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Single-session general anesthesia versus multiple-visit therapy for severe early childhood caries divergent short-term impacts on the oral–gut microbiome

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Research Article

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Single-session general anesthesia versus multiple-visit therapy for severe early childhood caries divergent short-term impacts on the oral–gut microbiome

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Abstract

Background: The oral-gut microbial axis mediates bidirectional regulation between oral and gut microbiota. Single general anesthesia treatment and conventional multiple-visit therapy are two core strategies for severe early childhood caries (S-ECC), yet their concurrent impacts on oral and gut microbiota remain insufficiently compared.

Objective: To compare the short-term effects of the two regimens on the diversity and community structure of salivary and gut microbiota in children with S-ECC from the oral-gut axis perspective.

Design: Fifty children aged 3–6 years with S-ECC were divided into single general anesthesia treatment group (C1, n=25) and a conventional multiple-visit group (C2, n=25) based on clinical indication, parental preference, and patient cooperation. 16S rRNA V3-V4 region high-throughput sequencing was applied to analyze microbial diversity and compositional changes before and after treatment.

Results: (1) Salivary microbiota: Group C1 exhibited significantly decreased observed features, shannon index and shifted community structure post-treatment (all $P<0.05$), with marked reductions in core cariogens including *Streptococcus mutans* (*S. mutans*) and *Prevotella denticola* (*P. denticola*)($P<0.05$). Group C2 showed no significant change in α-diversity but altered community structure ($P<0.05$); only *Scardovia wiggsiae* decreased significantly, while *S. mutans* presented a downward trend without statistical significance. (2) Gut microbiota: Neither group had significant α-diversity changes; only group C2 showed significantly altered gut community structure ($P<0.05$). Fecal *S. mutans* was downregulated in both groups, and the butyrate-producing genus *Anaerobutyricum* increased significantly in group C2 ($P<0.05$).

Conclusion: Single general anesthesia treatment effectively eliminates core cariogens

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and remodels oral microbiota with only transient diversity reduction and mild disturbance to gut microbial homeostasis. Conventional multiple-visit therapy has limited inhibitory effects on oral cariogens and causes more pronounced disruption to overall gut microbial stability. These findings highlight the differential short-term microbiological impacts of the two treatment strategies and warrant further investigation into their long-term implications.
Keywords: severe early childhood caries; single general anesthesia treatment; oral-gut microbial axis; salivary microbiota; gut microbiota; 16S rRNA sequencing

Introduction

Early childhood caries (ECC) is defined as the presence of one or more decayed, missing, or filled tooth surfaces in any primary tooth in children under 6 years of age[1]. S-ECC is diagnosed with decayed, missing and filled teeth (dmft) scores ≥4 (age 3), ≥5 (age 4) and ≥6 (age 5). According to China's Fourth National Oral Health Epidemiological Survey, caries prevalence reaches 50.8%, 63.6% and 71.9% in 3-, 4- and 5-year-old children, respectively[2]. Such high prevalence renders S-ECC a critical pediatric public health challenge in China, as it compromises children's eating, sleep, speech and physical-mental development, and imposes substantial long-term burdens on families and the public health system[1, 3].

Currently, two standardized interventions dominate S-ECC management: Single general anesthesia treatment and conventional multiple-visit therapy[4]. In young children, the prevalence of dental anxiety also varies widely, ranging from 4% to 98% [5]. Single-session DGA thoroughly removes all carious tissues and cariogenic biofilms in one appointment, yet anesthetic agents and operative manipulation may acutely perturb the native oral microbiota via microecological, immune, salivary protein and total antioxidant pathways[6–8]. Conversely, conventional multiple-visit therapy applies milder, controllable procedures suitable for compliant children, but its fragmented intervention intensity produces a markedly different oral microbiome remodeling effect. Most prior clinical studies focus on endpoints like restoration success, caries recurrence and treatment experience, while microecological comparisons of the two regimens' differential impacts on children's oral and gut microbiota remain scarce[9–11].

The oral-gut microbial axis acts as a bidirectional regulatory system bridging oral and gut microecology. Oral microbiota colonizes and remodels the intestinal microenvironment via continuous swallowing, humoral circulation and systemic

immune responses, while gut microbiota reciprocally modulates oral caries susceptibility through metabolites and immune feedback, serving as a critical mediator in childhood caries development[12]. Oral pathogens form biofilms with salivary mucins to resist gastric acid, migrate to the intestine and disrupt gut homeostasis; changes in beneficial gut microbes such as short-chain fatty acids, *Bifidobacterium* and *Lactobacillus* impair systemic immunity and tooth mineralization, further elevating caries risk[13–15]. Dominant gut probiotics preserve oral-gut homeostasis by competing for adhesion sites, inhibiting cariogenic biofilm formation and regulating systemic inflammation, whereas microbial dysbiosis exacerbates bidirectional microecological disturbances[16–20]. Moreover, S-ECC children show distinct oral and gut microbial richness from healthy children, suggesting a potential link between caries status and gut microecological alterations[21].

Accordingly, this study recruited 3–6-year-old children with S-ECC assigned to single general anesthesia or conventional multiple-visit therapy. Pre- and post-intervention saliva and fecal samples were analyzed via 16S rRNA high-throughput sequencing to characterize oral and gut microbial diversity and community structure, aiming to provide microecological evidence for individualized S-ECC management and the preservation of pediatric systemic microecological homeostasis.

Materials and methods

Ethics approval and patient recruitment

This study, approved by the Medical Ethics Committee of the Affiliated Stomatological Hospital of Zunyi Medical University (approval No. YJSKTLS-2023-2026-025H) and conducted in strict compliance with the Declaration of Helsinki, enrolled 3–6-year-old children with S-ECC from the hospital's Department of Preventive and Pediatric Dentistry between November 2024 and December 2025. Sample size calculated via G*Power 3.1.9.7 (α=0.05, power 1-β=0.8, effect size=0.7) was 21 cases per group at minimum. Accounting for a 10%–20% dropout rate, 25 children were finally included in each group, for a total of 50 participants[22]. The inclusion criteria were as follows:(1) Children aged 3–6 years, with primary dentition and no erupted permanent teeth, and a dmft score between 8 and 20;(2) No oral mucosal or periodontal diseases, no intestinal or systemic

diseases;(3) No prior history of caries treatment, professional fluoride application, pit and fissure sealants, or other caries-preventive interventions before enrolment. The exclusion criteria for patients were as follows: (1) Hormone or immunosuppressant use within 3 months before enrollment;(2) Antibiotic or topical oral medication (e.g., mouthwash) use within 2 weeks before enrollment;(3) Child/guardian unable to comply with oral examination, saliva/stool sampling or 1-month follow-up;(4) General anesthesia contraindications or dental treatment under general anesthesia within 3 months..

Oral examination

Oral examinations were conducted by a single paediatric dentist under natural light using standard mouth mirrors and explorers. Caries was diagnosed per WHO childhood caries criteria, with baseline dmft scores recorded for each child. Caries was defined as cavitated lesions, obvious subsurface enamel destruction, probe-detectable softened tissue, or temporary restorations/sealants with concurrent caries. Non-caries cases presented only white spot lesions, enamel abrasion, non-carious wedge-shaped defects, rough discolored surfaces, or moderate-to-severe fluorosis enamel changes, with no softened tissue or subsurface undermining.

Treatment and follow-up

This was a prospective, non-randomized, observational comparative study. Fifty children with S-ECC were allocated to a single general anesthesia group (C1, n=25) and a conventional multiple-visit group (C2, n=25) based on patient cooperation, parental preference, and clinical judgment, with all procedures performed by the same senior paediatric dentist. Group C1 underwent complete caries removal and restorative treatment of all carious teeth in one session under general anesthesia. Group C2 received partial dental treatment per visit at 5–7-day intervals, with all restorative procedures finished within 1 month. All children returned for 1-month follow-up and sample collection; for the conventional group, the 1-month timepoint was defined as one month after the final treatment session.

Samples were further subgrouped by type, treatment and timepoint: saliva groups (CO1, CO1.1M, CO2, CO2.1M) and faecal groups (CF1, CF1.1M, CF2, CF2.1M), where “1” denotes general anesthesia, “2” conventional therapy, “1M” 1-month post-treatment, “O” saliva, and “F” faeces.

Sample collection

First-morning faecal samples were collected with sterile paper placed in the toilet bowl to minimize contamination. The stool surface layer was discarded, and 5–10 g of the middle portion was transferred into sterile containers. Saliva samples were uniformly collected between 9:00 and 11:00 a.m. After 2 hours of fasting and no toothbrushing, 4 mL of unstimulated saliva was collected into sterile EP tubes with children's heads tilted slightly downward. All samples were stored in dry ice-packed foam boxes, transported to the laboratory within 2 hours, and frozen at −80 °C until further processing.

DNA extraction and sequencing

DNA extraction, nucleic acid purity testing, target fragment amplification, purification, and sequencing were completed by Novogene Co., Ltd. (Beijing, China). PCR amplification was performed using the universal bacterial 16S rRNA V3-V4 region primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3'). After purification of the amplification products, amplicon libraries were constructed and paired-end sequencing was carried out on the Illumina NovaSeq6000 platform. Raw reads were processed by assembly, quality filtering, chimera removal and DADA2 denoising to yield amplicon sequence variants (ASVs) with the following parameters: truncLen=c(240,200), maxEE=c(2,2), truncQ=2, and chimera removal using the "consensus" method. Taxonomies were annotated against the Silva 138.2 database.

Bioinformatics and statistical analysis

Based on ASV annotations, α-diversity indices (Observed Features, Chao1, Shannon, Simpson) were calculated. β-diversity matrices were constructed with weighted_unifrac distances, visualized by PCoA and NMDS, and tested for intergroup significance using MRPP. Within-group differential marker species were identified by Wilcoxon signed-rank test. Genus-level microbial co-occurrence networks were built via Spearman correlation to characterize microbial interactions. Statistical analyses were performed in SPSS 29.0. Categorical data were compared by χ^2 test. Normality of continuous variables was assessed with the Shapiro-Wilk test; normally distributed data were analyzed by independent or paired t-tests, and non-normal data by Wilcoxon signed-rank tests. Due to the exploratory nature of this analysis and the limited sample size (n=25 per group), P-values were not adjusted for multiple

comparisons; results are presented as hypothesis-generating and should be interpreted with caution. Co-occurrence networks were computed via Spearman correlation in R 4.3.3, with α set at 0.05.

Result

Baseline clinical characteristics of the participants

Baseline characteristics of the two groups are summarized in Table 1. Among the 50 enrolled S-ECC children (25 per group), sex distribution showed no significant difference ($P>0.05$), whereas age at first visit and dmft score differed significantly between groups ($P<0.05$). To eliminate confounding from baseline imbalances, age and dmft were included as covariates in all subsequent analyses of microbial diversity and species abundance.

Table 1 Basic characteristics of children in the two group

Group	Gender（male/female）	First visit age（months）	dmft
general anesthesia	10(10.5)/15(14.5)	52±9	12±3
conventional treatment	11(10.5)/14(14.5)	58±8	9±2
P value	0.77	0.01	0.00

Note: Sex was categorical data, while age (months) and dmft were continuous data, presented as mean ± standard deviation.

Sequencing and ASV basic statistics

For saliva samples, a total of 10,616,834 raw reads were generated. After quality control, 9,788,721 effective sequences were obtained, and denoising yielded 5,908 ASVs. Taxonomic annotation identified 27 phyla, 46 classes, 112 orders, 204 families, 479 genera, and 623 species. For faecal samples, 10,576,853 raw reads were obtained, with 9,356,196 effective sequences after quality control, and denoising produced 5,116 ASVs, annotated into 17 phyla, 31 classes, 71 orders, 127 families, 364 genera, and 496 species.

Rarefaction curve analysis

Rarefaction curves of saliva and faecal samples showed that Observed Features rose rapidly and then plateaued with increasing sequencing depth, indicating adequate coverage of microbial species and sufficient data volume for subsequent diversity and differential species analyses（Fig. 1A-B）.

Fig. 1 Rarefaction curves of saliva (A) and faecal (B) samples.

α-diversity analysis

Species richness was characterized by the Observed Features index and overall community diversity by the Shannon index. Paired within-group comparisons were performed using the Wilcoxon signed-rank test, with false discovery rate (FDR) correction for multiple comparisons (Fig. 2).

For salivary microbiota, both indices were significantly lower at 1 month post-treatment in the general anesthesia group (P<0.05), while no significant changes were detected in the conventional multiple-visit group (P>0.05), indicating only single-session general anesthesia transiently reduced oral microbial richness and diversity (Fig. 2A–B).

For gut microbiota, neither index showed significant alterations after treatment in either group (P>0.05), suggesting neither intervention exerted a notable short-term effect on gut microbial richness or overall diversity (Fig. 2C–D).

Fig. 2 Observed features index (A,C) and Shannon index (B,D) of salivary microbiota (A,B) and gut microbiota (C,D). (*, P < 0.05; ns, P > 0.05). Note: The top horizontal line of each box represents the maximum value.

β-diversity analysis

PCoA and NMDS of salivary microbiota showed more distinct spatial separation and lower species similarity between CO1 and CO1.1M, whereas CO2 and CO2.1M clustered more tightly. Adonis confirmed significant post-treatment alterations in salivary community structure in both groups (Fig. 3A–B, Table 2).

Fig. 3 Weighted PCoA (A,C) and NMDS (B,D) analyses of salivary microbiota (A,B) and gut microbiota (C,D).

For gut microbiota, CF1 and CF1.1M overlapped markedly with high compositional similarity, while CF2 and CF2.1M were clearly separated. Adonis indicated no significant structural shift in the general anesthesia group (P>0.05), but conventional multiple-visit treatment significantly changed overall gut microbial structure (P<0.05) (Table 3).

Table 2 Adonis analysis of inter-group differences in salivary microbiota community structure

Group	R^2	P

CO1-CO1.1M	0.04721	0.002
CO2-CO2.1M	0.03547	0.019

Table 3 Adonis analysis of inter-group differences in intestinal microbiota community structure

Group	R^2	P
CF1-CF1.1M	0.01504	0.842
CF2-CF2.1M	0.0349	0.044

Microbial species composition

At the phylum level, *Pseudomonadota*, *Bacillota*, *Bacteroidota*, *Actinomycetota* and *Fusobacteriota* dominated all four saliva groups (CO1, CO1.1M, CO2, CO2.1M) (Fig. 4A), while *Bacillota*, *Actinomycetota*, *Pseudomonadota* and *Bacteroidota* were the most abundant phyla in the four faecal groups (Fig. 4B).

At the genus level, the predominant salivary taxa included *Pseudomonas*, *Streptococcus*, *Neisseria*, *Prevotella*, *Rothia* and *Veillonella* (Fig. 4C), and the dominant faecal genera were *Bifidobacterium*, *Escherichia-Shigella*, *Bacteroides*, *Blautia* and *Faecalibacterium* (Fig. 4D).

Fig. 4 Top 10 taxa at the phylum level (A,B) and genus level (C,D) in salivary microbiota (A,C) and gut microbiota (B,D).

Within-group differential species analysis

Differential species in salivary microbiota

At the genus level, single general anesthesia treatment significantly enriched health-associated oral genera including *Gemella*, *Rothia* and *Streptococcus*, and reduced *Aggregatibacter*, *Prevotella*, *Veillonella*, *Fusobacterium* and *Campylobacter* (all $P<0.05$, Fig. 5A). Conventional multiple-visit therapy increased *Actinobacillus* and *Stenotrophomonas*, while decreasing *Leptotrichia* and *Pseudoleptotrichia* ($P<0.05$, Fig. 5C).

At the species level, single general anesthesia treatment markedly elevated *Rothia aeria* and lowered *S. mutans*, *P. denticola*, *Prevotella melaninogenica* and *Leptotrichia shahii* ($P<0.05$, Fig. 5B). Conventional multiple-visit therapy increased

Haemophilus parahaemolyticus and *Haemophilus parainfluenzae*, and reduced *Scardovia wiggsiae* and *Leptotrichia wadei* ($P<0.05$, Fig. 5D); *S. mutans* showed a downward trend without statistical significance ($P>0.05$).

Fig. 5 Differential taxa before and after treatment at the genus (A,C) and species (B,D) levels of salivary microbiota; general anaesthesia group (A,B) and conventional treatment group (C,D).

Differential species in gut microbiota

At the genus level, after single general anesthesia treatment, the relative abundances of opportunistic pathogens including *Klebsiella*, *Megasphaera*, and *Enterococcus* increased significantly ($P<0.05$), while that of *Olsenella* decreased significantly ($P<0.05$) (Fig. 6A). Following conventional multiple-visit therapy, the relative abundances of *Anaerobutyricum* and *Dorea* increased significantly ($P<0.05$), whereas those of *Agathobacter* and *Clostridium* decreased significantly ($P<0.05$) (Fig. 6C).

At the species level, after single general anesthesia treatment, the relative abundances of *S. mutans* and *Streptococcus anginosus* decreased significantly ($P<0.05$) (Fig. 6B). Following conventional multiple-visit therapy, the relative abundances of *S. mutans* and *Alistipes onderdonkii* decreased significantly ($P<0.05$) (Fig. 6D).

Fig. 6 Differential taxa before and after treatment at the genus (A,C) and species (B,D) levels of gut microbiota; general anaesthesia group (A,B) and conventional treatment group (C,D).

Microbial co-occurrence network analysis

Salivary microbiota co-occurrence network

Genera with relative abundance >0.5% and detection rate >30% were subjected to Spearman correlation analysis. Co-occurrence networks were constructed with pairs meeting $|r|>0.6$ and $P<0.05$, where positive and negative correlations represent synergistic and competitive interactions, respectively.

In the single general anesthesia treatment group, the pre-treatment salivary network comprised 25 edges (4 negative, 21 positive). Post-treatment, the network expanded to 85 edges (6 negative, 79 positive) with increased interaction complexity; *Fusobacterium* and *Prevotella* were core hub genera. The connectivity of *Streptococcus* dropped from 2 to 0, indicating reconfigured intergeneric interaction patterns (Fig. 7A–B).

In the conventional group, the pre-treatment network contained 82 edges (only 1

negative correlation). After treatment, edges increased to 142, with the negative correlation proportion rising to 9.86%, reflecting elevated overall network complexity. *Capnocytophaga* emerged as the core hub genus post-treatment (Fig. 7C–D).

Fig. 7 Correlation network analysis of salivary microbiota at the genus level: pre-operation (A) and post-operation (B) in the general anaesthesia group; pre-operation (C) and post-operation (D) in the conventional treatment group. Note: Each node represents one genus. Node size indicates the average relative abundance of the genus. Nodes belonging to the same phylum share the same colour. A thicker line between nodes indicates a larger absolute value of the correlation coefficient for species interaction. Red lines denote positive correlations, and blue lines denote negative correlations.

Gut microbiota co-occurrence network

Gut microbial co-occurrence networks were constructed using the same method as for salivary microbiota. In the single general anesthesia treatment group, gut network edges decreased from 69 at baseline to 49 post-treatment, with both positive and negative correlations reduced, indicating simplified interspecies interactions. The connectivity of *Streptococcus* remained stable at 2 (Fig. 8A–B).

In the conventional group, network edges rose sharply from 32 to 239 after treatment, and the proportion of negative correlations increased from 6.25% to 20.08%, reflecting markedly enhanced competitive and synergistic interactions. *Anaerobutyricum*, *Roseburia* and *Monoglobus* were identified as core high-connectivity genera post-treatment (Fig. 8C–D).

Fig. 8 Correlation network analysis of gut microbiota at the genus level: pre-operation (A) and post-operation (B) in the general anaesthesia group; pre-operation (C) and post-operation (D) in the conventional treatment group.

Discussion

The oral and gut microbiota interact bidirectionally via the oral–gut microbial axis through metabolites, bacterial translocation, inflammation and immune regulation, and the homeostasis of this axis is closely linked to children’s oral and systemic health[23]. S-ECC persistently disrupts oral microecological balance; abnormally proliferating cariogenic bacteria can enter the digestive tract via swallowing and further disturb gut microbiota structure and function. Conversely, gut dysbiosis may exacerbate dental caries susceptibility through immune and metabolic feedback. Thus, comparing the differential microecological effects of the two S-ECC treatment

modalities may provide new theoretical evidence for clinical decision-making.

The α-diversity results showed that single general anesthesia treatment significantly reduced salivary microbial richness and diversity, while conventional multiple-visit therapy had no significant effect. One-stage complete removal of carious tissues and plaque under general anesthesia directly disrupts cariogen-dominated dysbiotic microbiota, causing a transient diversity decline that recovers as healthy tooth surfaces are recolonized. In contrast, stepwise lesion removal in conventional therapy exerts milder, gradual interference without fully resolving pre-existing dysbiosis, leading to no marked diversity fluctuations — consistent with Ma et al.[22] , who observed rapid microbial shifts within 7 days after single general anesthesia treatment versus slower changes with multiple-visit therapy. Neither treatment altered gut α-diversity, indicating robust gut homeostasis; oral microbial changes affect the gut mainly via selective abundance shifts of specific genera rather than overall community diversity fluctuations.

The β-diversity analysis revealed complete separation of salivary samples before and after single general anesthesia treatment, but no significant separation of faecal samples, indicating the treatment markedly alters salivary microbial community structure in children with S-ECC while exerting relatively minor impact on gut microbiota. In contrast, both salivary and faecal samples in the conventional multiple-visit group showed distinct pre-treatment and post-treatment separation, suggesting significant shifts in both oral and gut microbial structures. These findings demonstrate that the two treatment modalities exert differential effects on gut microbial structure.

At the phylum level, the salivary microbiota of children with S-ECC was dominated by *Pseudomonadota*, *Bacillota*, *Bacteroidota*, *Actinomycetota*, and *Fusobacteriota*, which is consistent with previously reported baseline characteristics of the paediatric oral microbiome. This indicates that the dominant oral phyla remain relatively conserved under caries conditions, with differences mainly manifested as fluctuations in pathogenic species at the genus and species levels. The faecal samples from all four groups before and after treatment were predominantly composed of *Bacillota*, *Actinomycetota*, *Pseudomonadota*, and *Bacteroidota* at the phylum level, accounting for more than 98% of the total[24, 25].

Genus- and species-level analyses showed that single general anesthesia treatment significantly downregulated core cariogenic bacteria including *S. mutans*, *P.*

denticola, *Prevotella melaninogenica*, *Veillonella* and *Fusobacterium*, while enriching beneficial commensals such as *Rothia* and *Rothia aeria*, consistent with previous report[26]. In vitro evidence confirms that *P. denticola* synergizes with *S. mutans* to enhance biofilm cariogenicity and accelerate caries progression[27] ; one-stage elimination of pathogenic communities under general anesthesia effectively disrupts this cariogenic synergistic chain and rapidly reverses oral dysbiosis. Following conventional multiple-visit treatment, the relative abundance of *Scardovia wiggsiae* significantly decreased. Studies have found that *Scardovia wiggsiae* is significantly enriched in the saliva of children with ECC compared with caries-free children, promoting caries progression[28]. Although conventional treatment did not alter salivary microbial richness or diversity, it modified community structure and reduced certain cariogenic species, likely by lowering plaque biomass to partially modulate oral microecological balance[29].

Oral pathogens can enter the gut via swallowing and contribute to gut dysbiosis and low-grade inflammation. The two treatments differed notably in regulating gut beneficial bacteria: conventional multiple-visit therapy significantly enriched butyrate-producing genera *Anaerobutyricum* and *Dorea*. As a typical beneficial butyrate producer, *Anaerobutyricum* synthesizes short-chain fatty acids to maintain intestinal barrier integrity, suppress inflammation and regulate immune homeostasis[30]. This may result from reduced salivary cariogenic bacteria after conventional treatment, which lowers pathogen influx into the gut via swallowing and subsequently modulates gut microenvironment and microbial colonization through inflammatory and immune signals. In contrast, single general anesthesia treatment caused mild increases in a few gut opportunistic pathogens, presumably transient microecological fluctuations induced by anesthesia-related stress and short-term dietary changes. Given unaltered overall community structure, its impact on gut homeostasis is limited with low long-term risks.

Furthermore, our study confirmed synchronous reduction of *S. mutans* across oral and gut compartments. Previous animal studies have shown that *S. mutans* impairs intestinal health by secreting digestion-resistant lantibiotics, inducing intestinal pro-inflammatory factors and disrupting gut microbial diversity[31–34]. These findings indicate that both treatments can reduce *S. mutans* abundance in saliva and the gut of children with S-ECC, which not only lowers caries risk but also decreases swallowed *S. mutans* load, thereby alleviating its interference with gut

microecology, improving intestinal inflammatory status and promoting bidirectional improvement of oral and gut health.

Microbial communities form dynamic networks through complex synergistic, competitive and antagonistic interactions. Both treatments increased the complexity of salivary microbial interactions, indicating that therapeutic interventions disrupted the pre-existing dysbiotic network and drove the establishment of a new microecological balance. Interactions dominated by *Fusobacterium*, *Pseudomonas* and *Prevotella* prevailed after single-session general anesthesia, whereas *Capnocytophaga*-centered interactions became dominant after conventional multiple-visit therapy, suggesting distinct intergeneric interaction patterns induced by different modalities. As *Capnocytophaga* is reportedly positively associated with dental caries and may possess cariogenic potential[35]. This difference indicates that the post-treatment network under general anesthesia is closer to a healthy state, while the network after conventional therapy still retains certain cariogenic risk, requiring longer follow-up and stricter oral hygiene maintenance.

Gut microbial co-occurrence networks showed opposite post-treatment trends between the two groups. In the single general anesthesia group, reduced network complexity and weakened microbial interactions indicated decreased ectopic invasion of oral pathogens, enabling gut homeostasis and microecological stabilization. In contrast, the conventional multiple-visit group developed a highly complex gut network with significantly enhanced positive and negative interactions. A possible explanation, though speculative as our study did not measure stress-related biomarkers, is that repeated dental visits may trigger chronic stress via dental anxiety, psychological strain or dietary changes, potentially activating the hypothalamic-pituitary-adrenal axis and altering intestinal permeability and colonization niches [36–40]. However, this interpretation requires validation in future studies incorporating cortisol and other stress measures.

It is important to note that our network analyses are exploratory. Without a caries-free reference group, we cannot conclude that post-treatment networks are “closer to a healthy state.” Furthermore, the term “transient” for diversity reduction is used cautiously, as no subsequent timepoint was available to demonstrate recovery.

Limitations

This study has several limitations. First, the study design was non-randomized and allocation was based on clinical indication and patient/parental preference,

introducing potential selection bias. Baseline imbalances in age and dmft score existed between groups; although these were adjusted for as covariates in the LMM, residual confounding cannot be fully excluded. Second, the follow-up timepoints are not perfectly equivalent: the 1-month sample for the conventional group was calculated from the final treatment session, but total treatment duration could extend up to one month, introducing variability in the post-treatment interval. Third, dietary control relied only on verbal instructions without quantitative assessment, and post-operative diet may have mildly influenced microbial remodeling. Fourth, the 1-month follow-up only captures short-term changes; without a later timepoint, we cannot conclude that observed changes are “transient” or stable. Fifth, as an observational sequencing study, it only reveals correlational patterns without metabolomic or experimental validation of underlying mechanisms. Sixth, without a caries-free control group, interpretations of “health-associated” taxa or networks are assumptions rather than direct findings. Seventh, the network analysis was based on a small sample (n=25 per group) and did not apply multiple-testing correction, making it exploratory and hypothesis-generating. Eighth, the interpretation that repeated visits cause gut changes via HPA axis activation is speculative, as no cortisol, anxiety, or stress biomarkers were measured. Future large-sample randomized controlled cohorts with long-term follow-up, multi-omics approaches, and stress-related biomarker measurements are warranted to further elucidate the regulatory pathways of S-ECC treatments on the oral-gut axis..

Conclusions

In the short term, single-session general anesthesia significantly reduces salivary microbial richness, diversity and cariogenic bacteria in children with S-ECC, with minimal disturbance to gut microbiota. By contrast, conventional multiple-visit therapy exerts no significant effect on salivary microbial diversity but markedly disrupts gut microbial structure. Nevertheless, treatment selection requires comprehensive assessment of child cooperation, caries extent, family preferences and available medical resources.

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Author contributions

CRediT: Yuanjin Chen and: Writing – original draft, Methodology, Formal analysis; Xiaohong Zhou: Project administration, Funding acquisition; Shuangyi Zhang:Resources; Jianguo Liu: Writing – review & editing, Conceptualisation; Hu Li: Writing – review & editing, Supervision, Funding acquisition. All authors read and approved the final manuscript.

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Data availability

Raw sequencing data during the current study are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (accession number PRJNA1534637). Patient data are available from the the first author upon reasonable request.

Declarations

Ethics statement

This study was approved by the Medical Ethics Committee of the Affiliated Stomatological Hospital of Zunyi Medical University (approval No. YJSKTLS-2023-2026-025H) and was conducted in strict accordance with the ethical principles of the Declaration of Helsinki. All guardians of the enrolled children were fully informed of the study procedures and sample collection risks, and voluntarily signed written informed consent forms.

Consent for publication

Not applicable.

Disclosure statement

No potential conflict of interest was reported by the authors.

Athor details

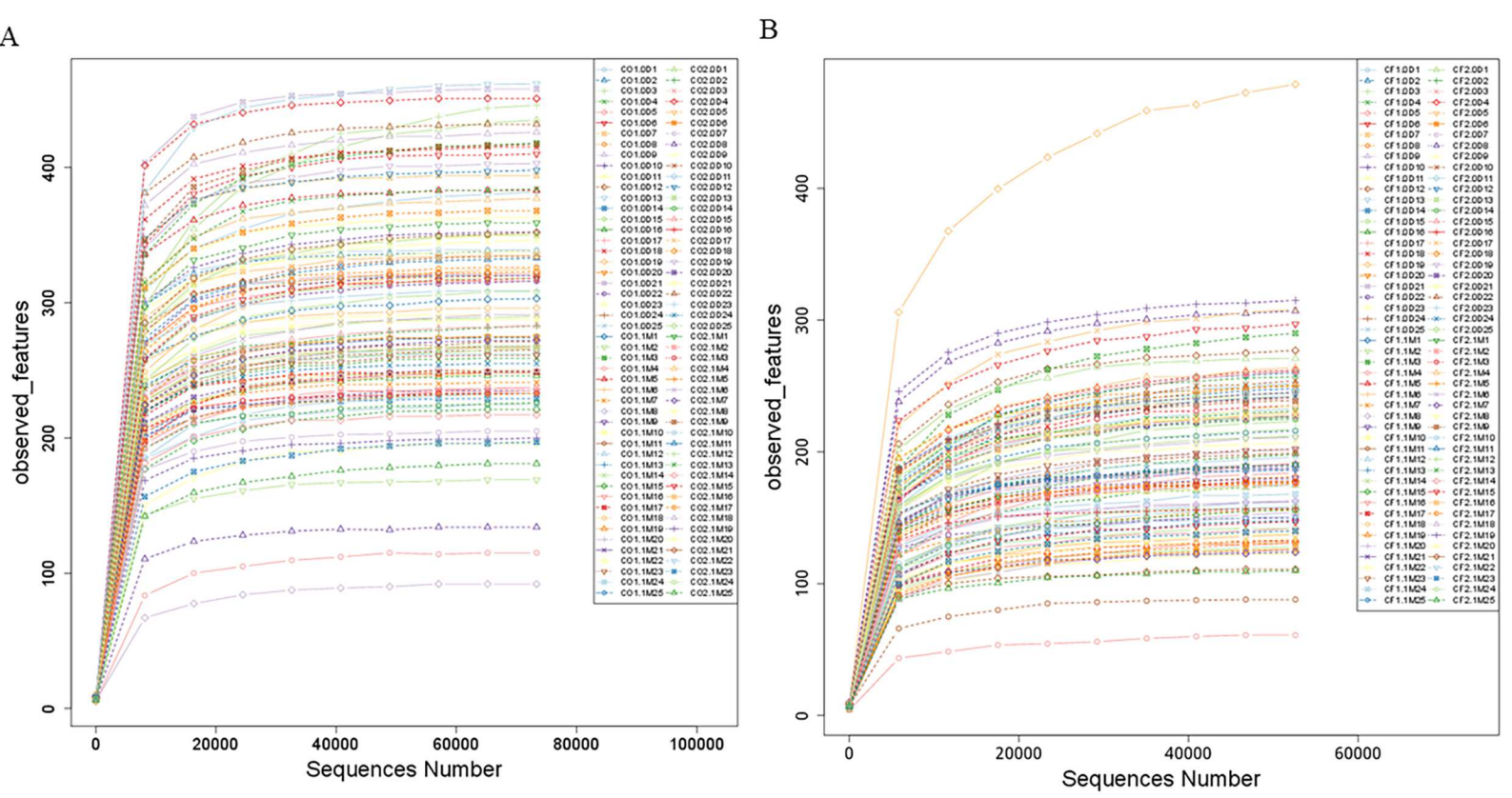
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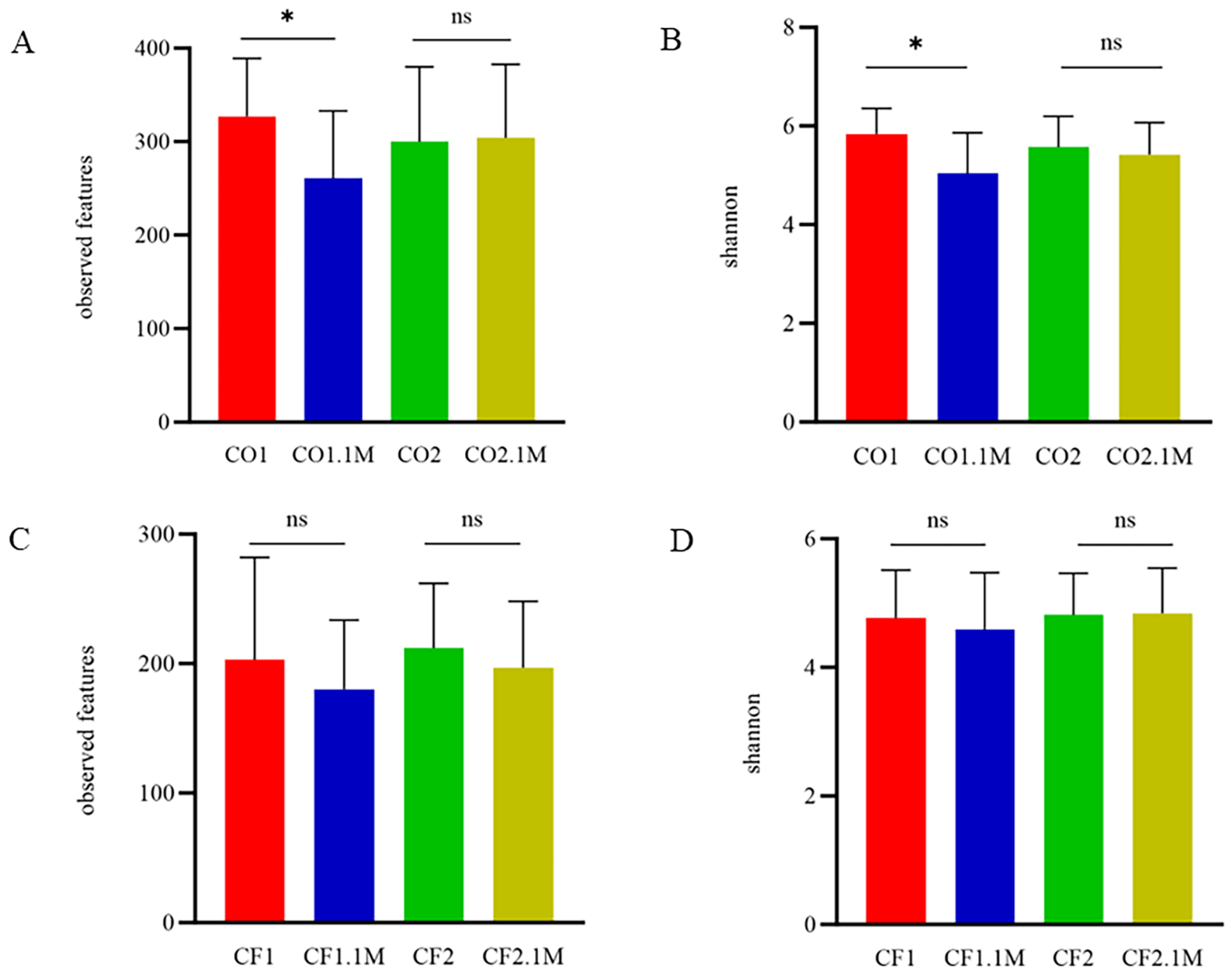
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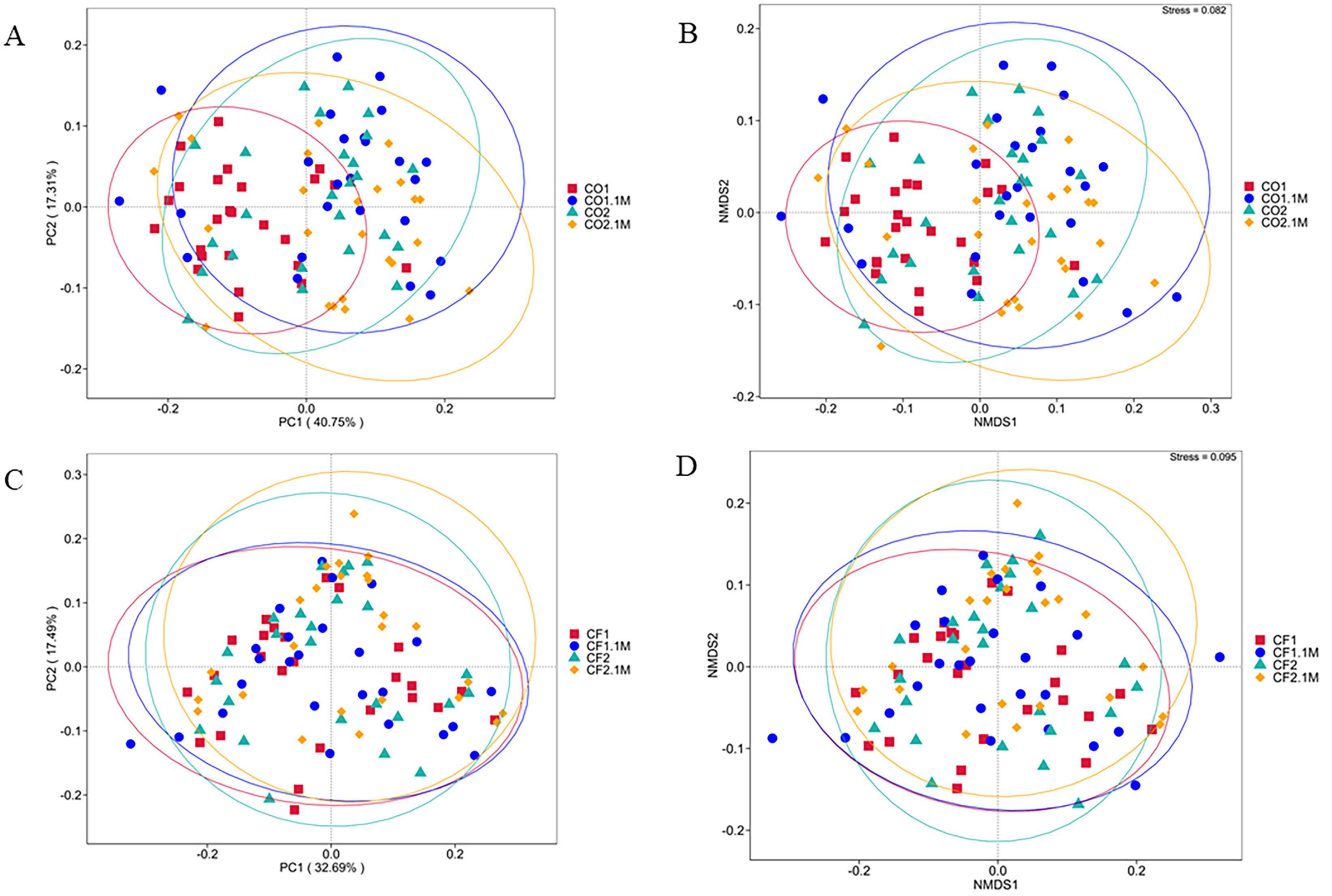
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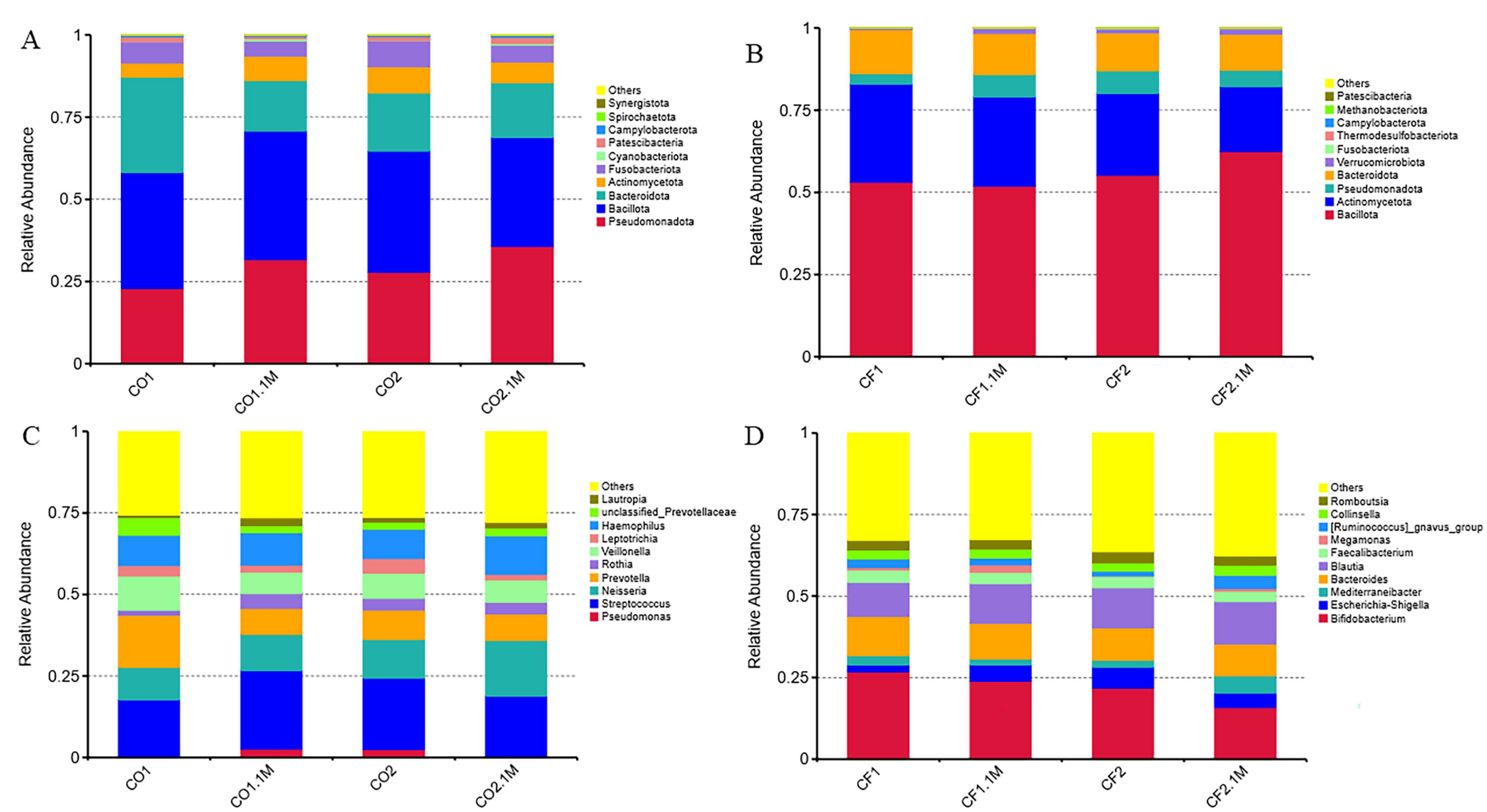
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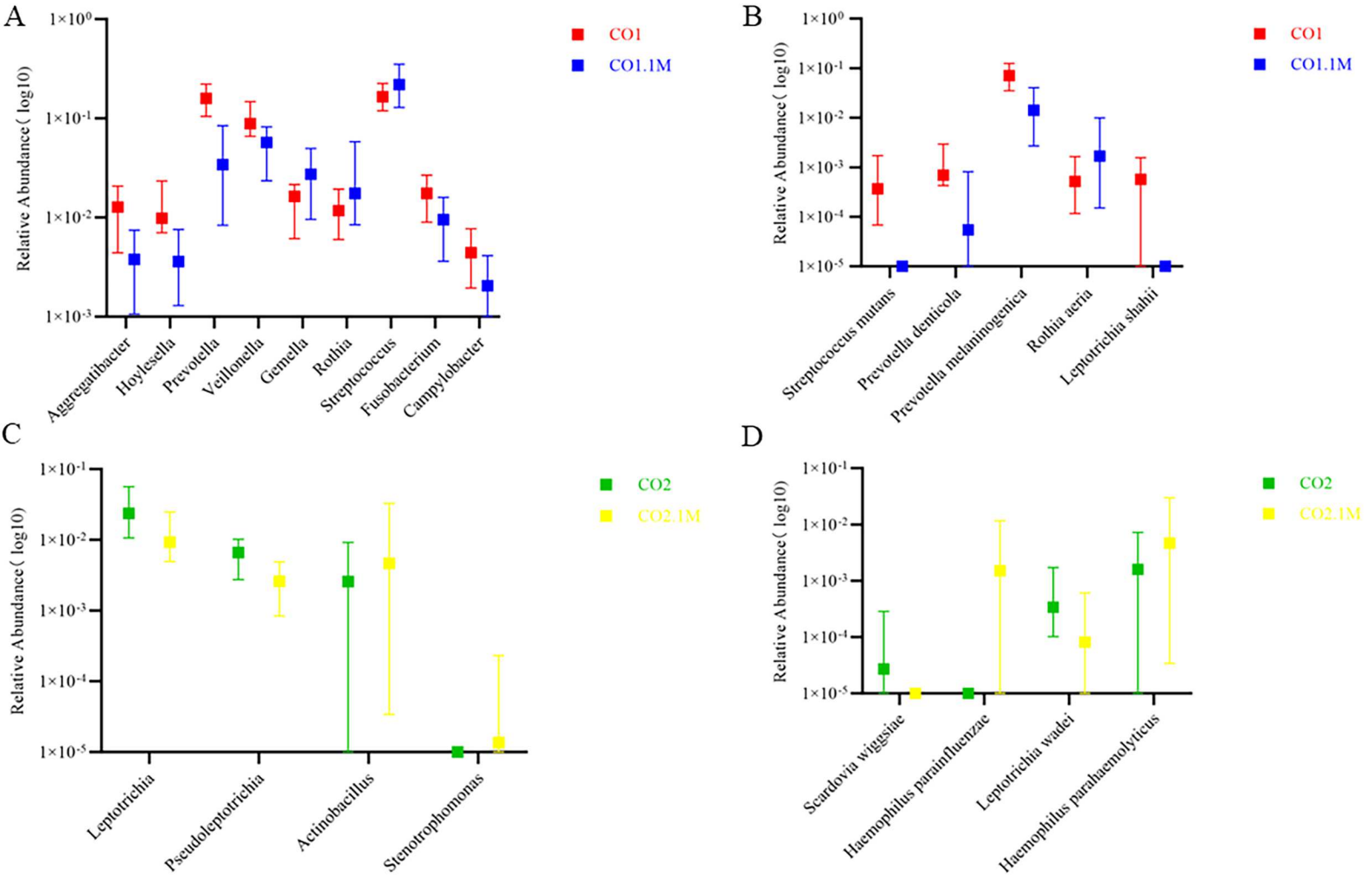
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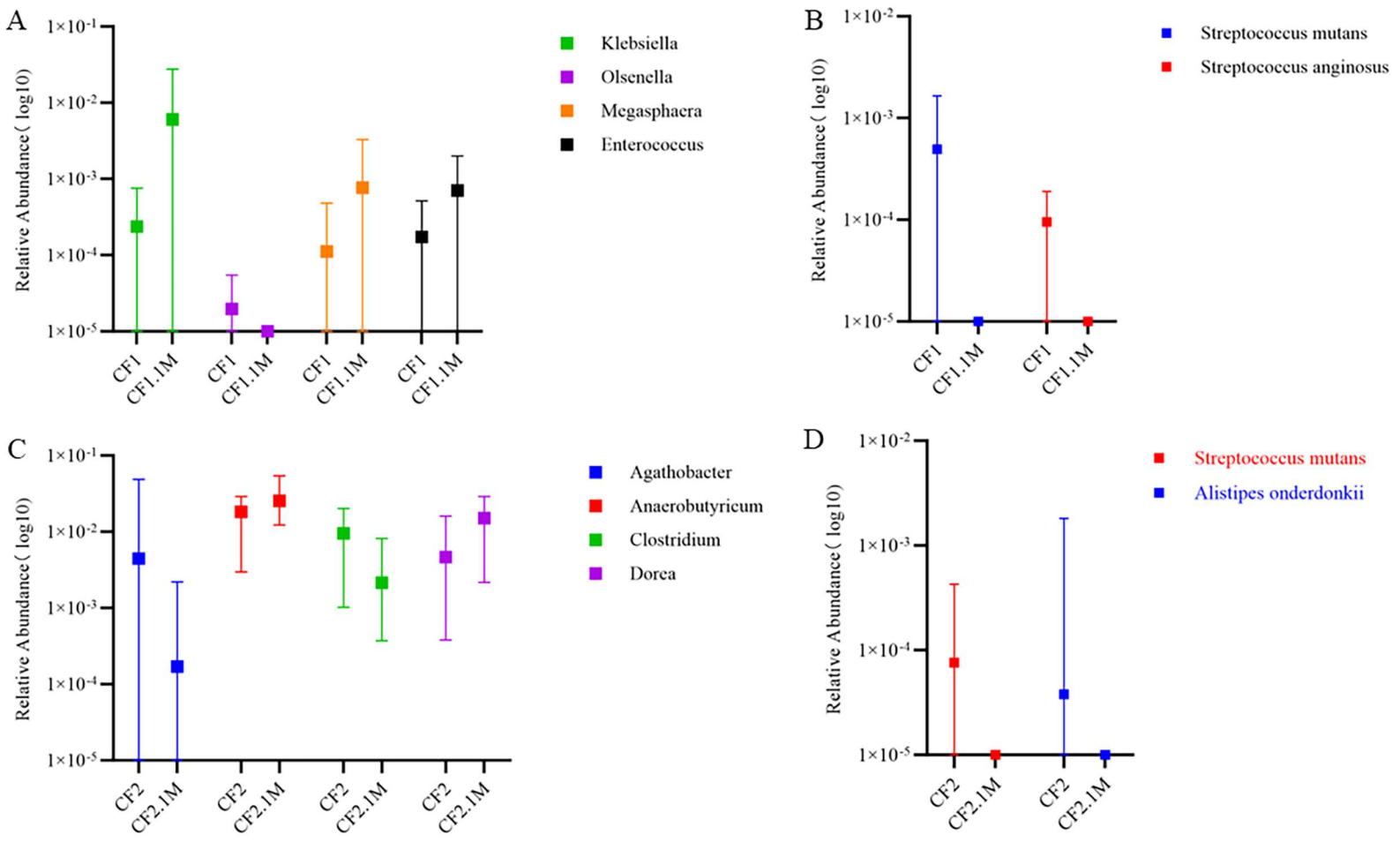

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60000
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300
400

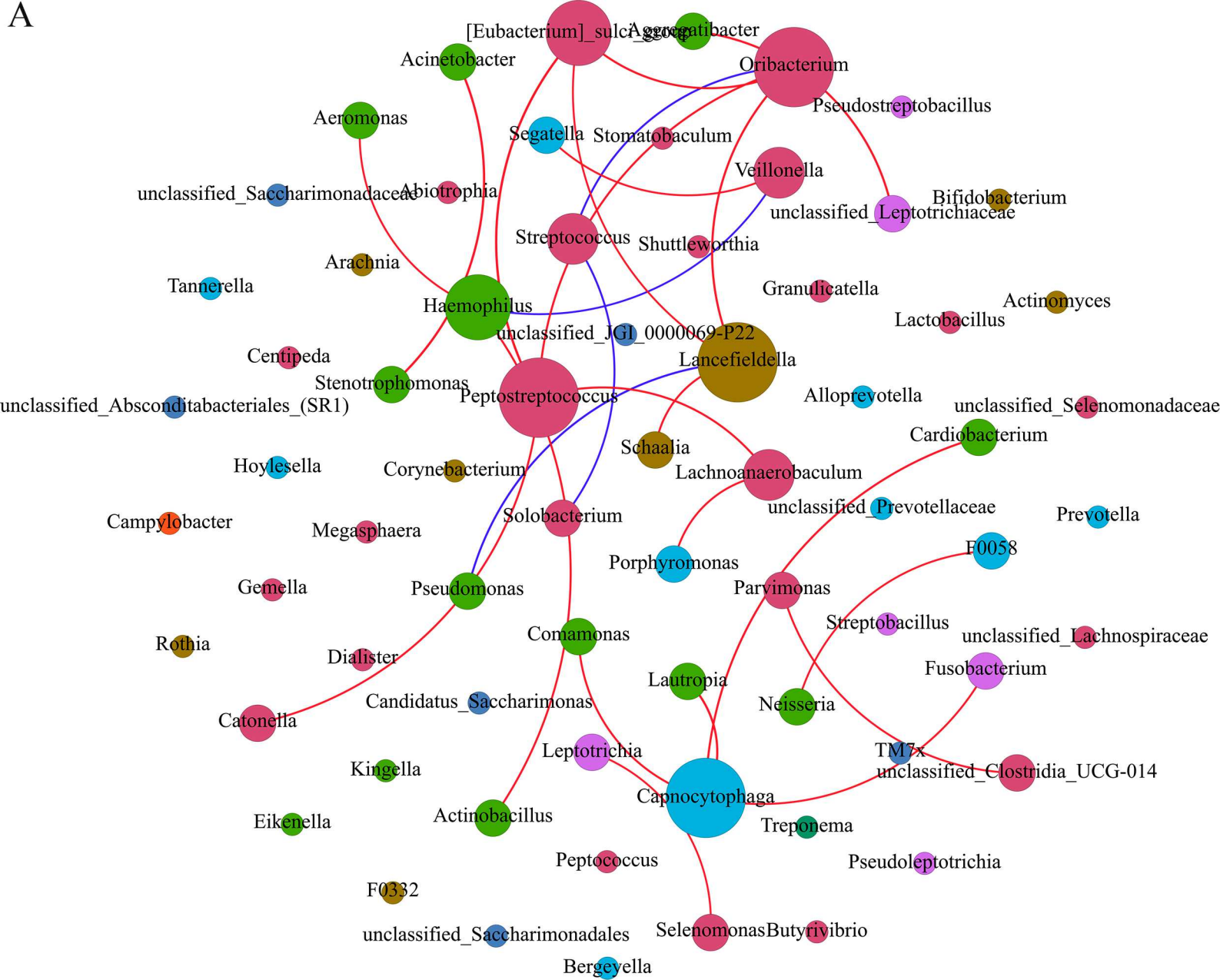

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observed features
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300
200
100
0
CO1
CO1.1M
CO2
CO2.1M
B
*
ns
shannon
8
6
4
2
0
CO1
CO1.1M
CO2
CO2.1M
C
ns
ns
observed features
300
200
100
0
CF1
CF1.1M
CF2
CF2.1M
D
ns
ns
shannon
6
4
2
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CF1
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CF2
CF2.1M

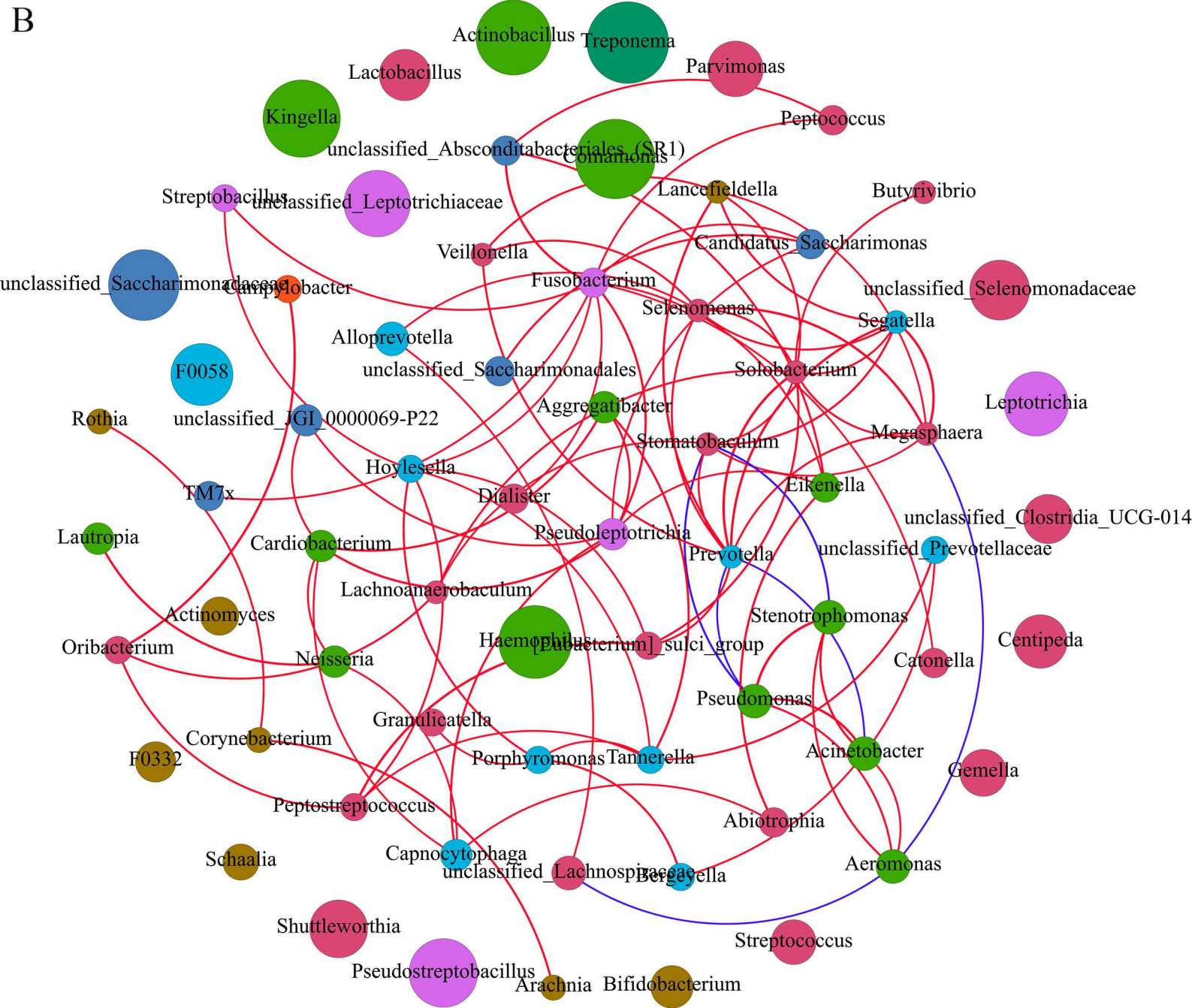

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PC2 (17.31%)
PC1 (40.75%)
CO1
CO1.1M
CO2
CO2.1M
B
Stress = 0.082
NMDS2
NMDS1
CO1
CO1.1M
CO2
CO2.1M
C
PC2 (17.49%)
PC1 (32.69%)
CF1
CF1.1M
CF2
CF2.1M
D
Stress = 0.095
NMDS2
NMDS1
CF1
CF1.1M
CF2
CF2.1M

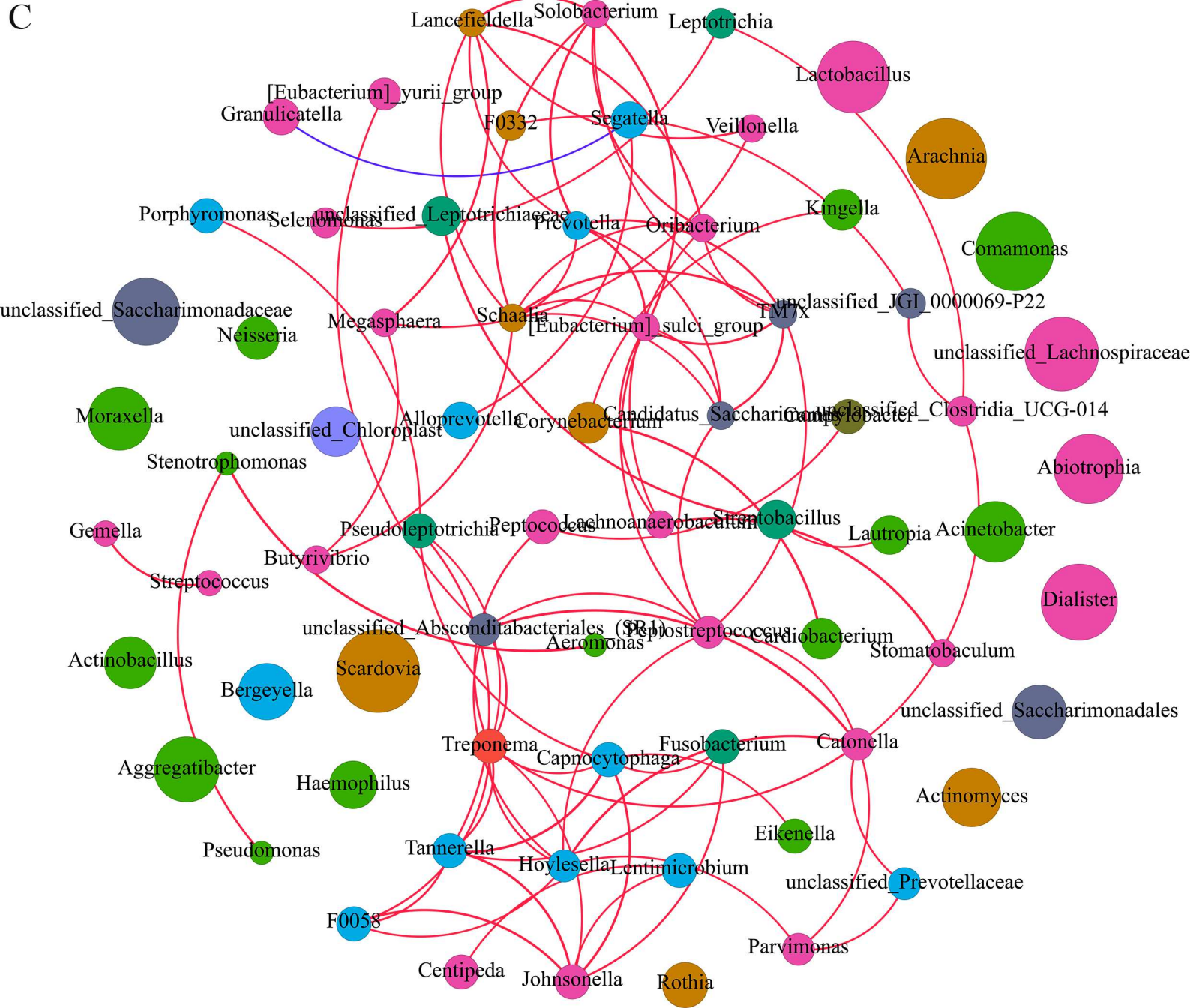

A
Relative Abundance
1
0.75
0.5
0.25
0
CO1
CO1.1M
CO2
CO2.1M
Others
Synergistota
Spirochaetota
Campylobacterota
Patescibacteria
Cyanobacteriota
Fusobacteriota
Actinomycetota
Bacteroidota
Bacillota
Pseudomonadota
B
Relative Abundance
CF1
CF1.1M
CF2
CF2.1M
Others
Patescibacteria
Methanobacteriota
Campylobacterota
Thermodesulfobacteriota
Fusobacteriota
Verrucomicrobiota
Bacteroidota
Pseudomonadota
Actinomycetota
Bacillota
C
Relative Abundance
CO1
CO1.1M
CO2
CO2.1M
Others
Lautropia
unclassified_Prevotellaceae
Haemophilus
Leptotrichia
Veillonella
Rothia
Prevotella
Neisseria
Streptococcus
Pseudomonas
D
Relative Abundance
CF1
CF1.1M
CF2
CF2.1M
Others
Romboutsia
Collinsella
[Ruminococcus]_gnavus_group
Megamonas
Faecalibacterium
Blautia
Bacteroides
Mediterraneibacter
Escherichia-Shigella
Bifidobacterium

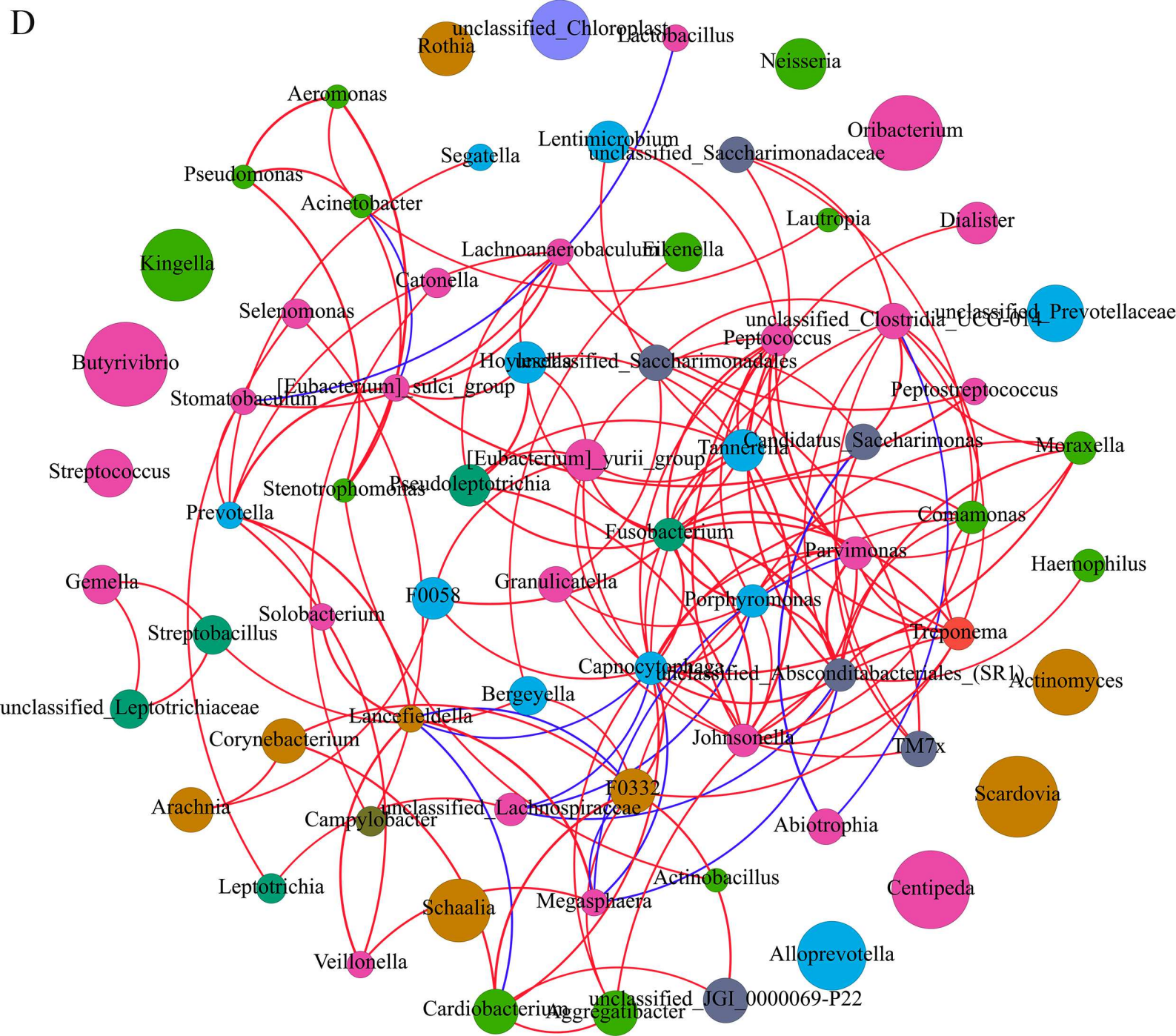

A
Relative Abundance (log10)
CO1
CO1.1M
Aggregatibacter
Hoylesella
Prevotella
Veillonella
Gemella
Rothia
Streptococcus
Fusobacterium
Campylobacter
B
Relative Abundance (log10)
CO1
CO1.1M
Streptococcus mutans
Prevotella denticola
Prevotella melaninogenica
Rothia aeria
Leptotrichia shahii
C
Relative Abundance (log10)
CO2
CO2.1M
Leptotrichia
Pseudoleptotrichia
Actinobacillus
Stenotrophomonas
D
Relative Abundance (log10)
CO2
CO2.1M
Scardovia wiggsiae
Haemophilus parainfluenzae
Leptotrichia wadei
Haemophilus parahaemolyticus

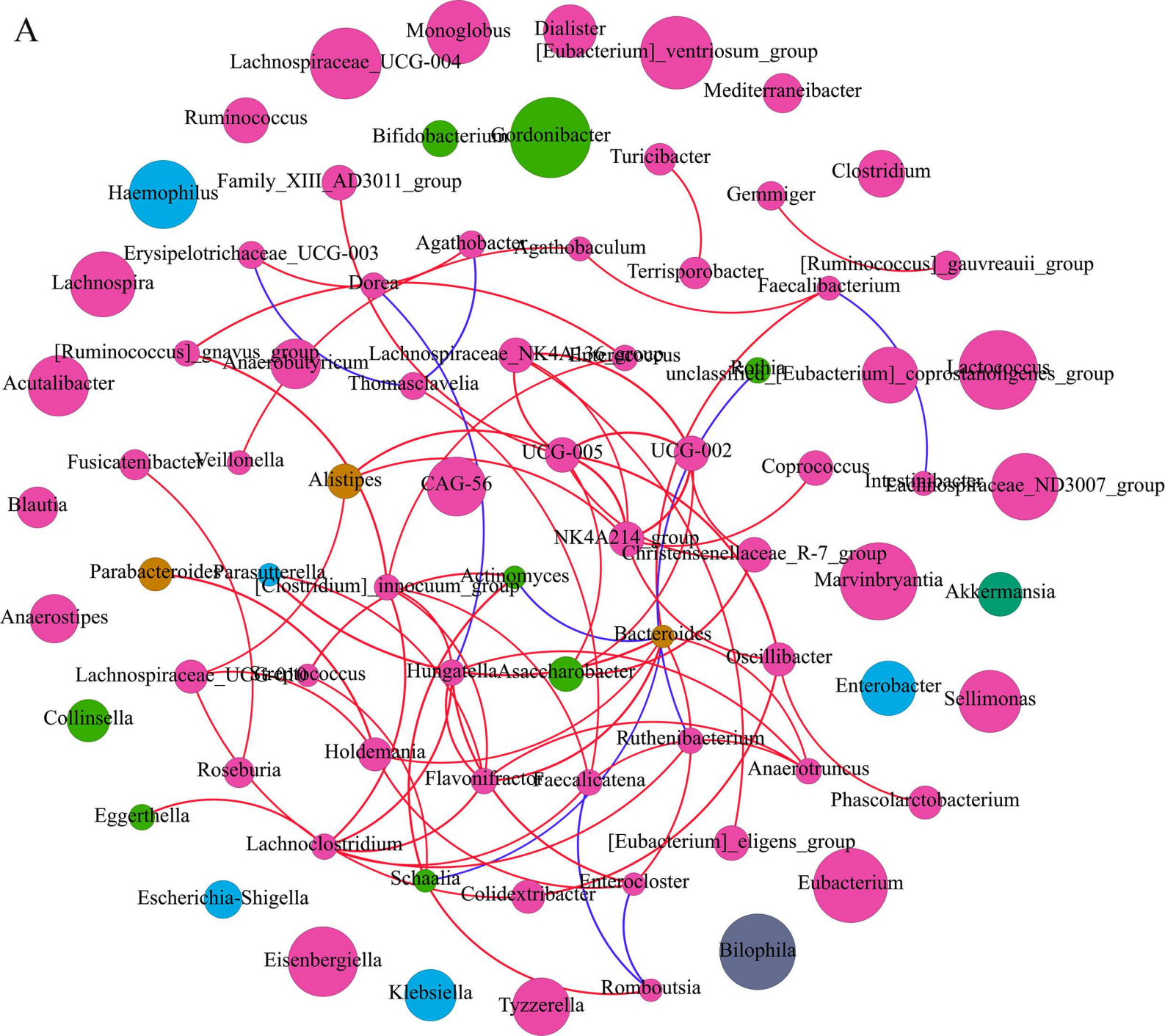

A
Relative Abundance (log10)
1×10-1
1×10-2
1×10-3
1×10-4
1×10-5
CF1
CF1.1M
Klebsiella
Olsenella
Megasphaera
Enterococcus
B
Relative Abundance (log10)
1×10-2
1×10-3
1×10-4
1×10-5
Streptococcus mutans
Streptococcus anginosus
C
Relative Abundance (log10)
CF2
CF2.1M
Agathobacter
Anaerobutyricum
Clostridium
Dorea
D
Relative Abundance (log10)
Streptococcus mutans
Alistipes onderdonkii

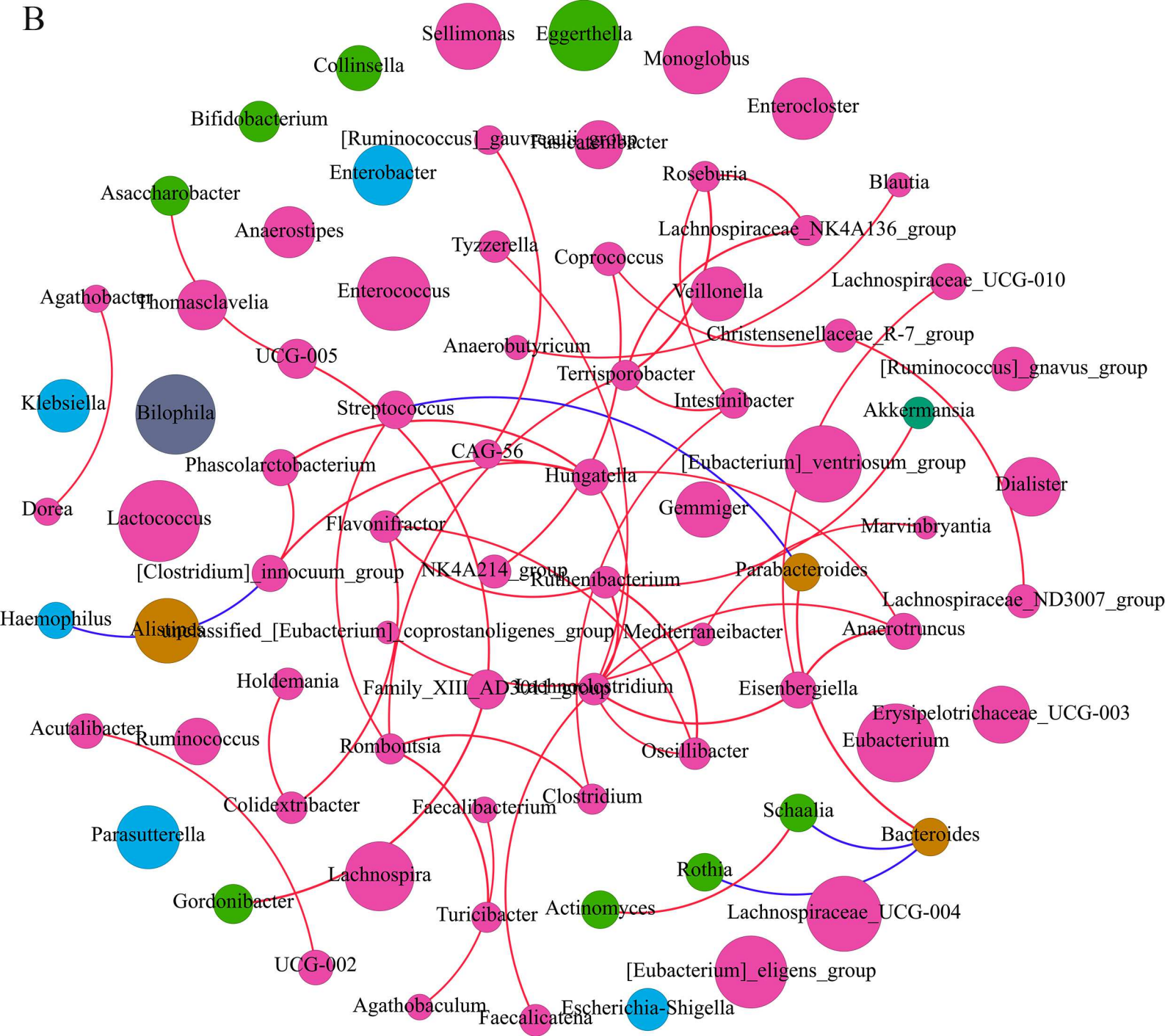

A
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Acinetobacter
Oribacterium
Pseudostreptobacillus
Aeromonas
Segatella
Stomatobaculum
Veillonella
unclassified_Saccharimonadaceae
Abiotrophia
Bifidobacterium
unclassified_Leptotrichiaceae
Streptococcus
Shuttleworthia
Arachnia
Tannerella
Granulicatella
Actinomyces
Haemophilus
Lactobacillus
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Lancefieldella
Stenotrophomonas
Peptostreptococcus
Alloprevotella
unclassified_Absconditabacteriales_(SR1)
unclassified_Selenomonadaceae
Cardiobacterium
Schaalia
Hoylesella
Corynebacterium
Lachnoanaerobaculum
Solobacterium
unclassified_Prevotellaceae
Prevotella
Campylobacter
Megasphaera
F0058
Porphyromonas
Gemella
Pseudomonas
Parvimonas
Streptobacillus
Comamonas
unclassified_Lachnospiraceae
Rothia
Dialister
Fusobacterium
Lautropia
Candidatus_Saccharimonas
Neisseria
Catonella
TM7x
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Kingella
Capnocytophaga
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Treponema
Peptococcus
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SelenomonasButyrivibrio
Bergeyella

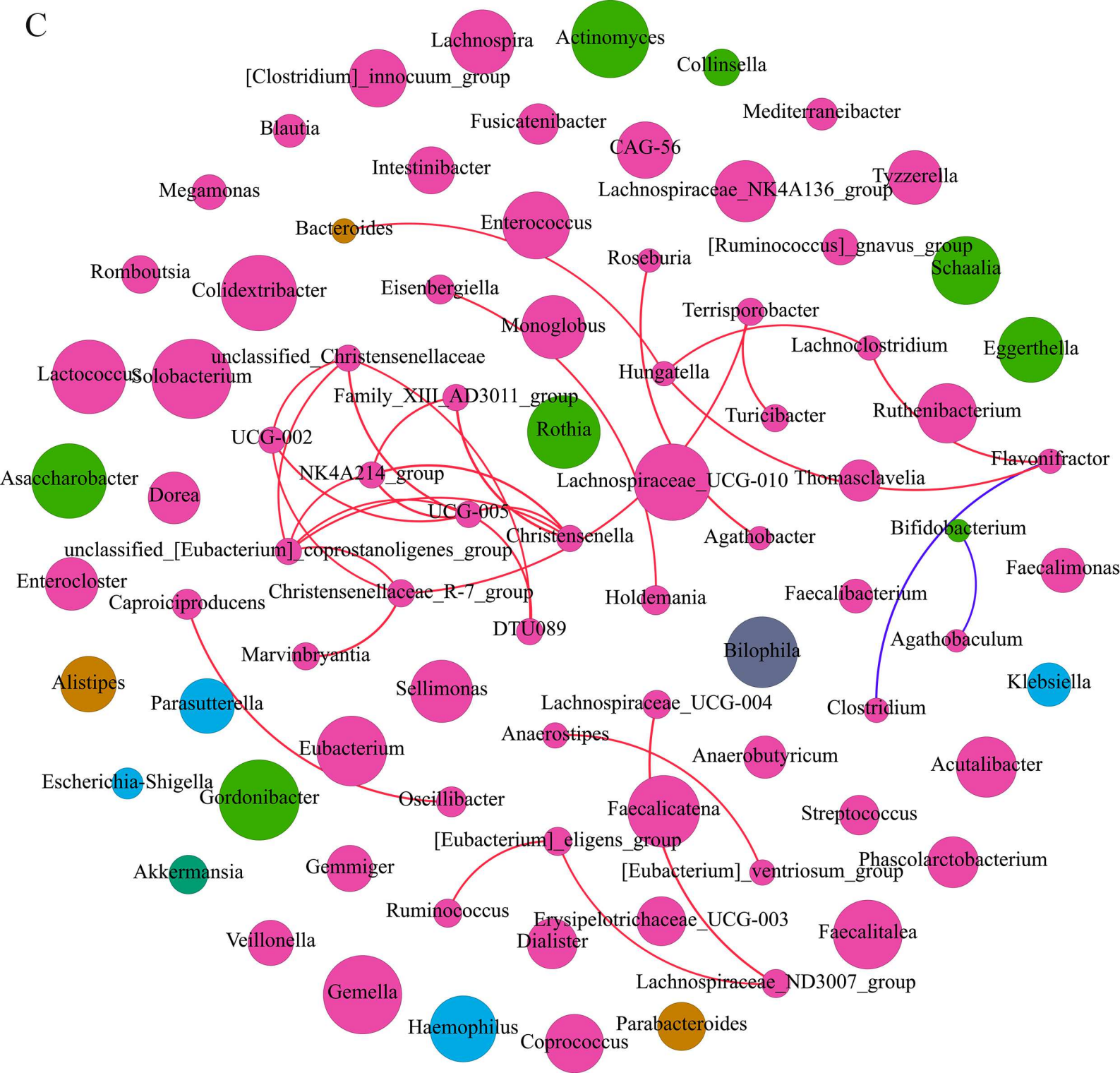

B
Actinobacillus
Treponema
Lactobacillus
Parvimonas
Kingella
Peptococcus
unclassified_Absconditabacteriales_(SR1)
Colmanomas
Lancefieldella
Butyrivibrio
Streptobacillus
unclassified_Leptotrichiaceae
Veillonella
Candidatus_Saccharimonas
unclassified_Saccharimonadaceae
Campylobacter
Fusobacterium
unclassified_Selenomonadaceae
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Cardiobacterium
Pseudoleptotrichia
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Stenotrophomonas
Oribacterium
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F0332
Porphyromonas
Tannerella
Acinetobacter
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Peptostreptococcus
Abiotrophia
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Bifidobacterium

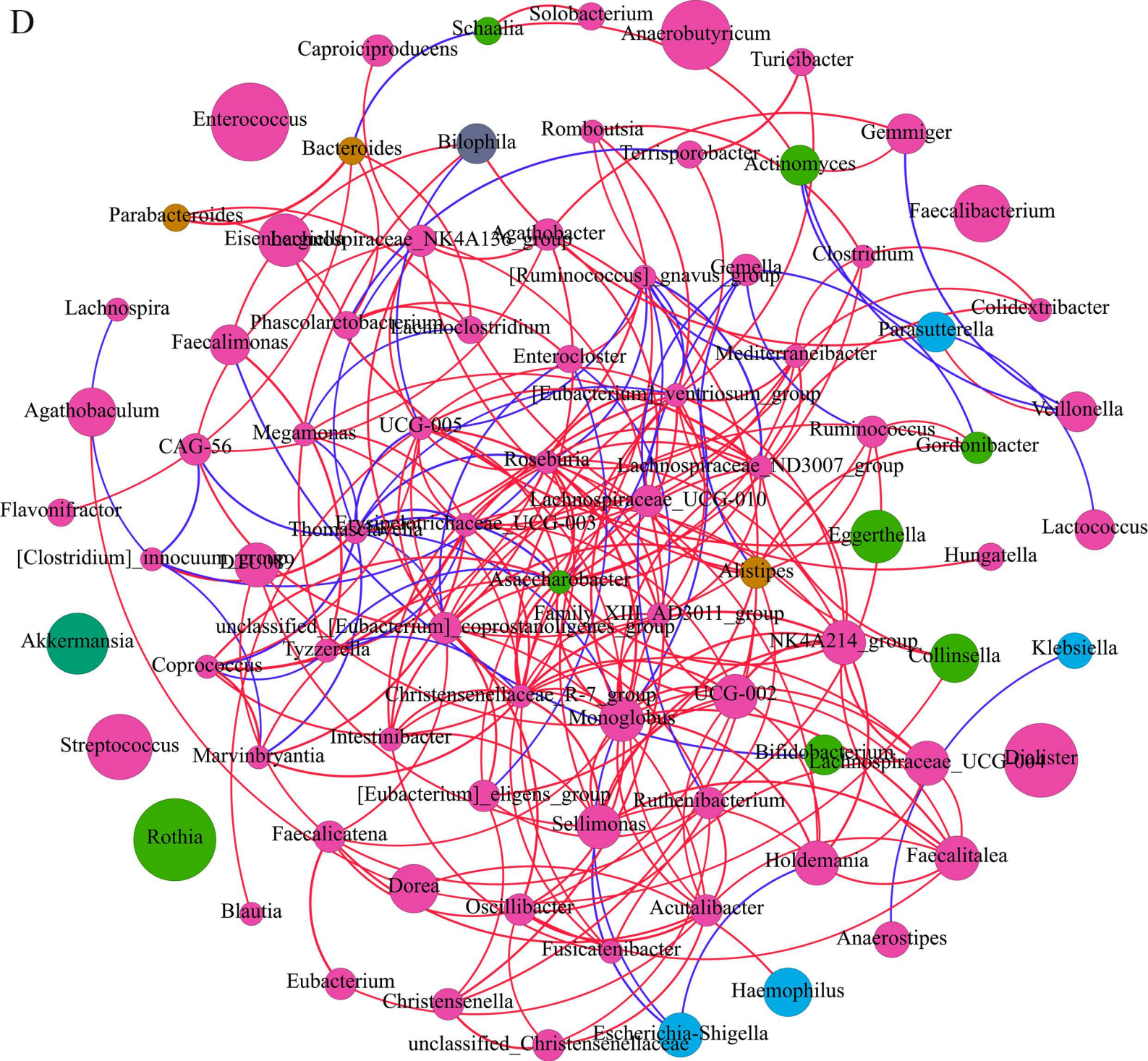

C
Lancefieldella
Solobacterium
Leptotrichia
Lactobacillus
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Granulicatella
F0332
Segatella
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Arachnia
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Stenotrophomonas
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Rothia


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Eisenbergiella
Erysipelotrichaceae_UCG-003
Acutalibacter
Ruminococcus
Romboutsia
Oscillibacter
Eubacterium
Colidextribacter
Clostridium
Faecalibacterium
Schaalia
Parasutterella
Bacteroides
Lachnospira
Rothia
Gordonibacter
Turicibacter
Actinomyces
Lachnospiraceae_UCG-004
UCG-002
[Eubacterium]_eligens_group
Agathobaculum
Escherichia-Shigella
Faecalicatena


C
Lachnospira
Actinomyces
[Clostridium]_innocuum_group
Collinsella
Blautia
Fusicatenibacter
Mediterraneibacter
CAG-56
Intestinibacter
Megamonas
Lachnospiraceae_NK4A136_group
Tyzzerella
Bacteroides
Enterococcus
[Ruminococcus]_gnavus_group
Roseburia
Schaalia
Romboutsia
Colidextribacter
Eisenbergiella
Monoglobus
Terrisporobacter
Lachnoclostridium
Eggerthella
Lactococcus
Solobacterium
unclassified_Christensenellaceae
Hungatella
Family_XIII_AD3011_group
Rothia
Turicibacter
Ruthenibacterium
UCG-002
Flavonifractor
Asaccharobacter
NK4A214_group
Lachnospiraceae_UCG-010
Thomasclavelia
Dorea
UCG-005
Christensenella
Agathobacter
Bifidobacterium
unclassified_[Eubacterium]_coprostanoligenes_group
Faecalimonas
Enterocloster
Christensenellaceae_R-7_group
Holdemania
Faecalibacterium
Caproiciproducens
DTU089
Agathobaculum
Bilophila
Marvinbryantia
Alistipes
Klebsiella
Sellimonas
Parasutterella
Lachnospiraceae_UCG-004
Clostridium
Anaerostipes
Eubacterium
Anaerobutyricum
Acutalibacter
Escherichia-Shigella
Gordonibacter
Oscillibacter
Faecalicatena
Streptococcus
[Eubacterium]_eligens_group
Akkermansia
Gemmiger
[Eubacterium]_ventriosum_group
Phascolarctobacterium
Ruminococcus
Erysipelotrichaceae_UCG-003
Faecalitalea
Veillonella
Dialister
Lachnospiraceae_ND3007_group
Gemella
Haemophilus
Parabacteroides
Coprococcus


D
Solobacterium
Schaalia
Anaerobutyricum
Caproiciproducens
Turicibacter
Enterococcus
Romboutsia
Gemmiger
Bacteroides
Bilophila
Terrisporobacter
Actinomyces
Parabacteroides
Faecalibacterium
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Agathobacter
Clostridium
[Ruminococcus]_gnavus_group
Gemella
Lachnospira
Colidextribacter
Phascolarctobacterium
Faecalimonas
Lachnoclostridium
Parasutterella
Enterocloster
Mediterraneibacter
[Eubacterium]_ventriosum_group
Agathobaculum
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Fusicatenibacter
Eubacterium
Haemophilus
Christensenella
Escherichia-Shigella
unclassified_Christensenellaceae