Title

Changes in Treponeme community after non-surgical therapy in patients with chronic periodontitis

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Changes in Treponeme community after non-surgical therapy in patients with chronic periodontitis

by
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Changes in Treponeme community after non-surgical therapy in patients with chronic periodontitis

Nicholas Arroyo

ABSTRACT

Periodontitis is a chronic, multifactorial inflammatory disease affecting 42% of US adults over the age of 30, characterized by progressive destruction of tooth-supporting structures associated with dysbiotic subgingival biofilms. *Treponema denticola*, a member of the Red complex, is one of the identified pathogenic bacteria that is strongly associated with the progression of periodontal disease. Advances in full-length 16S rRNA sequencing now allows for high-resolution taxonomic identification of *Treponema* species, including uncultivable phylotypes previously undetectable by traditional methods. The aim of this study was to investigate the relationship between subgingival *Treponema* community composition and clinical treatment outcomes following non-surgical periodontal therapy, with the hypothesis that greater reductions in *Treponema* relative abundance would be associated with improved clinical outcomes.

Twenty-eight patients were initially enrolled in this study. Subjects over the age of 18 years-old with a diagnosis of Localized Stage 3 or 4, Grade B or C Periodontitis were eligible for this study. Subjects were excluded if they had taken any antibiotics, steroids, or immunosuppressive medications or received a professional dental cleaning within three months of the baseline examination. Six subjects did not return for non-surgical therapy, and three subjects did not return for the one-month follow-up evaluation appointment scheduled 4–6 weeks

after scaling and root planning (SRP), leaving nineteen patients included in the final analysis (n = 19). Subgingival plaque samples were collected at baseline (T1) and at one-month follow-up (T3) from three teeth that presented with at least one site with CAL ≥ 5mm and PPD ≥ 6 mm. Full-length 16S rRNA sequencing was performed using the Oxford Nanopore platform and processed with the EMU pipeline to characterize the subgingival *Treponema* community. The primary outcome was the association between Treponema abundance the percentage of sites with clinical attachment loss ≥ 5mm. The secondary outcomes were the association between Treponema abundance and the percentage of sites with probing depth ≥ 6mm and percentage of sites with bleeding on probing. All statistical analyses were conducted in R (version 4.3.1) with significance set at $\alpha = 0.05$.

SRP produced significant improvements in all three clinical outcomes, with significant reductions in the percentage of sites with CAL ≥5 mm (55.6% to 33.3%, p = 0.0003), PPD ≥6 mm (27.8% to 16.7%, p = 0.0002), and bleeding on probing (BOP) (44.4% to 16.7%, p = 0.0027). Despite these clinical improvements, SRP did nots produce a statistically significant shift in overall *Treponema* community composition (PERMANOVA, p = 0.061), total *Treponema* abundance (p = 0.072), alpha diversity, or individual species abundance after FDR correction, suggesting that non-surgical periodontal therapy alone may not be sufficient in diminishing the relative abundance of the *Treponema* community. Among the 13 variables tested, BOP was the only variable to remain significant after Benjamini-Hochberg FDR correction in the PERMANOVA ($R^2 = 0.104$, F = 4.16, p = 0.001, p-adj = 0.013). Total *Treponema* relative abundance at follow-up was strongly positively correlated with BOP at follow-up ($\rho = 0.841$, $p < 0.001$), and the change in *Treponema* abundance from baseline to follow-up was positively correlated with the change in BOP over the same period ($\rho = 0.644$, p =

0.003). Patients in the High BOP tertile at follow-up retained significantly greater *Treponema* abundance compared to those in the Low BOP tertile ($\chi^2 = 6.63$, $p = 0.036$). Notably, two subjects had no detectable *Treponema* at either timepoint yet demonstrated clinically significant improvements in CAL, PD, and BOP, indicating that *Treponema*-independent pathways of periodontal disease and recovery exist.

These findings suggest that *Treponema* abundance is more closely linked to active periodontal inflammation than to cumulative tissue destruction, and that resolution of inflammation following SRP may be the primary driver of *Treponema* reduction. Sociodemographic factors, particularly education level and race/ancestry, explained substantial variance in *Treponema* community composition, highlighting the potential role of social determinants of health in shaping the subgingival microbiome. Future studies with larger sample sizes and longer follow-up periods are needed to confirm and extend these observations.

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INTRODUCTION

Periodontal disease encompasses a group of pathologic processes that affect the periodontium, most commonly manifesting as gingivitis or periodontitis. The American Academy of Periodontics defines periodontitis as a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and is characterized by progressive destruction of the tooth supporting structures: gingiva, cementum, periodontal ligament, and alveolar bone.[1] Clinically, periodontitis is characterized by the formation of periodontal pockets, loss of attachment, and alveolar bone resorption. It represents the most prevalent form of periodontitis, primarily affecting adults but can occur at any age, prevalent in 42% of the US population over the age to 30-years-old had periodontitis.[2] The condition is often associated with the accumulation of microbial plaque, which serve as a primary etiologic factor in the initiation and progression of disease.[3]

The human oral cavity harbors a remarkably complex microbial ecosystem. The National Institutes of Health (NIH) has reported that over 700 bacterial species can be detected in the mouth, many of which contribute to maintaining oral health by preventing colonization by pathogenic species and supporting host defense mechanisms.[4] However, shifts in microbial composition can disrupt this homeostasis, leading to a transition from health to disease. This dysbiosis underlies the microbial etiology of periodontitis.[5]

In a seminal series of studies, Socransky and Haffajee categorized oral bacteria into complexes based on their virulence and ecological interactions within the biofilm.[6] Among these, the Red complex, comprising *Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola*, was identified as being strongly associated with increasing probing depths[7] and

bleeding on probing (BOP)[8], a clinical marker of inflammation. Studies have found that the Red complex species are rarely found in absence of the Orange complex, which includes *Fusobacterium nucleatum*, a key bridging species facilitating the transition from early (health-associated) to late (disease-associated) microbial communities.[9,10] One of the notable microbial shifts during this transition is the increase in spirochetes (motile bacteria) and the corresponding decrease in non-motile cocci and straight rods. In health, the ratio of motile to non-motile microorganisms is approximately 1:49, which increases to nearly 1:1 in sites with active disease.[11] Among the members of the Red complex, the only motile spirochete is *Treponema denticola.*[12] The increase in spirochetes in disease progression coupled with the increase of *Treponema* in periodontal pockets leads to the inference of the importance of *Treponema* in disease progression.

Treponema genus are anaerobic, proteolytic, spiral-shaped, Gram-negative bacteria that are frequently detected in subgingival plaque from periodontitis patients. *Treponema denticola*, the most extensively studied species, exhibits several virulence factors that may contribute to tissue destruction and immune modulation. These include adherence to fibroblasts and epithelial cells from the virulence factor major surface protein (Msp)[13], degradation of extracellular matrix components, and production of outer-sheath-associated peptidases and proteinases with chymotrypsin- and trypsin-like activities. *T. denticola* also expresses hemolytic and hemagglutinating activities, adhesins that bind host proteins, and pore-forming outer-sheath proteins that facilitate host tissue invasion.[14,15]

To date, seven species of oral Treponema have been cultured and named: *T. denticola, T. pectinovorum, T. socranskii* (with three subspecies), *T. vincentii, T. maltophilum, T. medium, and T. amylovorum*, [16] However, molecular analyses of 16S rRNA gene sequences have revealed a

far greater diversity of oral Treponema species than previously recognized. In one study, 615 Treponema 16S rRNA sequences were identified, of which 521 (84.7%) were unique, corresponding to 110 distinct operational taxonomic units (OTUs) at 99% similarity. Among these, 93 OTUs were detected in subjects with periodontitis, with several showing statistically significant associations with disease status. Furthermore, the diversity and abundance of Treponema communities were found to be significantly higher in diseased periodontal pockets than in healthy or gingivitis sites.[17]

Interestingly, Treponema species are not exclusive to disease. High levels of treponeme taxon richness have also been detected in periodontally healthy subjects, suggesting that the presence of Treponema alone does not necessarily indicate pathogenicity. Instead, it is the specific taxa composition, relative abundance, and community interactions that appear to modulate disease activity and clinical outcomes.[18] Several species of the Treponema species have been found exclusively in health, in disease, and in both health and disease. A study by Willis *et al.* found *T. amylovorum, T. denticola, T. maltophilum, T. medium, and T. sockranskii* were detected in plaque samples from both healthy and disease sites. This study also found that were significantly less healthy sites with *T. pectinovorum* and there were significantly more disease sites with *T. vincentii*.[16]

Despite their recognized presence in periodontal biofilms, the precise contribution of individual Treponema species to disease progression and treatment response remains poorly understood. Advances in full-length 16S rRNA sequencing now allow for high-resolution taxonomic identification, including differentiation of closely related Treponema species, subspecies, and phylotypes, many of which are uncultivable using traditional methods. Understanding how these organisms change in abundance following periodontal therapy could

provide valuable insights into microbial signatures associated with clinical improvement or persistence of disease.

The aim of this study is to investigate the relationship between the Treponema genus and clinical treatment outcomes following non-surgical periodontal therapy. It is hypothesized that. Specifically, this research will use full-length, long-read 16S rRNA sequence data to evaluate the relative abundance of Treponema species before and after treatment and correlate these microbial shifts with changes in key clinical parameters. The primary outcome was the association between Treponema abundance the percentage of sites with clinical attachment loss $\geq$ 5mm. The secondary outcomes were the association between Treponema abundance and the percentage of sites with probing depth $\geq$ 6mm and percentage of sites with bleeding on probing.

MATERIALS AND METHODS

This study represents a longitudinal analysis of a prospective cohort of patients with periodontitis treated at the University of California, San Francisco's School of Dentistry Department of Periodontics. For the purposes of this study, only patients diagnosed with periodontitis (Localized or Generalized Stage 3 Grade B or C Periodontitis) at initial examination were included. Outcomes were evaluated before and after non-surgical periodontal therapy which included scaling and root planing (SRP), oral hygiene instruction, and one month evaluation. Clinical and microbiome data were analyzed at two time points: baseline (prior to scaling and root planing and at a one-month follow-up appointment (4–6 weeks post-SRP).

Patient Selection

Eligible participants were adults aged 18 years or older with severe, periodontitis defined as having at least two non-adjacent sites with clinical attachment loss (CAL) greater than or equal to 6 mm with periodontal probing depth (PD) greater than or equal to 5 mm. Individuals were excluded if they had used systemic antibiotics, and local antimicrobial agents (e.g. chlorhexidine), within the previous three months, had received professional dental cleaning within three months, or were taking immunosuppressive medications or systemic steroids within three months of the study.

This study was reviewed and approved by the University of California, San Francisco Institutional Review Board (IRB Protocol No. 22-36849). Prior to sample collection, consent was obtained from each subject and a self-answered questionnaire on demographic information was recorded. Subjects were asked to complete a survey asking for their age, biological sex,

race/ancestry, highest level of schooling completed, employment status, household income, marital status, brushing frequency, and flossing frequency.

Study Procedures

Clinical measurements were recorded at baseline and at the one-month follow-up visit (4-6 weeks). For each subject, three teeth were selected for plaque sampling, and six-site periodontal probing depth and clinical attachment levels (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) were recorded for each tooth, yielding 18 total sites per subject. Teeth that had at least one site of clinical attachment loss $\geq$ 5mm and probing depth $\geq$ 6 were selected. Both single-rooted and multi-rooted teeth were included. Periodontal risk markers bleeding on probing (BOP) was also recorded at 6 sites per tooth. Furcation involvement and mobility were not included in this analysis due to limited effect of non-surgical periodontal treatment. All outcomes were calculated at the patient level as percentages of sites per patient. Subgingival plaque samples were collected from 6–8 sites per patient using sterile Gracey curettes. Samples from selected sites were pooled into a single specimen tube per patient at each time point. Therefore, microbiome analyses represent pooled subgingival microbial communities per subject per visit. Plaque samples were placed into DNA/RNA Shield™ - a nucleic acid preservation and stabilization solution. All clinical examinations, subgingival sample collections, and non-surgical periodontal treatments were performed by one of three examiners. All three examiners were calibrated by demonstrating accuracy in reproducing the same probing depths on preselected teeth in the same patient. These values were compared to the probing depths recorded by the most senior clinician and acting principal investigator of this study. Examiners were considered calibrated if 100% of probing depth readings matched the readings of the master clinician.

Microbiome Analysis

Nucleic acid was extracted using the ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research, USA) according to manufacturer instructions with a modified bead-beating protocol. Samples underwent bead beating using an MP Bio FastPrep-24 instrument at 5.5 m/s for three one-minute cycles with five-minute intervals between cycles. DNA and RNA concentrations were quantified using Qubit fluorometry and stored at −80°C until library preparation. Full-length 16S rRNA gene amplification was performed using the Zymo Quick-16S Full-Length Library Prep Kit. DNA was normalized to 0.5 ng/μL, and 2 μL was used per reaction. Amplification was performed for 30 cycles. Following amplification, individual reaction cleanup was conducted per manufacturer supplemental protocol. Libraries underwent downstream processing using Oxford Nanopore Technologies (ONT) Ligation Sequencing Amplicons V14 (SQK-LSK114) protocol and were sequenced on an ONT PromethION platform.

Sequencing reads were processed using the EMU pipeline (v3.6.2) with the HOMD 16S rRNA reference database, optimized for full-length Oxford Nanopore sequencing reads. Taxonomic classification was performed at the species level, with a primary focus on the genus *Treponema*. Read depth was evaluated per sample using fastp prior to analysis, and samples sequenced in duplicate had the higher read depth run retained for analysis. Relative abundance data generated by EMU were used for all downstream microbiome statistical analyses. A filtered phyloseq object was used for all microbiome analyses.

Statistical Analysis

All statistical analyses were conducted in R (version 4.3.1, GUI 1.79 Big Sur Intel build), and statistical significance was set at $\alpha = 0.05$. Due to the small sample size (n = 19) and non-

normal distribution of microbiome and clinical data, non-parametric statistical methods were employed throughout. A formal power analysis was not conducted for this study. This investigation was exploratory in nature, and the sample size was determined by the availability of patients meeting eligibility criteria who completed both the baseline and follow-up visits within the study period. The small sample size is acknowledged as a limitation.

Clinical outcomes were defined as the percentage of 18 pooled sites (3 teeth × 6 sites of periodontal measurements) meeting each threshold per patient per timepoint. The primary outcome was the association between Treponema abundance the percentage of sites with clinical attachment loss $\geq$ 5mm. The secondary outcomes were the association between Treponema abundance and the percentage of sites with probing depth $\geq$ 6mm and percentage of sites with bleeding on probing. Paired Wilcoxon signed-rank tests were used to compare baseline (T1) and post-SRP follow-up (T3) values for each outcome. Clinical severity was further categorized into tertiles (Low, Medium, High) based on the 33rd and 67th percentiles of the T1 baseline distribution for each clinical outcome. Tertile boundaries were fixed at baseline and applied to both T1 and T3 to assess patient movement between severity categories following SRP.

Demographic associations were assessed using Fisher's Exact test, with chi-square used as a fallback when all expected cell counts were $\geq$ 5. All pairwise combinations of 9 categorical demographic and behavioral variables (sex, race/ancestry, tobacco use, brushing frequency, flossing frequency, education level, employment, household income, and marital status) were tested.

Microbiome community composition (beta diversity) was assessed using PERMANOVA (adonis2, vegan package) with Aitchison distance, computed as the Euclidean distance of

centered log-ratio (CLR) transformed relative abundances with a pseudocount of 0.0001 to handle zeros. Each variable was tested individually in a separate PERMANOVA model with 999 permutations. Models testing timepoint and clinical outcomes were stratified by patient to account for repeated measures. Demographic variables were tested without stratification as between-subject factors.

Within-sample diversity (alpha diversity) of the *Treponema* community was assessed using Shannon diversity index, Chao1 species richness, and Pielou's evenness, compared between T1 and T3 using paired Wilcoxon signed-rank tests. Ordination was performed using Principal Coordinates Analysis (PCoA) on the Aitchison distance matrix and visualized in two dimensions. Differential abundance testing of individual *Treponema* species was performed using two approaches. First, paired Wilcoxon signed-rank tests were applied per species. Second, DESeq2 (negative binomial model) was applied using estimated read counts derived by multiplying relative abundances by sample read depth and rounding to integers. Size factors were estimated using the poscounts method to accommodate zero-inflated data, and taxa were retained if they had ≥ 5 estimated counts in ≥ 3 samples.

Associations between *Treponema* abundance and clinical outcomes were assessed using Spearman rank correlation coefficients at T1, T3, and for change scores (Δ = T3 − T1). Kruskal-Wallis tests with post-hoc Dunn tests were used to compare total *Treponema* relative abundance across clinical severity tertile groups (Low, Medium, High) for CAL, PD, and BOP at both timepoints. Multiple comparison correction was applied using the Benjamini-Hochberg FDR procedure in all analyses involving more than one simultaneous test, including per-species Wilcoxon tests, PERMANOVA, Spearman correlations, Kruskal-Wallis post-hoc tests, and DESeq2.

RESULTS

Study Population and Demographics

Twenty-eight patients who were diagnosed with severe, periodontitis (Localized or Generalized Stage 3 or 4 Periodontitis were enrolled in this study and sample collections were taken at initial exam (T1) between October 2024 until January 2026. Six subjects did not return for non-surgical therapy and three subjects did not return for the follow-up one month evaluation appointment (T3) scheduled 4-6 weeks after scaling and root planing. Nineteen patients were included in the final analysis (n=19). A summary of the demographic information can be found in Table 1. Pairwise associations between demographic variables were assessed using Fisher's Exact test and are presented as a heatmap matrix (Figure 1). Tobacco use was uniform across all patients and was therefore excluded from subsequent microbiome analyses. Of 36 pairwise comparisons, only one reached statistical significance: brushing frequency was associated with employment status ($p < 0.001$). No other significant associations were identified.

Table 1: Patient Demographic Summary

n = 19 | One patient (0050) did not complete the demographic survey (reported as missing)

Characteristic	n	(%)
Sex		
Female	12	(63.2%)
Male	7	(36.8%)
Age		
35–44 years	2	(10.5%)

Characteristic	n	(%)
45–54 years	6	(31.6%)
55–64 years	2	(10.5%)
65–74 years	7	(36.8%)
75+ years	2	(10.5%)
Race/Ancestry		
Hispanic	7	(36.8%)
White or Caucasian	6	(31.6%)
Asian/Pacific Islander	3	(15.8%)
Black or African American	2	(10.5%)
Multiple ethnicities	1	(5.3%)
Education		
No school completed	2	(10.5%)
Nursery school to 8th grade	3	(15.8%)
Some high school, no diploma	3	(15.8%)
High school graduate/GED	1	(5.3%)
Some college, no degree	3	(15.8%)
Associate degree	2	(10.5%)
Bachelor's degree	4	(21.1%)
Master's degree	1	(5.3%)
Employment		
Employed	10	(52.6%)

Characteristic	n	(%)
Retired	5	(26.3%)
Not employed	3	(15.8%)
Missing	1	(5.3%)
Household Income		
$0–$30,000	11	(57.9%)
$31,000–$60,000	4	(21.1%)
$61,000–$90,000	2	(10.5%)
$91,000–$120,000	1	(5.3%)
Missing	1	(5.3%)
Marital Status		
Single	12	(63.2%)
Married	6	(31.6%)
Missing	1	(5.3%)
Tobacco Use		
No	18	(94.7%)
Missing	1	(5.3%)
Brushing Frequency		
Once daily	2	(10.5%)
Twice daily	15	(78.9%)
Few times per week	1	(5.3%)
Missing	1	(5.3%)

Characteristic	n	(%)
Flossing Frequency		
Once daily	8	(42.1%)
Twice daily	5	(26.3%)
Few times per week	2	(10.5%)
Never	3	(15.8%)
Missing	1	(5.3%)

Percentages calculated from total n = 19. Missing data represents one patient (0050) who did not complete the demographic survey.

Figure 1: Pairwise categorical association heatmap - demographic variables

Associations between Treponema Abundance and Clinical Outcomes

The primary outcome investigated was the change in *Treponema* relative abundance and its effect on the percentage of sites with CAL $\geq$ 5 mm after non-surgical periodontal treatment. No Significant associations were found between *Treponema* and CAL at either timepoint. Spearman rank correlations revealed that there was a strong positive association between total *Treponema* relative and abundance and BOP at follow-up ($\rho = 0.841$, $p < 0.001$, p-adj < 0.001), which indicates that patients with a greater number sites of bleeding had significantly higher *Treponema* abundance after SRP. Additionally, the change in *Treponema* abundance from T1 to T3 was positively correlated with the change in BOP ($\rho = 0.644$, $p < 0.003$, p-adj < 0.013), suggesting that subjects who had resolution in inflammation also showed the greatest reductions in *Treponema* levels. Total *Treponema* abundance at T3 was also positively associated with the percentage of sites with PPD $\geq$ 6 mm at T3 ($\rho = 0.538$, $p = 0.017$), although this did not survive FDR correction (p-adj = 0.052) (Table 2).

Table 2: Spearman rank correlations between *Treponema* abundance and clinical outcomes. *† p<0.05 uncorrected. BH-FDR correction applied across 9 tests.*

Spearman Correlations: Treponema vs Clinical Outcomes

ρ = Spearman rho | BH-FDR correction applied

Treponema Variable	Clinical Variable	n	ρ	p-value	p-adj (FDR)	
Baseline (T1)						
Δ Treponema (T3-T1)	**Δ BOP% (T3-T1)**	**19**	**0.644**	**0.003**	**0.013**	*****
Total Treponema (T1)	BOP% (T1)	19	0.426	0.069	0.155	ns
Δ Treponema (T3-T1)	Δ CAL% (T3-T1)	19	0.276	0.253	0.455	ns
Total Treponema (T1)	% sites CAL ≥5 (T1)	19	0.207	0.395	0.592	ns
Δ Treponema (T3-T1)	Δ PD% (T3-T1)	19	−0.065	0.792	0.891	ns
Total Treponema (T1)	% sites PD ≥6 (T1)	19	−0.025	0.920	0.920	ns
Follow-up (T3)						
Total Treponema (T3)	**BOP% (T3)**	**19**	**0.841**	**0.000**	**0.000**	*******
Total Treponema (T3)	**% sites PD ≥6 (T3)**	**19**	**0.538**	**0.017**	**0.052**	**†**
Total Treponema (T3)	% sites CAL ≥5 (T3)	19	0.111	0.652	0.839	ns

† p<0.05 uncorrected. * p-adj<0.05. FDR: Benjamini-Hochberg correction.

Kruskal-Wallis tests comparing *Treponema* abundance across clinical severity tertile groups revealed significant differences at follow-up for both BOP tertile ($\chi^2 = 6.63$, $p = 0.036$) and PD tertile ($\chi^2 = 6.09$, $p = 0.048$) (Figure 2). Post-hoc Dunn testing identified a significant difference between the Low and High BOP tertile groups at T3 ($p = 0.030$), with subjects in the High BOP tertile retaining a significantly greater *Treponema* abundance following SRP. No significant tertile differences were observed at baseline for any clinical variable, which suggests that the divergence in *Treponema* abundance between severity groups occur as a result of treatment as opposed to being present at baseline. No significant differences were found in the CAL tertile at either timepoint.

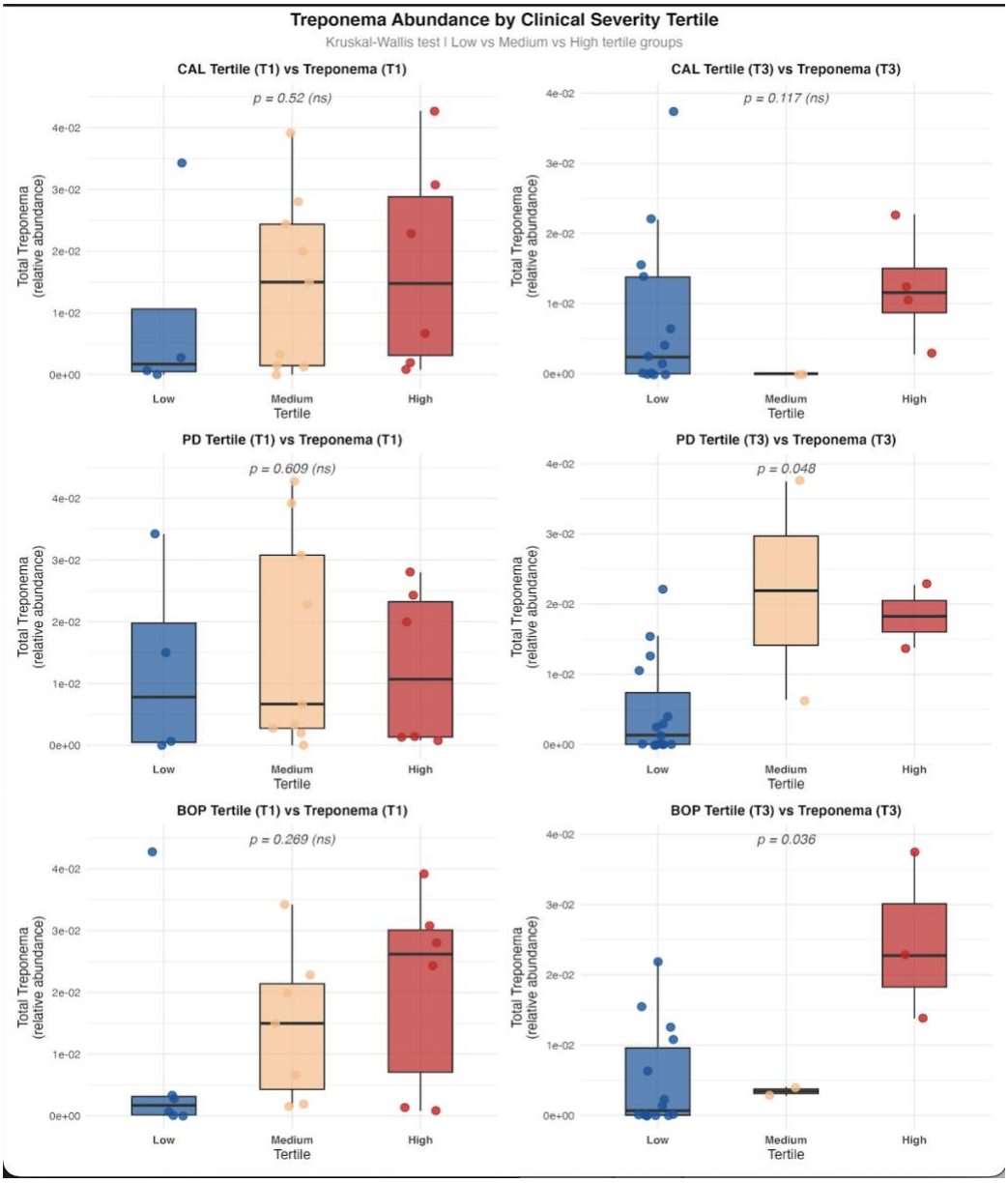

Figure 2: Boxplots – Total *Treponema* abundance stratified by CAL, PD, and BOP tertile at T1 and T3. Each box represents the median as the black line inside of the box and the height of the box represents IQR

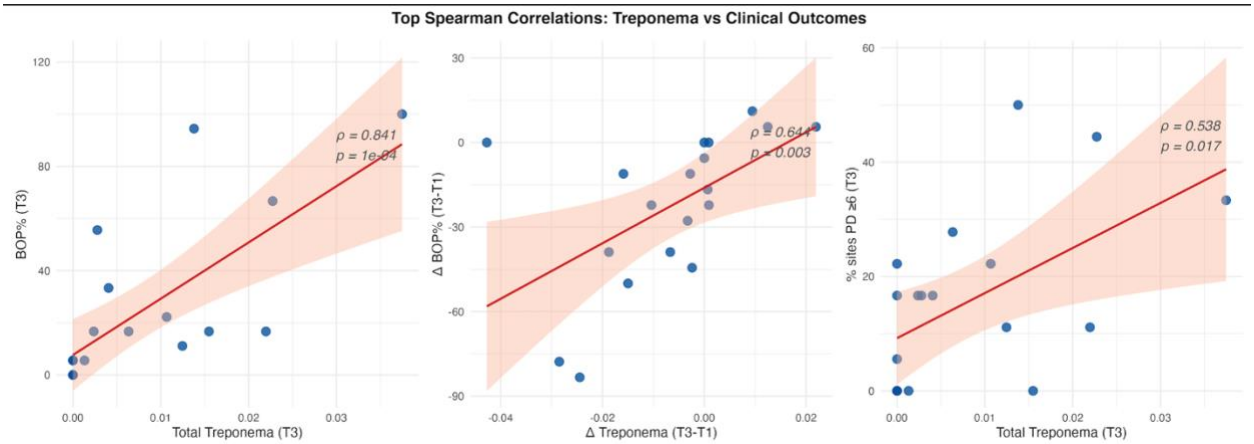

Figure 3: Scatter plots of percentage of sites with bleeding on probing (BOP %) and differences in percentage of sites with BOP (Delta BOP%) and % of sites with PD ≥ 6mm vs total treponema count- top Spearman correlations with regression lines

Sequencing Quality Control

Read depth was assessed across all 38 study samples prior to microbiome analysis (Figure 4). The majority of the samples had adequate read depth (median ~25,000 reads). Two samples had critically low read depths (< 100 reads), one sample had a low read depth (< 5,000 reads), and two samples had borderline read depths (< 10,000 reads). For samples sequenced in duplicate, the run with the higher read depth was used for analysis. These quality metrics are noted as a limitation in the interpretation of the microbiome findings for the affected samples. Figure 5 represents the read depth of T1 vs T3 on a patient level.

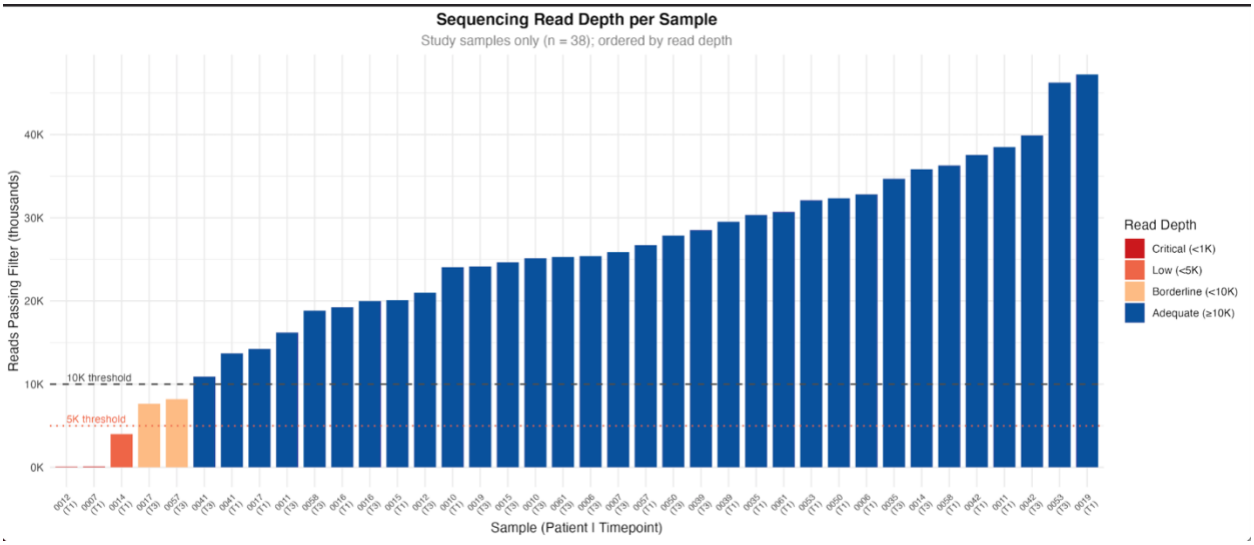

Figure 4: QC read depth plot per sample

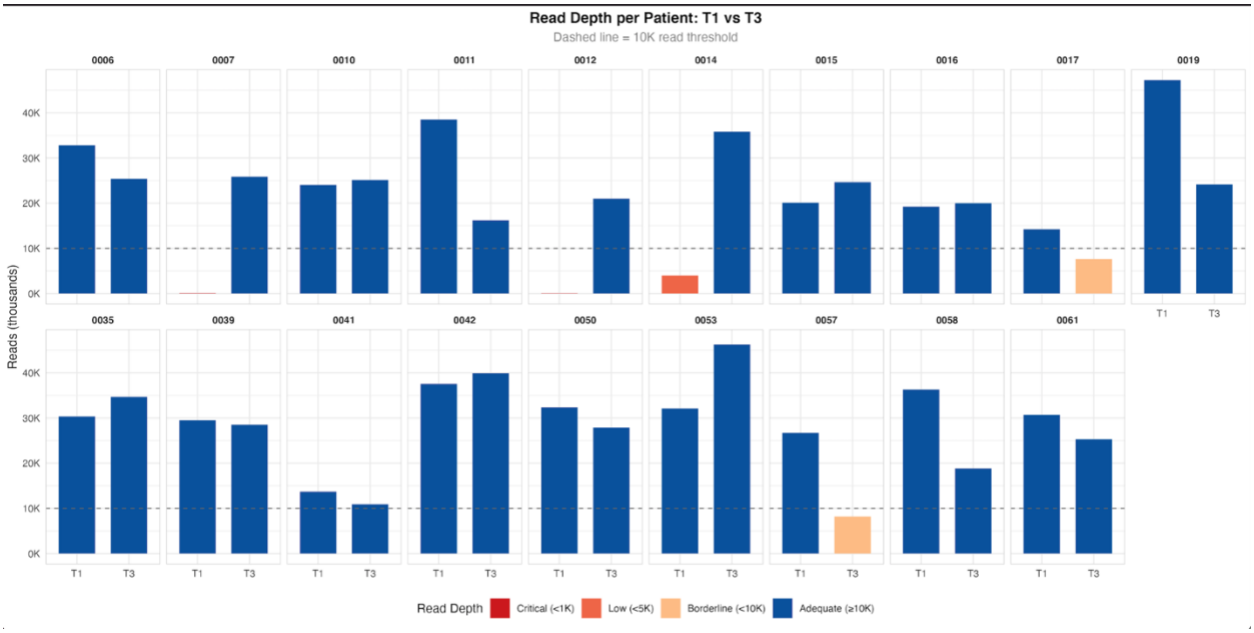

Figure 5: QC read depth plot T1 vs T3. Data is presented per patients.

Treponema Community Overview

Following taxonomic classification and quality filtering, 37 unique *Treponema* species/OTUs were detected across the 38 study samples. The total *Treponema* relative abundance was highly variable across different subjects at different timepoints (Figure 6 and figure 7). At baseline, *Treponema* were detected in 17/19 samples (89.5%) and deceases to detection in 12/19 samples (63.2%). The three most abundant species detected were *Treponema socranskii, Treponema denticola,* and *Treponema species HMT-237* (Figure 8). At T1, *T. socranskii* was detected in 12/19 (63.2%) samples with a median relative abundance of 0.0010 (range: 0 - 0.0063). At T3, *T. socranskii* was detected in 7/19 (36.8%) samples with a medial relative abundance of 0.0000 (range: 0.0000 - 0.0034). The second most abundant species, *T. denticola* was detected in 11/19 (57.9%) of subjects at T1 with a median relative abundance of 0.0000 (range: 0.0000 - 0.0117) which reduced to 5/19 (26.3%) subjects at T3 with a median relative abundance of 0.0000 (range: 0.0000 - 0.0057). For the third most prominent species, *T. sp. HMT-237* was detected in 9/19 (47.4%) samples at T1 with a median relative abundance of 0.0000 (range; 0.0000 - 0.0109) and 7/19 (38.6%) samples at T3 with a median relative abundance of 0.0000 (range: 0.0000 - 0.0067). Interestingly two subjects had no detectable *Treponema* at either timepoint despite displaying clinically significant improvements in CAL, PD, and BOP.

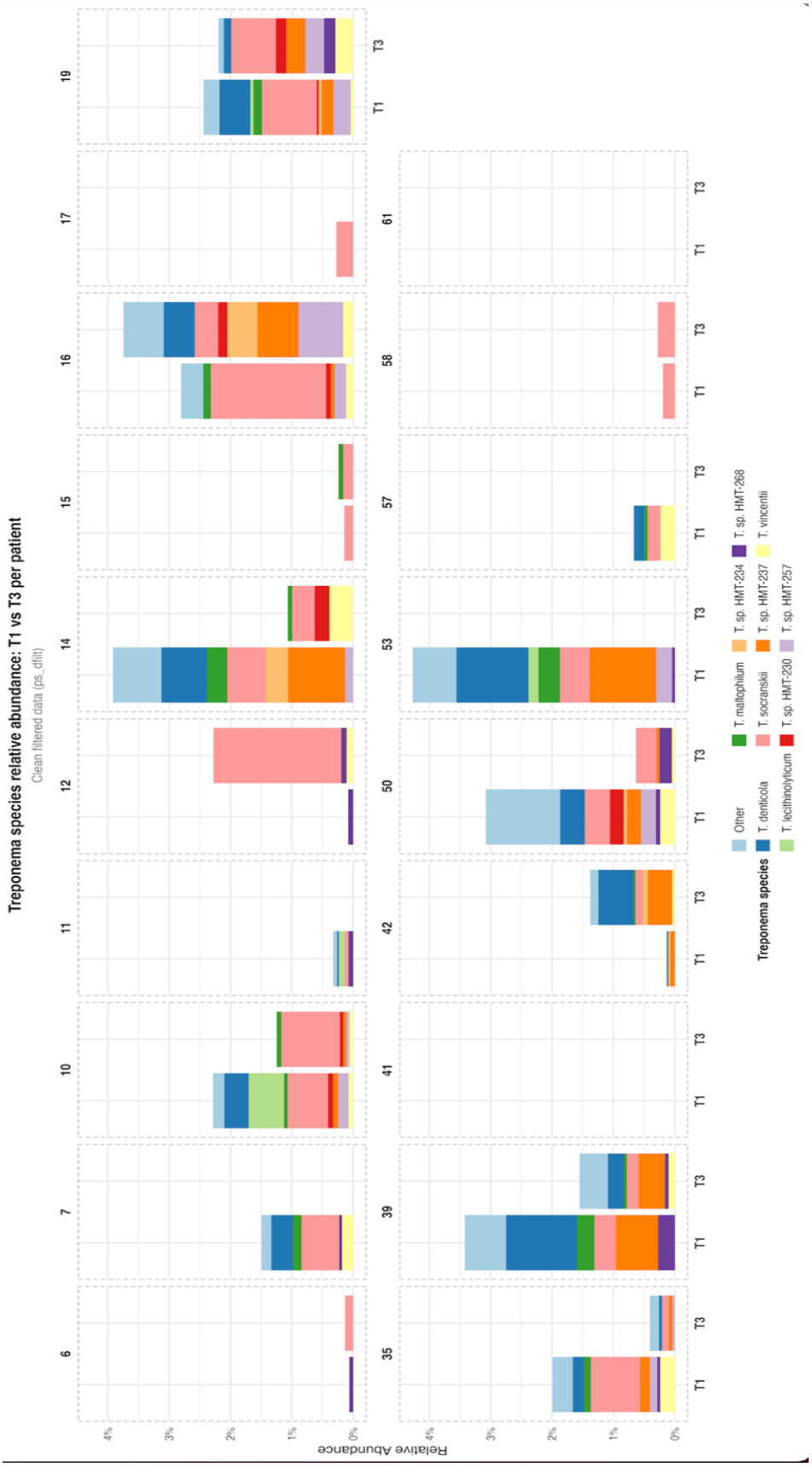

Figure 6: Stacked bar chart – treponema species relative abundance T1 vs T3 per patient

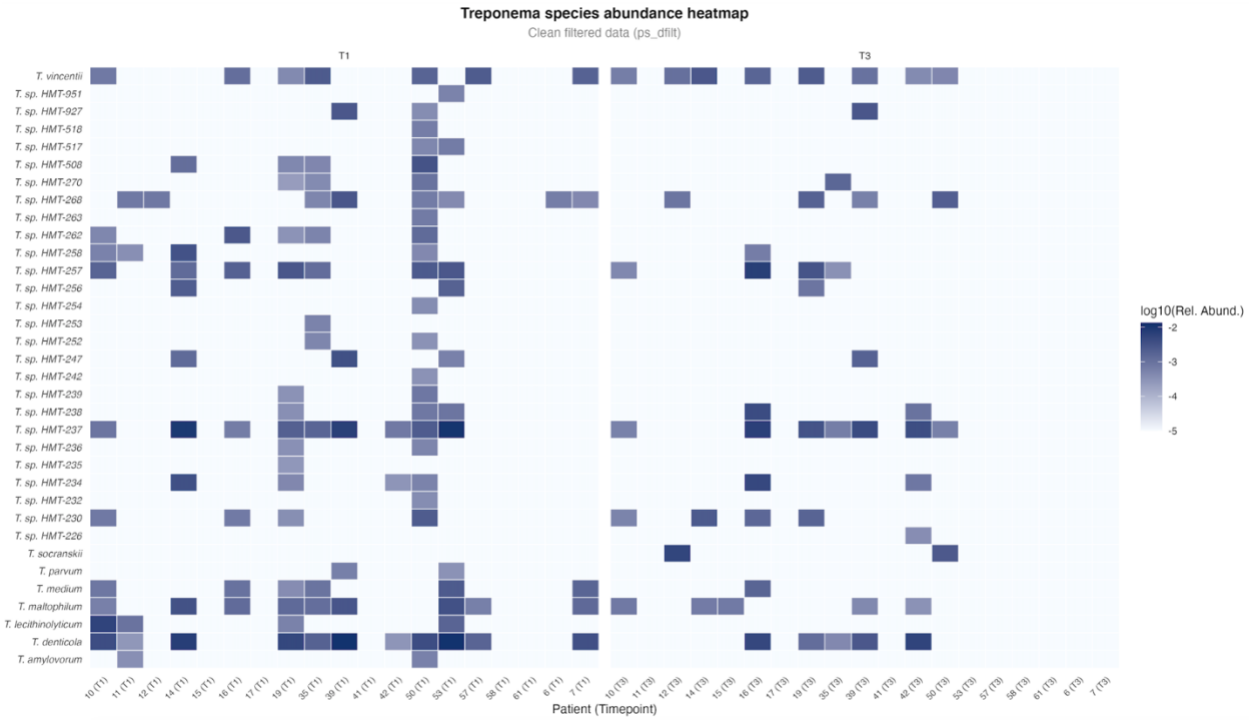

Figure 7: Heatmap - Treponema species x sample abundance at patients' level stratified based on appointment time point

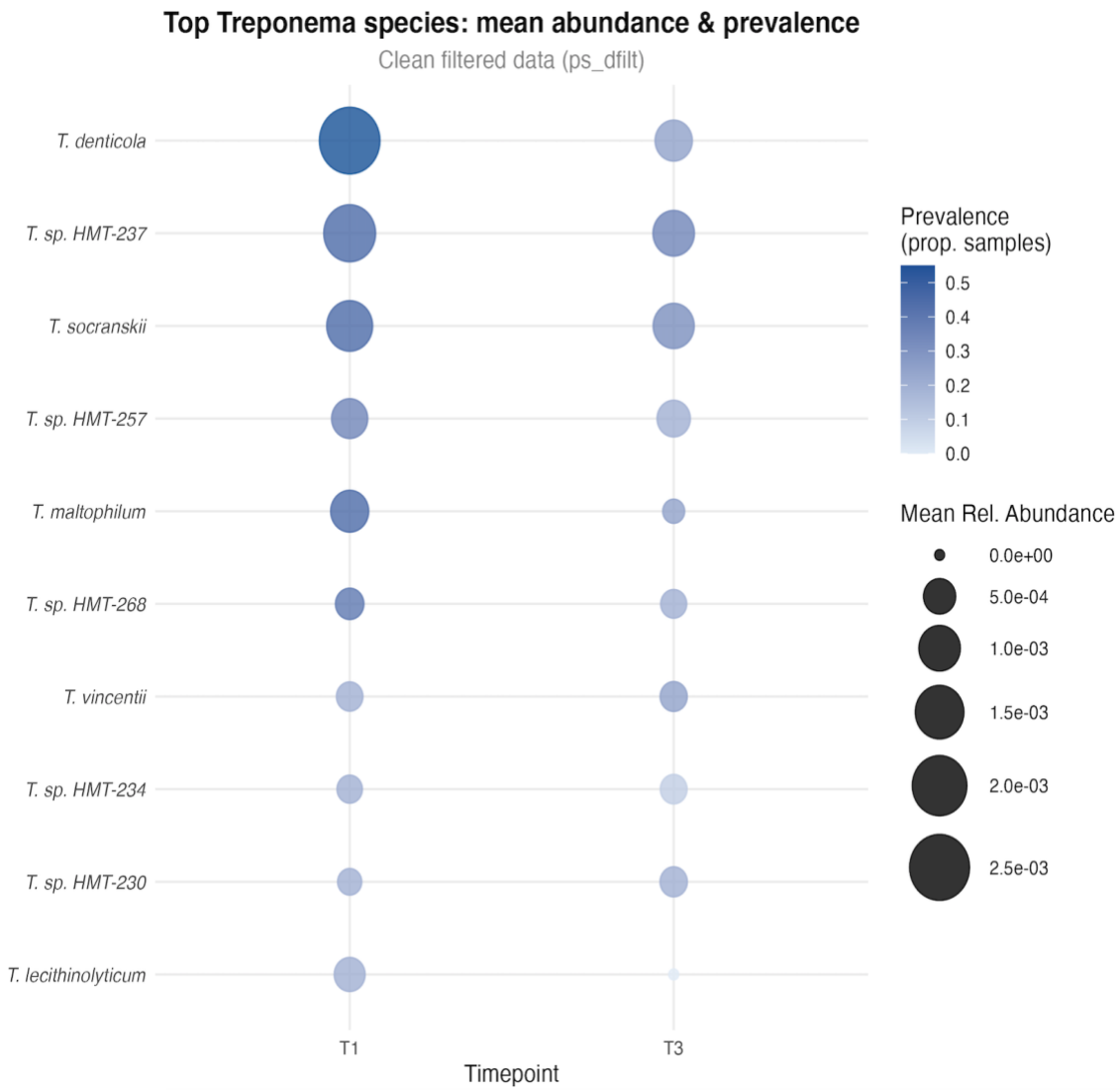

Figure 8: Bubble chart - top 10 *Treponema* species by mean abundance and prevalence in different timepoints (T1, T3). The size of the circle represents mean relative abundance. The larger circle signifies a higher count of that species. The color of the circle represents the prevalence, meaning the darker the color the higher proportion of samples that species can be found.

Differential Abundance of Treponema Species

Total *Treponema* relative abundance showed a non-significant trend toward decrease following SRP (median T1: 0.0067, IQR: 0.0014–0.0262; median T3: 0.0028, IQR: 0.0000–0.0131; W = 115.0, p = 0.072), with detectable Treponema decreasing from 17 of 19 patients at

baseline to 12 of 19 subjects at follow-up. No individual *Treponema* species demonstrated significant changes in abundance from T1 to T3 after Benjamin-Hochberg FDR correction in either the per-species paired Wilcoxon analysis (all p-adj $\geq$ 0.53) or the DEEqe1 negative binomial model (all p-adj $\geq$ 0.96) (Figure 9). Before correction, *T. maltophilum* (p = 0.023) and *T. socranskii* (p = 0.033) showed slight trend towards decrease after SRP. In the DESeq2 analysis, *T. sp. HMT-262* ($\log_2$FC = −4.2, p = 0.103), *T. lecithinolyticum* ($\log_2$FC = −4.5, p = 0.136), and *T. denticola* ($\log_2$FC = −1.1, p = 0.327) trended toward decrease, although no statistical significance was reached. The volcano plot demonstrates that the majority of species trended towards decreased abundance following SRP, although the effect sizes were insufficient to reach significance given the small sample size and zero-inflation of the data.

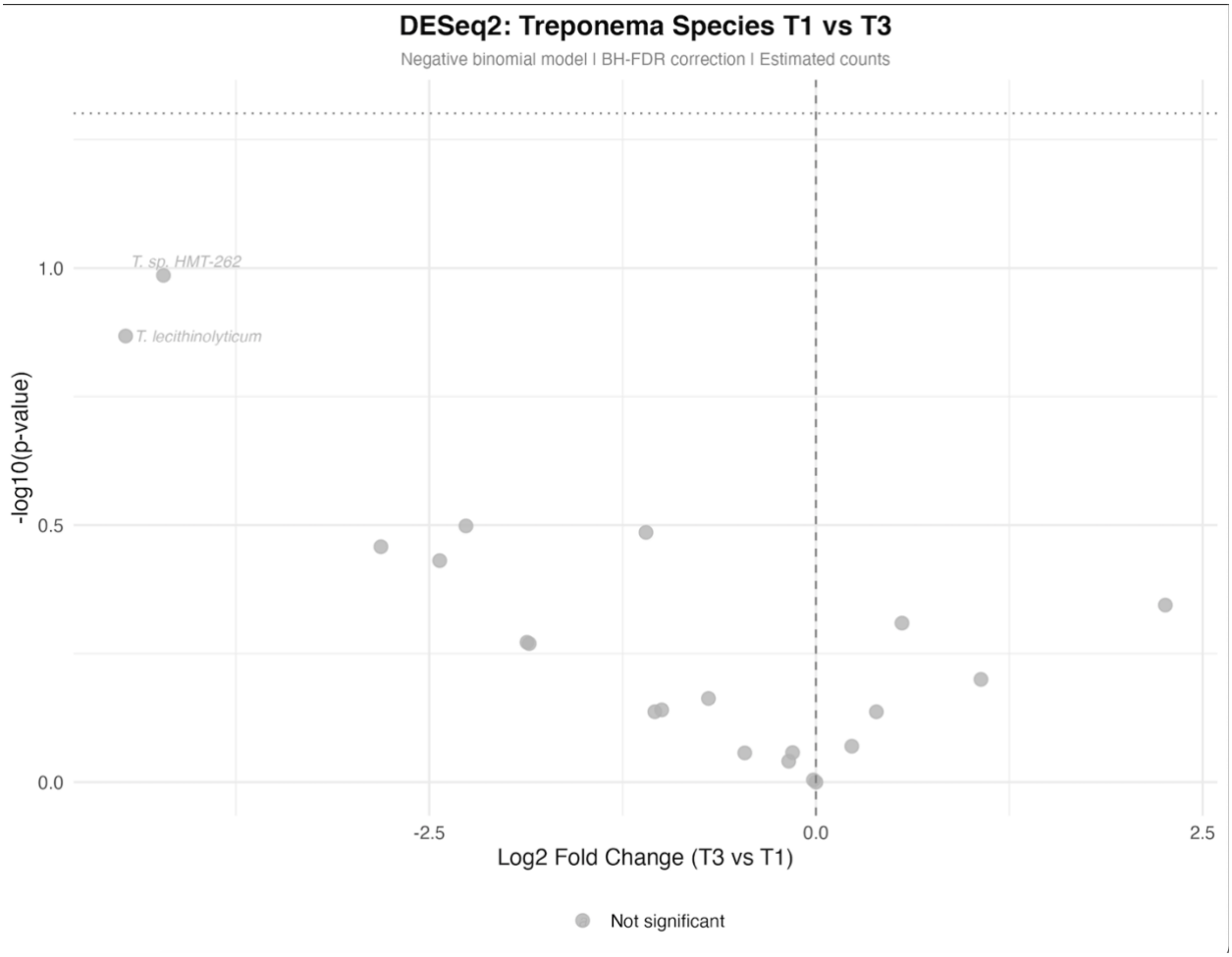

Figure 9: Volcano plot - DESeq2 results, log2 fold change vs -log10(p-value)

Alpha Diversity

The alpha diversity, the within-sample diversity, of the *Treponema* community was assessed using Shannon diversity index, Chao1 species richness, and Pielou's evenness (Figure 10). Shannon diversity showed a non-significant trend toward decreased diversity following SRP (median T1: 1.67, IQR: 0–2.12; median T3: 1.04, IQR: 0–1.52; W = 91, p = 0.083). Shannon diversity was only greater than zero in 13 of the 19 subjects at baseline and 10 of the 19 at follow-up, which indicates that the majority of the subjects did not have detectable *Treponema* diversity at either timepoint. Chao1 richness was uninformative due to extreme zero-inflation,

with a median of zero at both timepoints. Pielou's evenness did not change significantly between T1 and T3 (median T1: 0.792, IQR: 0–0.892; median T3: 0.705, IQR: 0–0.903; p = 0.478). The IQR lower bound of zero for both Shannon and Pielou's evenness at both timepoints reflects that at least 25% of patients had no detectable Treponema at a given visit, consistent with the zero-inflation observed throughout the microbiome analyses. No significant changes in alpha diversity were observed following SRP.

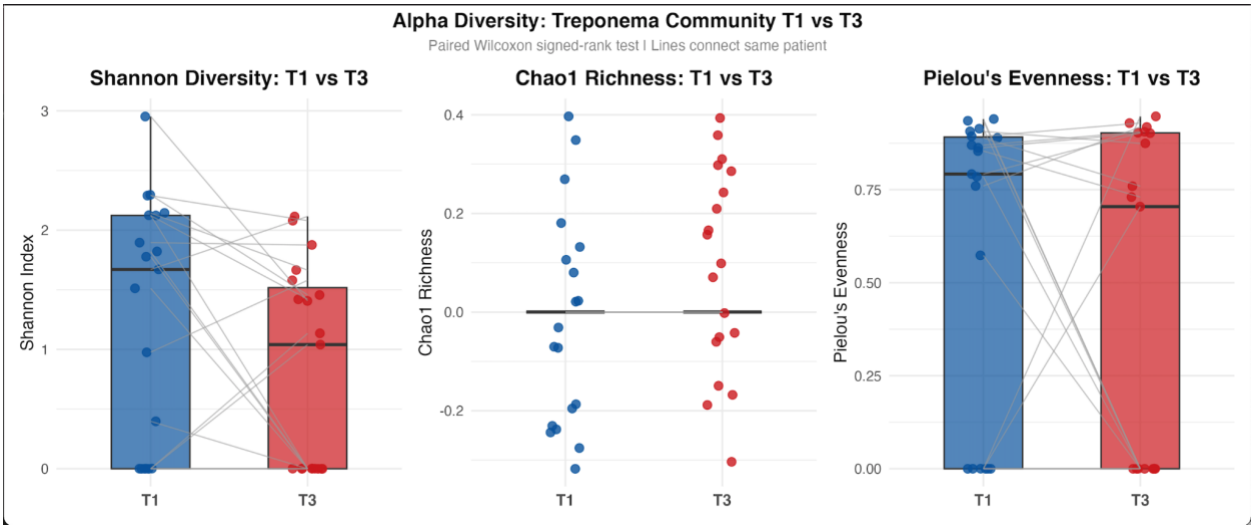

Figure 10: Alpha diversity boxplots - Shannon, Chao1, Pielou's evenness T1 vs T3. The boxplots represent the median value and the IQR.

Beta Diversity (PERMANOVA)

Community composition of the *Treponema* microbiome was assessed using PERMANOVA with Aitchison distance. Each variable was tested individually, with timepoint and clinical variables stratified by patient to account for repeated measures (Table 3). Among the 13 variables that were tested, BOP (percentage of positive sites) was the only variable to remain significant after Benjamin-Hochberg FRD correction ($R^2 = 0.104$. $F = 4.16$, $p = 0.001$, p-adj = 0.013). This means that BOP explained 10.4% of the difference in *Treponema* community composition between samples. Timepoint (T1 vs T3) approached but did not reach significance

($R^2 = 0.039$, p = 0.061), which indicates that SRP did not have a statistically significant shift in *Treponema* community composition. Several variables showed nominal trends before FDR correction but did not survive multiple comparison adjustment: education level ($R^2 = 0.270$, F = 1.59, p = 0.021), percentage of sites with PD ≥6 ($R^2 = 0.048$, F = 1.80, p = 0.035), race/ancestry ($R^2 = 0.156$, F = 1.53, p = 0.046), age ($R^2 = 0.150$, F = 1.46, p = 0.060), and flossing frequency ($R^2 = 0.149$, F = 1.44, p = 0.073). The primary outcome of this investigation, percentage of sites with CAL ≥ 5 mm, was not associated with *Treponema* community composition ($R^2 = 0.025$, p = 0.288).

Table 3: PERMANOVA results for *Treponema* community composition. BH-FDR correction applied across all 13 tests. *† p<0.05 uncorrected only.*

Variable	R^2	F	p-value	p-adj	Strata	Result
BOP+ % sites (secondary)	0.104	4.16	0.001	0.013	Patient	* Significant
% sites PD ≥6 (secondary)	0.048	1.80	0.035	0.132	Patient	† Trending
Education	0.270	1.59	0.021	0.132	None	† Trending
Race/Ancestry	0.156	1.53	0.046	0.132	None	† Trending
Timepoint (T1 vs T3)	0.039	1.44	0.061	0.132	Patient	ns
% sites CAL ≥5 (primary)	0.025	0.921	0.288	0.320	Patient	ns
Flossing frequency	0.149	1.44	0.073	0.136	None	ns
All other variables	—	—	>0.17	>0.25	—	ns

PCoA ordination of Aitchison distances showed that PC1 explained 29.5% and PC2 explained 16.3% of variance in Treponema community composition (Figure 11). T1 and T3 samples were broadly intermixed with no clear directional separation by timepoint, visually

consistent with the non-significant PERMANOVA result for timepoint. When samples were colored by BOP site count and BOP tertile, no clear visual separation can be observed in ordination space, despite the significant PERMANOVA association between BOP and *Treponema* community composition (Figures 12-13). This discrepancy likely reflects the low variance explained by BOP ($R^2 = 0.104$) and the high inter-patient variability characteristic of the microbiome data, which can limit visual separation in the PCoA even when statistically significant relationships exist.

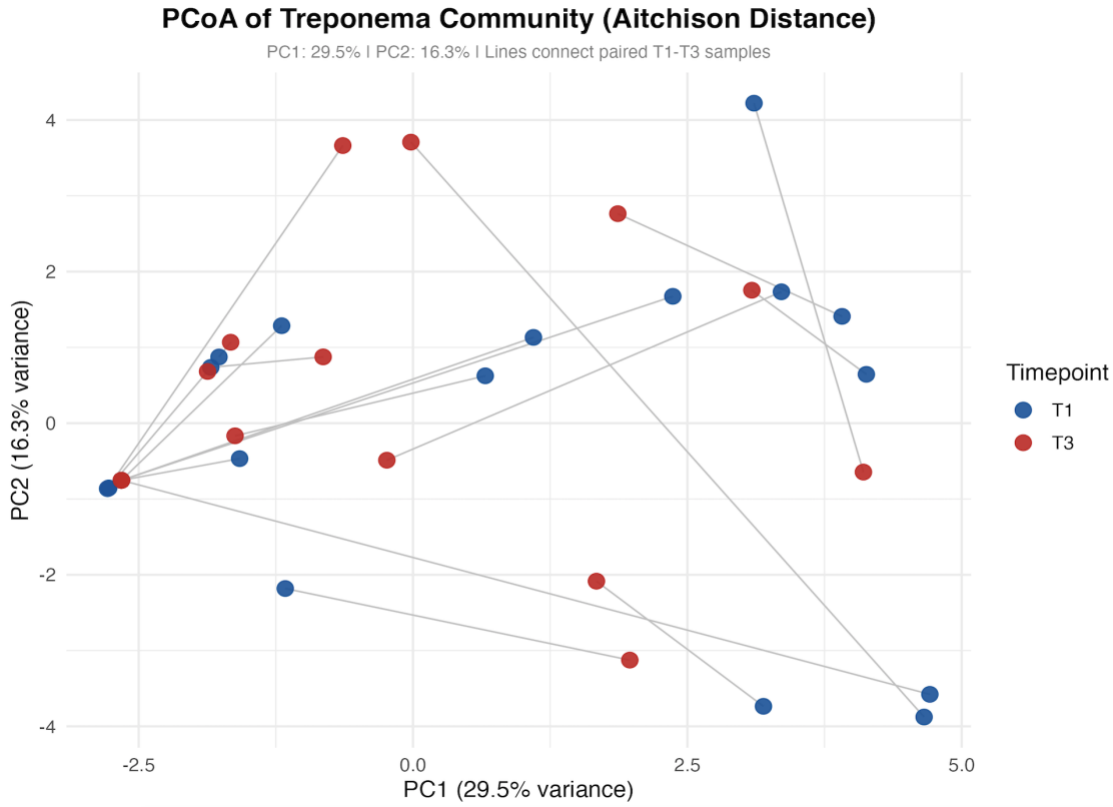

Figure 11: PCoA colored by timepoint - lines connect paired T1/T3 samples

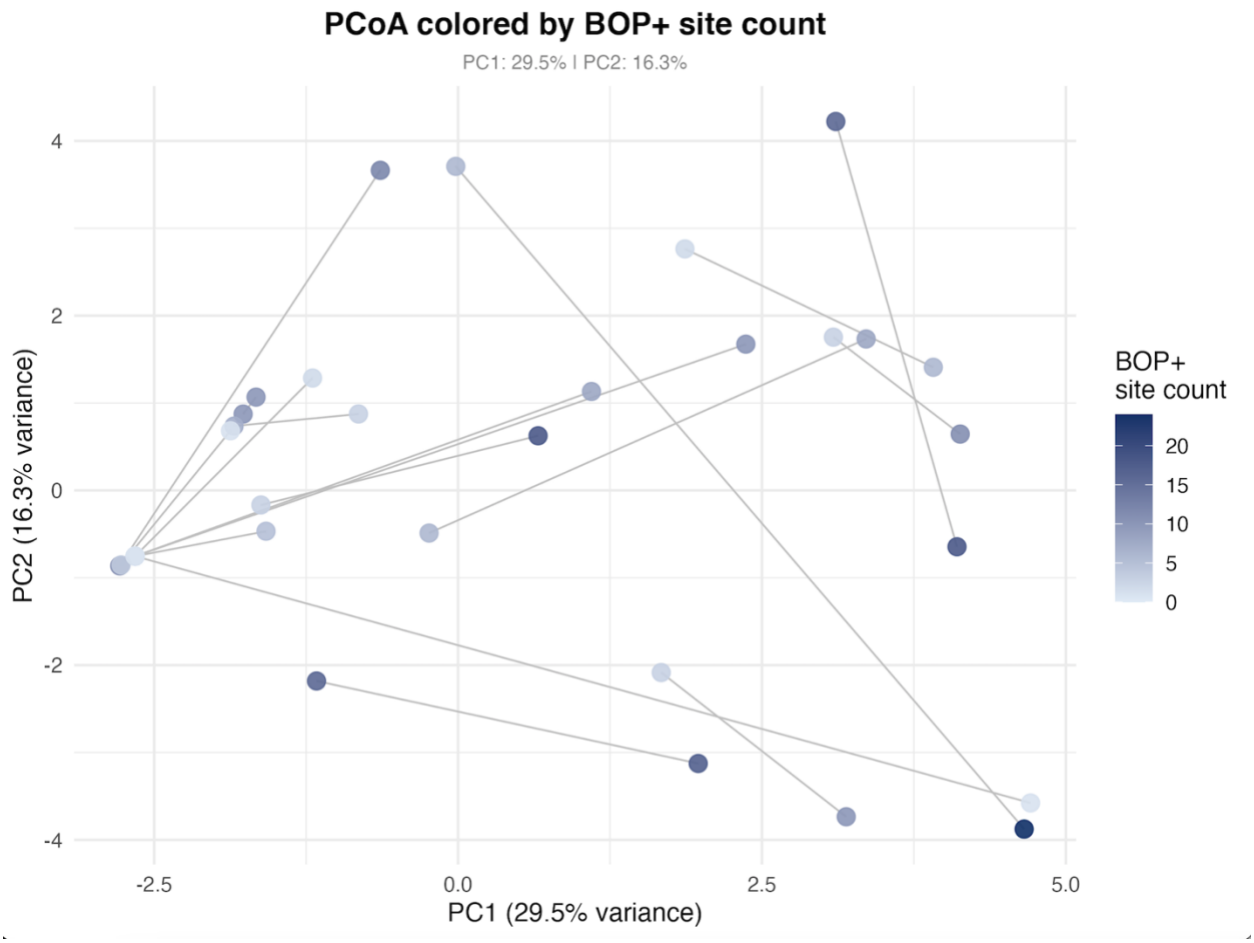

Figure 12: PCoA colored by BOP site count (continuous)

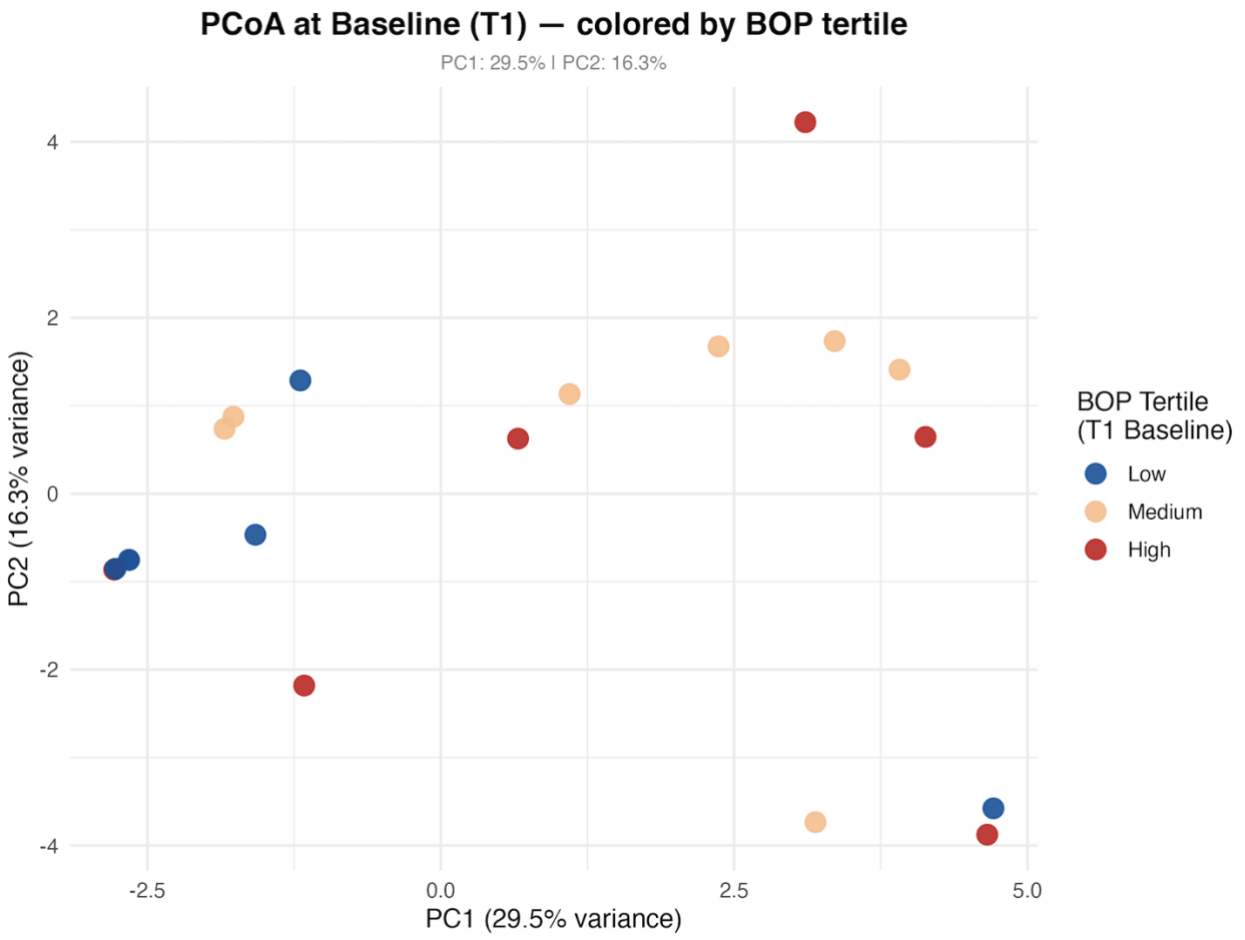

Figure 13: PCoA at T1 baseline colored by BOP tertile

Clinical Outcomes Following SRP

All three clinical outcomes investigated in this study demonstrated significant improvement from baseline (T1) to follow-up (T3) (Figure 14). Clinical outcomes were evaluated using both continuous and categorical measures. For the continuous measures, the median CAL decreased significantly from 4.44 mm (IQR: 4.17 - 5.25 mm) at baseline to 3.83 mm (IQR: 3.60 – 4.39 mm) at follow-up ($W = 186.0$, $p = 0.0003$),and the median PPD decreased significantly from 4.39 mm (IQR: 4.08 – 5.00 mm) to 3.83 mm (IQR: 3.44 – 4.14 mm) ($W = 190.0$, $p < 0.0001$). Site-level threshold of CAL $\geq$ 5 mm and PPD $\geq$ 6 mm represent clinically meaningful cutoffs consistent with the 2017 AAP classification criteria for Stage 3 periodontitis

used to define the study population. The percentage of sites with CAL $\geq$ 5 mm which significantly decreased from 55.6% (IQR: 44.4 - 67.7%) at T1 to 33.3% (IQR 22.2-44.4%) at T3 (W = 169, p = 0.0003). Percentage of sites with PPD $\geq$ 6 mm similarly decreased significantly from a median of 27.8% (IQR: 27-8-44.4%) to 16.7% (IQR: 0-22.2%) (W = 171, p = 0.0002). The percentage of sites positive for BOP which was reduced from 44.4% (IQR: 25.0 - 61.1%) at baseline to 16.7% (IQR: 5.6% - 27.8%) at follow-up (W = 126.5, p = 0.0027). Individual outcomes and tables are provided in Table 4.

Table 4: Clinical outcomes at baseline and follow-up. Values shown are median and IQR; paired Wilcoxon signed-rank tests

Clinical Outcomes at Baseline and Follow-up

Values shown are median (IQR) | Paired Wilcoxon signed-rank tests

Measure	Baseline T1 Median (IQR)	Follow-up T3 Median (IQR)	W	p-value
CAL				
Average (mm)	4.44 (4.17–5.25)	3.83 (3.60–4.39)	186.0	0.0003
% sites ≥5 mm	55.6 (44.4–66.7)	33.3 (22.2–44.4)	169.0	0.0003
PPD				
Average (mm)	4.39 (4.08–5.00)	3.83 (3.44–4.14)	190.0	0.0001
% sites ≥6 mm	27.8 (27.8–44.4)	16.7 (0–22.2)	171.0	0.0002
BOP				
% sites positive	44.4 (25.0–61.1)	16.7 (5.6–27.8)	126.5	0.0027

CAL = clinical attachment level; PPD = periodontal probing depth; BOP = bleeding on probing. IQR = interquartile range. Categorical thresholds (CAL ≥5 mm, PPD ≥6 mm) are consistent with the 2017 AAP Stage 3 Periodontitis classification criteria.

All p-values remain significant at α = 0.05 after Wilcoxon signed-rank testing.

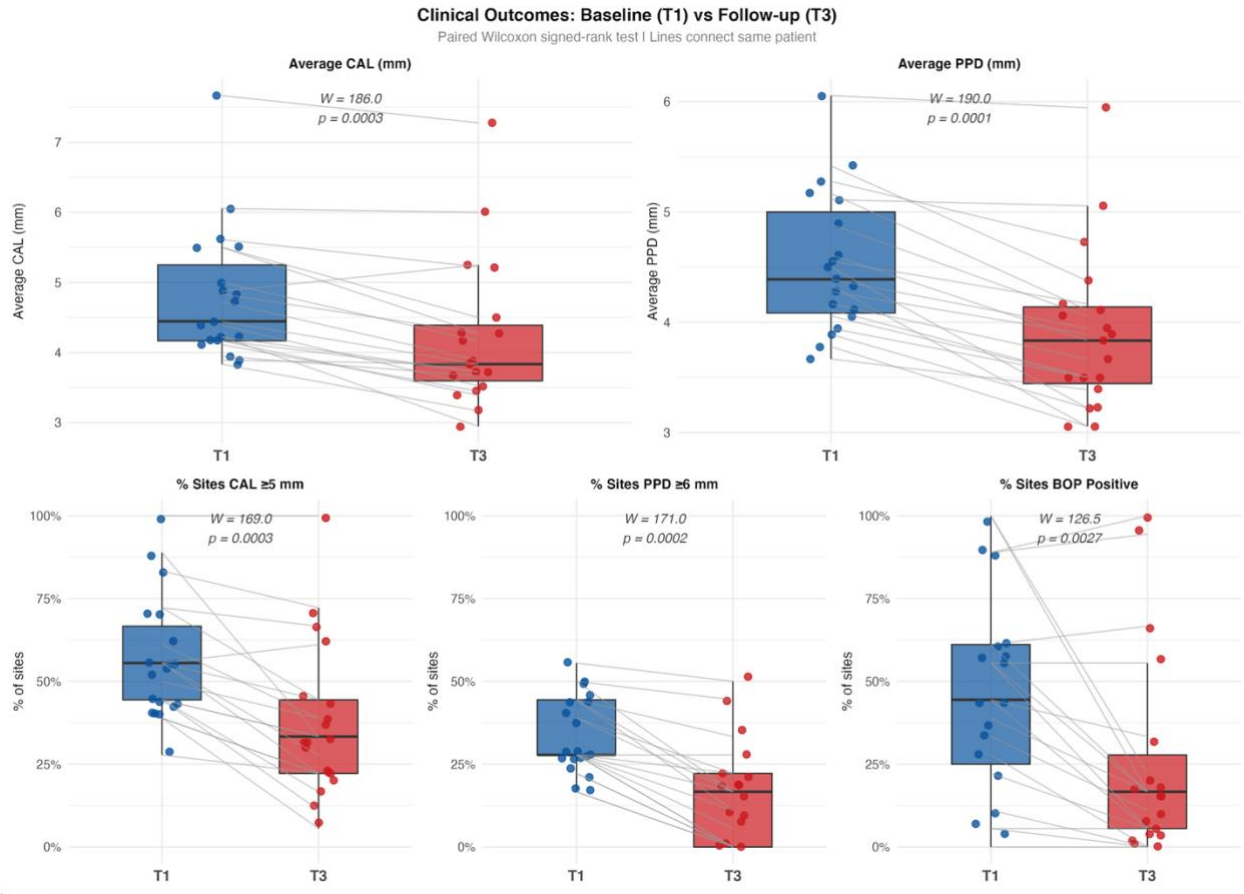

Figure 14 : Clinical Outcomes: Baseline (T1) vs Follow-up (T3). Box plots representing the median change and IQR of average CAL and PPD and the percentage of sites with CAL $\geq$ 5 mm, PPD $\geq$ 6 mm, and BOP positive sites.

When subjects were stratified into tertiles based on baseline disease severity, notable shifts in severity category were observed following SRP (Figures 15 - 17). For CAL, 7 of the 9 subjects in the Medium tertile at baseline improved to the Low tertile at follow-up, while 3 of the 6 High tertile subjects remained in the High tertile. BOP demonstrated the most heterogeneous response, with all 6 Low tertile patients remaining stable, 5 of 7 Medium tertile patients improving to Low, and 3 of the 6 High tertile patients remaining in the High tertile.

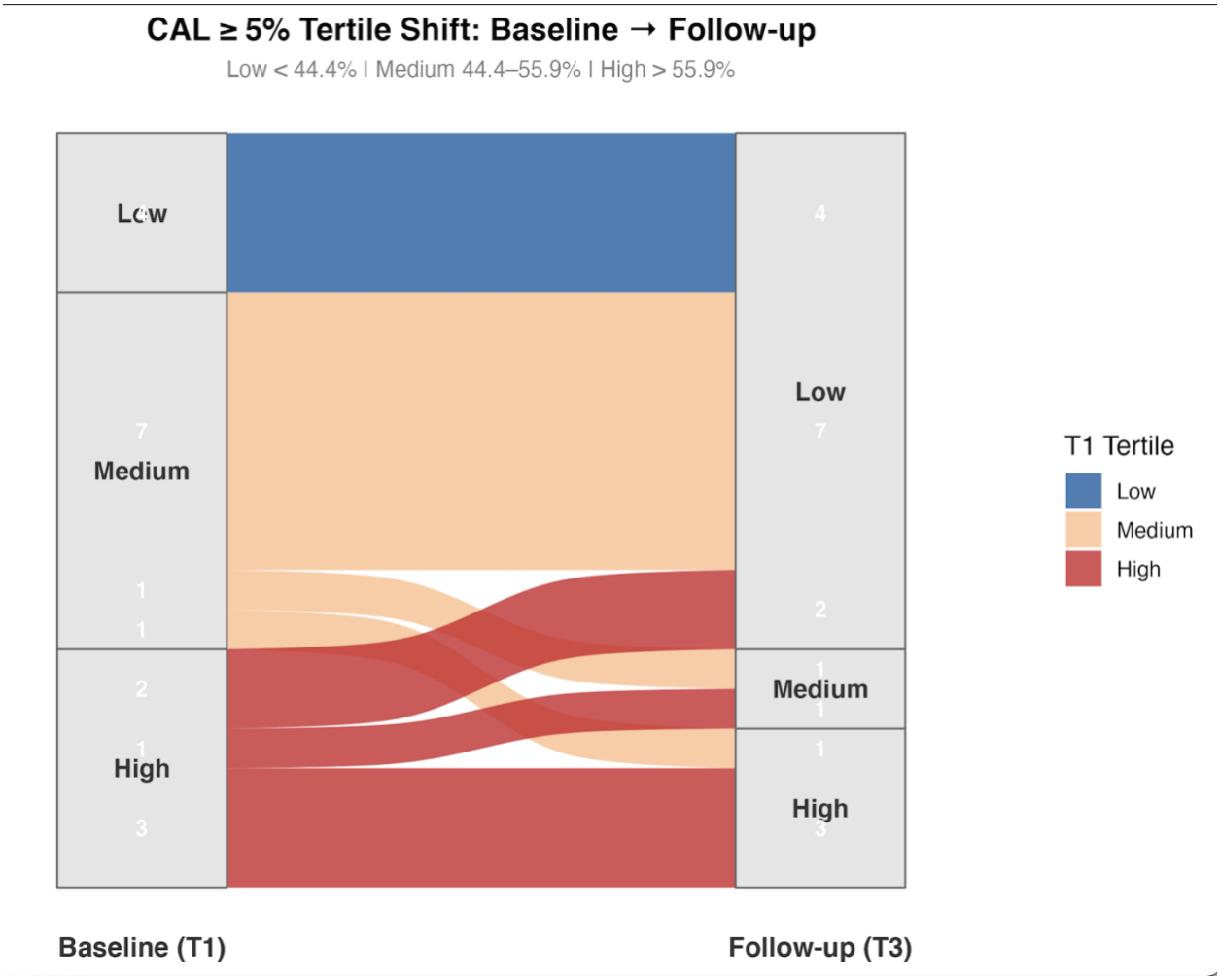

Figure 15: Alluvial plot showing tertile shifts for CAL from base line (T1) to follow up (T3)

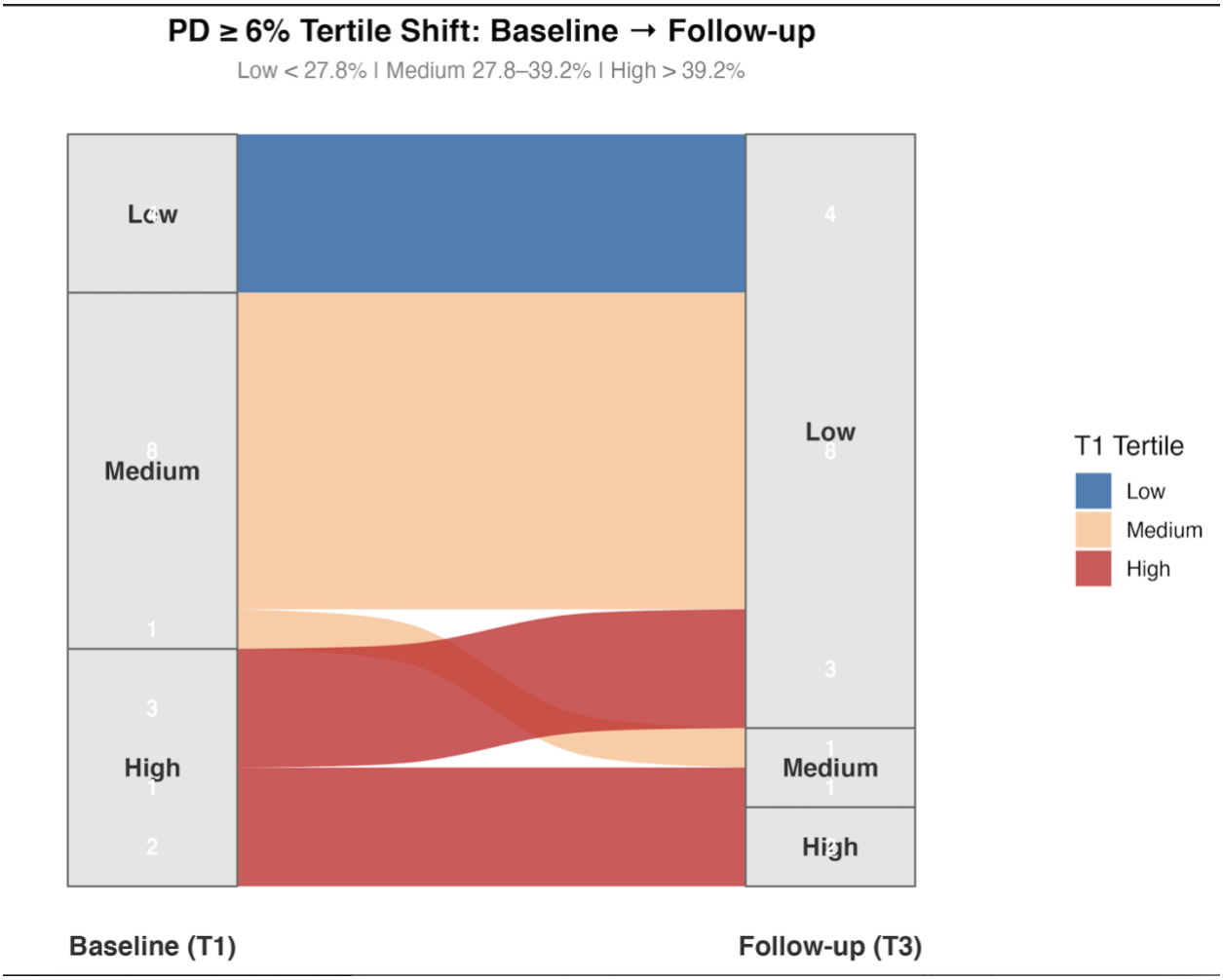

Figure 16: Alluvial plot showing tertile shifts for PD from base line (T1) to follow up (T3)

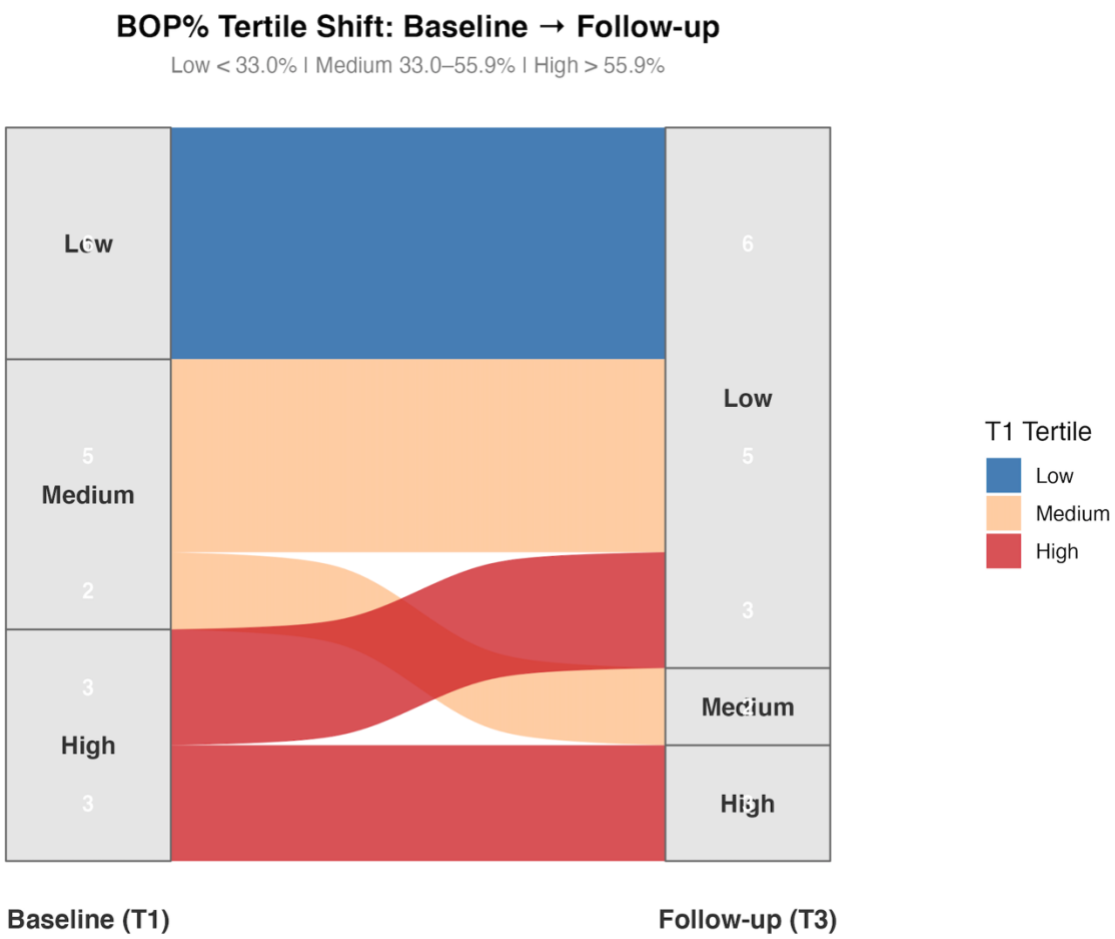

Figure 17: Alluvial plot showing tertile shift for BOP from base line (T1) to follow up (T3)

DISCUSSION

This study examined changes in the subgingival *Treponema* community and clinical periodontal parameters following non-surgical periodontal therapy (scaling and root planing - SRP) in 19 patients diagnosed with periodontal disease. The results show that SRP led to significant improvements among all three clinical outcomes: clinical attachment level (CAL), periodontal probing depth (PPD), and bleeding on probing (BOP). The primary outcome was the association between Treponema abundance the percentage of sites with clinical attachment loss $\geq$ 5mm. The secondary outcomes were the association between Treponema abundance and the percentage of sites with probing depth $\geq$ 6mm and percentage of sites with bleeding on probing. The 2017 World Workshop described the periodontitis as categorized by the microbially-associated, host-mediated inflammation that results in the loss of periodontal attachment detected as clinical attachment loss.[19] The CAL cutoff of 5 mm is based on the Armitage 1999 classification where chronic, severe periodontitis was defined as a CAL $\geq$ 5 mm.[20] This translates into the 2017 World Workshop classification as patients with either Stage 3 or Stage 4 periodontitis.[19] Secondary outcomes for this study were the *Treponema* abundance with percentage of sites with PPD $\geq$ 6 mm and percentage of sites with BOP. A periodontal pocket of 6 mm or more will also place a patient into Stage 3 or 4 periodontitis. 6 mm was also chosen as it is often considered to be one of the main factors in deciding what treatment is performed to treat periodontal disease. Non-surgical treatment is the mainstay for initial phase therapy to treat periodontal disease, but at the one month evaluation appointment, if a pocket still remains $\geq$ 6 mm surgical intervention is often indicated for treatment. One rationale for this cut-off comes from the limitation of a curette for subgingival plaque removal being 5.52 mm.[21] . BOP is considered a risk marker for periodontitis- associated with increased risk of disease. Lang *et al.*

showed that BOP 29% sensitivity and 88% specificity in predicting the progression of periodontal disease. Thus, it was suggested that the absence of BOP is a reliable predictor of stable periodontium.[22]

In this study, SRP did not produce a statistically significant shift in overall *Trepomona* community composition (PERMANOVA, $p = 0.061$), total *Treponema* abundance ($p = 0.072$), alpha diversity (Shannon, $p = 0.083$), or individual species abundance after FDR correction. This differs from the findings from the systematic review and meta-analysis by Krajewski *et al.* which found there was a significant decrease in relative abundance of *T. denticola* in patients with periodontitis after non-surgical periodontal therapy.[23]

Looking at the primary outcome, there was also no significant difference noted in the *Treponema* community in terms of percentage of sites with CAL $\geq$ 5 mm from baseline to follow-up. Total *Treponema* abundance at T3 was positively associated with the percentage of sites with PPD $\geq$ 6 mm after SRP ($p = 0.0017$), however the significance did not survive FDR correction (p-adj = 0.0052). Although not statistically significant a trend can be acknowledged that there was a trend towards decreased Treponeme abundance when there are less sites with PPD $\geq$ 6 mm.

BOP was the only clinical variable to remain significantly associated with *Treponema* community composition after FDR correction (PERMANOVA, $R^2 = 0.104$, p-adj = 0.013), explaining approximately 10.4% of variance in the *Treponema* community structure. At the individual patient level, total *Treponema* abundance at follow-up was strongly positively correlated with BOP at follow-up ($\rho = 0.841$, $p < 0.001$), indicating that patients with greater residual inflammation following SRP retained significantly higher Treponema abundance. This correlated with the findings of Bertelsen *et al* which found that frequent gingival bleeding was

associated with higher abundance of *Treponema denticola*.[24] Furthermore, the change in Treponema abundance from baseline to follow-up was significantly correlated with the change in BOP over the same period ($\rho = 0.644$, $p = 0.003$), suggesting that the degree to which inflammation resolved following SRP was mirrored by a corresponding reduction in Treponema. This relationship was further supported by the Kruskal-Wallis analysis, in which patients in the High BOP tertile at follow-up retained significantly greater Treponema abundance compared to those in the Low BOP tertile ($\chi^2 = 6.63$, $p = 0.036$), with post-hoc Dunn testing confirming a significant difference between the Low and High groups ($p = 0.030$). No such differences in BOP and *Treponema* abundance were observed at T1, suggesting that BOP severity at follow-up, rather than at baseline, is the key clinical correlate of *Treponema* abundance. This also suggests that the divergence between patients emerges as a function of treatment response rather than pre-existing disease characteristics.

Despite minimal changes noted in the *Treponema* community, SRP did lead to a statistically significant reduction in the percentage of sites with CAL $\geq$ 5 mm from 55.6% to 33.3% ($p = 0.0003$), percentage of sites with PPD $\geq$ 6 mm from 27.8% to 16.7% ($p = 0.0002$), and percentage of sites with BOP decreasing from 44.4% to 16.7% ($p = 0.0027$). These findings coincide with literature supporting non-surgical therapy as a first line of treatment for periodontal disease. A series of studies by Badersten found scaling and root planing with either ultrasonic or hand instrumentation led to reductions in plaque, PD, CAL, and BOP.[25] Another study found that there is no upper limit of periodontal probing depth which would render non-surgical therapy ineffective.[26]

From the PERMANOVA, patient demographics as a combined model explained 58.7% of variance in Treponema community composition ($R^2 = 0.589$, $p < 0.005$). Of the 9 categorical demographic and categorical data variables (sex, race/ancestry, tobacco use, marital status, household income, level of education, employment status, brushing frequency, and flossing frequency), education appeared to be the strongest driver of variance ($R^2 = 0.270$, $p = 0.021$) followed by race/ancestry ($R^2 = 0.156$, $p = 0.046$). Subjects with mid-to-lower education levels (GED, nursery school through 8th grade, and some high school) tended to have higher median Treponema abundance at baseline compared to those with higher education levels, though not statistically significant after FDR correlation. This finding correlates with literature which states that individuals with lower education have a greater risk of periodontal disease. A study by Boillot *et al* found that relative to a higher education group, people with low education attainment experience a greater risk of chronic periodontitis with an odds ratio of 1.86.[27] In this study, 8 of the 19 subjects (50%) have less than a high school diploma which could explain this finding. Race and ethnicity have also been considered with increasing prevalence of periodontal disease in Hispanic and non-Hispanic Black people followed by non-Hispanic white people and Asian people.[28] Looking more closely at Treponemes, Yang *et al.* found *T. denticola* to be more significantly prevalent in African Americans when compared to European Americans.[29] In this study 7 participants were Hispanic, 6 were White/Caucasian, 3 were Asian or Pacific Islander, 2 were Black or African American, and 1 subject identified with multiple ethnicities. The wide distribution of race/ancestry and suggested differences of the oral microbiome among persons of different ethnicities can explain the variance in the Treponeme communities found in this study. Neither level of education nor race/ancestry survived FDR correction after individual testing, so it was not deemed necessary to correct for these when processing the data. Subject's age trended

towards significance, but was found not to reach statistical significance after correction (R^2 = 0.150, p = 0.060). This correlates with current literature that states that evidence indicates that the composition of the oral microbiome changes with age, although findings on diversity are inconsistent with reports of both increased and decreased diversity in older adults. It appears these shifts are more greatly influenced by factors such as diet, oral hygiene, and immune function rather than age alone.[30] From the pairwise demographic heatmap, brushing frequency was significantly associated with employment status. Kim *et al.* also found that brushing frequency was lower in people who work part-time jobs as opposed to those fully employed. Interestingly, none of the subjects in this study were tobacco users.[31] Tobacco use has been identified as one of the primary etiologies of periodontal disease and is known to have an effect on the composition of the oral microbiome. Al Kawas *et al.* found that the relative abundance for *Treponema* species were higher in smokers versus non-smokers.[32] With this information it can be presumed that tobacco use would have had a significant impact on the diversity of Treponemes.

At baseline, *Treponema* species were found in 17/19 subjects which is 89% of the study sample. A study by Absuleme *et al.* found *Treponema* to be the most abundant genus in patients with periodontitis making up for approximately 20% of the sequence reads using 16S rRNA sequencing.[33] Despite being identified in the majority of the sample, 2 subjects with severe periodontitis who did not have detectable levels of this putative pathogen in their subgingival microbiome. Periodontal disease is a complex polymicrobial etiologic disease that is typically categorized by an increase in anaerobic bacteria. It is important to understand that periodontitis is not caused by a single pathogen. A study by Wilson *et al.* looked at the prevalence of *Treponema* in periodontally-healthy and periodontally-disease sites and found Treponemes in 43% of healthy sites and 73% of diseased sites which means 27% of diseased sites in their 157 samples

did not contain any species of the *Treponema* genus.[34] The three most abundant species found at baseline in this study were *T. denticola*, *T. sp. HMT-270*, *T. socranskii*. *Treponema denticola* has been extensively associated with periodontal disease, however *T. sp. HMT-270* and *T. socranskii* are less commonly noted species. This study however, found that *T. socranskii* was the species found in the largest number of subjects (12/19) while *T. denticola* was found in 11/19. Moter *et al.* investigated the molecular epidemiology of Treponemes in patients with periodontitis and in periodontitis-resistant subjects and found that there was no significant difference in the presence of *T. denticola* in either group but did find there was significantly more *T. socranskii* found in the periodontitis group.[35] Future studies should be conducted to investigate the role of *T. socranskii* in periodontal disease progression.

Limitations

There were several limitations to this study that should be noted. The small sample size (n = 19) represents the most significant constraint by limiting statistical power across all microbiome analysis and detection of modest effect sizes after FDR correction. Due to the small sample size, confounders such as oral hygiene, race, education, and other variables were unable to be controlled for. The small sample size also proved to be a limitation when looking at the per-species Wilcoxon and DESeq2 analysis, where no individual *Treponema* species reached significance after multiple comparison and adjustment despite consistent trends toward decreased abundance following SRP. The extreme zero-inflation of *Treponema* abundance data further compounded this issue making standard summary statistics such as median and Chao1 richness uninformative for the majority of species, and preventing the inclusion of subject-level blocking in the DESeq2 model due to rank-deficiency of the design matrix. Three samples had critically low read depth which may have resulted in the under detection of *Treponema* at baseline. This

could potentially be attributed to sampling error when collecting the subgingival plaque. Raw read counts were unavailable for this study, therefore estimated counts were derived from relative abundance multiplied by read depth for DESeq2 modeling. Additionally, subgingival plaque samples were pooled from three teeth per subject, so site specific analysis could not be performed. Being able to determine *Treponema* abundance from a site-specific level could give more information on what specific species of *Treponemes* are found in higher abundance in sites with residual CAL $\geq$ 5 mm, PPD $\geq$ 6 mm, or BOP after treatment, thus aiding in the identification of the more pathogenic species Future studies should include a larger sample size and additional follow-up sample to determine if there is a rebound in the *Treponema* community. Other future studies can explore how the addition of local antibiotics as a combination therapy can affect *Treponema* relative abundance. Another future study can investigate changes in *Treponema* relative abundance interplays with clinical results of surgical periodontal therapy which can be used to determine if surgical or non-surgical treatment is more effective in reducing the relative abundance.

The strong association between BOP and *Treponema* raises the question of directionality between the two variables. Due to the observational design of this study, it is not possible to determine whether *Treponema* species actively drives gingival inflammation as evident by BOP, or whether the presence of BOP reflects an inflammatory environment that preferentially supports *Treponema* colonization. Future experimental or longitudinal studies would be needed to establish the temporal relationship between *Treponema* abundance and BOP. Other limitations to this study design are the lack of power analysis and inter-examiner reliability testing which can impact the strength of the findings of this investigation.

CONCLUSION

This study investigated the relationship between subgingival Treponema community composition and clinical periodontal outcomes following non-surgical periodontal therapy in 19 patients with chronic, severe periodontitis. SRP produced significant improvements in all three clinical outcomes, with reductions in the percentage of sites with CAL ≥5 mm, PPD ≥6 mm, and BOP at 4–6 weeks post-treatment. Despite these clinical improvements, SRP did not produce a statistically significant shift in overall Treponema community composition, total Treponema abundance, alpha diversity, or individual species abundance after FDR correction, suggesting that non-surgical periodontal therapy alone may not be sufficient in diminishing the relative abundance of the Treponema community.

The association between Treponema abundance and BOP was the only variable to remain significant after FDR correction in the PERMANOVA ($R^2 = 0.104$, p-adj = 0.013), and the strongest Spearman correlation observed ($\rho = 0.841$, $p < 0.001$). Patients who retained higher Treponema abundance at follow-up had significantly more BOP-positive sites, and those whose Treponema decreased most showed the greatest reductions in BOP. These findings suggest that Treponema abundance is more closely linked to active periodontal inflammation than to cumulative tissue destruction, and that resolution of inflammation following SRP may be the primary driver of Treponema reduction. Notably, two patients had no detectable Treponema at either timepoint yet demonstrated clinical improvement, indicating that Treponema-independent pathways of periodontal disease and recovery exist.

Sociodemographic factors, particularly education level and race/ancestry, explained substantial variance in Treponema community composition, highlighting the potential role of

social determinants of health in shaping the oral microbiome. These findings should be interpreted in the context of study limitations including small sample size, extreme zero-inflation of Treponema abundance data, and short follow-up time. Future studies with larger sample sizes, longer follow-up periods, and raw sequencing count data would be needed to confirm and extend these observations. This study provides evidence that BOP may serve as a clinical correlate of Treponema abundance following periodontal therapy, with implications for the use of microbial biomarkers in monitoring treatment response in periodontitis.

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