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1 **Variability of bone preservation in a confined environment: the case of the**  
2 **catacomb of Sts Peter and Marcellinus (Rome, Italy)**

3  
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21  
22 **Keywords:** diagenesis; stable isotopes, collagen; apatite; radiocarbon; Fourier-transform  
23 infrared spectroscopy

24  
25 **Abstract**

26 Most of the studies investigating the diagenetic trajectory of fossil bones focus on  
27 open-air sites and very little work have been published in confined environments such as  
28 catacombs. While the stable thermal history of catacombs should favor bone preservation, the  
29 accumulation of corpses over a short period of time could favor their destruction. The aim of  
30 this study is to describe the diagenetic trajectory of 128 human bone samples coming from six  
31 different burial chambers of the catacomb of Sts Peter and Marcellinus (SSPM, Rome, Italy).  
32 A multi-proxy approach was undertaken to provide an assessment of the molecular  
33 preservation as well as a direct record of the isotopic composition itself. Collagen yield,  
34 carbon and nitrogen abundances, C:N ratio, FT-IR based collagen and carbonate contents and  
35 crystallinity index, radiocarbon dating and stable isotope analysis of bone collagen and  
36 carbonate indicate that both the mineral and the organic fractions are impacted by diagenesis  
37 to various degrees, and that bones originating from the small burial chambers are more  
38 affected than those coming from the large ones. While some of the bones were strongly  
39 recrystallized, the impact of bone diagenesis on the stable isotope values of bone carbonate  
40 was limited. Comparison with contemporary sites from the Latium showed that conditions  
41 prevailing in catacombs seem overall to favor, rather than disadvantage bone preservation.

42

43

#### 44 **1. Introduction**

45 Excavations conducted from 2004 to 2010 in the central area of the catacomb of Sts  
46 Peter and Marcellinus (hereafter the SSPM catacomb) located in the south-east of Rome  
47 revealed several mass graves dated to between the first and the third century AD based on  
48 radiocarbon and artefact (coins) dating (Castex et al., 2007; Castex et al., 2011). They  
49 contained tens to hundreds of articulated human skeletons laid together according to well-  
50 reasoned management (Blanchard et al., 2007; Castex et al., 2007; Castex et al., 2009; Castex

51 et al., 2011). A significant number of these individuals received a specific funerary treatment  
52 characterized, in particular, by the use of gypsum to partially or entirely cover the corpses  
53 (Castex and Blanchard, 2011; Castex et al., 2009; Devière et al., 2010; Vanhove, 2006). A  
54 mortality crisis following an epidemic episode is thought to be at the origin of these mass  
55 graves suggesting that they have been used for a relatively limited period of time (Blanchard  
56 et al., 2007; Castex and Blanchard, 2011; Castex et al., 2009; Castex et al., 2011; Kacki et al.,  
57 2013). Unfortunately, the study of the biological identity (sex, age at death, stature, non-  
58 metric traits, pathological conditions, *etc.*) of the individuals was restricted due to the state of  
59 skeletal preservation (Blanchard et al., 2007; Castex and Blanchard, 2011; Castex et al., 2007;  
60 Castex et al., 2009). Many bones and teeth have lost their anatomical integrity even though  
61 the preservation quality differs strongly among burial chambers, different deposit levels  
62 within a chamber, and different skeletons within the same level (Blanchard et al., 2007;  
63 Castex and Blanchard, 2011; Castex et al., 2007; Castex et al., 2009). Stable isotope  
64 approaches offer invaluable assistance when the macroscopic anthropological observations  
65 are limited by the poor quality of skeletal preservation (*e.g.* Katzenberg et al., 2009; Oelze et  
66 al., 2012; Wright et al., 2010). Measurements of the stable isotope composition of the SSPM  
67 population would allow us to better understand their life history, provided that *in vivo* isotopic  
68 compositions are preserved.

69         Checking whether the original isotope compositions of biomineral fractions are not  
70 altered by diagenesis is an essential prerequisite to interpret isotope analyses (Bocherens et  
71 al., 2005; Bocherens et al., 2008; Brady et al., 2008; Shin and Hedges, 2012). Most of the  
72 studies investigating the diagenetic trajectory of fossil bones focus on open-air sites. To our  
73 knowledge, very little work has been published in confined environments. The advantage of  
74 such an approach was demonstrated by Bocherens et al. (2008) in Chauvet cave. In this  
75 Palaeolithic site, the authors showed that the preservation of bear bones exposed on the floor

76 of the cave varied widely probably due to spatial variation in local conditions. The Chauvet  
77 cave results cannot be directly translated to man-made cavities such as catacombs because the  
78 age and taphonomic contexts are quite different. Like in caves, the thermal history of  
79 catacombs is more stable than in open-air sites which should *a priori* favor their preservation.  
80 On the other hand, the accumulation of corpses over a short period of time in catacombs could  
81 have an effect on the soil pH and therefore favor the destruction of human bones. The aim of  
82 this study is to describe the diagenetic trajectory of the human bone samples from the SSPM  
83 catacomb in order to assess their preservation and compare it with the preservation observed  
84 in contemporary open-air sites from the region of Rome. A multi-proxy approach  
85 (measurement of classical collagen preservation indicators; Fourier-transform infrared  
86 spectroscopy (FT-IR) based collagen preservation indicators, carbonate content and  
87 crystallinity,  $^{14}\text{C}$  measurements, and stable isotope analysis of the collagen and carbonate  
88 fractions) was undertaken to provide a record of the preservation of the molecules. Our results  
89 show that both the mineral and the organic fractions are affected by diagenesis to various  
90 degrees, and that bones originating from the small burial chambers are the most diagenetically  
91 altered. While some of the bones were strongly recrystallized, the stable isotope values of  
92 bone carbonate remained reliable. Comparison with contemporary sites from the Latium  
93 showed that conditions prevailing in catacombs seem overall to favor, rather than  
94 disadvantage bone preservation

95

## 96 **2. Study area and material**

97 A total of 128 individuals were selected for this study. The material comes from six of  
98 the seven burial chambers discovered in 2003 in a previously unexplored area (region X) of  
99 the SSPM catacomb (Blanchard et al., 2007; Castex et al., 2007) (Figure 1). These burial  
100 chambers are part of a vast funerary complex that covers approximately 3 ha with 4.5 km of

101 underground galleries (Guyon, 1987). Four of them (X78/T15, X80/T16, X81 and X82/T18)  
102 are small in size ( $< 7\text{m}^2$ ), while the remaining two (X83 and X84) are larger in size ( $> 9\text{m}^2$ )  
103 (Castex et al., 2007; Castex et al., 2009). Different phases of multiple deposits separated by a  
104 layer of sediment were identified in the small chambers (Blanchard et al., 2007; Castex et al.,  
105 2011; Kacki et al., 2013; Sachau-Carcel et al., 2013). At least three phases were recognized in  
106 X78/T15 (Pagni and Burdassi, 2004) whereas 9 and 12 phases were distinguished in X80/T16  
107 and X82/T18, respectively (Blanchard et al., 2007; Castex et al., 2007). In contrast, no  
108 stratum of soil was discovered between the skeletons in X81, X83 and X84 (Castex and  
109 Blanchard, 2011; Castex et al., 2009; Castex et al., 2011).

110 For each individual, a fragment of long (*i.e.* humerus, radius, ulna, metacarpus, femur  
111 and tibia), flat (*i.e.* cranial vault, rib and hip bone), irregular (*i.e.* mandible and maxilla) or  
112 short (calcaneus) bone was sampled. Due to the poor skeletal representation of bone remains  
113 of five individuals from the burial chamber X82/T18, several fragments of different skeletal  
114 parts were selected in order to obtain enough material to achieve the required analyses  
115 (hereafter named miscellaneous samples). Modern reference material required for FT-IR  
116 analysis was composed of bones of modern ox, calf, horse and sheep in order to take into  
117 account variation due to species and age at death.

118

### 119 **3. Methods**

#### 120 3.1. Bone carbonate preparation and analysis

121 Between 30 and 36 mg of bone powder (grain size  $< 0.3\text{ mm}$ ) was first soaked in 2 to  
122 3% sodium hypochlorite at room temperature for 48 h to remove organic matter then rinsed  
123 five times with distilled water. The remaining fraction was treated with 1 M acetic acid for 1 h  
124 to remove exogenous carbonate then rinsed five times with distilled water. Sodium  
125 hypochlorite and acetic acid solutions were renewed half-way through each process. Samples

126 were oven-dried for 18 h at 65°C following pretreatment. The purification process resulted in  
127 a weight loss comprising between 10 and 80%. Bone carbonate  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values were  
128 measured at the Service de Spectrométrie de Masse Isotopique of the MNHN (SSMIM).  
129 Samples weighing from 580 to 630 mg were reacted for 360 s with 100% phosphoric acid at  
130 70°C in individual vessels in an automated cryogenic distillation system (Kiel IV device),  
131 interfaced with a ThermoScientific Delta V Advantage isotope ratio mass spectrometer. The  
132 stable isotope results are reported in per mil (‰) relative to VPDB. Over the period of  
133 analysis, the analytical precision calculated based on repeated measurements of our internal  
134 carbonate standard (Marble LM) was 0.02‰ for  $\delta^{13}\text{C}_{\text{carb}}$  and 0.05‰ for  $\delta^{18}\text{O}_{\text{carb}}$  values.

135

### 136 3.2. Bone collagen extraction and analysis

137 Bone collagen extraction followed Longin's (1971) protocol modified by DeNiro and  
138 Epstein (1981) and Bocherens et al. (1988; 1991). Between 0.1 and 1.1 g of bone powder  
139 (grain size comprised between 0.3 and 0.7 mm) was soaked in 40 ml of 1 M hydrochloric acid  
140 for 20 min at room temperature then filtered for decalcification using 5  $\mu\text{m}$  cellulose filters.  
141 The residue was soaked into 0.125 M sodium hydroxide for 20 h at room temperature to  
142 oxidize fulvic and humic acids then filtered. The gelatin was then solubilized in 0.01 M  
143 hydrochloric acid at 100°C for 17 h, and filtered again to remove impurities. The solubilized  
144 collagen was then freeze-dried for 48 to 72 h and extraction yields calculated. Stable isotope  
145 composition was measured at the environmental isotope laboratory at James Cook  
146 University's Advanced Analytical Centre (Cairns, Australia) using samples weighing between  
147 200 and 400  $\mu\text{g}$ . Carbon and nitrogen abundance and isotope composition ( $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$   
148 values) were determined using a Costech Elemental Analyzer 4010 fitted with a zero-blank  
149 auto-sampler coupled via a ConFlo IV to a Thermo Scientific Delta V<sup>PLUS</sup> using Continuous-  
150 Flow Isotope Ratio Mass Spectrometry. The  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$  values are reported in per mil

151 (‰) deviation relative to VPDB and AIR, respectively. Over the period of analysis, the  
152 carbon and nitrogen abundances were  $\pm 5\%$  of the value for laboratory internal standards and  
153 the analytical precision for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was better than  $\pm 0.1\%$  and  $\pm 0.2\%$ , respectively.  
154 Weight percent carbon and nitrogen were used to calculate atomic C:N ratios.

155

### 156 3.3. FT-IR analysis

157 Attenuated total reflexion mode (ATR) FT-IR analysis was performed on bone powder  
158 (grain size  $< 0.3$  mm) before and after pretreatment. Infrared spectra were collected by  
159 accumulation of 64 scans with a spectral resolution of  $2\text{ cm}^{-1}$  on a Bruker Vector 22 FT-IR  
160 spectrometer equipped with a diamond-ATR accessory (Specac Golden Gate). Spectral data  
161 analyses were performed using OPUS software (Bruker). Crystallinity was evaluated using  
162 the Infrared Splitting Factor (IRSF) (Figure 2). The sum of the two  $\nu_4\text{PO}_4$  bands at 565 and  
163  $600\text{ cm}^{-1}$  were divided by the valley at  $590\text{ cm}^{-1}$  following the protocol described by Weiner  
164 (1990). Relative carbonate content was measured by the intensity ratio of the  $\nu_3\text{CO}_3$  band at  
165  $1415\text{ cm}^{-1}$  over the  $\nu_4\text{PO}_4$  band at  $600\text{ cm}^{-1}$  (adapted from Wright and Schwarcz 1996).  
166 Relative collagen content was measured by the intensity ratio of the Amide I band at  $1660\text{ cm}^{-1}$   
167 over the  $\nu_4\text{PO}_4$  band at  $600\text{ cm}^{-1}$  (adapted from Trueman et al., 2004). The precipitation of  
168 secondary calcite was monitored by the presence of its specific band at  $712\text{ cm}^{-1}$ .

169

### 170 3.4. Radiocarbon dating

171 The collagen and apatite fraction of five individuals from burial chambers X80/T16,  
172 X82/T18 and X83 were radiocarbon dated. An aliquot of collagen (between 8 and 13 mg) was  
173 combusted for 15 to 60 min at  $800^\circ\text{C}$ . Carbonate was purified following a modified version of  
174 Krueger et al. (1991). About 2 g of bone powder (grain size  $< 0.1$  mm) was first soaked in 2

175 to 3% sodium hypochlorite at room temperature for six days to oxidize the organic matter.  
176 Bone powder was then rinsed several times with distilled water and dried for 3 h at 100°C. It  
177 was then placed in 100 ml of 2 M acetic acid for 24 h at room temperature under weak  
178 vacuum. The evolution of fine gas bubbles usually slows down drastically after 1 h, and stops  
179 after 10+ h, suggesting that all secondary calcite as well as the smallest apatite crystals had  
180 been dissolved. The acetic acid treatment resulted in weight losses between 40 and 80%. An  
181 aliquot of purified bioapatite weighing between 220 and 280 mg was then reacted in  
182 orthophosphoric acid for 12 min at 70°C under vacuum. CO<sub>2</sub> derived from the collagen and  
183 apatite samples was cryogenically trapped and about 1 mg C was sealed in a glass tube for  
184 radiocarbon dating. Graphitization and AMS measurement were conducted at Queen's  
185 University's <sup>14</sup>Chrono Centre (Belfast, Northern Ireland).

186

## 187 **4. Bone diagenesis evaluation**

### 188 4.1. Macroscopic preservation

189 Bone samples were classified into three categories according to their macroscopic  
190 characteristics as well as their strength and resistance when cutting: (1) “good preservation”  
191 characterizes a solid and heavy bone difficult to cut and crush; (2) “poor preservation”  
192 corresponds to a bone which is fragile, soft and easy to cut and crush; and finally (3) “medium  
193 preservation” refers to a bone with intermediate characteristics (Figure 3).

194

### 195 4.2. Collagen quality

196 Bone collagen preservation was evaluated based on three criteria: bone collagen  
197 extraction yield (wt%), carbon and nitrogen abundances in collagen (%), and atomic C:N ratio  
198 (Table 1). Collagen concentration in modern bone is around 20.4 wt% (SD ±3.9 wt%)  
199 (Bocherens et al., 1991), and values <1 wt% are usually considered as being unreliable

200 (Dobberstein et al., 2009; van Klinken, 1999). In modern bones, carbon and nitrogen  
201 abundances range from 13 to 47% and from 4.8 to 17.3% respectively (Ambrose, 1990), and  
202 values below or above these thresholds indicate alteration or contamination (Garvie-Lok,  
203 2001; Iacumin et al., 1998; Reitsema, 2012, van Klinken, 1999). Average atomic C:N ratio in  
204 modern bones is 3.2 to 3.3 (Ambrose, 1990; van Klinken, 1999), and vary between 2.9 and  
205 3.6 (DeNiro, 1985). Atomic ratios below or above these thresholds indicate alteration or  
206 contamination (Ambrose, 1990; DeNiro and Weiner, 1988; Grupe et al., 2001). Samples that  
207 fail to pass any of these three criteria were considered to be poorly-preserved.

208

#### 209 4.3. Infrared spectroscopy

210 Bone recrystallization promotes the formation of a more stable mineral phase  
211 characterized by a higher crystallinity (*i.e.* crystal size and lattice perfection) and lower  
212 carbonate content. These changes are related to both microbial activities (bioerosion) and  
213 interaction of bone mineral with the burial environment (*i.e.* pH) that control dissolution-  
214 recrystallization processes (Berna et al., 2004; Hedges and Millard, 1995; Smith et al., 2007).  
215 Crystallinity and carbonate content can be evaluated by FT-IR using the IRSF and CO<sub>3</sub>/PO<sub>4</sub>  
216 ratio, respectively. IRSF values vary between 2.5 and 3.3 for modern bones (Stiner et al.,  
217 1995; Wright and Schwarcz, 1996; Nielsen-Marsh and Hedges, 2000; Berna et al., 2004;  
218 Munro et al., 2007; Asscher et al., 2011) and usually reaches values between 3.4 and 4.5 for  
219 archaeological samples (Weiner and Bar-Yosef, 1990; Stiner et al., 1995; Wright and  
220 Schwarcz, 1996; Munro et al., 2007). Bones are usually considered altered for values above  
221 3.8 to 4.0 (Shemesh, 1990; Stiner et al., 1995; Berna et al., 2004). In modern animal bones,  
222 CO<sub>3</sub>/PO<sub>4</sub> ratio is comprised between 0.7 and 1.0. This ratios decreases to less than 0.2 for  
223 altered samples (Lebon, 2008). The fate of the mineral phase is mainly dependent on the  
224 degradation of the organic phase (Collins et al., 2002; Lebon et al., 2011; Trueman et al.,

225 2008). FT-IR analyses offer also the ability to measure the relative collagen content of a bone  
226 by measuring the AmideI/PO<sub>4</sub> ratio. This ratio is comprised between 0.9 and 1.2 for modern  
227 bones and decreases as collagen decays. The AmideI/PO<sub>4</sub> and CO<sub>3</sub>/PO<sub>4</sub> ratios are usually  
228 correlated (Lebon, 2008; Lebon et al., 2011).

229

#### 230 4.4. Radiocarbon dating of bone collagen-carbonate pairs

231 The <sup>14</sup>C/<sup>12</sup>C and <sup>13</sup>C/<sup>12</sup>C ratios differ by several orders of magnitude. This results in a  
232 higher sensitivity of the <sup>14</sup>C/<sup>12</sup>C ratio over the <sup>13</sup>C/<sup>12</sup>C ratio to post-mortem isotope exchange.  
233 Therefore, <sup>14</sup>C is a useful proxy to assess the influence of diagenesis on the carbon stable  
234 isotope composition of carbonate in apatite (Shin and Hedges, 2012; Zazzo, in press). To  
235 quantify carbon isotope exchange between bone carbonate and dissolved inorganic carbon  
236 during diagenesis, we measured the radiocarbon age difference between the collagen and  
237 carbonate fraction (<sup>14</sup>C<sub>col-carb</sub>) from the same bone. If bone collagen passes the three tests  
238 outlined above, any deviation should be the result of carbon isotope exchange in the carbonate  
239 fraction. This difference can then be transformed in percent of carbon isotope exchange  
240 assuming that exchange takes place with modern carbon (Hedges et al., 1995a). This is  
241 probably a reasonable approximation as an extensive review of the literature and a recent <sup>14</sup>C  
242 dating program of several archaeological sites worldwide demonstrated that diagenetically  
243 altered apatites are always younger than the reference age for the site (Zazzo, in press; Zazzo  
244 and Saliège, 2011). Radiocarbon ages are presented as BP and fraction modern (F<sub>m</sub>).

245

## 246 **5. Results**

### 247 5.1. Collagen preservation

248 Collagen preservation varies greatly between different samples (Table 2). Collagen  
249 yields differ according to bone location within the catacomb, the anatomical part category and

250 the macroscopic aspect (Figure 4). In total, 29 (23%) bone collagen samples do not pass at  
251 least one of the three tests defined (collagen yield, carbon and nitrogen abundances, and C:N  
252 ratio (Table 3). Within this group, all but two were classified as macroscopically poorly-  
253 preserved (Table 3). These samples all originate from three of the the four lowest levels of the  
254 chamber X80/T18 and from all the levels of the chamber X82/T18 (Table 3). They consist  
255 largely of long and flat bones (31 and 38%, respectively). Additionally, 46% of the flat bones  
256 and 100% of the miscellaneous samples have poorly-preserved collagen (Table 3).  
257 Macroscopic preservation was variable among the 99 (77%) bone collagen samples with  
258 reliable collagen (*e.g.* see Figure 5 for the variations of atomic carbon and nitrogen  
259 abundances). Poor scores were obtained for macroscopically poorly-preserved specimens  
260 coming from two small chambers (X80/T16 and X82/T18).

261

## 262 5.2. FT-IR

263 The modern animal reference samples have relatively low IRSF values (Mean = 3.4;  
264 SD  $\pm$ 0.1) and high CO<sub>3</sub>/PO<sub>4</sub> ratios (Mean = 0.85; SD  $\pm$ 0.10), consistent with values  
265 previously published (Lebon et al., 2010). With the exception of three samples, all the  
266 archaeological human bone samples present higher IRSF values and lower CO<sub>3</sub>/PO<sub>4</sub> ratios  
267 than modern animal reference samples (Figure 6). IRSF values measured on the  
268 archaeological samples before pretreatment range from 3.6 to 5.1 (Mean = 4.3; SD  $\pm$ 0.4),  
269 while CO<sub>3</sub>/PO<sub>4</sub> ratios vary between 0.15 and 1.01 (Mean = 0.49; SD  $\pm$ 0.20). IRSF values are  
270 negatively correlated with bone CO<sub>3</sub>/PO<sub>4</sub> ratios ( $R^2 = 0.75$ ) (Figure 6). No trace of calcite was  
271 detected (detection limit 1.5 %) in bone samples before pretreatment. Amide I/PO<sub>4</sub> ratios vary  
272 from 0.07 to 0.86 (Mean = 0.35; SD  $\pm$ 0.25) and are lower for the archaeological bones than  
273 for the modern animal reference samples (Figure 7). Carbonate and collagen relative contents  
274 are positively correlated ( $R^2 = 0.76$ ) (Figure 7). Small chamber samples are significantly

275 different from large chambers samples with respect to IRSF values, CO<sub>3</sub>/PO<sub>4</sub> and Amide  
276 I/PO<sub>4</sub> ratios (Mann-Whitney U tests; p-values < 0.03). Samples from small chambers have  
277 lower relative carbonate content, lower relative collagen content, and higher IRSF values  
278 compared to samples from larger chambers. Following chemical pretreatment of bone  
279 carbonate, IRSF values increase (Mean = 4.6; SD ±0.3) and relative carbonate content  
280 decrease (Mean = 0.28; SD ±0.10). CO<sub>3</sub>/PO<sub>4</sub> ratios remain negatively correlated to IRSF  
281 values (R<sup>2</sup> = 0.59) (Figure 8).

282

### 283 5.3. Stable isotope analysis

284 Bone collagen carbon and nitrogen isotope values were measured for all specimens  
285 that yielded a collagen extract (Table 2). There was no significant difference in δ<sup>13</sup>C<sub>col</sub>  
286 between the well-preserved and the poorly-preserved collagen samples (Mann-Whitney U  
287 test; p-value = 0.52). In contrast, the well- and poorly-preserved collagen samples have  
288 significantly different δ<sup>15</sup>N<sub>col</sub> values (Mann-Whitney U test; p-value = 0.00). There was no  
289 relationship between the δ<sup>13</sup>C<sub>col</sub> or δ<sup>15</sup>N<sub>col</sub> values and the collagen yield (R<sup>2</sup> < 0.05), the  
290 atomic carbon and nitrogen abundances (R<sup>2</sup> < 0.2) or the atomic C:N ratios (R<sup>2</sup> < 0.07). There  
291 was no significant difference between the isotope values and the burial chambers, the skeletal  
292 part categories, or the macroscopic appearances when either one of the two groups or the  
293 totality of the corpus is considered (Mann-Whitney U tests; p-values >> 0.05). We found one  
294 exception, however. When samples with well-preserved collagen were considered (n = 99),  
295 δ<sup>15</sup>N<sub>col</sub> values measured in macroscopically poorly- and well-preserved samples differed  
296 significantly (Mann-Whitney U test; p-value = 0.04).

297 Bone carbonate carbon and oxygen stable isotope values were measured for the 128  
298 individuals (Table 2). Bone δ<sup>18</sup>O<sub>carb</sub> values in X82/T18 differed significantly from bone  
299 δ<sup>18</sup>O<sub>carb</sub> values in X80/T16 and X84 (Mann-Whitney U test; p-value = 0.01). When the totality

300 of the corpus was considered,  $\delta^{13}\text{C}_{\text{carb}}$  values of irregular and flat bones, irregular and long  
301 bone, and long and miscellaneous bones differed significantly (Mann-Whitney U tests; p-  
302 values  $< 0.04$ ). The same conclusion was reached (excepted for long vs. miscellaneous bones)  
303 when only samples with well-preserved collagen were considered (Mann-Whitney U tests; p-  
304 values  $< 0.04$ ). Moreover, when the totality of the corpus was considered bone  
305 macroscopically identified as well-preserved had  $\delta^{13}\text{C}_{\text{carb}}$  and  $\delta^{18}\text{O}_{\text{carb}}$  values that differed  
306 significantly from those measured in medium and poorly-preserved bones, respectively  
307 (Mann-Whitney U tests; p-values  $\leq 0.015$ ). The samples which yielded well- ( $n = 99$ ) and  
308 poorly-preserved ( $n = 29$ ) collagen samples also had significantly different  $\delta^{13}\text{C}_{\text{carb}}$  and  
309  $\delta^{18}\text{O}_{\text{carb}}$  values (Mann-Whitney U test; p-value = 0.01 and 0.02, respectively). There was no  
310 relationship between the  $\delta^{13}\text{C}_{\text{carb}}$  or  $\delta^{18}\text{O}_{\text{carb}}$  values and the IRSF values ( $R^2 < 0.01$ ), the  
311  $\text{CO}_3/\text{PO}_4$  ratio ( $R^2 < 0.01$ ) or the AmideI/ $\text{PO}_4$  ratio ( $R^2 < 0.01$ ) before or after bone carbonate  
312 pretreatment.

313

#### 314 5.4. Radiocarbon dating

315 The  $^{14}\text{C}_{\text{col}}$  ages range from  $1779 \pm 27$  BP to  $1965 \pm 31$  BP whereas the  $^{14}\text{C}_{\text{carb}}$  ages  
316 range from  $1690 \pm 27$  BP to  $1848 \pm 22$  BP for the five individuals (Table 4). The  $^{14}\text{C}_{\text{col-carb}}$  age  
317 differences vary between  $-1 \pm 40$  and  $153 \pm 46$   $^{14}\text{C}$  yr (Table 4). Except for one individual in  
318 chamber X83 where the age measured in the two fractions are identical, the  $^{14}\text{C}_{\text{carb}}$  age is  
319 always younger than the  $^{14}\text{C}_{\text{col}}$  age. The  $^{14}\text{C}_{\text{col}}$  ages are positively correlated with the  $\delta^{13}\text{C}_{\text{col}}$   
320 values ( $R^2 = 0.95$ ), negatively correlated with the  $\delta^{13}\text{C}_{\text{carb}}$  values ( $R^2 = 0.68$ ) but are not  
321 correlated with the  $\delta^{15}\text{N}_{\text{col}}$  values ( $R^2 = 0.24$ ). The  $^{14}\text{C}_{\text{carb}}$  ages are negatively correlated with  
322 the  $\delta^{13}\text{C}_{\text{carb}}$  values ( $R^2 = 0.86$ ) but are not correlated with the  $\delta^{18}\text{O}_{\text{carb}}$  values ( $R^2 = 0.01$ ). The  
323  $^{14}\text{C}_{\text{col-carb}}$  age difference is not correlated with the  $\delta^{13}\text{C}_{\text{col}}$ ,  $\delta^{13}\text{C}_{\text{carb}}$ , or  $\delta^{18}\text{O}_{\text{carb}}$  values ( $R^2 <$   
324  $0.42$ ), but is negatively correlated with bone  $\delta^{15}\text{N}_{\text{col}}$  values ( $R^2 = 0.88$ ). In terms of  $^{14}\text{C}$

325 activity, Fm values of bone collagen varies between  $78.30 \pm 0.31$  and  $80.13 \pm 0.26$  pMC  
326 while bone apatite is about 1% more active on average and varies between  $79.45 \pm 0.22$  and  
327  $81.02 \pm 0.27$  pMC (Table 4).

328

## 329 **6. Discussion**

### 330 6.1. Diagenetic trajectory

331 The high IRSF values displayed by bone apatite from the SSPM catacomb, ranging  
332 between 3.6 and 5.1, are indicative of a moderate to high degree of recrystallization for these  
333 samples (Table 2, Figure 6). The presence of a  $\text{PO}_4$  peak at  $1093 \text{ cm}^{-1}$ , and of shoulders at  
334  $3570 \text{ cm}^{-1}$  and  $630 \text{ cm}^{-1}$  in the most severely altered samples argue for a partial  
335 recrystallization in hydroxylapatite. The inverse relationship between mineral crystallinity and  
336 carbonate content is typical of dissolution/recrystallization processes that take place following  
337 burial (Nielsen-Marsh and Hedges, 2000). Such an alteration of the mineral phase is  
338 frequently linked to the loss of the organic matter. This is confirmed by the correlation  
339 observed between collagen and carbonate relative contents (Figure 7). This relationship can  
340 be explained by the intimate association of the apatite crystals and collagen fibrils at the  
341 nanoscale in the bone structure. Mineral dissolution exposes the collagen to microbial or  
342 physico-chemical alterations while, in turn, the loss of the organic matter increases micro-  
343 porosities and interactions of bone minerals with surrounding water (Collins et al., 2002). The  
344 combination of collagen loss, decrease in carbonate content and increase in crystallinity could  
345 have been driven by collagen hydrolysis and/or microbial activity (Smith et al., 2007).  
346 Bacterial attacks generally result in a loss of the chemical integrity of collagen and a loss of  
347 collagen in bone (Balzer et al., 1997; Child, 1995; Grupe, 2001; Harbeck and Grupe, 2009)  
348 and begin quickly following burial (Hedges, 2002). Chemical hydrolysis of collagen is  
349 another, slower mechanism (Child, 1995; Smith et al., 2002). The lack of data regarding the

350 histological preservation and the porosity of samples does not allow us to distinguish between  
351 the two processes. However, archaeological evidence suggests that both could have occurred.  
352 The simultaneous or quasi-simultaneous decomposition of the high number of bodies in the  
353 mass graves from the SSPM catacomb could have been conducive to microorganism  
354 development. Moreover, the burial chambers from the SSPM catacomb were carved in a  
355 pozzolan (Funicicillo et al., 2008), which presents an alkaline pH ranging from 7.5 to 7.9  
356 (measurements carried out by Zitelli (2013) on samples from ceiling of X82/T18 and X83).  
357 These results are consistent with the pH of environmental water recorded in other catacombs  
358 in Rome varying between 7.5 and 7.8 (Sánchez-Moral et al., 2005a). However, a much lower  
359 pH ranging from 5.9 to 6.4 has been found at the floor level of the X82/T18 and X83  
360 chambers (Zitelli 2013). This decrease of the rock pH at the bottom of the stratigraphic  
361 sequences could be caused by the decomposition fluids (principally organic acids; Vass et al.  
362 (2002)) released from the corpses in putrefaction (Carter and Tibbett, 2008; Gill-King, 1997;  
363 Schotsmans et al., 2014). In addition, the gypsum used as part of the funerary practices inside  
364 these graves could have also contributed to the acidification of the burial environment. In  
365 environmental management, gypsum is frequently incorporated alone or with other  
366 amendments in alkaline soils in order to reduce soil pH (Carter, 1986; Courtney and Kirwan,  
367 2012; Kordlaghari and Rowell, 2006; Wong et al., 2009). Bone apatite is known to have a low  
368 solubility in alkaline environments ( $\text{pH} > 7.5$ ) while its solubility becomes relatively high in  
369 acidic environments ( $\text{pH} < 6.5$ ) (Berna et al., 2004; Nielsen-Marsh and Hedges, 2000; Pate et  
370 al., 1989). The drop in pH of the burial environment in the chambers could have favored  
371 dissolution/recrystallization processes leading to the high IRSF values that we have measured.  
372 Finally, the high porosity of the pozzolan (Sánchez-Moral et al., 2005a; Sánchez-Moral et al.,  
373 2005b) could have facilitated water drainage. These particular conditions, associated with the  
374 absence of sediments between the different strata of corpses in certain burial chambers may

375 have limited the capacity of the burial environment to buffer water and increase interaction of  
376 bone with groundwater (Nielsen-Marsh et al., 2007; Smith et al., 2002). These conditions  
377 could then have accelerated the recrystallization of bone apatite and the subsequent alteration  
378 of the collagen matrix.

379

## 380 6.2. Spatial heterogeneity of bone preservation

381 Bone preservation in the SSPM catacomb is highly variable. While some of the  
382 archaeological samples present IRSF values and  $\text{CO}_3/\text{PO}_4$  ratios very close to those observed  
383 for the modern samples, most of them display higher IRSF values and lower  $\text{CO}_3/\text{PO}_4$  ratios.  
384 Collagen extraction yields are also variable (from 0 to 100%). Time, burial environment  
385 (temperature, hydrogeology, soil pH, etc.) and taphonomic processes (inhumation type, filling  
386 of the grave, anthropogenic or animal disturbance, etc.) are the three main parameters  
387 controlling the state of preservation of bones in archaeological contexts (Collins et al., 2002;  
388 Hedges, 2002). These different parameters interplay in a complex manner between and within  
389 archaeological sites (Hedges et al., 1995b; Nielsen-Marsh and Hedges, 2000; Pestle and  
390 Colvard, 2012). In the SSPM catacomb the organization and taphonomic evolution of the  
391 deposition levels suggests massive accumulation of corpses (Castex et al., 2007; Castex et al.,  
392 2009; Castex et al., 2011). Despite the fact that contemporary inhumation of 2000-3000  
393 corpses *stricto sensu* is unlikely given the dimensions of the burial chambers (Sachau-Carcel,  
394 2012; Sachau-Carcel et al., 2013), sepulchral areas appear to have been used during a  
395 relatively short period of time (Castex and Blanchard, 2011; Kacki et al., 2013). Therefore,  
396 time cannot be invoked to account for the strong heterogeneity of the conservation of the  
397 remains and other parameters need to be discussed.

398 The heterogeneity of bone preservation is not randomly distributed. With the  
399 exception of the small chamber X81 in which bone samples were well-preserved, most of the

400 well-preserved samples come from the large chambers (X83 and X84) while the poorly-  
401 preserved samples are found mostly in the small chambers (X80/T16 and X82/T18). In  
402 chamber X78/T15, the limited sampling (n= 2) did not allow us to conclude on the quality of  
403 collagen preservation in this room. This result is in keeping with anthropological observations  
404 performed during the excavation although local variations can occur depending on the  
405 disposition of the bones within a chamber (see below). Like in other great Roman catacombs,  
406 the SSPM catacomb has a relatively stable climate throughout the year. Sánchez-Moral et al.  
407 (2005a) reported a number of micro-environmental parameters in the catacombs of Domitilla  
408 and St. Callistus that can easily be extrapolated to the SSPM catacomb given the similar  
409 characteristics and geographical proximity (a few kilometers away) between these sepulchral  
410 complexes. A high humidity close to saturation ( $\geq 97\%$ ) and a constant temperature of 15-  
411 17°C were recorded in the two catacombs. Given the small size of the central sector of the  
412 SSPM catacomb and the existence of large openings between chambers, differences in  
413 microclimate between rooms are highly unlikely. During the excavation, visual inspection of  
414 bones in the large chambers indicated that bones close to the chamber walls were more altered  
415 than in the center (Castex et al., 2009). We propose that runoff against the walls and higher  
416 humidity locally could explain this difference in preservation between the center and the  
417 periphery of the large chambers. In the smaller chambers, all the bones remain relatively close  
418 to the walls and would be equally affected by the water runoff. In the large chambers,  
419 buffering conditions (constant humidity and temperature) would have favored exceptional  
420 bone collagen preservation for bones situated further away from the walls. Due to its central  
421 position, the small chamber X81 has several openings (Figure 1). This has probably restricted  
422 runoff against the walls, which could explain the better preservation of the bones in this  
423 chamber in general. Finally, trampling of the human remains by the grave diggers, families of  
424 the deceased and/or pilgrims along the walls of the burial chambers, especially in the large

425 ones, could be another explanation. A circulation area along the west wall of the chamber  
426 X83 has been identified causing the loss of the anatomical integrity of the bone in this sector  
427 of the chamber (Castex et al., 2009).

428

### 429 6.3. Testing for the preservation of the isotopic composition of bone apatite using radiocarbon 430 dating

431 A crucial point for the use of bone apatite isotope values to infer diet and mobility  
432 patterns of past populations is to establish whether bone recrystallization was associated with  
433 carbon and oxygen isotope exchange. We found apatite to be slightly more  $^{14}\text{C}$  active  
434 (between 0 and 1.5 pMC) than collagen from the same individual. This is in keeping with  
435 differences calculated for bones in archaeological sites ranging in age between 0 and 9.5 kyr  
436 BP (Zazzo, in press). The positive  $^{14}\text{C}_{\text{col-carb}}$  age difference together with the correlation  
437 between  $^{14}\text{C}_{\text{carb}}$  and  $\delta^{13}\text{C}_{\text{carb}}$  values, indicate that carbon isotope exchange between bone  
438 apatite and dissolved inorganic carbon took place with a younger source of carbon with higher  
439  $\delta^{13}\text{C}$  values. The  $^{14}\text{C}_{\text{col}}$  age offset can be used to calculate a percent contamination of the  
440 carbonate fraction with modern carbon if this value represents the true age of an individual.  
441 However, the positive correlation found between  $\delta^{13}\text{C}_{\text{col}}$  and  $^{14}\text{C}_{\text{col}}$  values suggests that the  
442 individuals with the highest  $\delta^{13}\text{C}$  values could have consumed marine foods as these usually  
443 have more positive  $\delta^{13}\text{C}$  values than terrestrial foods (Craig et al., 2013; Richards et al., 2005;  
444 Schoeninger et al., 1983). The lack of correlation between the  $\delta^{15}\text{N}_{\text{col}}$  values and the  $^{14}\text{C}_{\text{col}}$   
445 ages could be explained by the consumption of low trophic level fish (e.g., anchovies,  
446 sardines, mackerel, *etc.*) or bivalves (e.g., oysters, cockles, clams, *etc.*) (Carlier et al., 2007;  
447 Garvie-Lok, 2001). This result is coherent with the significant (ca. 15-20%) proportion of  
448 marine food found in the diet of the victims of the 79 AD Vesuvius eruption at Herculaneum  
449 (Craig et al., 2013). According to the equation in Figure 3A from Craig et al. (2013),  $\delta^{13}\text{C}_{\text{col}}$

450 values measured for the five individuals could correspond to  $^{14}\text{C}$  age excesses between 60 (for  
451 Individual US216/Mand1) and 133 (for Individual 64)  $^{14}\text{C}$  yr. This would affect the collagen  
452 age more than the apatite age because collagen records the protein fraction of the diet, while  
453 apatite carbon records the whole diet, leading to an overestimation of the age differences  
454 between the two fractions. A large dependence on marine food in individual 64 could explain  
455 the large age difference ( $180 \pm 40$   $^{14}\text{C}$  yr) measured between the two individuals dated in the  
456 burial chamber X82/T18 (Table 4). This hypothesis is also supported by the fact that in  
457 chamber X80/T16, the lack of  $^{14}\text{C}_{\text{col}}$  age difference between individuals 24 and 79  
458 corresponds to identical  $\delta^{13}\text{C}_{\text{col}}$  values for these specimens, suggesting a similar amount of  
459 marine food in their diet. For these reasons, the value of 1.5 pMC contamination must be  
460 considered as a maximum estimate.

461         The central area of the entire SSPM catacomb and numerous others roman catacombs  
462 (e.g. the Domitilla and St Callixtus catacombs) is carved through pozzolan, a tuff layer of the  
463 Villa Senni formation resulting from an eruptive event of the Colli Albani volcano  
464 (Funicicillo et al., 2008; Giordano, 2010). Bulk measurements on samples coming from the  
465 Domitilla and St Callixtus catacombs indicate that pozzolan can contain 1-5% calcite  
466 (Sánchez-Moral et al., 2005a; Sánchez-Moral et al., 2005b). To our knowledge, there is no  
467 published stable isotope data for this ignimbrite. However,  $\delta^{13}\text{C}$  of carbonate in lavas and  
468 ejecta from the same volcanic complex have been measured and range from -19.8 to 3.5‰  
469 (Fornaseri and Turi, 1969). If we use the highest  $\delta^{13}\text{C}$  value as an estimate for the diagenetic  
470 endmember, a simple mass balance calculation taking into account the intra-individual  
471 differences in  $^{14}\text{C}$  activity allows us to estimate a  $\delta^{13}\text{C}$  values shift between 0.00‰ (SD  
472  $\pm 0.07$ ‰) and 0.27‰ (SD  $\pm 0.08$ ‰) as a result of diagenesis. Therefore, the intake of  
473 exogenous carbon in bone carbonate is limited and does not modify its carbon isotope  
474 composition to any great extent. This is in keeping with the slightly acidic conditions in the

475 SSPM catacomb. As previously mentioned, the correction corresponds to a maximum  
476 estimate. Thus, the isotopic composition of the carbonate fraction of bone can be considered  
477 as reliable as that of the collagen fraction and can be used to reconstruct the life histories of  
478 the individuals buried in the central area of the catacomb. This is beyond the scope of this  
479 study and will be the focus of another article.

480 Finally, the significant carbon and oxygen isotope difference of apatite found between  
481 samples whose collagen was well- and poorly-preserved is intriguing and deserves attention.  
482 Samples which did not pass the collagen preservation tests had  $\delta^{13}\text{C}_{\text{carb}}$  values 0.3‰ lower  
483 and  $\delta^{18}\text{O}_{\text{carb}}$  0.3‰ higher on average than those with well-preserved collagen (Table 2). The  
484 difference between the two groups, although small, was significant for  $\delta^{13}\text{C}_{\text{carb}}$  and  $\delta^{18}\text{O}_{\text{carb}}$   
485 (Mann-Whitney U tests; p-values  $\ll 0.05$ ). Because of the negative correlation found  
486 between  $^{14}\text{C}_{\text{carb}}$  ages and  $\delta^{13}\text{C}_{\text{carb}}$  values, lower  $\delta^{13}\text{C}_{\text{carb}}$  are an unlikely result of a contaminant  
487 with a more negative  $\delta^{13}\text{C}$  value. We propose a different interpretation for the difference  
488 found between the two groups. It is striking that the direction and magnitude of the isotopic  
489 shifts that we observed are similar to those measured by Koch et al. (1997) between untreated  
490 and acetic-acid treated modern enamel and bone samples. The two sub-groups of bones from  
491 the SSPM mimic the untreated and treated samples from the Koch et al. (1997) experiment.  
492 Moreover, bones with low collagen yields are also highly recrystallized and have lost part of  
493 their structural carbonate. Carbonates in apatite are present in two different positions, A and  
494 B, A-bonds being the weakest. We found OH<sup>-</sup> ions in some of the spectra, possibly  
495 substituting for A-carbonates. As postulated by Koch et al. (1997) it is plausible to assume  
496 that these two sites with different chemical bonds discriminate stable isotopes differently.  
497 Since A/B type carbonate ratios are likely different in recrystallized and preserved apatites,  
498 the slight isotopic difference measured at the population level could well reflect this

499 difference in isotope fractionation. This difference would then result from recrystallization,  
500 not isotope exchange.

501

#### 502 6.4. Regional variability in bone preservation: catacombs vs. open-air sites

503 Rutgers et al. (2009) hypothesized that the microclimate of catacombs affects  
504 negatively bone collagen preservation. To further investigate this issue, we compared bone  
505 collagen preservation in the catacombs and in contemporary open air sites from Italy (Figure  
506 9). The choice of sites included in this comparison is dependent on the collagen extraction  
507 protocol used on each site. Some methods, such as ultra-filtration, are known to drastically  
508 reduce collagen extraction yields. Because of this possible methodological bias, our  
509 comparison only includes sites from central Italy – all located in Rome’s region, also known  
510 as Latium - (Killgrove and Tykot, 2013; Prowse et al., 2004; Prowse, 2001; Prowse et al.,  
511 2007; Prowse et al., 2005; Rutgers et al., 2009), but excludes sites from southern Italy (Craig  
512 et al. 2009; Craig et al. 2013). With an average collagen extraction yield close to 50%, bones  
513 from the SSPM appear to be better preserved than most of the contemporary sites of the  
514 Latium. Comparison indicates that the quantity of extracted collagen is similar (50% or  
515 below) in the small chambers (with the exception of X81) but much higher (between 60-90%  
516 on average) in the large chambers. It is also interesting to note that the two sites presenting the  
517 highest (although highly variable) average extraction yields in the Latium are catacombs.  
518 Despite the limitations described above, the relatively stable climatic conditions prevailing in  
519 catacombs seem overall to have favored, rather than disadvantaged bone collagen  
520 preservation and therefore contradicts Rutgers et al. (2009)’s assumption. However, an  
521 opposite pattern seems to emerge if one looks at the mineral fraction of bone. The comparison  
522 of the crystallinity values calculated for the bone samples from the SSPM catacomb with  
523 those published for open air sites from different Roman Empire provinces clearly shows that

524 the mineral phase of bones is much more altered in the SSPM catacomb than in the other sites  
525 (Figure 10). Although we are aware that the comparison of IRSF values reported in different  
526 studies can be problematic due to variances in sample preparation protocols and measurement  
527 methods (KBr vs. ATR mode), the IRSF values calculated for the SSPM catacomb are similar  
528 to those observed for highly degraded bones (Berna et al., 2004; Lebon et al., 2010). Overall,  
529 bones found in catacomb contexts seem to present *a Janus face*<sup>1</sup>, showing both a well-  
530 preserved organic and a poorly-preserved mineral phase. But as previously shown, this high  
531 degree of recrystallization is not correlated with isotope exchange of the mineral phase with  
532 the surrounding environment, possibly due to the relatively low pH reigning in the catacomb.  
533 Based on this extensive study, we can conclude that it will be possible to examine traits of the  
534 life history of the individuals buried in the catacomb based on the combined analysis of bone  
535 collagen (when preserved) and apatite isotope values.

536

## 537 **7. Conclusion**

538 This study - the first focusing on diagenesis in a catacomb context – illustrates the  
539 effect of post-depositional processes on bone preservation. Our multi-proxy approach showed  
540 that extreme differences in preservation can be found between contemporary individuals  
541 within a small area, highlighting the influence of localized variations on taphonomy. We  
542 found that the heterogeneity of bone preservation was not randomly distributed in the SSPM  
543 catacomb and that bone was better preserved in the large chambers than in the small ones.  
544 This is in keeping with anthropological observations performed during the excavation and we  
545 propose that the combination of acidic conditions, together with condensation of air humidity  
546 and runoff against the walls could explain this differential preservation. Comparison with  
547 contemporary sites from the Latium indicates the two sites presenting the highest (although

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<sup>1</sup> named after the Roman deity Janus who had two faces, one looking forward, the other looking backward. By extension, this term refers to a deceitful person or an object having or containing contrasting characteristics.

548 highly variable) average extraction yields are catacombs, and that bone collagen in large  
549 chambers is much better preserved than in other open air contemporary sites. Therefore, the  
550 relatively stable climatic conditions prevailing in catacombs seem overall to have favored,  
551 rather than disadvantaged bone preservation.

552         Despite high rates of recrystallization observed for some of the bone samples, coherent  
553 radiocarbon ages measured in the carbonate and collagen fractions of several individuals  
554 demonstrates a lack of significant (>1%) isotopic exchange. We noticed a slight (0.3‰)  
555 difference in both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values between the well-preserved and the most severely  
556 recrystallized samples. This difference is similar in magnitude and direction with differences  
557 observed on modern samples pretreated with acetic acid and could be due to selective  
558 leaching of A vs. B-type carbonate in apatite during recrystallization. This shift is close to  
559 analytical precision and does not preclude reliable reconstructions of the individual history of  
560 these specimens based on stable isotope analysis of the carbonate fraction. Therefore, further  
561 dietary reconstructions can include all the individuals of the population, even those for which  
562 bone collagen is no longer preserved.

563

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577  
578

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869

870 **Figure Captions**

871

872 **Figure 1:** Plan of the burial chambers in the region X from the SSPM catacomb. After M.

873 Ricciardi, modified.

874

875 **Figure 2:** FT-IR spectrum of a fresh bone showing the regions of interest as well as the IRSF

876 calculation procedure.

877

878 **Figure 3:** Photographs showing examples of good (A), medium (B) and poor (C) preservation

879 of the SSPM bones, based on their macroscopic characteristics

880

881 **Figure 4:** Collagen yield of the SSPM bone samples grouped by the burial chamber, skeletal

882 part category and macroscopic appearance. Boxplots represent the 25<sup>th</sup>, 50<sup>th</sup> (median) and 75<sup>th</sup>

883 percentiles in the distribution of scores. Whiskers indicate the variability outside the upper

884 and lower quartiles. Outliers are identified by individual points. The shaded area corresponds

885 to the collagen yield measured in modern bones (after Bocherens et al. 1991).

886

887 **Figure 5:** Carbon and nitrogen abundances of the SSPM bone samples. Samples which fail

888 one of the collagen preservation criteria defined in Table 1 are considered poorly-preserved

889 (closed symbols) while those passing all the preservation criteria are considered well-

890 preserved (open symbols).

891

892 **Figure 6:** IRSF plotted against CO<sub>3</sub>/PO<sub>4</sub> ratio of the SSPM bone samples. Modern bone

893 samples are shown for reference.

894

895 **Figure 7:** CO<sub>3</sub>/PO<sub>4</sub> ratio plotted against Amide1/ PO<sub>4</sub> ratio of the SSPM bone samples.

896 Modern bone samples are shown for reference.

897

898 **Figure 8:** IRSF plotted against Amide1/ PO<sub>4</sub> ratio of the SSPM bone samples. Modern bone  
899 samples are shown for reference.

900

901 **Figure 9:** Inter-site comparison of the collagen extraction yields measured on contemporary  
902 human bone samples from the Latium. The Isola Sacra necropolis is located near Ostia and  
903 Portus Romae and dated from the 1<sup>st</sup> to 3<sup>rd</sup> c. AD (Prowse et al., 2004; Prowse, 2001; Prowse  
904 et al., 2007; Prowse et al., 2005). The ANAS cemetery is located between Ostia and Rome  
905 and dated from the 1<sup>st</sup> to 3<sup>rd</sup> c. AD (Prowse et al., 2004; Prowse, 2001). The necropolises of  
906 Casal Bertone and Castellaccio Europarco are located in Rome and dated from the 2<sup>nd</sup> to 3<sup>rd</sup>  
907 and from the 1<sup>st</sup> to 2<sup>nd</sup> c. AD, respectively (Killgrove, 2010; Killgrove and Tykot, 2013). The  
908 St Callixtus catacomb is located in Rome and dated from the 3<sup>rd</sup> to 5<sup>th</sup> c. AD (Rutgers et al.,  
909 2009). Boxplots represent the 25<sup>th</sup>, 50<sup>th</sup> (median) and 75<sup>th</sup> percentiles in the distribution of  
910 scores. Whiskers indicate the variability outside the upper and lower quartiles. Outliers are  
911 identified by individual points. The shaded area corresponds to the collagen yield measured in  
912 modern bones (after Bocherens et al. 1991). OAS stands for open air site and SS stands for  
913 subterranean site.

914

915 **Figure 10:** Inter-site comparison of the IRSF values measured on human bone apatite  
916 samples from the Roman Empire. The Kellis 2 cemetery is located near Mut in the Dakhleh  
917 Oasis in Egypt and dated from the 1<sup>st</sup> to 5<sup>th</sup> c. AD (Dupras, 1999; Dupras and Schwarcz,  
918 2001). The Leptiminus cemeteries are located in the modern day town of Lamta in Tunisia  
919 and dated from the 2<sup>nd</sup> to 6<sup>th</sup> century AD (Keenleyside et al. 2009). The Poseidonia

920 necropolises are located near Capaccio-Paestum in Italy and dated from the 7<sup>th</sup> c. BC to the  
921 2<sup>nd</sup> c. AD (Alfano et al. 2009). The Isola Sacra necropolis is located near Ostia and Portus  
922 Romae and dated from the 1<sup>st</sup> to 3<sup>rd</sup> c. AD (Prowse et al., 2004; Prowse, 2001; Prowse et al.,  
923 2007; Prowse et al., 2005). Boxplots represent the 25<sup>th</sup>, 50<sup>th</sup> (median) and 75<sup>th</sup> percentiles in  
924 the distribution of scores. Whiskers indicate the variability outside the upper and lower  
925 quartiles. Outliers are identified by individual points. The hatched area corresponds to the  
926 IRSF values found for modern bones. The shaded area corresponds to the samples mildly and  
927 highly recrystallized. OAS stands for open air site and SS for subterranean site.  
928