

Nitrogen isotopic composition of tooth enamel organic matter records trophic position in modern and fossil ecosystems

Jennifer Leichliter (✉ Jennifer.Leichliter@mpic.de)

Max Planck Institute for Chemistry <https://orcid.org/0000-0001-9373-8634>

Tina Lüdecke

Max Planck Institute for Chemistry <https://orcid.org/0000-0002-9165-2586>

Alan Foreman

Max Planck Institute for Chemistry <https://orcid.org/0000-0002-5082-5786>

Nicolas Bourgon

Max Planck Institute for Evolutionary Anthropology <https://orcid.org/0000-0001-8538-1903>

Nicolas Duprey

Max Planck Institute for Chemistry

Hubert Vonhof

Max Planck Institute for Chemistry <https://orcid.org/0000-0002-0897-8244>

Viengkeo Souksavatdy

Department of Heritage, Ministry of Information, Culture and Tourism

Anne-Marie Bacon

Paris Descartes University

Daniel Sigman

Princeton University <https://orcid.org/0000-0002-7923-1973>

Thomas Tütken

University of Mainz <https://orcid.org/0000-0002-2590-8600>

Alfredo Martinez-Garcia

Max Planck Institute for Chemistry <https://orcid.org/0000-0002-7206-5079>

Article

Keywords: nitrogen isotopes, tooth enamel, paleodiet, trophic level, paleoecology, African mammals

Posted Date: September 12th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1942250/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

1
2
3 **Nitrogen isotopic composition of tooth enamel organic matter**
4 **records trophic position in modern and fossil ecosystems**

5 Jennifer N. Leichliter^{a,b,c*}, Tina Lüdecke^{a,b,d*}, Alan D. Foreman^a, Nicolas Bourgon^f,
6 Nicolas N. Duprey^a, Hubert Vonhof^e, Viengkeo Souksavatdy^g, Anne-Marie Bacon^h,
7 Daniel M. Sigmanⁱ, Thomas Tütken^c, Alfredo Martínez-García^a

8
9 ^aOrganic Isotope Geochemistry Group, Climate Geochemistry Department, Max Planck
10 Institute for Chemistry, 55128 Mainz, Germany.

11
12 ^bEmmy Noether Group for Hominin Meat Consumption, Max Planck Institute for
13 Chemistry, 55128 Mainz, Germany.

14
15 ^cInstitute of Geosciences, Department of Applied and Analytical Paleontology, Johannes
16 Gutenberg University, 55128 Mainz, Germany.

17
18 ^dSenckenberg Biodiversity and Climate Research Centre, 60325 Frankfurt, Germany.

19
20 ^eInorganic Gas Isotope Geochemistry Group, Climate Geochemistry Department, Max
21 Planck Institute for Chemistry, 55128 Mainz, Germany.

22
23 ^fDepartment of Human Evolution, Max Planck Institute for Evolutionary Anthropology,
24 04103 Leipzig, Germany.

25
26 ^gDepartment of Heritage, Ministry of Information, Culture and Tourism, 0100
27 Setthathirath Road, Vientiane Capital, Lao People's Democratic Republic.

28
29 ^hUniversité Paris Cité, CNRS, BABEL, 75012 Paris, France.

30
31 ⁱDepartment of Geosciences, Princeton University, Princeton, New Jersey 08544, U.S.A.

32
33 * Jennifer Leichliter and Tina Lüdecke co-first author

34 **Email:** Jennifer.Leichliter@mpic.de, Tina.Luedecke@mpic.de

35
36 **Keywords:** nitrogen isotopes, tooth enamel, paleodiet, trophic level, paleoecology,
37 African mammals

38 **This document includes:**

39 Abstract

40 Main Text

41 Display Items (8 of 10)

42 References

43 **Abstract**

44 Nitrogen isotopes are widely used to study the trophic position of animals in
45 modern food webs, however, their application in the fossil record is severely limited by
46 degradation of organic material during fossilization. In this study, we show that the
47 nitrogen isotopic composition of organic matter preserved in mammalian tooth enamel
48 ($\delta^{15}\text{N}_{\text{enamel}}$) records diet and trophic position in modern and fossil ecosystems. The
49 $\delta^{15}\text{N}_{\text{enamel}}$ of modern African mammals shows a trophic enrichment of 3.7 ‰ between
50 herbivores and carnivores, as well as a strong positive correlation between $\delta^{15}\text{N}_{\text{enamel}}$ and
51 $\delta^{15}\text{N}_{\text{bone-collagen}}$ values from the same individuals. $\delta^{15}\text{N}_{\text{enamel}}$ values of Late Pleistocene
52 fossil teeth record expected dietary patterns, despite complete diagenetic loss of collagen
53 in the same specimens. We demonstrate that $\delta^{15}\text{N}_{\text{enamel}}$ represents a powerful new
54 paleodietary proxy that could help delineate major dietary transitions in ancient
55 vertebrate lineages, such as the onset and intensification of animal resource use in early
56 hominins.

57 **Main Text**

58

59 **Introduction**

60

61 Diet is a fundamental driver of evolution, and the development of geochemical
62 proxies that can be used to reconstruct past food webs has been a central focus of
63 paleontological research over the last several decades. While traditional stable isotope
64 analyses of tooth enamel (e.g., carbon and oxygen) and trace element ratios (e.g., Sr/Ca,
65 Ba/Ca) have significantly advanced our understanding of the dietary ecology of fossil
66 organisms^{1,2}, these proxies do not provide clear information about trophic level. Recent
67 efforts to develop new trophic proxies have significantly expanded the geochemical tools
68 at our disposal (e.g., calcium and zinc isotopes) and have contributed to our
69 understanding vertebrate feeding behaviors and past ecosystems^{3,4}. However, while
70 promising, data documenting baseline variation and variability in the isotopic
71 fractionation of these elements across taxa and tissue types is limited. As such, a reliable,
72 well-characterized geochemical proxy for determining trophic position in deep time
73 remains elusive.

74 In contrast to these novel systems, the nitrogen (N) isotope ratio of organic
75 material (expressed as $\delta^{15}\text{N}$ versus AIR in ‰, where $\delta^{15}\text{N} = [({}^{15}\text{N}/{}^{14}\text{N}_{\text{sample}}/{}^{15}\text{N}/{}^{14}\text{N}_{\text{reference}})$
76 $- 1] * 1000$) is a widely used proxy for the dietary and trophic behavior of animals in
77 terrestrial and marine food webs. More than 40 years of research shows that, in well-
78 constrained systems, consumer tissues are typically enriched by 3 to 4 ‰ in ^{15}N relative
79 to their diet, and $\delta^{15}\text{N}$ analyses have been applied across a broad range of ecosystems⁵⁻¹².

80 Despite the potential of nitrogen isotopes for trophic reconstruction, their
81 application in the paleontological record has been limited as a result of poor preservation
82 of N-containing organic matter. The analysis of $\delta^{15}\text{N}$ in fossil vertebrates has largely been
83 restricted to collagen extracted from relatively young (e.g., <100 ka), well-preserved
84 bone or dentin samples¹³⁻¹⁶. Enamel, bone, and dentin - the three hard tissues most often
85 preserved in the fossil record - are composed of an inorganic bioapatite fraction (hydrated
86 calcium phosphate mineral with structural and adsorbed carbonate ions;
87 $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) and an organic matrix (i.e., proteins and lipids that vary in
88 composition depending on the tissue)¹⁷⁻²⁰. Relative to enamel, bone and dentin are poorly
89 mineralized (~60 to 70 % wt.), with smaller bioapatite crystals, and a higher organic
90 fraction (30 %) made up predominantly of collagen. The organic matter in bone and
91 dentin is, as a result, susceptible to diagenetic alteration, particularly in unfavorable
92 depositional contexts (e.g., humid environments, acidic soils). As such, reliable
93 $\delta^{15}\text{N}_{\text{collagen}}$ values are rare for fossil material older than tens of thousands of years²¹⁻²⁴. In
94 contrast, tooth enamel is more resistant to diagenetic alteration, and preserves well across
95 long (e.g., million-year) timescales^{20,25-30}. The high density, crystalline structure of
96 mature tooth enamel (~85 to 95 % wt.²⁰), effectively encloses and protects organic matter
97 within the biomineral matrix itself. Until recently, however, efforts to measure nitrogen
98 isotopes in the organic matter of tooth enamel have been hampered by the low N content
99 of enamel (~ 0.5 to 2 % wt. depending on taxon^{20,27,30,31}) and the concomitantly large
100 sample size required for isotope analysis via traditional combustion methods (i.e.,
101 Elemental Analyzer - Isotope Ratio Mass Spectrometry or EA-IRMS). While a modified

102 EA-IRMS system known as a nano-EA has been developed³², analysis of enamel-bound
103 nitrogen isotopes using this system have not been reported. More generally, sample size
104 requirements for the nano-EA-IRMS method are still prohibitive for many applications
105 (i.e., sampling of small and/or precious fossil teeth), and analytical precision remains
106 relatively low. The ‘*oxidation-denitrification method*’³³ drastically improves analytical
107 precision from ~1.0 ‰ 2σ standard deviation for nano-EA measurements at 25 nmol of N
108 ^{32,34} to <0.2 ‰ at 5 nmol of N. Moreover, the rigorous pre-treatment protocol of the
109 ‘*oxidation-denitrification method*’, conducted in a dedicated nitrate-free clean lab,
110 ensures that only endogenous, enamel-bound nitrogen is measured with low N blanks³⁵.

111 Leichliter et al.³⁵ measured nitrogen isotopes of organic matter in the tooth
112 enamel ($\delta^{15}\text{N}_{\text{enamel}}$) of rodents from a controlled feeding experiment using the *oxidation-*
113 *denitrification method* and established that $\delta^{15}\text{N}_{\text{enamel}}$ records the nitrogen isotope
114 composition of the diet. In that study, experimental animals that received plant-based
115 diets had significantly lower $\delta^{15}\text{N}_{\text{enamel}}$ values than those fed meat-based diets. These
116 findings suggest that the $\delta^{15}\text{N}_{\text{enamel}}$ values of herbivores and carnivores living in natural
117 ecosystems should also reflect trophic level differences. While feeding experiments are
118 conducted under controlled conditions, both abiotic and biotic variables (e.g., aridity,
119 feeding behavior, digestive physiology) are known to influence the $\delta^{15}\text{N}$ of animals in the
120 wild. Thus, to further establish $\delta^{15}\text{N}_{\text{enamel}}$ as a trophic level proxy, investigation of
121 $\delta^{15}\text{N}_{\text{enamel}}$ in modern natural ecosystems is needed to ground-truth this method for
122 reconstructing ancient food webs.

123 Here we evaluate whether $\delta^{15}\text{N}_{\text{enamel}}$ records dietary and trophic level information
124 in modern fauna living in natural ecosystems. We measured the $\delta^{15}\text{N}_{\text{enamel}}$ values of 57
125 modern mammals from several locations in Africa (Fig. S1, Tables 1 and S1). Herbivores
126 (including browsers, grazers, and mixed feeders), omnivores, and carnivores were
127 selected to ensure that different trophic levels and feeding behaviors were represented. In
128 addition, paired $\delta^{15}\text{N}_{\text{bone-collagen}}$ values were measured for a subset ($n = 34$) of the same
129 individuals from each trophic level to evaluate whether enamel and bone collagen (the
130 material in which $\delta^{15}\text{N}$ has most often been measured in archeological and fossil contexts
131 ³⁶) record similar dietary information (Fig. 1 and Table 1).

132 Recent evidence from Martínez-García et al.³⁷ demonstrates that the $\delta^{15}\text{N}$ values
133 and N content of modern and fossil tooth enamel remain stable under experimentally
134 induced conditions that favor organic matter degradation, including oxidative attack,
135 dissolution, and heating. Taken together, these observations suggest that enamel-bound
136 organic matter is highly protected and therefore has strong potential to preserve an *in vivo*
137 nitrogen isotope signal even in diagenetically altered settings. Further evidence for this
138 comes from the work of Kast et al.³⁸, who successfully reconstructed the trophic behavior
139 of extinct megatooth sharks across the Cenozoic (~66 to 3.5 Ma) using $\delta^{15}\text{N}$ values
140 obtained from analysis of the enameloid of fossil shark teeth. It remains an open question,
141 however, if fossil mammalian enamel preserves diet and trophic level information in a
142 similar manner to the enameloid of shark teeth, given both the differences in mineral
143 structures between mammalian enamel (hydroxylapatite) and shark enameloid
144 (fluorapatite), as well as the differences in depositional environments.

145 As such, tooth enamel $\delta^{15}\text{N}$ values have not yet been measured for fossil
146 mammals or any other terrestrial vertebrate. Here we apply for the first time the

147 *oxidation-denitrification* method to fossil mammalian teeth from a Late Pleistocene
148 (38.4–13.4 ka) terrestrial assemblage from Tam Hay Marklot Cave (THM), Laos
149 southeast (SE) Asia (Fig. S2). Fossils from this site are characterized by excellent enamel
150 preservation, but poor preservation of bone and dentin, thus precluding $\delta^{15}\text{N}$ analysis of
151 collagen for the vast majority of samples³⁹. As such the THM site represents an ideal test
152 for the application of $\delta^{15}\text{N}_{\text{enamel}}$ to the fossil record. We test whether fossil enamel N
153 content is in the same range as modern tooth enamel, consistent with good enamel-bound
154 organic matter preservation, and evaluate whether the $\delta^{15}\text{N}_{\text{enamel}}$ values of fossil
155 mammalian teeth preserve dietary and trophic level information in a setting where the
156 degradation of collagen prevents analysis of $\delta^{15}\text{N}_{\text{collagen}}$.

157

158 **Results**

159

160 $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{13}\text{C}_{\text{enamel}}$ were measured in all modern ($n = 57$) and fossil ($n = 10$)
161 teeth (primarily third molars from adult individuals). In addition, stable isotope values
162 from
163 mandibular collagen ($\delta^{15}\text{N}_{\text{bone-collagen}}$ and $\delta^{13}\text{C}_{\text{bone-collagen}}$) were measured in a subset ($n =$
164 34) of the modern African fauna. One juvenile elephant was also measured, but is
165 excluded from $\delta^{15}\text{N}$ analyses. Fossil dentin $\delta^{15}\text{N}_{\text{dentin-collagen}}$ and $\delta^{13}\text{C}_{\text{dentin-collagen}}$ values (n
166 = 4) are from Bourgon et al.³⁹. Isotopic data for all samples are reported in Tables 1 and
167 2.

168

169 Modern African Mammals

170

171 *Nitrogen isotopes*

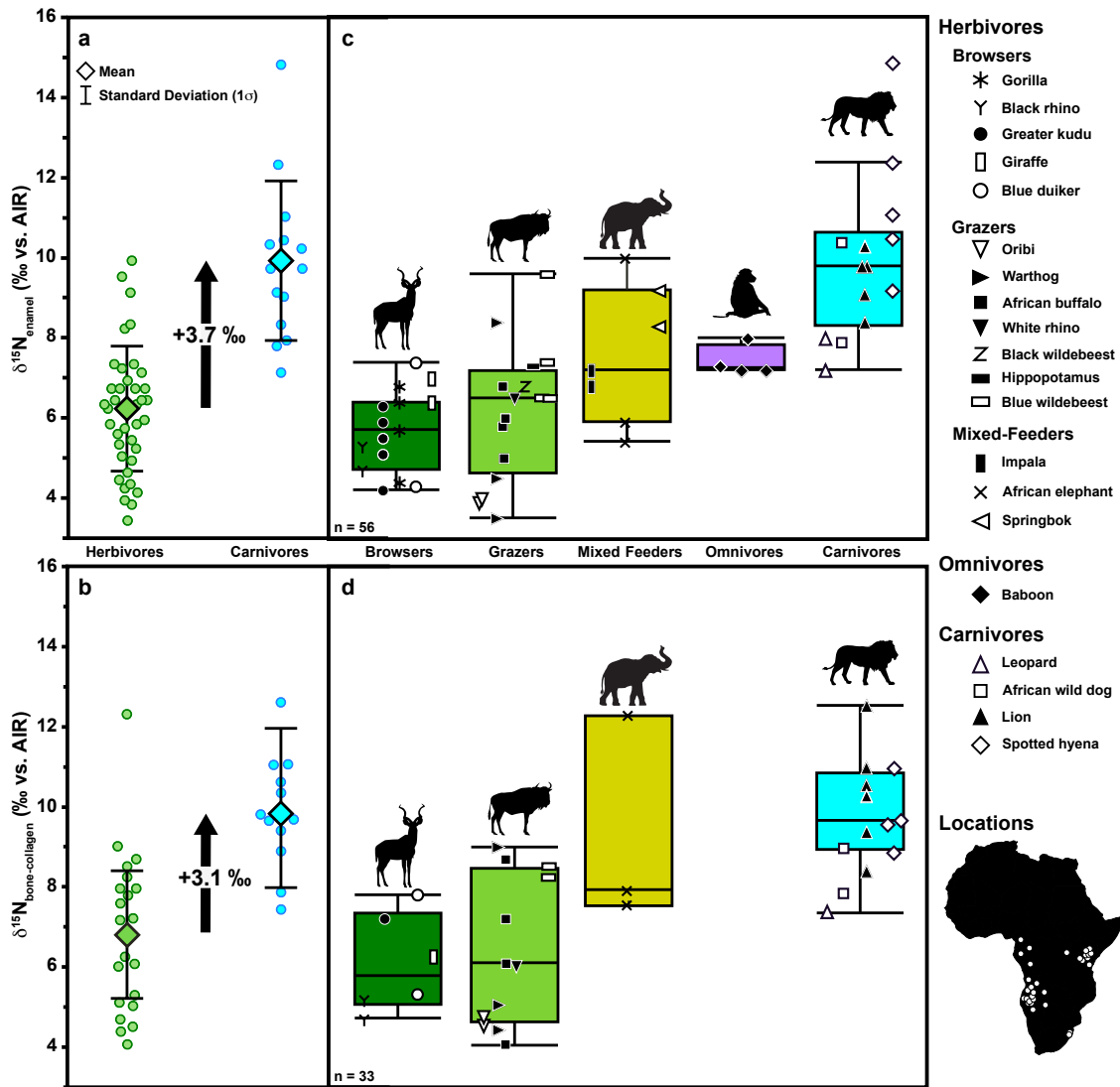
172

173 $\delta^{15}\text{N}_{\text{enamel}}$ values of modern African mammals ranged from 3.5 to 14.9 ‰ ($n = 56$)
174 (Fig. 1a and 1c; Table 1) and differ significantly according to diet ($F(2,55) = 26.05$,
175 $p = <0.0001$). Mean $\delta^{15}\text{N}_{\text{enamel}}$ values were lowest in herbivores (6.2 ± 1.6 ‰; $n = 38$)
176 and highest in carnivores (9.9 ± 2.0 ‰; $n = 14$), while omnivores had intermediate values
177 (7.4 ± 0.4 ‰; $n = 4$). Herbivores and carnivores differ significantly ($p = <0.0001$).
178 Differences were also observed between herbivores and the other diet groups according
179 to feeding behavior ($F(4,55) = 15.83$, $p = <0.0001$). Across ecosystems, browsing taxa
180 typically had the lowest $\delta^{15}\text{N}_{\text{enamel}}$ values (5.7 ± 1.0 ‰; $n = 15$), followed by grazing
181 (6.1 ± 1.7 ‰; $n = 16$) and then mixed feeding taxa (7.5 ± 1.7 ‰; $n = 7$; Fig. 1). All
182 herbivorous dietary groups differ significantly from carnivores ($p = <0.0001$ for grazers
183 and browsers; $p = 0.015$ for mixed feeders).

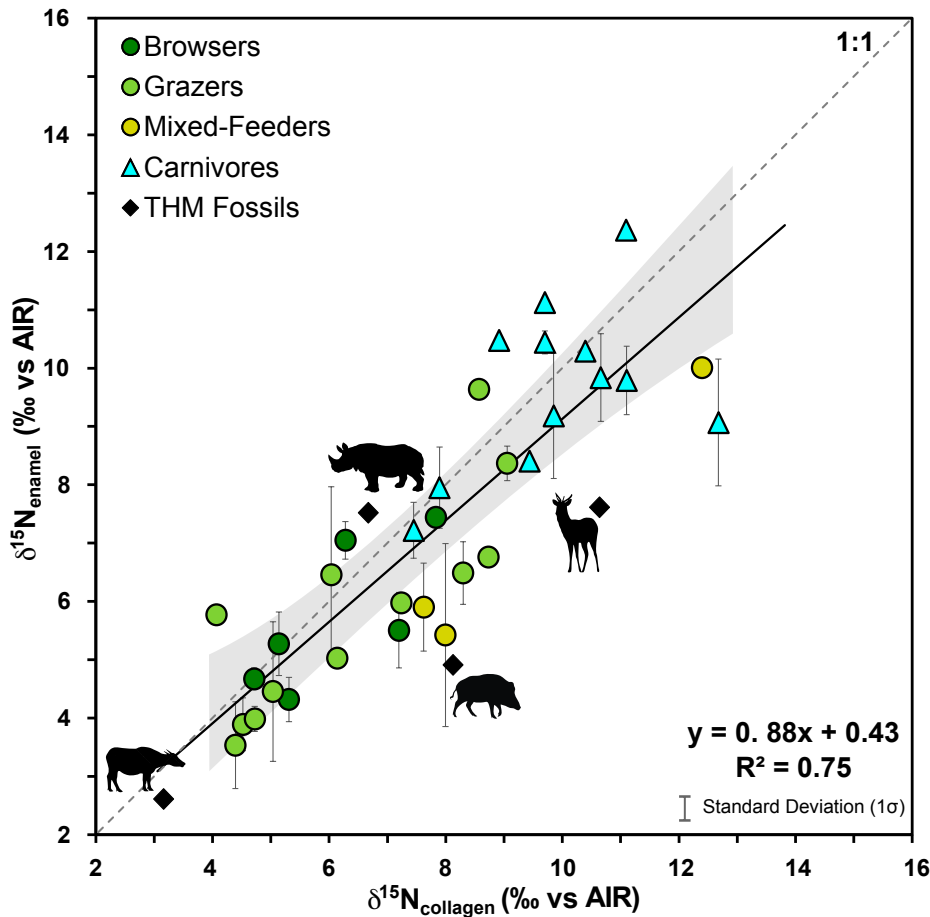
184 $\delta^{15}\text{N}_{\text{bone-collagen}}$ values ranged from 4.1 to 12.6 ‰ ($n = 33$) (Fig. 1b and 1d; Table
185 1) and differ significantly according to diet ($F(3,29) = 11.66$, $p = <0.0001$). As in enamel,
186 mean $\delta^{15}\text{N}_{\text{bone-collagen}}$ values were lowest in herbivores (6.7 ± 2.0 ‰; $n = 21$) and highest in
187 carnivores (9.8 ± 1.4 ‰; $n = 12$), and herbivores and carnivores differ significantly ($p = <$
188 0.0001). Among herbivores, $\delta^{15}\text{N}_{\text{bone-collagen}}$ values were similar to $\delta^{15}\text{N}_{\text{enamel}}$. Browsers
189 had the lowest values (6.0 ± 1.2 ‰; $n = 6$), followed by grazers (6.4 ± 1.9 ‰; $n = 12$),
190 and mixed feeders (9.3 ± 2.6 ‰; $n = 3$). Grazers and browsers differ significantly from
191 carnivores ($p = <0.0001$), but, in contrast to the enamel dataset, the mixed feeders do not
192 ($p = 0.95$).

193 We found a significant, positive correlation between $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{15}\text{N}_{\text{bone-collagen}}$
194 values (Pearson's correlation $r(31) = 0.865$, $p = <0.001$; Fig. 2). An ordinary least
195 squares regression yields the following relationship; $\delta^{15}\text{N}_{\text{enamel}} \sim 0.88$ [95% confidence
196 interval (CI): 0.66 to 1.1] $\times \delta^{15}\text{N}_{\text{bone-collagen}} + 0.43$ ‰ [95% confidence interval (CI): -0.8
197 to 2.0] with no consistent, directional offset in $\delta^{15}\text{N}$ values.

198 Trophic enrichment between herbivores and carnivores ($\Delta^{15}\text{N} = \delta^{15}\text{N}_{\text{carnivore}} -$
199 $\delta^{15}\text{N}_{\text{herbivore}}$) is apparent in both datasets but is larger in enamel (3.7 ‰) compared to bone
200 collagen (3.1 ‰) from the same individuals (Fig. 1).



201 **Fig. 1. Nitrogen isotope ratios measured in enamel (top) and bone collagen (bottom).**
 202 (a) $\delta^{15}\text{N}_{\text{enamel}}$ and (b) $\delta^{15}\text{N}_{\text{bone-collagen}}$ values for all modern African herbivores and
 203 carnivores measured in this study, with mean and standard deviation (1σ) indicated. The
 204 bolded black arrow illustrates the average trophic enrichment between herbivores and
 205 carnivores, which is similar in both datasets. Box plots of (c) $\delta^{15}\text{N}_{\text{enamel}}$ and (d) $\delta^{15}\text{N}_{\text{bone-collagen}}$
 206 values for all dietary groups. Data points for all taxa are plotted separately for each
 207 dietary group. Relative patterns of ^{15}N enrichment for dietary groups are similar in both
 208 datasets.



209 **Fig. 2.** Regression of paired $\delta^{15}\text{N}_{\text{bone-collagen}}$ versus $\delta^{15}\text{N}_{\text{enamel}}$ values ($\bar{x} \pm 1\sigma$) for all
 210 modern African mammals ($n = 33$). The dashed line represents a 1:1 regression between
 211 bone collagen and enamel values. The solid line indicates the true regression, with a
 212 bootstrapped 95% CI illustrated by the shaded gray region. The equation for the
 213 regression as well as the R^2 value are indicated in the lower right of the figure. $\delta^{15}\text{N}_{\text{dentin-}}$
 214 collagen versus $\delta^{15}\text{N}_{\text{enamel}}$ values for the four fossil Tam Hay Marklot (THM) specimens
 215 (see Table 2) are also plotted for reference (black diamonds) but were not included in
 216 regression calculation. There is clear positive correlation between $\delta^{15}\text{N}_{\text{bone-collagen}}$ and
 217 $\delta^{15}\text{N}_{\text{enamel}}$.

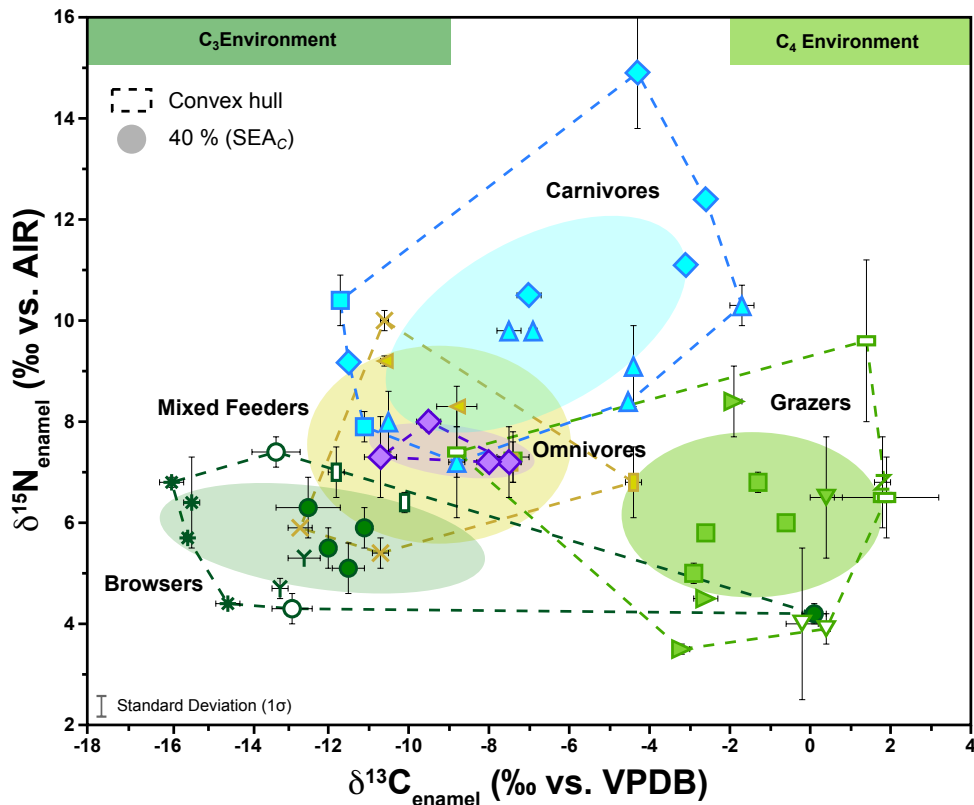
218 *Carbon Isotopes*

219

220 $\delta^{13}\text{C}_{\text{enamel}}$ values ranged from -15.9 to +1.9 ‰ ($n = 57$; Fig. 3, Table 1) and
221 differed significantly according to diet ($\chi^2(4) = 35.57, p = < 0.0001$). Mean $\delta^{13}\text{C}_{\text{enamel}}$
222 values were -7.3 ± 6.0 ‰ for herbivores ($n = 39$), and -6.8 ± 3.5 ‰ for carnivores ($n=14$).
223 Browsers had the lowest $\delta^{13}\text{C}_{\text{enamel}}$ values (-12.2 ± 3.8 ‰; $n = 15$), followed by mixed
224 feeders (-10.1 ± 3.3 ‰; $n = 8$) and then grazers (-1.5 ± 3.1 ‰; $n = 16$). Omnivores ($-8.9 \pm$
225 1.5 ‰; $n = 4$) had intermediate values. Carbonate content was typically 4 to 7 wt. %.

226 $\delta^{13}\text{C}_{\text{bone-collagen}}$ values ranged from -22.2 to -5.5 ‰ ($n = 33$; Fig. S4). Mean
227 $\delta^{13}\text{C}_{\text{bone-collagen}}$ values were -13.9 ± 6.3 ‰ ($n = 21$) for herbivores and -11.2 ± 4.3 ‰
228 ($n = 12$) for carnivores. Browsers and mixed feeders had low $\delta^{13}\text{C}_{\text{bone-collagen}}$ values (-21.0
229 ± 0.9 ‰; $n = 6$ and -20.4 ± 1.4 ‰; $n = 3$, respectively), and grazers significantly higher
230 ones ($- 8.7 \pm 1.7$ ‰; $n = 12$).

231 $\delta^{13}\text{C}_{\text{enamel}}$ was positively correlated with $\delta^{13}\text{C}_{\text{bone-collagen}}$ ($R_s(34) = 0.878, p =$
232 < 0.001). The positive correlation between $\delta^{13}\text{C}_{\text{enamel}}$ and $\delta^{13}\text{C}_{\text{bone-collagen}}$ is stronger within
233 each dietary group (Pearson's correlation $r(19) = 0.976, p = < 0.001$ for herbivores and
234 $r(10) = 0.97, p = < 0.001$ for carnivores, respectively; see Fig. S4). The offset between
235 $\delta^{13}\text{C}_{\text{enamel}}$ and $\delta^{13}\text{C}_{\text{bone-collagen}}$ was higher (8.2 ‰) in herbivores than in carnivores (4.5 ‰).



236

237 **Fig. 3.** Cross plot of $\delta^{13}\text{C}_{\text{enamel}}$ versus $\delta^{15}\text{N}_{\text{enamel}}$ values for all modern African mammals.

238 Browsing (dark green), grazing (light green), and mixed feeding (brown-green)

239 herbivores, omnivores (purple), and carnivores (blue) are indicated (see legend in Fig. 1

240 for taxon-specific symbols). Dashed lines for convex hulls represent the full range of

241 variation, and shaded ellipses indicate 40 % estimated standard ellipse areas (SEAC).

242 Herbivores and omnivores have relatively low $\delta^{15}\text{N}_{\text{enamel}}$ values and thus plot in the lower

243 half of the figure, while carnivores have higher $\delta^{15}\text{N}_{\text{enamel}}$ values and plot in the top half

244 of the figure. Green shaded bars at the top of the figure indicate tooth enamel $\delta^{13}\text{C}$

245 isotopic ranges of modern herbivores (after Cerling et al. ⁴⁰) corresponding to pure C₃ and

246 C₄ resource utilization.

247 Fossil Mammals

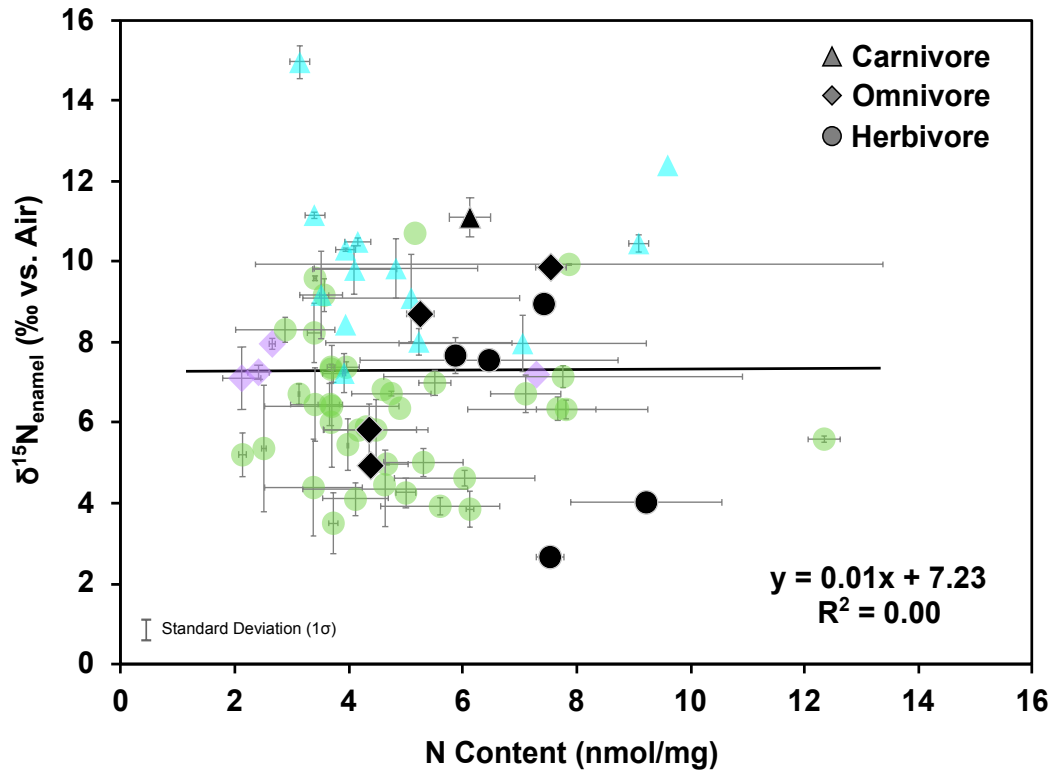
248

249 *Nitrogen Content*

250

251 The nitrogen content of modern African mammalian enamel was typically
252 between 2 to 10 ($\bar{x} = 4.8 \pm 2.0$; $n = 56$) nmol/mg after reductive-oxidative cleaning (see
253 Materials and Methods for details). Fossil tooth enamel N content was between 4 and 10
254 ($\bar{x} = 6.4 \pm 1.6$; $n = 10$) nmol/mg after cleaning, within the range of the modern enamel
255 samples (Fig. 4; Table 1 and 2). No differences were observed between dietary or
256 taxonomic groups in either modern or fossil datasets (Fig. S3) and, importantly, no
257 significant correlation was observed between $\delta^{15}\text{N}_{\text{enamel}}$ and N content (Pearson's
258 correlation $r(65) = 0.00741$, $p = 0.953$).

259



260
 261 **Fig. 4.** Nitrogen content of modern African mammals (colored symbols) and Tam Hay
 262 Marklot fossil mammals (black symbols). The solid black line illustrates the regression
 263 between N content and $\delta^{15}\text{N}_{\text{enamel}}$, with the equation for the regression as well as the R^2
 264 value are indicated in the lower right of the figure. Fossil tooth enamel N content falls
 265 within the range of modern mammalian tooth enamel N content. $\delta^{15}\text{N}_{\text{enamel}}$ and N content
 266 are not significantly correlated.
 267

268 *Nitrogen Isotopes*

269

270 The Late Pleistocene mammals from SE Asia exhibited a range of $\delta^{15}\text{N}_{\text{enamel}}$
271 values (2.6 to 11.1 ‰, $n = 10$) similar to those of the modern African mammals. When
272 added to the regression of the modern African mammal $\delta^{15}\text{N}_{\text{bone-collagen}}$ and $\delta^{15}\text{N}_{\text{enamel}}$
273 values, the four fossil specimens show a moderate positive correlation, and the regression
274 residuals are within the range of modern mammals (Fig. 2 and S6). As observed in the
275 modern dataset, $\delta^{15}\text{N}_{\text{enamel}}$ values were the lowest in the herbivores (6.1 ± 2.7 ‰; $n = 5$),
276 the highest in the carnivore (a leopard, *Panthera pardus*; 11.1 ‰), and intermediate in the
277 omnivores (7.3 ± 2.4 ‰; $n = 4$; Fig. 5). The grazers, including an Asian water buffalo
278 (*Bubalus bubalis*) and an unspecified bovine (*Bos* sp.), had the lowest values (4.0 and 2.6
279 ‰, respectively), while the mixed feeding cervid (*Muntiacus muntjak*; 7.6 ‰), the
280 browsing rhinoceros (*Rhinoceros sondaicus*; 7.5 ‰), and the goat-like Sumatran serow
281 (*Capricornis sumatraensis*; 8.9 ‰) all had higher values. Among the omnivores, the two
282 pigs (*Sus scrofa*; 5.4 ± 0.6 ‰) had the lowest values, while the macaque (*Macaca* sp.;
283 9.9 ‰), and the Asian black bear (*Ursus thibetanus*; 8.8 ‰) had higher values.

284 Paired $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{66}\text{Zn}_{\text{enamel}}$ values (data from Bourgon et al.³⁹) for the Tam
285 Hay Marklot ($n = 10$) are negatively correlated (Pearson's correlation $r(8) = 0.671$, $p =$
286 0.034; Fig. 6).

287

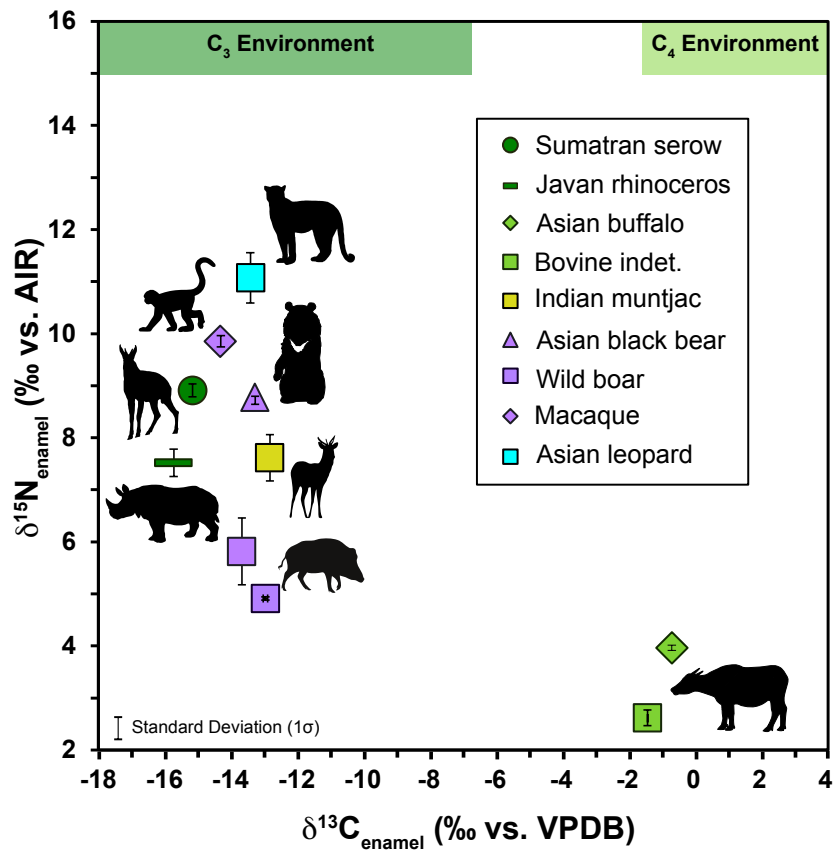
288 *Carbon Isotopes*

289

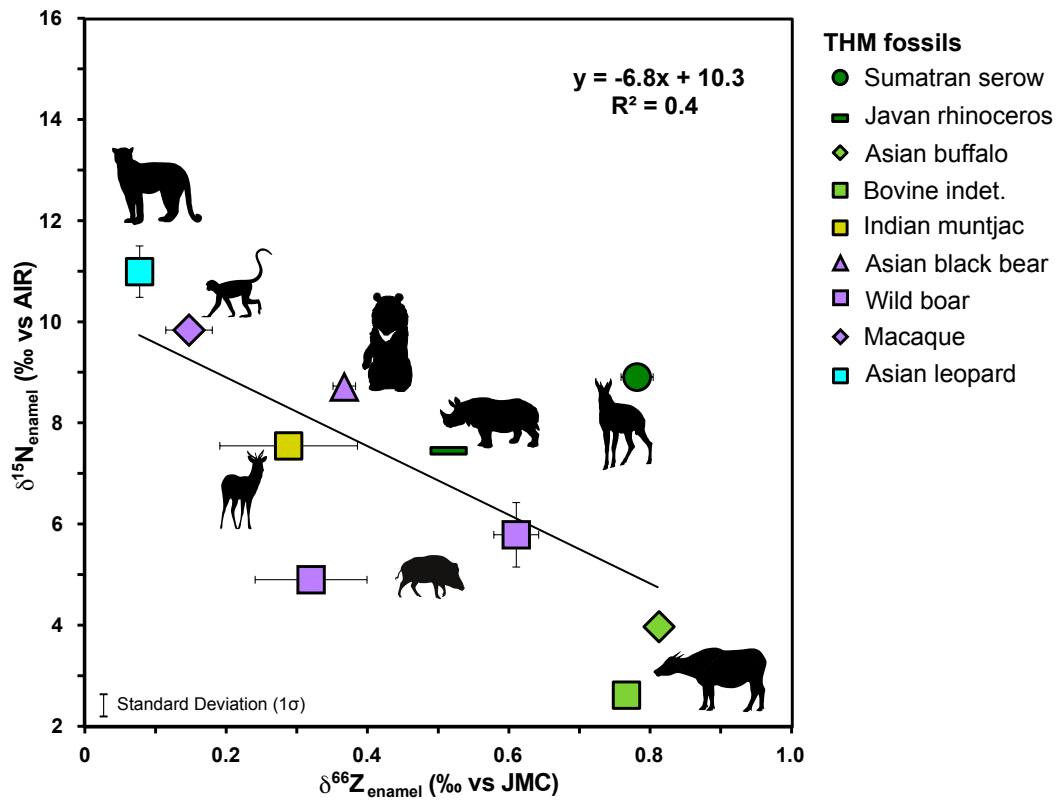
290 Fossil $\delta^{13}\text{C}_{\text{enamel}}$ values ranged from -15.7 to -0.8 ‰ (Fig. 5). $\delta^{13}\text{C}_{\text{enamel}}$ values
291 were the lowest in the rhinoceros (-15.7 ‰) and serow (-15.2 ‰). The mixed feeding
292 cervid had an intermediate value of -12.9 ‰. The bovine (indet.) and Asian water
293 buffalo, both grazers, have the two highest values (-1.5 ‰ and -0.8, respectively). The
294 suids, macaque, bear, and leopard all have similar $\delta^{13}\text{C}_{\text{enamel}}$ values, ranging between -
295 14.3 and -13.3 ‰ (see Table 2). As with the modern mammals, carbonate content in the
296 fossil enamel samples was typically between 4 to 7 wt. %.

297 Only four THM specimens yielded sufficient collagen preservation for $\delta^{13}\text{C}_{\text{dentin-}}$
298 collagen analysis (data from Bourgon et al.³⁹) but they show a positive relationship with
299 $\delta^{13}\text{C}_{\text{enamel}}$ data from the same specimens, consistent with the regression obtained for
300 modern specimens (Fig. S4).

301



302 **Fig. 5.** Cross plot of $\delta^{13}\text{C}_{\text{enamel}}$ versus $\delta^{15}\text{N}_{\text{enamel}}$ for all fossil tooth samples from Tam Hay
 303 Marklot Cave. Herbivore (green), omnivore (purple), and carnivore (blue) taxa are
 304 indicated. The isotopic ranges for C₃ and C₄ environments, after Cerling, et al.⁴⁰ and
 305 corrected for the fossil-fuel-induced atmospheric CO₂ shift of ~1.5 ‰⁴¹, are indicated by
 306 green shaded bars at the top of the figure. Herbivores and omnivores have lower
 307 $\delta^{15}\text{N}_{\text{enamel}}$ values compared to the single carnivore, which has the highest value (*Panthera*
 308 *pardus*). Most of the THM fauna lived and foraged in predominantly C₃
 309 habitats. Grazing taxa are clearly distinguished from the rest of the fauna by their higher
 310 $\delta^{13}\text{C}_{\text{enamel}}$ values.



311 **Fig. 6:** Regression of paired $\delta^{66}\text{Zn}_{\text{enamel}}$ (from Bourgon et al.³⁹) versus $\delta^{15}\text{N}_{\text{enamel}}$ values
 312 (this study) for the Late Pleistocene fossil teeth from Tam Hay Marklot ($\bar{x} \pm 1\sigma$; $n = 10$).
 313 The black line indicates the regression for all fossils, and equation for the regression as
 314 well as the R^2 value are indicated in the upper right of the figure. The two trophic level
 315 proxies are negatively correlated.

316 **Discussion**

317

318 Paired N and C Isotopes in Tooth Enamel Record Diet and Trophic Level Information in
319 Modern African Mammals

320

321 We observed an average difference in $\delta^{15}\text{N}_{\text{enamel}}$ of 3.7 ‰ between herbivores and
322 carnivores (Fig. 1a). This agrees well with the average trophic enrichment of ~ 3.5 ‰
323 documented in numerous large-scale ecological studies^{7,12,13}. Moreover, the observed
324 $\delta^{15}\text{N}_{\text{enamel}}$ values of the different dietary groups – specifically the low values of browsers
325 and grazers, intermediate values of mixed feeders, and high values of carnivores (Fig. 1c)
326 – agree well with published $\delta^{15}\text{N}$ data for bone collagen^{8-10,42,43}.

327 Previous research has shown that both abiotic (e.g., aridity, altitude, soil
328 chemistry) and biotic (e.g., digestive physiology, protein intake, water dependence)
329 factors can cause significant variation in $\delta^{15}\text{N}$ between different habitats and within
330 trophic levels, sometimes to such a degree that the overall trophic level effect in nitrogen
331 isotope ratios is obscured^{10,42,44-48}. Although the samples included in this study are drawn
332 from multiple localities across Africa (see Fig. S1; Tables 1 and S1), trophic level
333 patterns are nonetheless clearly discernible in the $\delta^{15}\text{N}_{\text{enamel}}$ values of animals belonging
334 to different dietary groups. Thus, while regional differences between sampling localities
335 (i.e., baseline variation) may have contributed to observed intra-group variation in
336 $\delta^{15}\text{N}_{\text{enamel}}$ values, their effects are not strong enough to obscure the overall trophic level
337 effect recorded in $\delta^{15}\text{N}_{\text{enamel}}$.

338 Additionally, the same individuals' paired $\delta^{15}\text{N}_{\text{enamel}}$ and mandibular $\delta^{15}\text{N}_{\text{bone-}}$
339 collagen values are positively correlated (Fig. 2), confirming that, when collagen is well
340 preserved, enamel and collagen record very similar isotopic information. This finding is
341 significant because collagen is the most frequently measured material in nitrogen-based
342 paleodietary studies, and diet-related nitrogen isotope fractionation in bone collagen is
343 well-understood⁶⁻⁸. Thus, demonstrating a clear link between $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{15}\text{N}_{\text{bone-}}$
344 collagen represents an important step in establishing $\delta^{15}\text{N}_{\text{enamel}}$ as a new geochemical
345 archive for reconstructing past diets of fossil vertebrates.

346 The correlation between bone collagen and enamel $\delta^{15}\text{N}$ values is not expected to
347 be perfect because there are differences both in when these two hard tissues form as well
348 as in the composition of their organic matter. Bone is a living, dynamic, and actively-
349 growing tissue, which turns over on a time scale of multiple years, incorporating the
350 isotopic composition of an animal's diet throughout its lifetime⁴⁹. In contrast,
351 mammalian teeth mineralize during a discrete period relatively early in an animal's life
352 (i.e., weeks to months or even years, depending on taxon and tooth type and size⁵⁰), after
353 which the enamel becomes metabolically inactive and hence isotopically inert⁵¹. Thus,
354 the two hard tissues represent different periods in an animal's lifetime and may
355 potentially record different diets; the isotopic composition of mandibular bone usually
356 records the last three to five years before death⁴⁹, whereas tooth enamel reflects discrete
357 periods ranging from infancy to juvenile stages to early adulthood, depending on the
358 tooth type under consideration⁵⁰. In order to capture the adult diet, and avoid enrichment
359 in ^{15}N as a result of the consumption of breastmilk⁵², we targeted the latest forming tooth
360 in each taxon (typically a molar; Tables 1 and 2). In addition to different tissue formation

361 times, bone and enamel also differ in the composition of their organic matter. While the
362 organic fraction of bone consists mainly of collagen (90 %), the organic matter in enamel
363 is comprised of enamel-specific proteins (predominantly amelogenin) and proteases^{27,53-}
364⁵⁵. There should not thus necessarily be a perfect 1:1 correlation between $\delta^{15}\text{N}_{\text{enamel}}$ and
365 $\delta^{15}\text{N}_{\text{bone-collagen}}$. Nevertheless, the positive correlation between enamel and bone collagen
366 from the same individuals and the clear enrichment in $\delta^{15}\text{N}_{\text{enamel}}$ across trophic levels,
367 confirm that $\delta^{15}\text{N}_{\text{enamel}}$ records diet and trophic level in a manner similar to $\delta^{15}\text{N}_{\text{bone-}}$
368 collagen.

369 Importantly, our dataset also includes measurements of nitrogen and carbon
370 isotope values using the same aliquot of tooth enamel. When $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{13}\text{C}_{\text{enamel}}$
371 values are plotted together in Fig. 3, they reveal clear, interpretable patterns that are in
372 good agreement with expected dietary habits for the modern taxa included in this study.
373 Next, we discuss these data in more detail.

374

375 Herbivores:

376 While herbivores have a lower overall mean $\delta^{15}\text{N}_{\text{enamel}}$ value than carnivores, they
377 exhibit a high degree of variation in $\delta^{15}\text{N}_{\text{enamel}}$ as a dietary group. Numerous factors have
378 been proposed to drive this variation, including environment (e.g., precipitation,
379 temperature, soil chemistry, and their effect on soil N cycling), physiology (e.g., water
380 conservation, digestive physiology, nutritional status of the animal^{47,48}), and diet (e.g.,
381 feeding strategy, foraging microhabitat)^{10,42,44,56-61} (see Supplementary Information for
382 more details). Ultimately, the $\delta^{15}\text{N}_{\text{enamel}}$ values of herbivore body tissues are driven
383 primarily by the isotopic composition of the plants they consume, which is in turn
384 controlled by abiotic factors specific to each ecosystem⁵⁶. The observed variation in
385 herbivore tissue $\delta^{15}\text{N}$ values are discussed further below.

386 Amongst the herbivores in this study, we observed no difference between taxa
387 grouped according to their water dependence (high, low, and none; after Hempson, et al.
388⁶²; $\chi^2(2) = 3.78$, $p = 0.151$; see Fig. S5). When grouped according to feeding behavior,
389 browsing and grazing taxa do not differ in $\delta^{15}\text{N}_{\text{enamel}}$, but mixed feeders (springbok,
390 impala, and elephants) had significantly higher $\delta^{15}\text{N}_{\text{enamel}}$ values, even overlapping with
391 those of the carnivores (Fig. 1c). This pattern is consistent with previously published
392 herbivore data^{8,10,25,43,44,63}. For instance, Ambrose⁴² observed relatively high $\delta^{15}\text{N}_{\text{bone-}}$
393 collagen values (also overlapping with those of co-occurring carnivores) in mixed feeding
394 herbivores from eastern Africa; Sealy et al.¹⁰ observed higher $\delta^{15}\text{N}_{\text{bone-collagen}}$ values in
395 mixed feeding springbok compared to other herbivorous taxa in southern Africa, and
396 Codron et al.⁴³ measured the highest herbivore $\delta^{15}\text{N}$ values in the feces of mixed feeders
397 (impala, nyala) in their study of herbivores in Kruger National Park. African elephant
398 tissue $\delta^{15}\text{N}$ values vary widely, depending on which region of Africa the animals inhabit,
399 but our observed $\delta^{15}\text{N}_{\text{enamel}}$ values (5.4 to 10.0 ‰) fit well within the large range of
400 published bone collagen values (2.0 to 16.0 ‰) for this taxon^{10,64-66}. It remains unclear
401 why mixed feeders tend to have higher $\delta^{15}\text{N}$ values than other herbivores overall, and our
402 $\delta^{15}\text{N}_{\text{enamel}}$ dataset for one given locality in Africa is too small to test if this holds true
403 amongst mixed feeders more broadly. Moreover, it is worth noting that additional factors,
404 including consumed plant part (e.g., leaf, fruit, stem, root) and plant nutritional quality
405 (e.g., protein content, fiber content) as well as the digestive physiology (ruminant *versus*

406 non-ruminant) and the nutritional status (e.g., starvation, pregnancy, lactation) of the
407 animal itself also can impact herbivore $\delta^{15}\text{N}$ values^{48,59-61,67-71}. In our dataset, the mixed
408 feeders are significantly different from carnivores in $\delta^{15}\text{N}_{\text{enamel}}$, although not in $\delta^{15}\text{N}_{\text{bone-}}$
409 collagen. However, this difference may have been driven, at least in part, by the smaller size
410 of the collagen dataset.

411

412 Omnivores:

413 For this study, we analyzed enamel from baboons (*Papio cynocephalus*), which
414 are of particular interest for the application of this method to important questions about
415 human evolution, as baboons live in open savanna ecosystems and have been proposed as
416 a model taxon for early hominins. Baboons are well-known ecological and dietary
417 generalists that consume a wide variety of foods in an opportunistic manner⁷²⁻⁷⁵,
418 including many types of plants, as well as insects, small animals, eggs, etc. Indeed,
419 DeVore and Hall⁷⁶ write that, "...it is almost easier to list the items which they do not eat
420 than to describe the items which they do" (p. 43).

421 The $\delta^{15}\text{N}_{\text{enamel}}$ values we obtained for baboons are consistent with those of a
422 dietary generalist and in good general agreement with existing stable isotope data for
423 these primates⁷⁷. Baboon $\delta^{15}\text{N}_{\text{enamel}}$ values were significantly lower than those of
424 carnivores, and were instead comparable to those of herbivores (Fig. 1). The teeth
425 sampled for this study would have formed just at the end of the juvenile period, and
426 young, low-ranking baboons rarely consume meat, a behavior that has primarily been
427 observed in dominant adult males^{78,79}. While some studies have found that baboons tend
428 to have low $\delta^{15}\text{N}$ values compared to sympatric herbivores^{8,77}, perhaps related to the
429 consumption of N_2 -fixing plants and/or underground storage organs, no such tendency
430 was observed in our $\delta^{15}\text{N}_{\text{enamel}}$ data. This is not particularly surprising considering the
431 complexity of baboon feeding behavior and given that our specimens derived from
432 different sampling localities across Africa.

433

434 Carnivores:

435 $\delta^{15}\text{N}_{\text{enamel}}$ values were the highest for the carnivores, evidencing clear trophic
436 enrichment compared to herbivores and falling into the range of $\delta^{15}\text{N}_{\text{bone-collagen}}$ values
437 typical for carnivores. Within the carnivore guild, we also observed differences in
438 $\delta^{15}\text{N}_{\text{enamel}}$ between taxa. Leopards (*Panthera pardus*) and wild dogs (*Lycaon pictus*), for
439 instance, had lower $\delta^{15}\text{N}_{\text{enamel}}$ values than spotted hyenas (*Crocuta crocuta*) and lions
440 (*Panthera leo*). Overall, the $\delta^{15}\text{N}_{\text{enamel}}$ values of spotted hyenas are higher than those of
441 other carnivore species, and, in two individuals, substantially higher than all other $\delta^{15}\text{N}$
442 values measured for this species in this study. It is probable that these higher values
443 incorporate a nursing signal, as the permanent dentition in spotted hyenas erupts at the
444 same time that weaning occurs⁸⁰. If these two outliers are excluded from the carnivore
445 $\delta^{15}\text{N}_{\text{enamel}}$ dataset, $\Delta^{15}\text{N}_{\text{carnivore-herbivore}}$ decreases to 3.1 ‰, but trophic enrichment between
446 herbivores and carnivores nevertheless remains significant and equal that of $\delta^{15}\text{N}_{\text{bone-}}$
447 collagen.

448 Although the observed differences between carnivore $\delta^{15}\text{N}_{\text{enamel}}$ values are
449 potentially related to niche separation and differential habitat use/prey preference, no
450 strong conclusions can be drawn from the differences in $\delta^{15}\text{N}_{\text{enamel}}$ values alone,

451 especially when considering that these samples are drawn from disparate localities.
452 However, carbon isotope data help shed further light on niche separation between
453 carnivore taxa. For instance, while all carnivores included in this study are typical
454 savanna dwellers, $\delta^{13}\text{C}_{\text{enamel}}$ values for leopards and wild dogs in this dataset indicate that
455 these two taxa relied more heavily on browsing prey (i.e., C_3 -consuming prey) compared
456 to spotted hyenas and lions (Fig. 3). Most carnivores selectively hunt specific taxa and
457 their diets can be strongly influenced by competition with other carnivores. Leopards, for
458 example, prefer relatively small prey (e.g., body mass 10 to 40 kg) which occur in dense
459 habitats; for example, impala, bushbuck, and common duiker, while larger prey and
460 species restricted to open vegetation are generally avoided⁸¹. Similarly, wild dogs tend to
461 hunt in areas of denser vegetation and target browsing and mixed feeding herbivores in
462 regions where they co-occur with hyenas and lions⁸². Lions and hyenas, in contrast, have
463 higher $\delta^{13}\text{C}_{\text{enamel}}$ values, consistent with the consumption of a greater proportion of
464 grazing taxa (i.e., C_4 -consuming prey) which occupy more open environments. These
465 carnivore data illustrate the potential to refine reconstructions of trophic niches using
466 combined $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{13}\text{C}_{\text{enamel}}$ analyses.

467 Importantly, this study was not designed to explicitly test the effect of the
468 potential confounding abiotic or biotic factors that may be driving variation in
469 herbivore/carnivore $\delta^{15}\text{N}$ values, and our $\delta^{15}\text{N}_{\text{enamel}}$ dataset for any one given locality in
470 Africa is too small to do so properly. Rather, we set out to demonstrate that $\delta^{15}\text{N}_{\text{enamel}}$
471 values record diet in a robust manner comparable to other commonly measured tissue
472 types, especially collagen, the material most frequently measured in the archeological and
473 fossil record. Significantly, our data show that tooth enamel organic matter clearly
474 records dietary information, confirming that $\delta^{15}\text{N}_{\text{enamel}}$ is a powerful trophic level proxy.

475
476

477 Nitrogen and Carbon Isotopes in Late Pleistocene Fossil Teeth

478

479 In their study of zinc isotopes in fossil tooth enamel from THM, Bourgon et al.³⁹
480 attempted to extract collagen from dentin for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. Of 72 samples, 23
481 were suitable for extraction, and only four specimens had sufficient collagen preservation
482 (i.e., C:N between 2.9 and 3.6^{83,84}) for stable isotope analysis. Collagen yield was also
483 relatively poor (<1 % for all samples). Thus, reconstruction of trophic levels based
484 $\delta^{15}\text{N}_{\text{collagen}}$ values was not feasible for this assemblage. In contrast, all THM fossil teeth
485 analyzed using the *oxidation-denitrification method* ($n = 10$) have nitrogen contents that
486 are comparable to both modern tooth enamel measured both in this study (Fig. 4) and to a
487 controlled feeding experiment with rodents (5.0 ± 1.0 nmol/mg; $n = 36$ ³⁵). If additional
488 exogenous N would have been added during fossilization, we would expect fossil
489 samples to have a higher N content than their modern counterparts. Alternatively, if
490 organic matter was degraded over time, we would expect a clear decrease in N content
491 with respect to modern samples^{37,85}. Both scenarios, i.e., addition of exogenous N or
492 degradation of organic matter, could result in corresponding, directional changes in
493 $\delta^{15}\text{N}_{\text{enamel}}$ values in relation to N content. However, the N content and $\delta^{15}\text{N}_{\text{enamel}}$ values of
494 the fossil samples are within the range of modern specimens and show no correlation (see
495 Fig. 4), indicating good enamel-bound organic matter preservation in our fossil dataset.

496 These observations are consistent with results of laboratory degradation experiments³⁷,
497 and with measurements of million-year-old marine microfossils^{38,86,87}, which suggest
498 that biomineral structures act as an effective physical barrier that protect organic matter
499 from degradation. Although it is a small dataset, the paired $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{15}\text{N}_{\text{dentin-collagen}}$
500 values for the four fossil specimens are also positively correlated, with a relationship
501 similar to that between $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{15}\text{N}_{\text{bone-collagen}}$ values in modern mammals (see Fig.
502 2 and Fig. S6).

503 Within the THM assemblage, fossil $\delta^{15}\text{N}_{\text{enamel}}$ values record trophic enrichment in
504 ^{15}N (i.e., low values for herbivores, intermediate values for omnivores, and a high value
505 for the carnivore; Fig. 5). While the current fossil dataset is too small to draw any strong
506 conclusions regarding the trophic structure of the THM fossil assemblage, these patterns
507 corroborate the good preservation of dietary N-isotope signals in tooth enamel for
508 samples in which the collagen is already degraded. Additionally, we observed some
509 interesting results that may be unique to this ecosystem. For example, the $\delta^{15}\text{N}_{\text{enamel}}$
510 values of the browsers were relatively high compared to those of the other herbivores,
511 deviating from the pattern of low $\delta^{15}\text{N}_{\text{enamel}}$ values observed for browsers in the modern
512 African fauna. African herbivores living in forests generally exhibit lower $\delta^{15}\text{N}_{\text{collagen}}$
513 values than herbivores from more open (i.e., grassland) environments^{8,42}. Thus, it is
514 plausible that the relatively higher $\delta^{15}\text{N}_{\text{enamel}}$ values of the browsers from THM may
515 reflect selective feeding behavior in the two analyzed individuals, as plant $\delta^{15}\text{N}$ values
516 are known to vary according to plant taxon, parts, and position in the forest canopy^{88,89}.
517 The $\delta^{13}\text{C}_{\text{enamel}}$ values reveal that, except for the two grazers, the analyzed fossil taxa from
518 THM lived and foraged in predominantly C_3 environments. This is consistent with the
519 ecology of the fauna present in the assemblage, and with what is known about the
520 environment in this region of Southeast Asia during the Late Pleistocene, which was
521 probably predominantly forested, albeit not as densely as a closed low-light tropical
522 rainforest^{39,90,91}.

523 $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{66}\text{Zn}_{\text{enamel}}$ values from the same individuals show a negative
524 correlation (Fig. 6). This is expected as $\delta^{66}\text{Zn}_{\text{enamel}}$ has been shown to decrease with
525 increasing trophic level^{39,92-94}. Encouragingly, reconstructed trophic positions for
526 omnivorous taxa based on the two isotope systems are in good agreement. Specifically,
527 $\delta^{15}\text{N}_{\text{enamel}}$ value(s) for the pigs are low, the bear is intermediate, and the macaque is high,
528 while the inverse is true for $\delta^{66}\text{Zn}_{\text{enamel}}$. Future studies that incorporate both $\delta^{15}\text{N}_{\text{enamel}}$ and
529 $\delta^{66}\text{Zn}_{\text{enamel}}$ may help us better resolve omnivores' dietary behavior, a task that is
530 particularly challenging given their broad resource use.

531

532 Conclusions

533

534 In this study, we present paired organic nitrogen and inorganic carbon isotopic
535 values measured in a single aliquot (5 to 7 mg) of tooth enamel. Importantly, our study
536 demonstrates that the $\delta^{15}\text{N}_{\text{enamel}}$ values of mammals from natural ecosystems record diet
537 and trophic behavior in the same manner as the classical dietary proxy of $\delta^{15}\text{N}_{\text{collagen}}$. The
538 $\delta^{15}\text{N}_{\text{enamel}}$ values of carnivores are elevated by 3 to 5 ‰ relative to herbivores and
539 omnivores in both modern and fossil food webs, which is comparable to the well-
540 established 3 to 4 ‰ enrichment in $\delta^{15}\text{N}$ per trophic level along food chains^{5-11,95}.

541 Furthermore, paired $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{15}\text{N}_{\text{bone-collagen}}$ values from the same individuals are
542 positively correlated. While more studies comparing bone, dentin, and enamel $\delta^{15}\text{N}$ from
543 the same individual will be useful in characterizing the exact nature of the relationship
544 between these different tissue types, the results of these analyses conclusively
545 demonstrate that $\delta^{15}\text{N}_{\text{enamel}}$ and mandibular $\delta^{15}\text{N}_{\text{bone-collagen}}$ record similar diet and trophic
546 information. Indeed, given what is known about the inherent variability in $\delta^{15}\text{N}$ related to
547 abiotic and biotic factors, the coherence of our modern dataset and the consistency of the
548 enrichment in ^{15}N between trophic levels in samples drawn from disparate localities is
549 remarkable, and demonstrates that $\delta^{15}\text{N}_{\text{enamel}}$ is a robust trophic proxy with great potential
550 for application in paleodietary studies.

551 The results of our analysis of $\delta^{15}\text{N}_{\text{enamel}}$ of enamel samples from modern and fossil
552 teeth are highly promising. In the archeological and paleontological record, the
553 degradation of organic matter (specifically collagen) is a fundamental limitation⁹⁶
554 restricting measurements to relatively young, well-preserved samples. Therefore, the
555 ability to measure the nitrogen isotope composition of the organic matter preserved in
556 diagenetically robust tooth enamel (for example, at THM) has the potential to be used to
557 investigate the trophic ecology of ancient or even extinct animals over time periods far
558 beyond the limit of collagen preservation. For example, dental wear, stone tools, and cut-
559 marks on fossil bones associated with early hominins suggest that members of the genus
560 *Australopithecus* may have engaged in meat consumption as early as 3 million years ago
561⁹⁷. However, no direct geochemical data exists to evaluate this claim, and the inference
562 that *Australopithecus* shaped and used tools to access animal resources prior to the
563 emergence of *Homo* is heavily debated⁹⁸. $\delta^{15}\text{N}_{\text{enamel}}$ values of early hominins and
564 associated fauna have the potential to shed new light on this debate by providing
565 geochemical evidence for the onset and intensification of animal-resource consumption
566 throughout human evolution.

567

568 **Materials and Methods**

569

570 Experimental Design

571

572 Tooth enamel ($n = 57$) and mandibular bone ($n = 33$) were sampled from 20
573 modern African mammalian taxa housed in the zoological collection at the University of
574 Hamburg. We targeted herbivores (including browsers, grazers, and mixed feeders),
575 omnivores, and carnivores to evaluate the effect of trophic level on $\delta^{15}\text{N}$ values. A
576 minimum of three individuals were sampled per taxon. Enamel from the third molar (M3)
577 was preferentially used whenever possible to avoid the effect of milk consumption which
578 typically results in ~2 to 3 ‰ higher $\delta^{15}\text{N}$ values in the tissues of nursing individuals
579 compared to the mother's tissues⁵². Specimens were sampled using a hand-held Dremel
580 with a diamond burr tip. For fossil specimens ($n = 10$; 9 taxa) from THM, a chip of tooth
581 enamel was crushed and ground to a fine powder in an agate mortar and pestle. All data
582 and associated information are presented in Tables 1, 2 and S1.

583

584 Tooth Enamel Nitrogen Isotope Measurement

585

586 Tooth enamel samples (5 to 7 mg) were measured for $\delta^{15}\text{N}_{\text{enamel}}$ in eight analytical
587 batches using the oxidation-denitrification method. $\delta^{15}\text{N}_{\text{enamel}}$ values of bacterially
588 converted N_2O were measured via gas chromatography-isotope ratio mass spectrometry
589 at the Max Planck Institute for Chemistry (MPIC, Mainz, Germany). The method used to
590 measure $\delta^{15}\text{N}$ values of tooth enamel is described only briefly here; for a detailed
591 description, Leichliter, et al. ³⁵ and references therein. The procedure consists of four
592 main steps: 1) 5 to 7 mg of tooth enamel powder is subjected to a reductive-oxidative
593 cleaning to remove exogenous organic matter ⁹⁹; 2) samples are demineralized, and all
594 endogenous organic matter (i.e., intra-and inter-crystalline bound N) is oxidized to nitrate
595 using a persulfate oxidizing reagent (0.67–0.70 g of four times re-crystallized potassium
596 persulfate added to 4 ml of 6.25 N NaOH solution in 95 ml Milli-Q water) ¹⁰⁰, 3) nitrate
597 is quantitatively converted to N_2O via the ‘denitrifier’ method ¹⁰¹, and 4) sample-derived
598 N_2O is extracted, and its nitrogen isotopic composition is measured on a custom system
599 online to a Thermo ScientificTM 253 Plus isotope ratio mass spectrometer (IRMS).
600 Isobaric interference with CO_2 , is dealt with both using cryo-isolation and two sets of gas
601 chromatography columns as detailed in ^{101,102}. These processes result in full separation of
602 the CO_2 peak from the N_2O peak, which can be seen in each sample chromatogram.
603 International (USGS 40, USGS 65, USGS 41, USGS 34, IAEA-NO-3) and in-house
604 standards (PO-1, LO-1, AG-Lox, Noto-1; see ³⁵ for details) are included in every run and
605 each step of this process, allowing us to monitor instrument stability, and evaluate the
606 possibility of matrix-based effects during cleaning. Blank N concentration and $\delta^{15}\text{N}$ were
607 measured for each batch, and the sample N content and $\delta^{15}\text{N}$ values were corrected using
608 the blank measurements of the associated batch. Samples were measured in duplicate or
609 triplicate (resulting in a total of 115 individual measurements) and in separate batches
610 whenever possible. Blank N content was between 0.3–0.5 nmol/ml, resulting in an
611 average blank contribution of 3 % or less. Inter-batch precision ($\pm 1\sigma$) in $\delta^{15}\text{N}$ for in-
612 house standards was 0.3 ‰ for coral standards ($n = 43$) and 0.5 ‰ for tooth enamel
613 standards ($n = 41$) across all analytical batches.

614

615 Tooth Enamel Carbon Isotope Measurement

616

617 Small aliquots (50–100 μg) of untreated enamel powder were measured using
618 high-precision continuous-flow mass spectrometry. $\delta^{13}\text{C}_{\text{enamel}}$ analyses were performed at
619 the MPIC on a Thermo Delta-V continuous flow mass spectrometer coupled to a
620 Gasbench II gas preparation system, equipped with a liquid nitrogen cryogenic trap (i.e.,
621 the ‘cold trap method’) ¹⁰³. Untreated enamel powder was reacted with >99 % H_3PO_4 for
622 90 minutes at 70 °C before the resulting CO_2 was introduced to the continuous flow
623 system. Isotope data are calculated by direct comparison to ten replicates of a tooth
624 enamel standard (AG-Lox) analyzed in each batch. Samples were analyzed in a total of
625 seven batches, and samples were measured in duplicate or triplicate (where possible) in
626 different batches. A logarithmic fit through the isotope ratios *versus* peak size for the
627 AG-Lox replicates was used to eliminate fractionation effects due to sample size. After
628 these corrections, the reproducibility of international and in-house carbonate standards
629 (IAEA-603, NBS 18, VICS) as well as sedimentary phosphate (NIST SRM 120c) was
630 better than 0.1 ‰ (1σ).

631

632 Bone Collagen Carbon and Nitrogen Isotope Measurement

633

634 Up to 150 mg mandibular bone powder was demineralized with 0.5 M HCl for at
635 least 24 hours at 4 °C. Samples were then centrifuged, the supernatant discarded, and the
636 remaining collagen was rinsed three times with deionized water. Afterward, a pH of 2–3
637 was obtained by adding a few drops of 0.5 M HCl. The sample was then gelatinized by
638 heating collagen to 70 °C for 48 hours. After 48 hours, gelatinized collagen was filtered
639 (using 0.55 µm pore size midi-filters), centrifuged, the supernatant was discarded, and the
640 remaining collagen was freeze-dried. Collagen yield was between 5–25 %.

641 $\delta^{13}\text{C}_{\text{bone-collagen}}$ and $\delta^{15}\text{N}_{\text{bone-collagen}}$ were measured in the same aliquot of collagen at the
642 Institute for Organic Chemistry, Johannes Gutenberg University Mainz, Germany, using
643 an IsoPrime™ High Performance Stable Isotope Ratio Mass Spectrometer, GV
644 Instruments. Each run included the following standards; 10 Sulfanilamide replicates,
645 three replicates each of IAEA-N1, IAEA-N2, IAEA-CH6, and IAEA-CH7, and two
646 replicates of bovine liver (NIST SRM 1577). For collagen measurements, 1 to 2 mg of
647 extracted collagen was analyzed in replicate for each specimen. Analytical precision was
648 better than ± 0.25 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

649

650 Statistical Analyses

651

652 Isotopic datasets were evaluated to determine if the data was normally distributed
653 and if variance was equal between groups using Levene's test. A non-parametric
654 Kruskal-Wallis test followed by a Dunn's post-hoc test with a Bonferroni correction
655 for pairwise comparisons was used when the requirements for ANOVA were not
656 satisfied, otherwise Analysis of variance (ANOVA; one-tailed) was used to identify
657 statistically significant differences in isotopic values between groups for modern African
658 fauna. Where ANOVA indicated statistical significance, pairwise comparisons were
659 made using a Tukey-Kramer HSD post-hoc test to determine which groups differed from
660 one another in their isotope values. Statistically significant relationships between paired
661 isotopic values from the same individuals were determined using Pearson's correlation
662 (two-tailed; $\delta^{15}\text{N}_{\text{enamel}}$ and $\delta^{15}\text{N}_{\text{collagen}}$) and Spearman Rank correlation (two-tailed;
663 $\delta^{13}\text{C}_{\text{enamel}}$ and $\delta^{13}\text{C}_{\text{collagen}}$). Statistical analyses were performed using Paleontological
664 Statistics Version 4.09 (PAST4) and JMP®, Version 16 statistical software using an
665 alpha level for significance of 0.05. Detailed results of pairwise comparisons can be
666 found in Table S2, S3, and S4.

667

668 Data Availability Statement

669

670 SI Methods includes additional information on analytical procedures, data
671 processing, sampling locations, and ecological information for the analyzed taxa used in
672 this work. All $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values are provided in the main text as well as in
673 Supplementary Information and an associated excel database.

674

675 **Acknowledgments**

676

677 We thank F. Rubach, S. Brömme, M. Schmitt, and B. Hinnenberg (Climate Geochemistry
678 Department, Max Planck Institute for Chemistry, Germany) for technical support, D.
679 Winkler (Department of Natural and Environmental Studies, University of Tokyo, Japan)
680 and T. Kaiser (Center of Natural History, University of Hamburg, Germany) for
681 assistance in sampling the modern African fauna, as well as J. Broska and P. Held for
682 collagen preparation and $\delta^{13}\text{C}/\delta^{15}\text{N}$ analysis of bone collagen. We also thank S.
683 Luangaphay (Department of National Heritage, Ministry of Information and Culture in
684 Vientiane, Laos) for the authorization to study the published fauna of Tam Hay Marklot,
685 A.-M. Bacon and P.-O. Antoine who analyzed the fauna, and researchers who are part of
686 the Laos project and participated to the fieldwork (F. Demeter, L. Shackelford, P.
687 Düringer, J.-L. Ponche, Q. Boesch, E. Patole-Edoumba, T.E. Dunn, A. Zachwieja, E.
688 Suzzoni, S. Frangeul, S. Duangthongchit, T. Sayavonkhamdy, P. Sichanthongtip, D.
689 Sihanam, and V. Souksavatdy). This study was funded by the Max Planck Society (A.
690 Martínez-García), the European Research Council (ERC) under the European Union's
691 Horizon 2020 Research and Innovation Programme (Grant Agreement 681450) (ERC
692 Consolidator Grant Agreement to T. Tütken); the Paul Crutzen Nobel Prize fellowship of
693 the Max Planck Society to N.N. Duprey; and the Deutsche Forschungsgemeinschaft
694 (DFG) Grant LU 2199/1-2 and the Emmy Noether Fellowship LU 2199/2-1 to T.
695 Lüdecke. Prior method development work was supported by the Scott Fund of the
696 Department of Geosciences, Princeton University (to D. Sigman).

697

698 **Author Contributions:** Author contributions: J.L., T.L., T.T., and A.M.G. designed the
699 research; J.L. and T.L. performed enamel N isotope analyses in the laboratory of A.M.G
700 with assistance from A.F. and N.D.; TT contributed the bone collagen C and N isotope
701 data; J.L. and T.L. performed C and O isotope analyses in the laboratory of H.V., J.L. and
702 T.L. analyzed the data; S.V. and A.M.B. are part of the LAOS project; J.L. and T.L. wrote
703 the paper. All authors contributed to the interpretation of the data and provided input to
704 the final manuscript.

705 References

706

- 707 1 Cerling, T. E. & Harris, J. M. Carbon isotope fractionation between diet and bioapatite in
 708 ungulate mammals and implications for ecological and paleoecological studies.
 709 *Oecologia* **120**, 347-363, doi:10.1007/s004420050868 (1999).
- 710 2 Balter, V. *et al.* Ecological and physiological variability of Sr/Ca and Ba/Ca in mammals
 711 of West European mid-Würmian food webs. *Palaeogeography, Palaeoclimatology,*
 712 *Palaeoecology* **186**, 127-143 (2002).
- 713 3 Jaouen, K. & Pons, M.-L. Potential of non-traditional isotope studies for bioarchaeology.
 714 *Archaeological and Anthropological Sciences* **9**, 1389-1404, doi:10.1007/s12520-016-
 715 0426-9 (2017).
- 716 4 Martin, J., Tacail, T. & Balter, V. Non-traditional isotope perspectives in vertebrate
 717 palaeobiology. *Palaeontology* **60**, 485-502, doi:10.1111/pala.12300 (2017).
- 718 5 Schoeninger, M. J. Stable Isotope Analyses and the Evolution of Human Diets. *Annual*
 719 *Review of Anthropology* **43**, 413-430, doi:10.1146/annurev-anthro-102313-025935
 720 (2014).
- 721 6 Minagawa, M. & Wada, E. Stepwise enrichment of ¹⁵N along food chains: further
 722 evidence and the relation between ¹⁵N and animal age. *Geochimica et Cosmochimica*
 723 *Acta* **48**, 1135-1140 (1984).
- 724 7 Schoeninger, M. J. & DeNiro, M. J. Nitrogen and carbon isotopic composition of bone
 725 collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta* **48**, 625-
 726 639, doi:10.1016/0016-7037(84)90091-7 (1984).
- 727 8 Ambrose, S. H. & DeNiro, M. J. The isotopic ecology of East-African mammals.
 728 *Oecologia* **69**, 395-406, doi:10.1007/bf00377062 (1986).
- 729 9 Ambrose, S. H. Stable carbon and nitrogen isotope analysis of human and animal diet in
 730 Africa. *Journal of Human Evolution* **15**, 707-731 (1986).
- 731 10 Sealy, J. C., van der Merwe, N. J., Thorp, J. A. L. & Lanham, J. L. Nitrogen isotopic
 732 ecology in southern Africa: Implications for environmental and dietary tracing.
 733 *Geochimica et Cosmochimica Acta* **51**, 2707-2717, doi:10.1016/0016-7037(87)90151-7
 734 (1987).
- 735 11 DeNiro, M. J. & Epstein, S. Influence of diet on the distribution of nitrogen isotopes in
 736 animals. *Geochimica et Cosmochimica Acta* **45**, 341-351, doi:10.1016/0016-
 737 7037(81)90244-1 (1981).
- 738 12 Caut, S., Angulo, E. & Courchamp, F. Variation in discrimination factors ($\Delta^{15}\text{N}$ and
 739 $\Delta^{13}\text{C}$): The effect of diet isotopic values and applications for diet reconstruction. *Journal*
 740 *of Applied Ecology* **46**, 443-453, doi:10.1111/j.1365-2664.2009.01620.x (2009).
- 741 13 Bocherens, H. & Drucker, D. Trophic level isotopic enrichment of carbon and nitrogen in
 742 bone collagen: case studies from recent and ancient terrestrial ecosystems. *International*
 743 *Journal of Osteoarchaeology* **13**, 46-53 (2003).
- 744 14 Britton, K., Gaudzinski-Windheuser, S., Roebroeks, W., Kindler, L. & Richards, M. P.
 745 Stable isotope analysis of well-preserved 120,000-year-old herbivore bone collagen from
 746 the Middle Palaeolithic site of Neumark-Nord 2, Germany reveals niche separation
 747 between bovids and equids. *Palaeogeography, Palaeoclimatology, Palaeoecology* **333-**
 748 **334**, 168-177, doi:10.1016/j.palaeo.2012.03.028 (2012).
- 749 15 Jaouen, K. *et al.* Exceptionally high $\delta^{15}\text{N}$ values in collagen single amino acids confirm
 750 Neandertals as high-trophic level carnivores. *Proceedings of the National Academy of*
 751 *Sciences of the United States of America* **116**, 4928-4933, doi:10.1073/pnas.1814087116
 752 (2019).

- 753 16 DeNiro, M. J. Postmortem preservation and alteration of in vivo bone collagen isotope
754 ratios in relation to palaeodietary reconstruction. *Nature* **317**, 806-809 (1985).
- 755 17 Krueger, H. W. Exchange of carbon with biological apatite. *Journal of Archaeological*
756 *Science* **18**, 355-361, doi:[https://doi.org/10.1016/0305-4403\(91\)90071-V](https://doi.org/10.1016/0305-4403(91)90071-V) (1991).
- 757 18 Driessens, F. C. M., van Dijk, J. W. E. & Borggreven, J. M. P. M. Biological calcium
758 phosphates and their role in the physiology of bone and dental tissues I. Composition and
759 solubility of calcium phosphates. *Calcified Tissue Research* **26**, 127-137,
760 doi:10.1007/BF02013247 (1978).
- 761 19 He, L. H. & Swain, M. V. Understanding the mechanical behaviour of human enamel
762 from its structural and compositional characteristics. *Journal of the Mechanical Behavior*
763 *of Biomedical Materials* **1**, 18-29, doi:<https://doi.org/10.1016/j.jmbbm.2007.05.001>
764 (2008).
- 765 20 Teruel, J. d. D., Alcolea, A., Hernández, A. & Ruiz, A. J. O. Comparison of chemical
766 composition of enamel and dentine in human, bovine, porcine and ovine teeth. *Archives*
767 *of Oral Biology* **60**, 768-775, doi:<https://doi.org/10.1016/j.archoralbio.2015.01.014>
768 (2015).
- 769 21 Ostrom, P. H., Macko, S. A., Engel, M. H. & Russell, D. A. Assessment of trophic
770 structure of Cretaceous communities based on stable nitrogen isotope analyses. *Geology*
771 **21**, 491-494, doi:10.1130/0091-7613(1993)021<0491:AOTSOC>2.3.CO;2 (1993).
- 772 22 Ostrom, P., Macko, S., Engel, M., Silfer, J. & Russell, D. Geochemical characterization
773 of high molecular weight material isolated from Late Cretaceous fossils. *Organic*
774 *Geochemistry* **16**, 1139-1144 (1990).
- 775 23 Palmqvist, P., Gröcke, D. R., Arribas, A. & Fariña, R. A. Paleocological reconstruction
776 of a lower Pleistocene large mammal community using biogeochemical ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$,
777 Sr:Zn) and ecomorphological approaches. *Paleobiology* **29**, 205-229, doi:10.1666/0094-
778 8373(2003)029<0205:PROALP>2.0.CO;2 (2003).
- 779 24 Jones, A. M. *et al.* Biogeochemical data from well preserved 200 ka collagen and skeletal
780 remains. *Earth and Planetary Science Letters* **193**, 143-149,
781 doi:[https://doi.org/10.1016/S0012-821X\(01\)00474-5](https://doi.org/10.1016/S0012-821X(01)00474-5) (2001).
- 782 25 Lee-Thorp, J. & Van der Merwe, N. J. Carbon isotope analysis of fossil bone apatite.
783 *South African Journal of Science* **83**, 712-715 (1987).
- 784 26 Wang, Y. & Cerling, T. E. A model of fossil tooth and bone diagenesis: implications for
785 paleodiet reconstruction from stable isotopes. *Palaeogeography, Palaeoclimatology,*
786 *Palaeoecology* **107**, 281-289, doi:[https://doi.org/10.1016/0031-0182\(94\)90100-7](https://doi.org/10.1016/0031-0182(94)90100-7) (1994).
- 787 27 Robinson, C., Kirkham, J., Brookes, S. J., Bonass, W. A. & Shore, R. C. The chemistry
788 of enamel development. *International Journal of Developmental Biology* **39**, 145-152,
789 doi:10.1387/ijdb.7626401 (1995).
- 790 28 Koch, P. L., Tuross, N. & Fogel, M. L. The effects of sample treatment and diagenesis on
791 the isotopic integrity of carbonate in biogenic hydroxylapatite. *Journal of Archaeological*
792 *Science* **24**, 417-429, doi:10.1006/jasc.1996.0126 (1997).
- 793 29 Zazzo, A., Lécuyer, C., Sheppard, S. M. F., Grandjean, P. & Mariotti, A. Diagenesis and
794 the reconstruction of paleoenvironments: A method to restore original $\delta^{18}\text{O}$ values of
795 carbonate and phosphate from fossil tooth enamel. *Geochimica et Cosmochimica Acta*
796 **68**, 2245-2258, doi:10.1016/j.gca.2003.11.009 (2004).
- 797 30 Robinson, C. Enamel maturation: A brief background with implications for some enamel
798 dysplasias. *Frontiers in Physiology* **5**, doi:10.3389/fphys.2014.00388 (2014).
- 799 31 Savory, A. & Brudevold, F. The Distribution of Nitrogen in Human Enamel. *Journal of*
800 *Dental Research* **38**, 436-442, doi:10.1177/00220345590380030301 (1959).

801 32 Polissar, P. J., Fulton, J. M., Junium, C. K., Turich, C. C. & Freeman, K. H. Measurement
802 of ^{13}C and ^{15}N Isotopic Composition on Nanomolar Quantities of C and N. *Analytical*
803 *Chemistry* **81**, 755-763, doi:10.1021/ac801370c (2009).

804 33 Knapp, A. N., Sigman, D. M. & Lipschultz, F. N isotopic composition of dissolved
805 organic nitrogen and nitrate at the Bermuda Atlantic Time-series study site. *Global*
806 *Biogeochemical Cycles* **19**, 1-15, doi:10.1029/2004GB002320 (2005).

807 34 Fulton, J. M., Arthur, M. A., Thomas, B. & Freeman, K. H. Pigment carbon and nitrogen
808 isotopic signatures in euxinic basins. *Geobiology* **16**, 429-445,
809 doi:<https://doi.org/10.1111/gbi.12285> (2018).

810 35 Leichliter, J. N. *et al.* Nitrogen isotopes in tooth enamel record diet and trophic level
811 enrichment: Results from a controlled feeding experiment. *Chemical Geology* **563**,
812 120047-120047, doi:10.1016/j.chemgeo.2020.120047 (2021).

813 36 Lee-Thorp, J. On Isotopes and Old Bones. *Archaeometry* **50**, 925-950,
814 doi:10.1111/j.1475-4754.2008.00441.x (2008).

815 37 Martinez-Garcia, A. *et al.* Laboratory Assessment of the Impact of Chemical Oxidation,
816 Mineral Dissolution, and Heating on the Nitrogen Isotopic Composition of Fossil-bound
817 Organic Matter. *Geochemistry, Geophysics, Geosystems* (2022).

818 38 Kast, E., R. *et al.* Cenozoic megatooth sharks occupied extremely high trophic positions.
819 *Science Advances* **8**, eabl6529, doi:10.1126/sciadv.abl6529 (2022).

820 39 Bourgon, N. *et al.* Zinc isotopes in Late Pleistocene fossil teeth from a Southeast Asian
821 cave setting preserve paleodietary information. *Proceedings of the National Academy of*
822 *Sciences of the United States of America* **117**, 4675-4681, doi:10.1073/pnas.1911744117
823 (2020).

824 40 Cerling, Harris, J. M. & Passey, B. H. Diets of East African bovidae based on stable
825 isotope analysis. *Journal of Mammalogy* **84**, 456-470, doi:10.2307/1383890 (2003).

826 41 Friedli, H., Löttscher, H., Oeschger, H., Siegenthaler, U. & Stauffer, B. Ice core record of
827 the $^{13}\text{C}/^{12}\text{C}$ ratio of atmospheric CO_2 in the past two centuries. *Nature* **324**, 237-238,
828 doi:10.1038/324237a0 (1986).

829 42 Ambrose, S. H. Effects of diet, climate and physiology on nitrogen isotope abundances in
830 terrestrial foodwebs. *Journal of Archaeological Science* **18**, 293-317, doi:10.1016/0305-
831 4403(91)90067-Y (1991).

832 43 Codron, D., Codron, J., Sponheimer, M. & Clauss, M. Within-population isotopic niche
833 variability in savanna mammals: disparity between carnivores and herbivores. *Frontiers*
834 *in Ecology and Evolution* **4**, 15, doi:10.3389/fevo.2016.00015 (2016).

835 44 Heaton, T. H. E., Vogel, J. C., Von La Chevallerie, G. & Collett, G. Climatic influence
836 on the isotopic composition of bone nitrogen. *Nature* **322**, 822-823,
837 doi:10.1038/322822a0 (1986).

838 45 Amundson, R. *et al.* Global patterns of the isotopic composition of soil and plant
839 nitrogen. *Global Biogeochemical Cycles* **17**, 5, doi:10.1029/2002GB001903 (2003).

840 46 Männel, T., Auerswald, K. & Schnyder, H. Altitude gradients of grassland carbon and
841 nitrogen isotope composition are recorded in hair of grazers. *Global Ecology and*
842 *Biogeography* **16**, 583-592, doi:10.1111/j.1466-8238.2007.00322.x (2007).

843 47 Hartman, G. Are elevated $\delta^{15}\text{N}$ values in herbivores in hot and arid environments caused
844 by diet or animal physiology? *Functional Ecology* **25**, 122-131, doi:10.1111/J.1365-
845 2435.2010.01782.X (2011).

846 48 Codron, J. *et al.* Taxonomic, anatomical, and spatio-temporal variations in the stable
847 carbon and nitrogen isotopic compositions of plants from an African savanna. *Journal of*
848 *Archaeological Science* **32**, 1757-1772, doi:10.1016/j.jas.2005.06.006 (2005).

849 49 Manolagas, S. C. Birth and death of bone cells: basic regulatory mechanisms and
850 implications for the pathogenesis and treatment of osteoporosis. *Endocrine reviews* **21**,
851 115-137 (2000).

852 50 Ungar, P. S. *Mammal Teeth: Origin, Evolution, and Diversity*. (Johns Hopkins
853 University Press, 2010).

854 51 Balasse, M., Bocherens, H. & Mariotti, A. Intra-bone variability of collagen and apatite
855 isotopic composition used as evidence of a change of diet. *Journal of Archaeological*
856 *Science* **26**, 593-598 (1999).

857 52 Fuller, B. T., Fuller, J. L., Harris, D. A. & Hedges, R. E. M. Detection of breastfeeding
858 and weaning in modern human infants with carbon and nitrogen stable isotope ratios.
859 *American Journal of Physical Anthropology* **129**, 279-293,
860 doi:<https://doi.org/10.1002/ajpa.20249> (2006).

861 53 Castiblanco, G. A. *et al.* Identification of proteins from human permanent erupted
862 enamel. *European Journal of Oral Sciences* **123**, 390-395, doi:10.1111/eos.12214 (2015).

863 54 Lacruz, R. S., Habelitz, S., Wright, J. T. & Paine, M. L. Dental enamel formation and
864 implications for oral health and disease. *Physiological Reviews* **97**, 939-993,
865 doi:10.1152/physrev.00030.2016 (2017).

866 55 Welker, F. *et al.* The dental proteome of *Homo antecessor*. *Nature* **580**, 235-238,
867 doi:10.1038/s41586-020-2153-8 (2020).

868 56 Murphy, B. P. & Bowman, D. M. J. S. Kangaroo metabolism does not cause the
869 relationship between bone collagen $\delta^{15}\text{N}$ and water availability. *Functional Ecology* **20**,
870 1062-1069, doi:10.1111/J.1365-2435.2006.01186.X (2006).

871 57 Ambrose, S. H. & DeNiro, M. J. Reconstruction of African human diet using bone
872 collagen carbon and nitrogen isotope ratios. *Nature* **319**, 321-324, doi:10.1038/319321a0
873 (1986).

874 58 Sponheimer, M. *et al.* Nitrogen isotopes in mammalian herbivores: Hair $\delta^{15}\text{N}$ values
875 from a controlled feeding study. *International Journal of Osteoarchaeology* **13**, 80-87,
876 doi:10.1002/oa.655 (2003).

877 59 Fuller, B. T. *et al.* Nitrogen balance and $\delta^{15}\text{N}$: why you're not what you eat during
878 pregnancy. *Rapid Communications in Mass Spectrometry* **18**, 2889-2896,
879 doi:10.1002/rcm.1708 (2004).

880 60 Robbins, C. T., Felicetti, L. A. & Sponheimer, M. The effect of dietary protein quality on
881 nitrogen isotope discrimination in mammals and birds. *Oecologia* **144**, 534-540,
882 doi:10.1007/s00442-005-0021-8 (2005).

883 61 Cantalapiedra-Hijar, G. *et al.* Diet–animal fractionation of nitrogen stable isotopes
884 reflects the efficiency of nitrogen assimilation in ruminants. *British Journal of Nutrition*
885 **113**, 1158-1169, doi:10.1017/S0007114514004449 (2015).

886 62 Hempson, G., Archibald, S. & Bond, W. A continent-wide assessment of the form and
887 intensity of large mammal herbivory in Africa. *Science* **350**, 1056-1061,
888 doi:10.1126/science.aac7978 (2015).

889 63 Ambrose, S. H. in *Biogeochemical Approaches to Paleodietary Analysis* 243-259
890 (2002).

891 64 Vogel, J. C., Talma, S., Hall-Martin, A. J. & Viljoen, P. J. Carbon and nitrogen isotopes
892 in elephants. *South African Journal of Science* **86**, 147-150 (1990).

893 65 Tieszen, L., Boutton, T., Ottichilo, W., Nelson, D. & Brandt, D. An assessment of long-
894 term food habits of Tsavo elephants based on stable carbon and nitrogen isotope ratios of
895 bone collagen. *African Journal of Ecology* **27**, 219-226, doi:10.1111/j.1365-
896 2028.1989.tb01015.x (2008).

- 897 66 Koch, P. L. *et al.* Isotopic tracking of change in diet and habitat use in african elephants.
898 *Science* **267**, 1340-1343, doi:doi:10.1126/science.267.5202.1340 (1995).
- 899 67 Sponheimer, M. *et al.* Nitrogen isotopes in mammalian herbivores: hir $\delta^{15}\text{N}$ values from a
900 controlled feeding study. *International Journal of Osteoarchaeology* **13**, 80-87,
901 doi:10.1002/oa.655 (2003).
- 902 68 Codron, D., Lee-Thorp, J. A., Sponheimer, M., Ruitter, D. d. & Codron, J. Inter- and
903 intrahabitat dietary variability of chacma baboons (*Papio ursinus*) in South African
904 savannas based on fecal $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\%N$. *American Journal of Physical*
905 *Anthropology* **129**, 204-214, doi:10.1002/AJPA.20253 (2006).
- 906 69 Robinson, T. F. *et al.* Digestibility and nitrogen retention in llamas and goats fed alfalfa,
907 C_3 grass, and C_4 grass hays. *Small Ruminant Research* **64**, 162-168 (2006).
- 908 70 Mekota, A. M., Grupe, G., Ufer, S. & Cuntz, U. Serial analysis of stable nitrogen and
909 carbon isotopes in hair: Monitoring starvation and recovery phases of patients suffering
910 from anorexia nervosa. *Rapid Communications in Mass Spectrometry* **20**, 1604-1610,
911 doi:10.1002/rcm.2477 (2006).
- 912 71 Reynard, L. & Tuross, N. The known, the unknown and the unknowable: weaning times
913 from archaeological bones using nitrogen isotope ratios. *Journal of Archaeological*
914 *Science* **53**, 618-625, doi:10.1016/j.jas.2014.11.018 (2015).
- 915 72 Swedell, L. in *Primates in Perspective* (ed C.J. Campell) 241-277 (Oxford University
916 Press, 2011).
- 917 73 Altman, S. A. *Foraging for survival: yearling baboons in Africa* (The University of
918 Chicago Press, 1998).
- 919 74 Byrne, R. W., Whiten, A., Henzi, S. P. & McCulloch, F. M. Nutritional constraints on
920 mountain baboons (*Papio ursinus*): Implications for baboon socioecology. *Behavioral*
921 *Ecology and Sociobiology* **33**, 233-246, doi:10.1007/BF02027120 (1993).
- 922 75 Barton, R. A., Whiten, A., Strum, S. C., Byrne, R. W. & Simpson, A. J. Habitat use and
923 resource availability in baboons. *Animal Behaviour* **43**, 831-844, doi:10.1016/S0003-
924 3472(05)80206-4 (1992).
- 925 76 DeVore, I. & Hall, K. R. L. in *Primate Behavior. Field Studies of Monkeys and Apes* (ed
926 I. De Vore) (Holt, Rinehart and Winston, 1992).
- 927 77 Codron, D., Lee-Thorp, J. A., Sponheimer, M., de Ruitter, D. & Codron, J. Inter- and
928 intrahabitat dietary variability of chacma baboons (*Papio ursinus*) in South African
929 savannas based on fecal $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\%N$. *American journal of physical anthropology*
930 **129**, 204-214, doi:10.1002/ajpa.20253 (2006).
- 931 78 Hamilton, W. J. & Busse, C. Social dominance and predatory behavior of chacma
932 baboons. *Journal of Human Evolution* **11**, 567-573, doi:[https://doi.org/10.1016/S0047-
933 2484\(82\)80003-1](https://doi.org/10.1016/S0047-2484(82)80003-1) (1982).
- 934 79 Rhine, R. J., Norton, G. W., Wynn, G. M., Wynn, R. D. & Rhine, H. B. Insect and meat
935 eating among infant and adult baboons (*Papio cynocephalus*) of Mikumi National Park,
936 Tanzania. *American Journal of Physical Anthropology* **70**, 105-118,
937 doi:<https://doi.org/10.1002/ajpa.1330700115> (1986).
- 938 80 Binder, W. J. & Van Valkenburgh, B. Development of bite strength and feeding
939 behaviour in juvenile spotted hyenas (*Crocuta crocuta*). *Journal of Zoology* **252**, 273-283
940 (2000).
- 941 81 Hayward, M. W. *et al.* Prey preferences of the leopard (*Panthera pardus*). *Journal of*
942 *Zoology* **270**, 298-313 (2006).
- 943 82 Crossey, B., Chimimba, C., du Plessis, C., Ganswindt, A. & Hall, G. African wild dogs
944 (*Lycaon pictus*) show differences in diet composition across landscape types in Kruger

945 National Park, South Africa. *Journal of Mammalogy* **102**, 1211-1221,
946 doi:10.1093/jmammal/gyab087 (2021).

947 83 Van Klinken, G. J. Bone collagen quality indicators for palaeodietary and radiocarbon
948 measurements. *Journal of Archaeological Science* **26**, 687-695,
949 doi:10.1006/jasc.1998.0385 (1999).

950 84 DeNiro, M. J. Postmortem preservation and alteration of in vivo bone collagen isotope
951 ratios in relation to palaeodietary reconstruction. *Nature* **1985 317:6040** **317**, 806-809,
952 doi:10.1038/317806a0 (1985).

953 85 Lueders-Dumont, J. A., Wang, X. T., Jensen, O. P., Sigman, D. M. & Ward, B. B.
954 Nitrogen isotopic analysis of carbonate-bound organic matter in modern and fossil fish
955 otoliths. *Geochimica et Cosmochimica Acta* **224**, 200-222,
956 doi:10.1016/J.GCA.2018.01.001 (2018).

957 86 Kast, E. R. *et al.* Nitrogen isotope evidence for expanded ocean suboxia in the early
958 Cenozoic. *Science* **364**, 386-389, doi:10.1126/science.aau5784 (2019).

959 87 Auderset, A. *et al.* Enhanced ocean oxygenation during Cenozoic warm periods. in press.
960 Doi: 10.1038/s41586-022-05017-0. *Nature*, doi:10.1038/s41586-022-05017-0 (2022).

961 88 Roberts, P. *et al.* Stable carbon, oxygen, and nitrogen, isotope analysis of plants from a
962 South Asian tropical forest: Implications for primatology. *American Journal of*
963 *Primatology* **79**, doi:10.1002/ajp.22656 (2017).

964 89 Blumenthal, S. A., Rothman, J. M., Chritz, K. L. & Cerling, T. E. Stable isotopic
965 variation in tropical forest plants for applications in primatology. *American Journal of*
966 *Primatology* **78**, 1041-1054, doi:10.1002/AJP.22488 (2016).

967 90 Milano, S. *et al.* Environmental conditions framing the first evidence of modern humans
968 at Tam Pà Ling, Laos: A stable isotope record from terrestrial gastropod carbonates.
969 *Palaeogeography, Palaeoclimatology, Palaeoecology* **511**, 352-363,
970 doi:10.1016/j.palaeo.2018.08.020 (2018).

971 91 Louys, J. & Roberts, P. Environmental drivers of megafauna and hominin extinction in
972 Southeast Asia. *Nature* **586**, 402-406, doi:10.1038/s41586-020-2810-y (2020).

973 92 Bourgon, N. *et al.* Trophic ecology of a Late Pleistocene early modern human from
974 tropical Southeast Asia inferred from zinc isotopes. *Journal of Human Evolution* **161**,
975 103075, doi:<https://doi.org/10.1016/j.jhevol.2021.103075> (2021).

976 93 McCormack, J. *et al.* Zinc isotopes from archaeological bones provide reliable trophic
977 level information for marine mammals. *Communications Biology* **4**, 683,
978 doi:10.1038/s42003-021-02212-z (2021).

979 94 Jaouen, K. *et al.* Dynamic homeostasis modeling of Zn isotope ratios in the human body.
980 *Metallomics* **11**, 1049-1059, doi:10.1039/c8mt00286j (2019).

981 95 Caut, S., Angulo, E. & Courchamp, F. Variation in discrimination factors ($\Delta^{15}\text{N}$ and
982 $\Delta^{13}\text{C}$): The effect of diet isotopic values and applications for diet reconstruction. *J. Appl.*
983 *Ecol.* **46**, doi:10.1111/j.1365-2664.2009.01620.x (2009).

984 96 Grupe, G. & Turban-Just, S. Amino acid composition of degraded matrix collagen from
985 archaeological human bone. *Anthropologischer Anzeiger*, 213-226 (1998).

986 97 McPherron, S. P. *et al.* Evidence for stone-tool-assisted consumption of animal tissues
987 before 3.39 million years ago at Dikika, Ethiopia. *Nature* **466**, 857-860,
988 doi:10.1038/nature09248 (2010).

989 98 Sahle, Y., Zaatari, S. E. & White, T. D. Hominid butchers and biting crocodiles in the
990 African plio-pleistocene. *Proceedings of the National Academy of Sciences of the United*
991 *States of America* **114**, 13164-13169, doi:10.1073/PNAS.1716317114/-
992 /DCSUPPLEMENTAL (2017).

993 99 Ren, H. *et al.* Foraminiferal isotope evidence of reduced nitrogen fixation in the ice age
994 Atlantic ocean. *Science* **323**, 244-248, doi:10.1126/science.1165787 (2009).
995 100 Knapp, A., Sigman, D. & Lipschultz, F. N isotopic composition of dissolved organic
996 nitrogen and nitrate at the Bermuda Atlantic time-series study site. *Global*
997 *Biogeochemical Cycles* **19(1)**, doi:10.1029/2004GB002320 (2005).
998 101 Sigman, D. M. *et al.* A bacterial method for the nitrogen isotopic analysis of nitrate in
999 seawater and freshwater. *Analytical Chemistry* **73**, 4145-4153, doi:10.1021/ac010088e
1000 (2001).
1001 102 Weigand, M. A., Foriel, J., Barnett, B., Oleynik, S. & Sigman, D. M. Updates to
1002 instrumentation and protocols for isotopic analysis of nitrate by the denitrifier method.
1003 *Rapid Communications in Mass Spectrometry* **30**, 1365-1383, doi:10.1002/rcm.7570
1004 (2016).
1005 103 Vonhof, H. *et al.* High-precision stable isotope analysis of <5 µg CaCO₃ samples by
1006 continuous-flow mass spectrometry. *Rapid Communications in Mass Spectrometry* **34**,
1007 e8878, doi:10.1002/rcm.8878 (2020).
1008 104 J. Hamilton III, W. Namib desert chacma baboon (*Papio ursinus*) use of food and water
1009 resources during a food shortage. *Madoqua* **1986**, 397-407,
1010 doi:doi:10.10520/AJA10115498_477 (1986).
1011 105 Bothma, J. d. P. Water-use by southern Kalahari leopards. *South African Journal of*
1012 *Wildlife Research* **35**, 131-137 (2005).
1013 106 Wilson, D. E. & Mittermeier, R. A. *Handbook of the Mammals of the World*. Vol. 1
1014 (Lynx Wsicions, 2009).

1015

1016 **Table 1.** List of all 57 analyzed modern African mammal specimens including diet, common name, species attribution, sample ID,
 1017 nitrogen content in nmol/mg and carbon and nitrogen isotope values in ‰ with mean values grouped by diet. Water dependency after
 1018 62,104-106. All but one specimen were adult individuals (a juvenile African elephant, indicated with asterisk next to sample ID, was
 1019 excluded from calculations of the $\delta^{15}\text{N}$ averages to avoid the effect of weaning). Number of analyses, typically duplicates, are given in
 1020 brackets.

| Diet | Common name | Taxon | ZMH Sample ID | Locality | Water dependency | Tooth | $\delta^{13}\text{C}_{\text{bone-collagen}}$ (‰ vs. VPDB) | $\delta^{15}\text{N}_{\text{bone-collagen}}$ (‰ vs. AIR) | $\delta^{13}\text{C}_{\text{enamel}}$ (‰ vs. VPDB) | $\delta^{15}\text{N}_{\text{enamel}}$ (‰ vs. AIR) | N content (nmol/mg) |
|-------------------------------------|-------------------|-----------------------------------|---------------|--------------------------------|------------------|-------|---|--|--|---|-----------------------|
| Browsers | Duiker, blue | <i>Philantomba monticola</i> | S-4484 | Dondo, Angola | None | M3 | -21.3 ± 0.1 (2) | 7.8 ± 0.1 (2) | -13.3 ± 0.6 (2) | 7.4 ± 0.1 (2) | 3.7 ± 0.1 (2) |
| | Duiker, blue | <i>Philantomba monticola</i> | S-4483 | Dondo, Angola | None | M3 | -21.5 ± ± 0.0 (2) | 5.3 ± 0.1 (2) | -12.9 ± 0.6 (2) | 4.3 ± 0.4 (2) | 5.0 ± 0.2 (2) |
| | Giraffe | <i>Giraffa camelopardalis</i> | S-1869 | Tanzania | Low | M3 | -20.8 ± 0.0 (2) | 6.2 ± 0.0 (2) | -11.8 ± 0.2 (2) | 7 ± 0.3 (2) | 5.5 ± 0.3 (2) |
| | Giraffe | <i>Giraffa camelopardalis</i> | S-9816 | Wamba, Kenya | Low | M3 | - | - | -10.1 ± 0.1 (2) | 6.4 ± 0.3 (2) | 7.7 ± 1.6 (2) |
| | Gorilla | <i>Gorilla gorilla</i> | S-7902 | Gabon | High | M3 | - | - | -15.5 ± 0.1 (2) | 5.7 ± 0.1 (2) | 12.3 ± 0.3 (2) |
| | Gorilla | <i>Gorilla gorilla</i> | S-8192 | Cameroon | High | M3 | - | - | -15.4 ± 0.2 (2) | 6.4 ± 0.2 (2) | 7.8 ± 0.5 (2) |
| | Gorilla | <i>Gorilla gorilla</i> | S-7113 | Sanga, Congo | High | M3 | - | - | -14.5 ± 0.3 (2) | 4.4 ± 0.9 (2) | 4.6 ± 1.6(2) |
| | Gorilla | <i>Gorilla gorilla</i> | S-1857 | Congo | High | M3 | - | - | -15.9 ± 0.3 (2) | 6.8 ± 0.5 (2) | 7.1 ± 0.6 (2) |
| | Greater kudu | <i>Tragelaphus strepsiceros</i> | S-8014 | Chitado, Angola | Low | M3 | - | - | 0.1 ± 0.3 (2) | 4.2 ± 0.4 (2) | 4.1 ± 0.6 (2) |
| | Greater kudu | <i>Tragelaphus strepsiceros</i> | S-4510 | Angola | Low | M3 | - | - | -11.5 ± 0.4 (2) | 5.1 ± 0.4 (2) | 5.3 ± 0.7 (2) |
| | Greater kudu | <i>Tragelaphus strepsiceros</i> | S-4509 | Rio Caporello, Angola | Low | M3 | - | - | -12.5 ± 0.8 (2) | 6.3 (1) | 4.9 (1) |
| | Greater kudu | <i>Tragelaphus strepsiceros</i> | S-5648 | Ruacana, Angola | Low | M3 | -19.6 ± 0.0 (2) | 7.2 ± 0.1 (2) | -12 ± 0.2 (2) | 5.5 ± 0.6 (2) | 4.0 ± 0.0 (2) |
| | Greater kudu | <i>Tragelaphus strepsiceros</i> | S-4508 | Taka, Angola | Low | M3 | - | - | -11.1 (1) | 5.9 (1) | 4.3 (1) |
| | Rhino, black | <i>Diceros bicornis</i> | S-2553 | Hluhluwe Reserve, South Africa | High | M3 | -20.5 ± 0.0 (2) | 5.1 ± 0.0 (2) | -12.6 ± 0.4 (2) | 5.3 ± 0.5 (2) | 2.1 ± 0.1 (2) |
| | Rhino, black | <i>Diceros bicornis</i> | S-1865 | East Africa | High | M3 | -22.2 ± 0.0 (2) | 4.7 ± 0.2 (2) | -13.2 ± 0.2 (2) | 4.7 ± 0.2 (2) | 6.0 ± 1.2 (2) |
| Browser mean values (n = 15) | | | | | | | -21.0 ± 0.9 (6) | 6.0 ± 1.1 (6) | -12.1 ± 3.8 (15) | 5.7 ± 1.0 (15) | 5.6 ± 2.4 (15) |
| Grazers | African buffalo | <i>Syncerus caffer</i> | S-6773 | Aberdare, Kenya | High | M3 | -12.9 ± 0.0 (2) | 6.1 ± 0.1 (2) | -2.9 (1) | 5 ± 0.0 (2) | 4.7 ± 0.4 (2) |
| | African buffalo | <i>Syncerus caffer</i> | S-5649 | Dirico, Angola | High | M3 | -9.5 ± 0.0 (2) | 7.2 ± 0 (2) | -0.6 ± 0.1 (2) | 6 (1) | 3.7 (1) |
| | African buffalo | <i>Syncerus caffer</i> | S-3913 | Dondo, Angola | High | M3 | -9.2 ± 0.0 (2) | 4.1 ± 0.1 (2) | -2.6 ± 0.1 (2) | 5.8 (1) | 4.2 (1) |
| | African buffalo | <i>Syncerus caffer</i> | S-9566 | Tanzania | High | M3 | -10.4 ± 0.1 (2) | 8.7 ± 0.2 (2) | -1.3 ± 0.2 (2) | 6.8 ± 0.1 (2) | 4.7 ± 0.7 (2) |
| | Hippo | <i>Hippopotamus amphibius</i> | S-9588 | no information available | High | M3 | - | - | -7.4 ± 0.4 (2) | 7.3 ± 0.2 (2) | 3.7 ± 0.2 (2) |
| | Oribi | <i>Ourebia ourebi</i> | S-7943 | Cambembe, Angola | None | M3 | -7.9 (1) | 4.5 (1) | 0.4 ± 0.1 (2) | 3.9 ± 0.5 (2) | 6.1 ± 0.1 (2) |
| | Oribi | <i>Ourebia ourebi</i> | S-7944 | Chana, Angola | None | M3 | -9.3 ± 0.0 (2) | 4.7 ± 0 (2) | -0.2 ± 0.4 (2) | 4 ± 0.2 (2) | 5.6 ± 1.0 (2) |
| | Rhino, white | <i>Ceratotherium simum</i> | S-2552 | Umfolozi Reserve, South Africa | High | M2 | -8.2 ± 0.0 (2) | 6 ± 0 (2) | 0.4 ± 0.4 (2) | 6.5 ± 1.5 (2) | 3.7 ± 1.2 (2) |
| | Warthog | <i>Phanocochoerus aethiopicus</i> | S-4511 | Cubal, Angola | Low | M3 | -8.2 ± 0.1 (2) | 4.4 ± 0 (2) | -3.2 ± 0.2 (2) | 3.5 ± 0.7 (2) | 3.7 ± 0.1 (2) |
| | Warthog | <i>Phanocochoerus aethiopicus</i> | S-4490 | Cubal, Angola | Low | M2 | -8.1 ± 0.0 (2) | 5 ± 0.1 (2) | -2.6 ± 0.3 (2) | 4.5 ± 1.2 (2) | 3.4 ± 0.9 (2) |
| | Warthog | <i>Phanocochoerus aethiopicus</i> | S-6739 | Nanyuki, Kenya | Low | M3 | -7.3 ± 0.0 (2) | 9 ± 0.0 (2) | -1.9 ± 0.2 (2) | 8.4 ± 0.3 (2) | 2.9 ± 0.9 (2) |
| | Wildebeest, black | <i>Connochaetes gnou</i> | S-7938 | no information available | High | M3 | - | - | 1.8 ± 0.2 (2) | 6.8 (1) | 4.6 (1) |

| | | | | | | | | | | | |
|---|---------------------------------------|--|---------|-------------------------------|------|-------|-------------------------|-------------------------|------------------------|------------------------|-----------------------|
| | Wildebeest, blue | <i>Connochaetes taurinus</i> | S-6775 | Kajiado, Kenya | High | M3 | -6.6 ± 0.1 (2) | 8.2 ± 0.0 (2) | 1.9 ± 1.3 (2) | 6.5 ± 0.5 (2) | 3.7 ± 0.2 (2) |
| | Wildebeest, blue | <i>Connochaetes taurinus</i> | S-6774 | Kenya | High | M3 | - | - | 1.8 ± 0.2 (2) | 6.5 ± 0.9 (2) | 3.4 ± 0.4 (2) |
| | Wildebeest, blue | <i>Connochaetes taurinus</i> | S-5669 | no information available | High | M3 | - | - | -8.8 ± 0.2 (2) | 7.4 ± 0.1 (2) | 4.0 ± 0.2 (2) |
| | Wildebeest, blue | <i>Connochaetes taurinus</i> | S-6776 | Narok/Maasai-Mara, Kenya | High | M3 | -6.8 ± 0.0 (2) | 8.5 ± 0.2 (2) | 1.4 (1) | 9.6 ± 0.1 (2) | 3.4 ± 0.0 (2) |
| Grazer mean values (n = 16) | | | | | | | -8.7 ± 1.7 (12) | 6.4 ± 1.9 (12) | -1.5 ± 3.1 (16) | 6.1 ± 1.7 (16) | 4.1 ± 0.9 (16) |
| Mixed feeders | African elephant | <i>Loxodonta africana</i> | S-8397 | Cunene, Angola | High | M? | -19 ± 0.1 (2) | 7.9 ± 0.0 (2) | -10.7 ± 0.2 (2) | 5.4 ± 1.6 (2) | 2.5 ± 0.0 (2) |
| | African elephant | <i>Loxodonta africana</i> | S-8233* | Virunga National Park, Uganda | High | M1/2? | -25 ± 0.0 (2) | [10.2 ± 0.0 (2)] | -15.2 ± 0.2 (2) | [10.7 (1)] | 5.2 (1) |
| | African elephant | <i>Loxodonta africana</i> | S-8232 | Virunga National Park, Uganda | High | M? | -21.8 ± 0.0 (2) | 7.6 ± 0.1 (2) | -12.7 ± 0.3 (2) | 5.9 ± 0.8 (2) | 4.5 ± 0.9 (2) |
| | African elephant | <i>Loxodonta africana</i> | S-8398 | Cunene, Angola | High | M? | -20.4 ± 0.0 (2) | 12.3 ± 0.0 (2) | -10.6 ± 0.1 (2) | 10 ± 0.0 (2) | 7.9 ± 5.5 (2) |
| | Impala | <i>Aepyceros melampus</i> | S-5687 | Secadiva, Angola | High | M3 | - | - | -4.4 ± 0.2 (2) | 6.78 ± 0.2 (2) | 3.1 ± 0.0 (2) |
| | Impala | <i>Aepyceros melampus</i> | S-5688 | Secadiva, Angola | High | M3 | - | - | -7.4 ± 0.2 (2) | 7.2 ± 0.3 (2) | 7.8 ± 3.1 (2) |
| | Springbok | <i>Antidorcas marsupialis angolensis</i> | S-4539 | Capolopoppo, Angola | Low | M3 | - | - | -10.6 ± 0.1 (2) | 9.2 ± 0.4 (2) | 3.6 ± 0.1 (2) |
| | Springbok | <i>Antidorcas marsupialis angolensis</i> | S-3776 | Capolopoppo, Angola | Low | M3 | - | - | -8.8 ± 0.5 (2) | 8.3 ± 0.7 (2) | 3.4 ± 0.1 (2) |
| Mixed feeder mean values (n = 7) | | | | | | | -21.6 ± 2.6 (4) | 9.3 ± 2.6 (3) | -10.1 ± 3.3 (8) | 7.5 ± 1.7 (7) | 4.7 ± 2.1 (8) |
| Herbivore mean values (n = 39) | | | | | | | -14.4 ± 6.6 (22) | 6.7 ± 2.0 (21) | -7.3 ± 6.0 (39) | 6.2 ± 1.6 (38) | 4.8 ± 1.9 (39) |
| Omnivores | Baboon, yellow | <i>Papio cynocephalus</i> | S-6791 | Lake Baringo, Kenya | High | M3 | - | - | -10.7 ± 0.4 (2) | 7.3 ± 0.2 (2) | 2.4 ± 0.2 (2) |
| | Baboon, yellow | <i>Papio cynocephalus</i> | S-6790 | Lake Baringo, Kenya | High | M3 | - | - | -9.5 ± 0.3 (2) | 8 ± 0.1 (2) | 2.6 ± 0.1 (2) |
| | Baboon, yellow | <i>Papio cynocephalus</i> | S-6795 | Lake Baringo, Kenya | High | M3 | - | - | -7.5 ± 0.3 (2) | 7.2 ± 0.8 (2) | 2.1 ± 0.3 (2) |
| | Baboon | <i>Papio sp.</i> | S-10960 | Makania, Tanzania | High | M3 | - | - | -8 ± 0.3 (2) | 7.2 (1) | 7.3 (1) |
| Omnivore mean values (n = 4) | | | | | | | - | - | -8.9 ± 1.5 (4) | 7.4 ± 0.4 (4) | 3.6 ± 2.5 (4) |
| Carnivores | African wild dog | <i>Lycaon pictus</i> | S-3797 | Capelongo, Angola | High | M2 | -18.1 ± 0.0 (2) | 7.8 ± 0.1 (2) | -11.1 ± 0.2 (2) | 7.9 ± 0.7 (3) | 7.1 ± 2.2 (3) |
| | African wild dog | <i>Lycaon pictus</i> | S-4678 | Capelongo, Angola | High | M2 | -16.3 ± 0.0 (2) | 9.6 ± 0.0 (2) | -11.7 ± 0.2 (2) | 10.4 ± 0.2 (2) | 9.1 ± 0.2 (2) |
| | Leopard | <i>Panthera pardus</i> | S-4682 | Luati, Angola | Low | M1 | -13.4 ± 0.7 (2) | 7.4 ± 0.1 (2) | -8.8 ± 0.2 (2) | 7.2 ± 0.5 (2) | 3.9 ± 0.0 (2) |
| | Leopard | <i>Panthera pardus</i> | S-4683 | Angola | Low | M1 | - | - | -10.5 ± 0.0 (2) | 8 ± 0.3 (2) | 5.2 ± 1.6 (2) |
| | Lion | <i>Panthera leo</i> | S-4677 | Angola | Low | M1 | -12.6 ± 0.0 (2) | 10.6 ± 0.1 (2) | -7.5 ± 0.3 (2) | 9.8 ± 0.8 (3) | 4.8 ± 1.4 (3) |
| | Lion | <i>Panthera leo</i> | S-5178 | Angola | Low | M1 | -11.6 ± 0.0 (2) | 11 ± 0.0 (2) | -6.9 ± 0.1 (2) | 9.8 ± 0.6 (3) | 4.1 ± 0.7 (3) |
| | Lion | <i>Panthera leo</i> | S-8028 | Dondo, Angola | Low | M1 | -6.8 ± 0.0 (2) | 9.4 ± 0.1 (2) | -4.5 (1) | 8.4 (1) | 3.9 (1) |
| | Lion | <i>Panthera leo</i> | S-8666 | Etosha Pan, Namibia | Low | M1 | -9.8 ± 0.0 (2) | 12.6 ± 0.0 (2) | -4.4 (1) | 9.2 ± 1.1 (2) | 5.1 ± 1.9 (2) |
| | Lion | <i>Panthera leo</i> | S-7888 | Koma-Region, Tanzania | Low | M1 | -6.6 ± 0.1 (2) | 10.3 ± 0.1 (2) | -1.7 ± 0.3 (2) | 10.3 ± 0.0 (2) | 3.9 ± 0.2 (2) |
| | Spotted hyena | <i>Crocuta crocuta</i> | S-3236 | Kenya | High | M3 | -7.1 ± 0.0 (2) | 9.6 ± 0.0 (2) | -3.1 ± 0.2 (2) | 11.1 ± 0.1 (2) | 3.4 ± 0.2 (2) |
| | Spotted hyena | <i>Crocuta crocuta</i> | S-949 | Uganda | High | M3 | - | - | -4.3 ± 0.2 (2) | 14.9 ± 0.4 (2) | 3.2 ± 0.2 (2) |
| | Spotted hyena | <i>Crocuta crocuta</i> | S-5629 | Dirico, Angola | High | M3 | -16.5 ± 0.0 (2) | 9.8 ± 0.0 (2) | -11.5 ± 0.1 (2) | 9.2 ± 1.1 (2) | 3.5 ± 0.4 (2) |
| | Spotted hyena | <i>Crocuta crocuta</i> | S-4675 | Dondo, Angola | High | M3 | -10.8 ± 0.1 (2) | 8.9 ± 0.1 (2) | -7 ± 0.3 (2) | 10.5 ± 0.1 (2) | 4.2 ± 0.2 (2) |
| | Spotted hyena | <i>Crocuta crocuta</i> | S-8034 | Kirawira/Serengeti, Tanzania | High | M3 | -5.5 ± 0.0 (2) | 11 ± 0.0 (2) | -2.6 ± 0.1 (2) | 12.4 (1) | 9.6 (1) |
| | Carnivore mean values (n = 14) | | | | | | | -11.2 ± 4.3 (12) | 9.8 ± 1.4 (12) | -6.8 ± 3.5 (14) | 9.9 ± 2.0 (14) |

1021 **Table 2.** Summary data for fossil (Tam Hay Marklot) specimens including diet, common name, species attribution, sample ID, and
 1022 carbon nitrogen and zinc isotope values in ‰ with mean values grouped by diet. Zinc values taken from Bourgon et al.³⁹. All
 1023 individuals were adults. Number of analyses is given in brackets.

| Diet | Common name | Taxon | SEVA Sample ID | Tooth | $\delta^{13}\text{C}_{\text{dentin-collagen}}$ (‰ vs. VPDB) | $\delta^{15}\text{N}_{\text{bone-collagen}}$ (‰ vs. AIR) | $\delta^{13}\text{C}_{\text{enamel}}$ (‰ vs. VPDB) | $\delta^{15}\text{N}_{\text{enamel}}$ (‰ vs. AIR) | $\delta^{66}\text{Zn}_{\text{enamel}}$ (‰ vs. JMC) | N content (nmol/mg) |
|---|------------------|--------------------------------|----------------|--------|---|--|--|---|--|----------------------|
| Browsers | Sumatran serow | <i>Capricornis sumatrensis</i> | 34493 | M3 | - | - | -15.2 (1) | 8.9 ± 0.1 (2) | 0.78 ± 0.02 (2) | 7.4 ± 0.2 (2) |
| | Javan rhinoceros | <i>Rhinoceros sondaicus</i> | 34556 | M3 | -24.0 (1) | 6.6 (1) | -15.7 ± 0.1 (2) | 7.5 ± 0.3 (2) | 0.52 ± 0.01 | 6.5 ± 2.3 (2) |
| Browser mean values (n = 2) | | | | | -24.0 (1) | 6.6 (1) | -15.4 ± 0.4 (2) | 8.2 ± 1.0 (2) | 0.65 ± 0.18 (2) | 7.0 ± 0.7 (2) |
| Grazers | Asian buffalo | <i>Bubalus bubalis</i> | 34524 | P2/P3? | - | - | -0.8 (1) | 4.0 ± 0.0 (2) | 0.81 ± 0.01 (2) | 9.3 ± 1.3 (2) |
| | Bovine indet. | <i>Bos</i> sp. | 34527 | P2 | -9.2 (1) | 3.2 (1) | -1.5 (1) | 2.6 ± 0.1(2) | 0.77 (1) | 7.6 ± 0.2(2) |
| Grazer mean values (n = 2) | | | | | -9.2 (1) | 3.2 (1) | -1.2 ± 0.5 (2) | 3.3 ± 1.0 (2) | 0.70 ± 0.03 (2) | 8.4 ± 1.2 (2) |
| Mixed feeders | Indian muntjac | <i>Muntiacus muntjak</i> | 34517 | M3 | -21.0 (1) | 10.6 (1) | -12.9 ± 0.0 (2) | 7.6 ± 0.4 (2) | 0.29 ± 0.10 (2) | 5.9 ± 0.2 (2) |
| Mixed feeder mean values (n = 1) | | | | | 21.0 (1) | 10.6 (1) | -12.9 (1) | 7.6 (1) | 0.29 (1) | 5.9 (1) |
| Herbivore mean values (n = 5) | | | | | -18.98 ± 6.7 (3) | 7.1 ± 3.1 (3) | -9.2 ± 7.4 (5) | 6.1 ± 2.7 (5) | 0.63 ± 0.22 (5) | 7.3 ± 1.3 (5) |
| Omnivores | Asian black bear | <i>Ursus thibetanus</i> | 34501 | M2 | - | - | 13.3 (1) | 8.8 ± 0.1 (2) | 0.37 ± 0.02 (2) | 5.3 ± 0.2 (2) |
| | Wild boar | <i>Sus cf. scrofa</i> | 34537 | P4 | -21.7 (1) | 8.1 (1) | 13.3 ± 0.0 (2) | 4.9 ± 0.0 (2) | 0.32 ± 0.08 (2) | 4.4 ± 0.0 (2) |
| | Wild boar | <i>Sus cf. scrofa</i> | 34538 | P4 | - | - | 13.7 (1) | 5.8 ± 0.6 (2) | 0.61 ± 0.03 (2) | 4.4 ± 0.8 (2) |
| | Macaque | <i>Macaca</i> sp. | 34548 | M1/M2? | - | - | 14.3 (1) | 9.9 ± 0.1 (2) | 0.15 ± 0.03 (2) | 9.9 ± 0.1 (2) |
| Omnivore mean values (n = 4) | | | | | -21.7 (1) | 8.1 (1) | -13.6 ± 0.5 (4) | 7.3 ± 2.4 (4) | 0.36 ± 0.19 (4) | 5.4 ± 1.5 (4) |
| Carnivores | Asian leopard | <i>Panthera pardus</i> | 34505 | P4 | - | - | -13.4 (1) | 11.1 ± 0.5 (2) | 0.08 ± 0.02 (2) | 6.1 ± 0.4 (2) |
| Carnivore mean values (n = 1) | | | | | - | - | -13.4 (1) | 11.1 (1) | 0.08 (1) | 6.1 (1) |

1024

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [LeichliterLuedeckeSI.pdf](#)
- [LeichliterLuedeckelsotopeData.xlsx](#)