MATURATION OF THE ORAL MICROBIOME IN CARIES-FREE TODDLERS:

A LONGITUDINAL STUDY

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APPENDIX

MATERIALS AND METHODS

ETHICAL CONSENT AND STUDY DESIGN

Approval for this study was obtained from the Medical Ethics Committee of the University of Michigan Medical School [Institutional Review Board (IRBMED) approval no. HUM00071519. Date of approval: 14-03-2013], Duke University [Duke Medicine Institutional Review Board approval no. PRO00044905. Date of approval: 13-06-2013], Indiana University [Indiana University Institutional Review Board approval no. 1303010908. Date of approval: 15-04-2013] and University of Iowa [University of Iowa Institutional Review Board approval no. 201302810. Date of approval: 5-03-2013].

CLINICAL EXAMINATION

Children underwent clinical oral examination conducted by calibrated dentists or dental hygienists at Duke University, Indiana University and University of Iowa. After collection of saliva and plaque samples, the teeth and mucosal tissue were assessed. Teeth were cleaned with a toothbrush, air or gauze dried, and assessed under light (Orascoptic Endeavour headlamps, Middleton, WI), without magnification, using the International Caries Detection and Assessment

System (ICDAS II) criteria for scoring (ICDAS Coordinating Committee 2012). No dental radiographs were obtained. Primary caregivers were informed of any conditions requiring treatment.

QUESTIONNAIRES

A self-reported 53-item questionnaire (DCR-007-Primary Caregiver Questionnaire) was used for the parental study. Only general questions (demographics, delivery mode, Medicaid) about the child and the caregiver were included in the current study (Daly et al. 2016; Eckert et al. 2010; Fontana et al. 2019; Fontana et al. 2011). Medicaid is a United States state and federal program that provides health coverage for some low-income families and children, pregnant women, the elderly, and people with disabilities. The ethnicity terms “White” and “Black” which were used in the questionnaire, have been replaced with “Caucasian” and “African American” in the current text.

CURRENT STUDY POPULATION

In total, 266 children completed the longitudinal collection (T1, T2, and T3) of salivary and dental plaque samples. Among the 266 participants at the third visit (T3), 127 (47.7%) children had dental caries (ICDAS≥1), 20 (7.5%) children had experienced remineralization of dental caries (ICDAS=1 at T2 and ICDAS=0 at T3), while 119 (44.7%) children maintained caries-free status during all time points (ICDAS=0). Only children who did not present with clinical signs of dental caries by visual observation until the age of 4 years (n=119) and their caregivers (n=116) were included in the current study (Appendix Fig. 1). Among these were three twin pairs and their caregivers. None of the caries-free (n=119) participants had fillings during the 3 years of the study. Five children had received sealants at some point during the study.

SAMPLE COLLECTION AND STORAGE

Sample collection in children

Unstimulated saliva and pooled dental plaque samples were collected from children at three time points: at baseline or T1, when children were 1-year-old, at T2 (2.5 years of age) and at T3 (4 years of age). The collection of the samples varied in time of the day. No recommendation was given for food intake or tooth brushing before sampling. The participants received recommendation to use Fluoride toothpaste in their daily oral hygiene after each visit. From all children, saliva samples were collected at each visit prior to the dental examination, in an OM-505 collection tube (OMNIgene®ORAL, USA). The mouth of the child was swabbed holding two sponges (Puritan PurFlock Ultra, Guilford, Maine, USA) together in the cheek pouch, by gently moving sponges around, sampling in one slow sweep the left pouch, the floor of the mouth and right pouch during 30 sec. From this point onwards saliva swab sample is referred to as saliva sample. The sponges where placed in the OM-505 tubes after breaking off the handle (at the breaking point close to the sponge). If teeth were present, a pooled plaque sample was taken prior to the ICDAS exam by swabbing all buccal surfaces of the child teeth with a sterile microbrush (Microtip micro-applicator fine size; Microbrush International, Grafton, Wisconsin, USA). After the sample was collected the entire microbrush with plaque sample was dropped into Liquid dental transport medium (LDT) vial (Anaerobe Systems, Morgan Hill, California, USA).

Sample collection in caregivers

Unstimulated saliva was collected at T1 from all primary caregivers, while caregivers from the University of Iowa (*n*=66) were sampled at all three visits. Saliva collection was performed by drooling 1 mL saliva into the funnel (OM-505 tube) without active spiting.

All samples (from children and their caregivers) were placed on ice immediately after collection. Samples were transported on dry ice and stored at -80^{o}C.

SAMPLE PROCESSING

DNA isolation

Samples were subjected to DNA isolation in batches of 84 samples per sample type. All samples belonging to the same child and caregiver were processed in one batch as they were compared longitudinally. Additionally, each isolation batch contained samples originating from the three recruitment sites (Duke, Indiana and Iowa Universities). This was done to minimize the possible differences in isolation between batches of the same kit, buffers, and daily variations. To control for potential contaminations, the isolation blanks (kit chemicals) and the following separate sample blank controls were added to each of the batch: 1) unused brushes; 2) LDT fluid; 3) swabs; 4) stabilizing fluid from unused OM-505 tubes.

For plaque, the LDT vials were thawed, the microbrush was removed using sterile forceps and, together with 100 µL of the solution, transferred to an assigned well in a 1.1 mL deep-well plate (Axygen scientific Inc., CA, USA). For adult saliva samples, the vials were thawed and vortexed extensively and 200 µL of the solution was transferred to an assigned well. For child saliva samples, the vials were thawed and one swab was transferred using sterile forceps; additionally, 200 µL of the solution was added to the same well as the swab. Each 1.1 mL deep-well plate

contained 250 µL 0.1-mm Zirconia beads, 200 µL of phenol (Rotiphenol, Carl Roth GMBH&Co. KG, Germany) and 200 µL of lysis buffer (MagMini DNA isolation kit, LGC Genomics Ltd, UK). The deep well plate was sealed with a silicone lid and placed in a Mini-BeadBeater-96 (BioSpec Products, Bartlesville, OK, USA) for 2 min at 2,100 oscillations/min.

DNA was extracted and purified using the MagMini DNA Isolation Kit (MagMini DNA isolation kit, LGC Genomics Ltd, UK). Bacterial DNA concentration in the samples after purification was determined by qPCR, with universal primers specific to the bacterial 16S rRNA gene (Ciric et al. 2010).

PCR amplification & sequencing

The V4 hypervariable region of the 16S rRNA gene was amplified using 1 ng DNA with 1 µM of each barcoded forward and reverse primer used and performing 30 amplification cycles (Kozich et al. 2013). Samples were mixed into a pool in an equimolar fashion; PCR products of isolation blanks (kit chemicals only), sample blanks (kit chemicals and unused brushes (plaque) or LDT fluid (plaque) or swabs (child saliva) or stabilizing fluid (child and caregiver saliva) and negative PCRs (PCR mix with DNA free water) were included. Furthermore, PCR products of three designated samples and a bacterial mock community sample (HM-782D, BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Genomic DNA from Microbial Mock Community B (Even, Low Concentration), v5.1L, for 16S rRNA Gene Sequencing) were added to each sample pool to serve as run-to-run controls. This was done in order to assess the potential batch effects of sequencing. Each sequencing run included replicate templates of one sample from different individuals.

Paired-end sequencing of the DNA was conducted on the MiSeq platform (Illumina, San Diego, CA, USA) in 6 runs, using a V3 kit and 2x251 nt at the AUMC Cancer Center Amsterdam (Amsterdam, the Netherlands). For each run, the flow cell was loaded with 12 pmol DNA containing 25% PhiX. The data are available in the NCBI BioProject database under accession number PRJNA575641.

DATA PROCESSING

All samples, from all runs were processed together. The 16S rDNA reads were merged, quality-filtered and checked for possibly remaining PhiX reads as previously described (Koopman et al. 2016) with the following exception: a maximum of 25 mismatches (still 10%) was used in the overlap region during read merging. Next, the quality-filtered sequences (max. expected error 0.5) were denoised using UNOISE3 (usearch v10.0.240, 32-bit; (Edgar 2016)). Mapping of the sequences to the "zero-radius OTUs" (zOTUs) was carried out using usearch_global with –maxaccepts 128 –maxrejects 1024 –maxhits 1, for higher sensitivity during mapping. The representative most abundant zOTU sequences were assigned a taxonomy as previously described (Koopman et al. 2016), however, a trimmed version of HOMD v14.51 (Chen et al. 2010) was used as taxonomic reference database for the RDP naïve Bayesian classifier with minimum confidence of 80% (Wang et al. 2007). The zOTU-table was subsampled at a depth of 7,000 reads per sample, using the single_rarefaction.py script of QIIME v1.8.0, in order to allow comparisons among the different samples.

QUANTIFICATION OF FUNGAL DNA

After DNA extraction, the fungal abundance in the samples was determined using quantitative PCR (qPCR), as previously described (Vollmer et al. 2008). These primers cover the following

fungi: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Candida dubliniensis*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Microsporum canis*, *Mucor flavus*, *Trichophyton rubrum*, *Cryptococcus neoformans,* and *Aureobasidium pullulans*. The fungal load was calculated as the relative percentage of fungal DNA to the concentration of bacterial (16S) DNA. Detection limit for fungal qPCR was 0.00188 ng/µl and for 16S qPCR: 0.041649 ng/µl.

STATISTICAL ANALYSES

Microbial profile analyses of unrelated samples

For multivariate analyses of microbial profile data and assessment of the clustering of samples, the zOTU-table was log-2 transformed and ordinated into principal components using Principal Component Analysis (PCA). To preclude negative values, log-2 transformation was performed on read counts+1. Differences in microbial profiles among the sample groups (beta diversity) were assessed with One-way Permutational Multivariate Analysis of Variance (PERMANOVA; 9999 permutations) using the Bray-Curtis similarity. The *P*-values were corrected for multiple testing using Bonferroni correction. These analyses were performed using PAST software version 3.18 (Hammer et al. 2001).

Microbial profile analyses of related samples

In case of dependent samples, such as samples of the same subject over time, PERMANOVA was performed using adonis (vegan v.2.4-6 (Oksanen et al. 2018); R v.3.4.3 (R-Core 2017) with a restriction on the permutations within the subject. R^2 values in adonis gives the effect size showing the variation in distances is explained by the variables being tested. Due to relatively

large sample size, Bonferroni correction was used when overall microbial profiles were compared, to rule out sporadic differences.

Similarity and microbial diversity analyses

Similarity in microbiome profiles between different types of sample or different time points of the same child and between the child and his/her caregiver was assessed using Bray-Curtis similarity. Alpha diversity was assessed using Shannon Diversity Index and Species richness (number of taxa/sample). All analyses were performed using PAST software version 3.18 (Hammer et al. 2001).

Assessment of shared taxa

The Venn diagram layout from Venny (Oliveros 2007) was used to illustrate number of shared zOTUs between time points or between saliva and plaque samples. The calculations were processed either per group or per child. For the pairwise Venn analysis, a threshold of 70 reads per zOTU accounting for 0.1% of the reads per sample was used. For unpaired group-wise Venn analysis of zOTUs, the reads were not filtered for abundance, in order to allow comparisons with previously published work on so called 'core taxa'. Next, the unique zOTUs and overlaps were calculated for each group or sample (separately, child saliva and plaque over time, child and caregiver saliva, also plaque-saliva comparison) and summarized per set in a median value and range (min-max).

Assessment of differentially abundant zOTUs

To assess which zOTUs had a statistically different relative abundance among the sample groups, the linear discriminant analysis effect size (LEfSe) biomarker discovery tool (Segata et

al. 2011) was used. For LEfSe analysis, a 100-read cut-off per zOTU in the respective subset of samples was used to allow consistency while working with different time points and sample types.

Univariate statistical tests

The distribution of the univariate continuous variables was tested using the Kolmogorov-Smirnov test for normality. The Friedman test and Wilcoxon rank-sum test was applied for non-normally distributed variables of related samples. For normally distributed variables of related samples General Linear Model Repeated Measures test was used. To compare unrelated samples, Kruskal-Wallis test followed by Mann-Whitney test were used. All tests above were performed in SPSS version 25. False Discovery Rate (FDR) correction of *P*-values for multiple comparisons was performed in R v. 3.4.3. The FDR was set to 5%.

RESULTS

STUDY POPULATION

In total, 119 children who were caries-free at all study time points (T1: 1 year; median: 11.3, range 9-15.9 months, T2: 2.5 years; 29 (25.1-35.7) months and T3: 4 years; 47 (42.9-53.8) months) were included in the study (Table). Each child was paired with their own primary caregiver forming a child-caregiver pair. In case of twins (*n*=3 pairs), one caregiver was paired with each of his or her twin child separately, creating two child-caregiver pairs. Among the caregivers, 110 (92%) were mothers.

OVERALL SEQUENCING OUTPUT

The entire dataset consisting of 266 children and their caregivers resulted in 2,412 zOTUs. This table, including all samples and controls, was used to check for possible contaminants. zOTUs which had a relatively higher presence in controls vs samples, in total 42, were removed from the dataset. The vast majority (33 zOTUs) of these zOTUs had a very low total abundance of less than 400 reads (in all samples and all controls).

In total, 22,910,789 reads (average 24,373 reads per sample, SD 6,216, range 5-42,617), obtained from the clinical sample sequencing, passed quality filtering and were assigned to 2,345 zOTUs. After subsampling, 2,320 zOTUs remained in the dataset.

In order to assess potential batch effects of sequencing, run-to-run control samples were analyzed using PCA and PERMANOVA. These control samples clustered together according to their sample origin as three designated samples (P=0.0001), but not according to their run number (P=0.99).

From 940 samples, 925 samples passed the subsampling depth to 7,000 reads per sample. These included 246 unstimulated saliva samples from the caregivers (115 - at T1, 66 - at T2 and 65 - at T3), 323 pooled dental plaque samples (93 - at T1, 114 - at T2 and 116 - at T3) and 356 saliva samples from the children (119 - at T1, 118 - at T2 and 119 - at T3) (Appendix Fig. 1).

Saliva samples from the caregivers collected at T1 were used for all analyses below, while the caregiver samples from all three time points (T1, T2, and T3) were only used for taxonomy summary and to assess the overtime stability of salivary microbial profiles.

TAXONOMY AND CHANGES AT PHYLUM AND GENUS LEVEL OVER TIME

The zOTUs of the subsampled dataset were classified in 12 phyla (Fig. 1, Appendix Fig. 2) and in 163 genera or higher taxa (Appendix Table 1A, B). Of all zOTUs, 32 zOTUs (1,142 reads or 0.02% of all reads) could only be assigned to kingdom Bacteria.

The most dominant genera

Child saliva samples were dominated by genus *Streptococcus* (T1: 40%, T2: 36%, T3: 33%), followed by *Haemophilus* (T1: 11%, T2: 11%, T3: 13%) and *Neisseria* (T1: 8%, T2: 9%, T3: 9%), while most reads in saliva of the caregivers were classified as *Streptococcus* (33%), *Prevotella* (21%), *Veillonella* (8%), *Haemophilus* (7%) and *Neisseria* (6%). The top three genera in child plaque samples were *Streptococcus* (T1: 25%, T2: 22%, T3: 21%), *Neisseria* (T1: 18%, T2: 12%, T3: 13%) and *Actinomyces* (T1: 8%, T2: 11%, T3: 12%) (Appendix Table 1B). The predominance of the genera mentioned above was consistent among all three time points.

Changes in bacterial phyla over time

At the phylum level, salivary microbiome of children changed the most from T1 (1 year of age) to T2 (2.5 years), with significant decrease in relative abundance of Firmicutes and Bacteroidetes, and increase in Proteobacteria, Fusobacteria, Actinobacteria and Saccharibacteria (TM7) (Appendix Fig. 3). In plaque, only Actinobacteria increased significantly with time, while Bacteroidetes and Proteobacteria decreased (Appendix Fig. 3).

Changes in bacterial genera over time

At the genus level, *Leptotrichia*, *Fusobacterium*, *Actinomyces*, *Corynebacterium* and *Rothia* increased significantly with time both in saliva and in plaque (Fig. 2) of children. Genus *Streptococcus*, *Veillonella*, *Granulicatella*, *Porphyromonas* and *Alloprevotella* decreased

significantly with time in saliva of children, while in plaque decrease was observed with *Capnocytophaga* and *Neisseria* (Fig. 2).

CHANGES IN MICROBIAL PROFILES OVER TIME

Microbial composition of children over time

Microbial profile analyses on saliva (Fig. 3A) and dental plaque samples (Fig. 3B) of children showed that samples collected at the earliest time point (T1: 1 year of child age) formed a separate cluster from the other samples. Although no clear separation was discernible between the later time points - T2 (2.5 years) and T3 (4 years), the differences were significant among all three time points (P=0.0001, R^2=0.1 for saliva, and P=0.0001, R^2=0.07 for plaque, restricted PERMANOVA), also between T2 and T3 in saliva (P=0.006, R^2=0.009, Bonferroni corrected restricted PERMANOVA) and in plaque (P=0.006, R^2=0.007, Bonferroni corrected restricted PERMANOVA).

Microbial composition of caregivers over time

Microbial profiles of the caregivers from whom saliva was collected at all three time points (n=66), changed significantly (P=0.01, restricted PERMANOVA) across the three years (Appendix Fig. 4A). Explained variation of changes over time in salivary composition of the caregivers was 0.08% (R^2 =0.008).

Microbial composition of children over time and their caregivers at T1

Comparison of the salivary microbiome profiles of children and their caregivers showed that samples of the caregivers (n=115) clustered clearly separately from those of children collected at

all time points (P=0.0001, R^2= 0.21, restricted PERMANOVA) (Fig. 3C, Appendix Fig. 4B, C, D).

Similarity of the microbial composition

Although microbial composition of children and adults remained significantly different throughout the three years of the study, the similarity in salivary microbiome between each child and his or her caregiver (child-caregiver pair) increased significantly from the age of 1 (T1) until 2.5 years (T2) (P<0.01, FDR corrected general linear model repeated measures test).

Comparisons of similarity of the samples within individual child, collected at two different time points, showed that microbial composition of samples collected at T2 and T3 were more similar to each other than composition of samples between the first and the later time points of the same child both for saliva and for plaque samples. In other words, microbial composition changed the most between the first time point and the rest (Fig. 3D).

Microbial diversity

Alpha Diversity (Shannon Diversity index and Species Richness) of both saliva and plaque of children collected at T1 (1-year-old) was significantly lower than at the later time points (T2, T3), while there was no change in diversity from T2 (2.5 years) to T3 (4 years) (Appendix Fig. 5A, B).

Salivary microbiome of the caregivers was significantly more diverse (median 400 (244-641) zOTUs/sample) than that of children at any time point (at T1: 235 (112-457), T2: 369 (141-518), T3: 359 (161-573) zOTUs/sample) (Appendix Fig. 5B).

CHANGES OF INDIVIDUAL TAXA (zOTUs) OF CHILDREN OVER TIME AND IN COMPARISON WITH THE CAREGIVERS

Proportion of the shared taxa (zOTUs)

Of all 2,320 zOTUs, the zOTU1, classified as *Streptococcus* (*S. dentisani /infantis /mitis /oralis /tigurinus* /several unclassified oral taxons) was present in all saliva and plaque samples (*n*=925) of the study (16% of the reads).

All saliva samples of children and their caregivers (*n*=471) shared three zOTUs: zOTU1, zOTU32 (*Streptococcus*) and zOTU9 (*Gemella haemolisans /morbillorum /sanguinis*) with 22%, 0.7% and 3% of the reads, respectively (Appendix Table 1C). There was a 76% overlap in zOTUs (zOTUs > 0 reads included) in the overall child saliva composition at the three time points, and a 60% overlap between the overall child and caregiver saliva (Appendix Fig. 6A). A large variation in the proportion of the shared zOTUs was observed within an individual child over time (2-42% of the zOTUs≥70 reads) or within a child-caregiver pair throughout all time points (2-17%) (Appendix Fig. 6B).

In plaque, there was a 69% overlap in zOTUs in the overall composition at the three time points (Appendix Fig. 6A). All plaque samples collected at T1 (1 year of age, *n*=93) contained reads of zOTU1 and zOTU32 (streptococci) and zOTU7 (*Neisseria flavescens /subflava*) with 10%, 0.5% and 1.2% of reads, respectively. At the T2 (2.5 years of age), the zOTU7 was found in 112 (98%) of the 114 samples, while zOTU1 and zOTU32, together with three other zOTUs also classified as streptococci, zOTU3 (*Haemophilus parainfluenzae*), zOTU5 (*Actinomyces*) and zOTU9 (*Gemella*) were found in all plaque samples of the time point. At the T3 (4 years of age), all plaque samples (*n*=116) contained the same two zOTUs classified as streptococci (zOTU1,

zOTU4) and single *Neisseria* zOTU (zOTU2), with all but one sample sharing zOTU32 (*Streptococcus*), zOTU20 (*Lautropia mirabilis*), zOTU36 (*Abiotrophia defectiva*), three zOTUs classified as *Neisseriaceae* (zOTU22), *Haemophilus parainfluenzae* (zOTU3) and *Gemella* (zOTU9) (Appendix Table 1C). At the individual subject level, again, as in saliva, large inter-individual variation (2.5-38%) in the proportion of the shared zOTUs among the time points was observed (Appendix Fig. 6B).

Changes of individual taxa (zOTUs)

Next, we assessed which zOTUs changed significantly in their relative abundance both in time and in relation to the caregivers. To identify potentially differential zOTUs we applied a biomarker discovery tool LEfSe, followed by pairwise comparisons.

Of zOTUs that were included in LEfSe analyses, the relative abundance of over 200 zOTUs was significantly higher in saliva of the caregivers compared to the children, while the number of zOTUs that were significantly higher in children in comparison to the caregivers increased with time: 113 zOTUs at T1, 183 zOTUs at T2 and 252 zOTUs at T3 (Appendix Table 2A).

In children, over 180 and 130 taxa increased in their relative abundance with time in saliva and plaque, respectively, including *Fusobacterium* (zOTU70), *Actinomyces* (zOTU61) and *Corynebacterium* (zOTU17) (Fig. 4AB), while over 40 taxa in saliva and over 20 taxa in plaque showed significant decrease over time (Appendix Table 2A). For instance, at T1 (1 year of age) children had the highest proportion of *Alloprevotella* (zOTU11) in their saliva and *Capnocytophaga* (zOTU29) in their plaque, compared to a later age (Fig. 4A, B).

zOTUs CLASSIFIED AS *STREPTOCOCCUS MUTANS* AND *PORPHYROMONAS GINGIVALIS*

To address the question if specific microbial taxa – *Streptococcus mutans* and *Porphyromonas gingivalis*, traditionally associated with dental caries and periodontal diseases, respectively, - are present in orally-healthy children between 1 and 4 years of age, we assessed the presence and relative abundance of these two taxa in both child and caregiver samples. In the entire dataset (925 samples), 7 zOTUs were classified as *S. mutans*, while only single zOTU (zOTU 209) was classified as *P. gingivalis*. The latter was found at a very low prevalence (1-2 reads or 0.01-0.02%) in only 2 saliva samples (both at the age of 4 years) and in two plaque samples (at T1 and T3) in four different children. In caregivers, *P. gingivalis* zOTU was present in 13 saliva samples at a 0.01-2.4% relative abundance. None of these caregivers matched the four children with *P. gingivalis*. On the other hand, reads classified as *S. mutans* zOTUs were found in saliva of 12 child-caregiver pairs, of which 11 pairs shared the same zOTUs, suggesting transmission from caregiver to the child. In total, *S. mutans* zOTUs were identified in saliva of 40 (34.8%) caregivers, in saliva of 18 children and in plaque of 21 children. In 11 children *S. mutans* was present in both sample types (saliva and plaque) and in 9 cases *S. mutans* was found at two time points - T2 and T3 - of the same child. Of four children who had *S. mutans* in their samples at the age of 1 year, all presented it at a very low relative abundance (0.01%), and none of them remained positive for this taxon at the later time points.

DIFFERENCES BETWEEN SALIVARY AND PLAQUE MICROBIAL COMPOSITION OF CHILDREN

Differences in the microbial profiles

Next, we compared the microbial composition of two types of samples, unstimulated saliva and pooled dental plaque, over time. As expected, microbial profiles of saliva were significantly

different from those of plaque (T1: P=0.0001, R^2=0.23; T2: P=0.0001, R^2=0.13; T3: P=0.0001, R^2=0.15, restricted PERMANOVA) (Appendix Fig. 7A).

Similarity and diversity of microbial composition

The composition of saliva and plaque was less similar (Bray-Curtis similarity) at 1 year of age than at the later time points (P<0.0001, FDR corrected Wilcoxon test). Only at T1, saliva samples had significantly higher species richness (more zOTUs/sample) than plaque (P<0.0001, FDR corrected Friedman test), while Shannon Diversity index did not differ between two types of samples at any of the time points.

Proportion of the shared taxa (zOTUs)

There was a 72%, 84% and 83% overlap in zOTUs between the overall child saliva and plaque composition at T1, T2 and T3, respectively (Appendix Fig. 7B). A large inter-individual variation in the proportion of the shared zOTUs was observed within a child between the two types of samples: 10% (range 2.6-65%) of the zOTUs at T1, 25% (0-60%) – at T2 and 18% (2.7-56%) – at T3 (Appendix Fig. 7C).

Changes of individual taxa (zOTUs)

Of the zOTUs ≥ 100 reads, 349, 373 and 373 were significantly different between two sample types at T1, T2 and T3, respectively (Appendix Table 2B). Among the 349 zOTUs from T1, 214 zOTUs were at a significantly higher proportion in saliva compared to plaque. For example, zOTU1 (*Streptococcus*), zOTU11 (*Alloprevotella*), zOTU7 (*Neisseria*), *Haemophilus* (zOTU13, zOTU3) and zOTU9 (*Gemella*) differed the most (had the highest LDA scores by the LEfSe

biomarker discovery tool) (Appendix Fig. 8A). These taxa remained in top ten most discriminatory zOTUs also at later time points.

In plaque at T1, 135 zOTUs were significantly higher in their relative abundance than in saliva at T1. Among these, *Neisseria* (zOTU2), *Streptococcus sanguinis* (zOTU4), *Actinomyces* (zOTU5), *Capnocytophaga sputigena* (zOTU29), *Rothia aeria* (zOTU21) and *Corynebacterium durum* (zOTU14) differed the most between two sample types (Appendix Fig. 8B; Appendix Table 2B). These taxa remained in top ten most discriminatory zOTUs also at later time points (Appendix Fig. 8).

MICROBIOME DIFFERENCES BY AGE AND TOOTH ERUPTION STATUS OF THE CHILDREN AT T1

The age of the children at the start of the study (T1) varied from 8.97 to 15.9 months and included both predentate (n=18) and dentate (n=101) children (median 4 teeth, range 0-12) (Table, Fig. 1). Since the age at T1 correlated significantly with the number of the erupted teeth (P<0.0001, Spearman's Rho=0.6), we assessed the relation of age and the teeth eruption status of the children at T1 with their salivary and plaque microbiome composition. For this, we stratified the children into three age subgroups: 1) below 11 months old (n=54), 2) 11-13.9 months old (n=40) and 3) 14 months old or older (n=25). Both, salivary and plaque microbiome of the youngest age subgroup differed significantly from the two older subgroups, but there were no significant differences between middle and the oldest subgroups (Appendix Fig. 9A, B). The species richness of the youngest children was significantly lower than in the older children, both in saliva and plaque, while Shannon Diversity index was lower only in plaque and not in saliva of the youngest children (Appendix Fig. 9E, F).

Children also were divided in three subgroups based on their teeth eruption status: 1) predentate (*n*=18); 2) 1-4 teeth erupted (*n*=56) and 3) 5-12 teeth erupted (*n*=45). Saliva of the predentate children and children with 1-4 teeth erupted differed significantly from the microbial profile of saliva from children with 5-12 teeth erupted (Appendix Fig. 9C). These differences were reflected in the diversity data (Appendix Fig. 9G, H). Microbial profiles and diversity of plaque from children with 1-4 teeth differed significantly from those with 5 or more teeth erupted (Appendix Fig. 9D, G, H).

SALIVARY AND PLAQUE MICROBIOME BY DEMOGRAPHIC DATA AND EXPOSURE TO ANTIBIOTICS

We found no differences in the composition of both salivary and plaque microbiome by delivery mode, while plaque at T2 differed by gender (P=0.007, PERMANOVA), and at T3 by race/ethnicity (Caucasian vs African American, P=0.002, Bonferroni corrected PERMANOVA). Microbial profiles of saliva collected at T2 (results not shown) and plaque collected at all three time points from children who were part of the Medicaid program differed from those who were not (P<0.01, PERMANOVA). Plaque samples collected at T3 from the Medicaid group showed higher diversity (Shannon Diversity index, P=0.007, Kruskal-Wallis test) than plaque from the non-Medicaid group. At all time points, multiple zOTUs significantly discriminated between the two groups (Appendix Table 2C). Microbial composition of both saliva and plaque samples of children who were exposed to antibiotics within 4 weeks of sample collection differed significantly from those who were not (P<0.05, Bonferroni corrected PERMANOVA).

TOTAL BACTERIAL AND FUNGAL LOAD IN SALIVA AND PLAQUE SAMPLES

Microbial DNA and fungal DNA in the samples was quantified using universal qPCR for 16S rRNA gene (thus bacterial DNA) and for 28S region, a specific for large group of fungi, including multiple *Candida* species (further called fungal DNA). Saliva samples collected at T1 (1-year-old) and those collected from caregivers had higher bacterial DNA concentration than saliva from the older children (Appendix Fig. 10A).

At T1, fungal DNA was found in saliva of 98% of the caregivers and in 98% of the children, while at T2 and T3 92% and 89% of the children, respectively, had detectable fungi in their saliva. Over time, the youngest children had the highest and the oldest – the lowest fungal DNA concentration in their saliva (Appendix Fig. 10B). Salivary fungal load, calculated as a relative abundance of fungal DNA over bacterial DNA in a sample, was also the highest at the youngest age (median: 0.013%, range: 0-11%) (Appendix Fig. 10C). There was no difference in fungal DNA concentration or in fungal load between saliva of the caregivers and their children at the age of 1 year, while the caregivers had significantly higher fungal concentration and fungal load than their children at 2.5 years and 4 years of age.

In plaque, only 66% of the samples had detectable fungi at T1, while at T2 fungi were present in 90% of the samples and at T3 in 68% (Appendix Fig. 10B, C). The fungal load in plaque at T3 was significantly lower in comparison with T2 (T1: median 0.007%; T2: 0.009%; T3: 0.003%), and it remained significantly lower than in saliva at T1 and T3.

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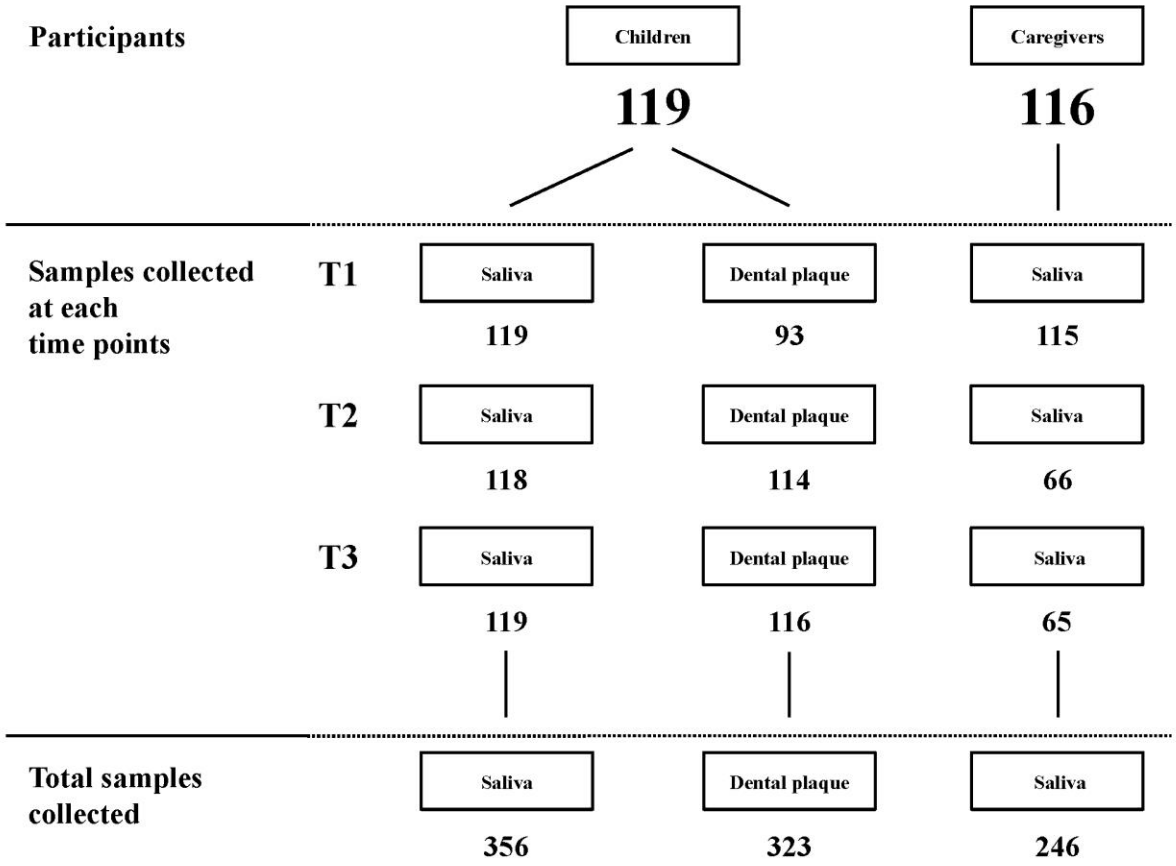
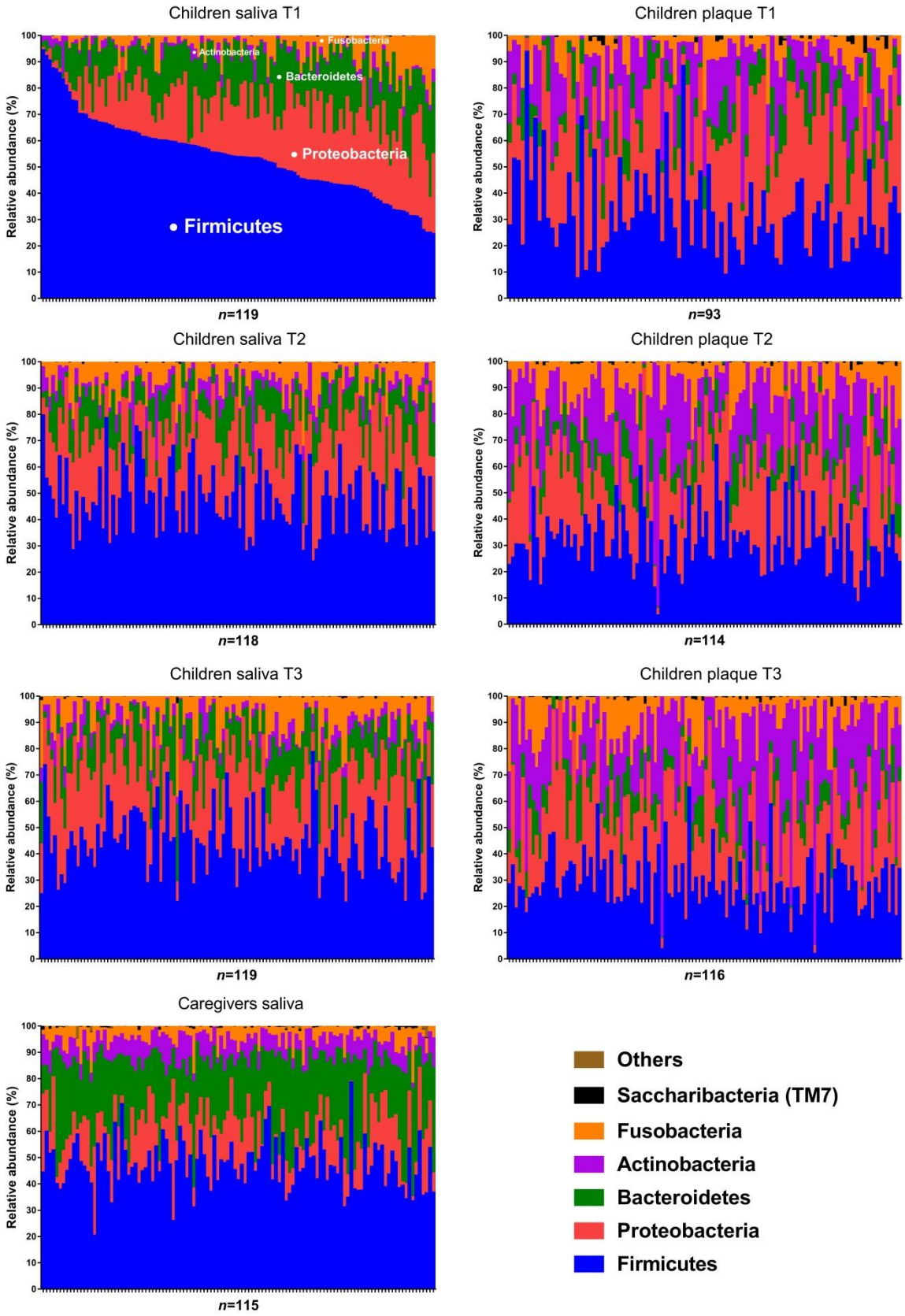
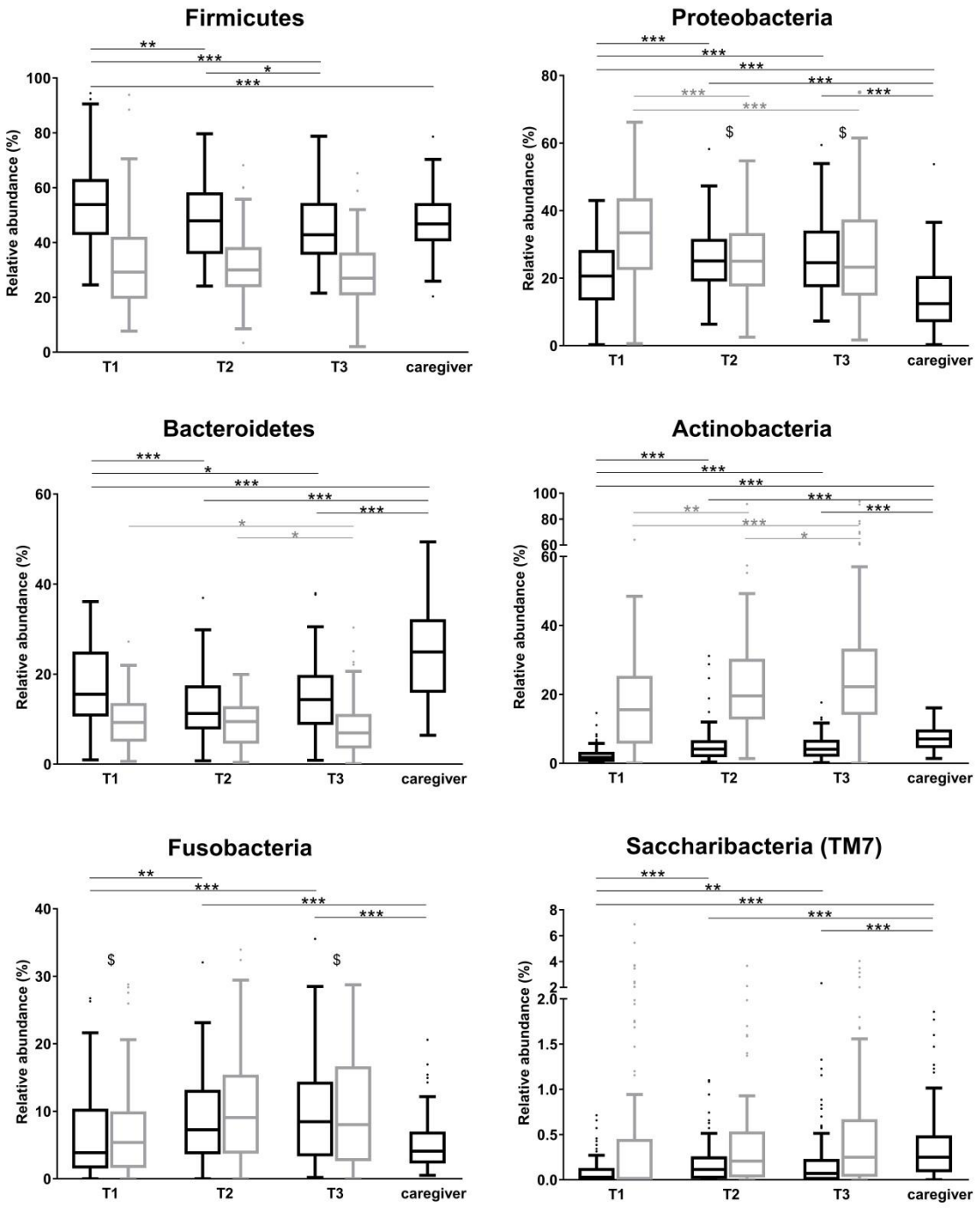
APPENDIX FIGURES:



Appendix Figure 1: Current study population.

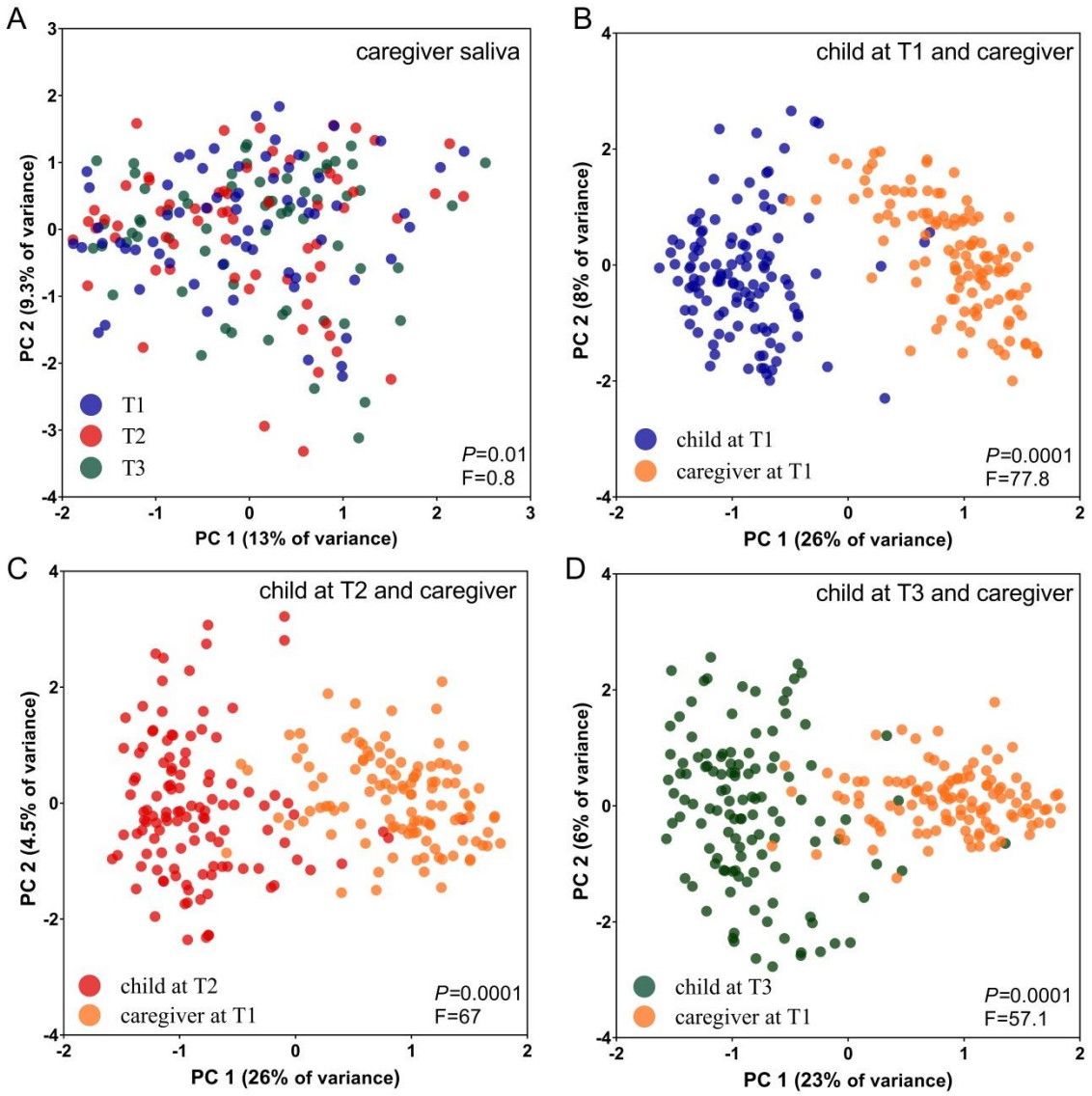

Children saliva T1
Children plaque T1
Children saliva T2
Children plaque T2
Children saliva T3
Children plaque T3
Caregivers saliva
Relative abundance (%)
Fusobacteria
Actinobacteria
Bacteroidetes
Proteobacteria
Firmicutes
n=119
n=93
n=118
n=114
n=119
n=116
n=115
Others
Saccharibacteria (TM7)
Fusobacteria
Actinobacteria
Bacteroidetes
Proteobacteria
Firmicutes

Appendix Figure 2: Taxonomic distribution of the relative abundance of reads (%) of major bacterial phyla in salivary (left panel) and plaque samples (right panel) of children per time point (T1 - 1, T2 - 2.5, T3 – 4-year-old) and in salivary samples of their caregivers at the start of the study (T1). The samples are ordered according to the relative abundance of phylum Firmicutes in salivary profiles of children at T1. All remaining plots follow the same child ID order.

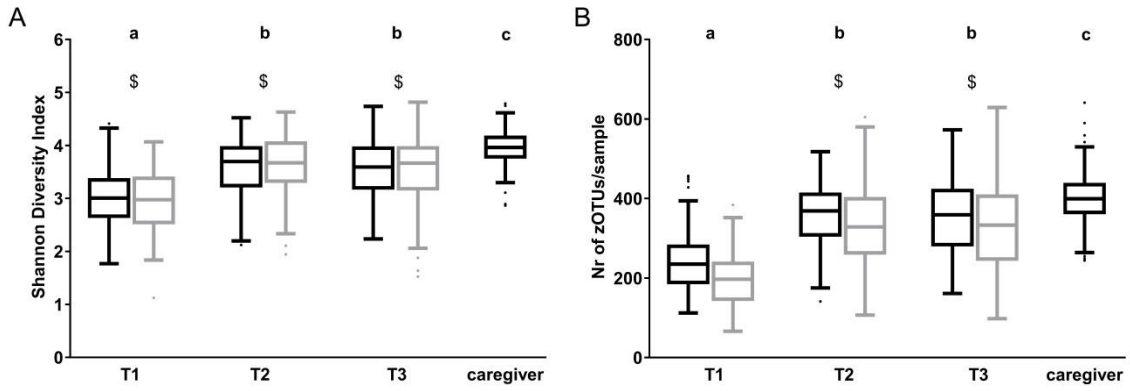


Appendix Figure 3: The relative abundance of major bacterial phyla (%) in salivary (black boxes) and plaque (grey boxes) samples over time. The boxplots are plotted using Tukey's method. Significant differences over time within the respective sample type are indicated by

asterisks: *P<0.05, **P<0.01, and ***P<0.001 (paired samples Friedman test followed by Wilcoxon rank-sum test with FDR correction). Lines connect the time points with the respective difference. The pairwise comparisons of the two types of samples (saliva vs plaque) were significant (P<0.05) in all but those time points indicated with $.

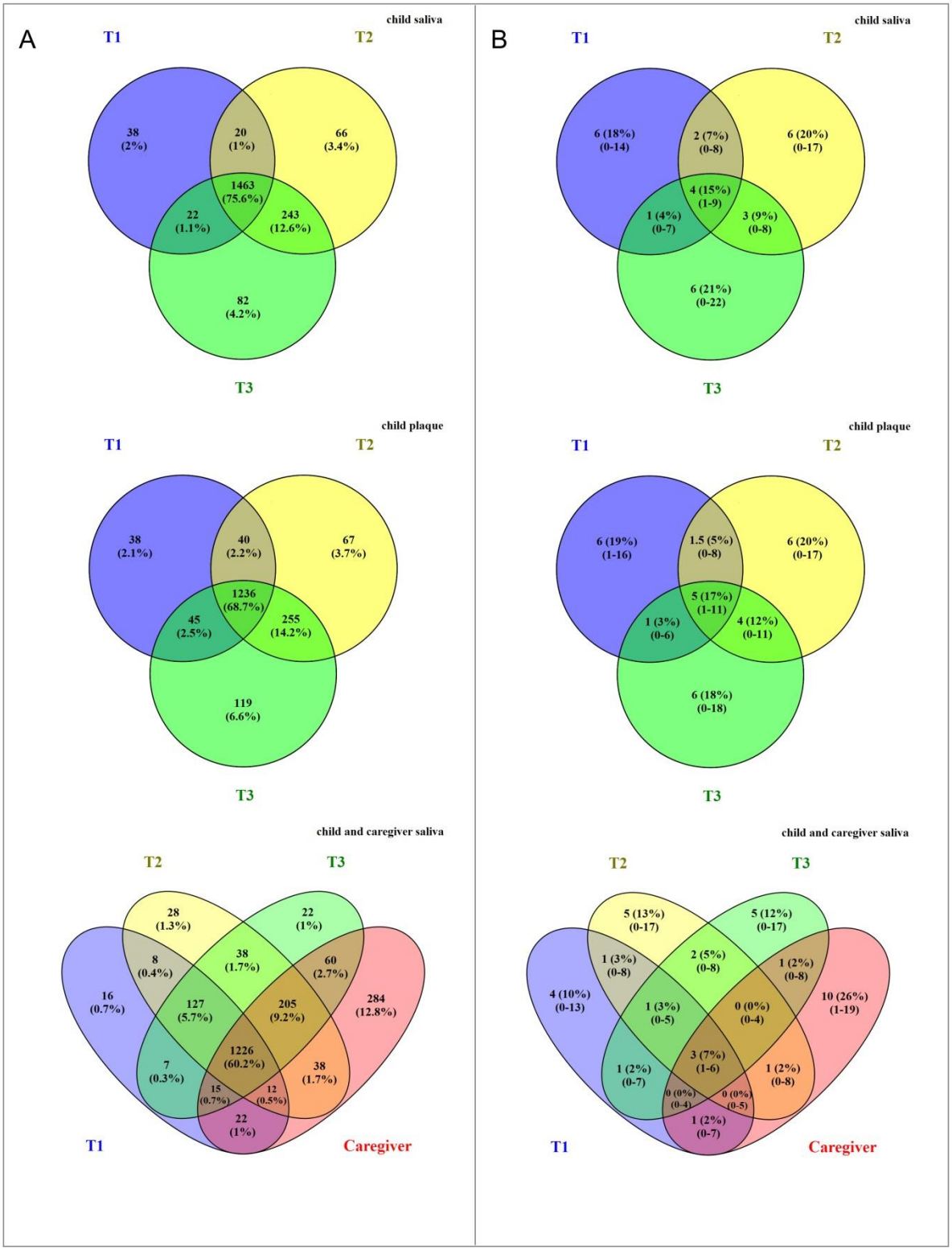


Appendix Figure 4: Principal Component Analysis (PCA) plots of salivary microbiome profiles of (A) the caregivers at three time points (T1, T2, T3) and (B-D) the children at different time points (B – child at T1; C – child at T2 and D – child at T3) in comparison with the salivary microbiome profiles of their caregivers at the start of the study (T1). Blue dots –samples collected from caregivers at T1 (A) and children at T1 (1 year of child age) (B), red dots – samples collected from caregivers at T2 (A) and children at T2 (2.5 years of age) (C), and green dots – samples collected from caregivers at T3 (A) and children at T3 (4 years of age) (D); orange dots – saliva samples of caregivers at T1 (BCD). PCA was performed on subsampled and log2 transformed zOTU data. Axis shows the first two greatest principal components (PCs) explaining the highest intersample variation (% of variance). The *P*- and F-values indicate the output of PERMANOVA analyses, using Bray-Curtis similarity.

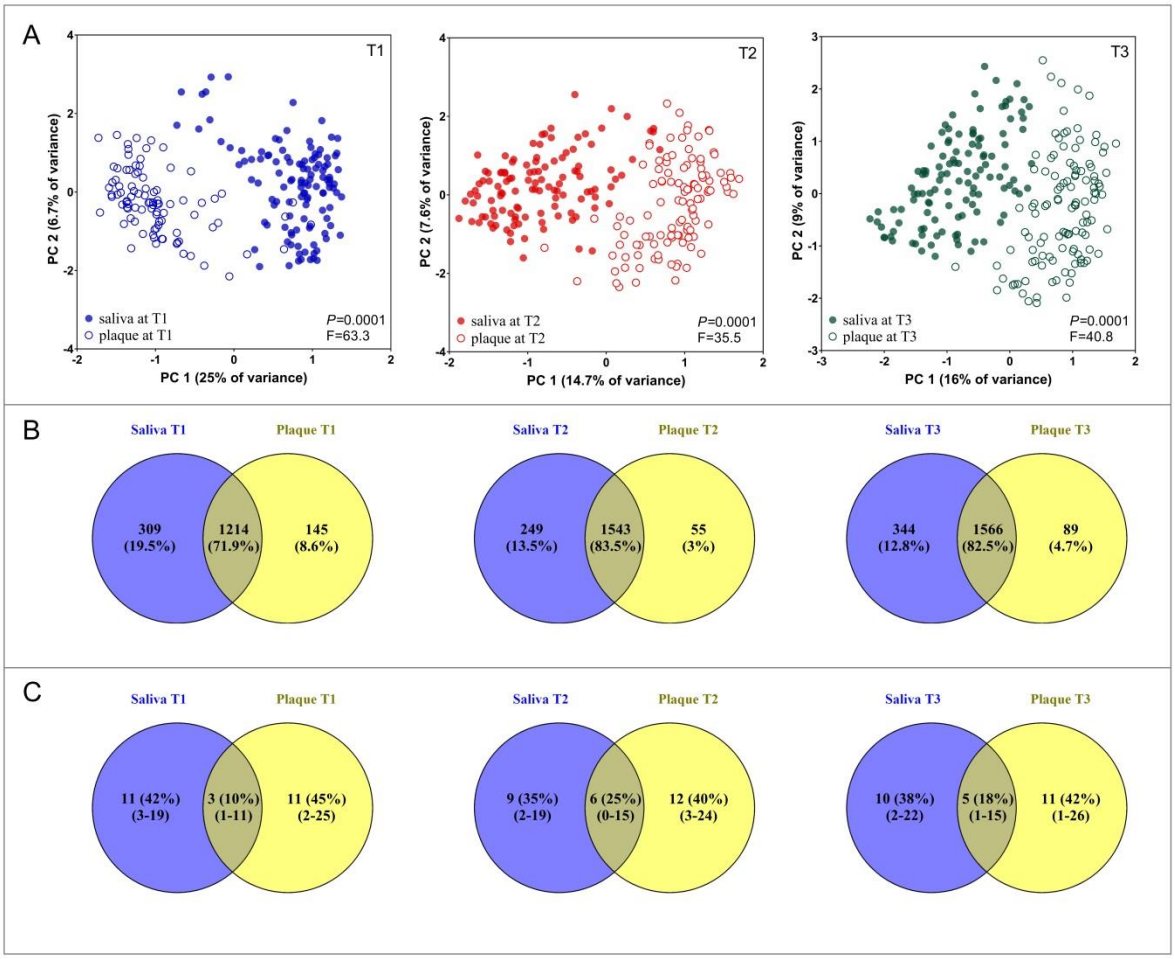


Appendix Figure 5: Alpha diversity presented as (A) Shannon Diversity Index and as (B) Species richness of the microbial composition in both salivary (black boxes) and plaque (grey boxes) samples over time. The boxplots are plotted using Tukey's method. Different letters indicate statistically significant differences ($P<0.001$, paired samples Friedman test followed by Wilcoxon rank-sum test with FDR correction) within each sample type over time. Saliva (black)

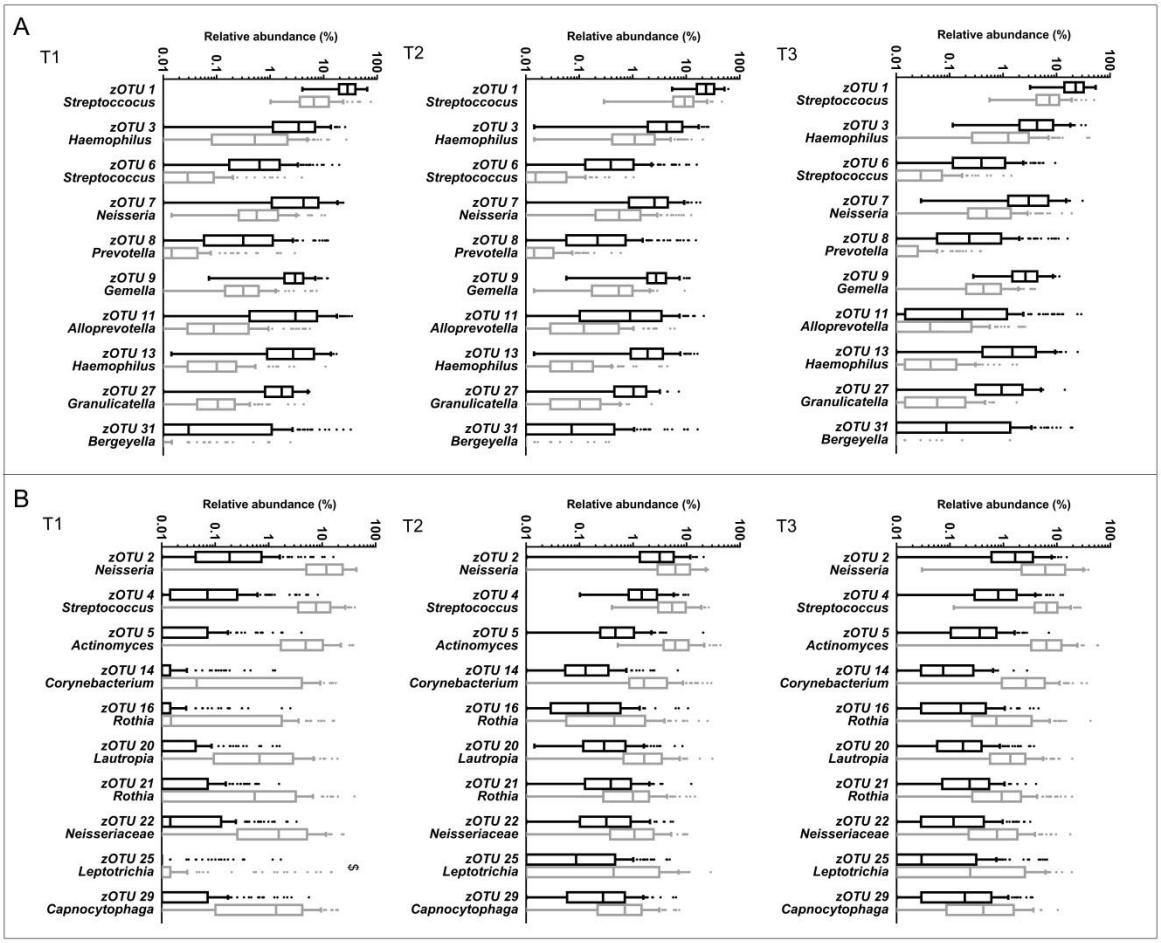
and plaque (grey) samples were tested separately and plotted together as they showed the same pattern of changes. The pairwise comparisons of two types of samples (saliva vs plaque) were significant ($P<0.05$) in all but those time points indicated with $.


A
child saliva
T1
T2
T3
38 (2%)
20 (1%)
66 (3.4%)
1463 (75.6%)
22 (1.1%)
243 (12.6%)
82 (4.2%)
child plaque
38 (2.1%)
40 (2.2%)
67 (3.7%)
1236 (68.7%)
45 (2.5%)
255 (14.2%)
119 (6.6%)
child and caregiver saliva
T2
T3
28 (1.3%)
22 (1%)
8 (0.4%)
38 (1.7%)
60 (2.7%)
16 (0.7%)
127 (5.7%)
205 (9.2%)
284 (12.8%)
1226 (60.2%)
7 (0.3%)
38 (1.7%)
15 (0.7%)
12 (0.5%)
22 (1%)
T1
Caregiver
B
child saliva
T1
T2
T3
6 (18%) (0-14)
2 (7%) (0-8)
6 (20%) (0-17)
4 (15%) (1-9)
1 (4%) (0-7)
3 (9%) (0-8)
6 (21%) (0-22)
child plaque
6 (19%) (1-16)
1.5 (5%) (0-8)
6 (20%) (0-17)
5 (17%) (1-11)
1 (3%) (0-6)
4 (12%) (0-11)
6 (18%) (0-18)
child and caregiver saliva
T2
T3
5 (13%) (0-17)
5 (12%) (0-17)
1 (3%) (0-8)
2 (5%) (0-8)
1 (2%) (0-8)
4 (10%) (0-13)
1 (3%) (0-5)
0 (0%) (0-4)
10 (26%) (1-19)
3 (7%) (1-6)
1 (2%) (0-7)
1 (2%) (0-8)
0 (0%) (0-4)
0 (0%) (0-5)
1 (2%) (0-7)
T1
Caregiver

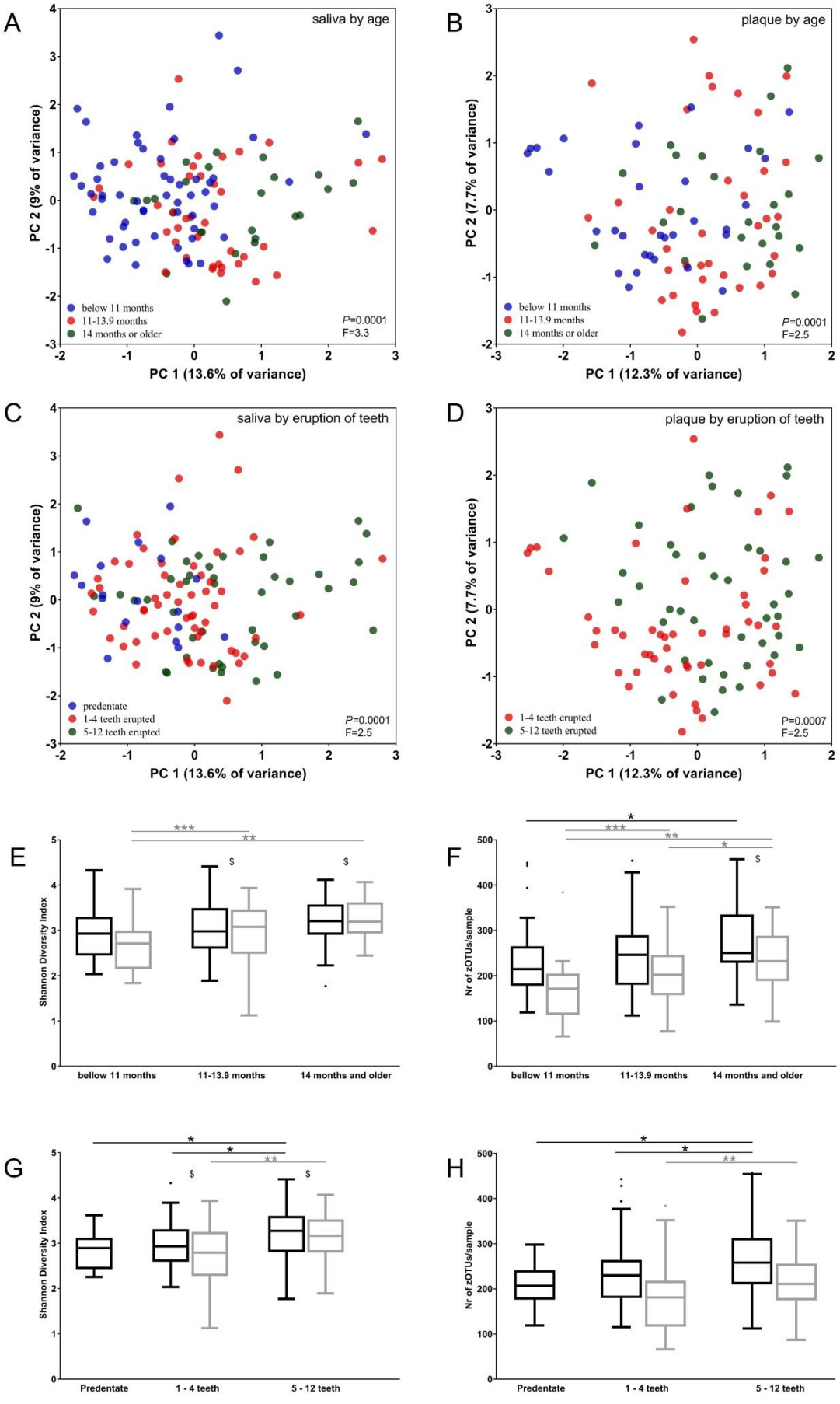
Appendix Figure 6: Venn diagrams of number and proportion (%) of the shared zOTUs (A) groupwise among saliva and plaque samples of the children over time and saliva samples of the children and the caregivers. All zOTUs were included. (B) The median of the number and of the relative abundance and the range (minimum – maximum) of the shared zOTUs within an individual child over time in saliva and in plaque samples and the shared zOTUs between the respective child at the three time points and his or her caregiver. Only zOTUs ≥ 70 reads in the subsampled dataset were included in these analyses.



Appendix Figure 7: (A) Principal Component Analysis (PCA) plots of microbiome profiles of children by sample type (saliva – dot, plaque – circle) over time (blue - T1, red - T2, green - T3). (B) Number and % of the shared zOTUs in saliva and plaque samples, groupwise. (C) The median of the number and of the relative abundance and the range (minimum – maximum) of the shared zOTUs in two types of sample (saliva and plaque) within an individual child over time. Top 50 list of significantly different zOTUs is shown in Appendix Table 2B.

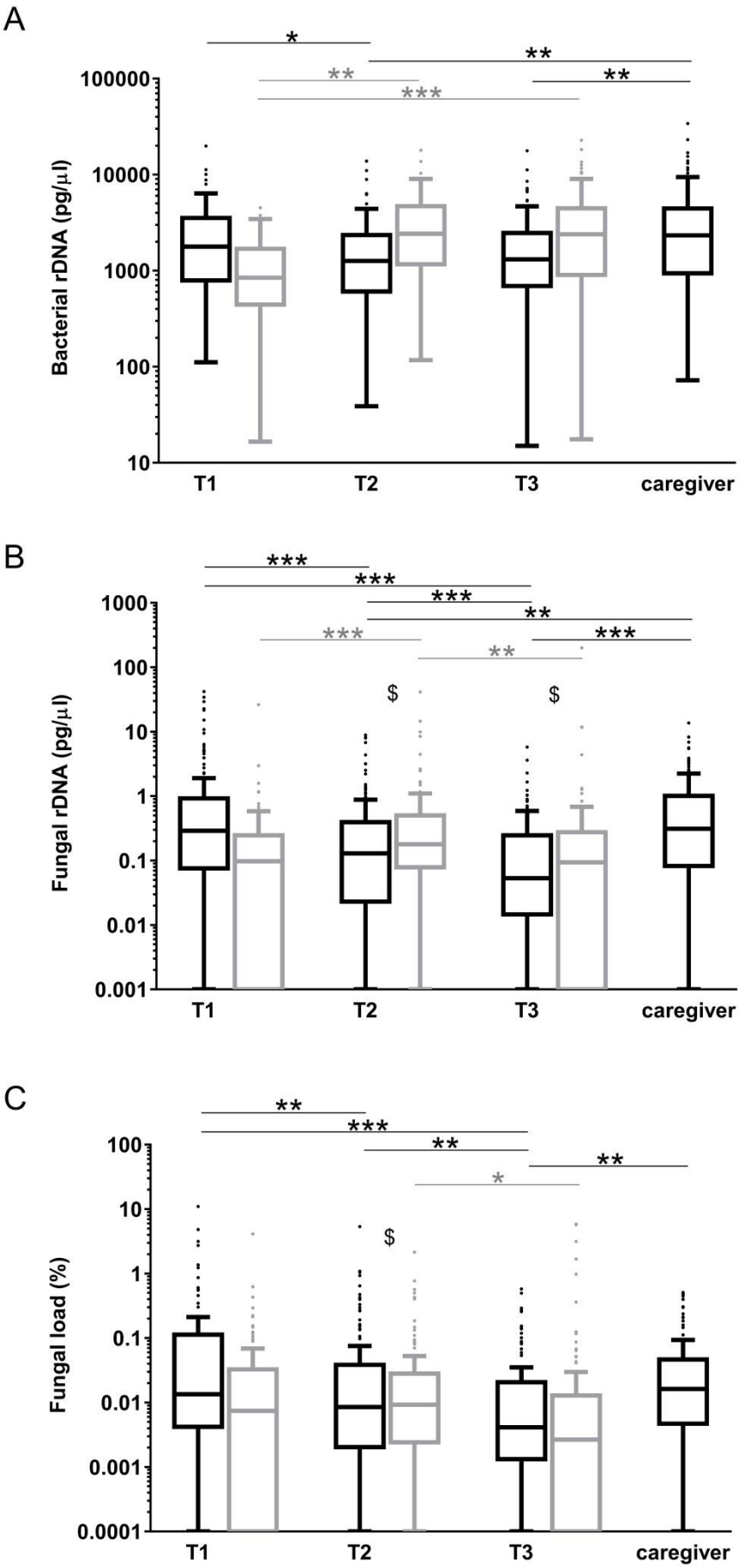


Appendix Figure 8: The top ten most discriminatory zOTUs between saliva (black boxes) and plaque (grey boxes) samples that were higher in (A) saliva or in (B) plaque samples over time. T1 – samples collected at 1 year, T2 – at 2.5 years, T3 – at 4 years of child age. The boxplots are plotted using Tukey's method. All zOTUs except those indicated with $ differed significantly between saliva and plaque samples at all time points (P<0.001, paired samples Friedman test).


A
saliva by age
PC 2 (9% of variance)
PC 1 (13.6% of variance)
below 11 months
11-13.9 months
14 months or older
P=0.0001
F=3.3
B
plaque by age
PC 2 (7.7% of variance)
PC 1 (12.3% of variance)
below 11 months
11-13.9 months
14 months or older
P=0.0001
F=2.5
C
saliva by eruption of teeth
PC 2 (9% of variance)
PC 1 (13.6% of variance)
predentate
1-4 teeth erupted
5-12 teeth erupted
P=0.0001
F=2.5
D
plaque by eruption of teeth
PC 2 (7.7% of variance)
PC 1 (12.3% of variance)
1-4 teeth erupted
5-12 teeth erupted
P=0.0007
F=2.5
E
Shannon Diversity Index
bellow 11 months
11-13.9 months
14 months and older
F
Nr of zOTUs/sample
bellow 11 months
11-13.9 months
14 months and older
G
Shannon Diversity Index
Predentate
1 - 4 teeth
5 - 12 teeth
H
Nr of zOTUs/sample
Predentate
1 - 4 teeth
5 - 12 teeth

Appendix Figure 9: Principal Component Analysis (PCA) plots of microbiome profiles of samples collected at T1 (AB) by age (blue dots – below 11 months; red – 11–13.9 months, green – 14 months or older) of the children and (CD) the eruption of teeth (blue – predentate; red – 1–4 teeth erupted; green – 5–12 teeth erupted). Both saliva (A) and plaque (B) of children younger than 11 months (blue dots) differed significantly from the older groups. Salivary (C) and plaque (D) microbiome profiles of children, who were predentate or had 1–4 teeth erupted differed from those with 5 or more teeth present.

Alpha diversity of microbial profiles of saliva (black boxes) and plaque (grey boxes) of children at T1 as Shannon Diversity Index (EG) and Species richness (FH) by the child age (EF) and number of the teeth erupted (GH). The boxplots are plotted using Tukey's method. Significant differences within the respective sample type are indicated by asterisks: *P<0.05, **P<0.01, and ***P<0.001 (Kruskal Wallis test and Mann-Whitney test). Lines connect the subgroups with the respective difference (black – for saliva, grey – for plaque).


A
Bacterial rDNA (pg/µl)
100000
10000
1000
100
10
T1
T2
T3
caregiver
B
Fungal rDNA (pg/µl)
1000
100
10
1
0.1
0.01
0.001
$
C
Fungal load (%)
100
10
1
0.1
0.01
0.001
0.0001

Appendix Figure 10: Concentration of bacterial (A) and fungal DNA (B) and fungal load (C) in salivary (black boxes) and plaque (grey boxes) samples. The boxplots are plotted using Tukey's method. Significant differences over time within the respective sample type are indicated by asterisks: *P<0.05, **P<0.01, and ***P<0.001 (paired samples Friedman test followed by Wilcoxon rank-sum test with FDR correction). Lines connect the time points with the respective difference. The pairwise comparisons of the two types of samples (saliva vs plaque) were significant (P<0.05) in all but those time points indicated with $.

Appendix Table 1A: Relative abundance (%) of the reads at the phylum level per sample type and timepoint.

Phyla ordered from the most to the least abundant in child saliva at T1.

Phyla	Child saliva T1; N=119	Child saliva T2; N=118	Child saliva T3; N=119	Child plaque T1; N=93	Child plaque T2; N=114	Child plaque T3; N=116	Caregiver saliva T1; N=115	Caregiver saliva T2; N=66	Caregiver saliva T3; N=65
Firmicutes	53.9	47.7	44.7	32.6	31.6	29	47.5	47.9	49
Proteobacteria	20.2	25.3	25.7	33.0	25.7	25.6	14.3	15.3	14.6
Bacteroidetes	17.2	13.1	14.7	9.9	9.1	8	25.3	23.2	22.8
Actinobacteria	2.3	5.2	4.7	16.6	22.6	26.7	7.3	7.3	7.1
Fusobacteria	6.3	8.4	9.9	7.2	10.5	10.1	5	5.8	5.9
Saccharibacteria (TM7)	0.1	0.2	0.2	0.6	0.4	0.5	0.4	0.3	0.4
SR1	0.04	0.1	0.05	0.1	0.1	0.04	0.1	0.1	0.1
Spirochaetes	0.001	0.01	0.03	0.0002	0.01	0.02	0.2	0.1	0.1
Gracilibacteria	0.003	0.02	0.02	0.1	0.1	0.1	0.01	0.01	0.01
Unclassified Bacteria	0.02	0.03	0.02	0.005	0.008	0.01	0.02	0.03	0.03
Synergistetes	0	0	0	0.0002	0	0.0001	0.02	0.01	0.01
Chloroflexi	0	0	0	0	0	0	0	0	0.0002

Appendix Table 1B: Relative abundance of the reads at the genus level per sample type and timepoint.
From 163 genera or higher taxa top 30 are shown.

Genera	Child saliva T1; N=119	Child saliva T2; N=118	Child saliva T3; N=119	Child plaque T1; N=93	Child plaque T2; N=114	Child plaque T3; N=116	Caregiver saliva T1; N=115	Caregiver saliva T2; N=66	Caregiver saliva T3; N=65
Streptococcus	39.86	35.48	32.73	25.15	22.31	21.07	32.60	32.94	33.81
Haemophilus	10.75	11.43	12.46	2.53	2.84	3.70	6.98	6.10	5.99
Neisseria	8.08	9.27	8.96	17.88	12.23	12.93	5.46	7.42	6.66
Alloprevotella	6.36	2.99	2.42	0.69	0.61	0.38	2.02	1.91	1.69
Veillonella	6.07	4.38	3.76	3.38	4.84	3.47	8.27	7.93	8.61
Porphyromonas	5.21	3.63	4.45	0.91	2.11	1.90	1.62	1.64	1.31
Leptotrichia	4.04	5.45	6.08	6.00	8.13	7.70	2.61	2.95	3.33
Gemella	3.97	4.05	3.79	0.76	0.88	0.80	2.63	2.39	2.29
Granulicatella	2.96	2.23	2.67	0.75	0.82	0.76	0.95	0.84	0.83
Prevotella	2.93	3.35	3.82	0.54	1.97	1.68	21.04	19.07	19.15
Bergeyella	2.06	1.30	2.02	0.87	0.42	0.43	0.21	0.15	0.17
Fusobacterium	1.73	2.63	3.61	1.08	2.34	2.35	2.40	2.78	2.48
Rothia	1.69	2.62	2.46	4.73	3.58	5.11	4.58	4.89	4.45
Capnocytophaga	0.58	1.69	1.78	6.88	3.81	3.43	0.31	0.36	0.37
Sneathia	0.46	0.27	0.15	0.07	0.04	0.02	0.03	0.02	0.07
Actinomyces	0.43	1.43	1.10	8.00	10.77	11.59	2.18	1.87	1.96
uncl. Neisseriaceae	0.42	1.28	0.81	5.08	2.91	2.38	0.27	0.001	0.001
Aggregatibacter	0.30	1.04	1.51	1.06	1.50	1.33	0.48	0.37	0.45
uncl. Bacilli	0.29	0.29	0.27	0.03	0.05	0.04	0.16	0.001	0.01
uncl. Lactobacillales	0.22	0.18	0.21	0.08	0.11	0.11	0.19	0.03	0.03
Kingella	0.17	0.47	0.43	1.72	1.33	1.28	0.07	0.08	0.07
Corynebacterium	0.15	1.01	0.95	3.79	7.30	9.33	0.17	0.15	0.18
uncl. Pasteurellaceae	0.12	0.27	0.41	0.02	0.08	0.08	0.30	0	0
Lachnoanaerobaculum	0.11	0.16	0.21	0.13	0.30	0.41	0.25	0.28	0.31
Abiotrophia	0.10	0.46	0.38	2.01	1.25	1.30	0.11	0.14	0.10
Lautropia	0.10	0.80	0.50	2.31	3.02	2.44	0.18	0.28	0.36
uncl. Leptotrichiaceae	0.09	0.05	0.03	0.003	0.004	0.003	0.002	0.006	0.004
Cardiobacterium	0.09	0.29	0.30	1.19	1.07	0.87	0.04	0.04	0.06
Campylobacter	0.08	0.19	0.24	0.18	0.32	0.25	0.39	0.46	0.48
Ruminococcaceae[G-1]	0.06	0.08	0.06	0.03	0.04	0.04	0.07	0.04	0.06

Appendix Table 1C: Prevalence (%) of the microbial taxa (zOTUs) in the respective samples type and timepoints.

From 2320 top 50 zOTUs are shown. zOTUs are ordered from to the most to the least prevelant.
The data on saliva of caregivers at T2 and T3 is not shown.

zOTUs		Child saliva T1; N=119	Child saliva T2; N=118	Child saliva T3; N=119	Child plaque T1; N=93	Child plaque T2; N=114	Child plaque T3; N=116	Caregiver saliva T1; N=115
zOTU 1	Streptococcus; (s dentisani / s infantis/s mitis/s oralis/s HOT 058/ 061/ 064/ 070/ 423/ 431/s tigurinus)	100	100	100	100	100	100	100
zOTU 9	Gemella; (s haemolysans/s morbillorum/s sanguinis)	100	100	100	96.77	100	99.14	100
zOTU 2336	unclassified Streptococcus	100	99.15	100	78.49	78.07	81.90	100
zOTU 32	unclassified Streptococcus	100	100	100	100	100	99.14	100
zOTU 309	Streptococcus; (several species)	100	98.31	94.96	62.37	83.33	76.72	99.13
zOTU 75	unclassified Streptococcus	100	100	99.16	78.49	94.74	87.07	99.13
zOTU 13	unclassified Haemophilus	100	100	97.48	84.95	91.23	86.21	93.04
zOTU 2100	Streptococcus; (several species)	100	98.31	94.12	30.11	48.25	39.66	87.83
zOTU 7	Neisseria; (s flavescens/s subflava)	99.16	97.46	100	100	98.25	97.41	97.39
zOTU 2396	Streptococcus; (several species)	99.16	95.76	95.80	20.43	48.25	40.52	91.30
zOTU 1469	unclassified Streptococcus	99.16	100	99.16	87.10	98.25	94.83	88.70
zOTU 33	Granulicatella; s adiacens	98.32	100	99.16	94.62	99.12	98.28	100
zOTU 2323	Gemella; (s haemolysans/s morbillorum/s sanguinis)	98.32	96.61	100	47.31	59.65	57.76	96.52
zOTU 27	Granulicatella; s elegans	98.32	98.31	96.64	94.62	90.35	86.21	59.13
zOTU 47	unclassified Streptococcus	97.48	98.31	99.16	82.80	95.61	89.66	100
zOTU 52	unclassified Streptococcus	97.48	99.15	100	97.85	100	97.41	99.13
zOTU 2276	Gemella; (s haemolysans/s morbillorum/s sanguinis)	97.48	94.92	93.28	34.41	52.63	56.90	90.43
zOTU 200	unclassified Streptococcus	97.48	96.61	96.64	70.97	84.21	70.69	80.00
zOTU 28	Veillonella; HOT 780	97.48	91.53	85.71	87.10	85.96	65.52	58.26
zOTU 122	Granulicatella; s elegans	97.48	92.37	90.76	58.06	57.02	40.52	36.52
zOTU 1942	Gemella; (s haemolysans/s morbillorum/s sanguinis)	96.64	96.61	95.80	56.99	64.04	68.10	86.09

zOTU 1725	Streptococcus; (several species)	96.64	98.31	96.64	24.73	41.23	31.03	79.13
zOTU 870	unclassified Streptococcus	96.64	92.37	92.44	81.72	81.58	75.00	72.17
zOTU 12	Streptococcus; (several species)	95.80	98.31	98.32	87.10	77.19	88.79	100
zOTU 6	Streptococcus; (s salivarius/s vestibularis)	95.80	96.61	93.28	68.82	68.42	72.41	99.13
zOTU 1944	Streptococcus; (several species)	95.80	98.31	99.16	43.01	78.07	66.38	92.17
zOTU 1746	unclassified Bacilli	95.80	96.61	93.28	33.33	56.14	49.14	87.83
zOTU 1826	unclassified Bacilli	95.80	94.92	99.16	32.26	48.25	40.52	80.87
zOTU 38	Porphyromonas; HOT930	95.80	90.68	89.08	59.14	57.02	47.41	34.78
zOTU 26	unclassified Streptococcus	94.96	99.15	99.16	97.85	100	98.28	100
zOTU 140	Granulicatella; s elegans	94.96	87.29	85.71	51.61	45.61	31.03	26.96
zOTU 11	Alloprevotella; s HOT473	94.12	90.68	84.03	83.87	85.09	68.97	76.52
zOTU 3	Haemophilus; s parainfluenzae	93.28	100	100	93.55	100	99.14	100
zOTU 1458	Streptococcus; (several species)	93.28	83.90	80.67	27.96	56.14	47.41	88.70
zOTU 10	unclassified Veillonella	91.60	94.07	89.92	63.44	70.18	72.41	99.13
zOTU 566	unclassified Granulicatella	91.60	92.37	93.28	41.94	53.51	54.31	76.52
zOTU 1490	Streptococcus; (several species)	90.76	87.29	77.31	45.16	64.91	56.03	93.91
zOTU 663	Haemophilus; s parainfluenzae	90.76	90.68	96.64	43.01	65.79	58.62	88.70
zOTU 1665	unclassified Bacilli	90.76	94.07	94.12	23.66	44.74	40.52	80.00
zOTU 60	Streptococcus; (s salivarius/s vestibularis)	89.92	88.98	86.55	26.88	23.68	18.10	98.26
zOTU 1958	unclassified Streptococcus	89.92	98.31	97.48	86.02	92.11	88.79	81.74
zOTU 1809	Gemella; (s haemolysans/s morbillorum/s sanguinis)	89.92	88.98	84.87	18.28	28.95	27.59	73.04
zOTU 1962	Gemella; (s haemolysans/s morbillorum/s sanguinis)	89.92	86.44	84.03	15.05	29.82	17.24	55.65
zOTU 80	unclassified Streptococcus	89.08	94.07	93.28	38.71	40.35	42.24	100
zOTU 8	unclassified Prevotella	89.08	91.53	91.60	54.84	50.88	44.83	98.26
zOTU 1231	Neisseria; (s flavescens/s subflava)	89.08	81.36	84.87	41.94	48.25	43.10	79.13
zOTU 411	unclassified Veillonella	88.24	77.97	80.67	46.24	73.68	64.66	99.13
zOTU 106	unclassified Haemophilus	87.39	96.61	97.48	25.81	47.37	36.21	79.13
zOTU 62	unclassified Porphyromonas	87.39	83.05	76.47	54.84	65.79	43.97	32.17
zOTU 46	Porphyromonas; HOT 930	87.39	94.07	81.51	60.22	53.51	45.69	26.96

Appendix Table 2A: zOTUs by their relative abundances in timepoints and in relation to the caregiver: Output from LEfSe.

The top 10 zOTUs by their LDA score are shown.

Saliva samples													
Child T1 and T2 (total 288 significantly discriminative zOTUs)					**Child T1 and T3 (N=total 297 significantly discriminative zOTUs)**				**Child T2 and T3 (total 95 significantly discriminative zOTUs)**				
zOTUs		Time points	LDA score	*p* value	zOTUs		Time points	LDA score	*p* value	zOTUs		Time points	LDA score	*p* value
zOTU 1	Streptococcus several species	T1	4.3	1.25E-02	zOTU 1	Streptococcus several species	T1	4.4	8.28E-04	zOTU 2	Neisseria	T2	3.9	4.29E-04
zOTU 11	Alloprevotella HOT 473	T1	4.2	2.87E-04	zOTU 11	Alloprevotella HOT 473	T1	4.3	1.26E-09	zOTU 11	Alloprevotella HOT 473	T2	3.5	1.19E-03
zOTU 7	Neisseria flavescens/subflava	T1	4.0	6.51E-03	zOTU 28	Veillonella HOT 780	T1	4.0	1.53E-11	zOTU 4	Streptococcus sanguinis	T2	3.4	1.03E-04
zOTU 28	Veillonella HOT 780	T1	4.0	1.93E-07	zOTU 2336	Streptococcus	T1	4.0	5.19E-09	zOTU 5	Actinomyces	T2	3.3	2.30E-02
zOTU 2336	Streptococcus	T1	4.0	6.88E-11	zOTU 13	Haemophilus	T1	3.7	1.39E-02	zOTU 21	Rothia aeria	T2	3.2	3.66E-03
zOTU 46	Porphyromonas HOT 930	T1	3.7	1.68E-03	zOTU 10	Veillonella	T1	3.6	2.99E-05	zOTU 22	Neisseriaceae	T2	3.2	3.54E-05
zOTU 38	Porphyromonas HOT 930	T1	3.7	2.16E-02	zOTU 46	Porphyromonas HOT 930	T1	3.6	1.08E-04	zOTU 20	Lautropia mirabilis	T2	3.2	6.10E-04
zOTU 10	Veillonella	T1	3.5	3.75E-05	zOTU 38	Porphyromonas HOT 930	T1	3.5	1.58E-02	zOTU 28	Veillonella HOT 780	T2	3.0	2.68E-02
zOTU 27	Granulicatella elegans	T1	3.4	1.15E-04	zOTU 6	Streptococcus salivarius/vestibularis	T1	3.5	4.96E-02	zOTU 34	Streptococcus	T2	2.9	3.91E-02
zOTU 101	Neisseria s flavescens/s subflava	T1	3.3	1.20E-03	zOTU 27	Granulicatella elegans	T1	3.2	1.45E-03	zOTU 14	Corynebacterium durum	T2	2.9	1.33E-02
zOTU 2	Neisseria	T2	4.2	1.28E-21	zOTU 3	Haemophilus parainfluenzae	T3	4.0	5.21E-03	zOTU 7	Neisseria flavescens/subflava	T3	3.8	4.31E-02
zOTU 4	Streptococcus sanguinis	T2	3.9	3.60E-26	zOTU 2	Neisseria	T3	3.9	4.99E-14	zOTU 30	Fusobacterium periodonticum	T3	3.1	4.01E-02
zOTU 3	Haemophilus parainfluenzae	T2	3.8	2.74E-02	zOTU 4	Streptococcus sanguinis	T3	3.8	8.21E-16	zOTU 42	Rothia mucilaginosa	T3	3.1	1.28E-02
zOTU 15	Veillonella dispar	T2	3.7	1.74E-20	zOTU 37	Fusobacterium	T3	3.6	2.47E-20	zOTU 77	Prevotella nigrescens	T3	2.9	3.92E-03
zOTU 5	Actinomyces	T2	3.6	1.60E-26	zOTU 49	Aggregatibacter segnis/HOT 458, 512	T3	3.6	3.12E-20	zOTU 33	Granulicatella adiacens	T3	2.8	2.87E-02
zOTU 21	Rothia aeria	T2	3.5	5.76E-25	zOTU 18	Porphyromonas pasteri/HOT 278)	T3	3.5	8.74E-08	zOTU 62	Porphyromonas	T3	2.8	4.23E-02
zOTU 37	Fusobacterium	T2	3.5	2.53E-24	zOTU 15	Veillonella dispar	T3	3.5	2.60E-14	zOTU 103	Leptotrichia	T3	2.7	4.96E-02
zOTU 20	Lautropia mirabilis	T2	3.5	2.41E-29	zOTU 59	Haemophilus	T3	3.4	1.22E-05	zOTU 54	Rothia mucilaginosa	T3	2.7	1.35E-02
zOTU 24	Leptotrichia hongongensis	T2	3.5	1.23E-14	zOTU 17	Corynebacterium matruchotii	T3	3.4	1.28E-20	zOTU 181	Fusobacterium	T3	2.7	3.23E-02
zOTU 22	Neisseriaceae	T2	3.4	3.16E-16	zOTU 5	Actinomyces	T3	3.4	4.93E-20	zOTU 178	Leptotrichia	T3	2.6	4.99E-02

Saliva samples														
Child T1 and caregiver (total 407 significantly discriminative zOTUs)					**Child T2 and caregiver (total 488 significantly discriminative zOTUs)**				**Child T3 and caregiver (total 498 significantly discriminative zOTUs)**					
zOTUs		Groups	LDA score	*p* value	zOTUs		Groups	LDA score	*p* value	zOTUs		Groups	LDA score	*p* value
zOTU 6	Streptococcus salivarius/vestibularis	care-giver	4.4	2.43E-21	zOTU 6	Streptococcus salivarius/vestibularis	care-giver	4.5	1.75E-23	zOTU 6	Streptococcus salivarius/vestibularis	care-giver	4.5	1.96E-26
zOTU 8	Prevotella	care-giver	4.4	1.73E-24	zOTU 8	Prevotella	care-giver	4.4	2.13E-26	zOTU 8	Prevotella	care-giver	4.4	3.94E-26
zOTU 12	Streptococcus several species	care-giver	4.2	2.15E-32	zOTU 10	Veillonella	care-giver	4.2	6.27E-19	zOTU 10	Veillonella	care-giver	4.3	1.23E-20
zOTU 10	Veillonella	care-giver	4.1	3.54E-12	zOTU 12	Streptococcus several species	care-giver	4.2	3.95E-31	zOTU 12	Streptococcus several species	care-giver	4.2	5.65E-34
zOTU 23	Prevotella	care-giver	4.0	1.17E-24	zOTU 23	Prevotella	care-giver	4.0	2.07E-20	zOTU 23	Prevotella	care-giver	4.0	8.48E-21
zOTU 39	Prevotella veroralis	care-giver	3.8	3.35E-23	zOTU 2336	Streptococcus	care-giver	3.9	2.34E-21	zOTU 2336	Streptococcus	care-giver	3.8	2.17E-19
zOTU 110	Prevotella	care-giver	3.8	2.03E-30	zOTU 39	Prevotella veroralis	care-giver	3.8	2.87E-23	zOTU 39	Prevotella veroralis	care-giver	3.8	1.26E-23
zOTU 40	Leptotrichia	care-giver	3.7	5.40E-21	zOTU 42	Rothia mucilaginosa	care-giver	3.7	6.83E-17	zOTU 40	Leptotrichia	care-giver	3.7	5.73E-16
zOTU 19	Veillonella parvula	care-giver	3.7	1.39E-21	zOTU 40	Leptotrichia	care-giver	3.7	8.50E-15	zOTU 110	Prevotella	care-giver	3.7	3.79E-21
zOTU 3	Haemophilus parainfluenzae	care-giver	3.7	6.59E-03	zOTU 110	Prevotella	care-giver	3.7	5.25E-22	zOTU 51	Prevotella veroralis	care-giver	3.6	1.71E-18
zOTU 1	Streptococcus several species	child at T1	5.0	7.99E-26	zOTU 1	Streptococcus several species	child at T2	4.9	1.16E-19	zOTU 1	Streptococcus several species	child at T3	4.8	1.29E-16
zOTU 11	Alloprevotella HOT 473	child at T1	4.4	4.82E-18	zOTU 2	Neisseria	child at T2	4.2	2.31E-23	zOTU 13	Haemophilus	child at T3	4.1	4.24E-19
zOTU 13	Haemophilus	child at T1	4.3	5.21E-24	zOTU 13	Haemophilus	child at T2	4.2	1.16E-24	zOTU 2	Neisseria	child at T3	4.0	1.06E-14
zOTU 28	Veillonella HOT 780	child at T1	4.2	2.62E-31	zOTU 11	Alloprevotella HOT 473	child at T2	4.0	2.43E-09	zOTU 11	Alloprevotella HOT 473	child at T3	3.9	1.22E-02
zOTU 38	Porphyromonas HOT 930	child at T1	4.0	6.12E-33	zOTU 4	Streptococcus sanguinis	child at T2	3.8	2.01E-13	zOTU 31	Bergeyella	child at T3	3.9	7.72E-11
zOTU 27	Granulicatella elegans	child at T1	3.9	6.57E-36	zOTU 9	Gemella haemolysans/ morbillorum/ sanguinis	child at T2	3.8	2.16E-06	zOTU 27	Granulicatella elegans	child at T3	3.9	1.43E-27
zOTU 46	Porphyromonas HOT 930	child at T1	3.9	1.15E-27	zOTU 27	Granulicatella elegans	child at T2	3.8	1.03E-30	zOTU 38	Porphyromonas HOT 930	child at T3	3.8	4.79E-27
zOTU 31	Bergeyella	child at T1	3.9	7.73E-08	zOTU 38	Porphyromonas HOT 930	child at T2	3.7	1.57E-28	zOTU 9	Gemella haemolysans/ morbillorum/ sanguinis	child at T3	3.7	1.16E-04
zOTU 7	Neisseria flavescens/subflava	child at T1	3.9	1.34E-02	zOTU 28	Veillonella HOT 780	child at T2	3.7	1.24E-20	zOTU 4	Streptococcus sanguinis	child at T3	3.7	4.68E-03
zOTU 43	Leptotrichia	child at T1	3.9	6.85E-16	zOTU 31	Bergeyella	child at T2	3.6	1.51E-08	zOTU 37	Fusobacterium	child at T3	3.6	2.96E-11

Plaque samples														
Child T1 and T2 (total 283 significantly discriminative zOTUs)					**Child T1 and T3 (total 302 significantly discriminative zOTUs)**					**Child T2 and T3 (total 55 significantly discriminative zOTUs)**				
zOTUs		Time points	LDA score	*p* value	zOTUs		Time points	LDA score	*p* value	zOTUs		Time points	LDA score	*p* value
zOTU 2	Neisseria	T1	4.5	4.67E-04	zOTU 2	Neisseria	T1	4.4	3.20E-03	zOTU 1	Streptococcus several species	T2	3.9	1.24E-02
zOTU 4	Streptococcus sanguinis	T1	4.3	5.43E-03	zOTU 22	Neisseriaceae	T1	4.0	6.82E-03	zOTU 11	Alloprevotella HOT 473	T2	3.1	2.40E-03
zOTU 29	Capnocytophaga sputigena	T1	4.0	2.54E-02	zOTU 29	Capnocytophaga sputigena	T1	4.0	5.80E-03	zOTU 45	Capnocytophaga leadbetteri	T2	3.0	4.00E-02
zOTU 57	Capnocytophaga	T1	3.4	1.47E-03	zOTU 21	Rothia aeria	T1	3.5	2.31E-02	zOTU 126	Neisseriaceae	T2	2.9	1.63E-02
zOTU 65	Bergeyella HOT 322	T1	3.2	3.48E-03	zOTU 58	Neisseria oralis	T1	3.4	1.54E-06	zOTU 2248	Actinomyces	T2	2.9	4.81E-02
zOTU 2336	Streptococcus	T1	3.1	6.06E-03	zOTU 57	Capnocytophaga	T1	3.3	1.33E-02	zOTU 780	Veillonella dispar	T2	2.9	2.71E-03
zOTU 34	Streptococcus	T1	3.1	3.02E-03	zOTU 28	Veillonella HOT 780	T1	3.3	1.54E-05	zOTU 2377	Cardiobacterium hominis	T2	2.9	3.87E-02
zOTU 48	Cardiobacterium hominis	T1	3.1	1.57E-02	zOTU 11	Alloprevotella HOT 473	T1	3.2	3.94E-02	zOTU 13	Haemophilus	T2	2.8	2.05E-02
zOTU 45	Capnocytophaga leadbetteri	T1	3.0	1.20E-02	zOTU 65	Bergeyella HOT 322	T1	3.2	1.53E-02	zOTU 28	Veillonella HOT 780	T2	2.8	3.31E-04
zOTU 170	Neisseria oralis	T1	2.9	9.38E-03	zOTU 34	Streptococcus	T1	3.1	1.34E-02	zOTU 166	Leptotrichia HOT 225	T2	2.7	2.63E-02
zOTU 17	Corynebacterium matruchotii	T2	4.0	1.85E-15	zOTU 17	Corynebacterium matruchotii	T3	4.1	4.23E-16	zOTU 16	Rothia dentocariosa	T3	3.8	6.06E-03
zOTU 25	Leptotrichia shahii	T2	3.9	9.98E-11	zOTU 14	Corynebacterium durum	T3	4.1	9.75E-08	zOTU 103	Leptotrichia	T3	2.7	2.16E-02
zOTU 15	Veillonella dispar	T2	3.8	1.76E-07	zOTU 5	Actinomyces	T3	4.0	2.00E-02	zOTU 137	Actinobaculum HOT 183	T3	2.7	4.39E-04
zOTU 1	Streptococcus several species	T2	3.8	2.78E-02	zOTU 3	Haemophilus parainfluenzae	T3	3.8	2.99E-03	zOTU 2268	Rothia dentocariosa	T3	2.7	2.82E-02
zOTU 14	Corynebacterium durum	T2	3.7	3.74E-06	zOTU 25	Leptotrichia shahii	T3	3.8	1.93E-09	zOTU 30	Fusobacterium periodonticum	T3	2.7	2.01E-02
zOTU 61	Actinomyces massiliensis	T2	3.7	1.32E-20	zOTU 16	Rothia dentocariosa	T3	3.7	2.79E-07	zOTU 2209	Rothia dentocariosa	T3	2.7	1.74E-02
zOTU 18	Porphyromonas pasteri/HOT 278	T2	3.6	1.15E-14	zOTU 61	Actinomyces massiliensis	T3	3.7	1.87E-25	zOTU 706	Fusobacterium	T3	2.5	2.08E-02
zOTU 37	Fusobacterium	T2	3.6	9.97E-13	zOTU 18	Porphyromonas pasteri/HOT 278	T3	3.7	2.50E-12	zOTU 336	Tannerella HOT 286/ 808	T3	2.5	2.92E-02
zOTU 58	Neisseria oralis	T2	3.6	2.21E-08	zOTU 15	Veillonella dispar	T3	3.6	2.04E-06	zOTU 12	Streptococcus several species	T3	2.5	1.69E-02
zOTU 94	Actinobaculum HOT 848	T2	3.5	2.26E-13	zOTU 37	Fusobacterium	T3	3.5	1.95E-11	zOTU 232	Actinomyces dentalis	T3	2.5	3.80E-02

Appendix Table 2B: zOTUs by their relative abundances in two sample types per time points: Output from LEfSe.

The top 25 zOTUs by their LDA score are shown.

Saliva vs plaque at T1 (total 349 significantly discriminative zOTUs)					**Saliva vs plaque at T2 (total 373 significantly discriminative zOTUs)**				**Saliva vs plaque at T3 (total 373 significantly discriminative zOTUs)**				
zOTUs		Sample type	LDA score	*p* value	zOTUs		Sample type	LDA score	*p* value	zOTUs		Sample type	LDA score	*p* value
zOTU 2	Neisseria	plaque	4.8	5.07E-22	zOTU 5	Actinomyces	plaque	4.6	1.37E-34	zOTU 5	Actinomyces	plaque	4.6	3.53E-34
zOTU 4	Streptococcus sanguinis	plaque	4.7	6.22E-28	zOTU 4	Streptococcus sanguinis	plaque	4.4	1.92E-18	zOTU 2	Neisseria	plaque	4.5	2.98E-13
zOTU 5	Actinomyces	plaque	4.5	2.11E-25	zOTU 2	Neisseria	plaque	4.3	6.94E-07	zOTU 4	Streptococcus sanguinis	plaque	4.5	4.33E-25
zOTU 22	Neisseriaceae	plaque	4.2	1.03E-18	zOTU 14	Corynebacterium durum	plaque	4.2	1.45E-23	zOTU 14	Corynebacterium durum	plaque	4.4	6.71E-28
zOTU 21	Rothia aeria	plaque	4.1	1.90E-08	zOTU 17	Corynebacterium matruchotii	plaque	4.1	3.93E-16	zOTU 17	Corynebacterium matruchotii	plaque	4.2	1.37E-14
zOTU 29	Capnocytophaga sputigena	plaque	4.1	1.92E-15	zOTU 20	Lautropia mirabilis	plaque	4.0	4.58E-16	zOTU 16	Rothia dentocariosa	plaque	4.1	2.84E-11
zOTU 14	Corynebacterium durum	plaque	4.1	2.39E-10	zOTU 25	Leptotrichia shahii	plaque	3.9	8.59E-04	zOTU 21	Rothia aeria	plaque	4.0	4.87E-12
zOTU 20	Lautropia mirabilis	plaque	4.0	2.54E-17	zOTU 15	Veillonella dispar	plaque	3.9	1.82E-06	zOTU 20	Lautropia mirabilis	plaque	4.0	2.04E-18
zOTU 36	Abiotrophia defectiva	plaque	4.0	6.89E-23	zOTU 16	Rothia dentocariosa	plaque	3.8	4.51E-04	zOTU 25	Leptotrichia shahii	plaque	3.8	6.20E-04
zOTU 24	Leptotrichia hongongensis	plaque	4.0	8.51E-08	zOTU 22	Neisseriaceae	plaque	3.8	2.02E-08	zOTU 15	Veillonella dispar	plaque	3.8	4.41E-08
zOTU 16	Rothia dentocariosa	plaque	3.9	3.50E-08	zOTU 21	Rothia aeria	plaque	3.7	1.83E-05	zOTU 22	Neisseriaceae	plaque	3.8	2.00E-12
zOTU 15	Veillonella dispar	plaque	3.7	1.01E-07	zOTU 61	Actinomyces massiliensis	plaque	3.7	5.23E-13	zOTU 61	Actinomyces massiliensis	plaque	3.7	2.26E-21
zOTU 48	Cardiobacterium hominis	plaque	3.7	7.46E-15	zOTU 44	Kingella oralis	plaque	3.6	1.66E-07	zOTU 36	Abiotrophia defectiva	plaque	3.7	2.31E-15
zOTU 45	Capnocytophaga leadbetteri	plaque	3.7	4.25E-08	zOTU 36	Abiotrophia defectiva	plaque	3.6	2.78E-11	zOTU 34	Streptococcus	plaque	3.5	6.41E-13
zOTU 34	Streptococcus	plaque	3.7	2.51E-10	zOTU 48	Cardiobacterium hominis	plaque	3.5	1.91E-14	zOTU 29	Capnocytophaga sputigena	plaque	3.5	4.09E-04
zOTU 57	Capnocytophaga	plaque	3.6	2.47E-06	zOTU 34	Streptococcus	plaque	3.5	1.85E-08	zOTU 44	Kingella oralis	plaque	3.4	1.91E-11
zOTU 74	Capnocytophaga gingivalis	plaque	3.6	1.34E-13	zOTU 94	Actinobaculum HOT 848	plaque	3.5	6.25E-03	zOTU 48	Cardiobacterium hominis	plaque	3.4	6.80E-13
zOTU 66	Leptotrichia HOT 212	plaque	3.4	1.53E-03	zOTU 45	Capnocytophaga leadbetteri	plaque	3.5	1.83E-07	zOTU 128	Actinomyces	plaque	3.3	1.25E-05
zOTU 44	Kingella oralis	plaque	3.4	4.60E-09	zOTU 29	Capnocytophaga sputigena	plaque	3.4	2.00E-05	zOTU 45	Capnocytophaga leadbetteri	plaque	3.2	8.21E-04

zOTU 65	Bergeyella HOT 322	plaque	3.4	1.43E-13	zOTU 53	Leptotrichia HOT 225	plaque	3.3	4.12E-02	zOTU 53	Leptotrichia HOT 225	plaque	3.1	1.98E-02
zOTU 71	Leptotrichia HOT 212	plaque	3.4	1.69E-04	zOTU 37	Fusobacterium	plaque	3.2	1.91E-03	zOTU 26	Streptococcus	plaque	3.1	1.78E-02
zOTU 111	Neisseriaceae	plaque	3.4	3.06E-19	zOTU 57	Capnocytophaga	plaque	3.1	4.40E-04	zOTU 368	Actinomyces	plaque	3.0	1.79E-24
zOTU 116	Kingella HOT 012	plaque	3.3	3.65E-02	zOTU 26	Streptococcus	plaque	3.1	2.30E-02	zOTU 1215	Corynebacterium durum	plaque	3.0	1.69E-26
zOTU 98	Capnocytophaga	plaque	3.3	3.84E-02	zOTU 105	Streptococcus intermedius	plaque	3.1	1.07E-08	zOTU 94	Actinobaculum HOT 848	plaque	3.0	1.15E-02
zOTU 265	Neisseriaceae	plaque	3.2	9.65E-16	zOTU 76	Prevotella HOT317	plaque	3.1	4.72E-02	zOTU 57	Capnocytophaga	plaque	3.0	1.62E-03
zOTU 1	Streptococcus several species	saliva	5.0	1.73E-23	zOTU 1	Streptococcus several species	saliva	4.9	3.47E-22	zOTU 1	Streptococcus several species	saliva	4.9	2.64E-24
zOTU 11	Alloprevotella HOT 473	saliva	4.4	4.41E-15	zOTU 3	Haemophilus parainfluenzae	saliva	4.3	4.24E-13	zOTU 7	Neisseria flavescens/subflava	saliva	4.2	3.28E-16
zOTU 7	Neisseria flavescens/subflava	saliva	4.3	1.35E-12	zOTU 13	Haemophilus	saliva	4.1	3.18E-28	zOTU 3	Haemophilus parainfluenzae	saliva	4.2	9.41E-12
zOTU 13	Haemophilus	saliva	4.3	6.54E-24	zOTU 9	Gemella haemolysans/morbilloru m/sanguinis	saliva	4.1	1.03E-28	zOTU 13	Haemophilus	saliva	4.2	1.27E-28
zOTU 3	Haemophilus parainfluenzae	saliva	4.2	2.45E-08	zOTU 7	Neisseria flavescens/subflava	saliva	4.0	5.75E-11	zOTU 9	Gemella haemolysans/morbillorum /sanguinis	saliva	4.1	1.09E-28
zOTU 9	Gemella haemolysans/morbillorum /sanguinis	saliva	4.1	1.20E-26	zOTU 11	Alloprevotella HOT 473	saliva	4.0	5.55E-08	zOTU 11	Alloprevotella HOT 473	saliva	3.9	2.45E-05
zOTU 28	Veillonella HOT 780	saliva	4.1	5.64E-16	zOTU 6	Streptococcus salivarius/ vestibularis	saliva	3.8	3.14E-25	zOTU 31	Bergeyella	saliva	3.8	3.63E-17
zOTU 2336	Streptococcus	saliva	4.0	4.84E-20	zOTU 8	Prevotella	saliva	3.7	5.13E-22	zOTU 27	Granulicatella elegans	saliva	3.8	9.41E-23
zOTU 38	Porphyromonas HOT 930	saliva	4.0	3.81E-21	zOTU 27	Granulicatella elegans	saliva	3.7	6.09E-24	zOTU 38	Porphyromonas HOT 930	saliva	3.8	1.19E-23
zOTU 31	Bergeyella	saliva	3.9	1.94E-08	zOTU 10	Veillonella	saliva	3.7	1.19E-13	zOTU 8	Prevotella	saliva	3.8	1.02E-23
zOTU 27	Granulicatella elegans	saliva	3.9	1.73E-26	zOTU 35	Haemophilus	saliva	3.7	1.56E-22	zOTU 35	Haemophilus	saliva	3.7	6.10E-17
zOTU 46	Porphyromonas HOT 930	saliva	3.9	2.39E-17	zOTU 31	Bergeyella	saliva	3.6	5.92E-15	zOTU 6	Streptococcus salivarius/ vestibularis	saliva	3.7	7.60E-21
zOTU 43	Leptotrichia	saliva	3.9	5.76E-05	zOTU 38	Porphyromonas HOT 930	saliva	3.6	1.42E-20	zOTU 10	Veillonella	saliva	3.6	7.72E-13
zOTU 6	Streptococcus salivarius/ vestibularis	saliva	3.8	2.83E-18	zOTU 28	Veillonella HOT 780	saliva	3.6	1.30E-11	zOTU 46	Porphyromonas HOT 930	saliva	3.6	4.69E-15
zOTU 10	Veillonella	saliva	3.8	1.40E-17	zOTU 41	Rothia mucilaginosa	saliva	3.5	7.27E-15	zOTU 30	Fusobacterium periodonticum	saliva	3.6	3.57E-20
zOTU 8	Prevotella	saliva	3.8	8.15E-15	zOTU 12	Streptococcus several species	saliva	3.5	5.00E-21	zOTU 28	Veillonella HOT 780	saliva	3.6	1.13E-10

zOTU 35	Haemophilus	saliva	3.7	9.16E-09	zOTU 43	Leptotrichia	saliva	3.5	9.02E-07	zOTU 43	Leptotrichia	saliva	3.5	1.40E-06
zOTU 30	Fusobacterium periodonticum	saliva	3.6	1.08E-13	zOTU 46	Porphyromonas HOT 930	saliva	3.5	7.84E-18	zOTU 41	Rothia mucilaginosa	saliva	3.5	1.08E-11
zOTU 12	Streptococcus several species	saliva	3.5	9.19E-14	zOTU 30	Fusobacterium periodonticum	saliva	3.5	4.92E-21	zOTU 59	Haemophilus	saliva	3.5	3.67E-14
zOTU 62	Porphyromonas	saliva	3.4	8.33E-13	zOTU 2336	Streptococcus	saliva	3.4	3.68E-26	zOTU 2336	Streptococcus	saliva	3.5	6.95E-27
zOTU 18	Porphyromonas pasteri/HOT 278	saliva	3.4	3.13E-08	zOTU 59	Haemophilus	saliva	3.4	1.08E-06	zOTU 12	Streptococcus several species	saliva	3.5	1.35E-22
zOTU 75	Streptococcus	saliva	3.4	1.94E-33	zOTU 62	Porphyromonas	saliva	3.3	9.78E-11	zOTU 42	Rothia mucilaginosa	saliva	3.4	5.72E-18
zOTU 81	Leptotrichia	saliva	3.3	6.78E-07	zOTU 81	Leptotrichia	saliva	3.3	7.88E-06	zOTU 62	Porphyromonas	saliva	3.3	2.64E-09
zOTU 90	Sneathia	saliva	3.3	5.77E-06	zOTU 106	Haemophilus	saliva	3.2	4.55E-32	zOTU 49	Aggregatibacter segnis/HOT 458/512	saliva	3.3	7.35E-03
zOTU 42	Rothia mucilaginosa	saliva	3.3	3.04E-09	zOTU 75	Streptococcus	saliva	3.1	3.76E-24	zOTU 81	Leptotrichia	saliva	3.3	3.23E-06

Appendix Table 2C: zOTUs by their relative abundances in children plaque samples per Medicaid over time: Output from LEfSe.

The top 10 zOTUs by their LDA score are shown.

Plaque T1 (total 35 significantly discriminative zOTUs)					Plaque T2 (total 81 significantly discriminative zOTUs)					Plaque T2 (total 90 significantly discriminative zOTUs)				
zOTUs		Medicaid status	LDA score	*p* value	zOTUs		Medicaid status	LDA score	*p* value	zOTUs		Medicaid status	LDA score	*p* value
zOTU 16	Rothia dentocariosa	Yes	3.9	1.74E-03	zOTU 17	Corynebacterium matruchotii	Yes	3.9	7.56E-03	zOTU 25	Leptotrichia shahii	Yes	3.9	2.27E-03
zOTU 25	Leptotrichia shahii	Yes	3.7	2.85E-03	zOTU 25	Leptotrichia shahii	Yes	3.9	3.68E-03	zOTU 34	Streptococcus	Yes	3.6	9.27E-03
zOTU 11	Alloprevotella HOT473	Yes	3.6	7.12E-03	zOTU 40	Leptotrichia	Yes	3.7	2.48E-03	zOTU 70	Fusobacterium	Yes	3.5	2.94E-02
zOTU 34	Streptococcus	Yes	3.4	4.98E-02	zOTU 26	Streptococcus	Yes	3.3	3.78E-02	zOTU 26	Streptococcus	Yes	3.4	1.61E-03
zOTU 17	Corynebacterium matruchotii	Yes	3.4	3.94E-02	zOTU 220	Leptotrichia HOT 498	Yes	3.2	3.44E-03	zOTU 40	Leptotrichia	Yes	3.2	2.00E-03
zOTU 10	Veillonella	Yes	3.4	3.24E-02	zOTU 143	Leptotrichia HOT 498	Yes	3.1	3.34E-04	zOTU 57	Capnocytophaga	Yes	3.2	8.15E-03
zOTU 9	Gemella haemolysans/morbillorum/sanguinis	Yes	3.4	8.18E-03	zOTU 76	Prevotella HOT 317	Yes	3.1	1.15E-02	zOTU 84	Leptotrichia	Yes	3.2	1.33E-03
zOTU 28	Veillonella HOT 780	Yes	3.4	8.50E-03	zOTU 86	Prevotella	Yes	3.0	1.65E-02	zOTU 49	Aggregatibacter segnis/ HOT 458/512	Yes	3.1	1.04E-02
zOTU 179	Neisseriaceae	Yes	3.1	2.45E-02	zOTU 177	Selenomonas	Yes	2.9	8.75E-05	zOTU 128	Actinomyces	Yes	3.1	3.57E-04
zOTU 61	Actinomyces massiliensis	Yes	3.1	1.18E-02	zOTU 56	Actinomyces odontolyticus/HOT 180	Yes	2.9	3.78E-02	zOTU 143	Leptotrichia HOT 498	Yes	3.0	1.26E-04
zOTU 20	Lautropia mirabilis	No	4.1	9.36E-04	zOTU 4	Streptococcus sanguinis	No	3.9	4.63E-02	zOTU 14	Corynebacterium durum	No	4.1	1.61E-02
zOTU 36	Abiotrophia defectiva	No	3.6	2.83E-02	zOTU 22	Neisseriaceae	No	3.8	4.71E-03	zOTU 4	Streptococcus sanguinis	No	4.1	5.01E-03
zOTU 65	Bergeyella HOT 322	No	3.3	1.74E-02	zOTU 104	Neisseria oralis	No	3.6	3.70E-02	zOTU 20	Lautropia mirabilis	No	3.9	2.11E-03
zOTU 454	Lautropia mirabilis	No	2.9	1.04E-03	zOTU 29	Capnocytophaga sputigena	No	3.6	5.61E-03	zOTU 58	Neisseria oralis	No	3.5	2.37E-02
zOTU 704	Betaproteobacteria	No	2.8	1.15E-02	zOTU 20	Lautropia mirabilis	No	3.5	1.19E-02	zOTU 93	Leptotrichia wadei	No	3.0	2.11E-03
zOTU 2223	Lautropia mirabilis	No	2.8	5.72E-04	zOTU 48	Cardiobacterium hominis	No	3.3	2.56E-02	zOTU 65	Bergeyella HOT 322	No	2.9	1.56E-02
zOTU 1426	Betaproteobacteria	No	2.8	1.39E-03	zOTU 73	Aggregatibacter aphrophilus/paraphrophilus	No	3.3	9.96E-03	zOTU 454	Lautropia mirabilis	No	2.6	3.51E-02
zOTU 1109	Neisseria	No	2.7	7.17E-03	zOTU 74	Capnocytophaga gingivalis	No	3.0	1.95E-02	zOTU 2239	Lautropia mirabilis	No	2.4	4.67E-04
zOTU 536	Neisseriaceae	No	2.7	1.56E-02	zOTU 65	Bergeyella HOT 322	No	2.8	5.74E-04	zOTU 942	Neisseria oralis	No	2.4	7.96E-03
zOTU 920	Neisseria	No	2.7	9.82E-03	zOTU 265	Neisseriaceae	No	2.8	2.04E-02	zOTU 1426	Betaproteobacteria	No	2.4	2.44E-03