

# Limits of calcium isotopes diagenesis in fossil bone and enamel

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1	Limits of calcium isotopes diagenesis in fossil bone and enamel
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#### 22 Abstract

23 Diagenesis has been recognized for decades to significantly alter the trace elements biogenic signatures in fossil tooth enamel and bone that are routinely used for paleobiological and 24 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 leaching secondary minerals, but this technique is inefficient for a substitutive-like, or 28 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 hydroxylapatite, which stable isotope composition ( $\delta^{44/42}$ Ca) also conveys biological and 31 32 environmental information. We present a suite of leaching experiments for monitoring  $\delta^{44/42}$ Ca values in artificial and natural fossil enamel and bone from different settings. The 33 results show that enamel  $\delta^{44/42}$ Ca values are insensitive to an additive-like diagenesis that 34 35 involves the formation of secondary Ca-carbonate mineral phases, while bone shows a consistent offset toward <sup>44</sup>Ca-enriched values, that can be restored to the biogenic baseline 36 37 by a leaching procedure. In the context of a substitutive-like diagenesis, bone exhibits constant  $\delta^{44/42}$ Ca values, insensitive to leaching, and shows a REE pattern symptomatic of 38 extensive diagenesis. Such a REE pattern can be observed in fossil enamel for which  $\delta^{44/42}$ Ca 39 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 significantly modify the isotopic composition of the enamel Ca pool. 43

#### 45 1. Introduction

46 Bone, dentin, and enamel are valuable archives for recording information about the life history and environment of fossil and extant vertebrates. The information is embedded 47 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 laser ablation measurements (Kohn and Moses, 2013; Kral et al., 2022), highlighting the 51 52 complex interplay of the diffusion, adsorption, and transport reaction processes. However, while sophisticated techniques are now deployed to fully integrate all the hallmarks of 53 54 diagenesis (Suarez and Kohn, 2020; Weber et al., 2021; Kral et al., 2022), the results still recognize the central importance of porosity which was already suspected in the 2000's 55 56 (Hedges, 2002; Trueman and Tuross, 2002; Kral et al., 2021). Porosity mediates fluid circulation and secondary mineral deposition, and increases as a result of collagen 57 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 pretreatment (Sillen and LeGeros, 1991; Koch et al., 1997; Nielsen-Marsh and Hedges, 2000; 66 67 Balter et al., 2002a; Wathen et al., 2022). When formation of secondary mineral phases is thought to have occurred (additive-like diagenesis), leaching is potentially efficient at 68 removing these minerals. One issue is that leaching is often performed in batch, leading 69 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.

All told, these considerations hold for trace elements, but the situation is unclear for major elements. Traditionally, the  $\delta^{13}$ C and  $\delta^{18}$ O values of CO<sub>3</sub> present in HAp (~5 wt%) are measured in leached samples due to the widespread presence of calcite in the porosity, but 77 the  $\delta^{18}$ O value of HAp PO<sub>4</sub> (~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 (Tacail et al., 80 2017; Hassler et al., 2021; Koutamanis et al., 2021; Li et al., 2022), but deserves an assessment of potential diagenetic effects on  $\delta^{44/42}$ Ca values in bone and tooth enamel. Simple mass 81 balance calculations already show that changes of the original  $\delta^{44/42}$ Ca values in bone and 82 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).

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

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# 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. These samples consist of mixtures of HAp and calcite powder which  $\delta^{44/42}$ Ca value are distinct 95 and constrained. An artificial fossil bone was prepared using the bone ash certified reference 96 material (SRM-1400, NIST;  $\delta^{44/42}$ Ca = -1.21 ± 0.04‰, ± 2SD, n = 3) to which was added various 97 proportions of the calcite certified reference material SRM-915b (NIST,  $\delta^{44/42}$ Ca = -0.28 ± 98 99 0.04%, ± 2SD, n = 21). The SRM-1400 certified reference material has been ashed and is devoid of organic matter and has a high crystallinity, both being characteristics of fossil bone. 100 101 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 modern elephant (*Elephas maximus*;  $\delta^{44/42}$ Ca = -1.43 ± 0.07‰, ± 2SD, n = 10) with various 103 proportions of SRM-915b. Second, we analyzed a suite of natural samples from different 104 105 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 Pleistocene (Mousterian period, 35.1 ± 2 ka BP; (Balter et al., 2002b). Taxonomic information 110 is given in Table S2. This material is characterized by trace elements (Sr/Ca and Ba/Ca) 111 112 distributions that reflect a trophic pattern (Balter et al., 2002b). A suite of fossil enamel samples (n = 14) from the Plio-Pleistocene of the Shungura Formation in the Lower Omo 113 Valley (Ethiopia, sample age bracketed between 3.6 and 1.76 Ma; Boisserie et al., 2008) 114 115 represents the second set of material. Here, the material is composed by only two extinct genera of large African suids (Metridiochoerus sp. and Notochoerus sp., Table S2). Leached 116 fossil bone at Shungura exhibit Sr/Ca ratios characteristic of a trophic pattern (Sillen, 1986). 117 A third set of samples gather vertebrate (fish and tetrapod) fossil bone (n = 25, Table S2) from 118 119 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 calcite. The samples were dissolved in distilled concentrated HNO3 and an aliquot measured 126 127 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 allowing the recovery of Ca, Fe and Sr only, the second to purify Ca from Fe and the third to 132 133 remove Sr (Table 1). Blanks for the procedure did not exceed 100 ng of Ca.

#### 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 HCI	10
Load	1 M HCI	2+1
Elution (matrix)	1 M HCI	55
Ca elution (Ca, Sr, Fe)	6 M HCI	10

#### 2. Fe elimination if necessary

AG1-X8 resin (100-200 mesh) ~ 2 mL				
Step	Eluent	Vol. (mL)		
Condition	6 M HCI	10		
Load	6 M HCI	1+1		
Elution (Ca, Sr)	6 M HCI	6		
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 HNO3	0.5+0.5		
Elution (Ca)	3 M HNO <sub>3</sub>	6		

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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
 Neptune Plus multi-collector ICP-MS using the standard-sample-standard bracketing method.
 All Ca isotope compositions are expressed using the delta notation, calculated as follows:

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

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

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# 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  $\delta^{44/42}$ Ca value of the certified reference 151 material SRM-1400 and found no difference after leaching ( $\delta^{44/42}$ Ca = -1.21 ± 0.03‰, ± 2SD, 152 n = 3) compared to unleached material ( $\delta^{44/42}$ Ca = -1.21 ± 0.04‰, ± 2SD, n = 3). Absence of 153 difference between the  $\delta^{44/42}$ Ca values of unleached and leached enamel is also observed as we measured a constant  $\delta^{44/42}$ Ca value of -1.43‰ (Table S1). Because of the very small amount of processed material (~ 5 mg), the remaining bone or enamel residue after leaching is so small that it is impossible to weigh it, so Ca concentration for leached material is underestimated. Nevertheless, using in 0.1 M acetic acid for 30 min generally leads to a loss of about 30% of initial material. The absence of Ca isotope fractionation during leaching therefore indicates that the dissolution of HAp is quantitative and that leaching *per se* does not affect fossil bone and enamel  $\delta^{44/42}$ Ca values.

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



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173**Fig. 1**: Effect of leaching on the Ca concentration (A), Ca/P ratio (B), and the  $\delta^{44/42}$ Ca (C) value of artificial fossil174bone material (SRM-1400 + SRM-915b). Percentage of added calcite is relative to the initial total mass of the175mixture. Error bars are typically < 5% in panel A. Dotted lines in panel B represent the natural range of the Ca/P</td>176ratio from 1.9 to 2.7 in biological HAp. Dotted lines in panel C represent the 2 standard deviations of the mean177 $\delta^{44/42}$ Ca value of raw material. The  $\delta^{44/42}$ Ca value is reported relative to ICP-Ca Lyon.

In the mixture of enamel with increasing proportion of calcite, the Ca concentration 179 180 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  $\delta^{44/42}$ Ca value is detected with  $\geq$  10% added calcite, which follows the theoretical balance between the HAp and the calcite end-members, and leaching restores the initial enamel 184  $\delta^{44/42}$ Ca value even up to 80% of added calcite (Fig. 1C). Porosity in bone and enamel is 185 variable (see Kral et al., 2021 for a discussion) but is grossly 40% in bone and one order of 186 magnitude less (~1%) in enamel (Wang and Cerling, 1994). Given that bone and enamel can 187 188 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 detected in the bone  $\delta^{44/42}$ Ca value but not for that enamel, except for unrealistic calcite 190  $\delta^{44/42}$ Ca value or important secondary porosity (e.g., post burial enamel surface cracks). This 191 192 assumption is confirmed with results obtained on natural fossil bone and enamel.



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

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



**Fig. 3**: Effect of leaching on the Ca/P ratio and  $\delta^{44/42}$ Ca value of natural fossil bone from Camiac (A and B) and 212 213 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  $\delta^{44/42}$ Ca value is reported relative to ICP-Ca Lyon.

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



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

- Taken together, the results of leaching experiments suggest that Ca isotopes in bone, 230 231 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  $\delta^{44/42}$ Ca value. In case of clear additive-like diagenesis (artificial fossil bone and enamel, 233 Camiac fossil bone) the original HAp  $\delta^{44/42}$ Ca value is restored by leaching. However, in the 234 case of the Strud fossil bones, the original HAp  $\delta^{44/42}$ Ca value cannot be restored by leaching 235 as suggested by the absence of trophic pattern. At Strud, fossil bone probably experienced a 236 237 substitutive-like diagenesis that was sufficiently pervasive to reset original HAp  $\delta^{44/42}$ Ca 238 values.
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#### 240 4. Discussion

The type of fossil bone and enamel diagenesis, additive or substitutive, can be depicted by the REE pattern, and therefore help to detect whether the Ca isotope composition is potentially reset or not. REE trapping in fossil bone or enamel is a post-mortem 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 (La) or heavy (Yb) REE. The REE pattern can thus typify the nature of diagenetic processes 247 248 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 mechanism controlled by surface crystal-chemical properties. Those mechanisms are 251 252 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 only, the efficiency of leaching is questionable in case of protracted diagenesis during which 255 256 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 transformation of HAp into francolite (Trueman and Tuross, 2002). The effects of this late-262 263 stage substitutive-like diagenesis are anticipated to be insensitive to leaching.

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

enamel are open systems and accumulate REE during diagenesis, being through the adsorption and/or the substitution processes. The REE concentration ( $\Sigma$ REE)<sub>n</sub> thus increases during diagenesis, spanning four orders of magnitude between Shungura enamel samples (0.009 ± 0.020 µg/g, ± 2SD, n = 28, Table S2) and Strud bone samples (1028 ± 724 µg/g, ± 2SD, n = 25, Table S2, Fig. 4C). Dinosaurs enamel samples from Morocco (417 ± 581 µg/g, ± 2SD, n = 23) and from Niger (237 ± 282 µg/g, ± 2SD, n = 49) display high ( $\Sigma$ REE)<sub>n</sub> concentrations, but still variable  $\delta^{44/42}$ 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 bulk Ca. This assumption holds providing a realistic  $\delta^{44/42}$ Ca value of the diagenetic pool (fluid 288 289 or mineral), which must stand close to the value of river water, i.e., ~0‰ (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‰ different from the HAp to modify the  $\delta^{44/42}$ Ca 292 value by  $\pm 0.1$ %. In bone, the situation is clearly different because the porosity (~40%) grossly 293 equals the HAp Ca content, so the diagenetic pool can have realistic  $\delta^{44/42}$ 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.

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#### 297 5. Conclusion

298 We demonstrate that an addition of a diagenetic phase containing Ca in the form of Ca-carbonate significantly modifies the bulk  $\delta^{44/42}$ Ca value of artificial fossil bone and enamel, 299 300 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  $\delta^{44/42}$ Ca value. 303 For fossils that have undergone extensive diagenesis, as typified by the REE patterns, leaching is unable to restore the initial bone  $\delta^{44/42}$ Ca value, while enamel can still contain non-304 significantly modified  $\delta^{44/42}$ Ca values, even in the absence of leaching. The diagenetic Ca 305 306 content that can be added in the porosity during early diagenesis of enamel is too low and is further unable to modify its bulk  $\delta^{44/42}$ Ca value by substitution. The REE pattern of fossil 307

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

310

# 311 Declaration of competing interest

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

314

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329

#### 330 Appendix A. Supplementary material

331 Supplementary figure 1: Mass fractionation in three-isotope space  $\delta^{43/42}$ Ca vs  $\delta^{44/42}$ 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.

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1	Limits of calcium isotopes diagenesis in fossil bone and enamel
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#### 22 Abstract

23 Diagenesis has been recognized for decades to significantly alter the trace elements biogenic signatures in fossil tooth enamel and bone that are routinely used for paleobiological and 24 25 paleoenvironmental reconstructions. This signature is modified during diagenesis according to a complex continuum between two main processes, addition, and substitution. For an 26 27 additive-like, or early diagenesis, the trace elements biogenic profiles can be restored by leaching secondary minerals, but this technique is inefficient for a substitutive-like, or 28 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 hydroxylapatite, which stable isotope composition ( $\delta^{44/42}$ Ca) also conveys biological and 31 32 environmental information. We present a suite of leaching experiments for monitoring  $\delta^{44/42}$ Ca values in artificial and natural fossil enamel and bone from different settings. The 33 results show that enamel  $\delta^{44/42}$ Ca values are insensitive to an additive-like diagenesis that 34 35 involves the formation of secondary Ca-carbonate mineral phases, while bone shows a consistent offset toward <sup>44</sup>Ca-enriched values, that can be restored to the biogenic baseline 36 37 by a leaching procedure. In the context of a substitutive-like diagenesis, bone exhibits constant  $\delta^{44/42}$ Ca values, insensitive to leaching, and shows a REE pattern symptomatic of 38 extensive diagenesis. Such a REE pattern can be observed in fossil enamel for which  $\delta^{44/42}$ Ca 39 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 significantly modify the isotopic composition of the enamel Ca pool. 43

#### 45 1. Introduction

46 Bone, dentin, and enamel are valuable archives for recording information about the life history and environment of fossil and extant vertebrates. The information is embedded 47 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 laser ablation measurements (Kohn and Moses, 2013; Kral et al., 2022), highlighting the 51 52 complex interplay of the diffusion, adsorption, and transport reaction processes. However, while sophisticated techniques are now deployed to fully integrate all the hallmarks of 53 54 diagenesis (Suarez and Kohn, 2020; Weber et al., 2021; Kral et al., 2022), the results still recognize the central importance of porosity which was already suspected in the 2000's 55 56 (Hedges, 2002; Trueman and Tuross, 2002; Kral et al., 2021). Porosity mediates fluid circulation and secondary mineral deposition, and increases as a result of collagen 57 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 pretreatment (Sillen and LeGeros, 1991; Koch et al., 1997; Nielsen-Marsh and Hedges, 2000; 66 67 Balter et al., 2002a; Wathen et al., 2022). When formation of secondary mineral phases is thought to have occurred (additive-like diagenesis), leaching is potentially efficient at 68 removing these minerals. One issue is that leaching is often performed in batch, leading 69 samples to be leached more than necessary, therefore dissolving the most soluble HAp 70 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.

All told, these considerations hold for trace elements, but the situation is unclear for major elements. Traditionally, the  $\delta^{13}$ C and  $\delta^{18}$ O values of CO<sub>3</sub> present in HAp (~5 wt%) are measured in leached samples due to the widespread presence of calcite in the porosity, but 77 the  $\delta^{18}$ O value of HAp PO<sub>4</sub> (~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 (Tacail et al., 80 2017; Hassler et al., 2021; Koutamanis et al., 2021; Li et al., 2022), but deserves an assessment of potential diagenetic effects on  $\delta^{44/42}$ Ca values in bone and tooth enamel. Simple mass 81 balance calculations already show that changes of the original  $\delta^{44/42}$ Ca values in bone and 82 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).

Here, we attempt to resolve this issue by studying how Ca isotopes behave during controlled experiments of artificial fossil bone and enamel leaching and in different diagenetic case studies. The Ca isotope compositions are measured along with Ca concentrations and Ca/P ratios to monitor the origin of Ca, and Rare Earth Elements (REE) to decipher the type (additive or substitutive) and extrapolate the extend of diagenetic alteration (Reynard et al., 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. These samples consist of mixtures of HAp and calcite powder which  $\delta^{44/42}$ Ca value are distinct 95 and constrained. An artificial fossil bone was prepared using the bone ash certified reference 96 material (SRM-1400, NIST;  $\delta^{44/42}$ Ca = -1.21 ± 0.04‰, ± 2SD, n = 3) to which was added various 97 proportions of the calcite certified reference material SRM-915b (NIST,  $\delta^{44/42}$ Ca = -0.28 ± 98 99 0.04%, ± 2SD, n = 21). The SRM-1400 certified reference material has been ashed and is devoid of organic matter and has a high crystallinity, both being characteristics of fossil bone. 100 101 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 modern elephant (*Elephas maximus*;  $\delta^{44/42}$ Ca = -1.43 ± 0.07‰, ± 2SD, n = 10) with various 103 proportions of SRM-915b. Second, we analyzed a suite of natural samples from different 104 105 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 Pleistocene (Mousterian period, 35.1 ± 2 ka BP; (Balter et al., 2002b). Taxonomic information 110 is given in Table S2. This material is characterized by trace elements (Sr/Ca and Ba/Ca) 111 112 distributions that reflect a trophic pattern (Balter et al., 2002b). A suite of fossil enamel samples (n = 14) from the Plio-Pleistocene of the Shungura Formation in the Lower Omo 113 Valley (Ethiopia, sample age bracketed between 3.6 and 1.76 Ma; Boisserie et al., 2008) 114 115 represents the second set of material. Here, the material is composed by only two extinct genera of large African suids (Metridiochoerus sp. and Notochoerus sp., Table S2). Leached 116 fossil bone at Shungura exhibit Sr/Ca ratios characteristic of a trophic pattern (Sillen, 1986). 117 A third set of samples gather vertebrate (fish and tetrapod) fossil bone (n = 25, Table S2) from 118 119 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 calcite. The samples were dissolved in distilled concentrated HNO3 and an aliquot measured 126 127 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 allowing the recovery of Ca, Fe and Sr only, the second to purify Ca from Fe and the third to 132 133 remove Sr (Table 1). Blanks for the procedure did not exceed 100 ng of Ca.

#### 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 HCI	10
Load	1 M HCI	2+1
Elution (matrix)	1 M HCI	55
Ca elution (Ca, Sr, Fe)	6 M HCI	10

#### 2. Fe elimination if necessary

AG1-X8 resin (100-200 mesh) ~ 2 mL				
Step	Eluent	Vol. (mL)		
Condition	6 M HCI	10		
Load	6 M HCI	1+1		
Elution (Ca, Sr)	6 M HCI	6		
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 HNO3	0.5+0.5		
Elution (Ca)	3 M HNO <sub>3</sub>	6		

136

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
 Neptune Plus multi-collector ICP-MS using the standard-sample-standard bracketing method.
 All Ca isotope compositions are expressed using the delta notation, calculated as follows:

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

142

143The ICP Ca Lyon standard, used routinely in Lyon, was used as the bracketing standard.144The 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  $\delta^{44/42}$ Ca value of the certified reference 151 material SRM-1400 and found no difference after leaching ( $\delta^{44/42}$ Ca = -1.21 ± 0.03‰, ± 2SD, 152 n = 3) compared to unleached material ( $\delta^{44/42}$ Ca = -1.21 ± 0.04‰, ± 2SD, n = 3). Absence of 153 difference between the  $\delta^{44/42}$ Ca values of unleached and leached enamel is also observed as we measured a constant  $\delta^{44/42}$ Ca value of -1.43‰ (Table S1). Because of the very small amount of processed material (~ 5 mg), the remaining bone or enamel residue after leaching is so small that it is impossible to weigh it, so Ca concentration for leached material is underestimated. Nevertheless, using in 0.1 M acetic acid for 30 min generally leads to a loss of about 30% of initial material. The absence of Ca isotope fractionation during leaching therefore indicates that the dissolution of HAp is quantitative and that leaching *per se* does not affect fossil bone and enamel  $\delta^{44/42}$ Ca values.

161 In the mixture of bone HAp with increasing proportion of added calcite, the slight theoretical increase of Ca concentration is barely detected in unleached mixtures, probably 162 due to the ~5% error inherent to ICPMS measurements (Fig. 1A), but the decrease of Ca 163 content is inambiguous in leached mixture (Fig. 1A). In leached mixtures, the Ca content is 164 165 generally lower than predicted particularly for low calcite content (Fig. 1A). This suggests partial dissolution of HAp that further occurs after complete dissolution of calcite (Balter et 166 al., 2002b). The leaching restores the Ca/P ratio of the mixture to the pure bone value (Fig. 167  $^{1
m B}$ ). The leaching also restores the original bone  $\delta^{44/42}$ Ca value even up to 80% of added calcite 168 (Fig. 1C). The deviation of the bulk  $\delta^{44/42}$ Ca value can be detectable with  $\geq$  10% added calcite 169 170 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  $\delta^{44/42}$ Ca (C) value of artificial fossil174bone material (SRM-1400 + SRM-915b). Percentage of added calcite is relative to the initial total mass of the175mixture. Error bars are typically < 5% in panel A. Dotted lines in panel B represent the natural range of the Ca/P</td>176ratio from 1.9 to 2.7 in biological HAp. Dotted lines in panel C represent the 2 standard deviations of the mean177 $\delta^{44/42}$ Ca value of raw material. The  $\delta^{44/42}$ Ca value is reported relative to ICP-Ca Lyon.

In the mixture of enamel with increasing proportion of calcite, the Ca concentration 179 180 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  $\delta^{44/42}$ Ca value is detected with  $\geq$  10% added calcite, which follows the theoretical balance between the HAp and the calcite end-members, and leaching restores the initial enamel 184  $\delta^{44/42}$ Ca value even up to 80% of added calcite (Fig. 1C). Porosity in bone and enamel is 185 variable (see Kral et al., 2021 for a discussion) but is grossly 40% in bone and one order of 186 magnitude less (~1%) in enamel (Wang and Cerling, 1994). Given that bone and enamel can 187 188 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 detected in the bone  $\delta^{44/42}$ Ca value but not for that enamel, except for unrealistic calcite 190  $\delta^{44/42}$ Ca value or important secondary porosity (e.g., post burial enamel surface cracks). This 191 192 assumption is confirmed with results obtained on natural fossil bone and enamel.



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

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



**Fig. 3**: Effect of leaching on the Ca/P ratio and  $\delta^{44/42}$ Ca value of natural fossil bone from Camiac (A and B) and 212 213 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  $\delta^{44/42}$ Ca value is reported relative to ICP-Ca Lyon.

215

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



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

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  $\delta^{44/42}$ Ca value. In case of clear additive-like diagenesis (artificial fossil bone and enamel, 233 Camiac fossil bone) the original HAp  $\delta^{44/42}$ Ca value is restored by leaching. However, in the 234 case of the Strud fossil bones, the original HAp  $\delta^{44/42}$ Ca value cannot be restored by leaching 235 as suggested by the absence of trophic pattern. At Strud, fossil bone probably experienced a 236 237 substitutive-like diagenesis that was sufficiently pervasive to reset original HAp  $\delta^{44/42}$ Ca 238 values.

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#### 240 4. Discussion

The type of fossil bone and enamel diagenesis, additive or substitutive, can be depicted by the REE pattern, and therefore help to detect whether the Ca isotope composition is potentially reset or not. REE trapping in fossil bone or enamel is a post-mortem 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 (La) or heavy (Yb) REE. The REE pattern can thus typify the nature of diagenetic processes 247 248 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 mechanism controlled by surface crystal-chemical properties. Those mechanisms are 251 252 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 only, the efficiency of leaching is questionable in case of protracted diagenesis during which 255 256 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 transformation of HAp into francolite (Trueman and Tuross, 2002). The effects of this late-262 263 stage substitutive-like diagenesis are anticipated to be insensitive to leaching.

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

enamel are open systems and accumulate REE during diagenesis, being through the adsorption and/or the substitution processes. The REE concentration ( $\Sigma$ REE)<sub>n</sub> thus increases during diagenesis, spanning four orders of magnitude between Shungura enamel samples (0.009 ± 0.020 µg/g, ± 2SD, n = 28, Table S2) and Strud bone samples (1028 ± 724 µg/g, ± 2SD, n = 25, Table S2, Fig. 4C). Dinosaurs enamel samples from Morocco (417 ± 581 µg/g, ± 2SD, n = 23) and from Niger (237 ± 282 µg/g, ± 2SD, n = 49) display high ( $\Sigma$ REE)<sub>n</sub> concentrations, but still variable  $\delta^{44/42}$ 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  $\delta^{44/42}$ Ca value of the diagenetic pool (fluid 289 or mineral), which must stand close to the value of river water, i.e., ~0‰ (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‰ different from the HAp to modify the  $\delta^{44/42}$ Ca 292 value by  $\pm 0.1$ %. In bone, the situation is clearly different because the porosity (~40%) grossly 293 equals the HAp Ca content, so the diagenetic pool can have realistic  $\delta^{44/42}$ 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 Ca-carbonate significantly modifies the bulk  $\delta^{44/42}$ Ca value of artificial fossil bone and enamel, 299 300 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  $\delta^{44/42}$ Ca value. 303 For fossils that have undergone extensive diagenesis, as typified by the REE patterns, leaching is unable to restore the initial bone  $\delta^{44/42}$ Ca value, while enamel can still contain non-304 significantly modified  $\delta^{44/42}$ Ca values, even in the absence of leaching. The diagenetic Ca 305 306 content that can be added in the porosity during early diagenesis of enamel is too low and is further unable to modify its bulk  $\delta^{44/42}$ Ca value by substitution. The REE pattern of fossil 307

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

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# 311 Declaration of competing interest

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

314

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329

#### 330 Appendix A. Supplementary material

331 Supplementary figure 1: Mass fractionation in three-isotope space  $\delta^{43/42}$ Ca vs  $\delta^{44/42}$ 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.

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