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Pyrosequencing of the subgingival microbiome in peri-implantitis after non-surgical mechanical debridement therapy

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Abstract

Background and Objectives: Peri-implantitis remains a challenge for dental implant therapy, and the prognosis of non-surgical therapy for peri-implantitis is unsatisfactory. In order to reveal the impact of non-surgical mechanical debridement therapy on microbial communities, we investigated the subgingival microbial communities of healthy implants and implants with peri-implantitis, both before and after the therapy.

Material and Methods: Subgingival plaques were collected from patients with healthy dental implants (HC; n = 10) and from patients with peri-implantitis (n = 13) before and after non-surgical mechanical debridement therapy. The treatment was conducted using curettes for submucosal debridement followed by irrigation with 0.2% (*w/v*) chlorhexidine, with re-examination 1 month later. 16S rRNA pyrosequencing was used to analyze the subgingival microbiome, and co-occurrence networks were adopted to explore the interactions between pathogens in the microbial communities.

Results: A total of 506 955 high-quality reads were generated, and 2222 operational taxonomic units were finally detected using a 97% similarity cutoff, with a mean of 249 ± 69 per sample. The peri-implantitis sites harbored similar microbial communities before and after the treatment, as demonstrated by the microbial diversity, relative abundance, and prevalence of bacteria. Most importantly, the microbial community structures were stable before and after non-surgical therapy based on the microbial diversity and bacterial composition, as well as the interactions between key pathogens, including Enterobacteriaceae, *Selenomonas sputigena*, Parvimonas, *Eubacterium infirmum*, *Campylobacter gracilis*, *Tannerella forsythia*, and *Fusobacterium*, which were measured using a co-occurrence network analysis. Periodontal pathogens were also detected in subgingival plaque after the treatment. Distinct microbial communities were found between the healthy and peri-implantitis sites.

Conclusion: Our results demonstrate that non-surgical mechanical debridement therapy did not significantly affect the subgingival microbial communities in peri-implantitis, and the stable microbial networks created via interactions among pathogens may be responsible for the poor prognosis of peri-implantitis treatment.

KEYWORDS

chlorhexidine, non-surgical mechanical debridement therapy, peri-implantitis, pyrosequencing

Nie and Zhang contributed equally to the work as first authors.

1 | INTRODUCTION

Dental implants play an increasingly important role in dental practice. Although a high success rate has been achieved for dental implant therapy, infections at peri-implant sites remain a challenge.[1-3] Peri-implantitis is an inflammatory process around an implant characterized by soft tissue inflammation (probing depths ≥4 mm) and a progressive loss of supporting bone (≥2 mm).[4] It has been previously reported that approximately 28%-56% of patients who have dental implants suffer from peri-implantitis, and 12%-43% of implants eventually fail due to peri-implantitis.[5]

The development of biofilms on the surface of dental implants is an important feature of peri-implantitis.[6] Treatments are based on the general principles of periodontitis therapy, including eliminating the bacterial microbiota, preventing bacterial colonization, and creating an ecology capable of suppressing subgingival anaerobic flora.[7] In a survey conducted in the United States, providing oral hygiene instructions and non-surgical therapy, including an antimicrobial rinse/irrigation and mechanical debridement, was the treatment of choice for the majority of clinicians when treating peri-implantitis.[8]

To achieve better biofilm control, chlorhexidine is most commonly used along with mechanical debridement as a chemical plaque control agent.[9] Chlorhexidine is an effective anti-plaque and anti-inflammatory agent that exhibits a broad spectrum of antibacterial activity without systemic toxic effects or microbial resistance when delivered orally.[10] However, only a small subset of periodontists in the United States consider peri-implantitis treatment to be effective. Studies have also shown that mechanical debridement alone has limited effects,[1,3,11,12] and the additional use of chlorhexidine did not increase the effectiveness of treatment.[10] While chlorhexidine is commonly used, however, its clinical efficacy and ideal regimes are unclear. In order to address the unsatisfactory prognosis, it is necessary to elucidate the microbial changes after the therapy.

The current non-surgical mechanical debridement therapy for peri-implantitis is derived from the therapy for periodontitis; however, the surface texture and composition of implants differ from those of teeth.[13] As a result, the microbiome of peri-implants is significantly different from that of the periodontal community in health and disease.[14] Recent studies have indicated that peri-implantitis may be caused by a shift in the microbial community instead of a limitation in pathogens, and the unique microbial structures associated with both healthy and failing dental implants have been revealed.[14,15] Conventional culture and molecular hybridization methods, which are time-consuming, are unable to identify previously uncultivated or unknown bacteria.[16] 16S rRNA pyrosequencing is a revolutionary method that can identify a broad microbiome structure in a given ecosystem. It has previously been used to characterize the oral, peri-implant, and periodontal microbiomes in states of health and disease.[14,15,17] To improve the prognosis of non-surgical mechanical debridement therapy for peri-implantitis, it is crucial to identify the microbial communities present before and after the treatment.

The aim of the present study was to analyze the subgingival microbial communities of healthy implants and of patients with peri-implantitis before and after non-surgical mechanical debridement therapy using 16S rRNA pyrosequencing and co-occurrence network analysis to reveal the impact of the treatment on microbial communities. The hypothesis of the study was that the non-surgical mechanical debridement therapy could not affect the short-term microbiome structure of peri-implantitis.

2 | MATERIAL AND METHODS

2.1 | Study design and ethical considerations

A total of 23 patients with one dental implant (Straumann) placed at least 1 year prior at the Third Dental Center of Peking University School and Hospital of Stomatology were enrolled in this study. All patients were medically healthy non-smokers. The exclusion criteria were as follows: uncontrolled periodontal disease; use of antibiotics, steroids, or immunosuppressive medications in the past 6 months; and pregnancy. This study was approved by the Peking University Biomedical Ethics Committee (PKUSSIRB-201735067). All patients provided written informed consent before treatment.

The patients underwent an oral examination by an experienced dentist using a periodontal probe. The dentist was trained before the examination to conduct with a light probing force (approximately 0.2 N) consistently, and the clinical parameters were recorded at six sites per implant. Thirteen patients were diagnosed with peri-implantitis, with probing depths ≥4 mm, bleeding on probing with/without suppuration, and marginal bone loss ≥2 mm according to radiographs. The reference point chosen for determining marginal bone loss was the radiograph of the marginal bone taken after placing the crown. Intra-oral radiographs were taken using a standardized holder, which allow for the clear identification of the reference point and distinct visualization of implant threads. All the radiographs were assessed by the same examiner. Ten patients had healthy implants, with probing depths ≤3 mm and without visual signs of inflammation or marginal bone loss.[5] The clinical parameters are presented in Table 1.

TABLE 1 Demographic and clinical characteristics of the patients

Characteristics		Healthy (n = 10)	Peri-implantitis (n = 13)
Male/Female		3/7	8/5
Age (years± SD)		42.6 ± 3.6	47.1 ± 5.8
Probing depth	Mean (mm ± SD)	2 ± 0.9	4.5 ± 0.7
	Deepest (mm)	3	6
Bone loss (mm ± SD)		0	2.2 ± 0.4
Bleeding on probing (±)		0/10	13/13

2.2 | Sampling

The patients with peri-implantitis received supragingival prophylaxis 2 weeks before the non-surgical mechanical debridement therapy. The treatment was conducted by the same dentist using carbon fiber curettes for submucosal debridement under local anesthesia until the operator felt that the implant surfaces were properly debrided. After that, the pockets around the implant were irrigated with 0.2% (*w*/*v*) chlorhexidine (Nanyue Pharmaceutical) for 1 minute. The patients were re-examined 1 month later. Submucosal plaque samples were collected from the peri-implant sulci of patients with peri-implantitis using the probes before therapy (BT; n = 13) and 1 month after therapy (AT; n = 13), according to a standardized sampling protocol. The implants were isolated with cotton rolls, and the saliva and supragingival deposits were removed; then, the plaque samples at the peri-implant site with the deepest pocket were collected and repeated two times, which were pooled together and stored. Plaque samples from the healthy implants were also collected as healthy controls (HC; n = 10). Each sample was suspended in a separate 1-mL sterile tube containing 200 μL of TE buffer (20 mmol/L Tris and 2 mmol/L EDTA; pH = 7.4) and frozen at −80°C prior to DNA extraction.

2.3 | DNA extraction and 16S rRNA pyrosequencing

DNA extraction was conducted using a TIANamp Bacteria DNA Kit (Tiangen Biotech) after initial treatment with lysozyme (20 mg/mL, 37°C for 1 hour). The quantity and quality of DNA were measured using a Qubit Fluorometer (Invitrogen) and 1% agarose gel electrophoresis. The high-quality DNA with OD_{260}/OD_{280} = 1.8-2.0 and concentration higher than 50 ng/μL were selected for sequencing.

The v1-v3 hypervariable regions of bacterial 16S rRNA genes were amplified for 454-pyrosequencing. The primers were as follows: forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse (5′-ACTCCTACGGGAGGCAGCAG-3′). The 10-nucleotide (nt) barcodes were tagged to the 5′ position to distinguish each sample. PCR was performed according to the GS FLX Amplicon DNA library preparation method (Roche). The libraries were then pyrosequenced on the 454 GS FLX sequencing platform (Life Sciences) at the BGI Institute. The sequence data have been submitted to the Sequence Read Archive with the accession number PRJNA487121.

2.4 | Data processing

In total, 36 samples were sequenced, and the raw data were analyzed using the pipeline tools MOTHUR[18] and QIIME.[19] Sequences were disposed based on a unique barcode assigned to each sample.

The sequences were trimmed with the exclusion criteria: primer mismatch, average quality scores ≤25, or minimum lengths <200 nt, more than one barcode mismatch and six ambiguous bases. After singletons were discarded, the high-quality trimmed reads were clustered into operational taxonomic units (OTUs) with a 97% similarity cutoff using the de novo OTU strategy and classified with RPD Classifier (release 11)[20]; meanwhile, chimera were removed.

2.5 | Statistical analysis

To avoid the deviation from sequencing depths, we randomly selected the same number of reads from each sample for alpha and beta diversity estimators. Alpha diversity indexes including observed OTUs and Chao1 were calculated by fixed reads. The beta diversity metrics of weighted UniFrac distances were calculated based on OTUs and phylogenetic trees by a matrix of pairwise distances between all samples.[21] All the alpha diversity results were compared using Student's *t* test (independent *t* test for HC-BT/HC-AT and paired *t* test for BT-AT; $P < .05$). For beta diversity, PCoA plot with ANOSIM test ($P < .05$) was also conducted. And the Procrustes analysis based on OTU was adopted to depict the divergence of the BT and AT communities. Pairs of BT and AT samples were linked with bars. *P*-values were generated using a Monte Carlo simulation.

The microbial taxa at each of the phylum, class, order, family, genus, and species level were clustered based on the OTUs. Wilcoxon rank-sum test was used to compare the significant different OTUs and taxa between every two groups. A bubble chart was computed to compare the significantly different OTUs between healthy implants and peri-implantitis sites before treatment (Wilcoxon rank-sum test, $P < .05$). A Venn diagram was constructed based on the OTUs with high prevalence (>80% in each group). All the comparisons were calibrated by false discovery rate (FDR) using "p.adjust" function in R package. We defined the core microbiome as OTUs that were presented in all three groups.

Co-occurrence networks were constructed using CYTOSCAPE (v 3.6.1) based on the relative abundance of the major OTUs (prevalence >80%) to explore the interactions between microbial taxa in the three groups. The Pearson correlation coefficients (PCCs) for each pair of OTUs were calculated, and the statistical significance was analyzed by Permutation test in MATLAB in all samples randomly. PCC recalculating was set to 10 000 times, and *P* value cutoff was set to .01.[22] Pairs of OTUs with significant correlation (permutation test, $P < .01$) were connected with edges. Modules consisting of OTUs with at least five edges, defined as hub OTUs, were constructed.[23]

3 | RESULTS

A total of 506 955 high-quality reads were generated after processing, with a mean of 14 082 ± 8803 reads per sample. In total, 2222 OTUs were finally detected using a 97% similarity cutoff, with a mean of 249 ± 69 per sample.

3.1 | Peri-implantitis sites demonstrated stable microbial communities before and after non-surgical mechanical debridement therapy

Non-surgical mechanical debridement therapy had a limited influence on the microbial diversity of peri-implantitis sites. Observed OTUs and the Chao1 index, which describe OTU richness, showed that the OTU richness was similar in peri-implantitis sites before

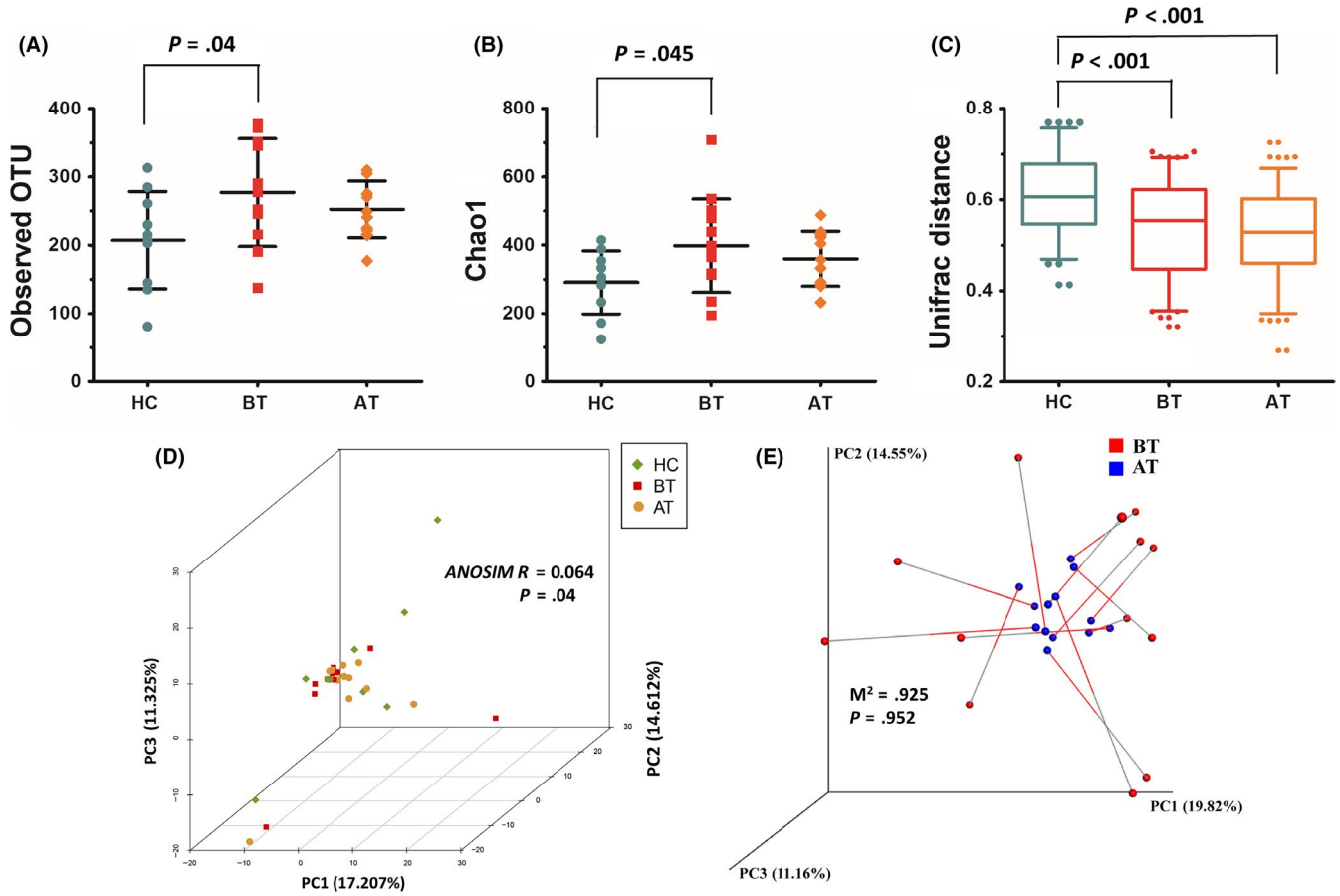

FIGURE 1 The microbial community diversity of healthy implant and peri-implantitis sites. A-B, Observed OTUs and Chao1 index of the three groups (independent *t* test for HC-BT/HC-AT and paired *t* test for BT-AT; $P < .05$). C, The variation in the microbial community among three groups based on weighted UniFrac distance. D, The PCoA plot of the three groups. E, Procrustes analysis of the BT and AT communities. Pairs of samples with lower M^2 were linked with shorter bars showing a more similar relationship ($P > .05$). AT, after treatment; BT, before treatment; HC, healthy control; OUT, operational taxonomic unit

and after treatment (Figure 1A,B). We used the weighted UniFrac distance to analyze variation in the microbial community composition between samples, and no significant differences were found between the groups before and after treatment, as well as showed in PCoA (Figure 1C,D). Procrustes analysis also indicated that there were no significant differences between BT and AT groups (Figure 1E).

The major genera (relative abundance >2%) influenced by peri-implantitis treatment were *Streptococcus*, *Capnocytophaga*, *Leptotrichia*, *Actinomyces*, *Prevotella*, *Fusobacterium*, *Neisseria*, *TM7-genus incertae sedis*, *Veillonella*, *Corynebacterium*, *Selenomonas*, *Campylobacter*, *Porphyromonas*, *Treponema*, and *Eubacterium* (Figure S2). The predominant species-level OTUs (relative abundance >1%) that decreased after treatment were *Campylobacter gracilis*, *Campylobacter showae*, *Capnocytophaga gingivalis*, *Capnocytophaga leadbetteri*, *Lactobacillus vaginalis*, *Leptotrichia hofstadii*, *Leptotrichia wadei*, *Prevotella loescheii*, *Prevotella tannerae*, *Rothia aeria*, and *Streptococcus sanguinis*. In contrast, *Actinomyces dentalis*, *Actinomyces naeslundii*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Corynebacterium matruchotii*, *Eikenella corrodens*, *Granulicatella adiacens*, *Leptotrichia hongkongensis*, *Ottowia thiooxydans*, *Porphyromonas catoniae*, *Porphyromonas endodontalis*, and *Porphyromonas gingivalis* increased. However, no differences were found between the BT and AT groups (Figure 2A). The relative abundance of OTUs at different levels is shown in Figures S1 and S2. The data are also shown in a bubble chart, which demonstrates a similar pattern of the relative abundance of OTUs between the two groups distinct from that of the healthy implant sites (Figure 2B).

A Venn diagram was constructed consisting of the 90 OTUs with high prevalence, and the core microbiome was constructed using the 27 OTUs detected in all three groups. Most of these OTUs were predominant in all groups with a relative abundance >1%, including *C gingivalis*, *C showae*, *C matruchotii*, *Fusobacterium* (genus level), *L hongkongensis*, *L wadei*, *P catoniae*, *R aeria*, *S sanguinis*, *Streptococcus* (genus level), *TM7-genus incertae sedis*, and *Veillonella* (genus level). The peri-implantitis sites shared 42 OTUs (ie, nearly half of the total) before and after treatment (Figure 3).

The interactions between the microbial taxa were also stable before and after therapy. Eighty-nine nodes (OTUs) with significantly different PCC values were included in the co-occurrence networks, which contained nine hub OTUs (highlighted in yellow and

green). The hub OTUs before therapy included Enterobacteriaceae, *Selenomonas sputigena*, Parvimonas, and *Eubacterium infirmum* (yellow), together with the hub OTUs with six edges, including *C gracilis*, *Tannerella forsythia*, and *Fusobacterium* (green). The microbial communities possessed the same hub OTUs and almost the same module structure after therapy (Figure 4).

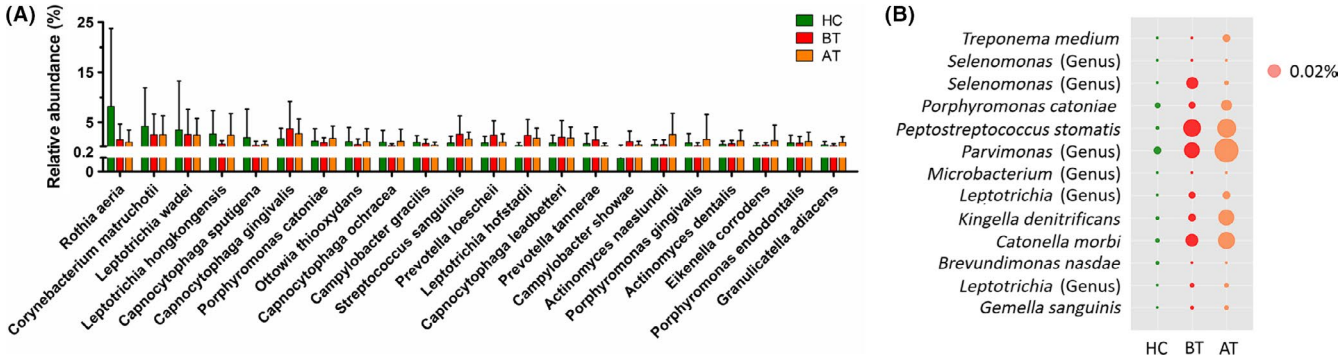

FIGURE 2 The bacterial taxonomic profiles compared with the relative abundance of OTUs. A, The bacterial taxonomic profiles show the predominant species-level OTUs (with relative abundance >1%) in the three groups. B, The bubble chart shows the OTUs that differed significantly in relative abundance between HC and BT (Wilcoxon rank-sum test, $P < .05$). The related OTUs in AT group were also noted. AT, after treatment; BT, before treatment; HC, healthy control; OUT, operational taxonomic unit

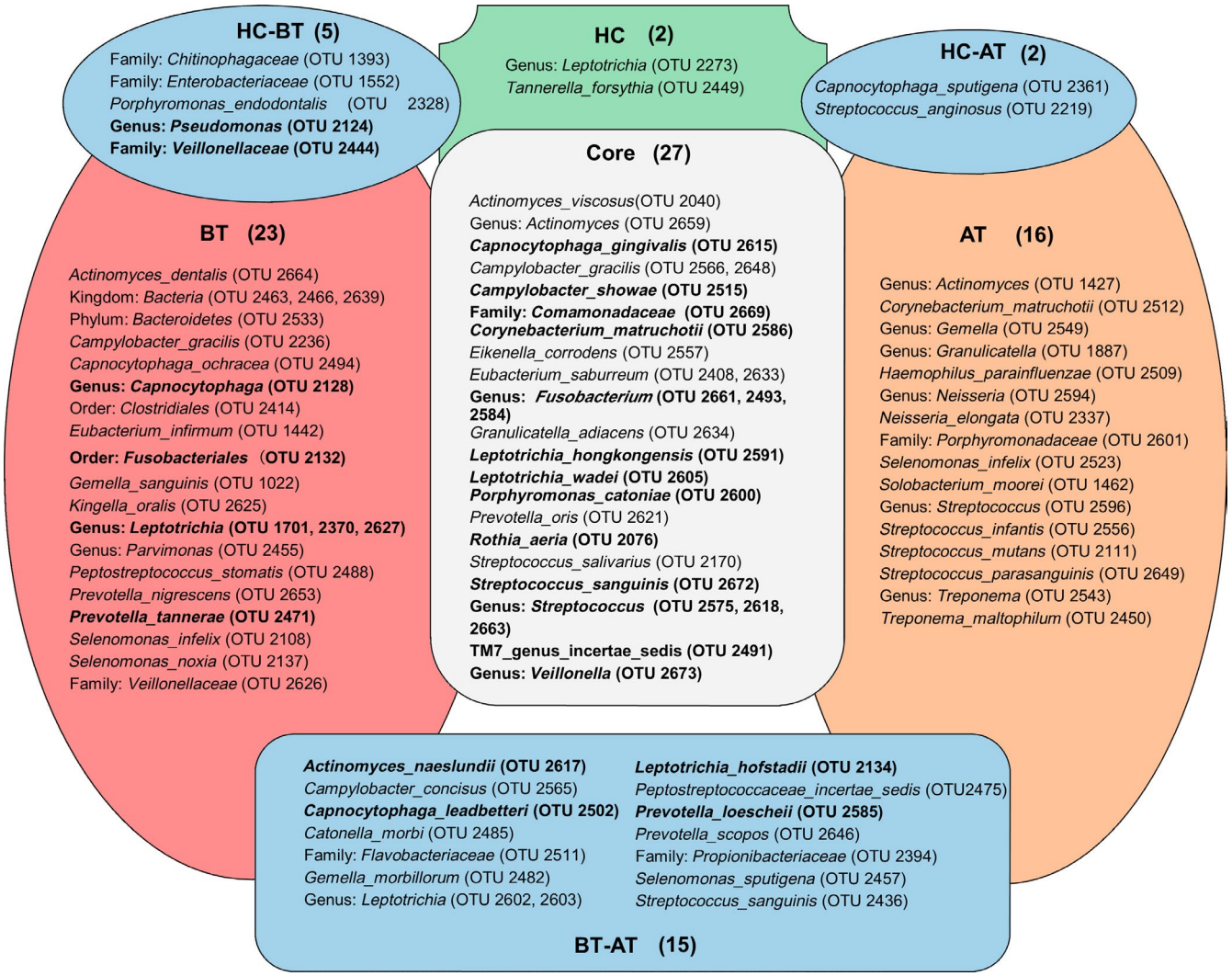

FIGURE 3 Venn diagram of OTUs with high prevalence in healthy implant (green) and peri-implantitis sites (BT: red; AT: orange). The core microbiome (gray part) contains the OTUs shared by all three groups, and the blue part contains the OTUs shared by either two groups. OTUs with relative abundance > 1% were shown in bold. AT, after treatment; BT, before treatment; HC, healthy control; OUT, operational taxonomic unit

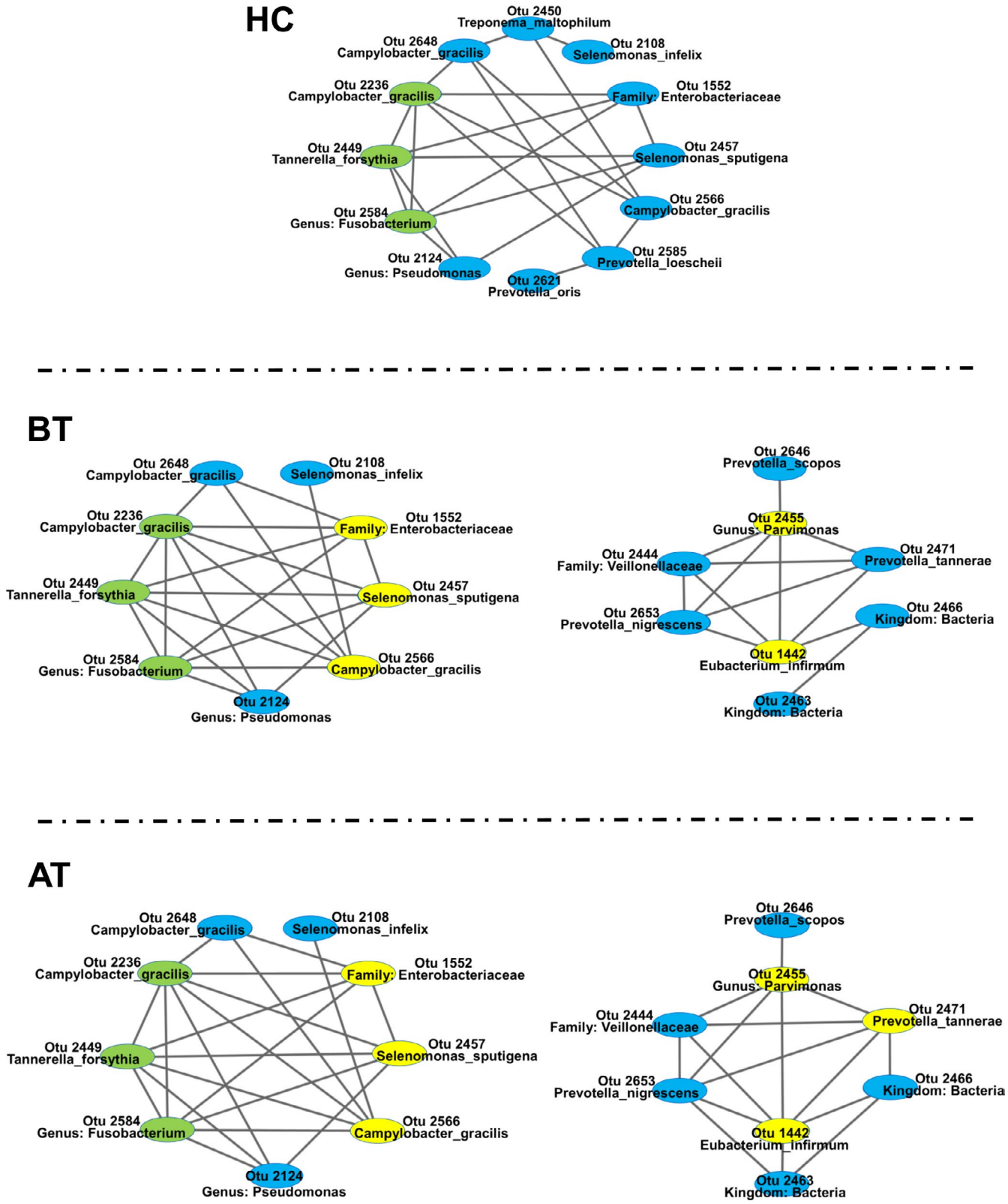

FIGURE 4 The co-occurrence networks of the three groups. Pairs of OTUs with significant correlation (blue) were connected with edges. The hub OTUs highlighted in green were found in all three groups. The hub OTUs unique in peri-implantitis sites were highlighted in yellow. The network modules of AT were similar to BT without unique OTUs (permutation test, $P < .01$). AT, after treatment; BT, before treatment; HC, healthy control; OUT, operational taxonomic unit

3.2 | Healthy and peri-implantitis implants had distinct microbial communities

The microbial diversity of peri-implantitis sites differed significantly from those of healthy implants. The observed OTUs and Chao1 were higher in peri-implantitis sites than in healthy implants (Figure 1A,B). The weighted UniFrac distance also showed that the microbial community of healthy implants had greater variations than the peri-implantitis sites (Figure 1C).

The predominant species-level OTUs with a relative abundance >1%, such as *C showae*, *C gingivalis*, *C leadbetteri*, *L hofstadii*, *P loescheii*, *P tannerae*, and *S sanguinis*, increased in peri-implantitis sites compared with healthy implants (Figure 2A). The bubble chart also demonstrated distinct bacterial community patterns between the healthy and peri-implantitis sites (Figure 2B). As shown in the co-occurrence networks, although the three hub OTUs in healthy implants (*C gracilis*, *T forsythia*, and *Fusobacterium*) were also detected in the peri-implantitis implants, the module structure of the networks of healthy implants differed significantly from that of the peri-implantitis implants (Figure 4).

4 | DISCUSSION

Non-surgical mechanical debridement therapy should always be performed prior to other treatments for peri-implantitis, as dentists can monitor the soft tissue healing process as well as guide patients to achieve effective oral hygiene.[24] However, the prognosis of the treatment for peri-implantitis remains poor.[9,25] Studies have attempted to determine why non-surgical mechanical debridement therapy exerts only minimal effects in the treatment of peri-implantitis. Persson et al[26] found that the bacterial counts in peri-implantitis sites were reduced immediately after non-surgical mechanical debridement therapy with curettes or an ultrasonic device, but no differences were found in 6-month recall for any species. Most of the treatments are performed with scalers or ultrasonic devices, but decontamination using these instruments is ineffective because of the specific shape and surface texture of implants. To suppress anaerobic bacteria on implant surfaces and improve prognosis, antimicrobials have been combined with mechanical treatment, including chlorhexidine. This combination has been shown to improve the probing depth and bleeding on probing in the short-term, but clinical indices remained poorly improved.[27] Paolantonio et al[28] reported that the application of a 1% chlorhexidine gel could reduce bacterial counts, without interfering with specific pathogens. These findings imply that the number of bacteria present cannot be used to predict the prognosis of peri-implantitis.[29]

Emerging evidence indicates that oral microbiomes are tightly related to human health and oral disease, and current molecular methods have enabled us to determine the composition and function of the oral microbiome.[30] In this study, we determined the structure of microbial communities in peri-implantitis sites before and after treatment using a 16S high-throughput sequencing technique. The microbial diversity, relative abundance, and microbial composition of microbial communities at peri-implantitis sites remained stable after non-surgical mechanical debridement therapy, which may explain the poor prognosis associated with the treatment. Furthermore, we compared the structures of microbial communities on healthy implants and implants with peri-implantitis, which were distinct from each other. The microbial diversity of the peri-implantitis sites was higher than that of the healthy sites, consistent with previous findings.[15,17,31] However, some studies have demonstrated that implants with peri-implantitis have relatively low microbial diversity compared with healthy implants.[14] These results can be explained in a variety of ways. First, different sampling protocols were employed in these studies. We obtained plaque from the deepest pockets of the diseased sites using a periodontal probe. In contrast, previous studies employed the paper point sampling method and collected the superficial region of submucosal biofilms.[32] Second, dental implant systems with different surface characteristics, including roughness, free energy, and material composition, could impact bacterial attachment and microbial composition, which may also have resulted in high microbial diversity in the peri-implantitis sites in this study.[13,33]

Peri-implantitis is associated with shifts in the microbial community, and it is important to understand the interactions among microbes. In this study, we used co-occurrence networks to investigate the coexistence patterns of microorganisms based on relative abundance. Compared with healthy implant sites, co-occurrence networks were significantly more complex, and more bacteria were correlated. These findings indicate that at diseased implant sites pathogenic microorganisms had to correlate with each other to promote peri-implantitis. More importantly, the co-occurrence networks of the peri-implantitis sites remained stable before and after therapy, both in composition and structure. The hub OTUs were the same in the two groups and were located in the same node in the networks. These results imply that the pathogenesis of peri-implantitis depends on the interactions of correlated pathogens rather than individual microbes.[34] In the quorum sensing theory, bacteria communicate and cooperate with certain microbes, acting similarly to a multicellular organism. In this way, groups of bacteria can colonize a host, regulate biofilm formation, and even express their pathogenic potential.[35]

The elimination of biofilms on implant surfaces is one of the most important factors to promote proper healing as any residual pathogens may be eliminated by the host's immune system.[24] In this study, periodontal pathogens were found, which may play an important role in the pathogenesis of peri-implantitis. It has been reported that periodontal pathogens, including *P gingivalis*, *Treponema denticola*, *T forsythia*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Fusobacterium*, and *Campylobacter*, are associated with diseased implants.[36-39] In the present study, these pathogens were all detected at peri-implantitis sites, but with very low relative abundance. Indeed, only *P gingivalis* from the red complex and *C showae* and *C gracilis* from the orange complex showed a relative abundance >1%. Importantly, these periodontal pathogens were tightly connected with other species to form stable microbial networks, in which pathogens such as *T forsythia*, *C gracilis*, and *Fusobacterium* played a hub role in the microbial

communities both before and after therapy. Non-surgical mechanical debridement therapy for peri-implantitis treatment was derived from that for chronic periodontitis, by which the subgingival microbiome of chronic periodontitis may be destroyed and removed after treatment, and the highly correlated microorganisms in periodontitis before treatment became poorly correlated after treatment.[40] However, our results indicate that non-surgical mechanical debridement therapy may not affect microbial communities in peri-implantitis sites significantly, as discussed above. Compared with natural teeth, dental implants lack periodontal ligaments, resulting in a poor physical barrier against bacterial invasion, restricting the blood supply, and reducing the number of immune system cells.[41] These features may render implants more susceptible to infection than natural teeth. Moreover, the microbes may be "protected" in the micro gaps, pits, and grooves of the rough surfaces of implants. These areas are inaccessible for curettage; as a result, conventional non-surgical mechanical debridement therapy may only disrupt parts of biofilms, without disturbing the structure of microbial communities or the cooperative interactions among pathogens.[42-44]

Because of the purpose of this study, several limitations should be acknowledged. First, the criterions of peri-implantitis used in this study were commonly used; however, according to AAP and EFP, the latest clinical definition of peri-implantitis is based on the following criteria: (a) presence of peri-implant signs of inflammation, (b) radiographic evidence of bone loss following initial healing, and (c) increasing probing depth as compared to probing depth values collected after placement of the prosthetic reconstruction.[45] All of which emphasizes that the loss of supporting bone is progressive, and the disease progresses in a non-linear and accelerating pattern, which highlights the importance to monitor the changes of clinical parameters around implant sites. Second, we strictly enrolled the peri-implantitis patients according to probing depth, bone loss, and BOP; as a result, the sample size was relatively small. Besides, we did not divide the patients into mild, moderate, or severe peri-implantitis based on the clinical status, and mild to moderate peri-implantitis were included in this study, with relatively shallow probing depth. The future study should include samples from all severity of peri-implantitis in order to unveil the relationship between the microbiome composition and the clinical parameters. Third, we conducted a short-term study on the microbiome composition change of peri-implantitis sites after standard non-surgical mechanical treatment. However, the microbiome composition change may be associated with the clinical improvement in a long-term observation; moreover, it is also very important to conduct a comparative longitudinal analysis of the healthy sites' microbiomes, which might reveal the time-stable and variable components of the microbiomes. Fourth, the non-surgical mechanical debridement therapy also included other methods, such as the application of laser, air-abrasion, and local/systemic antimicrobials. Whether these methods could influence the microbial communities should also be investigated in future studies.

In conclusion, within the limitation of the present study, we described the microbial communities of healthy implants and implants with peri-implantitis before and after non-surgical mechanical debridement therapy. The community diversity of peri-implantitis sites was higher than that of healthy sites, and the change in community structure produced a shift away from a healthy status to diseased. Most importantly, in this study, non-surgical mechanical debridement was not efficient to decrease or even alter the microbiome of peri-implantitis, which may underlie the pathogenesis of peri-implantitis and result in the poor prognosis of the treatment. Because of the relatively small sample size and lack of correlation with clinical healing indices, such as improvement in probing depth and negative bleeding on probing, future studies should correlate the microbiome shift with clinical improvements, as well as the cooperative interactions of pathogens.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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