Subgingival Microbiome Composition and Diversity in Plaque-Induced Gingivitis: Insights from 16S rRNA Gene Sequencing Studies

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Abstract

Background: Plaque-induced gingivitis is associated with subgingival microbiome alterations, but their consistency and clinical relevance remain unclear.
Aim: To summarize recent evidence on subgingival microbiome composition and diversity in gingivitis based on 16S rRNA sequencing studies.
Methods: A narrative synthesis of experimental, observational, and interventional studies assessing subgingival microbiome profiles in health and gingivitis was performed.
Results: Experimental models showed increased Bacteroidota and Fusobacteriota and decreased Bacillota, Pseudomonadota, and Actinomycetota. Genera such as Prevotella, Fusobacterium, Capnocytophaga, and Porphyromonas were enriched during plaque accumulation. Alpha diversity findings were inconsistent, whereas beta diversity analyses consistently detected compositional shifts. Clinical and interventional studies reported limited changes in overall diversity despite improvement in gingival inflammation. Longitudinal data indicated incomplete return to baseline microbial composition after short-term resolution.
Conclusions: Gingivitis is associated with reproducible shifts in subgingival microbial composition, primarily reflecting changes in relative abundance within existing communities. Microbiome alterations may persist beyond clinical recovery, and diversity metrics show variable sensitivity. Further standardized longitudinal studies are required. (**TCM-GMJ August 2026; 11 (3): P3-P7).**

Keywords: Subgingival Microbiome; Gingivitis; 16S rRNA; Microbial Diversity; Periodontal Disease; Dysbiosis; Oral Microbiology

Introduction

The subgingival sulcus represents a biologically constrained ecological niche in which microbial communities are shaped by host-derived substrates, oxygen gradients, and mechanical forces. Within this environment, biofilm formation is a dynamic process involving structured microbial interactions and continuous adaptation to local conditions. Contemporary models of periodontal disease emphasize that disease expression is associated with shifts in microbial community composition rather than the presence of a single pathogenic species, consistent with the concept of polymicrobial synergy and dysbiosis [1,2, 10, 13].

Plaque-induced gingivitis is defined as an inflammatory condition of the gingival tissues resulting from the accumulation of dental biofilm, characterized clinically by signs such as bleeding on probing in the absence of clinical attachment loss [3, 15]. It is considered a reversible condition and represents a key stage in the prevention of periodontitis. Current classification frameworks distinguish gingivitis from periodontitis based on the absence of irreversible periodontal tissue destruction, while recognizing gingivitis as the most prevalent inflammatory gingival condition worldwide [1, 15].

From a microbiological perspective, gingivitis is increasingly interpreted as an intermediate ecological state within the oral microbiome. Subgingival microbial communities in gingivitis may show features associated with periodontal health or may demonstrate compositional characteristics observed in periodontitis. This variability suggests that gingivitis does not correspond to a single microbial profile but rather represents a spectrum of community configurations within a broader dysbiosis continuum [2]. Reanalysis of sequencing datasets and integrative microbiome studies have shown that taxa commonly associated with periodontitis can be detected in gingivitis at low abundance, supporting the concept of gradual ecological transitions rather than discrete disease states [2,3].

The application of 16S rRNA gene sequencing has sub-

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stantially expanded the characterization of subgingival microbial communities. Amplicon-based sequencing enables culture-independent detection of diverse bacterial taxa, including those that are difficult to cultivate, and allows for the assessment of microbial composition and diversity within complex biofilms [4]. This approach has been widely used in studies of gingival health and disease, including experimental gingivitis models that examine temporal changes in microbial communities during controlled plaque accumulation [1,2].

Despite these advances, findings across studies remain variable. Differences in targeted hypervariable regions of the 16S rRNA gene, sequencing platforms, and bioinformatics pipelines can influence taxonomic resolution and diversity estimates [4,6]. In addition, variability in subgingival sampling strategies, including site selection and collection methods, may affect the detection of microbial taxa and the comparability of results across studies [14]. These methodological factors limit the ability to directly compare findings and complicate the interpretation of microbial patterns associated with gingivitis.

Although the microbiome of periodontitis has been extensively investigated, the subgingival microbiome in plaque-induced gingivitis remains less consistently characterized, particularly in studies focusing on early inflammatory changes. There is a need to synthesize recent sequencing-based evidence to clarify patterns of microbial composition and diversity in gingivitis and to identify consistent findings across heterogeneous study designs.

The aim of this review is to summarize current evidence from 16S rRNA gene sequencing studies on subgingival microbiome composition and diversity in plaque-induced gingivitis, with attention to methodological variability and its impact on reported findings.

Methods

Study design

This study was conducted as a narrative review of published literature examining subgingival microbiome composition and diversity in plaque-induced gingivitis using 16S rRNA gene sequencing.

Search strategy

A structured literature search was performed using PubMed and Scopus databases. The search strategy included combinations of the following terms: "gingivitis", "plaque -induced gingivitis", "subgingival microbiome", "subgingival plaque", "16S rRNA", "amplicon sequencing", and "oral microbiome".

The search was limited to studies published between 2020 and 2026. Only articles published in English were considered. Additional relevant studies were identified through manual screening of reference lists of selected publications.

Eligibility criteria

Studies were included if they investigated plaque-induced gingivitis, included analysis of subgingival plaque samples, applied 16S rRNA gene sequencing, and reported outcomes related to microbial composition and/or diversity. Studies were excluded if they focused exclusively on periodontitis, analyzed only supragingival samples, did not use sequencing-based methods, or included non-human subjects.

Data extraction

Initial database search yielded **186** articles. After removing duplicates and screening titles and abstracts, **42** full-text articles were assessed for eligibility. Finally, **14** studies were included in the narrative synthesis. Data from eligible studies were extracted and reviewed descriptively. Extracted variables included study design, sample size and population characteristics, subgingival sampling methods, targeted 16S rRNA gene regions, sequencing platforms, bioinformatics pipelines, and reported outcomes related to microbial composition and diversity.

Data synthesis

Given the heterogeneity in study design, sampling protocols, sequencing regions, and analytical approaches, a quantitative synthesis was not performed. Findings were synthesized qualitatively, with emphasis on patterns that were consistently reported across studies.

Methodological considerations

Methodological variability across studies was considered during interpretation. Differences in subgingival sampling techniques, sequencing regions, and bioinformatics workflows may influence taxonomic resolution and diversity estimates. Variability in sampling depth, site selection, and pre-sampling procedures may also affect the detection of microbial taxa and the comparability of findings across studies.

Results and discussion

Experimental gingivitis models consistently demonstrate reproducible subgingival microbiome shifts following plaque accumulation. In Hall et al. [1], suspension of oral hygiene for three weeks resulted in increased relative abundance of Bacteroidota (21.63% to 40.12%) and Fusobacteriota (10.55% to 21.47%), alongside decreases in Bacillota (34.63% to 24.48%), Pseudomonadota (21.29% to 8.42%), and Actinomycetota (9.09% to 2.84%). At the genus level, baseline communities dominated by Streptococcus, Neisseria, Prevotella, Fusobacterium, and Veillonella shifted toward higher proportions of Prevotella (21.00%), Fusobacterium (17.38%), Capnocytophaga (6.73%), Veillonella (4.85%), and Porphyromonas (4.54%) [1].

Keijser et al. [2] reported longitudinal subgingival sampling across multiple time points, including baseline, oral hygiene cessation, and resolution phases. Subgingival communities demonstrated compositional divergence from baseline during induction, which remained detectable after one week of resumed oral hygiene [2].

Clinical case–control data remain limited. Vishnu et al. [4] analyzed subgingival plaque samples from healthy and gingivitis subjects and reported no significant differences among the most abundant taxa. However, Synergistetes were detected in gingivitis but not in healthy samples, and higher relative abundance of Dialister and Aneroglobus was observed in gingivitis [4].

Spectrum analyses across periodontal states provide additional comparative data. Iniesta et al. [3] identified significant differences between periodontal health, gingivitis, and periodontitis groups ($p<0.05$). Genera associated with

periodontal health included Granulicatella, Streptococcus, Haemophilus, Bergeyella, and Capnocytophaga, whereas increased detection frequency of Filifactor, Fretibacterium, and Porphyromonas/Prevotella/Tannerella complexes was observed in periodontitis [3].

Alpha diversity findings were heterogeneous. In Hall et al. [1], alpha diversity metrics (Shannon index and observed ASVs) increased during gingivitis induction but did not reach statistical significance ($p>0.05$). In Keijser et al. [2], significant increases in alpha diversity were observed at later induction stages (day 9 and day 14). In interventional studies, Iniesta et al. [6] reported no significant changes in richness or evenness over six weeks, and Furlaneto et al. [7] reported no significant differences in Shannon diversity between baseline and follow-up ($p>0.05$).

Beta diversity analyses consistently detected compositional differences. Hall et al. [1] reported higher inter-individual variability in subgingival plaque (Bray–Curtis dissimilarity ~0.738±0.084) compared to saliva (~0.608±0.113). Keijser et al. [2] reported increased compositional distance from baseline during gingivitis induction, with incomplete return to baseline after recovery. Iniesta et al. [6] and Furlaneto et al. [7] reported limited overall community restructuring in interventional settings despite detectable changes in specific taxa. A summary of the divergent trends between alpha and beta diversity across the included studies is presented in Table 1.

Across studies, changes in relative abundance of several genera were reported. Experimental gingivitis models showed increases in Prevotella, Fusobacterium, Capnocytophaga, and Porphyromonas [1]. Case–control data identified presence of Synergistetes and increased Dialister and Aneroglobus in gingivitis [4]. Health-associated genera reported in comparative analyses included Streptococcus, Granulicatella, and Haemophilus [3].

Santi-Rocca et al. [9] analyzed subgingival microbiome profiles across health, gingivitis, and periodontitis and identified 10 microbial complexes based on probabilistic taxonomic grouping. Complex 6 was associated with health, whereas Complex 10 was associated with disease-associated taxa, including red-complex species and Treponema. Gingivitis samples were distributed across intermediate clusters. Lafaurie et al. [5] reported significant separation between combined health/gingivitis and periodontitis groups in beta diversity analyses ($p\leq0.01$), with lower richness and evenness observed in health/gingivitis compared to periodontitis.

Methodological heterogeneity was observed across studies. Sampling approaches included curette-based and paper point collection, with variation in site selection and pooling strategies. Sequencing approaches included ASV-based pipelines (DADA2/QIIME2), OTU-based classification, and entropy-based MED or probabilistic UTG frameworks [1,2,4,9,14].

The findings of this study align with experimental and clinical evidence indicating that plaque accumulation is associated with structured and reproducible shifts in the subgingival microbiome rather than random compositional fluctuation. Experimental gingivitis models provide the clearest evidence for temporal dynamics, demonstrating that short-term disruption of oral hygiene leads to rapid enrichment of taxa associated with biofilm maturation, particularly Bacteroidota and Fusobacteriota, alongside a reduction in health-associated phyla [1,2]. These changes occur within a defined time window and are consistent across controlled settings, supporting the reproducibility of early microbiome responses to plaque accumulation.

A notable observation across studies is the incomplete reversibility of subgingival microbial composition following reintroduction of oral hygiene. Longitudinal data indicate that, although clinical inflammation may resolve within days, microbial communities retain a degree of compositional divergence from baseline [2]. This temporal dissociation between clinical and microbiological recovery is consistent with broader frameworks describing periodontal disease as an ecological shift rather than a transient imbalance [10,13]. In this context, gingivitis may represent a state of altered microbial equilibrium that is not immediately restored by short-term plaque control.

Diversity metrics further illustrate the complexity of this transition. Alpha diversity findings are inconsistent across studies, with some reporting no significant changes during gingivitis induction [1], while others demonstrate increases at later stages [2]. Interventional trials similarly report stability of alpha diversity despite clinical improvement [6,7]. These discrepancies suggest that commonly used diversity indices may not fully capture functionally relevant compositional changes, particularly when shifts occur within existing taxa rather than through the introduction of new taxa. In contrast, beta diversity measures consistently detect changes in community structure, indicating that gingivitis is associated with redistribution of taxa rather than uniform expansion of diversity.

At the taxonomic level, the observed increase in genera such as Prevotella, Fusobacterium, and Porphyromonas across experimental and clinical studies reflects patterns of biofilm maturation described in ecological models of periodontal disease [12,13]. These taxa are frequently present at low abundance in health and increase in relative proportion during plaque accumulation, supporting the concept that gingivitis involves quantitative shifts within an existing microbial community rather than the emergence of entirely distinct organisms. Case–control findings further support this pattern, with detection of taxa such as Synergistetes and increased abundance of Dialister and Aneroglobus in gingivitis, despite limited differences among dominant taxa [4].

The position of gingivitis within the continuum from health to periodontitis remains complex. Large-scale subgingival sequencing studies indicate that gingivitis does not represent a single uniform microbial state but instead encompasses multiple community configurations. Santi-Rocca et al. identified distinct microbial complexes and clustering patterns, with gingivitis samples distributed across intermediate states rather than forming a discrete group [9]. Similarly, comparative analyses across disease

stages demonstrate partial overlap between gingivitis and both health- and disease-associated communities [3,5]. These findings support the concept of multiple potential trajectories rather than a single linear progression pathway.

Conceptual models of periodontal disease provide a framework for interpreting these observations. The IMPEDE model and related ecological theories describe gingivitis as an early inflammatory stage that facilitates shifts in microbial composition, which may subsequently progress toward dysbiosis under permissive conditions [10]. Importantly, this transition is not deterministic, and the presence of intermediate microbial configurations suggests that gingivitis may follow different trajectories depending on host and environmental factors.

Methodological variability across studies remains a significant limitation for direct comparison. Differences in sampling strategies, sequencing regions, and analytical pipelines influence observed diversity and taxonomic resolution. The use of ASV-based approaches such as DADA2 has improved reproducibility and taxonomic resolution compared to OTU-based methods [14], yet variability in primer selection and sequencing depth continues to affect comparability across datasets. In addition, differences in site selection and pooling strategies may obscure localized ecological variation within the subgingival niche.

From a translational perspective, the findings highlight a partial dissociation between clinical outcomes and microbiome structure. Interventional studies demonstrate that reductions in gingival inflammation can occur without major restructuring of the overall subgingival microbiome, with detectable changes often limited to specific taxa [6-8]. This observation is consistent with the concept that clinical improvement may be achieved through reduction of microbial load and inflammatory drivers without complete restoration of baseline community composition.

Finally, emerging approaches to quantify dysbiosis provide a potential framework for integrating microbiome data into clinical assessment. The Subgingival Microbial Dysbiosis Index (SMDI) has demonstrated high discriminatory performance for periodontitis [11], suggesting that similar composite indices could be developed for gingivitis if validated in longitudinal datasets. Such approaches may allow more precise characterization of microbial states associated with increased risk of progression, although further evidence is required to establish predictive value.

Limitations

Several limitations should be considered when interpreting these findings. First, as a narrative review, this study is susceptible to selection bias, as a formal systematic review protocol (e.g., PRISMA) was not fully applied, which may influence the comprehensiveness of the included literature.

Second, heterogeneity in study design across included publications limits direct comparability. Differences in sampling approaches, including curette versus paper point collection, site selection, and pooling strategies, may influence the detected microbial composition and diversity measures. Variation in sequencing targets (e.g., V1–V2, V3–V4, V4–V5 regions) and analytical pipelines, including ASV-, OTU-, and MED-based approaches, further contributes to methodological inconsistency and may affect taxonomic resolution and relative abundance estimates [1,2,4,9,14].

Third, the majority of available data are derived from experimental gingivitis models or short-term interventional studies, which may not fully represent real-world heterogeneity in plaque accumulation patterns, host response, and behavioral factors. Controlled experimental conditions improve internal validity but reduce external validity for broader populations [1,2].

Fourth, several clinical studies lack comprehensive reporting of diversity metrics or effect sizes, particularly in case–control designs, limiting the ability to compare findings across studies and to quantify the magnitude of microbiome changes [4]. In addition, pooling of periodontal health and gingivitis categories in some analyses restricts gingivitis-specific interpretation and may obscure subtle microbial differences between these states [5].

Fifth, 16S rRNA gene sequencing provides taxonomic information but limited functional insight. The absence of strain-level resolution and direct assessment of gene expression or metabolic activity constrains interpretation of biological relevance, particularly in distinguishing active contributors from commensal taxa [12,13].

Finally, most studies are cross-sectional or short-term, and longitudinal evidence directly linking gingivitis-associated microbiome profiles with progression to periodontitis remains limited. As a result, inferences regarding disease trajectories and predictive microbial signatures should be interpreted cautiously.

Conclusion

Subgingival microbiome changes associated with plaque-induced gingivitis are characterized by reproducible shifts in relative abundance of existing taxa rather than the emergence of distinct microbial communities. Experimental and clinical data demonstrate enrichment of taxa associated with biofilm maturation and anaerobic conditions, alongside variability in diversity metrics across study designs. Beta diversity analyses consistently indicate compositional restructuring, whereas alpha diversity findings remain heterogeneous.

The available evidence supports the interpretation that gingivitis represents a transitional ecological state within the spectrum of periodontal conditions, with overlapping features of both health and disease-associated microbiomes. Microbial changes may persist beyond short-term clinical resolution, indicating that restoration of baseline community structure does not necessarily occur concurrently with improvement in clinical parameters.

Despite increasing use of high-resolution sequencing approaches, methodological variability and limited longitudinal data constrain definitive conclusions regarding progression pathways. Future studies incorporating standardized sampling, longitudinal designs, and integrative functional analyses are required to clarify the role of subgingival microbiome dynamics in disease transition and risk stratification.

Table 1: Comparison of Alpha and Beta Diversity Shifts in Plaque-Induced Gingivitis

Study / Design	Alpha Diversity	Beta Diversity
Hall et al. [1] (Experimental model)	Increased during gingivitis induction, though the change did not reach statistical significance ($p > 0.05$).	Demonstrated significant compositional shifts and high inter-individual variability.
Keijser et al. [2] (Experimental / Longitudinal)	Significantly increased only during the later stages of induction (days 9 and 14).	Revealed substantial structural divergence from baseline and incomplete microbiome restoration following the resumption of oral hygiene.
Iniesta et al. [6] & Furlaneto et al. [7] (Interventional studies)	No statistically significant changes observed (in either taxonomic richness or evenness), despite notable clinical improvement.	Showed limited overall structural restructuring of the microbial community, alongside detectable shifts in specific target taxa.
Lafaurie et al. [5] (Cross-sectional study)	Recorded lower diversity metrics (richness and evenness) in the combined health/gingivitis group compared to the periodontitis cohort.	Demonstrated a significant structural separation between the health/gingivitis and periodontitis groups ($p \leq 0.01$).
Overall Trend (Summary)	Heterogeneous and inconsistent (Frequently fails to reflect clinical status, as overall taxonomic richness undergoes minimal alteration).	Consistent and sensitive (Reliably captures proportional redistribution and structural shifts within the microbial community).

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