

## Maintaining oral health for a hundred years and more? - An analysis of microbial and salivary factors in a cohort of centenarians

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### ABSTRACT

**Aim:** To investigate associations between oral health-related conditions and the oral microbiome in a representative study sample of centenarians.

**Materials and methods:** Clinical and microbial parameters from 54 centenarians were assessed in the Heidelberg Dental Centenarian Study. Plaque and salivary samples were collected, and the microbiota was characterized by 16S rRNA gene sequencing.

**Results:** Diversity and structure of the oral microbiome were mainly influenced by the presence of natural teeth and the number of decayed, missing, and filled teeth ( $0.028 \leq p \leq 0.001$  in plaque and salivary samples). Centenarians with less caries experience possessed a more diverse oral microbiome. Moreover, the number of dental visits also showed a significant influence on the microbial composition. Most centenarians presented with hyposalivation (mean stimulated flow rate =  $0.84 \pm 0.55$  ml/min), a low buffering capacity, and an acidic pH. The latter was between 5.0 and 5.8 in 46.3% of cases, and we observed that an increased salivary pH correlated with higher alpha-diversity in both salivary and plaque samples.

**Conclusion:** The microbiome diversity correlated significantly with successful oral aging. In addition, regular dental visits were a beneficial factor. However, diversity can be negatively influenced by hyposalivation, associated with pH changes due to aging effects.

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### Introduction

As the population in most industrialized nations grows older, including the rapidly expanding group of those reaching the remarkable age of 100 years or more, efforts are being made to gain insights into the effects of aging on oral health. Next to well-known influencing factors such as oral hygiene, healthy diet, and adequate fluoridation, recent research has also focused on salivary and microbial factors as promising indicators for oral health monitoring and disease diagnostics [1,2].

The oral microbiome consists of complex microbial communities forming on and around the teeth and on all soft tissues and can be determined on an individual basis, e.g. in supragingival dental plaque and saliva. The shift from homeostasis to dysbiosis in the dental plaque microbiota, in particular, is associated with two major oral diseases, caries, and periodontitis [3,4]. Oral health is reflected in the balance between host and oral microorganisms. The ecological plaque hypothesis [5–8] describes the currently most widely accepted theory of the etiology and pathogenesis of dental caries. This hypothesis combines the specific plaque hypothesis (acknowledging

the role of cariogenic bacteria such as *Streptococcus mutans* and lactobacilli [9–13]) and the unspecific plaque hypothesis, assuming that a shift in the described equilibrium (dysbiosis) enables the caries process. Frequent consumption of fermentable carbohydrates increases the proportions of acidogenic and aciduric species in saliva, which gradually displace the non-pathogenic microbiota [7,14,15].

Periodontal diseases are associated with special, well-known pathogenic species, most recognized from a pivotal study by Socransky et al. in 1998 [3]. For instance, bacteria identified to be key pathogens in periodontitis are *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Aggregatibacter actinomycetemcomitans*, and *Prevotella intermedia* [16]. More recent research has led to the discovery of other, putative periodontal pathogens [17], such as *Synergistetes* phylum members [18], resulting in the understanding that periodontitis is a complex dysbiotic disease. [19,20]

Saliva plays multiple important roles in maintaining oral health. Firstly, it protects against caries [21], and a reduction in the salivary flow rate predisposes

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to the development of dental decay, a problem common in higher age groups due to frequent medication and impaired salivary gland function [22]. Other physicochemical properties such as pH and buffering capacity are additional influencing factors [23]. Secondly, although bacteria in a planktonic state are not generally regarded as direct causal agents of oral diseases, saliva is a major vector for the intraoral transmission of pathogenic bacteria [24,25]. It is known that the composition of the intraoral microbiome changes with age [26], and research into the gut microbiome has proposed possible determinants of healthy aging with a special health-related composition [27,28]. It is therefore, possible that these factors may equally play a role in the oral cavity and in retaining one's natural teeth for 100 years or more.

Because most centenarians escape life-threatening conditions such as cancer and circulatory diseases and remain functionally independent for most of their lives [29,30], their secret of longevity and health has long been of great scientific interest. However, their oral health status has not yet been widely studied [31]. It is for this reason that the Heidelberg Dental Centenarian Study (HD100Z) was initiated, the first comprehensive population-based study on the epidemiology of centenarians' oral health in Germany. In our previous publications, we could show that the majority of examined centenarians still had natural teeth, and although the prevalence of severe periodontal disease was low, the caries experience increased steadily with aging. [32,33]

Given the aforementioned associations of salivary and microbial associations with oral health, we aimed to investigate associations between oral health-related conditions and the oral microbiome of supragingival dental plaque and saliva in a representative study sample of centenarians in order to identify detrimental or beneficial factors in maintaining good oral health at high age.

## Methods

The Heidelberg Dental Centenarian Study (HD100Z) was designed as a prospective, cross-sectional, population-based survey and clinical examination among persons born before 1920, living in South-Western Germany. The study was approved by the Ethics Committee of the Medical Faculty of the Heidelberg University (S-168/2019) and registered with the German Clinical Trials Register (DRKS 00017128, date of registration: 20/05/2019). Considering sample sizes reached by previously published studies on centenarians [30,34,35] and the exploratory nature of the study, the target for patient recruitment was set at 50 study participants. Informed written consent was obtained from all study participants. This study

followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [36]. Contact information on all persons born before 1920 was obtained via the 183 registries in the catchment area (from Karlsruhe in the south to Darmstadt in the north and from the Rhine-Palatinate to the Neckar-Odenwald district in its east-west extension) in April/May 2019. Fifty-five centenarians agreed to take part in the study, however, one centenarian refused microbial sampling. Consequently, 54 centenarians could be included in this analysis and were visited at their residence between May and October 2019. In three cases, xerostomia was so severe that the collection of salivary samples was not possible, and only supragingival plaque samples were collected and analyzed.

## Clinical investigation

Before entering the interview or examination, a shortened version of the Mini-Mental State Examination (short MMSE, max. 21 points) [37] was performed. A score below 5 was considered as a termination criterion. Sociodemographic items and dental health behaviours (i.e. disability, nursing care levels, frequency of tooth brushing, etc.) were assessed using the standardized sociodemographic survey for older adults aged 75–100 years used in the 5<sup>th</sup> German Oral Health Study (DMS V) [38]. Caries experience was recorded via the Decayed, Missing, and Filled Teeth Index (DMFT), according to WHO basic methods [39]. For further information regarding interview and clinical examination methods, dental health behaviors, caries experience, functional capacity, periodontal, and peri-implant conditions, please see our previous publications. [32,33]

## Sample collection

Supragingival plaque and salivary samples were collected using OMNIgene ORAL OMR-110 and OMNIgene ORAL OM-505 (DNA Genotek) Kits, following the manufacturer's collection instructions [40,41]. The salivary sample was expectorated into the funnel of the OMNIgene ORAL OM-505 kit and thus collected separately from the saliva used for physicochemical analysis (see below). In case of edentulous participants, the plaque was swabbed along the teeth of their complete dentures. Samples were stored at  $-80^{\circ}\text{C}$  until DNA extraction. DNA extractions were performed using the MasterPure™ Complete DNA and RNA Purification Kit (Lucigen Corp. Middleton, WI). Subsequently, the 16S rDNA sequencing library was prepared as previously

described [42]. In short, the library was prepared using two PCR reactions to amplify the V4 region (primers 515 F and 806 R) of the *16S rRNA* gene and to ligate to Illumina adapters and barcodes to be sequenced on a MiSeq instrument (2\*300 cycles). Salivary pH, stimulated salivary flow rate and buffering capacity were assessed using the Saliva-Check Buffer-Kit (GC, Leuven, Belgium), according to the manufacturer's protocols [43]. The pH was measured by placing the provided pH strip into a sample of resting saliva for 10 s and then assessing the resulting color. To measure the stimulated salivary flow, the participant was asked to chew the supplied piece of wax and expectorate after 30 s, then continue chewing for 5 min, expectorating every 15–20 s. The flow rate was subsequently calculated in ml/min. To test the buffering capacity of stimulated saliva, one drop of the collected stimulated saliva was applied on each of the three test pads of the buffer test strip, excess saliva was drained onto a tissue, and the color was then assessed after 2 min. Salivary flow rates were grouped according to Ericsson and Hardwick [44,45].

### Statistical analyses

Data analysis of the cohort was performed with SPSS, Version 24.0 [46]. Characteristics of the study population were analyzed through descriptive statistics. Mean  $\pm$  SD of continuous variables and proportion and frequency of categories of factor variables are reported.

16S data were analyzed using R 3.1.4 with the R package *dada2* as previously described [42]. Descriptive indices as alpha-diversity (Shannon index), richness (numbers of ASVs observed), evenness (Pielou index), and dominance (Berger-Parker index) were calculated using the package *microbiome*. The impact of clinical parameters and sources of sampling on these indices was analyzed using the Mann-Whitney-Wilcoxon test for categorical data and the Spearman correlation for ranked, scored or measured clinical data. Beta-diversity was assessed by calculating weighted Unifrac distances. Principal coordinate analysis (PCoA) was used to illustrate clustering of samples. PERMANOVA was performed to assess the statistical significance of differences between the two samples (plaque and saliva) and the impact of the different clinical parameters on the microbial structure. Differential abundance between groups was evaluated using *Deseq2* [47]. *p* values are purely descriptive and regarded considerable if  $\leq 0.05$ .

## Results

### Study population

Table 1 presents the demographic, dental health and behavioral characteristics of the study population. The mean age of study participants was  $101.1 \pm 1.6$  years, 83.3% were female. Most centenarians were non-smokers (75.0%) and not diabetic (77.8%). The number of drugs taken for various diseases ranged from 0 to 13, with a median intake of 6. Regarding oral hygiene habits and capabilities, few were still capable of performing adequate oral hygiene (18.5%), and approximately half of the participants did not adhere to common recommendations (frequency of tooth brushing < twice a day, last dental visit > 1 year, complaint-oriented utilization of dental services). The DMFT ranged between 11 and 28 (mean: 25.2, SD 4.0), natural teeth were present in 63.0% of centenarians.

### Caries experience

We observed a significant difference between the microbial structure in saliva and plaque (PERMANOVA:  $R^2 = 0.05$ ,  $p < 0.001$ ). Figure 1 shows the abundance on genus level in centenarians with different DMFT values, arranged in the order of increasing DMFT. The diversity of the oral microbiome significantly correlated with the DMFT index (Spearman correlation:  $\rho = 0.43$ ,  $p = 0.002$  (in saliva),  $\rho = 0.49$ ,  $p < 0.001$  (in plaque), i.e. centenarians with less caries experience possessed a more diverse oral microbiome.

### Dentate vs. edentulous

Analogously, as shown in Figure 1, the diversity was higher in dentate individuals than in edentulous individuals ( $p < 0.001$  in plaque and saliva). The results of PCoA illustrated the differences in the microbial structure (Figure 2). This difference could also be seen when examining those with successful oral aging, i.e. fulfilling the World Health Organization goal of retaining  $\geq 20$  teeth (Figure 3(a,b)). These individuals showed a significantly more diverse oral microbiome in both saliva and plaque. Similarly, an analysis of the type of denture worn showed a significant difference between edentulous centenarians with total prosthesis and those centenarians with remaining teeth, whilst differences between the group of centenarians with remaining teeth and with or without removable dentures were small (Figure 3(c,d)). Table 2 shows the results of the PERMANOVA analysis in plaque and in saliva: the DMFT index, remaining natural teeth and the frequency of dental

**Table 1.** Characteristics of the study population for microbial analysis (n = 54).

Variable		Mean (SD)	n (%)
Age (years)		101.1 (1.6)	
Sex	Male		9 (16.7)
	Female		45 (83.3)
Level of education	Low		29 (53.7)
	Medium		13 (24.1)
	High		12 (22.2)
Recognized disability	No		26 (48.1)
	Yes		28 (51.9)
Degree of disability		Mean (SD)	83.8 (20.6)
Residence	Care facility		25 (46.3)
	At home		29 (53.7)
Recognized nursing care level	No care level		7 (13.0)
	Slight 1		2 (3.7)
	2		12 (22.2)
	3		20 (37.0)
	4		12 (22.2)
	Most severe 5		1 (1.9)
Smoking status	Non-smoker		41 (75.9)
	Former smoker		8 (14.8)
	Pack years	Mean (SD)	17.8 (19.4)
	Years since quitting	Mean (SD)	54 (11.3)
	Active smoker		1 (1.9)
	No response		4 (7.4)
Diabetes	No		42 (77.8)
	Yes		12 (22.2)
Number of drugs taken		Mean (SD)	5.5 (3.1)
Dentate	No		20 (37.0)
	Yes		34 (63.0)
DMFT		Mean (SD)	25.2 (4.0)
Capability of oral hygiene [48]	Normal		10 (18.5)
	Slightly reduced		21 (38.9)
	Greatly reduced		18 (33.3)
	Non-existent		5 (9.3)
Frequency of tooth/ denture brushing	≥ Twice a day		27 (50.0)
	Once a day		21 (38.9)
	Once a week		5 (9.3)
	Never		1 (1.9)
Dental service utilization	Control-oriented		27 (50.0)
	Complaint-oriented		26 (48.1)
Last dental visit	In the last year		28 (51.9)
	In the last two years		6 (11.1)
	In the last five years		4 (7.4)
	More than five years ago		16 (29.6)

visits were the most important clinical parameters influencing the microbiome structure in both models.

By using Deseq2 for differential abundance analysis, we could show that this difference in diversity was due to a number of bacteria differentially enriched or depleted between dentate and edentulous centenarians (Figure 4(a,b)). At the level of amplicon variants, a higher relative abundance of 43 ASVs could be found for dentate individuals in salivary samples (vs. 2 ASVs classified as *Streptococcus* spp. which were more often found in edentulous participants). Similar results were found in plaque samples, where a total of 74 ASVs had a higher relative abundance in dentate individuals, vs. 11 ASVs with a higher relative abundance in edentulous centenarians. Of the latter, *Streptococcus* spp. also had the highest relative abundance. However, the Log<sub>2</sub> (fold change) values regarding differences in the abundance of *Streptococcus* spp. between dentate and edentulous individuals in both plaque and saliva were moderate (Log<sub>2</sub> (fold change) between -1.14 and -3.43, p adj. ≤ 0.001, Figure 4b). Among the

many species with a relative higher abundance in dentate individuals, anaerobic *Prevotella* spp., *Campylobacter* spp., *Anaeroglobus* spp., *Selenomonas* spp., *Porphyromonas endodontalis* and *Fusobacterium* spp. had the highest Log<sub>2</sub> (fold change) in both plaque and salivary samples (Log<sub>2</sub> fold change > 20 for all species listed, p adj. < 0.001, see Figure 4b).

At the genus level, a higher relative abundance of 16 genera was found in the salivary samples and of 23 genera in the plaque samples of dentate individuals. Among those with known associations to oral diseases, the higher abundance of *Aggregatibacter* (Log<sub>2</sub> fold change = 6.29 and 6.30 in saliva and plaque, respectively, p adj. < 0.001) in both sample types, as well as of bifidobacteria and *Scardovia* in plaque samples of dentate individuals (Log<sub>2</sub> fold change = 4.18 and 9.83 respectively, p adj. < 0.001, Figure 4b) may be noted, whereas the genus *Streptococcus* was more frequently found in the edentulous in both sample types (Log<sub>2</sub> fold change = -1.14 (saliva) and -2.44 (plaque), p adj. ≤ 0.001).

At the phylum level, *Actinobacteria* and *Firmicutes* were more common in edentate individuals, whereas *Spirochaetes* and *Synergistetes* were more frequent in dentate individuals, both in plaque and in salivary samples.

**Periodontal pathogens**

The abundance of several periodontal pathogens, e.g. *P. gingivalis*, *T. forsythia*, and *P. intermedia*, also varied strongly; they were significantly more frequently found in plaque samples of dentate centenarians (please see Figure 4(a,b) for further details). This also included *Fusobacterium nucleatum*, which has coaggregation properties allowing it to transport periodontopathogenic bacteria [49]. However, the periodontitis diagnosis according to the Periodontal Disease Surveillance Project by the Centers for

Disease Control and Prevention and the American Academy of Periodontology (CDC/AAP) case definitions [50] or the Community Periodontal Index (CPI) [51] did not show a significant impact on the structure or the alpha-diversity of the oral microbiome.

**Additional potential risk factors**

Neither the degree of disability, nursing care/type of residence nor the presence of diabetes nor smoking showed a significant impact on the structure or the alpha-diversity of the oral microbiome.

**Salivary parameters**

The results of the salivary examinations are shown in Table 3. The viscosity of the unstimulated saliva was normal in approximately half of centenarians and was

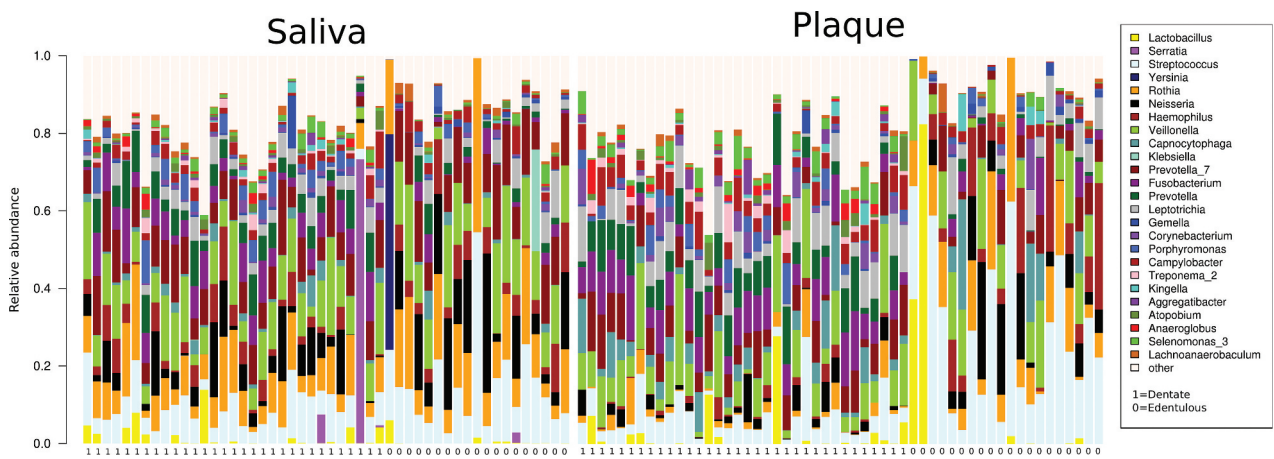


Figure 1. Microbial composition in salivary and in plaque samples, in order of increasing DMFT (only the 25 most abundant genera are represented).

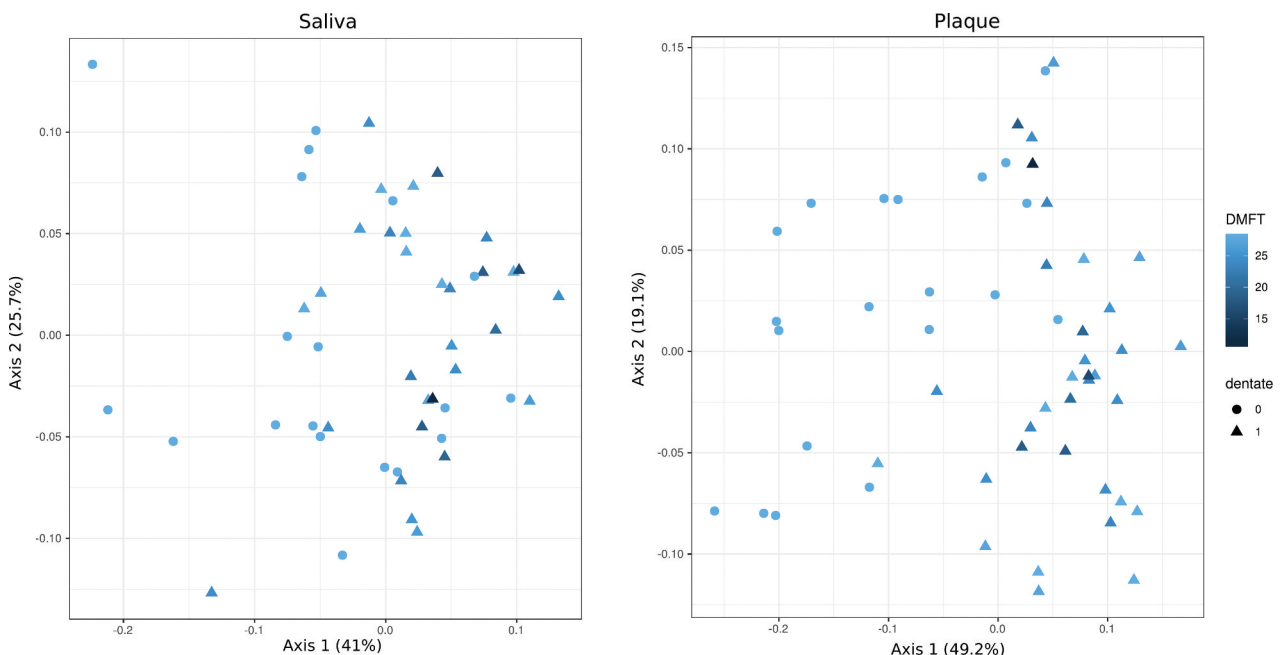
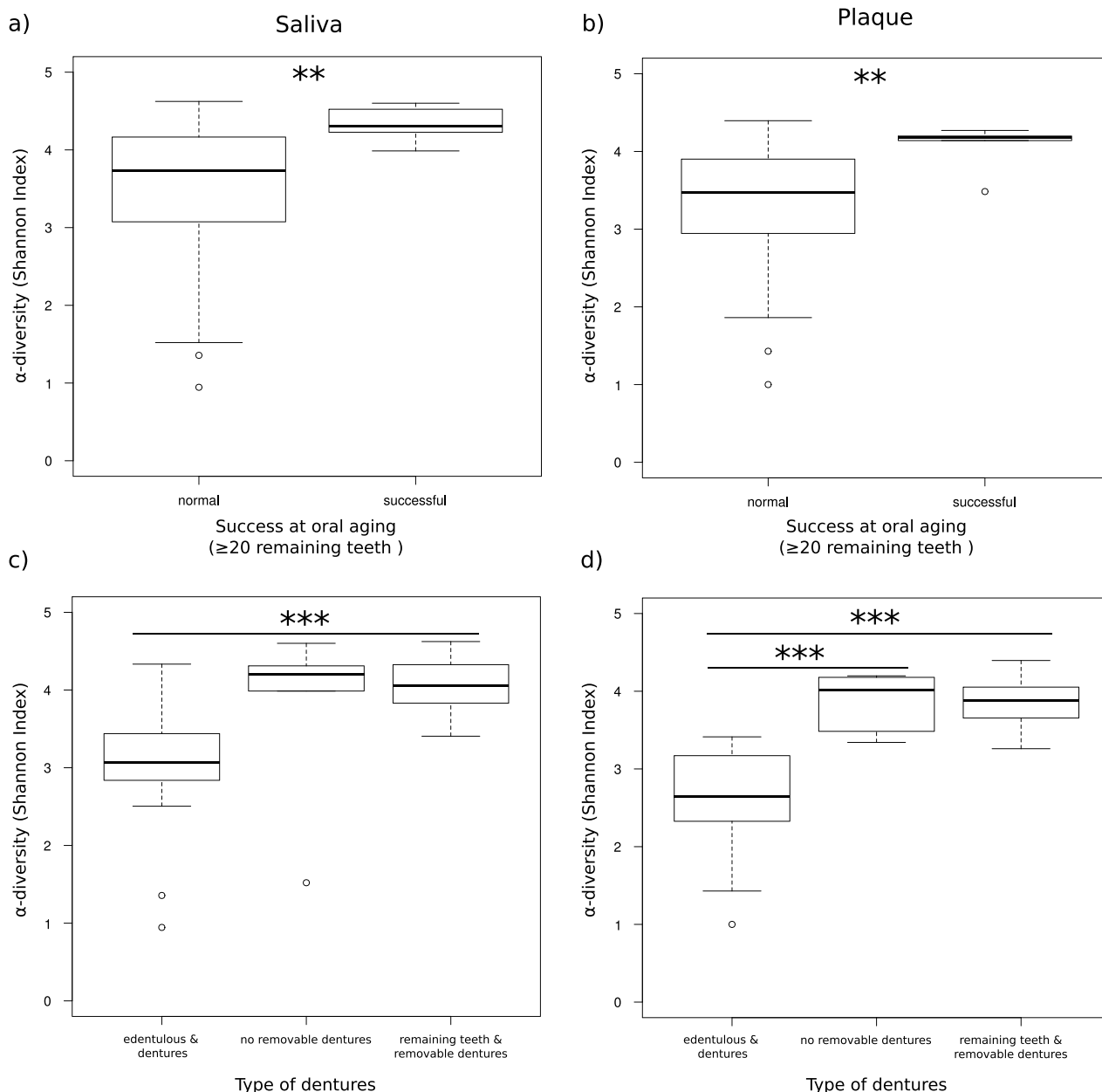


Figure 2. PCoA analysis of microbiota in dentate and edentulous centenarians with different DMFT.



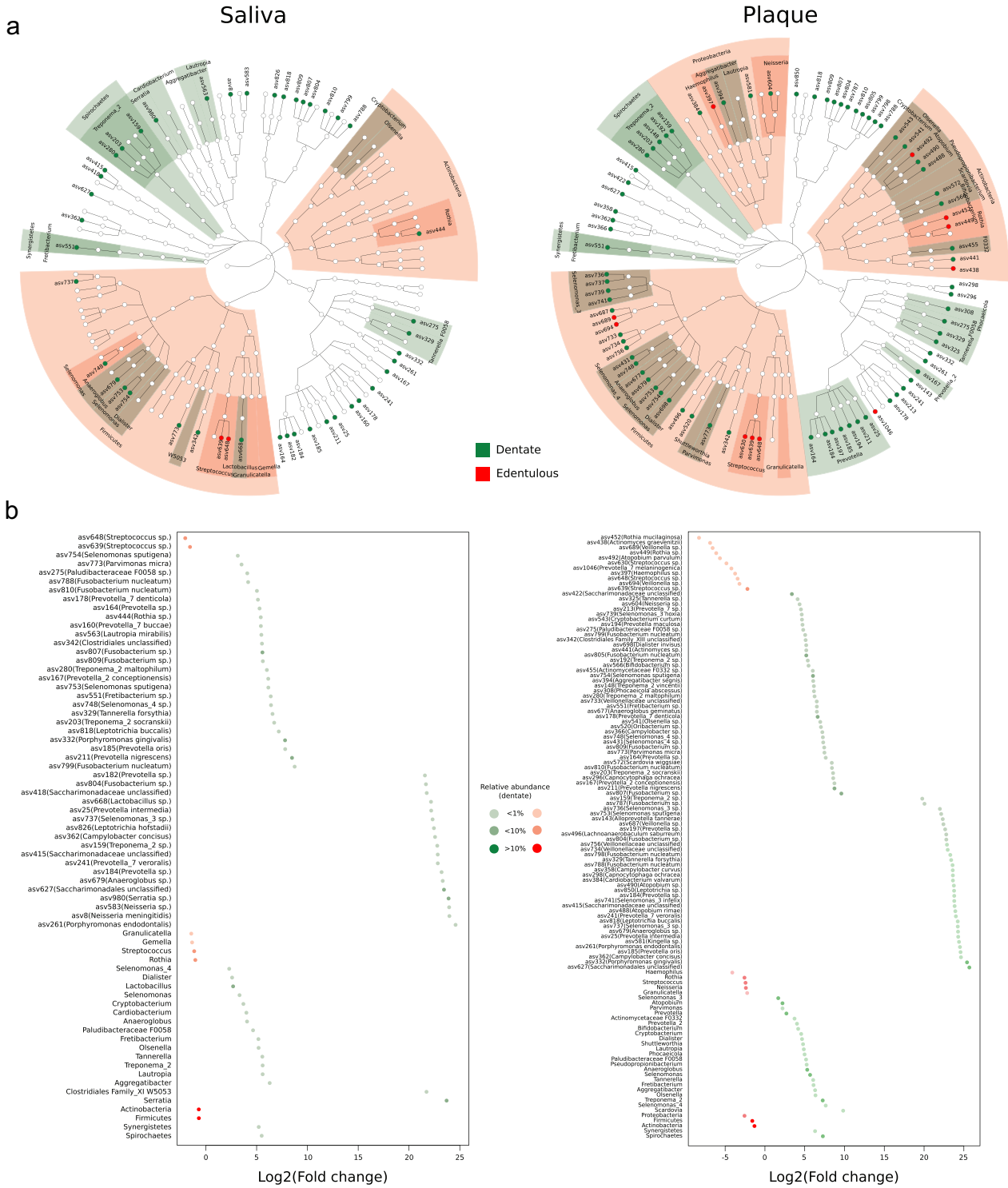
**Figure 3.** Microbial diversity in successful oral ages ( $\geq 20$  remaining teeth) and others, in (a) salivary and in (b) plaque samples. Impact of the type of denture on the microbial diversity in (c) salivary and in (d) plaque samples.

**Table 2.** Influence of clinical parameters on the microbiome structure and variance (PERMANOVA).

Parameter	Saliva		Plaque	
	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value
Dentate	<b>0.07</b>	<b>&lt;0.001</b>	<b>0.14</b>	<b>&lt;0.001</b>
DMFT	<b>0.04</b>	<b>0.028</b>	<b>0.05</b>	<b>&lt;0.001</b>
Capability of oral hygiene [48]	0.02	0.451	0.02	0.604
Frequency of tooth/ denture brushing	0.01	0.843	0.01	0.89
Frequency of dental visits	<b>0.03</b>	<b>0.038</b>	<b>0.04</b>	<b>0.015</b>
Dental service utilization	<b>0.03</b>	<b>0.044</b>	0.03	0.053
Residence	0.03	0.154	0.02	0.299
Disability	0.02	0.333	0.02	0.205
Degree of disability	0.02	0.263	0.02	0.207
Nursing care	0.01	0.853	0.01	0.925
Degree of nursing care	0.03	0.131	0.02	0.635
Sex	0.02	0.477	<b>0.03</b>	<b>0.01</b>
Age	0.02	0.329	0.02	0.251
Educational level	0.02	0.353	0.02	0.209

frothy-bubbly or sticky-frothy in the other half, showing an increased viscosity. In 46.3% of the study participants, the resting saliva was very acid

(pH = 5.0–5.8). Only in one-third of the centenarians, the pH was within a normal range (pH = 6.8–7.8). The mean stimulated salivary flow rate was low



**Figure 4.** Differential abundance of taxa between edentulous and dentate patients. The differential analysis was performed with Deseq2 at the level of amplicon variants, genus and phylum. Taxa over-represented in one group are color-coded (green: dentate, red: edentulous). (a) Cladograms showing the differentially abundant taxa between edentulous and dentate patients in salivary and plaque samples. Overlapping shading is due to significant differences in the genus or phylum, in cases where a genus is more abundant in the dentate group but the whole phylum is more abundant in the edentulous group. (b) Relative abundance and log<sub>2</sub> (fold change) of the differentially abundant taxa between edentulous and dentate patients. The differential analysis is shown at the level of amplicon variants (top part), genus (middle part) and phylum (bottom part).

(0.84 ± 0.55 ml/min). Only 26.4% had a normal salivary flow rate of 1–3 ml/min. No significant influence of the number of drugs taken on the salivary flow rate could be found. Furthermore, the buffering capacity was low or very low in most centenarians (84.9%).

While the buffering capacity had a minor impact on the structure of the salivary microbiome ( $R^2 = 0.04$ ,  $p$ -value = 0.012), and the salivary flow rate showed no influence on its structure or diversity, the salivary pH significantly influenced the alpha-diversity of the oral

microbiome. Centenarians with a higher pH also presented with a higher diversity (Figure 5).

## Discussion

In this study, combining salivary and microbial analyses with a detailed clinical examination of 54 people with a mean age of 101 years, we showed that the oral microbiome structure and diversity differed significantly in those fulfilling the criteria of successful oral aging. When interpreting the results, it should be noted in advance that this is a cross-sectional study of a relatively small, but nevertheless representative cohort. It can therefore be assumed that the occurrence of oral diseases such as caries or periodontal disease in subgroups only allows an exploratory description and further studies are necessary to clarify disease-related microbial relationships. For the first time, however, this study provided highly valuable insights into the oral microbiome of very old people and gave indications of relevant scientific topics that should be further investigated with special regard to the oral diseases caries and periodontitis.

We could show that dentate centenarians, in particular those with 20 or more remaining natural teeth ('successful oral agers'), possessed a more diverse oral microbiome. The higher relative abundance of several species, such as *Prevotella* spp., *Selenomonas* spp., *Fusobacterium* spp. and *Campylobacter* spp. in dentate individuals may be due to their capacity for biofilm formation [52–54], which is easier on inert surfaces such as natural teeth. For instance, it was found that *Selenomonas* spp. made a relevant contribution to the structural organization of the biofilm [55], and that *F. nucleatum* coaggregated with *Prevotella* spp. [53]. Moreover, it is known that many of the bacteria associated with periodontal disease, usually assessed in subgingival plaque, can also be found supragingivally [56]. As the supra- and subgingival microbiota are linked, higher relative abundance of (facultative) periodontopathogens may also be due to the lack of subgingival niches in edentulous participants. In addition, some species generate ammonia, which may provide resilience to acidification and thus be a health-maintaining mechanism [57] resulting in a higher relative abundance in dentate individuals. Interestingly, the genera *Bifidobacterium* and *Scardovia* were also more common in dentate individuals. While these bacteria are known to have health-promoting effects in the gut microbiome [58], oral species have rather been found connected to diseases such as root caries, a consequence of their ability to produce acid and survive in the acidic environment of carious lesions [59,60]. Their presence may thus be due to the high prevalence of root caries among the dentate centenarians [33]. Conversely, streptococci, of which some

species, particularly *S. mutans*, are also strongly associated with caries, were more frequently found in edentulous participants, which is in line with previous findings that these bacteria remain in the oral cavity even after the loss of all teeth if hard surfaces in form of dentures are present [61–63]. Their relative decrease in dentate centenarians may be a positive factor in maintaining more natural teeth up to old age, given their influence on dental decay. However, not all streptococci are cariogenic [64,65], and interpretations are thus limited without further data on a taxonomic level.

Some of the species more frequently found in dentate individuals were known periodontopathogens, confirming previous observations that these bacteria are reduced after the elimination of subgingival niches [66]. Surprisingly, the periodontal status of centenarians did not influence the structure or diversity of the oral microbiome, although periodontal disease is known to influence the subgingival microbiota [67]. This may have been due to our choice of the extraction site, as only supragingival plaque and saliva were collected, and no subgingival sampling took place. The microbial environment can differ based on location in the same oral cavity, including tongue, palate, buccal, gingival, or tooth surfaces as well as other tissues [68,69]. Periodontopathogens are usually more abundant subgingivally, as they favor an environment with lower oxygen tension [70].

It must thus be highlighted that the higher relative abundance of periodontopathogens in the supragingival plaque of dentate centenarians and accompanying lower relative proportions of cariogenic streptococci and lactobacilli is an unusual microbial composition, particularly in individuals with a high prevalence of caries [33], a low salivary pH and a relatively low prevalence of severe periodontitis [32]. Further studies are necessary to explore the reasons for this phenomenon, possibly also assessing centenarians' oral immunity to see whether an altered or diminished immune reaction to these bacteria could play a role.

In contrast to our results regarding the benefits of microbial diversity, higher bacterial diversity in subgingival plaque has been shown to be associated with periodontal disease [71]. Moreover, in an epidemiological study among 2,343 adult residents of Hisayama town, Japan, the authors concluded that a lower phylogenetic diversity was associated with better conditions for oral health since it interrelated with a lower plaque index, less gingival bleeding, shallower periodontal pockets and less decayed teeth [72]. However, this conclusion must be regarded with caution as a less diverse oral microbiome was also significantly associated with increased tooth loss – often the final outcome of



**Table 3.** Salivary parameters in centenarians.

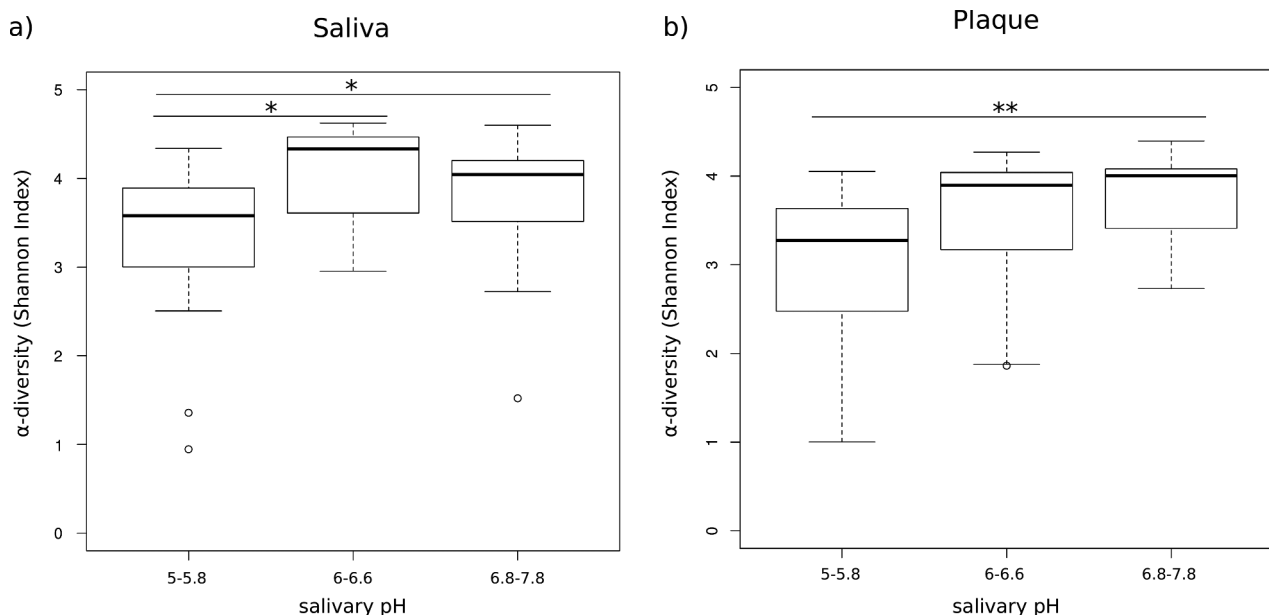
Variable	n (%)	
Viscosity	strongly increased	17 (31.5)
	increased	11 (20.4)
	normal	26 (48.1)
Stimulated salivary flow rate (ml/min)	Mean (SD)	0.84 (0.55)
pH	very low (< 0.7)	20 (37.7)
	low (0.7–1.0)	19 (35.8)
	normal (> 1.0)	14 (26.4)
Buffering capacity	5.0–5.8	25 (46.3)
	6.0–6.6	11 (20.4)
	6.8–7.8	18 (33.3)
very low		21 (39.6)
	low	24 (44.4)
	normal	8 (14.8)

periodontal and cariological diseases and the most important factor in dental health. The latter result is supported by a number of recent studies, showing that bacterial diversity is lower in edentulous persons [73–75]. Moreover, other studies assessing supragingival microbiome diversity support our results by showing that higher diversity was associated with less caries [42,76]. Further studies assessing both the supragingival and subgingival microbiota are necessary to elucidate which of these opposing trends remains beneficial for the maintenance of oral health in the long term.

The degree of disability, nursing care, or type of residence did not play a considerable role, although several studies have suggested associations between general frailty and the microbiome [2,77–79], some of which have also assessed the oral microbiota. For instance, Ogawa et al [2]. observed a different salivary microbiota between elderly individuals living in

a nursing home and those living independently. However, it is not clear whether oral dysbiosis influences general frailty or if general frailty induces oral dysbiosis, for instance via the mediation of local factors, such as reduced oral care and increased tooth loss. The centenarians examined in this study, although many still resided at home, were all in need of care and support, which may have alleviated differences in this regard.

On the contrary, salivary parameters, a decreased pH in particular, did play a role in the microbiome diversity and thus also in the centenarians’ oral health status. For one thing, regarding the development of caries, microbiome dysbiosis is associated with an overgrowth of acidogenic bacteria. Then again, increased salivary acidity is associated with a reduced buffering capacity, consequently reducing the ability to restore a healthy pH. As a result, this may lead to further dysbiosis and decrease in microbiome diversity. These factors may thus be mutually reinforcing. While this effect has been extensively studied in relation to dental caries [57], less is known about metabolic pH effects in relation to gingivitis and periodontitis. Gingivitis increases the secretion of inflammatory factors into the gingival crevice. [80] This leads to a more protein-rich environment, supporting the growth of proteolytic and amino acid-degrading periodontopathogens, the alkaline products of which typically raise the average pH to neutral or slightly alkaline values. [81] However, other studies have found great variations in the pH of periodontal pockets [82,83], including more acidic environments. Further studies are thus necessary to evaluate the effect of salivary acidity on gingivitis and periodontitis in an age cohort where this condition is so highly prevalent.



**Figure 5.** Influence of the salivary pH on the microbial diversity in (a) saliva and in (b) plaque samples.

Moreover, salivary acidity is also associated with hyposalivation [84], a major problem in this cohort. Although this is often attributed to polypharmacy in the elderly [85], a correlation with the number of drugs taken daily could not be established. This lack of correlation might have occurred due to the relatively small size of the centenarian cohort, which, however, has to be accepted in this group of the oldest old. Other influencing factors are circadian deviations in the saliva flow rate, and whether the patient is dehydrated at the time of the examination [86]. This may have played a role, as it is known that the aging process alters important physiological control systems associated with thirst and satiety, often resulting in a reduced fluid intake [87]. Moreover, a significant cause of reduced saliva flow is the age-related degeneration of the salivary glands [88–90]. The results of this centenary study suggested that the influence of age could outweigh the impact of other common factors such as medication or frailty. The high frequency of salivary problems may have been a cause of the overall high burden of caries and root caries in the study population [33].

Interpreting results in this cross-sectional setting bears limitations, mainly due to the exploratory nature of the study. As it is the first of its kind, comparisons with other scientific data are therefore limited. Furthermore, the cross-sectional design does not allow for the deduction of causality and the population-based sample is only representative of a defined geographic area. Moreover, the age of the biofilm was unknown.

In conclusion, we could show that centenarians with good oral health, i.e. with less caries experience and more natural teeth possess a more diverse oral microbiome. Regular dental visits also have a positive influence on the microbiome diversity to that effect. However, diversity can be influenced by hyposalivation, a frequent impairment among centenarians which can have a negative impact through pH changes. It is thus a major problem that should be addressed.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability

Raw data were generated at the Heidelberg University Hospital. The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Author contributions

C.S.: contributed to data acquisition, analysis and interpretation, drafted and critically revised the manuscript. E.L.: contributed to data acquisition, analysis and interpretation and critically revised the manuscript. D.W.: contributed to data analysis and interpretation, and critically revised the manuscript. S.B.: contributed to data acquisition, analysis and interpretation and critically revised the manuscript. C.F.: contributed to conception, design, data analysis and interpretation and critically revised the manuscript. All authors gave their final approval and agree to be accountable for all aspects of the work.

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