Comparative isotope ecology of western Amazonian rainforest mammals

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Closed-canopy rainforests are important for climate (influencing atmospheric circulation, albedo, carbon storage, etc.) and ecology (haborng the highest biodiversity of continental regions). Of all rainforests, Amazonia is the world’s most diverse, including the highest mammalian species richness. However, little is known about niche structure, ecological roles, and food resource partitioning of Amazonian mammalian communities over time. Through analyses of δ13Cbioapatite, δ13Cchair, and δ15Nchair, we isotopically characterized aspects of feeding ecology in a modern western Amazonian mammalian community in Peru, serving as a baseline for understanding the evolution of Neotropical rainforest ecosystems. By comparing these results with data from equatorial Africa, we evaluated the potential influences of distinct phylogenetic and biogeographic histories on the isotopic niches occupied by mammals in analogous tropical ecosystems. Our results indicate that, despite their geographical and taxonomic differences, median δ13Cdiet values from closed-canopy rainforests in Amazonia (−27.4‰) and equatorial Africa (−26.9‰) are not significantly different, and that the median δ13Cdiet expected for mammalian herbivores in any closed-canopy rainforest is −27.2‰. Amazonian mammals seem to exploit a narrower spectrum of dietary resources than equatorial African mammals, however, as depicted by the absence of highly negative δ13Cdiet values previously proposed as indicative of rainforests (<−31‰). Finally, results of keratin and bioapatite δ13C indicate that the predictive power of trophic relationships, and traditional dietary ecological classifications in bioapatite-protein isotopic offset expectations, must be reconsidered.

Significance

Closed-canopy rainforests are important for climate and ecology, yet identifying this ecosystem in the fossil record is challenging. An existing paradigm for identification of closed-canopy rainforests using fossil mammal carbon isotope data is the presence of highly negative δ13Cdiet values (<−31‰) in the herbivore community, as observed in modern equatorial African rainforest ecosystems. Our data from western Amazonian mammals, however, show that the absence of these values is not evidence for absence of closed-canopy rainforests. Our results also document that the proposed relationship between carbon isotope spacing variables and traditional dietary ecological classifications is not straightforward, and that better characterizations of the mixture of nutrients in animal diets are necessary to fully understand diet-tissue isotopic fractionation patterns.

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South American and African Mammalian Communities. The history of South American and African mammals is intimately linked to the fate of these two landmasses after the break-up of the long-lasting southern supercontinent Gondwana. South America and Africa, once part of Gondwana, fully separated by the mid-Cretaceous (~110 to 100 Ma [7, 8]). South America, although briefly connected with North America at the end of the Cretaceous and with Antarctica until the early Paleogene, ultimately became and remained an island continent for most of the Cenozoic (7, 9). The phylogenetic structure of modern South American mammals is the result of tens of millions of years of geographic isolation, with a few exceptional trans-Atlantic dispersal events from Africa during the late Paleogene, major faunal exchanges with North America during the late Neogene, and a dramatic extinction event at the end of the Pleistocene, in which more than 80% of mammals above 40 kg became extinct in South America (10, 11). Africa, on the contrary, remained connected to Arabia after the Gondwanan breakup and drifted slowly northeastward, culminating in a collision with Eurasia in the late Eocene (12). What followed were largely continental and often more violent events than those that have affected South America, which has remained an island continent for most of the Cenozoic (7, 9). The phylogenetic structure of modern South American mammals is the Miocene

mammals. Notable within the evolutionary history of African mammals is the Miocene–Pliocene radiation of endemic groups, including various bats, primates (e.g., hominids), hyrachyidae, carnivores, proboscideans, etc. (13). In contrast to the dramatic events in South America and most other continents, Africa was the continent least affected by the Pleistocene extinction event, with only eight genera of megamammals disappearing (10).

Sampling Localities and \(\delta^{13}C\) of Dietary Sources of Amazonian Mammals. The \(\delta^{13}C\) values of terrestrial and aquatic C3 plants from western Amazonia fall within a range of \(-36.9\%e\) to \(-24.1\%e\) (14-17). Of these, leaves show the most negative values \((-32.1\%e\) \), compared to bole \((-28.4\%e\) \) or litter \((-28.7\%e\) \) (14). Although the data are not exhaustive, fruits and seeds \((-29.3\%e\) \) show higher \(\delta^{13}C\) values than leaves (18). In a vertical profile of the forest, Amazonian plants show a decrease in their \(\delta^{13}C\) values with proximity to the ground, known as the canopy effect (19). Indeed, leaf samples of plant species have average \(\delta^{13}C\) values of \(-35.2\%e\) if growing within 1 m above the ground, \(-33.4\%e\) in the lower canopy (2 to 10 m), and \(-30.5\%e\) in the upper canopy (>20 m) (16). These plant \(\delta^{13}C\) values and the leaf \(\delta^{13}C\) gradient are the same as those observed in African and other rainforests (4, 20), which is expected given the similar climatic and vegetational criteria that define all tropical rainforests (1). Grass species are present in the Amazon rainforest, and most of them utilize C4 photosynthesis (21).

All mammals sampled in this study, except for two specimens, are from localities in Peruvian western Amazonia (mainly from the Madre de Dios, Ucayali, and Loreto regions). Although some of these sampling areas (e.g., Manu National Park in Madre de Dios) span a large altitudinal gradient (Andean highland, cloud forest, and lowland rainforest), our sampling has been restricted to localities below 700 m above sea level (i.e., lowland rainforest), with the exception of four samples coming from the cloud forest (SI Appendix). All samples therefore come from wet forest localities exhibiting analogous and relatively homogeneous environmental conditions. Aiming to include all available data from the literature for other western Amazonian sites, two specimens of the largest extant Amazonian mammal, Tapirus terrestris, from Colombia and Bolivia have also been included (22). Our Amazonian localities span a latitudinal gradient of about 13° (from 0°40'S [Rio Curaray, the northernmost locality] to 13°6'S [Manu, the southernmost locality], although 68% of the samples are from Madre de Dios and Ucayali, encompassing a narrower 4° latitudinal range). Virtually all samples are from undisturbed habitats in national parks. Selection criteria for data from African and Amazonian localities were the same (SI Appendix). Therefore, the

African mammals assessed in this study were sampled from equatorial lowland rainforests (primarily from the Democratic Republic of Congo, but also from Uganda and Gabon). We have not included data from any other African ecosystem (e.g., savannas or woodlands). The African localities span \(<5\%e\) of latitude (from 0.5° N [Kibale, Uganda] to 3.3° S [Mwenga, Congo]).

In addition to terrestrial plants, other dietary sources for various Amazonian mammal species include aquatic plants, fishes, insects, or nonmammalian vertebrates. Aquatic systems in Amazonia are dominated by C4 macrophytes (mean \(\delta^{13}C\) = \(-13.1\%e\), primarily represented by aquatic grasses); however, western Amazonian fishes show a marked predilection for consuming C3 plants, with \(\delta^{13}C\) values ranging from \(-37\%e\) to \(-21\%e\) (15, 23). Herbivorous insects in central Amazonia also exhibit a broad range of \(\delta^{13}C\) values \((-29.5\%e\) to \(-15\%e\) \), generally mirroring their consumption of C3 or C4 plants (18, 24). Bird data from Amazonian lowland rainforests indicate preferential consumption of C3 plants, whereas frogs and lizards derive at least half of their carbon from C4 sources (24, 25).

Results

Mammals analyzed herein (Fig. 1) were collected from as long ago as 1912 to as recently as 2015, and \(\delta^{13}C_{\text{diet}}\) has decreased by 1.7% during that time interval. Consequently, all data described are normalized to the preindustrial atmospheric \(\delta^{13}C\) of the year 1750 \((-6.3\%e\) \), subsequently referred as \(\delta^{13}C_{\text{PDB}}\); the SI Appendix provides details on this “Suess Effect correction”). Data for African mammals are used for broader comparative interpretations (Discussion), but, as these data have been published previously (Dataset S1), the results herein described focus on the Amazonian data (Table 1 and Fig. 2 also summarize prior results for African taxa).

\(\delta^{13}C_{\text{diet}}\) of Western Amazonian mammals. The taxa examined in this study included representatives of all nonvolant mammal groups present in western Amazonia (terrestrial: Artiodactyla, Carnivora, Didelphimorphia, Lagomorpha, Perissodactyla, Primates, Rodentia, Xenarthra [Pilosa, and Cingulata]; and aquatic: Cetacea and Sirenia). This sampling yields the most complete \(\delta^{13}C\) isotopic characterization of a closed canopy rainforest mammalian community to date. Our results document that western Amazonian herbivores have a median \(\delta^{13}C_{\text{diet}}\) of \(-27.4\%e\), ranging from \(-30.4\%e\) to \(-12.3\%e\). This \(18\%e\) range of variation is bracketed by the red titi monkey Plectrocebus discolor and the tapir T. terrestris at the lower end and by the capybara Hydrochoerus at the upper end, the latter being the only C4 consumer of all of the Amazonian mammals analyzed (Figs. 1 and 2).

Amazonian artiodactyls (4 species, \(n = 16\); deer and peccaries, median \(\delta^{13}C_{\text{diet}} = -24.9\%e\) \) show an isotopic span of 3%e, bracketed by the red brocked deer Mazama americana and the collared peccary Pecari tajacu at the lower and upper ends, respectively. Individuals of the Amazonian tapir T. terrestris, the only perissodactyl occurring in the study area, show little isotopic variation (<3%e), with a median \(\delta^{13}C_{\text{diet}} = -28.6\%e\) \((n = 7)\). In contrast, the only lagomorph in this study (the tapeti, Sylvilagus brasiliensis), shows a large intraspecific isotopic variation (5%e, median \(\delta^{13}C_{\text{diet}} = -28.2\%e\) \), even though the individuals sampled come from the same area \((n = 3)\). Rodents (9 species, \(n = 38\)) overall show \(\delta^{13}C_{\text{diet}}\) values ranging from \(-28.8\%e\) to \(-12.3\%e\). This represents the largest range of \(\delta^{13}C_{\text{diet}}\) span (16.5%e) of all western Amazonian herbivore clades, driven by the presence of the sole C4 consumer, the capybara \((\text{median } \delta^{13}C_{\text{diet}} = -15.5\%e)\). Excluding the capybara, the range of \(\delta^{13}C_{\text{diet}}\) for rodents is much narrower, at 5.5%e. Primates, the group best represented in our sampling \((13\text{ species, } n = 54)\), show an isotopic span of 4.6%e, with \(\delta^{13}C_{\text{diet}}\) values ranging from \(-30.2\%e\) (red titi monkey, P. discolor)
to −25.6‰ (black-headed night monkey, *Aotus nigriceps*). Herbivorous xenarthrans (i.e., sloths) show a δ¹³Cdiet range of 3.6‰ and have a median δ¹³Cdiet of −27.8‰ (*n* = 15). The only fully aquatic herbivore in our study, the Amazonian manatee (*Sirenia: Trichechus inunguis*), has a median δ¹³Cdiet falling within the average values of terrestrial Amazonian C₃ consumers (−26.6‰), and has a narrow intraspecific δ¹³Cdiet variation (2.3‰, *n* = 5).

The only member of Carnivora that actually is a primary consumer, the frugivore *Potos flavus* (kinkajou, *n* = 5), has a median δ¹³Cdiet of −27.4‰, which differs significantly from that of the other terrestrial carnivoran species (Figs. 1 and 2 and SI Appendix).

Although this contribution focuses on mammalian herbivores, we also report the δ¹³C₁⁷⁵⁰ of 12 secondary consumers (carnivores, piscivores, and insectivores). The δ¹³C₁⁷⁵⁰ values of these secondary consumers span 13.2‰, ranging from −25.4‰ to −12.2‰ (Fig. 1 and Table 2). The lowest δ¹³C₁⁷⁵⁰ values of all taxa are observed among the piscivorous species: the Amazon River dolphin (*Inia geoffrensis*; median δ¹³C₁⁷⁵⁰ = −24.4‰, *n* = 2) and the giant otter (*Carnivora: Pteronura brasiliensis*; median δ¹³C₁⁷⁵⁰ = −20.3‰, *n* = 6). Terrestrial carnivores (*Puma, Galictis, Atelocynus*, and *Leopardus*) show a median δ¹³C₁⁷⁵⁰ of −15‰ (SD = 1.1‰, *n* = 13) and no significant differences among them (SI Appendix). Among insectivores, the four-eyed opossum *Philander* sp. shows a median δ¹³C₁⁷⁵⁰ of −15.4‰ (*n* = 4) and low variation among specimens (0.6‰). Armadillos and anteaters, the insectivorous xenarthrans, have a median δ¹³C₁⁷⁵⁰ of −15.1‰ and a similarly small variation among individuals (SD = 0.7‰, *n* = 8).

### δ¹⁵Nhair and Hair-Bioapatite δ¹³C Enrichment

Fig. 1. δ¹³Cdiet (colored box plots, below) for Amazonian herbivores only and δ¹³C₁⁷⁵⁰ (white, sepia-bordered boxplots, above) for all western Amazonian mammals analyzed. Box plots represent the distribution of the data as explained in the key in the upper left corner. δ¹³Cdiet values for herbivores (calculated from dental bioapatite) represent the vegetation on which these primary consumers feed. Taxa inside colored framing rectangles in the δ¹³C₁⁷⁵⁰ plots are secondary consumers (lilac, insectivores; yellow, carnivores; blue, piscivores). Numbers below the box plots represent the number of samples analyzed per taxon.

δ¹⁵Nhair and Hair-Bioapatite δ¹³C Enrichment. Sampling hair (keratin) in addition to dental bioapatite provided new measures of δ¹⁵Nhair variation across Amazonian mammals and enabled us to analyze natural variations in δ¹⁵Nhair as well as a more nuanced view of dietary δ¹³C values (δ¹³Chair and δ¹³Cbioapatite) among Amazonian mammals (Fig. 3 and Table 3). Indeed, these data enabled us to test whether the “expected” offset between the δ¹³C of bioapatite and that of a proteinaceous tissue (keratin in this study, e²bioapatite-keratin), proposed as indicative of trophic level (26, 27), is met in this mammalian assemblage. δ¹⁵Nhair values for 37 mammalian species span 10.0‰, ranging from Rodentia (*Dinomys branickii*, δ¹⁵Nhair = 2.3‰) to Carnivora (*P. brasiliensis*, median δ¹⁵Nhair = 11.8‰). The δ¹⁵Nhair values of the Amazonian primary consumers are significantly lower than those of secondary consumers. Primary vs. secondary consumers show no significant differences
Table 1. Summary of δ¹³C results from dental bioapatite

| Group                | No. species/no. specimens | δ¹³C₁₇⁵₀ % | δ¹³C₁₇⁵₀ span | Reconstructed δ¹³C₆₆υ % | δ¹³C₆₆υ span |
|----------------------|---------------------------|------------|---------------|--------------------------|-------------|
| Western Amazonia     |                           |            |               |                          |             |
| All mammals          | 45/176                    | −15.9 ± 3.1| −25.4 to 0.3  | 25.7                     | —           |
| Herbivores           | 33/143                    | −16.0 ± 3.1| −19.3 to 0.3  | 19.6                     | −27.4 ± 2.8 | −30.4 to −12.3 | 18.1 |
| Only C3 herbivores   | 32/137                    | −16.0 ± 1.7| −19.3 to −11.4| 7.9                      | −27.5 ± 1.5 | −30.4 to −23.3 | 7.1 |
| Artiodactyla         | 4/16                      | −13.1 ± 0.7| −15.0 to −12.2| 2.8                      | −24.9 ± 0.8 | −27.0 to −24.0 | 3.0 |
| Primates             | 13/54                     | −16.8 ± 0.9| −19.3 to −14.7| 4.6                      | −28.2 ± 0.9 | −30.2 to −25.6 | 4.6 |
| Rodentia             | 9/38                      | −15.0 ± 4.9| −17.6 to 0.3  | 17.9                     | −26.1 ± 4.4 | −28.8 to −12.3 | 16.5 |
| Lagomorpha           | 1/3                       | −17.3 ± 2.8| −17.7 to −12.7| 5.0                      | −28.2 ± 2.7 | −28.6 to −23.6 | 5.0 |
| Perissodactyla       | 1/7                       | −15.7 ± 1  | −17.5 to −14.6| 2.9                      | −28.6 ± 1   | −30.4 to −27.6 | 2.8 |
| Sirenia              | 1/5                       | −13.2 ± 1.2| −13.8 to −11.4| 2.4                      | −26.5 ± 1.1 | −27.1 to −24.8 | 2.3 |
| Xenarthra            | 8/23                      | −15.6 ± 0.8| −17.2 to −13.9| 3.3                      | —           | —               | —   |
| Sloths only          | 3/15                      | −15.9 ± 0.6| −17.2 to −15.1| 2.1                      | −27.8 ± 1.1 | −29.4 to −25.8 | 3.6 |
| Equatorial Africa    |                           |            |               |                          |             |
| All mammals          | 30/137                    | −14.1 ± 3.7| −24.5 to −0.9 | 23.6                     | —           | —               | —   |
| Herbivores           | 29/135                    | −14.1 ± 3.7| −24.5 to −0.9 | 23.6                     | −26.9 ± 3.6 | −35.1 to −13.7 | 21.4 |
| Only C3 herbivores   | 27/123                    | −14.3 ± 2.3| −24.5 to −9.8 | 14.7                     | −27.2 ± 3.7 | −35.1 to −22.7 | 13.8 |
| Artiodactyla         | 19/84                     | −13.9 ± 4.6| −24.5 to −0.9 | 23.6                     | −25.8 ± 4.3 | −35.1 to −13.7 | 21.4 |
| Primates             | 7/18                      | −15.1 ± 0.7| −16.1 to −13.2| 2.9                      | −27 ± 0.6   | −28.1 to −25.9 | 2.2 |
| Proboscidea          | 2/32                      | −13.7 ± 1.2| −16.6 to −11.1| 5.5                      | −28 ± 1.2   | −30.9 to −25.4 | 5.5 |
| Rodentia             | 1/1                       | −16.3      | —             | —                       | −27.5       | —               | —   |

The top half of the table shows the data from western Amazonian mammals presented in the present study, and the bottom half is a compilation of published data from mammals in equatorial Africa (see refs. in SI Appendix). δ¹³C₁₇⁵₀ refers to raw values corrected for anthropogenic CO₂ set to preindustrial values (the year 1750) ± 1 SD. δ¹³C₆₆υ refers to the reconstructed diet, which, for herbivores, refers to the δ¹³C of the vegetation on which they feed. δ¹³C₆₆υ was not calculated for secondary consumers (Table 2 shows secondary consumer δ¹³C₁₇⁵₀ values only). C₃ taxa consuming C₃ plants; span, total range in ‰.

in δ¹³C enrichment between bioapatite and keratin (ε*bioapatite-keratin, Fig. 3A and SI Appendix). Amazonian folivores, however, do have significantly larger ε*bioapatite-keratin values than frugivores, carnivores, and omnivores (at the 0.05 level), but do not differ significantly from insectivores (SI Appendix, Fig. S4). Differences between other dietary groups were not statistically significant (SI Appendix, Figs. S4 and S5). Primary consumers drive the large variation in the range of δ¹³C ε*bioapatite-keratin values across this Amazonian mammalian community (>7%ε from 4.8%ε to 12.1%ε; Fig. 3A and Table 3). In contrast, secondary consumers cluster within a narrow range of ε*bioapatite-keratin values (2%ε from 6.4%ε to 8.4%ε; Table 3). Frugivores show the smallest ε*bioapatite-keratin values of the entire Amazonian mammalian community (range = 4.8 to 8.2%ε). When δ¹³C enrichment between diet and keratin (ε*diet-keratin) is assessed instead of ε*bioapatite-keratin, we observe the expected clustering of carnivores at the lower extreme of ε*diet-keratin values and primary consumers with significantly larger ε*diet-keratin values than carnivores (SI Appendix, Figs. S6 and S15). The ε*diet-keratin among primary consumers spans 6%ε, ranging from 0.2%ε to 6.3%ε (Fig. 3B). Significant differences were only found in the ε*diet-keratin between folivores and frugivores.

Discussion
Comparisons of δ¹³C₆₆υ of Mammalian Herbivore Communities from Western Amazonia and Equatorial Africa. On comparing the data from Amazonian mammals described here to published information on analogous tropical rainforest mammals from African sites, only one C₄ consumer was identified in western Amazonia, whereas at least three C₄ specialists exist in African rainforests (Figs. 1 and 2). None of the Amazonian species analyzed (representing >90% of all herbivores above 1 kg body mass in the study area) fills the carbon isotopic niche occupied by African understory forest dwellers. The breadth of isotope δ¹³C₆₆υ values exhibited by herbivorous mammals in both continents is comparable (21%ε vs. 18%ε in Africa and SA, respectively), but while the isotopic range in Amazonia is driven primarily by the high δ¹³C₆₆υ values of the only C₄ consumer (the capybara), in equatorial Africa, this similarly broad range is driven instead by the extremely negative δ¹³C₆₆υ values (<−30%ε) observed in the few species feeding in the subcanopy stratum of the forest (the antelope Neotragus batesi [Bates’s pygmy antelope], the giraffe Okapia johnstoni [okapi], and some individuals of the suid Hylochoerus meinertzhageni [giant forest hog] among artiodactyls, and the forest elephant Loxodonta cyclotis). In Amazonia, no terrestrial mammal exhibits such extreme negative δ¹³C₆₆υ values, even though species living and feeding in the subcanopy stratum are represented in our analysis, and plants with δ¹³C values as negative as the most negative plants in Africa do exist in western Amazonia (14, 15). Within a forest, the most negative δ¹³C values are found in leaves growing in the understory, where light-deprived conditions increase isotope discrimination (28, 29). In fact, a δ¹³C difference up to almost 5%ε can be seen within a single plant species, and a range of 10%ε can occur across a vertical profile of the canopy, depending on the amount of light received (20, 28). Therefore, for mammals to record such negative isotopic values, they have to be selective in what they eat, but most importantly, where they forage. This foraging selectivity limits this extremely negative δ¹³C₆₆υ niche to selective-feeding forest dwelling herbivores, i.e., animals that almost exclusively consume leaves growing deep in the understory. That mammals with median δ¹³C₆₆υ values <=−30%ε are absent in Amazonia, and rare even in Africa, the only modern ecosystem where these values have so far been identified, indicates that these values can no longer be considered “expected” for all mammals living in the subcanopy stratum of any rainforest, nor be used as an indispensable indicator of rainforests. Subcanopy-feeding herbivorous mammals in western Amazonia today, therefore, are consuming vegetation falling from the upper layers of the canopy (e.g., fruits) or consuming items with a wide range of isotopic values (e.g., leaves growing under different degrees of
canopy closure or incorporating a variety of food items in their diets), thereby averaging the $\delta^{13}C$ of their energy pool to yield values closer to the typical median for rainforests.

The median $\delta^{13}C_{\text{diet}}$ values of artiodactyls in South America and Africa are not significantly different (Table 1 and SI Appendix). The isotope range exhibited by artiodactyls in Africa ($>21‰$, ranging from $-21.35$ to $-13.7‰$), however, is significantly larger than in South America ($5‰$, ranging from $-29.06$ to $-24.06‰$). This dramatic difference may result from the disparity in diversity of the artiodactyl clade samples across the two continents ($4$ vs. $19$ species in our dataset, in South America and Africa, respectively), but we note that artiodactyls also are much more diverse in Africa due to their long history on that continent and the extremely recent arrival of the group in South America. The $\delta^{13}C_{\text{diet}}$ of primates in both continents differs significantly, but the difference in means is only $4‰$ (mean $\delta^{13}C_{\text{diet}} = -28.06‰$ for SA rainforest primates, $-27.06‰$ for African rainforest primates; Table 1). Moreover, fewer species were sampled in Africa, so this difference could be reduced or disappear with a more extensive sampling of other African rainforest primate species. Rodents are poorly sampled in equatorial Africa (data were available for only one species, the phiomorph Atherurus africanus [African brush-tailed porcupine]). In contrast, in western Amazonia, rodents are well sampled and span a broad $\delta^{13}C_{\text{diet}}$ range (16.5‰), mainly driven by the $\delta^{13}C_{\text{diet}}$ of the capybara, whose exclusion decreases the breadth of $\delta^{13}C_{\text{diet}}$ variation in Amazonian rodents to 5.5‰. This wide $\delta^{13}C_{\text{diet}}$ niche occupation might also reflect the ecological diversity resulting from a relatively long evolutionary history of caviomorphs on the continent (described in the previous section of the Discussion). With this rationale, we also might expect Xenarthra (along with the marsupials, all of which are secondary consumers) to occupy a large range of $\delta^{13}C_{\text{diet}}$ values because these are the only other surviving groups from the original pool of mammals that existed in South America before the faunal immigration waves that occurred throughout the mid-late Cenozoic. Modern herbivorous xenarthrans (i.e., sloths), however, represent only 2% of the earlier diversity of the group in the fossil record, and our results document that modern sloths are restricted to a very narrow $\delta^{13}C_{\text{diet}}$ range ($<4‰$), consistent with their low modern taxonomic and ecological diversity (limited to three species in western Amazonia).

Fig. 2. $\delta^{13}C_{\text{diet}}$ values and distributions of the herbivores in mammalian communities of western Amazonia (Left) and equatorial Africa (Right). Histograms on the right axis represent the worldwide distribution of $\delta^{13}C$ values for plants with C3 and C4 photosynthesis (modified from ref. 52). Numbers below box plots represent the number of samples per taxon.

The $\delta^{13}C_{1750}$ for terrestrial Amazonian carnivores (median = $-15.06‰$, excluding the frugivore Potos and the semiaquatic Pteronura) matches the raw $\delta^{13}C_{1750}$ values of most herbivores in our study (Fig. 1). Although a study showed $\varepsilon^{\text{predator-prey}}$ for specialized terrestrial hypercarnivores (26), this $\varepsilon^{\text{predator-prey}}$ value ($-1.3‰$) has not been thoroughly examined in non-specialized terrestrial carnivores (like Amazonian predators) and is likely not applicable to Amazonian mammalian predators, which are rather opportunistic in their feeding behaviors (including insects, other nonmammalian vertebrates, and plant elements in their diets). Indeed, use of this $\varepsilon^{\text{predator-prey}}$ results in the reconstructed $\delta^{13}C_{\text{diet}}$ of Amazonian carnivores not matching that of most sympatric herbivores. The same applies for the African genet Genetta, for which a $\varepsilon^{\text{predator-prey}}$ of $-1.3‰$ may not reflect the omnivorous feeding behavior of this species.
Table 2. Summary of $\delta^{13}C_{1750}$ results of secondary consumers only (i.e., carnivores, insectivores, piscivores)

| Group                     | No. species/specimens | $\delta^{13}C_{1750}$ (‰) | Range       | $\delta^{13}C_{1750}$ span |
|---------------------------|-----------------------|-----------------------------|-------------|-----------------------------|
| Western Amazonia          |                       |                             |             |                             |
| All secondary consumers   | 12/33                 | $-15.4 \pm 3$              | -25.4 to -12.2 | 13.2                        |
| Xenarthra (nonherbivores) | 5/8                   | $-15.1 \pm 0.7$            | -16.0 to -13.9 | 2.1                         |
| Carnivora                 | 5/19                  | $-15.4 \pm 2.4$            | -21.1 to -12.2 | 8.9                         |
| Terrestrial carnivores    | 4/13                  | $-15.0 \pm 1.1$            | -16.4 to -12.2 | 4.2                         |
| Ceteacea                  | 1/2                   | $-24.4 \pm 1.5$            | -25.4 to -23.3 | 2.1                         |
| Didelphimorphia           | 1/4                   | $-15.4 \pm 0.6$            | -15.9 to -14.4 | 1.5                         |
| Equatorial Africa         | 1/2                   | $-11.7 \pm 1.2$            | -12.5 to -10.9 | 1.6                         |

Carnivora in this table excludes Potos (frugivore). *Terrestrial carnivores* excludes Pteronura (semiaquatic, piscivore) and Potos (frugivore).

The aquatic herbivore *T. inunguis* (Amazonian manatee) exhibits $\delta^{13}C_{\text{diet}}$ values corresponding to exclusive consumption of food with carbon sources of C3 plant origin. The two piscivorous species (Ceteacea: *I. geoffrensis* [river dolphin]; and Carnivora: *P. brasiliensis* [giant otter]) show significantly different $\delta^{13}C_{1750}$ values (medians of $-20.3\%e$ and $-24.4\%e$, respectively), with *Inia* being the most negative of all sampled species. The difference in $\delta^{13}C_{1750}$ values of these two species might be due to *Inia* being an exclusive piscivore, whereas *Pteronura* is not. Indeed, *Pteronura* incorporates other vertebrates and invertebrates in its diet (30), both of which have higher $\delta^{13}C$ values than usually observed in Amazonian fishes (23). This broader feeding choice in *Pteronura* also is consistent with a larger intraspecific $\delta^{13}C_{1750}$ variation (Fig. 2).

One key question in our study was whether or not it is possible to define a closed-canopy rainforest from mammalian isotope data. Our results show that the median $\delta^{13}C_{\text{diet}}$ of terrestrial herbivores in South America ($-27.4\%e$) and Africa ($-26.9\%e$) are not significantly different (Fig. 4 and SI Appendix, Fig. S3). The median $\delta^{13}C_{\text{diet}}$ for these two rainforests is $-27.2\%e$ (SD = 3.2\%e, SE = 0.2\%e), a value that we propose could be expected for mammalian herbivores living in any closed-canopy tropical rainforest. Although this $-27.2\%e$ value for closed-canopy tropical rainforests is nearly identical to the global mean $\delta^{13}C$ for plants (31), the median $\delta^{13}C_{\text{diet}}$ of nonrainforest mammalian communities seems to be more positive than $-27.2\%e$ (SI Appendix, Fig. S7). Furthermore, the median $\delta^{13}C_{\text{plants}}$ of tropical rainforests is more negative than the global average ($-31\%e$; comparative data of rainforest plant communities provided in SI Appendix, Figs. S9–S13). That both Amazonian and African herbivores show an offset in their $\delta^{13}C_{\text{diet}}$ ($-27.2\%e$) relative to the overall rainforest $\delta^{13}C_{\text{plants}}$ values ($-31\%e$) documents complexities in the incorporation of carbon from diet to tissues and the necessity of comprehensive baseline studies to understand the processes underlying this offset and better characterize the isotopic structure of these ecosystems.

$\delta^{13}C$ Enrichment between Proteinaceous Tissues and Bioapatite. Increases in position within the trophic chain have been linked to stepwise rises in $\delta^{13}C$ values, with $3\%e$ to $4\%e$ as the constant value usually invoked for each change in trophic level (32, 33). However, substantial variation in $\delta^{15}N$ values across trophic guilds, and unexpectedly high $\delta^{15}N$ values in some herbivorous species (overlapping that of carnivores) also have been identified (26, 33), making the use of $\delta^{15}N$ alone a potentially imprecise or even misleading proxy to identify trophic levels (without knowledge of $\delta^{15}N$ baseline values and especially if comparing taxa among habitats). Instead, case studies document that apparent fractionation between the $\delta^{13}C$ of bioapatite and a proteinaceous tissue ($\epsilon^{\text{bioapatite-protein}}$) could be a more reliable method for assessing food chain relationships (26, 27). Carnivores and herbivores are expected to have extreme values within the spectrum of $\epsilon^{\text{bioapatite-protein}}$ (low and high, respectively), while omnivores show intermediate values. The rationale behind this expectation is that the type of digestive system influences the degree to which food is degraded by fermentation or by endogenous enzymes (affecting the subsequent degree of $\delta^{13}C$ transformation of bioapatite relative to diet), and that the contribution of different macronutrients (carbohydrates, proteins, and lipids) in the synthesis of bioapatite and proteinaceous tissues differs according to feeding behavior and food choice (27, 34, 35).

Our results show that this expectation is simplistic in a hyperdiverse natural environment like Amazonia, revealing implicacies associated with the breakdown of dietary macromolecules in a community of primary consumers mostly characterized by generalist taxa (i.e., “herbivores” that incorporate a wide variety of plants and even some animal elements in their diets). Indeed, of the 36 species of primary consumers analyzed, only 6 can be classified as obligate folivores (i.e., animals whose fundamental niche precludes them from incorporating any animal tissues in their diets: *Bradyus*, *Trichechus*, *Syvilarus*, *Hydrochoerus*, and two species of *Coendou*). Of these, only *Bradyus* and *Coendou* are classifiable as obligatory specialists (i.e., very narrow realized niche and diet [36]), corroborated by their narrow intraspecific $\delta^{13}C_{\text{diet}}$ Values. Contrary to findings in other studies (e.g., ref. 26), frugivores rather than secondary consumers had the lowest $\epsilon^{\text{bioapatite-keratin}}$ Values (keratin is representative of values for protein), a result that could be explained by the high lipid content of Amazonian fruits and seeds (37). Body protein tracks dietary protein, whereas bioapatite tracks bulk diet (i.e., the combination of all three macronutrients: carbohydrates, proteins, and lipids). Diets with high lipid content will decrease the $\delta^{13}C$ of the whole diet because lipids are $13C$-depleted relative to other macronutrients. This more negative $\delta^{13}C_{\text{diet}}$ will then be imprinted in the $\delta^{13}C_{\text{bioapatite}}$ (thus now closer in its $\delta^{13}C$ value to proteinaceous tissues). Even though low $\epsilon^{\text{bioapatite-keratin}}$ among some frugivores might also be interpreted as reflecting omnivory, in the current paradigm, omnivores would not be expected to show lower $\epsilon^{\text{bioapatite-keratin}}$ values than carnivores or insectivores (e.g., in our dataset, some primate and rodent species have smaller $\epsilon^{\text{bioapatite-keratin}}$ Values than felids and canids; Fig. 3). Alternatively, these results can be explained with a utilization of macromolecules and energy ratios that are inconsistent with traditional herbivore macronutrient profiles, as recently identified in an obligate specialist herbivore (38). Indeed, that study (38) revealed that, by switching foraging areas associated with asynchronous phenomenologies of two bamboo species, giant pandas

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maximized their protein intake and minimized their fiber ingesta. This resulted in a dietary macronutrient composition that equates to that of hypercarnivores because of similar reliance on proteins, albeit plant rather than animal, as the dominant macronutrient source. With such a high percentage of energy coming from dietary protein (instead of carbohydrates, as in most herbivores), we also predict that pandas will show low values in the e*bioapatite-protein spectrum when measured. The lower than expected values in e*bioapatite-keratin observed in some Amazonian primary consumers might be explained in a similar way. Other studies have also shown highly variable e*bioapatite-protein values within a free-ranging herbivore mammalian community (39), as well as e*bioapatite-protein poorly distinguishing trophic levels (40), suggesting that the e*bioapatite-protein within dietary categories (especially among primary consumers) should not be expected to be uniform.

The lack of trophic-level segregation in the e*bioapatite-keratin spectrum leads to reconsideration of three tacit assumptions underlying this expectation: (i) diet-bioapatite enrichment within the herbivore primary consumer guild is not significantly different among species, (ii) the general dietary macronutrient profile of herbivores and carnivores is always different, and/or (iii) δ13C enrichment between animals’ proteinaceous tissues and dietary protein is relatively constant.

In conflict with assumption i, diet-bioapatite enrichment differs significantly among herbivores. Indeed, after correcting for diet-bioapatite enrichment values, and plotting enrichment between diet and keratin (e*diet-keratin; Fig. 3B) rather than between bioapatite and keratin, we observe the expected segregation of carnivores at the lower extreme of e*diet-keratin values and primary consumers showing significantly higher e*diet-keratin values (SI Appendix, Fig. S15). Among primary consumers, the obligate herbivores Hydrochoerus and the two Coendou species (Rodentia) show the lowest e*diet-keratin values (Fig. 3B).

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Further, the specialized obligate folivore Bradypus, a species with a known controlled-feeding e*diet-bioapatite value (41), also shows e*diet-keratin Values at the lower end of that for all primary consumers, suggesting that dietary traits (in addition to or rather than physiological traits) might be involved instead.

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Amazonian data do not support the widely held assumption that carnivores-herbivores and specialists-generalists have necessarily distinct macronutrient profiles. Thus, a better characterization of the mixture of nutrients in an organism’s diet (rather than just the kinds of food or energy content) are necessary to fully understand diet-tissue isotopic fractionations (38, 42). Finally, the large range in the δ13C_diet-bioapatite values in obligate herbivores like Hydrochoerus, Coendou, Dinomys, or Bradypus suggest that dietary proteins are supplying their amino acid needs for keratin synthesis. In contrast, the large δ13C_diet-bioapatite values observed in most frugivore species suggest that these species are synthesizing amino acids de novo (likely from carbohydrates) to produce keratin (Fig. 3B).

In summary, many Amazonian mammals do not fall in the expected place along the δ13C_diet-keratin spectrum when classified by their feeding choice, which calls into question existing underlying assumptions and the predictive power of traditional dietary ecological classifications for bioapatite-protein isotopic offset expectations.

On Isotopic Niche Occupation of Amazonian Mammals. Amazonia is the world’s largest rainforest, and western Amazonia in particular is further considered to harbor the highest modern mammalian diversity on the planet (43, 44). Yet, the isotopic range of mammalian herbivores there is narrower than that of equatorial Africa, even though the sampled Amazonian localities span a wider latitudinal range and Amazon closed-canopy rainforest vegetation exhibits a similar δ13C range to that observed for African plants (14, 16, 17). Why do equatorial African mammals exploit a broader spectrum of resources than Western Amazonian mammals, or why do the latter not consume all available plant resources in the forest, instead occupying a comparatively narrower breadth of isotopic niches than in Africa? The δ13C_diet data from terrestrial equatorial African mammals indicate that four artiodactyl species exploit resources at the isotopic extremes in a closed-canopy rainforest; among these are two pure C4 consumers (Syncerus caffer nanus and Phacochoerus africanus) and two subcanopy dwellers with extremely negative δ13C_diet values (N. batesi and O. johnstoni, although some individuals of the suid H. meinertzhageni as well as the forest elephant L. cyclotis also show δ13C_diet ≤ −30‰). In South America, only one rodent species occupies the upper isotopic extreme (C4 consumer values), and no Amazonian mammal seems to be feeding (at least exclusively) on the most isotopically negative plants (i.e., in the isotopic space occupied by Neotragus and Okapia in Africa). Assessing these differences requires comparison of the herbivorous mammalian communities in South America and Africa in biological traits that might influence isotopic niche occupation. One important distinction is substrate occupation. In Africa, 73% of the species sampled are obligately terrestrial,

| Group          | No. of species | Median δ15N_hair % | Range of δ15N_hair | Median δ13C_hair % | Range of δ13C_hair | Median δ12C_hair % | Range of δ12C_hair | Range of δ13C_diet-keratin % | Range of ε*biopat-keratin % | Range of ε*diet-keratin % |
|----------------|----------------|--------------------|--------------------|--------------------|---------------------|--------------------|-----------------|-----------------------------|-----------------------------|---------------------------|
| All mammals    | 35             | 7.5                | 2.3–1.18           | −23.3              | 4.8–12.1            | —                  | —               | —                           | —                           | —                         |
| Folivores      | 9              | 7.0                | 2.3–8.1            | −23.4              | 7.1–12.1            | 0.2–5.2            | —               | —                           | —                           | —                         |
| Frugivores     | 13             | 5.4                | 3.8–8.6            | −23.4              | 4.8–8.2             | 2.9–6.3            | —               | —                           | —                           | —                         |
| Omnivores      | 3              | 7.6                | 6.6–7.8            | −23.6              | 6.1–7.1             | 3.3–5.5            | —               | —                           | —                           | —                         |
| Secondary      | 10             | 9.5                | 7.4–11.8           | −22.5              | 6.4–8.4             | —                  | —               | —                           | —                           | —                         |
| consumers      |                |                    |                    |                    |                     |                    | —               | —                           | —                           | —                         |
| Primates       | 13             | 4.6                | 3.8–8.6            | −23.6              | 5.2–8.2             | 2.9–6.3            | —               | —                           | —                           | —                         |
| Rodentia       | 8              | 7.6                | 2.3–8.2            | −22.9              | 4.8–12.1            | 0.2–6.3            | —               | —                           | —                           | —                         |
| Lagomorpha     | 1              | 3.1                | —                  | −25.3              | 8.2                 | 3.0                | —               | —                           | —                           | —                         |
| Xenarthra      | 7              | 7.9                | 7.0–9.5            | −22.8              | 6.4–8.4             | —                  | —               | —                           | —                           | —                         |
| Carnivora      | 5              | 10.4               | 5.4–11.8           | −21.8              | 6.4–7              | —                  | —               | —                           | —                           | —                         |
| Didelphimorpha | 1              | 9.5                | —                  | −23.0              | 7.8                 | —                  | —               | —                           | —                           | —                         |

The reported range of values refers to medians per species (not of individual specimens). No ε*predator-prey is available for Amazonian secondary consumers; thus, δ13C_diet and ε*diet-keratin values could not be calculated for those species. Secondary consumers: carnivores, piscivores, and insectivores.
whereas these represent only 39% of the Amazonian sample. This is relevant because terrestrial mammals are those most likely to feed on plants growing in the lowest stratum of the forest, the understory. The difference in the number of terrestrial Amazonian species is not a flaw in our sampling design; there simply are fewer exclusively terrestrial mammals in modern Amazonia than in Africa, and particularly in the much lower number of ungulate (artiodactyls, perissodactyls) species. Indeed, our study includes all but one of the six ungulates living in lowland western Amazonia, none of which have δ13Cbioapatite (median -28‰). A similar situation pertains to numbers of obligate herbivores (-14% in Amazonia vs. >60% in equatorial Africa), which also might influence the smaller breadth of δ13Cbioapatite values observed in Amazonian mammals. One variable that arguably encompasses the biological traits differing between the mammalian communities of these two tropical rainforests (e.g., substrate occupation, feeding niches, body mass), is the distinct evolutionary time, and therefore feeding guild scope, represented by clades in both continents (Fig. 4). Indeed, while the evolutionary history of most lineages of modern terrestrial African mammals can be traced back to the Paleogene, only caviomorph rodents can be considered as terrestrial herbivores native to South America prior to the late Pliocene-Pleistocene Great American Biotic Interchange (GABI). Caviomorphs represent, in fact, the group of mammals with the largest δ13Cbioapatite range in Amazonia. Given that species diversification is often followed by niche evolution (45), and that diversification is related to evolutionary time (46), time may be an important factor in determining the breadth and variety of isotopic fundamental niches that species within a mammalian herbivore community can exhibit. Thus, even though our sampling of extant Amazonian herbivores (terrestrial and nonterrestrial) better encompasses the total phylogenetic diversity of the ecosystem than does the African sample, the shorter evolutionary time and restricted phylogenetic breadth represented by modern South American mammals could explain the more restricted isotopic range compared to Africa (Fig. 4).

Indeed, the modern mammalian communities in equatorial South America and Africa are not strictly comparable ecologically because the former represents an ecosystem that experienced a relatively recent and large-scale extinction (particularly of large-bodied herbivores), whereas the latter was not comparably affected. Although it remains to be tested, this range of ecologies that does not currently occur in Amazonia could have been occupied by clades of terrestrial mammalian herbivores with no close extant relatives (e.g., notoungulates, litopterns) that are known to have gone extinct recently, groups of herbivores with unparalleled physiological traits (e.g., extinct giant ground sloths, the largest extant mammoths that have ever existed), and other mammals that occupied currently empty body mass categories (e.g., >200 kg). Analysis of the large-bodied Pleistocene herbivore *Toxodon* (a notoungulate, one of the 66 or more megafaunal species that became extinct in the Pleistocene of South America [47]) from a broad latitudinal range in the Americas showed exclusive consumption of C4 plants at high latitudes but C3 plants in the Amazon (48). The calculated δ13Cbioapatite of Amazonian toxodonts (median δ13Cbioapatite = -27‰) was found to be lower than modern Amazonian artiodactyls, although still not as negative as the African subcanopy feeders (i.e., δ13Cbioapatite <-30‰). Although other studies have isotopically characterized Pleistocene mammalian communities from closed-canopy rainforests has yet been done because of limited known localities from these habitats, where plants with δ13C <-30‰ are present and may have been consumed by recently extinct herbivore lineages, as is observed in equatorial Africa today. Isotopic characterization of the Pleistocene Amazonian mammalian community also could better inform understanding of the influence of the extinct megafauna on the realized niches of modem Amazonian mammals by revealing if surviving lineages shifted or expanded their feeding ecologies (and isotopic niche occupation) after the extirpation of those species from the ecosystem. Indeed, Pleistocene megafaunal extinction was suggested to be responsible for expansion of modern deer into their current δ13Cbioapatite niche within temperate North American habitats (51).

Alternatively, given the more extensive sampling of Amazonian mammals, the question may not be why they do not show δ13Cbioapatite values <-30‰, but rather why some African mammals do show these values, similar to the lower, highly negative values of those few African mammals only from the Iru Forest, both small- and large-bodied, and have not been recorded elsewhere. This observation would further highlight our conclusion that δ13Cbioapatite values <-30‰ cannot be used as an indispensable indicator of a rainforest. Other potential explanations for the restricted isotopic occupation observed in the modern Amazonian mammalian herbivore community might include inherent traits reflecting their narrower feeding diversity compared to equatorial African mammals, or a sampling bias (some Amazonian species are represented by small sample sizes, although the same is true for the African dataset). Given that isotopically similar plant resources are available in both rainforests (C4 grasses and plants with δ13C <-30‰), that Amazonian mammals might specifically avoid consuming these resources would be intriguing. Our sampling is limited for mammals <0.3 kg, which might be consuming the highly negative understory plants, but includes more than 80% of all larger species in the sampling area, spanning the body sizes of taxa with highly negative values in Africa. Excluding marsupials, which are omnivores, the only terrestrial herbivorous mammals <0.3 kg are rodents, and small rodents are not represented at all in the African sample. Future empirical geochemical sampling and paleontological field efforts in pre-Holocene deposits of Amazonia will permit testing of these ideas and should reveal new questions involving complexities of ecological interactions over time in what was perhaps the most biodiverse continental ecosystem in Earth history.

**Materials and Methods**

We analyzed δ13C from dental bioapatite (enamel for all mammals except xenarthrans [see below]) of 45 mammalian species (n = 176 individuals), δ13C and δ15N of hair keratin of 35 species (n = 125), and δ13Ccheekpouch, δ13Chair, and δ13Nhair from matched samples of a smaller subset of taxa (31 species, n = 82; Tables 1–3 and Dataset S1). All but four specimens sampled are from closed-canopy rainforest habitats in western Amazonian localities in Peru, including the well-known biodiversity hotspots of Tambopata National Reserve and Manu National Park, both in the Madre de Dios region. Specimens (only adults) were sampled from the mammalogy collections of the Museo de Historia Natural in Lima, Peru, and the American Museum of Natural History in New York, NY. With few exceptions, only late-erupting molars, ever-growing teeth, or canines were sampled. Our criteria for species selection are described in the SI Appendix. All samples were analyzed at the Stable Isotope Research Facility at the University of Utah. For the two extant sloth genera, the δ13Ccheekpouch was sampled from the orthodentine, whereas, for the five species of anteaters and armadillos (toothless or with small teeth that sampling was not possible), the proxy δ13C of their "enamel" was projected from bone δ13C values. We transformed bone δ13C data to corresponding dental enamel values using regression equations obtained from a separate analysis of the matched samples (SI Appendix, Fig. S1). All raw δ13C data (for bioapatite, hair, and comparative plant values for both South American and African samples) were corrected for anthropogenic CO2 and set to preindustrial values (to the baseline year of 1750; δ13C1750). In order to make data comparable among herbivorous taxa, the CO2-corrected bioapatite δ13C (i.e., δ13Cbioapatite) was converted to dietary δ13C (δ13Cdiet). This was done by using the body mass-dependent equations determined by a previous study (41) to calculate the diet-bioapatite δ13C enrichment (Δ13Cdiet-bioapatite) specific to each species (the calculated Δ13Cdiet-bioapatite values for the herbivores in our study range from 10.3% to 13.7%). Reported δ13Cdiet values for secondary consumers reflects the δ13Cdiet (and not the reconstructed Δ13Cdiet) pending development of reliable methods for estimating Δ13Cdiet-predator, that allow
confident dietary reconstructions for these feeding guilds. $\delta^{15}N_{\text{hair}}$ data are presented as raw values (Fig. 3). All raw data and equations are presented in Tables 1–3 and Dataset S1. Data from African mammals (S–S) also were standardized following the criteria described above. The same criteria were used for selecting data from African and Amazonian mammals (SI Appendix). Mammals were classified into six dietary categories: folivores (including browsers and grazers), frugivores, omnivores, carnivores, piscivores, and insectivores. Except for omnivores, animals were binned into one of these categories when a dietary component (e.g., fruits for frugivores) represented >50% of the total diet. The term “frugivorous” includes both folivores and frugivores. Statistical analyses, within and between orders, across continents, and between dietary guilds, were conducted with both parametric (t test, ANOVA) and nonparametric (Mann–Whitney, Kruskal–Wallis) tests for significance (SI Appendix). Bonferroni corrections were applied for all multiple pairwise comparisons. Unless otherwise noted, we report differences as statistically significant when $P$ values of pairwise comparisons for both parametric and nonparametric tests are $<0.01$.

**Data Availability.** All data are available as Dataset S1.

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