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Burial-associated microbial communities in Early Modern human remains: evidence for postmortem ecological restructuring revealed by 16S rRNA sequencing

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Abstract

Microbial communities preserved in archaeological human remains represent dynamic ecological systems shaped by host-associated microbiota, local environmental conditions, and postmortem succession. To investigate the persistence and structure of these communities, bacterial assemblages associated with Early Modern burials (1650–1800 CE) from two Early Modern burial sites in the Czech Republic two sites in Bohemia (the Czech Republic) were analysed using Illumina sequencing of the V3–V4 region of the 16S rRNA gene. Samples included teeth, abdominal sediments, environmental control soils, a non-human bone control, and cultivation-derived isolates. Community composition was evaluated using diversity indices, principal coordinate analysis (PCoA), and multivariate statistics. Burial-associated samples exhibited pronounced heterogeneity and several specimens were characterized by extreme dominance of individual genera. Despite this variability, significant differences in community composition were detected between the two sites, whereas no significant differences were observed between dental samples and abdominal sediments. Filtering analyses based on human-associated taxa revealed clearer separation between burial-associated samples and environmental controls, indicating that dominant soil bacteria partially obscure biologically meaningful patterns. Cultivation-derived communities formed distinct assemblages, demonstrating the strong selective effect of laboratory cultivation. Overall, the results suggest that archaeological microbial communities do not represent simple reflections of surrounding soils or direct preservation of ante-mortem microbiota, but rather dynamic ecological systems shaped by postmortem processes, local environmental conditions, and long-term ecological filtering.

Introduction

Microbial signatures preserved in archaeological remains represent a potentially valuable source of information about past human biology, health, and environmental interactions. In recent years, most archaeomicrobiological research has relied on the recovery of ancient DNA (aDNA), which has provided important insights into past microbiomes, pathogens, and aspects of diet. However, ancient DNA recovered from archaeological material is typically highly fragmented, chemically modified, and frequently overwhelmed by environmental contamination introduced during burial and post-excavation handling (Warinner et al., 2017; Weyrich et al., 2019). Consequently, distinguishing microbial signals associated with human remains from surrounding soil microbiota remains a major methodological challenge in archaeogenomics.

Approaches that analyse microbial community structure and ecological differentiation between burial contexts may therefore provide an additional perspective for interpreting archaeomicrobiological datasets. In the present study, the focus is placed on community-level ecological patterns rather than strict authentication of ancient DNA damage signatures. Instead of attempting formal aDNA authentication, the analytical design relies on comparative environmental controls and multivariate community analyses to assess whether burial-associated microbial signals can be distinguished from surrounding soil microbiota.

An alternative perspective considers not only degraded nucleic acids but also the ecological fate of microbial communities after death. Following burial, the human body enters part of a complex decompositional environment shaped by tissue breakdown, oxygen gradients, soil chemistry, moisture availability, and microbial competition. These factors drive microbial succession, during which some bacterial taxa decline while others persist due to physiological resilience, ecological advantage, or the ability to enter dormant states. Many microorganisms can survive for extended periods through mechanisms such as sporulation or other forms of dormancy that protect cellular structures and genetic material from degradation (Nicholson et al., 2000; Lennon and Jones, 2011). Dormant or spore-forming bacteria have been documented to survive for centuries or even millennia in sediments, permafrost, and other environments (Cano and Borucki, 1995; Johnson et al., 2007). Archaeological burials, therefore, represent complex ecological systems in which microbial communities may reflect both ante-mortem host microbiota and postmortem environmental filtering.

Microbial assemblages preserved in archaeological contexts may also contain taxa linked to past human activities. Fermentative microorganisms associated with dairy production, meat preservation, or plant processing have been reported from archaeological materials and may reflect aspects of diet or domestic practices (Şanlier et al., 2017; Warinner et al., 2015a). When detected in dental remains or sediments associated with the abdominal cavity, such taxa may provide indirect evidence of dietary habits or household environments rather than simple environmental contamination, although such interpretations remain context-dependent.

Recent studies suggest that archaeological teeth, bones, and abdominal sediments can retain microbial communities reflecting both host-associated microbiota and postmortem ecological processes (Warinner et al., 2015b; Mann et al., 2018). However, the extent to which microbial assemblages preserved in skeletal material remain distinguishable from surrounding soil microbiota remains insufficiently explored. In many studies, environmental controls are limited or absent, making it difficult to determine whether detected taxa represent residual host-derived communities or secondary colonization from the burial matrix.

In the present study, we investigated microbial communities preserved in skeletal remains from two Early Modern burial sites in Central Bohemia and northeastern Bohemia (Czech Republic). Using Illumina sequencing of the V3–V4 region of the 16S rRNA gene, we analysed dental tissues and abdominal cavity sediments and compared them with surrounding control soils, a non-human bone control, and cultivation-derived isolates. Rather than focusing solely on fragmented ancient DNA, our approach emphasizes community-level structure and ecological differentiation.

Specifically, this study addressed three questions:

(1) whether microbial assemblages preserved in archaeological skeletal remains differ from surrounding burial soils,

(2) whether microbial patterns are associated with different anatomical contexts,

(3) and which bacterial taxa contribute to long-term persistence in burial environments.

By integrating controlled archaeological sampling with multivariate community analyses, this study evaluates whether detectable but potentially overlapping microbial signals associated with anatomical origin and postmortem processes can persist in archaeological skeletal remains after several centuries of burial.

Materials and Methods

Human-associated samples

Fourteen archaeological samples and four control samples consisting of teeth and abdominal sediments were collected from two Early Modern burial grounds in the Czech Republic) (Table 1). Three crushed tooth samples and one sediment sample from the abdominal cavity/pelvic region were obtained from an abandoned cemetery dated to the second half of the 17th century at Hostivice (Prague-West district Central Bohemia). The rescue excavation conducted between 2008 and 2011 by the Central Bohemian Museum in Roztoky near Prague uncovered 89 individuals buried in 73 grave pits excavated into loess subsoil (Daněček and Smíšek 2010). The cemetery lies on marl and acidic gravel sands (Culek et al. 1996) and was associated with a rural community.

Table 1
Overview of used samples

Sample	Sample name	Type	Context	Material	Sex	Age
HT1	Hostivice tooth 1399	Tooth	Oral cavity	Tooth	Male	Adult
HT2	Hostivice tooth 1487B	Tooth	Oral cavity	Tooth	Female	Adult
HT3	Hostivice tooth 1491	Tooth	Oral cavity	Tooth	Female	Adult
HS4	Hostivice soil 1487Bs	Sediment	Abdominal cavity	Abdominal sediment	Male	Adult
ST1	Semonice tooth 1	Tooth	Oral cavity	Tooth	Not estimated	Adult
ST2	Semonice tooth 62	Tooth	Oral cavity	Tooth	Male	Adult
ST3	Semonice tooth 107-1	Tooth	Oral cavity	Tooth	Male	Adult
ST4	Semonice tooth 107-3	Tooth	Oral cavity	Tooth	Not estimated	Adult
ST5	Semonice tooth 108-3	Tooth	Oral cavity	Tooth	Female	Adult
ST6	Semonice tooth 407	Tooth	Oral cavity	Tooth	Not estimated	Adult
SS7	Semonice soil 234	Sediment	Abdominal cavity	Abdominal sediment		Adult
SS8	Semonice soil 322	Sediment	Abdominal cavity	Abdominal sediment		Adult
SS9	Semonice soil 418_S17	Sediment	Abdominal cavity	Abdominal sediment		Adult
SB1	Semonice cow Bone 65	Bone	Bovid control	Bone		
HSC1	Hostivice soil Control	Sediment	Sediment control	Soil control		
SSC2	Semonice soil Control	Sediment	Sediment control	Soil control		
HTA3	Alive Hostivice tooth 1487B	LB medium	LB medium	Alive		
SSA4	Alive Semonice 1457B soil	LB medium	LB medium	Alive		

The remaining ten samples (six crushed teeth, three sediment samples from abdominal cavities/pelvic regions, and one bovid tooth recovered from a separate animal burial) were collected from an atypical burial ground at Semonice near Jaroměř (Náchod district, Northeastern Bohemia) (Drnovský and Průchová 2021). The excavation conducted between 2017 and 2019 by the Department of Archaeology at the University of Hradec Králové revealed 66 individuals buried in 33 grave pits located outside the regular cemetery near a roadside chapel. Archaeological evidence suggests that the individuals may represent members of a military camp who died during an epidemic event in the second half of the 18th century (Drnovský and Průchová 2021). The site is located on fluvisol developed on medium-grained alluvial sediments (Bičík et al. 2009; Kozák 2009; Tomášek 2014).

All samples were collected by anthropologist Erika Průchová, who also provided the anthropological profiles of the individuals. Age estimation was based on established osteological methods including tooth eruption and mineralization (Ubelaker 1989), ossification stages (Ferembach et al. 1980), auricular surface morphology of the ilium (Buckberry and Chamberlain 2002), and dental wear patterns (Lovejoy 1985). Sex estimation was primarily based on pelvic morphology (Bruzek 2002) and cranial traits (Ferembach et al. 1980; Buikstra and Ubelaker 1994).

Control samples and cultivation experiment

Two environmental control soil samples were collected approximately 70 m away from the burial areas at both sites at a depth of 40–50 cm corresponding to the transition between topsoil and subsoil.

Two burial samples were additionally subjected to cultivation experiments to revive potentially dormant bacteria. Samples were macerated in sterile water for three hours, briefly vortexed, and inoculated onto LB (Luria–Bertani) medium. This approach was intended to preferentially support the growth of bacteria capable of surviving prolonged burial while limiting the growth of many environmental soil bacteria, although the cultivation conditions inevitably introduce a bias because not all human-associated bacteria grow on LB medium (37°C, room temperature, 48 hours, normal CO_2 concentration).

Samples were categorized into five analytical groups (see Material column in Table 1):

1. Tooth – DNA extracted from human teeth
2. Abdominal sediment – DNA extracted from sediment collected from abdominal cavities/pelvic regions
3. Bone – DNA extracted from non-human bone used as an additional control
4. Soil control – DNA extracted from soil collected outside burial contexts
5. Alive – DNA extracted bacterial taxa obtained through cultivation

The analytical strategy focused on comparing microbial community composition across sample categories rather than authenticating ancient DNA damage patterns. Therefore, the study design relied

primarily on comparative environmental controls and multivariate community analyses to differentiate burial-associated microbial assemblages from surrounding soil microbiota.

DNA extraction

Teeth samples were processed under aseptic conditions. External surfaces were cleaned using sterile paper to minimize surface contamination and then mechanically crushed into a fine powder. All instruments were disinfected and UV-irradiated prior to use. The other samples were handled to avoid contamination with DNA and microorganisms from the laboratory.
DNA extraction from both tooth powder and sediment samples was performed using a phenol–chloroform protocol followed by magnetic bead purification. Approximately equal amounts of powdered material were mixed with 400 µl of distilled water and 700 µl of phenol/chloroform/isoamyl alcohol solution, vortexed for 3 min and centrifuged at 11,000 × g for 7 min. The aqueous phase was transferred to a new sterile tube while avoiding the interphase.

The extraction step was repeated to improve protein removal, followed by chloroform purification. DNA was then precipitated using NaCl and polyethylene glycol in the presence of CleanNGS magnetic beads. Beads were washed with 70% ethanol and DNA was eluted in 50 µl TE buffer.

Library preparation and sequencing

Amplification of the V3–V4 region of the 16S rRNA gene was performed using the primer pair described by Klindworth et al. (2012) with Illumina overhang adapters:

Forward primer:

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG

Reverse primer:

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC

PCR reactions contained 2.5 µl template DNA (5 ng/µl), 5 µl of each primer (1 µM), and 12.5 µl KAPA HiFi HotStart Ready Mix. Cycling conditions consisted of 95°C for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min.

PCR products were verified using the Agilent D1000 ScreenTape system. Following PCR clean-up using AMPure XP beads, dual indices and Illumina sequencing adapters were added using the Nextera XT Index Kit. Libraries were purified again and quantified fluorometrically, normalized to 4 nM, pooled, and sequenced on an Illumina MiSeq platform (2 × 300 bp) using the MiSeq Reagent Kit v3.

All sequencing reads are publicly available in the European Nucleotide Archive (ENA) under accession PRJEB106075.

Bioinformatic processing

Raw reads were converted to FASTQ format and analysed using the SEED 2 sequence visualiser (Vetrovský et al. 2018). Taxonomic identification was performed using the SEED 2 16S reference database based on RDP release 11 (2022-03-29). Sequence fragments ranged from approximately 300 to 410 bp in length. Not all reads could be classified to the genus level. The proportion of unassigned sequences ranged from 0% to 28.6% across samples. For visualization purposes, taxa outside the six most abundant groups in the total sample were aggregated into the category “Other”, while sequences that could not be classified at the genus level were labelled “Unassigned.”

Environmental filtering

To facilitate interpretation of potential host-associated signals, taxa commonly associated with soil environments were identified by comparison with agricultural soil microbiome databases (MGnify 2020). These taxa were removed only in secondary analyses used for ecological interpretation. All statistical analyses were first performed on the complete, unfiltered dataset to avoid bias.

Remaining taxa were classified into ecological categories including commensals, opportunistic pathogens, gastrointestinal bacteria, respiratory bacteria, and diet-associated bacteria based on literature and reference databases including BacDive and the NCBI Taxonomy Browser.

Classification of bacterial genera into host-associated and environmental categories was based on published literature and curated ecological assignments (Supplementary Tables S2–S7).

Statistical analysis

Community composition analyses were based on genus-level relative abundance data. Alpha diversity at the genus level was quantified using the Shannon evenness index (SEI) calculated from relative abundance data, with values ranging from 0 to 1. Higher SEI values indicate a more even distribution of genera abundances, whereas lower values reflect dominance by a few genera. SEI is a normalization of the Shannon diversity index (SDI) that focuses exclusively on evenness, whereas SEI reflects overall diversity, including both richness and evenness. Differences among sample material categories (tooth, abdominal sediment, cow bone, soil control, and alive cultures) were evaluated descriptively and visualized using boxplots.

Beta diversity was assessed using Bray–Curtis distance based on relative abundance data. Differences in community composition were visualized using principal coordinate analysis (PCoA) based on Bray–Curtis distance matrix between samples. To identify taxa contributing to the observed ordination pattern,

two complementary approaches were applied. First, the envfit function was used to fit genus-level abundances onto the PCoA ordination, and only taxa with a significant association ($P < 0.05$) and a coefficient of determination ($R^2 > 0.2$) were retained. Second, similarity percentage (SIMPER) analysis was performed on sample groups defined according to their distribution in the PCoA plot to identify taxa contributing most to between-group dissimilarities. Taxa contributing cumulatively up to 60% of the dissimilarity were retained. Finally, the intersection of taxa identified by both approaches was used for downstream visualization in the PCoA space.

Statistical differences in community composition were tested using permutational multivariate analysis of variance (PERMANOVA) with 9,999 permutations. In addition, analysis of multivariate dispersion (PERMDISP) was performed to evaluate differences in within-group variability.

All analyses were first performed at the genus level, with taxa unassigned at that level removed prior to analysis. To assess the robustness of this pattern, additional analyses were then performed on datasets from which taxa commonly associated with soil environments were removed, leaving predominantly human-associated bacterial genera. The list of human-associated bacterial genera is provided in Table S4. Filtering of soil-associated taxa was applied only as a supplementary analysis to evaluate the robustness of observed patterns and was not used for primary statistical inference. All statistical analyses were conducted in R (version 4.5.2; R Core Team).

Results

Sequencing output and taxonomic overview

Amplicon sequencing of the V3–V4 region of the 16S rRNA gene generated an average of 255,000 reads (median 186,000) per sample after quality filtering and chimera removal (see Table S1 for details). Across all samples, bacterial communities were dominated by members of the phyla *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidota*. Genus-level abundances in the total dataset matrices are provided in Supplementary Table S2. Among the most abundant genera in the complete dataset were *Sphingomonas*, *Caulobacter*, *Propionibacterium*, *Bradyrhizobium*, and *Staphylococcus* (Supplementary Table S2).

Microbial community composition differed markedly among individual samples and no single taxonomic pattern characterized all burial-associated communities (Fig. 1). Control soil samples exhibited relatively balanced communities dominated by environmental genera. In contrast, burial-associated samples were considerably more heterogeneous and were frequently characterized by high relative abundances of genera such as *Propionibacterium*, *Staphylococcus*, *Caulobacter*, *Roseomonas*, and *Bradyrhizobium*, although the dominant genus differed among individual samples. Several samples were strongly dominated by a single genus, whereas others retained more diverse community compositions. In contrast to the highly variable Semonice samples, tooth samples from Hostivice exhibited relatively similar community compositions.

Several individual samples displayed highly uneven compositions. In particular, sample ST1 was characterized by extreme dominance of *Sphingomonas*, accounting for approximately 91% of reads, while sample ST3 was dominated by *Actinomadura*. These atypical profiles resulted in reduced evenness and contributed to the high heterogeneity observed among dental samples.

Abdominal sediment samples and the non-human bone control frequently contained high proportions of *Staphylococcus*. Species-level assignment suggested that many of these reads corresponded to *Staphylococcus epidermidis*, although species identification based on short 16S amplicons should be interpreted cautiously.

Cultivation-derived samples (“Alive”) differed markedly from the original burial communities and were dominated by genera capable of growth under laboratory conditions.

Alpha diversity and community evenness

Within-sample diversity assessed using the Shannon evenness index (SEI) by site and material of the sample is presented in Fig. 2 and Supplementary Table S3. Most samples exhibited intermediate levels of evenness, with SEI values generally ranging between 0.4 and 0.6.

The most extreme outlier was sample ST1, which exhibited very low evenness due to the overwhelming dominance of *Sphingomonas* (SEI = 0,08). In contrast, the Hostivice control soil sample (HSC1) showed the highest evenness values and represented the most balanced community structure in the dataset (SEI = 0.82).

Dental samples and abdominal sediments showed broadly comparable diversity patterns, indicating no systematic difference in community evenness between these sample types. Cultivation-derived samples displayed lower diversity, consistent with the selective conditions imposed by laboratory cultivation.

Overall, diversity patterns were characterized by pronounced sample-to-sample variability rather than by consistent differences among material categories.

Beta diversity and community structure

Differences in microbial community examined using principal coordinate analysis (PCoA) is shown in Fig. 3a. The first two ordination axes together explained approximately 41% of the total variation, indicating that a substantial proportion of the major gradients in community composition was captured within the two-dimensional ordination space.

The most pronounced pattern was the clear separation of cultivation-derived (“Alive”) samples from all archaeological samples along the first ordination axis, reflecting the strong effect of laboratory cultivation on microbial community composition. In addition, the second ordination axis largely separated samples originating from Hostivice and Semonice, suggesting that site-specific factors contributed substantially to community structure.

Several individual samples occupied outlying positions in ordination space. In particular, samples ST1 and ST3 differed markedly from the remaining Semonice samples, consistent with their unusual taxonomic composition characterized by the dominance of *Sphingomonas* and *Actinomadura*, respectively. Control soil samples from the two sites also showed distinct compositions and did not cluster closely together.

Permutational multivariate analysis of variance (PERMANOVA) performed on tooth and abdominal sediment samples revealed significant differences in community composition between Hostivice and Semonice ($R^2 \approx 0.30$, $P = 0.002$), indicating that sampling site is a strong factor on community composition and accounted for approximately 30% of the observed variation. In contrast, no significant differences were detected between tooth and abdominal sediments samples ($P = 0.77$). Analysis of multivariate dispersion (PERMDISP) showed no significant differences in within-group variability for either comparison (site: $P = 0.08$; material type: $P = 0.35$), although samples from Semonice tended to exhibit greater dispersion.

SIMPER and envfit analyses identified several genera contributing most strongly to the observed ordination structure, including *Staphylococcus*, *Propionibacterium*, and several environmental genera (Fig. 3b). These taxa showed strong correlations with the principal axes and accounted for much of the differentiation among sample groups.

Overall, beta diversity analyses indicate that microbial communities form heterogeneous assemblages influenced more strongly by site-specific conditions than by anatomical origin. Although considerable overlap among samples was observed, several burial-associated samples occupied regions of ordination space distinct from environmental controls, indicating detectable but heterogeneous ecological differentiation.

Host-associated versus environmental bacterial signal

To evaluate the persistence of host-derived microbial signatures, bacterial genera were classified into human-associated and environmental categories. The relative contributions of these groups are shown in Fig. 4, while the complete classification of genera and supporting abundance data are provided in Supplementary Tables S4-S5.

Control soil samples were dominated by environmental taxa, accounting for approximately 59–69% of the classified community. In contrast, burial-associated samples displayed substantial heterogeneity and contained varying proportions of both environmental and human-associated genera. No consistent enrichment of host-associated bacteria was observed across all archaeological samples. Instead, individual teeth and abdominal sediments exhibited markedly different ecological compositions, reflecting the combined influence of local environmental conditions and postmortem succession.

Filtered dataset analysis

To evaluate the influence of dominant soil-associated bacteria, an additional analysis was performed after excluding genera classified as typical environmental taxa (Supplementary Tables S4–S5 and Supplementary Figs. S1–S3).

Following filtering, differences between burial-associated samples and environmental controls became more pronounced than in the complete dataset. Principal coordinate analysis based exclusively on human-associated genera (Supplementary Fig. S3) revealed that most tooth and abdominal sediment samples formed a relatively compact cluster, whereas control soil samples occupied distinct regions of ordination space. Cultivation-derived (“Alive”) samples remained clearly separated from all archaeological samples. Two dental samples (ST3 and ST6) represented conspicuous outliers, reflecting their highly uneven community composition.

The enhanced separation observed after filtering indicates that dominant environmental taxa partly mask biologically relevant variation in the complete dataset. Removal of common soil-associated genera revealed underlying similarities between dental and abdominal samples and emphasized their distinction from environmental controls.

These findings suggest that differences between burial-associated communities and surrounding soils are not solely driven by dominant environmental bacteria. Instead, residual host-associated taxa and postmortem ecological processes contribute substantially to the observed community structure.

Cultivation experiments

Cultivation experiments recovered only a limited subset of taxa compared with sequencing-based community profiles. The resulting communities were dominated by genera capable of growth under standard laboratory conditions and differed markedly from the original burial-associated assemblages.

Principal coordinate analysis demonstrated that cultivation-derived (“Alive”) samples occupied regions of ordination space clearly separated from all archaeological samples (Fig. 3), indicating substantial shifts in community composition during laboratory cultivation. This pattern reflects the strong selective pressure imposed by nutrient-rich media and artificial growth conditions.

These observations highlight the well-established bias of cultivation-based approaches and indicate that only a limited fraction of the burial microbiome is recoverable under standard laboratory conditions. Consequently, culture-dependent and sequencing-based approaches should be regarded as complementary rather than interchangeable methods for the characterization of archaeological microbial communities.

Discussion

Microbial community structure in burial environments

A central expectation of archaeomicrobiological studies is that burial-associated microbial communities may differ from surrounding environmental microbiota in a systematic and detectable manner. In the present dataset, such differences are indeed observable, but not in the form of clearly separated clusters. Instead, burial-associated samples exhibit heterogeneous and partially overlapping community structures, suggesting that postmortem microbial assemblages are shaped by variable ecological trajectories rather than by a single consistent pattern.

Control soil samples consistently exhibited high diversity and relatively even community structure, reflecting the complexity of environmental microbiomes (Fierer and Jackson 2006; Lennon and Jones 2011). In contrast, skeletal samples showed greater variability and often more uneven taxonomic profiles. Rather than forming a distinct and uniform “burial microbiome”, these samples appear to reflect a spectrum of ecological outcomes driven by decomposition processes, local environmental conditions, and stochastic factors.

Among the factors examined, site-specific conditions represented the strongest determinant of microbial community composition. Samples from Hostivice and Semonice formed distinct assemblages, indicating that local environmental conditions exert long-term effects on postmortem microbial succession.

An interesting feature of the dataset is the presence of individual outlier samples exhibiting extreme dominance by single genera. Sample ST1 was overwhelmingly dominated by *Sphingomonas*, whereas sample ST3 was characterized by a strong predominance of *Actinomadura*. Such profiles suggest that individual teeth may follow highly specific ecological trajectories during long-term burial. Rather than representing analytical artefacts, these outliers may illustrate the stochastic nature of postmortem microbial succession and the importance of microenvironmental conditions operating at the level of individual specimens.

Postmortem ecological restructuring and dominance of Staphylococcus epidermidis

One of the most consistent patterns observed in the dataset is the high relative abundance of the genus *Staphylococcus* in burial-associated sediments and in the non-human bone control sample.

Species-level assignments suggest that many of these sequences correspond to *Staphylococcus epidermidis*, although this identification should be interpreted cautiously given the limitations of 16S rRNA resolution.

In living individuals, *S. epidermidis* typically represents a minor component of microbial communities and rarely reaches the high relative abundances observed here. Its dominance in burial-associated samples therefore most likely reflects postmortem ecological restructuring rather than direct preservation of ante-mortem microbiota composition.

Following death, the decomposition of soft tissues, changes in oxygen availability, and the breakdown of host immune barriers create ecological conditions that favour opportunistic and environmentally

resilient taxa (DeBruyn and Hauther 2017). As an aerotolerant, biofilm-forming organism, *S. epidermidis* may proliferate during early decomposition stages and subsequently persist as community diversity declines. Over time, ecological bottlenecking may lead to simplified communities dominated by a limited number of persistent taxa.

Although *Staphylococcus* was particularly abundant in several burial-associated samples, appreciable abundance of putatively *Staphylococcus epidermidis* were also detected in one control soil sample. This observation does not necessarily contradict a host-associated origin. Small vertebrate (e.g. *Cricetus cricetus* or *Microtus arvalis*) carcasses and other decomposing organic substrates are common components of natural soils and may provide local reservoirs of skin-associated microorganisms. Consequently, occasional detection of *Staphylococcus* in environmental controls should not be interpreted as evidence against postmortem expansion and long-term persistence of this taxon in burial environments.

The selective persistence of host-associated taxa further suggests that long-term preservation is restricted to only a subset of microorganisms. Notably, genera expected to dominate the intestinal microbiota of living individuals, such as Escherichia, were largely absent from abdominal sediments, indicating that many gut-associated bacteria do not persist over archaeological timescales. In contrast, environmentally resilient taxa and spore-forming genera appear to have a greater capacity for long-term survival. Consequently, the microorganisms detected in archaeological samples are unlikely to represent active communities and more probably reflect a combination of dormant forms, highly resistant cells, biofilm-associated microorganisms, and preserved DNA originating from taxa that withstood postmortem ecological filtering.

This selective preservation is consistent with the concept of microbial seed banks and long-term dormancy described in modern microbial ecology (Lennon and Jones 2011). However, the persistence observed here appears to be taxon-specific rather than universal. The near absence of typical intestinal bacteria, together with the enrichment of environmentally robust taxa, suggests that postmortem succession and long-term ecological filtering progressively eliminate many originally abundant microorganisms while favouring a limited subset capable of prolonged persistence.

Persistence of host-associated microbial signals

Filtration analyses revealed that the tooth samples contained genera such as *Neisseria* and *Leptotrichia* (Supplementary Tables S5), which are well-established components of the human oral microbiome (Warinner et al. 2015b). Their presence suggests that elements of the original microbiota may persist in archaeological material over extended timescales.

However, these signals do not represent intact or unchanged microbiomes. Instead, burial-associated communities reflect the combined effects of biological origin and subsequent ecological filtering. Reduced diversity, uneven community structure, and the dominance of selected taxa indicate that postmortem processes substantially reshape microbial composition.

Exploratory filtering analyses further suggested that the observed community patterns were not solely attributable to dominant soil-associated taxa, supporting the interpretation that burial-associated microbial signals reflect both residual host-derived taxa and postmortem ecological restructuring (Supplementary Table S3).

The near absence of *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* (so-called “red complex” of periodontal pathogens) contrasts with numerous dental calculus studies and suggests either extensive ecological filtering or genuine differences in the oral ecology of early modern populations. Previous archaeomicrobiological studies of dental calculus have often reported the persistence of these taxa in historical populations (Adler et al. 2013; Warinner et al. 2015b). Their near absence in the present dataset may reflect differences in sample type, postmortem ecological filtering, or dietary structure. In particular, the limited availability of refined carbohydrates in early modern populations may have reduced the selective conditions favouring extensive cariogenic and periodontopathic microbial communities, although occasional exposure to natural sugars such as honey cannot be excluded.

Comparison with previous archaeomicrobiological studies

Most archaeomicrobiological studies have focused on ancient DNA recovered from dental calculus, bone, or sediments (Adler et al. 2013; Warinner et al. 2015a; Mann et al. 2018), often emphasizing molecular authenticity and taxonomic identification. While these approaches provide valuable insights into past microbiomes, they do not fully capture the ecological processes shaping microbial communities after death.

The present study adopts a complementary perspective by focusing on community-level ecological patterns. The results demonstrate that even without strict ancient DNA authentication, meaningful ecological structure can be detected in burial-associated microbial assemblages (Lennon and Jones 2011). The observed prominence of *Staphylococcus* is consistent with previous reports of skin-associated taxa in archaeological contexts (Warinner et al. 2015a; DeBruyn and Hauther 2017). However, the strong dominance observed in some samples highlights the importance of postmortem ecological restructuring, which may amplify specific taxa beyond their original abundance in living microbiomes.

Interpretation of commensal and potentially pathogenic taxa

The detection of taxa commonly associated with the human microbiome does not necessarily indicate disease. Taxonomic resolution based on short 16S amplicons is often insufficient to distinguish pathogenic from non-pathogenic strains, and many genera include both commensal and opportunistic species.

For example, *Alloiococcus* has been associated with clinical conditions such as otitis media (Harimaya et al. 2006), yet its presence in archaeological samples cannot be interpreted as evidence of disease

without independent osteological or historical data. Similarly, other opportunistic taxa detected in this study may reflect environmental persistence or postmortem colonization rather than pathological processes. These limitations highlight the need for cautious interpretation when linking archaeomicrobiological data to health or disease in past populations.

Environmental and domestic microbial signals

In addition to host-associated taxa, the dataset includes microorganisms commonly linked to environmental and domestic settings. Some taxa may represent environmentally resilient organisms capable of surviving long-term burial, while others could reflect interactions between human activity and microbial exposure.

For example, food-associated genera such as *Leuconostoc*, as well as taxa linked to animal or aquatic environments, may reflect aspects of diet or domestic ecology. The detection of *Snodgrassella alvi*, a symbiont of honey bees, is compatible with historical honey consumption, although such interpretations remain speculative.

Some thermotolerant and environmentally resilient taxa detected in the present study are compatible with prolonged exposure to stable warm and humid domestic reservoirs. Although speculative, such habitats may have included fermentation vessels, heated water containers, and household systems maintaining relatively constant temperatures (Fig. S4). Although such interpretations remain speculative and cannot be directly verified from the present dataset, they illustrate the potential complexity of microbial inputs contributing to burial-associated communities beyond simple soil contamination.

Methodological considerations

This study prioritizes community-level ecological analysis over strict authentication of ancient DNA damage. While this approach allows detection of structured microbial patterns, it also introduces several limitations.

Taxonomic resolution is constrained by short-read 16S sequencing, particularly at the species level. In addition, DNA-based detection does not provide information on microbial viability (Cano and Borucki 1995; Johnson et al. 2007). Cultivation experiments confirmed that only a subset of detected taxa can be recovered under laboratory conditions, reflecting strong cultivation bias.

Despite pronounced heterogeneity, multivariate analyses revealed significant site-specific structure, whereas anatomical origin contributed comparatively little to community differentiation. This limits the strength of categorical interpretations but provides insight into the complexity of postmortem microbial dynamics.

Implications for archaeomicrobiology

The results demonstrate that microbial communities preserved in archaeological remains retain detectable yet variable ecological signals reflecting both host-associated origin and postmortem

processes. Rather than representing simple contamination or direct preservation, burial microbiomes appear to emerge from the interaction of biological, environmental, and stochastic factors.

These findings highlight the value of community-level analyses as a complement to traditional archaeogenetic approaches. When interpreted cautiously and supported by appropriate controls, such analyses can contribute to understanding both past human-associated microbiota and the ecological processes governing decomposition and preservation.

Conclusion

This study demonstrates that microbial communities preserved in Early Modern burial contexts retain detectable ecological structure despite several centuries of burial. Multivariate analyses revealed significant site-specific differences, whereas anatomical origin contributed comparatively little to overall community differentiation. Filtering analyses further indicated that host-associated microbial signals remain detectable despite the strong influence of environmental taxa. At the same time, the pronounced dominance of particular genera in individual specimens highlights the importance of stochastic processes and microenvironmental conditions during long-term postmortem succession. Rather than representing either direct reflections of ante-mortem microbiota or simple environmental contamination, burial-associated microbial communities constitute heterogeneous assemblages resulting from the interaction of environmental bacteria, residual host-associated microorganisms, and long-term postmortem ecological processes. These findings demonstrate that community-level archaeomicrobiological analyses provide a valuable ecological perspective on burial environments and complement traditional ancient DNA approaches in reconstructing postmortem microbial succession and the long-term preservation of host-associated microbial signatures.

Declarations

Competing interests:

The authors declare no competing interests.

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Author Contribution

Jaroslav Pavelka conceived the study, performed laboratory analyses, conducted data interpretation, and wrote the manuscript.Erika Průchová collected archaeological samples and provided anthropological context and interpretation.Patrik Galeta developed and interpreted all the statistics used and wrote the manuscript.Luboš Chroustovský manuscript proofreading and archaeological manuscript processingMarie-Dorothe Klaisnerová sample processing, DNA isolationErika Průchová sampling, anthropological identification of materialDavid Daněček archaeological material collection, site processing, dating, etc.Pavel Drnovský archaeological material collection, site processing, dating, etc.Radek Šíma DNA sequencing and manuscript proofreadingKateřina Černá DNA sequencing and manuscript proofreading

Data Availability

All sequencing reads are publicly available in the European Nucleotide Archive (ENA) under accession PRJEB106075.

References

1. Adler CJ, Dobney K, Weyrich LS, Kaidonis J, Walker AW, Haak W, Bradshaw CJA, Townsend G, Sołtysiak A, Alt KW, Parkhill J, Cooper A (2013) Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. Nat Genet 45(4):450–455. https://doi.org/10.1038/ng.2536
2. Bičík I, Hauptman I, Kukal Z, Pošmourný K (2009) Půda v České republice / Soil in the Czech Republic. Consult, Praha
3. Bruzek J (2002) A method for visual determination of sex using the human hip bone. Am J Phys Anthropol 117:157–168
4. Buckberry JL, Chamberlain AT (2002) Age estimation from the auricular surface of the ilium: a revised method. Am J Phys Anthropol 119:231–239
5. Buikstra JE, Ubelaker DH (eds) (1994) Standards for data collection from human skeletal remains. Arkansas Archaeological Survey, Fayetteville
6. Cano RJ, Borucki MK (1995) Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. Science 268(5213):1060–1064. https://doi.org/10.1126/science.7538699
7. Culek M, Grulich V, Povolný D (1996) Biogeografické členění České republiky / Biogeographical division of the Czech Republic. Enigma, Praha
8. Daněček D, Smíšek K (2011) Hostivice, k. ú. Hostivice (př. č. 20/2010). Středočeský vlastivědný sborník 29:170–172
9. DeBruyn JM, Hauther KA (2017) Postmortem succession of gut microbial communities in deceased human subjects. PeerJ 5:e3437. https://doi.org/10.7717/peerj.3437

10. Drnovský P, Průchová E (2021) Problematika vícečetných pohřbů v novověku: případová studie pohřebiště z 18. století v Semonicích u Jaroměře. Multiple burials in the Early Modern Period: the case study of an 18th -century burial ground in Jaroměř-Semonice. Památky archeologické 112:385–431. https://doi.org/10.35686/PA2021.8
11. European Nucleotide Archive (ENA) (2021) https://www.ebi.ac.uk/ena/browser/home. Accessed 12 May 2026
12. Ferembach D, Schwidetzky I, Stloukal M (1980) Recommendations for age and sex diagnoses of skeletons. J Hum Evol 9:517–549
13. Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the USA 103(3):626–631. https://doi.org/10.1073/pnas.0507535103
14. Harimaya A, Takada R, Hendolin PH, Fujii N, Ylikoski J, Himi T (2006) High incidence of Alloiococcus otitidis in children with otitis media, despite treatment with antibiotics. J Clin Microbiol 44(3):946–949. https://doi.org/10.1128/JCM.44.3.946-949.2006
15. Johnson SS, Hebsgaard MB, Christensen TR, Mastepanov M, Nielsen R, Munch K, Brand T, Gilbert MTP, Zuber MT, Bunce M, Rønn R, Gilichinsky D, Froese D, Willerslev E (2007) Ancient bacteria show evidence of DNA repair. Proceedings of the National Academy of Sciences USA 104(36):14401–14405. https://doi.org/10.1073/pnas.0706787104
16. Kozák J et al (2009) Atlas půd České republiky / Soil atlas of the Czech Republic. Czech University of Life Sciences, Praha
17. Lennon JT, Jones SE (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat Rev Microbiol 9(2):119–130. https://doi.org/10.1038/nrmicro2504
18. Lovejoy CO (1985) Dental wear in the Libben population: its functional pattern and role in the determination of adult skeletal age at death. Am J Phys Anthropol 68:47–56
19. Mann AE, Sabin S, Ziesemer K, Vågene ÅJ, Schroeder H, Ozga AT, Sankaranarayanan K, Hofman CA, Fellows Yates JA, Salazar-García DC, Frohlich B, Aldenderfer M, Hoogland M, Read C, Milner GR, Stone AC, Lewis CM Jr, Krause J, Hofman C, Bos KI, Warinner C (2018) Differential preservation of endogenous human and microbial DNA in dental calculus and dentin. Sci Rep 8:9822. https://doi.org/10.1038/s41598-018-28091-9
20. MGnify (2020) Soil metagenome from healthy and unhealthy agricultural soil. https://doi.org/10.15468/rguc4r
21. Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P (2000) Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev 64(3):548–572. https://doi.org/10.1128/MMBR.64.3.548-572.2000
22. Şanlier N, Gökcen BB, Sezgin AC (2019) Health benefits of fermented foods. Crit Rev Food Sci Nutr 59(3):506–527. https://doi.org/10.1080/10408398.2017.1383355
23. Tomášek M (2014) Půdy České republiky / Soils in the Czech Republic. Czech Geological Survey, Praha

24. Ubelaker DH (1989) Human skeletal remains, 2nd edn. Taraxacum, Washington DC
25. Vetrovský T, Baldrian P, Morais D (2018) SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. Bioinformatics 34(13):2292–2294. https://doi.org/10.1093/bioinformatics/bty071
26. Warinner C, Speller C, Collins MJ, Lewis CM Jr (2015a) Ancient human microbiomes. J Hum Evol 79:125–136. https://doi.org/10.1016/j.jhevol.2014.10.016
27. Warinner C, Speller C, Collins MJ (2015b) A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. Philosophical Trans Royal Soc B 370(1660):20130376. https://doi.org/10.1098/rstb.2013.0376
28. Weyrich LS, Duchene S, Soubrier J, Arriola L, Llamas B, Breen J, Morris A, Alt KW, Caramelli D, Dresely V, Farrell M, Farrer AG, Francken M, Gully N, Haak W, Hardy K, Harvati K, Held P, Holmes E, Kaidonis J, Lalueza-Fox C, de la Rasilla M, Rosas A, Semal P, Soltysiak A, Townsend G, Usai D, Wahl J, Wroe S, Yong R, Cooper A (2017) Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus. Nature 544(7650):357–361. https://doi.org/10.1038/nature21674

Figures



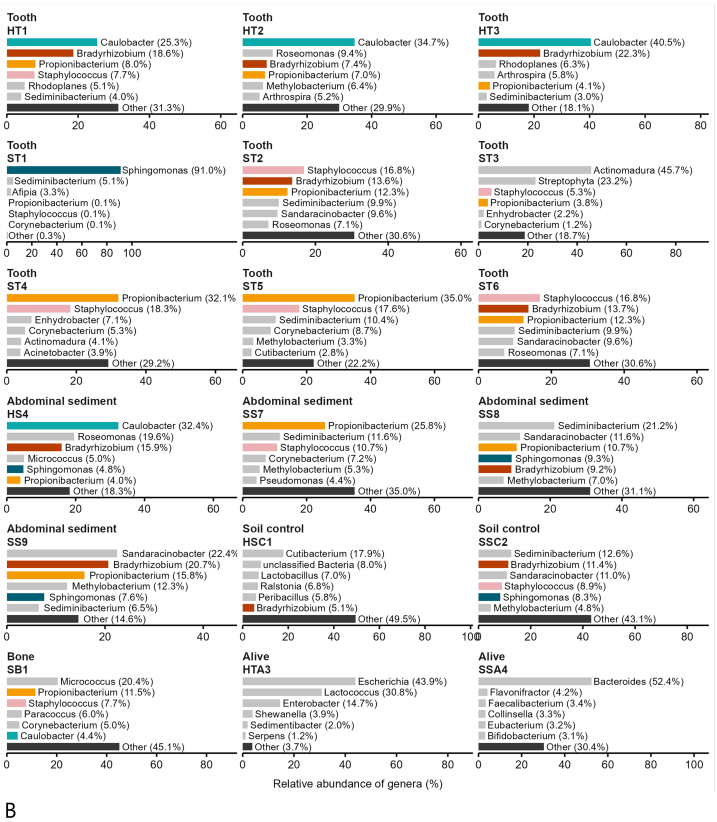


Figure 1

Relative abundance of the six most abundant genera in each sample by material of the sample. Colors indicate the six most abundant genera in the total dataset. Light grey represents genera that are

abundant within individual samples but not among the top six in the total dataset.

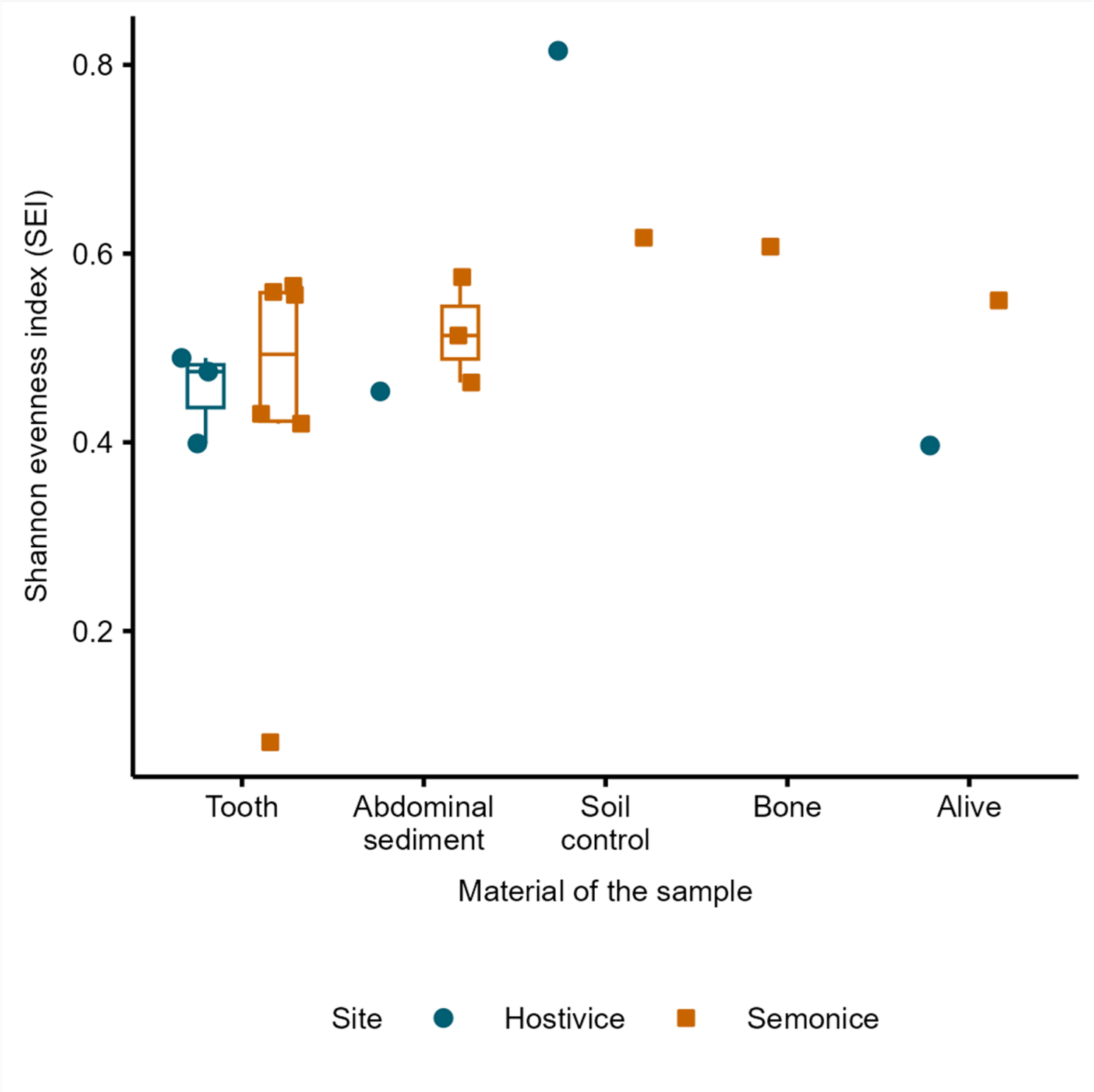


Figure 2

Bacterial diversity measured by Shannon evenness index (SEI) across samples and their contexts. SEI ranges from 0 (extreme dominance) to 1 (perfect evenness). Sequences unassigned at the genus level were excluded from the plot.

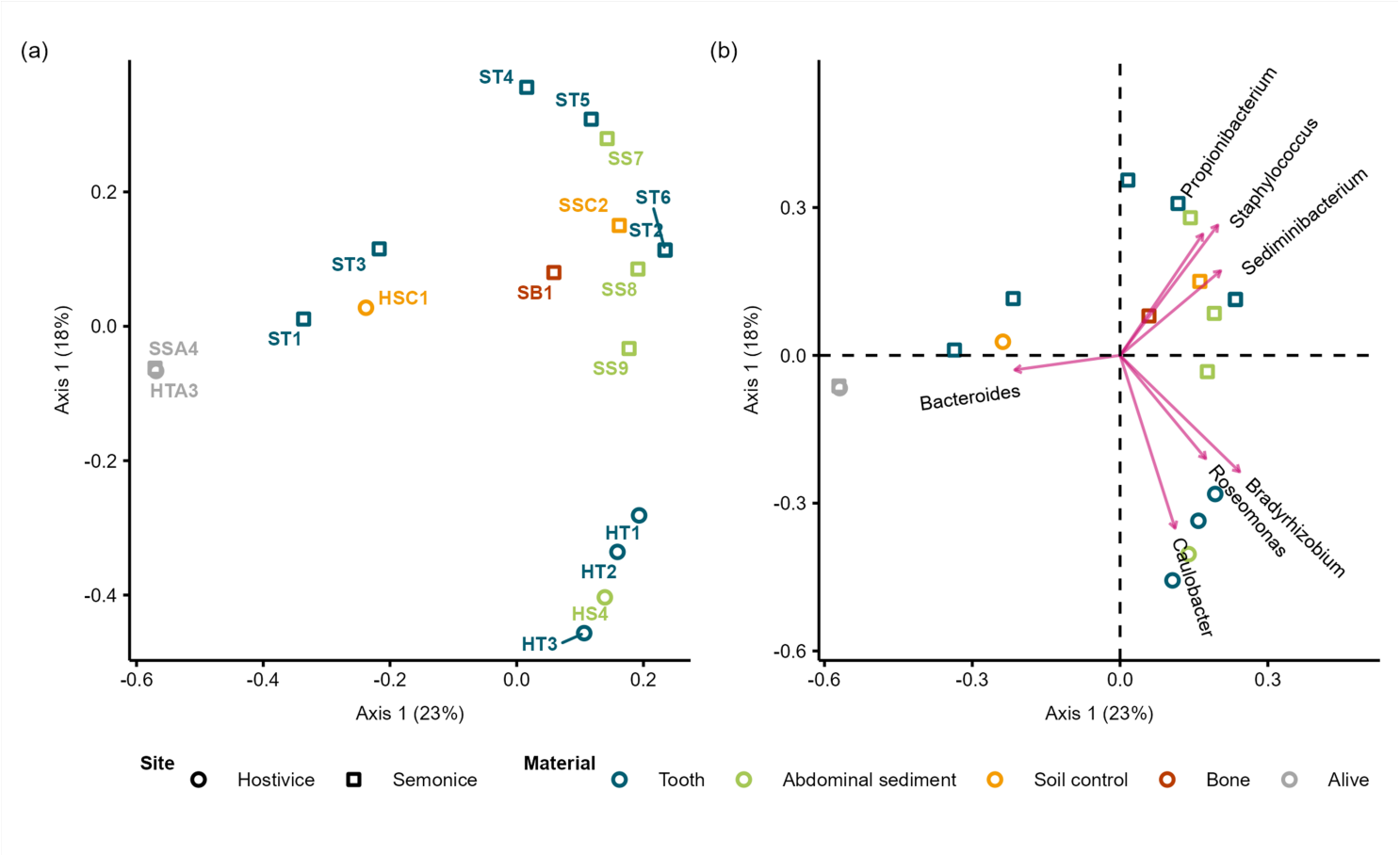


Figure 3

Principal coordinate analysis (PCoA) ordination based on Bray–Curtis dissimilarities of bacterial genera relative abundances. Samples are colored by material type. The proportion of variance explained by each axis is shown in parentheses. Only genera with relative abundance >0.1% in at least one sample were included in the analysis. Sequences unassigned at the genus level were excluded from the plot. (a) Ordination of samples. (b) Genus-level taxa contributing to the observed ordination pattern.

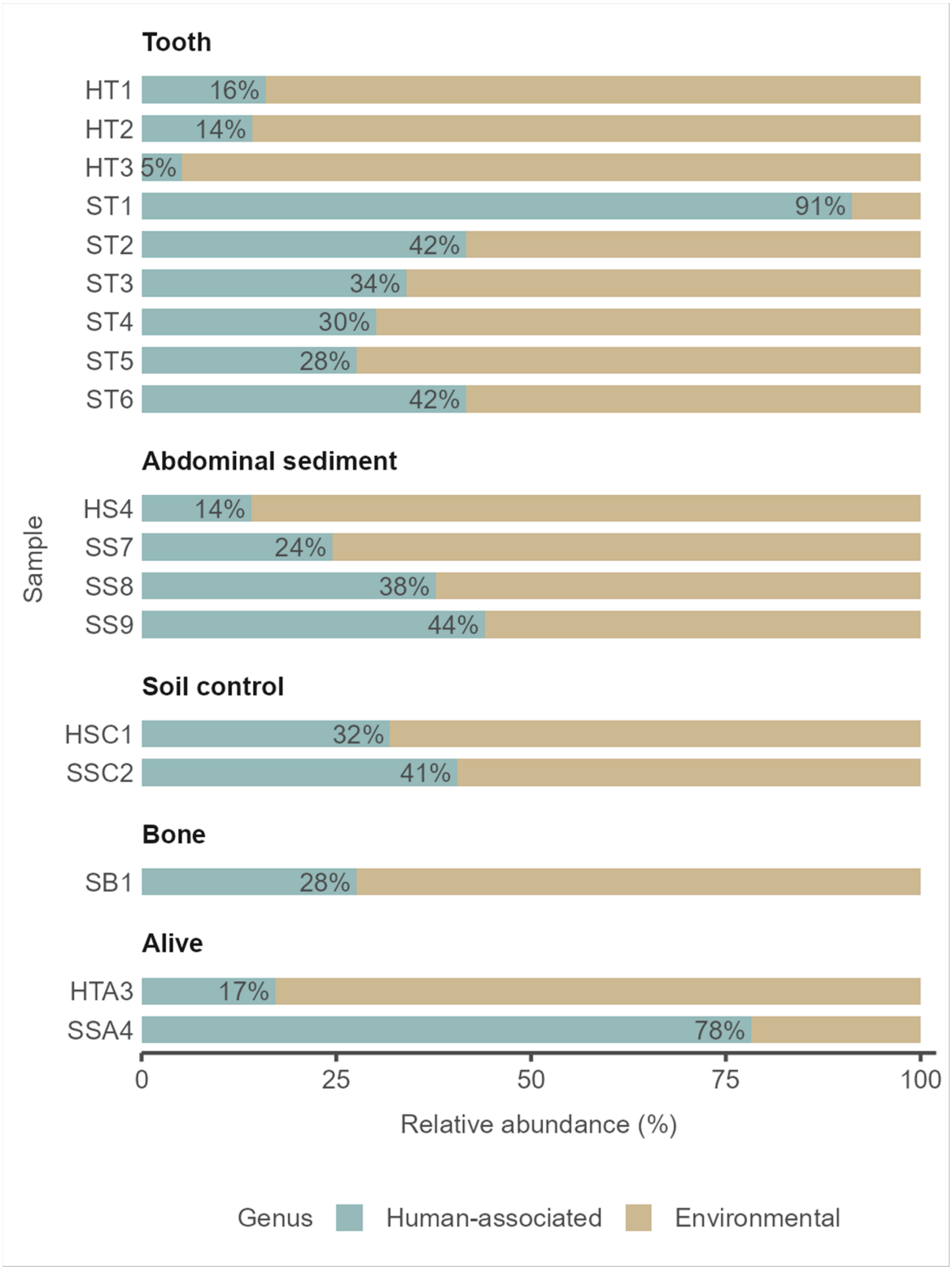


Figure 4

Relative abundance (%) of human-associated and environmental genera across samples and sample materials. Genera not assigned at the genus level were excluded.

Supplementary Files

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