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Papilla preservation flap surgery and endoscope-assisted subgingival debridement for Step 3 therapy: A 12-month pilot study on subgingival microbial community dynamics in deep periodontal pockets

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ABSTRACT

Objectives: This study aimed to assess subgingival microbial changes following papilla preservation flap surgery (PPFS) or endoscope-assisted subgingival debridement (EASD) in patients with residual periodontal intrabony defects requiring Step 3 therapy.

Methods: Nineteen periodontitis participants requiring Step 3 periodontal therapy were randomly allocated to either the PPFS ($n = 11$) or EASD ($n = 8$) treatment group and were conveniently sampled. Subgingival plaque samples were collected at baseline, days 3, 7, 14, and months 1, 3, 6, 9, and 12 post-treatment. DNA extraction and 16S rRNA sequencing were used to analyze microbial communities through bioinformatics tools, assessing diversity and differential abundance.

Results: Both treatments were effective, with no significant difference in clinical outcomes. Pocket resolution was achieved in 100% of PPFS sites and 87.5% of EASD sites. The overall microbiome composition was similar between groups. However, microbial diversity dynamics differed: PPFS exhibited stable shifts over time (PERMANOVA, $p = 0.004$), whereas EASD showed more variability in species richness and abundance, indicating less predictable microbial reorganization (Procrustes, $p = 0.030$). Notably, *Fusobacterium nucleatum* subsp. *vincentii*/*Fretibacterium fastidiosum* and *Treponema socranskii* were significantly associated with bleeding on probing/probing pocket depth, respectively ($p < 0.05$).

Conclusions: Both PPFS and EASD effectively treat residual intrabony defects, with PPFS showing more consistent microbial modulation. This 12-month pilot study offers new insights into the changes in microbiota following surgical and minimally invasive periodontal therapies, highlighting potential microbial biomarkers associated with clinical outcomes.

Clinical Significance: This 12-month study, for the first time, profiled the subgingival microbiota over the management of residual intrabony defects using either PPFS or EASD in Step 3 therapy. The study also revealed possible associations between specific microbes and clinical parameters during the post-operative period, which warranted further investigation.

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1. Introduction

Periodontal tissue destruction results from the complex interaction between host responses and dysbiotic subgingival microbial challenges. Suboptimal plaque control by the host allows changes in the dental biofilm, enriching periodontal disease-associated species such as *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola*, as well as novel or putative periodontopathogens like *Fretibacterium* sp. HMT 362, *Eubacterium nodatum, Peptoniphilaceae* sp. HMT 113 and so on [1,2]. When allowed to grow, interact, and multiply, these pathogenic species trigger the susceptible host's overreactive or inappropriate immune and inflammatory responses, destroying the periodontal apparatus and deepening periodontal pockets.

The acquisition of optimal oral hygiene and the successful delivery of non-surgical periodontal therapy (NSPT) are the most critical initial steps in periodontal disease management, which the European Federation of Periodontology categorizes as essential elements in Step 1 or 2 therapy, respectively [3]. Due to the limitations of NSPT, not all patients achieve the full resolution or closure of periodontal pockets after Steps 1 and 2 therapy, often requiring Step 3 therapy, i.e., the repeated NSPT or surgical interventions [3]. Repeated non-surgical subgingival instrumentation has limited effectiveness in closing residual periodontal pockets [4]. Thus, periodontal surgery is often indicated, especially for the deep pockets. It allows better visualization of the defect/lesion and, hence, improved calculus removal and modification of defect morphology to enhance healing [5–7]. While previous studies have explored the effects of surgical and non-surgical periodontal therapies on subgingival microbiota, the results are inconsistent [8,9].

A recent single-blinded randomized controlled trial from this group showed that endoscope-assisted subgingival debridement (EASD) or minimally invasive periodontal operation with papillary-preservation flap surgery (PPFS) showed similar clinical outcomes in residual periodontal intrabony defects management [10]. Given the above study, it is imperative to investigate and comprehend the corresponding changes in subgingival microbiota over the post-treatment period to appreciate the microbial influence of the EASD protocol, in which the visualization/access protocol was an endoscopic flapless rather than a flap approach. EASD's 'closed' nature might, by itself, restrict clinicians from biofilm disruption and removal of diseased tissue, including the periodontopathogen-infected periodontal tissue. This may pose a potential hazard for the further development of periodontal endoscope-assisted, minimally invasive, nonsurgical periodontal treatment, especially when applied in conjunction with periodontal regeneration materials. Therefore, the current project aims to evaluate the changes in subgingival microbiota at isolated residual periodontal pockets after management with periodontal surgery using the papillary preservation technique or periodontal endoscopic instrumentation.

2. Materials and methods

2.1. Study design

This follow-up prospective observational study assesses the changes in subgingival microbiota up to 12 months post-PPFS/EASD treatment, using a convenience sample of 22 participants from the previously published non-inferiority randomized controlled clinical trial [10]. The study sample size was determined with reference to similar reports [11,12]. The study was approved by the Hong Kong Hospital Authority West Cluster Ethics Committee (UW 19-086).

2.2. Clinical intervention

The current project is part of the published randomized, examiner-blind, parallel-group, controlled non-inferiority trial which was conducted in the Periodontology Clinic / Clinical Research Center of Prince Philip Dental Hospital in the period between 1 June 2020 to 30 Sept 2022 [10]. In brief, participants with residual periodontal intrabony defects, 6–12 weeks after completing Step 1 and 2 periodontal therapies, were recruited. Persistent deep pocket ($\geq$ 5 mm with bleeding on probing (BOP) or $\geq$ 6 mm irrespective of bleeding status), clinical attachment loss of $\geq$ 6 mm, $\geq$ 3 mm intrabony defect, a neighboring tooth with probing depths $\leq$ 3 mm, an adequate level of oral hygiene (full-mouth plaque score $\leq$ 30 %) at periodontal re-evaluation were listed as inclusion criteria. Subjects were excluded if the defects extended into a furcation or the subject was presented with self-reported cigarette smoking ($\geq$ 10/day), systemic comorbidities or medications known to alter the manifestation or outcome of periodontal therapy, pregnancy or intention to become pregnant, history of under systemic antibiotics in the previous 3 months or the need for antibiotic prophylaxis for dental treatment.

2.2.1. Randomization, masking, and study site selection

A Blinded Assessor BA (KLRH) initially screened the subjects, and baseline records were measured. Randomization was carried out with a balanced, permuted-block approach (in blocks of four patients) as described in the previous trial [10]. Treatment assignments were concealed in opaque envelopes and revelated to the therapist on the day the treatment was administered. BA was masked throughout the study period. One site will be recruited for the study. If more than one site is suitable for inclusion, the site for study purposes will be randomly selected by coin tossing. The site(s) not selected for the study were treated with surgical periodontal therapy according to the clinical indication. The participants were scheduled for the study operation, which required at least six weeks of recovery after a non-study-related operation. On the day of the operation, the subjects were randomly assigned to receive standard papilla preservation flaps surgery (PPFS group) or endoscopy-assisted subgingival debridement (EASD group) with block randomization. The assignment was concealed in opaque envelopes and revealed to the operator (KLDH) on the day of operation. The first 11 participants from PPFS or EASD group consenting to the microbiological arm of the study were recruited follow by baseline microbiological samples collection before the surgery or the endoscope-assisted re-instrumentation.

2.2.2. Interventions

For PPFS, open flap debridement with a minimally invasive periodontal surgical approach was applied [13]. Under local anesthesia, the defect-associated interdental papilla will be incised with a buccal approach horizontal incision at the base of the papilla [14]. A full-thickness mucoperiosteal flap was raised to expose the intrabony defects. The elevation was limited to exposing the sound bone margin maximally up to 2 mm apically. Granulation tissue was removed, and the root surface was debrided mechanically. The flap was repositioned and sutured with 4/0 silk (Surgisilk, Sutures Limited).

For EASD, the participants were anaesthesized with local anesthesia and subgingival atraumatic mechanical instrumentation through the orifice of the periodontal pocket was carried out with the use of slim ultrasonic tips (PS, PL4/5 tip, Piezon Master 700 EMS, Switzerland) and with the aid of a periodontal endoscope to visualize the subgingival portion of the root surface (Perioscope, Perioscopy Inc., USA) and a pair of $\times$ 2.5 magnification loupes (TP-720, Sandy Grendel, Switzerland). The atraumatic subgingival instrumentation was performed within the periodontal probing depth, maintaining the gingival margin and interdental papilla intact, avoiding excess soft-tissue retraction with the endoscope, and removing calcified deposits in the subgingival root surface without intentional removal of the cementum [15]. At the end of the operation, the study sites were gently compressed with gauze to achieve hemostasis.

2.2.3. Follow-up appointments

All operations were performed by a single operator (KLDH), and the subjects were followed up at 3-, 7-, 14-, 30-, and 60-day post-operative

intervals to ensure uneventful healing of the soft tissue and resolution of the surgical marks, which affected the blindness of the study. During this period, KLDH would be responsible for collecting microbial samples. BA (KLRH) evaluated the subjects at follow-ups, during which microbial sampling was followed by recording of clinical parameters and provision of supportive periodontal care. The locations for microbial sampling were passed on to KLRH in written format. The end-point of the therapy was defined as no deep residual pocket with PPD $\geq$ 5 mm with bleeding on probing at 360-day recall. The primary investigator re-evaluated the prognosis of any site that did not achieve the therapy end-point, and appropriate therapy was delivered accordingly.

2.2.4. Clinical data recording

The presence of plaque and bleeding on gentle probing was recorded dichotomously at six sites per tooth. The full-mouth plaque (FMPS) and bleeding (BOP) scores (FMBS) were calculated, as previously described [16]. PPD and recession of the gingival margin (REC) were recorded to the nearest millimeter with a standardized periodontal probe (color--coded UNC15, PCPUNC156, Hu-Friedy). Clinical attachment level (CAL) was calculated as PPD plus REC. The intraclass consistency coefficient for CAL for the calibrated examiner (KLRH) was 0.976 (Cronbach's α, two-way mixed model with absolute agreement).

2.3. Subgingival microbial sample collection

Subgingival plaque samples were collected from the study sites at baseline, 3 days, 7 days, 14 days, 30 days, 3 months, 6 months, 9 months, and 12 months post-operation. The site was first isolated with a sterile cotton roll and then air-dried. Supragingival plaque was removed with a sterile sickle curette, and five #30 sterile paper points (Dentsply Sirona, USA) were inserted into the periodontal pocket at the study site. After 10 s, the paper points were transferred and pooled into sterilized, dry Eppendorf tubes and stored at −80 °C.

2.4. 16S rRNA sequencing and data analysis

Total bacterial genomic DNA was extracted from paper point plaque samples using QIAamp DNA Mini Kits (Qiagen, Hilden, Germany) following the manufacturer's protocol, as previously described [12]. The V3–V4 hypervariable region of the 16S ribosomal RNA (rRNA) gene was amplified via PCR using universal bacterial primer pairs 341F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR amplification and subsequent library construction were performed according to DNBSEQ's standard protocol for 16S Metagenomic Sequencing Library Preparation [17]. Paired-end sequencing (300 bp × 2) was conducted on a DNBSEQ platform at the Beijing Genomics Institute (BGI, Shenzhen, China). Raw sequencing reads were processed into amplicon sequence variants (ASVs) using the DADA2 workflow in R [18], with script details and parameter settings provided in the Materials and Methods section of the Supplementary Information.

2.5. Statistical analysis

Statistical analyses were performed in the R environment. Periodontal parameter values were used in their original form, while taxonomic data were aggregated to the species level. Microbiome data were transformed to relative abundances for alpha and beta diversity analyses and scaled to z-scores for model fitting. A significant threshold of $p < 0.05$ was applied to all statistical tests. Details of the R packages and parameter configurations used are summarized in the Materials and Methods section of the Supplementary Information.

3. Results

A total of 22 participants (11 PPFS and 11 EASD) were recruited. Two participants in the EASD group withdrew after the baseline without attending any of the subsequent follow-up appointments, and one refused microbial sampling from day 30 and was subsequently dropped. The remaining 19 participants, who suffered from Stage III to IV periodontitis, completed the entire study period, and subgingival plaque samples were collected at all study time points (Supplementary Fig.1). The FMPS, FMBS, PPD, REC, and CAL between groups did not show a significant difference at any time point (Supplementary Table 2) [10]. Uneventful healing of the soft tissue of all sites and no participants reported any severe adverse event during the healing period (0 – 90 days post-operation). At the end of the study, all sites in the PPFS group (100.0 %) and seven out of the eight study sites in the EASD group (87.5 %) had achieved the therapy endpoint. The one site from the EASD participant was immediately followed up on after the study's conclusion.

3.1. Subgingival microbial composition between the PPFS and EASD treatment groups

The clinical samples that failed to meet the downstream sequencing standards and could not be analyzed are listed in Supplementary Table 3. Overall, 86.6 % of the samples collected could be scrutinized.

Subgingival microbiome sequencing demonstrated comparable biodiversity and community structure between PPFS and EASD groups throughout the study. Rarefaction curves were generated for microbial samples from both the PPFS and EASD groups, confirming sufficient sequencing depth and comparability between the groups (Supplementary Fig. 2). Alpha and beta diversity analyses at baseline revealed no significant differences in subgingival microbiota composition between the PPFS and EASD groups (PERMANOVA $p = 0.108$), indicating similar initial microbial community structures (Fig. 1A and B). Alpha-diversity analyses indicated similar species diversity characteristics between the groups, with some variations at specific time points. The Chao1 and Observed indices suggested potential differences in species richness at particular intervals. In contrast, the Gini-Simpson and Shannon indices indicated comparable species evenness and overall diversity, with minor fluctuations (Fig. 1A). Over the 12-month follow-up, beta diversity analysis revealed no significant differences in microbial composition between the two treatments at any time point (PERMANOVA $p > 0.05$ for days 3, 7, 14, 30, 3 months, 6 months, 9 months, and 12 months; Fig. 1B). Venn diagrams further illustrated the overlapping proportions of microbial species between groups, supporting consistent community structures post-intervention (51.1–61.7 %, Fig. 1C and Supplementary Fig. 3).

3.2. Potential immediate post-treatment microbial shifts of PPFS and EASD groups

To delve deeper into the microbial alterations, a time-series analysis was conducted for the two groups, aiming to provide a more comprehensive comparison of the changes in their microbial communities. To compare the immediate post-treatment microbial changes, subgingival microbiomes were collected on day 3, and the results showed that while both the PPFS and EASD methods effectively removed subgingival microbiota (Fig. 1A, with observed alpha diversity decreasing), their impacts on the microbial community differed (Fig. 2). On day 3, in the PPFS group, a significant difference in beta diversity was observed compared to the baseline (PERMANOVA, $p = 0.001$; Fig. 2A), indicating that the PPFS method induced substantial changes in microbial composition. In contrast, for the EASD group, although a change in the microbiome was also observed, the beta-diversity change compared to the baseline was not significant (PERMANOVA $p = 0.120$; Fig. 2B), yet it still indicated a certain impact on the microbial composition.

By comparing the Venn diagrams of the microbes identified from PPFS and EASD samples, variations in the numbers of shared and unique bacterial species at months' time points can be observed

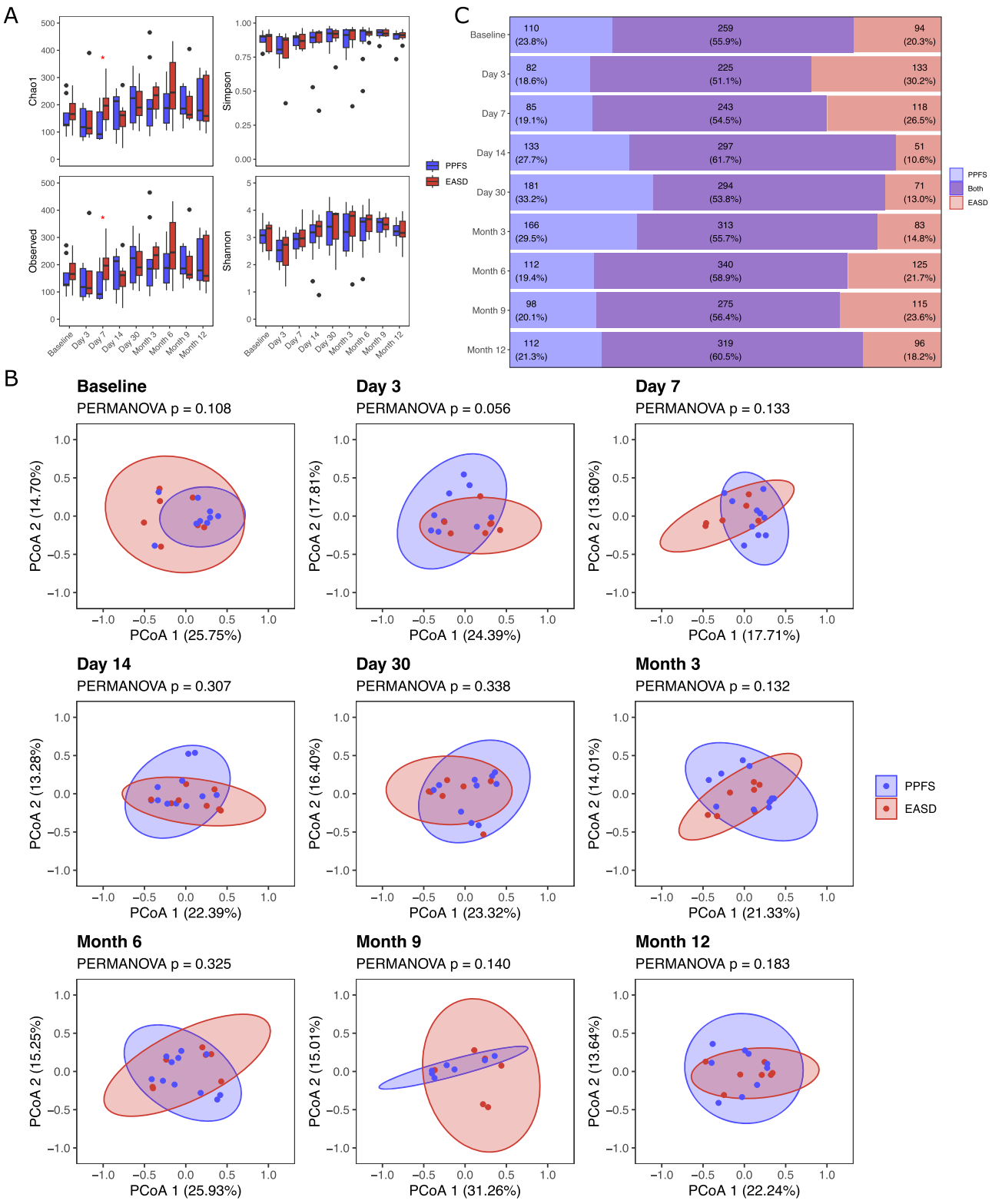


Fig. 1. Diversity, composition, and overview of microbial communities in residual periodontal intrabony defects treated by papilla preservation flap surgery (PPFS) or endoscope-assisted subgingival debridement (EASD). **A.** Microbial alpha-diversity indices of the PPFS (blue) and EASD (orange) samples at different time-points. Except for Choa 1 and the observed alpha-diversity on day 7. **B.** Principal Coordinates Analysis (PCoA) plots based on unweighted UniFrac distances of the microbial communities from the PPFS (blue) and EASD (orange) groups at a particular sampling time point. PERMANOVA (permutational multivariate analysis of variance) *p*-values are shown. **C.** Venn diagrams showing the bacteria species detectable only from PPFS (blue), EASD (orange) sample, or in both PPFS and EASD (purple) samples at the time-point of concern. * $p < 0.05$.

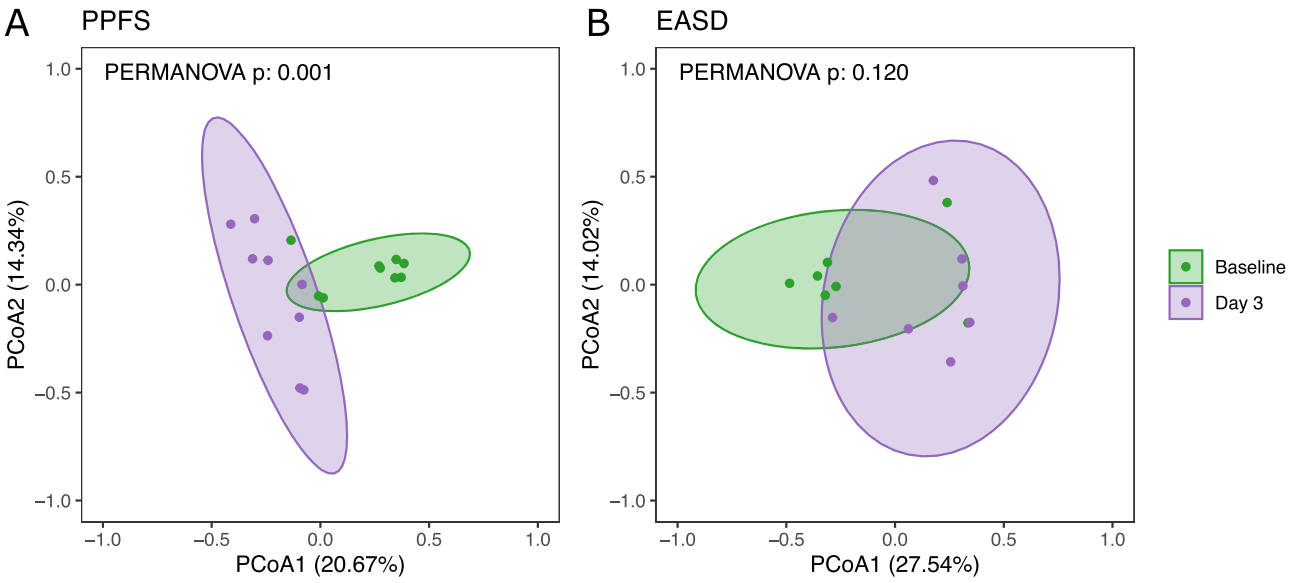


Fig 2. Baseline and immediate post-treatment (day 3) microbial composition in residual periodontal intrabony defects treated by papilla preservation flap surgery (PPFS) or endoscope-assisted subgingival debridement (EASD). Principal-Coordinates Analysis (PCoA) plots based on unweighted UniFrac distances of the microbial communities showing the changes in subgingival microbiota composition from baseline (green) to day 3 (purple) for the PPFS (**A**) and EASD (**B**) groups. Permutational multivariate analysis of variance (PERMANOVA) revealed a significant change in microbial composition in the PPFS samples at day 3.

(Supplementary Fig. 3). In terms of the number of shared bacterial species across all time points, the PPFS (235) and the EASD (219) groups were comparable. Regarding the number of unique bacterial species, a similar pattern was observed (PPFS = 161, EASD = 125).

3.3. Longitudinal changes in subgingival microbial composition following PPFS and EASD treatments

When analyzing the relative abundance of the top 20 bacterial taxa (which collectively accounted for over 50 % of the microbial community at baseline), it was observed that they together accounted for over 50 % abundance at all time points examined, indicating their dominant position within the community (Fig. 3A & B). Concerning the known putative periodontopathic [1,19] (Fig. 3C & D) or periodontal health [1] (Fig. 3E & F) bacteria, which are at any time <0.2 or 0.07 relative abundance, respectively, minimal differences can be observed between that of the PPFS vs. the EASD group at any time point (Supplementary Fig. 5).

Further analysis of pathogenic bacteria revealed that, at post-treatment, the average relative abundance of putative periodontopathogens in the PPFS and EASD groups was approximately 60 % that of the baseline (Fig. 3C and D). Immediately after the therapy, on day 3, the relative abundances of pathogenic bacteria in the PPSF/EASD group decreased to approximately 50/30 %, indicating the effectiveness of the two surgical procedures in removing these pathogens. However, by one month, a 50–60 % relative abundance of periodontopathogens compared to baseline had already been established (Fig. 3C & D). Considering the three identifiable 'periodontal health' [1] species, there is also a roughly 40 % (PPSF) or 90 % (EASD) relative abundance reduction over the post-treatment period (Fig. 3E & F), indicating that they are also affected by the periodontal intervention.

3.4. Microbial community stability and community function following PPFS and EASD treatments

The microbial community in the EASD group exhibited minimal variation across multiple time points compared to the PPFS groups (Fig. 4A & B). This result indicates that there were no statistically significant changes in the microbial community structure over time within the EASD group (PERMANOVA $p = 0.324$) (Fig. 4B). Despite fluctuations observed at intermediate time points, the overall microbial composition remained stable.

The Procrustes analysis aligns microbial community structures from different time points. The results showed that the EASD 12-month microbial composition was statistically similar to the baseline (Procrustes SS = 0.033, $p = 0.030$) (Fig. 4D). In contrast, the PPFS microbial community at 12 months remained significantly different from the baseline (Procrustes SS = 0.207, $p = 0.100$, Fig. 4C).

3.5. Correlation between the predominant post-treatment microbial profile and periodontal parameters

Considering the generally successful therapy by PPFS or EASD, and the relatively small sample sizes for both groups, the research team chose to consolidate all data for this part of the study and focus only on microbes with ≥50 % prevalence ($n = 14$). Subsequent correlation analysis between post-treatment periodontal clinical parameters (PI, BOP, and PPD; 3- to 12-month) and microbial taxa [11] revealed distinct associations at the site level (Fig. 5A). Specifically, five commensal species exhibited negative correlations with PI, BOP, and PPD, whereas nine pathogenic taxa showed positive correlations. In all samples, mixed-effects modeling revealed that BOP was positively associated with *Fusobacterium nucleatum* subsp. *vincentii*, while PPD correlated with *Fretibacterium fastidiosum* and *Treponema socranskii* (Fig. 5B).

4. Discussion

PPFS and EASD demonstrated comparable clinical outcomes on the management of residual periodontal pockets with intrabony defects up to 12 months post-operation in the previous non-inferiority study [10]. The underlying therapeutic mechanisms and the anticipated healing dynamics induced are distinct.

This study elucidates the subgingival microbiota dynamics following PPFS and EASD in residual periodontal intrabony defects. During the one-year observation period, the absence of significant differences in overall microbiome composition between PPFS and EASD aligns with their comparable clinical outcomes (Supplementary Tables 1 & 2) [10, 20]. The microbial results showed that both PPFS and EASD achieved

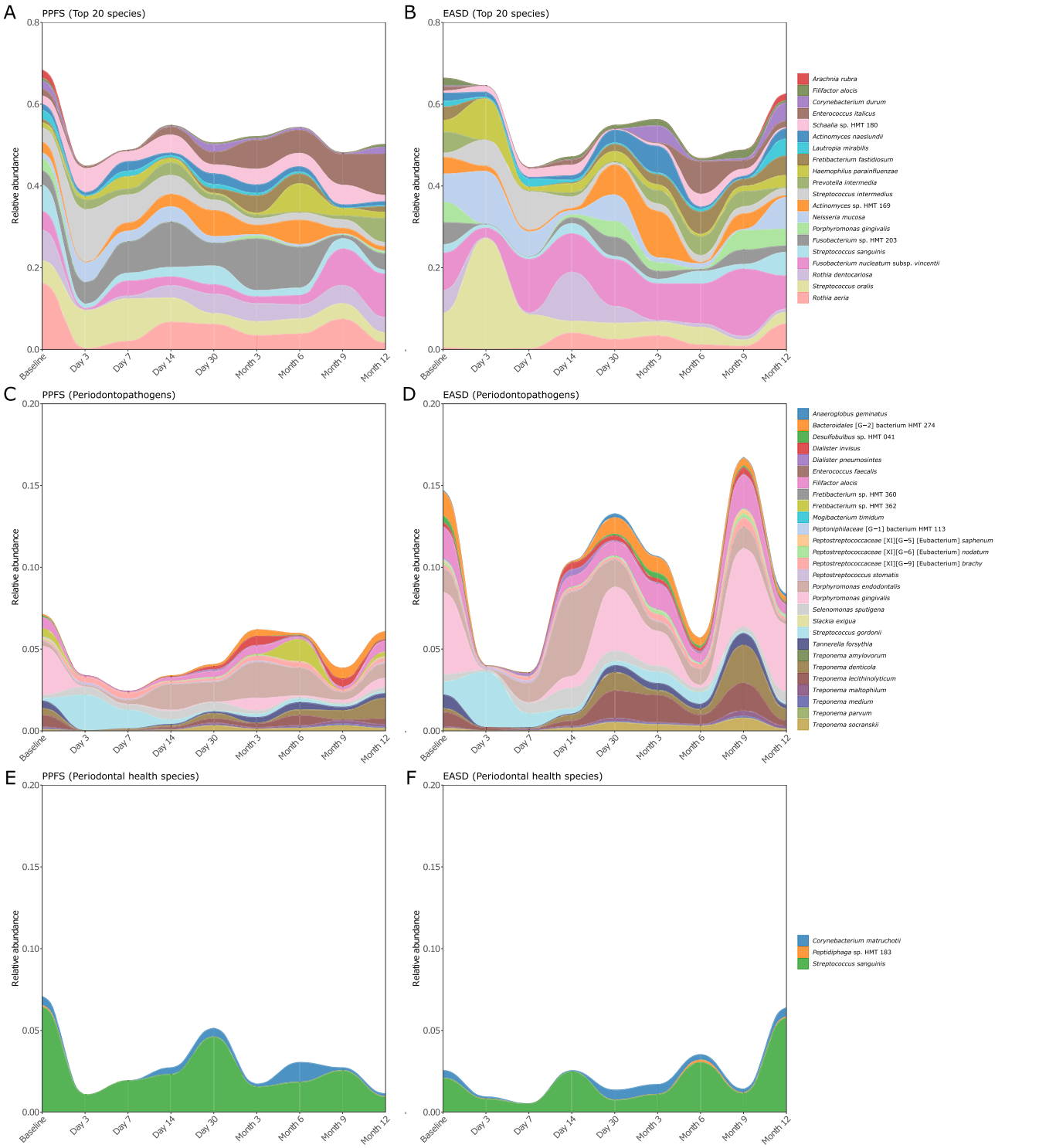


Fig. 3. Longitudinal changes in subgingival microbial composition following papilla preservation flap surgery (PPFS) or endoscope-assisted subgingival debridement (EASD) in residual periodontal intrabony defects. Relative abundance of the top 20 species at baseline/periodontopathogenic species [putative species [1] and red complex species [19] (n = 28)]/periodontal health species [1] (n = 3) over the study timeline for the PPFS (**A**/**C**/**E**) or EASD (**B**/**D**/**F**) groups, respectively. Please note that the currently used culture-independent microbial detection system cannot unequivocally identify *Streptococcus mitis;* therefore, the corresponding information under periodontal health species was not included.

comparable overall influences in the predominantly detectable species and periodontopathogenic bacteria profiles (Fig. 3, Supplementary Fig. 3 & Fig. 4), consistent with the shared therapeutic objective of disrupting subgingival biofilms—the primary etiological factor in periodontitis [3,21]. A noteworthy point is that, including the prior Step 1 and 2 therapies, neither the surgical approach nor the endoscope-assisted treatment could eradicate the putative or established periodontopathogens, nor could we see a massive increase in abundance of any "health-related" species (Fig. 3, Supplementary Fig. 3 & Fig. 4). This finding is concurred by the previous report that suppressing periodontal pathogen in deep periodontal pocket with intrabony component by subgingival debridement may not be sufficient [22]. All in all, the

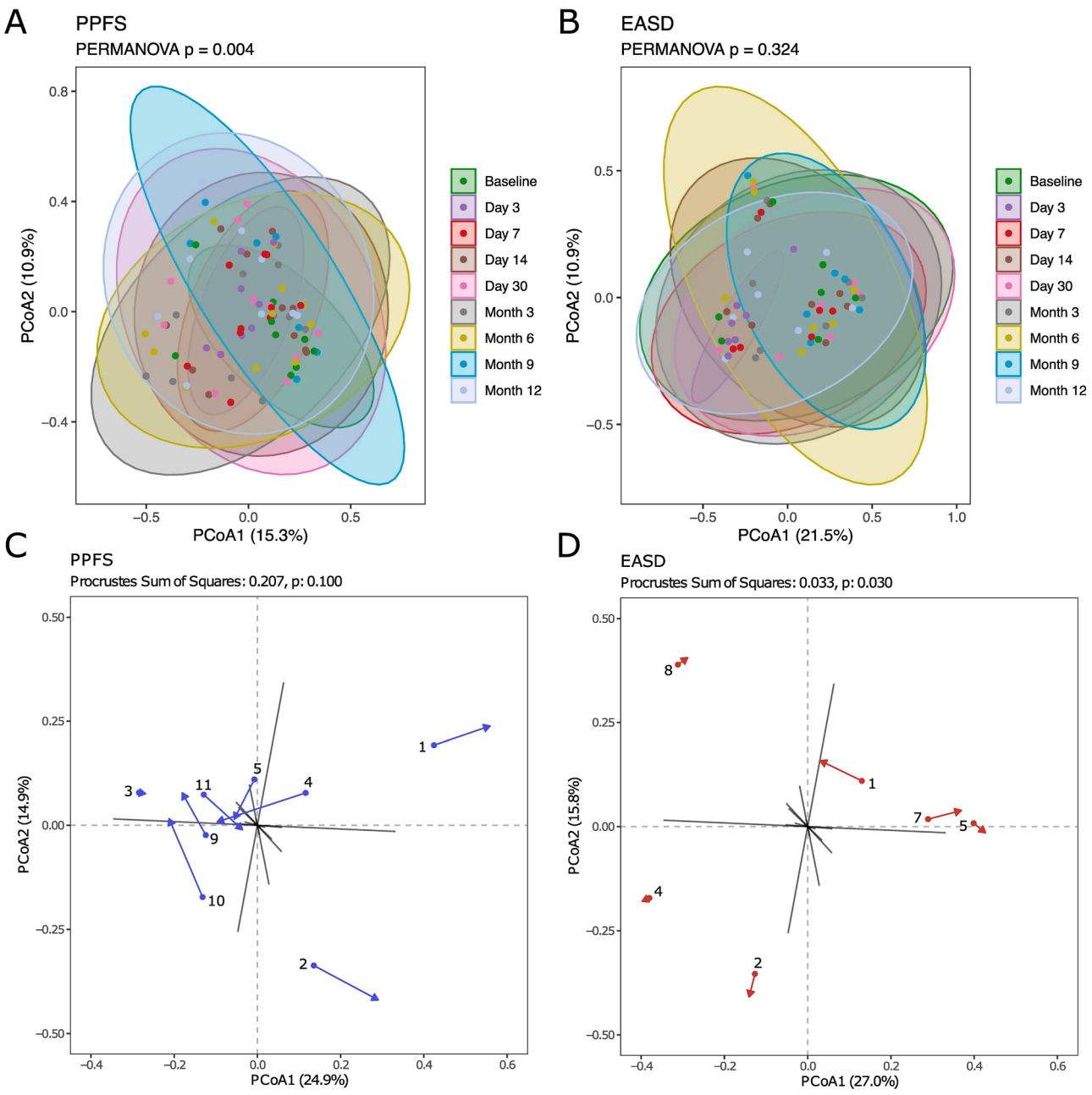


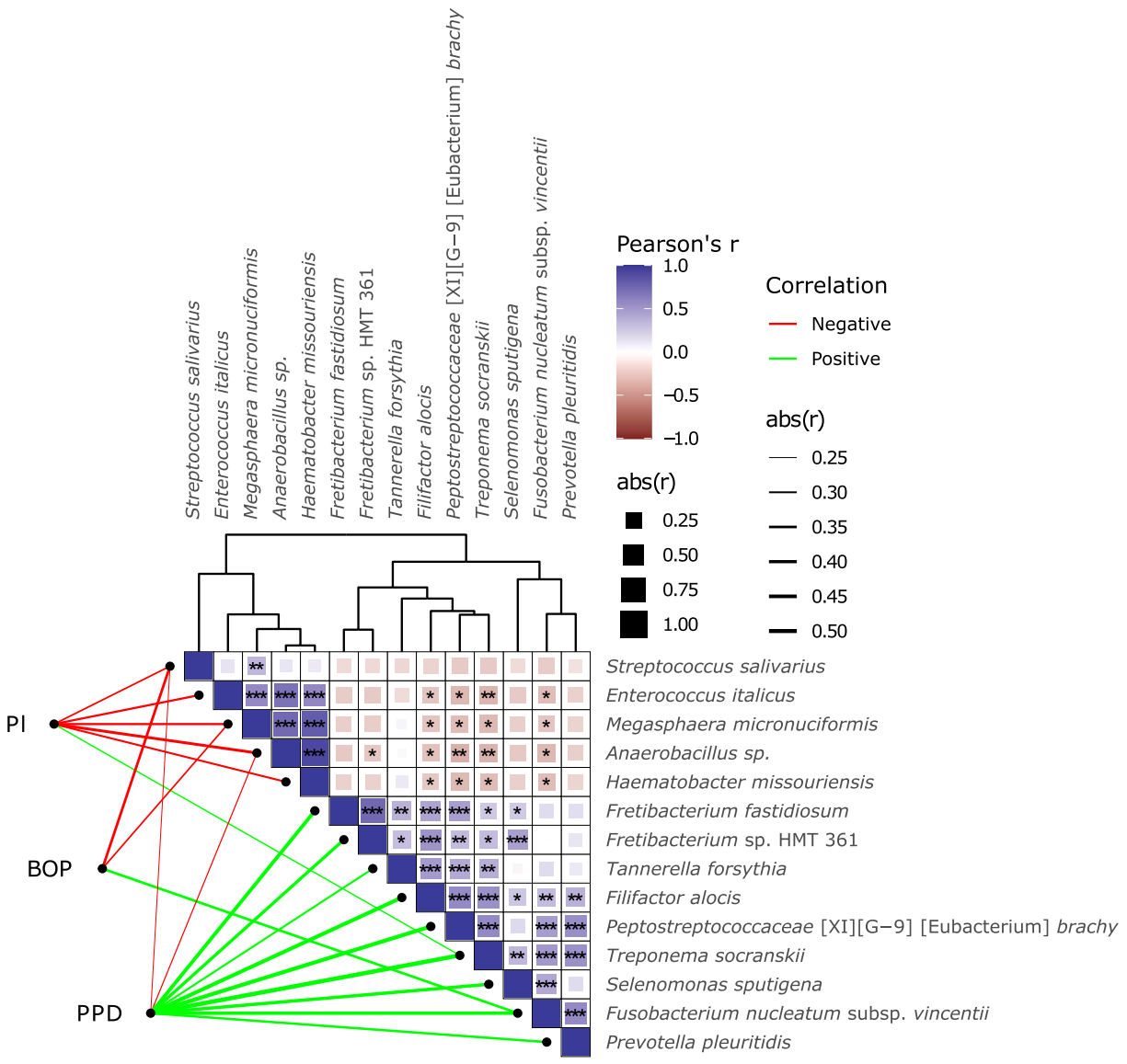
Fig. 4. Microbial composition following papilla preservation flap surgery (PPFS) or endoscope-assisted subgingival debridement (EASD) in residual periodontal intrabony defects over all study time points. Principal Coordinates Analysis (PCoA) plots based on unweighted UniFrac distances of the microbial communities comparing the subgingival microbiota composition of samples in PPFS (**A**) or EASD (**B**) groups, from baseline to 12 months, permutational multivariate analysis of variance (PERMANOVA) indicated a statistically significant difference ($p = 0.004$) was observed in the PPFS group. When focusing on the microbial profile variations between the baseline and 12-month (**C** and **D**) time points, the Procrustes analysis (sums of squares approach) indicated significant intra-participant microbial profile similarity in the EASD group (D). Numbers in the plots (**C and D**) represent individual participants as shown in Supplementary Table 1. Please note that either the baseline or 12-month microbial samples from participants 6, 7, and 8 in the PPFS group, as well as participants 3 and 6 in the EASD group, did not meet the required standards for downstream sequencing and were therefore not analyzed (Supplementary Table 3).

observation perhaps reinforces the notion regarding the importance of coexistence between periodontopathogens and oral commensals, as well as the opportunistic nature of the pathogen-caused periodontal infection [23].

Reductions in putative pathogenic bacteria, including the red-complex (*P. gingivalis, T. denticola, T. forthysia*), observed in both groups within the first week of the Step 3 treatment, support the efficacy of the mechanical biofilm disruption, whether via PPFS direct surgical access or EASD closed-endoscopic root surface debridement (Fig. 3C & D) [7,24]. Nevertheless, various levels of relative abundance rebound of the total periodontopathogen were observed from 3 to 12 months, despite the almost 100 % elimination or minimization of the periodontal pocket concern (Supplementary Table 1).

Despite the persistent inflammation and inability of the test sites to heal after the Steps 1 and 2 therapy, not all samples from all study time points, including three from baseline, had sufficient microbial material required for downstream analysis (Supplementary Table 3). This could perhaps be explained, at least in part, by the relatively good home care delivered by the participants (Supplementary Table 2). Moreover, there were 3 subjects dropped out from the EASD group which affects the

A



B

	Pl				BOP				PPD			
	b	*SE*	*z value*	*p*	*b*	*SE*	*z value*	*p*	*b*	*SE*	*t value*	*p*
(Intercept)	0.262	0.286	0.914	NS	0.986	0.435	2.269	*	3.797	0.107	35.569	***
Filifactor alocis	---	---	---	---	-1.002	0.539	-1.861	NS	---	---	---	---
Fretibacterium fastidiosum	---	---	---	---	---	---	---	---	0.331	0.113	2.922	**
Fusobacterium nucleatum subsp. *vincentii*	---	---	---	---	1.361	0.680	2.002	*	---	---	---	---
Haematobacter missouriensis	-0.674	0.348	-1.940	NS	---	---	---	---	---	---	---	---
Megasphaera micronuciformis	---	---	---	---	-0.667	0.417	-1.599	NS	---	---	---	---
Selenomonas sputigena	---	---	---	---	---	---	---	---	0.224	0.117	1.918	NS
Streptococcus salivarius	-0.743	0.465	-1.597	NS	-0.625	0.499	-1.251	NS	---	---	---	---
Tannerella forsythia	---	---	---	---	0.928	0.647	1.434	NS	---	---	---	---
Treponema socranskii	---	---	---	---	---	---	---	---	0.426	0.117	3.647	***

Fig. 5. Correlation between subgingival species ($n = 14$) with prevalence of ≥50 % post-treatment [papilla preservation flap surgery (PPFS) and endoscope-assisted subgingival debridement (EASD) in residual periodontal intrabony defects; 3-, 6-, 9-, and 12-month] and their corresponding periodontal parameters. **A.** The correlation plots depicting the strength of association (Pearson Correlation) between the 14 species of concern and periodontal parameters followed: plaque (Pl, no/yes), bleeding on probing (BOP, no/yes), or probing pocket depth (PPD, in mm) measured at the four study time points. Correlations between bacteria are displayed in *rmcorr*'s r (boxes: blue = positive, red = negative; size = absolute r value). Correlations between bacteria and periodontal parameters are displayed in green (positive) and red (negative) lines of varying thickness, indicating the absolute r value. **B.** A linear mixed-effects model (for continuous outcome PPD) and a logistic mixed-effects model (for binary outcomes Pl and BOP) were used to analyze the relationship between periodontal outcomes and the 14 bacterial species. The bacterial variables were selected using a stepwise model selection. Estimates of the coefficients (b) and the associated standard error (SE) are provided for the fixed effects. Statistical analysis refers to a *t*-test (PPD) or a Chi-square test (Pl and BOP). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

comparison between the test and control group. These are the limitations of the current study; researchers may need to consider this fact to improve microbial specimen collection, increase the sample size and improve retention of subjects in future investigations.

In the current study, patients diagnosed with Stage III and IV periodontitis who presented with persisting residual periodontal intrabony defects which require attention of Step 3 periodontal therapy were recruited [10]. The highly standardized inclusion criteria, the well-executed Step 1 and 2 periodontal therapies and the Step 4 periodontal therapy at 3-month interval have largely controlled the inflammatory burden of the subjects in two groups. To further minimize confounding factors, all 19 included subjects were non-smokers and had no relevant medical history, such as diabetes mellitus. While four subjects were on medication for hypertension, one was being monitored for osteoporosis, and one had a history of breast cancer, these conditions are not directly linked to periodontal inflammation in the same way as smoking or diabetes. This rigorous selection and pre-treatment protocol ensured that the microbiological evaluation was conducted on a patient cohort with controlled inflammation, providing a more accurate representation of the microbial community at the specific intrabony defect sites. Furthermore, with comparable baseline clinical and microbiological features of the subjects (Supplementary Table 1 & 2), the difference of the impact of the inflammatory burden between two groups should have been kept insignificant and the findings of the current study is reflecting the subgingival microbial community induced by the test and control therapy in the 12-month study period.

Concerning a recent systematic review and meta-analysis, the microbiological changes after periodontal surgery observed were, however, heterogeneous, despite the clinical improvement [25]. None of the included studies appropriately reported the completion of Step 2 therapy before the study commencement, i.e., with a reported Pl % >40 % or BOP % approximately 50 % at the study's baseline. The findings of the current study provided valuable information on the effect of microbial shifts during the initial healing phase of periodontal tissue following both surgical and non-surgical periodontal procedures. It may be potentially crucial for emerging minimally invasive treatment protocols, including non-incisional periodontal regeneration and minimally invasive non-surgical therapy, to seek further improvements.

Only minimal studies comprehensively reported the microbes associated with clinical parameters before or during active periodontal therapy [11]. That recent report from this group focused on 21 species that exhibited significant changes in relative abundance over the study period [11]. The current study investigated the relationship between clinical parameters and microbes that could be identified more than or equal to twice in the 3- to 12-month samples (Fig. 5).

It further validated correlations between changes in the microbiome and clinical parameters through site-specific analysis of samples. Bleeding on probing, a key marker of inflammation, exhibited a positive association with *F. nucleatum* subsp. *vincentii* whereas PPD correlated with the detection of *F. fastidiosum* and *T. socranskii* (Fig. 5).

Periodontal pocket depth showed significant positive correlations with nine putative periodontopathogens pre-treatment [1]: *F. fastidiosum, Fretibacterium* sp. HMT 361, *T. forsythia, Filifactor alocis, Peptostreptococcaceae* [XI][G-9] [Eubacterium] brachy, *T. socranskii, Selenomonas sputigena, F. nucleatum*, and *Prevotella pleuritidis*. A similar observation was reported in pre-treatment periodontitis patients, where *F. fastidiosum, T. forsythia*, and *Campylobacter rectus* were identified as key drivers of subgingival plaque alteration, contributing to periodontitis [26], or the former two pathogens together forming tentative oral microbiome signatures relevant to periodontitis [27]. Similarly, it was also reported to be the case for *T. socranskii* [28], with implications or correlations with clinical parameters, including PPD [29]. The association between BOP and *F. nucleatum* subsp. *vincentii* relative abundance, however, remained to be elucidated.

The current pilot study observations, in line with previous studies, suggesting that clinicians may need to be cautious about the potential association between deeper PPD and an increased relative abundance of the gram-negative anaerobic motile *F. fastidiosum* during the healing process of residual pockets after Step 3 therapy, whether with surgery or EASD (Fig. 5). Indeed, *F. fastidiosum* was reported, together with *S. sputigena*, to contribute to the upregulation of bacterial chemotaxis, flagellar assembly, and two-component system proteins upon transcriptional activities analysis of the periodontal pocket microbiota from all domains of life as well as that of humans [30]. Although EASD demonstrated non-inferior clinical outcomes compared to PPFS up to 12 months post-operation, the current study revealed a subtle difference in microbiological responses and a tendency for baseline reversion within the subgingival plaque of the EASD group.

In conclusion, both PPFS and EASD demonstrate comparable efficacy in reducing pathogens and achieving similar long-term functional outcomes in residual periodontal intrabony defects. However, their microbial stability and ecological trajectories subtly diverge: PPFS creates sustained shifts in microbial composition. At the same time, EASD exhibits slightly greater variability in species richness and pathogen abundance during intermediate healing phases, reflecting relatively less stable ecological restructuring. EASD's tendency toward baseline microbial reversion and its dependency on operator skill for closed visualization may impact clinical reliability in deep pocket management. Future research should prioritize larger sample cohorts, evaluations of inter-operator variability, longitudinal microbial monitoring, investigations into adjunctive therapies for EASD, and efforts to enhance and validate the reliability of endoscope-assisted subgingival debridement.

Data availability

The sequence reads and metadata were deposited in NCBI Short Reads Achieve with BioProject number PRJNA1288221 while other data supporting the findings of this study are available on request from the corresponding author. These data are not publicly available due to privacy or ethical restrictions.

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Human research ethic

This study received approval from the Hong Kong Hospital Authority West Cluster Ethics Committee (approval number UW 19–086) and was prospectively registered in the Chinese Clinical Trial Registry (ChiCTR-INR-16,008,407).

CRediT authorship contribution statement

King-Lun Dominic Ho: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Miao Wang:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Ka-Leong Ryan Ho:** Investigation, Data curation, Conceptualization. **Wai Man Tong:** Software, Methodology, Investigation, Formal analysis, Data curation. **Maurizio S. Tonetti:** Methodology, Conceptualization. **Wai Keung Leung:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jdent.2025.106058.

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