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CLINICAL RESEARCH:

From Stability to Dysbiosis: Ecological Profiling of Subgingival Biofilms in Costa Rican Adults with Periodontal Health and Disease-A Pilot Study

De la estabilidad a la disbiosis: Perfil ecológico del biofilm subgingival en adultos costarricenses en salud y enfermedad periodontal-Estudio piloto

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ABSTRACT: This pilot study characterized the subgingival microbiota in Costa Rican adults with and without periodontitis, using 16S rRNA gene sequencing and ecological diversity analysis. The study sample consisted of 20 participants, including 10 with periodontal health and 10 diagnosed with periodontitis, from whom subgingival samples were collected for DNA extraction and sequencing of the V3-V4 region. Amplicon sequence variants (ASVs) were analyzed to assess alpha and beta diversity, identify indicator taxa, and explore microbial co-occurrence patterns. Rarefaction curves indicated adequate sequencing depth. Although alpha diversity indices were higher in the periodontitis group, the differences were not statistically significant ($p>0.05$), but showed moderate effect sizes. Beta diversity revealed significant differences between groups (PERMANOVA, $p<0.05$), with greater dispersion observed in the periodontitis group (Betadisper, $p<0.05$). *P. gingivalis* and *T. denticola* were identified as indicator taxa for the periodontitis group. Microbial networks showed distinct ecological organization between groups. These findings support the ecological model of dysbiosis and provide preliminary evidence on the subgingival microbiota in the Costa Rican population, laying the groundwork for future longitudinal studies on the progression or resolution of periodontal disease.

KEYWORDS: Periodontitis; Microbiota; Ecology; Subgingival space.

RESUMEN: Este estudio piloto caracterizó la microbiota subgingival en adultos costarricenses con y sin periodontitis, mediante secuenciación del gen 16S rRNA y análisis ecológicos de diversidad. Se incluyeron 20 participantes (10 sanos, 10 con periodontitis), a quienes se les recolectaron muestras subgingivales para extracción de ADN y secuenciación de la región V3-V4. Se analizaron variantes de secuencia de amplicón (ASVs) para evaluar diversidad alfa y beta, identificar taxones indicadores y explorar patrones de coocurrencia microbiana. Las curvas de rarefacción indicaron una profundidad adecuada. Aunque los índices de diversidad alfa fueron mayores en el grupo con periodontitis, las diferencias no fueron estadísticamente significativas ($p>0.05$), pero mostraron tamaños del efecto moderados. La diversidad beta reveló diferencias significativas entre grupos (PERMANOVA, $p<0.05$), con mayor dispersión en el grupo con periodontitis (Betadisper, $p<0.05$). Se identificaron como taxones indicadores del grupo con periodontitis, *P. gingivalis* y *T. denticola*. Las redes microbianas evidenciaron una organización ecológica distinta entre grupos. Estos hallazgos respaldan el modelo de disbiosis ecológica y ofrecen evidencia preliminar sobre la microbiota subgingival en población costarricense, aportando una base para futuros estudios longitudinales sobre progresión o resolución de la enfermedad periodontal.

PALABRAS CLAVE: Periodontitis; Microbiota; Ecología; Espacio subgingival.

INTRODUCTION

Periodontitis is a highly prevalent chronic inflammatory disease that affects the supporting tissues of the teeth. It is one of the leading causes of tooth loss in adults. Worldwide, it is estimated that more than 45% of the population has some form of periodontal disease, and about 10% develop severe forms that significantly impair oral function and quality of life (1). In addition to its local effects, periodontitis has been linked to systemic diseases such as type 2 diabetes mellitus, cardiovascular diseases and neurodegenerative processes, in a context of chronic inflammation (2).

The etiologic understanding of periodontitis has evolved from the concept of specific infection, focused on a few pathogenic microorganisms, to a broader model of polymicrobial dysbiosis. This model proposes that disease emerges from alterations in the composition, structure and functional interactions of complex microbial communities in response to changes in the host and oral microenvironment (3). In this context, the subgingival biofilm is no longer a simple sum of pathogens but is understood as a dynamic ecosystem, in which processes such as inflammation, competition for nutrients and ecological coadaptation shape its architecture (4).

Sequencing the 16S rRNA gene has enabled to characterize in greater depth the bacterial diversity present in the subgingival sulcus, allowing the detection of previously unculturable organisms (5). This technique has been key to describe patterns of alpha (intraindividual) and beta (between individuals or groups) diversity, revealing that the microbial composition can be differentiated according to periodontal health status (6).

Recent studies have highlighted the importance of analyzing not only the oral microbiota composition, but also its ecological and functional organization. Rather than focusing exclusively on individual taxa, these approaches consider aspects such as diversity, community stability, and species interactions, aspects that could be related to periodontal status (2,5). Although it has been proposed that these characteristics vary between periodontally healthy individuals and those with

periodontitis, the findings are not uniform, and may depend on methodological, clinical and population factors (7,8).

Most of the available studies have been conducted in populations of European descent, which limits the applicability of their findings to other populations (9). In Latin America, and particularly in Costa Rica, there is a notable lack of research exploring the oral microbiota from an ecological perspective (10).

In this context, this study aimed to characterize and compare the subgingival microbiota of periodontally healthy adults and those with periodontitis treated at the Faculty of Dentistry of the University of Costa Rica, using 16S sequencing-based analyses and ecological diversity metrics. This effort attempts to provide local evidence on oral microbial ecology and lay the groundwork for future clinical and epidemiological studies.

MATERIALS AND METHODS

PATIENT CLASSIFICATION AND SAMPLE COLLECTION

During 2022, at the Periodontal Clinic of the Faculty of Dentistry, University of Costa Rica, two groups of adult participants were recruited: a control group (n=10) with no clinical signs of periodontal disease, and a group with periodontitis (n=10), diagnosed according to standard periodontal clinical criteria. The control group consisted of dentists and dental faculty members. All participants provided informed consent prior to inclusion. Periodontitis was diagnosed based on the 2017 Classification of Periodontal and Peri-Implant Diseases and Conditions. Individuals exhibiting a clinically healthy periodontium, in the absence of biofilm-induced gingivitis or periodontitis, were considered periodontally healthy. All samples were obtained by a calibrated periodontal expert (GR-G) to ensure consistency and reliability in the collection process. Subgingival samples were collected by sterile swabbing of the deepest periodontal sulcus or pocket of each patient. Samples were placed in sterile 1X PBS and refrigerated or frozen.

DNA PRESERVATION AND EXTRACTION

After collection, samples were sent within 24 hours to the laboratory, and stored at -80°C until processing. Bacterial genomic DNA extraction was performed with a protocol based on chemical lysis, using the QIAamp DNA Micro Kit (QIAGEN, Germany). DNA quality and quantity were verified by spectrophotometry and electrophoresis.

DNA SEQUENCING AND DATA ANALYSIS

Samples were sequenced (16S rRNA gene, V3-V4 region: primers 341F-805R) by Macrogen (Seoul, South Korea). Raw data were processed by the bioinformatics service of the same company employing the ASV analysis pipeline (DADA2) and the NCBI database.

ANALYSIS OF MICROBIAL DIVERSITY

Preliminary results submitted by the company were analyzed and statistical tests were performed. The depth of sequencing coverage was analyzed using rarefaction curves as a preliminary to the analysis. Then, alpha diversity was assessed using Shannon, Gini-Simpson, ASVs richness and phylogenetic diversity (PD_whole_tree) indices. Statistical differences were analyzed with the Wilcoxon test, and classical thresholds were used to determine the magnitude of the effect. Next, beta diversity was explored using Bray-Curtis, weighted and unweighted UniFrac distances, and represented by principal coordinate analysis (PCoA) and non-metric scaling (NMDS). Differences between groups were evaluated using PERMANOVA and Betadisper tests. Finally, core taxa (based on

prevalence in ≥9 of 10 samples from at least one of the groups and abundance ≥5% in the corresponding group), indicator species (indicator value index, IndVal with significance assessed by permuntations test and correction by FDR), and co-occurrence patterns (Spearman's correlations $|\rho| \geq 0.7$) were identified.

ETHICS AND DATA

The protocol number of this study (440-C1-364) was approved by the Ethical Review Committee of the University of Costa Rica (CEC-589-2020) and registered in the National Health Research Council (CONIS-38-2022). All participants signed an informed consent.

RESULTS

In this pilot study, subgingival bacterial diversity was evaluated in two groups, periodontally healthy individuals and periodontitis patients. The analysis started with the evaluation of sequencing depth by means of rarefaction curves. The average rarefaction curves per group showed an increasing trend in the detection of ASVs as the sequencing depth increased (Figure 1.A). It was observed that both groups reached a plateau, indicating that the sequencing depth was sufficient to capture most of the bacterial diversity present in the samples. The maximum number of ASVs observed per sample did not present significant differences between the control and periodontitis groups (Figure 1.B). Similarly, the estimated depth of stabilization, which represents the number of readings needed to reach the plateau in the rarefaction curve, was comparable between groups (Figure 1.C). No statistically significant differences were observed between groups: Welch's t-test for maximum ASVs and Mann-Whitney test for depth of stabilization (Table S1). These results suggest that sequencing coverage was adequate and homogeneous between groups, allowing reliable comparison of subsequent diversity metrics.

Alpha diversity in the subgingival samples was studied using the Shannon index, the Gini-Simpson index, the richness of unique variants (ASVs) and the phylogenetic diversity index (whole tree). In the four indexes analyzed, the samples from the control group presented lower median values than those from the periodontitis group (Figure 2). This suggests a trend towards greater subgingival microbial diversity in the presence of periodontal disease. Although the differences were not statistically significant, according to the Wilcoxon test for independent samples, the calculated effect sizes were of moderate magnitude in all indices (Table S2).

To explore differences in the overall composition of the subgingival microbiota between the periodontitis groups and controls, we assessed: i. beta diversity using distance-based metrics, ii. visualizations with principal coordinate analysis (PCoA) and non-metric scaling (NMDS), and iii. statistical analyses (PERMANOVA and Betadisper). In all cases, PCoA analyses showed some degree of overlap between groups, although separating trends were observed. The first two principal components explained about 30-65% of the variance (Figure 3). NMDS analyses showed acceptable stress values for all metrics (<0.15), indicating reliable representations of dissimilarities between samples (Figure 3). PERMANOVA testing revealed significant differences in microbial composition between groups in all three metrics analyzed (Table S3), these results suggest that differences in microbial composition between periodontitis subjects and controls explain about 9-16% of the observed variation.

Multivariate dispersion analysis (Betadisper) indicated that only for the Bray-Curtis metric there were significant differences in dispersion within groups, with the periodontitis group showing the greatest variability (Figure 3 and Table S3).

Finally, to characterize the main taxa and ecological patterns associated with each group, core genera, taxa with significant indicator value, and subgingival microbial co-occurrences were analyzed (Figure 4). Eight core genera were identified that met the criteria of prevalence (≥90%) and average relative abundance (≥5%) in at least one of the groups (Figure 4.A). Although some genera, such as *Streptococcus*, *Fusobacterium*, and *Veillonella*, were present in both groups, notable differences in their relative distribution were observed. In particular, *Porphyromonas* and *Treponema* showed higher prevalence and abundance in the periodontitis group.

The indicator value index (IndVal) analysis allowed the identification of nine taxa significantly associated with the periodontitis group (p FDR<0.05) and none for the control group, distributed among phylum, family, genus and species levels (Figure 4.B). Among the most prominent were *Porphyromonas gingivalis* and *Treponema denticola*, both recognized for their role in periodontal pathogenesis.

The cooccurrence network showed strong associations between microbial species ($|\rho| \geq 0.7$), both positive and negative, differentiated by group (Figure 4.C). The periodontitis group exhibited a dense cluster of positive interactions between pathogens such as *P. gingivalis*, *T. denticola* and *Prevotella intermedia*, as well as negative correlations with commensals such as *Streptococcus sanguinis*. In contrast, the control group exhibited positive interactions between typically commensal genera, such as *Actinomyces*, *Corynebacterium* and *Rothia*.

Finally, significant group-specific co-occurrences were identified ($|\rho| \geq 0.7$, $p<0.05$), i.e., species pairs whose association was observed exclusively in one of the groups (Figure 4.D). In the periodontitis group, associations between species with pathogenic potential predominated, whereas in the controls, interactions typical of more structured and mutualistic communities were observed. Thus, positive associations between periodontopathogenic species and dissociation with commensals were observed in the periodontitis group. All taxa analyzed at the species level are summarized in Table S4.

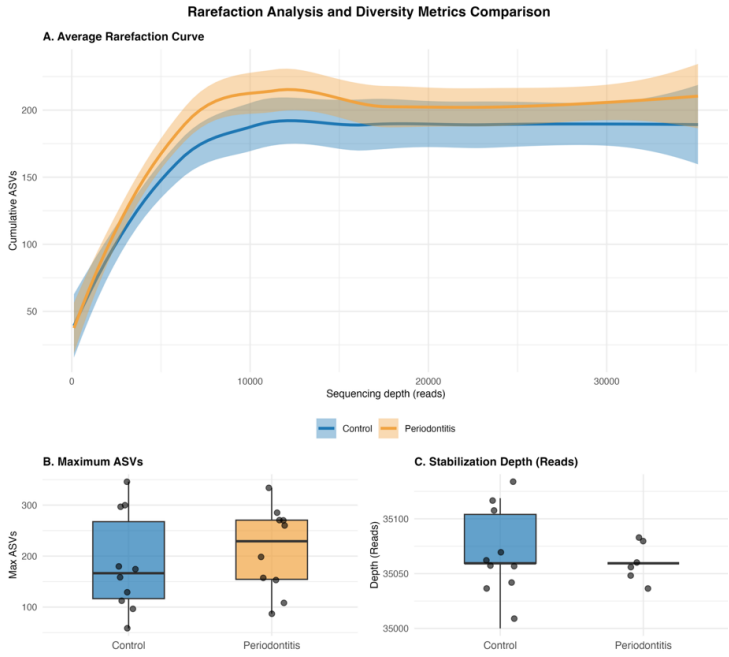

Figure 1. Evaluation of the rarefaction process. (A) Average rarefaction curves per group, with 95% confidence intervals. (B) Comparison of the maximum number of amplicon sequence variants (ASVs) observed per sample in each group. (C) Comparison of the estimated depth of stabilization in each group.

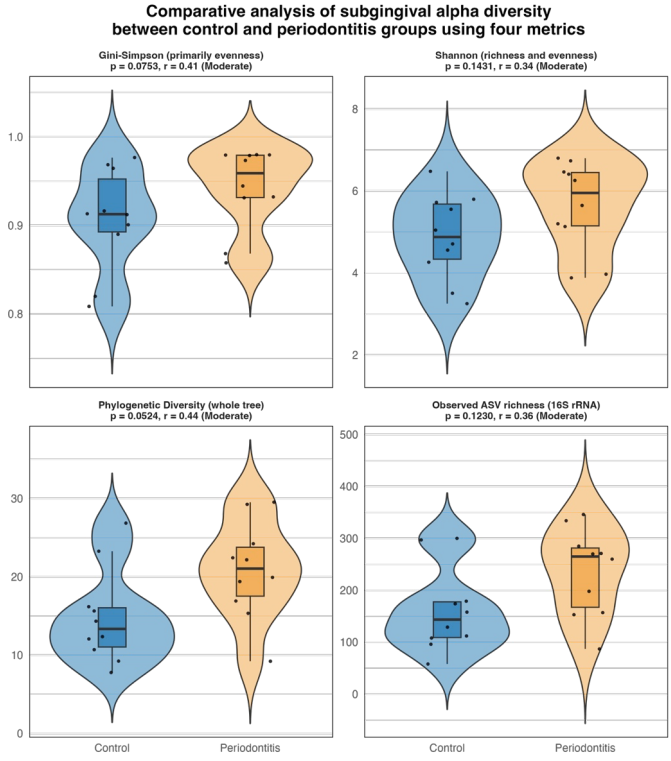

Figure 2. Subgingival alpha diversity in controls and patients with periodontitis. Comparison of four indices: Shannon, Gini-Simpson, phylogenetic diversity (whole tree) and ASV richness. The periodontitis group presented higher values in all cases. Differences were not significant (p>0.05), but with moderate effect sizes.

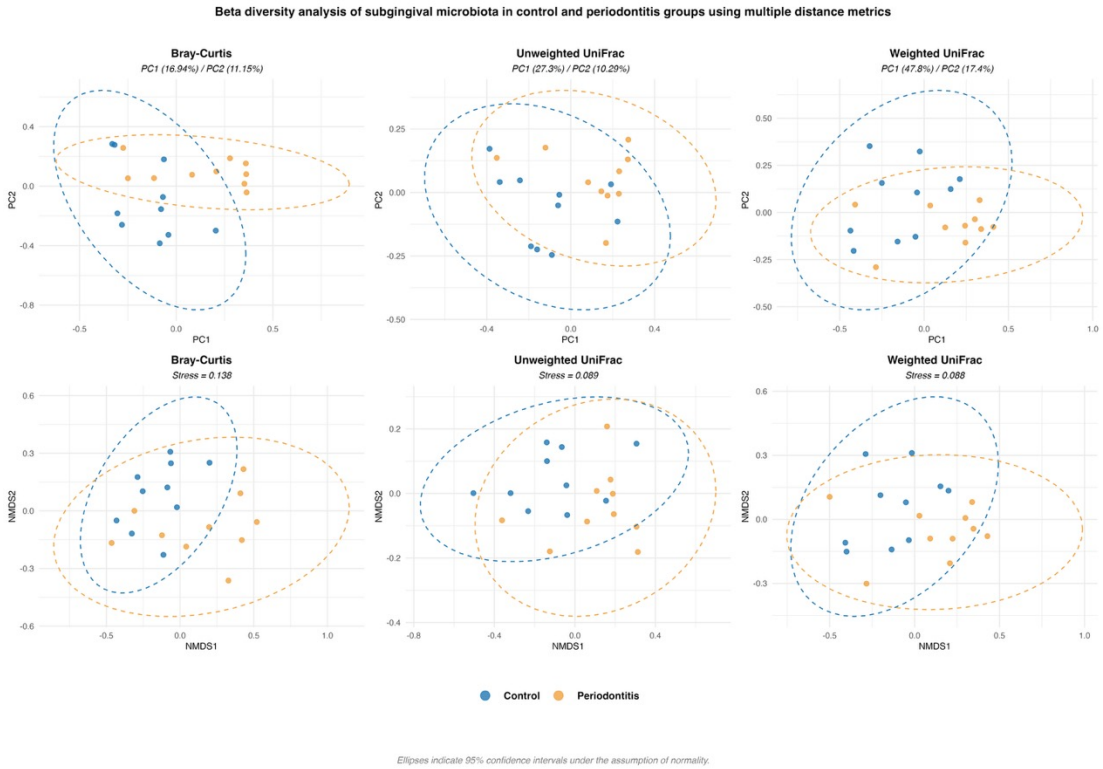

Figure 3. Subgingival beta diversity in controls and patients with periodontitis. Visualization by PCoA and NMDS using three metrics: Bray-Curtis, Unweighted and Weighted UniFrac. Significant differences were observed between groups (PERMANOVA, $p<0.05$). Greater dispersion in the periodontitis group for Bray-Curtis.

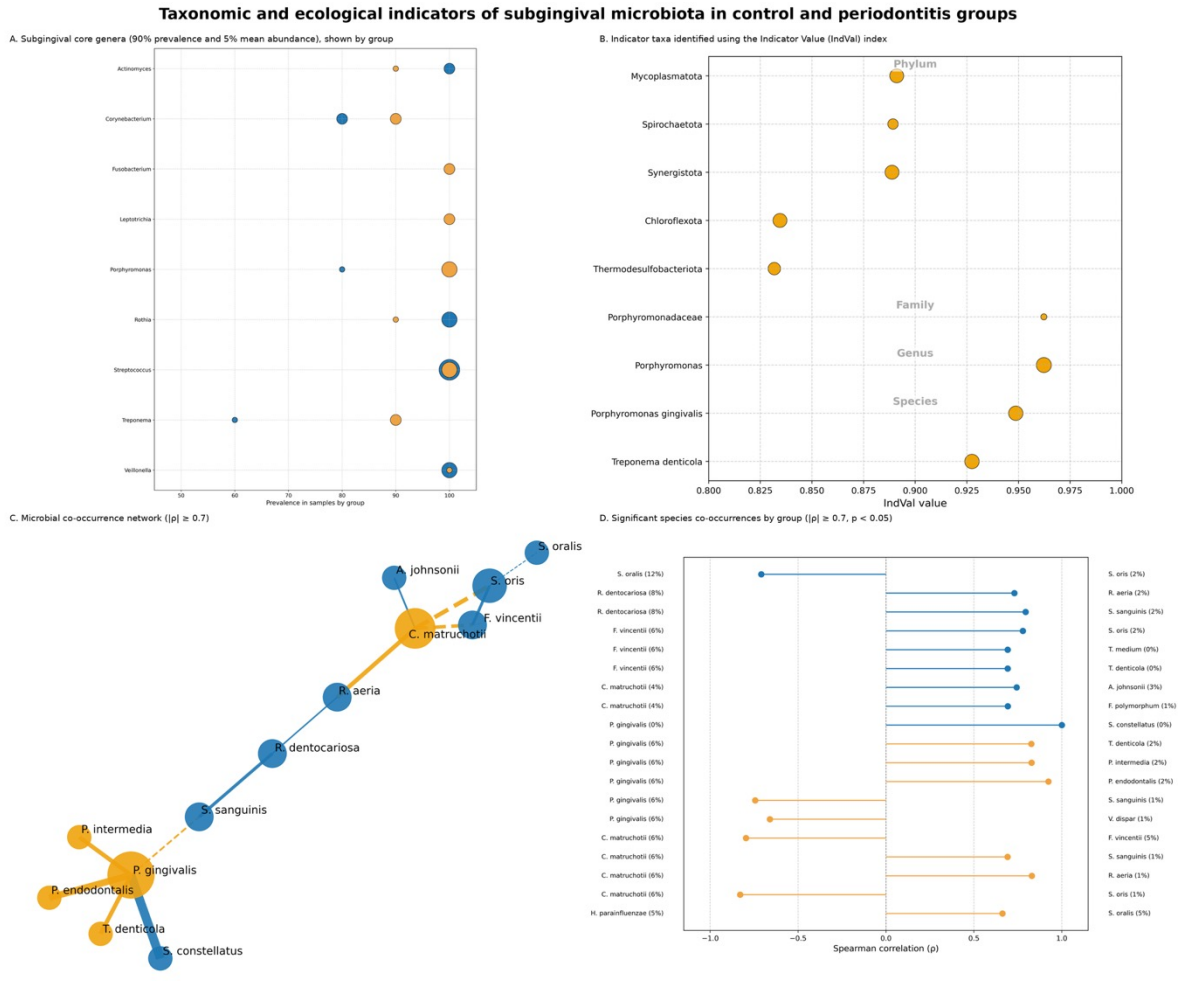

Figure 4. Subgingival microbial indicators in controls and patients with periodontitis. (A) Core genera with ≥90% prevalence and ≥5% mean relative abundance. (B) Taxa significantly associated with the periodontitis group (IndVal index, p FDR<0.05). (C) Network of cooccurrences among species (|ρ|≥0.7), differentiated by type and group. (D) Species pairs with significant group-specific cooccurrences (|ρ|≥0.7, p<0.05).

DISCUSSION

This pilot study explored the subgingival microbial composition in periodontally healthy and with periodontitis adults. Despite the small sample size (n=10 per group), rarefaction curves indicated adequate coverage of the diversity present, which supports the interpretation of the observed patterns. Similar studies have shown that even with modest sample sizes robust differences in oral microbiota can be identified (11).

One of the most notable findings was the trend toward greater alpha diversity in the periodontitis group, observed in all the indices used. Although the differences were not statistically significant (p>0.05), the effect sizes were moderate, suggesting possible biological relevance. One possible reason explaining the lack of a statistically significant difference may related to the small sample size. This result contrasts with the traditional paradigm associating health with increased diversity, but is consistent with recent observations

showing that periodontitis may be accompanied by functional diversification of the microbial community (11,12). In fact, studies in several populations have reported increases in alpha diversity from health to periodontitis, possibly reflecting an expansion of ecological niches in an inflamed environment (11).

The differences between groups were more marked at the beta diversity level. PERMANOVA analysis confirmed a significant separation between the bacterial communities of both groups, and dispersion analysis (Betadisper) showed greater internal variability in the periodontitis group. This suggests not only a change in composition, but also greater ecological instability, in line with the dysbiosis model according to which an unbalanced microbial community can promote inflammation and tissue damage (3). Beta diversity thus suggests a deeper ecological dysregulation in cases of periodontitis (13).

Analysis of microbial indicators revealed a significant association between *P. gingivalis* and *T. denticola* with the periodontitis group. These findings do not represent a taxonomic novelty, but they validate the ability of the analytical approach employed to detect relevant associations. Both microorganisms have historically been defined as members of the "red complex", strongly associated with advanced forms of periodontal disease (14). Their persistent identification in modern studies, including those in Latin America, reinforces their role as functional nuclei of dysbiotic communitinies (15).

In addition of the individual presence of taxa, this study examined the ecological organization of subgingival microbial communities. In periodontally healthy subjects, microbial networks characterized by positive interactions between commensal genera such as *Actinomyces* and *Rothia* were observed, suggesting a stable mutualistic structure. In contrast, the networks of the periodontitis group were denser and more complex, with numerous co-occurrences between pathogens, including both positive and negative interactions. This pattern is consistent with recently proposed models of dysbiotic networks, in which disease is not only associated with particular taxa, but also with a profound reorganization of ecological interactions (16).

This change in community architecture has not only ecological but also clinical implications. Cooccurrence networks could reflect functional dynamics of microbial competition or synergy that modulate inflammation. This perspective has motivated proposals to use not only the presence of taxa, but also their interactions as potential diagnostic or progression biomarkers (17). Some authors have postulated that these patterns may even precede clinical changes and thus be useful for early intervention (6,18).

In this regard, the findings of the present study could be the starting point for developing broader hypotheses in prospective studies. The identification of unstable and pathogen-dominant communities could inform periodontal treatment monitoring schemes or predict clinical response. Longitudinal research analyzing how the microbial ecology changes during disease progression or resolution is required, ideally incorporating host factors as well.

Finally, this study represents the first analysis of the subgingival microbiota in health and periodontitis in the Costa Rican population, at the Faculty of Dentistry of the University of Costa Rica. Its relevance lies not only in the specific findings, but also in its contribution to a scarce database in Latin American contexts. Studies in Colombia and Mexico have shown significant geographic variability in subgingival composition (6,11,15), which reinforces the need to develop local profiles to improve the diagnosis and treatment of periodontitis in our populations.

CONCLUSIONS

This pilot study provides an initial characterization of the subgingival microbiota in Costa Rican adults with and without periodontitis, showing differences in diversity, community structure and presence of indicator taxa. Despite the small sample size, the analyses suggest ecological patterns consistent with contemporary models of dysbiosis. The identification of associated taxa and distinct microbial networks between groups reinforces their potential diagnostic value. Future longitudinal studies with larger sample sizes will allow us to evaluate the use of microbial profiles and their interactions as biomarkers of progression, resolution or response to periodontal treatment in local clinical contexts.

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DISCLOSURE STATEMENT: The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION STATEMENT: Study concept and design: C.B.S. and L.A.A.; Data acquisition: C.B.S.; Drafting of the manuscript: C.B.S. and L.A.A.; Clinical evaluation: G.R.G.; Critical revision of the manuscript: C.B.S., G.R.G., K.R.C. and L.A.A.; Analysis and interpretation of data: L.A.A.; Statistical analysis: L.A.A.; Supervision: L.A.A.; Administrative support: C.B.S.

DATA AVAILABILITY: Data will be made available on request.

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SUPPLEMENTARY DATA

Table S1. Descriptive statistics and statistical test results for rarefaction metrics.

Diversity Metric	Control (Mean ± SD)	Periodontitis (Mean ± SD)	p-value
Maximum ASVs Observed	185.13 ± 97.07	212.41 ± 83.10	0.508
Stabilization Depth (Reads)	35071.31 ± 37.58	34715.73 ± 880.61	0.464

ASVs observed and the depth of stabilization for the control and periodontitis groups are presented. The p-values correspond to the tests comparing the groups (Welch's t-test for maximum ASVs and Mann-Whitney U test for stabilization depth). No significant differences were observed between groups ($p>0.05$), indicating comparable sequencing coverage.

Table S2. Comparison of subgingival alpha diversity between controls and patients with periodontitis.

Diversity Metric	Control (Mean)	Periodontitis (Mean)	p-value	Effect size (r)	Interpretation
Shannon	4.89	5.65	0.143	0.34	Moderate
Gini-Simpson	0.91	0.94	0.075	0.41	Moderate
Phylogenetic diversity (whole tree)	20.83	14.84	0.052	0.44	Moderate
ASVs	161.10	236.10	0.123	0.36	Moderate

Averages of alpha diversity indices: Shannon index, Gini-Simpson index, phylogenetic diversity (PD_whole_tree), and ASV richness. P values (Wilcoxon test), effect size (r), and qualitative interpretation are presented. Although the differences were not statistically significant ($p>0.05$), all indices showed moderate effect sizes.

Table S3. Comparison of subgingival beta diversity between controls and patients with periodontitis.

Distance Metric	Test	R^2	F-value	p-value	Significance
Bray-Curtis	PERMANO-VA	0.091	1.797	0.011	Significant
Unweighted UniFrac	PERMANO-VA	0.115	2.348	0.009	Significant
Weighted UniFrac	PERMANO-VA	0.158	3.382	0.021	Significant
Bray-Curtis	Betadisper	N/A	5.532	0.027	Significant
Unweighted UniFrac	Betadisper	N/A	0.053	0.811	Not Significant
Weighted UniFrac	Betadisper	N/A	0.005	0.955	Not Significant

*N/A: not applicable

Results of PERMANOVA and Betadisper analyses for three distance metrics: Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac. R^2, F, and p values are included. Significant differences between groups were detected according to PERMANOVA in all metrics. Only Bray-Curtis showed significant differential dispersion between groups (Betadisper, $p=0.028$).

Table S4. Subgingival taxa at the species level identified in core microbiota analyses, indicator value (IndVal), and co-occurrences.

Taxon or Taxon Pair	Associated Group	Analysis Type	Summary
Actinomyces johnsonii – C. matruchotii	Control	Cooccurrence	ρ=0.740
Actinomyces johnsonii – Rothia aeria	Control	Cooccurrence	ρ=0.700
Actinomyces johnsonii – Streptococcus sanguinis	Control	Cooccurrence	ρ=0.640
Corynebacterium matruchotii	Both	Core	Abundance: 4.5% (control), 5.8% (periodontitis)
Corynebacterium matruchotii – A. johnsonii	Control	Cooccurrence	ρ=0.740
Corynebacterium matruchotii – Actinomyces johnsonii	Control	Cooccurrence	ρ=0.743
Corynebacterium matruchotii – F. vincentii	Periodontitis	Cooccurrence	ρ=-0.800
Corynebacterium matruchotii – Fusobacterium polymorphum	Control	Cooccurrence	ρ=0.693
Corynebacterium matruchotii – Fusobacterium vincentii	Periodontitis	Cooccurrence	ρ=-0.796
Corynebacterium matruchotii – R. aeria	Periodontitis	Cooccurrence	ρ=0.830
Corynebacterium matruchotii – Rothia aeria	Periodontitis	Cooccurrence	ρ=0.829
Corynebacterium matruchotii – S. oris	Periodontitis	Cooccurrence	ρ=-0.830
Corynebacterium matruchotii – Segatella oris	Periodontitis	Cooccurrence	ρ =-0.829
Corynebacterium matruchotii – Streptococcus sanguinis	Periodontitis	Cooccurrence	ρ=0.691
Fusobacterium polymorphum – Neisseria elongata	Control	Cooccurrence	ρ=0.877
Fusobacterium polymorphum – Prevotella intermedia	Control	Cooccurrence	ρ=0.664
Fusobacterium vincentii	Both	Core	Abundance: 5.8% (control), 4.7% (periodontitis)
Fusobacterium vincentii – Actinomyces johnsonii	Periodontitis	Cooccurrence	ρ=-0.731
Fusobacterium vincentii – C. matruchotii	Periodontitis	Cooccurrence	ρ=-0.800
Fusobacterium vincentii – Porphyromonas endodontalis	Periodontitis	Cooccurrence	ρ=0.644
Fusobacterium vincentii – Rothia aeria	Periodontitis	Cooccurrence	ρ=-0.869
Fusobacterium vincentii – S. oris	Control	Cooccurrence	ρ=0.780
Fusobacterium vincentii – Segatella oris	Periodontitis	Cooccurrence	ρ=0.900
Fusobacterium vincentii – Segatella oris	Control	Cooccurrence	ρ=0.778
Fusobacterium vincentii – Streptococcus sanguinis	Periodontitis	Cooccurrence	ρ=-0.890
Fusobacterium vincentii – Treponema denticola	Control	Cooccurrence	ρ=0.692
Fusobacterium vincentii – Treponema medium	Control	Cooccurrence	ρ=0.692
Haemophilus parainfluenzae – Streptococcus oralis	Periodontitis	Cooccurrence	ρ=0.663
Haemophilus parainfluenzae – Streptococcus sanguinis	Periodontitis	Cooccurrence	ρ=0.654
Porphyromonas endodontalis – P. gingivalis	Periodontitis	Cooccurrence	ρ=0.920
Porphyromonas endodontalis – Prevotella intermedia	Control	Cooccurrence	ρ=0.694
Porphyromonas endodontalis – Streptococcus sanguinis	Periodontitis	Cooccurrence	ρ=-0.737
Porphyromonas endodontalis – Treponema denticola	Control	Cooccurrence	ρ =0.902
Porphyromonas endodontalis – Treponema medium	Control	Cooccurrence	ρ=0.902
Porphyromonas gingivalis	Periodontitis	IndVal	IndVal=0.949 (p=0.001, FDR=0.019)
Porphyromonas gingivalis	Both	Core	Abundance: 0.0% (control), 6.0% (periodontitis)

Taxon or Taxon Pair	Associated Group	Analysis Type	Summary
Porphyromonas gingivalis – P. endodontalis	Periodontitis	Cooccurrence	ρ=0.920
Porphyromonas gingivalis – P. intermedia	Periodontitis	Cooccurrence	ρ=0.830
Porphyromonas gingivalis – Porphyromonas endodontalis	Periodontitis	Cooccurrence	ρ=0.924
Porphyromonas gingivalis – Prevotella intermedia	Periodontitis	Cooccurrence	ρ=0.828
Porphyromonas gingivalis – S. constellatus	Control	Cooccurrence	ρ=1.000
Porphyromonas gingivalis – S. sanguinis	Periodontitis	Cooccurrence	ρ=-0.740
Porphyromonas gingivalis – Streptococcus constellatus	Control	Cooccurrence	ρ= 1.000
Porphyromonas gingivalis – Streptococcus sanguinis	Periodontitis	Cooccurrence	ρ=-0.744
Porphyromonas gingivalis – T. denticola	Periodontitis	Cooccurrence	ρ=0.830
Porphyromonas gingivalis – Treponema denticola	Periodontitis	Cooccurrence	ρ=0.827
Porphyromonas gingivalis – Veillonella dispar	Periodontitis	Cooccurrence	ρ=-0.661
Prevotella intermedia – P. gingivalis	Periodontitis	Cooccurrence	ρ=0.830
Prevotella intermedia – Porphyromonas endodontalis	Periodontitis	Cooccurrence	ρ=0.794
Prevotella intermedia – Treponema denticola	Control	Cooccurrence	ρ=0.796
Prevotella intermedia – Treponema medium	Control	Cooccurrence	ρ=0.796
Prevotella intermedia – Veillonella dispar	Periodontitis	Cooccurrence	ρ=-0.681
Rothia aeria – Actinomyces johnsonii	Periodontitis	Cooccurrence	ρ=0.798
Rothia aeria – C. matruchotii	Periodontitis	Cooccurrence	ρ=0.830
Rothia aeria – Neisseria elongata	Control	Cooccurrence	ρ=0.650
Rothia aeria – R. dentocariosa	Control	Cooccurrence	ρ=0.730
Rothia aeria – Segatella oris	Periodontitis	Cooccurrence	ρ=-0.921
Rothia aeria – Streptococcus sanguinis	Control	Cooccurrence	ρ=0.669
Rothia dentocariosa	Both	Core	Abundance: 8.1% (control), 2.6% (periodontitis)
Rothia dentocariosa – Actinomyces oris	Periodontitis	Cooccurrence	ρ=0.753
Rothia dentocariosa – R. aeria	Control	Cooccurrence	ρ=0.730
Rothia dentocariosa – Rothia aeria	Control	Cooccurrence	ρ=0.730
Rothia dentocariosa – S. sanguinis	Control	Cooccurrence	ρ=0.790
Rothia dentocariosa – Streptococcus sanguinis	Control	Cooccurrence	ρ=0.794
Segatella oris – C. matruchotii	Periodontitis	Cooccurrence	ρ=-0.830
Segatella oris – F. vincentii	Control	Cooccurrence	ρ=0.780
Segatella oris – S. oralis	Control	Cooccurrence	ρ=-0.710
Streptococcus constellatus – P. gingivalis	Control	Cooccurrence	ρ=1.000
Streptococcus constellatus – Porphyromonas endodontalis	Periodontitis	Cooccurrence	ρ=0.705
Streptococcus oralis	Both	Core	Abundance: 11.7% (control), 4.7% (periodontitis)
Streptococcus oralis – S. oris	Control	Cooccurrence	ρ=-0.710
Streptococcus oralis – Segatella oris	Control	Cooccurrence	ρ=-0.709
Streptococcus sanguinis – Actinomyces johnsonii	Periodontitis	Cooccurrence	ρ=0.748
Streptococcus sanguinis – P. gingivalis	Periodontitis	Cooccurrence	ρ=-0.740

Taxon or Taxon Pair	Associated Group	Analysis Type	Summary
Streptococcus sanguinis – R. dentocariosa	Control	Cooccurrence	ρ=0.790
Streptococcus sanguinis – Rothia aeria	Periodontitis	Cooccurrence	ρ=0.844
Streptococcus sanguinis – Segatella oris	Periodontitis	Cooccurrence	ρ=-0.820
Treponema denticola	Periodontitis	IndVal	IndVal=0.927 (p=0.002, FDR=0.019)
Treponema denticola – P. gingivalis	Periodontitis	Cooccurrence	ρ=0.830
Treponema denticola – Porphyromonas endodontalis	Periodontitis	Cooccurrence	ρ=0.951
Treponema denticola – Prevotella intermedia	Periodontitis	Cooccurrence	ρ=0.646
Treponema denticola – Streptococcus sanguinis	Periodontitis	Cooccurrence	ρ=-0.743
Treponema medium – Treponema denticola	Control	Cooccurrence	ρ=1.000
Treponema medium – Treponema denticola	Periodontitis	Cooccurrence	ρ=0.747
Veillonella dispar	Both	Core	Abundance: 10.5% (control), 0.6% (periodontitis)

Species that were part of the core microbiota (criteria: ≥90% prevalence and ≥5% average relative abundance in at least one of the groups), species significantly associated with periodontitis using the indicator value index (IndVal, p FDR<0.05), and species included in pairs with significant co-occurrences ($\rho \geq 0.7$, $p<0.05$), both in the global network and in specific associations by group, are included in this table. The summary column shows the relative abundance value per group (core analysis), the IndVal index value (with p and FDR), or the correlation coefficient ρ. The associated group is indicated when applicable and the type of analysis is specified in the last column.