Comparative isotope ecology of western Amazonian rainforest mammals

Julia V. Tejada[a,b,c,1], John J. Flynn[a,b], Pierre-Olivier Antoine[d], Victor Pacheco[c], Rodolfo Salas-Gismondi[b,c,e], and Thure E. Cerling[f,g]

[a]Department of Earth and Environmental Sciences, Lamont-Doherty Earth Observatory, Columbia University, New York, NY 10027; [b]Division of Paleontology, American Museum of Natural History, New York, NY 10024; [c]Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima 15072, Peru; [d]Institut des Sciences de l'Évolution, UMR 5554, Université de Montpellier, CNRS, Institut de Recherche pour le Développement, Ecole Pratique des Hautes Etudes, Montpellier 34095, France; [e]BioGeoCiencias Lab, Facultad de Ciencias y Filosofía, Laboratorios de Investigación y Desarrollo (LID), Centro de Investigación para el Desarrollo Integral y Sostenible (CIDIS), Universidad Peruana Cayetano Heredia, Lima 15102, Peru; [f]Department of Geology and Geophysics, University of Utah, Salt Lake City, UT 84112; and [g]Department of Biology, University of Utah, Salt Lake City, UT 84112

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Closed-canopy rainforests are important for climate (influencing atmospheric circulation, albedo, carbon storage, etc.) and ecology (harboring 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 $\delta^{13}C_{bioapatite}$, $\delta^{13}C_{hair}$, and $\delta^{15}N_{hair}$, 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 $\delta^{13}C_{diet}$ values from closed-canopy rainforests in Amazonia (−27.4‰) and equatorial Africa (−26.9‰) are not significantly different, and that the median $\delta^{13}C_{diet}$ 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 $\delta^{13}C_{diet}$ values previously proposed as indicative of rainforests (<−31‰). Finally, results of keratin and bioapatite $\delta^{13}C$ indicate that the predictive power of trophic relationships, and traditional dietary ecological classifications in bioapatite-protein isotopic offset expectations, must be reconsidered.

western Amazonia | closed canopy rainforests | mammals | stable isotopes | isotope ecology

Climate and vegetation are traits defining tropical rainforests (1), but confident characterization and quantification of those archetypal traits of modern rainforests become increasingly challenging when analyzing ancient ecosystems. Closed-canopy rainforests have been proposed to occur in the area now occupied by Amazonia since at least the Eocene, some 50 million years ago, but their extent, and the influence of the active Cenozoic geologic history in South America (with Andean uplift as the major driver) on these forests, is equivocal due to the sparse plant fossil record in the tropics and the variable interpretations from sedimentology and paleobotanical-based modeling (1, 2). Isotopic evidence ($\delta^{13}C$) from mammalian herbivores can be a reliable proxy for reconstructing ancient ecosystems because the isotopic signals of vegetation (which tracks environmental factors and plant physiology) are recorded in primary consumers and are passed along the trophic chain to higher-level consumers. Thus, isotopic analysis is also a way to quantify vegetational criteria, which otherwise are primarily qualitative. To date, the isotopic structure of extant equatorial African mammals (3–6) has been the only broadly sampled system for understanding tropical ecosystems. Although phylogenetically and size-biased, this African system has become the de facto model to infer closed-canopy rainforests from fossil mammal data on all continents. However, the absence of comprehensive isotopic data from other closed-canopy mammalian communities impedes confident attribution of an isotopic range for this type of ecosystem across landmasses and over time. Thus, is there a unique mammalian isotope signal for all closed-canopy rainforest ecosystems? Do different phylogenetic, geologic, and biogeographic histories influence the isotopic structure of these ecosystems? To answer these questions, a broad suite of modern western Amazonian mammalian taxa were sampled for isotopic analyses. Western Amazonian mammalian communities, the most diverse on the planet, exhibit phylogenetic structures distinct from their African counterparts, resulting from tens of millions of years of geographic isolation over most of the Cenozoic in addition to distinct and complex biotic interactions over time. By comparing the isotopic structure of mammalian communities from tropical rainforests in western Amazonia and equatorial Africa, this study aims to define the $\delta^{13}C$ range for closed-canopy rainforests across continents and to provide a baseline for understanding changes in the Amazonian ecosystem through time.

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 $\delta^{13}C_{diet}$ 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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[1]To whom correspondence may be addressed. Email: jtejada@ldeo.columbia.edu.

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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 intertwined evolutionary histories between African and Eurasian mammals. Notable within the evolutionary history of African mammals is the Miocene–Pliocene radiation of endemic groups, including various bats, primates (e.g., hominids), hyracoids, carnivorans, 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‰ to −24.1‰ (14–17). Of these, leaves show the most negative values (−32.1‰), compared to bole (−28.4‰) or litter (−28.7‰) (14). Although the data are not exhaustive, fruits and seeds (−29.3‰) show higher $\delta^{13}C$ values than leaves (18). In a vertical profile of the forest, Amazonian plants show a decrease in their leaf $\delta^{13}C$ 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‰ if growing within 1 m above the ground, −33.4‰ in the lower canopy (2 to 10 m), and −30.5‰ 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 ~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° 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‰, 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‰ to −21‰ (15, 23). Herbivorous insects in central Amazonia also exhibit a broad range of $\delta^{13}C$ values (−29.5‰ to −15‰), 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 $\delta^{13}C_{atm}$ has decreased by ca. 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‰, subsequently referred as $\delta^{13}C_{1750}$; 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).

$\delta^{13}C_{diet}$ 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 mammalian herbivores have a median $\delta^{13}C_{diet}$ of −27.4‰, ranging from −30.4‰ to −12.3‰. This 18‰ range of variation is bracketed by the red titi monkey *Plecturocebus 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 $\delta^{13}C_{diet}$ = −24.9‰) show an isotope span of 3‰, bracketed by the red brocket 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 isotope variation (<3‰), with a median $\delta^{13}C_{diet}$ of −28.6‰ (n = 7). In contrast, the only lagomorph in this study (the tapeti, *Sylvilagus brasiliensis*), shows a large intraspecific isotopic variation (5‰, median $\delta^{13}C_{diet}$ = −28.2‰), even though the individuals sampled come from the same area (n = 3). Rodents (9 species, n = 38) overall show $\delta^{13}C_{diet}$ values ranging from −28.8‰ to −12.3‰. This represents the largest range of $\delta^{13}C_{diet}$ span (16.5‰) of all western Amazonian herbivore clades, driven by the presence of the sole C4 consumer, the capybara (median $\delta^{13}C_{diet}$ = −15.5‰). Excluding the capybara, the range of $\delta^{13}C_{diet}$ for rodents is much narrower, at 5.5‰. Primates, the group best represented in our sampling (13 species, n = 54), show an isotopic span of 4.6‰, with $\delta^{13}C_{diet}$ values ranging from −30.2‰ (red titi monkey, *P. discolor*)

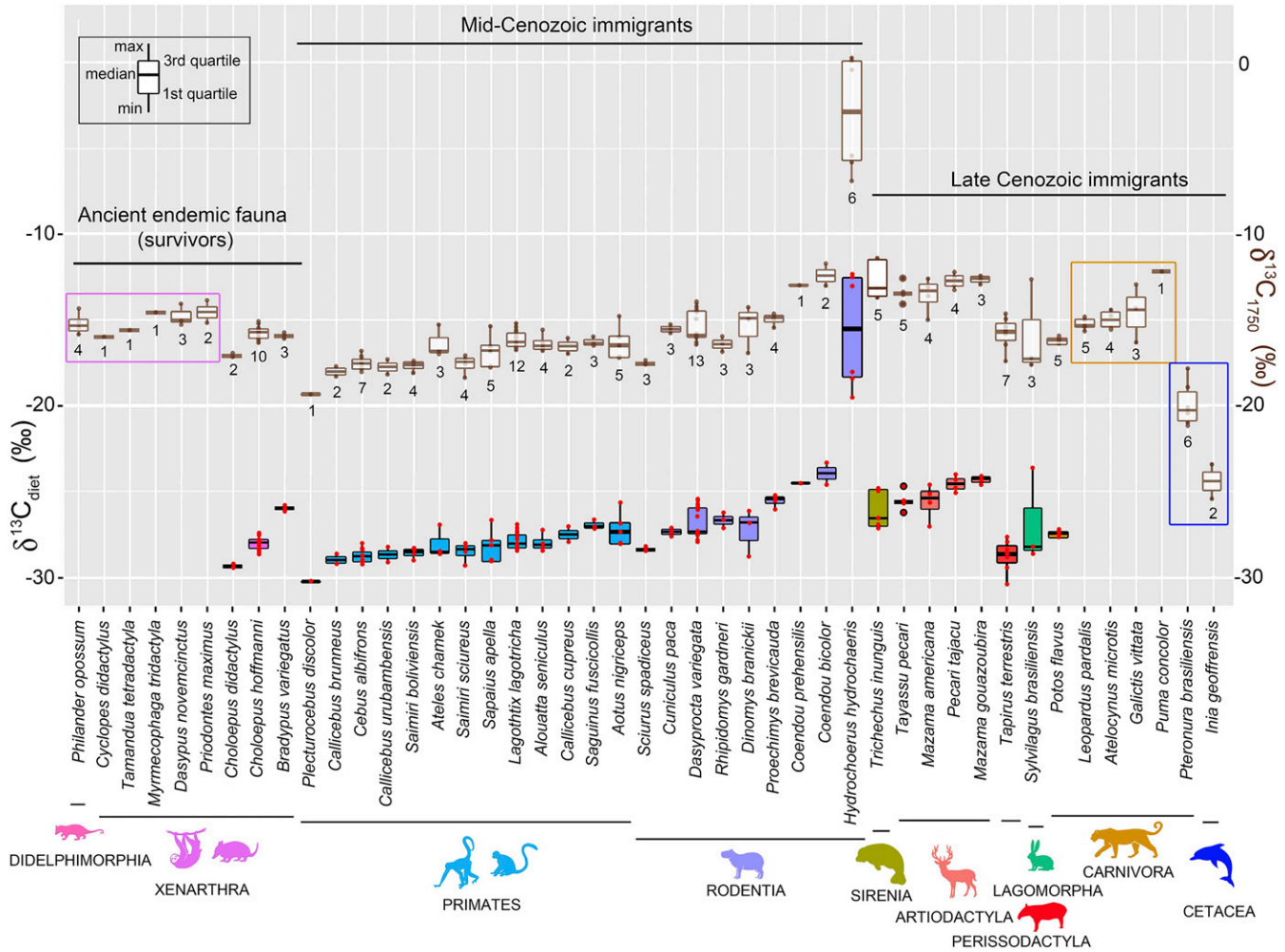

Fig. 1. $\delta^{13}C_{diet}$ (colored box plots, below) for Amazonian herbivores only and $\delta^{13}C_{1750}$ (white, sepia-bordered boxplots, above) for all western Amazonian mammals analyzed. Box plots represent the distribution of the data as explained in the key in the upper left corner. $\delta^{13}C_{diet}$ values for herbivores (calculated from dental bioapatite) represent the vegetation on which these primary consumers feed. Taxa inside colored framing rectangles in the $\delta^{13}C_{1750}$ plots are secondary consumers (lilac, insectivores; yellow, carnivores; blue, piscivores). Numbers below the box plots represent the number of samples analyzed per taxon.

to −25.6‰ (black-headed night monkey, *Aotus nigriceps*). Herbivorous xenarthrans (i.e., sloths) show a $\delta^{13}C_{diet}$ range of 3.6‰ and have a median $\delta^{13}C_{diet}$ of −27.8‰ ($n = 15$). The only fully aquatic herbivore in our study, the Amazonian manatee (Sirenia: *Trichechus inunguis*), has a median $\delta^{13}C_{diet}$ falling within the average values of terrestrial Amazonian C3 consumers (−26.6‰), and has a narrow intraspecific $\delta^{13}C_{diet}$ 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 $\delta^{13}C_{diet}$ 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 $\delta^{13}C_{1750}$ of 12 secondary consumers (carnivores, piscivores, and insectivores). The $\delta^{13}C_{1750}$ values of these secondary consumers span 13.2‰, ranging from −25.4‰ to −12.2‰ (Fig. 1 and Table 2). The lowest $\delta^{13}C_{1750}$ values of all taxa are observed among the piscivorous species: the Amazon River dolphin (*Inia geoffrensis*; median $\delta^{13}C_{1750} = -24.4‰$, $n = 2$) and the giant otter (Carnivora: *Pteronura brasiliensis*; median $\delta^{13}C_{1750} = -20.3‰$, $n = 6$). Terrestrial carnivores (*Puma*, *Galictis*, *Atelocynus*, and *Leopardus*) show a median $\delta^{13}C_{1750}$ 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 $\delta^{13}C_{1750}$ of −15.4‰ ($n = 4$) and low variation among specimens (0.6‰). Armadillos and anteaters, the insectivorous xenarthrans, have a median $\delta^{13}C_{1750}$ of −15.1‰ and a similarly small variation among individuals (SD = 0.7‰, $n = 8$).

$\delta^{15}N_{hair}$ and Hair-Bioapatite $\delta^{13}C$ Enrichment. Sampling hair (keratin) in addition to dental bioapatite provided new measures of $\delta^{15}N_{hair}$ variation across Amazonian mammals and enabled us to analyze natural variations in $\delta^{15}N_{hair}$ as well as a more nuanced view of dietary $\delta^{13}C$ values ($\delta^{13}C_{hair}$ and $\delta^{13}C_{bioapatite}$) among Amazonian mammals (Fig. 3 and Table 3). Indeed, these data enabled us to test whether the "expected" offset between the $\delta^{13}C$ of bioapatite and that of a proteinaceous tissue (keratin in this study, $\varepsilon^*_{bioapatite\text{-}keratin}$), proposed as indicative of trophic level (26, 27), is met in this mammalian assemblage. $\delta^{15}N_{hair}$ values for 37 mammalian species span 10‰, ranging from Rodentia (*Dinomys branickii*, $\delta^{15}N_{hair} = 2.3‰$) to Carnivora (*P. brasiliensis*, median $\delta^{15}N_{hair} = 11.8‰$). The $\delta^{15}N_{hair}$ 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 $\delta^{13}C$ results from dental bioapatite

Group	No. species/no. specimens	$\delta^{13}C_{1750}$ ‰			Reconstructed $\delta^{13}C_{diet}$ ‰		
		Median ± 1 SD	Range	$\delta^{13}C_{1750}$ span	Median ± 1 SD	Range	$\delta^{13}C_{diet}$ 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	−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 Dataset). $\delta^{13}C_{1750}$ refers to raw values corrected for anthropogenic CO_2 set to preindustrial values (the year 1750) ± 1 SD. $\delta^{13}C_{diet}$ refers to the reconstructed diet, which, for herbivores, refers to the $\delta^{13}C$ of the vegetation on which they feed. $\delta^{13}C_{diet}$ was not calculated for secondary consumers (Table 2 shows secondary consumer $\delta^{13}C_{1750}$ values only). C3, taxa consuming C3 plants; span, total range in ‰.

in $\delta^{13}C$ enrichment between bioapatite and keratin ($\varepsilon^*_{bioapatite\text{-}keratin}$; Fig. 3*A* and *SI Appendix*). Amazonian folivores, however, do have significantly larger $\varepsilon^*_{bioapatite\text{-}keratin}$ 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 $\delta^{13}C$ $\varepsilon^*_{bioapatite\text{-}keratin}$ values across this Amazonian mammalian community (>7‰, from 4.8‰ to 12.1‰; Fig. 3*A* and Table 3). In contrast, secondary consumers cluster within a narrow range of $\varepsilon^*_{bioapatite\text{-}keratin}$ values (2‰, from 6.4‰ to 8.4‰; Table 3). Frugivores show the smallest $\varepsilon^*_{bioapatite\text{-}keratin}$ values of the entire Amazonian mammalian community (range = 4.8 to 8.2‰). When $\delta^{13}C$ enrichment between diet and keratin ($\varepsilon^*_{diet\text{-}keratin}$) is assessed instead of $\varepsilon^*_{bioapatite\text{-}keratin}$, we observe the expected clustering of carnivores at the lower extreme of $\varepsilon^*_{diet\text{-}keratin}$ values and primary consumers with significantly larger $\varepsilon^*_{diet\text{-}keratin}$ values than carnivores (*SI Appendix*, Figs. S6 and S15). The $\varepsilon^*_{diet\text{-}keratin}$ among primary consumers spans 6‰, ranging from 0.2‰ to 6.3‰ (Fig. 3*B*). Significant differences were only found in the $\varepsilon^*_{diet\text{-}keratin}$ between folivores and frugivores.

Discussion

Comparisons of $\delta^{13}C_{diet}$ 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 $\delta^{13}C_{diet}$ values exhibited by herbivorous mammals in both continents is comparable (21‰ vs. 18‰ in Africa and SA, respectively), but while the isotopic range in Amazonia is driven primarily by the high $\delta^{13}C_{diet}$ values of the only C4 consumer (the capybara), in equatorial Africa, this similarly broad range is driven instead by the extremely negative $\delta^{13}C_{diet}$ values (<−30‰) observed in the few species feeding in the subcanopy stratum of the forest (the antelope *Neotragus batesi* [Bates's pygmy antelope], the giraffid *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 $\delta^{13}C_{diet}$ values, even though species living and feeding in the subcanopy stratum are represented in our analysis, and plants with $\delta^{13}C$ values as negative as the most negative plants in Africa do exist in western Amazonia (14, 15). Within a forest, the most negative $\delta^{13}C$ values are found in leaves growing in the understory, where light-deprived conditions increase isotope discrimination (28, 29). In fact, a $\delta^{13}C$ difference up to almost 5‰ can be seen within a single plant species, and a range of 10‰ 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 $\delta^{13}C_{diet}$ niche to selective-feeding forest dwelling herbivores, i.e., animals that almost exclusively consume leaves growing deep in the understory. That mammals with median $\delta^{13}C_{diet}$ values <−30‰ 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

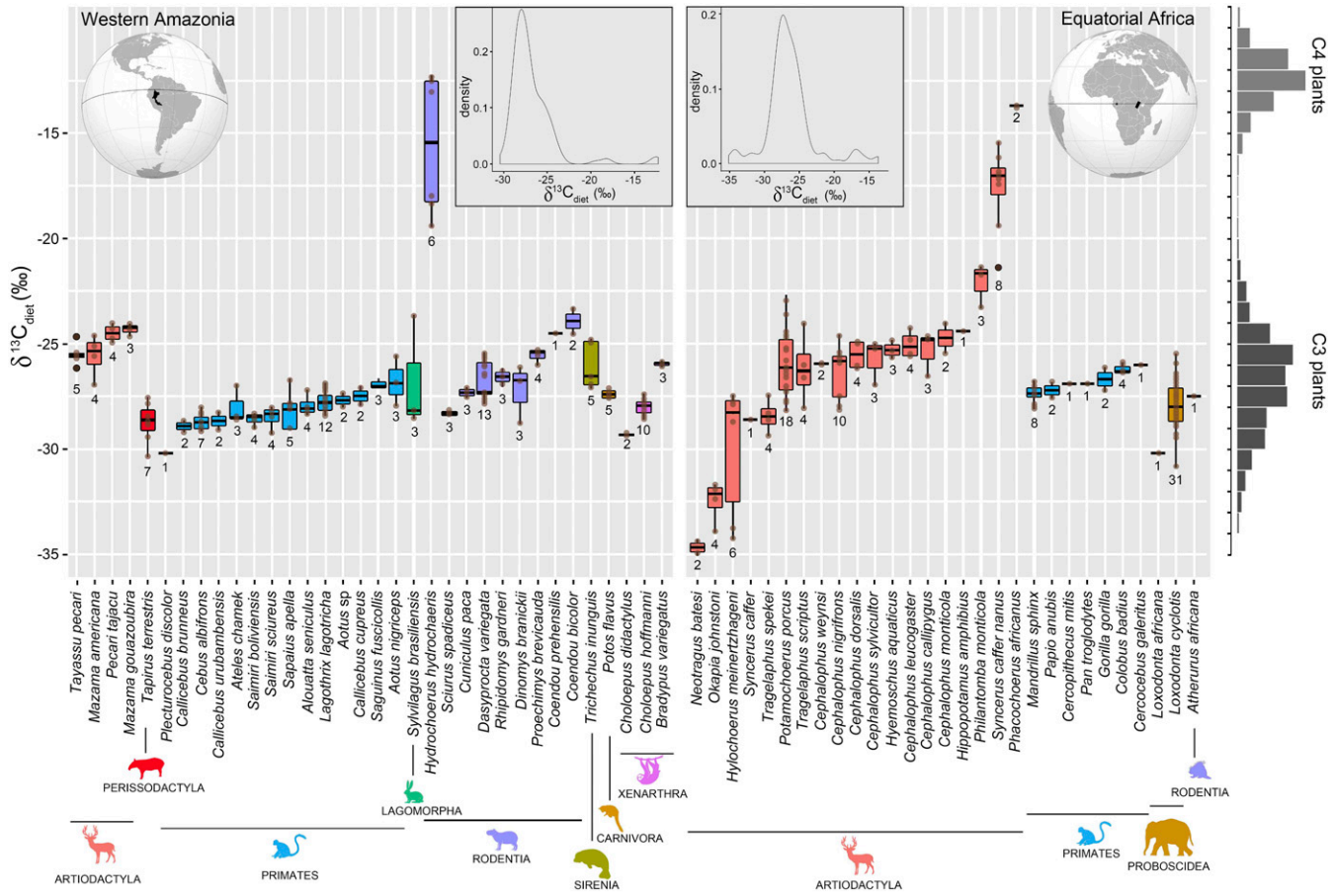

Fig. 2. $\delta^{13}C_{diet}$ values and distributions of the herbivores in mammalian communities of western Amazonia (*Left*) and equatorial Africa (*Right*). Histograms on the right axis represent the worldwide distribution of $\delta^{13}C$ values for plants with C3 and C4 photosynthesis (modified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

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 $\delta^{13}C_{diet}$ 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 −35.1‰ to −13.7‰), however, is significantly larger than in South America (5‰, ranging from −29‰ to −24‰). 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 $\delta^{13}C_{diet}$ of primates in both continents differs significantly, but the difference in means is only 1‰ (mean $\delta^{13}C_{diet}$ = −28‰ for SA rainforest primates, −27‰ 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 $\delta^{13}C_{diet}$ range (16.5‰), mainly driven by the $\delta^{13}C_{diet}$ of the capybara, whose exclusion decreases the breadth of $\delta^{13}C_{diet}$ variation in Amazonian rodents to 5.5‰. This wide $\delta^{13}C_{diet}$ 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 $\delta^{13}C_{diet}$ 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 $\delta^{13}C_{diet}$ range (<4‰), consistent with their low modern taxonomic and ecological diversity (limited to three species in western Amazonia).

The $\delta^{13}C_{1750}$ for terrestrial Amazonian carnivores (median = −15‰, 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 $\varepsilon^*_{predator\text{-}prey}$ for specialized terrestrial hypercarnivores (26), this $\varepsilon^*_{predator\text{-}prey}$ value (−1.3‰) has not been thoroughly examined in nonspecialized 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 $\varepsilon^*_{predator\text{-}prey}$ results in the reconstructed $\delta^{13}C_{diet}$ of Amazonian carnivores not matching that of most sympatric herbivores. The same applies for the African genet *Genetta*, for which a $\varepsilon^*_{predator\text{-}prey}$ 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	$\delta^{13}C_{1750}$, ‰		
		Median ± 1 SD	Range	$\delta^{13}C_{1750}$ span
Western Amazonia				
All secondary consumers	12/33	−15.4 ± 3	−25.4 to −12.2	13.2
Xenarthra (nonherbivores)	5/8	−15.1 ± 0.7	−16.0 to −13.9	2.1
Carnivora	5/19	−15.4 ± 2.4	−21.1 to −12.2	8.9
Terrestrial carnivores	4/13	−15.0 ± 1.1	−16.4 to −12.2	4.2
Cetacea	1/2	−24.4 ± 1.5	−25.4 to −23.3	2.1
Didelphimorphia	1/4	−15.4 ± 0.6	−15.9 to −14.4	1.5
Equatorial Africa				
Carnivora	1/2	−11.7 ± 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 $\delta^{13}C_{diet}$ values corresponding to exclusive consumption of food with carbon sources of C3 plant origin. The two piscivorous species (Cetacea: *I. geoffrensis* [river dolphin]; and Carnivora: *P. brasiliensis* [giant otter]) show significantly different $\delta^{13}C_{1750}$ values (medians of −20.3‰ and −24.4‰, 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 $\delta^{13}C_{diet}$ of terrestrial herbivores in South America (−27.4‰) and Africa (−26.9‰) are not significantly different (Fig. 4 and *SI Appendix*, Fig. S3). The median $\delta^{13}C_{diet}$ for these two rainforests is −27.2‰ (SD = 3.2‰, SE = 0.2‰), a value that we propose could be expected for mammalian herbivores living in any closed-canopy tropical rainforest. Although this −27.2‰ value for closed-canopy tropical rainforests is nearly identical to the global mean $\delta^{13}C$ for plants (31), the median $\delta^{13}C_{diet}$ of nonrainforest mammalian communities seems to be more positive than −27.2‰ (*SI Appendix*, Fig. S7). Furthermore, the median $\delta^{13}C_{plants}$ of tropical rainforests is more negative than the global average (−31‰; comparative data of rainforest plant communities provided in *SI Appendix*, Figs. S9–S13). That both Amazonian and African herbivores show an offset in their $\delta^{13}C_{diet}$ (−27.2‰) relative to the overall rainforest $^{13}C_{plant}$ values (−31‰) 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 $\delta^{15}N$ values, with 3 to 4‰ 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 ($\varepsilon^*_{bioapatite\text{-}protein}$) 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 $\varepsilon^*_{bioapatite\text{-}protein}$ (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 intricacies 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: *Bradypus*, *Trichechus*, *Sylvilagus*, *Hydrochoerus*, and two species of *Coendou*). Of these, only *Bradypus* and *Coendou* are classifiable as obligatory specialists (i.e., very narrow realized niche and diet [36]), corroborated by their narrow intraspecific $\delta^{13}C_{diet}$ values. Contrary to findings in other studies (e.g., ref. 26), frugivores rather than secondary consumers had the lowest $\varepsilon^*_{bioapatite\text{-}keratin}$ 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 ^{13}C-depleted relative to other macronutrients. This more negative $\delta^{13}C_{diet}$ will then be imprinted in the $\delta^{13}C_{bioapatite}$ (thus now closer in its $\delta^{13}C$ value to proteinaceous tissues). Even though low $\varepsilon^*_{bioapatite\text{-}keratin}$ among some frugivores might also be interpreted as reflecting omnivory, in the current paradigm, omnivores would not be expected to show lower $\varepsilon^*_{bioapatite\text{-}keratin}$ values than carnivores or insectivores (e.g., in our dataset, some primate and rodent species have smaller $\varepsilon^*_{bioapatite\text{-}keratin}$ values than felids and canids; Fig. 3*A*). 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 phenologies of two bamboo species, giant pandas

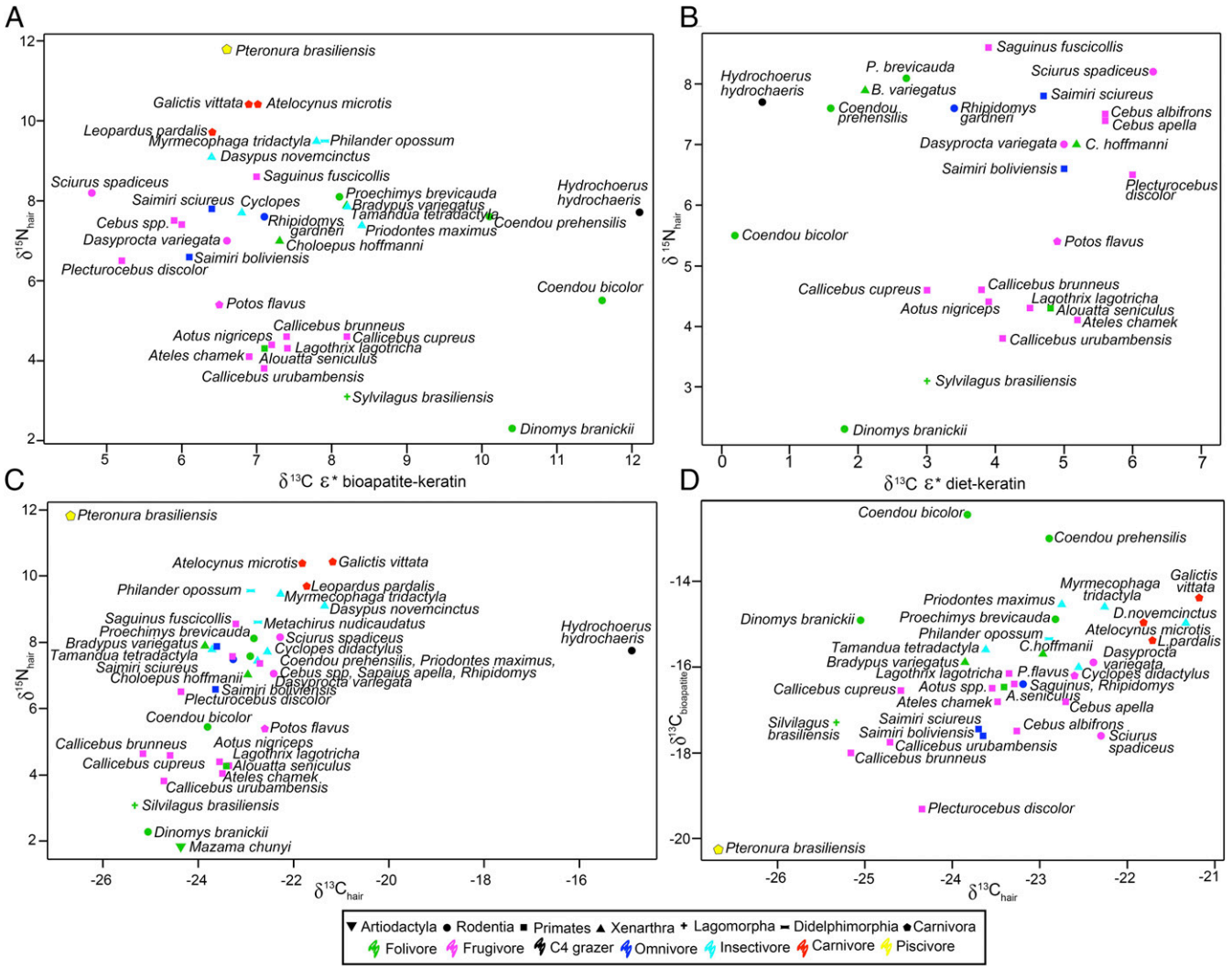

Fig. 3. (*A*) $\delta^{15}N_{hair}$ vs. $\delta^{13}C\ \varepsilon^*_{bioapatite\text{-}keratin}$ of all Amazonian mammals and (*B*) $\delta^{13}C\ \varepsilon^*_{diet\text{-}keratin}$ ($\delta^{13}C_{diet}$ from bioapatite) for Amazonian primary consumers only; (*C*) $\delta^{13}C_{hair}$ vs. $\delta^{15}N_{hair}$ and (*D*) $\delta^{13}C_{bioapatite}$ (both standardized only to atmospheric $\delta^{13}C_{1750}$ values). Each point represents the median value per species. Taxonomic symbols and dietary categorization colors indicated in the boxed legend at the bottom of the figure.

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 $\varepsilon^*_{bioapatite\text{-}protein}$ spectrum when measured. The lower than expected values in $\varepsilon^*_{bioapatite\text{-}keratin}$ observed in some Amazonian primary consumers might be explained in a similar way. Other studies have also shown highly variable $\varepsilon^*_{bioapatite\text{-}protein}$ values within a free-ranging herbivore mammalian community (39), as well as $\varepsilon^*_{bioapatite\text{-}protein}$ poorly distinguishing trophic levels (40), suggesting that the $\varepsilon^*_{bioapatite\text{-}protein}$ within dietary categories (especially among primary consumers) should not be expected to be uniform.

The lack of trophic-level segregation in the $\varepsilon^*_{bioapatite\text{-}keratin}$ 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*) $\delta^{13}C$ 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 ($\varepsilon^*_{diet\text{-}keratin}$; Fig. 3*B*) rather than between bioapatite and keratin, we observe the expected segregation of carnivores at the lower extreme of $\varepsilon^*_{diet\text{-}keratin}$ values and primary consumers showing significantly higher $\varepsilon^*_{diet\text{-}keratin}$ values (*SI Appendix*, Fig. S15). Among primary consumers, the obligate herbivores *Hydrochoerus* and the two *Coendou* species (Rodentia) show the lowest $\varepsilon^*_{diet\text{-}keratin}$ values (Fig. 3*B*). Even using a higher $\varepsilon^*_{diet\text{-}bioapatite}$ value (e.g., 14‰) than our body mass-corrected values to reconstruct the dietary $\delta^{13}C$ (11.8‰ for 4-kg *Coendou* and 12.7‰ for 50-kg *Hydrochoerus*) does not place these species at the uppermost extreme of the $\varepsilon^*_{diet\text{-}keratin}$ spectrum, as would be expected for obligate herbivores. Furthermore, the specialized obligate folivore *Bradypus*, a species with a known controlled-feeding $\varepsilon^*_{diet\text{-}bioapatite}$ value (41), also shows $\varepsilon^*_{diet\text{-}keratin}$ 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. The

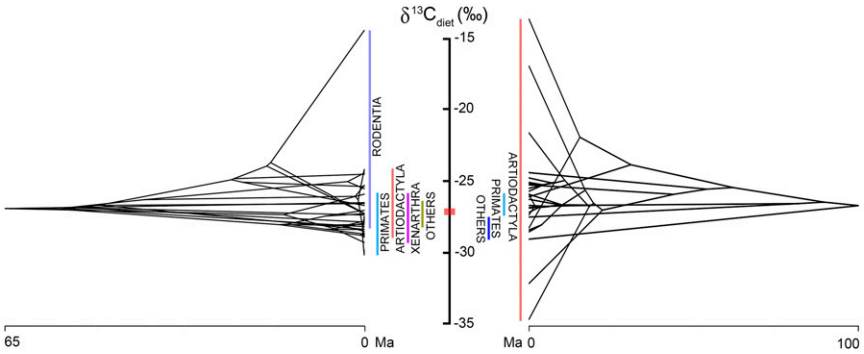

Fig. 4. $\delta^{13}C_{diet}$ values for western Amazonian and equatorial African herbivorous mammals plotted into their respective phylogenies. Although the medians for $\delta^{13}C_{diet}$ of herbivores in western Amazonia (−27.4‰) and equatorial Africa (−26.9‰) are not significantly different (median for the two = −27.2‰; red box on the $\delta^{13}C_{diet}$ axis), the ranges of $\delta^{13}C_{diet}$ exhibited by the herbivores in these mammalian clades differ dramatically in each rainforest.

Amazonian data do not support the widely held assumption *ii* 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 $\varepsilon^*_{diet\text{-}keratin}$ among Amazonian primary consumers illustrates previously unexpected complexities associated with routing of macronutrients for protein synthesis. The low $\varepsilon^*_{diet\text{-}bioapatite}$ 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 $\varepsilon^*_{diet\text{-}bioapatite}$ values observed in most frugivore species suggest that these species are synthesizing amino acids de novo (likely from carbohydrates) to produce keratin (Fig. 3*B*).

In summary, many Amazonian mammals do not fall in the expected place along the $\varepsilon^*_{bioapatite\text{-}keratin}$ 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 $\delta^{13}C$ 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 $\delta^{13}C_{diet}$ 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 $\delta^{13}C_{diet}$ values (*N. batesi* and *O. johnstoni*, although some individuals of the suid *H. meinertzhageni* as well as the forest elephant *L. cyclotis* also show $\delta^{13}C_{diet} < -30‰$). 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,

Table 3. Summary of results for $\delta^{15}N_{hair}$ and $\delta^{13}C_{hair}$ (standardized to $\delta^{13}C_{1750}$ values) and $\delta^{13}C$ $\varepsilon^*_{diet\text{-}keratin}$ for modern western Amazonian mammals

Group	No. of species	Median $\delta^{15}N_{hair}$, ‰	Range of $\delta^{15}N_{hair}$, ‰	Median $\delta^{13}C_{hair\ 1750}$, ‰	Range of $\varepsilon^*_{bioapat\text{-}keratin}$, ‰	Range of $\varepsilon^*_{diet\text{-}keratin}$, ‰
All mammals	35	7.5	2.3–11.8	−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
Secondary consumers	10	9.5	7.4–11.8	−22.5	6.4–8.4	—
Primates	13	4.6	3.8–8.6	−23.6	5.2–8.2	2.9–6
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	—
Didelphimorphia	1	9.5	—	−23.0	7.8	—

The reported range of values refers to medians per species (not of individual specimens). No $\varepsilon^*_{predator\text{-}prey}$ is available for Amazonian secondary consumers; thus, $\delta^{13}C_{diet}$ and $\varepsilon^*_{diet\text{-}keratin}$ 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 $\delta^{13}C_{diet}$ <−30‰ (the tapir, the only modern Amazonian perissodactyl, has the lowest $\delta^{13}C_{diet}$ [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 $\delta^{13}C_{diet}$ 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 $\delta^{13}C_{diet}$ 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 isotope 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 foregut fermenters 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 $\delta^{13}C_{diet}$ of Amazonian toxodonts (median $\delta^{13}C_{diet}$ = −27‰) was found to be lower than modern Amazonian artiodactyls, although still not as negative as the African subcanopy feeders (i.e., $\delta^{13}C_{diet}$ <−30‰). Although other studies have isotopically characterized Pleistocene taxa at high latitudes in South America (49, 50), no isotopic characterization of Pleistocene mammalian communities from closed-canopy rainforests has yet been done because of limited known localities from these habitats, where plants with $\delta^{13}C$ <−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 modern Amazonian mammals by revealing if surviving lineages shifted or expanded their feeding ecologies (and isotope 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 $\delta^{13}C_{diet}$ 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 $\delta^{13}C_{diet}$ values <−30‰, but rather why some African mammals do. Indeed, those highly negative values are only observed in a few African mammal species only from the Ituri Forest, both small- and large-bodied, and have not been recorded elsewhere. This observation would further highlight our conclusion that $\delta^{13}C_{diet}$ <−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 $\delta^{13}C$ <−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 $\delta^{13}C$ from dental bioapatite (enamel for all mammals except xenarthrans [see below]) of 45 mammalian species (n = 176 individuals), $\delta^{13}C$ and $\delta^{15}N$ of hair keratin of 35 species (n = 125), and $\delta^{13}C_{bioapatite}$, $\delta^{13}C_{hair}$, and $\delta^{15}N_{hair}$ 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 $\delta^{13}C_{bioapatite}$ was sampled from the orthodentine, whereas, for the five species of anteaters and armadillos (toothless or with such small teeth that sampling was not possible), the proxy $\delta^{13}C$ of their "enamel" was projected from bone $\delta^{13}C$ values. We transformed bone $\delta^{13}C$ data to corresponding dental enamel values using regression equations obtained from a separate analysis of the matched samples (*SI Appendix*, Fig. S1). All raw $\delta^{13}C$ data (for bioapatite, hair, and comparative plant values for both South American and African samples) were corrected for anthropogenic CO_2 and set to preindustrial values (to the baseline year of 1750; $\delta^{13}C_{1750}$). In order to make data comparable among herbivorous taxa, the CO_2-corrected bioapatite $\delta^{13}C$ (i.e., $\delta^{13}C_{1750}$) was converted to dietary $\delta^{13}C$ ($\delta^{13}C_{diet}$). This was done by using the body mass-dependent equations determined by a previous study (41) to calculate the diet-bioapatite $\delta^{13}C$ enrichment ($\varepsilon^*_{diet\text{-}bioapatite}$) specific to each species (the calculated $\varepsilon^*_{diet\text{-}bioapatite}$ values for the herbivores in our study range from 10.3‰ to 13.7‰). Reported $\delta^{13}C$ values for secondary consumers reflects the $\delta^{13}C_{1750}$ (and not the reconstructed $\delta^{13}C_{diet}$) pending development of reliable methods for estimating $\varepsilon^*_{predator\text{-}prey}$ that allow

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confident dietary reconstructions for these feeding guilds. $\delta^{15}N_{hair}$ 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 (3–5) 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 "herbivore" 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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