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Citation

Velsko, I. M., Gallois, S. L., Stahl, R., Henry, A. G., & Warinner, C. (2023). High conservation of the dental plaque microbiome community across populations with differing subsistence strategies and levels of market integration. *Molecular Ecology*, 32(14), 3872-3891. doi:10.1111/mec.16988

Version: Publisher's Version

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Downloaded from: <https://hdl.handle.net/1887/3674026>

Note: To cite this publication please use the final published version (if applicable).

High conservation of the dental plaque microbiome across populations with differing subsistence strategies and levels of market integration

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Funding information

Deutsche Forschungsgemeinschaft, Grant/Award Number: EXC 2051, Project-ID 390713860; H2020 European Research Council, Grant/Award Number: STG-677576 ("HARVEST"); Werner Siemens Stiftung

Handling Editor: Tatiana Giraud

Abstract

Industrialization—including urbanization, participation in the global food chain and consumption of heavily processed foods—is thought to drive substantial shifts in the human microbiome. While diet strongly influences stool microbiome composition, the influence of diet on the oral microbiome is largely speculative. Multiple ecologically distinct surfaces in the mouth, each harbouring a unique microbial community, pose a challenge to assessing changes in the oral microbiome in the context of industrialization, as the results depend on the oral site under study. Here, we investigated whether microbial communities of dental plaque, the dense biofilm on non-shedding tooth surfaces, are distinctly different across populations with dissimilar subsistence strategies and degree of industrialized market integration. Using a metagenomic approach, we compared the dental plaque microbiomes of Baka foragers and Nzime subsistence agriculturalists in Cameroon ($n = 46$) with the dental plaque and calculus microbiomes of highly industrialized populations in North America and Europe ($n = 38$). We found that differences in microbial taxonomic composition between populations were minimal, with high conservation of abundant microbial taxa and no significant differences in microbial diversity related to dietary practices. Instead, we find that the major source of variation in dental plaque microbial species composition is related to tooth location and oxygen availability, which may be influenced by toothbrushing or other dental hygiene measures. Our results support that dental plaque, in contrast to the stool microbiome, maintains an inherent stability against ecological perturbations in the oral environment.

KEYWORDS

Baka, dental plaque, diet, industrialization, Nzime, oral microbiome, subsistence

1 | INTRODUCTION

The human mouth is a complex environment, containing up to seven distinct surfaces that host distinct microbiomes (Aas et al., 2005; Human Microbiome Project Consortium, 2012). Dental plaque, which forms on tooth surfaces, is a dense biofilm containing predominantly bacteria, the species composition of which is strongly linked to oral and systemic health and disease (Beck et al., 2019; Teles et al., 2013). The dental plaque microbial community has been relatively well-characterized for individuals in heavily industrialized countries, particularly in North America and Europe, where heavily processed diets are consumed (Aas et al., 2005; Dewhirst et al., 2010). However, little is known about the structure and function of microbial dental plaque communities among individuals outside of industrialized systems.

As microbiome research has expanded to include more diverse human groups, increased attention has been paid to the role of industrialization in influencing the human microbiome. For example, the stool microbiome of industrialized and non-industrialized societies consistently shows distinct compositional differences, with stool from non-industrialized groups being characterized by higher microbial diversity, as well as higher proportions of *Prevotella* (De Filippo et al., 2010; Gomez et al., 2016; Morton et al., 2015; Obregon-Tito et al., 2015; Rubel et al., 2020; Schnorr et al., 2014; Stagaman et al., 2018) and the presence of treponemes (Gomez et al., 2016; Morton et al., 2015; Obregon-Tito et al., 2015; Rubel et al., 2020; Schnorr et al., 2014), eukaryotic intestinal parasites (Morton et al., 2015; Rubel et al., 2020) and unique bacteriophages (Devoto et al., 2019). Such distinctions have largely been linked to dietary and subsistence differences, as well as lifestyle changes with respect to sanitation, antibiotic exposure, and urbanization (Lokmer et al., 2020; Morton et al., 2015; Obregon-Tito et al., 2015; Ou et al., 2013; Rubel et al., 2020; Stagaman et al., 2018). The clear difference in stool sample taxonomic composition between industrialized and non-industrialized populations is often extrapolated to represent a generalized shift in the human microbiome associated with industrialization, yet similarly consistent differences have not been reported in oral microbiome studies.

Of the studies to date that have investigated the role of diet on oral microbiome composition, simple sugar intake has been associated with microbial shifts (Anderson et al., 2020), but broader microbial changes related to differences in subsistence or urbanization, industrialization, and/or hygiene are subtle and often inconsistent (Kato et al., 2017). For example, for hunter-gatherers in the Amazon basin, the Philippines and Central Africa, within-sample diversity of oral bacteria in saliva and oral mucosa has been reported to be both similar to those of industrialized populations (Clemente et al., 2015; McCall et al., 2020) and higher than those of subsistence farmers or more urbanized populations living in close geographic proximity (Lassalle et al., 2018; Lokmer et al., 2020; Nasidze et al., 2011). Likewise, taxonomic composition and between-sample microbial diversity of these populations have been reported to differ from industrialized populations (Clemente et al., 2015; Nasidze et al., 2011)

but also to be highly similar (Lassalle et al., 2018; Lokmer et al., 2020; McCall et al., 2020). Although these contradictory findings may be related to a variety of study-specific factors (e.g. differences in study design, sample collection and preparation, and data processing and analysis), similar methodological heterogeneity also characterizes gut microbiome studies, which in contrast have yielded consistent findings. Terminology describing the groups under study may be another factor in the inconsistencies of reported findings. The dichotomy of industrialized/non-industrialized populations is a misrepresentation of continuous scales of integration into the market economy, consumption of industrially processed foods, and level of urbanization (Mangola et al., 2022). In particular, the term non-industrial is broadly used to refer to groups with substantial heterogeneity in their placement across these scales, and therefore, across numerous studies, populations described as non-industrial cannot be equated (Mangola et al., 2022).

One study-design issue that may be preventing oral microbiome studies from detecting consistent differences is the variety of sampling sites in the mouth. Saliva, oral mucosa, tongue and dental plaque harbour distinct microbial communities, and the species profiles of these different sites cannot be directly compared (Aas et al., 2005; Human Microbiome Project Consortium, 2012; Mark Welch et al., 2016, 2019). Saliva, which is easy and non-invasive to collect, is not a true microbiome, but rather a dynamic assemblage of microbes shed from other oral sites, especially the tongue, oral mucosa and throat (Belström, 2020; Segata et al., 2012; Zaura et al., 2021) that is open to the environment. Therefore, saliva studies may be more prone to stochastic results than studies using solid sites that allow bacterial adherence, such as dental plaque on tooth surfaces or keratinized gingiva of the gums or buccal mucosa, potentially making it difficult to discern whether novel salivary microbial diversity is truly host-associated or environmentally derived.

Dental plaque, a dense and highly structured biofilm, is relatively well-characterized in North American and European populations (Dewhirst et al., 2010; Teles et al., 2013), and is the microbial community directly responsible for two of the most common microbial diseases world-wide, caries and periodontal disease. Further, these two oral diseases, and the microbes that cause them, are epidemiologically linked to numerous systemic inflammatory diseases including atherosclerosis, rheumatoid arthritis and Alzheimer's (Hajishengallis, 2022). Therefore, the study of this biofilm community in global populations is of considerable interest and population-level comparisons of dental plaque microbiomes rather than saliva microbiomes may lead to more consistent results related to the impact of integration into the global food chain, urbanization and industrialization on oral bacteria, as well as oral and systemic health.

Dental plaque biofilms develop over several days in a consistent succession of species with different metabolic requirements, making it possible to distinguish early and late-stage plaque biofilms by the species composition. Biofilm growth is initiated by oxygen-tolerant, saccharolytic species, while anaerobic, proteolytic taxa are not readily detectable until several days of biofilm growth (Diaz et al., 2006; Nyvad & Kilian, 1987; Ramberg et al., 2003; Ritz, 1967; Socransky

et al., 1977; Theilade et al., 1966; Tinanoff et al., 1976). Mechanical dental plaque removal such as through tooth brushing and flossing disrupt biofilm development, prolonging the early biofilm community stage of development, as the microbes re-establish metabolic dependencies and reproduce microenvironments that favour colonization and proliferation of late-stage species. In addition, dental plaque periodically spontaneously calcifies into dental calculus, also known as tooth tartar, encasing the entire plaque biofilm in a mineral matrix, which is subsequently re-colonized and used as the substratum for new dental plaque biofilm formation. This cycle may occur numerous times throughout an individual's life, resulting in a build-up of calculus as visible deposits on tooth surfaces. Although uncommon in populations today practicing regular dental hygiene, dental calculus was common historically, evidenced by the prevalence and abundance of dental calculus deposits on archaeological human remains (Warinner, 2016; Warinner et al., 2014). Archaeological dental calculus represents a lifetime accumulation of dental plaque biofilms (Warinner, 2016; Warinner et al., 2014), and is the only host-associated microbiome for which there are historic and pre-historic representatives. Thus, dental calculus potentially enables long-term inferences to be made about factors influencing the evolution, ecology and function of the oral microbiome (Fellows Yates, Velsko, et al., 2021), particularly related to cultural and social interactions throughout human evolution and history.

Here, we investigate the dental plaque composition of individuals from two ethnic groups in Cameroon whose livelihoods are based on foraging and small-scale farming, the Baka and the Nzime, in order to better understand oral microbiome diversity within these groups, and between these groups and extensively characterized North American and European dental plaque biofilms. While both Baka and Nzime practice varying degrees of foraging and small-scale farming, Baka are historically more nomadic, depending more on foraging, and incorporate more wild and foraged foods into their diet, while Nzime are more sedentary, relying mostly on agriculture, and incorporate more domesticated and cultivated foods into their diet. Nevertheless, despite these differences in subsistence and lifestyle, we find that the dental plaque microbial communities of Baka and Nzime individuals (hereafter BN) are highly similar to those of both dental plaque and dental calculus from heavily industrialized populations in the US and Europe (hereafter USE). Further, no associations between dental plaque taxonomic composition and subsistence strategy were identified. These findings are consistent with recent archaeogenetics studies showing the long-term stability of dental calculus microbial communities within diverse human populations extending back to the late Pleistocene (Fellows Yates, Velsko, et al., 2021; Ottoni et al., 2021), as well as experimental studies examining dental plaque changes over short and medium time frames (Tamashiro et al., 2021; Zaura et al., 2015).

Collectively, these findings support a characteristic stability of oral microbiota that is distinct from the gut (Wade, 2021) and suggest that the human microbiome does not undergo monolithic changes in response to major social, economic and lifestyle changes. Rather, each body site is affected and constrained by distinct factors.

Characterizing the diversity and evolutionary history of the oral microbiome may be key to both understanding and treating microbially mediated diseases of the oral cavity.

2 | MATERIALS AND METHODS

2.1 | Samples and study populations

This study was conducted as part of a broader investigation of foraging strategies among the Baka people of southeastern Cameroon, with a specific focus on the importance of plant dietary resources in forested tropical environments (Gallois et al., 2020; Gallois & Henry, 2021). For details see Methods S1. Dental plaque samples and metadata were collected by author S.G. between October–November 2018, with the assistance of a Nzime research assistant and interpreter who spoke the local dialects of both studied populations. This occurred during the major rainy season, when many Baka individuals reside in forest camps and participate in major hunting and wild edible-foraging excursions in the forest (Gallois et al., 2020). A total of 47 individuals (30 Baka and 17 Nzime) from five different communities (three Baka and two Nzime villages) in the Messok and Lomie districts of the Haut Nyong Division of southeastern Cameroon participated in the study. These Baka communities and Nzime villages were located along the logging road, with the exception of one Baka forest camp, which was located at a distance of about an hour and a half by foot from the logging road. Based on location, access to the market economy and to health and school services differed among the communities in this study.

2.2 | Informed consent and ethical review

Before the onset of the research, we received the free prior informed consent from both the local authorities of the villages and the participants. The study received the research permit from the Ministry of Scientific Research and Innovation (MINRESI n°000115/MINRESI/B00/C00/C10/C12 and n°000116/MINRESI/B00/C00/C10/C12). Members of Baka and Nzime communities were contacted by project members about the possibility of participating in a study on dental health and diet. Prior to sample and metadata collection, the study and its goals and methods were explained to prospective participants in the five communities. Study volunteers provided informed consent and agreed to a dental health assessment and dental cleaning, followed by an analysis of their dental biofilm. To ensure representation of the general adult population as well as age and gender balance in the study, we considered all community adults to be eligible, including individuals with missing teeth, periodontitis and visible caries. Participant demographic metadata and oral health status (as determined at the time of the dental cleaning) were recorded for each individual (Figure S1). Individuals who had taken antibiotics within the past 6 months were excluded from the study. The women who participated verbally confirmed that they were not pregnant. After the completion of the study,

project member S.G. met with local authorities and village members in different meetings to share the results of the study and distribute a booklet describing a summary of the findings. The study design was approved by the Ethical Committee from the Ministry of Health of Cameroon (n°2018/06/1049/CE/CNERSH/SP) and the ethics committee of Leipzig University (196-16/ek). It also adheres to the Code of Ethics of the International Society of Ethnobiology (The ISE Code of Ethics, 2011).

2.3 | Sample collection and storage

Sample collection proceeded as follows: first, the participants' teeth were brushed with a dampened toothbrush to remove food debris and loosely attached dental plaque. Next, the overall condition of the teeth was visibly inspected, and decayed, missing, filled or altered teeth were recorded, along with any instances of loose teeth, bleeding gums and teeth with visible root exposure (Table S1, using the FDI World Dental Federation notation). Using a sterilized (autoclaved) dental scaler, we collected two supragingival biofilm samples from each individual where possible, one from the anterior teeth (incisors or canines, depending on availability, and from both mandible and maxilla, depending on availability) and one from the posterior teeth (premolars and molars, again where available, and from both mandible and maxilla, depending on availability). This sampling strategy was adopted because previous studies have found small but significant differences between microbial communities on anterior and posterior teeth (Fagernäs et al., 2022; Haffajee et al., 2009; Proctor et al., 2018; Simón-Soro et al., 2013). When plaque was visible on teeth in both the upper and lower jaws, plaque was sampled off teeth in both jaws with the same scaler, and collected on the same piece of gauze as a pooled sample. Each biofilm sample was placed onto a piece of sterile gauze, dried and individually packaged into small sealed plastic bags containing a silica gel desiccant to reduce humidity. The dried biofilm samples were stored at ambient temperature (10–28°C) for several weeks until the completion of fieldwork, after which they were transported to the laboratory facility at the Max Planck Institute for the Science of Human History (now the Max Planck Institute for Evolutionary Anthropology), where they were stored at –20°C. This collection method was adopted to account for our remote sampling location, where it was not feasible to use standard clinical collection methods (Kim et al., 2017). Previous work on oral swab samples has found that their microbial communities are stable at room temperature for more than a week if stored in a dry condition (Kim et al., 2017). Likewise, faecal samples have been shown to maintain fidelity for room temperature storage if dried on paper cards (Song et al., 2016) or with silica (Schnorr et al., 2014).

2.4 | DNA extraction and next generation sequencing

DNA was extracted from samples using the Qiagen PowerSoil kit. For each sample, a small piece of gauze with visible dental plaque was

cut from the main piece and placed in a PowerSoil bead tube. Two controls were included for each extraction batch: a gauze control and an empty tube control. Both controls were processed alongside the samples throughout the entire extraction and library building process. For details, see the Methods S1. DNA was extracted following the detailed protocol "DNA Extraction from Modern Dental Plaque on Gauze" (Aron & Velsko, 2021).

Illumina library preparation followed the detailed protocol "Non-UDG treated double stranded modern DNA library preparation for Illumina sequencing" (Aron et al., 2020), and included a library blank control, which used water as input instead of sample. The libraries were indexed following the detailed protocol "Illumina double-stranded DNA dual indexing for ancient DNA" (Stahl et al., 2020), adapted from (Kircher et al., 2012), with the modification that the entire process was performed in a modern DNA lab. All libraries were sequenced on an Illumina NextSeq500 with 2×150bp chemistry. Each library was sequenced on 2 or 3 flow cells to a depth of approximately 10 million reads per sample (Table S1). Sequencing across multiple flow cells allowed us to reduce potential batch effects. Sequencing data were demultiplexed with an in-house pipeline.

2.5 | Comparative data

Previous oral microbiome studies of non-industrial populations have focused primarily on saliva, and to a lesser degree buccal mucosa (cheek swabs; Aas et al., 2005; Human Microbiome Project Consortium, 2012). To our knowledge, no studies of dental plaque from non-industrial populations have been published to date. For this study, we selected as comparative datasets dental plaque and dental calculus from industrialized populations, buccal mucosa samples from both non-industrialized and industrialized populations, and saliva from non-industrialized and industrialized populations. For industrial food consumers, both dental plaque and dental calculus were included because they have been shown to contain distinct species profiles (Velsko, Fellows Yates, et al., 2019). The six comparative datasets consisted of (1) 20 dental plaque samples (10 supragingival and 10 subgingival) of industrial food consumers in the United States from the Human Microbiome Project (HMP; PRJNA48479; Human Microbiome Project Consortium, 2012); (2) 18 dental calculus samples of industrial food consumers in Spain (PRJEB31185 and PRJEB34569; Fellows Yates, Velsko, et al., 2021; Velsko, Fellows Yates, et al., 2019); (3) 14 buccal mucosa samples of Yanomami hunter-gatherers in the Amazon rainforest of Venezuela (PRJNA245336; Clemente et al., 2015); (4) 14 buccal mucosa samples from industrial food consumers in the United States from the HMP (PRJNA4847; Human Microbiome Project Consortium, 2012); (5) 24 saliva samples from hunter-gatherers and traditional farmers in the Philippines (PRJEB14383; Lassalle et al., 2018); and (6) five saliva samples from industrial food consumers in the United States from the HMP (PRJNA4847; Human Microbiome Project Consortium, 2012). A full list of sample accession IDs is provided in Table S1. While supra- and subgingival plaque samples have distinct community compositions and minimal overlap in PCA space

(Figure S2A), the distinction between the two sample types is difficult to frame in the context of the BN dental plaque samples, which are predominantly supragingival plaque, yet do not cluster closer to supragingival than to subgingival industrial dental plaque (Figure S2A,B). Metabolic pathway composition does not distinguish supra- and subgingival plaque samples (Figure S2C), and the metabolic pathway composition of BN dental plaque samples likewise does not appear to more closely resemble that of either supragingival or subgingival industrial dental plaque. The Yanomami comparative dataset (Clemente et al., 2015) contained the only samples that were treated with multiple displacement amplification after extraction. Directly comparing the microbial species profiles of dental plaque, buccal mucosa and saliva of industrial and non-industrial populations allowed us to investigate whether stronger differences exist between oral sites or between industrial and non-industrial populations.

2.6 | Data processing

All sequencing data produced for this study, as well as the comparative datasets, were processed with the nf-core/eager pipeline (Fellows Yates, Lamnidis, et al., 2021). This included adapter trimming, read merging and quality filtering with AdapterRemoval (Schubert et al., 2016), and mapping against the human genome (HG19) with bwa aln -n 0.02 -l 1024 (Li & Durbin, 2009) to remove human reads prior to analysis. All sequencing files for each library were concatenated during the eager run to create a single fastq file per library, which was mapped against the human genome. All reads that did not map to the human genome were used for taxonomic profiling.

2.7 | Taxonomic profiling and clean-up

For taxonomic profile exploration, we used MALT version 0.4 (Vågene et al., 2018) within the nf-core/eager pipeline, using a RefSeq database (RefSeq_malt) that included bacteria, archaea and the human genome (Fellows Yates, Velsko, et al., 2021). The complete MALT table is available as Table S4. To assess post-collection environmental contamination, sample integrity was checked with the R package CUPERDEC (<https://github.com/jfy133/cuperdec>; Fellows Yates, Velsko, et al., 2021). To remove possible contaminants introduced to the samples during laboratory processing, we used the R package DECONTAM (Davis et al., 2018). All species that were identified as contaminants were removed from the species table before performing further analyses. We tested for batch effects in our extraction data by performing diversity analyses and comparing the extraction batches.

2.8 | Alpha-diversity

To assess within-sample diversity, we calculated the number of observed species and the Shannon index. Taxa present at less than

0.001% abundance were removed from the table, as recommended for MALT (Velsko et al., 2018), which produces a high number of low-abundance false-positive taxonomic assignments. Heatmaps of the top 50 most abundant species were generated by determining the 50 most abundant species in each group (Baka and Nzime dental plaque, industrial dental plaque and industrial dental calculus) and then these species were subsetted from the species table.

2.9 | Factor analysis

Exploratory factor analysis was performed to reduce the number of variables (species) for beta-diversity analyses, rather than using an abundance-based cut-off, with the R package PSYCH (Revelle, 2001) with oblimin rotation and minres factoring method. A species table containing only the species that were in the first factor (123 species for BN and USE samples, or 117 species for only BN samples), minimum residual 1 (MR1), which explains a majority of the variation in the data, was used for further diversity analyses. We used linear discriminant analysis to confirm the high potential of the species in the first factor to discriminate the samples in each group (for details see Methods S1). Heatmaps of the CLR-transformed read counts for each species in MR1 were generated with ggplot (Figures S12 and S13).

2.10 | Beta-diversity

Beta-diversity analyses were performed on a species table including only the species in minimum residual 1 from the factor analysis. A value of +1 was added to all species read counts, which were then CLR-transformed and a principal components analysis (PCA) performed on a Euclidean distance matrix using the R package MIXOMICS (Rohart et al., 2017), and plots generated with ggplot. To determine if sample groups have significantly different species profiles, a PERMANOVA was performed on the Euclidean distance matrix with the function ADONIS2 and homogeneity of multivariate dispersions was tested with the function betadisper, both from the R package VEGAN (Oksanen, 2007). We additionally separated the samples by anterior and posterior tooth collection site, as this difference was the strongest explanation of variation between groups in the PERMANOVA, and repeated the beta-diversity analyses on only anterior and only posterior samples.

2.11 | Differential abundance

Species differential abundance was tested with ANCOM-BC (Lin & Peddada, 2020), which was designed to work with compositional microbiome data and to account for the biases inherent in this kind of data. ANCOM-BC was performed following the examples provided on the ANCOM-BC github page, and plots were generated with ggplot.

2.12 | Functional profiling with HUMAnN3

We performed functional profiling of all samples with HUMAnN3 (Beghini et al., 2021) with default settings. We used the path abundance output table with paths classified with MetaCyc pathways (Caspi et al., 2020; Karp et al., 2002), and the gene families output table classified with UniProt90, both normalized by copies per million reads (CPM). For pathway and gene family analysis, we selected the total abundance lines from the gene families table and the pathways table, (this removed all species-level assignments), for diversity analyses. The variation in pathways and gene families between groups were assessed using PCA and differential abundance following the same steps as for species analysis, see Methods S1 for details.

2.13 | Taxonomic profiles with and without metagenome-assembled metagenomes

To determine if there are taxa present in the Baka and Nzime samples that are not represented in the NCBI RefSeq database, we used Kraken2 (Wood et al., 2019) and built two databases, one containing only RefSeq genomes (here, RefSeq_kraken) and the other containing those same RefSeq genomes and the metagenome-assembled genomes (MAGs) published by Pasolli et al. (2019; here, RefSeq_Pasolli_kraken). Taxonomic profiling using Kraken2 was applied to all Baka and Nzime dental plaque, industrial dental plaque and industrial calculus datasets for both databases. The number of reads that were classified and unclassified in each sample in each run were determined from the Kraken2 output file. Samples were grouped by market integration (Logging road, Forest camp, Farming) for BN samples and by sample type (plaque, calculus) for USE samples, and a Wilcoxon rank-sum test was used to determine if there was a significant difference between the number of reads assigned by each database within each group. To determine if there are differences in the number of reads assigned to known oral taxa between the two databases, we calculated the log fold change in relative abundance of the 14 phyla described in the human oral cavity (Dewhirst et al., 2010) when profiling with RefSeq_Pasolli_kraken vs RefSeq_kraken. For details see Methods S1.

2.14 | Taxonomic ratios

We calculated the ratio of the relative abundance of the phyla Bacteroidetes versus Firmicutes and the ratio of the relative abundance of the genera *Prevotella* versus *Bacteroides* within the Baka and Nzime samples, as well as within industrial dental plaque and calculus from the complete MALT-generated species profiles. The table was not restricted to the species in the first factor of factor analysis performed for beta-diversity analysis. These taxa are generally the top two most explanatory taxonomic annotations for the gut microbiome, and we wanted to test whether they are explanatory for the oral microbiome.

3 | RESULTS

3.1 | Subsistence and lifestyle in southeastern Cameroon

We partnered with neighbouring members of two distinct ethnic groups, the Baka, a Ubangian speaking people who practice foraging and small-scale cultivation, and the Nzime, a Bantu-speaking people who practice subsistence-level agriculture (Patin et al., 2009; Verdu et al., 2009), in order to better understand the influence of diet and lifestyle on oral microbial communities. Although living in the same area (Figure 1), Baka and Nzime lifeways greatly differ.

The Nzime rely on subsistence agriculture, cultivating mostly cassava and plantains as major crops, and also producing cacao for trade. The Baka rely on a more mixed subsistence strategy, combining foraging (hunting, gathering and fishing) with small-scale farming (Reyes-García et al., 2019). The Baka and Nzime have long had an economic relationship based on the exchange of foraged forest and farmed agricultural products. The diets of both the Baka and the Nzime are primarily plant-based, with a focus on cassava and plantains, supplemented by bushmeat and cultivated or wild vegetables. While the main components of Baka and Nzime diets overlap (Table S2), Baka diets have a higher degree of dietary seasonality and they consume a wider diversity of wild products in their daily meals, although such diversity tends to decrease with higher integration into the market economy (Gallois et al., 2020; Reyes-García et al., 2019).

Both the Baka and Nzime have limited access to market economy goods through small local shops and traders travelling along logging roads, but the Nzime, due to their higher income from agricultural surplus, have greater access to market goods and integrate a higher proportion of processed foods obtained from local shops into their diet. Toothpaste is not typically available, and dental cleaning is uncommon, particularly among the Baka. The Nzime participants were from two farming villages situated along logging roads. The Baka participants were from three villages, two of which were situated along logging roads, and one of which was a forest camp. Individuals living in the forest camp have much lower consumption of market economy goods than those living along the logging roads. Therefore, for analysis we grouped the dental plaque samples by village location (logging road and forest camp for Baka, and farming for Nzime; Figure 1a–d).

3.2 | Generation of metagenomic data from Baka and Nzime dental plaque

From 46 individuals, we successfully built 85 shotgun DNA libraries: 57 libraries from 29 Baka individuals (27 paired anterior and posterior samples, two unpaired samples and one additional anterior sample from a paired set), and 28 libraries from 17 Nzime individuals (11 paired anterior and posterior samples and six unpaired samples; Figure 1e; Table S1). The average sequencing depth across samples

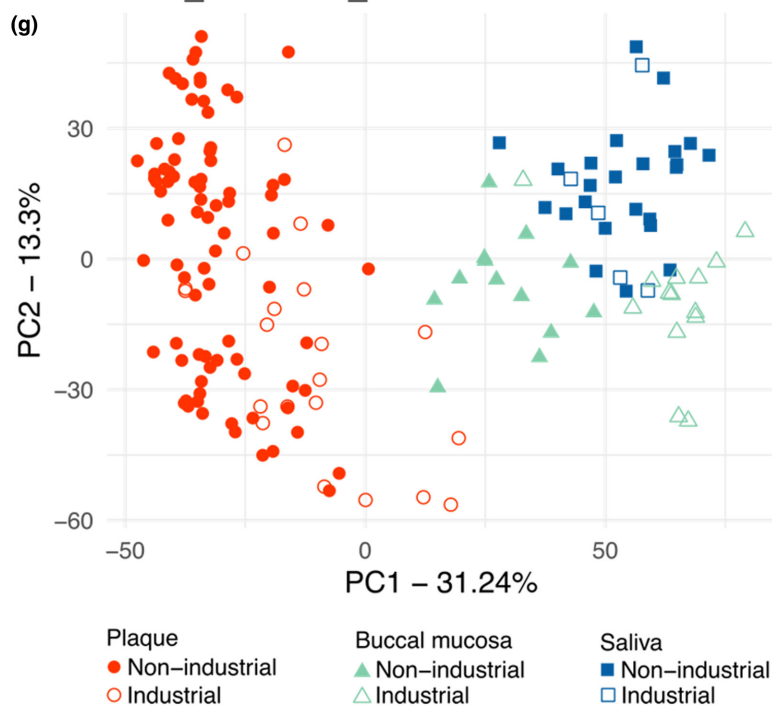
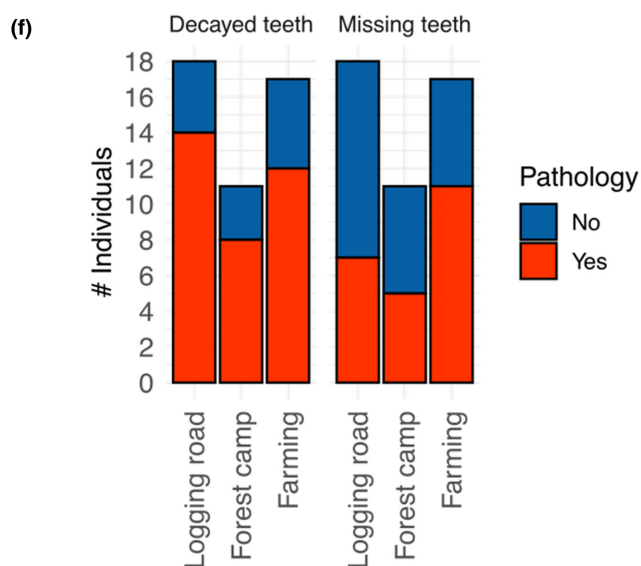
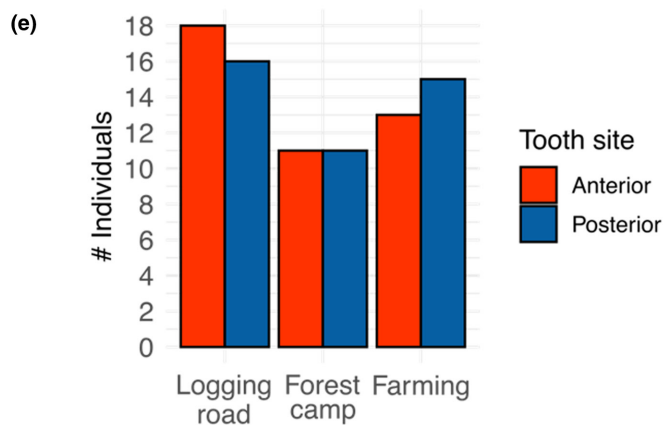
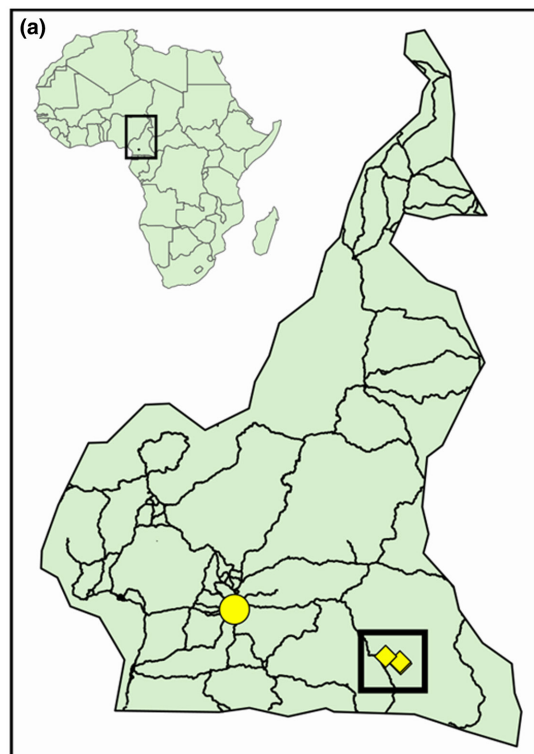


FIGURE 1 Sample collection locations. (a) Location of the villages included in this study. The inset of Cameroon shows the major roadways, and the capital Yaounde marked as a yellow circle. The locations of the villages are indicated by yellow diamonds in the highlighted box. The villages are in close proximity and the diamonds overlap. (b) Baka village along a logging road. (c) Baka forest camp village. (d) Nzime village along a logging road. (e) Number of individuals with missing or decayed teeth. No significant differences were found between groups. (f) Number of individuals with anterior and posterior samples. No significant differences were found between groups. (g) PCA plot of plaque, buccal mucosa, and saliva microbial composition from groups with high market integration (industrial) and low market integration (non-industrial). Photo credits: Sandrine Gallois.

was 14.5 ± 3 million paired-end reads, which is sufficient to obtain an accurate species profile (Hillmann et al., 2018). Eighty-four of 85 libraries contained predominantly known oral bacteria, indicating that they were not subject to overgrowth of environmental taxa during storage and shipping (Figure S6); one library was built from insufficient biological material and was removed for resembling blanks (see Section 2). No significant differences were observed in diversity between extraction or library batches (Figure S7).

3.3 | Oral health comparison of Baka and Nzime individuals

Dental health is known to influence dental plaque species composition, such that differences in dental health between comparison groups can confound the results of comparative microbiological studies by obscuring differences stemming from other factors. Therefore, we compared the dental health of the Baka and Nzime participants in this study. We found that dental health was not substantially different between the Baka and Nzime with respect to the number of decayed teeth, missing teeth (Figure 1f) or teeth with exposed roots ($p > .05$; Figures S3 and S4). Moreover, dental plaque species composition did not cluster samples based on these measures (Figure S5).

3.4 | Comparison of oral site communities across populations with differing levels of market integration

While microbial profiles have been shown to differ across oral sites in North American and European populations, this has not yet been investigated in more diverse global populations. Several oral sites, such as buccal mucosa and saliva, have been individually studied in groups with differing levels of market integration (Clemente et al., 2015; Lassalle et al., 2018; Lokmer et al., 2020; McCall et al., 2020; Nasidze et al., 2011); however, to our knowledge, this is the first microbiome investigation of dental plaque in populations practicing foraging or small-scale agriculture. We tested whether microbiome communities are distinctive between three oral sites—dental plaque, saliva and buccal mucosa—by performing PCA on samples from groups with high and low levels of market integration. The microbial community structure clustered the samples by oral site rather than by population or market integration status (Figure 1g), indicating that the distinct microbial community structure of samples from different oral sites is a general feature across

human populations, and samples from different oral sites cannot be directly compared.

3.5 | Microbial composition of Baka and Nzime dental plaque

To determine the composition and similarity of microbial communities in Baka and Nzime dental plaque compared to dental biofilms previously reported from heavily industrialized populations (Fellows Yates, Velsko, et al., 2021; Velsko, Fellows Yates, et al., 2019), we first compared the presence and abundance of the 50 most abundant microbial species in these groups (88 species in total; Figure 2a; Figure S9; Table S3). Of the 88 species, 22 were among the top 50 most abundant in all three groups, indicating high conservation of the most abundant species. USE dental calculus and BN dental plaque shared 29 of their top 50 most abundant species, while USE dental plaque and BN dental plaque shared 30 of their top 50 species (Table S3). BN dental plaque samples appear to have an intermediate species profile that falls between USE dental calculus and USE dental plaque, suggesting that the absence of a frequent method to reduce biofilm accumulation (i.e. toothbrushing) may contribute to the formation of a more mature dental plaque biofilm among the Baka and Nzime.

3.6 | Alpha-diversity of the Baka and Nzime dental plaque microbial communities

Higher species diversity has been reported in both saliva (Nasidze et al., 2011) and stool (Obregon-Tito et al., 2015) samples of populations with lower levels of market integration compared to European and North American populations, so we next looked at whether there is a higher within-sample diversity in dental plaque from Baka and Nzime individuals than in USE dental plaque or dental calculus. At the species level, we found few significant differences between groups in any metadata tested for the number of taxa or the Shannon Index (Figure 3; Figure S10; Table S5). BN dental plaque has on average more species than USE dental plaque and dental calculus, but the difference is not significant (Figure 3a). We further investigated whether there are differences in the microbial diversity between Baka and Nzime individuals, who differ slightly in their consumption of wild, farmed and processed foods, and have differences in diet based on gender (Duda et al., 2018; Gallois & Duda, 2016).

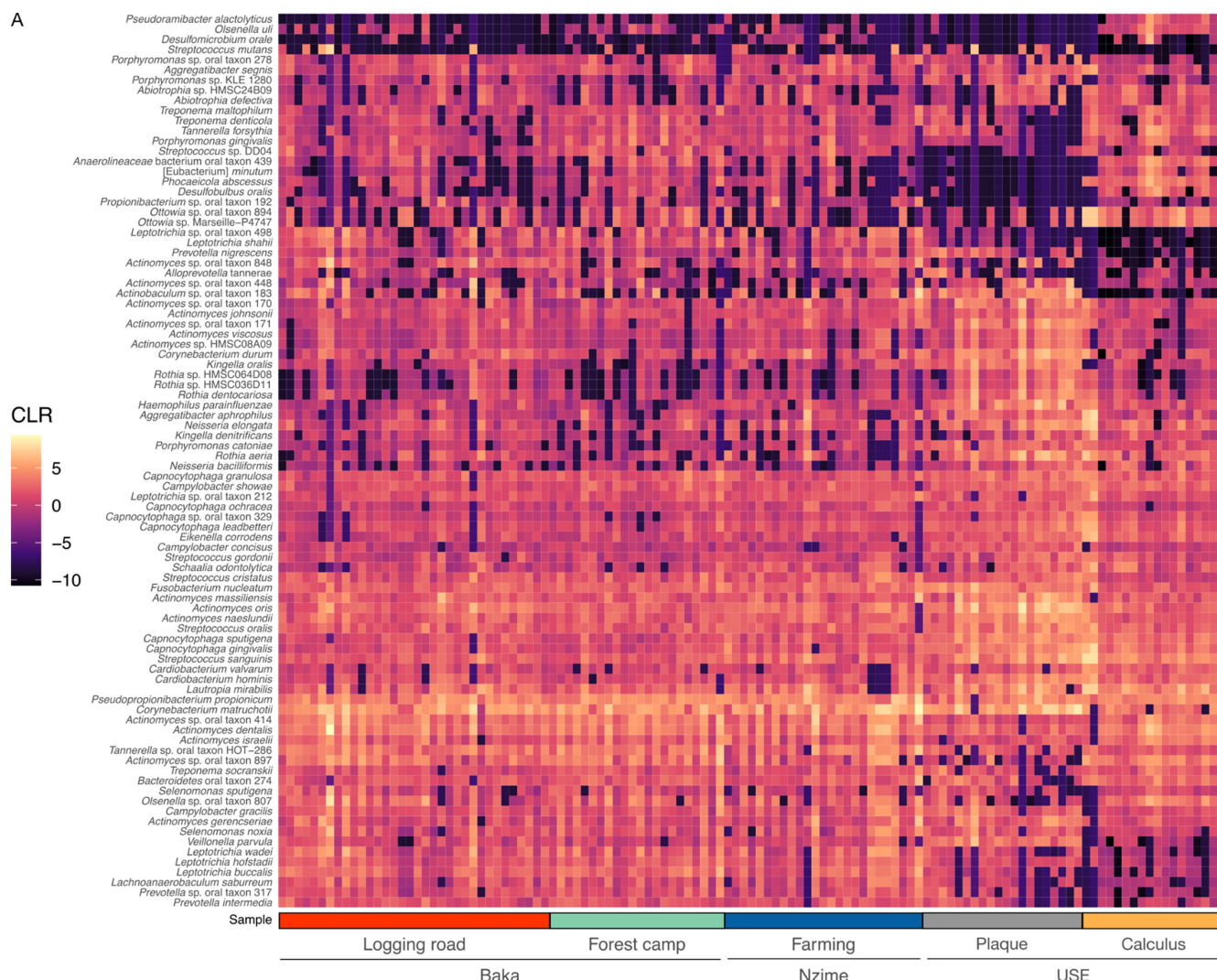


FIGURE 2 Comparison of abundant oral microbial species across groups. Heatmap showing the CLR-transformed abundance of the top 50 most abundant species in each group (BN dental plaque, USE calculus, USE dental plaque). Species are hierarchically clustered but samples are not.

The only significant difference in alpha-diversity within these groups was between anterior and posterior teeth (Figure 3b,c). Taken together, microbial diversity in the dental plaque of diverse human groups appears much less affected by dietary and lifestyle differences than in stool.

3.7 | Comparison to diet- and lifestyle-associated changes in the gut microbiome

In previous gut microbiome studies of diverse human populations, notable differences in the most abundant gut taxa were reported between populations with high and low levels of market integration, particularly an inverse relationship between the phyla Bacteroidetes and Firmicutes, and of the genera *Prevotella* and *Bacteroides* within the phylum Bacteroidetes (Obregon-Tito et al., 2015). To determine

whether these differences are also seen in dental biofilms, we calculated the ratio of Bacteroidetes versus Firmicutes and of *Prevotella* versus *Bacteroides* within the Baka and Nzime plaque samples, as well as within industrial dental plaque and dental calculus (Figure 4). The ratio of Bacteroidetes versus Firmicutes is close to one for all sample groups, with some dispersion in all groups except for USE dental calculus, in which the samples cluster tightly around one (Figure 4a). No significant differences were detected between groups. In contrast, the *Prevotella*:*Bacteroides* ratio is much higher than the Bacteroidetes:Firmicutes ratio, averaging above 100 but with substantial dispersion for all sample groups. Again USE calculus, in which the samples cluster more tightly than in the other groups (Figure 4b), is the exception, and all three BN groups are significantly different from USE dental calculus despite the within-group dispersion. *Prevotella* are prevalent and abundant in dental plaque, which is reflected in the ratio observed in these samples,

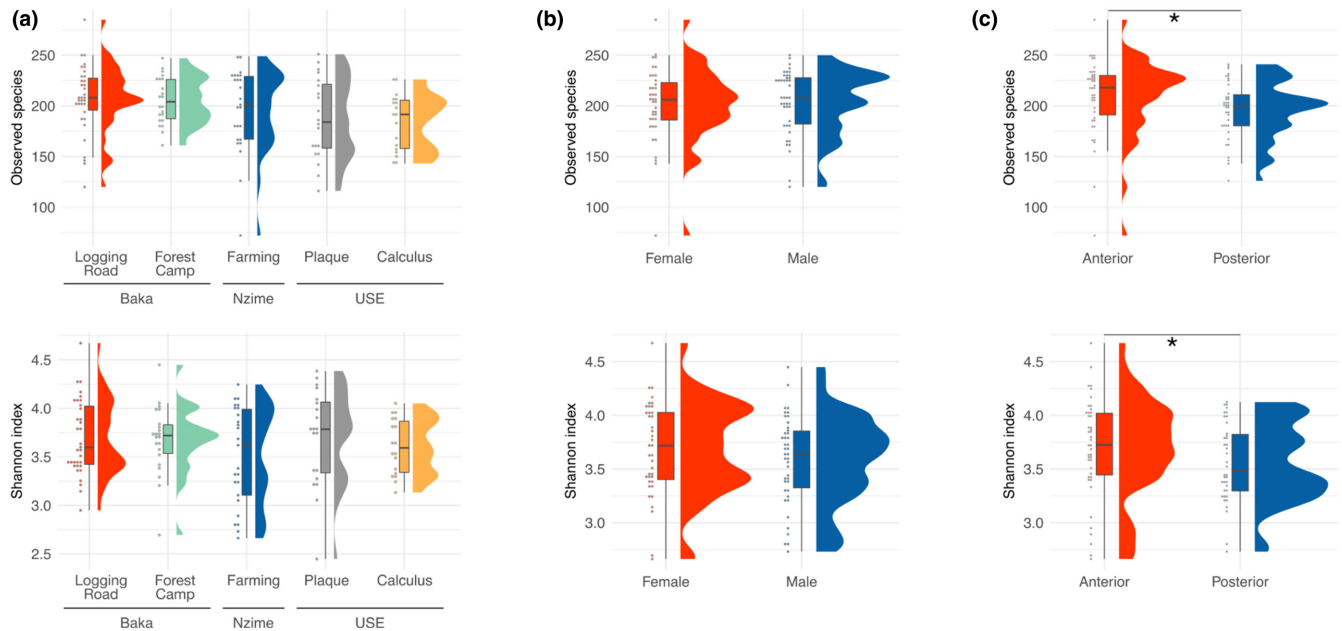


FIGURE 3 Minimal differences in species diversity exist between Baka, Nzime, and USE oral microbiomes. Alpha-diversity metrics number of species and Shannon index by (a) market integration and comparison sample source (USE dental plaque and USE calculus). (b) Baka and Nzime plaque samples only, by gender. (c) Baka and Nzime plaque samples only, by tooth collection site. $p < .05$.

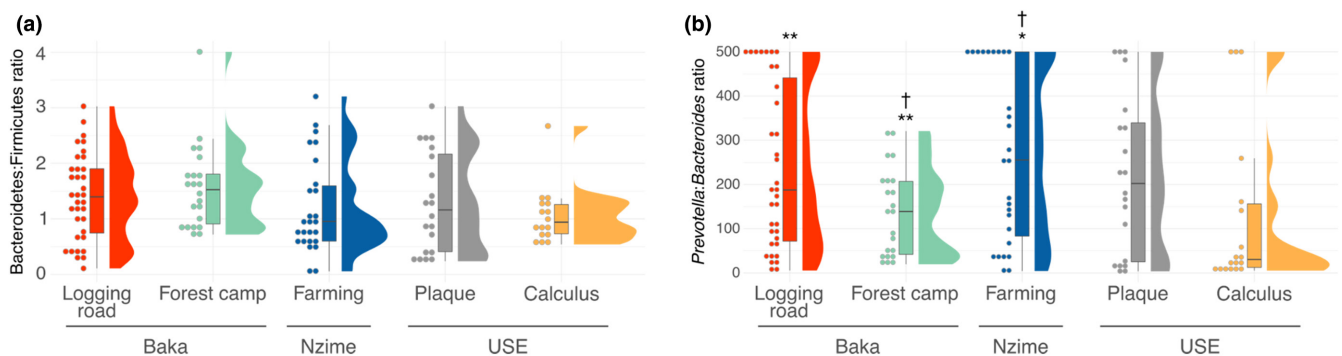


FIGURE 4 Taxon ratios with distinctive differences in gut microbiomes of populations with high and low levels of market integration. (a) Ratio of assignments to the phylum Bacteroidetes over assignments to the phylum Firmicutes. (b) Ratio of assignments to the genus *Prevotella* over assignments to the genus *Bacteroides*. All samples with no *Bacteroides* were assigned a ratio of 500 for plotting purposes, and were excluded from statistical testing. USE - US and European. $**p < .01$, $*p < .05$ versus Industrial calculus. $†p < .05$ within the group between anterior and posterior samples.

yet few species in *Bacteroides* have been described in dental plaque (Dewhirst et al., 2010).

The tight clustering of USE dental calculus samples indicates that dental calculus profiles are much more similar to each other than the dental plaque samples are to each other within each group, suggesting that the mature biofilm community observed in dental calculus is highly consistent across individuals. Based on the substantial within-group dispersion of these taxonomic ratios in both BN and USE dental plaque, it appears that the reported inverse relationship in ratios of Bacteroidetes versus Firmicutes and *Prevotella* versus *Bacteroides*, which are clear in gut microbiomes of populations with high and low levels of market integration, is not a characteristic of dental plaque microbiomes.

3.8 | Presence and abundance of *Streptococcus mutans*

Streptococcus mutans is an oral species strongly associated with dental caries (Lemos et al., 2019; Moye et al., 2014). We observed high counts of *S. mutans* in several BN dental plaque samples, and investigated the abundance of this species within our dataset related to the number of decayed and missing teeth, both of which are dental pathologies associated with dental caries. *S. mutans* was found in 17 of 46 BN dental plaque samples (37%), eight of 20 USE dental plaque samples (40%), and no USE dental calculus samples (Figure 5). Most of the Baka and Nzime individuals with detectable levels of *S. mutans* were over 40 years of age and were female, while all of the USE

individuals were under 40 years of age (due to the HMP study inclusion criteria) and were female. In general, for Baka and Nzime individuals with higher proportions of *S. mutans* (Figure 5a), *S. mutans* was the most abundant *Streptococcus* species (Figure 5b), and these individuals had more decayed and missing teeth (Figure 5c), but this was not a consistent pattern. Although *S. mutans* is strongly associated with dental caries, these data support recent studies that have found a highly diverse dental plaque microbiome associated with dental caries, in which *S. mutans* may not be a central component (Aas et al., 2008; Gross et al., 2012; Johansson et al., 2016; Richards et al., 2017), possibly explaining the difference in pathology observed in samples with *S. mutans*.

3.9 | Microbial community structure in Baka and Nzime dental plaque

We next asked whether the species diversity profiles of BN dental plaque and USE dental plaque and dental calculus are distinct, as has been shown for the stool microbiome of groups with different levels of market integration. To address this, we restricted our analysis to the species that contribute most to the variation in our dataset (Figures S11 and S12; Table S6), as determined by factor analysis, and performed beta-diversity analysis followed by PCA (Figure 6; Figure S14). Within the PCA plot, USE dental plaque and dental calculus samples separate along PC2, which is consistent with

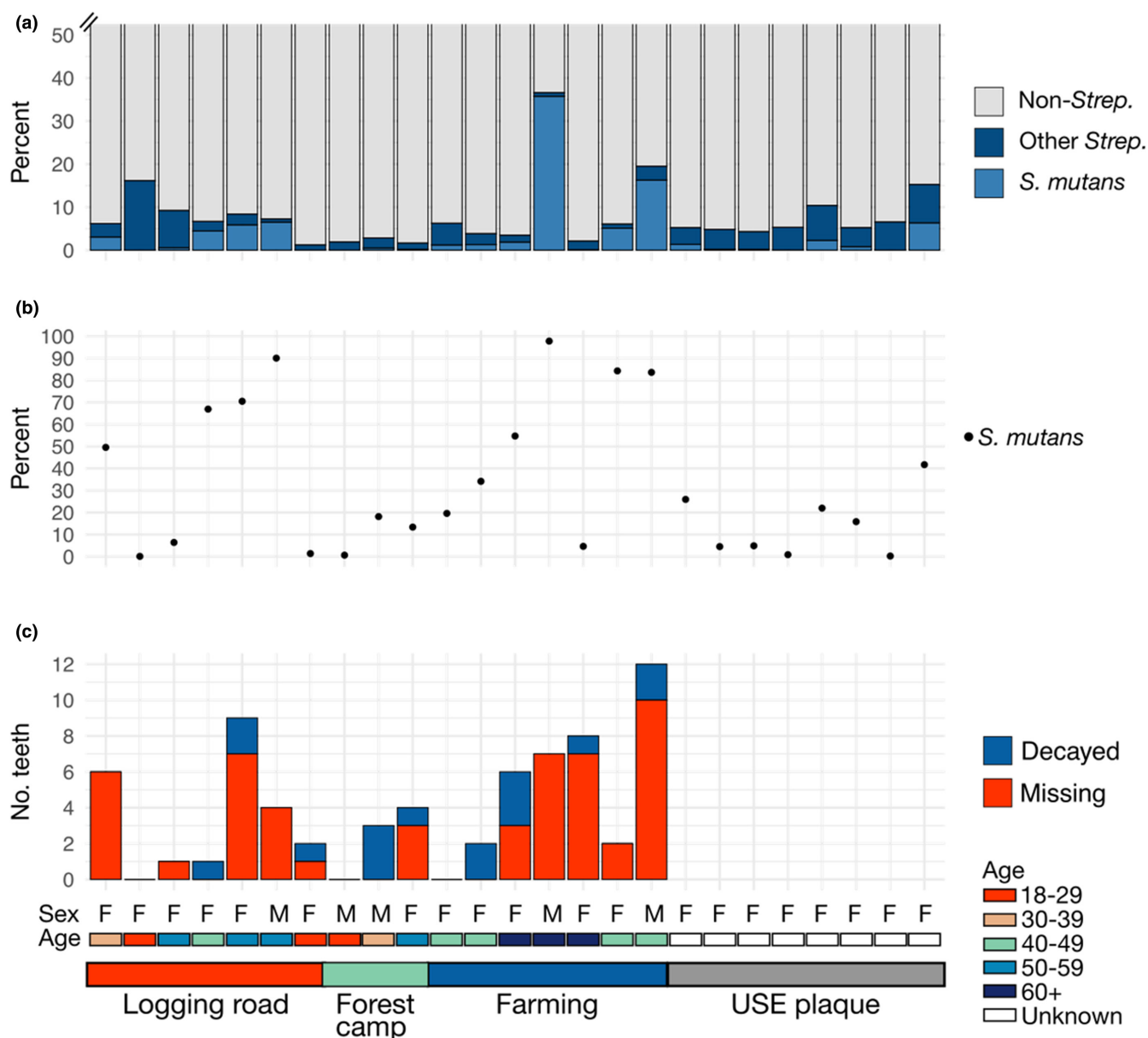


FIGURE 5 *Streptococcus mutans* proportions in dental plaque. (a) Percent of reads assigned to all genera except *Streptococcus* (grey), all *Streptococcus* species except *S. mutans* (dark blue), and *S. mutans* (light blue). (b) Percent of reads assigned to *S. mutans* out of reads assigned to all *Streptococcus* species. (c) Number of missing and decayed teeth. Data is not available for the USE plaque individuals, but exclusion criteria for the HMP study excluded all individuals with teeth missing for dental disease-related causes and individuals over 40 years old. Only individuals for which *S. mutans* is present are shown.

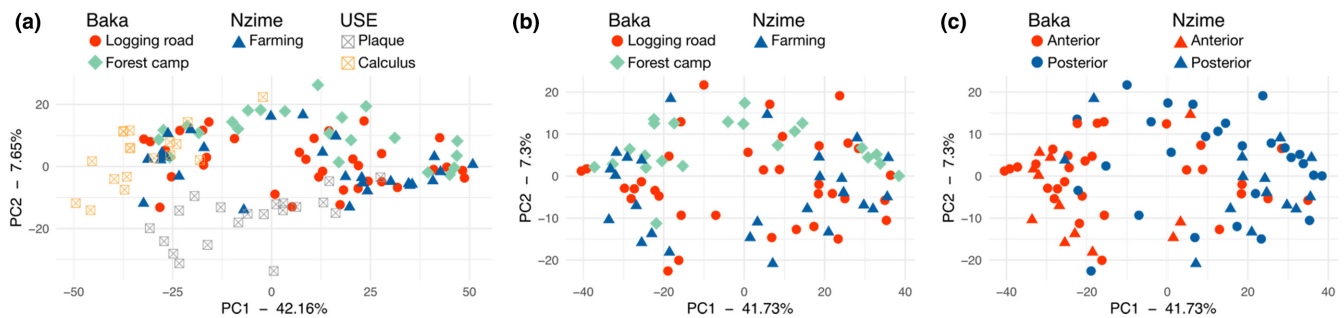


FIGURE 6 Species community structure of dental plaque from Baka and Nzime differs by tooth sampling location and oxygen tolerance. (a) PCA of microbial communities from Baka, Nzime, industrial dental plaque and industrial calculus show the structure of these groups is different. Points coloured and shaped by village location, which corresponds to ethnic group, or industrial sample type. (b) PCA of Baka and Nzime samples with points coloured and shaped by village location, which corresponds to ethnic group. (c) Same as (b) with shapes corresponding to ethnic group and colours corresponding to tooth collection site.

known species compositional differences between the two sample types (Velsko, Fellows Yates, et al., 2019). Baka and Nzime dental plaque beta-diversity, and thus species composition, falls intermediate between USE dental plaque and calculus samples, falling neither entirely within either group, nor entirely separately (Figure 6a). Of the metadata categories examined (Figure 6; Figure S14), only tooth site (anterior vs. posterior) shows patterning, and this appears to be driven predominantly by the BN dental plaque samples (Figure S14D). The BN anterior plaque samples plot closer to USE dental calculus than posterior dental plaque, a trend that is maintained when the PCA is repeated using BN dental plaque samples from only a single tooth site (Figure S15). This sample clustering suggests that there may be taxa that are more prevalent and/or abundant in the posterior BN plaque than either the anterior BN plaque or the USE dental calculus samples, which separates them out from the anterior BN and USE samples along PC1.

When considering variation only within BN dental plaque, the samples appear to form two clusters along PC1, with no distinction by village, market integration, ethnic background, age group, or sex (Figure 6b; Figure S14). Instead, they separate by tooth site (anterior vs. posterior teeth, Figure 6c), which is significant by PERMANOVA ($F=13.0$, $R^2=.141$, $p=.001$), and for which there was no significant difference in the homogeneity of multivariate dispersions ($p>.05$). This suggests that the oral microbiome profile of Baka and Nzime are more strongly influenced by differences in oral environment than by ethnic group, lifestyle factors, or diet.

To determine the species most influencing compositional differences between sample types, we performed differential abundance analysis focused on the differences between USE and BN samples, as well as between anterior and posterior samples of BN dental plaque. Compared to USE dental plaque, the BN dental plaque samples were enriched in anaerobic species, while compared to USE dental calculus, the BN dental plaque samples were enriched in aerotolerant species (Figures 7a,b; Tables S7 and S8). This indicates that the biofilm maturation stage of BN dental plaque is intermediate between that of USE dental plaque and USE dental calculus, which may be related to the absence of frequent biofilm reduction through toothbrushing. Within Baka and Nzime dental

plaque, anterior samples were enriched in aerobic and facultatively anaerobic taxa compared to posterior samples (Figure 7c; Table S9), which may be driving the separation of these sample types in the PCA. From this distinction, there appears to be a gradient of species oxygen tolerance that aligns with the sampling site, where anterior samples have a profile characterized by more aerotolerant species, and posterior samples have a profile characterized by more anaerobic species. This is consistent with anterior teeth having greater exposure to air during breathing, speaking, and eating compared to posterior teeth.

3.10 | Functional characteristics of Baka and Nzime dental plaque

While the taxonomic composition of dental biofilms among the Baka, Nzime, and USE populations were similar, oral taxa may have large pangenomes (Ibrahim et al., 2017; Utter et al., 2020; Velsko, Perez, & Richards, 2019), the collection of genes found in all strains of a species, and it is possible that there are distinctive differences in the gene content of the taxa present. We used HUMAnN3 functional classification to generate a metabolic pathway abundance table for functional analysis. The amount of data that could not be classified by HUMAnN3 was significantly higher in the BN samples compared to USE dental plaque, suggesting more uncharacterized and uncategorized gene content in the BN dental plaque samples (Figure S16A). This is reflected in the lower number of pathways detected in BN dental plaque compared to USE dental plaque and USE dental calculus (Figure S16B,C). To determine if the metabolic pathways distinguish sample groups more distinctly than taxonomic composition, we performed a PCA on the metabolic pathways (Figure 8). Sample group separation based on metabolic pathway content was less distinct between BN dental plaque, USE dental plaque, and USE dental calculus (Figure 8a) than by species taxonomic composition (Figure 6). However the BN dental plaque again separates along PC1 by anterior/posterior tooth collection site, which is significant by PERMANOVA ($R^2=.114$, $F=10.5$, $p=.001$), rather than by ethnic group or village (Figure 8b,c; Figure S16), similar to the species

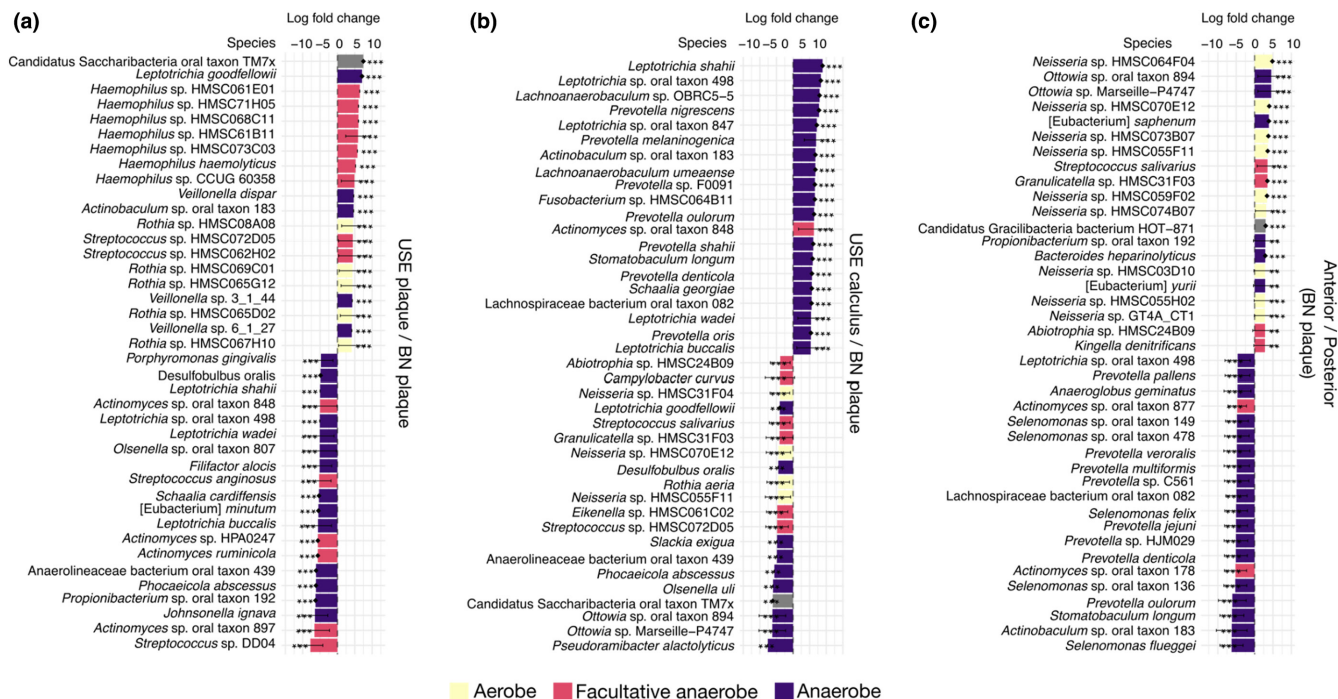


FIGURE 7 Differentially abundant taxa suggest different levels of oxygen tension in USE and BN dental plaque biofilms. Bars are coloured by aerotolerance of each species. (a) Top 40 species enriched in USE dental plaque (positive values) and BN dental plaque (negative values). (b) Top 40 species enriched in USE calculus (positive values) and BN dental plaque (negative values). (c) Top 40 species enriched in BN dental plaque anterior collection sites (positive values) and posterior collection sites (negative values). * $p < .05$, ** $p < .01$, *** $p < .001$; dots on bars indicate structural zeros determined by ANCOM-BC.

profile. Repeating the analysis with the gene families produced a similar sample clustering (Figure S17).

We assessed whether there are distinct metabolic pathways that characterize the sample groups by performing differential abundance analysis. No clear patterns were discernible in the types of metabolic pathways that characterized each group (Figure 8d–i), with one exception. Within BN dental plaque, anterior tooth samples were enriched in pathways related to oxidation–reduction reactions (Figure 8h,i) including PWY-5855, PWY-5856, PWY-5857, PWY-6708, PWY-7371, UBISYN-PWY and SO4ASSIM-PWY (Tables S10–S12), which may be involved in dealing with available oxygen, such as maintaining a reduced environment for the biofilm in a location with higher oxygen exposure.

3.11 | Identification of additional taxonomic diversity in Baka and Nzime dental plaque

Previous studies suggest that there is diversity in human oral and intestinal microbiomes that is not currently represented in databases that are typically used for taxonomic profiling, such as those maintained by the NCBI (Pasolli et al., 2019; Zhu et al., 2021). This trend is also indicated in the BN dental plaque samples analysed here by the lower percentage of reads assigned taxonomy (Figure S8) and the lower number of classified metabolic pathways and genes (Figures S16 and S17) compared to USE plaque and calculus. To determine whether the dental plaque of Baka and Nzime individuals

contain uncharacterized taxonomic diversity, we performed an additional taxonomic profiling using a database containing MAGs that include taxa not represented in NCBI RefSeq from Pasolli et al. (2019). We found that more reads were assigned taxonomy when using a database that includes MAGs than when using a database restricted to RefSeq genomes alone (Figure 9a), although the increase within each group was small and not significant. The unexpectedly low number of classified reads in the USE calculus group is likely because they have a shorter read length (75 bp) than BN plaque (150 bp) and USE plaque (100 bp), which is a bias we observe in Kraken2 classification.

To determine if there were substantial changes in the proportions of known oral phyla identified by profiling with a database including MAGs, we looked at the difference in proportion of assignments to the 14 phyla previously described in the oral cavity (Dewhirst et al., 2010; Figure 9b). Five of the phyla, Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria and Proteobacteria, which include the most prevalent and best-characterized oral taxa, showed little difference in their abundance between profiling with the two databases for all sample groups. These are also the most abundant phyla in supragingival dental plaque (Figure 9c). *Candidatus Gracilibacteria* (formerly GN02) and *Euyarchaeota* showed high variability across samples in each group. Four of the phyla that include less-well characterized oral taxa were uniformly more abundant when profiling with a database including MAGs [Spirochaetes, *Candidatus saccharibacteria* (formerly TM7), Synergistetes, and Chlamydiae], while *Candidatus absconditabacteria* (formerly SR1), *Tenericutes* and *Chloroflexi* were uniformly less

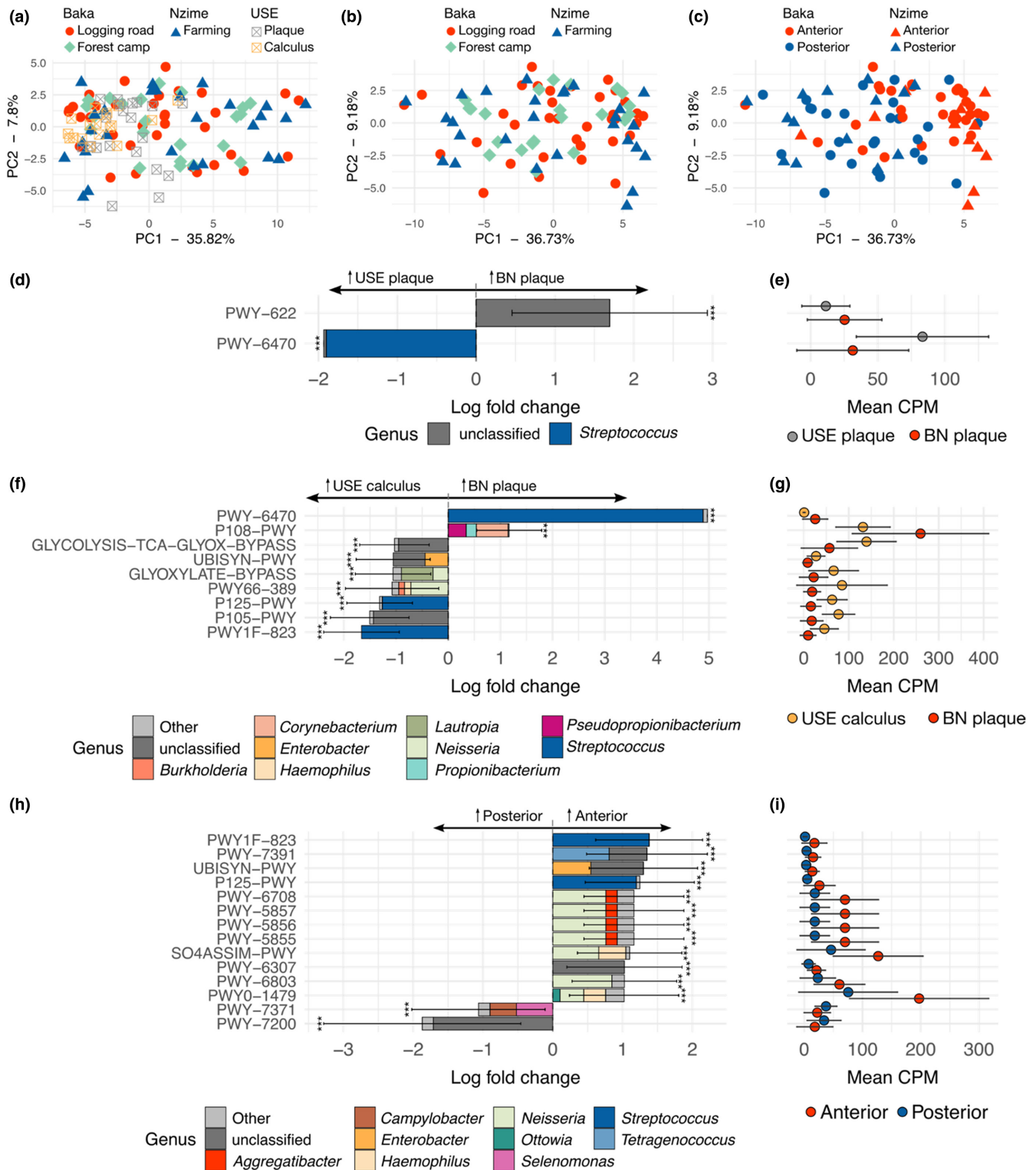


FIGURE 8 Functional differences of dental plaque communities from Baka, Nzime and industrial dental biofilms are not clearly delineated. (a) PCA of metabolic pathway content from Baka, Nzime, USE dental plaque, and USE calculus show the pathway content is similar between these oral microbiome sources. Points coloured and shaped by market integration, corresponding to ethnic group, and industrial sample type. PCA of metabolic pathway content in BN samples with points coloured and shaped by market integration (b) and tooth collection location (c). (d, f, h) Metabolic pathways that are 1-fold more or less abundant between groups are shown. Colours indicate the proportion of the metabolic pathway attributed to each genus present at $\geq 5\%$ abundance in each sample group. (e, g, i) Mean copies per million (CPM) per sample group of the differentially abundant metabolic pathways. (d, e) BN samples compared to USE dental plaque samples. (f, g) BN samples compared to industrial calculus samples. (h, i) Anterior samples compared to posterior samples of Baka and Nzime individuals. *** $q < 0.001$.

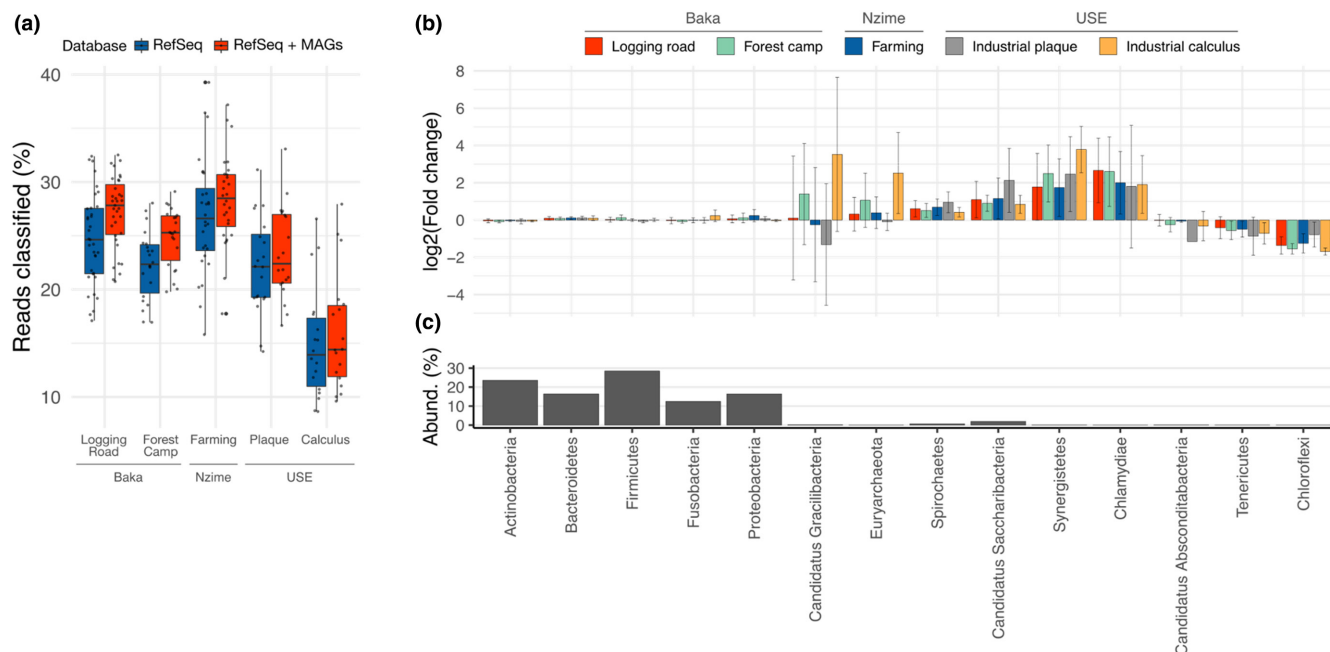


FIGURE 9 Novel taxa are present in dental plaque samples regardless of market integration status. (a) The percent of reads assigned taxonomy increases per sample when using a database that excludes (RefSeq) or includes (RefSeq + MAGs) MAGs. (b) Enrichment or depletion of taxa in the 14 phyla known to exist in the mouth, when profiled with the databases in (a) including MAGs compared to that without MAGs. (c) Relative abundance in supragingival dental plaque of the 14 phyla known to exist in the mouth, averaged from abundance-based tables catalogued on the Human Oral Microbiome Database (hombd.org). The five phyla with least change in abundance between databases (b) are the most abundant (c).

abundant when profiling with a database including MAGs. These results indicate that there is a similar set of taxa being missed in both USE and BN samples, and that these are largely present in the nine less-abundant and less-well studied phyla occupying the oral cavity.

4 | DISCUSSION

The dental plaque microbiome shows high conservation across populations with distinct diets, lifestyles, and different levels of market integration. While the variation in composition of species profiles of BN dental plaque samples does not fall completely within that of previously described USE oral biofilms (dental plaque and dental calculus), the dominant taxa are largely the same as those in North American and European populations. This is in contrast to the gut microbiome, which has been shown to have a highly variable and distinct microbial community structure between groups that consume industrially-processed diets and those that rely more heavily on hunting, foraging, or small-scale farming (Brito et al., 2016; Gomez et al., 2016; Lokmer et al., 2020; Morton et al., 2015; Obregon-Tito et al., 2015; Schnorr et al., 2014). Our results highlight that the distinct characteristics of microbiomes from independent body sites cannot be broadly generalized as pertaining to “the human microbiome”, and care should be taken to refer to the specific site under study.

While several studies have reported pronounced differences between stool microbiomes of North Americans and Europeans

compared to those of individuals with lower market integration from southwest Cameroon and neighbouring Central African Republic, previous studies of saliva found differences that were less pronounced (Gomez et al., 2016; Lokmer et al., 2020; Morton et al., 2015). We found that in dental plaque differences are even less apparent. Instead, dental plaque microbial composition is primarily structured by oxygen tolerance. The high degree of conservation between dental plaque of BN communities, and between BN dental plaque compared to USE dental biofilms, supports that diet has only a limited influence on the dental plaque microbiome. As the major nutrient source for many oral microbes is salivary mucins (Wade, 2013), diet-based fluctuations in oral species abundance may be a minor contributor to overall species profiles. In this way, the oral cavity contrasts sharply with the gut, where digestive passage times are considerably longer and host diet is the major nutrient source for taxa that make up the stool microbiome. Although microbes colonizing gut mucosa, in contrast to stool, have not been extensively studied in diverse populations, we speculate that they may likewise show reduced variation across populations with distinct diets and lifestyles due to their higher reliance on host mucins as a primary source of nutrition (Jones et al., 2018).

One limitation of this study is the reliance on USE samples as a comparative dataset of dental plaque and calculus from a highly urbanized context, rather than samples from individuals in Cameroon living in metropolitan areas with high levels of market integration and urbanization. This leaves a gap in our understanding of the extent to which the differences we observe may have geographic structure. Currently,

there is limited evidence of geographic structuring of oral microbiomes at a community taxonomic level, and only a few geographic differences have been described concerning the prevalence and abundance of particular species in saliva (Gupta et al., 2017). As saliva is a dynamic and open system, and these results have not been replicated in additional studies, the results therefore may be inherent to the conditions of the study in which they were reported. Further, most studies of populations with high market integration and urbanization have sampled from Europe and North America, in contrast to the more global profile of populations with lower levels of market integration and urbanization. This makes it difficult to assess the extent to which oral microbiomes differ based on geographic differences, and inclusion of geographically close populations across a range of market integration and urbanization, such as (McCall et al., 2020) are needed to resolve the extent to which geography shapes oral microbiome taxonomic composition.

Dental plaque microbiomes of USE populations are notably different between healthy teeth and those experiencing dental disease, particularly dental caries or periodontal disease (Nascimento et al., 2017; Socransky & Haffajee, 2005). Therefore, it is important for studies of oral microbiomes to take into account study participant dental health. We found similar rates of dental pathologies between Baka and Nzime individuals, so it is unlikely that there are microbial composition differences between the two groups stemming from differences in dental health. However, there are clinical aspects that could be investigated in future studies, such as site-specific microbial profile differences, or subgingival versus supragingival plaque profiles, which this study was not designed to investigate. These may provide insight into processes of dental biofilm development that sustain health or promote disease, particularly in the absence of regular oral hygiene.

The overall species profile of BN dental plaque samples appears to be intermediate between USE dental calculus and USE dental plaque, a point of caution for future comparative studies. While the most abundant dental plaque taxa of the Baka and Nzime individuals are all well-known oral taxa, they are enriched in anaerobic taxa compared to industrial dental plaque but enriched in aerobic taxa compared to industrial dental calculus. The distinction in aerotolerance of differentially abundant taxa suggests the BN dental plaque biofilms are in a more mature stage of biofilm development than is typical in dental plaque from groups with heavy market integration. This may be due to differences in daily oral hygiene regimens that reduce dental biofilm buildup (i.e. toothbrushing). As dental plaque biofilms develop and mature according to a predictable species succession (Marsh, 2000), the maturation stage can be distinguished by species composition (Ritz, 1967; Socransky et al., 1977). Thus, hygiene practices and biofilm maturation stage are important factors that need to be considered in future dental plaque studies.

It has been proposed that some microbial taxa present in populations with limited market integration may preserve diversity that has been lost in heavily integrated populations (Obregon-Tito et al., 2015; Tett et al., 2019). We present evidence that the dental biofilm communities of not only Baka and Nzime individuals, but also

North American and European individuals, contain taxonomic diversity not currently represented in existing databases derived from NCBI. This suggests that the mouth, in addition to the gut, may be able to sustain higher levels of diversity than can currently be identified; however, the unidentified taxa may have similar prevalence across a range of market integration levels.

It will be of interest in future studies to identify the taxa in oral samples that are not presently in our databases, to characterize their genomic content, and to determine if they are stably and commonly present in oral microbiomes, and whether they are associated with oral health or disease. Further, description and characterization of novel taxa, especially at the strain-level, may help us study co-evolution of microbial species and communities through deep time using ancient DNA (Warinner et al., 2017). One major question faced by ancient metagenomics researchers is whether the microbial taxa currently present in databases are sufficient representatives of those in ancient samples. Including taxa from populations with low levels of market integration in databases for profiling ancient microbiomes may improve taxonomic representation, and therefore taxonomic identification, and aid in clarifying the extent of historical microbiome diversity.

Here, we show that subsistence strategy minimally shapes the dental plaque microbiome based on the taxonomic diversity currently represented in large databases such as NCBI. While there are small differences in species profiles between dental plaque from groups with high and low levels of market integration, they appear to be largely related to biofilm maturation and dental hygiene practices that delay maturation (i.e. toothbrushing). Consequently, dental calculus, which represents a more mature and less disturbed oral biofilm, may be a more appropriate comparative material for future studies of dental biofilms from populations practicing limited dental plaque removal. We show that diet and lifestyle factors influence dental plaque microbial communities to a much lower extent than they do the gut microbiome, indicating that different forces shape the taxonomic composition at these two body sites. Such distinctions have implications for understanding how human microbiomes respond to industrialization-related changes (diet, hygiene, urbanization) and how this relates to health and disease—both in populations currently undergoing these changes, and in populations that experienced them in the past.

AUTHOR CONTRIBUTIONS

C.W. and A.H. conceived the project. S.G. contacted involved communities, collected samples and returned results to the communities. R.S. performed data generation. I.M.V. performed data analysis and wrote the manuscript, with contributions from all authors.

ACKNOWLEDGEMENTS

We thank Ernest Simpo for assistance with interpretation during participant recruitment and sample donation, Antje Wissgot for the sequencing assistance of the dental plaque samples, and Alexander Hübner for constructing the Kraken2 databases. This research was supported by the European Research Council under the

European Union's Horizon 2020 research and innovation program, grant agreement number STG-677576 ("HARVEST"), the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy (EXC 2051, Project-ID 390713860), the Werner Siemens Stiftung ("Paleobiotechnology"), and the Max Planck Society. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts to declare.

DATA AVAILABILITY STATEMENT

All raw sequencing data is deposited in ENA under the project code PRJEB42572. All scripts for analysis can be found in the github page for the project https://github.com/ivelsko/Cameroon_plaque.

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SUPPORTING INFORMATION

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How to cite this article: Velsko, I. M., Gallois, S., Stahl, R., Henry, A. G., & Warinner, C. (2023). High conservation of the dental plaque microbiome across populations with differing subsistence strategies and levels of market integration. *Molecular Ecology*, 32, 3872–3891. <https://doi.org/10.1111/mec.16988>