Ancient dental calculus reveals oral microbiome shifts associated with lifestyle and disease in Great Britain

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Abstract

The prevalence of chronic, noncommunicable diseases has risen sharply in recent decades, especially in industrialized countries. While several studies implicate the microbiome in this trend, few have examined the evolutionary history of industrialized microbiomes. Here, we sampled 235 ancient dental calculus samples from individuals living in Great Britain (~2200 BCE to 1853 CE), including 127 well-contextualized London adults. We reconstructed their microbial history spanning the transition to industrialization. After controlling for oral geography and technical biases, we identified multiple oral microbial communities that co-existed in Britian for millennia, including a community associated with *Methanobrevibacter*, an anaerobic Archaea not prevalent in the oral microbiome of modern industrialized societies. Calculus analysis suggests that oral hygiene contributed to oral microbiome composition, while microbial functions reflected past differences in diet, specifically in dairy and carbohydrate consumption. In London samples, *Methanobrevibacter*-associated microbial communities are linked with skeletal markers of systemic diseases (e.g., periostitis and joint pathologies), and their disappearance is consistent with temporal shifts, including the arrival of the Second Plague Pandemic. This suggests pre-industrialized microbiomes were more diverse than previously recognized, enhancing our understanding of chronic, noncommunicable disease origins in industrialized populations.

Main Text

Introduction

Modern, industrialized microbiomes are linked to a wide-range of noncommunicable, chronic diseases, including obesity, cardiovascular disease, allergies, and poor mental health[1,2], which are increasing rapidly in industrialized countries and are predicted to rise in low- and middle-income countries in the future[3,4]. As such, determining the evolutionary background of these microbial communities is critical to understanding the origins and aetiologies of these diseases. To date, the origins and evolution of industrialized microbiomes are primarily investigated by examining so-called "pre-industrialized" microbiomes of other primates or extant Indigenous peoples who practise traditional subsistence lifeways, (i.e., hunting and foraging)[5–9]. Such research has suggested that shifts in diet (e.g., reductions in dietary fibre[8]) and the loss of microbes (e.g., *Helicobacter pylori*) have shaped industrialized gut microbiomes[5], alongside changes in environmental and social factors[10]. Studies tracking gut microbiomes of immigrants to industrialized countries, such as the United States of America, have similarly demonstrated a decrease in diversity and a loss of certain species upon the adoption of 'Western' lifestyles[11,12], confirming that industrialization has significant impacts on the human gut microbiome. As a response, scientists have called for the biobanking of Indigenous people's microbes before those microbes become extinct[13].

Despite these findings, the extent and rate of microbial extirpations in industrialized societies remain poorly understood, as the approaches used to describe pre-industrialized microbiota are problematic. First, each population has a unique evolutionary history with distinct genetics, environments, diets and selection pressures that shape its microbiome in unique ways[14]. Consequently, modern non-industrialized populations or immigrants may not accurately reflect the microbes that existed in the ancestors of industrialized peoples today[15,16]. Second, this research places unnecessary responsibilities and obligations on Indigenous communities to participate in microbiome research, where the benefits of these studies may not directly serve Indigenous peoples[17]. Therefore, a more direct path towards reconstructing pre-industrialized human microbiomes is needed. One such approach is to utilise the available bioarchaeological record of communities that predate industrialized populations through the analysis of ancestral archaeological human remains. Although this approach involves a number of complex challenges in recovering gut microbiomes[18,19], reconstructing ancient oral microbiomes preserved within calcified dental plaque (i.e., calculus) is an established way of tracing past oral microbial histories[20,21].

Results

Filtering and Authentication

We performed the largest ancient dental calculus study conducted to date (n=235 samples assessed), reconstructing authenticated oral microbiota using shotgun metagenomics from 183 pre-industrialized individuals who were excavated across 27 archaeological sites in England and Scotland (Figure 1; Table S1) from ~2,200 BCE to 1853 CE, to directly describe the history of a pre-industrialized population's microbiome. These samples originate from eight geographic regions and include individuals who resided in Britain before Roman colonization through to the 'Industrial Revolution' (Supplemental Text (Archaeological context and sample descriptions); Table S1). We used a multi-tiered assessment procedure to authenticate and control for contamination in this data set (Supplemental Text (Data processing, quality filtering, and authentication); summarized in Figure S3; Tables S2, S3, S4, S5, S13). First, we only included high quality samples with >100,000 taxonomically assigned sequences[22] and more than five phyla[20]. We authenticated ancient DNA fragmentation with a novel, reference-free DNA damage program called ChangePoint [23] (Table S5) and the gold-standard, reference-based approach called MapDamage2.0 [24]; damage consistent with these archaeological ages was present in known oral species (*Streptococcus sanguinis*, *Porphyromonas gingivalis*, *Actinomyces oral taxon 414*, *Anaerolineacea bacterium oral taxon 439*, and *Methanobrevibacter oralis*) and less in common contaminant species (*Burkholderia multivorans*, *Comamonas 5estosterone*, *Escherichia coli*, and *Flavobacteriaceae* bacterium; Table S13). We were conservative and limited the effects of potential laboratory and environmental contaminant DNA by removing samples whose microbial composition was similar to that of laboratory controls (Figure S2; Table S2) and conservatively filtering contaminant species identified in environmental and laboratory controls (Figure S4; Table S4). Lastly, we verified the presence of oral taxa from known modern and ancient oral microbiomes using SourceTracker1.0 (MALTx results) and SourceTracker2.0 (MALTn results) (Supplemental Text (data processing, quality filtering, and authentication); average 85% oral; Figure S2 and S13) and confirmed the highly abundant taxa were present in the Human Oral Microbiome Database (HOMD; Figure S13C).

Oral Geography Biases

As oral geography (i.e., position within the oral cavity) plays a role in oral microbiome composition[25–28], we tested and identified oral geography biases in the dataset; calculus sample size and gingival location influenced taxonomic composition in molars, while tooth

surface drove compositional variation in incisors (Figure 2A-C; Supplemental Text (Microbial composition analysis). As a result, we stratified our taxonomic and functional data according to tooth type and included oral geography (i.e., tooth type, surface, gingival region, and calculus size) in our statistical analyses (Table S6-S9). As these biases likely reflect biological and ecological differences in the mouth[25], these differences attributed to oral geography raise questions about the interpretations of previous paleomicrobiome studies that used calculus samples collected from a mixed dentition[29–31] and suggest that future studies should control for oral geography during sampling and analysis (Figure S18). Overall, 954 microbial species were identified across all ancient British calculus samples, predominantly spanning Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes phyla (Figure S4).

Drivers of Variation

To explore drivers of variation in pre-industrialized oral microbiome composition, we performed a Principal Coordinates Analysis (PCoA) ordination of Bray-Curtis dissimilarities with BiPlots (Figure 3A and S5A) using microbial genera in the samples. We observed a 'U' shaped curve indicative of distinct ecologies[32] (Figure 3A; Axis 1=41.25% variation; Table S10), and the Biplots indicated that *Methanobrevibacter* and *Streptococcus* genera were associated with the 41.2% of the variation explained by Axis 1, while *Actinomyces*-dominated communities drove variation on Axis 2 (13.8%; Figure 3A). As such, we grouped the samples according to whether or not *Methanobrevibacter*, *Streptococcus*, or *Actinomyces* genera were most dominant in each sample. We then compared the two communities on the extremes of Axis 1 (i.e., *Streptococcus*- and *Methanobrevibacter*-associated) to assess major factors that drive oral microbial diversity (Figure 3A). *Streptococcus*- and *Methanobrevibacter*-associated samples contained distinct community assemblages (Dominant category; R2=>0.20, p = <0.05; Table S12). Co-occurrence analysis of the *Streptococcus*- or *Methanobrevibacter*-associated communities using CCLasso positively associated *Streptococcus* with *Leptotrichia*, *Neisseria*, *Gemella*, *Capnocytophaga*, *Granulicatella*, *Lautropia*, *Kingella*, *Aggregatibacter*, *Lachnoanaerobaculum*, and *Rothia* genera (Figures 3B-C; Table S11), as seen in modern industrialized oral microbiomes from Spain (Figure S6). In contrast, *Methanobrevibacter* positively co-occurred with genera not often described in the industrialized oral cavity, including *Methanosphaera*, *Peptoniphilus*, *Anaerofustis*, *Syntrophomonas*, *Shuttleworthia*, *Subdoligranulum*, *Pseudoramibacter*,

Synergistes, *Hungatella*, and *Butyrivibrio* taxa (Figures 3B-C; Table S11). Several different oral *Methanobrevibacter* species have now been described in ancient mouths[33], and at least three of the co-occurring genera have been previously characterized in the mouth[34–36] and not as contaminants[20,26,30]. We confirmed via a literature review that species within these co-occurring genera can cohabitate with *Methanobrevibacter* oral species, as all are anaerobic and can produce metabolic by-products that support methanogenesis[37]. However, this *Methanobrevibacter*-associated community has not yet been described in studies of modern dental calculus[20,31,38], suggesting that a *Methanobrevibacter*-associated community may represent a unique oral microbial ecology not typically found in modern industrialized societies.

To explore the origins of these two microbial communities, we tested whether physiological, cultural, and temporal factors previously thought to drive ancient oral microbiome composition were associated with this signal in ancient British microbiomes[39]. Demographic variables (i.e., sex and age), broad cultural classifications (i.e., religion, class, or urban/rural locations; Table S1), or major biocultural or socio-political events (e.g., Civil War or plague outbreaks, such as the Second Plague Pandemic, also known as the Black Death (caused by *Yersinia pestis*); Table S1) did not explain significant levels of taxonomic or functional compositional variation across Great Britain (ADONIS; p=>0.05; Table S12). A mild association between the location where individuals were buried (i.e., cemetery) and microbial genera composition was observed (ADONIS; R2=0.158; p=0.031; Table S12), but this was not true when examining the data at the species level (ADONIS of contaminant species-filtered data; p=>0.05; Table S12). Surprisingly, these findings suggest that these large scale cultural and social factors that occurred across Britain over 2,200 years were not significantly drivers of oral microbiome composition at a population scale in this data set.

Associations with Oral Health

We next examined if these microbial communities were linked to known signatures of oral disease, as *Methanobrevibacter* taxa have been linked to severe periodontitis in modern populations[40]. As indicated by an ADONIS test (p=<0.05), oral microbiome composition in all individuals was not linked to the occurrence of periodontal disease, nor other known oral pathologies (i.e., caries or apical abscesses; Table S12). Surprisingly, species and functions linked to periodontal disease in modern populations (e.g., *Porphyromonas gingivalis* and *Tanerella forsythia*) were more likely to be found in *Streptococcus*-associated communities than those dominated by *Methanobrevibacter* (Table S13), suggesting that the modern

aetiology of industrial-age periodontal disease may in fact originate from the *Streptococcus*-associated communities. However, oral microbiome composition was directly linked with size of the calculus sample analysed (Figure 2B; Table S6-S9). While it would be reasonable to infer that *Methanobrevibacter*-associated communities may thrive within larger, potentially more mature calculus deposits, consistent with the anaerobic requirements of these taxa, we did not find this to be the case. The separation of samples into *Streptococcus*- or *Methanobrevibacter*-associated communities did not explain calculus sample size (adonis; p=0.101), nor were the size of calculus samples driven by *Methanobrevibacter*-associated species. Rather, *Streptococcus* community-associated taxa (based on the correspondence analysis in Table S11), including *Gemella* and *Lautropia*, were associated with larger sample sizes (ALDEx2; Figure S17). Nevertheless, modern oral hygiene practices can reduce dental plaque and calculus formation[41,42] and lead to smaller deposits, so it is possible that compositional shifts in ancient British communities are linked to dental hygiene practices.

Exploring Diets

As dietary changes are proposed to be a main driver of oral microbiome evolution through time[20,30,39], we further explored if differences in diet could underpin these two distinct microbial ecologies. Direct dietary DNA signals were explored in six deeply sequenced calculus samples (i.e., >100 million sequences per sample; three samples each from *Streptococcus*-associated or *Methanobrevibacter*-associated communities), but after careful consideration[43,44], no verifiable DNA fragments could be robustly confirmed from either plant or non-host animals. As oral microbes in dental plaque can ferment sugars, starch molecules, and amino acids in the mouth[45], we then developed a novel approach to explore if indirect dietary signals were present. We assembled a list of microbial genetic functional differences linked to dietary changes in the gut[8,20,46,47], as a proxy for predicting broad dietary differences. We included 42 amino acid metabolism functions linked to either carnivorous or herbivorous diets[46]; 17 functions linked to high or low-dietary fibre digestion[8]; 124 carbohydrate metabolism gene families; and 30 lactose and galactose metabolism functions linked to milk consumption (Table S14). We first validated this approach by examining the presence of these microbial functions in calculus from a modern, industrialized Spanish population (Table S1). Our results were consistent with an omnivorous diet with high sugar (e.g., lactate fermentation, fructose utilization, galactose degradation, and glucose utilization) and low dietary fibre intake (e.g., glycan degradation) (Figure 4; Table S15). Notably, microbial functions associated with dairy consumption (i.e., beta-galactosidase or lactase; EC

3.2.1.23) were highly abundant in these modern human oral microbiomes (Figure 4; Table S15).

In our ancient dataset, we identified 81.0% (34 of 42) of the amino acid metabolism pathways associated with herbivory or carnivory in ancient samples (Table S15). Whilst 88% were significantly more abundant in one of the two microbial communities (Figure 4, Figure S7), both microbiomes possessed functions consistent with an omnivorous diet (Figure 4, Figure S8). *Streptococcus*-associated communities contained more microbial functions significantly linked with low-fibre (70% of the 16 identified fibre digestion functions; galactose metabolism, glycosphingolipid biosynthesis, and glycan degradation; Figure 4; Figure S9) and high carbohydrate diets (46.8% of the 124 carbohydrate metabolism associated genes, compared to 22.6% in *Methanobrevibacter*-associated communities), including pathways linked to fructose, sucrose, trehalose, mannose, beta-glucosides, and maltose metabolism (Figure 4; Figure S10). In contrast, *Methanobrevibacter*-associated communities were enriched for functions linked to methanogenesis, gluconeogenesis, and xylose utilization (Figure 4; Figure S10). Lastly, *Streptococcus*-associated individuals showed increased abundances of microbial functions linked to dairy consumption (26.7% of lactose metabolism genes were significantly enriched compared to 6.7% in *Methanobrevibacter*-associated communities; Figure 4 and S12), including a significant enrichment in alpha and beta galactosidases, similar to observations in modern individuals (Figure S12; Table S15). This observation may reflect differences in dairy consumption or access across Britain, which is consistent with the presence of milk proteins in only one-third of Medieval calculus samples from Britain (800 BCE to 1895 CE)[48], even though dairy products were widely available[49]. Together, this analysis represents a powerful instrument in a growing toolbox of biomolecular approaches to reconstruct past diets and suggests that past carbohydrate and dairy consumption may be reflected in the oral microbiome of past British populations.

London-specific Analyses

As large-scale geographic differences across Great Britain may mask factors that drive oral microbiome variation through time[49], we further narrowed our analysis to 127 medieval and post-medieval (1066 to 1853 CE) individuals from London that have been extensively studied to further explore the origin of these two distinct oral communities, while again controlling for oral geography effects (Table S16-S18). As seen in the above Britain-wide analysis, the physiological or cultural factors considered did not appear to significantly

contribute to past Londoners' oral microbiome composition (p = >0.05; Table 1). However, the presence of disease, as defined by 14 detailed oral and other systemic health indicators previously recorded on the bones and teeth of the individuals included in this study in the Wellcome Osteological Research Database (WORD), did potentially explain some of the differences in oral microbiome composition. In London, periodontal disease (but not caries, abscesses, or dental developmental defects) was significantly linked to oral microbiome composition (R2 > 0.05; p <0.05; Table 1). Again, microbial species positively associated with periodontal disease were, rather surprisingly, more commonly found in *Streptococcus*-associated communities, rather than *Methanobrevibacter*-associated ones (Table S19). However, the *Methanobrevibacter*-associated communities were associated with the presence of several skeletal markers of systemic disease, including non-specific periostitis, joint porosity, osteophytic lipping, and overall scores for joint pathologies (R2 > 0.05; p <0.05; Table 1). While there could be multiple causes for these skeletal markers, most are thought to be related to inflammatory-associated conditions. Each of these disease-markers was also linked to specific genera within the *Methanobrevibacter*-associated community (Table S19), such as *Methanobrevibacter*, *Eubacterium*, *Pseudoramibacter*, *Mogibacterium*, and *Peptoniphilus* taxa (Table S19). While not all systemic diseases contribute to morphological changes in the skeleton, the association between oral microbiomes and systemic diseases has been clearly demonstrated in modern individuals, often through inflammatory pathways[50,51]. While cause and effect remain unclear, or may be the result of indirect associations (e.g., socioeconomic status), this is the first time that ancient microbiomes have been linked to systemic diseases that manifest in the skeleton, and therefore, provides a new model to examine the origins of modern chronic, noncommunicable diseases.

Methanobrevibacter Over Time

Lastly, we examine why this distinct *Methanobrevibacter*-associated oral ecology is not commonly found today in industrialized populations by exploring its presence over time. In all of Britain, the *Methanobrevibacter*-associated community was first observed in individuals ~2,200 years ago and was still present in London until at least ~1853 (Figure S10). We then examined compositional shifts over time in just London. Oral microbiome composition significantly shifted across 300- and 400-year intervals (p= <0.05; Table 1), and significantly shifted after the arrival of the Second Plague Pandemic, in London in 1348. The arrival of *Y. pestis* was verified in plague cemeteries (e.g., East Smithfield)[52] and resulted in the deaths of over 30-50% of Londoners between 1348 to 1351 alone[53], changing the

population structure and ways of life in the city substantially. As temporal differences can be confounded by taphonomy, we examined these compositional shifts in the context of the Second Plague Pandemic more closely by fitting a Bayesian multinomial logistic-normal linear model to the overall oral microbiome composition from historic London, which included the arrival of the Second Plague Pandemic as a covariate and minimized the impacts of oral geography and cemetery location. Our results show that 10.88% of the total variation in microbiome composition can be explained by temporal changes, including the arrival of the Second Plague Pandemic (95% credible interval: 4.98% to 19.47%), while only 65.41% of that signal could be equally explained by other factors (Supplemental Text (Bayesian modelling)). Additionally, only 1.5% of the variation explained by burial location was attributed to DNA damage patterns (deltaD) associated with oral taxa *Anaerolineaceae, Methanobrevibacter oralis, Porphyromonas gingivalis,* and *Streptococcus sanguinis* (Table S13; Supplemental Text (Bayesian modelling)), suggesting this observation is not driven by taphonomy. While this finding needs further examination, temporal shifts in oral microbiome composition coinciding with the Second Plague Pandemic in London could be the result of disease selection and susceptibility during the pandemic[54,55], subsequent advancements in public health and hygiene[56], dietary shifts, and/or cultural shifts that were a consequence of this significant pandemic on the citizens of London.

Discussion

Our study reveals the existence of a now rare or potentially extinct oral microbial ecosystem that was present in British populations over at least 2,200 years alongside other oral microbiome communities. This oral microbial community persisted through major bio-cultural transitions and historically significant socio-political events, only to diminish in recent history – a phenomenon associated with the rise of industrialization. Why this *Methanobrevibacter*-associated community disappeared in Britain or has not yet been described in healthy modern, industrialized societies remains unknown, but reports of broad spread *Methanobrevibacter* species in the ancient calculus literature suggest that this community may have once been widespread[33]. Our findings suggest that advancements in modern dentistry (e.g., the routine removal of large calculus deposits and the use of modern oral hygiene products), shifts in dairy and carbohydrate consumption, and medical care may have additionally contributed to its perceived absence today, although further work should explore additional lifestyle changes post-1900s, including migration and nutrition. This finding establishes a new paradigm to explore the foundations and origins of chronic,

noncommunicable disease in living populations and opens the door to identify unknown (now extinct) microbial diversity in past, pre-industrialized human populations.

Methods

Sample Information and Collection

For calculus samples obtained from individuals who lived in Britain, access was provided by the Natural History Museum (NHM), Royal College of Surgeons of England, Oxford Archaeology East, and the Aberdeen Museum (Table S1). For samples from historic London, 160 archaeological samples were collected from individuals buried at eight different cemeteries in a 10-square mile section of London (16 km^2), which formed a continuous temporal sequence from ~1000 – 1853 CE, from the curated archaeological skeletal remains collection stored at the Museum of London. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to and generally exceed those reported in previous publications[20,49]. Detailed information on each sample is provided in Table S1. Samples were handled using sterile procedures as previously outlined[20]. Intact calculus samples were stored in labelled, sterile, plastic bags and transported to the ancient DNA facility at the Australian Centre for Ancient DNA (ACAD), University of Adelaide, Australia.

Decontamination, DNA extraction, and Library Preparation

Careful consideration was given to the risk of laboratory and environmental contamination, as endogenous signals can be easily obscured or misinterpreted due to contaminating microbial DNA[26,27]. To minimize contamination, samples were processed in an ultra-sterile, specialized ancient DNA laboratory and underwent a decontamination protocol, as previously published[39]. Samples underwent an in-house, silica-based DNA extraction, as previously described[20,57,58]. Extraction blank controls (EBCs) were incorporated throughout to monitor laboratory and reagent contamination at a ratio of two EBCs to ten samples, as well as no template controls (NTCs) during the amplification process[59]. Shotgun libraries were generated using a previous protocol[20]. Samples were sequenced on an Illumina NextSeq using a high output 2 x 150 bp kit. Further details about the methods are available in the Supplemental Text.

Bioinformatic and Statistical Analysis

DNA sequences were demultiplexed, trimmed, and merged using AdapterRemoval2.0 with a 5 bp overlap[60]. Taxonomic and functional information was derived from analysis-ready reads using MALTx[20] against the 2014nr database. Taxonomic comparisons were also completed using MALTn against the 2017 RefSeqGCS database (Supplemental Text (Data processing, quality filtering, and authentication)). Only collapsed reads were used because fragments greater than 300 bp were considered more likely to be modern DNA contamination[19]. 14 samples with a Bray-Curtis dissimilarity value > 0.72 (the similarity of negative controls to themselves) in comparison to negative control were excluded. Next, all species identified within the negative control samples were conservatively removed from all samples; genera and higher classifications of data were not filtered. Four samples with <100,000 DNA sequences were also removed, as these are unlikely to capture overall microbial community structures[22]. We also authenticated the oral signal in our samples using a range of ancient calculus, modern calculus and plaque, soil, and laboratory controls SourceTracker2.0[61], and we verified DNA damage consistent with known ages of samples using both a reference free approach using ChangePoint analysis[23] and the reference based gold-standard approach MapDamage2.0[24] against oral and contaminant species (Supplemental Text (Data processing, quality filtering, and authentication)).

To identify correlations with metadata and taxonomic data, taxonomic information from modern and ancient samples were exported from MEGAN6 CE and imported into QIIME2 (V2020.2.0)[62], and singletons were removed. We rarefied the dataset to the maximum number of sequences available (100,000 for all taxa; 60,000 for genera; and 30,000 for species per sample). Bray-Curtis dissimilarity was calculated with BiPlots, and the ADONIS test was applied (9999 permutations) to identify factors that shaped beta diversity in the data set. As each ADONIS test incorporates multiple variables, a correction for multiple tests may not be necessary. Nevertheless, we report p-values of p=<0.05 as significant, and we highlight which results are also significant when applying a Bonferroni correction to account for multiple ADONIS tests (i.e., p-value of p-0.002) (Table 1). LefSe analysis was conducted to identify specific species that increased in abundance with select metadata fields[63]. To explore the functional potential of the microbiomes, functional tables were exported from MEGAN6 CE into QIIME2. Amino acid functions matching the Enzyme Commission numbers identified as distinguishing of carnivores and herbivores[46] were exported. For carbohydrate and dairy metabolism, all Level 4 functional groups were exported. Fiber metabolism functions[64] were exported from the KEGG database within MEGAN5. ADONIS tests were run on functional data as done with taxonomic data, and

LefSE analysis was conducted to identify specific functions that increased in abundance with select metadata fields.

Project Outreach

To promote this project and provide ways for non-scientists to engage with this work, we engaged visitors to the Museum of London in conversations during the sampling period of this study. A stall was set-up in the public galleries of the Museum for one afternoon within the five-day sampling visit. The stall consisted of a single table, using skulls and models from the Museum's Centre for Human Bioarchaeology teaching collection, and a conference poster. The teaching collection and poster served to attract attention and trigger conversation. Conversational engagement allowed the public to ask their own questions and query the topics that interested them. Discussions included the active project, background to the field, broader anthropological questions, and discussions of our team member's career path. In doing this, we offered an opportunity to engage with the public and support diverse and active learning in museums, enhancing Science Capital[65], while promoting the active research and ongoing partnerships of these institutions.

Ethics Statement

Ancient human samples are not subject to institutional review board approvals; however, this study was reviewed by the University of Adelaide Human Research Ethics Committee and received approval (H-2012-108).

Data Availability

All trimmed and merged DNA sequences (fastq) are available on NCBI's SRA database (BioProject PRJNA780005). The 2017 NCBI nr database and the 2017 NCBI RefSeq GCS databases were both used in this study. Unmerged reads can be made available upon request.

Code Availability

The analysis pipelines are available on the microARCH github page (@microARCHlab/BritishDentalCalculus_2021) as well as on https://github.com/michellepistner/ancientDNA.

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Author Contributions

A.G.F., N.G., A.C., K.D., and L.S.W. conceived of the study and developed the experimental design. A.C., A.G.F., C.A., K.B., K.D. and L.S.W. worked on sample acquisition. A.G.F. completed the laboratory analysis. A.S.G., A.G.F., S.W. and L.A. completed the bioinformatics and computational analysis. A.S.G., M.N., E.D., J.S., and L.S.W. performed the statistical analysis. A.S.G., A.G.F., and L.S.W. wrote the manuscript, and all authors edited and commented on the manuscript.

Competing Interests

The authors declare no competing interests.

458 **Figure Legends**

459

460 **Figure 1:** (A) Map showing the locations of the archaeological sites examined in this study
461 across the British Isles. Each site is represented by a circle, and the size of the circle
462 corresponds to the number of dental calculus samples examined from that site. A total of 235
463 individuals and 27 sites were sampled. (B) The number of dental calculus samples from each
464 time period is shown. The broad time periods and associated dates are: Pre-Roman Britain (–
465 43 CE), Roman Britain (43 CE – 410 CE), Anglo-Saxon/early medieval Britain (410 – 1066
466 CE), Norman Britain and the Middle Ages (1066 CE – 1547 CE), Reformation (1547 CE –
467 1750 CE), and Industrial (1750 CE – 1900 CE).

468

469 **Figure 2:** The contributions of oral geography on bacterial and archaeal taxonomic
470 compositions are displayed by performing a Principal Coordinates of Analysis (PCoA) of
471 Bray-Curtis distances for all teeth. The oral microbiota composition from each calculus
472 sample is colored according to (A) the tooth that was sampled; (B) the approximated size of
473 the dental calculus sample obtained for DNA extraction as described in the SI; or (C) the
474 surface of the tooth that was sampled shown for molar teeth only.

475

476 **Figure 3:** (A) A Principal Coordinates of Analysis (PCoA) plot displays differences in Bray-
477 Curtis distances of microbial genera present in each sample. Biplots are also displayed using
478 arrows for the top five most significant genera, with the length of the arrow proportional to its
479 magnitude. Samples are colored according to which the top three genera identified via
480 Biplots (*Actinomyces*, *Streptococcus*, or *Methanobrevibacter*) were most dominant within
481 each sample; samples that contained either more *Actinomyces*, *Streptococcus*, or
482 *Methanobrevibacter* are colored in grey, green, or pink, respectively. (B-C) CCLasso was
483 utilized to identify genera that positively (B) or negatively (C) cooccurred in all samples; the
484 top three genera identified using Biplots are colored as in (A): *Actinomyces*-associated (grey);
485 *Streptococcus*-associated (green), and *Methanobrevibacter*-associated (pink).

486

487 **Figure 4:** Normalized relative abundances of dietary microbial functions found to be
488 differentially abundant using a Benjamini-Hochberg corrected p value of a two-tailed
489 Welch's t test (p=<0.05) in ALDEx2 are displayed for *Streptococcus*-associated,
490 *Methanobrevibacter*-associated, and modern oral microbiomes. Red coloring represents high

abundances, yellow is medium, and while blue represents low; the coloring is normalized within each dietary function category. A full list of functions tested is shown in Table S14, and a full version of this differential abundance analysis is shown in Table S15.

Table Legend

Table 1: The results from ADONIS analysis on the beta diversity (Bray-Curtis) of oral microbiota from all London individuals is shown in a table. The fit of the test (R^2) and the p-value for each test are displayed for all species, genera, or all taxa present in each sample, after accounting for oral geography. Significant results are highlighted in red (p=<0.05). Results significant after implementing a Bonferroni correction are demarcated with *. Taxa driving these shifts (Figure S19) and linkages to the dominant associated taxa (Table S23) are presented in the Supplemental Text.

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Main Text Table: ADONIS of Culture and Health Variables For All Teeth and Molars, With Factors Based On Oral Geography Tables Accounted Into Models for MoL Samples Only

	SPECIES		GENERA		ALL TAXA	
Variable (All Teeth)	**R2**	**Pr(>F)**	**R2**	**Pr(>F)**	**R2**	**Pr(>F)**
Black Death (1346-1353)	0.042271	0.013	0.030313	0.048	0.025943	0.115
Civil War (Pre, Post, or During)	0.016108	0.135	0.011964	0.283	0.01031	0.342
Date (300 year intervals)	0.034836	0.048	0.024156	0.198	0.022078	0.221
Date (400 year intervals)	0.029446	0.013	0.016392	0.12	0.013127	0.203
Overall Pathology	0.006137	0.755	0.008607	0.449	0.004815	0.737
Blood Disorder	0.004924	0.874	0.006369	0.667	0.003584	0.875
Non Specific Periostitis	0.011312	0.376	0.008351	0.485	0.006533	0.55
Overall Vertebral Pathology	0.007189	0.668	0.006596	0.6	0.002856	0.936
Vertebral Pathology (Facets)	0.011992	0.398	0.011231	0.326	0.008289	0.482
Vertebral Pathology (Schmorls Nodes)	0.01889	0.163	0.014865	0.201	0.012520	0.248
Vertebral Anomaly	0.003516	0.975	0.00074	0.999	0.003231	0.928
Overall Joint Score	0.014469	0.339	0.013742	0.278	0.008986	0.476
Osteophitic Lipping (Joints)	0.016424	0.252	0.015658	0.2	0.013047	0.293
Porosity (Joint)	0.018879	0.2	0.016178	0.216	0.008034	0.561
Dental Abscess	0.007348	0.802	0.006688	0.831	0.008883	0.689
Caries	0.013519	0.365	0.010129	0.552	0.006764	0.742
Hypoplasia	0.016002	0.315	0.014795	0.32	0.012569	0.413
Periodontitis	0.007565	0.738	0.006348	0.791	0.003939	0.936
Class	0.050463	0.817	0.047845	0.842	ND	ND
Hospital	0.008254	0.583	0.006867	0.68	0.003706	0.89
Rank	0.024601	0.375	0.022691	0.419	0.013996	0.698
Religion	0.069326	0.37	0.065258	0.523	ND	ND
Empire	0.022583	0.161	0.024645	0.121	0.009261	0.296
Sex	0.012235	0.229	0.010149	0.346	0.007244	0.506
Cemetery	0.078145	0.066	0.06099	0.307	0.056177	0.38
Rural vs. Urban	0.015152	0.454	0.013429	0.55	0.008919	0.73
Medieval vs. Post Medieval	0.013773	0.126	0.013609	0.107	0.009232	0.233
Age	0.0334	0.14	0.040031	0.053	0.039874	0.074
Dominant Community	0.295798	**0.001**	0.278079	**0.001**	0.270359	**0.001**
	SPECIES		GENERA		ALL TAXA	

Variable (Molars)	R2	Pr(>F)	R2	Pr(>F)	R2	Pr(>F)
Black Death (1346-1353)	0.051076	0.141	0.034373	0.529	ND	ND
Civil War (Pre, Post, or During)	0.030608	0.245	0.025953	0.287	0.22388	0.33
Date (300 year intervals)	0.045842	0.356	0.040998	0.438	0.033105	0.646
Date (400 year intervals)	0.033868	0.137	0.027913	0.238	0.023156	0.329
Overall Pathology	0.036885	0.071	0.053478	0.119	0.047454	0.014
Blood Disorder	0.029438	0.135	0.04632	0.157	0.029031	0.121
Non Specific Periostitis*	0.073858	**0.001**	0.068745	0.04	0.066492	**0.002**
Overall Vertebral Pathology	0.032115	0.101	0.022347	0.595	0.027613	0.146
Vertebral Pathology (Facets)	0.017843	0.464	0.034455	0.369	0.011874	0.801
Vertebral Pathology (Schmorls Nodes)	0.020959	0.367	0.0136	0.908	0.017336	0.485
Vertebral Anomaly	0.007946	0.931	0.005958	0.998	0.012164	0.737
Overall Joint Score	0.064592	**0.007**	0.100103	0.018	0.059902	0.012
Osteophitic Lipping (Joints)	0.064268	0.007	0.099268	0.022	0.060218	0.006
Porosity (Joint)	0.057578	0.014	0.099376	0.016	0.05229	0.022
Dental Abscess	0.034019	0.262	0.035143	0.531	0.036956	0.209
Caries	0.019593	0.573	0.031298	0.502	0.024434	0.334
Hypoplasia	0.027694	0.268	0.036616	0.442	0.026032	0.291
Periodontitis	0.011512	0.833	0.025481	0.633	0.015703	0.026032
Class	ND	ND	ND	ND	ND	ND
Hospital	0.023396	0.234	0.035948	0.274	0.026313	0.177
Rank	0.024147	0.82	ND	ND	0.022222	0.852
Religion	0.095904	0.117	ND	ND	0.093495	0.104
Empire	0.032085	0.089	0.026332	0.185	0.019894	0.321
Sex	0.0239	0.376	0.032519	0.187	0.023802	0.365
Cemetery	0.087245	0.159	0.088173	0.158	0.06858	0.385
Medieval or Post Medieval	0.030868	0.117	0.025388	0.169	0.019233	0.346
Rural vs. Urban	0.033662	0.503	0.062878	0.296	0.037808	0.393
Age	0.049095	0.619	0.058474	0.37	0.047467	0.573
Dominant Community	0.221255	**0.001**	0.237476	**0.001**	0.187197	**0.001**

Bolding indicates a variable with significant results.

p=<0.05

* Significantly associated with dominant category

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