

Gut Bacterial and Fungal Dysbiosis in Patients with Non-Statutory Infectious Diarrhea in Hohhot: A 16S rRNA and ITS Amplicon Sequencing Case–Control Study

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Abstract

Objective

Non-statutory infectious diarrhea remains a widespread intestinal disease under national surveillance in China. Although cross-kingdom imbalances between intestinal bacteria and fungi drive disease progression, the underlying dysbiosis profiles, host regulatory patterns, and diagnostic values of these microbial markers remain poorly defined. This case–control study investigated dual-kingdom microbial dysbiosis in affected individuals in Hohhot, evaluated age-dependent microecological shifts, and assessed the diagnostic utility of key gut microbes to support targeted therapies.

Methods

Fecal samples from 85 patients and 70 matched healthy controls were analyzed via high-throughput 16S rRNA (V3–V4) and ITS amplicon sequencing to evaluate bacterial and fungal diversity and taxonomic shifts. Gender and age stratification were applied to parse host-driven microbial alterations. Candidate diagnostic biomarkers were selected and validated using random forest modeling paired with receiver operating characteristic (ROC) curves.

Results

Infectious diarrhea severely disrupted gut microecological stability. Patients exhibited significantly reduced alpha diversity in both kingdoms, with bacterial lineages undergoing profound structural shifts ($P < 0.001$) compared to more modest alterations in fungal networks. Taxon-level profiling showed marked declines in Firmicutes and Bacteroidetes alongside expansions of Proteobacteria and Actinobacteriota. Key butyrate producers (*Faecalibacterium*, *Roseburia*, *Blautia*, and *Bacteroides*) were severely depleted, whereas opportunistic pathogens such as *Escherichia-Shigella* and *Streptococcus* proliferated. Fungal dysbiosis was driven by *Candida* and Sordariaceae enrichment alongside *Aspergillus* depletion. While host gender did not affect microbial composition, age emerged as a key determinant. Bacterial diversity followed an age-dependent trajectory, lowest in infants, peaking in young and middle-aged adults, and dropping in elderly subjects, accompanied by sharp compositional divergence. Conversely, fungal variations were primarily limited to species richness within a preserved core community architecture. Random forest models demonstrated exceptional diagnostic performance for *Faecalibacterium*, *Roseburia*, and *Ruminococcus* (AUC > 0.9), whereas fungal panel performance remained modest.

Conclusion

Patients in Hohhot suffering from non-statutory infectious diarrhea exhibit pronounced cross-kingdom dysbiosis marked by beneficial bacterial loss, opportunistic bacterial growth, and fungal expansion. Host age specifically shapes these dysbiotic phenotypes, producing distinct, age-dependent microecological profiles. Core butyrate-producing bacteria act as highly sensitive diagnostic biomarkers. These insights provide a foundational baseline for understanding disease mechanisms, refining age-stratified therapeutics, and optimizing targeted prevention.

1 Introduction

Non-statutory infectious diarrhea represents a major public health burden across China. Designated under national surveillance protocols, this diagnostic category encompasses infectious diarrheal conditions distinct from statutory diseases like cholera, amoebic dysentery, and typhoid or paratyphoid fevers [1]. Driven by diverse viral, bacterial, fungal, and parasitic pathogens, this complex disease affects all age cohorts, spreads rapidly, and recurs frequently [2]. Rising annual incidence rates underscore the urgent need to clarify its etiology and refine control strategies.

Recent advances in metagenomic and amplicon sequencing have clarified how the gut microbiome influences intestinal health. However, most research focuses exclusively on single microbial kingdoms. Critical knowledge gaps persist regarding the combined architecture, cross-kingdom dynamics, and authentic microecological profiles of co-existing bacterial and fungal populations [3]. Addressing these gaps is essential for unraveling how cross-kingdom interactions dictate disease pathogenesis.

The human gut functions as a complex, dynamic cross-kingdom ecosystem. Resident bacteria and fungi do not operate in isolation; rather, they form complex networks maintained by metabolic cross-feeding, physical co-assembly within biofilms, niche competition, and cooperative colonization. Together, these kingdoms protect mucosal barrier function, tune host immune responses, and sustain general homeostasis [4]. Consequently, single-kingdom analyses fail to capture the full spectrum of dysbiosis or highlight how trans-kingdom interactions drive infectious diarrhea.

To address these limitations, we conducted a cross-sectional case-control study on cohort participants from Hohhot, Inner Mongolia. Using high-throughput 16S rRNA gene and ITS amplicon sequencing on stool samples from 85 infected patients and 70 healthy controls, we comprehensively profiled dual-kingdom diversity, taxonomic shifts, and key biomarker taxa. We further analyzed host determinants, including age and sex, to determine how individual demographic factors shape dysbiotic phenotypes, and evaluated the diagnostic efficacy of candidate microbial markers. This work offers valuable insight into disease-driven microecological shifts, paving the way for targeted clinical therapies and improved public health interventions.

2 Materials and Methods

2.1 Study cohort

Case group fecal samples were obtained from the foodborne disease surveillance archives of the Hohhot Center for Disease Control and Prevention (2025). The cohort comprised 85 patients clinically diagnosed at Hohhot First Hospital and Inner Mongolia Maternal and Child Health Hospital. Inclusion criteria specified ≥ 3 bowel movements per day accompanied by abnormal stool consistency (e.g., loose, watery, mucoid, bloody, or purulent) [5]. The control group included 70 healthy volunteers recruited during routine health screenings at the Hohhot Center for Disease Control and Prevention. Demographics (age and sex) were balanced between groups to minimize confounding effects on microbiome composition (Table 1). This study received funding from Prevention and Control of Emerging and Major Infectious Diseases-National Science and Technology Major Project (2026ZD01909600) and ethical approval from the Institutional Review Board (approval number: I-26PJ0575). All participants or their legal guardians provided informed consent.

2.2 Sample collection and storage

Trained clinical staff collected fresh fecal specimens following standardized aseptic protocols. Samples were stored at 4°C immediately after collection and transported to the laboratory within 24 hours. Aliquots not targeted for immediate DNA extraction were frozen at -80°C to preserve community structure and prevent microbial degradation prior to sequencing.

2.3 Fecal DNA extraction and quality control

Total genomic DNA was extracted using a CretMag Power Fecal DNA Kit (Cretaceous Biology). Briefly, fecal material was transferred with a 10 μL sterile inoculating loop into 2 mL grinding tubes containing zirconia beads, 500 μL Buffer Lysis S1, and 100 μL Buffer Lysis S2. Mechanical lysis was performed via bead beating (60 Hz; 5 cycles of 1 min shaking and 1 min rest). RNA was digested by adding 4 μL of RNase A (100 mg/mL). Samples were centrifuged at 12,000 rpm ($13,400 \times g$) for 2 minutes, and the supernatants were transferred to clean 1.5 mL tubes, mixed with 200 μL of Buffer IRS by inversion, vortexed for 15 seconds, and incubated at 4°C for 10 minutes. Following a second centrifugation (12,000 rpm, 2 min), 450 μL of supernatant was transferred to pre-loaded reagent plates for automated extraction, purification, and elution according to the manufacturer's protocol.

DNA integrity and purity were evaluated via 1% agarose gel electrophoresis. Concentrations and absorbance ratios were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) to ensure samples met quality thresholds for downstream applications.

2.4 High-Throughput Sequencing: Amplification and Library Construction

Purified genomic DNA served as the template for targeted PCR amplification. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was targeted using forward primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') [6]. The fungal ITS region was amplified using primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') [7]. Amplicon size and specificity were confirmed by 2% agarose gel

electrophoresis, followed by gel purification and quantification using a Synergy HTX microplate reader (BioTek, USA).

Sequencing libraries were prepared from purified amplicons with a NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, USA), incorporating adapter ligation, bead-based removal of self-ligated products, PCR enrichment, and final library purification. Validated libraries were submitted to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. for paired-end sequencing (2×300) on an Illumina NextSeq 2000 platform.

2.5 Sequence processing and bioinformatic analysis

Raw reads were processed with fastp [8] for quality control and merged using FLASH. Base trimming was triggered when tail quality scores dropped below 20, and a 50 bp sliding window discarded sequences once the mean quality fell below 20. Reads < 50 bp or containing ambiguous bases (N) were excluded. Paired reads were assembled requiring a minimum overlap of 10 bp and a maximum mismatch rate of 0.2. Demultiplexing enforced exact barcode matches and allowed up to two primer mismatches.

Filtered reads were denoised, dereplicated, and error-corrected using the DADA2 plugin within QIIME2 to yield Amplicon Sequence Variants (ASVs) [9, 10]. Taxonomic assignment of ASVs was performed with the QIIME2 naive Bayes classifier against SILVA v138 for bacteria and UNITE v9 for fungi, using a 70% confidence threshold [11]. Sequences identified as chloroplasts or mitochondria were removed.

To account for variable sequencing depth, datasets were rarefied prior to diversity analyses. For the 155 matched samples, the fungal dataset was normalized to 11,424 reads per sample (yielding 3,092 ASVs), while the bacterial dataset was normalized to 21,134 reads per sample (yielding 14,583 ASVs).

Standardized matrices were used for downstream alpha and beta diversity comparisons and taxonomic profiling.

3 Results

3.1 Gut microbiota alpha diversity

We assessed bacterial and fungal alpha diversity using Chao1 (richness) and Shannon (diversity and evenness) indices across cohorts. Sex-stratified analyses revealed no significant differences in bacterial or fungal alpha diversity between male and female subjects (Bacteria: Chao1, $P = 0.8723$; Shannon, $P = 0.4854$; Fungi: Chao1, $P = 0.8025$; Shannon, $P = 0.9528$; Supplementary Fig. 1), indicating that sex does not drive variance in microbial richness or evenness within this population.

Disease status, however, induced kingdom-specific impacts on alpha diversity. Diarrheal patients exhibited marked reductions in bacterial Chao1 richness and Shannon diversity compared to healthy controls ($P < 0.001$, Fig. 1A, 1B), reflecting severe loss of species richness and community evenness. Fungi experienced a similar, albeit less severe, disruption: patients showed a significant drop in fungal Chao1 richness ($P < 0.01$, Fig. 1C) alongside a modest decline in Shannon diversity ($P < 0.05$, Fig. 1D).

These findings demonstrate that bacterial communities are more vulnerable to diarrhea-associated microecological disruption than fungal networks.

3.2 Beta diversity and community structure

Principal Coordinates Analysis (PCoA) revealed distinct clustering between case and control cohorts for both kingdoms. Structural divergence was highly pronounced in bacterial communities ($P < 0.001$, Fig. 1E). While fungal communities also differed significantly by disease status ($P < 0.05$, Fig. 1F), the small effect size ($R = 0.0264$) and extensive sample overlap indicate that disease exerts a limited impact on global mycobiome architecture. Thus, bacterial communities undergo more substantial microecological remodeling during infection. Sex-stratified beta diversity showed no significant structural segregation (Supplementary Fig. 2).

3.3 Taxonomic composition at phylum and genus levels

Venn diagrams demonstrated that patients and controls shared 254 bacterial taxa, while 60 taxa were unique to controls and 102 were exclusive to patients. In the fungal dataset, 172 taxa were shared, with 135 control-specific and 156 patient-specific taxa (Supplementary Fig. 3).

At the bacterial phylum level, both groups were dominated by Bacillota, Pseudomonadota, Actinomycetota, and Bacteroidota. Controls were enriched in Bacillota (59.1%) and Bacteroidota (21.5%), both of which decreased in patients (46.7% and 6.5%, respectively). Conversely, patients harbored higher proportions of Pseudomonadota (28.4% vs. 7.9%) and Actinomycetota (17.1% vs. 11.0%), signaling an expansion of potentially pathogenic lineages.

At the bacterial genus level, dominant taxa included *Bifidobacterium*, *Bacteroides*, *Escherichia-Shigella*, *Enterococcus*, *Faecalibacterium*, and *Blautia* (Figs. 2A, 2B). Controls maintained higher relative abundances of beneficial genera, including *Bacteroides* (15.6%), *Faecalibacterium* (11.5%), and *Blautia* (6.8%), all of which dropped markedly in patients (4.5%, 0.6%, and 3.8%, respectively). Conversely, patients exhibited expansions of *Bifidobacterium* (14.8% vs. 9.0%), *Escherichia-Shigella* (9.9% vs 5.4%), and *Enterococcus* (13.4% vs. 0%). The overrepresentation of *Escherichia-Shigella* and *Enterococcus* aligns with typical infectious dysbiosis [12]. The elevation of *Bifidobacterium* in patients, contrasting with classic adult dysbiosis, reflects the inclusion of infants in the case cohort, as *Bifidobacterium* naturally dominates the infant gut and wanes with age [13].

Fungal communities were overwhelmingly dominated by Ascomycota (82.7% in controls vs 85.2% in patients) and Basidiomycota (16.0% vs. 11.2%). At the genus level, *Candida*, *Saccharomyces*, and *Aspergillus* predominated (Figs. 2C, 2D). Patients were enriched in *Candida* (29.4% vs. 17.3%) and depleted in *Aspergillus* (8.4% vs. 14.0%), while *Saccharomyces* remained stable (14.3% vs. 12.6%). Overgrowth of *Candida* represents a key feature of fungal dysbiosis [14], whereas lower *Aspergillus* abundance may stem from competitive exclusion by expanding *Candida* populations alongside dietary factors [15].

3.4 Core differential taxa

Genus-level differential abundance testing highlighted key drivers of cohort separation (Fig. 3A). Controls were significantly enriched in protective, butyrate-producing bacterial genera including *Bacteroides*, *Faecalibacterium*, *Blautia*, *Agathobacter*, *Roseburia*, and *Ruminococcus* [16]. Patients displayed significant enrichment of opportunistic pathogens such as *Escherichia* and *Streptococcus*.

Fungal differential patterns revealed distinct shifts (Fig. 3B). Controls showed higher levels of *Aspergillus*, a transient fungal taxon typically derived from diet [15]. Patients exhibited notable enrichment of Sordariaceae. Because Sordariaceae rarely colonizes healthy human guts, its accumulation in diarrheal samples indicates compromised mucosal barrier integrity and opportunistic fungal colonization [17].

3.5 Age-stratified diversity and succession

To assess age-dependent effects, patients were stratified into four cohorts: infants (< 3 years), children/adolescents (3–17 years), adults (18–59 years), and older adults (≥ 60 years). Bacterial alpha diversity showed a strong age-gradient ($P < 0.001$, Fig. 4A, 4B), reaching its nadir in infants, rising through childhood, peaking in adults, and declining in older adults. This trajectory reflects progressive microbiome maturation followed by age-related decline [17].

Fungal Chao1 richness followed a similar age trend ($P < 0.001$; Fig. 4C), lowest in infants and highest in adults. However, fungal Shannon diversity did not differ significantly across age groups ($P > 0.05$; Fig. 4D), indicating that host age primarily modulates fungal species richness rather than overall community evenness. PCoA confirmed these dynamics: bacterial communities segregated sharply by age group ($P < 0.001$; Fig. 4E), whereas fungal structures showed no significant age-based clustering ($P > 0.05$; Fig. 4F).

Taxonomic profiling revealed clear age-driven succession (Fig. 5). Infant patients were dominated by *Bifidobacterium*, consistent with immature gut ecology. *Bacteroides* expanded during childhood, marking the establishment of stable commensal communities. Older adults displayed increased abundances of *Blautia*, *Streptococcus*, and *Psychrobacter*; expansion of *Streptococcus* highlights heightened dysbiosis in older individuals due to declining colonization resistance [17].

Fungal profiles also varied by age. Adult patients maintained higher *Aspergillus* levels. Infants exhibited high Sordariaceae enrichment, underscoring fragile fungal homeostasis early in life. Adults showed increased abundances of *Rhodotorula* and *Wallemia*. Fecal *Rhodotorula* may provide metabolic benefits to the host [18], whereas *Wallemia* detection likely reflects impaired immune tolerance and environmental fungal transit [19].

Finally, random forest modeling combined with ROC analysis evaluated the diagnostic utility of differential genera. Stepwise logistic regression identified three key bacterial markers, *Faecalibacterium* (AUC = 0.94, 95% CI: 0.90–0.98), *Roseburia* (AUC = 0.94, 95% CI: 0.90–0.98), and *Ruminococcus* (AUC = 0.91, 95% CI: 0.86–0.96), all demonstrating strong diagnostic accuracy for identifying diarrheal status

(Fig. 6). In contrast, models constructed from five differential fungal genera yielded mean AUC values below 0.7 (Fig. 6), demonstrating limited diagnostic value.

4 Discussion

Maintaining gut microbial homeostasis is fundamental to preserving intestinal barrier function, immune tolerance, and metabolic stability. Dysbiosis across bacterial and fungal kingdoms directly drives the onset and progression of infectious diarrhea. By characterizing alpha diversity, beta diversity, and taxonomic profiles between healthy individuals and diarrheal patients, this study confirms that non-statutory infectious diarrhea induces distinct, taxon-specific microecological shifts. While global species richness remained relatively stable, overall community architecture underwent significant restructuring. Patients exhibited reduced alpha diversity across both kingdoms, though structural shifts were far more severe in bacterial communities than in fungal networks. Furthermore, host sex did not account for inter-individual variance in bacterial or fungal diversity, demonstrating that baseline diversity is independent of sex in this cohort.

Integrating age-stratified profiles with established lifespan microbiome dynamics clarifies the age-dependent dysbiosis patterns observed during infection. The age gradient detected in bacterial diversity, nadir in infancy, progressive accumulation through childhood and adolescence, peak in adulthood, and decline in older adults, closely mirrors developmental trajectories described in healthy populations [12, 20]. Host age thus acts as a primary master regulator of gut bacterial maturation and homeostatic set points, establishing baseline configurations that persist even under pathological stress.

Healthy gut ecosystems rely on core bacterial taxa to sustain microecological stability. We identified marked enrichment of beneficial genera, including *Faecalibacterium*, *Roseburia*, *Blautia*, *Bacteroides*, *Agathobacter*, and *Ruminococcus*, in healthy controls. Comparative profiling isolated three genera (*Faecalibacterium*, *Roseburia*, and *Agathobacter*) that robustly distinguish diarrheal patients from healthy subjects, expanding our understanding of clinical dysbiosis and highlighting promising diagnostic biomarkers. These three taxa are well-established primary producers of short-chain fatty acids, particularly butyrate. As the main energy source for colonic epithelial cells, butyrate supports mucosal barrier repair, suppresses local inflammation, tunes immune responses, and enhances host resistance to enteric pathogens [21, 22]. Similarly, *Blautia* secretes specific bacteriocins to block opportunistic colonization while regulating host glycolipid metabolism and anti-inflammatory pathways [23]. *Bacteroides* further underpins nutrient digestion, mucosal immunity, and pathogen exclusion [24]. Domination by these commensals creates a resilient, anti-inflammatory environment essential for normal gut physiology.

Conversely, diarrheal patients presented severe bacterial dysbiosis marked by the depletion of core commensals and the expansion of opportunistic pathogens. The profound loss of butyrate producers such as *Faecalibacterium* and *Roseburia* compromises short-chain fatty acid synthesis, weakens barrier integrity, and reduces local immune defenses [25]. Concurrently, opportunistic lineages including

Escherichia, *Streptococcus*, and *Enterococcus* proliferated. Normally restricted to low abundances, these taxa invade disrupted mucosal niches, amplify inflammatory signaling, and exacerbate clinical symptoms, establishing a self-reinforcing cycle of dysbiosis, tissue damage, and disease progression [26].

While bacterial dynamics are well documented, the functional contribution of the gut mycobiome during infection remains less clear, representing a key contribution of this work. Ascomycota and Basidiomycota dominated the healthy mycobiome, maintaining balanced taxon proportions to preserve basal fungal homeostasis [27]. Controlled colonization by *Aspergillus* in healthy guts restrains pathogenic fungi via nutrient competition and metabolic crosstalk [15]. In contrast, *Candida*, an opportunistic pathogen normally suppressed under homeostatic conditions, expanded markedly in patients. This expansion signals a collapse of fungal equilibrium, disrupting the beneficial bacterial-fungal cross-talk that stabilizes the holistic gut ecosystem.

Age-stratified analyses revealed striking kingdom-specific responses to host aging. Bacterial communities underwent comprehensive structural shifts across age groups, encompassing richness, diversity, and beta diversity. Fungal communities, however, varied primarily in species richness, maintaining stable evenness and global architectures across age groups. This contrast highlights the lower homeostatic plasticity of gut fungi relative to bacteria. Because gut fungi consist largely of transient, environmentally derived organisms, they are less tightly regulated by host physiological aging and more heavily influenced by diet and environment [27]. In our diarrheal cohort, host age primarily governed baseline bacterial stability and global microecological tolerance, whereas fungal age differences manifested chiefly as variations in opportunistic colonization load rather than structural remodeling.

Several limitations warrant consideration. First, as a single-center cross-sectional study, this work identifies associations between dysbiosis, host age, and diarrhea, but cannot establish direct causality. Second, our analysis relied on amplicon profiling; functional metabolic pathways, immune regulatory mechanisms, and direct physical trans-kingdom interactions require further experimental validation using strain isolations and animal models. Third, cohort recruitment was localized to Hohhot, meaning regional dietary, climatic, and lifestyle factors may limit geographic generalizability. Finally, unmeasured variables such as precise dietary intake, recent medication history, and micro-environmental exposures could introduce minor confounding effects.

In summary, patients in Hohhot with non-statutory infectious diarrhea exhibit cross-kingdom gut microecological dysbiosis. Patients display reduced alpha diversity and structural remodeling in both kingdoms, with bacterial networks experiencing the most severe disruption. Bacterial dysbiosis is driven by the depletion of protective, short-chain fatty acid-producing anaerobes (*Faecalibacterium*, *Roseburia*, *Blautia*, *Agathobacter*, and *Bacteroides*) combined with the expansion of facultative opportunistic pathogens (*Escherichia-Shigella*, *Enterococcus*, and *Streptococcus*). Fungal dysbiosis is characterized by the overgrowth of opportunistic and environmental taxa such as *Candida* and Sordariaceae, reflecting

compromised mucosal barriers and altered physical niches during infection. The core bacterial markers identified here provide a strong foundation for future mechanistic studies, multi-center validation cohorts, and the development of age-stratified, microbiota-targeted diagnostics and therapeutics.

Table 1
Baseline demographic and clinical characteristics of healthy controls and patients with non-statutory infectious diarrhea.

Characteristic	Classification	Healthy controls	Patient
Number of samples	-	70	85
Gender	Male	31 (44.3%)	50 (58.8%)
	Female	39 (55.7%)	35 (41.2%)
Age	< 3	0	23 (14.8%)
	3–17	7 (4.5%)	23 (14.8%)
	18–59	56 (36.1%)	23 (14.8%)
	≥ 60	7 (4.5%)	16 (10.3%)

A and B, Bacterial genus-level richness (Chao1 index) and diversity (Shannon index), respectively. C and D, fungal genus-level richness (Chao1 index) and diversity (Shannon index), respectively. E and F, Genus-level principal coordinate analysis (PCoA) of bacterial and fungal communities based on Bray-Curtis distances. Statistical significance: $0.01 < *P \leq 0.05$; $0.001 < **P \leq 0.01$; $***P \leq 0.001$.

A and B, relative abundances of bacterial phyla and genera, respectively. C and D, relative abundances of fungal phyla and genera, respectively.

A, relative abundance distributions for the top 10 bacterial genera. B, relative abundance distributions for the top 10 fungal genera. $0.01 < *P \leq 0.05$; $0.001 < **P \leq 0.01$; $***P \leq 0.001$.

A and B, bacterial genus-level richness (Chao1 index) and diversity (Shannon index), respectively. C and D, fungal genus-level richness (Chao1 index) and diversity (Shannon index), respectively. E and F, genus-level Bray-Curtis PCoA ordinations for bacterial and fungal communities across age strata.

$0.01 < *P \leq 0.05$; $0.001 < **P \leq 0.01$; $***P \leq 0.001$.

A and B, genus-level relative abundances for bacterial and fungal communities across age subgroups, respectively. C and D, abundance shifts for the top 5 bacterial and fungal genera, respectively.

$0.01 < *P \leq 0.05$; $0.001 < **P \leq 0.01$; $***P \leq 0.001$.

A, model performance using candidate bacterial genus biomarkers. B, model performance derived from candidate fungal genus features.

Supplementary Fig. 1. Sex-stratified alpha diversity of fecal microbiota in diarrheal patients and healthy controls.

A and B, bacterial richness (Chao1) and diversity (Shannon) by sex in controls. C and D, corresponding bacterial metrics in patients. E and F, fungal richness (Chao1) and diversity (Shannon) by sex in controls. G and H, corresponding fungal metrics in patients. $0.01 < *P \leq 0.05$; $0.001 < **P \leq 0.01$; $***P \leq 0.001$.

Supplementary Fig. 2. Sex-stratified β -diversity and overall community ordinations of fecal microbiota.

A and B, bacterial Bray-Curtis PCoA by sex in patients and controls, respectively. C and D, fungal Bray-Curtis PCoA by sex in patients and controls, respectively. E and F, non-metric multidimensional scaling (NMDS) plots of total bacterial and fungal communities based on Bray-Curtis distances.

Supplementary Fig. 3. Shared and unique fecal microbial genera between diarrheal patients and healthy controls.

A and B, Venn diagrams of shared and exclusive bacterial and fungal genera between cohorts, respectively. Numbers inside regions indicate genus counts.

Declarations

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Ethics declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of the funding's lead institution, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, with the approval number I-26PJ0575. Sample collection and data handling strictly adhered to the approved ethical guidelines. Informed consent was obtained from all subjects involved in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

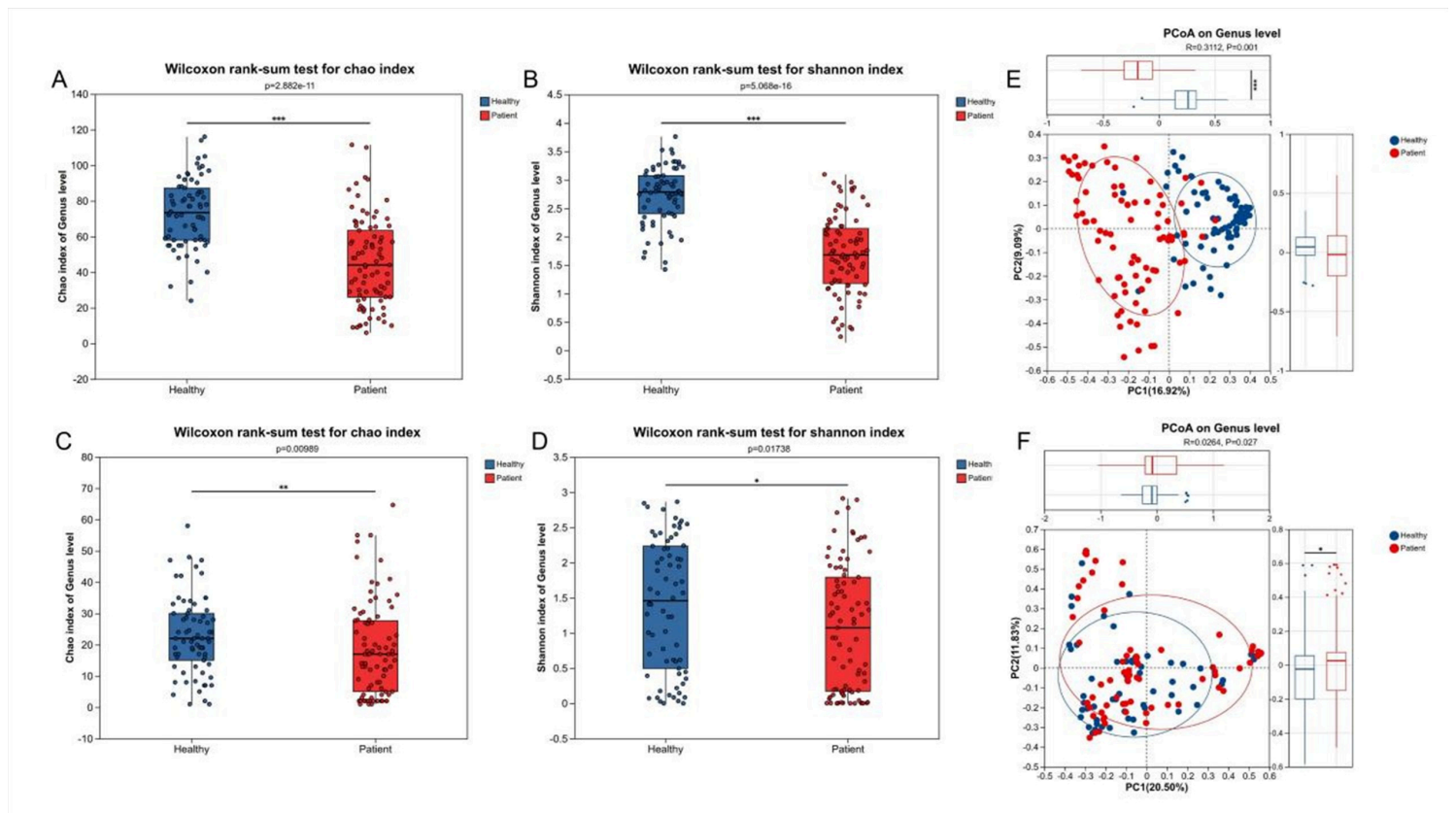


Figure 1

Fecal microbiota alpha diversity and β -diversity in patients with non-statutory infectious diarrhea and healthy controls

A and B, Bacterial genus-level richness (Chao1 index) and diversity (Shannon index), respectively. C and D, fungal genus-level richness (Chao1 index) and diversity (Shannon index), respectively. E and F, Genus-level principal coordinate analysis (PCoA) of bacterial and fungal communities based on Bray-Curtis distances. Statistical significance: $0.01 < *P \leq 0.05$; $0.001 < **P \leq 0.01$; $***P \leq 0.001$.

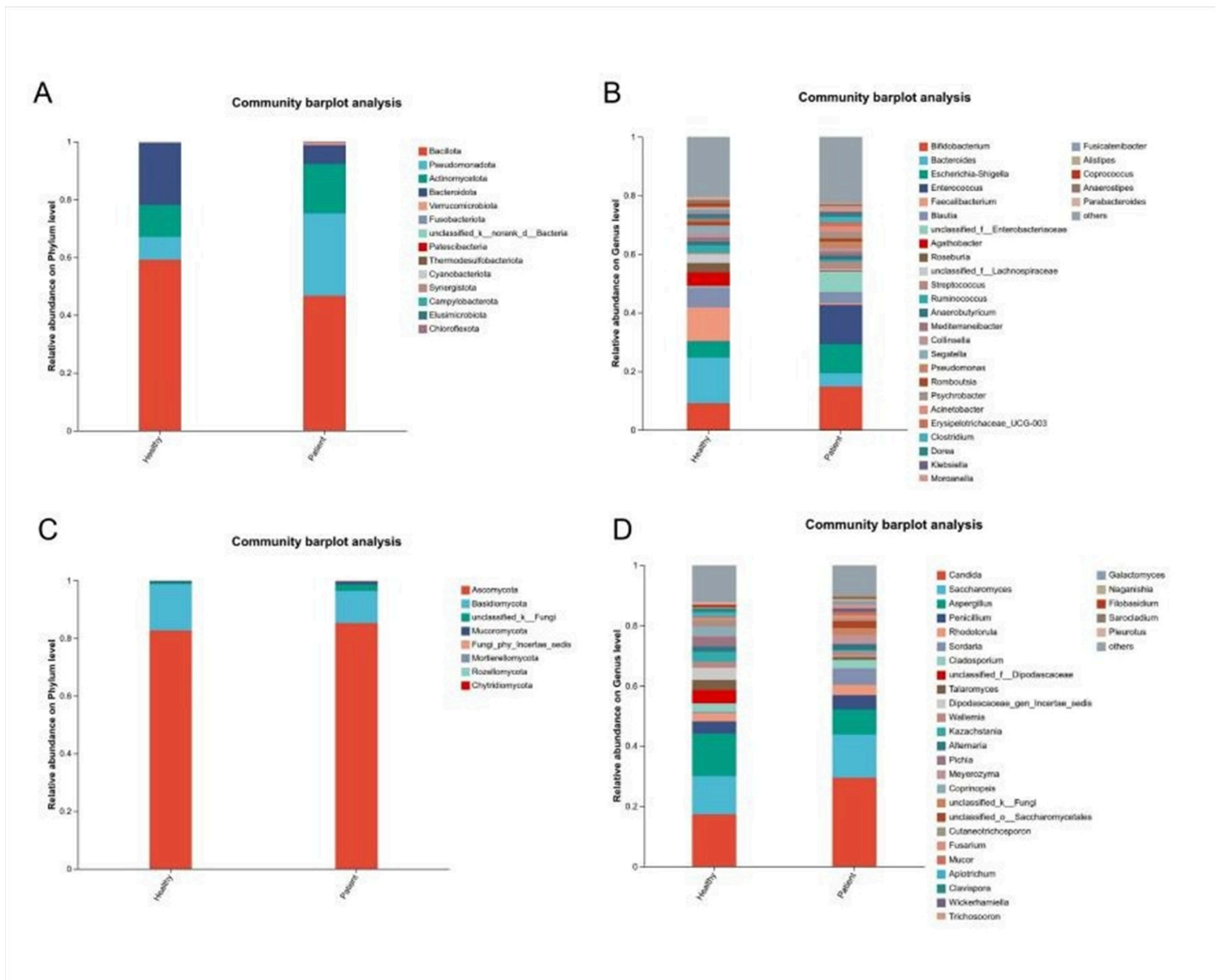


Figure 2

Relative taxonomic abundance of fecal microbiota in diarrheal patients and healthy controls.

A and B, relative abundances of bacterial phyla and genera, respectively. C and D, relative abundances of fungal phyla and genera, respectively.

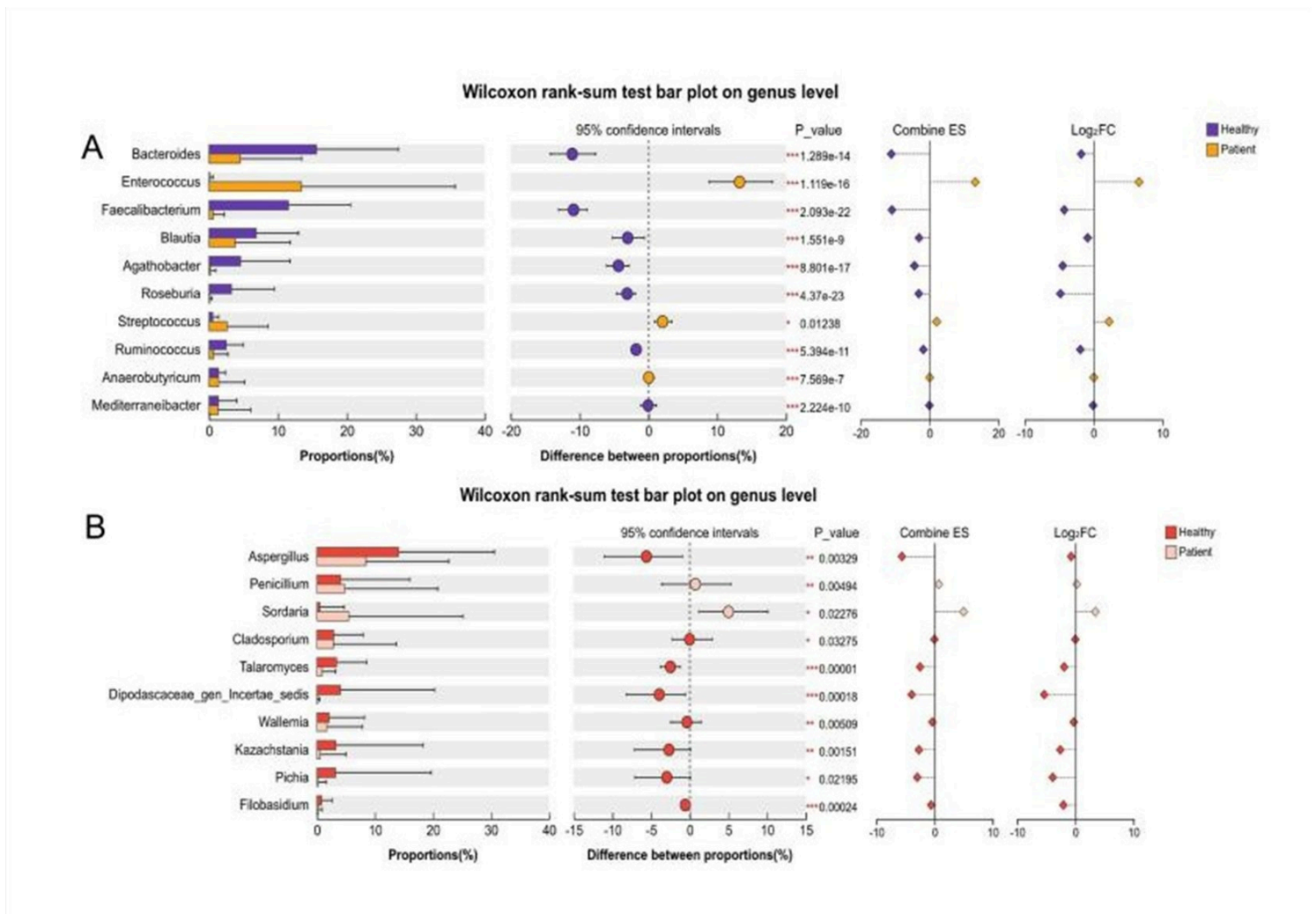


Figure 3

Abundance comparisonsof predominant fecal microbial genera between diarrheal patientsand healthy controls.

A, relative abundance distributions for the top 10 bacterial genera. B, relative abundance distributions for the top 10 fungal genera. $0.01 < *P \leq 0.05$; $0.001 < **P \leq 0.01$; $***P \leq 0.001$.

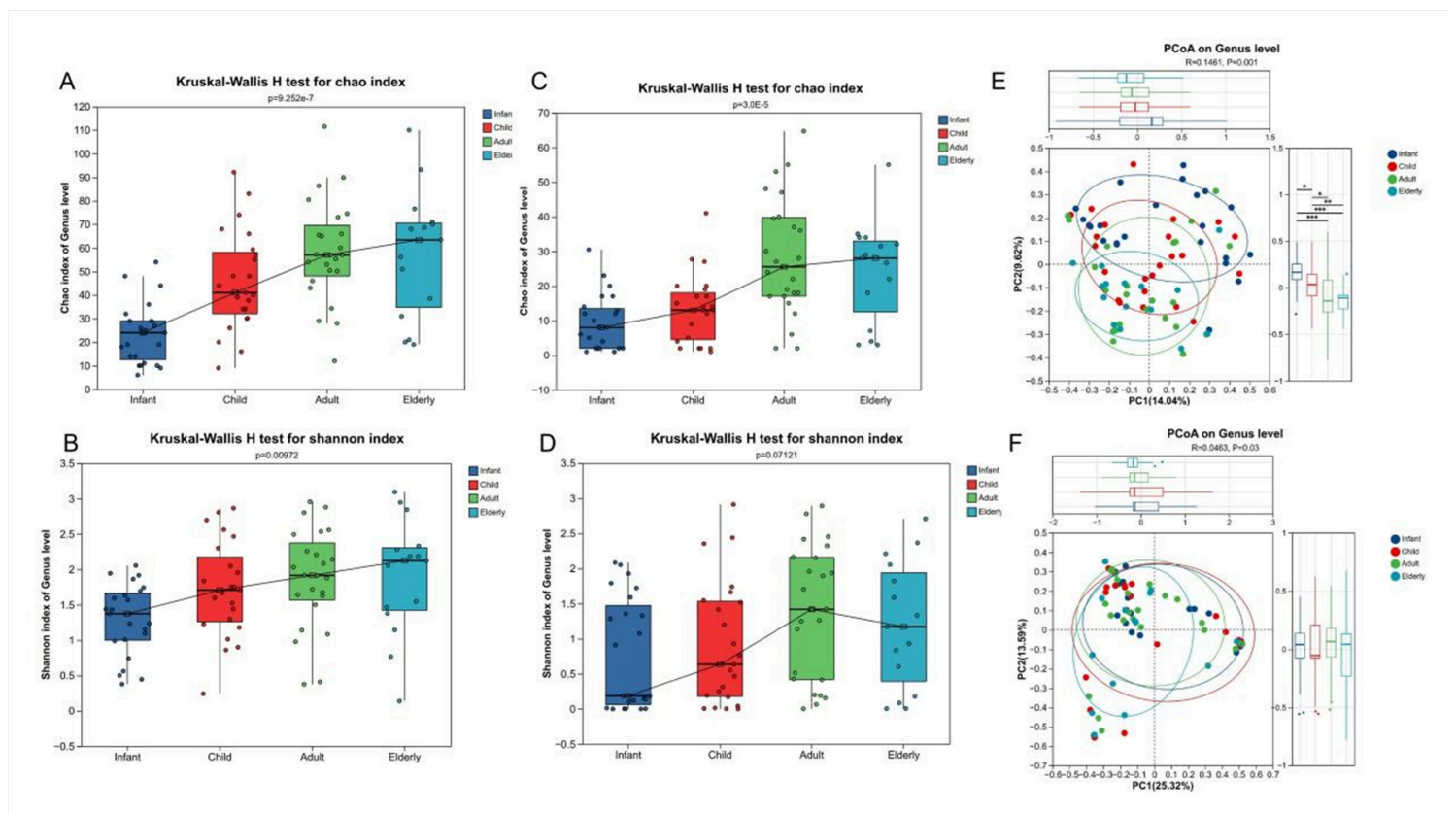


Figure 4

Fecal alpha diversity and β -diversity across age cohorts of diarrheal patients.

A and B, bacterial genus-level richness (Chao1 index) and diversity (Shannon index), respectively. C and D, fungal genus-level richness (Chao1 index) and diversity (Shannon index), respectively. E and F, genus-level Bray-Curtis PCoA ordinations for bacterial and fungal communities across age strata.

$0.01 < *P \leq 0.05$; $0.001 < **P \leq 0.01$; $***P \leq 0.001$

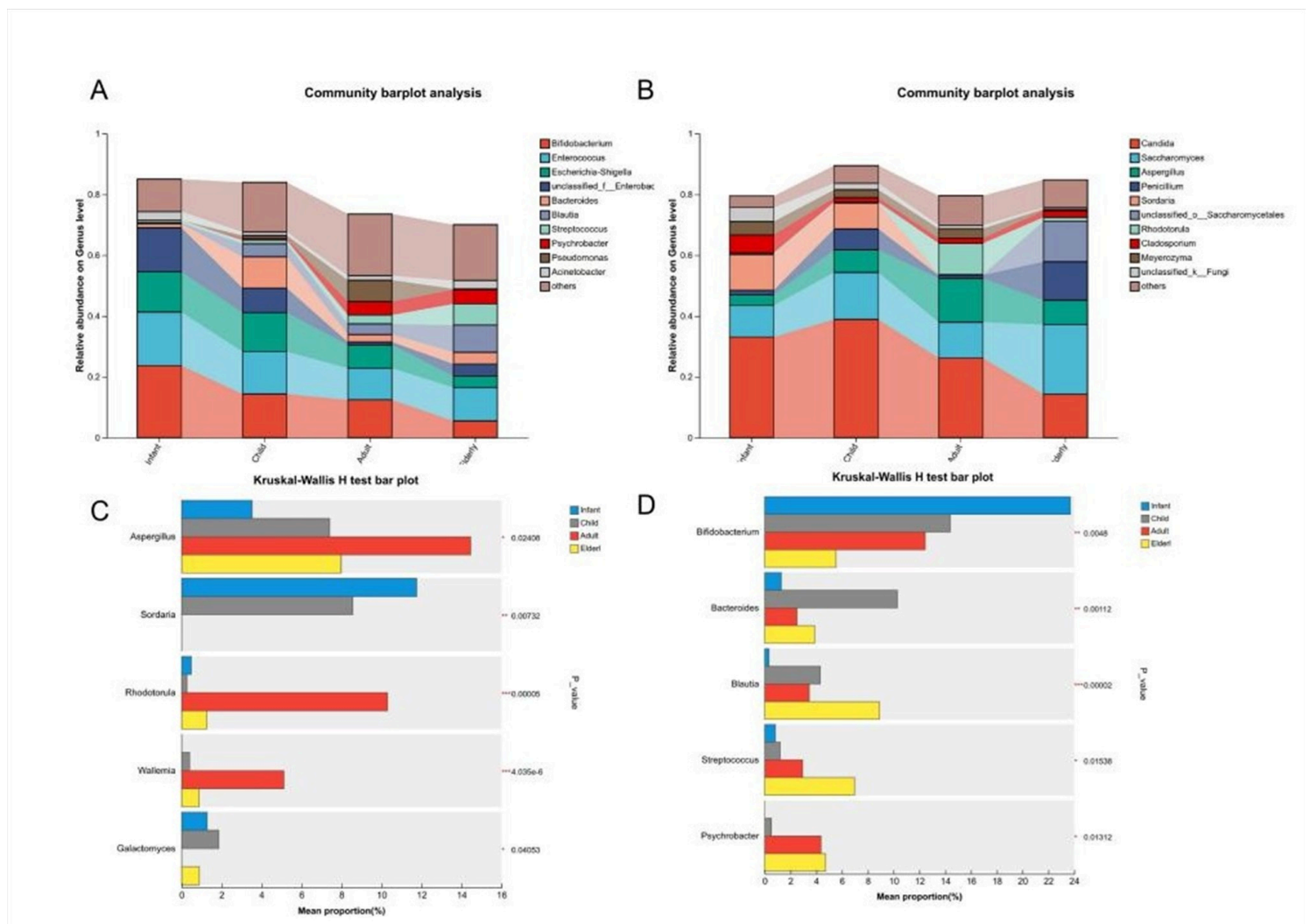


Figure 5

Microbial community profiles and core genus distribution across age cohorts in diarrheal patients.

A and B, genus-level relative abundances for bacterial and fungal communities across age subgroups, respectively. C and D, abundance shifts for the top 5 bacterial and fungal genera, respectively.

$0.01 < *P \leq 0.05$; $0.001 < **P \leq 0.01$; $***P \leq 0.001$.

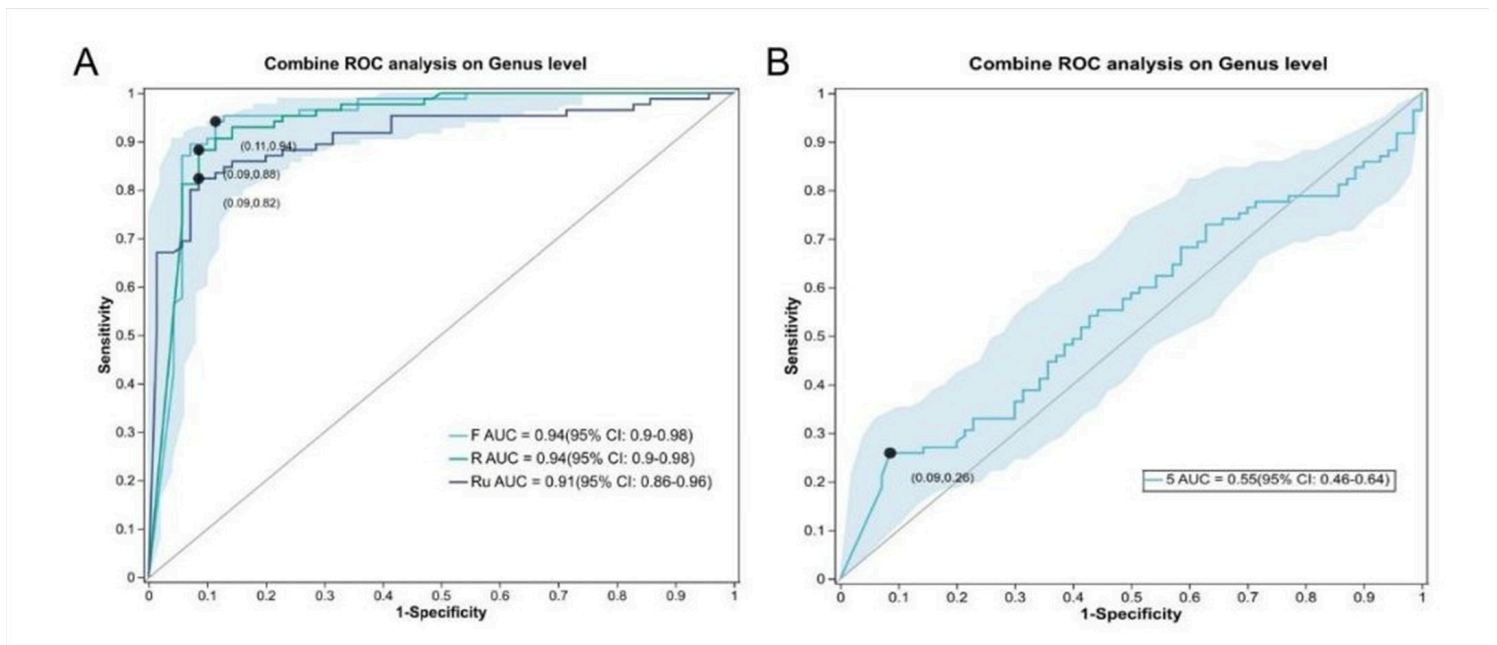


Figure 6

Receiver operating characteristic (ROC) curves evaluating random forest models for diarrhea diagnosis

A, model performance using candidate bacterial genus biomarkers. B, model performance derived from candidate fungal genus features.

Supplementary Files

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