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Experimental investigation of histotaphonomic changes in human bone from whole-body donors demonstrates limited effects of early post-mortem change in bone

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ABSTRACT

In recent years histological analysis has become widely used for reconstructing mortuary treatment in archaeological contexts. Interpretations rely on the degree and nature of microstructural taphonomic changes, particularly bacterial attack, but there is considerable disagreement on how these changes should be interpreted. Some researchers believe the origin of bacteria to be endogenous (i.e. from the gut) and others consider it to be exogenous (i.e. from the soil), with the two scenarios pursuing different interpretative pathways. In addition, the timing and duration of bacterial attack and other microscopic modifications is poorly understood. A paucity of experimental research, especially on whole-body human cadavers, has proved a barrier to confident interpretation of histotaphonomic data and as such research has often relied on received wisdom and inferential patterns.

This study makes progress towards addressing these issues through controlled experimental research on five human cadavers in different burial scenarios at the Forensic Anthropology Center at Texas State. The burial conditions comprised 1) buried in soil, 2) buried in a coffin, 3) semi-buried in a coffin, 4) exposed on the ground surface, and 5) exposed in an unfilled trench all for a duration up to 30 months. Contrary to expectations, the different burial scenarios produced very little variation in histological preservation. In addition, very little bioerosion occurred on any of the remains throughout the duration of the study. Crucially, this suggests that bioerosion may not relate to the early post-mortem period, as has often been considered and means some previous interpretations may require reconsideration. Further work is required to clarify the variables impacting varied preservation.

1. Introduction

Histological analysis of bone is applied in various contexts for wide-ranging purposes such as age-at-death estimation (Gocha et al., 2019), paleohistopathology (Schultz, 2001; Assis et al., 2015), human vs nonhuman differentiation (Mulhern and Ubelaker, 2001; Dominguez and Crowder, 2012), and histotaphonomic interpretation (e.g. Hackett, 1981; Garland, 1987; Bell, 1990; Hedges and Millard, 1995; Hollund et al., 2012). In recent years, the use of histotaphonomy to interpret archaeological material has become increasingly popular (Mulville et al., 2012; White and Booth, 2014; Booth, 2016; Booth and Madgwick, 2016, Brönnimann et al., 2018, Brönnimann et al., 2020; Booth et al., 2022), although interpretation of histotaphonomic signatures has varied. The signature of histological alteration can differ from generalized destruction down to single foci of modification visible in 2D cross-sections. Histotaphonomists assign these alterations to type according to whether they are caused by fungi or bacteria (Hackett, 1981) with bacterial attack being the most predominant form of bioerosion in archaeological material (Booth, 2016). In archaeological contexts, the extent of the attack is then quantified through the application of various indices which describe the percentage of the cross-section affected by bioerosion (see Hollund et al., 2012). Through this process, histotaphonomy has challenged long-standing beliefs about mortuary practices (e.g. excarnation and violence, see Booth et al., 2015; Booth and Madgwick, 2016). However the interpretations are impeded by the ambiguity surrounding histological diagenesis, including questions

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regarding (i) whether the bacterial attack visible in bone originates within the decaying organism itself (endogenous) or from the surrounding depositional environment (exogenous), although there is an increasing recognition that the origins of bacteria that attack bone may be both endogenous and exogenous (Emmons et al., 2022), and (ii) the point at which, during the postmortem interval, histological diagenesis appears in bone.

1.1. Bacterial origin

An unresolved issue in histotaphonomic studies which has substantive implications for histotaphonomic interpretation of archaeological material is whether the bacterial attack visible in bone is due to endogenous or exogenous bacteria at/near the time of death or to post-decomposition processes. For researchers who interpret histotaphonomic changes from the endogenous model view, meaning the bacteria attacking bone are originating from the gut microbiome of the deceased, the presence and intensity of bacterial attack has been interpreted as a proxy for early post-mortem treatments (i.e. mortuary treatment of the body during the putrefactive stage of decomposition, before skeletonization occurs). Histotaphonomic signatures from the endogenous viewpoint have recently been used to interpret a diverse array of mortuary treatments in archaeological contexts, including mummification, partial exposure, and exhumation (e.g. Booth et al., 2015; Booth and Madgwick, 2016; Brönnimann et al., 2018; Bricking et al., 2022; Madgwick and Bricking, 2023 in press).

Initially, the assignment of endogenous origins of bacterial attack achieved prominence due to butchered animal remains frequently having well preserved histological structures while fully articulated human remains (buried with gut bacteria in situ) having more poorly preserved histological structures (e.g. Jans et al., 2004). Since these initial observations, one of the most compelling arguments for an endogenous bacterial origin is the reduction or absence of attack seen in neonate remains. This pattern was observed experimentally in pigs by White and Booth (2014), and also noticed in neonatal human remains in archaeological contexts by Booth (2016) and Booth et al. (2016). The absence of bacterial attack in the neonatal pigs (White and Booth, 2014), as well as the reduced instances of the presence of bacterial attack in human infants when compared with the adult individuals in the same burial environment (Booth, 2016), lead the authors of both studies to conclude that the neonatal piglets and infants had not yet developed the microbiome necessary for microbial bioerosion. The use of micro CT by Booth et al. (2016) examining neonatal remains from Romano-British contexts also supports these findings. Further work by Kontopoulos et al. (2016) support this endogenous model through porcine experiments which found that buried pigs wrapped in synthetic carpet, when compared to buried pigs wrapped in cotton and cotton-based material, exhibited the highest levels of microbial attack within the bone microstructure. Kontopolous and colleagues (2016) suggested that this demonstrated a relationship between the slower soft tissue decomposition and prolonged exposure to putrefactive endogenous bacteria, due to the low-permeability of the synthetic material wrapping, resulting in higher levels of microbial attack. Most recently, Brönnimann et al. (2020) examined butchered animal bone as a proxy for remains without endogenous bacteria, and found that the butchered remains showed less bacterial attack than contemporaneous human bone in temperate European contexts, which supported their hypothesis that bacterial degradation observed in archaeological samples from Basel-Gasfabrik was caused by putrefactive gut bacteria.

Although these previous experimental studies support an endogenous origin, other experimental studies have contested this idea. Notably, a recent study by Turner-Walker (2019) which showed minor microbial attack on butchered disarticulated animal bone buried in flower pots in Taiwan indicates that osteolytic bacteria may originate exogenously, in this case from the soil placed in the pots. An exogenous origin of bacteria was also suggested by Fernández-Jalvo et al. (2010) who looked at long-term exposed animal bones in Wales and concluded that the bioerosion signatures observed, which were not consistent within or between animal carcasses, were likely due to soil bacteria. More recent studies such as Turner-Walker et al. (2022) and Eriksen et al. (2020) have also indicated that bone biodeterioration may be related to exogenous bacteria from the soil, instead of from the individual. The inconsistencies across these experimental animal studies and archaeological interpretations discussed above highlights the necessity for experimental studies to further understand the origin of bacterial bioerosion in order to correctly interpret early post-mortem mortuary practices in archaeological contexts.

1.2. Timing of diagenesis

Experimental research concerning human remains can answer questions on the relationship between time and diagenesis, specifically how long it takes for substantive bacterial attack to appear in bone as visualized in a histological cross-section. Knowing when after death to expect visible diagenesis in human skeletal remains directly informs the interpretation of archaeological burial evidence. Previous research by Bell et al. (1996) has suggested that microfocal changes in bone can take as little as 3 months, while Yoshino et al. (1991) demonstrated identifiable bioerosion 2.5 years post deposition. The mechanism by which endogenous bacteria can appear in bone within days of death is outlined by Bell et al. (1996). Bell et al. (1996) explain that the transmigration of gut bacteria to other organs occurs within 15 h of the moment of death. It is therefore feasible for the bacteria to continue to move through the Haversian systems of cortical bone and appear histologically within days after death during the early post-mortem period. Since human and nonhuman animals have basic differences in vascularization and cortical bone microstructure, the use of whole-body human donors instead of nonhuman animals to examine bacterial bioerosion timing is ideal, since it eliminates any potential influence these basic differences (e.g. the abundance of plexiform vs. secondary osteonal bone) might have on the progression of bacterial attack through bone.

Given the importance of experimental human studies to understanding the origin and time depth of bacterial attack on bone microstructure, this paper presents the first (to our knowledge) experimental whole-body human decomposition study to address histotaphonomic changes (N=5). This study is thus a novel contribution in using samples of whole-body donors with known decomposition stages and depositional histories to deliver on the following objectives (i) characterize the nature and intensity of bacterial attack on bone microstructure using fresh human cadavers in various burial environments (e.g. buried, exposed, partially exposed) for different periods of time (see Table 1 for details) and (ii) identify the origin of microbial attack on fresh human cadavers on different elements from the same body.

2. Materials and methods

This actualistic study was made possible through the body donation program run by the Forensic Anthropology Center at Texas State (FACTS) in San Marcos, Texas, USA. FACTS accepts full body donations of deceased individuals for scientific research in compliance with the Texas Anatomical Gift Act (ethical approval covered by the Texas Health and Safety Title 8, Chapter 691.001 and 692.001, and the Texas Administrative Code Title 25, Part 4, Chapters 477–485). All donors are either self-pre-registered as donors to FACTS or were donated by the legal next of kin to the research facility. Preceding body donation, written authorization for the remains to be utilized for scientific taphonomic, forensic, and biological anthropological research is required. As part of the body donation program, donors are studied in various decomposition scenarios at the Forensic Anthropology Research Facility (FARF), a karst 26-acre fenced decomposition facility located in the Texas Hill Country. While at FARF, the donors experience a climate that is marked by hot summers and cool winters, with temperatures ranging

Table 1
A summary of each donor experiment including demographics, deposition type, length of deposition, and month of initial deposit.

Donor	Sex	Age (Years)	Deposition Type	Covering	Duration of Deposition Months (Days)	Date of placement
D1	Male	82	Open Trench	Tarp & metal- wire-net covered wooden frame	29.5 (901)	March 5th, 2019
D2	Male	88	Ground Surface	Tarp covered metal-wire-net cage	20.5 (626)	December 3rd, 2019
D3	Female	86	Semi-buried in Coffin	Coffin lid	30 (909)	February 26th, 2019
D4	Male	72	Buried in Coffin	Coffin lid and soil	29.5 (898)	March 7th, 2019
D5	Female	67	Buried in Soil	Soil	27 (774)	May 7th, 2015

from subzero to over 38 °C. Flash floods resulting from heavy rains can occur during spring and fall. The FARF soils (Rumple-Comfort Association, Undulating, and Comfort-Rock Outcrop Complex, Undulating) overlie limestone. These clay soils are shallow and rocky, contain low organic matter, are in general neutral to mildly alkaline, and are well-drained but of low permeability to air and water (Carson, 2000). After decomposition at FARF, the skeletal remains of the donors are processed and curated into the Texas State Donated Skeletal Collection where they are kept in perpetuity and used in additional skeletal research (Gocha et al., 2022). For histological research, all samples taken for histological analysis are curated within the Texas State Comparative Histological Collection (TXSTCHC), and are kept in perpetuity, and are available for additional skeletal research.

For this study, a total of five individuals (henceforth 'donors') donated to FACTS were placed unclothed in various depositional environments at FARF. These environments include an open (i.e. unfilled) trench, a semi-buried coffin, a buried coffin, and a soil burial. Extreme variations in donor body mass were avoided in this study to control for the effect of body mass on histological changes. Donor body mass index (BMI), a standard measure of body size, ranged from 22.7 to 25.8 based on postmortem measurements of height and weight. A complete record of the antemortem medical treatment for each donor was unavailable and therefore not presented in this study. The variation in demographics and placement timing for each donor is illustrated in the summary data below (Table 1).

2.1. Donor depositional environments

The various depositional environments used in this study include an open trench, a semi-buried coffin, a buried coffin, and a soil burial. Three of the donors (D1, D2, D3) were observed on a regular basis throughout the decomposition process, while the fully buried donors (D4, D5) were not disturbed between placement and excavation. The variation in depositional environment for each donor allowed for examining the effects of exogenous or endogenous bacterial bioerosion since it was hypothesized that the donors directly on or in soil would have more exposure to exogenous soil bacteria than the donors protected by artificial structures like coffins. If the bacteria are endogenous in origin, it was also hypothesized that the structures which retain decomposition fluid (the coffins) would result in increased bacterial bioerosion when compared to the donors directly on the soil.

Donors D1 and D2 were placed in an extended supine position (Fig. 1b,d). D1 was placed supine in an open trench (measuring 1.95 m long x 1.55 m wide x 0.5 m deep) covered with a wooden frame with

Fig. 1. a. This wooden frame with wire-net and tarp attached covered the trench where D1 was placed to decompose. 1 b. The remains of D1 upon recovery. Desiccated skin covers the majority of the remains with local skeletonization present. 1c. A wire-net cage covered with tarp was placed over D2 who decomposed on the ground surface. 1 d. The remains of D2 upon recovery, desiccated skin covers parts of the otherwise skeletonized remains.

wire-net coverage and with tarps attached to avoid scavenging and direct sunlight (Fig. 1a–b). D2 was placed on the ground and covered with a wire-net cage with tarps attached to avoid scavenging and direct sunlight (Fig. 1c–d). The coverings on both D1 and D2 were not in direct contact with the donors.

D3 was placed in a semi-buried coffin to the extent that the lid was at ground level thus allowing regular observation of the decomposition progression (Fig. 2). The three structures (for D1–D3) were opened for documentation (approximately between 5 and 15 min per occasion) three times per week for the first six months, once weekly for five months, once every second week for four months, and once monthly until disarticulation. Disarticulation occurred at two and a half years post-placement for D1 and D3, and at one year and eight months following placement of D2 (Table 1). Due to local COVID-19 pandemic restrictions from mid March – early June 2020, the donors could not be monitored during this time (c. one year following placement for D1 and D3, c. three and a half months following placement of D2).

D4 was placed in a coffin and buried in soil at a depth of 90 cm at its deepest point (40 cm of soil covered the top of the coffin). This coffin burial was not disturbed throughout the experiment. Initial photo documentation took place through a camera mounted inside the coffin. The camera took a photo daily for the first three months of the experiment until the battery of the camera was exhausted. The coffins for D3 and D4 were built by one of the authors (CA) and made from untreated Southern Yellow Pine shiplap boards and untreated pine wooden joists (Fig. 2, consult Alfsdotter et al., 2022 for details on coffin construction).

D5 was placed in a flexed supine position in a soil burial at a depth of 70 cm and covered with soil (Fig. 3). The burial was not disturbed or uncovered for the entire length of the experiment. The state of decay for each donor was noted throughout the postmortem interval when monitored, and at the time of disarticulation for all donors.

Fig. 3. D5 was buried in a flexed supine position and covered with soil. The image shows the skeletonized remains at recovery shown here as a photogrammetry composite from the excavation. (Image courtesy of Dr. Hayley Mickleburgh).

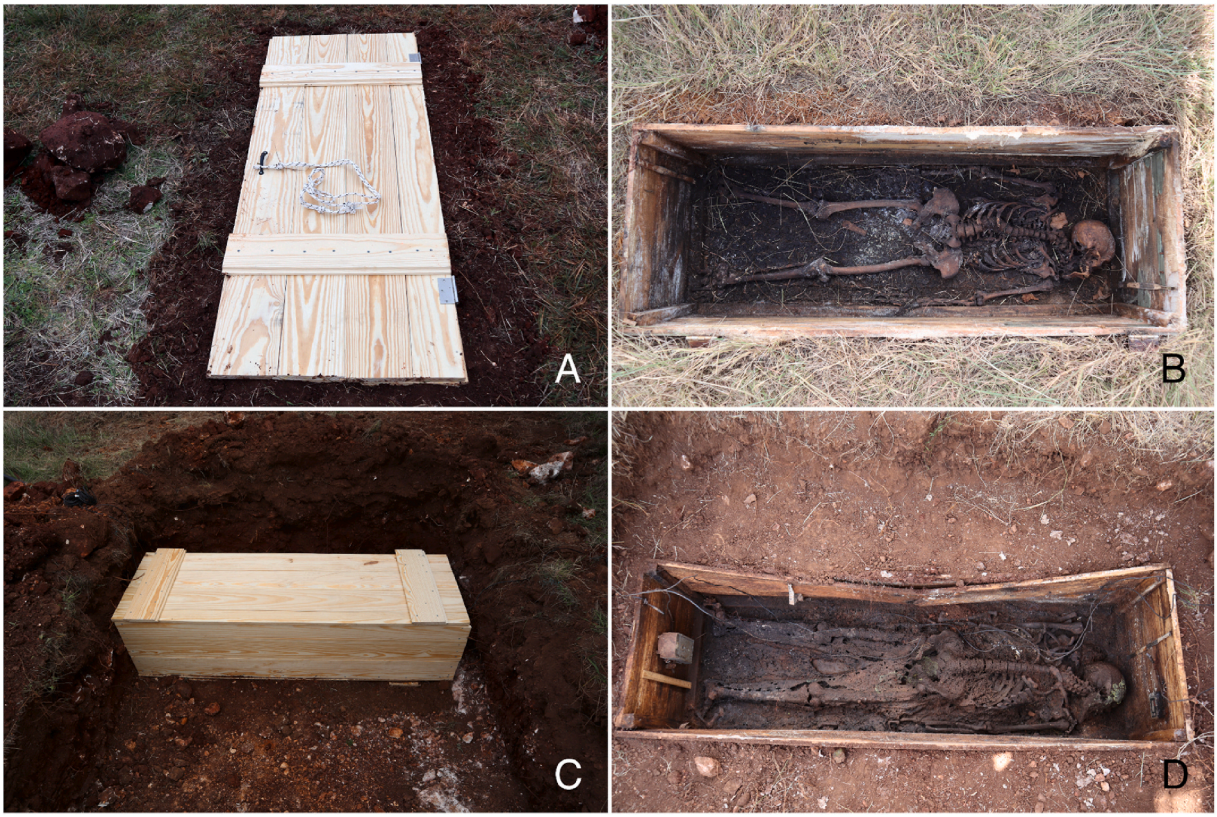

Fig. 2. a. The wooden coffin built for D3 was buried to the extent that the lid was at ground level which allowed regular observation throughout decomposition. 2 b. The skeletonized remains of D3 upon recovery. 2c. The wooden coffin built for D4 was buried in a trench dug with a small excavator. 2 d. The remains of D4 upon recovery. The remains are skeletonized but remnants of saponified tissue are retained in the coffin.

2.2. Histological sampling

The histological samples for this study were chosen to maximize intra-individual skeletal sampling where possible. The variation in sampling location (rib, tibia, and metatarsal) was chosen to examine if there was any measurable effect on bone microstructure dependent on the distance of the bone sample from the gut microbiome, where the endogenous bacteria should originate. The rib sample was chosen to be closest to the gut, followed by the tibia, and then the metatarsal. These sampling locations also allowed for maximum utility of histological samples since the rib is used in histological age-at-death estimation (Cho et al., 2002), the tibia mimics previous histotaphonomic sampling locations for cross-study comparisons (Bell et al., 1996), and the metatarsal has also been used in histotaphonomic research on burned remains (Mavroudas et al., 2022), the effects of which can be compared to this study in future research.

Following the excavation and recovery of the remains from their various depositional scenarios, 1–2 cm^2 samples of bone were removed with a Dremel tool from four of the five donors from the midshaft of the sixth rib, anterior tibia midshaft, and midshaft of a metatarsal. One donor (D5) was sampled only from the midshaft of the sixth rib due to sampling restrictions imposed by FACTS. The bone samples were prepared using standard histological protocols (Crowder et al., 2012) summarized below. The samples were embedded in Buehler Epothin 2 Epoxy Resin to facilitate processing. Once cured for at least 24 h, the blocks were cut on a Buehler Isomet 1000 saw with a diamond blade and ground using diamond-coated discs on a Buehler Ecomet 4000 variable speed grinder/polisher to the desired thickness (ranging from 30 to 80 μm). The samples were mounted on glass slides using Eukitt and cover-slipped to facilitate imaging. Each sample was imaged on a Leica DM6M light microscope and evaluated through a combination of digital imaging and live microscopic observation using 10x-400x magnification for 100% of the cross-section under both polarized and standard transmitted light.

For each sample, the scores for the following indices were collected: Cracking Index (CI), Birefringence Index (BI), Oxford Histological Index (OHI), and the General Histological Index (GHI). A description of each index and their associated scores are listed in Table 2. Detailed descriptions for each stage of OHI and GHI are listed in Table 3. Generalized notes were also recorded for each sample (e.g. on infiltrations, inclusions, staining and general section character). After recording all indices' scores, the results were qualitatively analyzed, and images of any histotaphonomic change were recorded. The scores for all indices and the visible histotaphonomic changes are reported below.

Table 2
List and descriptions of each index employed in the study along with the source information.

Index Name	Scale	Description	Reference
Cracking Index (CI)	% of Cracked Osteons	Percentage of cracked vs non-cracked osteons observed in five microscopic fields performed at 200X magnification	Reported in Hollund et al. (2012), developed by Jans (2005)
Birefringence Index (BI)	0–1	Measurement of birefringence visible in bone sample with 0 indicating no birefringence, 0.5 indicating reduced birefringence, and 1 indicating like fresh bone	As described in Jans et al. (2002)
Oxford Histological Index (OHI)	0–5	Measure of microbial bioerosion of a bone sample where 0 is complete destruction and 5 represents absence of diagenetic alteration.	Developed by Millard (2001) based on Hedges and Millard (1995)
General Histological Index (GHI)	0–5	Measure of unaltered bone microstructure where 0 represents complete destruction of bone and 5 represents a general absence of alteration due to any diagenetic factor, biotic or abiotic	Developed by Hollund et al. (2012)

Table 3
Oxford Histological and General Histological Index Scores as following Millard (2001).

OHI/GHI Score	Percent of Microstructure Remaining	Description
5	>95%	Very well preserved, fresh bone
4	>85%	Fairly-well preserved with minor amounts of destroyed areas
3	>50%	Large areas of well-preserved bone present
2	<50%	Some well-preserved bone still present
1	<15%	Small areas of well-preserved bone are still present or lamellar structure is still preserved by pattern of destruction
0	<5%	No original features identifiable except Haversian canals

3. Results and discussion

3.1. Donor decomposition

The state of decomposition for each donor is presented in Table 4 and visualized in Figs. 1–3.

As decomposition of a human body is affected by the immediate context in which it decomposes (Swift et al., 1979; Mann et al., 1990; Sorg and Haglund, 2002), differences resulting from above and below ground decomposition factors were expected between donors in this study. These factors include (but are not limited to) oxygen and insect access, moisture retention, ambient temperature, and temperature stability. This will in turn affect soft tissue decomposition, including susceptibility to desiccation, saponification, and rate of decomposition (e.g. Mann et al., 1990; Sorg and Haglund, 2002; Fiedler and Graw, 2003; Carter et al., 2007; Schotsmans et al., 2017). Such differences were reflected in the overall gross decomposition indicators of the current dataset. The remains of D1 and D2 (placed in open structures if discounting the animal protection coverings) skeletonized and desiccated to the extent that there were remains of skin and some connective tissue following initial moist decomposition. D1 reached a state of desiccation/skeletonization after four months (in July 2019), while D2 reached a state of desiccation/skeletonization after six months (in June 2020). This desiccation is typical of donors left on the surface at FARF and protected from scavenging (Bates and Wescott, 2016), as seen in over 400 decomposition experiments with donors (unpublished data). However, the trench containing D1 was in addition affected by occasional waterlogging from heavy rains during early decomposition. D3, the donor placed in the semi-buried coffin, skeletonized following

Table 4
Summary of the state of decomposition for each donor at the time of disarticulation.

Donor Number	State of decay at disarticulation
D1	Desiccation and partial skeletonization
D2	Desiccation and partial skeleltonization
D3	Skeletonization
D4	Skeletonization with some remaining saponified tissue
D5	Skeletonization

periods of partial drying and rehydration with modest amounts of desiccated connective tissue remaining. This stage was reached after five months of moist decomposition (in July 2019). D4, the donor placed in the fully buried coffin, was skeletonized with remnants of saponified tissue present in the coffin upon excavation. D5, the donor placed in the soil, was completely skeletonized upon excavation. Details of temperature and precipitation data during the decomposition process for all donors are available in Supplement 1.

The three donors that were observed during decomposition (D1, D2, D3) were all affected macroscopically by fungi at some point during the postmortem interval, D3 to the greatest extent (Fig. 4). The retention of a few centimeters of 'semi-fluid mass' of decomposition byproducts in coffins (e.g. Mant, 1987; Garland and Janaway, 1989) was observed in the cases of D3 and D4 throughout the experiment (Alfsdotter et al., 2022). Additionally, the D3 coffin contained some straw which likely infiltrated the coffin during documentation sessions, but no soil infiltration was observed. In the D4 coffin, modest amounts of soil from outside the coffin was observed along one of the coffin walls, seemingly due to the beginnings of breakdown of one of the right side-planks.

Invertebrate activity was ample in the three semi-exposed deposition environments (D1-D3). Large quantities of flies and larvae were observed in all three cases, as well as the presence of beetles, ants, spiders, and scorpions. Additionally, millipedes were observed on D2 while a butterfly was recorded on D3. For D4, the fully buried coffin, flies, fly larvae, and beetles were recorded in pictures from the stop motion camera mounted inside the coffin. Upon excavation, spider webs and ants were also recovered on D4 (see Alfsdotter et al., 2022 for further details on the coffin burials). No invertebrate activity was observed during the excavation of D5 who was buried directly in the soil (Dr. Hayley Mickleburgh, personal communication 2/3/2021).

Vertebrate activity was also recorded when present for the monitored donations (D1-D4). D1 vertebrate activity included a frog, snake, and a mouse which had nested within the empty thorax of the remains. No vertebrate activity was recorded for D2. Upon excavation of D3, a living frog and a mouse skeleton were recovered.

3.2. Histotaphonomic indices

A total of thirteen thin sections were analyzed, three (one each for the rib, tibia, and metatarsal) from each Donor 1–4, and one rib slide for Donor 5. The scores for each index are presented in Table 5 by sample and donor.

Table 5
Scores for the cracking index (CI), birefringence index (BI), Oxford histological index (OHI), and general histological index (GHI) for each sample (rib, metatarsal (MT), tibia) organized by donor.

Donor	Element	CI (%)	BI	OHI	GHI[a]
D1	Rib	0	1	5	4
	MT	0	1	5	5
	Tibia	0	1	5	5
D2	Rib	0	1	5	4
	MT	0	1	5	5
	Tibia	0	1	5	5
D3	Rib	0	1	5	4.5
	MT	0	1	5	5
	Tibia	0	1	5	5
D4	Rib	0	1	5	3.5
	MT	0	1	5	5
	Tibia	0	1	5	5
D5	Rib	0	1	5	3.5

[a] All GHI scores were influenced by the presence of enlarged canaliculi in the sample.

Fig. 4. Examples of fungi present on D3 at different stages of decomposition. 4a. Fungi is visible in the torso and hip region during decomposition of soft tissue. 4 b. Fungi covers several ribs and part of the coxae after skeletonization of the majority of the soft tissue. 4c. Fungi covers the base of the coffin where a semi-fluid mass formed largely by decomposition byproducts from the remains. 4 d. 'Cotton wool like' fungi (Forbes et al., 2005) covers parts of the skeletal remains and the coffin base.

Despite variation in the progression of decomposition stages as well as variation in the final state of decay at the time of disarticulation, the histological samples for each donor exhibited little variation in index scores across elements. As seen in Table 5, all samples exhibited CI values of 0%, BI scores of 1, and OHI scores of 5, all of which indicate no change. The OHI specifically measures degradation due to bioerosion, which in this sample was not advanced enough to be measurable through the application of this index. The longest postmortem interval observed in this study was 29.5 months, which suggests it takes more than this amount of time to measure biotic diagenetic changes through established indices in human remains. It should be noted that, variations in environment, climate and sedimentology between this study and previous ones are likely to impact on the rapidity of histological modification. Additionally, variation in medical histories and medical treatment before death could also have a substantial impact. Thus, the timescales observed in this experiment cannot be considered universal.

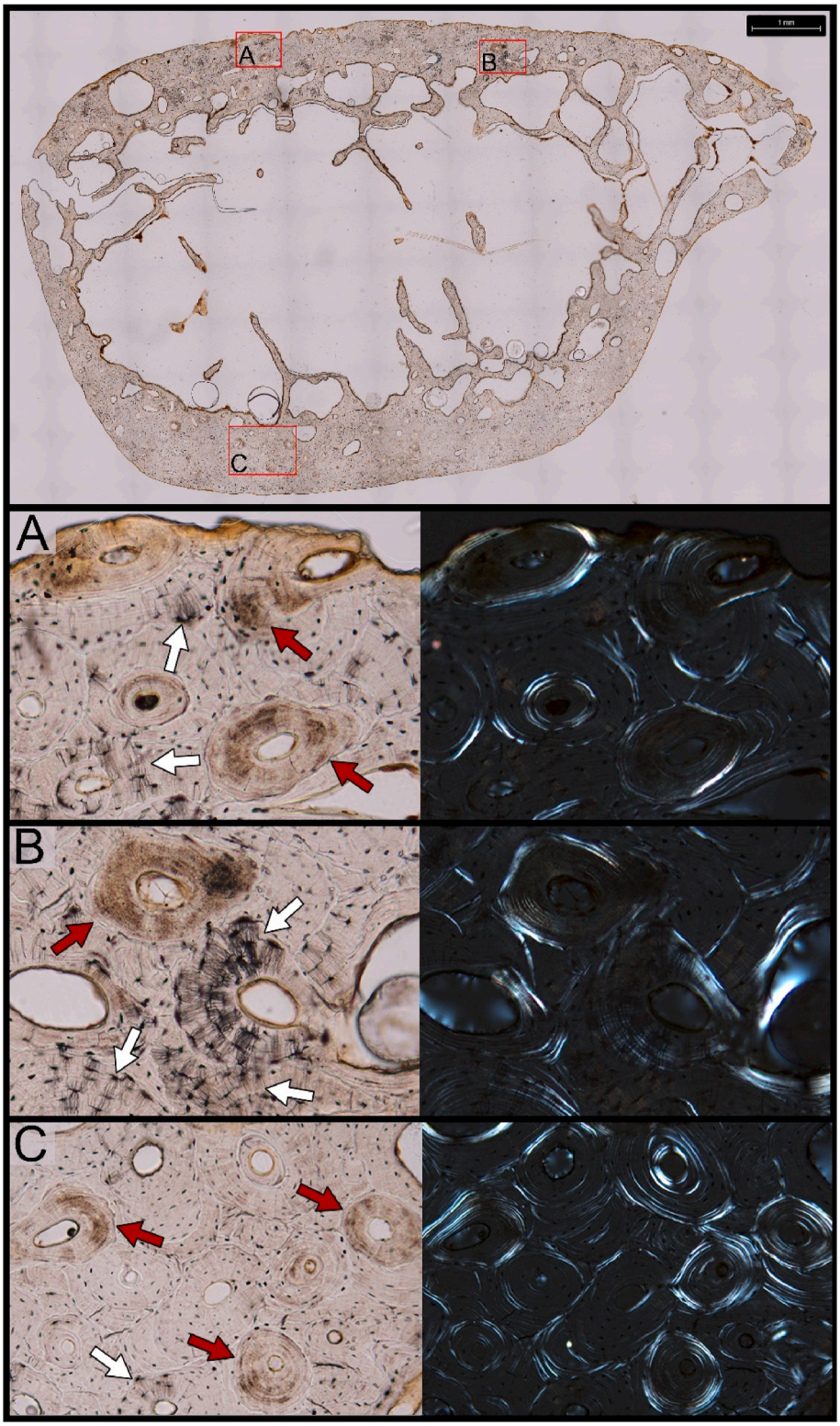

Fig. 5. Cross-section of D5 rib with areas of potential microscopic focal destruction (MFD) outlined in red boxes and annotated in images A, B, and C below the cross-sectional image. Within each inlay the red solid arrows point to potential MFD while the white solid arrows point to enlarged canaliculi. Each inlay also shows the same area of bone in both bright and polarized light. The superior edge of the rib is on the left side of the image and the cutaneous cortex is at the top of the image. Box A shows the superior cutaneous cortex inlay, Box B shows the inferior cutaneous cortex inlay, and Box C shows the pleural cortex inlay. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

However, this does not mean that no bioerosion occurred in the sample, but rather that it was too limited/localised to affect OHI scores in the elements tested.

Only the GHI scores, which includes biotic and abiotic degeneration, showed variation between donors and elements with GHI values ranging from 3 to 5. The most important factor influencing GHI score in this experimental study was the presence or absence of enlarged canaliculi in the sample as seen in Fig. 5. When looking at the GHI scores, every rib sample exhibited low GHI scores, due to enlarged canaliculi, compared to the same donor's tibia and metatarsal score when available (D1-4). While enlarged canaliculi can look like a specific type of bioerosion referred to as Wedl II tunneling (Trueman and Martill, 2002), the enlarged canaliculi in these samples did not cross microstructural boundaries and therefore were not considered definitive Wedl II tunnels. An important finding in this dataset was the absence of fungal tunneling in the thin-sections despite documented fungal growth on the remains as presented in Fig. 4.

A key concept of histotaphonomic interpretation in archaeological contexts has been that the time spent in the putrefaction stage of decomposition has a direct effect on the presence and extent of bioerosion in bone (Booth, 2016). The decomposition monitoring results show that each of the donors in this study experienced putrefactive changes and were in advanced states of decay (mummification or skeletonization) at the time of disarticulation. Undergoing putrefaction should have resulted in bacterial bioerosion for all donors if the bacteria were endogenous. Interpreted through this lens, the 'semi-fluid mass' consisting of decomposition byproducts present in the coffins of D3 and D4 should have also contributed to more extensive bacterial erosion than that found in the desiccated samples. Instead, none of the donors exhibited measurable bacterial bioerosion through the indices applied and D3 and D4 did not exhibit markedly higher levels of bioerosion than the other donors.

3.3. Examples of histotaphonomic change in D5

Although none of the samples exhibited diminished OHI scores, the rib sample from D5 did exhibit a few instances of noticeable diagenetic change through enlarged canaliculi and discoloration within secondary osteons (Fig. 5). These osteons were found on both cortices (pleural and cutaneous), intra-cortically, along the periosteal surface, and the endosteal surface. The random placement of the discoloured osteons across the cortex as well as the darkened canaliculi visible in Fig. 5b within the discolored osteon suggests these are examples of microscopic focal destruction (MFD) within the rib. This is a qualitative assessment, and the interpretation cannot be confirmed using transmitted light microscopy alone (Fig. 5). Some of these discolored areas are similar in appearance to bacterial MFD, especially budded and lamellate types. Birefringence is reduced in some, but not all, of the affected areas (Fig. 5), suggesting collagen loss, potentially resulting from microbial attack. The overall birefringence of the sample, however, is relatively normal and therefore the index score was not affected. For those osteons that exhibit potential MFD, the appearance of the affected osteons appears to be random within the cross-section and shows no patterns of orientation favoring either the periosteal or endosteal surface. Instead, the attack appears relative to the Haverisan canals and osteocyte lacunae. Future analysis employing scanning electron microscopy (SEM) may be able to confirm that MFD is present in this rib sample.

The fact that the presence of some MFD, as well as the associated loss of birefringence in D5, is potentially visible but not captured through the use of traditional histotaphonomic indices suggests that, for the early post-mortem period, these indices are not unequivocally or universally appropriate measures of histological change. Instead, a detailed descriptive approach of each sample may be necessary to more accurately interpret early post-mortem treatment of remains and mortuary practices in archaeological contexts. Generalised indices such as the OHI, though useful, may often provide too coarse an overview for precise interpretation.

The potential presence of some microfocal attack in D5 is especially interesting considering this was the only individual buried directly in soil with no structure. The fact that the limited amount of bioerosion present derives from the only donor buried directly in soil suggests exogenous bacterial origin. Confoundingly, the pattern of this possible MFD around Haversian systems and osteocyte lacunae correspond to Bell et al.'s (1996) hypothesis that supports an endogenous origin. In addition, given that bones are not fractured, the random organisation of bioerosion does not support an interpretation of exogenous origin, as if this were the case the periosteal surface would be expected to be most affected as seen in some experimental studies (Turner-Walker and Jans, 2008; Turner-Walker, 2019). Unfortunately, restrictions prevented sampling the anterior tibia and metatarsal from this donor, which may have helped elucidate the extent of bacterial erosion throughout the skeleton and possible differences in timing of diagenesis. More studies with longer durations of deposition are required to explore the potential MFD further to see at what point in the post-mortem interval the microstructure would be completely destroyed, which would directly influence how bioerosion is used to reconstruct mortuary practices.

Recent research by Emmons et al. (2022) used human cadavers in microbiome decomposition experiments to demonstrate a difference in microbial community throughout the body. In this study, intra-skeleton sampling sites further apart from one another showed greater variation in microbiota than sampling sites that were closer together. Emmons et al. (2022) also showed that microbiome bone samples taken from individuals buried deeper in the soil were more consistent with the gut microbiome data than samples taken from individuals closer to the surface, which were consistent with the soil microbiome. These findings run counter to the assumptions histotaphonomists often make about early postmortem diagenetic changes, that individuals buried directly in soil will be affected by exogenous bacteria, and offer an explanation on how the fully buried individual in this study (D5) showed MFD likely associated with endogenous bacteria. This result may be influenced by the fact that individuals buried (deep) in the ground exhibit a much-reduced decomposition speed, thus retaining soft tissue for a longer period of time. It must be acknowledged that the process of bodily decomposition itself alters the burial microbiome, potentially influencing exogenous bacterial attack (see Keenan et al., 2018; Emmons et al., 2022). Although the Emmons et al. (2022) study was not histotaphonomy focused, it points to a potentially fruitful avenue for future experimental research. Future studies using human cadavers which combine deposition-dependent bone microbiome analysis like Emmons et al. (2022) with histotaphonomic investigation from multiple intra-skeletal sampling sites would help clarify the origin of bioerosion if any is observed in the samples.

4. Conclusions

This research represents the first published experimental study on histotaphonomy that uses whole-body human cadavers. As such, it marks substantial progress to our understanding of early postmortem microstructural taphonomy. Findings in this study demonstrate limited evidence for modifications in the first 24 months postmortem. This pattern persists across different skeletal elements, durations of burial, and deposition types (exposed, buried in soil, buried in a coffin). In short, histotaphonomic analysis has often been considered a window into early postmortem processes before skeletonization, but these data instead suggest that the first two years postmortem cannot be unequivocally accessed using this approach. This point must come with the caveat that exhaustive medical histories of the donors are not known and certain maladies or medications (e.g. gastrointestinal disease, antibiotics, chemotherapy) could have impacted the microbiome and/or the susceptibility of remains to bacterial attack (whether exogenous or endogenous). The results of this study cannot definitively support one model of bacterial origin (endogenous or exogenous), but the absence of

measurable bacterial impacts using established indices indicates visible diagenesis may take years rather than months to occur in this environment. Together, these results demonstrate the necessity for histological studies on archaeological bone samples to consider the context of each burial closely and not rely on general indices (GHI, OHI etc.) alone. While it must be taken into account that the study was qualitative and carried out in a homogenous climate, our results indicate that caution is warranted for interpretation of early mortuary treatment based on histotaphonomic bioerosion. This study makes an important contribution to our understanding of the rapidity and intensity of bioerosion.

Credit author

SM, CA, AB and RM: Conceptualization, Writing – Original draft, review, and editing. SM: Primary analysis, data curation. SM and CA: Investigation, data curation, resources, project administration, funding acquisition. SM, AB, and RM: Methodology and validation. CA and AB: Visualization. AB and RM: Secondary analysis.

Declaration of competing interest

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jas.2023.105789.

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