Discriminant microbial signatures and effect of periodontal treatment along the gum-gut axis

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Abstract *(English version)*

Aims: 1) To explore the oral-gut microbial signatures associated with periodontitis; and 2) to longitudinally evaluate the effect of periodontal treatment on the gut microbial composition.

Material and Methods: Stool and saliva samples from stage III-IV periodontitis patients (P; n=47) were collected and analyzed by 16S rRNA gene sequencing, before and 3 months after steps I-II of periodontal treatment. Periodontally healthy matched subjects (H; n=47) were used as controls. Principal component analysis (PCA) was carried out to identify oral-gut microbial profiles. P and H were compared in terms of PCA and microbial taxa; P were longitudinally compared before and after treatment.

Results: Gut microbial profiles of P significantly differed from H ($p<0.001$), being characterized by lower beta-diversity. Periodontal treatment was associated with a significant change in gut microbiota ($p<0.001$), with post-treatment profiles tending to H ($p>0.05$). Genera *Bacteroides, Faecalibacterium,* and *Lachnospiraceae* were the most represented in P fecal samples, whereas genus *Lactobacillus* was more enriched in H. Periodontal treatment significantly reduced gut genera *Bacteroides*, *Eubacterium*, *Lachnoclostridium*, *Lachnospira*, *Lachnospiraceae*, *Oscillospiraceae*, *Roseburia*, and *Ruminococcaceae.*

Conclusions: Discriminating oral-gut microbial signatures of periodontitis were found. Periodontal treatment significantly reversed the gut microbial composition, raising novel clinical implications about the relevance of the gum-gut axis.

Keywords: Gum-gut axis, Inflammation, Periodontitis, Diagnosis, Oral health.

Abstract *(Versione italiana)*

Obiettivi: 1) Esplorare i profili microbiologici oro-intestinali dei pazienti con parodontite di stadio III-IV e 2) valutare l'effetto del trattamento parodontale sulla composizione microbica intestinale.

Materiali e metodi: Sono stati raccolti campioni di feci e saliva di pazienti con parodontite di stadio III-IV (P=47), ed analizzati tramite tecnica del 16S rRNA prima e 3 mesi dopo gli step I-II della terapia parodontale. Soggetti con salute parodontale (H=47) sono stati selezionati come controlli. L'analisi delle componenti principali è stata utilizzata per identificare i profili relativi a salute e malattia. P sono stati comparati ad H in termini di PCA e taxa microbiologici, prima e dopo trattamento.

Risultati: I profili microbiologici intestinali dei P differivano in maniera significativa rispetto agli H ($p<0.001$), essendo caratterizzati da una minore beta diversità. Il trattamento parodontale ha modificato in modo significativo la composizione microbica intestinale e salivare, con i profili post-terapia che tendevano a sovrapporsi con H ($p>0.05$). I genera *Bacteroides*, *Faecalibacterium*, e *Lachnospiraceae* erano i più rappresentati nei campioni fecali dei P, mentre il genus *Lactobacillus* era l'unico più presente negli H. Il trattamento parodontale ha ridotto significativamente i genera *Bacteroides*, *Eubacterium*, *Lachnoclostridium*, *Lachnospira*, *Lachnospiraceae*, *Oscillospiraceae*, *Roseburia*, e *Ruminococcaceae* nell'intestino dei P.

Conclusioni: I profili microbiologici dei P si sono distinti da quelli degli H. Il trattamento parodontale è stato in grado di modificare la composizione del microbioma intestinale.

1. Introduction

In recent years, the gut microbiome has received an enormous scientific interest in relation to human health, and now gut dysbiosis is being regarded as a major driver for multiple non-communicable diseases (NCDs), including cardiovascular diseases, metabolic alterations (including diabetes), and cancer (Ribaldone et al. 2019; Metwaly et al. 2022). Indeed, microbial dysbiosis induced by a high fat diet, sedentary life, smoking, alcohol, and chronic infections may result in reduced production of short chain fatty acids and mucosal leakage, which in turn may cause a spill-out of pathogen-associated molecular patterns in the blood triggering low-grade systemic inflammation (LGSI) and insulin resistance (Fan and Pedersen 2021). Therefore, there is a great interest in understanding further determinants of gut dysbiosis, as well as actionable factors to target the microbiome in the global prevention and clinical management of NCDs.

Since the mouth is the gateway for the gastrointestinal tract, the oral cavity may represent a major reservoir for gut pathobionts, especially in patients affected by a dysbiosis-associated oral disease, as periodontitis (Kitamoto et al. 2020). Indeed, periodontitis is the 6th most prevalent disease of mankind, affecting more than 50% of the global population (Aimetti et al. 2015; Chen et al. 2021; Morales et al. 2022). It is characterized by a non-resolving inflammation resulting from a polymicrobial dysbiosis of the oral subgingival biofilm that may finally lead to tooth exfoliation secondary to severe alveolar bone resorption. Moreover, periodontitis has been linked to a broad spectrum of NCDs through blood translocation of gum pathogens (bacteremia), bacterial aspiration into the lower respiratory tract, and elevation of LGSI biomarkers (Hajishengallis and Chavakis 2021; Baima et al. 2022a; Botelho et al. 2022). When timely delivered, periodontal treatment is effective in obtaining clinical control of the disease, reducing LGSI markers, and restoring a healthy oral microbial ecosystem by decreasing the relative abundance of specific taxa and related byproducts (D'Aiuto et al. 2018; Romano et al. 2019; Johnston et al. 2021).

Periodontitis may also influence intestinal and systemic health through the enteral translocation of resident oral bacteria, via swallowing of saliva (Baima et al. 2022a, b). Indeed, periodontal pathobionts consistently induced intestinal inflammation in preclinical models (Atarashi et al. 2017; Kitamoto et al. 2020). In humans, an exploratory cross-sectional study has shown that periodontitis is associated with a gut enrichment of oral bacteria and a lower beta diversity, being the latter a prominent feature of dysbiosis (Lourenco et al. 2018). However, a deeper characterization of the microbiome in these two interconnected anatomical niches is currently lacking, preventing a thorough mechanistic understanding of the

physiopathological influences along the gum-gut axis. Moreover, no data is available to verify whether periodontal treatment may perturb the gut environment through its influence on the oral ecology.

Therefore, this study aimed at:

- comparing the oral-gut microbial signatures of stage III-IV periodontitis with periodontal health;
- exploring the longitudinal taxonomic changes occurring at the gut and salivary microbiota after periodontal treatment.

2. Material and methods

The protocol was approved by the local Institutional Ethical Review Board (protocol number 00066/2021), and written informed consent was obtained from all participants.

2.1 Study population

Patients with stage III-IV periodontitis were consecutively recruited from individuals seeking oral health consultation at the C.I.R. Dental School, University of Turin (Italy), from March 2021 to September 2022. Periodontally healthy, age- and gender-matched, participants were also selected (Papapanou et al. 2018).

The following additional exclusion criteria were applied: <20 teeth; diagnosis of systemic diseases, including gastrointestinal diseases; obesity (BMI ≥ 30 kg/m^2); food allergies; heavy smoking (> 10 cigarettes/day); periodontal treatment during the previous 12 months; consumption of antibiotics or probiotics within the 3 months before enrollment; pregnancy/lactation; intake of medications known to affect periodontal status. Periodontitis patients taking systemic antimicrobials between the baseline and the 3-month examination were additionally excluded.

2.2 Periodontal parameters, dietary questionnaire and clinical procedures

All participants underwent a periodontal examination performed by two experienced and previously calibrated EFP-specialists in periodontology (G.B., V.D.L.). Presence of plaque (PI) and bleeding on probing (BoP), probing pocket depth (PPD), and clinical attachment level (CAL) were collected at six sites/tooth by manual probing (PCP UNC 15, Hu-Friedy, Chicago, IL). Inter-examiner reliability was determined through dual measurements of 15 non-study patients compared to a third reference examiner (M.A); intra-examiner reproducibility was assessed by taking replicate measurements on the same patients with an interval of 24 hours

between the first and the second recording. The percentage of agreement within 1 mm of PPD and CAL ranged between 94% and 97% of the sites.

At the first visit, detailed dietary (Italian EPIC Food Frequency Questionnaire) and lifestyle (WHO) questionnaires were also administered to all participants.

Periodontitis patients then received Step I-II of periodontal treatment according to EFP clinical practice guidelines (Sanz et al. 2020). Briefly, after a behavioral phase aimed at controlling local (including treatment of caries and endodontic treatment) and systemic risk factors, all patients underwent quadrant-wise subgingival instrumentation (one session/week), using both hand instruments and ultrasonic scalers without any adjunctive (Suvan et al. 2020). Patients were then re-evaluated 3 months after the last treatment appointment and periodontal clinical parameters were again recorded, together with biological samples collection and questionnaires administration.

2.3 Collection of saliva and stool samples

Unstimulated saliva (4 ml) was collected the day following each clinical examination, between 8:00 and 10:00 hours. Patients were instructed to avoid food, sugar drinks, caffeine, toothpaste and mouthwashes on that morning before collection (Aimetti et al. 2012).

Fecal samples were self-collected by participants within 7 days after clinical examinations, by transferring them into a sterile fecal collection tube using a polypropylene spoon (3 tablespoons of approximately 10 g). Both sample types were stored at −80°C within 24 hours from the collection.

2.4 Illumina 16S rRNA gene sequencing and bioinformatics

DNA extraction from samples was firstly carried out, following the SOP07 guidelines and procedures developed by the International Human Microbiome Standard Consortium (www.microbiome-standards.org). Next-generation sequencing targeting the V3-V4 hypervariable regions of the 16S rRNA gene was then performed using a high-throughput system. DNA was quantified by using the QUBIT dsHS kit, and standardized at 5 ng/L. The V3-V4 regions were amplified using the primers 16SF (5'-TCGTCG GCAGCGTCAGATGTGTATAAGAGACAG-3') and 16SR (5'-GTCTCGTGGGCTCGG AGATGTGTATAAGAGACAG-3' 0) (Klindworth et al. 2013), according to the Illumina 16S Metagenomic Sequencing Library Preparation instructions. Amplicons were then purified, tagged, normalized, and pooled according to the Illumina protocols. DNA libraries were sequenced on the Illumina MiSeq platform, leading to 2X250 bp paired-end reads. After

sequencing, raw reads were imported in QIIME2 software (https://docs.qiime2.org/), and the chimera filtering step by the QIIME2 dada2 denoise-paired script was applied (Callahan et al. 2016). The amplicon-sequence variants (ASVs) obtained were then used for taxonomic assignment against the SILVA database.

2.5 Sample size calculation

Since no previous studies investigated the effect of periodontal treatment on gut microbial composition, the sample size was calculated on the basis of expected decrease of proinflammatory genera *Bacteroidetes*, *Clostridiales*, and *Lachnospiraceae* detection rates (±SD) in periodontitis patients (Lourenco et al. 2022). A power level of 80% with a significant value of 0.05 required a sample size of 41 patients for each group. Considering a possible 15% drop-out rate, a total sample size of 94 participants (47 in each group), was required.

2.6 Statistical analysis

Demographic and clinical data were computed for each patient and averaged across clinical groups. Data distribution was checked by the D'Agostino-Pearson test. For continuous variables, the Mann-Whitney U test was used to compare periodontitis and periodontal health groups, while the Wilcoxon test was used to assess the longitudinal differences pre and post therapy. For qualitative variables, the differences were analyzed through the chi-square test and McNemar test for unpaired and paired comparisons, respectively. Alpha and beta diversity were computed by using the diversity script of QIIME2. Differences between groups and sampling time in alpha diversity value were assessed by Kruskal-Wallis test, while the PERMANOVA test was performed on the Bray Curtis distance matrix. The ASV table was then imported in the R environment to perform pairwise comparisons by using the Wilcoxon rank-sum test, and to perform ANOSIM statistical test. P-values were adjusted for multiple testing using the Benjamini–Hochberg procedure, which assesses the false discovery rate (FDR). ASVs with a frequency of 100 in at least 20 samples were considered only. ASV table was used to build a principal-component analysis (PCA) as a function of the sampling time or treatment by using the made4 package of R. Box plots represented the interquartile range between the first and the third quartile, with the error bars showing the lowest and the highest value. Pairwise Spearman’s non-parametric correlations were also used to study the relationships between the frequency of oral ASVs and clinical/dietary parameters. P value less than 0.05 was considered as statistically significant.

3. Results

Fifty-two patients with stage III-IV periodontitis were initially selected for baseline examination and periodontal treatment. Of these, 5 subjects were excluded after study initiation, due to the intake of systemic antimicrobials during the follow-up period. A total of 94 individuals (47 cases and 47 controls) was finally included in the study.

3.1 Population characteristics and response to periodontal therapy

The characteristics of the study population are presented in Table 1. Mean age for the periodontitis group was 55.8 ± 11.7 years, whereas it was 53.6 ± 8.4 years in the control group. Groups were comparable for gender distribution, smoking habits, and diet. As expected, the periodontitis group displayed significantly higher levels of full-mouth bleeding score (FMBS), full-mouth plaque score (FMPS), and mean number of pathological pockets (both PPD ≥ 4 mm with BoP and PPD ≥ 6 mm). In the periodontitis group, step I-II of therapy was effective in improving all the considered clinical parameters at 3 months ($p<0.001$).

3.2 Oral-gut microbiota composition before and after treatment

3.2.1 Principal-component analysis

PCA approach was effective in discriminating the gut microbial profile of periodontally healthy subjects and periodontitis patients ($p<0.001$, Figure 1a), as well as of periodontal patients pre- and after treatment ($p<0.001$, Figure 1b). Conversely, no significant differences were detected between treated patients and healthy controls ($p>0.05$; Figure 1c). Oral microbiota demonstrated an excellent discriminatory potential at baseline ($p<0.001$), with post-treatment microbial profiles tending to overlap with periodontally healthy subjects ($p>0.05$; Figure 2).

3.2.2 Stool taxa

Figure 3 shows the main ASVs differentially detected between periodontitis patients and subjects with periodontal health. Despite the great inter-individual variability, specific taxa differed significantly among groups ($p<0.001$). In detail, *Bacteroides, Faecalibacterium,* and *Lachnospiraceae* were the most represented ASVs in fecal samples of patients with periodontitis stage III-IV compared to periodontally healthy controls. Similarly, *Alistipes, Anaerostipes, Barnesiella, Bifidobacterium, Blautia, Butyricicoccus, Christensenellaceae, Clostridium, Desulfovibrio, Dorea, Eubacterium, Lachnoclostridium, Lachnospira,* and *Roseburia* genera were identified with higher frequency in stool of periodontitis individuals

than controls. Conversely, the genus *Lactobacillus* was the only one more enriched in the periodontally healthy group.

After Step I-II of periodontal treatment, 12 ASVs were significantly reduced in the fecal samples of stage III-IV periodontitis patients, related to genera *Alistipes*, *Bacteroides*, *Barnesiella*, *Colidextribacter*, *Eubacterium*, *Lachnoclostridium*, *Lachnospira*, *Lachnospiraceae*, *Oscillibacter*, *Oscillospiraceae*, *Roseburia*, and *Ruminococcaceae* (Figure 4A). Specifically, *Alistipes*, *Bacteroides*, *Barnesiella*, and *Lachnospira* decreased to levels similar to controls after step I-II of periodontal treatment; conversely, although reduced after treatment, *Eubacterium*, *Lachnospiraceae*, and *Roseburia* remained significantly higher than in the periodontally healthy group (Figure 4B).

Notably, *Firmicutes*/*Bacteroidetes* ratio in stool significantly differed between periodontitis patients and healthy controls ($p<0.05$), with periodontal treatment significantly increasing the value ($p<0.001$).

3.2.3 Salivary taxa

In the salivary samples, relative abundances of *Dialister*, *Fretibacterium*, *Fusobacterium*, *Peptostreptococcus*, *Porphyromonas*, and *Treponema* significantly differed between periodontally healthy and periodontitis subjects ($p<0.001$). After periodontal therapy, all these ASVs reached levels comparable with the healthy group, except for *Porphyromonas* ($p>0.05$; Figure 5).

3.2.4 Alpha and beta diversity

Alpha diversity calculation showed no difference in gut samples between periodontitis and periodontally healthy samples, as well as before and after treatment in periodontitis subjects. However, a reduction in all the indexes ($p<0.001$) was observed in salivary samples after periodontal therapy.

Beta diversity was significantly higher in gut samples of periodontally healthy subjects compared with untreated periodontitis patients ($p=0.001$). The value tended to increase in periodontitis subjects after receiving the steps I-II of treatment. No differences were observed in the saliva samples.

3.3 Differences in microbial composition between saliva and stool samples

ASVs were analyzed by a Venn diagram analysis by sample type (saliva/stool), in order to detect unique or shared ASVs. Between-group comparisons showed that *Clostridia*,

Dialister, *Eubacterium*, *Lachnospiraceae*, *Prevotella*, and *Streptococcus* were common among gut and oral samples. Of these genera, *Eubacterium* and *Lachnospiraceae* significantly reduced after treatment to levels similar to those in the healthy group; whereas genus *Clostridia* remained higher in the periodontitis patients even after treatment. Conversely, 46 ASV were unique in oral samples and 36 ASV in the gut ones.

3.4 Oral and gut taxa correlation with periodontal parameters and dietary patterns

Correlations analysis between ASVs detected in fecal samples and periodontal parameters showed a direct relationship between *Faecalibacterium*, *Lachnospiraceae* and *Roseburia* with FMBS; *Butyricicoccus*, *Dorea*, and *Lachnospiraceae* with FMPS. Similarly, *Butyricicoccus*, *Faecalibacterium*, and *Lachnospiraceae* correlated with number of pathological sites ($P<0.05$, Figure 6A).

Regarding saliva samples, high frequency of *Campylobacter* and *Prevotella* were strongly correlated with number of teeth, while *Selenomonas* with FMPS and *Aggregatibacter* with the number of pathological sites ($P<0.05$, Figure 6B).

None of the discriminant genera was significantly correlated with dietary variables.

4. Discussion

The results from this study portrayed a distinct gut microbial signature between subjects with periodontal health and periodontitis, which significantly changed after periodontal treatment. Moreover, specific discriminating ASVs between the different clinical periodontal conditions were identified in stool, with periodontal treatment decreasing their level. Most of these genera belonged to pro-inflammatory taxa, particularly enriched from the oral cavity. Beta diversity was significantly lower in the fecal samples of periodontitis patients; while oral alpha diversity decreased after periodontal treatment.

To now, data on the gut microbial profile of individuals with periodontitis are still scarce. Our findings are somehow consistent with the study of Lourenco et al. (2022), which reported differences in the salivary and gut microbial composition in a smaller group of patients at baseline. However, no longitudinal data were previously available on the modifications occurring after periodontal therapy. Despite the intrinsic resilience of the gut microbiome in health and disease (Levy et al. 2017), our findings pointed to a tangible effect of step I-II of periodontal treatment on gut microbial composition, suggesting mechanistic evidence of oral-gut pathogen translocation in humans. Despite the oral and gut microbiota have traditionally been considered as two separate entities (Human Microbiome Project 2012), increasing

evidence is suggesting that the dysbiotic oral microbiota is likely to move to the gut and affect the gastrointestinal microbiome homeostasis toward inflammation (Kitamoto et al. 2020). Indeed, Atarashi et al. (2017) showed in germ-free mice that oral bacteria are capable of colonizing the gut, causing chronic inflammatory reactions in predisposed hosts. Under certain circumstances, pathobionts may translocate from the oral cavity to the gut, leading to gut dysbiosis and contributing to systemic inflammation.

Regarding taxonomical characterizations, *Roseburia*, *Bacteroides*, *Lachnospiraceae*, *Eubacterium*, *Clostridium* were at significantly higher frequency in the gut microbiota of diseased patients than individuals with periodontal health. Some recent studies (Kawamoto et al. 2021, Amado et al. 2020; Lourenco et al. 2022) reported an increased abundance of members belonging to *Clostridium* and *Lachnospiraceae* families, in accordance with our data. These potentially pathogenic strains present a broad range of virulence factors and are potent inflammatory response inducers. In particular, *Lachnospiraceae* is a heterogeneous taxon of the intestinal core microbiota that comprises several genera and unclassified strains of anaerobic organisms, associated with various chronic inflammatory diseases and type 2 diabetes mellitus (Vacca et al. 2020). Also, a higher dominance of bile-tolerant, obligate anaerobes *Alistipes*, *Bacteroides*, and *Ruminococcaceae* corresponded to an unhealthy gut environment, and was associated with higher levels of intestinal inflammatory markers (Bolte et al. 2021). Interestingly, levels of most proinflammatory taxa decreased after periodontal therapy.

Our data on saliva confirmed the presence of an oral dysbiotic state in diseased individuals, which showed greater species variability and more equitability than periodontally healthy individuals (Nibali et al. 2020). Findings after periodontal therapy may suggest that restoring health provides a less rich and more dominant microbial distribution, favoring a few taxa within the group. Based on the hypothesis that the increased salivary microbial load seen in periodontal diseases may result in higher rates of oral-gut microbial translocation, we sought for oral taxa in fecal samples. Of the genera that were found in common between the oral and gut ecosystem, *Eubacterium* and *Lachnospiraceae* significantly reduced after treatment to levels similar to those in the healthy group; whereas genus *Clostridia* remained higher in the periodontitis patients even after treatment. It may be hypothesized that periodontal treatment may have reduced the salivary levels influencing their gut translocation along the gastrointestinal tract. Even though step I-II of periodontal treatment was particularly effective in achieving the endpoint of therapy, it must be considered that some residual pockets still

persisted (Citterio et al. 2022). Indeed, *Eubacterium*, *Lachnospiraceae*, and *Roseburia* remained significantly higher than the periodontitis group.

To the best of authors' knowledge, this is the first study investigating the impact of periodontal treatment on the gum-gut microbiological axis. In absence of previous causal evidence in humans, an indication that oral microbiota perturbation can produce changes at the gut environment has emerged. Even though more mechanistic research is needed, we identified some bacterial genera common to both oral and gut ecosystems that significantly reduced their levels after treatment. The implications of the above-mentioned observations are relevant, considering that shifts in the gut microbial composition have a significant impact on local and systemic inflammatory and autoimmune disorders (Fan and Pedersen 2021). Strain-level metagenomics techniques and improvements in reference databases will deepen our understanding of the role of pathogen translocation along the enteral route, as well as functional analyses through metabolomics and meta-transcriptomics techniques.

5. Conclusion

The present study pointed out that the gut microbiota of patients with stage III-IV periodontitis significantly differed compared to subjects with periodontal health, and that step I-II of periodontal therapy was able to induce significant changes on the oral-gut bacterial profile. Our data also indicated that this post-treatment profile tended to overlap to the one of patients with no diagnosis of periodontitis. These novel findings raise a great potential for future diagnostic tools and therapeutic interventions considering the microbiological continuum along the gum-gut axis.

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Table 1. Socio-demographic and clinical parameters of the study population.

Parameters	**Periodontal Health (n = 47)**	**Periodontitis T0 (n = 47)**	**Periodontitis T1 (n = 47)**
Age (years) Mean (SD)	53.6 (8.4)	55.8 (11.7)	/
Gender n (%)			
Females	24 (51.1)	25 (53.2)	/
Males	23 (48.9)	22 (46.8)	

Smoking (%)			
Non-smokers	44 (93.6)	41 (87.2)	
Former smokers	0 (0.0)	1 (2.1)	
Current smokers	3 (6.4)	5 (10.6)	
BMI (Kg/m^2) Mean (SD)	23.6 (3.3)	25.4 (3.6)	
FMPS	22.4 (17.3)	74.4 (18.8)*	19.1 (12.9)**
FMBS	15.4 (13.3)	72.9 (19.3)*	25.8 (12.3)**
N of teeth Mean (SD)	27.3 (1.3)	26.2 (4.4)	23.8 (4.2)**
Mean (SD) % of sites with			
PPD ≥ 4 mm BoP+	4.6 (6.5)	65.1 (41.4)	21.3 (15.0)**
PPD ≥ 6 mm	0	28.5 (28.2)	8.5 (8.1)**
Periodontitis stage (%)			
Stage III, n (%)	/	29 (61.7)	
Stage IV, n (%)	/	18 (38.3)	

Legend. FMBS: full-mouth bleeding score; FMPS: full-mouth plaque score; PPD: Probing Pocket Depth; CAL: Clinical Attachment Level; BOP: Bleeding on Probing; BMI: body mass index. *Significant differences among groups at baseline ($p<0.05$); **Significant differences pre- and post- treatment ($p<0.05$).

Figure 1: Principal Component Analysis (PCA) based on amplicon-sequence variants (ASVs) relative frequency in gut samples. A) Periodontitis patients before treatment (blue) and periodontally healthy subjects (red). The profile of periodontitis patients is significantly different from that of the control group ($p<0.001$). B) Periodontitis patients before (red) and after (blue) treatment. The profile of periodontitis patients at baseline was statistically significantly different from re-evaluation after the conclusion of step 2 ($p<0.001$). C) Periodontally healthy subjects (red) and periodontitis patients after treatment (blue). Their profiles tended to overlap ($p>0.05$).

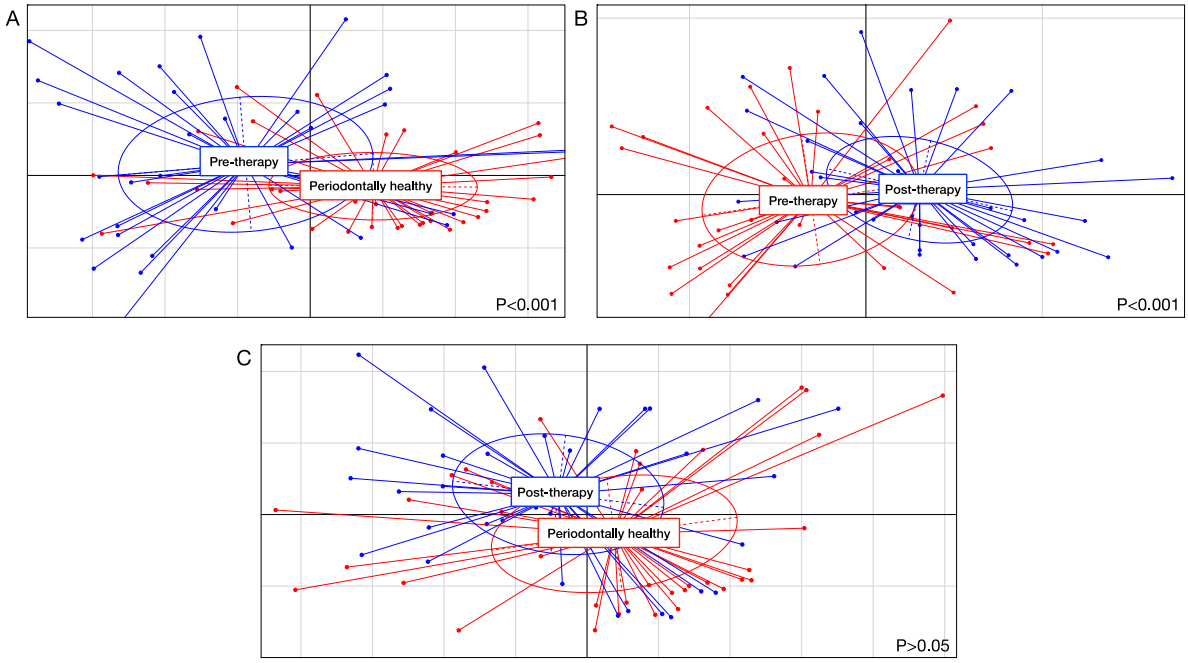

Figure 2: Principal Component Analysis (PCA) based on amplicon-sequence variants (ASVs) relative frequency in saliva samples of periodontally healthy subjects (red), periodontitis patients before treatment (blue), and after treatment (green). The profile of periodontitis patients at pre-treatment is significantly different from that of the control group, as well as from post-treatment ($p<0.001$). Profiles of periodontally healthy subjects and periodontitis patients after treatment markedly overlapped ($p>0.05$).

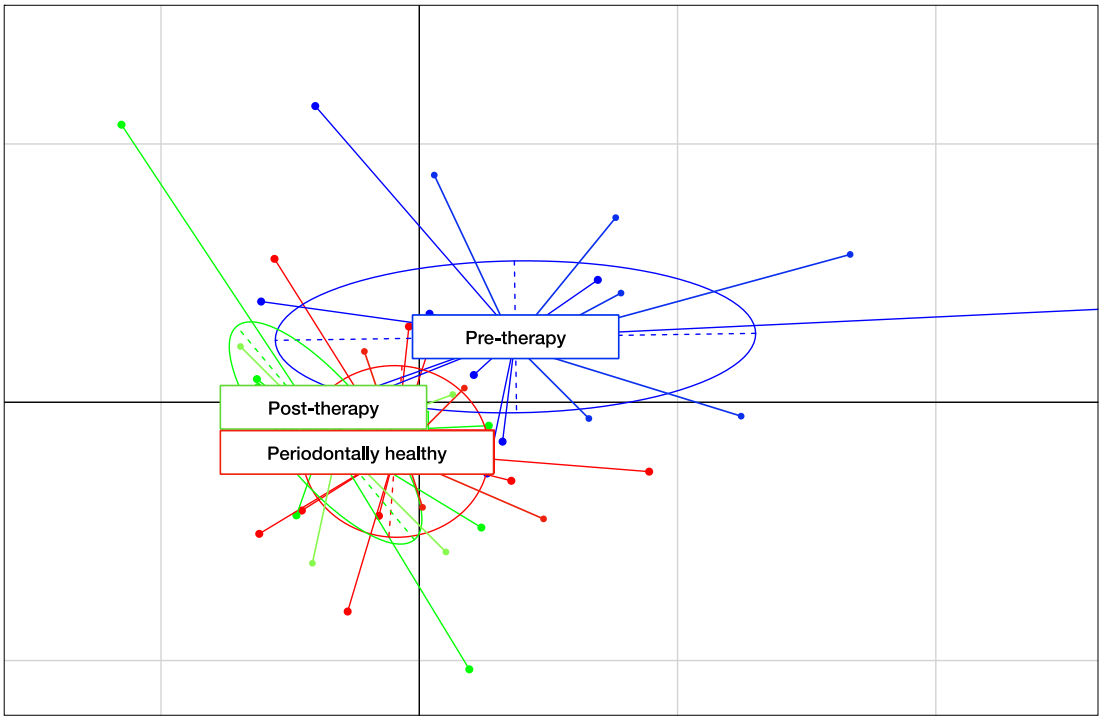

Figure 3: Predominant genera in the gut microbiota of periodontitis patients (yellow bars) and healthy controls (blue bars). Boxplots show the frequency at genus or family level of the ASVs in fecal samples. Only comparisons with statistically significant differences are shown ($p < 0.05$, Wilcoxon signed rank test with false discovery rate correction).

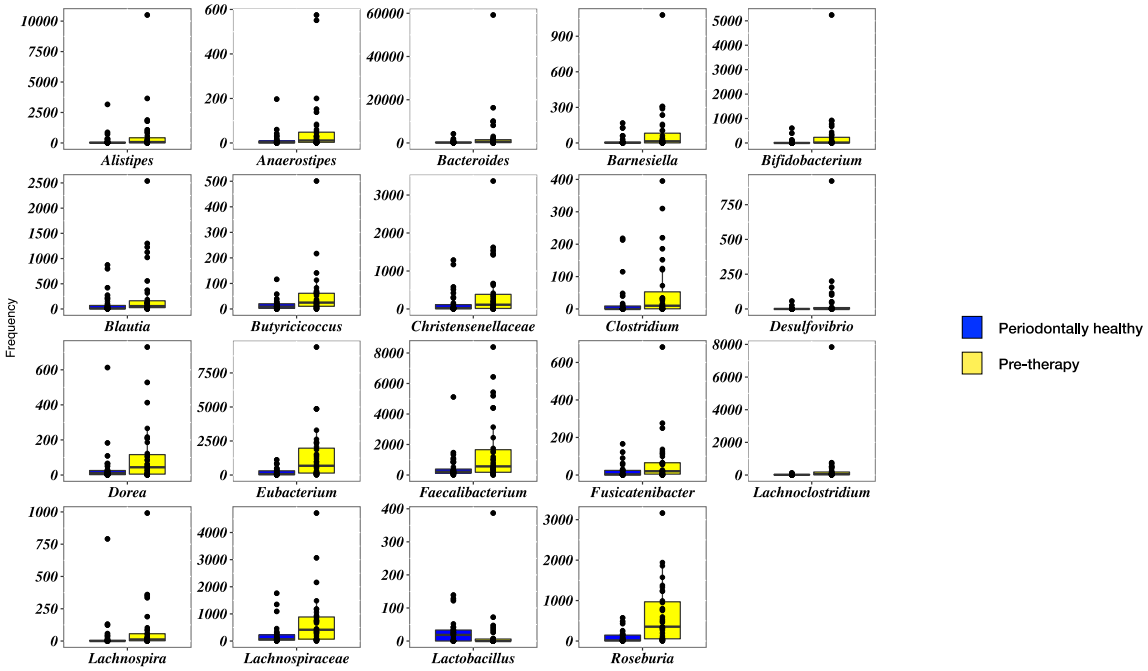

Figure 4: A) Predominant genera in the gut microbiota of periodontal patients before (T0; blue bars) and after step I-II of periodontal therapy (T1; green bars). B) Predominant genera in the gut microbiota of periodontitis patients after treatment (green bars) and healthy controls (blue bars). Boxplots show the frequency at genus or family level of the ASVs in fecal samples. Only comparisons with statistically significant differences are shown ($p < 0.05$, Wilcoxon signed rank test with false discovery rate correction).

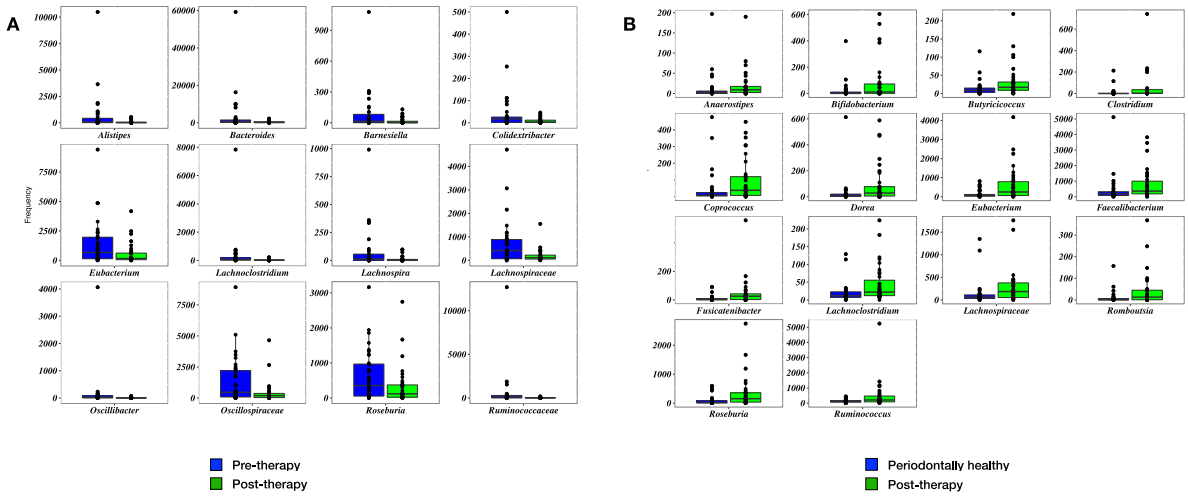

Figure 5: Predominant genera in the salivary microbiota of healthy controls (red bars), and periodontitis patients pre- (green bars) and post-treatment (blue bars). Boxplots show the frequency at genus or family level of the ASVs in salivary samples. Only comparisons with statistically significant differences are shown ($p < 0.05$, Wilcoxon signed rank test with false discovery rate correction).

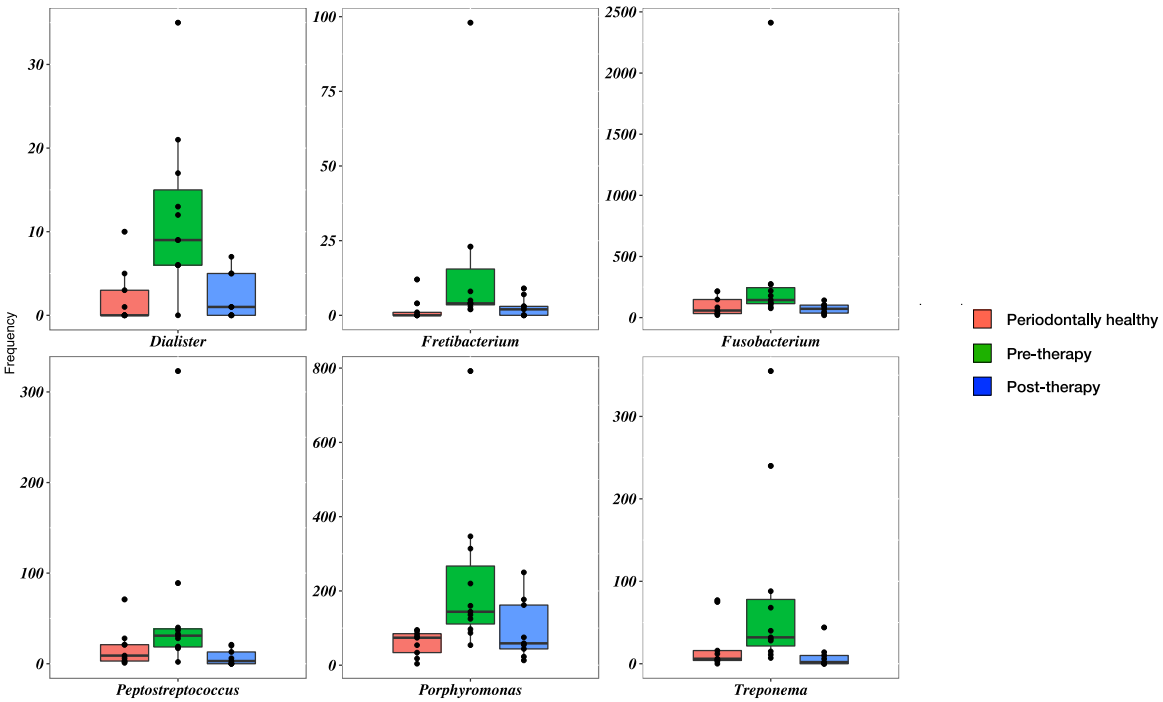

Figure 6: Spearman's correlation between oral amplicon sequence variants (ASVs) and clinical parameters in feces (A) and saliva (B). Only significant associations are shown (P < 0.01). The intensity of the colors represents the degree of correlation as measured by Spearman's correlation, where the blue color represents a positive degree of correlation and red a negative correlation.