal hypoxia or inflammation—may displace this commensal in favor of more pathogenic or anaerobic competitors. Alternatively, *H. influenzae* may engage in mutualistic interactions with other oral commensals that collectively support mucosal immune homeostasis.

4. Ecological Co-occurrence and Competitive Interactions Among Oral Core Taxa

Spearman correlation analysis revealed clear synergistic and competitive interactions between dominant oral microbes. The negative correlation between Thermus and LSQ-enriched anaerobic genera (Prevotella, Porphyromonas) provides a microbial ecological explanation for our taxonomic differential results: stress-resistant Thermus and inflammatory Bacteroidota anaerobes occupy mutually exclusive ecological niches, such that healthy sleep conditions favor Thermus proliferation while poor sleep drives Bacteroidota expansion.

Conversely, fermentative anaerobes including Prevotella, Nanosynbacter and Lancefieldella formed tight positive co-occurrence modules, consistent with their synchronous overgrowth in LSQ saliva. Rare phyla such as Gemmatimonadota also shared strong positive covariation, indicating that minor taxa with similar metabolic requirements tend to co-colonize the oral niche. These inter-taxa interaction patterns further validate the distinct microbial ecological landscapes of HSQ and LSQ cohorts.

5. Sleep Quality Does Not Induce Statistically Significant Shifts in Core Carbon Metabolic Functions

Although the row z-score KO heatmap displayed visually distinguishable group-biased functional clustering, quantitative validation of four core carbon-metabolism orthologs showed no statistically significant intergroup differences after FDR multiple testing correction. This result suggests that while sleep quality reshapes the relative abundance of specific microbial taxa, the core basal metabolic capacity of oral microbiota (central carbon metabolism, TCA cycle, glycolysis and lactic acid fermentation) remains functionally conserved. The mild visual functional divergence only reflects minor taxonomic compositional fluctuations without altering overall microbial metabolic potential.

16S rRNA sequencing only resolves taxonomic variation rather than precise functional gene profiles; future metagenomic shotgun sequencing will enable comprehensive characterization of functional shifts in neurotransmitter and short-chain fatty acid synthesis pathways linked to sleep regulation.

6. Mechanistic Framework: The Oral–Gut–Brain Axis Links Oral Dysbiosis and Sleep Disturbance

Our findings are best interpreted within the framework of the oral–gut–brain axis, a bidirectional communication network linking oral microbial ecology to central nervous system function via neural, immunological, and metabolic pathways(5, 7). The oral cavity serves as a gateway through which approximately 10 bacteria are swallowed daily(11), meaning that oral dysbiosis can directly seed and perturb gut microbial communities(5, 6). Sleep disturbances may therefore initiate a vicious cycle: poor sleep alters oral microenvironments (salivary flow, pH, oxygen tension), promoting anaerobic dysbiosis; dysbiotic oral taxa are swallowed and disrupt gut ecology; altered gut microbiota impairs production of

sleep-regulating metabolites such as short-chain fatty acids (SCFAs), GABA, and serotonin; and reduced microbial metabolite availability further compromises sleep quality(19).

SCFAs—particularly butyrate, propionate, and acetate—are critical mediators in this axis. Evidence indicates that serum butyrate levels are significantly decreased in individuals with insomnia, and oral butyrate supplementation can alleviate sleep disturbances in murine models by modulating hypothalamic neuronal activity and neurotransmitter homeostasis(19). Tryptophan metabolism, tightly regulated by gut microbiota, serves as a precursor for serotonin and melatonin synthesis, directly influencing the sleep–wake cycle(20).Furthermore, GABA produced by commensal bacteria such as *Lactobacillus* and *Bifidobacterium* can modulate the central GABAergic system via the vagus nerve, attenuating anxiety and insomnia-related symptoms(5, 20).

Circadian rhythmicity adds another layer of complexity. Takayasu et al.(7) demonstrated that salivary microbial composition and functional potential exhibit robust circadian oscillations, with specific taxa peaking during nocturnal or diurnal phases. Sleep disruption, particularly shift work or irregular schedules, can desynchronize these microbial rhythms, leading to temporal dysbiosis that persists even during attempted recovery sleep(10). Mei et al.(10) recently reported that mobile phone addicts with sleep disorders exhibited dysrhythmic salivary microbiota, characterized by the loss of circadian oscillations in *Rothia* and *Lautropia*—taxa that our study identified as enriched in good sleepers. These convergent findings suggest that circadian disruption may be a critical mechanistic link between sleep quality and oral microbial ecology.

Our results both complement and extend prior investigations of the oral microbiome–sleep relationship. In the NHANES 2009–2012 cohort, Hao et al.(14) reported that self-reported sleep disorders were associated with altered oral microbial diversity and composition, though effect sizes were modest and limited by the use of a single-item sleep disorder question. The NIH-AARP study by Dalton et al.(12) focused primarily on sleep duration and found associations with reduced diversity in short sleepers. Our study adds to this literature by: (i) employing the validated PSQI to capture multidimensional sleep quality rather than duration alone; (ii) identifying specific functional taxa (*Deinococcota, Thermus*) not previously linked to sleep in population-based studies; (iii) providing data from an Asian urban cohort, thereby addressing the geographic bias toward Western populations; and (iv) demonstrating that sleep quality may influence microbial ecology even in the absence of diagnosed sleep disorders or severe periodontal disease.

The enrichment of *Prevotella* and *Porphyromonas* in our LSQ group aligns with findings from obstructive sleep apnea (OSA) patients, in whom salivary dysbiosis is characterized by increased anaerobic taxa and their association with periodontitis(8). However, our participants were explicitly screened for severe periodontal disease and diagnosed sleep disorders, suggesting that subclinical sleep disturbances (as captured by PSQI) are sufficient to drive anaerobic enrichment independently of overt oral pathology.

If corroborated by longitudinal and interventional studies, our findings have several clinical implications. First, salivary microbial profiling could serve as a non-invasive biomarker panel for sleep-health risk

stratification, particularly in populations where polysomnography is inaccessible. Second, the identification of *Deinococcota* and *Thermus* as potential beneficial taxa associated with good sleep opens avenues for probiotic development. While these taxa are not traditionally considered probiotics, their stress-resistant properties make them attractive candidates for oral microbiome engineering. Third, the depletion of *B. adolescentis* and enrichment of LPS-producing anaerobes in poor sleepers suggests that microbiome-targeted interventions could modulate the oral-gut-brain axis to improve sleep quality.

Recent randomized controlled trials provide promising evidence for this approach. Murakami et al.(17) demonstrated that *B. adolescentis* supplementation improved objective sleep parameters (total sleep time, REM sleep) in adults with sleep complaints. A 2025 meta-analysis of 15 RCTs found that probiotic supplementation significantly reduced PSQI scores, with Bifidobacterium and Lactobacillus strains showing the most consistent effects(21). Similarly, *Bifidobacterium* animalis *subsp. lactis* BLa80 was shown to enhance sleep quality while increasing *Bifidobacterium* abundance and upregulating GABA production pathways(22). These findings collectively support the therapeutic potential of probiotics in sleep disorders, though strain specificity and delivery route (oral vs. gastrointestinal) remain critical considerations.

From a public health perspective, our findings reinforce the importance of oral hygiene as a modifiable lifestyle factor in sleep health. Given that sleep disturbances affect 20–30% of adults globally(3) and oral disease is among the most prevalent non-communicable diseases, integrated sleep–oral health interventions may offer a cost-effective strategy for reducing the burden of both conditions.

Strengths and Limitations

Strengths of this study include pulation-based cross-sectional design with standardized salivary sampling and 16S sequencing workflows; comprehensive adjustment for metabolic and thyroid clinical confounders; validated multi-dimensional PSQI sleep assessment rather than single-item sleep metrics; multi-layer microbial analyses covering alpha/beta diversity, differential species, LEfSe biomarkers, inter-taxa correlation and functional KO profiling.

Limitations

First, cross-sectional design cannot establish causal relationships between oral microbial shifts and sleep deterioration;Second, the modest sample size (n = 73) limits statistical power for rare taxa detection and may have contributed to the outlier-driven diversity patterns observed. Third, we relied solely on subjective PSQI scores rather than objective sleep measures such as polysomnography or actigraphy, which would have provided more precise phenotyping of sleep architecture and sleep-disordered breathing(15). Fourth, 16S rRNA gene sequencing provides taxonomic but not functional resolution; we cannot determine whether the observed taxonomic shifts translate into altered microbial metabolism (e.g., SCFA or GABA production). Finally, our findings are derived from a single urban center in China and may not generalize to rural populations or other ethnic groups.

Implications

If corroborated longitudinally, oral microbial profiling could supplement sleep-health risk stratification. Interventions (probiotics, prebiotics, antimicrobial peptides) that bolster SCFA producers or suppress LPS-rich taxa might offer novel therapeutic avenues.

Future directions

Longitudinal cohort studies with repeated microbiome sampling are urgently needed to disentangle temporal relationships between oral microbial shifts and sleep deterioration. Such studies should incorporate objective sleep monitoring (actigraphy, polysomnography), comprehensive dietary assessment, and serial inflammatory biomarker measurements (CRP, IL-6, TNF-α) to validate the proposed mechanistic pathways. Randomized controlled trials comparing oral probiotic formulations (e.g., B. adolescentis or Deinococcota-derived strains) versus placebo, with endpoints including PSQI scores, sleep architecture, and salivary cytokine profiles, would provide critical evidence for causality and therapeutic potential. Additionally, shotgun metagenomic and metabolomic analyses are warranted to elucidate the functional consequences of the taxonomic shifts observed in this study.

Conclusion

Among urban adults residing in Beijing, poor subjective sleep quality correlates with altered salivary microbial ecology, characterized by elevated alpha diversity, enrichment of pro-inflammatory anaerobic Bacteroidota lineages, and depletion of stress-resistant and sleep-protective commensals including Deinococcota and Bifidobacterium adolescentis. Although sleep status reshapes taxonomic relative abundance, core carbon metabolic functional capacity of oral microbiota remains statistically conserved. The oral microbiome represents a modifiable intermediate factor linking sleep physiology and systemic health via the oral–gut–brain axis, laying a foundation for future microbiome-targeted sleep intervention strategies.

Abbreviations

BMI	Body Mass Index
CRP	C-reactive protein
DBP	Diastolic Blood Pressure
DNA	Deoxyribonucleic Acid
FBG	Fasting Blood Glucose
FDR	False Discovery Rate
FT3	Free Triiodothyronine
FT4	Free Thyroxine
GABA	γ-aminobutyric acid
HPA	hypothalamic-pituitary-adrenal
HSQ	high sleep quality group
IBM	International Business Machines
IL-6	Interleukin-6
KO	KEGG Orthology
LDA	linear discriminant analysis
LDL-C	Low-Density Lipoprotein Cholesterol
LEfSe	linear discriminant analysis effect size
LSQ	low sleep quality group
NIH-AARP	National Institutes of Health-American Association of Retired Persons
NHANES	National Health and Nutrition Examination Survey
OSA	obstructive sleep apnea
OTU	operational taxonomic unit
PCoA	principal coordinate analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
PHQI	Pittsburgh Sleep Quality Index (appears in Keywords; likely a typo for PSQI)
PLA	People's Liberation Army
PSQI	Pittsburgh Sleep Quality Index
RCTs	randomized controlled trials
REM	rapid eye movement

SBP	Systolic Blood Pressure
SCFAs	short-chain fatty acids
SPSS	Statistical Package for the Social Sciences
TCHO	Total Cholesterol
TCA	tricarboxylic acid
TG	Triglycerides
TNF-α	Tumor Necrosis Factor-alpha
TSH	Thyroid-Stimulating Hormone
UA	Uric Acid

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of the ninth medical center of PLA general hospital (Approval Date: November 12, 2024), and All participants provided written informed consent prior to enrollment, and all study procedures complied with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analysed during the current study are available in the Genome Sequence Archive (GSA) of the National Genomics Data Center (NGDC), China National Center for Bioinformation repository, [PRJCA072537] (https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA072537). The raw 16S rRNA gene sequencing data and associated metadata have been deposited and will be released upon manuscript acceptance.

Competing interests

The authors declare no competing interests.

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Authors' contributions

Lintao Shi: Conceptualization, Methodology, Investigation, Writing – original draft. Aiyun Yu: Data curation, Formal analysis, Visualization. Weiwei Luo: Investigation, Data collection. Xiangrong Pu: Data collection. Chenghua Che: Data collection. Min Zhang, Zijie Huang: Resources, Supervision. Jianwen Gu: Validation, Project administration. Haiying Jia: Conceptualization, Writing – review and editing, Supervision. All authors have read and approved the final manuscript.

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Figures

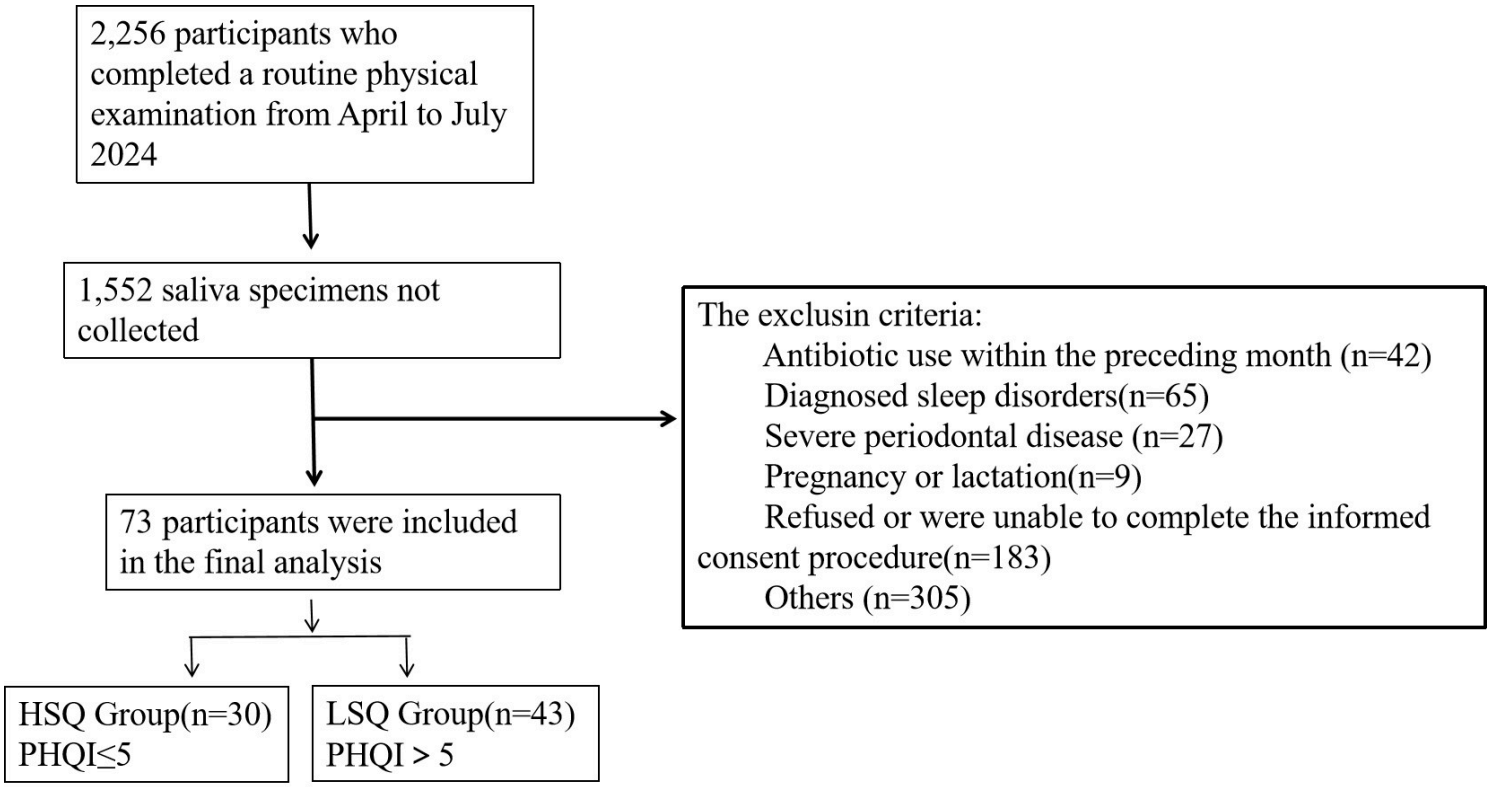


Fig 1 The flowchart of the population

Figure 1

See image above for figure legend

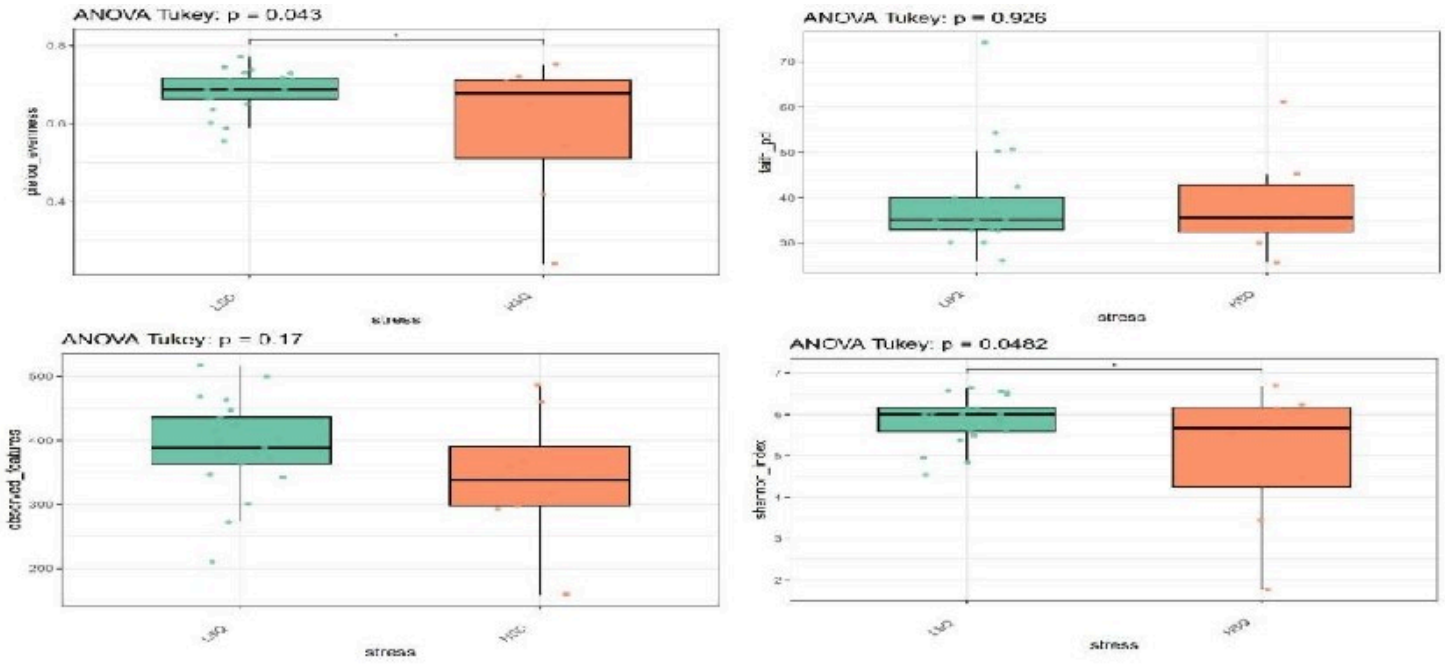


Fig 2 Comparison of alpha diversity metrics between HSQ and LSQ groups

Figure 2

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Fig 3A Bray-Curtis distance

Fig 3B Unweighted UniFrac distance

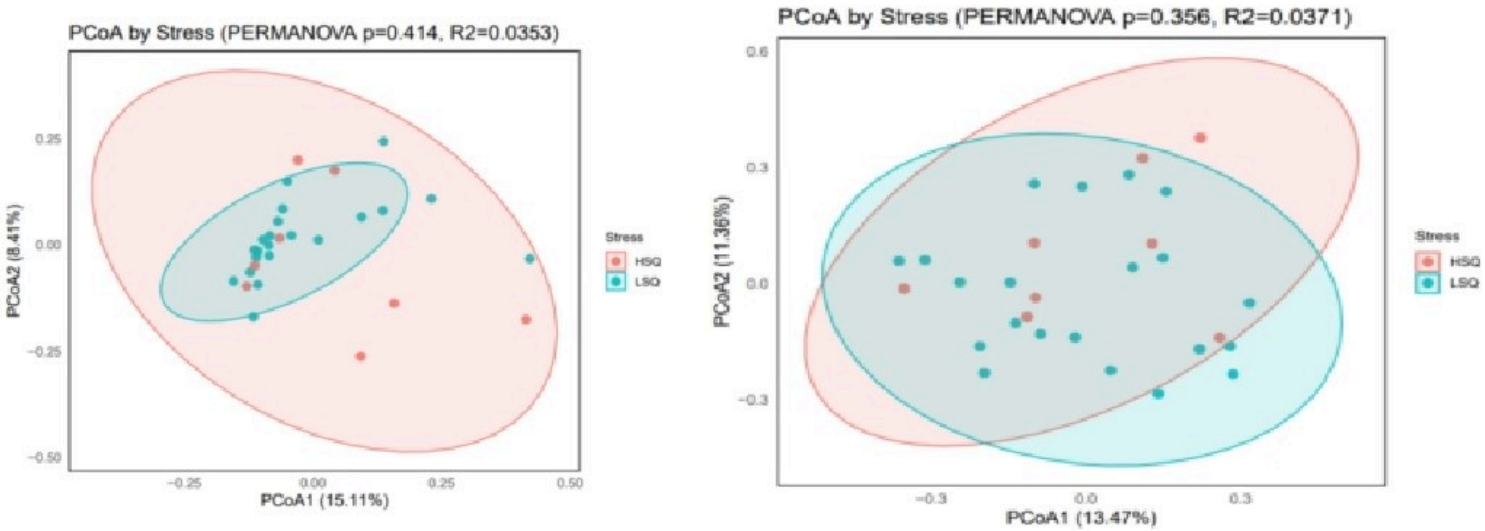


Fig 3 Beta diversity ordination plots based on Bray–Curtis (A) and unweighted UniFrac , (B) distances

Figure 3

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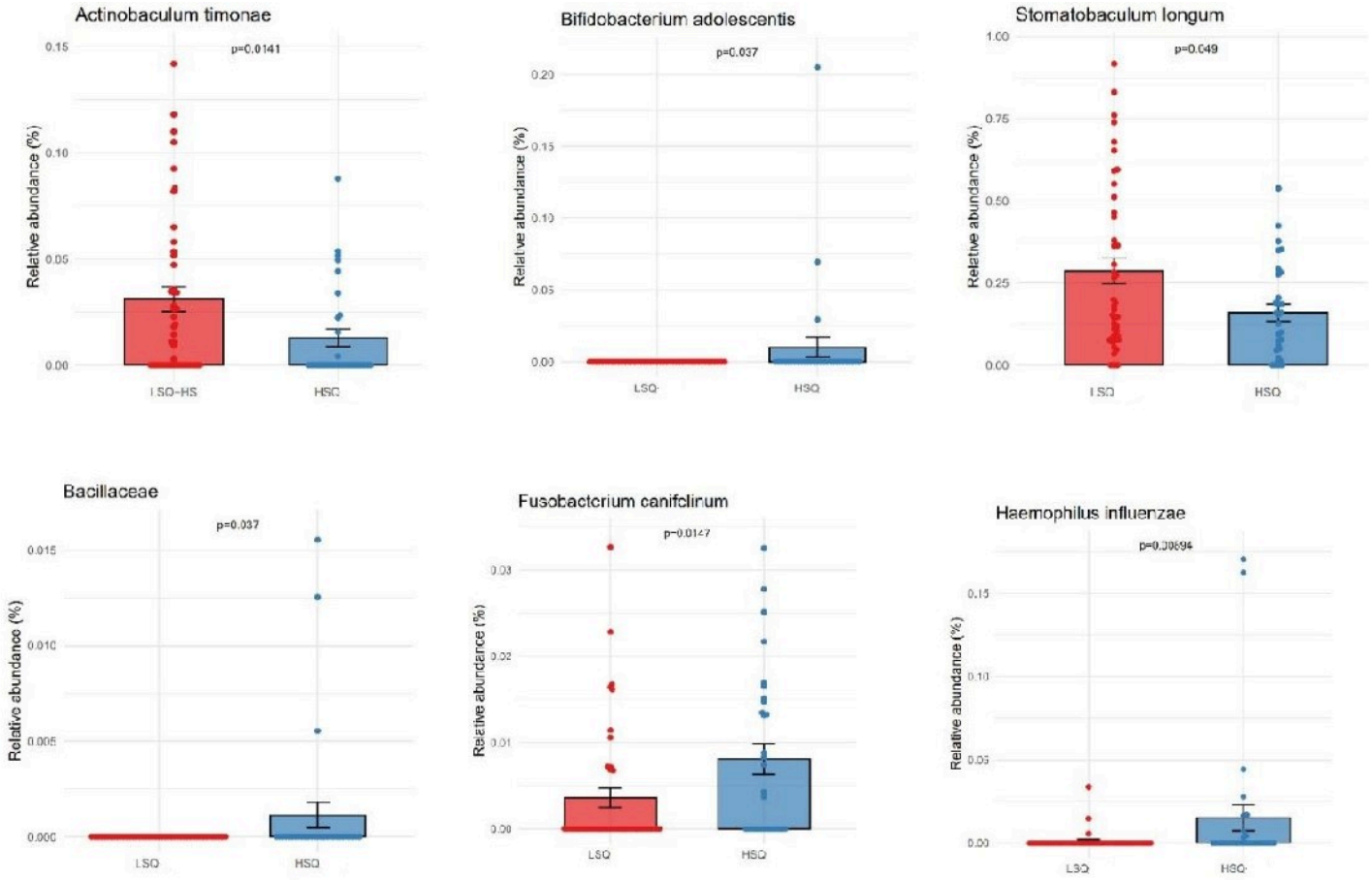


Fig 4 Differential relative abundance of six bacterial species between HSQ and LSQ groups

Figure 4

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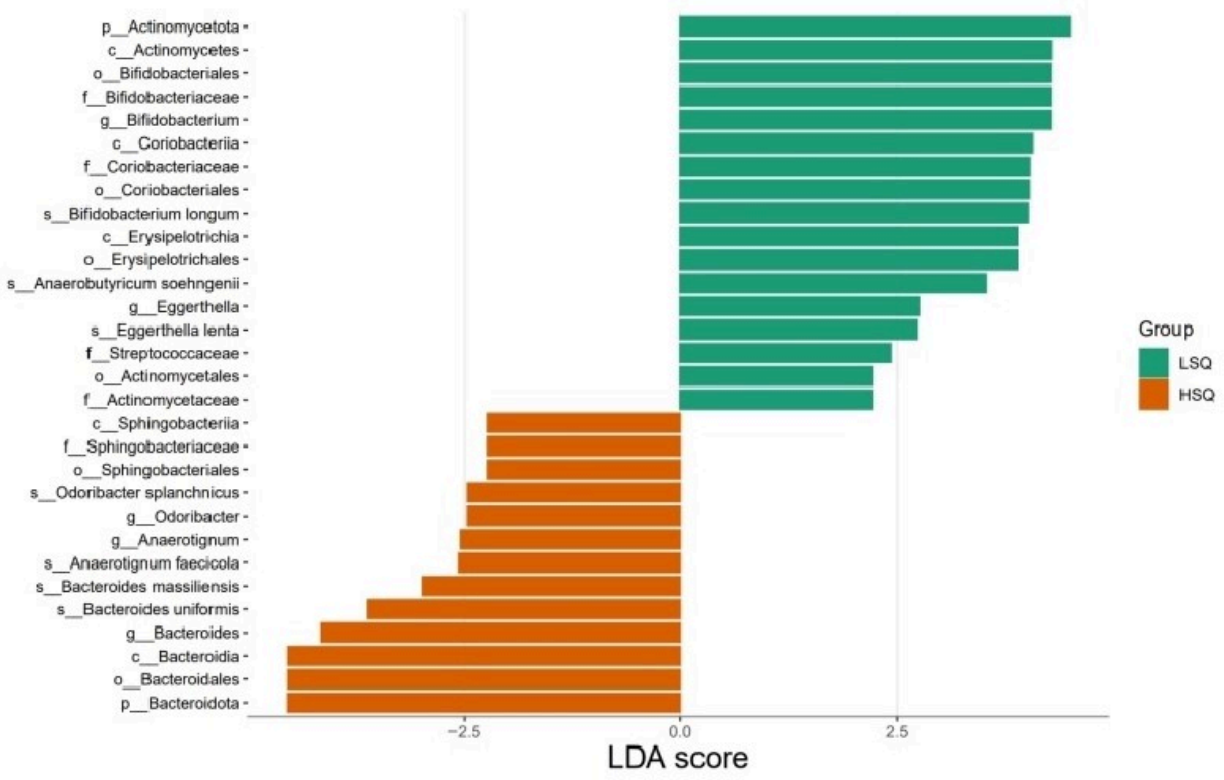


Figure 5 LEfSe analysis of differentially abundant taxa between two groups

Figure 5

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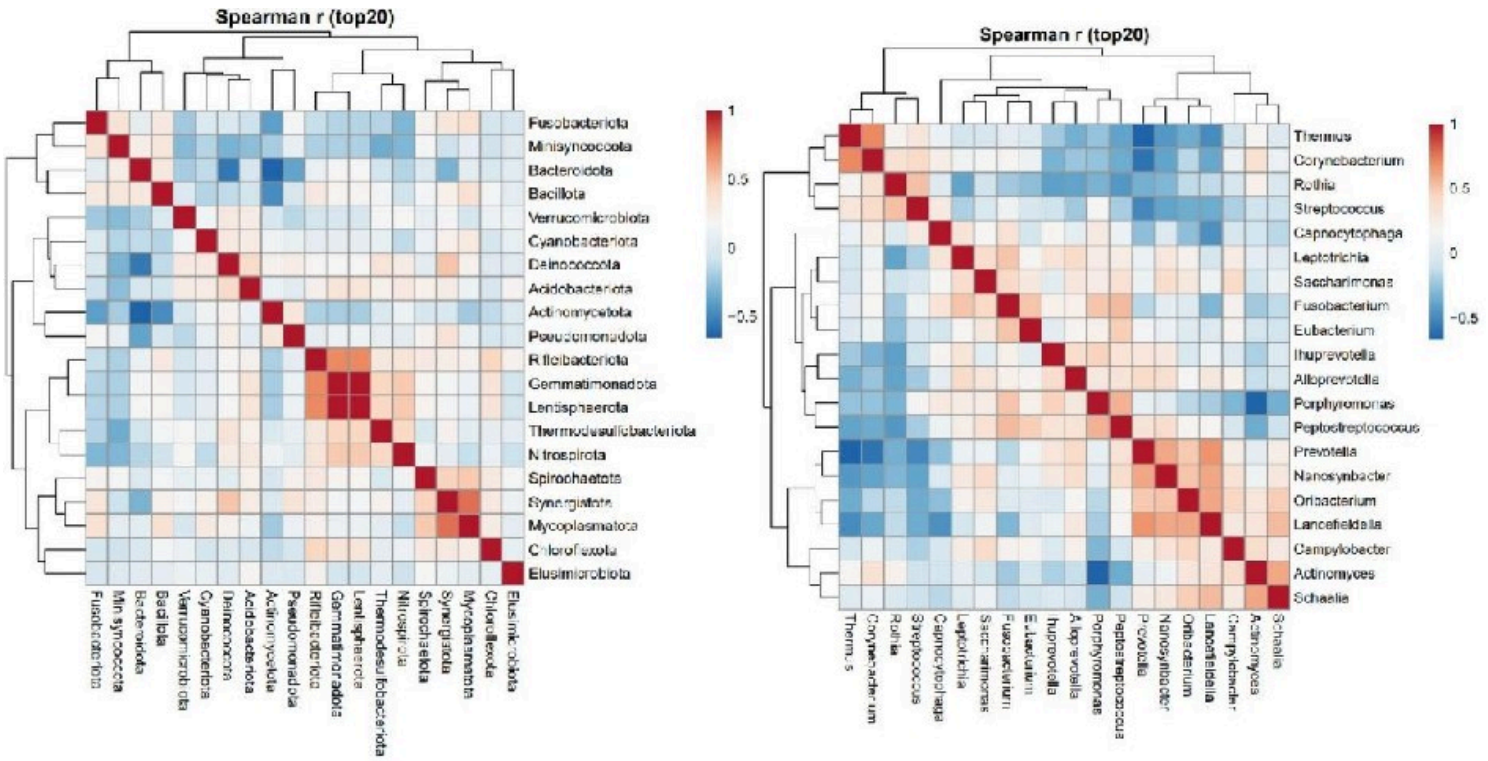


Fig 6 Spearman Correlation Heatmaps of Top 20 Taxa

Figure 6

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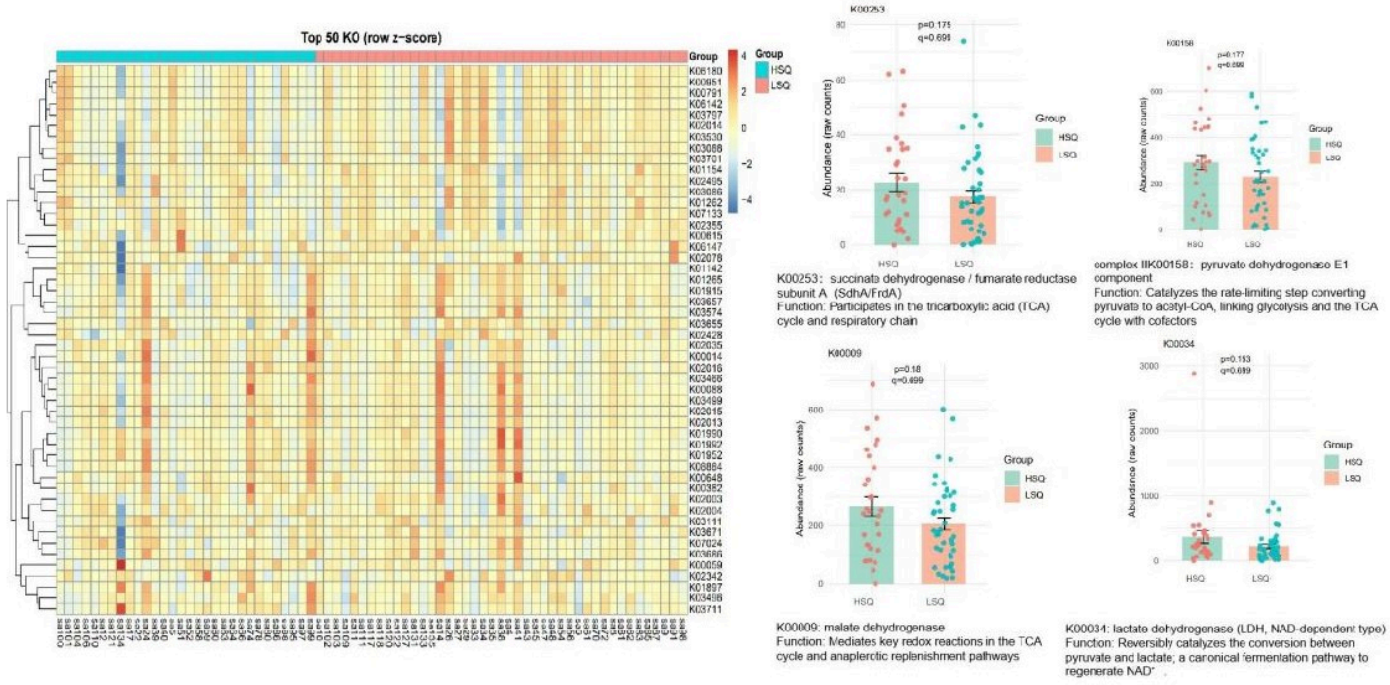


Fig 7 Top 50 KO Functional Profiles and Quantification of Core Carbon-Metabolic Orthologs (Left) Row z-score heatmap of the top 50 dominant KO functional orthologs; (Right) Scatter-box plots showing raw abundance of four core carbon-metabolic KO genes across HSQ and LSQ groups

Figure 7

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