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RESEARCH

Scratching the surface? A histotaphonomic study of human remains at Neolithic Çatalhöyük

Scott D. Haddow[1] · **Camilla Mazzucato**[1] · **Sıla Mangaloğlu-Votruba**[2] · **Barış Yağcı**[2] · **Thomas Booth**[3] · **Eline M. J. Schotsmans**[4,5] · **Christopher J. Knüsel**[5]

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Abstract
Recent bioarchaeological analyses at the Neolithic Anatolian site of Çatalhöyük have revealed considerable variation in skeletal completeness, preservation, articulation, and flexion among burials. Furthermore, organic remains from burnt contexts demonstrate that many bodies were tightly bound and wrapped using cordage, matting, textile, and animal hides. Some of the observed variation is suggestive of a period of delay between death and final burial for certain individuals, likely as part of a multi-stage funerary rite, perhaps seasonal in nature. It appears that some bodies may have been processed in such a way as to facilitate their temporary storage prior to burial. We examined bone samples from 57 Çatalhöyük individuals using light microscopy and scanning electron microscopy (SEM) imaging techniques to determine whether specific funerary treatments can be associated with specific patterns of microstructural preservation. As endogenous gut bacteria released into the body at the onset of putrefaction are believed by some researchers to be responsible for particular patterns of microscopical focal destruction (MFD) observed in cortical bone, the lack of such bio-erosive features has been used to infer anthropogenic treatments aimed at reducing soft tissue body mass. A previous study of skeletal material from Çatalhöyük claimed to identify bacterial bioerosion in rib thin sections but did not make use of SEM. In the present study, our analyses reveal limited evidence for bacterial MFD, which highlights the fact that standard light microscopy is insufficient for properly documenting microbial bioerosion. While there is a range of variation among other taphonomic variables observed in the current study, it is difficult to associate this variability with specific human interventions. Furthermore, the complex role of local environmental and depositional factors must also be taken into account. As such, caution must be taken when using the presence/absence of bioerosion in human bone alone to assess ancient funerary practices.

Keywords Neolithic · Funerary practices · Histology · Taphonomy · Anatolia

Introduction

One of the most striking features of Neolithic mortuary practices in Southwest Asia is the range of often-elaborate secondary burial treatments afforded to particular individuals or segments of the population. The practice of "skull retrieval" is the most well-known example of the phenomenon, which is observed archaeologically as deposits of disarticulated crania (and sometimes mandibles) in "caches" or as accompaniments to primary burials (e.g., Bienert 1991; Bocquentin et al. 2016; Haddow and Knüsel 2017). The occurrence of "headless" skeletons found in primary depositions at many Neolithic archaeological sites provides further evidence for this practice. Secondary treatments involving elements of the infra-cranial skeleton are also known, although they often receive less attention. Recent bioarchaeological observations

✉ Scott D. Haddow
scott.haddow@hum.ku.dk

[1] Department of Cross-Cultural and Regional Studies, University of Copenhagen, Copenhagen, Denmark

[2] Department of Archaeology and History of Art, Koç University, Istanbul, Türkiye

[3] Francis Crick Institute, London, UK

[4] Centre for Archaeological Science, University of Wollongong, Wollongong, Australia

[5] UMR 5199, PACEA, Université de Bordeaux, Bordeaux, France

at Çatalhöyük suggest that funerary practices at the site were even more complex and variable than previously considered, likely involving extended, multi-stage funerary treatments for at least some members of the Neolithic population (Haddow and Knüsel 2017; Haddow et al. 2021; Schotsmans et al. 2022). Whether these variable treatments reflect emergent social differentiation or some other consideration such as seasonal funerary cycles, such findings are especially relevant to our understanding of the ways in which Neolithic societies were organized and changed through time. In this study, bone samples from 57 Çatalhöyük individuals were examined using histotaphonomic techniques in order to determine whether specific funerary treatments can be associated with specific patterns of microstructural preservation. A previous study by Goren et al. (2020) using thin sections of ribs from Çatalhöyük documented various instances of bacterial bioerosion but did not employ backscatter scanning electron microscopy to confirm their observations. This analysis forms part of a larger program of research involving other Neolithic sites in Anatolia (Haddow et al. forthcoming) and Jordan (Haddow forthcoming). The present study is based on the analysis of femoral cortical bone using both transmitted light and SEM microscopic techniques.

Çatalhöyük

The Early Ceramic Neolithic (ECN) site of Çatalhöyük is situated on the Konya Plain of Türkiye. The region is a semi-arid steppe environment with hot, dry summers and cold, wet winters (de Meester 1970: 5; Kuzucuoğlu et al. 1999). As the plain represents a former lakebed, the soils are composed primarily of Quaternary marl sediments (de Meester 1970). The most recent palaeoenvironmental reconstructions demonstrate that the landscape at the time of the site's occupation was a mosaic of wet and dry land resulting from an anabranching river system associated with increasingly dry conditions from the early Holocene (Ayala et al. 2017).

Çatalhöyük consists of two separate mounds or "tells": the eastern mound, rising to a height of 17 m above the surrounding plain, covers an area of 13 ha and has been radiocarbon dated to ca. 7100–6000 cal BC (Bayliss et al. 2015). The western mound, much smaller in size, dates to the Early Chalcolithic and appears to have been occupied until the middle of the 6th millennium BCE (Orton et al. 2018). The cultivation of domesticated cereal crops and the keeping of domesticated sheep and goat was well established at Çatalhöyük from the beginning of the site's occupation (Bogaard et al. 2013; Russell et al. 2013). Wild animal species, including aurochs, continued to be hunted; in the later stages of occupation (6500–5950 cal BC), there is evidence for the introduction of domesticated cattle (Russell et al. 2013; Wolfhagen et al. 2021). The Neolithic East Mound is characterized by dense agglomerations of rectilinear domestic structures constructed of mudbrick that are interspersed with external spaces used for refuse disposal, animal penning, and other activities. At present, large-scale, clearly identifiable public structures have not been documented at the site. Individual houses at Çatalhöyük appear to have served as the focus for not only domestic activities such as craft production, food storage, and processing, but also ritual behaviors such as burials, wall paintings, and other architectural embellishments that reflect an elaborate system of symbolic representation (Hodder and Cessford 2004).

Under the direction of Professor Ian Hodder of Stanford University, the skeletal remains of at least 741 individuals were recovered from stratified Neolithic contexts during excavations between 1993 and 2017 (Larsen et al. 2019). Subfloor primary inhumations ($N = 471$ individuals) within houses are the dominant burial type on the East Mound (Boz and Hager 2013; Haddow et al. 2021). Individuals of both sexes were typically buried in a flexed position in narrow oval pits under the eastern or northern platform of the central room, although prenates, neonates, and infants were also interred within side rooms or near ovens and hearths in the central room (Boz and Hager 2013; Haddow et al. 2021). Secondary burials of loose or partially articulated skeletal remains, often interred with primary burials, are also well documented (MNI = 96) (Haddow and Knüsel 2017; Haddow et al. 2021). Towards the end of the occupation of the East Mound burials within houses became increasingly rare (Haddow et al. 2021; Marciniak et al. 2015), while burials within the settlement on the Chalcolithic West Mound are almost completely absent (Biehl 2012; Anvari et al. 2017).

During both the Mellaart and Hodder excavations, fully articulated skeletons, as well as loose and partially articulated skeletal elements, were recovered from beneath house platforms and floors (Haddow et al. 2016). Mellaart interpreted the presence of disarticulated and commingled remains as evidence for secondary burial practices involving the exposure and excarnation of bodies prior to intramural burial: "These burial habits … strongly suggest that secondary interment was practised involving the reburial of parts of the skeletons after complete or partial decomposition of the flesh" (Mellaart 1962: 51–52). Given the extremely tight flexion of some of the articulated skeletons, Mellaart also believed that these represented secondary burials as well: "Evidently care was taken to preserve the skeleton intact in anatomical position…" (Mellaart 1967: 204). During much of the Hodder excavations, the observed commingling of skeletal remains under house floors was instead interpreted primarily as the result of successive disturbances to earlier primary interments by later ones, while the tight flexion of some skeletons was no longer interpreted as reflecting secondary burial practices (Andrews et al. 2005; Andrews and Bello 2006; Boz and

Hager 2014). The prevailing interpretative model has been called into question once again, however, as recent excavations and a re-evaluation of previous findings have revealed substantial variation in skeletal completeness, preservation, articulation, and flexion among primary burials (Haddow and Knüsel 2017; Haddow et al. 2021). In addition, organic remains (especially from burnt contexts) demonstrate that bodies were often bound with cordage and wrapped with reed matting, textiles, and animal hides (Bender Jørgensen et al. 2021; Rast-Eicher et al. 2021; Ryan 2013; Wendrich 2005; Wendrich and Ryan 2012). These observations suggest a period of delay between death and final burial for certain individuals, likely as part of a multi-stage funerary rite, and that their corpses may have been processed in such a way as to facilitate these rites. Potential treatments include exposure, excarnation, or desiccation of the corpse prior to binding and wrapping.

Histotaphonomy

In archaeological science, taphonomy refers to the study of the environmental and anthropogenic processes that affect the preservation of organic material in the archaeological record. Such processes include biological, chemical, and physical modifications. By documenting the physical results of these processes in human skeletal assemblages, for example, we can better understand the sequence of events that occurred between death, burial, and subsequent recovery of once-living individuals. One of the most common factors affecting the preservation of archaeological bone is microscopical focal destruction (MFD) caused by microorganisms such as bacteria, fungi, and algae (Hackett 1981). Beginning with the work of Carl Wedl (1864), researchers have demonstrated that each of these microorganisms leave distinctive alterations to the microstructure of bone. Studies of these bio-erosive features, including their presence/absence and severity, have been successfully employed to characterize taphonomic changes associated with the burial of fleshed and unfleshed bodies (e.g., Bell 2012; Hollund et al. 2012; Jans 2005; Tjéllden et al. 2018; Turner-Walker and Jans 2008; Viani et al. 2021). Several studies have suggested that the bacteria responsible for specific patterns of MFD derive from the gut and are released during the early stages of putrefaction (e.g., Booth 2016; Brönnimann et al. 2018; Jans et al. 2004; White and Booth 2014). This is supported by the observation that bacterial attack is observed more frequently and at a more advanced rate in articulated human and animal bones than in disarticulated and fragmented animal bone (Brönnimann et al. 2018; Jans et al. 2004; Trueman and Martill 2002). Disarticulated and fragmented animal bones are likely to have been defleshed and cooked prior to being discarded and are thus less affected by enteric bacteria released during the putrefaction process. Studies by other researchers, however, suggest that the bacteria responsible for MFD derive from the soil, with MFD developing over decades rather than months (Eriksen et al. 2020; Kontopoulos et al. 2022; Turner-Walker 2019). A recent study by Turner-Walker and co-workers (2022) also calls into question the “enteric hypothesis.”

Regardless of the origins of the bacteria responsible for MFD in bone, histotaphonomic analyses have the potential to reveal important information about the period immediately following death, especially with regard to environmental conditions and human funerary interventions. For example, reduced levels of bacterial degradation of bone may indicate an anthropogenic intervention to reduce or halt the decomposition process, through the removal (i.e., excarnation) or reduction (i.e., desiccation) of soft tissue body mass. Several recent studies have attempted to associate the lack of bacterial MFD in archaeological human bone with specific funerary practices such as excarnation and mummification (Booth 2016; Booth and Madgwick 2016; Booth et al. 2015). However, before such conclusions can be drawn, it is essential to account for environmental and depositional factors that might also play a role in the development or hindrance of bacterial bioerosion. Other histotaphonomic indicators that can be used to characterize burial environments and potentially infer patterns of body treatment include collagen birefringence, microfissures, staining, and the presence of foreign inclusions within bone pores and cracks (Hollund et al. 2012; Brönnimann et al. 2018).

Materials

During the 2016 and 2017 excavation seasons, rectilinear sections of cortical bone (approx. 20 × 20 mm in size) were cut from 52 Neolithic Çatalhöyük individuals using a rotary electric saw (Dremel© 7750) with a diamond blade (Haddow et al. 2021). With the exception of one individual (CH35) whose lower limbs were missing, the bone samples were all taken from the anterior femoral midshaft. The Neolithic samples represents roughly 10% of the stratified burial assemblage ($N = 471$). In addition, five bone samples were taken from intrusive post-Neolithic burials recovered from the East Mound (one Roman and four early Islamic individuals). These burials are often encountered just below the modern surface of the East Mound, placed in pits that cut directly into the Neolithic occupation layers. As delayed burial is not thought to be a common feature of funerary practices in the Roman and Islamic periods, we can be fairly certain that these individuals were fully fleshed at the time they were interred. Apart from their shallower depth, the local environmental/soil conditions are essentially identical to that of the Neolithic burials. As such, they serve as a useful comparative group. Samples from the Roman and Islamic individuals were also taken from the femur except for one individual, CH30, whose bone sample derives from the humerus (because the lower limbs were not accessible).

In order to address the question of variable mortuary treatments, individuals from each major Neolithic occupation period (Early, Middle, Late, and Final) were selected, including individuals with signs of delayed burial (i.e., hyperflexion, partial articulation, missing elements), individuals with removed crania/mandibles and one individual from a secondary burial context. While most burials were located beneath houses, a selection of individuals from external contexts were also sampled. Both sexes and all age groups are represented, except for neonates and infants, whose bones are often too fragile for analysis (see Supplementary Table 1 for age/sex and contextual information). In addition to the bone samples taken from Çatalhöyük, cortical bone samples were also taken from the Neolithic Anatolian sites of Boncuklu Höyük and Barcın Höyük, as well as the Neolithic Jordanian site of Ba'ja. Samples from these sites will be compared with those from Çatalhöyük in future publications.

Methods

Transmission light and scanning electron microscopy

Standard transmission light and scanning electron microscopy were employed for each sample. Sample preparation and transmission light microscopy were carried out in the laboratory of the Department of Archaeology and History of Art at Koç University, Istanbul. Thin sections ranging between 40 and 125 µm in thickness were produced for each bone sample. Because many of the bone samples were particularly friable, each roughly 20 × 20 mm sample was coated in Araldite™ 2020 clear epoxy in order to prevent crumbling. Samples were then mounted and ground on a Metkon Geoform grinding machine and subsequently polished using a 3-µm diamond abrasive on a Metkon Forcipol 1 V polisher. Each sample was mounted on Isolab™ glass slides using Araldite™ 2020 adhesive without the use of coverslips. Each thin section was analyzed under plane-polarized and cross-polarized light with an Olympus BX51 fluorescence microscope at 50 ×, 100 ×, and 200 × magnification.

Each thin section was subsequently sputter-coated in gold (20 nm thickness) and examined using backscatter scanning electron microscopy (BSEM) with a Zeiss Ultra Plus field emission microscope. Backscatter SEM is especially well-suited for confirming the presence of bacterial MFD, as this type of bioerosion is characterized by an electron-dense hypermineralised rim (Fernández-Jalvo and Andrews 2016: 144). This analysis took place at the Surface Science and Technology Center (KUYTAM) at Koç University, Istanbul.

Scoring criteria

Based on observations of their microstructural preservation using standard light and SEM microscopy, each sample was scored for a series of taphonomic indices including bacterial attack (BAI), Wedl tunneling (WT), cyanobacterial attack (CAI), cracking (CRI), collagen birefringence (CBR), and inclusions (INC). With the exception of inclusions, which were developed for the present study, these indices and their scoring rationales are based on the scoring system developed by Brönnimann et al. (2018: Table 2).

Bacterial attack (BAI) results in a patterning of tunneling that tends to follow the lamellar structure of individual osteons. Hackett (1981) describes bacterial tunnels as either budded, lamellar, or linear longitudinal in form. In this study, bSEM was used as the primary means for identifying bacterial MFD, as light microscopy is not capable of visualizing the hypermineralized tunnel rims associated with these features.

Wedl tunneling (WT) is most often associated with exogenous fungal attack and characterized by very thin tunnels (5–10 µm in diameter) originating from the outer bone surfaces that do not respect osteon cement lines (Machiafava et al. 1974; Hackett 1981; Trueman and Martill 2002). However, Turner-Walker and co-workers (Turner-Walker and Jans 2008; Turner-Walker 2019) argue that Wedl tunnels are caused by cyanobacteria and thus only occur in aquatic environments. Typically, WT affects only the periosteal cortical layer (PCL). Cyanobacteria (CAI) (also referred to as blue-green algae) occur in aquatic and water-logged environments and produce narrow tunnels that typically do not penetrate beyond the PCL. Backscatter SEM was used as the primary means for identifying these features in this study.

Cracking (CRI) refers to micro-fissures (identified by bSEM) that occur within the bone tissue due to, e.g., wet/drying cycles and soil chemistry. Collagen birefringence (CBR) is assessed by observing the intensity of collagen fiber birefringence within osteon lamellae under cross-polarized transmitted light microscopy.

The scoring of BAI, WT, CAI, CRI, and CBR is based on the Oxford Histology Index (OHI) (Table 1), which records the percentage of intact bone microstructure (or collagen birefringence for CBR) on an ordinal scale of 0 to 5 (0 representing 0% preservation and 5 representing > 95% preservation) (Hedges et al. 1995; Millard 2001) (see also Brönnimann et al. 2018: Table 2, for scoring rationale). Thus, a sample with a high value will be less affected by the particular taphonomic variable being scored. Inclusions (INC) refer to the presence of foreign material such as mineral precipitations and soil particles within bone pores and cracks.

Table 1 Oxford histological index (OHI) after Hedges et al. (1995: Table 1)

OHI	% intact bone	Description
5	> 95	Very well preserved, virtually indistinguishable from fresh bone
4	> 85	Only minor amounts of destructive foci, otherwise generally well preserved
3	> 67	Clear preservation of some osteocyte lacunae
2	< 33	Clear lamellate structure preserved between destructive foci
1	< 15	Small areas of well-preserved bone, or some lamellar structure preserved by pattern of destructive foci
0	< 5	No original features identifiable, other than Haversian canals

Table 2 Scoring indices used in present study (modified from Brönnimann et al. 2018)

Taphonomic variable	Code	Scale	Description
Bacterial MFD	BAI	0–5	Degree of bacterial MFD (budded, linear longitudinal and lamellate) visually confirmed using bSEM. Based on the OHI (% of intact bone microstructure)
Wedl tunneling	WT	0–5	Degree of Wedl tunneling visually confirmed using bSEM (supposed fungal attack). Based on the OHI (% of intact bone microstructure)
Cyanobacterial attack	CAI	0–5	Degree of cyanobacterial attack visually confirmed using bSEM. Based on the OHI (% of intact bone microstructure)
Stained canaliculi	StC	0–5	Degree of stained canaliculi visually confirmed using transmitted light microscopy. Based on the OHI (% of intact bone microstructure)
Enlarged canaliculi	EC	0–5	Degree of enlarged canaliculi visually confirmed using bSEM. Based on the OHI (% of intact bone microstructure)
Cracking	CRI	0–5	Degree of crack formation visually confirmed using bSEM. Based on the OHI (% of intact bone microstructure)
Collagen birefringence	CBR	0–5	Degree of birefringence visually confirmed with transmitted light microscopy using a cross-polarized filter (% of intact birefringent collagen)
Inclusions	INC	0–3	Relative amount of foreign material such as mineral precipitations, soil particles, etc. within bone pores and cracks. Visually confirmed using bSEM

Inclusions (INC) were recorded on a scale of 0 to 3, with 0 representing no inclusions, and 3 representing frequent inclusions (Table 2). See figure 1 for scoring examples of BAI, StC, EC, CRI, CBR, and INC. While the indices used in this study are subjective in nature, testing by Hedges et al. (1995: 203) found that interobserver deviations in OHI scoring were minimal, with repeat assessments never differing by more than one grade.

Wedl type 2 tunneling, another commonly recorded histotaphonomic feature, is characterized by enlarged canaliculi emanating from osteocyte lacunae, sometimes creating a broad spider-web pattern. Wedl type 2 tunneling has been documented in a number of studies, but its etiology is not fully understood (Trueman and Martill 2002). Some researchers have attributed it to soil acidity and the action of moss, algae, and lichens (Fernández-Jalvo et al. 2010; Fernández-Jalvo and Andrews 2016), while others suggest that it is an early stage of bacterial MFD (Kontopoulos et al. 2016; White and Booth 2014). For the purposes of this study, the Wedl type 2 category has been split into two separate features, stained canaliculi (StC) and enlarged canaliculi (EC), to reflect the fact that what appear as widened canaliculi under light microscopy are often not visible with bSEM imaging and instead appear to represent staining of the canaliculi and the immediately surrounding bone tissue by intrusive compounds such as manganese (Mn) or iron (Fe) oxides. True canaliculi enlargement (EC), can only be confirmed by SEM. Recording these observations separately reflects the uncertain etiology of Wedl type II tunneling and the possibility that the two features reflect independent aetiologies.

Statistical analysis

In order to assess potential patterns and associations between the histological observations and the biological and archaeological characteristics of the skeletal samples (e.g. age-at-death, burial deposition and location, skeletal flexion), a multivariate statistical approach was employed. For this analysis, the *R* package *FactoMineR* (Lê et al. 2008) was used to produce PCA biplots showing the association of individual samples with the recorded histotaphonomic variables. Boxplots were also generated to illustrate the variation between histotaphonomic variables.

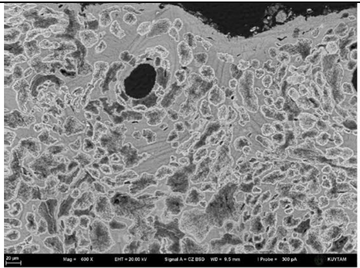
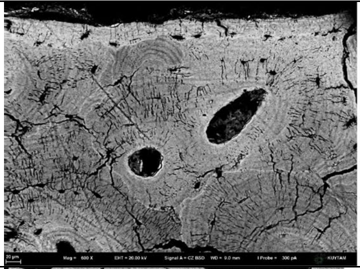
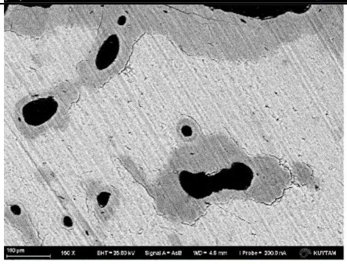
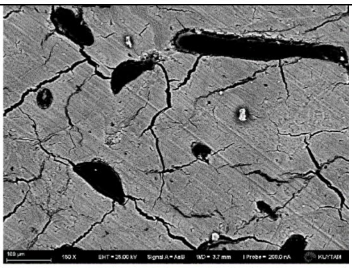
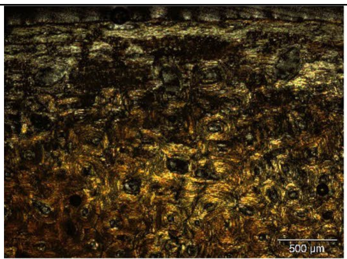
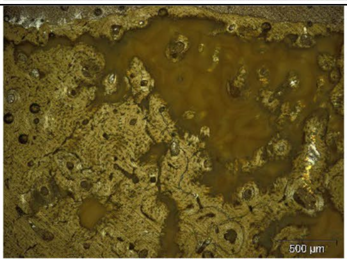
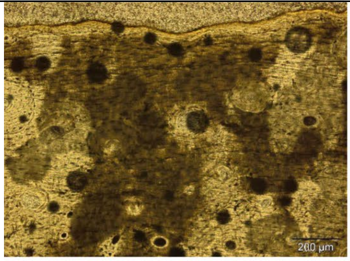
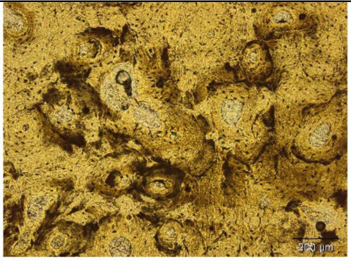
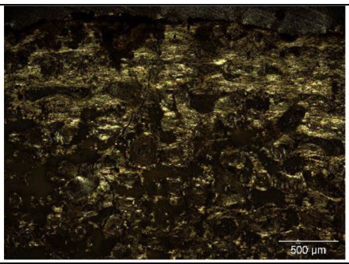
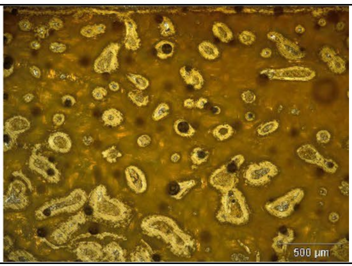
Taphonomic variable	Example	Description
Bacterial MFD (BAI)		Backscatter SEM image of a human bone sample from Boncuklu Höyük (Turkey) showing bacterial MFD (BAI) with distinctive hypermineralized (lighter colour) borders tracking the lamellar structure of individual osteons. Score = 2.
Enlarged canaliculi (EC)		Backscatter SEM image of a human bone sample from Boncuklu Höyük (Turkey) showing enlarged canaliculi (EC) emanating from osteon lacunae. Score = 3. Such features were not identified among the Çatalhöyük bone samples.
Cracking (CRI)		 (Left) Backscatter SEM image from Çatalhöyük sample CH41 with a cracking index (CRI) score of 4. (Right) Backscatter SEM image from Çatalhöyük sample CH28 with a cracking index (CRI) score of 1.
Collagen birefringence (CBR)		 (Left) Cross-polarized light microscopic image from Çatalhöyük human bone sample CH08 showing good collagen birefringence (CBR). Score = 4. (Right) Cross-polarized light microscopic image from Çatalhöyük human bone sample CH24 showing poor collagen birefringence (CBR). Score = 1.
Stained canaliculi (StC)		 (Left) Transmitted light microscopic image from Çatalhöyük sample CH31 with network of stained canaliculi (StC). These features are not visible in bSEM scans (see S. Table 2) and thus do not represent actual tunnelling. Score = 1. (Right) Transmitted light microscopic image from Çatalhöyük sample CH01 showing absence of stained canaliculi (StC). Score = 4.
Inclusions (INC)		 (Left) Cross-polarized light microscopic image from Çatalhöyük sample CH11 showing occasional foreign material (INC) within bone pores and cracks. Score = 1. (Right) Cross-polarized light microscopic image from Çatalhöyük sample CH26 showing frequent foreign material (INC) within bone pores and cracks. Score = 3.

Fig. 1 Scoring examples for histotaphonomic features observed in the present study

Results

Table 3 summarizes the scoring of the Çatalhöyük bone samples (including the five post-Neolithic samples) by taphonomic variable. Each variable is discussed below. Figure 2 presents the variation for each taphonomic variable. See Supplementary Table 2 for thin section and bSEM images of each sample as well as in situ contextual photos of each skeleton.

Table 3 Observed histotaphonomic scores for the Çatalhöyük bone samples (*n* = 57): *BAI*, Bacterial-Attack-Index; *WT*, Wedl Tunneling-index; *St*, Stained-Canaliculi-index; *EC*, Enlarged Canaliculi- Index; *CAI*, Cyanobacteria-Attack-Index; *CRI*, Crack-Index; *CBR*, Collagen-Birefringence-Index; *INC*, inclusions

Index	BAI		WT		StC		EC		CAI		CRI		CBR	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
5	56	98%	57	100%	0	0%	57	100%	57	100%	0	0%	0	0%
4	0	0%	0	0%	16	28%	0	0%	0	0%	8	14%	17	30%
3	1	2%	0	0%	14	25%	0	0%	0	0%	20	35%	20	35%
2	0	0%	0	0%	26	46%	0	0%	0	0%	20	35%	15	26%
1	0	0%	0	0%	1	1%	0	0%	0	0%	9	16%	5	9%
0	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%

Index	INC	
	n	%
0 (none)	16	28%
1 (occasional)	31	54%
2 (moderate)	5	9%
3 (frequent)	5	9%

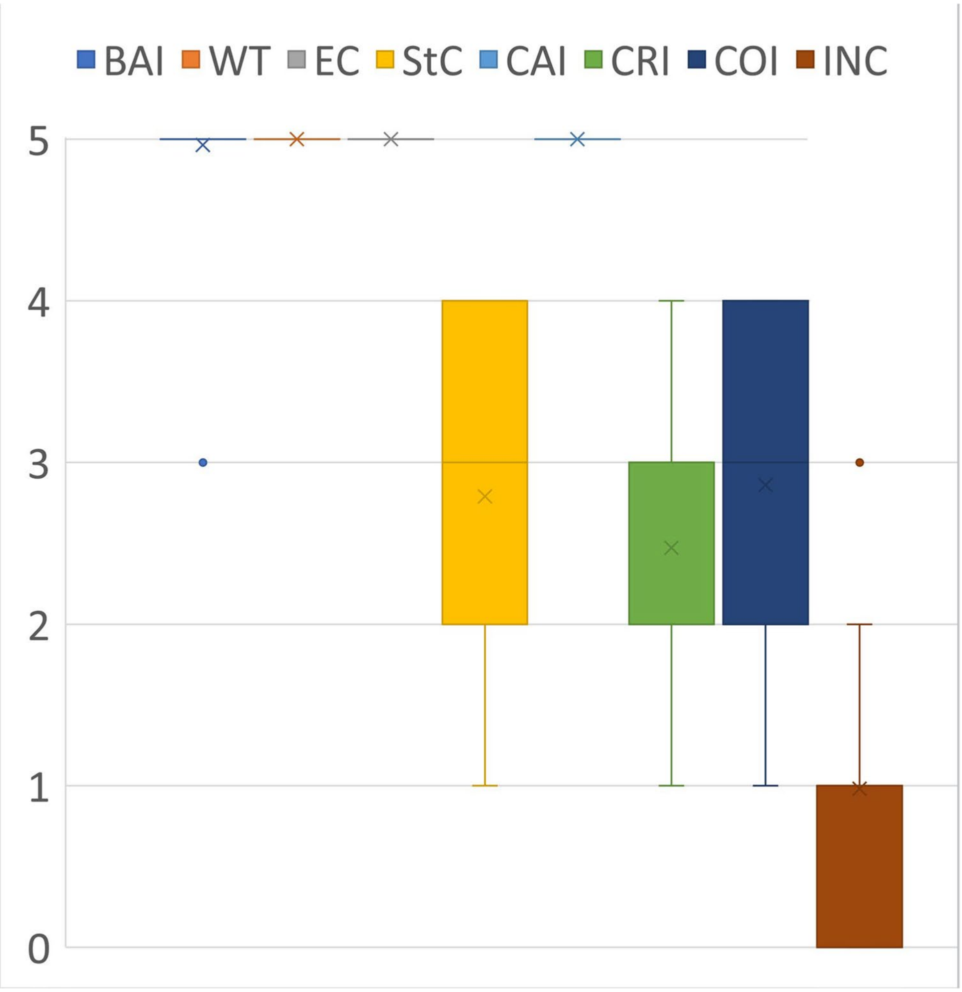


Fig. 2 Boxplots showing variation in taphonomic scores (note that the scoring range for INC is 0 to 3)

Taphonomic variables

Bacterial MFD (BAI)

A number of histotaphonomic studies have suggested that bones from intact, fleshed bodies are more likely to be affected by bacterial bioerosion than unburied or processed bones such as those from butchered animals (e.g., Jans et al. 2004; Nielsen-Marsh et al. 2007). It is thought that deviations from this general pattern are a reflection of anthropogenic factors rather than long-term environmental conditions (Hollund et al. 2012; Smith et al. 2007). Based on the archaeological evidence for delayed burial at Çatalhöyük, low levels of bacterial attack among some of the samples were anticipated. Intriguingly, however, only one sample, CH53 (skeleton 11,659), exhibits a series of tunnels that track with osteon lamellae but lack the hypermineralized rims typically associated with bacterial bioerosion (Supplementary Table 2). Turner-Walker (pers. comm.) suggests that the tell-tale hypermineralized rims associated with bacterial tunneling may have been dissolved into the surrounding soil due to leaching (Berna et al. 2004; Kendall et al. 2018). Sample CH53 has a BAI score of 3 (> 67% of observable bone structure affected). The boxplots in Fig. 2 show that sample CH53 is a statistical outlier within the BAI category and the PCA biplots. While the post-Neolithic samples derive from cultural contexts (Roman and early Islamic) in which immediate burial after death was the norm, none of them show signs of bacterial MFD either.

Wedl tunneling (WT)

Wedl tunnels are typically associated with fungal attacks originating from an external source (Hackett 1981; Trueman and Martill 2002; Fernández-Jalvo et al. 2010; Fernández-Jalvo and Andrews 2016) and may imply that the affected bones were exposed for a period above ground. However, Turner-Walker and co-workers (Turner-Walker and Jans 2008; Turner-Walker 2019) argue that they are caused by cyanobacteria rather than fungi and thus only occur in aquatic environments. A study by Jans and co-workers (2004) shows that Wedl tunneling (associated with fungal attack) is more common in archaeological animal bone assemblages than in archaeological human bone assemblages. In the present study, Wedl tunnels were not observed in any of the Çatalhöyük bone samples (Table 3).

Stained canaliculi (StC)

Within the Çatalhöyük assemblage, every sample (100%) showed some degree of stained canaliculi, with OHI scores ranging from 1 ($n = 1$) to 4 ($n = 16$), with a median score of 3 (Table 3, Fig. 2). These were most often observed via standard light microscopy, and occasionally with bSEM, in which the staining appears more electron-dense (i.e. lighter) than the surrounding bone tissue (e.g. sample CH47). Unlike the enlarged canaliculi described by Fernández-Jalvo and co-workers (Fernández-Jalvo et al. 2010: Figs. 5.4–5.6), the occurrence of stained canaliculi is not restricted to the periosteal cortical layer. Nevertheless, the frequency with which they occur suggests that site-wide soil chemistry has a role to play in their formation.

Enlarged canaliculi (EC)

While some degree of staining was observed throughout the Çatalhöyük sample, enlarged canaliculi (verified by backscatter SEM) are entirely absent in the Çatalhöyük assemblage (Table 3). As stained canaliculi are typically recorded as Wedl type II tunneling, this highlights the importance of using SEM to confirm histological observations made using standard light microscopy.

Cyanobacteria (CAI)

No evidence for cyanobacteria tunneling was observed in the Çatalhöyük assemblage (Table 3). Thus, while the surrounding landscape may have been a mixed wetland ecosystem, there is no evidence that the sampled individuals spent any length of time within an aquatic environment conducive to cyanobacterial growth.

Cracking (CRI)

Some degree of cracking, or microfissures, was observed in all Çatalhöyük bone samples, with OHI scores ranging between 1 (16%) and 4 (14%) (Table 3). The median score is 2 (Fig. 2).

Collagen birefringence (CBR)

Collagen birefringence ranged between OHI scores of 1 (9%) and 4 (30%) (Table 3). The median score is 3 (Fig. 2).

Inclusions (INC)

The occurrence of inclusions such as mineral precipitations and soil particles in bone pores and cracks is highly variable in the sample assemblage (Table 3). Some 28% ($n = 16$) of the sample showed no inclusions, 54% ($n = 31$) showed occasional inclusions, 9% ($n = 5$) showed moderate inclusions, and 9% ($n = 5$) showed frequent inclusions. The

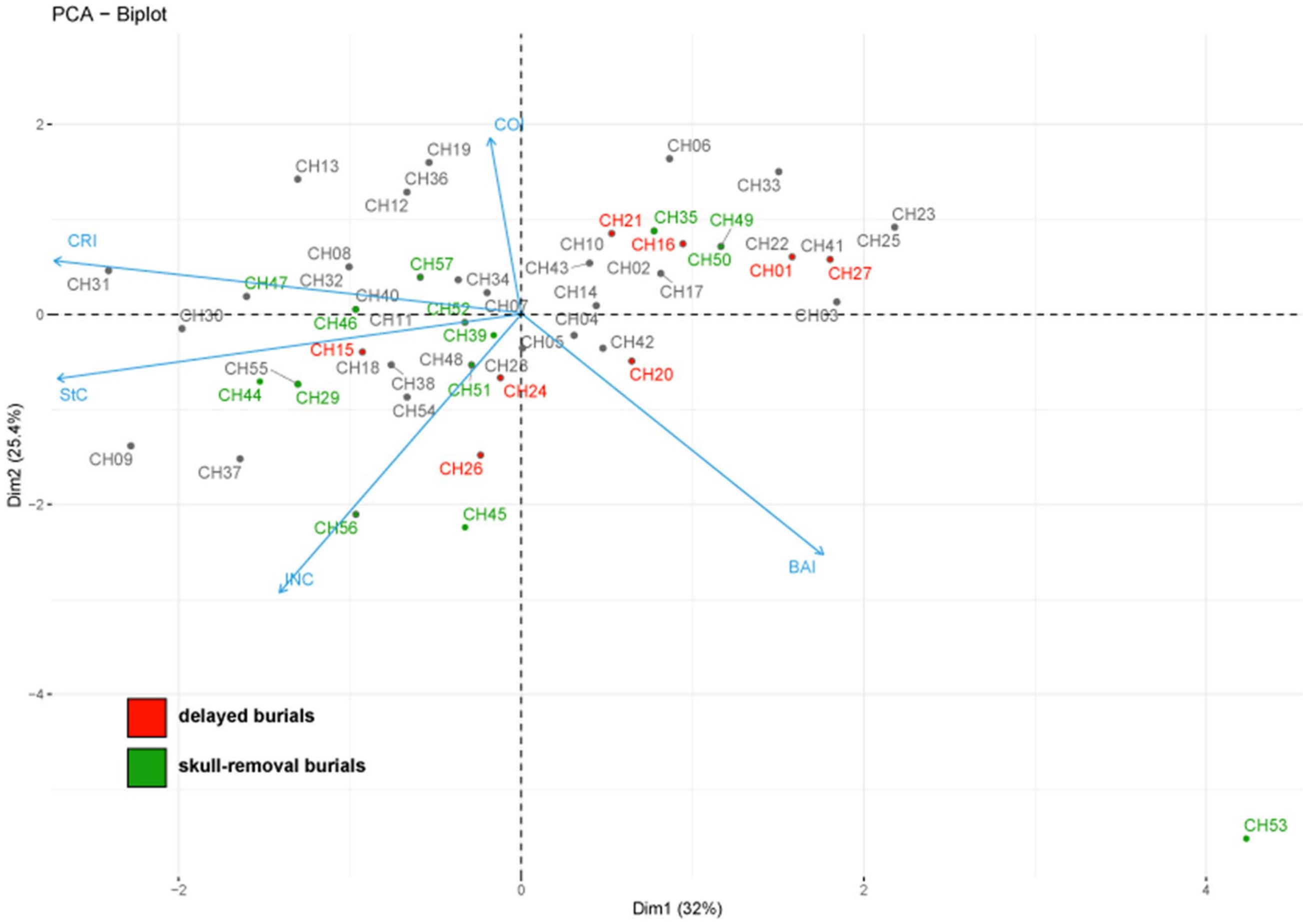


Fig. 3 PCA biplot of all Çatalhöyük samples. Taphonomic variables are represented by blue arrows. Delayed burials are in red, and skull-removal burials are in green

most common type of inclusion consisted of birefringent particles with high silicon content, likely reflecting soil particles. Less common inclusion types include gypsum crystals within the pores of a burial from Building 52 identified using EDS (sample CH29). The median score is 1 (Fig. 2).

Multivariate statistical analysis

Despite the lack of clear-cut bacterial MFD, the Çatalhöyük samples show a range of variability among the other taphonomic variables observed. For the most part, however, the multivariate statistical analyses do not reveal clear associations between these variables and the osteological, contextual, and chronological data (Supplementary Table 1). Most importantly, the samples from suspected delayed or skull-removal burials are indistinguishable from the rest of the assemblage (Fig. 3).

When the Neolithic, Roman, and early Islamic samples are plotted together (Fig. 4), it is noteworthy that the post-Neolithic individuals (labelled in red) do not cluster separately, although they tend to have higher levels of stained canaliculi and cracking. As with the majority of the Neolithic samples, bacterial MFD are entirely absent. For the remainder of the multivariate statistics presented below, the post-Neolithic samples have been removed.

One of the clearest results of the statistical analyses is that individuals from within the same building or space (e.g., Buildings 17, 49, 77, 80, 131, and Space 602) often have disparate taphonomic values, such that they do not cluster together in the overall multivariate plots (Fig. 4). This runs counter to what might be expected if these taphonomic variables were influenced primarily by the burial environment beneath individual houses (e.g., soil composition, pH, moisture levels). For example, two individuals (samples CH04 and CH05) were buried simultaneously within the same grave cut in Building 80, but CH04 has more cracking (CRI = 2) than CH05 (CRI = 4), while CH05 has higher levels of stained canaliculi (StC = 2) than CH04 (StC = 3). Perhaps the variation between the two individuals reflects subtle differences in post-mortem—but pre-burial—events/treatments (intentional or not). Similarly, two individuals

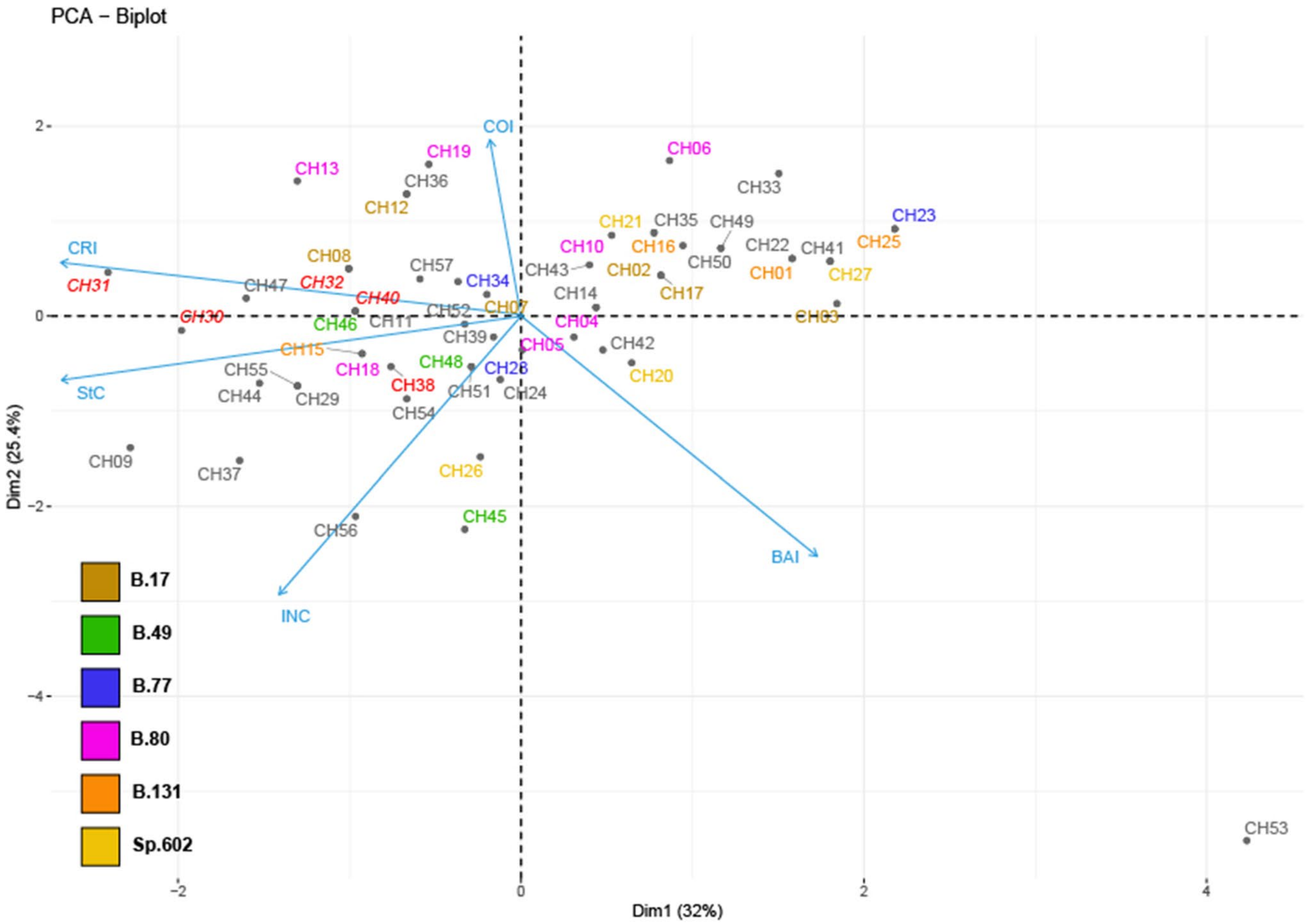
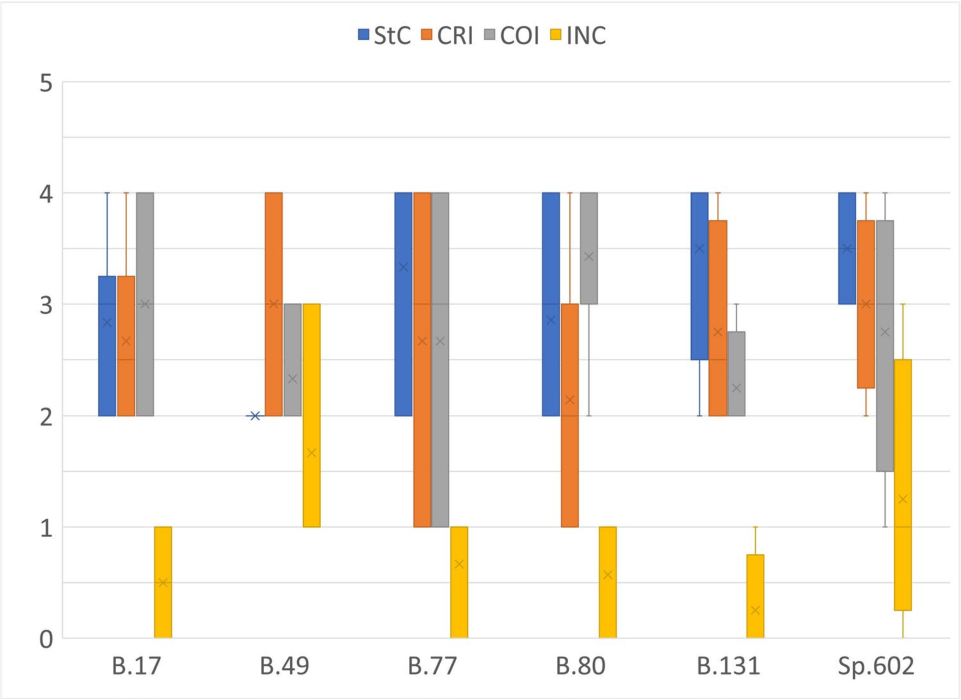


Fig. 4 PCA biplot of all Çatalhöyük samples, including post-Neolithic individuals (in *red*). Taphonomic variables are represented by blue arrows. Buildings and spaces with three or more samples have been color coded

Fig. 5 Boxplots showing variation in histotaphonomic scores within buildings and external spaces containing more than three individuals



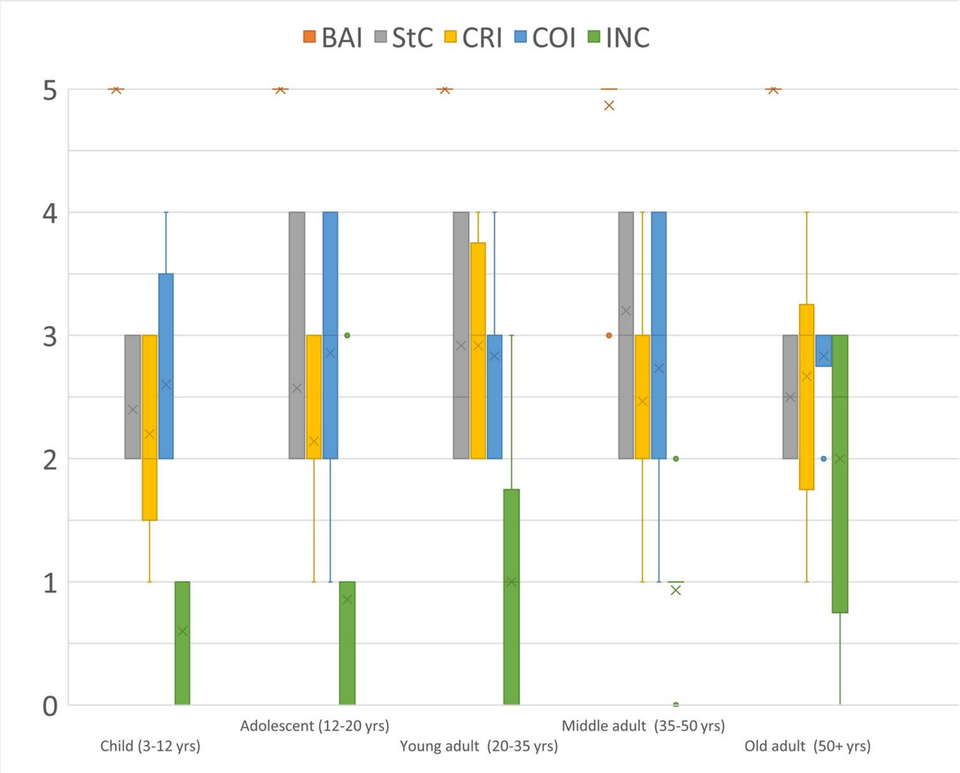
(samples CH18 and CH19) were buried in separate grave cuts within the eastern platform of Building 80, but CH18 has more stained canaliculi (StC = 2), lower collagen birefringence (CBR = 2), and more inclusions than CH19. Of three consecutive interments under the eastern platform of Building 131, sample CH15 has higher levels of stained canaliculi (StC = 2) and more inclusions (INC = 1) than samples CH01 and CH16 (INC = 0). Lastly, in Building 49, an old adult female (CH45) and child (CH46) buried separately but under the same northwest platform are strikingly different in terms of inclusions and cracking, with the adult having more inclusions (INC = 3) but less cracking (CRI = 4) than the child. Similarly, individuals from external space Sp.602, despite being located in the same soil context and sharing features associated with delayed burial (e.g., hyperflexion, paradoxical disarticulation, missing elements), have divergent histotaphonomic signatures. The degree of variation within buildings and spaces containing three or more individuals is also illustrated as boxplots in Fig. 5. Here, external space Sp.602 and Building 77, which was built above it, have the highest levels of taphonomic variation among co-burials. Variation in cracking, for example, may reflect the degree to which an individual body was skeletonized (and desiccated) prior to interment.

Another finding, although not strongly pronounced, is an age-based patterning observed in the multivariate plots (Fig. 6) adults (50 + years) tend to cluster in the lower left quadrant characterized by high inclusions (INC) and stained canaliculi (StC). Children (3–12 years) and, to a lesser extent, adolescents (12–20 years) tend to cluster along the negative axis of dimension 1 (*x*-axis), characterized by high cracking (CRI), stained canaliculi (StC), and inclusions. Young and middle adults are more evenly distributed. Figure 7 shows the overall variability in taphonomic scores by age category.

A weak temporal patterning of taphonomic features is discernible among the Neolithic Çatalhöyük samples. The majority of samples from the Early and Late periods have negative values on dimension 1 (*x*-axis) associated with higher levels of stained canaliculi (StC), cracking (CRI) and inclusions (INC) (Fig. 8). Samples from the Middle period appear to have the highest levels of variability in terms of taphonomic scores (Figs. 8 and 9).

Fig. 6 PCA biplot of bone samples showing age distribution. Taphonomic variables are represented by blue arrows

Fig. 7 Boxplots showing variability in taphonomic scores by age category



Discussion

The lack of bacterial MFD in archaeological human bone assemblages has been interpreted by some researchers as evidence for extended funerary treatments involving defleshing or desiccation of the corpse prior to burial (Booth et al. 2015; Booth 2016; White and Booth 2014). As such, we anticipated that samples from suspected delayed burials at Çatalhöyük would not show signs of bacterial MFD, but samples from regular contexts would. Instead, unequivocal signs of bacterial MFD (i.e., tunneling with hypermineralized rims) within the Çatalhöyük bone samples are completely lacking. This makes it difficult to conclude that this is a direct result of intentional funerary treatments, especially since the post-Neolithic samples also lack bacterial MFD. While early post-mortem interventions are thought to have a greater influence on the preservation of bone microstructure than the burial environment (Bell et al. 1996; Jans et al. 2004; Brönnimann et al. 2018), it is also important to account for environmental factors that may play a role in inhibiting bacterial bioerosion (Turner-Walker 2019). For example, if the bacteria responsible for patterns of MFD observed in bone originate in the soil, rather than the gut, what are the specific microenvironmental conditions beneath Çatalhöyük house floors that might inhibit them? Çatalhöyük is a tell/mound site that sits well above the water table. The mound is made up of the accumulation of successive occupation layers consisting primarily of mudbrick structures and midden deposits with a neutral to alkaline pH (Schotsmans et al. 2019). Thus, burials occur within anthropogenic soils that may contain different or reduced microbial populations than do natural soils. Furthermore, the high amounts of gypsum naturally present in the soil (Schotsmans et al. 2019) and the extensive use of ash in middens and elsewhere on the site (e.g. Shillito et al. 2013) may also have a role to play in suppressing microbial activity. Ethnographic and archaeological studies have demonstrated the use of ash as an insecticide (Hakbijl 2002) as well as to preserve foods and process hides (Lemorini et al. 2020); thus, the intentional use of ash in funerary contexts cannot be excluded as a factor in these results. Furthermore, previous studies indicate that bones recovered from organic-rich contexts such as middens have reduced levels of bioerosion (Jans 2005; Nicholson 1998). However, our multivariate tests could not differentiate between burials cut into midden deposits or more sterile contexts such as building infills, which consist primarily of mudbrick rubble. A larger study sample including skeletal remains from a wider range of contexts, including more samples from secondary burials, could help shed light on these issues.

The patterning related to the age-at-death of individuals observed in the multivariate plots is suggestive of selection biases in the post-mortem "histories" of individuals. Based

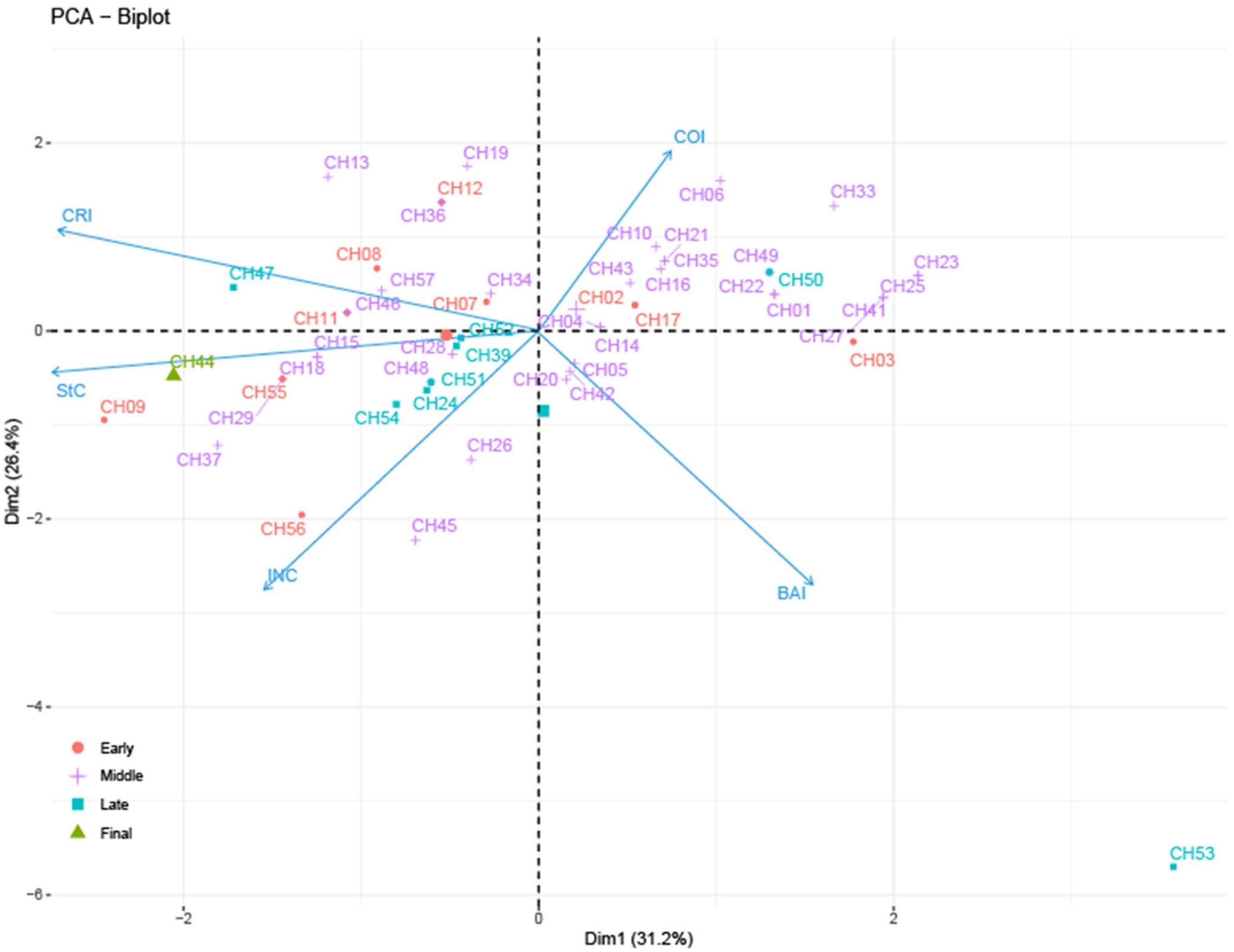


Fig. 8 PCA biplot of samples labelled according to temporal periods at Çatalhöyük. Taphonomic variables are represented by blue arrows

on the observed taphonomic variability, older adults, as well as children and adolescents, appear to have experienced distinctive post-mortem trajectories, while young and middle adults are more evenly distributed. While the incompletely mineralized bones of younger and older individuals may be differentially affected by diagenetic processes in comparison to those of young and middle adults, previous studies have revealed other age-related patterning at Çatalhöyük in terms of diet as well as grave good and figurine distribution (Nakamura and Meskell 2009, 2013a, b; Pearson and Meskell 2013; Pearson et al. 2015; Vasić et al. 2021). It is unclear, however, if this taphonomic variability reflects differences in body treatments, pre-burial storage of the body, duration of time between death and final burial, or some other factor. Perhaps it relates to mobility levels of different age groups, with young and middle adults more involved in resource acquisition and animal herding than older adults, children, and adolescents, and thus more likely to die away from the site and experience delays in being returned to site for burial.

In terms of temporal variation, samples from Middle period burials display higher intra-variable variation than do burials from the Early and Late periods (Figs. 8 and 9). The Middle period at Çatalhöyük is associated with peak population density at the site (Larsen et al. 2015), but beyond this, it is unclear what this patterning implies. It may be linked to population mobility, as previous studies suggest that individuals spent increasingly more time away from site as part of changing animal management and resource acquisition strategies (e.g., Pearson et al. 2007; Charles et al. 2014; Larsen et al. 2015). Perhaps fewer off-site deaths would account for the lower levels of taphonomic variability observed in the Early period samples, but it does not help to explain the Final period scores.

Similar to the concept of individual "life histories," the taphonomic variation observed within house burial assemblages at Çatalhöyük suggests that these individuals

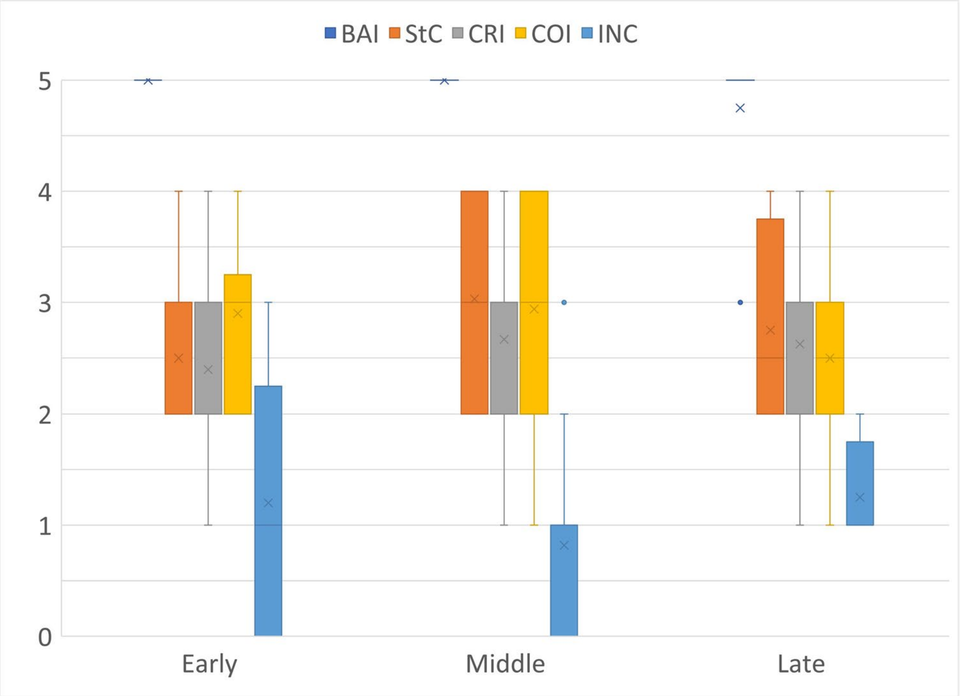


Fig. 9 Boxplots showing variability in taphonomic scores by temporal period

had diverse "post-mortem histories"; the bones of some individuals (sometimes within the same platform) are less affected by bioerosion and other taphonomic processes while others score much higher. This accords with macroscopic observations in the field that the preservation of skeletons interred under the same house floor (and presumably within the same soil matrix) are often highly variable (e.g., compare in situ photograph of skeleton CH02 with that of skeleton CH08 from Building 17 or CH24 with CH29 from Building 131—Supplementary Table 2). It is unlikely that differences in subfloor soil conditions alone could account for the variability observed between interments.

Based on the common assumption that households (however conceived for the Neolithic period) would share a "community of practice" in terms of mortuary practices, one might expect that individuals buried within the same house would be similar in terms of burial treatment, but this is not always the case. Previous researchers have suggested that burials at Çatalhöyük may have taken place on a seasonal basis (e.g., Mellaart 1967: 204–205; Haddow and Knüsel 2017; Haddow et al. 2021). Thus, rather than reflecting intentional treatments of the body, such as mummification or excarnation, these diverse post-mortem histories may reflect practical considerations related to seasonality or the length of time between death and final burial. Such histories may inform decisions regarding who is selected for burial in a particular house and who is excluded. Another potential factor is the differential use of textile, reed matting, or hides to wrap the body (Vasić et al. 2021). Forensic and experimental studies have shown that body covering will affect decomposition rates (Aturaliya and Lukasewycz 1999; Forbes et al. 2005; Mann et al. 1990) and thus may also affect histotaphonomic preservation.

Previous histotaphonomic studies at Çatalhöyük

A previous histotaphonomic study of human burials at Çatalhöyük using ribs (Goren et al. 2020: 126) assigned an OHI score of 0 or 1 (representing less than 15% preserved bone microstructure) to 90% of the sample ($N = 162$). This poor preservation is attributed by the authors to bacterial bioerosion. For example, in Fig. 6, Goren et al. (2020: 130) assign an OHI score of 0 (< 5% intact bone) to a rib sample (individual 4593) with dark staining and obscured microstructure. However, it is not clear that these alterations are the result of bacterial bioerosion and not some other taphonomic process. Femoral bone from the same individual (CH56: individual 4593) was analyzed in the present study and while stained canaliculi were visible, no evidence of bacterial tunneling was observed. This sample is only one of two samples shown in the Goren et al. (2020) paper, so it is difficult to evaluate their overall claims of high levels of bacterial bioerosion. Furthermore, without using more advanced imaging techniques such as backscatter SEM, it is not possible to identify the characteristic hypermineralized tunnel rims associated with bacterial bioerosion. In addition, by collapsing multiple taphonomic indicators such as bacterial and Wedl tunneling, staining, and cracking into a single OHI score, it is impossible to know how much of the poor microstructural preservation is attributable to any single factor. As such, the authors appear to have conflated poor overall bone microstructural preservation with high levels of bacterial bioerosion. The present study, albeit using a smaller sample, has not identified unambiguous signs of bacterial modification, even with the use of backscatter SEM.

Goren et al. (2020: 131) acknowledge, however, the limitations of using ribs, with their thin and fragile cortices, as opposed to long bones such as femora, which are more commonly used in histotaphonomic studies. It could be that ribs and other flat bones are more susceptible to taphonomic alteration than long bones, but until their samples can be analyzed with SEM, we remain skeptical of their identification of bacterial bioerosion. An interesting avenue for future research would be to systematically compare variation in taphonomic scores for several bones from the same individual. In an experimental study using domesticated pigs (Kontopoulos et al. 2016) and a subsequent study of medieval human burials (Kontopoulos et al. 2022), Kontopoulos and co-workers suggest that bioerosion may affect different parts of the skeleton at different rates.

Conclusions

With only one individual (sample CH53) showing clear signs of bacterial MFD, bioerosion is strangely lacking in the Çatalhöyük sample assemblage. Despite this, the present study has revealed variability in terms of the other taphonomic criteria observed and recorded. Apart from weak age-at-death and temporal associations, however, the statistical analyses show that this variability is not clearly associated with burial location or other biocultural factors. Excavators at Çatalhöyük have long observed that individuals buried under the same house floors often have dramatically different bone preservation, and this is confirmed by some of the samples examined here. It is unlikely that localized subfloor soil conditions could lead to such inter-individual variability. Rather, it appears that each individual buried within a specific house had its own postmortem—but preburial—history. This observation has important implications for studies of prehistoric kinship structures and social organization, as recent palaeogenomic studies have shown that co-burials (i.e., individuals buried within the same house) at early ceramic Neolithic Anatolian sites such as Çatalhöyük and Barcın Höyük are not as closely related genetically as individuals from earlier aceramic sites such as Boncuklu Höyük and Aşıklı Höyük (Yaka et al. 2021). This presents an interesting parallel between the diversity of kin relationships and the diversity of taphonomic signatures among co-burials.

This high degree of histotaphonomic variability, in tandem with previously observed variation in skeletal flexion and articulation (Haddow and Knüsel 2017; Haddow et al. 2021), suggests that subfloor inhumations may have taken place at prescribed intervals, perhaps on a seasonal basis. Thus, the observed variation may reflect differences in the length of time between death and burial. Other potential factors include the use of textiles, matting, and animal hides to wrap the dead. In the future, it is hoped that a larger sample of Çatalhöyük individuals—drawn from a wider range of contexts and age groups—will help clarify these issues, as well as resolve the uncertainty behind the lack of true bacterial MFD observed in the present study.

On a methodological note, we emphasize the need to supplement traditional light microscopy analysis of thin sections with more advanced imaging techniques in order to avoid conflating multiple taphonomic processes. Certain taphonomic features, such as bacterial tunneling and enlarged canaliculi can only be confirmed with backscatter SEM. In addition, non-destructive techniques such as microCT are becoming more common and provide greater coverage (Booth et al. 2015; Duffett Carlson et al. 2022; Mandl et al. 2022).

Lastly, an important outcome of this study is the recognition of the need for caution when using the presence/absence of bacterial MFD alone to make direct inferences about funerary practices. At Çatalhöyük, the archaeological and osteological evidence suggestive of delayed burial (e.g., tight flexion, paradoxical joint disarticulation, missing skeletal elements) is substantial, yet the histotaphonomic analyses cannot distinguish these individuals from the rest of the skeletal assemblage. The results of this study show that we are only scratching the surface when it comes to our ability to link histotaphonomic variability with anthropogenic interventions. There are clearly a host of factors involved, and far more work is required to disentangle them. An especially promising avenue of investigation would involve the role of ash in suppressing microbial activity.

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Author contribution The project was conceived by SDH, CJK, and ES. SDH and ES sampled the skeletal material. SDH, SMV, and BY carried out the sample preparation and microscopical analyses. CM conducted the statistical analysis. Interpretation of results was conducted by SDH and CM with the assistance of TB. SDH wrote the manuscript text. All authors reviewed the manuscript text.

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Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files). A representative selection of microscopic (transmitted light and bSEM) images for each bone sample is provided in Supplementary Table 3. The full set of images are available upon request.

Code availability N/A.

Declarations

Ethics approval All methods were carried out in accordance with relevant guidelines and regulations. No human or animal experiments were carried out for this study. Human skeletal remains were analyzed with the consent of the Turkish authorities under the permit from the Ministry of Culture and Tourism, General-Directorate of Cultural Heritage and Museums, provided to the Çatalhöyük Research Project under the direction of Prof. Ian Hodder. All protocols were approved by Koç University and the University of Copenhagen.

Consent to participate N/A.

Consent for publication N/A.

Competing interests The authors declare no competing interests.

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