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1           **Limits of calcium isotopes diagenesis in fossil bone and enamel**

2

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21

22 **Abstract**

23 Diagenesis has been recognized for decades to significantly alter the trace elements biogenic  
24 signatures in fossil tooth enamel and bone that are routinely used for paleobiological and  
25 paleoenvironmental reconstructions. This signature is modified during diagenesis according  
26 to a complex continuum between two main processes, addition, and substitution. For an  
27 additive-like, or early diagenesis, the trace elements biogenic profiles can be restored by  
28 leaching secondary minerals, but this technique is inefficient for a substitutive-like, or  
29 extensive diagenesis for which secondary trace elements are incorporated into the biogenic  
30 mineral. This scheme is however unclear for Ca, the major cation in tooth enamel and bone  
31 hydroxylapatite, which stable isotope composition ( $\delta^{44/42}\text{Ca}$ ) also conveys biological and  
32 environmental information. We present a suite of leaching experiments for monitoring  
33  $\delta^{44/42}\text{Ca}$  values in artificial and natural fossil enamel and bone from different settings. The  
34 results show that enamel  $\delta^{44/42}\text{Ca}$  values are insensitive to an additive-like diagenesis that  
35 involves the formation of secondary Ca-carbonate mineral phases, while bone shows a  
36 consistent offset toward  $^{44}\text{Ca}$ -enriched values, that can be restored to the biogenic baseline  
37 by a leaching procedure. In the context of a substitutive-like diagenesis, bone exhibits  
38 constant  $\delta^{44/42}\text{Ca}$  values, insensitive to leaching, and shows a REE pattern symptomatic of  
39 extensive diagenesis. Such a REE pattern can be observed in fossil enamel for which  $\delta^{44/42}\text{Ca}$   
40 values are still fluctuating and follow a trophic pattern. We conclude that Ca isotopes in fossil  
41 enamel are probably not prone to extensive diagenesis and argue that this immunity is due  
42 to the very low porosity of enamel that cannot accommodate enough secondary minerals to  
43 significantly modify the isotopic composition of the enamel Ca pool.

44

## 45 **1. Introduction**

46 Bone, dentin, and enamel are valuable archives for recording information about the  
47 life history and environment of fossil and extant vertebrates. The information is embedded  
48 as trace elements or isotopic ratios signature during the genesis of the mineralized tissue, a  
49 signature that is further obfuscated by diagenesis during fossilization. Regarding trace  
50 elements, the comprehension of diagenetic mechanisms has greatly benefited from in-situ  
51 laser ablation measurements (Kohn and Moses, 2013; Kral et al., 2022), highlighting the  
52 complex interplay of the diffusion, adsorption, and transport reaction processes. However,  
53 while sophisticated techniques are now deployed to fully integrate all the hallmarks of  
54 diagenesis (Suarez and Kohn, 2020; Weber et al., 2021; Kral et al., 2022), the results still  
55 recognize the central importance of porosity which was already suspected in the 2000's  
56 (Hedges, 2002; Trueman and Tuross, 2002; Kral et al., 2021). Porosity mediates fluid  
57 circulation and secondary mineral deposition, and increases as a result of collagen  
58 degradation, augmenting the exposure of carbonated hydroxylapatite (HAp) crystallites in the  
59 first stages of bone diagenesis. Because porosity is forty times higher in bone than in enamel  
60 (Wang and Cerling, 1994), the latter is widely accepted to be far more resistant to diagenesis  
61 than bone and is nowadays the tissue of reference for measuring trace elements  
62 concentration (Le Houedec et al., 2013; Joannes-Boyau et al., 2019; Nava et al., 2020; Funston  
63 et al., 2022) and/or isotope composition (Martin et al., 2014, 2015; Jaouen et al., 2017;  
64 McCormack et al., 2022).

65 In all types of tissue, some diagenetic overprints can be reverted using leaching  
66 pretreatment (Sillen and LeGeros, 1991; Koch et al., 1997; Nielsen-Marsh and Hedges, 2000;  
67 Balter et al., 2002a; Wathen et al., 2022). When formation of secondary mineral phases is  
68 thought to have occurred (additive-like diagenesis), leaching is potentially efficient at  
69 removing these minerals. One issue is that leaching is often performed in batch, leading  
70 samples to be leached more than necessary, therefore dissolving the most soluble HAp  
71 fraction that is the most pristine (Sillen and LeGeros, 1991; Balter et al., 2002a). The efficiency  
72 of the leaching method is even more questionable when diffusion of diagenetic elements  
73 (substitutive-like diagenesis) into the HAp has occurred.

74 All told, these considerations hold for trace elements, but the situation is unclear for  
75 major elements. Traditionally, the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of  $\text{CO}_3$  present in HAp (~5 wt%) are  
76 measured in leached samples due to the widespread presence of calcite in the porosity, but

77 the  $\delta^{18}\text{O}$  value of HAp  $\text{PO}_4$  (~55 wt%) is always measured in raw samples. The isotope  
78 composition of Ca of HAp (~40 wt%) varies according to trophic position (Skulan and DePaolo,  
79 1999; Heuser et al., 2011; Martin et al., 2018; Hu et al., 2022) and physiology (Tacaïl et al.,  
80 2017; Hassler et al., 2021; Koutamanis et al., 2021; Li et al., 2022), but deserves an assessment  
81 of potential diagenetic effects on  $\delta^{44/42}\text{Ca}$  values in bone and tooth enamel. Simple mass  
82 balance calculations already show that changes of the original  $\delta^{44/42}\text{Ca}$  values in bone and  
83 tooth enamel by secondary Ca minerals are either small or would require an uncommonly  
84 fractionated Ca isotopes diagenetic pool (Heuser et al., 2011; Martin et al., 2017).

85 Here, we attempt to resolve this issue by studying how Ca isotopes behave during  
86 controlled experiments of artificial fossil bone and enamel leaching and in different diagenetic  
87 case studies. The Ca isotope compositions are measured along with Ca concentrations and  
88 Ca/P ratios to monitor the origin of Ca, and Rare Earth Elements (REE) to decipher the type  
89 (additive or substitutive) and extrapolate the extend of diagenetic alteration (Reynard et al.,  
90 1999; Trueman and Tuross, 2002).

91

## 92 **2. Material and methods**

93 First, we analyzed the Ca isotope composition in leached and unleached artificial  
94 samples mimicking fossil bone and enamel that have undergone an additive-like diagenesis.  
95 These samples consist of mixtures of HAp and calcite powder which  $\delta^{44/42}\text{Ca}$  value are distinct  
96 and constrained. An artificial fossil bone was prepared using the bone ash certified reference  
97 material (SRM-1400, NIST;  $\delta^{44/42}\text{Ca} = -1.21 \pm 0.04\text{‰}$ ,  $\pm 2\text{SD}$ ,  $n = 3$ ) to which was added various  
98 proportions of the calcite certified reference material SRM-915b (NIST,  $\delta^{44/42}\text{Ca} = -0.28 \pm$   
99  $0.04\text{‰}$ ,  $\pm 2\text{SD}$ ,  $n = 21$ ). The SRM-1400 certified reference material has been ashed and is  
100 devoid of organic matter and has a high crystallinity, both being characteristics of fossil bone.  
101 Because enamel certified reference material does not exist, we prepared artificial fossil  
102 enamel by mixing a sample of extant enamel from an isolated and partial molar tooth of  
103 modern elephant (*Elephas maximus*;  $\delta^{44/42}\text{Ca} = -1.43 \pm 0.07\text{‰}$ ,  $\pm 2\text{SD}$ ,  $n = 10$ ) with various  
104 proportions of SRM-915b. Second, we analyzed a suite of natural samples from different  
105 localities and ages that were selected according to their potential degree of diagenetic  
106 alteration. Pleistocene bone and tooth enamel samples were chosen to be representative of  
107 weak diagenesis, with preserved trace elements compositions, and Devonian bone of more

108 pronounced diagenesis. A first set of material is composed by a suite of fossil bone samples  
109 (n = 13) from the Camiac cave (Gironde, France). The site is a hyena dens from the upper  
110 Pleistocene (Mousterian period,  $35.1 \pm 2$  ka BP; Balter et al., 2002b). Taxonomic information  
111 is given in Table S2. This material is characterized by trace elements (Sr/Ca and Ba/Ca)  
112 distributions that reflect a trophic pattern (Balter et al., 2002b). A suite of fossil enamel  
113 samples (n = 14) from the Plio-Pleistocene of the Shungura Formation in the Lower Omo  
114 Valley (Ethiopia, sample age bracketed between 3.6 and 1.76 Ma; Boisserie et al., 2008)  
115 represents the second set of material. Here, the material is composed by only two extinct  
116 genera of large African suids (*Metridiochoerus* sp. and *Notochoerus* sp., Table S2). Leached  
117 fossil bone at Shungura exhibit Sr/Ca ratios characteristic of a trophic pattern (Sillen, 1986).  
118 A third set of samples gather vertebrate (fish and tetrapod) fossil bone (n = 25, Table S2) from  
119 the Devonian locality of Strud (360 Ma, Belgium; Olive et al., 2015). To our knowledge, no  
120 geochemical studies has already been performed on this material.

121         The samples were processed in the clean room and analyzed on the facilities at the  
122 Laboratoire de Géologie de Lyon: Terre, Planètes, Environnements (LGL-TPE, ENS de Lyon).  
123 All the samples (ca. ~ 5 mg) were leached using 0.5 ml of 0.1 M acetic acid per mg of sample  
124 during 30 min in an ultrasonic bath and rinsed with 18.2 M $\Omega$ /cm grade MilliQ water. This  
125 represents 2.5-fold the quantity of acid necessary to dissolve an equivalent amount of pure  
126 calcite. The samples were dissolved in distilled concentrated HNO<sub>3</sub> and an aliquot measured  
127 for major elements (P and Ca) by ICP-OES (Thermo Scientific, iCap 6000 Radial) and trace  
128 elements (REE) by ICP-MS (Thermo Scientific, iCap-Q) according to Balter and Lécuyer (2004).  
129 Calcium from the remaining solution was purified by ion-exchange chromatography and Ca  
130 isotope composition measured by MC-ICP-MS (Thermo Scientific, Neptune Plus) according to  
131 Tacail et al. (2014). Briefly, three ion-exchange chromatography steps are necessary, first  
132 allowing the recovery of Ca, Fe and Sr only, the second to purify Ca from Fe and the third to  
133 remove Sr (Table 1). Blanks for the procedure did not exceed 100 ng of Ca.

134

135 **Table 1:** Detailed procedure for Ca extraction and purification.

1. Matrix elimination		
AG50W-X12 resin (200-400 mesh) ~ 2 mL		
Step	Eluent	Vol. (mL)
Condition	1 M HCl	10
Load	1 M HCl	2+1
Elution (matrix)	1 M HCl	55
<b>Ca elution (Ca, Sr, Fe)</b>	<b>6 M HCl</b>	<b>10</b>
2. Fe elimination if necessary		
AG1-X8 resin (100-200 mesh) ~ 2 mL		
Step	Eluent	Vol. (mL)
Condition	6 M HCl	10
Load	6 M HCl	1+1
<b>Elution (Ca, Sr)</b>	<b>6 M HCl</b>	<b>6</b>
3. Sr elimination		
Sr-Specific resin (Eichrom) ~ 0.7 mL		
Step	Eluent	Vol. (mL)
Condition	3 M HNO <sub>3</sub>	5
Load	3 M HNO <sub>3</sub>	0.5+0.5
<b>Elution (Ca)</b>	<b>3 M HNO<sub>3</sub></b>	<b>6</b>

136

137 Calcium isotope abundance ratios (<sup>44</sup>Ca/<sup>42</sup>Ca and <sup>43</sup>Ca/<sup>42</sup>Ca) were measured using a  
 138 Neptune Plus multi-collector ICP-MS using the standard-sample-standard bracketing method.  
 139 All Ca isotope compositions are expressed using the delta notation, calculated as follows:

140

$$141 \quad \delta^{44/42}\text{Ca} = \left( \frac{({}^{44}\text{Ca}/{}^{42}\text{Ca})_{\text{Sample}}}{0.5 \times ({}^{44}\text{Ca}/{}^{42}\text{Ca})_{\text{ICP-Ca-Lyon}}^{n-1} + 0.5 \times ({}^{44}\text{Ca}/{}^{42}\text{Ca})_{\text{ICP-Ca-Lyon}}^{n+1}} - 1 \right) \times 1000$$

142

143 The ICP Ca Lyon standard, used routinely in Lyon, was used as the bracketing standard.  
 144 The results can be converted relative to the SRM915a with an offset of -0.52‰.

145

### 146 3. Data presentation and interpretation

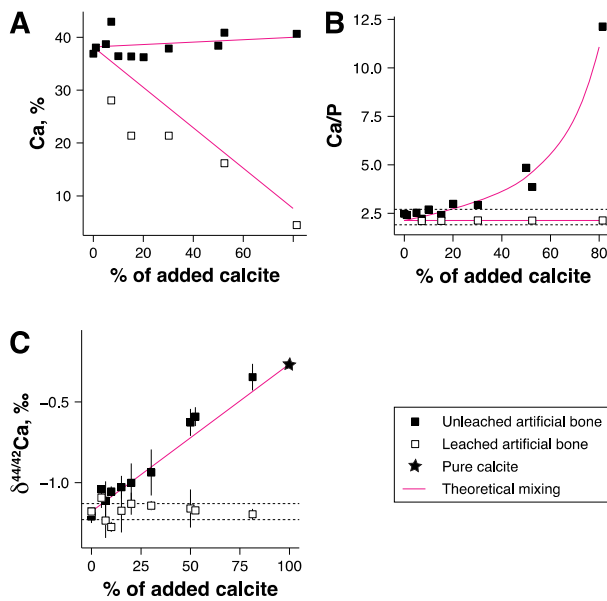
147 Results are given in **Table S1** for artificial fossil bone and enamel and in **Table S2** for  
 148 natural fossil bone and enamel. The mass fractionation measured in this study agrees with  
 149 the 0.5 slope predicted by the linear approximation of mass-dependent fractionation (**Fig. S1**).

150 We first test the effect of leaching on the  $\delta^{44/42}\text{Ca}$  value of the certified reference  
 151 material SRM-1400 and found no difference after leaching ( $\delta^{44/42}\text{Ca} = -1.21 \pm 0.03\text{‰}$ ,  $\pm 2\text{SD}$ ,  
 152  $n = 3$ ) compared to unleached material ( $\delta^{44/42}\text{Ca} = -1.21 \pm 0.04\text{‰}$ ,  $\pm 2\text{SD}$ ,  $n = 3$ ). Absence of  
 153 difference between the  $\delta^{44/42}\text{Ca}$  values of unleached and leached enamel is also observed as

154 we measured a constant  $\delta^{44/42}\text{Ca}$  value of -1.43‰ (Table S1). Because of the very small  
155 amount of processed material (~ 5 mg), the remaining bone or enamel residue after leaching  
156 is so small that it is impossible to weigh it, so Ca concentration for leached material is  
157 underestimated. Nevertheless, using in 0.1 M acetic acid for 30 min generally leads to a loss  
158 of about 30% of initial material. The absence of Ca isotope fractionation during leaching  
159 therefore indicates that the dissolution of HAp is quantitative and that leaching *per se* does  
160 not affect fossil bone and enamel  $\delta^{44/42}\text{Ca}$  values.

161 In the mixture of bone HAp with increasing proportion of added calcite, the slight  
162 theoretical increase of Ca concentration is barely detected in unleached mixtures, probably  
163 due to the ~5% error inherent to ICPMS measurements (Fig. 1A), but the decrease of Ca  
164 content is inambiguous in leached mixture (Fig. 1A). In leached mixtures, the Ca content is  
165 generally lower than predicted particularly for low calcite content (Fig. 1A). This suggests  
166 partial dissolution of HAp that further occurs after complete dissolution of calcite (Balter et  
167 al., 2002b). The leaching restores the Ca/P ratio of the mixture to the pure bone value (Fig.  
168 1B). The leaching also restores the original bone  $\delta^{44/42}\text{Ca}$  value even up to 80% of added calcite  
169 (Fig. 1C). The deviation of the bulk  $\delta^{44/42}\text{Ca}$  value can be detectable with  $\geq 10\%$  added calcite  
170 and generally follows the theoretical balance between the HAp and the calcite end-members.  
171





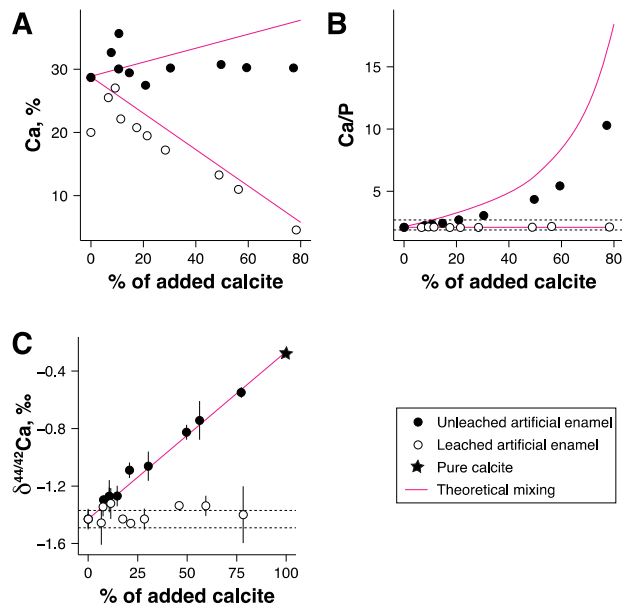
172

173 **Fig. 1:** Effect of leaching on the Ca concentration (A), Ca/P ratio (B), and the  $\delta^{44/42}\text{Ca}$  (C) value of artificial fossil  
 174 bone material (SRM-1400 + SRM-915b). Percentage of added calcite is relative to the initial total mass of the  
 175 mixture. Error bars are typically < 5% in panel A. Dotted lines in panel B represent the natural range of the Ca/P  
 176 ratio from 1.9 to 2.7 in biological HAP. Dotted lines in panel C represent the 2 standard deviations of the mean  
 177  $\delta^{44/42}\text{Ca}$  value of raw material. The  $\delta^{44/42}\text{Ca}$  value is reported relative to ICP-Ca Lyon.

178

179 In the mixture of enamel with increasing proportion of calcite, the Ca concentration  
 180 and Ca/P ratio between unleached and leached samples follow the same pattern than for  
 181 bone (Fig. 2A, 2B). Dissolution of enamel is also observed for low calcite content as measured  
 182 Ca concentrations stand below theoretical values (Fig. 2A). Similar to bone, deviation of the  
 183 bulk  $\delta^{44/42}\text{Ca}$  value is detected with  $\geq 10\%$  added calcite, which follows the theoretical balance  
 184 between the HAP and the calcite end-members, and leaching restores the initial enamel  
 185  $\delta^{44/42}\text{Ca}$  value even up to 80% of added calcite (Fig. 1C). Porosity in bone and enamel is  
 186 variable (see Kral et al., 2021 for a discussion) but is grossly 40% in bone and one order of  
 187 magnitude less ( $\sim 1\%$ ) in enamel (Wang and Cerling, 1994). Given that bone and enamel can  
 188 accommodate calcite to a magnitude equal to porosity, the results from artificial fossil bone  
 189 and enamel suggest that the incorporation of calcite in natural fossil materials can be  
 190 detected in the bone  $\delta^{44/42}\text{Ca}$  value but not for that enamel, except for unrealistic calcite  
 191  $\delta^{44/42}\text{Ca}$  value or important secondary porosity (e.g., post burial enamel surface cracks). This  
 192 assumption is confirmed with results obtained on natural fossil bone and enamel.

193



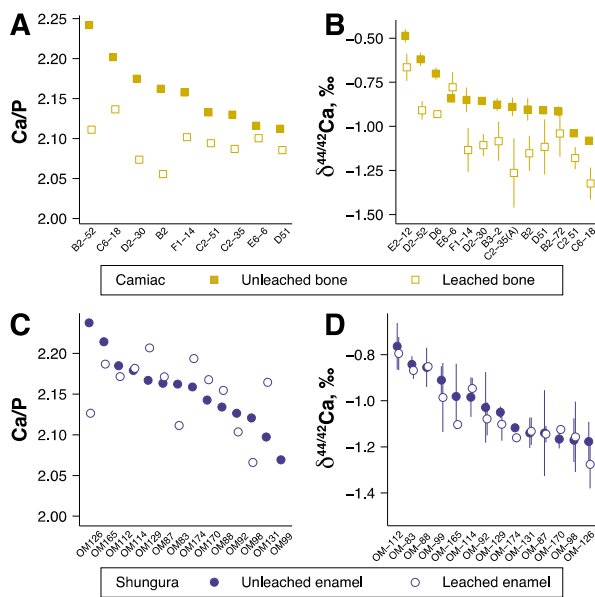
194

195 **Fig. 2:** Effect of leaching on the Ca concentration (A), Ca/P ratio (B), and the  $\delta^{44/42}\text{Ca}$  (C) value of artificial fossil  
 196 bone material (enamel + SRM-915b). Percentage of added calcite is relative to the initial total mass of the  
 197 mixture. Error bars are typically < 5% in panel A. Dotted lines in panel B represent the natural range of the Ca/P  
 198 ratio from 1.9 to 2.7 in biological HAp. Dotted lines in panel C represent the 2 standard deviations of the mean  
 199  $\delta^{44/42}\text{Ca}$  value of raw material. The  $\delta^{44/42}\text{Ca}$  value is reported relative to ICP-Ca Lyon.

200

201 Fossil bone from Camiac (Table S2) shows a Ca/P decrease indicative of efficient  
 202 removal of additive Ca of calcitic origin (Fig. 3A) along with a consistent decrease ( $-0.21 \pm 0.11$ ,  
 203  $n = 12$ ) of the  $\delta^{44/42}\text{Ca}$  values during the leaching (Fig. 3B), validating the assumption that the  
 204 added diagenetic Ca from calcite with a higher  $\delta^{44/42}\text{Ca}$  value than bone has been removed  
 205 accordingly. Fossil enamel unleached and leached samples from Shungura (Table S2) show a  
 206 Ca/P ratio that remains constant (Fig. 3C) and similar  $\delta^{44/42}\text{Ca}$  values (Fig. 3D), suggesting that  
 207 non-significant amount of calcite was present in raw samples and that a quantitative  
 208 fractionation of Ca isotopes occurred during leaching.

209



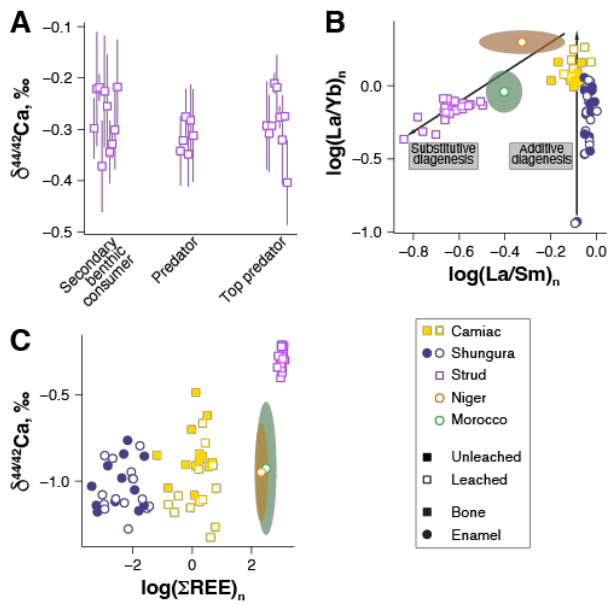
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211

212 **Fig. 3:** Effect of leaching on the Ca/P ratio and  $\delta^{44/42}\text{Ca}$  value of natural fossil bone from Camiac (A and B) and  
 213 fossil enamel from Shungura (C and D). The variability of the Ca/P ratio is in the natural range of the Ca/P ratio  
 214 in biological Hap. The  $\delta^{44/42}\text{Ca}$  value is reported relative to ICP-Ca Lyon.

215

216 Finally, the Devonian bone from Strud (Table S2) show invariable  $\delta^{44/42}\text{Ca}$  values with  
 217 no preserved trophic information (Fig. 4) despite the leaching procedure. Here, the range of  
 218 variation of the  $\delta^{44/42}\text{Ca}$  values (0.19‰, n = 25) is much more contracted than at Camiac  
 219 (0.84‰, n = 26) or Shungura (0.51‰, n = 28). Note that Shungura samples are represented  
 220 by two taxa of suids only (*Metridiochoerus* sp. and *Notochoerus* sp.), thus are not  
 221 representative of the possible full trophic variability. The Ca/P range of variation at Strud (2.12  
 222  $\pm 0.07$ ,  $\pm 2\text{SD}$ , n = 25) is in the variability of biological HAP and does not indicate any Ca excess.



223

224 **Fig. 4:** (A)  $\delta^{44/42}\text{Ca}$  values of leached bone at Strud. Trophic levels are assessed based on ecomorphological  
 225 features. (B)  $(\text{La}/\text{Sm})_n$  vs  $(\text{La}/\text{Yb})_n$  distribution of fossil samples analyzed in the study. Morocco and Niger samples  
 226 are from Hassler et al. (2018). REE have been normalized to PAAS. (C)  $(\Sigma\text{REE})_n$  vs  $\delta^{44/42}\text{Ca}$  distribution of fossil  
 227 samples analyzed in the study. Morocco and Niger samples are from Hassler et al. (2018). REE have been  
 228 normalized to PAAS. The  $\delta^{44/42}\text{Ca}$  value is reported relative to ICP-Ca Lyon.

229

230 Taken together, the results of leaching experiments suggest that Ca isotopes in bone,  
 231 but not in enamel, are sensitive to an additive-like diagenesis probably because bone porosity  
 232 is high enough to accommodate significant amount of calcite to modify the original HAp  
 233  $\delta^{44/42}\text{Ca}$  value. In case of clear additive-like diagenesis (artificial fossil bone and enamel,  
 234 Camiac fossil bone) the original HAp  $\delta^{44/42}\text{Ca}$  value is restored by leaching. However, in the  
 235 case of the Strud fossil bones, the original HAp  $\delta^{44/42}\text{Ca}$  value cannot be restored by leaching  
 236 as suggested by the absence of trophic pattern. At Strud, fossil bone probably experienced a  
 237 substitutive-like diagenesis that was sufficiently pervasive to reset original HAp  $\delta^{44/42}\text{Ca}$   
 238 values.

239

#### 240 4. Discussion

241 The type of fossil bone and enamel diagenesis, additive or substitutive, can be  
 242 depicted by the REE pattern, and therefore help to detect whether the Ca isotope  
 243 composition is potentially reset or not. REE trapping in fossil bone or enamel is a post-mortem  
 244 phenomenon that occurs through two main processes, namely adsorption and substitution.

245 The suite of REE differs by ionic radius, middle REE (Sm) having an ionic radius close to that of  
246 Ca (~ 100 pm) and thus more liable to incorporate the HAp crystal lattice compared to light  
247 (La) or heavy (Yb) REE. The REE pattern can thus typify the nature of diagenetic processes  
248 because the relative partitioning among REE will be characteristic of the incorporation  
249 process. A quantitative incorporation without relative REE partitioning first occurs during  
250 early diagenesis, followed during protracted diagenesis by a non-quantitative adsorption  
251 mechanism controlled by surface crystal-chemical properties. Those mechanisms are  
252 considered additive in that the REE content increases during diagenesis, constituting an extra  
253 burden of trace elements that can be theoretically removed by leaching. While this  
254 assumption can be easily accepted concerning fossils that have undergone early diagenesis  
255 only, the efficiency of leaching is questionable in case of protracted diagenesis during which  
256 recrystallization inevitably occurred (Trueman and Tuross, 2002). Note that Hap  
257 recrystallisation, as witnessed by the increase of crystallite size can occur within a few years  
258 post-mortem (Trueman et al., 2004), but is linked to the exposure of HAp crystallite surfaces  
259 during the breakdown of collagen (Trueman et al., 2008). Finally, a substitution mechanism,  
260 which is controlled by bulk crystal-chemical properties, can occur during extensive diagenesis,  
261 leading to strong REE partitioning (Reynard et al., 1999), along with progressive  
262 transformation of HAp into francolite (Trueman and Tuross, 2002). The effects of this late-  
263 stage substitutive-like diagenesis are anticipated to be insensitive to leaching.

264 As expected, the Late Pleistocene Camiac bone and Early Pleistocene Shungura  
265 enamel samples exhibit  $(La/Sm)_n$  and  $(La/Yb)_n$  ratios characteristic of early diagenesis,  
266 supporting the idea that well preserved  $\delta^{44/42}Ca$  values (Fig. 4B) can be obtained in leached  
267 samples, which is necessary for bone (Fig. 3B) but not for enamel (Fig. 3D) due to the presence  
268 of secondary Ca-carbonate in bone but not in enamel. Leaching has no influence on the  
269  $(La/Sm)_n$  and  $(La/Yb)_n$  ratios for both bone from Camiac and enamel from Shungura (Table  
270 S2). The REE pattern at Strud is indicative of a substitutive-like diagenesis (Fig. 4B), and despite  
271 leaching, the associated bone  $\delta^{44/42}Ca$ , which are contracted and invariable among trophic  
272 positions, are reset by diagenesis. The picture is however different for fossil enamel. For  
273 instance, Cretaceous dinosaur enamel samples from Hassler et al. (2018), which have not  
274 been leached, still exhibit highly variable  $\delta^{44/42}Ca$  values (from -1.6‰ to -0.4‰) with  
275 preserved trophic systematics, despite obvious signs of substitutive-like diagenesis, i.e., hat-  
276 shaped REE patterns (Hassler et al., 2018) and low  $(La/Sm)_n$  ratios (Fig. 4B). Fossil bone or

277 enamel are open systems and accumulate REE during diagenesis, being through the  
278 adsorption and/or the substitution processes. The REE concentration ( $\Sigma\text{REE}$ )<sub>n</sub> thus increases  
279 during diagenesis, spanning four orders of magnitude between Shungura enamel samples  
280 ( $0.009 \pm 0.020 \mu\text{g/g}$ ,  $\pm 2\text{SD}$ ,  $n = 28$ , Table S2) and Strud bone samples ( $1028 \pm 724 \mu\text{g/g}$ ,  $\pm 2\text{SD}$ ,  
281  $n = 25$ , Table S2, Fig. 4C). Dinosaurs enamel samples from Morocco ( $417 \pm 581 \mu\text{g/g}$ ,  $\pm 2\text{SD}$ ,  $n$   
282  $= 23$ ) and from Niger ( $237 \pm 282 \mu\text{g/g}$ ,  $\pm 2\text{SD}$ ,  $n = 49$ ) display high ( $\Sigma\text{REE}$ )<sub>n</sub> concentrations, but  
283 still variable  $\delta^{44/42}\text{Ca}$  values despite the absence of leaching.

284 The substitutive-like REE pattern is thus not a good proxy of Ca isotope extended  
285 diagenesis in fossil enamel. An explanation is that the enamel one percent of porosity is  
286 insufficient to accommodate enough Ca in diagenetic fluids and/or secondary minerals that  
287 will further substitute with HAp to significantly modify the isotopic composition of the enamel  
288 bulk Ca. This assumption holds providing a realistic  $\delta^{44/42}\text{Ca}$  value of the diagenetic pool (fluid  
289 or mineral), which must stand close to the value of river water, i.e.,  $\sim 0\text{‰}$  (Heuser et al., 2011;  
290 Martin et al., 2017). A simple mass balance calculation indicates that a 1% porosity full of Ca  
291 necessitates the diagenetic pool to be  $\pm 4\text{‰}$  different from the HAp to modify the  $\delta^{44/42}\text{Ca}$   
292 value by  $\pm 0.1\text{‰}$ . In bone, the situation is clearly different because the porosity ( $\sim 40\%$ ) grossly  
293 equals the HAp Ca content, so the diagenetic pool can have realistic  $\delta^{44/42}\text{Ca}$  value, i.e., close  
294 to that of HAp. Taking all the above results into account, we conclude that Ca isotopes are  
295 largely immune to diagenesis in fossil enamel, but not in fossil bone.

296

## 297 5. Conclusion

298 We demonstrate that an addition of a diagenetic phase containing Ca in the form of  
299 Ca-carbonate significantly modifies the bulk  $\delta^{44/42}\text{Ca}$  value of artificial fossil bone and enamel,  
300 whose initial composition can be restored using leaching. For fossils that have undergone  
301 weak diagenesis, this process is reproduced in fossil bone, but not in fossil enamel, which  
302 cannot accommodate enough diagenetic Ca in the porosity to modify the bulk  $\delta^{44/42}\text{Ca}$  value.  
303 For fossils that have undergone extensive diagenesis, as typified by the REE patterns, leaching  
304 is unable to restore the initial bone  $\delta^{44/42}\text{Ca}$  value, while enamel can still contain non-  
305 significantly modified  $\delta^{44/42}\text{Ca}$  values, even in the absence of leaching. The diagenetic Ca  
306 content that can be added in the porosity during early diagenesis of enamel is too low and is  
307 further unable to modify its bulk  $\delta^{44/42}\text{Ca}$  value by substitution. The REE pattern of fossil

308 enamel is thus not a reliable proxy for extensive diagenetic alteration of Ca isotopes, which  
309 remains to be potentially uncovered by crystal-chemical means.

310

### 311 **Declaration of competing interest**

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

314

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329

### 330 **Appendix A. Supplementary material**

331 Supplementary figure 1: Mass fractionation in three-isotope space  $\delta^{43/42}\text{Ca}$  vs  $\delta^{44/42}\text{Ca}$   
332 calculated from the measurements of the samples in this study.

333 Supplementary table 1: Table of results for artificial fossil bone and enamel samples.

334 Supplementary table 2: Table of results for natural fossil bone and enamel samples.

335

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- 457

1           **Limits of calcium isotopes diagenesis in fossil bone and enamel**

2

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21

22 **Abstract**

23 Diagenesis has been recognized for decades to significantly alter the trace elements biogenic  
24 **signatures** in fossil tooth enamel and bone that are routinely used for paleobiological and  
25 paleoenvironmental reconstructions. **This signature is modified during diagenesis according**  
26 **to a complex continuum between two main processes, addition, and substitution.** For an  
27 additive-like, or early diagenesis, the trace elements biogenic profiles can be restored by  
28 leaching secondary minerals, but this technique is inefficient for a substitutive-like, or  
29 extensive diagenesis for which secondary trace elements are incorporated into the biogenic  
30 mineral. This scheme is however unclear for Ca, the major cation in tooth enamel and bone  
31 hydroxylapatite, which stable isotope composition ( $\delta^{44/42}\text{Ca}$ ) also conveys biological and  
32 environmental information. We present a suite of leaching experiments for monitoring  
33  $\delta^{44/42}\text{Ca}$  values in artificial and natural fossil enamel and bone from different settings. The  
34 results show that enamel  $\delta^{44/42}\text{Ca}$  values are insensitive to an additive-like diagenesis that  
35 involves the formation of secondary Ca-carbonate mineral phases, while bone shows a  
36 consistent offset toward  $^{44}\text{Ca}$ -enriched values, that can be restored to the biogenic baseline  
37 by a leaching procedure. In the context of a substitutive-like diagenesis, bone exhibits  
38 constant  $\delta^{44/42}\text{Ca}$  values, insensitive to leaching, and shows a REE pattern symptomatic of  
39 extensive diagenesis. Such a REE pattern can be observed in fossil enamel **for which**  $\delta^{44/42}\text{Ca}$   
40 values are still fluctuating and follow a trophic pattern. We conclude that Ca isotopes in fossil  
41 enamel are probably not prone to extensive diagenesis and argue that this immunity is due  
42 to the very low porosity of enamel that cannot accommodate enough secondary minerals to  
43 significantly modify the isotopic composition of the enamel Ca pool.

44

## 45 **1. Introduction**

46 Bone, dentin, and enamel are valuable archives for recording information about the  
47 life history and environment of fossil and extant vertebrates. The information is embedded  
48 as trace elements or isotopic ratios signature during the genesis of the mineralized tissue, a  
49 signature that is further obfuscated by diagenesis during fossilization. Regarding trace  
50 elements, the comprehension of diagenetic mechanisms has greatly benefited from in-situ  
51 laser ablation measurements (Kohn and Moses, 2013; Kral et al., 2022), highlighting the  
52 complex interplay of the diffusion, adsorption, and transport reaction processes. However,  
53 while sophisticated techniques are now deployed to fully integrate all the hallmarks of  
54 diagenesis (Suarez and Kohn, 2020; Weber et al., 2021; Kral et al., 2022), the results still  
55 recognize the central importance of porosity which was already suspected in the 2000's  
56 (Hedges, 2002; Trueman and Tuross, 2002; Kral et al., 2021). Porosity mediates fluid  
57 circulation and secondary mineral deposition, and increases as a result of collagen  
58 degradation, augmenting the exposure of carbonated hydroxylapatite (HAp) crystallites in the  
59 first stages of bone diagenesis. Because porosity is forty times higher in bone than in enamel  
60 (Wang and Cerling, 1994), the latter is widely accepted to be far more resistant to diagenesis  
61 than bone and is nowadays the tissue of reference for measuring trace elements  
62 concentration (Le Houedec et al., 2013; Joannes-Boyau et al., 2019; Nava et al., 2020; Funston  
63 et al., 2022) and/or isotope composition (Martin et al., 2014, 2015; Jaouen et al., 2017;  
64 McCormack et al., 2022).

65 In all types of tissue, some diagenetic overprints can be reverted using leaching  
66 pretreatment (Sillen and LeGeros, 1991; Koch et al., 1997; Nielsen-Marsh and Hedges, 2000;  
67 Balter et al., 2002a; Wathen et al., 2022). When formation of secondary mineral phases is  
68 thought to have occurred (additive-like diagenesis), leaching is potentially efficient at  
69 removing these minerals. One issue is that leaching is often performed in batch, leading  
70 samples to be leached more than necessary, therefore dissolving the most soluble HAp  
71 fraction that is the most pristine (Sillen and LeGeros, 1991; Balter et al., 2002a). The efficiency  
72 of the leaching method is even more questionable when diffusion of diagenetic elements  
73 (substitutive-like diagenesis) into the HAp has occurred.

74 All told, these considerations hold for trace elements, but the situation is unclear for  
75 major elements. Traditionally, the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of  $\text{CO}_3$  present in HAp (~5 wt%) are  
76 measured in leached samples due to the widespread presence of calcite in the porosity, but

77 the  $\delta^{18}\text{O}$  value of HAp  $\text{PO}_4$  (~55 wt%) is always measured in raw samples. The isotope  
78 composition of Ca of HAp (~40 wt%) varies according to trophic position (Skulan and DePaolo,  
79 1999; Heuser et al., 2011; Martin et al., 2018; Hu et al., 2022) and physiology (Tacaïl et al.,  
80 2017; Hassler et al., 2021; Koutamanis et al., 2021; Li et al., 2022), but deserves an assessment  
81 of potential diagenetic effects on  $\delta^{44/42}\text{Ca}$  values in bone and tooth enamel. Simple mass  
82 balance calculations already show that changes of the original  $\delta^{44/42}\text{Ca}$  values in bone and  
83 tooth enamel by secondary Ca minerals are either small or **would require an uncommonly**  
84 **fractionated Ca isotopes diagenetic pool** (Heuser et al., 2011; Martin et al., 2017).

85 Here, we attempt to resolve this issue by studying how Ca isotopes behave during  
86 controlled experiments of artificial fossil bone and enamel leaching and in different diagenetic  
87 case studies. **The Ca isotope compositions are measured along with Ca concentrations and**  
88 **Ca/P ratios to monitor the origin of Ca**, and Rare Earth Elements (REE) to decipher the type  
89 (additive or substitutive) and extrapolate the extend of diagenetic alteration (Reynard et al.,  
90 1999; Trueman and Tuross, 2002).

91

## 92 **2. Material and methods**

93 First, we analyzed the Ca isotope composition in leached and unleached artificial  
94 samples mimicking fossil bone and enamel that have undergone an additive-like diagenesis.  
95 These samples consist of mixtures of HAp and calcite powder which  $\delta^{44/42}\text{Ca}$  value are distinct  
96 and constrained. An artificial fossil bone was prepared using the bone ash certified reference  
97 material (SRM-1400, NIST;  $\delta^{44/42}\text{Ca} = -1.21 \pm 0.04\text{‰}$ ,  $\pm 2\text{SD}$ ,  $n = 3$ ) to which was added various  
98 proportions of the calcite certified reference material SRM-915b (NIST,  $\delta^{44/42}\text{Ca} = -0.28 \pm$   
99  $0.04\text{‰}$ ,  $\pm 2\text{SD}$ ,  $n = 21$ ). The SRM-1400 certified reference material has been ashed and is  
100 devoid of organic matter and has a high crystallinity, both being characteristics of fossil bone.  
101 Because enamel certified reference material does not exist, we prepared artificial fossil  
102 enamel by mixing a sample of extant enamel from an isolated and partial molar tooth of  
103 modern elephant (*Elephas maximus*;  $\delta^{44/42}\text{Ca} = -1.43 \pm 0.07\text{‰}$ ,  $\pm 2\text{SD}$ ,  $n = 10$ ) with various  
104 proportions of SRM-915b. Second, we analyzed a suite of natural samples from different  
105 localities and ages that were selected according to their potential degree of diagenetic  
106 alteration. Pleistocene bone and tooth enamel samples were chosen to be representative of  
107 weak diagenesis, with preserved trace elements compositions, and Devonian bone of more

108 pronounced diagenesis. A first set of material is composed by a suite of fossil bone samples  
109 (n = 13) from the Camiac cave (Gironde, France). The site is a hyena dens from the upper  
110 Pleistocene (Mousterian period,  $35.1 \pm 2$  ka BP; Balter et al., 2002b). Taxonomic information  
111 is given in [Table S2](#). This material is characterized by trace elements (Sr/Ca and Ba/Ca)  
112 distributions that reflect a trophic pattern (Balter et al., 2002b). A suite of fossil enamel  
113 samples (n = 14) from the Plio-Pleistocene of the Shungura Formation in the Lower Omo  
114 Valley (Ethiopia, sample age bracketed between 3.6 and 1.76 Ma; Boisserie et al., 2008)  
115 represents the second set of material. Here, the material is composed by only two extinct  
116 genera of large African suids (*Metridiochoerus* sp. and *Notochoerus* sp., [Table S2](#)). Leached  
117 fossil bone at Shungura exhibit Sr/Ca ratios characteristic of a trophic pattern (Sillen, 1986).  
118 A third set of samples gather vertebrate (fish and tetrapod) fossil bone (n = 25, [Table S2](#)) from  
119 the Devonian locality of Strud (360 Ma, Belgium; Olive et al., 2015). To our knowledge, no  
120 geochemical studies has already been performed on this material.

121         The samples were processed in the clean room and analyzed on the facilities at the  
122 Laboratoire de Géologie de Lyon: Terre, Planètes, Environnements (LGL-TPE, ENS de Lyon).  
123 All the samples (ca. ~ 5 mg) were leached using 0.5 ml of 0.1 M acetic acid per mg of sample  
124 during 30 min in an ultrasonic bath and rinsed with 18.2 MΩ/cm grade MilliQ water. This  
125 represents 2.5-fold the quantity of acid necessary to dissolve an equivalent amount of pure  
126 calcite. The samples were dissolved in distilled concentrated HNO<sub>3</sub> and an aliquot measured  
127 for major elements (P and Ca) by ICP-OES (Thermo Scientific, iCap 6000 Radial) and trace  
128 elements (REE) by ICP-MS (Thermo Scientific, iCap-Q) according to Balter and Lécuyer (2004).  
129 Calcium from the remaining solution was purified by ion-exchange chromatography and Ca  
130 isotope composition measured by MC-ICP-MS (Thermo Scientific, Neptune Plus) according to  
131 Tacail et al. (2014). Briefly, three ion-exchange chromatography steps are necessary, first  
132 allowing the recovery of Ca, Fe and Sr only, the second to purify Ca from Fe and the third to  
133 remove Sr ([Table 1](#)). Blanks for the procedure did not exceed 100 ng of Ca.

134



135 **Table 1:** Detailed procedure for Ca extraction and purification.

1. Matrix elimination		
AG50W-X12 resin (200-400 mesh) ~ 2 mL		
Step	Eluent	Vol. (mL)
Condition	1 M HCl	10
Load	1 M HCl	2+1
Elution (matrix)	1 M HCl	55
<b>Ca elution (Ca, Sr, Fe)</b>	<b>6 M HCl</b>	<b>10</b>
2. Fe elimination if necessary		
AG1-X8 resin (100-200 mesh) ~ 2 mL		
Step	Eluent	Vol. (mL)
Condition	6 M HCl	10
Load	6 M HCl	1+1
<b>Elution (Ca, Sr)</b>	<b>6 M HCl</b>	<b>6</b>
3. Sr elimination		
Sr-Specific resin (Eichrom) ~ 0.7 mL		
Step	Eluent	Vol. (mL)
Condition	3 M HNO <sub>3</sub>	5
Load	3 M HNO <sub>3</sub>	0.5+0.5
<b>Elution (Ca)</b>	<b>3 M HNO<sub>3</sub></b>	<b>6</b>

136

137 Calcium isotope abundance ratios (<sup>44</sup>Ca/<sup>42</sup>Ca and <sup>43</sup>Ca/<sup>42</sup>Ca) were measured using a  
 138 Neptune Plus multi-collector ICP-MS using the standard-sample-standard bracketing method.  
 139 All Ca isotope compositions are expressed using the delta notation, calculated as follows:

140

$$141 \quad \delta^{44/42}\text{Ca} = \left( \frac{({}^{44}\text{Ca}/{}^{42}\text{Ca})_{\text{Sample}}}{0.5 \times ({}^{44}\text{Ca}/{}^{42}\text{Ca})_{\text{ICP-Ca-Lyon}}^{n-1} + 0.5 \times ({}^{44}\text{Ca}/{}^{42}\text{Ca})_{\text{ICP-Ca-Lyon}}^{n+1}} - 1 \right) \times 1000$$

142

143 The ICP Ca Lyon standard, used routinely in Lyon, was used as the bracketing standard.  
 144 The results can be converted relative to the SRM915a with an offset of -0.52‰.

145

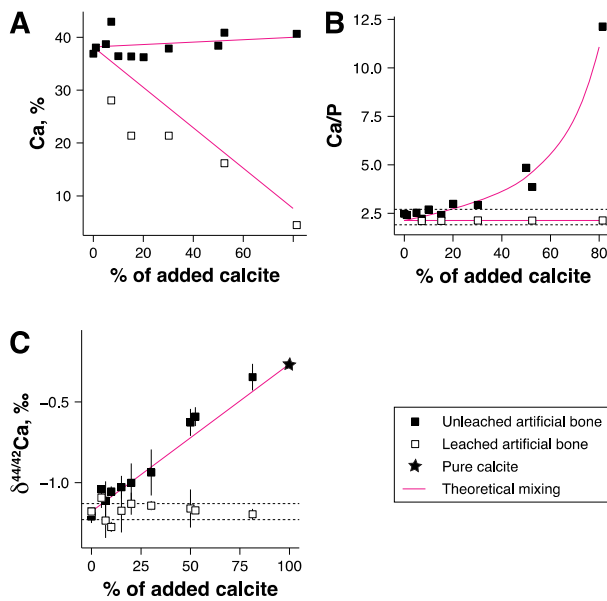
### 146 3. Data presentation and interpretation

147 Results are given in **Table S1** for artificial fossil bone and enamel and in **Table S2** for  
 148 natural fossil bone and enamel. The mass fractionation measured in this study agrees with  
 149 the 0.5 slope predicted by the linear approximation of mass-dependent fractionation (**Fig. S1**).

150 We first test the effect of leaching on the  $\delta^{44/42}\text{Ca}$  value of the certified reference  
 151 material SRM-1400 and found no difference after leaching ( $\delta^{44/42}\text{Ca} = -1.21 \pm 0.03\text{‰}$ ,  $\pm 2\text{SD}$ ,  
 152  $n = 3$ ) compared to unleached material ( $\delta^{44/42}\text{Ca} = -1.21 \pm 0.04\text{‰}$ ,  $\pm 2\text{SD}$ ,  $n = 3$ ). Absence of  
 153 difference between the  $\delta^{44/42}\text{Ca}$  values of unleached and leached enamel is also observed as

154 we measured a constant  $\delta^{44/42}\text{Ca}$  value of -1.43‰ (Table S1). Because of the very small  
155 amount of processed material (~ 5 mg), the remaining bone or enamel residue after leaching  
156 is so small that it is impossible to weigh it, so Ca concentration for leached material is  
157 underestimated. Nevertheless, using in 0.1 M acetic acid for 30 min generally leads to a loss  
158 of about 30% of initial material. The absence of Ca isotope fractionation during leaching  
159 therefore indicates that the dissolution of HAp is quantitative and that leaching *per se* does  
160 not affect fossil bone and enamel  $\delta^{44/42}\text{Ca}$  values.

161 In the mixture of bone HAp with increasing proportion of added calcite, the slight  
162 theoretical increase of Ca concentration is barely detected in unleached mixtures, probably  
163 due to the ~5% error inherent to ICPMS measurements (Fig. 1A), but the decrease of Ca  
164 content is inambiguous in leached mixture (Fig. 1A). In leached mixtures, the Ca content is  
165 generally lower than predicted particularly for low calcite content (Fig. 1A). This suggests  
166 partial dissolution of HAp that further occurs after complete dissolution of calcite (Balter et  
167 al., 2002b). The leaching restores the Ca/P ratio of the mixture to the pure bone value (Fig.  
168 1B). The leaching also restores the original bone  $\delta^{44/42}\text{Ca}$  value even up to 80% of added calcite  
169 (Fig. 1C). The deviation of the bulk  $\delta^{44/42}\text{Ca}$  value can be detectable with  $\geq 10\%$  added calcite  
170 and generally follows the theoretical balance between the HAp and the calcite end-members.  
171



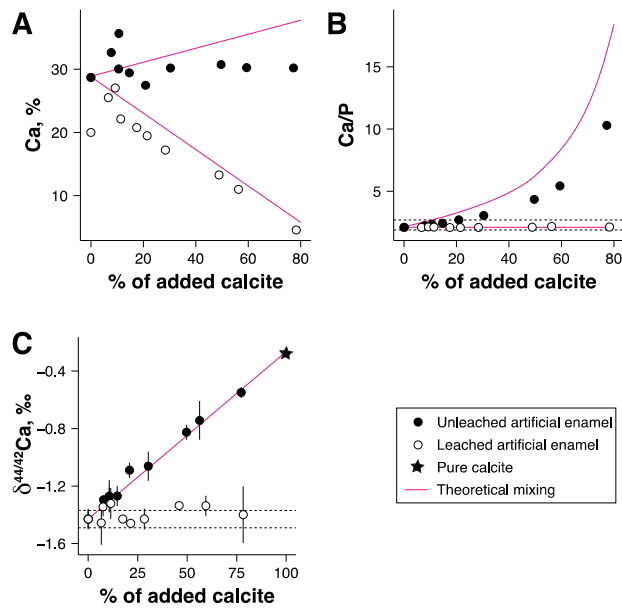
172

173 **Fig. 1:** Effect of leaching on the Ca concentration (A), Ca/P ratio (B), and the  $\delta^{44/42}\text{Ca}$  (C) value of artificial fossil  
 174 bone material (SRM-1400 + SRM-915b). Percentage of added calcite is relative to the initial total mass of the  
 175 mixture. Error bars are typically < 5% in panel A. Dotted lines in panel B represent the natural range of the Ca/P  
 176 ratio from 1.9 to 2.7 in biological HAP. Dotted lines in panel C represent the 2 standard deviations of the mean  
 177  $\delta^{44/42}\text{Ca}$  value of raw material. The  $\delta^{44/42}\text{Ca}$  value is reported relative to ICP-Ca Lyon.

178

179 In the mixture of enamel with increasing proportion of calcite, the Ca concentration  
 180 and Ca/P ratio between unleached and leached samples follow the same pattern than for  
 181 bone (Fig. 2A, 2B). Dissolution of enamel is also observed for low calcite content as measured  
 182 Ca concentrations stand below theoretical values (Fig. 2A). Similar to bone, deviation of the  
 183 bulk  $\delta^{44/42}\text{Ca}$  value is detected with  $\geq 10\%$  added calcite, which follows the theoretical balance  
 184 between the HAP and the calcite end-members, and leaching restores the initial enamel  
 185  $\delta^{44/42}\text{Ca}$  value even up to 80% of added calcite (Fig. 1C). Porosity in bone and enamel is  
 186 variable (see Kral et al., 2021 for a discussion) but is grossly 40% in bone and one order of  
 187 magnitude less ( $\sim 1\%$ ) in enamel (Wang and Cerling, 1994). Given that bone and enamel can  
 188 accommodate calcite to a magnitude equal to porosity, the results from artificial fossil bone  
 189 and enamel suggest that the incorporation of calcite in natural fossil materials can be  
 190 detected in the bone  $\delta^{44/42}\text{Ca}$  value but not for that enamel, except for unrealistic calcite  
 191  $\delta^{44/42}\text{Ca}$  value or important secondary porosity (e.g., post burial enamel surface cracks). This  
 192 assumption is confirmed with results obtained on natural fossil bone and enamel.

193



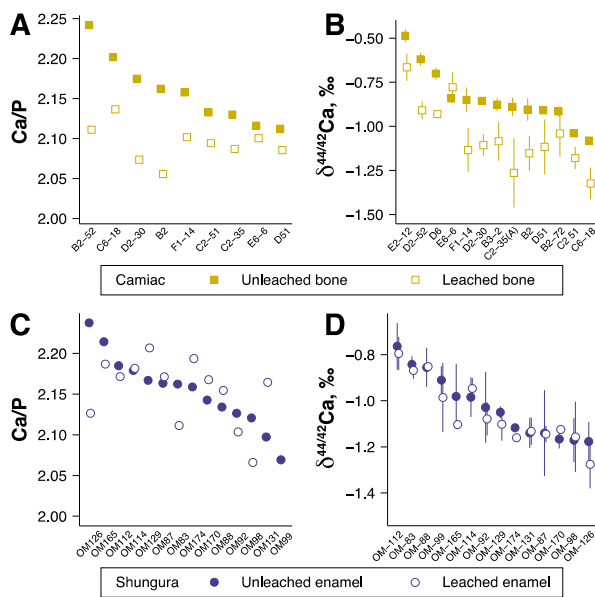
194

195 **Fig. 2:** Effect of leaching on the Ca concentration (A), Ca/P ratio (B), and the  $\delta^{44/42}\text{Ca}$  (C) value of artificial fossil  
 196 bone material (enamel + SRM-915b). Percentage of added calcite is relative to the initial total mass of the  
 197 mixture. Error bars are typically < 5% in panel A. Dotted lines in panel B represent the natural range of the Ca/P  
 198 ratio from 1.9 to 2.7 in biological HAp. Dotted lines in panel C represent the 2 standard deviations of the mean  
 199  $\delta^{44/42}\text{Ca}$  value of raw material. The  $\delta^{44/42}\text{Ca}$  value is reported relative to ICP-Ca Lyon.

200

201 Fossil bone from Camiac (Table S2) shows a Ca/P decrease indicative of efficient  
 202 removal of additive Ca of calcitic origin (Fig. 3A) along with a consistent decrease ( $-0.21 \pm 0.11$ ,  
 203  $n = 12$ ) of the  $\delta^{44/42}\text{Ca}$  values during the leaching (Fig. 3B), validating the assumption that the  
 204 added diagenetic Ca from calcite with a higher  $\delta^{44/42}\text{Ca}$  value than bone has been removed  
 205 accordingly. Fossil enamel unleached and leached samples from Shungura (Table S2) show a  
 206 Ca/P ratio that remains constant (Fig. 3C) and similar  $\delta^{44/42}\text{Ca}$  values (Fig. 3D), suggesting that  
 207 non-significant amount of calcite was present in raw samples and that a quantitative  
 208 fractionation of Ca isotopes occurred during leaching.

209



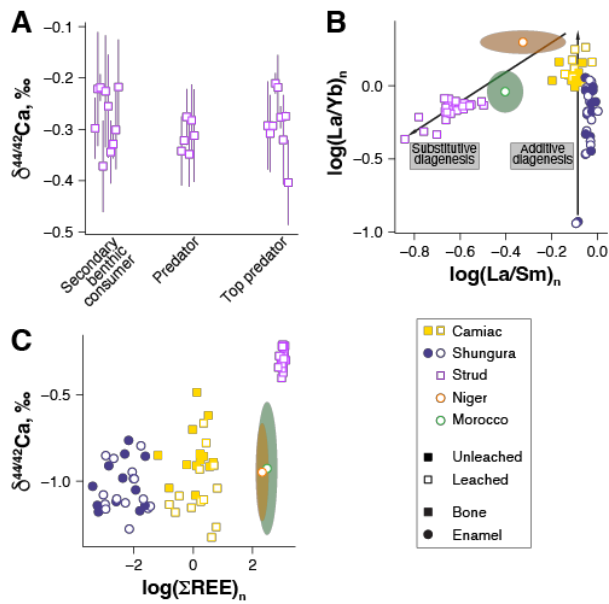
210

211

212 **Fig. 3:** Effect of leaching on the Ca/P ratio and  $\delta^{44/42}\text{Ca}$  value of natural fossil bone from Camiac (A and B) and  
 213 fossil enamel from Shungura (C and D). The variability of the Ca/P ratio is in the natural range of the Ca/P ratio  
 214 in biological Hap. The  $\delta^{44/42}\text{Ca}$  value is reported relative to ICP-Ca Lyon.

215

216 Finally, the Devonian bone from Strud (Table S2) show invariable  $\delta^{44/42}\text{Ca}$  values with  
 217 no preserved trophic information (Fig. 4) despite the leaching procedure. Here, the range of  
 218 variation of the  $\delta^{44/42}\text{Ca}$  values (0.19‰, n = 25) is much more contracted than at Camiac  
 219 (0.84‰, n = 26) or Shungura (0.51‰, n = 28). Note that Shungura samples are represented  
 220 by two taxa of suids only (*Metridiochoerus* sp. and *Notochoerus* sp.), thus are not  
 221 representative of the possible full trophic variability. The Ca/P range of variation at Strud (2.12  
 222  $\pm$  0.07,  $\pm$  2SD, n = 25) is in the variability of biological HAP and does not indicate any Ca excess.



223

224 **Fig. 4:** (A)  $\delta^{44/42}\text{Ca}$  values of leached bone at Strud. Trophic levels are assessed based on ecomorphological  
 225 features. (B)  $(\text{La}/\text{Sm})_n$  vs  $(\text{La}/\text{Yb})_n$  distribution of fossil samples analyzed in the study. Morocco and Niger samples  
 226 are from Hassler et al. (2018). REE have been normalized to PAAS. (C)  $(\Sigma\text{REE})_n$  vs  $\delta^{44/42}\text{Ca}$  distribution of fossil  
 227 samples analyzed in the study. Morocco and Niger samples are from Hassler et al. (2018). REE have been  
 228 normalized to PAAS. The  $\delta^{44/42}\text{Ca}$  value is reported relative to ICP-Ca Lyon.

229

230 Taken together, the results of leaching experiments suggest that Ca isotopes in bone,  
 231 but not in enamel, are sensitive to an additive-like diagenesis probably because bone porosity  
 232 is high enough to accommodate significant amount of calcite to modify the original HAp  
 233  $\delta^{44/42}\text{Ca}$  value. In case of clear additive-like diagenesis (artificial fossil bone and enamel,  
 234 Camiac fossil bone) the original HAp  $\delta^{44/42}\text{Ca}$  value is restored by leaching. However, in the  
 235 case of the Strud fossil bones, the original HAp  $\delta^{44/42}\text{Ca}$  value cannot be restored by leaching  
 236 as suggested by the absence of trophic pattern. At Strud, fossil bone probably experienced a  
 237 substitutive-like diagenesis that was sufficiently pervasive to reset original HAp  $\delta^{44/42}\text{Ca}$   
 238 values.

239

#### 240 4. Discussion

241 The type of fossil bone and enamel diagenesis, additive or substitutive, can be  
 242 depicted by the REE pattern, and therefore help to detect whether the Ca isotope  
 243 composition is potentially reset or not. REE trapping in fossil bone or enamel is a post-mortem  
 244 phenomenon that occurs through two main processes, namely adsorption and substitution.

245 The suite of REE differs by ionic radius, middle REE (Sm) having an ionic radius close to that of  
246 Ca (~ 100 pm) and thus more liable to incorporate the HAp crystal lattice compared to light  
247 (La) or heavy (Yb) REE. The REE pattern can thus typify the nature of diagenetic processes  
248 because the relative partitioning among REE will be characteristic of the incorporation  
249 process. A quantitative incorporation without relative REE partitioning first occurs during  
250 early diagenesis, followed during protracted diagenesis by a non-quantitative adsorption  
251 mechanism controlled by surface crystal-chemical properties. Those mechanisms are  
252 considered additive in that the REE content increases during diagenesis, constituting an extra  
253 burden of trace elements that can be theoretically removed by leaching. While this  
254 assumption can be easily accepted concerning fossils that have undergone early diagenesis  
255 only, the efficiency of leaching is questionable in case of protracted diagenesis during which  
256 recrystallization inevitably occurred (Trueman and Tuross, 2002). Note that Hap  
257 recrystallisation, as witnessed by the increase of crystallite size can occur within a few years  
258 post-mortem (Trueman et al., 2004), but is linked to the exposure of HAp crystallite surfaces  
259 during the breakdown of collagen (Trueman et al., 2008). Finally, a substitution mechanism,  
260 which is controlled by bulk crystal-chemical properties, can occur during extensive diagenesis,  
261 leading to strong REE partitioning (Reynard et al., 1999), along with progressive  
262 transformation of HAp into francolite (Trueman and Tuross, 2002). The effects of this late-  
263 stage substitutive-like diagenesis are anticipated to be insensitive to leaching.

264 As expected, the Late Pleistocene Camiac bone and Early Pleistocene Shungura  
265 enamel samples exhibit  $(La/Sm)_n$  and  $(La/Yb)_n$  ratios characteristic of early diagenesis,  
266 supporting the idea that well preserved  $\delta^{44/42}Ca$  values (Fig. 4B) can be obtained in leached  
267 samples, which is necessary for bone (Fig. 3B) but not for enamel (Fig. 3D) due to the presence  
268 of secondary Ca-carbonate in bone but not in enamel. Leaching has no influence on the  
269  $(La/Sm)_n$  and  $(La/Yb)_n$  ratios for both bone from Camiac and enamel from Shungura (Table  
270 S2). The REE pattern at Strud is indicative of a substitutive-like diagenesis (Fig. 4B), and despite  
271 leaching, the associated bone  $\delta^{44/42}Ca$ , which are contracted and invariable among trophic  
272 positions, are reset by diagenesis. The picture is however different for fossil enamel. For  
273 instance, Cretaceous dinosaur enamel samples from Hassler et al. (2018), which have not  
274 been leached, still exhibit highly variable  $\delta^{44/42}Ca$  values (from -1.6‰ to -0.4‰) with  
275 preserved trophic systematics, despite obvious signs of substitutive-like diagenesis, i.e., hat-  
276 shaped REE patterns (Hassler et al., 2018) and low  $(La/Sm)_n$  ratios (Fig. 4B). Fossil bone or

277 enamel are open systems and accumulate REE during diagenesis, being through the  
278 adsorption and/or the substitution processes. The REE concentration ( $\Sigma\text{REE}$ )<sub>n</sub> thus increases  
279 during diagenesis, spanning four orders of magnitude between Shungura enamel samples  
280 ( $0.009 \pm 0.020 \mu\text{g/g}$ ,  $\pm 2\text{SD}$ ,  $n = 28$ , Table S2) and Strud bone samples ( $1028 \pm 724 \mu\text{g/g}$ ,  $\pm 2\text{SD}$ ,  
281  $n = 25$ , Table S2, Fig. 4C). Dinosaurs enamel samples from Morocco ( $417 \pm 581 \mu\text{g/g}$ ,  $\pm 2\text{SD}$ ,  $n$   
282  $= 23$ ) and from Niger ( $237 \pm 282 \mu\text{g/g}$ ,  $\pm 2\text{SD}$ ,  $n = 49$ ) display high ( $\Sigma\text{REE}$ )<sub>n</sub> concentrations, but  
283 still variable  $\delta^{44/42}\text{Ca}$  values despite the absence of leaching.

284 The substitutive-like REE pattern is thus not a good proxy of Ca isotope extended  
285 diagenesis in fossil enamel. An explanation is that the enamel one percent of porosity is  
286 insufficient to accommodate enough Ca in diagenetic fluids and/or secondary minerals that  
287 will further substitute with HAp to significantly modify the isotopic composition of the enamel  
288 bulk Ca. This assumption holds providing a realistic  $\delta^{44/42}\text{Ca}$  value of the diagenetic pool (fluid  
289 or mineral), which must stand close to the value of river water, i.e.,  $\sim 0\text{‰}$  (Heuser et al., 2011;  
290 Martin et al., 2017). A simple mass balance calculation indicates that a 1% porosity full of Ca  
291 necessitates the diagenetic pool to be  $\pm 4\text{‰}$  different from the HAp to modify the  $\delta^{44/42}\text{Ca}$   
292 value by  $\pm 0.1\text{‰}$ . In bone, the situation is clearly different because the porosity ( $\sim 40\%$ ) grossly  
293 equals the HAp Ca content, so the diagenetic pool can have realistic  $\delta^{44/42}\text{Ca}$  value, i.e., close  
294 to that of HAp. Taking all the above results into account, we conclude that Ca isotopes are  
295 largely immune to diagenesis in fossil enamel, but not in fossil bone.

296

## 297 5. Conclusion

298 We demonstrate that an addition of a diagenetic phase containing Ca in the form of  
299 Ca-carbonate significantly modifies the bulk  $\delta^{44/42}\text{Ca}$  value of artificial fossil bone and enamel,  
300 whose initial composition can be restored using leaching. For fossils that have undergone  
301 weak diagenesis, this process is reproduced in fossil bone, but not in fossil enamel, which  
302 cannot accommodate enough diagenetic Ca in the porosity to modify the bulk  $\delta^{44/42}\text{Ca}$  value.  
303 For fossils that have undergone extensive diagenesis, as typified by the REE patterns, leaching  
304 is unable to restore the initial bone  $\delta^{44/42}\text{Ca}$  value, while enamel can still contain non-  
305 significantly modified  $\delta^{44/42}\text{Ca}$  values, even in the absence of leaching. The diagenetic Ca  
306 content that can be added in the porosity during early diagenesis of enamel is too low and is  
307 further unable to modify its bulk  $\delta^{44/42}\text{Ca}$  value by substitution. The REE pattern of fossil



308 enamel is thus not a reliable proxy for extensive diagenetic alteration of Ca isotopes, which  
309 remains to be potentially uncovered by crystal-chemical means.

310

### 311 **Declaration of competing interest**

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

314

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329

### 330 **Appendix A. Supplementary material**

331 Supplementary figure 1: Mass fractionation in three-isotope space  $\delta^{43/42}\text{Ca}$  vs  $\delta^{44/42}\text{Ca}$   
332 calculated from the measurements of the samples in this study.

333 Supplementary table 1: Table of results for artificial fossil bone and enamel samples.

334 Supplementary table 2: Table of results for natural fossil bone and enamel samples.

335

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