

Barrier-Mediated Niche Partitioning Maintains Distinct Plaque and Tissue Bacteriomes During Human Experimental Gingivitis

Anjali Bhagirath

University of Alberta

Andrew Gibb

University of Alberta

Anum Haider

University of Alberta

Umar Rekhi

University of Alberta

Johanna Redmond

University of Alberta

Brielle Winsor

University of Alberta

Lavanya Jain

University of Alberta

Monica Gibson

Indiana University – Purdue University Indianapolis

Khaled Altabtbaei

altabtba@ualberta.ca

University of Alberta

Research Article

Keywords: Niche partitioning, Community assembly, Dispersal limitation, 16S rRNA gene sequencing, Mucosal barrier, Microbiota, Host-microbe interactions

Posted Date: April 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9475542/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Microbial communities at mucosal surfaces are shaped by barrier-mediated ecological processes that constrain dispersal and impose selective filtering on community membership. How these processes operate at micrometer-scale host-microbe interfaces — where biofilm and tissue compartments coexist in intimate proximity — remains poorly understood. The human gingival sulcus, where dental plaque biofilm directly abuts junctional epithelium, offers an experimentally tractable system in which to dissect cross-niche assembly dynamics during a controlled inflammatory perturbation. Whether tissue-associated bacteriomes during early gingival inflammation represent passive spillover from adjacent plaque or reflect independent ecological assembly has not been systematically examined.

Results

In a split-mouth experimental gingivitis study of 22 periodontally healthy adults, we characterized paired plaque and gingival tissue bacteriomes by 16S rRNA gene amplicon sequencing across a 21-day induction timeline. Plaque and tissue communities were compositionally distinct at every timepoint ($F = 14.41$, $P = 0.001$), with cross-niche dissimilarity driven primarily by taxonomic turnover rather than nestedness (0.29 ± 0.089 vs. 0.11 ± 0.072 ; $P < 3.06 \times 10^{-14}$). Stegen framework assembly-process decomposition identified dispersal limitation as the dominant process maintaining niche separation (79.5%, 95% CI 70.0–86.7%), with smaller contributions from variable selection (12.5%) and ecological drift (8.0%). Tissue showed significant phylogenetic nepotism in recruitment ($D = -0.100$, $P = 0.015$), whereas plaque recruitment was consistent with neutrality ($D = -0.062$, $P = 0.129$). Cross-niche compositional distance remained stable throughout induction (mean PhILR separation ≈ 43.8 units; $P = 0.743$), and community-level dynamics were decoupled from clinical inflammation indices. Tissue was enriched for anaerobic, subgingival-associated taxa (*Segatella*, *Capnocytophaga*, *Treponema*, *Fusobacterium*), while plaque was enriched for early colonizers (*Streptococcus*, *Actinomyces*).

Conclusions

Plaque and gingival tissue operate as ecologically independent compartments during early gingivitis, with niche separation maintained by dispersal limitation across a functionally intact epithelial barrier. The persistence of niche independence — and its potential loss during disease progression — may represent a measurable ecological signature of the gingivitis-to-periodontitis transition, testable through cross-niche convergence metrics in future longitudinal studies.

Introduction

Microbial communities on mucosal surfaces are shaped by various ecological processes that determine which organisms can colonize, persist, and thrive. Mucosal surfaces like the host-microbe interfaces throughout the body provide physical and immunological barriers that partition microbial communities into distinct niches, even when those niches are physically congruous. Understanding how these barriers constrain community assembly, and what happens when they fail, are central questions in the mucosal microbiome ecology [1,2].

Ecological assembly theory provides a framework for decomposing the processes that shape community composition into deterministic components (environmental selection, phylogenetic filtering) and stochastic components (dispersal limitation, ecological drift) [3,4]. Although these frameworks were originally developed for soil ecology, they have been increasingly applied to host-associated microbiomes, revealing that the balance among assembly processes varies across body sites, health states, and perturbations. Yet their application to paired compartments within the same anatomical niche structures, especially where the biofilm and tissue communities coexist in intimate spatial proximity, remains under-explored.

The human gingival sulcus offers an ideal model system for this question. Dental plaque biofilm directly abuts the junctional epithelium, separated by a barrier whose integrity is actively maintained through constitutive neutrophil trafficking, antimicrobial peptide secretion, and complement activation [5]. The low-grade inflammation observed in clinically healthy gingiva is an immunological surveillance state, where a tonic host response to commensal biofilm maintains the barrier homeostasis through consistent immune engagement [1,2]. When undisturbed biofilm accumulates, this homeostatic balance shifts toward an inflammatory state (known as gingivitis) that is confined to the free and attached gingiva. This occurs as a reversible inflammation without irreversible tissue destruction [6,7]. Importantly, inflammation severity varies substantially across individuals under comparable biofilm challenge, underscoring that early disease is shaped by host and ecological factors beyond biofilm burden alone [8,9].

Whether tissue-associated bacteriomes during this early inflammatory state represent passive spillover from adjacent plaque, that is, a species-poor subset reflecting indiscriminate leakage of some of the microbial members, or instead reflect independent ecological assembly within the tissue niche has not been systematically examined. Bacterial tissue association is not restricted to established periodontitis; microorganisms have been detected within gingival tissues across the spectrum of clinical states, from clinically healthy individuals [10,11], advanced disease [12–14] through rapidly progressing lesions [15]. This suggests that tissue colonization is a general ecological behavior rather than a pathology-specific phenomenon. However, the community-level ecological processes governing tissue colonization during the earliest, reversible stages of gingival inflammation have never been characterized.

The human experimental gingivitis model, first formalized by Löe et al. [16] and subsequently refined through split-mouth designs and acrylic stent protocols [17–21], provides a controlled framework to study early disease dynamics. Its application to longitudinal tissue bacteriome characterization has

remained limited, largely because tissue sampling effectively terminates the experiment at the sampling timepoint. Consequently, it remains unknown what ecological processes govern plaque-tissue niche separation, whether tissue communities are assembled by the same or different rules as plaque, and whether niche independence is maintained or is eroded as inflammation develops.

To address these gaps, we employed a modified split-mouth experimental gingivitis design with integrated tissue sampling to characterize plaque and tissue bacteriomes in parallel across the induction timeline. We applied ecological assembly decomposition [22], phylogenetic recruitment analysis [22], tensor decomposition [23], temporal co-occurrence networks [24] and beta-diversity partitioning [25] to test whether plaque and tissue are governed by shared or distinct assembly rules during early gingivitis.

Materials and Methods

The study protocol received ethical approval at the University of Alberta (REB#00112019) and was conducted in accordance with the Helsinki declaration (1975, revised 2013). Written informed consent was obtained from each participant prior to any clinical examination or sample collection.

Participant Demographics

A total of 48 individuals were screened for eligibility. 26 were excluded either for not meeting eligibility criteria or declining participation. The final study population comprised 22 systemically and periodontally healthy adults who completed the study (Table 1). Participants were recruited from the Mike Petryk School of Dentistry, University of Alberta, at the Graduate Periodontics Clinic, Kaye Edmonton Clinic.

Table 1
Participant Demographics

Demographic Variable	N	Mean (SD) / Frequency (%)
Total Participants	22	
Age (years)		39.14 (7.18)
Gender		
- Female		12 (54.5%)
- Males		10 (45.5%)
Education Level		
- College Graduate		22 (100%)
Health Status		
- Systemically Healthy		22 (100%)
- Periodontally Healthy		22 (100%)

Inclusion criteria were that eligible participants must be systemically and periodontally healthy adults (≥ 18 years) with at least 20 natural teeth, no history of periodontitis, and a baseline Modified Gingival Index (MGI; a standardized measure of gingival inflammation severity scored from 0 [no inflammation] to 4 [severe inflammation with spontaneous bleeding]) of ≤ 1 . Exclusion criteria included antibiotic, anti-inflammatory, or anticoagulant use within 30 days prior to enrollment, need for antibiotic prophylaxis, current or recent tobacco use within the preceding 10 years, pregnancy or lactation, active orthodontic treatment, concurrent participation in another oral study, and any systemic or immunologic condition potentially influencing inflammatory or immune responses.

Study Design:

A schematic of the study design is presented in Fig. 1A. This study employed a modified split-mouth experimental gingivitis model comprising three phases: a baseline (pre-induction) phase, a time-staggered split-mouth induction phase (Day 0–Day 21), and a resolution phase, conducted over four scheduled visits.

Initial Visit

Participants meeting the inclusion criteria underwent a comprehensive periodontal examination conducted by a calibrated dentist (A.B.). Clinical parameters recorded included probing pocket depth (PPD; the distance in millimeters from the gingival margin to the base of the sulcus, measured at six sites per tooth using a UNC-15 periodontal probe), bleeding on probing (BOP; a dichotomous indicator of gingival inflammation recorded as present or absent upon gentle probing), the Modified Quigley-Hein Plaque Index (MQHPI; a semi-quantitative measure of dental plaque accumulation on tooth surfaces) [26,27], and the Papillary-Marginal-Attachment (PMA) Index (a composite measure that separately scores inflammation of the interdental papilla, the marginal gingiva, and the attached gingiva) [28]. Subgingival plaque samples were collected from the interproximal areas of the first molars (second molar substituted when the first was absent). Maxillary and mandibular impressions were recorded for custom acrylic stent fabrication. Full-mouth professional mechanical plaque removal (PMPR) and individualized oral hygiene instructions were provided.

Acrylic Stent Design and Delivery

Custom acrylic stents (removable intraoral appliances that shield selected tooth surfaces from oral hygiene procedures, thereby permitting controlled plaque accumulation) were fabricated to cover the occlusal and buccal surfaces of the posterior teeth (premolars to the distal-most molar) extending approximately 2 mm beyond the gingival margin. Stents were worn exclusively during daily oral hygiene routines and delivered within one week of the initial visit.

Induction Phase — Time-Staggered Split-Mouth Protocol

A staggered split-mouth design (in which different quadrants of the same mouth receive different durations of treatment, enabling within-participant comparison across multiple induction durations) enabled within-participant comparison across multiple induction durations. Quadrant assignment was determined using an online randomization tool (Randomizer.org; Research Randomizer); participants selected their own control quadrant based on personal preference. Beginning at Day 0, oral hygiene was withheld by the stent from the first assigned quadrant. At Days 7 and 14, stents were introduced to a second and third quadrant respectively. The fourth quadrant served as the control, with normal oral hygiene maintained throughout. By Day 21, the three test quadrants had accumulated 21, 14, and 7 days of oral hygiene withdrawal. All participants were provided with standardized oral hygiene supplies at Day 0 (Colgate Gum Comfort, soft-bristled toothbrush, Crest Gum Detoxify dentifrice) and instructed to use only these products throughout the study; use of additional oral hygiene aids was not permitted.

Subsequent Visits and Resolution Phase

Participants returned at Day 21 (Visit 3) for subgingival plaque, gingival tissue, and GCF sample collection from all quadrants, followed by full-mouth scaling and root planning (SRP) and reinstitution of oral hygiene. Participants returned at Day 28 (Visit 4) for subgingival plaque collection, and a further round of SRP. Subsequent weekly visits continued until the MGI score returned to 0 in all study quadrants. The analyses reported here focus on the induction phase.

Sample Collection

Subgingival Plaque Sampling

Subgingival plaque was collected from the interproximal sites of designated teeth at each visit by inserting sterile endodontic paper points (size 25; Dentsply Sirona) into the gingival sulcus for 15 seconds. Samples from each quadrant were pooled into a single microcentrifuge tube containing RNA/later (Thermo Fisher Scientific) and stored at – 20°C until processing.

Gingival Tissue Sampling

Gingival tissue samples were obtained only during the induction phase (control, Day 7, Day 14, and Day 21) under local anesthesia, excised from the mesial and distal interproximal areas of the designated first or second molars using the Excisional New Attachment Procedure [29] (ENAP; a surgical technique in which the sulcular epithelium and a thin layer of underlying connective tissue are excised from the inner wall of the gingival sulcus (also known as curettage [30]) as illustrated in Fig. 1B. Removal of these tissues occur during subgingival instrumentation, therefore, this is considered as a pre-emptive removal of tissues that will inadvertently be removed during normal professional cleaning. In all 22 participants, the primary tissue sample was allocated for 16S rRNA gene sequencing. In three participants where sufficient tissue volume permitted, a portion was additionally used for culture-based validation; in the remaining 19, the full sample volume was directed to DNA extraction.

Removal of Extracellular Bacteria and DNA from Tissue Samples

To eliminate extracellular and surface-adherent bacterial signal prior to DNA extraction, tissue samples underwent a sequential decontamination protocol adapted from published methods [31–33]. Briefly, samples were washed three times with 1 mL PBS, then incubated with gentamicin (100 µg/mL, 37°C, 1 hour) to eliminate surface-attached bacteria. The post-gentamicin wash was plated as a negative control. Samples were subsequently treated with DNase I to remove residual extracellular DNA, washed twice more with PBS, and homogenized by mechanical douncing in 0.025% saponin. Homogenates were used for both culture and DNA extraction.

Microbiological Proof-of-Concept Culture Testing

In 3 participants with sufficient tissue volume, homogenates were plated on selective media for 24–48 hours under taxon-specific conditions following decontamination. Post-gentamicin wash was plated in parallel as a negative control. Because tissue mass could not be standardized across specimens, these experiments were interpreted qualitatively as confirmation of viable bacterial growth following decontamination without CFU enumeration.

DNA Extraction and Processing:

Total DNA was extracted from tissue and plaque samples using the QIAamp AllPrep Kit, then purified using the DNA Clean & Concentrator-5 kit (Zymo Research, Irvine, CA, USA) and quantified fluorometrically using the Quant-iT dsDNA HS Assay Kit on a Qubit 2.0 fluorometer. Samples were stored at – 20°C until library preparation. 16S rRNA gene amplicon sequencing was performed targeting the V1-3 and V4-5 hypervariable regions at the Genome Quebec Innovation Centre (Montreal, QC, Canada) (primers listed in Table 2). Raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number **PRJNA1423792**. All analysis scripts and reproducible notebooks are available at github.com/khalidtab/tissueinvasion/.

Table 2: Primers used for 16S rRNA gene amplicon sequencing

Region	Sequence (5'–3')
V1-3 [27F/519R primers]	
Forward primer	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAAKRGTTYGATYNTGGCTCAG
Reverse primer	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGTNTBACCGCDGCTGCTG
V4-5 [515bF-926R primers]	
Forward primer	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGYCAGCMGCCGCGGTAA
Reverse primer	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGYCAATTYMTTTRAGTTT

Computational Preprocessing and Analyses

Clinical parameters were assessed using repeated-measures ANOVA, or the Friedman test where data were non-normally distributed. Beta diversity was quantified via PhILR transformation [34], a phylogeny-aware isometric log-ratio transform that accounts for the compositional nature of amplicon data. Cross-niche community assembly processes were quantified using the Stegen framework [4], implemented with custom parallelized code adapted from Zha [35]; briefly, β NTI and RCbray were calculated from 999 permutations to partition community dissimilarity into variable selection, homogeneous selection, dispersal limitation, homogenizing dispersal, and ecological drift. Phylogenetic nepotism during community recruitment was assessed using the Phylogenetic Recruitment Analysis framework [22], and alpha diversity was quantified using the phylogeny-aware metric D from the same framework. Temporal community trajectories were summarized using TEMPTED tensor decomposition [23] to collapse repeated-measures into a single dissimilarity measure per site. Linear mixed-effects models were fitted using the lme4 package [36]. Temporal co-occurrence networks were constructed using extended Local Similarity Analysis (eLSA; [24]) on robust centred log-ratio (rCLR) transformation [37], retaining $P < 0.05$ associations. Differential abundance analysis was performed using log-transformed LIMMA, selected based on spike-in benchmark tests conducted within the FALAPhyL pipeline [38]. Beta-diversity partitioning into turnover and nestedness components followed the Jaccard decomposition framework of Baselga and Orme [25].

Sample Size Calculation

Sample size was estimated a priori assuming a within-subject effect size of Cohen's $d = 0.65$ (two-sided paired t-test, $\alpha = 0.05$, 80% power), yielding a minimum of 21 completers; 22 participants were therefore targeted. This effect size is consistent with within-subject microbiome differences reported in comparable experimental gingivitis studies [9,39], though we note that power calculations for permutation-based microbiome analyses remain an active area of methodological development [40,41]. This manuscript was prepared in accordance with the STORMS Guidelines; the completed checklist is provided as a supplementary file.

Results

Proof-of-Concept Validation of Tissue Decontamination Protocol

Culture of decontaminated tissue homogenates from the subset of participants with sample bulk permissive of splitting yielded growth on selective media at induction timepoints, whereas post-gentamicin wash controls were negative, supporting both the effectiveness of the extracellular

decontamination protocol and the persistence of viable tissue-associated bacteria following processing (Appendix Fig. 1). These results provide the microbiological basis for interpreting the sequencing-based analyses that follow.

1. Clinical Parameters Confirm Successful Induction of Experimental Gingivitis

Cessation of oral hygiene produced progressive plaque accumulation and gingival inflammation during induction, with resolution following oral hygiene reinstitution (Fig. 2). MQHPI increased during induction, reaching peak levels at Day 21 significantly greater than both pre-induction and control quadrant values ($P < 0.001$), and returned to pre-induction levels during resolution. PPD remained largely stable, with a clinically negligible increase at Day 21 ($P < 0.05$) that did not persist. BOP% increased progressively, with significant elevations at Day 14 ($P < 0.01$) and Day 21 ($P < 0.001$) relative to control, returning to non-significant levels during resolution. Papillary and marginal components of the PMA Index increased significantly at all induction timepoints ($P < 0.01$) and resolved. The clinical data confirm that the time-staggered split-mouth protocol successfully induced quantifiable, reversible gingival inflammation consistent with published experimental gingivitis studies [9,39,42].

2. Gingival Tissue Harbors a Less Diverse but Compositionally Distinct Bacteriome Relative to Plaque

To determine whether gingival tissue contained a simple subset of the adjacent plaque community or instead harbored distinct bacterial members, we partitioned Jaccard dissimilarity into turnover (taxon replacement) and nestedness (subset) components (Fig. 3). Paired plaque-tissue dissimilarity was driven predominantly by taxonomic turnover rather than nestedness at all timepoints (Fig. 3A). This indicates that tissue communities were not simply plaque minus some taxa, but differed primarily through replacement by distinct organisms. Plaque consistently exhibited greater richness than tissue at all timepoints (mean observed features: 206 vs. 172; linear mixed-effects model, $P < 0.001$; Fig. 3B), and Shannon diversity was likewise higher in plaque throughout the study (mean: 4.08 vs. 3.73; $P < 0.001$; Fig. 3C). Neither richness nor Shannon diversity showed a significant temporal trend or niche $\times$ time interaction, indicating that the diversity contrast between compartments was established early and remained stable across the induction period.

3. Plaque and Gingival Tissue Remain Compositionally Distinct but Exhibit Coordinated Temporal Dynamics

Using distance-based redundancy analysis (db-RDA) of TEMPTED dissimilarity conditioned on participant, we observed clear compositional separation by niche (Fig. 4A). After accounting for inter-individual variation, niche identity explained a significant fraction of within-subject compositional variance ($F = 14.41$, $P = 0.001$). Despite this compartmentalization, within-niche temporal turnover was broadly similar across compartments. Sequential community change did not differ significantly by niche ($P = 0.139$), and there was no niche $\times$ time interaction ($P = 0.893$; Fig. 4B), indicating that plaque and tissue communities changed at comparable rates while maintaining persistent ecological separation.

Cross-niche PhILR distances remained stable over time (Fig. 4C; $P = 0.743$), with a mean plaque-tissue separation of approximately 43.8 PhILR units.

Neither plaque accumulation nor gingival inflammation was significantly associated with microbiome change over time. MQHPI change was not associated with qualitative or quantitative bacteriome dissimilarity ($\beta = 0.31$, $P = 0.557$; $\beta = 2.33$, $P = 0.422$), papillary inflammation was not associated with dissimilarity ($\beta = 0.72$, $P = 0.728$), and lagged analyses showed no predictive relationship in either direction. Composition of the plaque and tissue communities thus behaved as distinct yet temporally coordinated ecosystems whose dynamics were decoupled from the clinical indices measured here.

4. Phylogenetic Assembly Patterns Differ Between Plaque and Gingival Tissues

To test whether the two niches differed in their underlying assembly dynamics, we applied the phylogenetic recruitment analysis framework to assess whether newly detected taxa were more closely related to existing community members than expected under random recruitment (Fig. 4D). Tissue showed recruitment dynamics consistent with neutrality ($D = -0.15$, $P = 0.053$), suggesting no strong phylogenetic bias in the appearance of newly detected taxa during the induction period. In contrast, plaque showed significant phylogenetic nepotism ($D = -0.33$, $P = 0.005$), indicating that newly recruited taxa tended to be more closely related to residents than expected under a null model. Recruitment dynamics differed significantly between niches ($P = 1.87 \times 10^{-22}$), reinforcing that the two compartments operate under distinct ecological constraints: plaque permits phylogenetically nepotistic recruitment, which by virtue of being α -diverse, indicates that it is more permissive of colonization, whereas tissue imposes stronger phylogenetic filtering on incoming taxa, where even phylogenetically related members are not capable of occupying the same space.

5. Plaque and Tissue Bacteriomes Form Compartmentalized Ecological Networks with Distinct Niche-Enriched Taxa

Temporal association networks constructed using eLSA across the four sampling timepoints comprised 296 significant associations (Fig. 5), all of which sustained their connections across the entire induction time. The integrated network was strongly modular ($Q = 0.91$), indicating that taxa were organized into distinct ecological guilds with limited cross-module connectivity. Cross-niche associations accounted for 48.6% of all significant edges, indicating substantial temporal coupling between plaque and tissue even within an overall fragmented network. Network modules were biologically interpretable: taxa associated with proteolytic and anaerobic subgingival consortia — including *Porphyromonas*, *Fusobacterium*, *Treponema*, and *Prevotella/Segatella* — clustered separately from saccharolytic early colonizers including *Actinomyces*, *Streptococcus*, and *Rothia* (Figs. 6A–B), consistent with metabolic niche partitioning rather than a single undifferentiated community-wide shift.

To identify the processes that maintain the plaque-tissue differentiation despite their sustained temporal coupling, we applied the Stegen community assembly framework to cross-niche samples. Dispersal limitation was the dominant inferred process (79.5%, 95% CI 70.0%–86.7%), with smaller contributions

from variable selection (12.5%, 95% CI 7.1%–21.0%) and ecological drift (8.0%, 95% CI 3.9%–15.5%). This process-level finding offers a mechanistic explanation for the persistent plaque-tissue compositional separation observed in the diversity and ordination analyses above.

Differential abundance analysis identified consistent compositional contrasts between niches across all timepoints (Appendix Table 1). Plaque was enriched by canonical early colonizers including multiple *Streptococcus* species (*S. salivarius*, *S. intermedius*, *S. mitis*, *S. cristatus*, *S. oralis*), *Gemella*, *Actinomyces johnsonii*, and *Actinomyces naeslundii*, with *Enterococcus italicus* showing the strongest and most consistent plaque enrichment across contrasts. Gingival tissue was enriched for anaerobic, periodontal-associated taxa including *Segatella* spp., *Capnocytophaga ochracea*, *Capnocytophaga gingivalis*, *Corynebacterium matruchotii*, *Eikenella corrodens*, *Treponema socranskii*, *Selenomonas sputigena*, and *Fusobacterium* spp.

Discussion

This study provides the first longitudinal characterization of tissue-associated bacterial communities across the full timeline of human experimental gingivitis, and demonstrates that gingival tissue harbors a compositionally distinct bacteriome relative to paired plaque biofilms. Plaque and tissue formed separate microbial guild structures with limited compositional mixing at every timepoint despite micrometer-scale proximity, yet remained temporally synchronized, where each niche maintained its own ecological character while both responded to the gingivitis challenge. These findings establish bacterial niche independence as a defining ecological feature of early gingival inflammation and set the stage for a central question: what happens to that independence as disease progresses?

Tissue association versus plaque carryover

Two features in our workflow address the persistent challenge of distinguishing true tissue association from plaque carryover: tissue-specific sampling via ENAP, providing a bacteriome signal separated from adjacent plaque, and a sequential decontamination protocol. The proof-of-concept culture experiments (decontaminated tissue homogenates yielding growth on selective media) are consistent with tissue-associated separate from their plaque counterparts. We acknowledge these experiments were conducted in a subset of participants without quantitative CFU enumeration or spatial localization; they are therefore interpreted as qualitative support for the sequencing-based findings rather than independent evidence of intracellular invasion. Tissue communities differed from plaque primarily through taxon replacement rather than representing a reduced subset, which is inconsistent with passive spillover and instead consistent with selective ecological structuring.

Barrier-mediated bacterial sampling: parallels across other mucosal surfaces

Gingival tissue maintains a distinct bacterial community even in the absence of clinical disease, which invites comparison with a principle now established at other mucosal surfaces: controlled bacterial sampling is a feature of immune surveillance, not a failure of barrier function. In the gastrointestinal tract, dendritic cells sample luminal bacteria via transepithelial dendrites without disrupting barrier integrity [43,44], and live commensals reach mesenteric lymph nodes where they prime protective IgA while being contained from systemic dissemination [45,46]. The healthy periodontium operates under an analogous logic: the junctional epithelium actively participates in innate defense through constitutive neutrophil trafficking, antimicrobial peptide secretion, and complement activation [5]. Our finding that tissue harbors a distinct bacteriome even at baseline, with assembly dominated by dispersal limitation (79.5%), is consistent with this framework: the junctional epithelium does not exclude bacteria entirely but may selectively filter which organisms gain tissue access, functioning as a semipermeable barrier whose selectivity may itself constitute an immune function.

Gingivitis, then, may represent not a qualitative break from health but a quantitative exaggeration of this surveillance as demonstrated by the persistent niche independence regardless of the gingivitis stage. The barrier remained functionally intact, maintained dispersal limitation dominance, and produced different structured recruitment throughout the gingivitis timeline. The gastrointestinal literature clarifies what happens when such regulatory control is lost: in inflammatory bowel disease, pro-inflammatory cytokines disrupt tight junctions, converting controlled sampling into a permissive conduit [47], and increased intestinal permeability precedes clinical Crohn's disease [48,49]. The transition from controlled sampling to pathological permeability appears to be gradual, with barrier state determining disease trajectory.

Phylogenetic filtering and selective tissue colonization

The phylogenetic recruitment analyses provide evidence for the selective nature of tissue colonization. Tissue recruitment was consistent with neutrality, hosting only a specific group of bacteria during induction. Tissue showed significant phylogenetic nepotism, with recruitment dynamics differing strongly between niches. This suggests that tissue is only available as a colonization niche to a selective few bacteria who can adapt to its harsh environment (surface structures, stress-tolerance mechanisms, host interaction capacities) while plaque assembly tolerates a broader phylogenetic set. These findings extend the evidence by Duran-Pinedo et al. (2021), who found that stable periodontitis sites exhibited phylogenetically structured plaque recruitment while progressing sites followed a neutral model [50]. The interpretation of both studies converges into a theory: phylogenetic structure in recruitment may represent the ecologically organized signal, and its loss may accompany disease progression. That is, the features preserved in the gingival tissues are no longer contained in that niche. This is consistent with the Anna Karenina Principle (AKP) applied to the subgingival microbiome, where dysbiosis is characterized by compositional disorganization while health is defined by ecological order [51]. The modularity of proteolytic versus saccharolytic guilds within temporal co-occurrence networks in this study is consistent with this organization; functional barriers and habitat constraint reinforce taxonomic partitioning.

Tissue-enriched taxa and their ecological context

The tissue-enriched taxa, including *Segatella*, *Capnocytophaga*, *Eikenella corrodens*, *Treponema socranskii*, *Selenomonas sputigena*, and *Fusobacterium* spp., overlap substantially with organisms whose close relatives have been shown to possess capacities relevant to tissue colonization. Yost et al. [52] demonstrated that functional signatures enriched at baseline in plaque at sites that subsequently progressed (that is, before any detectable clinical attachment loss) included flagellar motility, iron acquisition, lipid A biosynthesis, and amino acid transport, each of which has been linked mechanistically to epithelial penetration or host-proximal survival in other systems. The compositional overlap between our tissue-enriched taxa and those activating such molecular virulence programs prior to clinical progression raises the possibility that the microbial preconditioning for barrier disruption may be established during reversible gingivitis. Validation of this inference requires functional validation beyond what 16S rRNA gene amplicon data can provide, but our method may serve as a framework for such studies.

Not all tissue-enriched taxa fit a straightforward pathobiont classification: *C. matruchotii*, for example, may arrive via co-aggregation rather than independent tissue tropism, underscoring that tissue enrichment reflects ecological assembly and polymicrobial context rather than a shared pathogenic program. Yost et al. also noted that organisms not conventionally regarded as pathogens (*Streptococcus* Spps.) were actively upregulating virulence factors during progression, further indicating that it is the community context, not individual taxon, that determines pathogenic potential.

The Niche Convergence Hypothesis

These converging observations motivate a proposal: gingivitis may represent exaggerated but controlled immunological surveillance with preserved niche independence. The loss of that independence, driven by progressive barrier breakdown, may constitute the defining ecological transition to periodontitis. The organisms enriched in tissue during gingivitis include taxa which have been seen to possess barrier-disrupting capabilities. That is, they are present within a compartment whose ecological independence is maintained by the very barriers those organisms are potentially equipped to dismantle. If those barriers are progressively compromised, then the community convergence should accompany or precede the transition. This may be measured as cross-niche turnover, reduced dispersal dominance, and compressed PhILR distances between niches.

This extends the keystone pathogen and polymicrobial synergy and dysbiosis models [53,54], which explain how low-abundance pathobionts drive community-wide dysbiosis within plaque. Our framework adds a spatial dimension: the dysbiosis that may matter for progression could depend critically on whether the tissue niche is colonized by organisms capable of dismantling barrier separation. Plaque-based sampling alone would be blind to this process. Duran-Pinedo et al. [50] provide complementary evidence: subgingival plaque in progressing periodontitis sites showed community convergence and loss of phylogenetic structure — the inverse of the organized niche independence we observe during gingivitis. The trajectory from our gingivitis state toward their progressing state may represent the

microbiological signature of the gingivitis-to-periodontitis transition. In our proposed framework, gingivitis is defined not solely by clinical inflammation severity or the associated plaque burden, but by the preservation of ecological independence between compartments despite them, while periodontitis is defined by the loss of that independence.

Limitations

16S rRNA gene amplicon sequencing provides relative compositional abundances with limited strain-level resolution; absolute bacterial burden in tissue cannot be inferred without complementary quantitative approaches. The absence of standardized tissue mass, quantitative enumeration, and spatial confirmation by imaging leaves open whether detected bacteria are intercellular, or intracellular. The quasi-longitudinal design introduces potential baseline site heterogeneity, and localized inflammation may exert contralateral intraoral effects complicating site-independence assumptions. Mock community and extraction blank sequencing controls were not included; although culture-based negative controls support the decontamination effectiveness, future studies should include sequenced controls. Critically, this study characterizes only one end of the disease spectrum, with the convergence hypothesis requiring direct testing in periodontitis using the same paired plaque-tissue sampling method, and the cross-niche ecological metrics defined here.

Future Directions

If niche convergence is the microbiological signature of the gingivitis-to-periodontitis transition, then cross-niche ecological metrics such as plaque-tissue turnover, dispersal limitation dominance, and PhILR distance may serve as an earlier and a more mechanistically meaningful indicators of disease progression than conventional plaque composition indices. Our conceptual framework proposal defines gingival health as a controlled bacterial sampling across an intact barrier, gingivitis by the exaggerated surveillance with preserved niche independence, and periodontitis by barrier collapse and compartment convergence. This aligns the gingival inflammatory spectrum with the broader mucosal immunology literature, where barrier-mediated partitioning is increasingly recognized as a unifying principle of mucosal homeostasis. Future work should apply this framework longitudinally across this disease spectrum, integrate host transcriptomic data to test whether tissue-enriched taxa associate with distinct barrier integrity signatures, and stratify analyses by inflammatory response phenotype. The inflamed gingival sulcus, accessible and experimentally tractable, represents a compelling model system for studying barrier-mediated niche partitioning at host-microbe interfaces, which is a question with relevance well beyond periodontal biology.

Declarations

Author Contribution

A.B. - design, acquisition, analysis, drafted work, revision for final version
A.G. - acquisition, analysis
A.H. - data interpretation, draft, and revisions
Y.R. - data interpretation, draft, and revisions
J.R. - acquisition,

analysisB.W. - acquisition, analysisL.J. - acquisition, analysisM.G. - conception, design, interpretation, draft, revision for final versionK.A. - conception, design, analysis, software code creation, interpretation, draft, revision for final versionAll authors have approved the submitted version, and have agreed to be personally accountable for their own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Data Availability

All fastq files used in this study are available in NCBI SRA (PRJNA1423792). Analyses used in this manuscript are available in github.com/khalidtab/tissueinvasion/

References

1. Moutsopoulos NM, Konkel JE. Tissue-Specific Immunity at the Oral Mucosal Barrier. *Trends Immunol.* 2018;39:276–87. <https://doi.org/10.1016/j.it.2017.08.005>
2. Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nat Rev Immunol.* 2021;21:426–40. <https://doi.org/10.1038/s41577-020-00488-6>
3. Global assembly of microbial communities. *mSystems* [Internet]. 2023 [cited 2026 Apr 10];8. <https://doi.org/10.1128/msystems.01289-22>
4. Stegen JC, Lin X, Fredrickson JK, Chen X, Kennedy DW, Murray CJ, et al. Quantifying community assembly processes and identifying features that impose them. *ISME J.* 2013;7:2069–79. <https://doi.org/10.1038/ismej.2013.93>
5. Bosshardt DD, Lang NP. The junctional epithelium: from health to disease. *J Dent Res.* 2005;84:9–20. <https://doi.org/10.1177/154405910508400102>
6. Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol.* 2018;89:S74–84. <https://doi.org/10.1002/JPER.17-0719>
7. Lang NP, Bartold PM. Periodontal health. *J Clin Periodontol.* 2018;45:S9–16. <https://doi.org/10.1111/jcpe.12936>
8. Kinane DF, Attström R, B on behalf of the EW in P group. Advances in the pathogenesis of periodontitis. *J Clin Periodontol.* 2005;32:130–1. <https://doi.org/10.1111/j.1600-051X.2005.00823.x>
9. Bamashmous S, Kotsakis GA, Kerns KA, Leroux BG, Zenobia C, Chen D, et al. Human variation in gingival inflammation. *Proc Natl Acad Sci U S A.* 2021;118:e2012578118. <https://doi.org/10.1073/pnas.2012578118>

10. Colombo AV, da Silva CM, Haffajee A, Colombo APV. Identification of intracellular oral species within human crevicular epithelial cells from subjects with chronic periodontitis by fluorescence in situ hybridization. *J Periodontal Res.* 2007;42:236–43. <https://doi.org/10.1111/j.1600-0765.2006.00938.x>
11. Rudney JD, Chen R, Sedgewick GJ. *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythensis* are components of a polymicrobial intracellular flora within human buccal cells. *J Dent Res.* 2005;84:59–63. <https://doi.org/10.1177/154405910508400110>
12. Manor A, Lebendiger M, Shifter A, Tovel H. Bacterial Invasion of Periodontal Tissues in Advanced Periodontitis in Humans. *J Periodontol.* 1984;55:567–73. <https://doi.org/10.1902/jop.1984.55.10.567>
13. Saglie R, Newman MG, Carranza FA, Pattison GL. Bacterial invasion of gingiva in advanced periodontitis in humans. *J Periodontol.* 1982;53:217–22. <https://doi.org/10.1902/jop.1982.53.4.217>
14. Saglie R, Elbaz JJ. Bacterial penetration into the gingival tissue in periodontal disease. *J West Soc Periodontol Abstr.* 1983;31:85–93.
15. Christersson LA, Slots J, Zambon JJ, Genco RJ. Transmission and colonization of *Actinobacillus actinomycetemcomitans* in localized juvenile periodontitis patients. *J Periodontol.* 1985;56:127–31. <https://doi.org/10.1902/jop.1985.56.3.127>
16. Loe H, Theilade E, Jensen SB. Experimental Gingivitis in Man. *J Periodontol.* 1965;36:177–87. <https://doi.org/10.1902/jop.1965.36.3.177>
17. Jones CL, Saxton CA, Ritchie JA. Microbiological and clinical effects of a dentifrice containing zinc citrate and Triclosan in the human experimental gingivitis model. *J Clin Periodontol.* 1990;17:570–4.
18. Morelli T, Stella M, Barros SP, Marchesan JT, Moss KL, Kim SJ, et al. Salivary Biomarkers in a Biofilm Overgrowth Model. *J Periodontol.* 2014;85:1770–8. <https://doi.org/10.1902/jop.2014.140180>
19. Offenbacher S, Barros SP, Paquette DW, Winston JL, Biesbrock AR, Thomason RG, et al. Gingival transcriptome patterns during induction and resolution of experimental gingivitis in humans. *J Periodontol.* 2009;80:1963–82. <https://doi.org/10.1902/jop.2009.080645>
20. Putt MS, Van der Weijden GA, Kleber CJ, Saxton CA. Validation of a 21-day, partial-mouth gingivitis model for evaluating chemotherapeutic dentifrices. *J Periodontal Res.* 1993;28:301–7. <https://doi.org/10.1111/j.1600-0765.1993.tb02097.x>
21. Yates RJ, Shearer BH, Morgan R, Addy M. A modification to the experimental gingivitis protocol to compare the antiplaque properties of two toothpastes. *J Clin Periodontol.* 2003;30:119–24. <https://doi.org/10.1034/j.1600-051x.2003.00205.x>
22. Darcy JL, Washburne AD, Robeson MS, Prest T, Schmidt SK, Lozupone CA. A phylogenetic model for the recruitment of species into microbial communities and application to studies of the human microbiome. *ISME J.* 2020;14:1359–68. <https://doi.org/10.1038/s41396-020-0613-7>
23. Shi P, Martino C, Han R, Janssen S, Buck G, Serrano M, et al. TEMPTED: time-informed dimensionality reduction for longitudinal microbiome studies. *Genome Biol.* 2024;25:317. <https://doi.org/10.1186/s13059-024-03453-x>

24. Xia LC, Steele JA, Cram JA, Cardon ZG, Simmons SL, Vallino JJ, et al. Extended local similarity analysis (eLSA) of microbial community and other time series data with replicates. *BMC Syst Biol*. 2011;5:S15. <https://doi.org/10.1186/1752-0509-5-S2-S15>
25. Baselga A, Orme CDL. betapart: an R package for the study of beta diversity: *Betapart package*. *Methods Ecol Evol*. 2012;3:808–12. <https://doi.org/10.1111/j.2041-210X.2012.00224.x>
26. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. *J Am Dent Assoc*. 1939; 1962;65:26–9. <https://doi.org/10.14219/jada.archive.1962.0184>
27. Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. *J Periodontol*. 1970;41:41–3. <https://doi.org/10.1902/jop.1970.41.41.41>
28. Schour I, Massler M. Gingival disease in postwar Italy (1945) prevalence of gingivitis in various age groups. *J Am Dent Assoc*. 1939; 1947;35:475–82. <https://doi.org/10.14219/jada.archive.1947.0266>
29. Yukna RA. Longitudinal Evaluation of the Excisional New Attachment Procedure in Humans. *J Periodontol*. 1978;49:142–4. <https://doi.org/10.1902/jop.1978.49.3.142>
30. The American Academy of Periodontology Statement Regarding Gingival Curettage. *J Periodontol*. 2002;73:1229–30. <https://doi.org/10.1902/jop.2002.73.10.1229>
31. Complex Intratissue Microbiota Forms Biofilms in Periodontal Lesions - K. Baek, S. Ji, Y. Choi, 2018 [Internet]. [cited 2026 Apr 10]. <https://journals.sagepub.com/doi/10.1177/0022034517732754>. Accessed 10 Apr 2026
32. Lai J, Coleman KK, Tai SS, German J, Hong F, Albert B, et al. Relative efficacy of masks and respirators as source control for viral aerosol shedding from people infected with SARS-CoV-2: a controlled human exhaled breath aerosol experimental study. *EBioMedicine*. 2024;104:105157. <https://doi.org/10.1016/j.ebiom.2024.105157>
33. Medapati MR, Singh N, Bhagirath AY, Duan K, Triggs-Raine B, Batista Jr EL, et al. Bitter taste receptor T2R14 detects quorum sensing molecules from cariogenic *Streptococcus mutans* and mediates innate immune responses in gingival epithelial cells. *FASEB J*. 2021;35:e21375. <https://doi.org/10.1096/fj.202000208R>
34. Silverman JD, Washburne AD, Mukherjee S, David LA. A phylogenetic transform enhances analysis of compositional microbiota data. *eLife*. 2017;6. <https://doi.org/10.7554/eLife.21887>
35. Zha Y, Lindström ES, Eiler A, Svanbäck R. Different Roles of Environmental Selection, Dispersal, and Drift in the Assembly of Intestinal Microbial Communities of Freshwater Fish With and Without a Stomach. *Front Ecol Evol*. 2020;8. <https://doi.org/10.3389/fevo.2020.00152>
36. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. *J Stat Softw*. 2015;67:1–48. <https://doi.org/10.18637/jss.v067.i01>
37. Martino C, Morton JT, Marotz CA, Thompson LR, Tripathi A, Knight R, et al. A Novel Sparse Compositional Technique Reveals Microbial Perturbations. *mSystems*. 2019;4. <https://doi.org/10.1128/mSystems.00016-19>
38. Faridoun A, Carvalho R, Smith J, Gibb A, Jain L, Zhang A, et al. FAVABEAN and FALAPhyL: Open-source pipelines for scalable 16s rRNA microbiome data processing and visualization. *PLOS ONE*.

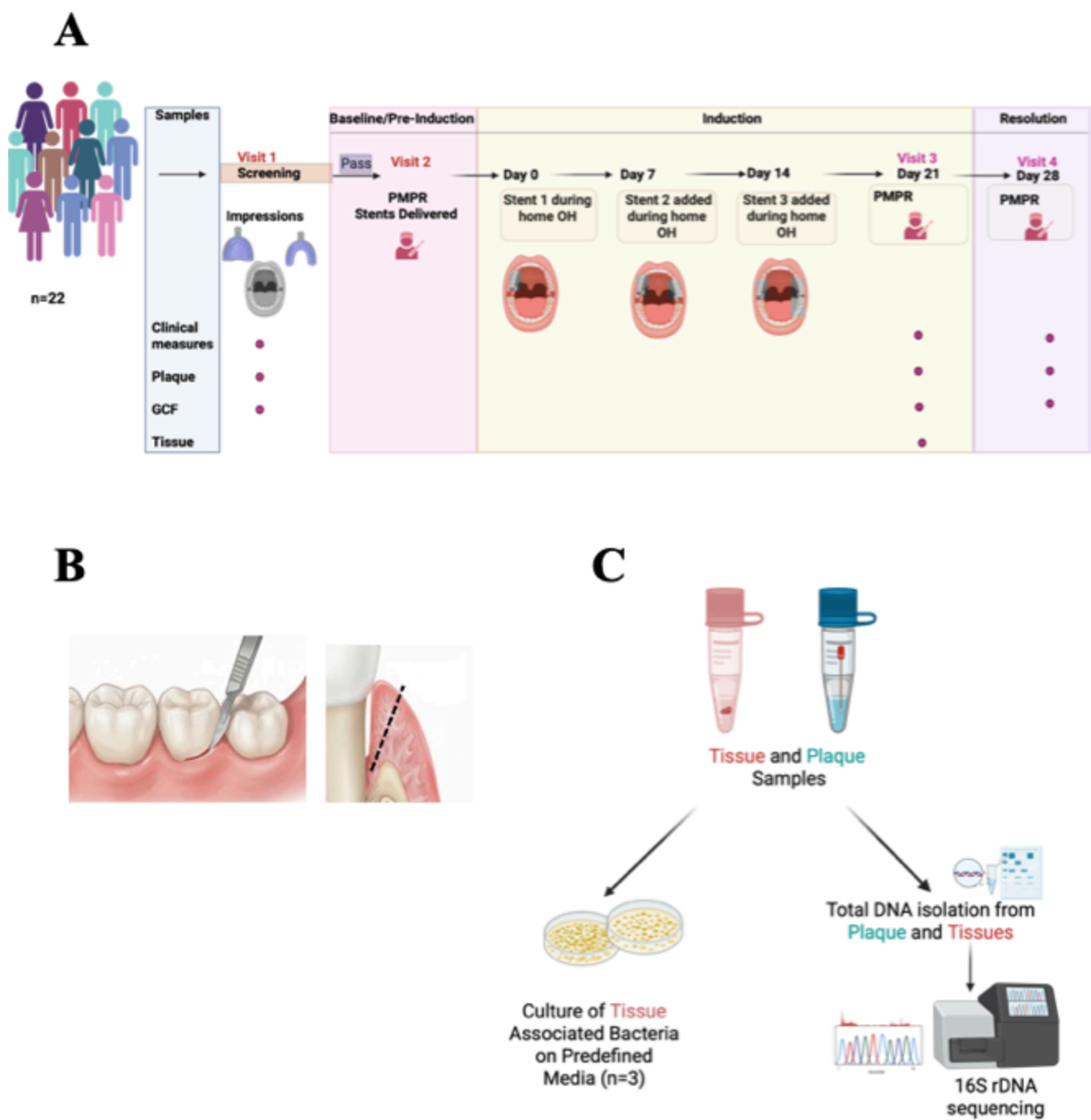
- Public Library of Science; 2026;21:e0331145. <https://doi.org/10.1371/journal.pone.0331145>
39. Kistler JO, Booth V, Bradshaw DJ, Wade WG. Bacterial Community Development in Experimental Gingivitis. PLOS ONE. Public Library of Science; 2013;8:e71227. <https://doi.org/10.1371/journal.pone.0071227>
40. Ferdous T, Jiang L, Dinu I, Groizeleau J, Kozyrskyj AL, Greenwood CMT, et al. The rise to power of the microbiome: power and sample size calculation for microbiome studies. Mucosal Immunol. 2022;15:1060–70. <https://doi.org/10.1038/s41385-022-00548-1>
41. Kelly BJ, Gross R, Bittinger K, Sherrill-Mix S, Lewis JD, Collman RG, et al. Power and sample-size estimation for microbiome studies using pairwise distances and PERMANOVA. Bioinformatics. 2015;31:2461–8. <https://doi.org/10.1093/bioinformatics/btv183>
42. Al-Kamel A, Baraniya D, Al-Hajj WA, Halboub E, Abdulrab S, Chen T, et al. Subgingival microbiome of experimental gingivitis: shifts associated with the use of chlorhexidine and N-acetyl cysteine mouthwashes. J Oral Microbiol. Taylor & Francis; 2019;11:1608141. <https://doi.org/10.1080/20002297.2019.1608141>
43. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol. 2001;2:361–7. <https://doi.org/10.1038/86373>
44. Chieppa M, Rescigno M, Huang AYC, Germain RN. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. J Exp Med. 2006;203:2841–52. <https://doi.org/10.1084/jem.20061884>
45. Sonnenberg GF, Monticelli LA, Alenghat T, Fung TC, Hutnick NA, Kunisawa J, et al. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. Science. New York, N.Y.; 2012;336:1321–5. <https://doi.org/10.1126/science.1222551>
46. Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science. New York, N.Y.; 2004;303:1662–5. <https://doi.org/10.1126/science.1091334>
47. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol. 2009;9:799–809. <https://doi.org/10.1038/nri2653>
48. Turpin W, Lee S-H, Raygoza Garay JA, Madsen KL, Meddings JB, Bedrani L, et al. Increased Intestinal Permeability Is Associated With Later Development of Crohn's Disease. Gastroenterology. 2020;159:2092-2100.e5. <https://doi.org/10.1053/j.gastro.2020.08.005>
49. Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. Ann Intern Med. 1986;105:883–5. <https://doi.org/10.7326/0003-4819-105-6-883>
50. Duran-Pinedo A, Solbiati J, Teles F, Teles R, Zang Y, Frias-Lopez J. Long-term dynamics of the human oral microbiome during clinical disease progression. BMC Biol. 2021;19:240. <https://doi.org/10.1186/s12915-021-01169-z>
51. Altatbaei K, Maney P, Ganesan SM, Dabdoub SM, Nagaraja HN, Kumar PS. Anna Karenina and the subgingival microbiome associated with periodontitis. Microbiome. 2021;9:97.

<https://doi.org/10.1186/s40168-021-01056-3>

52. Yost S, Duran-Pinedo AE, Teles R, Krishnan K, Frias-Lopez J. Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis. *Genome Med.* 2015;7:27. <https://doi.org/10.1186/s13073-015-0153-3>
53. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. *Nat Rev Microbiol.* 2012;10:717–25. <https://doi.org/10.1038/nrmicro2873>
54. Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Mol Oral Microbiol.* 2012;27:409–19. <https://doi.org/10.1111/j.2041-1014.2012.00663.x>

Figures

Figure 1



plaque accumulation. Oral hygiene was withheld from a first quadrant beginning at Day 0, from a second quadrant at Day 7, and from a third quadrant at Day 14, such that by Day 21 (Visit 3) the three test quadrants had accumulated 21, 14, and 7 days of hygiene withdrawal, respectively. A fourth quadrant served as the within-participant control with uninterrupted oral hygiene throughout. Comprehensive clinical assessment and paired subgingival plaque and gingival tissue sample collection were performed at Visit 3 (Day 21), followed by professional prophylaxis and reinstitution of oral hygiene. Resolution was assessed at Visit 4 (Day 28). **(B)** Illustration of gingival tissue collection by the excisional new attachment procedure (ENAP; Yukna et al. 1976) from the interproximal papillary tissues of first or second molars. **(C)** Overview of sample processing and sequencing workflow. Excised tissues were subjected to a sequential decontamination protocol (gentamicin treatment, DNase I and PBS washes) to eliminate extracellular and surface-adherent bacteria prior to DNA extraction. Subgingival plaque was collected by paper-point sampling. Both sample types underwent total DNA extraction, 16S rRNA gene amplicon library preparation targeting the V1–V3 and V4–V5 hypervariable regions, and Illumina sequencing (lower right). Study design and sampling procedures are described in the Methods.

Figure 2

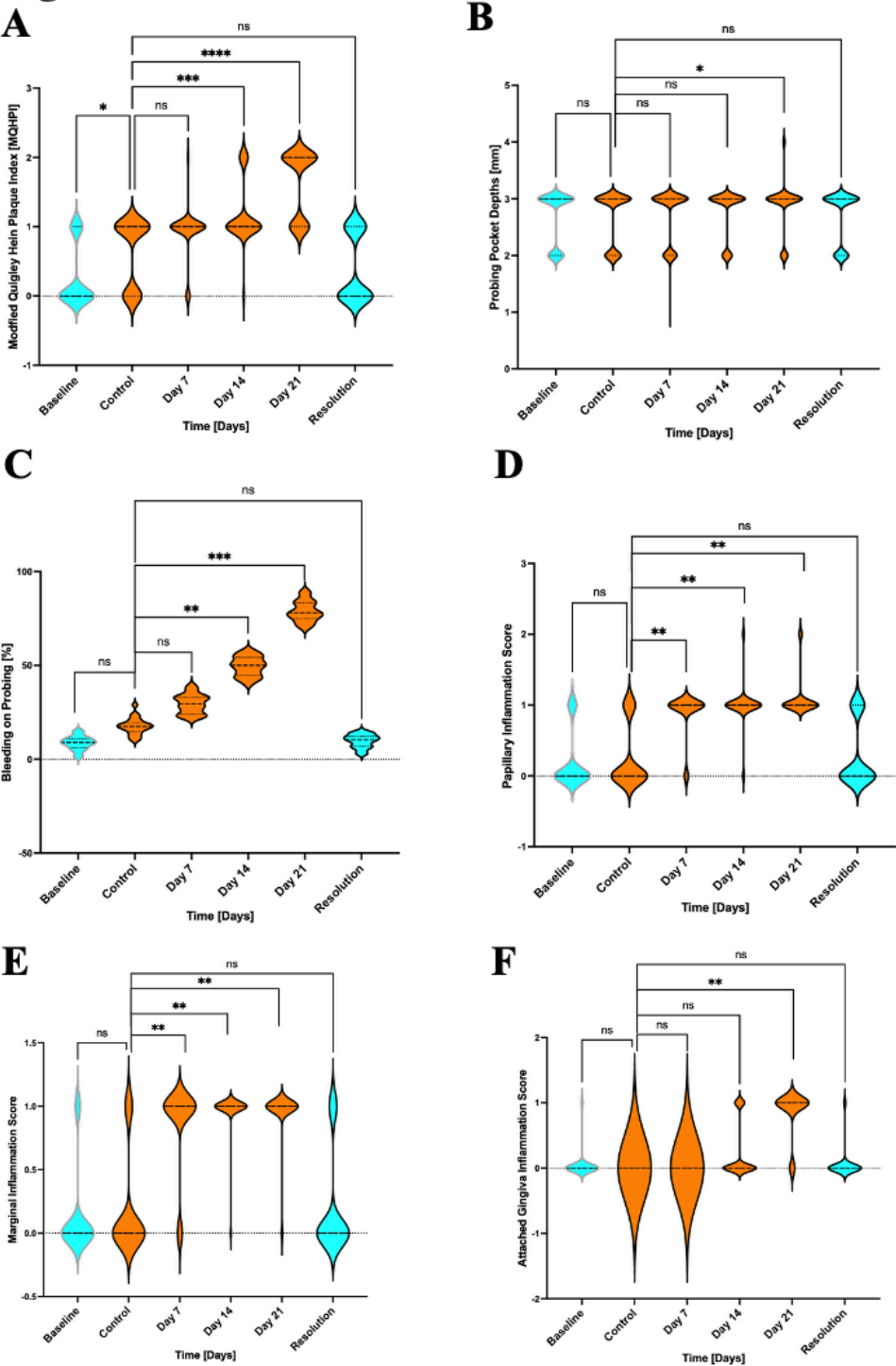


Figure 2

Dynamic changes in periodontal clinical parameters during induction and resolution of experimental gingivitis. Violin plots with embedded box plots (median and interquartile range) summarize longitudinal changes in clinical parameters across the study period. Timepoints spanning the induction phase (Days 0–21) are shown in orange; pre- and post-induction (Baseline and Resolution) timepoints are shown in turquoise. Control represents the hygiene-maintained quadrant sampled alongside Day 7, 14, 21 test

quadrants. **(A)** Dental plaque accumulation quantified by the Modified Quigley–Hein Plaque Index (MQHPI). **(B)** Mean probing pocket depth (PPD, mm). **(C)** Bleeding on probing expressed as the percentage of sites with bleeding (BOP%). **(D–F)** Gingival inflammation indices from the Papillary–Marginal–Attachment (PMA) Index, plotted as ranks: papillary **(D)**, marginal **(E)**, and attached **(F)** inflammation. Horizontal brackets indicate pairwise comparisons; significance is denoted as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). Longitudinal analyses were conducted using a mixed-effects framework with post hoc multiple comparisons; $n = 22$ participants throughout.

Figure 3

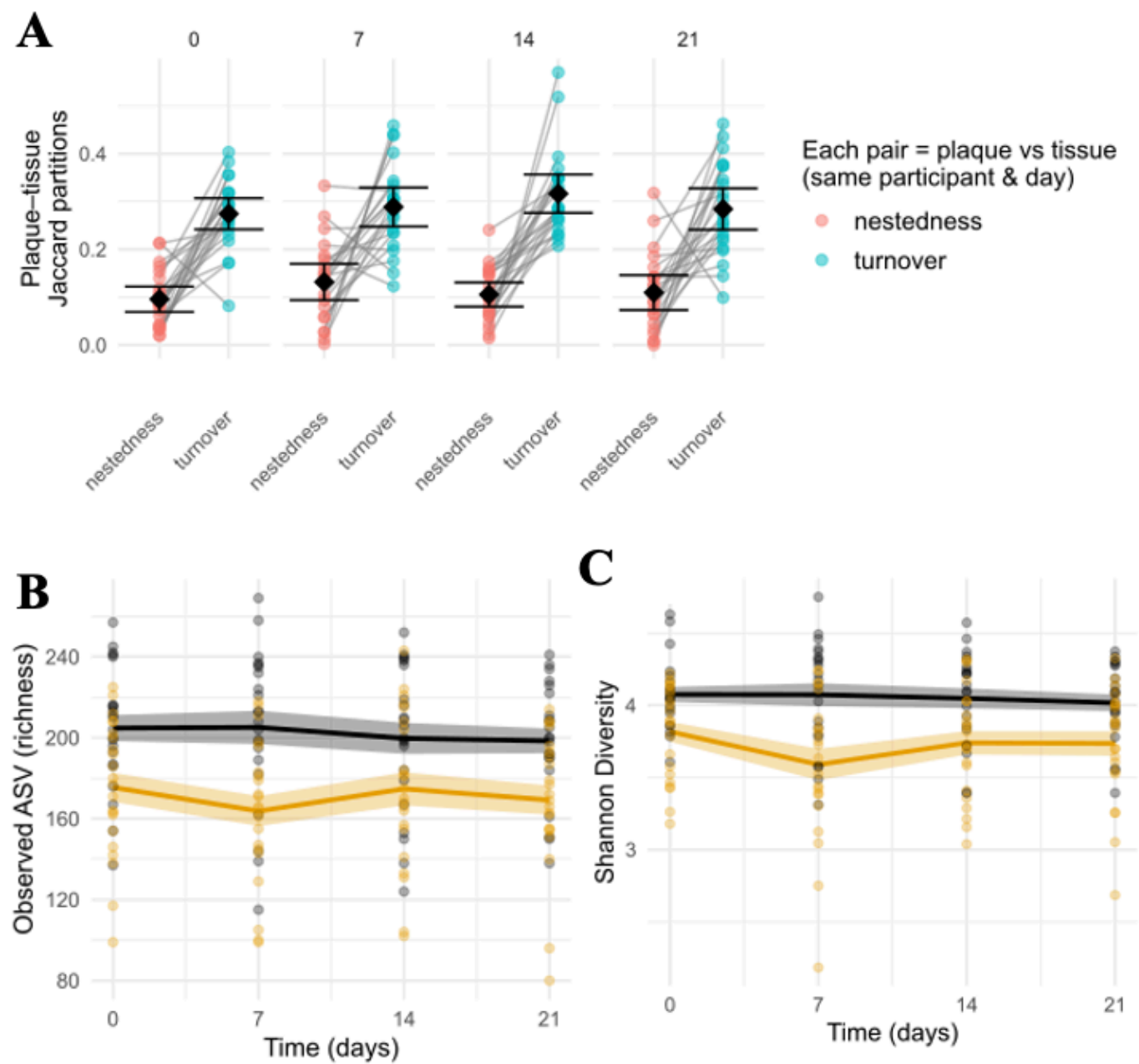


Figure 3

Alpha diversity and plaque-tissue dissimilarity partitioning across the experimental gingivitis time course. **(A)** Decomposition of paired plaque-tissue Jaccard dissimilarity into turnover and nestedness components at each study timepoint (Days 0, 7, 14, and 21). Each point represents a single participant; grey lines connect turnover (green) and nestedness (salmon) values derived from the same participant–timepoint comparison; black diamonds indicate mean component values per timepoint. Across all timepoints, the turnover component substantially exceeded the nestedness component (0.29 ± 0.089 vs. 0.11 ± 0.072 ; paired Wilcoxon test, $V = 3784$, $P < 3.063 \times 10^{-14}$), indicating that plaque-tissue compositional differences are driven primarily by taxon replacement rather than by tissue communities representing a species-poor subset of plaque. **(B)** Observed amplicon sequence variants (ASVs; richness) in plaque (black) and gingival tissue (orange) across timepoints. Individual participant values are shown as points; ribbons indicate $\pm$ SE. **(C)** Shannon diversity index in plaque and gingival tissue across timepoints, displayed as in **(B)**.

Figure 4

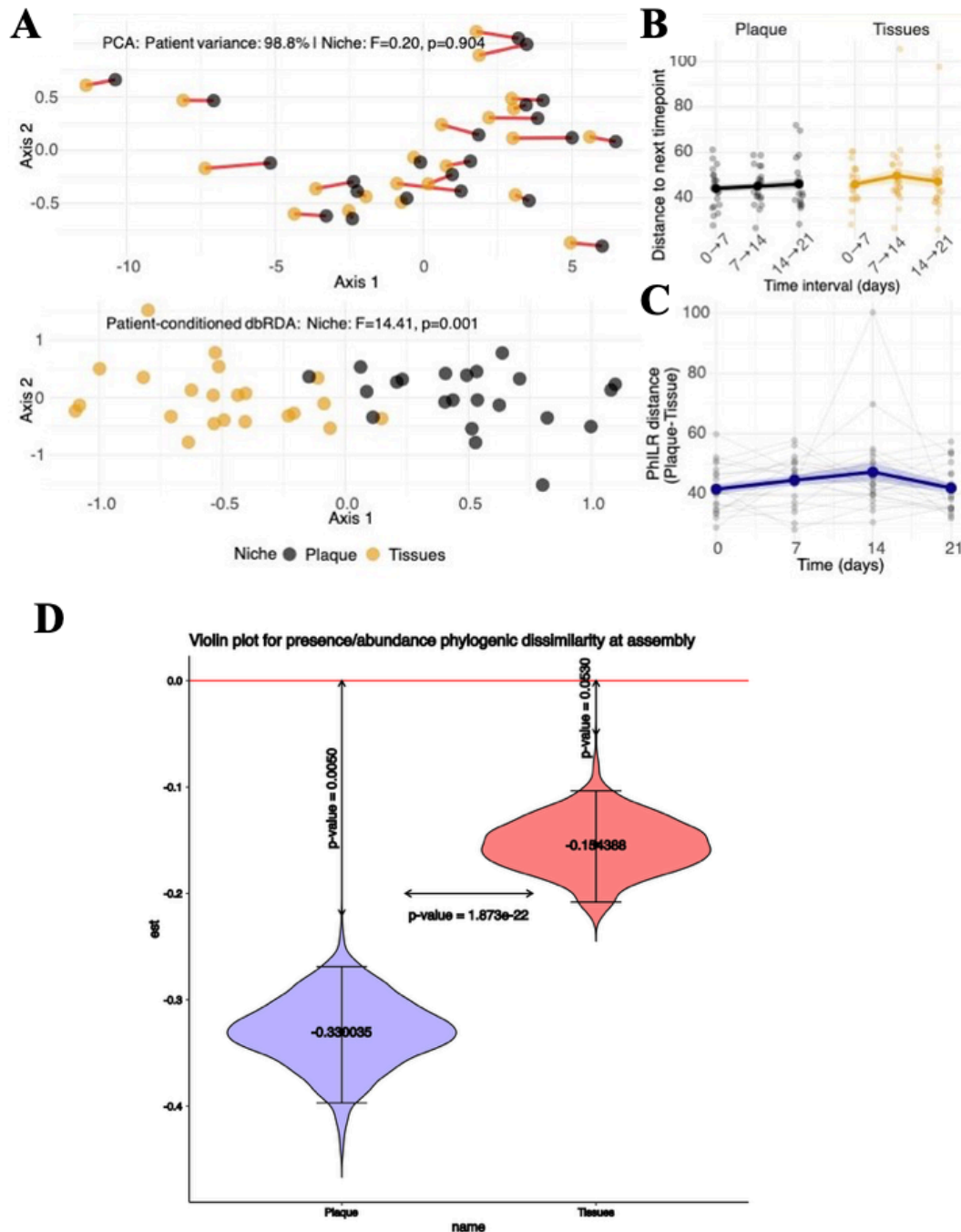


Figure 4

Plaque and gingival tissue bacteriomes maintain distinct compositions but exhibit coupled temporal dynamics and divergent recruitment processes. (A) Unconditioned PCA (top) and patient-conditioned db-RDA (bottom). Without controlling for participant, niche effect is not significant ($F = 0.20$, $P = 0.904$); distance-based redundancy analysis (db-RDA) of TEMPTED subject loadings, conditioned on participant, reveals significant within-subject compositional compartmentalization by niche ($F = 12.82$, $P = 0.001$).

(**B**) Within-niche compositional turnover rates, quantified as PhILR distance to the subsequent sampling timepoint, for plaque and tissue across the induction period. Turnover distributions were broadly comparable between niches (niche effect $P = 0.139$; niche $\times$ time interaction $P = 0.893$). (**C**) Cross-niche PhILR distance within participants across study timepoints. Plaque-tissue separation remained stable throughout induction (time effect $P = 0.743$; mean separation ≈ 43.8 PhILR units). (**D**) Phylogenetic recruitment analysis (assemblage D statistic) in plaque and gingival tissue during the induction window (Days 7–21). Presence/absence recruitment calculations showed that plaque communities had significant phylogenetic nepotism ($D = -0.33$, $P = 0.005$), consistent with phylogenetically clustered recruitment during biofilm development. Tissue communities showed a weaker signal ($D = -0.154$, $P = 0.053$), suggesting that recruitment in tissue do not follow phylogenetic bias. Recruitment dynamics differed significantly between niches under both weighting schemes ($P = 8.84 \times 10^{-10}$).

Figure 5

Cross-niche Network (eLSA $P < 0.05$, component size > 2 nodes)

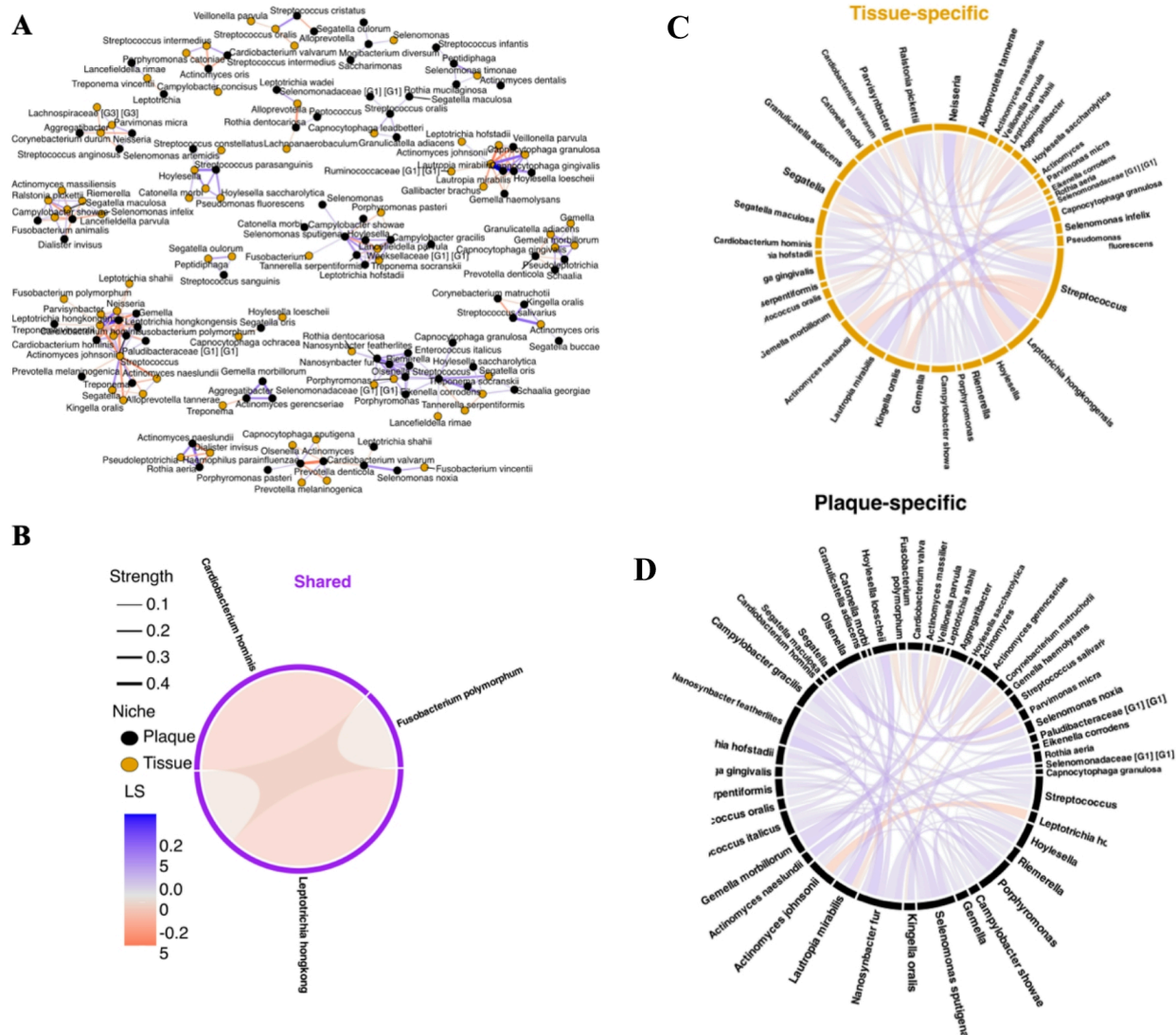


Figure 5

Integrated cross-niche temporal association network during experimental gingivitis. Co-occurrence networks were constructed using extended Local Similarity Analysis (eLSA) across four sampling timepoints (Days 0, 7, 14, and 21) following robust centered log-ratio (rCLR) transformation. Only edges with statistically significant associations ($P < 0.05$) were retained; the integrated network comprised 296 significant associations. **(A)** Arc diagrams illustrating within-niche co-occurrence networks for plaque (upper) and gingival tissue (lower) independently. **(B)** Combined cross-niche network integrating plaque (black nodes) and tissue (orange nodes) taxa. Edges are colored by the direction of association (blue = positive co-variation; red = negative co-variation) and scaled in thickness proportional to Local Similarity score magnitude (|LS|). The integrated network was strongly modular ($Q = 0.91$). Cross-niche

associations accounted for 48.6% of all significant edges. Community assembly analyses identified dispersal limitation as the dominant process maintaining niche separation (79.5%, 95% CI 70.0–86.7%), with smaller contributions from variable selection (12.5%, 95% CI 7.1–21.0%) and ecological drift (8.0%, 95% CI 3.9–15.5%). (C) Associations within gingival tissues. D. Associations within plaque.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile3STORMSChecklist.xlsx](#)
- [AppendixTables.docx](#)
- [AppendixFig.pptx](#)