Metagenomic Sequencing Reveals Distinct Microbial Community Structures in Healthy and Diseased Oral Microbiota

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Research

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Abstract

Background: Periodontitis and peri-implantitis are common biofilm-mediated infectious diseases affecting teeth and dental implants, and have been considered to be initiated with the adjacent microbial dysbiosis.

Study Aim: To further understand the essence of oral microbiota dysbiosis in terms of bacterial interactions, community structure and microbial stability.

Methods: We analyzed 64 plaque samples from 34 participants with teeth or implants under different health conditions using metagenomic sequencing. After taxonomical annotation, we computed the core microbiome, analyzed the bacterial co-occurrence networks, and calculated the microbial stability in supra- and sub-gingival plaques from hosts with different health conditions.

Result: When inflammation arises, the subgingival communities become less connective and competitive with fewer hub species. In contrast, the supragingival communities tend to be more connective and competitive with a increased number of hub species. Notably, periodontitis and peri-implantitis are associated with significantly increased microbial stability in subgingival plaques. In addition, we also observe similar core bacterial components yet distinct co-occurrence networks and community structures between the healthy and diseased hosts.

Conclusion: The findings indicated that the aberrant changes of the bacterial co-occurrence networks and community structures are the essence of dysbiosis in periodontal and peri-implant patients, while breaking the diseased equilibrium and reestablishing healthy equilibrium is crucial for the treatment of periodontitis and peri-implantitis.

Background

Periodontitis is a prevalent disease in the human oral cavity and the major cause of dentition defect[1]. It is a complex infectious disease resulting from infection-induced inflammation and hyperimmune response towards various microbial pathogens[2, 3]. Previous studies have proved that periodontitis is initiated with microbial dysbiosis in periodontium[4]. The prevalence of periodontitis is estimated from 4–76.0% in developed countries and from over 50% to almost 90% in developing ones[5]. Approximately over 700 million adults are suffering from periodontitis worldwide[6], which has become a severe burden in the oral health of humankind[7].

Peri-implantitis has been described as a pathological condition around dental implants where inflammation continuously affects connective tissue and finally leads to the loss of supporting bone matrix[8]. Similar to periodontitis, peri-implantitis is also caused by the hyper-inflammation in peri-implant tissue and the aberrant change in the microbial community[9–11]. A meta-analysis in 2017 indicated that the weighted mean prevalence of peri-implantitis was around 19.83% at patient level[12]. As implant-supported prostheses being more and more widely used to replace missing teeth[13], there will be an increasing number of patients suffering from peri-implantitis in the coming future.

Periodontitis and peri-implantitis share many clinical and etiological features, including biofilm-mediated infection, hyperinflammatory reaction, progressive absorption of alveolar bone, presence of bleeding on probing, and increase in probing depth[14–16]. Most importantly, the accumulation of dental plaque and the following microbial dysbiosis is considered to trigger diseases[17]. Given the shared nature as infectious diseases between periodontitis and peri-implantitis, it is necessary to delve into the microbial communities around teeth and implants to understand the two analogs further.

The stability of commensal microbial communities in human bodies has been proven essential to human health[18]. However, previous studies investigating oral microbiota using high-throughput sequencing approaches have mainly focused on the taxonomical profile or microbial functionalities[19–22]. Yet, the bacterial inter-species correlations, the community structure and the microbial stability have not been fully illustrated. In this scenario, we analyzed 64 microbial samples from plaque around teeth and implants in different health conditions using metagenomic shotgun sequencing. We annotated

taxonomical information at the species level, visualized the bacterial co-occurrence network, and calculated the microbial stability to further our understanding of periodontitis and peri-implantitis.

Methods

Subject recruitment

This study enrolled 34 participants, including 20 subjects with natural teeth (10 periodontal health and 10 periodontitis) and 14 subjects with dental implants (9 peri-implant health and 5 peri-implantitis). All participants were Chinese natives who seek care at the College of Stomatology, Xi'an Jiaotong University, and provided written consents. Natural teeth were considered periodontal health when there was no bleeding on probing (BOP), no clinical attachment loss (CAL) or radiographic bone loss (RBL) and the maximum probing depth (PD) was less than 3mm. Periodontitis was diagnosed with an increased PD of more than 4 mm, examinable RBL, and interdental CAL, which corresponded with the latest diagnostic criteria for Stage □-□ periodontitis[23]. As for implants, subjects were considered peri-implant health when peri-implant tissue showed no redness, suppuration, BOP, and no more than 1mm marginal RBL beyond bone remodeling. Peri-implantitis was diagnosed when there was clinical inflammation, increased PD of more than 6mm, and radiographic evidence of more than 3mm RBL compared to baselines[24]. Detailed inclusion and exclusion criteria are listed in Table 1.

Table 1
Detailed inclusion and exclusion criteria for subject recruitment.

Type	Health Condition	Inclusion Criteria	Exclusion Criteria
Teeth	Periodontal Health	• Individual normal occlusion with no less than 28 teeth left in dentition; • No RBL or examinable CAL; • Maximum PD ≤ 3 mm; • No BOP or redness examined.	• Diabetes mellitus or other severe systemic diseases; • HIV infection or other severe immune diseases; • A history of tobacco smoking; • A history of immunosuppressant therapy; • A history of bisphosphonates, steroids, or other therapy influencing bone metabolism; • Antibiotic therapy, oral antiseptic therapy, or oral prophylactic treatment undergoing or in recent 3 months; • Having other denture in any form besides the selected dental implant; • Pregnancy or lactation. • Over 60 years old or below 20 years old.
	Periodontitis	• Individual normal occlusion with no less than 20 teeth left in dentition; • Examinable interdental CAL ≥ 3 mm; • PD ≥ 4mm; • Examinable RBL; • Existing BOP and/or suppuration.	
Implants	Peri-implant Health	• A single implant with a single cement-retained crown seated to replace the missing tooth; • Implant in function for over 2 years; • Radiographic MBL ≤ 1 mm; • No redness, suppuration, or BOP examined around the implant.	
	Peri-implantitis	• A single bone-level implant with a single cement-retained crown seated to replace the missing tooth; • Implant in function for over 2 years; • Radiographic MBL ≥ 3mm compared to baseline; • PD ≥ 6mm around implant.	

Clinical examination and sample collection

Before sampling, full-mouth examination was conducted on all subjects by the same calibrated clinician (see Supplementary Methods) to record clinical and demographic features, including sex, age, PD, BOP and RBL. Especially for subjects with implants, we also recorded their implant type, location, and functional time (Supplementary Tables 1 and 2).

The selection of sampling sites followed the criteria in our Supplementary Methods. When sampling commenced, patients first gargled with distilled water for 1 minute. Then, we used cotton rolls to isolate the selected sites and sampled the supragingival plaque using sterile curettes by a single horizontal stroke on each site. Bacteria were washed off from the curettes by rinsing in 1.5mL microcentrifuge tubes containing phosphate-buffered saline (PBS). The remaining supragingival plaque was then removed. Afterward, we used sterile endodontic paper points for subgingival sampling[25], by inserting paper points as deep as possible into the periodontal or peri-implant sulcus and staying for 20 seconds. After taking out, paper points were transferred into 1.5mL microcentrifuge tubes containing PBS. All samples were stored at -80°C

and were then sent to BGI Insititute (BGI Group, Shenzhen, China) for genomic DNA extraction, metagenomic libraries preparation, and sequencing.

Metagenomic analysis

To obtain high-quality data, we firstly filtered the raw reads when they contained more than 10 low-quality bases (< Q20) or 15 bases of adapter sequences with self-constructed script. Using BWA software (version 0.7.17), we aligned the read data to the human genome (hg19) and filtered the reads when the alignment length exceeds 40% of the read length[26]. After the removal of host mapped reads, the clean metagenomic data was applied for the following metagenomic analysis.

Using MetaPhlAn3[27], we aligned the filtered reads to the microbial database of specific marker genes (mpa_v30_CHOCOPhlAn_201901) and obtained the taxonomical annotation results. Based on the microbial profiling, we calculated the relative abundances of bacteria at the phylum, class, order, family, genus, and species levels, respectively. After the taxonomical annotation, we performed Permutational Multivariate Analysis of Variance (PERMANOVA) to evaluate the impact of environmental factors on the microbiome, calculated alpha diversity using Chao1 and Shannon index, and detected the Spearman correlation coefficients among the species with relative abundance over 0.01% (see also Supplementary Methods). We kept the relations with Spearman correlation coefficients <-0.6 or > 0.6 ($p < 0.05$) to plot the bacterial co-occurrence networks by applying Gephi (version 0.4.2) and to construct the bacterial interacting matrix for further analysis.

Local stability analysis

Local stability measures the tendency of a community to return to its equilibrium after perturbation. The community is stable if it can return to its equilibrium after perturbation. Following May and Allesina's work[28–30], we used the community matrix to analyze the local stability (henceforth, stability) of oral microbiota. Local stability theory indicates that a stable system requires that all eigenvalues of the community matrix should have negative real parts. Moreover, the real part of the rightmost eigenvalue in the complex plane can be used to measure the extent of stability: the more negative its real part, the more stable the system. Based on experimental data, we performed a series of simulations to show the difference of stability among different groups and the effect of real network structure on stability (see also Supplementary Methods).

Result

Taxonomical annotation.

After low-quality filtration and host-reads removal, a total of 1,926,649,953 sequences were obtained from 64 samples, with an average of 30,103,906 sequences per sample (range from 1,004,522 to 77,090,552). Overall, 310 bacterial species have been identified (see Additional File 1). Table 2 summarizes the clinical and demographic characteristics of recruited subjects. There were no significant differences in mean age and sex distribution amongst all subjects and functional time between healthy and diseased implants ($p > 0.05$, ANOVA, Tukey HSD, Fisher Exact Test). However, average PD and RBL in diseased subjects were significantly higher than those in the corresponding healthy control subjects ($p < 0.05$, Mann-Whitney).

Table 2

Summarized clinical and demographical information. HT for healthy teeth, DT for teeth with periodontitis, HI for healthy implants and DI for implants with peri-implantitis.

Group Category	Mean Age	Sex Distribution	Average PD	Average RBL	Functional Time	Implant Location		Implant System		
	(years)	(Female%)	(mm)	(mm)	(years)	Anterior	Posterior	Osstem	Bego	ITI
Group T	**45.70**	**35.00**	**3.95**	**1.95**	**/**	**/**	**/**	**/**	**/**	**/**
HT	40.40	40.00	2.30	0.30	/	/	/	/	/	/
DT	51.00	30.00	5.60	3.60	/	/	/	/	/	/
Group I	**48.93**	**64.29**	**4.64**	**1.50**	**4.07**	**2**	**12**	**4**	**7**	**3**
HI	50.56	66.67	3.44	0.33	3.67	0	9	1	5	3
DI	46.00	60.00	6.80	3.60	4.80	2	3	3	2	0

We first performed the PERMANOVA to evaluate the differences in microbial communities contributed by several factors (Fig. 1A). The results showed that supragingival communities were significantly different from subgingival communities in both teeth and implants despite health conditions. Beta diversity between supragingival and subgingival communities was visualized using principal coordinate analysis (PCoA) (Fig. 1B). The 95% confidence ellipses of supragingival and subgingival microbiota were different. Afterward, we compared alpha diversity between two groups of samples based on Shannon and Chao1 indices (Fig. 1C). Chao1 index of supragingival communities was significantly higher than that of subgingival communities ($P < 0.05$), but no significant difference was observed in terms of the Shannon index.

We then computed the core microbiome of subgingival and supragingival communities from taxonomical taxa shared by at least 80% of the individuals in each group with a threshold of 0.1% in relative abundance (Fig. 1D). There were 28 and 13 core species in the supragingival and subgingival microbiome, respectively, and 12 core species were shared by both sample types. Bacteria from different “colored” microbial complexes defined by Socransky et al.[31, 32] were found present in the core (Supplementary Table 3). Further taxonomical information of the core species was compared in the healthy and diseased microbiome (Fig. 2). The result showed no significant differences in the relative abundance of core species between healthy and diseased microbiome in both supragingival samples (Fig. 2A) and subgingival samples (Fig. 2B), meaning that the core components of supra- and sub-gingival communities were constant and might not change with the shift of health conditions.

Bacterial co-occurrence network analysis.

We analyzed bacterial co-occurrence networks in healthy and diseased sites based on the Spearman correlation coefficient at the species level (Fig. 3, see also Additional File 1–2.). Subgingival plaques from periodontitis and peri-implantitis patients exhibited less connected and competitive bacterial networks. On the contrary, more connected and competitive bacterial networks existed in the patients' supragingival plaques when compared with healthy controls (Fig. 4A). We visualized the degree distribution of the networks using bar charts and calculated the connectance among groups to further dissect the structure of the networks (Fig. 4B). The degree of a species referred to the count of its correlations with other species. Connectance was defined as the fraction of non-zero off-diagonal elements of the community interaction matrix[28, 29], or briefly as the ratio of actual correlations to all topologically possible correlations.

Our study found that in subgingival microbiome, healthy communities had more high-degree species and higher connectance than diseased communities. Besides, healthy subgingival network had a larger proportion of negative correlations (22.51%, 208 of 924) than diseased subgingival network (9.97%, 67 of 672) ($p < 0.05$, Pearson Chi-Square). As for supragingival microbiome, differences were reverted where healthy communities exhibited a cluster in lower degrees and had lower connectance when compared with diseased communities. Also, healthy supragingival network showed a lower

proportion of negative correlations (11.38%, 56 of 492) than diseased supragingival network (16.52%, 116 of 702) ($p < 0.05$, Pearson Chi-Square).

Based on the degree distribution, we also selected those hub species with more than 25 correlations (degree > 25) in each group. These hub species represent those pivotal members in the co-occurrence networks which were highly connected with other species (Fig. 4C). The healthy subgingival microbiome had significantly more hub species (31 species) than the diseased subgingival microbiome (2 species). However, such difference is, on the contrary, reverted again in the supragingival group where diseased microbiome had more hub species (11 in diseased communities and 5 in healthy communities). The results above revealed distinct bacterial co-occurrence networks and community structures in different microbiome and built the foundation for further stability analysis.

Stability analysis.

The core of stability analysis is the construction of community matrix[28–30], which included three key features: the network structure, the direction of interactions, and the strength of interactions (both inter-species interactions and intra-species interactions). The first two features can be quickly drawn from our taxonomical information and the bacterial co-occurrence network, while the last feature usually requires a time-sequence analysis from a cohort study[33], which is not included in our work due to obvious ethical reasons. Therefore, we assign the strength of interactions following Allesina's assumptions (see Supplementary Methods). Thus, we mainly focused on the relative stability among different groups rather than numerically calculating the absolute stability value of a specific group. Stability analysis (Fig. 5) showed that healthy subgingival communities had the worst stability among four groups while diseased subgingival communities possessed the highest stability. As for the supragingival group, the healthy and diseased communities showed similar stability in our analysis. We performed a series of simulations using different parameter sets and concluded the same result, which proved its robustness (see also Supplementary Methods).

Discussion

Critical differences between supragingival and subgingival microbiome.

Our study found that in periodontal and peri-implant microbiota, supra- and subgingival communities were distinct in terms of alpha and beta diversity. Supragingival communities had a significantly higher Chao1 index but a similar Shannon index when compared with subgingival communities. These findings indicated that supragingival plaque contained more bacterial species, but a certain number of these species were either too little or too much in abundance, which resulted in greater species richness but poorer evenness. A possible explanation is that supragingival plaque was more prone to foreign bacterial attachment due to its exposed position in the oral cavity. Therefore, supragingival communities might have more passersby species that were absent in subgingival communities. Former studies on the plaque composition have shown that supragingival plaque may play a role as a reservoir of some pathogens for the spread of subgingival infection[34, 35], and some suspected pathogens could only be detected in supragingival plaque but not in subgingival plaque, which corroborated with our findings.

Similar core microbiome in healthy and diseased communities.

We computed the core bacterial species in supra- and sub-gingival communities and revealed similar core microbiome in healthy and diseased sites. Briefly, core species were those predominantly abundant in most samples, notably this core microbiome mainly consisted of species from genera *Streptococcus*, *Capnocytophaga*, *Actinomyces*, *Veillonella*, and *Fusobacterium*. According to Socransky's findings and other previous studies[32, 36–38], *Streptococcus* species from yellow complex, *Veillonella parvula* from purple complex, and *Actinomyces* speices from blue complex were considered to be early colonizers. These species were capable of rapid and firm attachment on teeth surface via expressing receptors for host ligands, and therefore modified the ecological environment for later succession. *Capnocytophaga* species from the green

complex were identified in the biofilm milieu and were considered to be associated with periodontal diseases by producing bacterial enzymes that may lead to periodontal destruction. *Fusobacterium* species belonged to the orange complex. This complex formed a co-aggregational “microbial bridge” by using and releasing nutrient substances in the biofilm and expressing certain structures that bind both early colonizers and pathogens from the red complex.

In our study, although the detailed lists of core members were different in supra- and sub-gingival microbiome, the predominant species in both cores were quite similar, as they were mainly from yellow, blue, and orange complexes. According to the relative abundance of core members between healthy and diseased samples, we found that the core microbiome in health and disease was not statistically different. This result indicated that members of the core microbiome, especially those from genera *Streptococcus*, *Actinomyces*, and *Capnocytophaga*, may constitute a general “background” in supra- and sub-gingival microbiome, and such bacterial background does not shift easily with the change of health conditions. We hypothesized that the common background referred to not only the core members and the correlations within themselves but also their interactions with hosts and biofilms to adjust and modify the bacterial habitat. One example is that *Streptococcus sanguinis* was believed to be essential in developing oral biofilms in both teeth and implants, as it first facilitated its attachment by fimbriae and adhesins, and then produced glucans to promote biofilm maturation[39]. Species from *Streptococcus* were also shown to have the ability to modulate host response and the expression of other bacteria species[40, 41]. Besides, *Actinomyces* were also among the earliest colonizers during biofilm formation and were found to attach directly to the acquired pellicle[42], which indicated their important role in regulating the microenvironment. The facts listed above are examples that the core species and their functionalities were of equal importance to both healthy and diseased conditions, as a general background for the formation, maturation, and further changes in the microbial communities.

Distinct bacterial networks between healthy and diseased communities.

The oral microbiome is structurally and functionally organized, which is to say, the properties of a microbial community are more than the sum of the components within it[43]. To study a microbial community, we are supposed to explore the whole structure and the aggregation of all interactions instead of focusing on single or pairwise species. Therefore, we investigated the bacterial co-occurrence network to learn the importance of interactions to the oral microbiome.

Our study revealed that when inflammation arose around teeth and implants, subgingival microbial networks tended to become less connected and less competitive, but, on the contrary, supragingival networks tended to become more connected and more competitive. We hypothesized that in a healthy subgingival microbiome around teeth and implants, an extensive competitive inter-species correlations played an essential role in the preservation of healthy subgingival equilibrium, where growth and metabolism of potential pathogens could be inhibited. In the diseased subgingival communities, such correlations were weakened, and the total connectivity amongst species was decreased. This might allow those pathogens to enlarge in abundance and upregulate in metabolism associated with periodontal and peri-implant destruction. In our study, the relative abundance of *Porphyromonas gingivalis* and *Treponema denticola* from the red complex was significantly higher in diseased subgingival microbiota than in healthy subgingival microbiota around teeth ($p < 0.05$, Man-Whitney), which agreed with our hypothesis. However, networks in diseased supragingival communities seemed to shift in the opposite direction, as there were more inter-species correlations, and the proportion of competitive correlations was, instead, increased when compared with healthy supragingival communities. This might be the consequence that the supragingival microbiome serving as a reservoir for potential pathogens and was more delicate to influences. The various influences made the taxonomical changes in supragingival communities far more complex than those in subgingival communities. We appealed that detailed mechanisms behind these changes require further exploration for better understanding.

Relationship between hub species and health conditions.

Hub species were those with a large number of inter-species correlations. Whether abundant or not, these species played roles as “traffic centers” in the bacterial network and were highly associated with microbial equilibrium, for changes in their

abundance might lead to a massive shift in the whole network as they were related to so many other species. Our study showed that the healthy subgingival network had the highest count of hub species, whereas the diseased subgingival network had the lowest. To be more specific, in the healthy supragingival network, species from the genus *Prevotella* made up a major part of the hub species. *Prevotella*, together with *Eubacterium nodatum* and *Campylobacter rectus* were considered members of the orange complex. Their presence in the hub nodes corresponded with their bridging function in the biofilm. Besides, *Streptococcus sanguinis* from yellow complex, *Capnocytophaga sputigena* from green complex, *Actinomyces massiliensis* from blue complex, and *Treponema denticola* from red complex were also found in the hub nodes of healthy subgingival network. In the diseased subgingival network, there were only two hub species, *Capnocytophaga granulosa* and *Selenomonas noxia*. These two species had been proven associated with calculus formation and periodontal disease[37, 44, 45]. Their emergence in the diseased hub nods indicated that their pivotal places in the bacterial network might contribute to their pathogenicity.

An interesting phenomenon is that *Streptococcus sanguinis* and *Capnocytophaga sputigena* were also from the core microbiome illustrated above, but they showed up only in healthy subgingival hub nodes but not in diseased subgingival hub nodes. This indicated that although their presence formed a general bacterial background in both healthy and diseased microbiome, the downregulation in their interactions with other species might be associated with the onset and progress of the inflammatory diseases.

As for the supragingival microbiome, differences between healthy and diseased networks were not as distinct as subgingival microbiome and seemed to change in an opposite direction where the diseased network had more hub species than the healthy one. *Spirochaetes*, or more specifically those in genus *Treponema*, took up most places in the hub nodes of healthy network. *Treponema socranskii and Treponema vincentii* had been reported in association with periodontal tissue breakdown[46–48]. Yet their presence in the pivots of healthy supragingival network also suggested that they might contribute to the equilibrium of healthy microenvironment. Besides, the proportion of *Prevotella* was much less than subgingival hub species, meaning their bridging function connecting early colonizers and red complex pathogens might be weakened. This inference was corroborated by the fact that relative abundance of red complex pathogens was significantly lower in supragingival microbiome than that in subgingival microbiome ($p < 0.05$, Mann-Whitney).

Former studies on the differences between healthy and diseased oral microbiota mainly focused on abundance and functionality variances. Here we revealed structural differences between healthy and diseased communities and suggested that the structure of bacterial networks and the hub species within them should be given more concern in later studies on the prevention and treatment of periodontal and peri-implant diseases.

Association between microbial stability and health conditions.

As we stressed above, patterns of the bacterial network in supra- and sub-gingival microbiome were associated with health and disease. And the multiple interactions gave the community a resilience to environmental perturbations. The capability of a microbial community to resist perturbations is defined as its stability. In our study, we found that diseased subgingival microbiome had the highest local stability among four groups while healthy subgingival microbiome had the lowest. This meant the equilibrium of healthy subgingival microbiome was more delicate and more prone to perturbations. When perturbations reached beyond resilience, equilibrium may break down with changes in microbial composition and shift in the structure of bacterial co-occurrence network. That could be where dysbiosis happened and be the essence of the initiation of periodontal and peri-implant diseases. On the other hand, the high local stability in diseased subgingival microbiome explained why, if without interventions, the periodontal and peri-implant microbiome could not spontaneously change back to health once infected by periodontitis or peri-implantitis. Previous studies found that cooperative correlations, enhanced interactions, and higher connectance tended to decrease stability[30, 49], which agreed with our calculation where healthy subgingival microbiome had the most amount of positive correlations and the most connectance in the bacterial network. We also proved that having more hub species in the network might destabilize the microbiome for changes in these species

could trigger a shift in the whole network (see supplementary materials). This meant that the hub species were in some way a weak point during the breakdown of the current equilibrium.

In this scenario, we hereby suggest that the key point in the treatment of periodontitis and peri-implantitis is to break the firm equilibrium of the diseased subgingival communities and try to reestablish the healthy equilibrium, for example by antibiotic therapy, total debridement, or even microbial therapy by introducing new species to oral microbiota and thereby restore a healthy structure of bacterial network.

Limitations and deficiencies of the study.

Despite the findings we put forward, there are also limitations and deficiencies in our study. One major limitation is that the sample size in our study, although equivalent to other similar studies[19, 20, 22], is too small to describe the oral microbiome of the whole population as the oral microbiome is considered to be highly individualized[50]. To generalize our findings and hypotheses, more bacterial samples are required for metagenomic studies. Besides, most of our findings are based on taxonomical information we annotated, which is to say, our work merely revealed those phenomena we observed yet did not verified the mechanisms in biochemistry or molecular view. Further studies on these mechanisms are required for the validation of our findings.

Conclusion

In conclusion, we revealed similar core components yet distinct microbial structures in healthy and diseased microbial communities around teeth and implants. We found that the subgingival microbiome tends to become less connective and competitive when inflammation arises, with decreased species and increased local stability. In contrast, the supragingival microbiome tends to become more connective and competitive, with increased species and similar local stability. These changes might be the essence of dysbiosis in the periodontal and peri-implant microbiome. Besides, we concluded that it was critical to break the aberrant microbial equilibirium and to reestablish the healthy microbial equilibrium during the treatment of periodontitis and peri-implantitis.

Abbreviations

BOP
bleed on probing;
CAL
clinical attachment loss;
RBL
radiographical bone loss;
PD
probing depth;
PBS
phosphate-buffered saline;
PERMANOVA
permutational multivariate analysis of variance;
PCoA
Principal co-ordinate analysis.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of College of Stomatology, Xi'an Jiaotong University (xjkqll[2020]NO.016) and conformed to the guidelines from STROBE (STrengthening the Reporting of OBservational studies in Epidemiology). Written consents to participate were obtained from all included participants.

Consent for publication

All participants have learned that their information including age, sex, health condition and relavent therapy will be recorded during the study. No other personal privacies are involved in the study. Written consents for publication were obtained from all included participants.

Availability of data and materials

All acquired data from our samples are provided with our Additional Files. Should any further data of our study be needed for reasonable causes, please kindly contact the corresponding e-mail for acquisition.

Competing interests

The authors declare that they have no competing interests.

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Authors Contribution

YZ designed the details of the study, conducted the statistical analysis, interpreted the analysis results and wrote this manuscript. YL performed the bioinformatics analyses, interpreted the analysis results, and revised the manuscript. YY conducted mathematical simulations and interpreted the results. YW helped perform statistical analysis and revised the menuscript. XC and YX helped with the collection of samples and the revision of the manuscript. QZ and SCL supervised the whole project and polished the manuscript. All authors reviewed and approved the manuscript.

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Figures

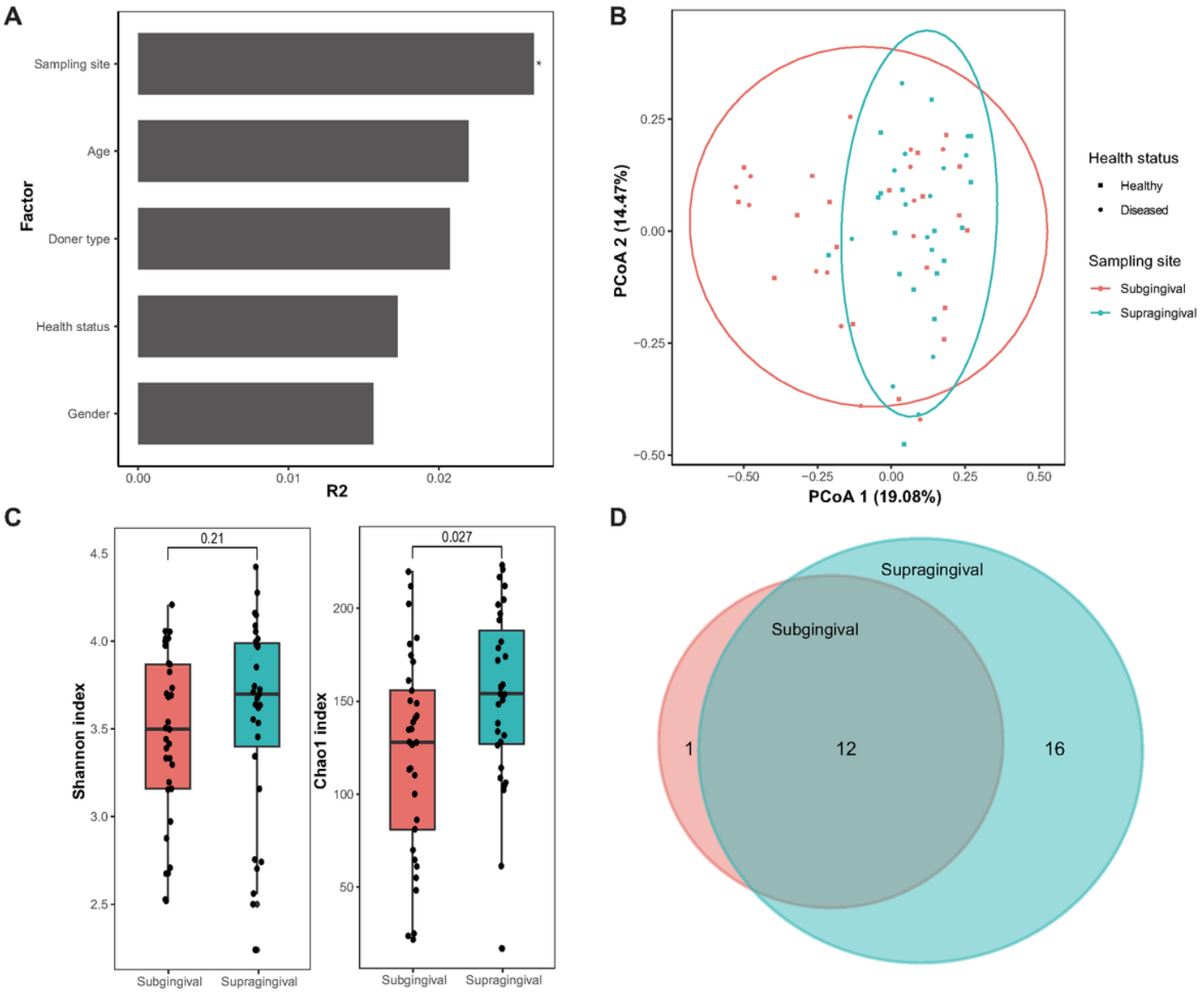

Figure 1

Comparison between supragingival and subgingival microbiome. (A) PERMANOVA showed that different sampling sites had distinct microbial communities. (B) Principal coordinate analysis showed a difference between supragingival and subgingival communities in terms of beta diversity. The red and green ellipses indicate the 95% confidence regions (C) Alpha diversity was analyzed using Shannon and Chao1 indices. No significant difference was observed in Shannon index. Chao1 index of supragingival samples was significantly higher than that of subgingival samples. (D) Vann diagram showed there were 28 and 13 core species in supragingival and subgingival microbiome, respectively, notably 12 species were shared by both cores.

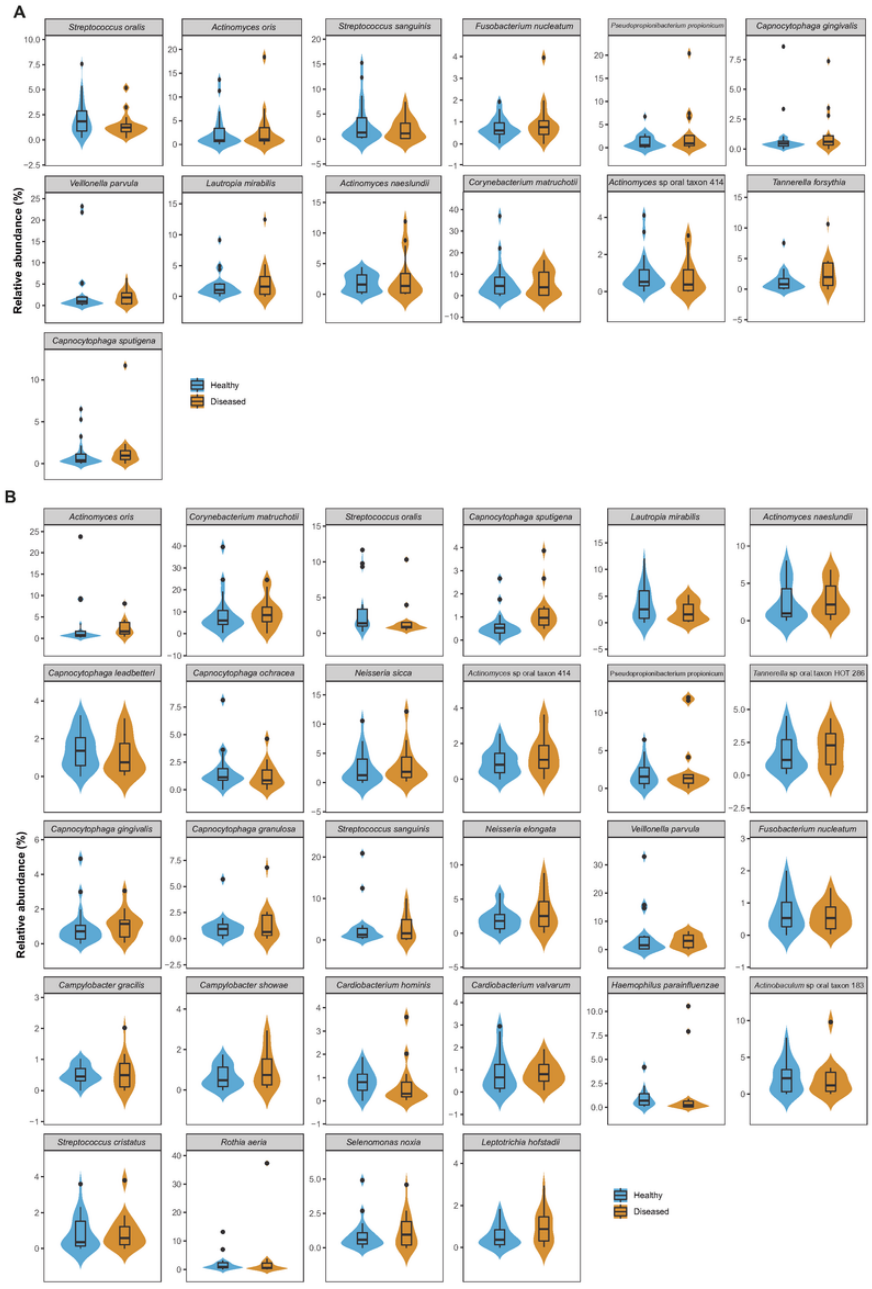

Figure 2

Comparison of relative abundance of the core species between healthy and diseased microbiome. (A) 13 subgingival core species (B) 28 supragingival core species. Blue violins and boxes stand for healthy microbiome while yellow violins and boxes stand for diseased microbiome. No significant differences are detected in the relative abundance of these core species. This indicates the core components of the supra- and sub-gingival microbiome do not change with the shift of health conditions.

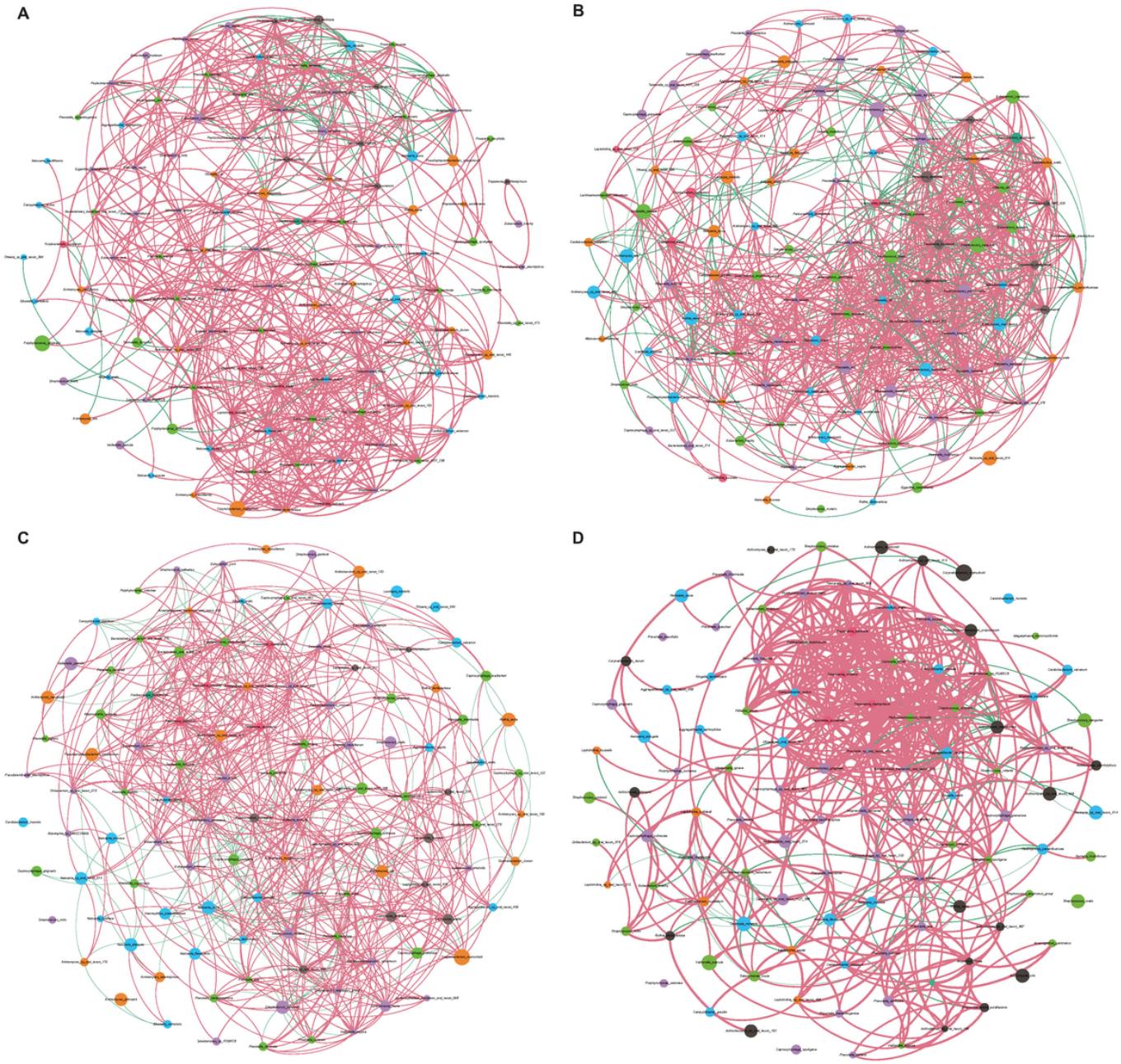

Figure 3

Bacterial co-occurrence networks. (A) Network of diseased subgingival microbiome. (B) Network of healthy subgingival microbiome. (C) Network of diseased supragingival microbiome. (D) Network of healthy supragingival microbiome. Different colors of the circles represent species from different phyla. The larger circles stand for the higher mean relative abundance of a species. We selected those interactions with Spearman correlation coefficient <-0.6 or >0.6 (adjusted p<0.05). Positive and negative correlations are shown in red and green lines respectively. Thicker lines mean higher absolute values in Spearman coefficient.

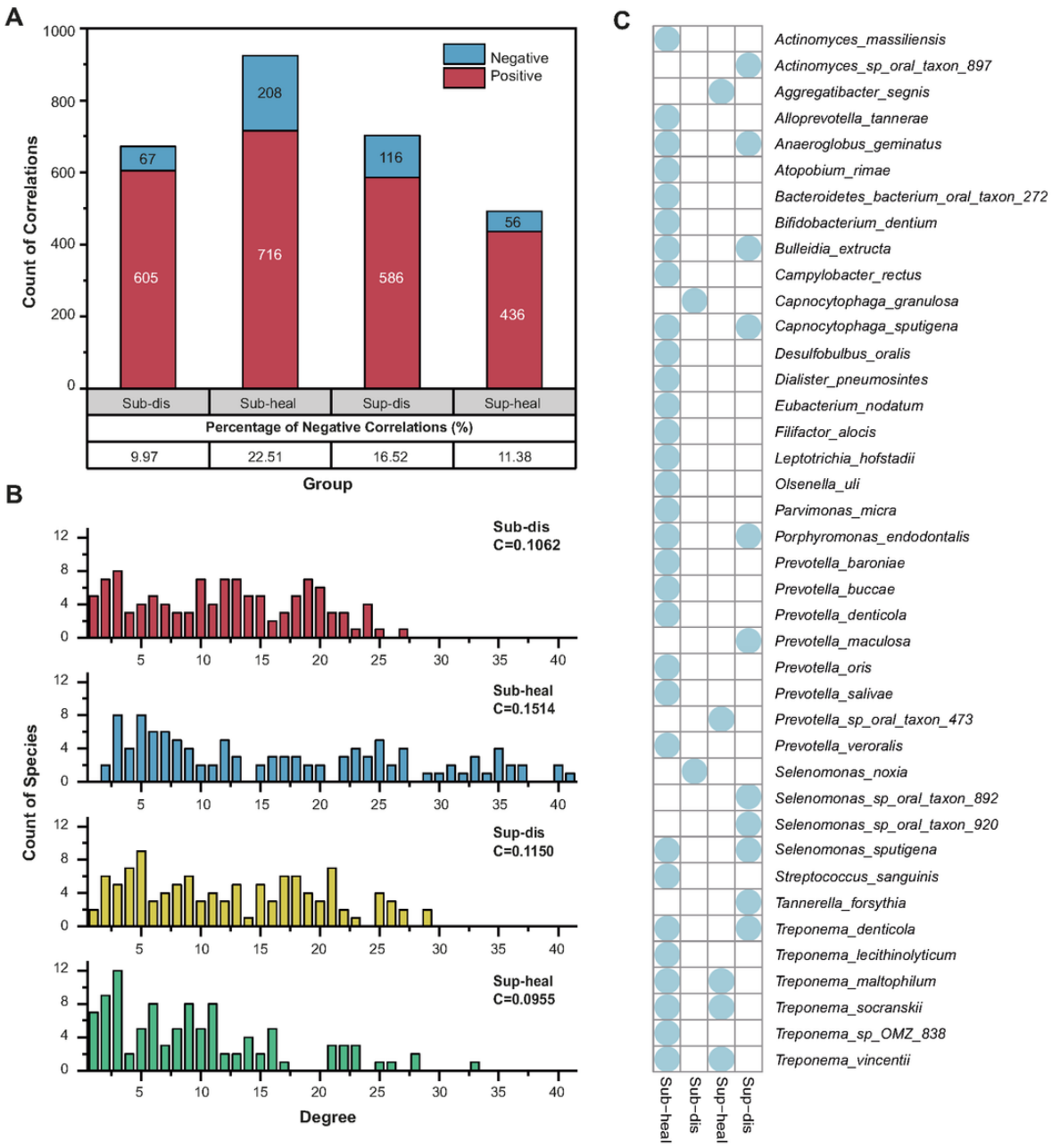

Figure 4

Further visualization of the community structures in different microbiome. (A) Bar charts showing the total counts of correlations (red for positive correaltions while blue for negative correlations) and the percentage of negative correlations. Positive correlations are predominant in all communities. The percentage of negative correlations in diseased subgingival communities is significantly lower than that in healthy subgingival communities ($p<0.05$, Pearson Chi-Square). However, such difference is reversed between diseased and healthy supragingival communities ($p<0.05$, Pearson Chi-Square). (B) Degree distributions of the diseased subgingival, healthy subgingival, diseased supragingival, and healthy supragingival networks are shown in red, blue, yellow, and green bars, respectively. C stands for connectance. A conspicuous difference can be observed in the degree distribution of healthy subgingival communities as there are significantly more high-degreed (degree>25) species ($p<0.05$ Pearson Chi-Square). (C) Hub species in the diseased subgingival, healthy subgingival, diseased supragingival, and healthy supragingival microbiome. A blue dot means the species has more than 25 inter-speceis correaltions in the corresponding microbiome

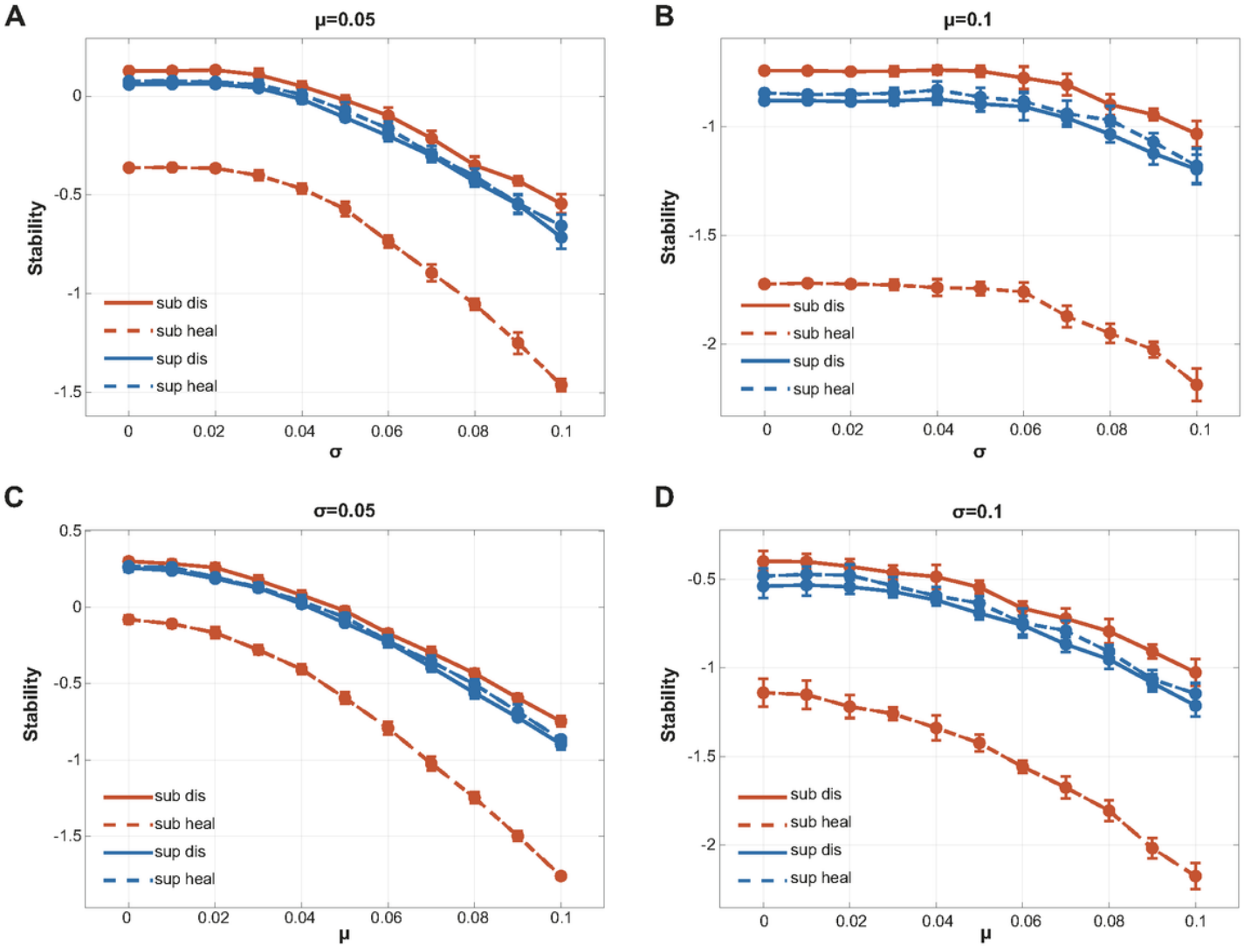

Figure 5

Calculation of local stability. Red lines stand for supragingival communities while blue lines stand for subgingival communities. Healthy and diseased communities were shown in dotted and solid lines, respectively. Connectance, interacting species richness, as well as bacterial correlations, were drawn directly from our interacting matrix. The strength of bacterial interactions was assumed to follow a normal distribution with mean μ and variance σ2. By changing the value of μ and σ, we performed a series of calculations to compare the stability of our communities (see also Supplementary Figure1). All calculations showed the same tendency that healthy subgingival communities had the worst local stability while diseased subgingival communities had the highest. However, the stability difference in supragingival communities was not as distinct as that in subgingival communities.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Taxonomicalannotation.xlsx
- Cooccurrencenetworkpvalue.xlsx
- Cooccurrencenetworkrvalue.xlsx

- SupplementaryInformation.docx
- SupplementaryFigure1.pdf
- SupplementaryFigure2.pdf