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Shift in the subgingival microbiome following scaling and root planing in generalized aggressive periodontitis

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Abstract

Aim: To investigate the shift in the subgingival microbiota under scaling and root planing (SRP) in patients with generalized aggressive periodontitis (GAgP).

Materials and Methods: After undergoing supragingival scaling, 12 individuals with GAgP were enrolled in this longitudinal study. Full-mouth SRP was accomplished in 3 weeks and re-evaluated 6 weeks later. Pooled subgingival samples (posterior-mesial, posterior-buccal, anterior-mesial, and anterior-buccal) were obtained from each patient before SRP (pre-treatment group) and at the time of re-evaluation (post-treatment group). 16S rRNA PCR products were generated and sequenced after DNA isolation.

Results: Under SRP, the diversity of the subgingival community was consistent, whereas genus-level biomarkers transformed from *Porphyromonas*, *Treponema*, and *Fretibacterium* to *Actinomyces*, *Streptococcus*, and *Haemophilus*. In a network analysis, pathogen-related and non-pathogen-related components were identified in both the pre- and post-treatment groups; the pathogen component was dramatically augmented, while the non-pathogen component shrank after treatment. Hubs were also distributed in both components pre-treatment and were confined to the pathogen component post-treatment.

Conclusions: Scaling and root planing decreased periodontal pathogens in the subgingival microbiota of patients with GAgP. However, the shift in the microbiota composition was characterized by the expansion of pathogen-related components and the contraction of non-pathogen-related components 6 weeks after SRP. Clinicaltrials.gov #NCT03090282.

KEYWORDS
aggressive periodontitis, microbiome, root planing, scaling, sequencing

1 | INTRODUCTION

Aggressive periodontitis (AgP) is characterized by rapid tissue destruction in an otherwise healthy individual (Armitage, 1999). Scaling and root planing (SRP) is an established standard in the treatment of AgP and chronic periodontitis (Cugini, Haffajee, Smith, Kent, & Socransky, 2000; Haffajee et al.,1997). Several studies have shown clinical improvements and microbiological changes in the subgingival bacteria after SRP (Cugini et al.,2000; Darby, Hodge, Riggio, & Kinane, 2005; Mestnik et al.,2010; Pihlstrom, Michalowicz, & Johnson, 2005; Serino, Rosling, Ramberg, Socransky, & Lindhe, 2001).

Studies have demonstrated that the microbial communities in periodontitis differ from those in periodontal health, and a framework of the "microbial complex" was proposed based on DNA–DNA

hybridization (Socransky & Haffajee, 2005; Socransky, Haffajee, Cugini, Smith, & Kent, 1998; Socransky, Haffajee, Smith, & Dibart, 1991). In recent years, the advent of open-ended 16S ribosomal RNA (16S rRNA) sequence analysis has supplemented our knowledge, suggesting that the transition from health to periodontitis is attributable to a shift in the global balance of the microbial flora rather than the appearance of specific periodontal pathogens (Abusleme et al., 2013; Camelo-Castillo et al., 2015; Griffen et al., 2012; Kistler, Booth, Bradshaw, & Wade, 2013; Li et al., 2014; Liu et al., 2012). This conclusion is primarily drawn from cross-sectional studies in chronic periodontitis, while pilot studies analysing subgingival plaques pre- and post-SRP in patients with AgP have failed to obtain consistent conclusions (Laksmana et al., 2012; Valenza et al., 2009).

To better understand the complexity of subgingival communities along with the changes in SRP, we used sequencing to investigate the subgingival microbiota in patients with generalized AgP (GAgP).

Clinical Relevance

Scientific rationale for the study: Although previous research has confirmed the association of specific bacteria with aggressive periodontitis (AgP), the pathogenicity of the whole microbiota is not fully understood.

Principal findings: This study revealed a shift in the microbiota composition after scaling and root planing in patients with GAgP. This shift was characterized by the expansion of pathogen-related components and the contraction of non-pathogen-related components.

Practical implications: This study reviewed changes in the structure of the microbiota in patients with AgP after scaling and root planing, providing an alternative method for the microbiological evaluation of treatment effectiveness and disease activity.

2 | MATERIALS AND METHODS

2.1 | Participant selection

This longitudinal study was reviewed and approved by the Ethics Committee of Peking University School and the Hospital of Stomatology (PKUSSIRB-201525102). From April 2016 to August 2016, twelve individuals with GAgP were recruited from Peking University Hospital of Stomatology (ClinicalTrials.gov #NCT03090282). All participants were individually informed and signed informed consent forms.

Inclusion criteria were as follows:

(i) Between 18 and 35 years old, diagnosed with GAgP according to the International Classification of Periodontal Diseases in 1999 (Armitage, 1999), with the following criteria:

(1) otherwise clinically healthy patient; (2) generalized proximal attachment loss involving at least three teeth, apart from the first molars and incisors; (3) the amount of microbial deposits is inconsistent with disease severity; (4) familial aggregation,

(ii) At least two teeth in each posterior region (upper-right, upper-left, lower-right, and lower-left) and at least four teeth in each anterior region (upper and lower) with clinical attachment loss (CAL) and probing depth (PD) ≥4 mm.

Individuals were excluded from this study if they were pregnant or lactating, were smokers, were in need of antibiotic coverage for periodontal treatment, or had undergone subgingival periodontal treatment or antibiotic therapy within the previous 6 months.

2.2 | Clinical examination and periodontal treatment

All candidates received supragingival scaling, oral hygiene instruction, and full-mouth peri-apical radiographs 1 week before baseline when qualified patients were recruited after full-mouth examination. Clinical parameters, including PD, CAL, plaque index (PII) (Silness & Loe 1964), and bleeding index (BI) (Mazza, Newman, & Sims, 1981), were recorded at six sites per tooth by a periodontist (LG) at baseline (Kappa values: .897, .780, .890, and .854 for PD, CAL, BI, and PII).

One week after baseline examination, the recruited patients received SRP under local anaesthesia for 3 weeks (one quadrant per visit at 1-week intervals). SRP was performed by the same periodontist (LG) with manual and ultrasonic instruments. Re-evaluation was performed 6 weeks after the completion of SRP.

2.3 | Sample collection

According to the baseline examination records, four pooled subgingival plaques were sampled from posterior-mesial (tooth #3, #4, #19, #20), posterior-buccal (tooth #13, #14, #29, #30), anterior-mesial (tooth #7, #8, #23, #24), and anterior-buccal (tooth #9, #10, #25, #26) sites with 3 mm < PD < 7 mm in each patient immediately prior to the beginning of SRP. In cases where the indicated teeth were not qualified, adjacent teeth were selected instead. Subgingival plaques were re-collected from those same sites at the time of re-evaluation.

Subgingival samples were collected by inserting filter papers (2*10 mm, Whatman Grade 3MM Chr; Whatman international Ltd., Maidstone, UK) into the sampling sites 30 s after isolation with cotton rolls and removing supragingival plaques. Then, samples obtained from each participant in the same location were pooled and transferred into a sterile Eppendorf tube. After adding 200 μl of phosphate-buffered saline to each tube and shaking for 1 hr, the samples were centrifuged, and the pellets were separated and stored at −80°C for further analysis (Yang, Xu, He, Meng, & Xu, 2016). Negative filter paper controls were set up throughout the study.

2.4 | DNA extraction and sequencing

DNA was isolated using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), and the final concentration was determined using a Nanodrop2000 (Thermo Fisher Scientific, MA, USA). For each sample, a 10-digit barcode sequence was added to the 5′ ends of the forward and reverse primers (provided by Auwigene Co., Beijing, China). PCR amplification of the 16S rRNA V3-V4 region was performed using universal primers (338F 5′-GTACTCCTACGGGAGGCAGCA-3′, 806R 5′-GTGGACTACHVGGGTWTCTAAT-3′), incorporating the sample barcode sequence. The following cycling conditions were used: initial temperature of 94°C for 5 min; 30 cycles consisting of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and elongation at 72°C for 40 s; and a final extension step at 72°C for 10 min. PCR products were separated by 2% agarose gel electrophoresis, and ~500-bp fragments were purified using a QIAquick Gel Extraction Kit (Qiagen), quantified by real-time PCR, and sequenced on a MiSeq platform (Auwigene Co., available at SRA of NCBI as SRP102224).

2.5 | Data analysis

The mean clinical parameters were determined by averaging the site data within each sample and across groups; the data were then compared via a paired Student's *t* test (PD, CAL) or Mann–Whitney *U* test (BI, PII). Sequences generated were analysed using the pipeline tools QIIME v 1.9.1 (Caporaso et al., 2012) and Mothur v 1.35.0 (Schloss et al., 2009). The multiplexed samples were deconvoluted based on their unique barcodes. Barcodes and primers were trimmed off, and raw reads with a quality value <20 were removed. High-quality reads were clustered into operational taxonomic units (OTUs) at 97% similarity by Usearch (Edgar, 2010). The Ribosomal Database Project Classifier tool (Cole et al., 2009) was used to classify all sequences into different taxonomic groups based on the Human Oral Microbiome Database with a default confidence threshold of 0.7 (Dewhirst et al., 2010). Alpha diversities were compared using the Mann–Whitney *U* test. Principal component analysis (PCA) was conducted according to the distance matrix. The UniFrac distance metric was generated based on the OTU phylogenetic tree and OTU relative abundance. Principal coordinate analysis (PCoA) was calculated according to the UniFrac distance using a weighted algorithm (Lozupone, Hamady, Kelley, & Knight, 2007; Lozupone, Hamady, & Knight, 2006). Analysis of similarities (ANOSIM) was calculated to compare the intra- and inter-group similarities based on the UniFrac distance. Linear discriminant analysis (LDA) Effect Size (LEfSe) was performed to determine differences in the relative abundances of the phylotypes (Segata et al., 2011) using an alpha value of .05 and an LDA threshold of 2.0. Microbiological data were transformed using Box–Cox transformations, and the significance of the differences in the relative abundance between groups was analysed by analysis of covariance (ANCOVA), adjusting for the sampling location. Mean counts were computed for each participant, and the differences in the relative abundances of the microbial taxa pre- and post-treatment were compared using the Mann–Whitney *U* test. All results were adjusted for multiple comparisons using R 3.3.2 (qvalue package; R Foundation for Statistical Computing, Vienna, Austria). Correlations between OTUs were generated using a Spearman correlation analysis. $p < .01$ and $|R| > .6$ were used to construct the networks using Cytoscape (ver. 3.3.1).

3 | RESULTS

Twelve Han Chinese patients, whose ages ranged from 26 to 35 years with a mean age of 30.75 years, participated in this study. There were no dropouts during the experimental period.

Significant reductions were observed in all clinical parameters. Before treatment, the mean PD of the sampling sites was 4.99 mm, and the mean had dropped to 3.20 mm (paired Student's *t* test, $p < .01$; 95% CI, 1.45-2.13) 6 weeks later at the time of re-evaluation. Moreover, the full-mouth mean PD decreased from 4.95 mm to 3.26 mm, and the median BI changed from 3.79 to 1.99. The demographic and clinical parameters of the participants are listed in Table 1.

Ninety-six samples were analysed using deep sequencing, of which one sample before treatment (the posterior-buccal sample of Pt. #10) and seven samples after treatment (all four samples of Pt. #5, the anterior-mesial and the anterior-buccal samples of Pt. #9, and the anterior-buccal samples of Pt. #10) failed to yield PCR products. Samples from the filter paper control yielded no PCR product. No significant differences were found in the number of tags before and after treatment. A total of 3, 172, 919 sequences and 683 OTUs were generated from the 88 samples, with a mean number of 184.39 OTUs/sample, and there was no difference between the two time point samples. In total, there were 12 phyla, 26 classes, 44 orders, 78 families, 147 genera, and 307 species-level taxa in these samples. Most of the

TABLE 1 Clinical parameters of individuals pre- and post-treatment (n = 12, male = 5, female = 7. Mean age: 30.75 ± 3.17)

Clinical parameters	Pre-treatment	Post-treatment	*p*-value*
BI (sampling teeth)	3.25 (2.81, 4.00)	2.00 (1.75, 2.25)	<.001
PD (sampling teeth, mm)	4.99 ± 1.41	3.20 ± 0.93	<.001
CAL (sampling teeth, mm)	5.13 ± 1.63	3.86 ± 1.15	.035
BI (full mouth)	3.79 (2.92, 4.00)	1.99 (1.76, 2.30)	<.001
PD (full mouth, mm)	4.95 ± 0.66	3.26 ± 0.21	<.001
PII (full mouth)	1.15 (0.88, 1.28)	0.78 (0.50, 0.98)	.002

BI, bleeding index; PD, probing depth; CAL, clinical attachment loss; PII, plaque index.

Values are the mean ± standard deviations or median value (inter-quartile range).

*Mann–Whitney *U* test for BI and PII and paired *t* test for PD and CAL.

bacteria were *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, and *Spirochaetes*.

3.1 | Bacterial diversity and community structure

We first investigated whether SRP changed the diversity metrics in the subgingival microbiota. The diversity in pre- and post-treatment groups was compared using the Shannon index, observed species, Chao1, and the Simpson index. There was no significant difference in alpha diversity among the four pooled subgingival samples from different locations ($p > .05$, Mann–Whitney *U* test) or between pre- and post-treatment groups ($p > .05$, Mann–Whitney *U* test, Figure 1).

Weighted PCA and PCoA indicate differences in the bacterial composition between the pre- and post-treatment groups, and the distances between each group were depicted based on similarities in the bacterial community structure. There was no significant difference in the community structure among the four pooled subgingival samples from different locations ($p = .651$ for pre-treatment and .766 for post-treatment, ANOSIM). Although there were indeed some overlaps in the distribution of the points in the two groups (Figure 2), the bacterial composition was found to be significantly different ($p = .001$, ANOSIM).

3.2 | Transition of the subgingival microbiota

Next, we compared the prevalence and relative abundances of the microbial taxa pre- and post-treatment.

High-dimensional biomarkers were detected using LEfSe (Figure 3a). Before treatment, most of the biomarkers were distributed in the phyla *Bacteroidetes*, *Synergistetes*, and *Chloroflexi* and the class *Clostridia*, including *Porphyromonadaceae* and *Bacteroidaceae*, which were the family-level biomarkers in *Bacteroidetes*. Conversely, the phyla *Actinobacteria* and *Proteobacteria* and the class *Bacilli* characterized the post-treatment-associated biomarkers. LEfSe was also used to detect OTUs with significantly different relative abundances at the two time points (Figure 3b). The genera *Porphyromonas*, *Treponema*, and *Fretibacterium* had higher LDA scores in the pre-treatment microbiota, while *Streptococcus*, *Lautropia*, *Haemophilus*, and *Actinomyces* had higher LDA scores after treatment.

We also used ANCOVA to compare these OTUs at the genus and species levels at both time points. Filtered by relative abundance threshold of 1%, we found six genera and 10 species that were increased and six genera and 11 species that were decreased after treatment (Figure 4). The genera *Treponema*, *Porphyromonas*, and *Fretibacterium* and the species *Porphyromonas gingivalis* showed greater decreases after treatment, while the genera *Lautropia* and *Actinomyces* and the species *Haemophilus parainfluenzae* and *Actinomyces naeslundii* increased significantly. These results are largely in agreement with the results of the LDA figures and Mann–Whitney test (Figure S1).

Although *Aggregatibacter actinomycetemcomitans* was always found in association with the localized AgP, its prevalence and relative abundance were 27.7% and 0.14%, respectively, in our patients with GAgP, and those parameters remained stable after SRP (29.3% and 0.15%; $p = .901$, ANCOVA).

3.3 | The core microbiome

Next, we searched for the core subgingival microbiota. Despite the high inter-sample variability, some OTUs were detected in either group sample, representing the core subgingival microbiome, that is

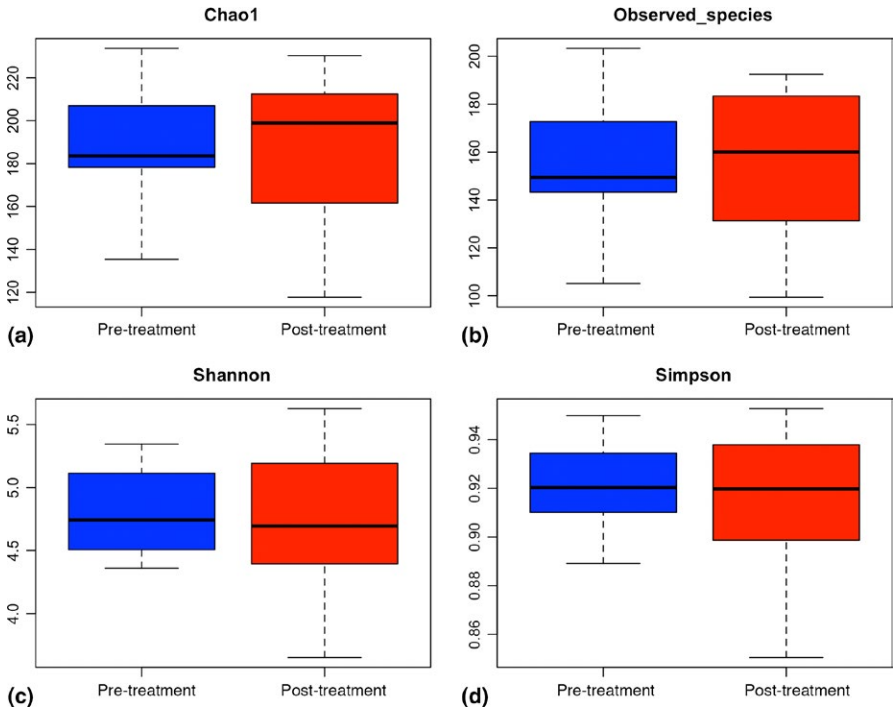


FIGURE 1 Phylogenetic diversity of subgingival samples pre- ($n = 12$) and post-treatment ($n = 11$). Box plots depict bacterial diversity according to the Chao1 (a), observed species (b), Simpson (c), and Shannon indexes (d) in the two groups. There was no statistically significant difference between the two time points ($p < .01$, Mann–Whitney *U* test)

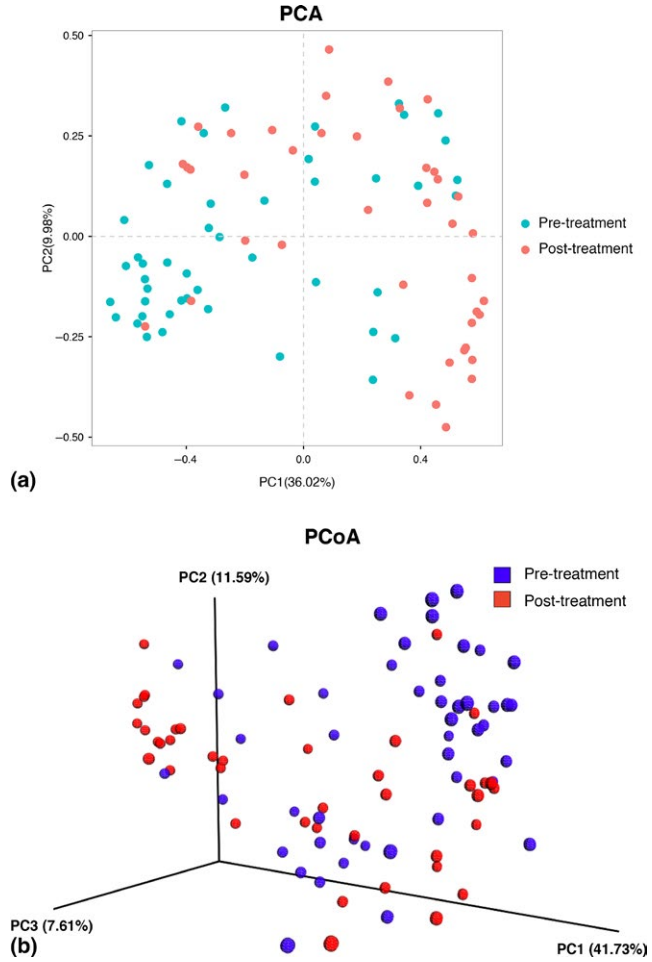


FIGURE 2 (a) Principal component analysis plots and (b) principal coordinate analysis plots of the subgingival samples pre- (n = 47) and post-treatment (n = 41). Each symbol represents one sample: blue (a) or green (b), pre-treatment communities; red, post-treatment

the taxa stably maintained during SRP in the subgingival environment of the patients with GAgP.

The core microbiome was defined as the OTUs with a prevalence >70% and relative mean abundance >2% in both pre- and post-treatment groups. Even after increasing the prevalence threshold from >70% to >75%, these OTUs remained the same (Figure S2). *Neisseria sicca*, *Streptococcus* sp. HOT 058, and two species of *Fusobacterium* were the most abundant members of the core microbiome, whereas 60 other OTUs that presented in most samples were less abundant (Figure 5). The pre- and post-treatment-associated microbiomes were defined using similar methods. The pre-treatment-associated microbiome included *P. gingivalis*, *Filifactor alocis*, *Eubacterium saphenum*, *Campylobacter rectus*, *Fretibacterium* sp. HOT 362, two species of *Treponema*, and the genus *Veillonella*. The five most abundant OTUs were associated with the post-treatment group: *Lautropia mirabilis*, *Neisseria elongate*, *H. parainfluenzae*, *Rothia aeria*, and *A. naeslundii*. Appearing in most samples, but less abundant, another 24 OTUs were associated with the pre-treatment microbiome, and 15 OTUs were associated with the post-treatment microbiome.

3.4 | Correlation networks

Finally, we compared the networks of the subgingival microbiota pre- and post-treatment. These networks revealed strongly connected microbial components.

In each group, OTUs with a prevalence >50% were selected for correlation analysis, and the corresponding networks were depicted (Figure 6a,b). As OTUs with a prevalence >50% increased dramatically after treatment (159 OTUs for the pre-treatment and 426 OTUs for the post-treatment group), the post-treatment-associated network was more complicated.

The pre-treatment network included 130 nodes and 666 edges, while the post-treatment network consisted of 359 nodes and 2,414 edges (network density and centralization: .079, .038 for density and .140, .117 for centralization). Two negatively correlated components constituted the networks both pre- and post-treatment, and one component was mainly composed of the family *Peptostreptococcaceae*, genus *Treponema*, and *Fretibacterium* spp., which was referred to as the "pathogen" component. The other consisted of the genera *Veillonella*, *Actinomyces*, *Streptococcus*, and *Neisseria*, and other health-related OTUs, which were referred to as the "non-pathogen" component. Before treatment, (a) the pathogen component contained few nodes, (b) nodes with high connectivity were distributed between the two components, and (c) most of these high-connectivity nodes had high abundance; after treatment, (a) the number of nodes in the pathogen component increased markedly, (b) all high-connectivity nodes were included in the pathogen component, except for *Granulicatella adiacens*, and (c) the majority of these high-connectivity nodes had a relatively low abundance.

We selected the top 10 connectivity OTUs in each group as hubs (13 for pre-treatment and 12 for post-treatment) and constructed correlation networks for them with typical periodontal pathogens (red and orange complex, Socransky et al., 1998). Pre-treatment-associated hubs were distributed in both the pathogen and non-pathogen components and came from various phyla, such as *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, and *Synergistetes*. Post-treatment-associated hubs all belonged to the pathogen component and were all *Firmicutes* and *Spirochaetes* spp. (Figure 6d) except three, even after the top 20 nodes were analysed in terms of their connectivity (Figure S3). Those hubs generated multiple indirect connections between typical periodontal pathogens in both the pre- and post-treatment groups, which were otherwise sparsely connected. Unlike the correlations described above, the negative connections between these nodes were mainly between host-compatible species and pathogens (Figure 6c). For example, *Veillonella parvula* had negative correlations with *Tannerella forsythia*, *Treponema denticola*, *Eubacterium nodatum*, *Fretibacterium fastidiosum*, and other suspected pathogens.

4 | DISCUSSION

The present study provides a comprehensive view of ecological shifts under mechanical treatment. *Porphyromonas gingivalis*,

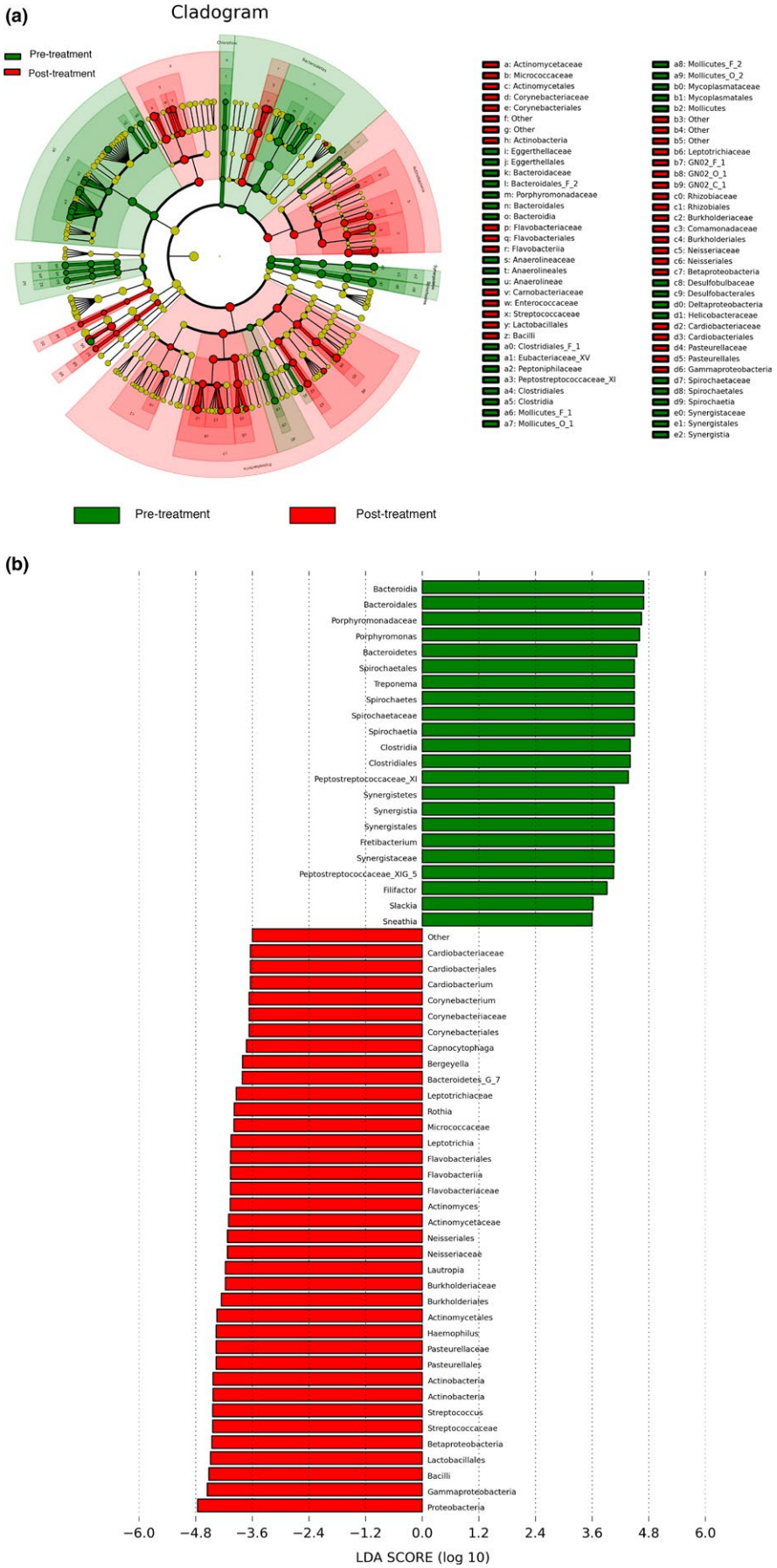


FIGURE 3 (a) Cladogram using the linear discriminant analysis effect size (LEfSe) method showing the phylogenetic distribution of the subgingival microbiota pre-treatment (green, *n* = 47) and post-treatment (red, *n* = 41). (b) LEfSe and linear discriminant analysis (LDA) based on the different relative abundances of bacterial taxa pre-treatment (green, *n* = 47) and post-treatment (red, *n* = 41). The bacteria are ranked according to their LDA scores (LDA score > 3.6)

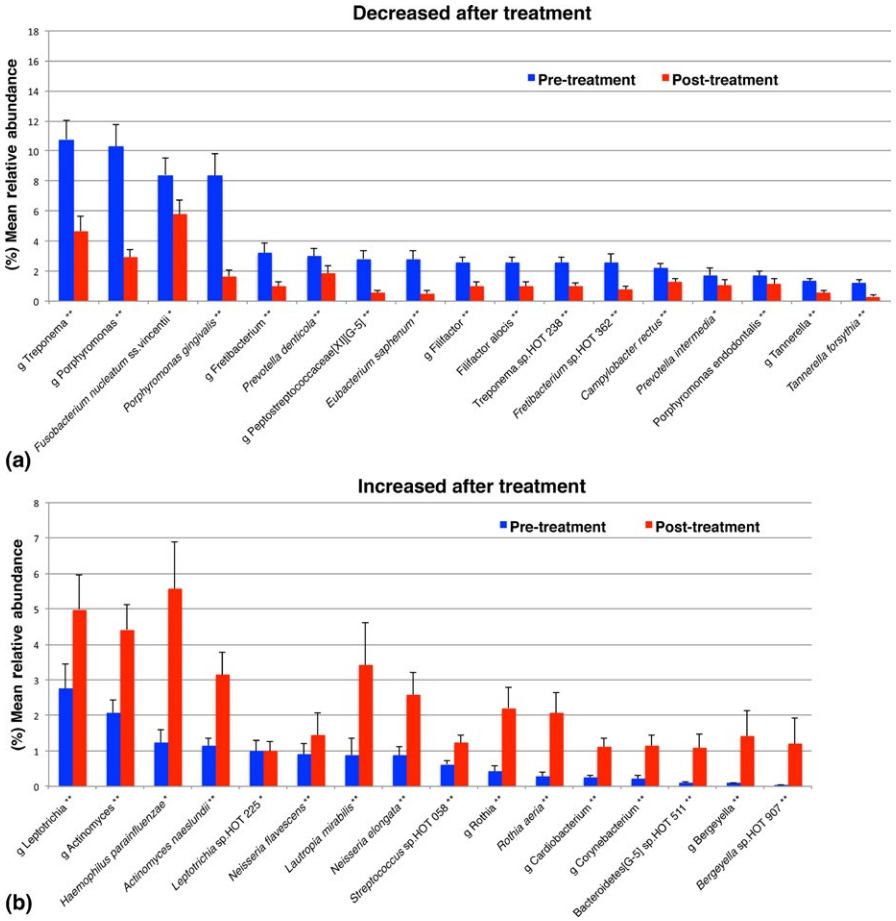
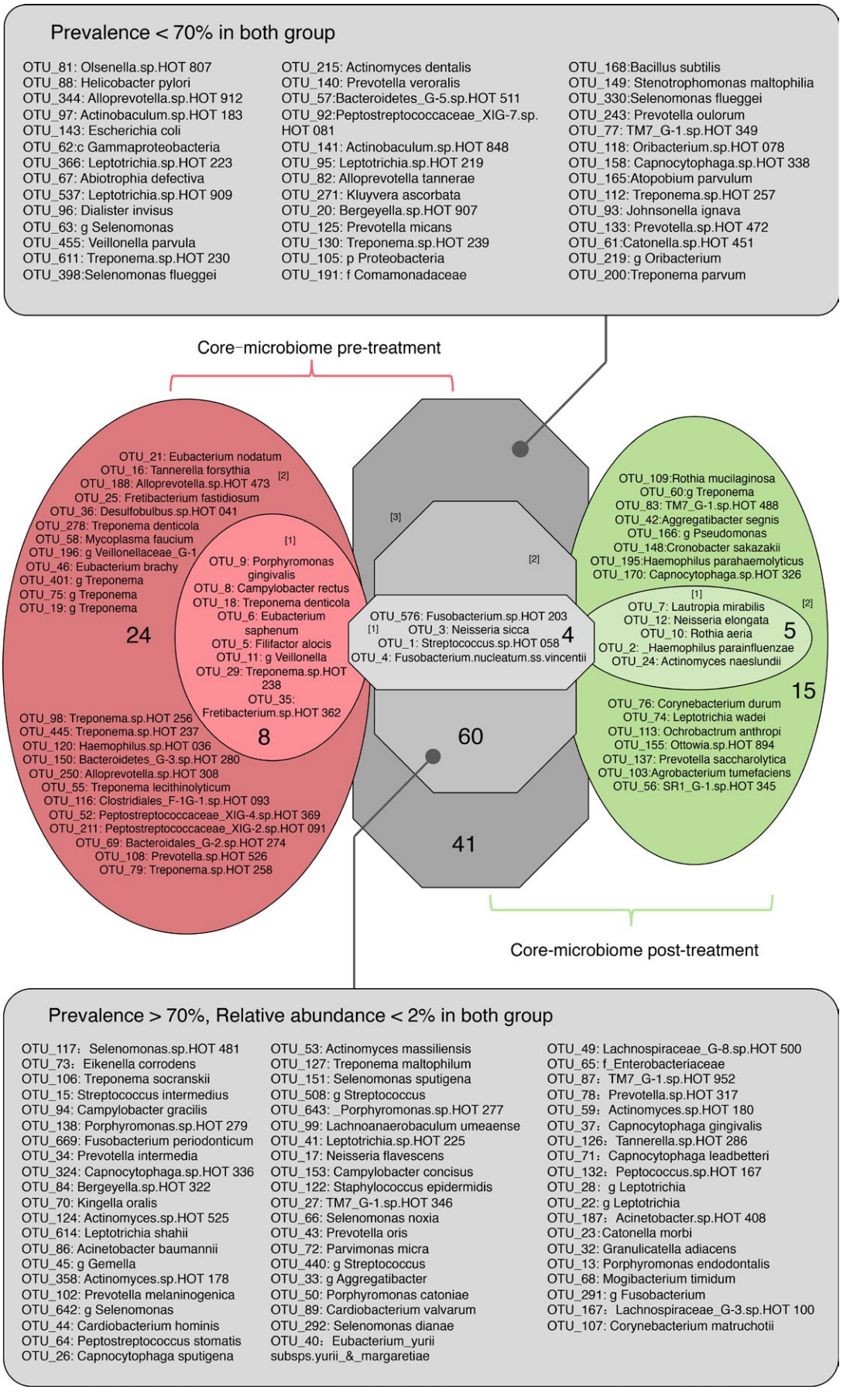


FIGURE 4 Mean (±*SEM*) relative abundances at the genus and species levels. (a) Genera and species that decreased after treatment. (b) Genera and species that increased after treatment (n = 47 for pre-treatment and n = 41 for post-treatment, mean relative abundance >1%, $^{*}p < .05$, $^{**}p < .01$, analysis of covariance)

T. denticola, and *T. forsythia*, designated the red complex (Socransky et al., 1991, 1998), were found to have high prevalences and relative abundances before treatment in our study (85.11%, 95.74%, and 91.49% for prevalence and 8.40%, 2.74%, and 1.23% for relative abundance). According to other studies, the abundance of *P. gingivalis* is associated with the depth of the pocket (Ge, Rodriguez, Trinh, Gunsolley, & Ping, 2013; Griffen et al., 2012; Riep et al., 2009) and the 16S hypervariable region selected for sequencing (Baker, Smith, & Cowan, 2003; Kumar, Brooker, Dowd, & Camerlengo, 2011; Lillo et al., 2006). This may explain the discrepancy between our study and another study carried out in patients of the same ethnicity (8.40% versus 35.88%, Li et al., 2015). After treatment, the abundance and prevalence of most classical periodontal pathogens were significantly reduced. This was consistent with previous studies using sequencing (Laksmana et al., 2012; Valenza et al., 2009) and non-sequencing methods (Darby et al., 2005; Mestnik et al., 2010). The prevalence and relative abundance of *A. actinomycetemcomitans* were maintained at relatively low levels, which may be due to ethnicity-related differences (Zambon et al. 1983, Bonta et al. 1985, Slot et al. 1988, Monteiro et al. 2015, Li et al., 2015; Feng et al., 2015). Some putative pathogens, such as *F. alocis* and *Selenomonas*, were also found to have high prevalences and abundances in our study, which was consistent with similar open-ended method studies in patients with AgP (Faveri et al. 2008, Li et al., 2015; Han, Wang, & Ge, 2016). These pathogens decreased dramatically after SRP with the exception of *Selenomonas* spp., which may have the ability to endure mechanical treatment. Additionally, some health-related bacteria (e.g., *H. parainfluenzae*

FIGURE 5 Venn diagram of the core subgingival microbiome at the two time points (n = 47 for pre-treatment and n = 41 for post-treatment). Each circle contains operational taxonomic units (OTUs) present in at least 50% of samples within a group. OTUs in grey represent the core subgingival OTUs, which are present with equal prevalence and relative abundance during treatment. OTUs in red represent the pre-treatment-associated core species, with increased prevalence and relative abundance before treatment; OTUs in green represent the post-treatment-associated core species, with increased prevalence and relative abundance after treatment. Inner circles labelled 1 contain highly prevalent and highly abundant OTUs (present in at least 70% of samples from each group and numerically dominant with a mean relative abundance of ≥2% of total sequences). Middle circles labelled 2 contain OTUs that are highly prevalent but present in low abundance (present in at least 70% of samples from the specific group but with a mean relative abundance of <2% of total sequences). Outer circles labelled 3 contain OTUs that are moderately prevalent and present in low abundance (present in 50%–70% of samples from each group)


Prevalence < 70% in both group
OTU_81: Olsenella.sp.HOT 807
OTU_88: Helicobacter pylori
OTU_344: Alloprevotella.sp.HOT 912
OTU_97: Actinobaculum.sp.HOT 183
OTU_143: Escherichia coli
OTU_62:c Gammaproteobacteria
OTU_366: Leptotrichia.sp.HOT 223
OTU_67: Abiotrophia defectiva
OTU_537: Leptotrichia.sp.HOT 909
OTU_96: Dialister invisus
OTU_63: g Selenomonas
OTU_455: Veillonella parvula
OTU_611: Treponema.sp.HOT 230
OTU_398:Selenomonas flueggei
OTU_215: Actinomyces dentalis
OTU_140: Prevotella veroralis
OTU_57:Bacteroidetes_G-5.sp.HOT 511
OTU_92:Peptostreptococcaceae_XIG-7.sp. HOT 081
OTU_141: Actinobaculum.sp.HOT 848
OTU_95: Leptotrichia.sp.HOT 219
OTU_82: Alloprevotella tannerae
OTU_271: Kluyvera ascorbata
OTU_20: Bergeyella.sp.HOT 907
OTU_125: Prevotella micans
OTU_130: Treponema.sp.HOT 239
OTU_105: p Proteobacteria
OTU_191: f Comamonadaceae
OTU_168:Bacillus subtilis
OTU_149: Stenotrophomonas maltophilia
OTU_330:Selenomonas flueggei
OTU_243: Prevotella oulorum
OTU_77: TM7_G-1.sp.HOT 349
OTU_118: Oribacterium.sp.HOT 078
OTU_158: Capnocytophaga.sp.HOT 338
OTU_165:Atopobium parvulum
OTU_112: Treponema.sp.HOT 257
OTU_93: Johnsonella ignava
OTU_133: Prevotella.sp.HOT 472
OTU_61:Catonella.sp.HOT 451
OTU_219: g Oribacterium
OTU_200:Treponema parvum
Core–microbiome pre-treatment
OTU_21: Eubacterium nodatum
OTU_16: Tannerella forsythia
[2]
OTU_188: Alloprevotella.sp.HOT 473
OTU_25: Fretibacterium fastidiosum
OTU_36: Desulfobulbus.sp.HOT 041
OTU_278: Treponema denticola
OTU_58: Mycoplasma faucium
OTU_196: g Veillonellaceae_G-1
OTU_46: Eubacterium brachy
OTU_401: g Treponema
OTU_75: g Treponema
OTU_19: g Treponema
24
[1]
OTU_9: Porphyromonas gingivalis
OTU_8: Campylobacter rectus
OTU_18: Treponema denticola
OTU_6: Eubacterium saphenum
OTU_5: Filifactor alocis
OTU_11: g Veillonella
OTU_29: Treponema.sp.HOT 238
OTU_35: Fretibacterium.sp.HOT 362
8
OTU_98: Treponema.sp.HOT 256
OTU_445: Treponema.sp.HOT 237
OTU_120: Haemophilus.sp.HOT 036
OTU_150: Bacteroidetes_G-3.sp.HOT 280
OTU_250: Alloprevotella.sp.HOT 308
OTU_55: Treponema lecithinolyticum
OTU_116: Clostridiales_F-1G-1.sp.HOT 093
OTU_52: Peptostreptococcaceae_XIG-4.sp.HOT 369
OTU_211: Peptostreptococcaceae_XIG-2.sp.HOT 091
OTU_69: Bacteroidales_G-2.sp.HOT 274
OTU_108: Prevotella.sp.HOT 526
OTU_79: Treponema.sp.HOT 258
[3]
[2]
OTU_576: Fusobacterium.sp.HOT 203
[1]
OTU_3: Neisseria sicca
OTU_1: Streptococcus.sp.HOT 058
OTU_4: Fusobacterium.nucleatum.ss.vincentii
4
60
41
OTU_109:Rothia mucilaginosa
OTU_60:g Treponema
OTU_83: TM7_G-1.sp.HOT 488
OTU_42:Aggregatibacter segnis
OTU_166: g Pseudomonas
OTU_148:Cronobacter sakazakii
OTU_195:Haemophilus parahaemolyticus
OTU_170: Capnocytophaga.sp.HOT 326
[1]
OTU_7: Lautropia mirabilis
OTU_12: Neisseria elongata
OTU_10: Rothia aeria
OTU_2: _Haemophilus parainfluenzae
OTU_24: Actinomyces naeslundii
5
[2]
15
OTU_76: Corynebacterium durum
OTU_74: Leptotrichia wadei
OTU_113: Ochrobactrum anthropi
OTU_155: Ottowia.sp.HOT 894
OTU_137: Prevotella saccharolytica
OTU_103:Agrobacterium tumefaciens
OTU_56: SR1_G-1.sp.HOT 345
Core-microbiome post-treatment
Prevalence > 70%, Relative abundance < 2% in both group
OTU_117：Selenomonas.sp.HOT 481
OTU_73：Eikenella corrodens
OTU_106: Treponema socranskii
OTU_15: Streptococcus intermedius
OTU_94: Campylobacter gracilis
OTU_138: Porphyromonas.sp.HOT 279
OTU_669: Fusobacterium periodonticum
OTU_34: Prevotella intermedia
OTU_324: Capnocytophaga.sp.HOT 336
OTU_84: Bergeyella.sp.HOT 322
OTU_70: Kingella oralis
OTU_124: Actinomyces.sp.HOT 525
OTU_614: Leptotrichia shahii
OTU_86: Acinetobacter baumannii
OTU_45: g Gemella
OTU_358: Actinomyces.sp.HOT 178
OTU_102: Prevotella melaninogenica
OTU_642: g Selenomonas
OTU_44: Cardiobacterium hominis
OTU_64: Peptostreptococcus stomatis
OTU_26: Capnocytophaga sputigena
OTU_53: Actinomyces massiliensis
OTU_127: Treponema maltophilum
OTU_151: Selenomonas sputigena
OTU_508: g Streptococcus
OTU_643: _Porphyromonas.sp.HOT 277
OTU_99: Lachnoanaerobaculum umeaense
OTU_41: Leptotrichia.sp.HOT 225
OTU_17: Neisseria flavescens
OTU_153: Campylobacter concisus
OTU_122: Staphylococcus epidermidis
OTU_27: TM7_G-1.sp.HOT 346
OTU_66: Selenomonas noxia
OTU_43: Prevotella oris
OTU_72: Parvimonas micra
OTU_440: g Streptococcus
OTU_33: g Aggregatibacter
OTU_50: Porphyromonas catoniae
OTU_89: Cardiobacterium valvarum
OTU_292: Selenomonas dianae
OTU_40：Eubacterium_yurii subsps.yurii_&_margaretiae
OTU_49: Lachnospiraceae_G-8.sp.HOT 500
OTU_65: f_Enterobacteriaceae
OTU_87：TM7_G-1.sp.HOT 952
OTU_78：Prevotella.sp.HOT 317
OTU_59：Actinomyces.sp.HOT 180
OTU_37：Capnocytophaga gingivalis
OTU_126：Tannerella.sp.HOT 286
OTU_71：Capnocytophaga leadbetteri
OTU_132：Peptococcus.sp.HOT 167
OTU_28：g Leptotrichia
OTU_22: g Leptotrichia
OTU_187：Acinetobacter.sp.HOT 408
OTU_23:Catonella morbi
OTU_32: Granulicatella adiacens
OTU_13: Porphyromonas endodontalis
OTU_68: Mogibacterium timidum
OTU_291: g Fusobacterium
OTU_167：Lachnospiraceae_G-3.sp.HOT 100
OTU_107: Corynebacterium matruchotii

in *Proteobacteria* and *A. naeslundii* in *Actinobacteria*) were also increased after treatment, suggesting that the post-treatment community is more host-compatible.

From the bacterial point of view, a more diverse community is associated with greater ecosystem resilience and healthy status (Feigelman et al., 2017; Lozupone et al., 2007; Proulx et al., 2012; Turnbaugh, 2008). However, bacterial diversity (both richness and evenness) is higher in chronic periodontitis than in health, which might result from a nutritionally richer environment or subversion of the host response in the disease group (Abusleme et al., 2013; Camelo-Castillo et al., 2015; Griffen et al.,2012). AgP individuals have a lower bacterial diversity than those with chronic periodontitis (Li et al., 2015). This may be due to an extremely high abundance of certain bacteria (e.g., *P. gingivalis*) that suppress other species below the detection threshold. Although SRP significantly reduces the total amount of subgingival bacteria (Uzel et al., 2011), it is controversial whether the community diversity changes in chronic periodontitis after SRP (Jünemann et al., 2012; Schwarzberg et al., 2014; Shi et al., 2015). The only study focused on community diversity in patients with AgP pre- and post-SRP simply showed an increased trend in diversity limited by the sample size (Han et al., 2016). In our study, stable diversity, including richness and evenness, under treatment was confirmed. Considering the highly organized spatial structures of dental plaques (Welch, Rossetti, Rieken, Dewhirst, & Borisy, 2016), the stability of the subgingival community may also be affected by the diversification of the structure. Furthermore, sampling methods should also be taken into account when we interpret diversity results in patients with AgP, as they may partially affect the outcomes of subgingival microbiological analysis (Belibasakis, Schmidlin, & Sahrmann, 2014; Jervøe-Storm, Alahdab, Koltzscher, Fimmers, & Jepsen, 2007; Renvert, Wikström, Helmersson, Dahlén, & Claffey, 1992).

As shown in this study, there were two components in subgingival communities. Positive connections prevailed in both components, indicating possible synergism among pathogens or non-pathogens. Notably, the two parts were negatively connected, which indicated the self-restraint of the ecosystem (Grenier, 1996). This subgingival community was always composed of two mutually suppressed components, even after disturbance from mechanical treatment. However, the density of the whole network decreased dramatically after treatment, suggesting a less stable community according to Duran-Pinedo, Paster, Teles, and Friaslopez (2011). Such communities are sensitive to external influences and are prone to stabilization within a certain period, which is either harmful or non-harmful to the host. These results further highlight the importance of periodontal maintenance or additional treatment for patients with GAgP after SRP, even at a time point when the soft tissue has been reconstructed (Ramfjord, Engler, & Hiniker, 1966; Wade, 1978). Meanwhile, hubs play pivotal roles in connections between bacteria. The pre-treatment hubs were distributed in multiple phyla and included the traditional pathogens *T. denticola* and *T. forsythia*. Novel pathogens (e.g., *E. saphenum*, *Fretibacterium* sp. HOT 362, and *F. fastidiosum*) were distributed in three phyla and comprised the majority of the pre-treatment hubs (7/13). There is limited information about the roles of these pathogens in periodontal destruction, as well as their impact on other substances in the subgingival microbiota, and further investigations are needed. Host-compatible bacteria were also included in the pre-treatment hubs (4/13), indicating a relatively coordinated non-pathogen component. After SRP, most of the hubs were novel pathogens (10/12), and host-compatible bacteria were not found in the hubs. Unlike the multiple phyla distributions in the pre-treatment hubs (six phyla), the post-treatment hubs were confined to two phyla, in which *Treponema* spp. predominated (6/12). Moreover, the two traditional pathogen hubs were all *T. denticola*. These results suggested that the reconstructed subgingival microbiota were immature after SRP. Regarding *Treponema* spp., their marked role in the hubs may be attributed to bacterial retention, re-colonization, and synergistic interactions with other periodontal pathogens after periodontal treatment (Dashper, Seers, Tan, & Reynolds, 2011; Inagaki, Kimizuka, Kokubu, Saito, & Ishihara, 2016; Kesavalu, Holt, & Ebersole, 1998; Yao, Lamont, Leu, & Weinberg, 1996).

Although changes in the prevalence and abundance of subgingival bacteria in patients with AgP were usually the most significant in the 2–3 months after SRP, some periodontal pathogens underwent regrowth in long-term observations (Aimetti, Romano, Guzzi, & Carnevale, 2012; Guarnelli, Franceschetti, Manfrini, & Trombelli, 2008; Guerrero et al., 2014; Heller et al., 2011; Xajigeorgiou, Sakellari, Slini, Baka, & Konstantinidis, 2006). Levels of the pivotal bacterium in the post-treatment network in our study, *T. denticola*, increase from 3 months after SRP until 12 months (Haas et al., 2012; Heller et al., 2011). Sample pooling may also attenuate the sensitivity and significance in this study. Longer observation times and site-specific sampling method may provide more information about the characteristics of the subgingival microbiota after SRP. Widely employed in patients with AgP, adjunctive antibiotic application improves both clinical and microbiological results (Aimetti et al., 2012; Ardila, Martelocadavid, Boderthacosta, Arizagarces, & Guzman, 2015; Guerrero et al., 2014; Mestnik et al., 2012). Such treatment was confirmed to further inhibit periodontal pathogens, including red and orange complexes, for more than 6 months (Ardila et al., 2015; Guerrero et al., 2014; Heller et al., 2011; Mestnik et al., 2010; Xajigeorgiou et al., 2006; Yek et al., 2010). Multiple-level studies are needed in future to assess the impact of antibiotics on bacterial correlations and community stability in patients with AgP.

In conclusion, there were mutual inhibitory relationships between periodontal pathogens and non-pathogens in the subgingival microbiota of patients with GAgP. This pattern was also observed after SRP, although the abundances of periodontal pathogens were dramatically reduced. At the re-evaluation time point after SRP, the structure of the reconstructed community was less coordinated than that in the pre-treatment community, especially for the non-pathogen components.

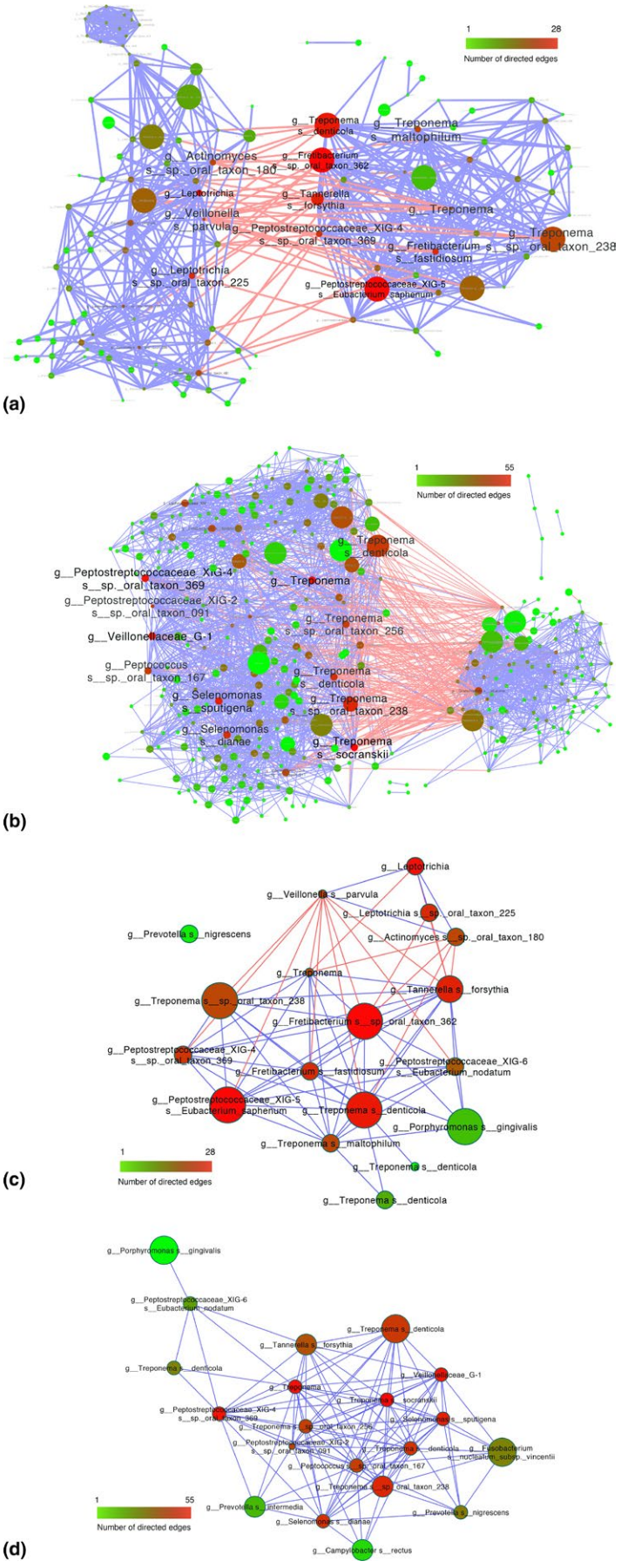


FIGURE 6 Correlation networks pre-treatment (n = 47) and post-treatment (n = 41). Edges between each pair of operational taxonomic units (OTUs) indicate significant correlations ($|R| > .6$, $p < .01$ by Spearman correlation analysis). The size of each node is determined by the mean relative abundance, edges in red indicate negative correlations, and blue edges indicate positive correlations. (a) Nodes in the pre-treatment network consist of OTUs with connectivity from 1 to 28, which are progressively labelled from green to red. (b) Nodes in the post-treatment network are coloured green to red with connectivity from 1 to 55. Networks of hubs (the first ten in terms of connectivity) and the red and orange complex pre-treatment (c) and post-treatment (d)

CONFLICT OF INTEREST

All authors declare that there are no conflict of interests in this study.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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