Received: 1 March 2022 | Revised: 24 March 2023 | Accepted: 27 March 2023

DOI: 10.1111/jcpe.13815

ORIGINAL ARTICLE

Journal of Clinical Periodontology WILEY

Parents with periodontitis drive the early acquisition of dysbiotic microbiomes in their offspring

Aurélio Amorim Reis[1] | Mabelle Freitas Monteiro[1] | Gabriela Martin Bonilha[1] | Luciana Saraiva[2] | Cassia Araújo[3] | Mauro Pedrine Santamaria[3] | Marcio Zaffalon Casati[1] | Purnima Kumar[4] | Renato Corrêa Viana Casarin[1]

[1]Piracicaba Dental School, State University of Campinas, São Paulo, Brazil

[2]School of Dentistry, University of São Paulo, São Paulo, Brazil

[3]Institute of Health Science, São Paulo State University, São Paulo, Brazil

[4]School of Dentistry, University of Michigan, Ann Arbor, Michigan, USA

Correspondence
Renato Corrêa Viana Casarin, Piracicaba Dental School, State University of Campinas São Paulo, Avenida Limeira 901, Areião, Piracicaba, São Paulo, Brazil.
Email: rcasarin@unicamp.br

Funding information
Coordination for the Improvement of Higher Education Personnel, Grant/Award Number: CAPES-001; National Council for Scientific and Technological Development, Grant/Award Number: 309429/2017-0

Abstract

Aim: To evaluate the microbial colonization in different dentition phases on individuals from 0 to 18 years of age belonging to families with a history of periodontitis compared to descendants of periodontally healthy parents.

Materials and Methods: The offspring of subjects with periodontitis ('Perio' group) and the offspring of periodontally healthy subjects ('Healthy' group), matched for gender and age, were included in this cross-sectional study and divided according to the dentition phase: pre-dentate, primary, mixed and permanent. The patients were clinically assessed, and their saliva was collected. DNA was extracted, and V1–V3 and V4–V5 regions of the 16S rRNA gene were sequenced.

Results: Fifty children of parents with periodontitis and 50 from healthy parents were included in the study and divided according to the dentition phase: pre-dentate ($n = 5$/group), primary dentition ($n = 15$/group), mixed dentition ($n = 15$/group) and permanent dentition ($n = 15$/group) in each group. The microbiome composition was different between dentitions for both groups. Children of the Perio group presented a microbial diversity different from that of the Healthy group in mixed and permanent dentitions. The more intense shift in the community occurred between primary and mixed dentition in the Perio group, while the transition between mixed and permanent dentition was the period with greater changes in the microbiome for the Healthy group. Furthermore, a pathogen-rich environment—higher prevalence and abundance of periodontitis-associated species such as *Prevotella* spp., *Selenomonas* spp., *Leptotrichia* spp., *Filifactor alocis*, *Prevotella intermedia*, *Treponema denticola* and *Tannerella forsythia*— was observed in the Perio group.

Conclusions: The parents' periodontal status significantly affects the microbiome composition of their offspring from an early age. The mixed dentition was the phase associated with establishing a dysbiotic and pathogen-rich microbiome in descendants of parents with periodontitis.

KEYWORDS
grade C periodontitis, microbiome, oral, teeth eruption, vertical transmission

Clinical Relevance

Scientific rationale for study: Subgingival microbiota in children at the mixed-dentition phase can be modulated by the parents' periodontal conditions, probably acting as a source of pathobionts during early ages. However, it is unclear at which dentition phase the microbiome alterations can be initially detected.

Principal findings: From early ages (pre-dentate and primary dentition), children from periodontitis-affected parents presented a different abundance of some species, and at later ages (mixed and permanent dentitions) a dysbiotic microbial community is established.

Practical implications: Parental periodontal health should be included as a factor that affects the microbial establishment in the oral cavity of their children, and an early preventive approach can be prioritized for this population.

1 | INTRODUCTION

The oral cavity is a virtually sterile niche before birth (Perez-Muñoz et al., 2017) but is sequentially colonized by different microorganisms over time (Mason et al., 2018; Schulz et al., 2019). However, a new concept of oral colonization has been considered, describing that the human microbiome commences earlier than birth (Chen et al., 2020). Some studies have described unique microbial colonization in the amniotic fluid in up to 70% of pregnant women (Prince et al., 2015). Interestingly, several oral microorganisms, such as those belonging to the gerera *Streptococcus*, *Fusobacterium*, *Neisseria*, *Prevotella*, *Veillonella* and *Porphyromonas*, are found to be present in the placental niche (Aagaard et al., 2014; Bearfield et al., 2002; Gomez-Arango et al., 2017).

Initially presenting only shedding mucosae in a pre-dentate phase, the oral niche undergoes a substantial modification after primary teeth eruption in early infancy and, subsequently, with permanent dentition, modulating the oral microbiome that evolves into a complex and diverse community (Escapa et al., 2018; Kennedy et al., 2019; Lif Holgerson et al., 2020; Mason et al., 2018). Several aspects can drive the colonization process and be responsible for determining the oral microbiota composition, such as the neonate's immunity (Wu et al., 2014; Yu et al., 2018), maternal transmission during childbirth, parental exposures, diet and horizontal transmission from caregivers and peers (Nelson-Filho et al., 2013; Sulyanto et al., 2019; Ward et al., 2018). These factors shape the oral microbiota and, consequently, the human host immune functions and physiological development, which influence future health (White et al., 2013; Xiao et al., 2020; Yatsunenko et al., 2012).

Recently, parental periodontitis has been presented as another factor altering their offspring's oral colonization. Toddlers and adolescents (6–12 years) who are descendants of younger grade C periodontitis-affected parents harbour a dysbiotic microbiome compared to periodontally healthy parents' descendants (Monteiro et al., 2021). Indeed, grade C periodontitis affecting systemically healthy youngsters (previously called aggressive periodontitis) is an immuno-inflammatory disease of the periodontium, occurring at an early age in systemically healthy individuals who present an accumulation of cases within the family, with descendants of a periodontitis-affected individual being 50% more likely to develop this disease than a non-related child (Michalowicz et al., 2000).

Because of the periodontitis severity and potential for extensive destruction at a very young age, along with familial aggregation of their cases, the knowledge of sequential colonization of this higher risk population and the comprehension of when oral dysbiosis is established could lead to a more predictable preventive therapy. Moreover, a significant part of the oral microbiome's maturation occurs during the first 2 years of life, and this development may be influenced by early life circumstances (Kennedy et al., 2019).

Therefore, this investigation aimed to examine the oral microbiome in each dentition cohort—pre-dentate, primary dentition, mixed dentition and permanent dentition—in a cross-sectional clinical study, comparing the descendants of periodontitis-affected parents with those of periodontally healthy ones.

2 | MATERIALS AND METHODS

2.1 | Study design

It is an age- and gender-matched cross-sectional study to assess the oral colonization in the different dentition phases of individuals from families with a history of periodontitis compared to children/adolescents of periodontally healthy parents. The study was approved by the University of Campinas Ethics Committee (70816017.6.000.5418) and was carried out from March 2017 to November 2018. The patients were recruited and evaluated at Piracicaba Dental School (Piracicaba, São Paulo, Brazil). The study inclusion criteria were different for each group:

Periodontitis-descendants (Perio) group: Subjects aged between 0 and 18 years, with at least one parent (father or mother) presenting grade C, stage III-IV, periodontitis (Papapanou et al., 2018). For the disease diagnosis of parents, individuals should be less than 35 years old at the time of diagnosis, have at least eight teeth with probing depth (PD) and clinical attachment loss (CAL) >5 mm (confirmed at radiographical exams), have at least 20 remaining teeth in the oral cavity and present good systemic health. At the time of examination, all parents with periodontitis should present periodontal pockets ≥5 mm associated with bleeding on probing (BoP), indicating still active

disease and need for periodontal treatment, in spite of previous treatment.

Healthy descendants (Healthy) group: Subjects aged between 0 and 18 years, with both parents (father and mother) periodontally healthy. Periodontally healthy individuals did not present any site with periodontal probing depth (PPD) ≥3 mm with BoP, absence of radiographic proximal bone loss, at least 20 remaining teeth and good systemic health.

Parents and their offspring with chronic disease, smokers or former smokers, pregnant or lactating women, antibiotic use within 3 months before the study and had received periodontal treatment within 6 months before the study were excluded from the study (Monteiro et al., 2014).

All children were segregated according to their dentition state into pre-dentate, primary, mixed and permanent dentition in each group. The sample size was based on previous studies from our group demonstrating sufficient power to identify differences in β-diversity and differential abundance of bacteria in periodontitis-associated microbiome using 16S sequencing (Mason et al., 2013; Paropkari et al., 2016; Queiroz et al., 2017).

Oral clinical exams were performed for all children when the gender and age information was collected. Then, the dmft (number of deciduous teeth decayed, missing or restored) and DMFT (number of permanent teeth decayed, missing or restored) were examined, as previously described (Díaz-Cárdenas & González-Martínez, 2010). The examination was performed by a single calibrated examiner (MFM). The examiner measured the dmft/DMFT of 13 children with different caries activities in four periods, obtaining an agreement of $\kappa = 0.86$. The same examiner who performed the children's clinical examination (MFM) examined the parents' periodontal status. This examiner was calibrated for the periodontal examination with an intra-class correlation of 92% for PPD. Parents' periodontal data were used only to evaluate the inclusion in the study but not included in the analysis. Children with mixed and permanent dentition were screened for periodontitis only to confirm the absence of active disease.

2.2 | Sample collection and DNA isolation

From each subject, unstimulated saliva was collected in Eppendorf microtubes (AXYGEN, USA); from pre-dentate subjects, saliva samples were collected using sterile swabs because of their inability to collect unstimulated saliva. The samples were collected between 7 AM and 9 AM and before the patients ate and brushed their teeth in the morning. After collection, all samples were stored in microtubes and frozen at −80°C until DNA isolation using the Qiagen MiniAmp kit (Valencia, CA) according to the manufacturer's instructions. DNA isolation was performed using 1 mL of saliva as input. DNA concentration was measured following the manufacturer's instructions (Qubit dsDNA HS Assay Kit; Life Technologies), and between 4.0 and 94.8 ng/μL of DNA was obtained per sample.

2.3 | Sequencing and bioinformatic analysis

The V1–V3 and V4–V5 regions of the 16S rRNA gene were sequenced using the Illumina Miseq platform, with 10 ng of DNA used per sequencing run. The raw sequences were deposited in the Sequence Read Archive (SRA) database under registration number PRJNA780174 (ncbi.nlm.nih.gov/sra). The sample processing protocol, library preparation and sequencing protocol have been described in a previous study (Monteiro et al., 2021). Analyses were conducted using QIIME (Caporaso et al., 2010) and PhyloToAST (Dabdoub et al., 2016). The Shannon method (Shannon, 1997) was used as an α-diversity estimator, and differences between α-diversities group-wise were measured using the one-way ANOVA test with the Tukey HSD test for multiple comparisons. The unweighted UniFrac distance was used to evaluate the β-diversity, and the differences between groups were analysed using principal coordinate analysis (PCoA) and tested using the Adonis test. Differences in the dispersion of samples between groups were tested using the PERMDISP test. The core species were characterized using Qiime's script (core_microbiome.py) when species were present in at least 75% of the patients in each group and visualized using PhyloToAST. The Bioconductor package for R, analysis of compositions of microbiomes with bias correction (ANCOM-BC) (Lin & Peddada, 2020), was used to perform differential analysis of the annotated taxa. This function estimates the unknown sampling fractions and corrects the bias induced by the differences among samples. The absolute abundance data are modelled using a linear regression framework. *p*-Values were adjusted for multiple testing (false discovery rate [FDR] <0.05, the Holm–Bonferroni method). The bacterial network correlations were determined significantly pairwise using the SparCC (Pylro et al., 2014) pipeline ($p < .01$, $r > 0.75$), and network graphs were drawn in Python (Networkx package) and visualized in Gephi (Bastian et al. 2009).

2.4 | Demographic and clinical analysis

Demographic and clinical data of children from Perio and Healthy groups were compared considering the dentition phase. Initially, the Shapiro–Wilk test was used to check for normalization of data distribution, the Chi-square test to evaluate the gender frequency in each group and the Student's *t*-test for age and dmft/DMFT comparisons. All analyses were done on the SIGMA plot program (Systat Software Inc., Microsoft), with a significance level of 5%.

3 | RESULTS

Table 1 shows the clinical and demographic data of all participants in the study. One hundred patients were included in the study, with 50 (5 pre-dentate, 15 in the primary dentition, 15 in the mixed dentition and 15 in the permanent dentition) in each group. None had periodontitis. The epidemiological indexes dmft for the primary, mixed and DMFT dentition for permanent dentition were applied. There was

TABLE 1 Clinical and demographic data of all participants in the study.

Pre-dentate	Perio group ($n = 5$)	Healthy group ($n = 5$)
Age mean (SD) (months)	2 (1)	1.6 (1.3)
Gender (%) M/F	66.6/33.3	80.0/20.0
Primary dentition	**($n = 15$)**	**($n = 15$)**
Age mean (SD) (years)	2.9 (1.1)	3.2 (1.3)
Gender (%) M/F	50.0/50.0	50.0/50.0
dmft (median)	0.5	0.8
Mixed dentition	**($n = 15$)**	**($n = 15$)**
Age mean (SD) (years)	9.6 (1.8)	9.6 (1.6)
Gender (%) M/F)	40.0/60.0	40.0/60.0
DMFT (median)	0.9	0
dmft (median)	0	0
Permanent dentition	**($n = 15$)**	**($n = 15$)**
Age mean (SD) (years)	15.7 (1.6)	15.2 (1.5)
Gender (%) M/F)	26.7/73.3	30.8/69.2
DMFT	0.3	0

Note: The epidemiological indexes dmft for the primary, mixed and DMFT dentition for permanent dentition were applied. There was no statistically significant difference between groups concerning clinical and demographic data. No difference between groups ($p > .05$).
Abbreviation: SD, standard deviation.

no statistically significant difference between groups concerning clinical and demographic data.

Figure 1 shows the overall characteristic of the included patients' salivary microbiome, highlighting the differences in the microbial diversity and the core microbiome for the Health and Perio groups in the pre-dentate, primary, mixed and permanent dentitions. Figure 1a shows the β-diversity represented in the PCoA of unweighted UniFrac distance. The microbial maturation process is seen as a curved line in the β-diversity graph, with the dentition status representing the most significant difference in PC1 for both groups (Figure 1a). However, the family history of periodontitis seems to modulate this process. More intense changes are observed from the mixed dentition in the Perio group when those samples were closely clustered to the Health group's permanent dentition. There was no statistically significant difference between the Perio and Health groups in the pre-dentate (Adonis, $p = .404$; PERMDISP, $p = .544$) or the primary dentition (Adonis, $p = .699$; PERMDISP, $p = .735$). In the mixed dentition, a different microbial diversity was observed between groups (Adonis, $p = .003$; PERMDISP, $p = .279$), which was also found in the permanent dentition (Adonis, $p = .007$; PERMDISP, $p = .884$). Regarding α-diversity, a similar trend of changes was also observed (Figure 1a) for both groups and all dentitions. No differences were observed between groups at pre-dentate and primary dentition (Student's *t*-test, $p > .05$), while in the mixed dentition a statistical difference was found between groups (Student's *t*-test, $p = .008$). Moreover, no statistical difference was identified in the permanent dentition (Student's *t*-test, $p = .56$). An increase in bacterial richness was demonstrated over dentition. However, the group differentially impacts the diversity increase, with the mixed dentition being similar to the primary dentition in the Healthy group (Anova/Tukey test, $p = .955$) and similar to the permanent dentition in the Perio group (Anova/Tukey test, $p = .964$). Furthermore, a similar trend of proximity between Perio mixed dentition and Health permanent dentition is also described for α-diversity (Figure 1a). Additionally, the colonization process was also described in the core microbiome (Figure 1c), which increased over dentition, with a more intense shift in the Perio group.

3.1 | Impact of dentition on the salivary microbiome

Figures 2 and 3 show the microbiome's differences according to distinct dentitions within groups. A statistical difference in the β-diversity between pre-dentate-primary (Figure 2a and 3a), primary-mixed (Figures 2b and 3b) and mixed-permanent (Figure 2c,f) was noted in both groups (Adonis, $p < .05$; PERMDISP, $p > .05$). Differential abundance analysis revealed an increase in the microbiome's complexity for both groups as the dentition transition occured. The more advanced the dentition stage, the higher the abundance of genera such as *Prevotella*, *Selenomonas*, *Capnocytophaga* and *Leptotrichia* and the species *Fusobacterium nucleatum*. However, differential abundance analysis also showed that this change occurred differently in each group, with a more intense shift in the Perio group and an increase in abundance of *Treponema*, *Tannerella*, *TM7*, *Mogibacterium* and *Peptostreptococcaceae*. Interestingly, the smallest difference between dentitions for diversity and differential abundance metrics in the Health group was the primary × mixed dentition comparison. In contrast, this comparison describes the most remarkable differences between dentition in the Perio group.

3.2 | Impact of familial periodontal status on the salivary microbiome

Figure 4 shows the differences in microbiome in the Perio and Health groups in the different stages of dentition. In pre-dentate, no significant statistical difference in the β-diversity (Adonis, $p = .404$) was seen between the groups (Figure 4a). Additionally, small species were differentially abundant between groups (Figure 4b). Similarly, in deciduous dentition, no difference was observed in the β-diversity (Figure 4c) (Adonis, $p = .699$), and a small number of species were differentially abundant between groups (Figure 4d).

The differences between Health and Perio are more remarkable in the mixed and permanent dentitions. The groups presented a different β-diversity (Figure 4e) (Adonis, $p = .003$), and 100 species were differentially abundant between groups (Figure 4f). The differences were maintained in the permanent dentition, and the groups presented different β-diversities (Adonis, $p = .007$) (Figure 4g) and 89 differentially abundant species (Figure 4h).

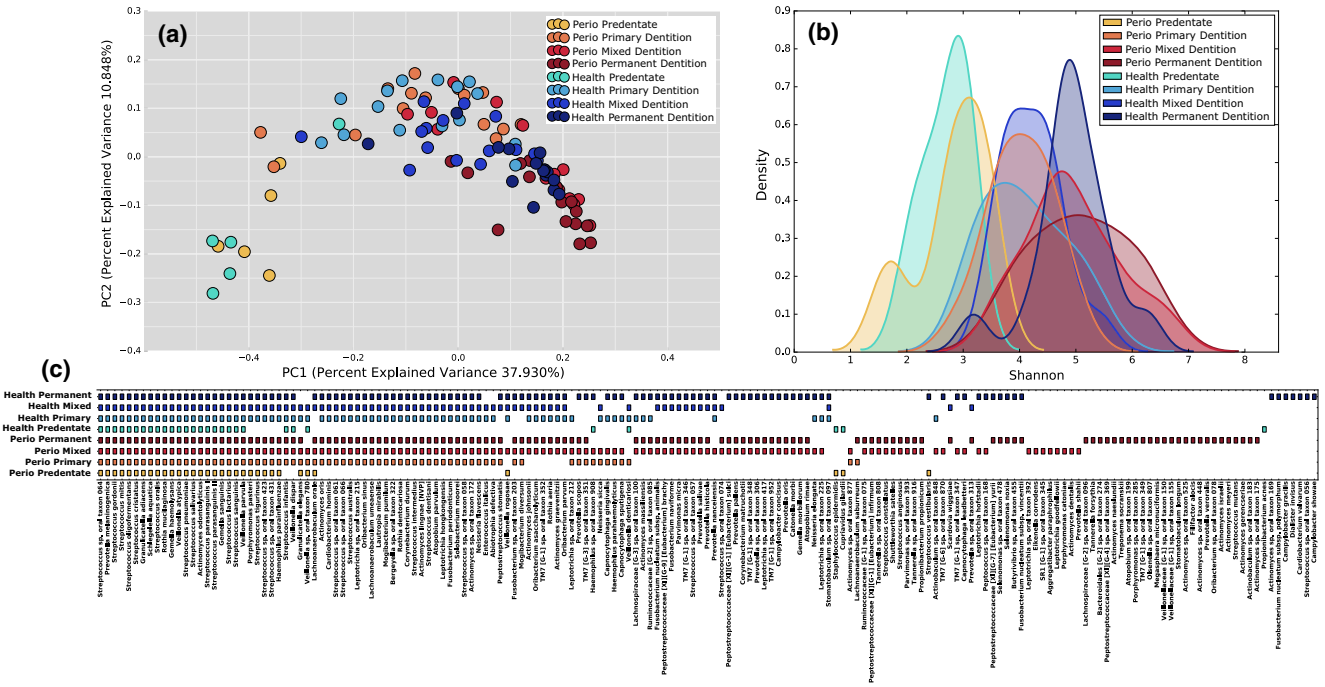

FIGURE 1 (a) β-diversity: PCoA of the unweighted unifrac distance for both groups and dentitions. (b) α-diversity: Shannon index for both groups and dentitions. (c) Core microbiome, considering species presented in at least 75% of the samples from a group.

Although no significant statistical difference was observed in the β-diversity of the pre-dentate and primary phases, differential abundance analysis showed that some species already differed. *Prevotella* spp. guided the differences in pre-dentate, and *Fusobacterium* and *Leptotrichia* genera, for example, were already more abundant in the primary dentition of the Perio group. The differences between the groups increased as the phases evolved. In the mixed dentition, a more pathogenic microbiome was observed in descendants of periodontitis patients, with species belonging to the genera *Prevotella*, *Selenomonas*, *TM7*, *Treponema*, *Leptotrichia* and *Tannerella* increased in the Perio group. In the permanent dentition, those differences were maintained, and many species related to periodontal destruction, such *as Prevotella intermedia*, *Treponema denticola* and *Fretibacterium* spp., were identified as more abundant in the Perio group, while *Streptococcus* spp., *Actinomyces* spp. and *Neisseria* spp. were more abundant in the Health group.

Besides microbial composition, substantial species–species co-occurrence network alteration was seen in the Perio group in the different dentition phases (Figure 5). An increase in the correlation number between species is observed in Health (Figure 5a) and Perio (Figure 5b) groups over time. However, the most intense increase in correlation number occurred from primary (42) to mixed (129) dentition in the Perio group and from mixed (68) to permanent (128) dentition in the Health group. The increase in correlation numbers is accompanied by the formation of complex hubs associated with higher inter-generic connections. Furthermore, the species members involved in each correlation were also descriptive for each group. The genera *Streptococcus* and *Actinomyces* were highly representative of all dentitions and groups, while *Mogibacterium* and *Oribacterium* assume protagonism in the Health group and *Fusobacterium*, *Veillonella* and *Prevotella* in the Perio group over dentition (Figure 5).

4 | DISCUSSION

Microbiota in the newborn undergoes rapid changes in composition during infancy, in a highly dynamic mode, towards a stable adult-like structure at each microbial community and a specific body site (Xiao et al., 2020; Yatsunenko et al., 2012), which is driven by several intrinsic and extrinsic factors. The present study, assessing the oral microbiome from pre-dentate to permanent dentition, confirmed the weight of parental periodontal diagnosis on the early alterations in the microbiome of their offspring, even during deciduous dentition.

The oral cavity is an environment abundant in bacteria, and colonization starts 8–16 h after birth. The primary transmission sources are diet, digital suction and vertical transmission (Mason et al., 2018), with saliva being the main contamination route (van Winkelhoff & Boutaga, 2005). Recently, our group demonstrated that oral condition, that is, periodontitis diagnosis of parents, drove the subgingival colonization of their offspring aged 6–12 years, introducing a higher number of dysbiotic-associated periodontal species to the subgingival environment in the mixed dentition (Monteiro et al., 2021). However, up to now, there has been little information

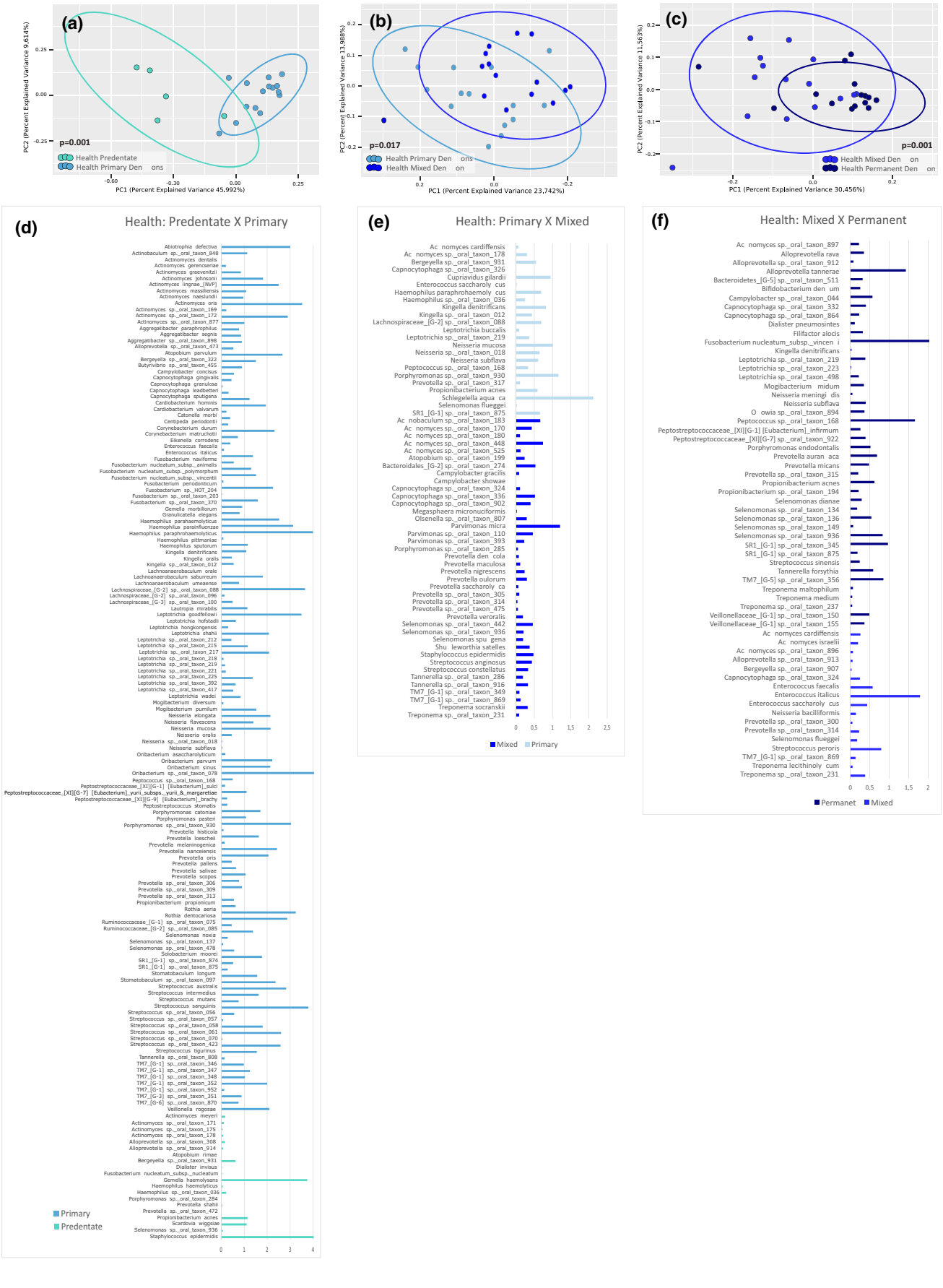

FIGURE 2 Legend on next page.

about how oral colonization occurs at early ages in families with periodontally affected parents.

4.1 | Different colonization was observed across the dentition stages

As expected, our study confirmed that salivary microbial characterization from pre-dentate to permanent dentition was highly impacted by teething. Previous studies have shown a natural and sequential alteration in the microbiota through ageing and oral development (Kennedy et al., 2019; Lif Holgerson et al., 2020; Mason et al., 2018). Lif Holgerson et al. (2020) longitudinally assessed the salivary microbiome from infants aged 2 days to 5 years, identifying an increase in genera across ageing, increasing α-diversity and changes in β-diversity, similar to previous studies (Chu et al., 2017; Mason et al., 2018). Their study found that the first colonization was dominated by *Streptococcus* spp. and *Gemella* spp., in particular *S. mitis* and *G. haemolysans*, when two-day-old babies were assessed. Moreover, while *Streptococcus* spp. were characteristic for 3 months, the genera *Capnocytophaga*, *Neisseria*, *Porphyromonas*, *Haemophilus* and *Fusobacterium* were the most representative of the 18-month community (Lif Holgerson et al., 2020), as was also seen in Dzidic et al. (2018) study. This timeframe also presented the greatest expansion of the predicted KO (2.028 additional Kegg Orthology from 3 to 18 months, while only 227 between 3 and 5 years), most of them associated with energy metabolism, cell motility, xenobiotic biodegradation and glycan biosynthesis. Our findings for the Healthy group (i.e., children from periodontally healthy parents) agree with those of previous studies when 82 OTUs were highly detected in primary dentition than in pre-dentate, around 20% of them being *Actinomyces* spp., *Fusobacterium* spp. (including *F. nucleatum*), *Gemella* spp. and *Streptococcus* spp. (as *S. mutans*, *S. sanguinis* and *S. infantis*). Interestingly, a dominant hub of the species–species networks already composed mainly of these genera (*Streptococcus*, *Actinomyces*, *Gemella* and *Mogibacterium*) characterizes the mixed dentition in the Healthy group. In contrast, a massive increase in species–species correlation and additional hubs composed of *Fusobacterium*, *Prevotella* and *Oribacterium* could be seen in permanent-dentition subjects.

However, although teething appears as the main stressor for oral microbial changes in the present (when α- and β-diversity were significantly changed and a huge increase in the core microbiome was seen) and previous studies (Kennedy et al., 2019; Lif Holgerson et al., 2020; Mason et al., 2018), other factors also impact on oral microbiome acquisition (Lif Holgerson et al., 2020; Ramadugu et al., 2021). Based on the vertical transmission phenomenon, the role of mother/caregivers in colonization is also well accepted and confirmed by previous studies, and the role of periodontal conditions of parents carrying a dysbiotic-associated microbiota has been recently considered (Monteiro et al., 2021).

4.2 | Periodontitis diagnosis of parents is associated with colonization of periodontitis-associated pathobionts and changes the colonization dynamics

Periodontitis is characterized by a marked taxonomical and functional change in the microbiome (Dabdoub et al., 2016; Duran-Pinedo, 2021; Reis et al., 2021). An increase in genera such as *Fusobacterium*, *Prevotella*, *Porphyromonas* and *Treponema* (Abusleme et al., 2013; Duran-Pinedo, 2021; Griffen et al., 2012; Kumar et al., 2011), along with functional changes, is mainly linked to the highly abundant pathobionts *P. gingivalis*, *Tannerella forsythia*, *T. denticola* and *Filifactor alocis* (Dabdoub et al., 2016; Duran-Pinedo, 2021; Hajishengallis, 2014). In young and systemically healthy subjects affected by periodontitis with rapid progression, previous studies have confirmed the role of those species (Schulz et al., 2019), along with *Aggregatibacter actinomycetemcomitans* (Casarin et al., 2010; Monteiro et al., 2021; Teles et al., 2010; Velsko et al., 2020), some *Selenomonas* spp. (Faveri et al., 2009), *Treponema lecithinolyticum* (Velsko et al., 2020) and *Deltaproteobacteria* (Amado et al., 2020). Thus, considering the presence of a complex dysbiotic community in the parents' oral cavity, there is an expected trend in vertically transmitting them to their offspring, as we have already shown in 6–12-year-old children (Monteiro et al., 2015, 2021). This precocious transmission of commensals and pathogenic species has also been described in other parent–children dyad studies (Drell et al., 2017; Jo et al., 2021; Monteiro et al., 2014; Ramadugu et al., 2021). The present study, enrolling from pre-dentate babies to adults with permanent dentition, also confirmed a pathogen-enriched community in the descendants from periodontitis-affected parents. Although this suggests a vertical transmission of bacteria, it cannot be confirmed in the present study because of the absence of microbiological data from periodontitis parents. This limitation should be evaluated in future studies focused on determining vertical transmission and other oral colonization aspects. Meanwhile, it is important to highlight that the present study is the first age-wise analysis showing that the disease-associated species (*F. alocis*, *P. gingivalis*, *A. actinomycetemcomitans*, *Streptococcus parasanguinis*, *F. nucleatum* and several species belonging to the genus

FIGURE 2 Microbial differences between dentitions in the Healthy group. β-Diversity: PCoA of the unweighted unifrac distances between pre-dentate × primary dentition (a); primary × mixed dentition (b); mixed × permanent dentitions (c). The ellipse of the 95% confidence interval is represented in the PCoA graphs. Differential abundance of species, tested with ANCOM-BC, comparing pre-dentate versus primary dentition (d), primary × Mixed dentition (e) and mixed × permanent dentitions (f). The bar size represents the fold change for each comparison, and the bar colour indicates the dentition in which the species are more abundant. Only species differentially abundant between groups were included in the graphs.

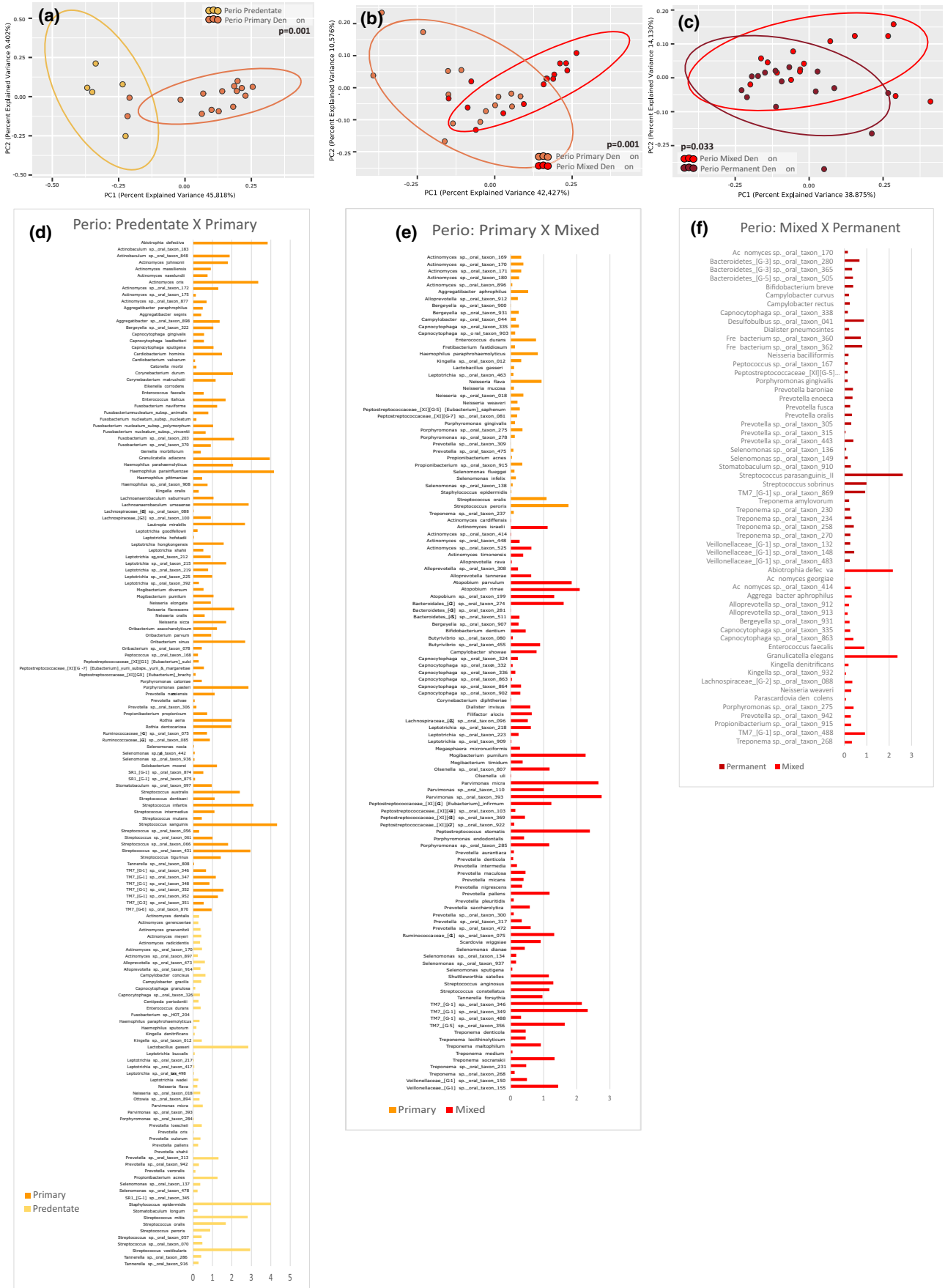

FIGURE 3 Legend on next page.

Selenomonas) consecutively colonize the oral microbiome of children, from pre-dentate stage to permanent dentition, when a parent is affected by periodontitis.

In the present study, as expected and previously discussed, the most abundant genera in pre-dentate babies were *Streptococcus* and *Lactobacillus*. However, even before teething, some *Prevotella* spp. were more abundant in babies from parents affected by periodontitis. As their parents presented a higher level of these microorganisms (Doğan et al., 2008; Monteiro et al., 2014), a precocious transmission and colonization may be suggested (Drell et al., 2017). However, differently from *S. mutans*, which presented transient colonization in the absence of non-shedding surfaces (Lif Holgerson et al., 2020), the transmission of *Prevotella* early in life could open an infection window for future colonizers. *Prevotella* spp. are gram-negative microorganisms able to co-aggregate by their protein or glycoprotein with carbohydrates or carbohydrate-containing molecules on the surface of the *Actinomyces* strains (Nesbitt et al., 1992), which is a common genus during this age. Indeed, the analysis of the inter-species network of pre-dentate babies from the Perio group was dominated by a central hub composed of *Prevotella melaninogenica*, *P. scopos* and *P._sp_oral_taxon_313* significantly correlated to *Actinomyces oris* and *A. odontolyticus*. Additionally, after colonizing the oral niches, *Prevotella* spp. could escape from the natural host response and alter the environment. Previous studies have observed that the Fc-binding activity of *P. intermedia* and *P. nigrescens* acts as an additional virulence factor by reducing IgG reactions with the bacterial cell (Jansen et al., 1995; Labbé & Grenier, 1995), which may explain their association with polymicrobial oral diseases. Meanwhile, it impacts not only *Prevotella* maintenance in the mouth but, as shown by Guentsch et al. (2013), cleavage of IgG1 may suppress antibody-dependent antibacterial activity in subgingival biofilms, instigating the colonization by pathobionts such as *P. gingivalis*. Although interesting, those results regarding pre-dentate stage should be seen with caution because of the small sample size included for this group. Pre-dentate babies are a hard-to-reach population, and future studies should include more subjects to confirm our results. However, these results highlight how maternal oral dysbiosis precociously impacts infants after and also before teething.

After teething, additional niches increase the dissimilarity between children from different parents' backgrounds. At primary dentition, children of parents with periodontitis remain with higher colonization of *Prevotella* spp., and the microbiome maturation was characterized by a higher abundance of *Tannerella* spp., *Neisseria flava*, *Campylobacter gracilis*, *Parvimonas micra* and *Leptotrichia* spp. in the Perio group than in the Health group. This remarkable result indicates an initial alteration in these subjects that could favour microbial succession and supports the establishment of later colonizers. However, the inter-species network was still driven by *Streptococcus* and *Actinomyces* hubs. Other pathobionts (such as *Treponema* spp., *Rothia* spp. and *Fusobacterium* spp.) only alter the co-occurrence network of the mixed and permanent dentition of the Perio group, suggesting the importance of this microorganism in the establishment of the community and the temporal effect of this initial colonization. This event was also associated with an abrupt microbial change between primary and mixed dentition in the Perio group (but did not occur in the Health group) and an increase in the differences in diversity, abundance and prevalence of species between Perio and Health from the mixed dentition.

In the present study, children from parents with periodontitis of mixed dentition, aged 9.6 years, presented a significantly different β-diversity and a higher abundance of disease-associated species compared to the Health group. Important inter-species network hubs of *TM7* genera, *Streptococcus* spp. and *Haemophilus-Mogibacterium-Prevotella* spp. were observed. Moreover, there was higher colonization of *Selenomonas* spp., *Leptotrichia* spp., *F. alocis*, *T. forsythia*, *P. nigrescens*, *T. denticola*, *P. intermedia* and *P. gingivalis*, all of which were found to be associated with periodontal breakdown previously (Hashim et al., 2017; Oliveira et al., 2016). As seen here and in our previous study enrolling youngers in mixed dentition (Monteiro et al., 2021), this cohort dentition phase is crucial for developing and maturing a more pathogenic biofilm (Fine et al., 2013; Mason et al., 2018; Umeda et al., 2004). Although the plaque index was not measured (though the dmft and DMFT scores were similar), several studies have indicated this phase as the one with a hard-to-control biofilm. Studies have listed some factors that can help understand this increase in plaque accumulation during this phase, such as malocclusion and positioning of teeth due to permanent eruption and the reduction in parental attention. Previous studies have shown that at this age, the parents stop assisting their children with toothbrushinng once they grow up and are presumably self-efficient. Although this does not mean the absence of brushing, its efficacy is reduced, thus increasing plaque accumulation (Gurunathan & Shanmugaavel, 2016; Lourenço et al., 2013). Meanwhile, the early acquired microbiome appears resilient to a shift in plaque and bleeding indexes. Monteiro et al. (2021) devised a strict oral hygiene programme for 3 months, and, unexpectedly, no significant changes in biofilm diversity were noted despite improvements in oral conditions. Indeed, in spite of using a chemical adjuvant in the toothpaste, children's microbiota appeared to be resilient to the shift, retaining most of their species and core (Monteiro et al., 2020, 2021).

FIGURE 3 Microbial differences between dentitions in the Perio group. β-Diversity: PCoA of the unweighted unifrac distances between pre-dentate × Primary dentition (a), primary × mixed dentition (b) and mixed x permanent dentitions (c). The ellipse of the 95% confidence interval is shown in the PCoA graphs. Differential abundance of species, tested with ANCOM-BC, comparing pre-dentate × primary dentition (d), primary × mixed dentition (e) and mixed × permanent dentitions (f). The bar size represents the fold change for each comparison, and the bar colour indicates the dentition in which the species is more abundant. Only species differentially abundant between groups were included in the graphs.

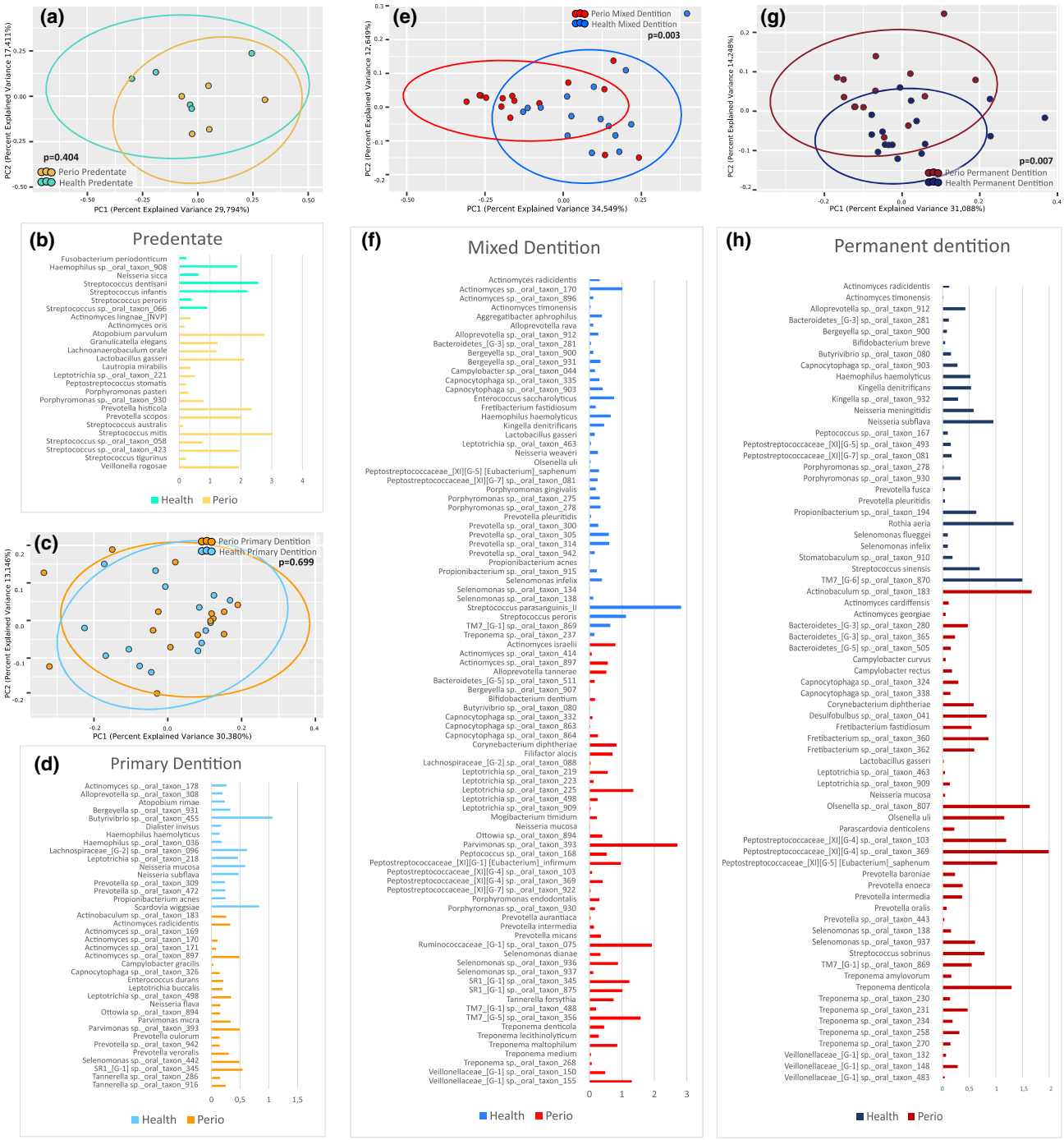

FIGURE 4 Microbial differences between groups in each dentition. β-Diversity: PCoA of the unweighted unifrac distances in pre-dentate (a) primary dentition (c), mixed dentition (e), and permanent dentitions (g). The ellipse of the 95% confidence interval is represented in the PCoA graphs. Differential abundance of species, tested with ANCOM-BC, in pre-dentate (b), primary dentition (d), mixed dentition (f) and permanent dentitions (h). The bar size represents the fold change for each comparison and the bar colour indicates the group in which the species is more abundant. Only species differentially abundant between groups were included in the graphs.

At the end of permanent teeth eruption and the establishment of complete permanent dentition, complex oral colonization is expected. Even though all subjects from periodontitis-affected parents did not present periodontitis and presented a DMFT score similar to that of the healthy group, a higher abundance of *Fretibacterium fastidious*, *P. micra*, *Campylobacter rectus*, *T. denticola* and several *Prevotella* spp. and members of the family *Veillonellaceae* were found in the Perio permanent-dentition group compared to the mixed-dentition group. Moreover, at the same time, a reduced abundance of *Streptococcus* spp., *Gemmella* spp. and *Leptotricha* spp. was seen in this group. This

FIGURE 5 Network co-occurrence analysis. The graphs describe the SparCC correlations between the species abundance ($r > 0.75$, $p < .01$) in different dentition stages for the Healthy (a) and Perio (b) groups. The green edges represent a positive correlation, and the red edges represent a negative correlation between the two nodes. Each node represents one bacterium, the node size is proportional to the number of correlations and the node colour corresponds to the colour assigned for each group the in the previous graphs.

difference in community results in a robust difference between the Perio and Healthy groups in permanent dentition. Besides the community composition, the species–species network also highlights the differences in the groups' microbial communities. Children from periodontitis parents showed an increase in the number of correlations of the gerera *Fusobacterium*, *Veillonella* and *Prevotella* since mixed

dentition also presented an intense inter-generic hub formation. On the other hand, less complex structures modulated for *Streptococcus*, *Actinomyces*, *Mogibacterium* and *Oribacterium* were descriptive in children from periodontally healthy patients. Altogether, it emphasizes that when severe grade C periodontitis affects a parent, their children seem to develop a dysbiotic oral community precociously (Herrero et al., 2018; Nibali et al., 2008). This community can stimulate a host response, which could initiate gingival inflammation and possibly start disease occurrence (Nibali, 2015). Meanwhile, it is important to consider that this trial assessed saliva samples and not the subgingival biofilm. Once pre-dentate babies were included, only saliva could be the sample for comparison between different ages. Saliva collection requires less time and participant burden and can be done remotely (Marotz et al., 2022). Some recent findings indicate that saliva could be an essential tool for detecting periodontitis (Belstrøm et al., 2017; Ma et al., 2021), presenting a significant correlation to subgingival niche for some microbial targets (Marotz et al., 2022). However, subgingival plaque is more diverse than saliva. Thus, although saliva is a major route for vertical transmission (Asikainen et al., 1997), future studies should also consider the assessment of subgingival niches along different dentitions.

4.3 | Early acquirement of oral species and systemic impacts

The recognition of early colonization and the impact of parental periodontal condition is more than one piece of the puzzle of microbiome acquisition. Recently, the concept of intra-uterine colonization has been introduced. Gomez-Arango et al. (2017) examined pregnant women's gut, oral and placental microbiome and found only three genera (i.e., *Prevotella*, *Streptococcus* and *Veillonella*) in all gut, oral and placenta samples. Although the placental microbiome does not harbour a unique core, indicating multiple sources of microorganisms, the placental microbiome resembles the oral microbiome of pregnant women (Aagaard et al., 2014).

One of the most critical implications of children's oral microbiome is oral disease occurrence and systemic health. Celiac disease, asthma, autism, paediatric inflammatory bowel disease and sleep alterations are systemic diseases linked to oral dysbiosis (Dzidic et al., 2018; Xiao et al., 2020). Higher levels of *Rothia*, *Porphyromonas endodontalis*, *S. sanguinis* and others, all commonly found in periodontitis samples, were linked to celiac disease and auto-immune diseases that alter gut barriers (Derrien et al., 2010). Hence, the confirmation of the impact of the parental oral condition on oral microbiome from very early ages, as well as the impact of local and systemic disease, sheds new light on the importance of controlling the oral microbiota during the gestational period and early colonization in childhood. Modulating the course of primary colonization should be a new focus of action for paediatricians and periodontists.

Significant results about oral colonization in descendants of periodontitis-affected individuals were presented in this study; however, some aspects of the study design and the clinical and microbiological data limited some wider conclusions. This study is a case–control study, with patients in different dentition stages and with a cross-sectional design; thus, temporal conclusions regarding the colonization process and a sequential increment of species in a patient during dentition stages are limited. These answers should be reached in a task force with long-term longitudinal trials including a large number of individuals and also periodontal clinical examination across dentitions, which was not possible in the present study because of the young ages of the children. Moreover, pre-dentate children included only very young babies prior to the first teeth eruption, around 3 months, making this population hard to reach. So, the limited size of this population should be considered. Additionally, another limitation is the absence of the parents' microbial analysis. Even though the present study was aimed at identifying the impact of parental periodontal disease on their offspring's microbiome across different dentitions, no parent–children comparison was performed, thus not allowing conclusions on vertical transmission of the microbial community. Thus, this aspect should be taken into account and evaluated in future studies to more deeply understand the clinical consequences of dysbiosis and identify factors related to periodontal breakdown. In spite of these limitations, the present results emphasize that the parent periodontal status is associated with a disease-associated microbiome in descendants of periodontitis-affected parents.

5 | CONCLUSIONS

In conclusion, parental periodontal condition impacts the oral microbiome from a very early age. The early colonization by pathobionts is initiated at pre-dentate and primary dentition. A dysbiotic-associated community could be seen already in mixed dentition and sustained at permanent dentition in children from periodontitis-affected parents.

AUTHOR CONTRIBUTIONS

Renato Corrêa Viana Casarin and Mauro Pedrine Santamaria designed the study; Aurélio Amorim Reis, Gabriela Martin Bonilha, Luciana Saraiva and Cassia Araújo were responsible for the selection of subjects and collection of samples; Mabelle Freitas Monteiro and Aurélio Amorim Reis were responsible for sample preparation; Mabelle Freitas Monteiro, Renato Corrêa Viana Casarin and Purnima Kumar carried out bioinformatic and data analysis. All authors contributed to writing or critically revising the manuscript.

FUNDING INFORMATION

No external funding was available for this study. Coordination for the Improvement of Higher Education Personnel (CAPES-001) funded the scholarship of Aurelio Reis, and the National Council for Scientific and Technological Development funded the scholarship of Renato Casarin (Grant 309429/2017-0).

CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest related to this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Sequence Read Archive at http://ncbi.nlm.nih.gov/sra, reference number PRJNA780174.

ORCID

Mabelle Freitas Monteiro https://orcid.org/0000-0001-9333-4349
Luciana Saraiva https://orcid.org/0000-0001-7592-7538
Purnima Kumar https://orcid.org/0000-0001-5844-1341
Renato Corrêa Viana Casarin https://orcid.org/0000-0003-1743-5855

REFERENCES

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science Translational Medicine*, *6*(237), 237ra65.

Abusleme, L., Dupuy, A. K., Dutzan, N., Silva, N., Burleson, J. A., Strausbaugh, L. D., Gamonal, J., & Diaz, P. I. (2013). The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. *The ISME Journal*, *7*(5), 1016–1025.

Amado, P. P. P., Kawamoto, D., Albuquerque-Souza, E., Franco, D. C., Saraiva, L., Casarin, R. C. V., Horliana, A. C. R. T., & Mayer, M. P. A. (2020). Oral and fecal microbiome in molar-incisor pattern periodontitis. *Frontiers in Cellular Infection Microbiology*, *10*, 583761. https://doi.org/10.3389/fcimb.2020.583761

Asikainen, S., Chen, C., Saarela, M., Saxén, L., & Slots, J. (1997). Clonal specificity of *Actinobacillus actinomycetemcomitans* in destructive periodontal disease. *Clinical Infectious Diseases*, *25*(Suppl 2), S227–S229. https://doi.org/10.1086/516211

Bastian, M., Heymann, S., & Jacomy, M. Gephi: An open-source software for exploring and manipulating networks. In Proceedings of the third international ICWSM conference 2009;361–362. https://doi.org/10.1136/qshc.2004.01003

Bearfield, C., Davenport, E. S., Sivapathasundaram, V., & Allaker, R. P. (2002). Possible association between amniotic fluid microorganism infection and microflora in the mouth. *BJOG: An International Journal of Obstetrics & Gynaecology*, *109*(5), 527–533.

Belstrøm, D., Sembler-Møller, M. L., Grande, M. A., Kirkby, N., Cotton, S. L., Paster, B. J., & Holmstrup, P. (2017). Microbial profile comparisons of saliva, pooled and site-specific subgingival samples in periodontitis patients. *PLoS ONE*, *12*(8), e0182992. https://doi.org/10.1371/journal.pone.0182992

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*, 335–336.

Casarin, R. C. V., Del Peloso, R. É., Mariano, F. S., Nociti, F. H., Casati, M. Z., & Gonçalves, R. B. (2010). Levels of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, inflammatory cytokines and species-specific immunoglobulin G in generalized aggressive and chronic periodontitis. *Journal of Periodontal Research*, *45*(5), 635–642.

Chen, T., Wu, D., Chen, H., Yan, W., Yang, D., Chen, G., Ma, K., Xu, D., Yu, H., Wang, H., Wang, T., Guo, W., Chen, J., Ding, C., Zhang, X., Huang, J., Han, M., Li, S., Luo, X., … Ning, Q. (2020). Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. *BMJ*, *368*, m1091. https://doi.org/10.1136/bmj.m1091

Chu, D. M., Ma, J., Prince, A. L., Antony, K. M., Seferovic, M. D., & Aagaard, K. M. (2017). Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nature Medicine*, *23*, 314–326.

Dabdoub, S. M., Fellows, M. L., Paropkari, A. D., Mason, M. R., Huja, S. S., Tsigarida, A. A., & Kumar, P. S. (2016). PhyloToAST: Bioinformatics tools for species-level analysis and visualization of complex microbial datasets. *Scientific Reports*, *6*, 29123. https://doi.org/10.1038/srep29123

Derrien, M., Van Passel, M. W. J., Van De Bovenkamp, J. H. B., Schipper, R. G., De Vos, W. M., & Dekker, J. (2010). Mucin-bacterial interactions in the human oral cavity and digestive tract. *Gut Microbes*, *1*(4), 254–268.

Díaz-Cárdenas, S., & González-Martínez, F. (2010). Prevalencia de caries dental y factores familiares en niños escolares de Cartagena de Indias, Colombia. *Revista de salud pública*, *12*, 843–851.

Doğan, B., Kipalev, A. Ş., Ökte, E., Sultan, N., & Asikainen, S. E. (2008). Consistent Intrafamilial transmission of *Actinobacillus actinomycetemcomitans* despite clonal diversity. *Journal of Periodontology*, *79*(2), 307–315.

Drell, T., Štšepetova, J., Simm, J., Rull, K., & Aleksejeva, A. (2017). The influence of different maternal microbial communities on the development of infant gut and oral microbiota. *Scientific Reports*, *7*, 9940.

Duran-Pinedo, A. E. (2021). Metatranscriptomic analyses of the oral microbiome. *Periodontology 2000*, *85*(1), 28–45.

Dzidic, M., Collado, M. C., Abrahamsson, T., Artacho, A., Stensson, M., Jenmalm, M. C., & Mira, A. (2018). Oral microbiome development during childhood: An ecological succession influenced by postnatal factors and associated with tooth decay. *ISME Journal*, *12*(9), 2292–2306. https://doi.org/10.1038/s41396-018-0204-z

Escapa, I. F., Chen, T., Huang, Y., Gajare, P., Dewhirst, F. E., & Lemon, K. P. (2018). New insights into human nostril microbiome from the expanded human oral microbiome database (eHOMD): A resource for the microbiome of the human aerodigestive tract. *mSystems*, *3*(6), e00187-18. https://doi.org/10.1128/mSystems.00187-18

Faveri, M., Figueiredo, L. C., Duarte, P. M., Mestnik, M. J., Mayer, M. P. A., & Feres, M. (2009). Microbiological profile of untreated subjects with localized aggressive periodontitis. *Journal of Clinical Periodontology*, *36*(9), 739–749.

Fine, D. H., Markowitz, K., Fairlie, K., Tischio-Bereski, D., Ferrendiz, J., Furgang, D., Paster, B. J., & Dewhirst, F. E. (2013). A consortium of *Aggregatibacter actinomycetemcomitans*, *Streptococcus parasanguinis*, and *Filifactor alocis* is present in sites prior to bone loss in a longitudinal study of localized aggressive periodontitis. *Journal of Clinical Microbiology*, *51*(9), 2850–2861.

Gomez-Arango, L. F., Barrett, H. L., McIntyre, H. D., Callaway, L. K., Morrison, M., & Nitert, M. D. (2017). Antibiotic treatment at delivery shapes the initial oral microbiome in neonates. *Scientific Reports*, *7*-(February), 1–10. https://doi.org/10.1038/srep43481

Griffen, A. L., Beall, C. J., Campbell, J. H., Firestone, N. D., Kumar, P. S., Yang, Z. K., Podar, M., & Leys, E. J. (2012). Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. *ISME Journal*, *6*(6), 1176–1185.

Guentsch, A., Hirsch, C., Pfister, W., Vincents, B., Abrahamson, M., Sroka, A., Potempa, J., & Eick, S. (2013). Cleavage of IgG1 in gingival crevicular fluid is associated with the presence of *Porphyromonas gingivalis*. *Journal of Periodontal Research*, *48*(4), 458–465.

Gurunathan, D., & Shanmugaavel, A. K. (2016). Dental neglect among children in Chennai.

Hajishengallis, G. (2014). The inflammophilic character of the periodontitis-associated microbiota. *Molecular Oral Microbiology*, *29*(6), 248–257. https://doi.org/10.1111/omi.12065

Hashim, N. T., Linden, G. J., Winning, L., Ibrahim, M. E., Gismalla, B. G., Lundy, F. T., & el Karim, I. A. (2017). Putative periodontal pathogens in the subgingival plaque of Sudanese subjects with aggressive periodontitis. *Archives of Oral Biology*, *81*(April), 97–102.

Herrero, E. R., Fernandes, S., Verspecht, T., Ugarte-Berzal, E., Boon, N., Proost, P., Bernaerts, K., Quirynen, M., & Teughels, W. (2018). Dysbiotic biofilms deregulate the periodontal inflammatory response. *Journal of Dental Research*, *97*(5), 547–555.

Jansen, H.-J., Grenier, D., & Van der Hoeven, J. S. (1995). Characterization of immunoglobulin G-degrading proteases of *Prevotella intermedia* and *Prevotella nigrescens*. *Oral Microbiology and Immunology*, *10*(3), 138–145.

Jo, R., Yama, K., Aita, Y., Tsutsumi, K., Ishihara, C., Maruyama, M., Takeda, K., Nishinaga, E., Shibasaki, K. I., & Morishima, S. (2021). Comparison of oral microbiome profiles in 18-month-old infants and their parents. *Scientific Reports*, *11*(1), 1–10. https://doi.org/10.1038/s41598-020-78295-1

Kennedy, B., Peura, S., Hammar, U., Vicenzi, S., Hedman, A., Almqvist, C., Andolf, E., Pershagen, G., Dicksved, J., Bertilsson, S., & Fall, T. (2019). Oral microbiota development in early childhood. *Scientific Reports*, *9*(1), 1–14.

Kumar, P. S., Brooker, M. R., Dowd, S. E., & Camerlengo, T. (2011). Target region selection is a critical determinant of community fingerprints generated by 16S pyrosequencing. *PLoS One*, *6*(6), 1–8.

Labbé, S., & Grenier, D. (1995). Characterization of the human immunoglobulin G Fc-binding activity in *Prevotella intermedia*. *Infection and Immunity*, *63*(7), 2785–2789.

Lif Holgerson, P., Esberg, A., Sjödin, A., West, C. E., & Johansson, I. (2020). A longitudinal study of the development of the saliva microbiome in infants 2 days to 5 years compared to the microbiome in adolescents. *Scientific Reports*, *10*(1), 9629.

Lin, H., & Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias correction. *Nature Communications*, *11*(1), 3514. https://doi.org/10.1038/s41467-020-17041-7

Lourenço, C. B., Saintrain, M. V., & Vieira, A. P. (2013). Child, neglect and oral health. *BMC Pediatrics*, *13*, 188. https://doi.org/10.1186/1471-2431-13-188.

Ma, J., Kageyama, S., Takeshita, T., Shibata, Y., Furuta, M., Asakawa, M., & Yamashita, Y. (2021). Clinical utility of subgingival plaque-specific bacteria in salivary microbiota for detecting periodontitis. *PLoS One*, *16*(6), e0253502. https://doi.org/10.1371/journal.pone.0253502. PMID: 34170942; PMCID: PMC8232462.

Marotz, C., Molinsky, R., Martino, C., Bohn, B., Roy, S., Rosenbaum, M., Desvarieux, M., Yuzefpolskaya, M., Paster, B. J., Jacobs, D. R., Colombo, P. C., Papapanou, P. N., Knight, R., & Demmer, R. T. (2022). Early microbial markers of periodontal and cardiometabolic diseases in ORIGINS. *npj Biofilms and Microbiomes*, *8*(1), 30.

Mason, M. R., Chambers, S., Dabdoub, S. M., Thikkurissy, S., & Kumar, P. S. (2018). Characterizing oral microbial communities across dentition states and colonization niches. *Microbiome*, *6*(1), 67.

Mason, M. R., Nagaraja, H. N., Camerlengo, T., Joshi, V., & Kumar, P. S. (2013). Deep sequencing identifies ethnicity-specific bacterial signatures in the oral microbiome. *PLoS One*, *8*(10), e77287. Erratum in: PLoS One. 2014;9(6):e99933.

Michalowicz, B. S., Diehl, S. R., Gunsolley, J. C., Sparks, B. S., Brooks, C. N., Koertge, T. E., Califano, J. V., Burmeister, J. A., & Schenkein, H. A. (2000). Evidence of a substantial genetic basis for risk of adult periodontitis. *Journal of Periodontology*, *71*(11), 1699–1707.

Monteiro, M. de F., Casati, M. Z., Taiete, T., Vale, H. F., Nociti, F. H., Jr, Sallum, E. A., Silvério, K. G., & Casarin, R. C. (2015). Periodontal clinical and microbiological characteristics in healthy versus generalized aggressive periodontitis families. *Journal of Clinical Periodontology*, *42*(10), 914–921. https://doi.org/10.1111/jcpe.12459

Monteiro, M. F., Altabtbaei, K., Kumar, P. S., Casati, M. Z., Ruiz, K. G. S., Sallum, E. A., Nociti-Junior, F. H., & Casarin, R. C. V. (2021). Parents with periodontitis impact the subgingival colonization of their offspring. *Scientific Reports*, *11*(1), 1–9. https://doi.org/10.1038/s41598-020-80372-4

Monteiro, M. F., Casati, M. Z., Taiete, T., Sallum, E. A., Nociti-Jr, F. H., Ruiz, K. G., & Casarin, R. C. V. (2014). Salivary carriage of periodontal pathogens in generalized aggressive periodontitis families. *International Journal of Paediatric Dentistry*, *24*(2), 113–121.

Monteiro, M. F., Tonelli, H., Reis, A. A., Casati, M. Z., Silvério, K. G., Nociti Junior, F. H., Sallum, E. A., & Casarin, R. C. V. (2020). Triclosan toothpaste as an adjunct therapy to plaque control in children from periodontitis families: A crossover clinical trial. *Clinical Oral Investigations*, *24*(4), 1421–1430. https://doi.org/10.1007/s00784-019-03121-6

Nelson-Filho, P., Borba, I. G., de Mesquita, K. S. F., Silva, R. A. B., de Queiroz, A. M., & Silva, L. A. B. (2013). Dynamics of microbial colonization of the oral cavity in newborns. *Brazilian Dental Journal*, *24*(4), 415–419.

Nesbitt, W. E., Beem, J. E., Leung, K. P., & Clark, W. B. (1992). Isolation and characterization of actinomyces viscosus mutants defective in binding salivary proline-rich proteins. *Infection and Immunity*, *60*(3), 1095–1100.

Nibali, L. (2015). Aggressive periodontitis: Microbes and host response, who to blame? *Virulence*, *6*(3), 223–228.

Nibali, L., Donos, N., Brett, P. M., Parkar, M., Ellinas, T., Llorente, M., & Griffiths, G. S. (2008). A familial analysis of aggressive periodontitis - clinical and genetic findings. *Journal of Periodontal Research*, *43*(6), 627–634.

Oliveira, R. R. D. S., Fermiano, D., Feres, M., Figueiredo, L. C., Teles, F. R. F., Soares, G. M. S., & Faveri, M. (2016). Levels of candidate periodontal pathogens in subgingival biofilm. *Journal of Dental Research*, *95*(6), 711–718.

Papapanou, P. N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D. H., Flemmig, T. F., Garcia, R., Giannobile, W. V., Graziani, F., Greenwell, H., Herrera, D., Kao, R. T., Kebschull, M., Kinane, D. F., Kirkwood, K. L., Kocher, T., Kornman, K. S., Kumar, P. S., … Tonetti, M. S. (2018). Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Periodontology*, *89*(Suppl 1), S173–S182. https://doi.org/10.1002/JPER.17-0721

Paropkari, A. D., Leblebicioglu, B., Christian, L. M., & Kumar, P. S. (2016). Smoking, pregnancy and the subgingival microbiome. *Scientific Reports*, *27*(6), 30388.

Perez-Muñoz, M. E., Arrieta, M. C., Ramer-Tait, A. E., & Walter, J. (2017). A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: Implications for research on the pioneer infant microbiome. *Microbiome*, *5*(1), 1–19.

Prince, A. L., Chu, D. M., Seferovic, M. D., Antony, K. M., Ma, J., & Aagaard, K. M. (2015). The perinatal microbiome and pregnancy: Moving beyond the vaginal microbiome. *Cold Spring Harbor Perspectives in Medicine*, *5*(6), 1–23.

Pylro, V. S., Roesch, L. F., Ortega, J. M., do Amaral, A. M., Tótola, M. R., Hirsch, P. R., Rosado, A. S., Góes-Neto, A., da Costa da Silva, A. L., Rosa, C. A., Morais, D. K., Andreote, F. D., Duarte, G. F., de Melo, I. S., Seldin, L., Lambais, M. R., Hungria, M., Peixoto, R. S., Kruger, R. H., … de Carvalho Azevedo, V. A. (2014). Brazilian microbiome project organization committee. Brazilian microbiome project: Revealing the unexplored microbial diversity–challenges and prospects. *Microbial Ecology*, *67*(2), 237–241. https://doi.org/10.1007/s00248-013-0302-4

Queiroz, L. A., Casarin, R. C. V., Dabdoub, S. M., Tatakis, D. N., Sallum, E. A., & Kumar, P. S. (2017). Furcation therapy with enamel matrix derivative: Effects on the subgingival microbiome. *Journal of Periodontology*, *88*(7), 617–625.

Ramadugu, K., Bhaumik, D., Luo, T., Gicquelais, R. E., Lee, K. H., Stafford, E. B., Marrs, C. F., Neiswanger, K., McNeil, D. W., Marazita, M. L., & Foxman, B. (2021). Maternal oral health influences infant salivary microbiome. *Journal of Dental Research*, *100*(1), 58–65.

Reis, A. A., Paz, H. E. S., Monteiro, M. F., Casati, M. Z., Steiner-Oliveira, C., Pascon, F. M., & Casarin, R. C. V. (2021). Early manifestation of periodontal disease in children and Its association with familial aggregation. *Journal of Dentistry for Children (Chicago)*, *88*(2), 140–143.

Schulz, S., Porsch, M., Grosse, I., Hoffmann, K., Schaller, H. G., & Reichert, S. (2019). Comparison of the oral microbiome of patients with generalized aggressive periodontitis and periodontitis-free subjects. *Archives of Oral Biology*, *99*(2018), 169–176.

Shannon, C. E. (1997). The mathematical theory of communication. 1963. *MD Computing*, *14*(4), 306–317.

Sulyanto, R. M., Thompson, Z. A., Beall, C. J., Leys, E. J., & Griffen, A. L. (2019). The predominant oral microbiota is acquired early in an organized pattern. *Scientific Reports*, *9*, 10550. https://doi.org/10.1038/s41598-019-46923-0

Teles, R., Sakellari, D., Teles, F., Konstantinidis, A., Kent, R., Socransky, S., & Haffajee, A. (2010). Relationships among gingival crevicular fluid biomarkers, clinical parameters of periodontal disease, and the subgingival microbiota. *Journal of Periodontology*, *81*(1), 89–98.

Umeda, M., Miwa, Z., Takeuchi, Y., Ishizuka, M., Huang, Y., Noguchi, K., Tanaka, M., Takagi, Y., & Ishikawa, I. (2004). The distribution of periodontopathic bacteria among Japanese children and their parents. *Journal of Periodontal Research*, *39*(6), 398–404.

van Winkelhoff, A. J., & Boutaga, K. (2005). Transmission of periodontal bacteria and models of infection. *Journal of Clinical Periodontology*, *32*-(Suppl 6), 16–27. https://doi.org/10.1111/j.1600051X.2005.00805.x

Velsko, I. M., Harrison, P., Chalmers, N., Barb, J., Huang, H., Aukhil, I., & Shaddox, L. (2020). Grade C molar-incisor pattern periodontitis subgingival microbial profile before and after treatment. *Journal of Oral Microbiology*, *12*(1), 1814674.

Ward, T. L., Dominguez-Bello, M. G., Heisel, T., Al-Ghalith, G., Knights, D., & Gale, C. A. (2018). Development of the human mycobiome over the first month of life and across body sites. *mSystems*, *3*(3), e00140-17. https://doi.org/10.1128/mSystems.00140-17

White, R. A., Bjørnholt, J. V., Baird, D. D., Midtvedt, T., Harris, J. R., Pagano, M., Hide, W., Rudi, K., Moen, B., Iszatt, N., Peddada, S. D., & Eggesbø, M. (2013). Novel developmental analyses identify longitudinal patterns of early gut microbiota that affect infant growth. *PLoS Computational Biology*, *9*(5), 1–9.

Wu, H., Fang, Y., Yu, J., & Zhang, Z. (2014). The quest for a unified view of bacterial land colonization. *The ISME Journal*, *8*(7), 1358–1369.

Xiao, J., Fiscella, K. A., & Gill, S. R. (2020). Oral microbiome: Possible harbinger for children's health. *International Journal of Oral Science*, *12*(1), 1–13. https://doi.org/10.1038/s41368-020-0082-x

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*(7402), 222–227.

Yu, J. C., Khodadadi, H., Malik, A., Davidson, B., Salles, É. D. S. L., Bhatia, J., Hale, V. L., & Baban, B. (2018). Innate immunity of neonates and infants. *Frontiers in Immunology*, *9*, 1759. https://doi.org/10.3389/fimmu.2018.01759

How to cite this article: Reis, A. A., Monteiro, M. F., Bonilha, G. M., Saraiva, L., Araújo, C., Santamaria, M. P., Casati, M. Z., Kumar, P., & Casarin, R. C. V. (2023). Parents with periodontitis drive the early acquisition of dysbiotic microbiomes in their offspring. *Journal of Clinical Periodontology*, *50*(7), 890–904. https://doi.org/10.1111/jcpe.13815