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Multifactorial approach to describe early diagenesis of bones: The case study of the Merovingian cemetery of Saint-Linaire (France)

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ABSTRACT

The excavation of the Merovingian cemetery of Saint-Linaire (France) was an opportunity to describe the completeness of the tombs preserved from soil erosion. An anthropobiological study was carried out on the osteological material and the different categories of graves. On the basis of a complete archaeo-anthropological corpus we have undertaken an analysis of the differential preservation of the bones according to the different archaeobiological parameters such as the type of bone, the individual age but also the type of grave such as earthen graves, coffin, or sarcophagus.

In order to establish whether and how funerary practices had impacted the early diagenesis of the bones, FTIR was used to investigate the carbonate and fluoride content of the bones, and the infra-red splitting factor (SF), while XRD analysis was performed to estimate the crystallinity index (CI). In addition, the micro, macro and total porosity of the bones were measured by water adsorption, and bone carbon and nitrogen amounts were obtained by EA-IRMS. These diagenetic variables were analysed with regards to environmental (grave filling: humus, calcareous, clay), cultural (type of grave) and biological (mature *versus* immature individuals) factors. While there is a tendency of a better preservation of the skeletons in humus, and of juvenile bones to show less *post-mortem* alteration than the adult skeletons, the most important factor which determines the fate of the skeleton is the type of bone, with a preferential preservation of the ribs at Saint-Linaire.

1. Introduction

The Merovingian cemetery of Saint-Linaire is located northwest of the town of Sainte Hermine in Vendée province, western France (Fig. 1). The Saint-Linaire cemetery is a rural funerary space that covers about 3000 m^2 and contains more than 200 primary burials with some ossuaries, buried in different type of tombs (in soil, coffin and sarcophagus). In comparison with a pre-Jennerian (i.e. archaic population) mortality curve with a life expectancy of 30 years at birth according to Ledermann (1969), the Saint-Linaire mortality curve shows a significant shortage of children under the age of five, and especially under one year old (Fig. 2; Table 1), a phenomenon which is commonly observed (Masset and Sellier, 1990; Lucy, 1994; Sellier, 1996; Guy et al., 1997; Erkske, 2020) and may originate from intrinsic properties of juveniles bones (Caruso et al., 2021). In addition, the general macroscopic preservation of the bones, varies from well-preserved to badly-preserved bones within the same cemetery (Fig. 3). The preservation of bones not only varies considerably from soil to soil but also from one area of burial to another, through minor soil differences (Brothwell, 1981). Bone preservation in gravels or sand depends on the soil acidity and permeability and on whether the deposit is anaerobic/aerobic and waterlogged/well drained. Owing to its permeable nature, bones in chalky soils may be considerably eroded and fragile, while a clay matrix may lead to corrosion through acidity and, in region where the subsoil

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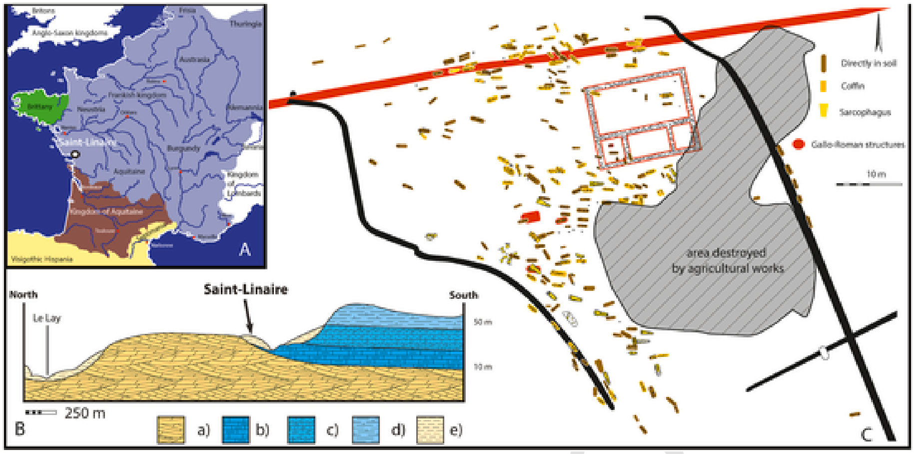

Fig. 1. A) Geographical location of the cemetery of Saint-Linaire; B) Geological substrate at Saint-Linaire: a) Brioverian schists and gneisses, b) Lias marl and limestone, c) Pliensbachian sandstone limestone, d) Toarcian marl, and e) Alluvial terraces. C) Map of the cemetery of Saint-Linaire showing the Gallo-Roman structures and the different burial types (directly in soil, coffin, sarcophagus).

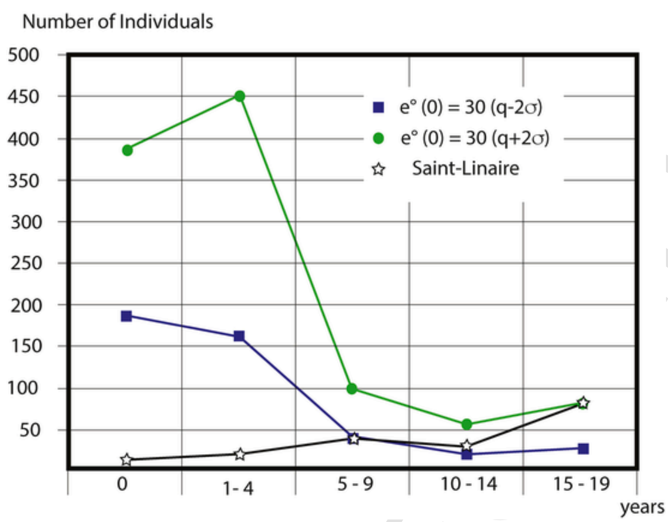

Fig. 2. Distribution by individual age group of the burials at Saint-Linaire compared with a pre-Jennerian mortality curve with a life expectancy of 30 years at birth according to Ledermann (1969). The age at death of the immature individuals was determined according to the tooth eruption tables and the degree of calcification (Ubelaker, 1984; Ferembach et al., 1979) was specified with the size of the diaphysis of the bones (Fazekas and Kosa, 1978; Stloukal and Hanákova, 1978), as well as with the ossification points (Birkner, 1980). For the adults, the methods proposed by (Ferembach et al., 1979; Lovejoy et al., 1985; Nemeskeri et al., 1960) have been used.

is marl, bone preservation is good. At Saint-Linaire the clay matrix of the soil covered by humus seems to lead to a globally bad preservation of the bone.

In order to understand the degradation pathway of bones at Saint-Linaire, we have undertaken a study of the differential preservation of the human skeletons according to several factors, including the type of tombs, the age of the individuals and the skeletal element.

In this study, the following variables were analysed to estimate the diagenetic state of the bones: bone crystallinity (CI), correlates of carbonate and fluoride content, infra-red-splitting factor (SF), were used for investigating *post-mortem* modifications of the mineral part of bones (bioapatite), while micro, macro, total porosity, as well as bone carbon and nitrogen content were examined to assess diagenetic modifications of the bone organics.

1.1. Geological settings

The cemetery of Saint-Linaire is situated on an ancient alluvial terrace 500 m east of the Lay valley, at an average altitude of 30 m, at the break of a metamorphosed Brioverian basement to the north and a Jurassic limestone plateau sitting on Lias marls to the south. The alluvial terrace is mainly made up of compact orange clay enriched with quartz pebbles, about 20 cm thick, resting on a compact clayey and yellow to grey sandy facies. The stratigraphy of the site shows the existence of a single layer of silty-clay cover in which burial pits were dug. The erosion of the site can be appreciated by the soil thickness from 20 to 80 cm above the graves at the time of the excavation. Ploughshare grooves are visible in the residues of the vats of three sarcophagi[1] and seven others[2] have been disaggregated. The northeastern portion of the cemetery and the large periphery of the building are less eroded. Children were found generally buried less deeply than adults, as commonly described by archaeologists (e.g. Lucy, 1994; Guy et al., 1997; Manifold, 2013) which may explain their deficit at Saint-Linaire, especially considering the signs of alteration of the site by ploughshares, and as observed with the dislocation of some sarcophagi.

1.2. Archaeological context

The cemetery of Saint-Linaire was initiated with a first Gallo-Roman phase around the end of the first century AD alongside the construction of a Gallo-Roman villa. Gallo-Roman tombs continued to be added until the middle of the fourth century AD. Although there seems to be a hiatus between this first phase of occupation and the following, there was nevertheless a continuity of the funerary practices on the site of Saint-Linaire, with burials until the end of the eighth century AD (Guérin, 1992, Table 2). According to historical data, while the Merovingian dynasty experienced many geopolitical changes in France with regional partitions and changes in political influences, the situation of Saint-Linaire remained stable throughout the Early Middle Ages.

Three Gallo-Roman tombs[3] are associated with a first E-W ditch whose filling is exclusively made up of *tegulae* and fragmented imbrices, a villa composed of four rooms, built parallel to this ditch and a pit whose filling has yielded ceramics dated to the first century AD. The building presents an elementary farm plan which seems to have been abandoned in the following century. The first burial space is diffuse and concomitant with the building between 75 and 140 AD. One tomb displayed a third century coin in the mouth of the buried individual.

In the Early Middle Ages, the cemetery was delimited by a first S-shaped ditch to the west and one N–S oriented ditch to the east, the fill-

Table 1
Comparison of the mortality quotients of the Saint-Linaire site with those established by the standard stable mortality for a stationary population according to Ledermann (1969), including the values of demographic evaluators (cf Bocquet and Masset, 1977). The natural mortality of pre-Jennerian populations is estimated using two values: the juvenility index (the ratio of 5–14 to 20 + years old, noted by convention: $D_{5\text{-}14}/D_{>20}$) greater than or equal to 0.10 and the ratio of 5–9 to 10–14 years old (noted: D5-9/D10-14) generally less than 2.

Age	< 1 year	1–4 years	5–9 years	10–14 years	15–19 years
mortality quotient	1q0	4q1	5q5	5q10	5q15
Saint-Linaire	14.04	22.33	38.81	30.37	83.33
e°(0) = 30 (q-2s) (according to Ledermann, 1969)	188.04	160.96	37.96	22.07	29.60
e°(0) = 30 (q + 2s) (according to Ledermann, 1969)	387.12	451.27	98.12	55.62	81.58
paleodemographic estimator at Saint Linaire according to Bocquet and Masset (1977)					
D(5–9)/D(10–14) = 1.30					
D(5–14)/D(> 20) = 0.10					

ing of which revealed only ceramics from the Early Middle Ages, suggesting a physical limit to the cemetery. Yellow dolomitized Hettangian limestone sarcophagi and several simple burials in the ground function in parallel, guided by the orientation of the S-shaped ditch. At that time, there are changes in the territorial expansion of the Frankish kingdom, but Saint-Linaire still belongs to the same region. During this second phase, which corresponds to the period between 550 and 650 AD, there are two sets of occupation: with earlier tombs from 550 to 600 AD, displaying no arrangement; while in a (from 600 to 650 AD) burial space located south of the Gallo-Roman Villa, tombs are aligned along rows. Bones in Bathonian white limestone sarcophagi and in burials that disturbed the walls of the villa have been dated after 650 AD. West-east oriented graves containing alignments of stone wedges or blocks are present to the north of the building. This reuse of material from the building's foundations ranges from the early seventh century to the late eighth century according to the dates provided by some burials. According to Gaillard de Semainville (1980) and Catalo (1988a, b), it is common for building ruins to be used for burial sites. The ruined state of the Gallo-Roman Villa at the end of the seventh century is confirmed by the date of a burial dug in the walls.

1.3. *Anthropological and archaeothanatological assessment*

The archaeothanatological approach (Duday, 1981, 2009; Duday et al., 1990) consists in the meticulous observation of the anatomical positions of the bones in a grave, to interpret how their original arrangement changed as the body decomposed. This allows inference of the nature of the original manner of burial of the different individuals and is a powerful tool for linking cultural (burial) practices with taphonomic processes. Indeed, structures made of perishable material, such as coffins or casings, can be recognised by analysing the consequences of the decomposition of corpses. Reconstructing the movement of the bones during the decomposition of soft tissue allows recognition of the initial presence of hollow volumes associated with a coffin, and thus suggests the intentional separation of the body from the earth using an initial container.

The cemetery of Saint-Linaire contains 214 primary burials and 34 ossuaries which are linked to the destruction of previous burials. While the archaeothanatological approach ultimately arrives at three categories in terms of mode of decomposition of the bodies – sarcophagus (n = 18), coffin (n = 76) and directly in soil (n = 120) – a greater variety of graves was recognised from an archaeological point of view.

They include 118 burials which did not show any trace of casing or funerary goods and the archaeothanatological analysis did not reveal the initial presence of a container. In addition, two anthropomorphic rock tombs were dug into a hard clay substrate (Fig. 4. 1 and 4.2). Such tombs are known further south, in Charente (Boissavit-Camus, 1989a) and in Aquitaine (Bizot, 1989), and further east in Orléans (Blanchard and Poitevin, 2012), and around Paris (Lafarge et al., 2013).

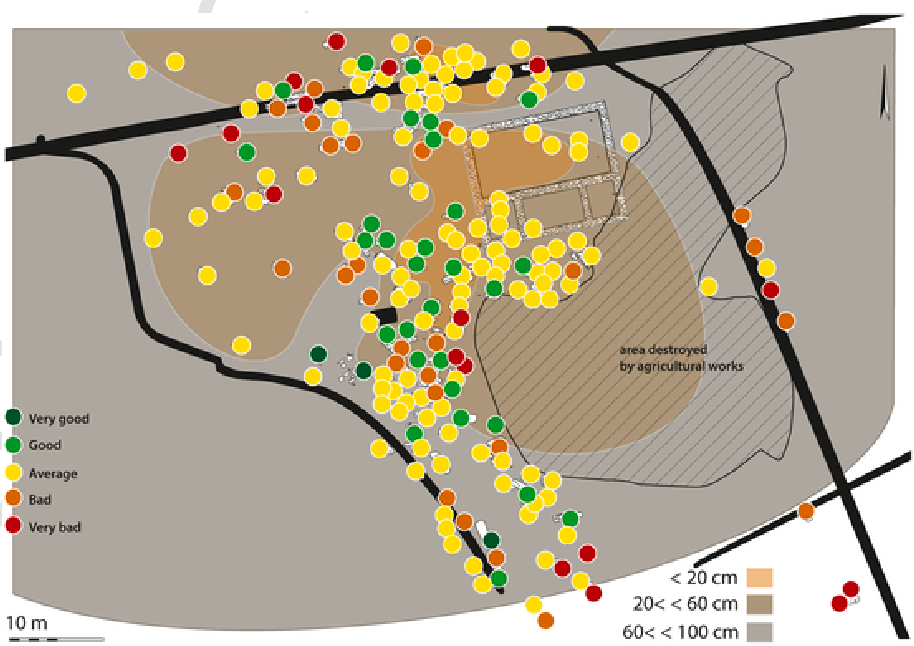

Fig. 3. Map of the distribution of the apparent state of preservation of the bones in the Saint-Linaire cemetery, with indication of residual sediment thickness (based on the plan made by Nogues, Rouzo and Zeitoun, in Guérin, 1992). Estimation of bone preservation was determined empirically according to a 5-point scale; from very good, when the cortical surface of the bone was completely preserved, to very poor, when it was totally degraded. The average state corresponds to a state where half of the cortical surface of the remaining bone is altered. Two intermediate states were rated between fair and very good on the one hand, and fair and very poor on the other. This is not a scale that takes into account the representation rate of the remaining bone (i.e. Anatomical Preservation Index) as in Bello et al. (2002).

Table 2
Radiocarbon dating of the main burials at Saint-Linaire. Data published in Person et al. (1995) and using the calibration from Pearson and Stuiver (1993) were re-evaluated using OxCal 4.4 (Bronk Ramsey, 2009) and IntCal20 (Reimer et al., 2020) and are presented rounded out to the nearest 10 years following standard conventions (Millard, 2014).

data from Person et al. (1995)				Pearson and Stuiver (1993)		OxCal 4.4 and IntCal20
Sample	**type of burial**	**Ref. Lab**	**14C age BP**	**Cal AD 68.3%**	**cal AD 95.4%**	**cal AD 95.4%**
SL 18	coffin	Pa 1217	1500 ± 40	543–621	448–649	430–650
SL 20	coffin	Pa 1165	1325 ± 60	657–774	630–86	600–880
SL 21	coffin	Pa 1167	1440 ± 60	594–660	537–682	430–780
SL 26	sarcophagus A9b	Pa 1189	1630 ± 80	346–541	243–612	250–600
SL 39	coffin	Pa 1183	1480 ± 40	550–536	536–655	480–660
SL 60	sarcophagus A0O	Pa 1187	1600 ± 60	410–545	338–607	260–600
SL 91	sarcophagus A5b	Pa 1185	1340 ± 60	653–767	615–955	590–870
SL 136	coffin	Pa 1171	1600 ± 80	396–554	255–636	250–640
SL 157	coffin	Pa 1211	1440 ± 40	603–654	549–666	560–660
SL 174	coffin	Pa 1172	1260 ± 40	688–790	669–884	660–880
SL 177	coffin	Pa 1192	1465 ± 80	542–658	424–689	410–780
SL 182	coffin	Pa 1184	1900 ± 40	74–142	24–232	20–240
SL 203	anthropomorphic rock tomb	Pa 1218	1460 ± 80	544–660	426–694	410–780
SL 223	coffin	Pa 1170	1895 ± 40	76–144	27–234	30–240

Also, for the 76 coffins or tombs made with perishable casings, several archaeological subcategories were recognised. In the absence of any archaeological structure, two coffins were identified through archaeothanatological analysis of the bones still present (Fig. 4.3). However, most of the coffins were recognisable by alignments of limestone blocks extracted from the foundations of the Gallo-Roman villa. These blocks correspond to the wedging of frames or structures in perishable materials. This interpretation is based on the observation of wall effects on the skeletons in the volume of the pits. Burials of this type have been described in the region (Boissavit-Camus, 1989a; Gallien, 2009) and in other regions in France (Gaillard de Semainville, 1980; Pétrequin et al., 1980; Raynaud, 1987b; Garnotel, 1998; Gallien and Langlois, 1998; Joly, 1998; Blanchard and Georges, 2004; Carré, 2012; Pecqueur, 2016). Amongst these graves with block alignments, three were also the object of cephalic arrangements (Fig. 4.4 and 4.5). One was a parallelepipedal limestone pillow (cf Boissavit-Camus, 1989b) and another, a limestone block and two large fragments of tegulae from the Gallo-Roman Villa. The head of a third individual rested on a coarser pillow of oolitic limestone. In this same category of block-lined grave, a child's burial used the wall of a sarcophagus vessel as a lateral wedge. Within the villa enclosure, a child's grave with adjoining block alignments yielded a limestone cover slab.

In addition, and despite of the intense erosion resulting from agricultural work prior to the excavation of the site, 18 incomplete and eroded sarcophagus vats enabled the recognition of three particular styles, according to the regional typology established by Bernard (1990) (Fig. 4.6): eight A0O type trapezoidal sarcophagi in yellow oolitic limestone, sometimes dolomitic, from the Hettangian, nine A5b type trapezoidal sarcophagi in chalky limestone from the Bathonian with cephalic compartment and low head, and one A9b type trapezoidal sarcophagi in yellow dolomite from the Hettangian, with cephalic compartment and anthropomorphic inner wall.

As previously mentioned, 34 ossuaries (i.e. body reduction) were additionally present and correspond to small piles of bones, for providing space in the tomb for the next dead by the destruction of the previous burial. Re-use of vats has been noted for some sarcophagi. This was either a patching up of the vats by adding repairing blocks to the feet in the case of an A5b sarcophagus; or a reuse of the vat with a rearrangement of the body inside the vat for the A5b and A9b types. Ossuaries have also been carefully carried out after recutting coffins between graves. According to Prigent (1986) and Raynaud (1987a), the reuse of graves and the establishment of ossuaries depending on them are rare at the end of the Antiquity and develop from the Early Middle Ages onwards to become predominant towards the 11th-12th centuries. Boissavit-Camus et al. (1996) point out that this is also the case in the region where Saint-Linaire is located, from the end of the 6th century until the 10th century. Such a practice may impact the fate of the bones by changing their environment over time. However, it is impossible to know whether the primary inhumation was in a sarcophagus, coffin or directly in soil.

2. Material and method

We were allowed by the Head of the Archaeological Service of Fontenay-le-Comte to collect 147 human bone samples from 113 individuals (i.e. 55 femur, 60 rib, 26 tibia, 3 humerus, 1 fibula, 1 acromion and 1 parietal fragment, Table 3) with the aim of quantifying intra-individual differences (for 29 skeletons) and inter-individual differences based on different class ages (non-adults n = 36 *versus* adults n = 111) and different types of burials (i.e. three distinct archaeothantological types: sarcophagi, earthen graves, and coffin or casing in perishable material). Samples were collected from broken bones, rinsed with water and dried. As cortical bone and trabecular bone being very different entities from a biochemical point of view (Ninomiya et al., 1990), all surfaces of the bones were cleaned from soil contaminant with a cutter blade and the trabecular part was mechanically removed.

2.1. X-ray powder diffraction

X-ray powder diffraction was performed on 3–10 μm fine powder of bone using a Siemens diffractometer fitted out with a rotating sample holder with a copper anticathode tube, a scintillation counter and a spinning holder rotating at 100 rpm. The diffractometer record obtained with Cu Kα radiation on a double-track analogue recorder was analysed to distinguish the apatite peaks corresponding to reflections 211, 112, 300 and 202. Height was measured between the average value at the top of a peak and the value of the valley separating it from the following peak. The sum of the height of peaks 112, 300 and 202 was divided by the height of the highest peak 211, subtracting the value of the baseline taken between 24° and 38° 2θ, in order to obtain the value of the semi-quantitative crystallinity index CI (Person et al., 1995).

2.2. Porosity

Porosity was measured using a protocol inspired by Pike (1993) and used by Hedges et al. (1995) which was based on standard soil science methods. Samples cut from the cortical bone were equilibrated with water vapour at a defined relative humidity. Porosity was measured by differential weighing of the water content after successive equilibration in those humidities. The bone sample of around 200 mg was dried to constant weight at 105 °C, and then weighed inside a thermostatically controlled environmental chamber in which the relative humidity was

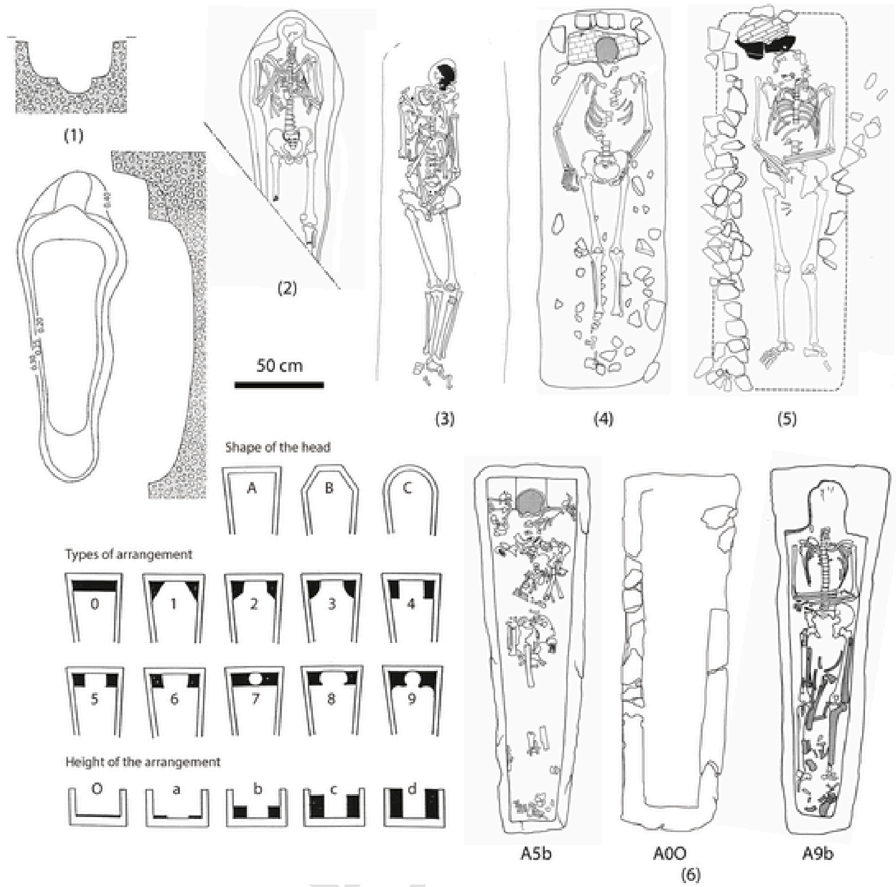

Fig. 4. Examples of graves in the Saint-Linaire cemetery. 1 and 2: anthropomorphic rock tomb; 3–5: coffin; 6: sarcophagi (according to Rouzo & Zeitoun, in Guérin, 1992) with typology of regional Merovingian sarcophagi according to Bernard (1990).

Table 3
Skeletal part analysed for immature and mature individuals, provided according to their burial type.

IMMATURE	Acromion	Femur	Fibula	Humerus	Parietal	Rib	Tibia
Directly in soil	0	2	0	0	0	7	0
Body reduction	0	1	0	0	0	0	0
Sarcophagus	0	1	0	0	0	1	0
Coffin	1	7	0	1	1	13	1
MATURE	Acromion	Femur	Fibula	Humerus	Parietal	Rib	Tibia
Directly in soil	0	6	0	1	0	3	1
Body reduction	0	2	0	0	0	1	2
Sarcophagus	0	7	0	0	0	5	3
Coffin	0	29	1	1	0	30	19

controlled using an open container of sulphuric acid at an appropriate concentration. H_2SO_4 concentrations of 3 M and 2.125M correspond to humidities of 76% and 90%, respectively. Several days were needed to equilibrate the bone sample with the water vapour. The total pore volume was measured by immersing the bone sample in distilled water under vacuum, and then rapidly weighing the saturated sample. This method was used to examine the microporosity (the volume of water absorption g^{-1} of dry bone at a relative humidity of 76%) corresponding to the volume of all pores up to a theoretical diameter of 40 Å, the macroporosity, corresponding to the volume $g^{-1}$of the bone of all pores over 100 Å in diameter (or equilibration at 90% RH), as well as the total porosity. Errors in these measurements were within 5%.

2.3. *Infra-red absorption*

Bone was ground to a fine powder with an agate mortar and pestle. Approximately 1 mg was mixed with potassium bromide and pressed into a disk. Spectra were measured on an ABB Bomem Michelson Fourier-Transform Infra-red (FTIR) spectrometer averaging 8 scans at a resolution of 4 cm^{-1}. Selected peak heights were measured after baseline correction and ratios calculated relating to various properties of the bone mineral, following the approach described in Weiner (2010). The peaks measured were:

- phosphate at approximately 1040 cm^{-1} (P);
- carbonate at 870-875 cm^{-1} (C);
- phosphate doublet at 603 cm^{-1} (S1) and 565 cm^{-1} (S2), and the minimum between the two peaks (M).

These were combined into the following measures:

- 'carbonate': C/P
- infra-red splitting factor (SF): (S1 + S2)/M (Shemesh, 1990)
- 'fluoride index': S1/S2 (based on the observation of Weiner (2010 p.289) that in carbonate apatites containing mixtures of hydroxide and fluoride this ratio increases with increasing fluoride content).

2.4. *Mass spectrometry (C, N, $\delta^{13}C$)*

EA-IRMS analyses were conducted on 10 mg of whole bone samples (Brock et al., 2012) powdered and weighed into tin capsules for elemental concentration (TOC and nitrogen amount, reported as %C and

Table 4
Summary of overall parameters measured for the 147 human bone samples of the individuals buried at Saint-Linaire.

	SF (FTIR)	F (FTIR)	Carbonate (FTIR)	CI (XRD)	%C	%N	C/Natom	microporosity	macroporosity	Total porosity	**d**13C ‰
modern bone	*2.68 ± 0.09*	*<1*	*0.33–0.39*	*0.00*	*11.6 (whole bone)*	*4.3 ± 0.6 (whole bone)*	*2.91 - 3.8 (whole bone)*	*0.0592*	*0.074*	*0.1332*	
	Hollund et al. (2013)	*Weiner (2010)*	*Ortiz-Ruiz et al. (2018)*	*Person et al. (1996)*	*Person et al. (1996)*	*Bocherens et al. (2005)*	*Kim et al. (2011)*	*Gutierrez (2001)*	*Gutierrez (2001)*	*Gutierrez (2001)*	
							Person et al. (1996)				
Saint-Linaire											
min	3.16	1.06	0.02	0.01	0.75	0.19	2.9	0.010	0.05	0.08	−22.7
mean	4.05	1.17	0.04	0.19	5.48	1.46	5.0	0.016	0.31	0.33	−19.7
median	4.03	1.17	0.04	0.19	4.85	1.17	4.7	0.016	0.27	0.28	−19.7
max	5.01	1.43	0.10	0.36	11	4.36	15.6	0.033	0.85	0.88	−17.9
standard dev	0.41	0.05	0.01	0.08	2.29	0.86	1.7	0.00	0.17	0.16	0.8

%N) and stable carbon isotopes ($\delta^{13}C$) measurements. These measurements were performed with a continous flow Elementar® VarioPyro cube elemental analyzer (EA) coupled to a Micromass® Isoprime Isotope Ratios Mass Spectrometer (IRMS) at the Institute of Ecology and Environmental Science of Paris. Temperatures were set to 850 °C and 1120 °C for the reduction and combustion furnaces. Analytical precision and repeatability were controlled with tyrosine ($\delta^{13}C$ = −23.2‰, %N = 7.78; %C = 59.66) samples calibrated against international standards (Coplen et al., 1983). In the current research, the mean uncertainties were 0.2% for %N, 0.2% for %C, and 0.1‰ for $\delta^{13}C$.

2.5. Data analysis and statistics

The whole dataset has been explored with a PCA to understand what are the main driving variables and correlations. We used the package *factominer* (Le et al., 2008) in R. To test differences of preservation depending on the various factors collected (e.g. burial, filling, skeletal elements), the structure of the data and our hypotheses would require linear modelling to quantify. Unfortunately, the sample sizes are low and very unbalanced thus precluding the use of classical parametric tests such as ANOVAs. We decided to use resampling methods to test several hypotheses. Comparisons between groups have been performed with permutation tests when the sample sizes are greater than 20 observations. These tests have been performed by 1) computing the difference between the average group values for a continuous variable, then 2) mixing groups attributions and 3) calculating again the difference between these new randomly assigned groups 4) A p-value is derived by counting how many times raw difference is more extreme than the ones with randomly assigned groups being what we except under the null hypothesis that there is no influence of the groups. For samples under this limit of 20 observations, we added a bootstrap sampling approach to inflate the sample size. Each bootstrap sample mimics an additional sample from the population. The main assumption we have to accept is that the real sample is representative of the population. Each bootstrap sample underwent the same permutation test as described above. Thus, each provides a raw difference and a p-value available in supplementary materials as histograms (Supplementary Materials E and F).

3. Results and discussion

All dataset obtained is available in Supplementary Material A.

3.1. General overview of the diagenetic trajectory at Saint-Linaire

Patterns of bone alteration depend on several intrinsic (type of bone, health, age at death etc.) and extrinsic factors (soil environment, pH, temperature, humidity, hydrology etc.) and may thus differ according to the archaeological site studied. However, modification of porosity, collagen loss, and mineralogical evolution is a common trend in archaeological bones, although the modalities of these changes vary from site to site, depending on the type of alteration the bones suffered (Hedges, 2002). At Saint-Linaire, the parameters used to monitor changes of the bone structure, bone organics and of the bone mineral, indicate such diagenetic alteration, with regards to the comparison to modern bones (Table 4). They show a decrease in microporosity, an increase in macroporosity and total porosity, a decrease in whole bone %C and %N, the degradation of the bone C/N ratio and an increase in crystallinity indicators (SF and CI), accompanied with an increase in fluoride index and the loss of carbonate. The relation between these diagenetic indicators can be used to estimate the type of alteration (Smith et al., 2007; Viera de Sousa et al., 2020) but does not always display clear patterns (Hedges and Millard, 1995). Here it is worth mentioning that the strong correlation between whole bone %C and %N (R = 0.94) very likely excludes any important contribution from structural carbonate, non-collagenous proteins (Sillen and Parkington, 1996) and/or humic acids, to whole bone %C and %N analysed. Such contamination might have occurred only for the two outliers excluded from that correlation (Supplementary Material B). In this study, bone %C and %N thus mostly derive from collagen.

In order to investigate the diagenetic processes prevailing at Saint-Linaire, principal component analysis (PCA) was conducted to project the 9 dimensions (including 8 of the diagenetic variables above-mentioned, as well as burial depth, but excluding C/N as well as total porosity to avoid redundancy) to principal components (PCs) and explore how variables are interconnected (Fig. 5). More than 60% of the total variance of the dataset is explained by the first two PCs (PC1: 43.9%, PC2: 21.2%). Including PC3-5, the PCA captures ~90% of the maximal variance of the presented dataset (with PC3: 10.5%; PC4: 7.9% and PC5: 5.9%). After the fifth dimension, the percentage of explained variance flattens with further dimensions reaching ≤5% (Supplementary Material C). According to the Broken Stick model approach (MacArthur, 1957; Kaiser, 1960) PC1 and PC2 are the principal components to interpret in this investigation (Supplementary Material D).

Eigenvectors corresponding to collagen degradation (%C, %N) and changes in microporosity mostly contribute (>15%) to PC1 (Fig. 5; Supplementary Material C). The contribution to PC1 of bone C/N ratio, used for screening collagen quality (Brock et al., 2012) is however lower (although not quantified to avoid any redundancy that would bias the PCA, regarding the inclusion of %C and %N as variables; see Fig. 5). This results from the fact that C/N ratio shows a linear correlation with %C and %N, up to a threshold where almost all collagen is gone and C/N rises exponentially from around 6 to 16 (Supplementary Material B). A threshold of %N = 0.7 and C/N of whole bone varying

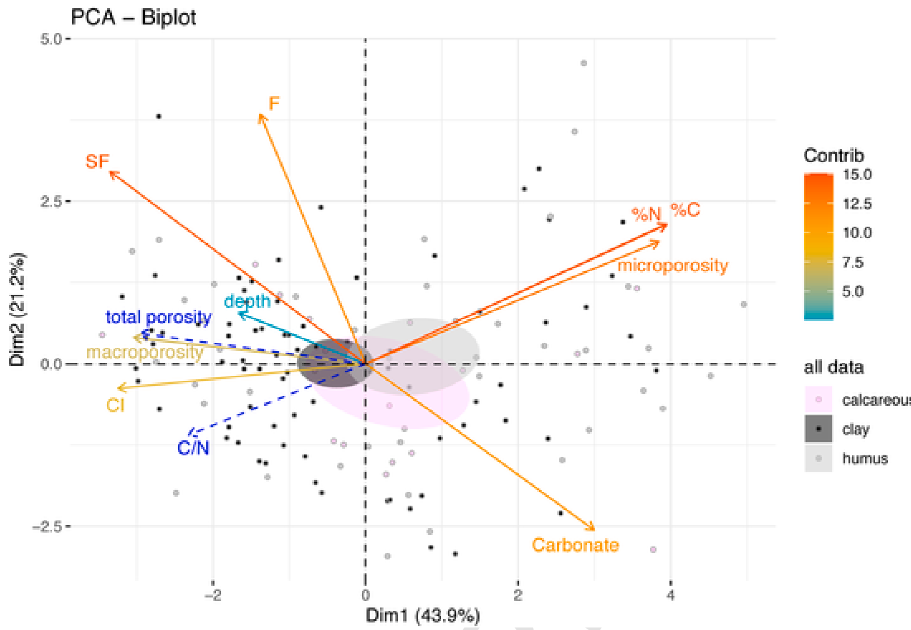

Fig. 5. PCA showing how the diagenetic variables (the eigenvectors) contribute to the two main Principal Components PC1 and PC2 (i.e. Dim1 and Dim 2). The influence of calcareous, humus and clay fillings of the tombs is indicated with 95% confidence ellipses around group means.

from 5 to 17 is often used for predicting success of radiocarbon dating (Tisnerat-Laborde et al., 2003; Kim et al., 2011; Brock et al., 2012). At Saint-Linaire, 16% of the samples yield %N ≤ 0.7.

The loss of organic matter (%C and %N) seems concomitant with a decrease in microporosity (nominal pore-diameter below 40 Å) but is not as strongly correlated to the increase in macro (diameter over 100 Å) and total porosity (Fig. 5; Supplementary Material B). In fact, macroporosity contributes to 10% to PC1 but reaches its highest contribution in the fourth dimension (>60%, Supplementary Material C). Such pattern between %N, micro and macroporosity was observed on human and animal bones from northwest Europe dated from the Pleistocene to the Medieval period (Nielsen-Marsh and Hedges, 2000).

The degradation of collagen fibrils of 0.1 μm diameter (Weiner & Traub, 1986; Sarathchandra et al., 1999) and biological attack from bacteria and fungi (>0.1 μm, Turner-Walker et al., 2002) are very likely responsible for the increase in macroporosity, while the loss of microporosity could be linked to the coarsening of the bone mineral (Nielsen-Marsh and Hedges, 2000; Hedges, 2002) having lost its protection, to the redistribution of porosity or biological mineralization (Hedges and Millard, 1995).

Unlike Nielsen-Marsh and Hedges (2000), we found no strong correlation (R = 0.42) between SF and macroporosity. The same holds true for CI (R = 0.28) which however, is better connected to microporosity (R = −0.52). Albeit not linear, there is a relation between the loss of bone organics, porosity and mineral changes which is illustrated by the contribution of SF and CI ~ 12% to PC1 (Fig. 5; Supplementary Material C).

SF (FTIR) and CI (XRD) are both commonly used for investigating bone mineral transformations and experiments conducted on synthetic hydroxylapatite show a strong linear correlation of these two crystallinity indicators with crystal size (Sa et al., 2017) making them useful for investigation of crystallinity. While FTIR informs about the geometry of the crystal bonds, and XRD, about the structural arrangement of the atomic plans, SF and CI relate to different crystal properties (Rogers and Daniels, 2002; Dal Sasso et al., 2018; Vargas-Becerril et al., 2019) which may explain the discrepancies observed between both crystallinity indicators with the other diagenetic variables, and the lack of a strong correlation – albeit co-varying- between them (R = 0.38). It is worth mentioning that although they correlate strongly in experimental heating studies (Sa et al., 2017; Greiner et al., 2019), soil percolation water and bacterial activity are not involved.

PC2 illustrates the low correlation between both empirical crystallinity parameters, indicating bone mineral transformations obtained using FTIR (with the highest contribution of SF, fluoride index F and carbonate, reaching more than 15%) and for which the contribution of CI is almost null. Besides its involvement in PC1, its main contribution only occurs in dimensions four and five (Fig. 5; Supplementary Material C).

SF and fluoride index are positively correlated (R = 0.68), while carbonate is negatively correlated to SF (R = −0.72) but not strongly with the fluoride index (R = −0.44). In fact, the fluoride index used in this study does not correlate with any of the other diagenetic variables. Although bone fluoride content does not necessarily correlate with CI if widespread recrystallization does not occur (Maurer et al., 2011), the lack of relation between fluoride index and the other (except those concomitantly measured by FTIR) diagenetic parameters at Saint-Linaire may also result from a methodological bias. The detection of the presence of fluoride in apatite using FTIR has been first suggested by Geiger and Weiner (1993) and highlighted by Weiner (2010). However, to our knowledge, only one published study (Toffolo et al., 2015) used the fluoride index empirically determined from FTIR spectra to discuss bone fluoridation. However, whether this index solely relates to the qualitative presence of fluoride (when >1) or if its variability corresponds to varying F concentrations and can therefore be used quantitatively (as is the case for carbonates) needs to be further validated via analyses of F concentrations in the bones.

In contrast, carbonate amounts are commonly estimated by FTIR, with however, a variability in the choice of the peaks. Whether $\nu_3(CO_3)$ (Dal Sasso et al., 2018; Gonçalves et al., 2018; Bayarı et al., 2020) or $\nu_2(CO_3)$ (this study and, Ortiz-Ruiz et al., 2018; Caruso et al., 2020, 2021; Foley et al., 2020; Viani et al., 2021) is used, a decrease in carbonate is observed during diagenesis and is validated by mass balance calculations (Saliège et al., 1995). This decrease occurs when calcite does not precipitate in the porosity, which is the case at Saint-Linaire, as assessed by the XRD diffractograms. However, the lack of correlation of carbonate with CI (R = −0.32; Fig. 5; Supplementary Material B) and the important loss of carbonate, deduced from the comparison with expected values for modern bones (Table 4), for all bones whatever

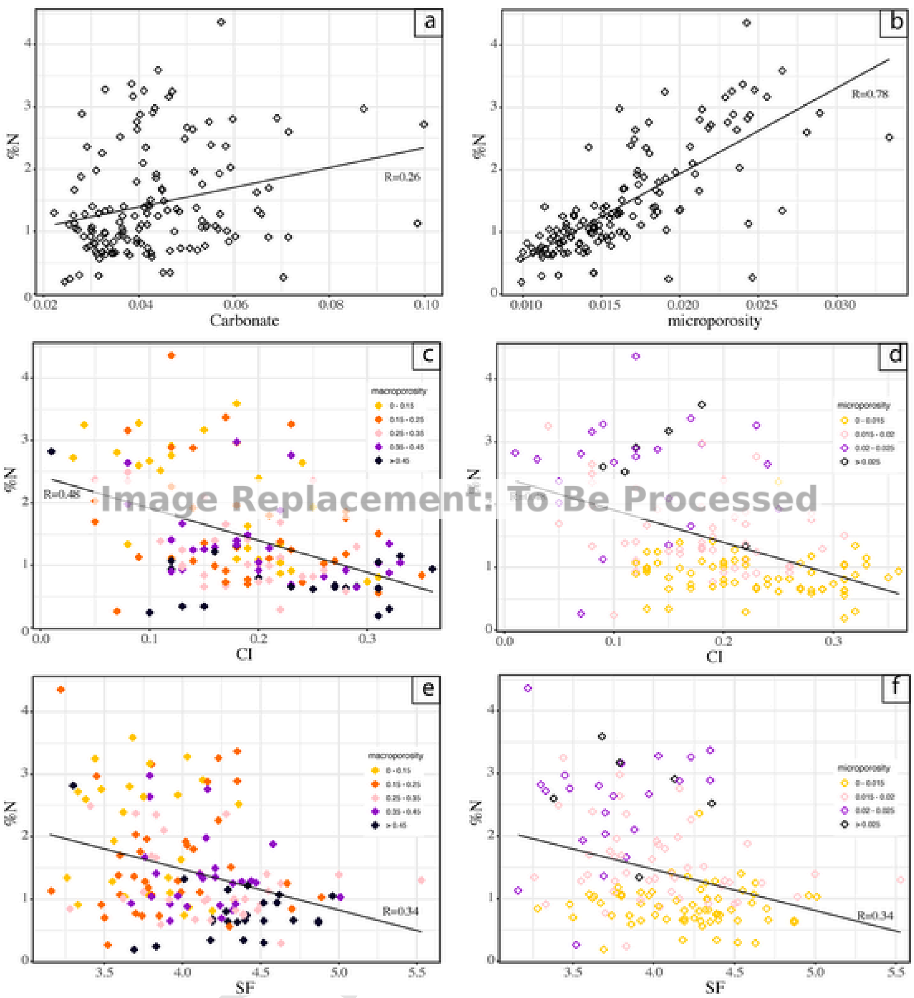

Fig. 6. Graphs showing bone carbonate (6a), microporosity (6b) and crystallinity (SF and CI, 6c-f) as a function of the amount of bone nitrogen (%N). For inspecting the relation between crystallinity and micro- and macro-porosity for bones with different amounts of organics, levels of micro- and macro-porosity are displayed for CI and SF, of bones with varying amounts of %N; 6c-f. Regression line considering all points (without including porosity categories) are provided for each plot and accompanied by their Pearson coefficient.

their preservation state, evidence that the observed significant correlation with SF ($R = -0.72$) may result from a FTIR effect, i.e. a dependency on peak broadening (Dal Sasso et al., 2018) rather than providing real estimates of the carbonate amounts whose correlation with SF still requires substantiation (Viani et al., 2021). The same may apply to F determined by FTIR, perhaps again explaining the significant correlation with SF.

To sum up the diagenetic history at Saint-Linaire, it is clear that carbonate has been lost before, or at the beginning of collagen degradation. Carbonate amount estimated via C/P ratio, is highly degraded for all bones ($C/P = 0.04 \pm 0.01$, Table 4) compared to modern samples (with $C/P \sim 0.3$–0.5, Ortiz-Ruiz et al., 2018; Caruso et al., 2020). This degradation occurs regardless of the amount of bone organics (whether close to fresh bone, with %N > 3 or highly altered, with %N < 1, Fig. 6a). Part of the microporosity is also already lost (considering microporosity of ~0.06 for modern bones, Gutierrez, 2001) for bones with still high amounts of nitrogen (~3–4%) and it continues to decrease as collagen degrades (Fig. 6b). Crystallinity changes assessed by the two crystallinity indicators (SF and CI which provide composite measures of crystal size and perfection), are also not strongly dependant on the amount of bone organics (although bones with less than 2% N can reach higher crystallinity), and can be quite important (SF: 4–4.5, and CI: 0.15–0.25), even for bones with N > 3%. These bone mineralogical transformations do not depend on modifications of bone porosity, as high macroporosity and low microporosity encompass most of the range displayed by CI and SF values (Fig. 6c–f; Supplementary Material B).

The lack of strong correlation between bone organics, bone mineral and bone porosity changes very likely results from the involvement of different processes, i.e. dissolution, dissolution/recrystallization and bioerosion, which may occur at different rates within the site. In all cases, depth of the burials had little to no influence on the preservation of the skeletons (Fig. 5). The main contribution of depth is visible in the third dimension (Supplementary Material C).

3.2. Environmental, "cultural" and biological factors affecting bone diagenesis

A key factor in bone preservation/alteration is water circulating around and through the skeleton (Hedges and Millard, 1995; Hedges, 2002; Kendall et al., 2018). Amongst other environmental parameters (i.e. acidity, oxygenation, temperature etc.), different types of soils have different draining conditions, going from stagnant (clay), to circulating (sand) water. At Saint-Linaire, skeletons were excavated in different substrates: humus, calcareous and clay. When these parameters are considered, it appears that samples excavated in humus tend to show an overall better preservation than those in clay (permutation tests: mean CI_{humus} < mean CI_{clay}, p-value = 0.083, mean

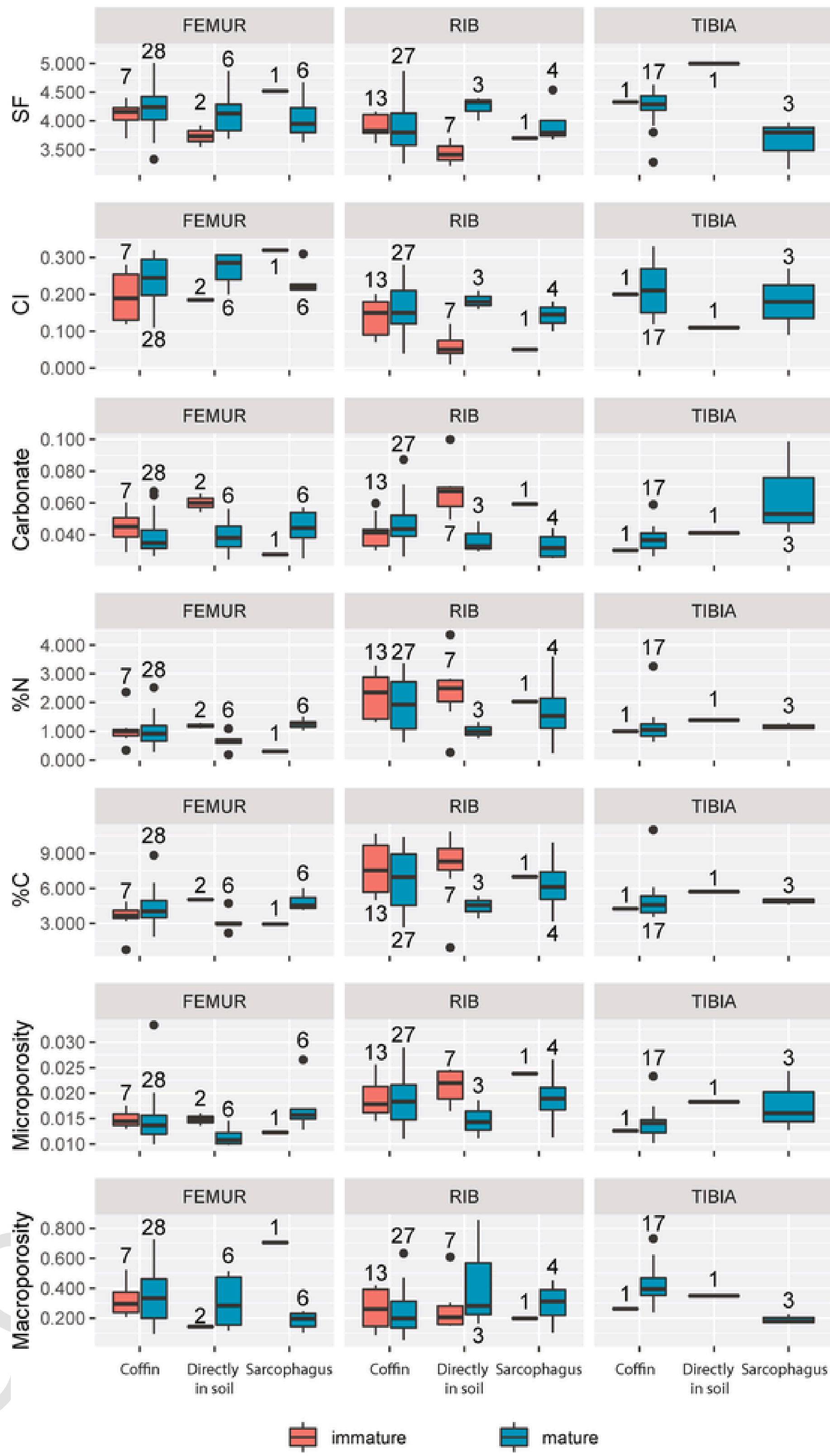

Fig. 7. Boxplots showing the crystallinity (SF and CI), bone carbonate, %N, %C, micro- and macro-porosity for different skeletal elements (femurs, ribs and tibia) of immature and mature individuals according to the nature of their tomb (in soil, in sarcophagus or in wood coffin). The number of samples for each category is mentioned near each boxplot.

$\%N_{humus}$ > mean $\%N_{clay}$, p-value = 0.03; mean $microporosity_{humus}$ > mean $microporosity_{clay}$, p-value = 0.07), while skeletons in calcareous environment display no trend and instead, varied diagenetic states (see Fig. 5; and none of the permutation tests between calcareous and humus as well as calcareous and clay provide a p-value lower than 0.135). Yet, the burial settings at Saint-Linaire are complicated by the utilisation of different types of tombs: directly in soil, in sarcophagus and in wood coffin. To determine whether the burial type influenced bone diagenetic processes, data were analysed according to these three sets of categories (Fig. 7) and including the age of the individuals (mature *versus* immature) as well as the type of bone analysed (rib, femur and tibia, which are the most represented bones in the series). Although no clear pattern emerges regarding the burial type, there is a tendency for the mature individuals to display more altered bones when buried with no casing. Their femurs and ribs tend to yield amongst the highest CI, the lowest %N and microporosity when buried in soil (Fig. 7), as compared to sarcophagus and wood coffin (the tendency is particularly strong for %N and microporosity; Supplementary Material E). Macroporosity is also much higher but only for the ribs. Bones of immature individuals do not display a constant pattern, but their ribs tend to be less crystallized, with high amounts of organics and carbonates, and a high microporosity when buried directly in soil. This opposite trend compared to the adults, results in a striking difference between mature and immature bones excavated from tombs with no casing where the bones of the children are always better preserved than those of the adults (Supplementary Material F). This is very surprising since the under-representation of juveniles of less than five years old (in this study, they compose 53.8% of the juveniles) within cemeteries is often explained by the greater susceptibility of their bones to *post-mortem* modifications (Gordon and Buikstra, 1981; Buckberry, 2000; Bello and Andrews, 2006; McCraw, 2014) because of their smaller size, higher collagen content, lower mineralization degree and higher porosity. However, while Caruso et al. (2020, 2021) found that juveniles bones display lower carbonate and higher SF than adult skeletons from the same Roman to Modern sites in Italy, the exact opposite is observed at Saint-Linaire (Fig. 7, Supplementary Material F). The common shallower burial depth of children in archaeological cemeteries is also often suggested for explaining the under-representation of juveniles, but at Saint-Linaire, depth did not influence any of the *post-mortem* processes (Fig. 5). To know whether bones from juveniles tend to degrade faster has strong implications for demography and societal reconstruction. If *post-mortem* processes do not particularly alter these bones, the "missing juveniles" of the demographic curves may have been buried in other locations with social implications (Manifold, 2013). It is impossible to say whether this was the case at Saint-Linaire, especially because of the intense erosion which may have dramatically destroyed the shallower graves. Another possibility would be that the preservation trajectory is biased by a sort of Osteological Paradox (Wood, 1992), where samples analysed survived *post-mortem* transformations, while the others did not because of their high susceptibility to diagenesis and were already totally dissolved. This does not however explain why the juvenile bones excavated present lower *post-mortem* transformations than the adults. The type of soil within the grave is not responsible for this pattern. Fig. 8 shows that mature individuals buried directly in wood coffins and in soil, respectively present none or contradictory (i.e., lower SF-higher CI; lower SF-lower %N etc.) and thus insignificant pattern whether buried in humus or clay. The tendency for a better preservation of the juvenile bones (compared to the adults) in humus is clear (particularly when buried in soil, Fig. 8) but most of the juvenile samples were ribs (58% of the juvenile bones), while femurs, tibias and humerus were also sampled on the adults (with ribs consisting in 35% of the mature bones).

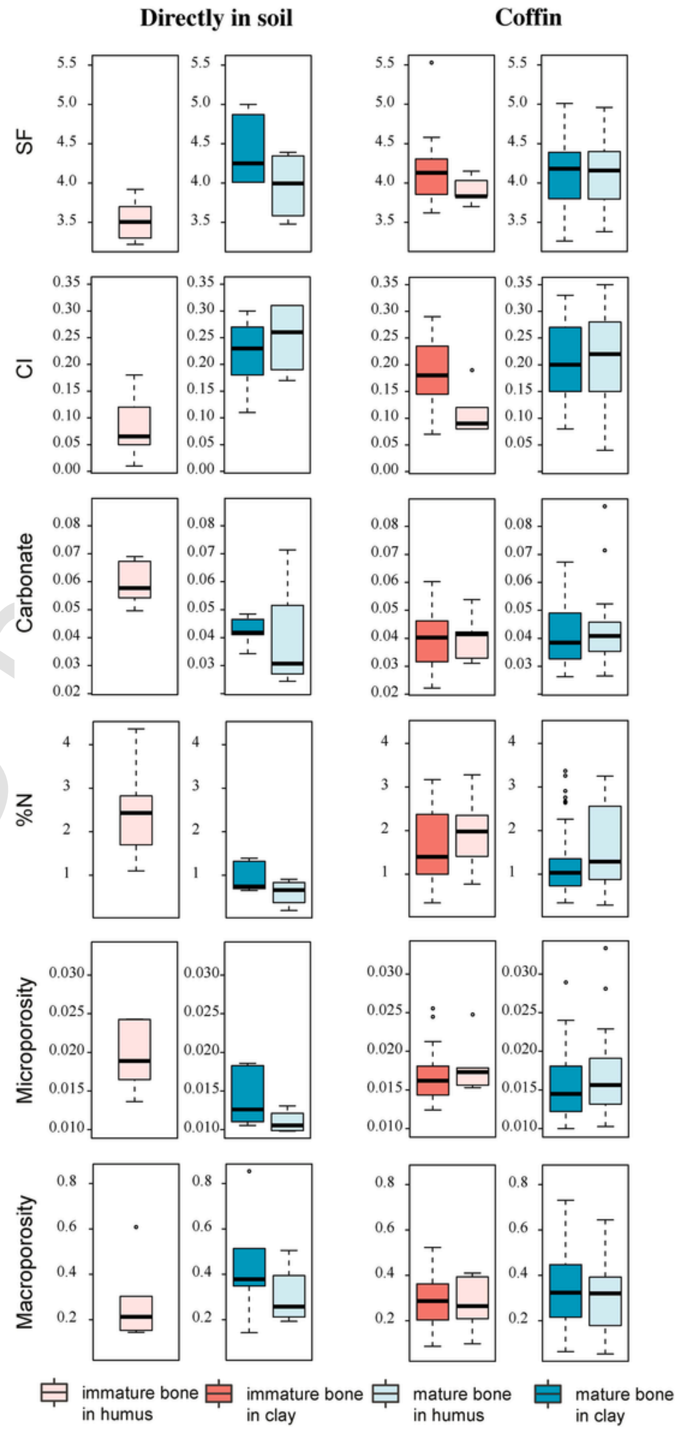

Fig. 8. Boxplots showing the crystallinity (SF and CI), bone carbonate, %N, %C, micro- and macro-porosity for bones of immature (red) and mature (blue) individuals, buried in soil or in coffin. Light colours refer to humus as grave filling while darker colours indicate clay filling.

As 29 individuals were the object of intraskeletal sampling (at least 2 bones, mostly rib and femur), it is possible to appreciate whether the intraskeletal variability is a function of the type of bones (Fig. 9) that would lead to the pattern seen above between juvenile bones and adults. Out of these 29 skeletons, 22 have ribs displaying lower crystallinity than their femurs (21 skeletons) and their tibia (1 skeleton). As for four skeletons ribs were not sampled, this results in the better preservation of the ribs in 88% of the individuals, including 4 immature and 18 mature. For ribs with CI less than 0.15, the difference in crys-

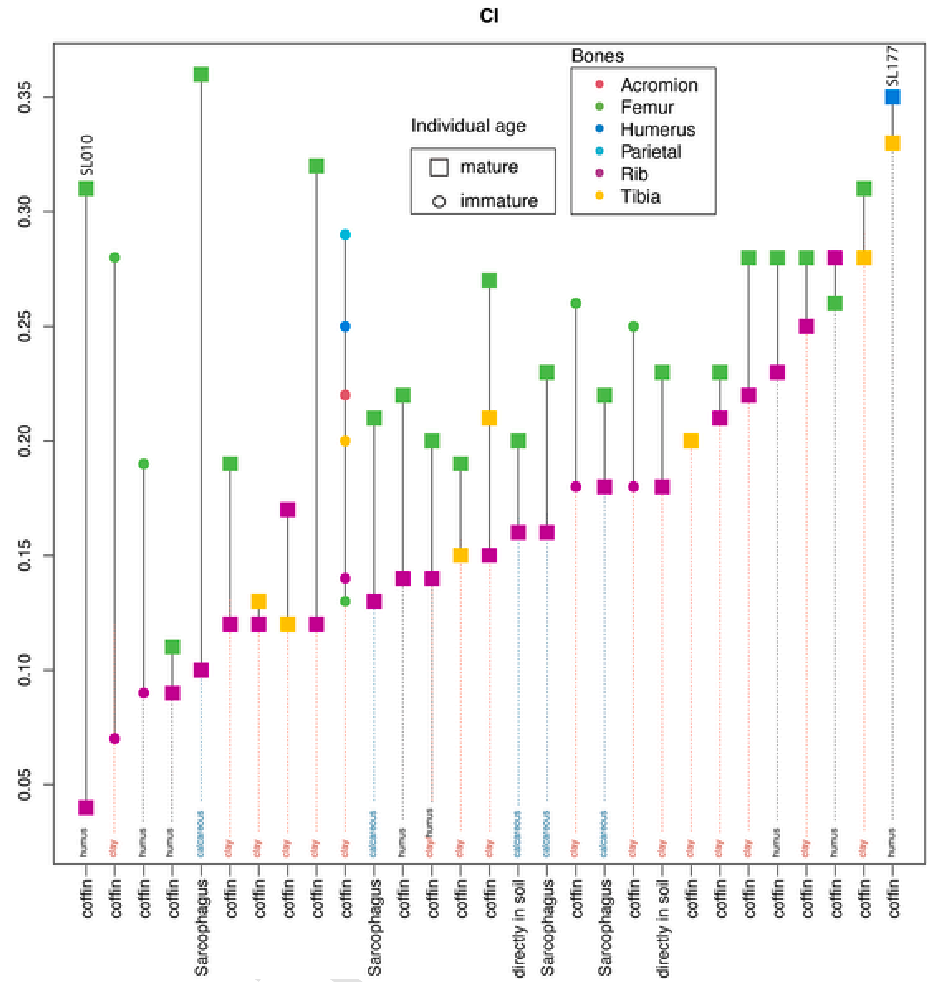

Fig. 9. Intraskeletal variability in bone crystallinity (CI) for the 29 individuals (mature: squares and immature: circles) who were the object of multi skeletal elements sampling. The type of burial (in soil, in sarcophagus, in coffin) as well as the grave filling (humus, calcareous, clay) are provided for each individual.

tallinity with their corresponding femur can be as high as 0.27, while after 0.15, the intraskeletal difference in crystallinity progressively decreases from 0.12 ± 0.08 (CIrib < 0.15) to 0.04 ± 0.02 (CIrib > 0.15). This pattern seems independent from the maturity of the individuals, the burial type (wooden coffin, sarcophagus, in soil) and from the sediment in which the individuals were excavated (humus, calcareous, clay). The highest $\Delta_{CIrib\text{-}femur}$ (0.27) and one of the lowest $\Delta_{CItibia\text{-}humerus}$ (0.02) relate to two mature individuals (SL010 and SL177) buried in wooden coffins filled with humus (Fig. 9). The other diagenetic variables indicate the same pattern. At the intraskeletal level, ribs show a lower SF (68% of the individuals), a lower C/N (75% of the individuals), higher %C (75% of the individuals), %N (79% of the individuals) and microporosity (79% of the individuals). It is also worth noting that while most femurs (84% of the total analysed) yield less than 1.5% N, 67% of rib samples analysed from the same individuals display more than 1.5% N. Finally, there is no pattern for carbonate (since most of the bones already lost this), and no striking evidence for a better preservation of the ribs deduced from the fluoride index and macroporosity (only 52% and 58% respectively, of the individuals show better preserved ribs according to these variables).

The fact that crystallinity, bone organics and microporosity all point towards an overall much better preservation of the ribs than the femurs is quite surprising. Ribs are often presumed to be more susceptible than femurs to *post-mortem* changes (Lambert et al., 1982; 1985; Zapata et al., 2006; Misner et al., 2009; López-Costas et al., 2016). It is however well known that bones weather at different rates (Behrensmeyer, 1978) and each bone may follow its own diagenetic pathway to degradation (Caruso et al., 2021). At Saint-Linaire, it seems that there is a preferential pathway for the ribs that is difficult to explain, as it does not relate to any biological (age), environmental (grave filing) or cultural (type of tomb) factor. Future studies combining a multi-analytical approach at the intra-skeletal scale will reveal whether this is a peculiarity of the site or if preferential preservation occurs at other sites for specific skeletal elements. This could allow for targeting samples for successful radiocarbon dating, DNA and chemical analysis.

3.3. Conclusion

Accurate inferences from elemental composition of human and faunal bone recovered from archaeological sites will depend on controls for *post-mortem* processes which involve the microbiota associated with the cadaver during its decomposition and may result in dissolution of the bone mineral, its recrystallization, authigenic precipitation, the complete loss of organic matter and ionic substitution. The initial conditions, i.e. the mode of burial and the evolution or inertia of the materials of which it is composed, the filling of the tombs, hydrological conditions etc., will orient the fate of the skeleton. Joint or antagonistic phenomena can occur and it is often difficult to assess which agents are predominant. Using our multi-analytical approach, we explored the impact of environmental (grave filling: humus, calcareous, clay), cultural (buried in soil, in sarcophagus, in wood coffin) and biological (mature *versus* immature) factors on the preservation of the skeletons of Saint-Linaire. While bones seemed better preserved in humus, and the juvenile bones looked to be better preserved than those of mature individuals when buried directly in soils, the multi-analytical approach conducted at the intra-skeletal level indicated that this image was biased by the confounding factor "skeletal element" where ribs followed a distinct path of degradation leading to a better preservation. Therefore, data obtained from Saint-Linaire go against some general assumptions (juvenile bones are badly preserved, ribs are more susceptible to diagenesis) that Alain Person, to whom we pay a tribute here, was always testing. The work presented here was initiated in 1992 in a friendly and collective trans-laboratory framework and remained unfinished for a long time. With this contribution, we want to acknowledge his teaching and illustrate the need to go on testing the variations of diagenetic signals within cemeteries and amongst individuals.

Author contributions

Valéry Zeitoun: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing; Anne-France Maurer: Formal analysis, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing; Jérémie Bardin: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft; Andrew R Millard: Funding acquisition, Investigation, Methodology, Resources, Writing – review & editing; Loïc Ségalen: Resources; Frédéric Guérin: Resources; Jean-François Saliège: Investigation, Methodology, Resources; Alain Person: Conceptualization, Investigation, Methodology, Resources.

Data availability

All data used and discussed in the manuscript are presented in the figures and tables, and the complete dataset, as well as five figures related to data treatment, are made entirely available within six supplementary online materials.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quaint.2023.03.003.

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