Carbon-isotope composition of artiodactyl tooth enamel and its implications for paleodiets

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The stable carbon-isotope composition of mammalian tooth enamel is a powerful tool for reconstructing paleodiet and paleoenvironment. Its application in the fossil record relies on a thorough understanding of the isotopic composition of mammalian diets in modern ecosystems. We compiled and evaluated a global dataset of the carbon-isotope values of artiodactyl tooth enamel, supplemented by new samples, for 79 extant species. After correcting for differences in atmospheric carbon-isotope composition, body mass, and digestive physiology, we compared the inferred carbon-isotope values of ingested forage ($\delta^{13}C_{diet}$) among seven feeding categories. The artiodactyl herbivore dietary spectrum is expressed through a wide range of $\delta^{13}C_{diet}$ values, with the most depleted mean value in frugivores and the most enriched in obligate grazers. In general, grazing species have a broader range of isotope values than browsing species, suggesting a wider dietary niche breadth. Notably, variable grazers exhibit a bimodal distribution of $\delta^{13}C_{diet}$ values, with North American and Asian taxa consuming C_3 diets and African taxa consuming C_4 diets, reflecting the amount of C_4 vegetation in the environment. Variation in $\delta^{13}C_{diet}$ values also occurs among terrestrial ecoregions and artiodactyl clades. Grassland ecoregions differ significantly from forest ecoregions. We detected a low but significant phylogenetic signal in the mean $\delta^{13}C_{diet}$ values of extant species, with some of the oldest ruminant lineages having maintained C_3 feeding and pure C_4 diets being restricted to two bovid clades. Determining variation in $\delta^{13}C_{diet}$ values in different feeding categories and lineages will help refine paleoecological and paleoenvironmental reconstructions from the rich fossil record of artiodactyls.

KEYWORDS

stable isotope, herbivore, ungulates, tooth enamel, paleodiet

Introduction

Stable isotopes are one of nature's great ecological recorders and have been widely used to study organisms and ecosystems across time and space (West et al., 2006; Clementz, 2012). Among the animal tissues that are commonly sampled for stable isotope analyses of mammals (tooth enamel, dentin, bone collagen, hair, blood), only tooth enamel is resistant to long-term fossilization processes and diagenesis (Wang and Cerling, 1994; Koch et al., 1997; Lee-Thorp, 2002). Therefore, data generated from modern mammal teeth can be readily applied to the interpretation of deep-time records. Isotope data from extant ungulates have contributed substantial insights into our understanding of herbivore dietary ecology and have laid the foundations for a large body of literature inferring paleodiet and paleoenvironment from fossil herbivore teeth (e.g., Koch et al., 1991; Bocherens et al., 1996; Cerling and Harris, 1999; Passey and Cerling, 2002; Cerling et al., 2003, 2010; Sponheimer et al., 2003; Sponheimer and Cerling, 2014). Extant terrestrial ungulates are represented by over 250 species of artiodactyls and 18 species of perissodactyls (Burgin et al., 2018). Artiodactyls are naturally widespread in the ecosystems of Africa, Eurasia, North America, and South America. The taxonomic and ecological diversity of this family renders them good modern analogs for many extinct ungulates.

Carbon isotopes in tooth enamel

The stable isotope composition of carbon (δ^{13}C) in the enamel of mammalian herbivores provides information about the animals' feeding ecology and vegetation present in the habitat. Plants that use different photosynthetic pathways differ in their fractionation of atmospheric CO_2 during photosynthesis. The resulting δ^{13}C values in plant tissues are lowest in plants using the C_3 pathway, intermediate in plants using the crassulacean acid metabolism (CAM) pathway, and highest in plants using the C_4 pathway (O'Leary, 1988; Cerling et al., 1997). When plants are consumed by mammalian herbivores and incorporated into their body tissues, tooth enamel bioapatites are systematically enriched in δ^{13}C relative to bulk plant diet with measurable enrichment factors (Lee-Thorp and van der Merwe, 1987; Cerling and Harris, 1999; Passey et al., 2005). Thus, the carbon isotope composition of enamel reliably reflects the values in the ingested plants. Pure C_3 and C_4 consumers have non-overlapping δ^{13}C values, while mammals with mixed C_3-C_4 diets have intermediate δ^{13}C values (Cerling et al., 1997, 2015; Koch, 1998). CAM plants commonly grow in xeric habitats (Ehleringer et al., 1991) and are not typically consumed by ungulates. Therefore, they are not usually considered when interpreting the carbon isotopic values of modern or fossil ungulates.

Isotopic variability among C_3 plants is generally greater than that in C_4 plants (e.g., Cerling et al., 1997). In general, higher (more enriched) values of the carbon isotope composition of tooth enamel of herbivorous mammals are representative of open-canopy, drier habitats (such as shrubland and grassland), while low values represent closed-canopy habitats (such as woodlands and forests) (O'Leary et al., 1992; Koch, 1998; Cerling and Harris, 1999; Feranec and MacFadden, 2006; Feranec, 2007; Secord et al., 2008). Resource partitioning in diet and habitat use may be revealed through stable isotope analysis (Feranec and MacFadden, 2006) and has been documented for medium-to large-bodied herbivores in both modern and ancient environments in which a mixture of C_3 and C_4 plants is present (e.g., Wang et al., 1994; MacFadden and Cerling, 1996; Koch, 1998; Feranec and MacFadden, 2006; Kita et al., 2014; Wang and Secord, 2020).

The browser–grazer spectrum

Most artiodactyls are herbivorous, and they are typically classified as browsers, grazers, or mixed feeders. Browsers primarily feed on dicotyledonous material, such as leaves, fruits, and twigs; grazers primarily feed on monocotyledonous material, such as grasses or sedges, which are generally more abrasive than dicotyledonous material; and mixed feeders consume a mixture of dicotyledonous and monocotyledonous materials across space and seasons (Hofmann and Stewart, 1972). Much of the existing literature classifies living and fossil herbivores using these categories. Finer dietary classifications have been used in some studies to capture more details in the foraging behavior of various artiodactyls (e.g., Janis and Ehrhardt, 1988; Spencer, 1995; Sponheimer et al., 1999; Mendoza et al., 2002). These studies show promise for differentiating artiodactyl feeding categories beyond three broad categories, giving consideration to the specialization or generality of species' diets, the canopy-cover of feeding environment, and sometimes the level (height from the ground) that species feed at. Other studies in recent years have used the percentage of grass in species' diets to place species quantitatively along a dietary continuum. This approach overcomes the potential weaknesses of categorical trait data and conceptualizes diet as a spectrum with two end members: browsers and grazers (e.g., Clauss et al., 2003).

Research questions

Recent research has identified gaps in our knowledge of the stable isotope ecology of large mammals and its application in the fossil record, such as what factors influence enrichment processes and how much variability in resource partitioning exists among different faunas (Tejada-Lara et al., 2018;
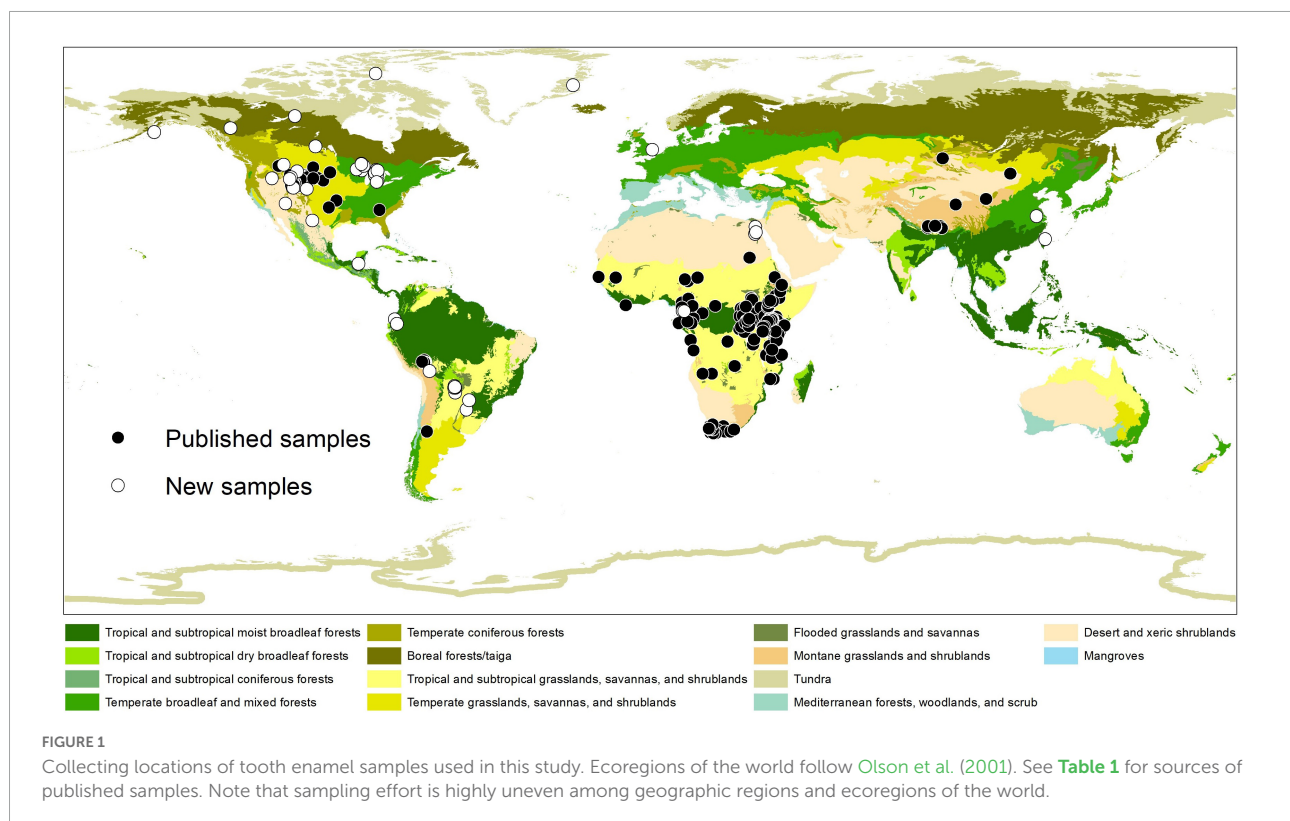
DeSantis et al., 2020; Tejada et al., 2020). These findings highlight the need for more data from a range of modern ecosystems for better understanding of processes and factors that affect the isotopic signatures in mammal tissues. In addition, stable-isotope ratios of ungulate tooth enamel can be compared to the dental hypsodonty index, dental mesowear (sharpness of tooth cusps), dental microwear (microscopic abrasion patterns on the occlusal surface), ecomorphological analysis of craniomandibular features, as well as stomach- and fecal contents and other types of data that are used to document ungulate dietary ecology. It has been shown that combining results from multiple methods improves accuracy of paleoecological reconstructions (e.g., Rivals and Ziegler, 2018; Sewell et al., 2019). Such cross-method comparisons capture dietary behaviors and adaptations recorded through different processes and over different time scales, thus providing more reliable and detailed dietary information for extant and fossil species (e.g., Sponheimer et al., 2003; Boisserie et al., 2005; Merceron et al., 2006; Louys et al., 2012; Bradham et al., 2018; Uno et al., 2018; Gong et al., 2020).

In this study, we compile and compare the carbon-isotope data published to date from the tooth enamel of extant artiodactyls. To build on existing data and increase the range and distribution of isotopic values across taxa and regions, we additionally sampled and analyzed 80 tooth specimens from 23 species of artiodactyls. Each species was chosen either because it had not been previously analyzed for stable isotopes of enamel or because existing isotope data for the species included small sample sizes from restricted locations. The resulting dataset includes published and new isotope data for 79 artiodactyl species, covering a range of habitats and dietary ecology. We adopt a more detailed classification scheme of herbivore diets than what is commonly used in the literature. This classification scheme includes seven feeding categories and provides more information about dietary habits than the three broad categories of browsing, grazing, and mixed feeding. Combining the isotope data from artiodactyl enamel, dietary data derived from other studies, and the environmental setting of localities, we address the following research questions: (1) How do species with different dietary habits compare in the mean and range of $\delta^{13}C$ values of their diet? (2) How do artiodactyl diets in different ecoregions of the world compare in the mean and range of $\delta^{13}C$ values? (3) How do phylogenetic groups of artiodactyls compare in the mean and range of $\delta^{13}C$ values?

Materials and methods

We compiled a global dataset of the carbon-isotope values of artiodactyl tooth enamel ($\delta^{13}C_E$) from the literature, supplemented by 80 newly analyzed samples to expand the taxonomic and geographic coverage of our data (**Figure 1** and **Table 1**). After correcting for differences in atmospheric carbon-isotope composition ($\delta^{13}C_{atm}$), body mass, and digestive

FIGURE 1
Collecting locations of tooth enamel samples used in this study. Ecoregions of the world follow Olson et al. (2001). See **Table 1** for sources of published samples. Note that sampling effort is highly uneven among geographic regions and ecoregions of the world.

TABLE 1 Eighty new samples of artiodactyl tooth enamel collected from specimens housed in the University of Michigan Museum of Zoology (UMMZ) and analyzed for carbon-isotope composition ($\delta^{13}C_E$).

Species	Collection No.	Tooth	Region	Country	Latitude	Longitude	$\delta^{13}C_E$
Alces americanus	UMMZ 60536	Lp4	Michigan	United States	48.10	−88.70	−14.4
Alces americanus	UMMZ 61782	Rm3	Michigan	United States	47.37	−88.11	−14.9
Alces americanus	UMMZ 64975	Lm3	Michigan	United States	48.03	−88.77	−14.3
Antilocapra americana oregona	UMMZ 44370	Lm3	Montana	United States	45.78	−108.50	−10.1
Antilocapra americana oregona	UMMZ 44372	Lm3	Montana	United States	45.78	−108.50	−9.2
Antilocapra americana oregona	UMMZ 65026	Rm3	Michigan	United States	42.46	−84.01	v13.9
Antilocapra americana oregona	UMMZ 65502	Rm3	Michigan	United States	42.46	−84.01	−12.2
Antilocapra americana oregona	UMMZ 67482	Lm3	Michigan	United States	42.46	−84.01	−11.2
Capra nubiana	UMMZ 163513	Lm3	Red Sea	Egypt	26.57	32.20	−6.2
Capra nubiana	UMMZ 164923	Lp4	Red Sea	Egypt	28.70	32.37	−7.9
Cv-apra nubiana	UMMZ 164942	Lm3	Red Sea	Egypt	27.15	32.53	−8.1
Capreolus capreolus	UMMZ 125684	Lm3	England	United Kingdom	52.40	0.70	−11.1
Cephalophus sp.	UMMZ 38376	Lm3	Kribi	Cameroon	2.95	9.92	−12.1
Cervus elaphus canadensis	UMMZ 57713	Lm3	Michigan	United States	42.81	−83.78	−11.8
Cervus elaphus canadensis	UMMZ 57755	Lm3	Michigan	United States	44.85	−83.96	−12.0
Cervus elaphus canadensis	UMMZ 59189	Lm3	Utah	United States	40.85	−109.89	−11.0
Cervus elaphus canadensis	UMMZ 59798	Rm3	Wyoming	United States			−11.2
Cervus elaphus canadensis	UMMZ 59799	Rm3	Wyoming	United States			−10.8
Cervus elaphus canadensis	UMMZ 62121	Lm3	Wyoming	United States	44.00	−110.41	−11.3
Cervus elaphus canadensis	UMMZ 62122	RM3	Wyoming	United States	43.48	−110.76	−10.6
Gazella dorcas	UMMZ 158959	Lm3	Wadi	Egypt			−12.1
Gvazella dorcas	UMMZ 158960	Lm3	Wadi	Egypt			−10.1
Gazella dorcas	UMMZ 158962	Rm3	Wadi	Egypt			−11.0
Gazella dorcas	UMMZ 158966	Lm3	Wadi	Egypt			−8.7
Gazella dorcas	UMMZ 158967	Lm3	Wadi	Egypt			−10.6
Gazella dorcas	UMMZ 158969	Rm3	Wadi	Egypt			−10.1
Gazella dorcas	UMMZ 158970	Lm3	Wadi	Egypt			−10.1
Gazella dorcas	UMMZ 158972	Lp4	Wadi	Egypt			−12.3
Hydropotes inermis inermis	UMMZ 56527	Rm3	Kiang-su	China	32.07	118.78	−14.2
Mazama americana	UMMZ 126128	Rm3	Canindeyu	Paraguay	−24.45	−55.65	−14.8
Mazama americana	UMMZ 126854	Lm3	La Paz	Bolivia	−15.52	−67.82	−14.0
Mazama americana	UMMZ 146493	Rm3	Canindeyu	Paraguay	−24.45	−55.65	−14.5
Mazama americana	UMMZ 146494	RM3	Canindeyu	Paraguay	−24.45	−55.65	−15.0
Mazama americana gualea	UMMZ 77816	RM3	Imbabura	Ecuador	0.35	−78.53	−14.2
Mazama americana zamora	UMMZ 82862	Rm3	Napo	Ecuador	−0.98	−77.82	−14.1
Mazama gouazoubira	UMMZ 124699	Lm3	Nueva asuncion	Paraguay	−22.10	−59.90	−6.6
Mazama gouazoubira	UMMZ 124700	Lm3	Chaco	Paraguay	−20.63	−60.32	−10.7
Mazama gouazoubira	UMMZ 124701	Lm3	Nueva asuncion	Paraguay	−20.70	−60.00	−9.0
Mazama gouazoubira	UMMZ 125569	Lm3	Chaco	Paraguay	−20.63	−60.32	−10.0
Mazama gouazoubira	UMMZ 125572	Lm3	Chaco	Paraguay	−20.13	−60.15	−9.6
Mazama gouazoubira	UMMZ 125573	Lm3	Chaco	Paraguay	−20.40	−60.10	−11.9
Mazama rufina	UMMZ 126126	Lm3	Itapua	Paraguay	−27.33	−56.42	−15.8
Mazama temama cerasina	UMMZ 63500	Lm3	Peten	Guatemala	17.39	−89.63	−13.6
Mazama temama cerasina	UMMZ 63502	Lm2	Peten	Guatemala	17.39	−89.63	−13.2
Mazama temama cerasina	UMMZ 76637	Rm2	Peten?	Guatemala			−14.4
Muntiacus reevesi roberti	UMMZ 97617	Lm3	Taiwan	China	25.02	121.45	−11.2
Neotragus batesi	UMMZ 39516	Rm3	Kribi	Cameroon	2.78	10.53	−24.6
Neotragus batesi	UMMZ 39517	Rm3	Kribi	Cameroon	2.78	10.53	−19.4
Odocoileus hemionus crooki	UMMZ 46190	Rm3	Texas	United States	30.60	−103.88	−7.5

(Continued)

TABLE 1 (Continued)

Species	Collection No.	Tooth	Region	Country	Latitude	Longitude	$\delta^{13}C_E$
Odocoileus hemionus crooki	UMMZ 79419	Lm3	Texas	United States	30.60	−103.89	−10.1
Odocoileus hemionus hemionus	UMMZ 59187	Rm3	Utah	United States	40.64	−109.72	−12.3
Odocoileus hemionus hemionus	UMMZ 59638	Lm3	Arizona	United States	35.92	−112.05	−11.5
Odocoileus hemionus sitkensis	UMMZ 103357	Lm3	Alaska	United States	57.86	−152.41	−14.4
Odocoileus virginianus borealis	UMMZ 5240	Rm3	Michigan	United States			−12.5
Odocoileus virginianus borealis	UMMZ 59029	Rm3	Michigan	United States	44.66	−84.71	−13.4
Odocoileus virginianus borealis	UMMZ 60964	Lm3	Michigan	United States	46.24	−84.18	−13.7
Odocoileus virginianus borealis	UMMZ 61004	Lm3	Michigan	United States	46.09	−88.64	−12.2
Odocoileus virginianus borealis	UMMZ 61038	Lm3	Michigan	United States	45.27	−84.58	−13.8
Odocoileus virginianus borealis	UMMZ 61048	Lm3	Michigan	United States	46.00	−83.85	−14.5
Odocoileus virginianus borealis	UMMZ 61147	Lm3	Michigan	United States	46.46	−90.17	−13.7
Odocoileus virginianus borealis	UMMZ 80213	Lm3	Michigan	United States	42.46	−84.01	−14.1
Odocoileus virginianus thomasi	UMMZ 76630	Rm3	Peten?	Guatemala			−13.2
Odocoileus virginianus thomasi	UMMZ 76631	Rm3	Peten?	Guatemala			−11.9
Odocoileus virginianus thomasi	UMMZ 76632	Rm3	Peten?	Guatemala			−12.4
Odocoileus virginianus thomasi	UMMZ 76634	Rm3	Peten?	Guatemala			−12.7
Odocoileus virginianus thomasi	UMMZ 76638	Rm3	Peten?	Guatemala			-14.1
Odocoileus virginianus thomasi	UMMZ 76641	Rm3	Peten?	Guatemala			−12.5
Odocoileus virginianus thomasi	UMMZ 76648	Rm3	Peten?	Guatemala			−14.1
Odocoileus virginianus thomasi	UMMZ 76654	Rm3	Peten?	Guatemala			−13.2
Oreamnos americanus missoulae	UMMZ 60546	Rm3	Alberta	Canada	53.50	−102.90	−9.8
Oreamnos americanus missoulae	UMMZ 87772	Rm3	Montana	United States	47.77	−112.70	−10.5
Oryx beisa	UMMZ 124068	Rm3					3.2
Ovibos moschatus	UMMZ 112377	Lm3	Greenland	Denmark	72.18	−23.75	−10.1
Ovibos moschatus	UMMZ 116376	Lm3	Northwest Territories	Canada	75.70	−84.40	−2.3
Ovis canadensis canadensis	UMMZ 167428	RM3	Colorado	United States	40.38	−105.52	−2.9
Ovis canadensis canadensis	UMMZ 42316	RM3	Idaho	United States	43.61	−116.20	−10.8
Ovis dalli stonei	UMMZ 53659	Lm3	British Columbia	Canada	59.00	−129.00	−10.2
Philantomba monticola monticola	UMMZ 39515	Lm3	Kribi	Cameroon	2.78	10.53	−14.8
Rangifer tarandus groenlandicus	UMMZ 97462	Rm3	Northwest Territories	Canada	62.71	−109.20	−8.6
Rangifer tarandus osborni	UMMZ 53658	Lm3	British Columbia	Canada	59.00	−129.00	−8.8

L, left; R, right; M, upper molar; m, lower molar; p, lower premolar.

physiology, the inferred carbon-isotope values of the vegetation that artiodactyls fed on ($\delta^{13}C_{diet}$) were compared among seven feeding categories, 11 terrestrial ecoregions, and phylogenetic groups.

Published $\delta^{13}C_E$ data

We assembled published $\delta^{13}C_E$ values of extant artiodactyls from 24 primary sources (Table 2). Data from paleontological or archeological sites or from other body tissues were not included. In most instances, we considered only samples from wild animals. Two exceptions are studies of free-range livestock (Wang et al., 2008; Lazzerini et al., 2021), included to increase the sample size from Asia. Along with published $\delta^{13}C_E$ data, we collected the following information from the literature whenever available: taxonomic identification, sample identification number (field number or museum catalog number), provenance (locality name, geographic coordinates, elevation), year of collection from the field, sampled element (tooth position), method of sampling (serial or bulk), and whether or not samples were pretreated before analysis. If multiple samples were taken from the same tooth or duplicates were run for the same bulk sample, then average values for the tooth were used. If multiple teeth were sampled from an individual animal, then samples taken from teeth that erupted late in the sequence (more posteriorly positioned in the premolar or molar row) were used, as these teeth are among the last ones to develop (Hillson, 2005), thereby avoiding a weaning signal. Some data have appeared in multiple studies or review papers since they were first published, in which case we traced them back to the original publication. Only studies that reported

TABLE 2 Summary statistics for calculated $\delta^{13}C_{diet}$ values of 79 species of artiodactyls documented in this study.

Family	Species	Count	Min.	Max.	Mean	Median	S.D.
Antilocapridae	*Antilocapra americana*	36	−25.6	−21.1	−23.1	−23.2	0.82
Bovidae	*Aepyceros melampus*	63	−23.5	−8.9	−15.8	−16.3	2.95
Bovidae	*Alcelaphus buselaphus*	49	−14.0	−8.2	−9.8	−9.5	1.28
Bovidae	*Antidorcas marsupialis*	7	−22.9	−20.7	−21.6	−21.5	0.80
Bovidae	*Beatragus hunteri*	2	−11.2	−10.6	−10.9	−10.9	0.38
Bovidae	*Bison bison*	88	−25.6	−13.4	−20.9	−22.3	3.44
Bovidae	*Bos grunniens*	7	−24.3	−21.3	−22.9	−22.8	1.29
Bovidae	*Capra hircus*	17	−23.1	−19.8	−21.4	−21.4	0.74
Bovidae	*Capra nubiana*	3	−19.3	−17.5	−18.6	−19.1	1.00
Bovidae	*Capra walie*	1	−23.9	−23.9	−23.9	−23.9	
Bovidae	*Cephalophus* sp.	9	−27.2	−22.2	−25.5	−26.1	1.68
Bovidae	*Cephalophus callipygus*	4	−26.5	−17.7	−23.4	−24.6	3.90
Bovidae	*Cephalophus dorsalis*	4	−26.1	-24.9	−25.5	−25.5	0.71
Bovidae	*Cephalophus leucogaster*	4	−25.5	−24.1	−24.9	−25.1	0.65
Bovidae	*Cephalophus nigrifrons*	10	−28.1	−24.6	−26.3	−25.8	1.26
Bovidae	*Cephalophus silvicultor*	2	−26.9	−24.9	−25.9	−25.9	1.42
Bovidae	*Cephalophus weynsi*	1	−25.9	−25.9	−25.9	−25.9	
Bovidae	*Connochaetes gnou*	10	−10.9	−8.9	−9.8	−10.0	0.69
Bovidae	*Connochaetes taurinus*	40	−12.9	−8.3	−10.2	−9.9	1.24
Bovidae	*Damaliscus lunatus*	15	−13.4	−8.3	−10.2	−9.8	1.34
Bovidae	*Eudorcas thomsonii*	16	−18.7	−10.9	−13.7	−13.0	2.49
Bovidae	*Gazella dorcas*	8	−22.6	−19.1	−21.0	−20.7	1.16
Bovidae	*Hippotragus equinus*	5	−15.1	−8.3	−11.1	−10.1	2.65
Bovidae	*Hippotragus niger*	3	−11.4	−9.5	−10.3	−9.9	1.02
Bovidae	*Kobus ellipsiprymnus*	75	−14.2	−8.8	−11.3	−11.0	1.24
Bovidae	*Kobus kob*	11	−12.5	−8.8	−10.7	−10.4	1.11
Bovidae	*Litocranius walleri*	8	−24.2	−21.1	−23.2	−23.4	1.00
Bovidae	*Madoqua guentheri*	2	−18.8	−18.3	−18.6	−18.6	0.35
Bovidae	*Madoqua kirkii*	25	−24.3	−17.6	−21.8	−21.9	1.61
Bovidae	*Madoqua saltiana*	5	−24.2	−19.7	−21.7	−21.7	1.78
Bovidae	*Nanger granti*	57	−27.9	−13.5	−21.3	−22.1	3.02
Bovidae	*Nanger soemmerringii*	1	−22.6	−22.6	−22.6	−22.6	
Bovidae	*Neotragus batesi*	4	−35.0	−29.5	−33.3	−34.4	2.56
Bovidae	*Oreamnos americanus*	2	−22.5	−21.9	−22.2	−22.2	0.45
Bovidae	*Oreotragus oreotragus*	3	−24.6	−19.9	−22.5	−23.1	2.37
Bovidae	*Oryx beisa*	27	−17.0	−9.4	−12.0	−11.9	1.71
Bovidae	*Ourebia ourebi*	1	−16.3	−16.3	−16.3	−16.3	
Bovidae	*Ovibos moschatus*	2	−22.8	−15.1	−19.0	−19.0	5.47
Bovidae	*Ovis* sp.	3	−22.6	−20.7	−21.5	−21.3	0.94
Bovidae	*Ovis canadensis*	2	−23.1	−15.2	−19.1	−19.1	5.62
Bovidae	*Ovis dalli*	1	−22.1	−22.1	−22.1	−22.1	
Bovidae	*Philantomba monticola*	7	−25.4	−21.3	−23.4	−23.3	1.60
Bovidae	*Raphicerus campestris*	8	−25.0	−18.4	−22.2	−23.2	2.33
Bovidae	*Redunca fulvorufula*	2	−11.0	−10.3	−10.7	−10.7	0.47
Bovidae	*Redunca redunca*	13	−17.9	−7.7	−10.5	−9.3	2.76
Bovidae	*Sylvicapra grimmia*	5	−25.9	−21.4	−23.5	−23.1	1.84
Bovidae	*Syncerus caffer*	116	−28.5	−9.6	−13.2	−12.1	3.52
Bovidae	*Taurotragus oryx*	29	−25.2	−18.0	−22.1	−22.4	1.81
Bovidae	*Tragelaphus buxtoni*	5	−25.7	−23.5	−24.6	−24.3	1.00

(Continued)

TABLE 2 (Continued)

Family	Species	Count	Min.	Max.	Mean	Median	S.D.
Bovidae	*Tragelaphus euryceros*	2	−27.3	−26.7	−27.0	−27.0	0.40
Bovidae	*Tragelaphus imberbis*	6	−24.5	−19.5	−22.9	−23.3	1.72
Bovidae	*Tragelaphus scriptus*	16	−28.1	−21.7	−24.8	−25.2	1.59
Bovidae	*Tragelaphus spekii*	4	−29.3	−26.5	−27.9	−27.9	1.21
Bovidae	*Tragelaphus strepsicerus*	13	−27.9	−20.6	−23.6	−23.2	1.94
Camelidae	*Llama guanaco*	4	−26.7	−25.9	−26.4	−26.5	0.37
Cervidae	*Alces americanus*	3	−28.0	−27.4	−27.7	−27.6	0.31
Cervidae	*Capreolus capreolus*	1	−22.2	−22.2	−22.2	−22.2	
Cervidae	*Cervus elaphus*	32	−28.3	−23.2	−24.6	−24.6	0.96
Cervidae	*Hydropotes inermis*	1	−25.1	−25.1	−25.1	−25.1	
Cervidae	*Mazama americana*	10	−26.9	−24.5	−25.4	−25.4	0.66
Cervidae	*Mazama gouazoubira*	9	−24.5	−17.2	−21.6	−21.3	2.43
Cervidae	*Mazama rufina*	1	−25.9	−25.9	−25.9	−25.9	
Cervidae	*Mazama temama*	3	−25.6	−24.4	−24.9	−24.7	0.64
Cervidae	*Muntiacus reevesi*	1	−21.8	−21.8	−21.8	−21.8	
Cervidae	*Odocoileus hemionus*	29	−26.4	−19.5	−24.2	−24.4	1.44
Cervidae	*Odocoileus virginianus*	23	−28.2	−17.0	−25.4	−25.8	2.15
Cervidae	*Rangifer tarandus*	2	−21.0	−20.7	−20.9	−20.9	0.22
Giraffidae	*Giraffa camelopardalis*	51	−28.6	−20.6	−25.0	−25.2	1.59
Giraffidae	*Okapia johnstoni*	2	−32.4	−31.7	−32.0	−32.0	0.54
Hippopotamidae	*Choeropsis liberiensis*	1	−28.3	−28.3	−28.3	−28.3	
Hippopotamidae	*Hippopotamus amphibius*	182	−25.5	−10.7	−16.8	−16.2	2.36
Suidae	*Hylochoerus meinertzhageni*	13	−34.2	−24.6	−28.6	−27.8	2.82
Suidae	*Phacochoerus aethiopicus*	58	−24.3	−8.9	−12.9	−12.0	2.89
Suidae	*Phacochoerus africanus*	33	−14.9	−9.8	−12.6	−12.1	1.21
Suidae	*Potamochoerus larvatus*	14	−26.0	−13.2	−20.7	−20.9	4.62
Suidae	*Potamochoerus porcus*	23	−28.2	−19.6	−25.6	−26.1	1.96
Tayassuidae	*Pecari tajacu*	4	−24.8	−23.9	−24.4	−24.4	0.42
Tayassuidae	*Tayassu pecari*	4	−26.1	−24.6	−25.4	−25.5	0.60
Tragulidae	*Hyemoschus aquaticus*	3	−25.6	−24.7	−25.2	−25.2	0.45

Data sources: Bocherens et al. (1996), Cerling and Harris (1999), Cerling et al. (1999), Harris and Cerling (2002), Cerling et al. (2003), Cerling et al. (2004), Boisserie et al. (2005), Hoppe et al. (2006), Feranec (2007), Cerling et al. (2008), Fenner (2008), Copeland et al. (2008), Levin et al. (2008), Wang et al. (2008), Copeland et al. (2009), Cerling et al. (2011), Kingston (2011), Nelson (2013), van der Merwe (2013), Cerling et al. (2015), Martin et al. (2015), Luyt and Sealy (2018), Rivera-Araya and Birch (2018), Lazzerini et al. (2021), and this study.

original $\delta^{13}C_E$ data of extant artiodactyls were cited as primary sources.

New $\delta^{13}C_E$ data

Eighty enamel samples from 23 species of extant artiodactyls were gathered from specimens housed in the University of Michigan Museum of Zoology (UMMZ). We chose samples with consideration for their prior taxonomic representation and geographic coverage in the literature, as well as the availability and abundance of specimens in the UMMZ collection.

The general method for sampling and pretreating tooth enamel followed Koch et al. (1997). Bulk samples were gathered by drilling approximately 5 mg of pristine enamel powder on the lateral surface of the tooth parallel to the growth axis.

Sampling was done using a portable dental drill with a 1-mm diamond burr. All samples were taken from third molars or fourth premolars. Samples were treated with 3% reagent grade NaOCl for 24 h to remove organic matter and with 1M buffered acetic acid for 24 h to remove non-structural carbonate. Each treatment was followed by centrifuging and rinsing five times with deionized water. Samples were dried by lyophilization. At the University of Michigan Stable Isotope Laboratory, samples were reacted at 77° ± 1°C with anhydrous phosphoric acid for 8 min in a Thermo Scientific Kiel IV preparation device coupled directly to the inlet of a Thermo Delta V triple collector isotope ratio mass spectrometer, which measured the resultant CO_2. Analytical precision was better than ± 0.1‰ (1 S.D.), based on international standards for carbonate (NBS-18, NBS-19). Isotope values are expressed in standard δ-notation: $\delta^{13}C_E = [(R_{sample}/R_{standard}) - 1] \times 1000$,

where R = $^{13}C/^{12}C$. The $\delta^{13}C_E$ values are reported relative to the Vienna PeeDee Belemnite (VPDB) standard.

Correcting for the Suess effect and calculating $\delta^{13}C$ of dietary sources

Sampled tooth specimens in our data compilation were collected from the field as long ago as 1891 to as recently as 2017 (**Supplementary Table 1**). During this time interval, $\delta^{13}C_{atm}$ has decreased by ~1.8‰ due to anthropogenic activities (Suess effect). To account for this effect, all $\delta^{13}C_E$ data were corrected to the preindustrial $\delta^{13}C_{atm}$ level of the year 1750 ($\delta^{13}C_{1750}$, taken to be –6.3‰). Correction values were based on $\delta^{13}C$ data from Antarctic ice cores, fern samples, and direct measurements of air (Rubino et al., 2013; Keeling et al., 2017). These records show that change in $\delta^{13}C_{atm}$ since 1750 occurred in three stages (**Supplementary Table 2**). The first 130 years witnessed a slow decrease, bringing $\delta^{13}C_{atm}$ from –6.3 to –6.7‰ by 1880. Another 0.4‰-decrease took only over 80 years, with a rate of ~0.005‰ per year during this interval. From the early 1960s to the early 2010s, $\delta^{13}C_{atm}$ dropped by 1.3‰, meaning an accelerated annual decrease of 0.025‰. Based on these observed trends, we corrected the $\delta^{13}C_E$ value of each sample with the offset in $\delta^{13}C_{atm}$ between its collection year and 1750. For some samples, the exact year of collection was not reported, so the correction value could not be accurately determined. In these cases, we estimated the correction value based on year of publication or other information provided in the primary study. These samples constitute a small proportion (2.5%) of the dataset and should not affect the overall analytical results.

The $\delta^{13}C$ values of the ingested vegetation ($\delta^{13}C_{diet}$) were calculated from $\delta^{13}C_{1750}$ to facilitate cross-species comparison of forage selection. Conventionally, an average enrichment factor between diet and enamel ($\varepsilon^*_{diet-bioapatite}$) of 14.1 ± 0.5‰, derived by Cerling and Harris (1999), have been used in paleodietary reconstructions of large ungulate mammals. Recently, Tejada-Lara et al. (2018) found that fractionation between diet and enamel increases with species' body mass and is also affected by digestive physiology (foregut vs. hindgut fermentation). Because our dataset covers artiodactyls with a range of body sizes, we use equations derived by Tejada-Lara et al. (2018) to determine the $\varepsilon^*_{diet-bioapatite}$ for each species based on its average body mass and digestive physiology. For foregut fermenters, $\varepsilon^*_{diet-bioapatite} = 2.34 + 0.05(BM)$, where (BM) is the natural log of body mass in kg. For hindgut fermenters, $\varepsilon^*_{diet-bioapatite} = 2.42 + 0.032(BM)$. Resulting $\varepsilon^*_{diet-bioapatite}$ values range from 11.0 (*Neotragus batesi*, 3 kg) to 15.0‰ (*Hippopotamus amphibius*, 1500 kg). When comparing the calculated $\delta^{13}C_{diet}$ values, we use -17‰ to separate C_3 and C_4 vegetation, based on plant data collected since the late 20th century (Cerling and Ehleringer, 2000;

Sponheimer and Cerling, 2014). Mixed C_3-C_4 feeders would have $\delta^{13}C_{diet}$ close to this value.

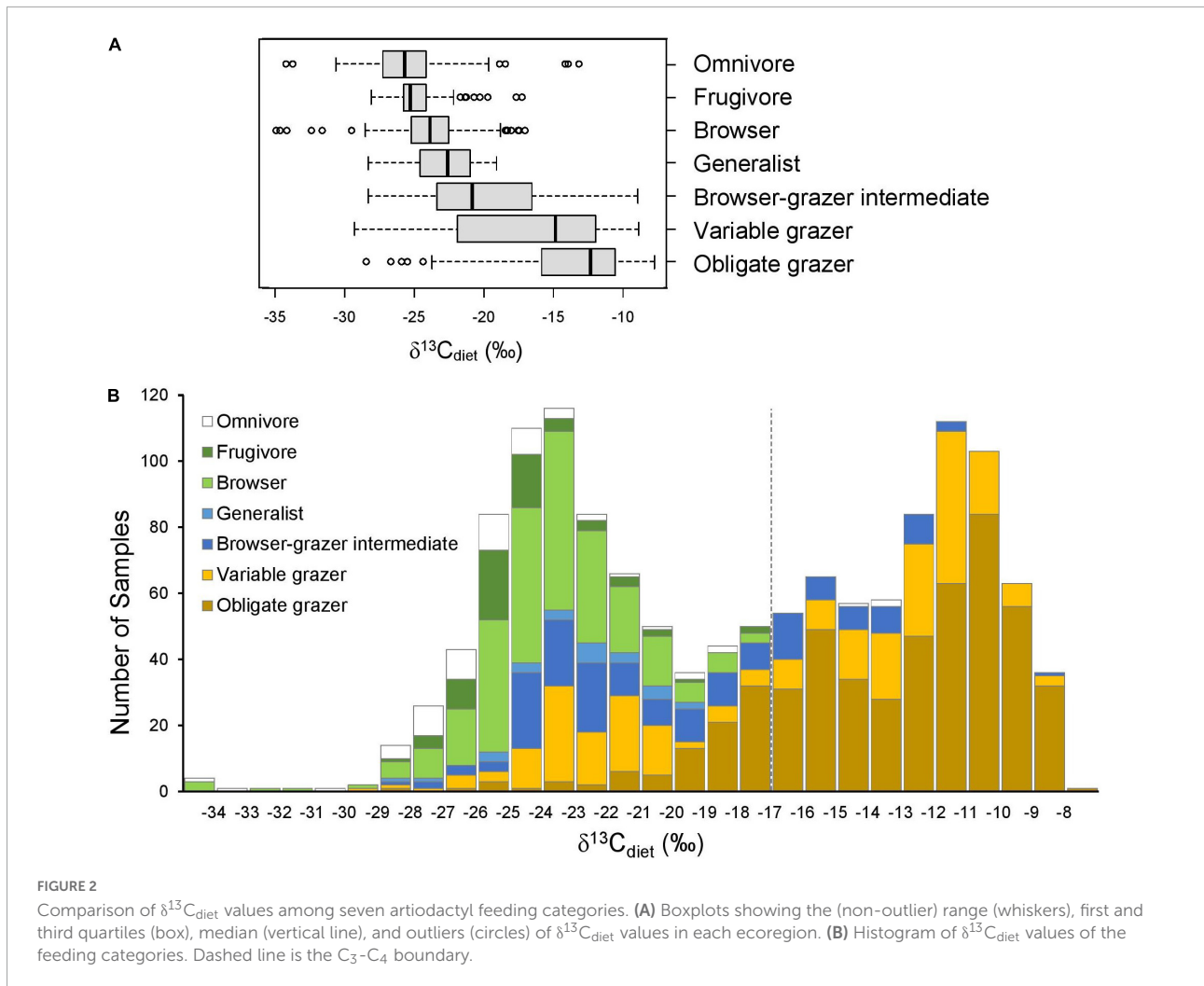## Comparing $\delta^{13}C_{diet}$ values among feeding categories and ecoregions

We assigned species to one of seven feeding categories based on published dietary information (**Supplementary Table 3**). The feeding categories include one omnivorous category (omnivore) and six herbivorous categories (frugivore, browser, browser–grazer intermediate, variable grazer, and obligate grazer). Assignment of species into herbivorous categories is based on the relative abundance of fruit, dicots, and monocots in the species' average diet, following criteria from Gagnon and Chew (2000) and Wang et al. (2022). The diets of frugivores and browsers consist of > 70% fruit and > 70% dicots, respectively. Variable grazers and obligate grazers have diets that include 60–90% and > 90% monocots, respectively. Browser–grazer intermediates consume < 70% dicots, < 60% monocots, and < 20% fruit, while generalists consumed > 20% of all three food types. This six-category classification scheme captures more information about the dietary preference and dietary selectivity of herbivores than the traditional three-category classification of browsers, mixed feeders, and grazers.

Each sampled locality was assigned to one of the Global 200 terrestrial ecoregions, which were established on the bases of biodiversity dynamics and environmental conditions (Olson et al., 2001). Different ecoregions provide different habitats and different plants for artiodactyls to feed on. Therefore, $\delta^{13}C_{diet}$ values are expected to vary among ecoregions.

Box and whisker plots were used to illustrate the summary statistics for feeding categories (**Figure 2**) and for ecoregions (**Figure 3**). Histograms of $\delta^{13}C_{diet}$ values illustrate the total dataset for each feeding category and for each ecoregion with sufficiently large sample size (> 100 samples, **Figures 2–5**). These diagrams allow for comparison of the differences and similarities in the range and frequency of $\delta^{13}C_{diet}$ values that each feeding category or ecoregion represents. Parametric (ANOVA) and non-parametric statistical tests (Kruskal–Wallis) were used to compare the difference between group-means, using PAST v4.03 (Hammer et al., 2001). Because some feeding categories and ecoregions are more geographically widespread than others, we also evaluate the composition of continental origin of the samples.

Since sampling is highly uneven among geographic regions (**Figure 1**) and among taxa (**Table 2**), the $\delta^{13}C_{diet}$ values of well-sampled species from certain locations could have an oversize impact on the overall distribution of $\delta^{13}C_{diet}$ values. Therefore, we repeated the comparison among feeding categories using mean $\delta^{13}C_{diet}$ values of species, both with and without controlling for phylogeny. Non-phylogenetic analyses are the same as detailed above. A phylogenetic ANOVA was

FIGURE 2
Comparison of $\delta^{13}C_{diet}$ values among seven artiodactyl feeding categories. **(A)** Boxplots showing the (non-outlier) range (whiskers), first and third quartiles (box), median (vertical line), and outliers (circles) of $\delta^{13}C_{diet}$ values in each ecoregion. **(B)** Histogram of $\delta^{13}C_{diet}$ values of the feeding categories. Dashed line is the C_3-C_4 boundary.

conducted using the "phylANOVA" function from the R package "phytools" (Revell, 2012).

Phylogenetic signal of $\delta^{13}C_{diet}$ values

To examine the $\delta^{13}C_{diet}$ variation among phylogenetic groups, we obtained a sample of 1000 node-dated consensus trees of artiodactyls from Upham et al. (2019) and generated a maximum clade credibility tree using *TreeAnnotator* (Drummond et al., 2012). The phylogeny and the isotope dataset share 76 species. We then estimated the phylogenetic signal in species' mean $\delta^{13}C_{diet}$ values with Blomberg's K (1000 permutations) using the function "phylosig" from "phytools". The K value can be either less than 1, equal to 1, or greater than 1. A K < 1 suggests that $\delta^{13}C_{diet}$ values are less similar in closely related species than expected under neutral Brownian model, while K > 1 suggests that $\delta^{13}C_{diet}$ values are more similar in phylogenetically closely related species than expected under a Brownian motion (Blomberg et al., 2003). We also mapped

the mean $\delta^{13}C_{diet}$ values of species onto the pruned artiodactyl phylogeny, using the function "contMap" from "phytools". Because species with similar mean $\delta^{13}C_{diet}$ values could have different range and variance of $\delta^{13}C_{diet}$ values that reflect difference in dietary niche breadths, we additionally aligned box and whisker plots of species with the phenogram (**Figure 6**).

Results

Including the new samples from this study (**Table 1**), the $\delta^{13}C_{diet}$ compilation results in a dataset of 1366 carbon-isotope values from 25 primary sources (**Table 2**). The tooth-enamel samples come from 79 species of artiodactyls sampled from Africa, Eurasia, North America, and South America, with the highest number of samples from Africa (**Figure 1**). Thirteen of the 23 species sampled from UMMZ (**Table 1**) have not been previously analyzed for $\delta^{13}C_E$ values. Other specimens were chosen to expand the geographic range of sampled species.
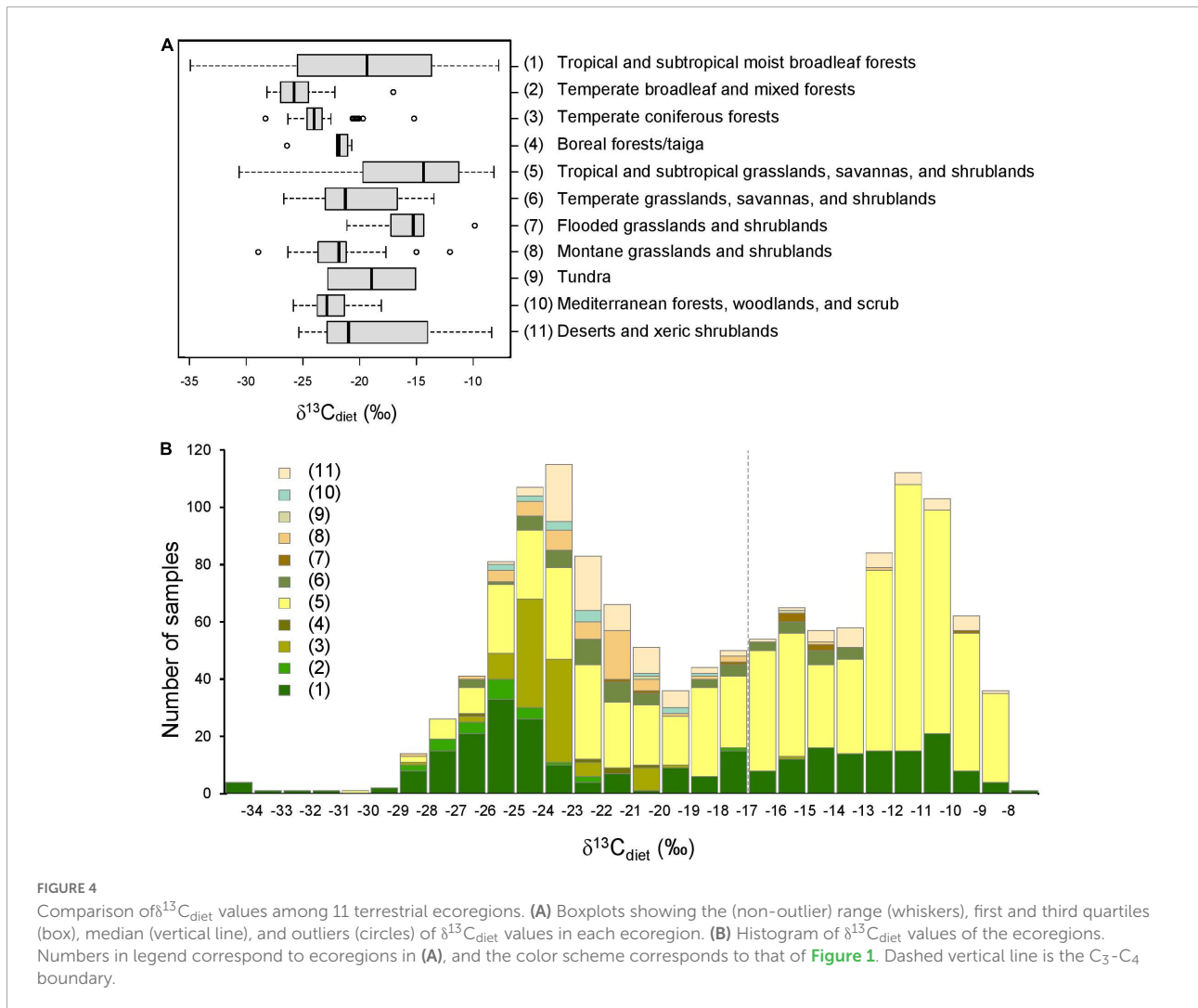
FIGURE 3

Histograms of $\delta^{13}C_{diet}$ values in seven artiodactyl feeding categories in relation to **(A–G)** taxonomic group and **(H)** continent. Dashed line is the C_3-C_4 boundary.
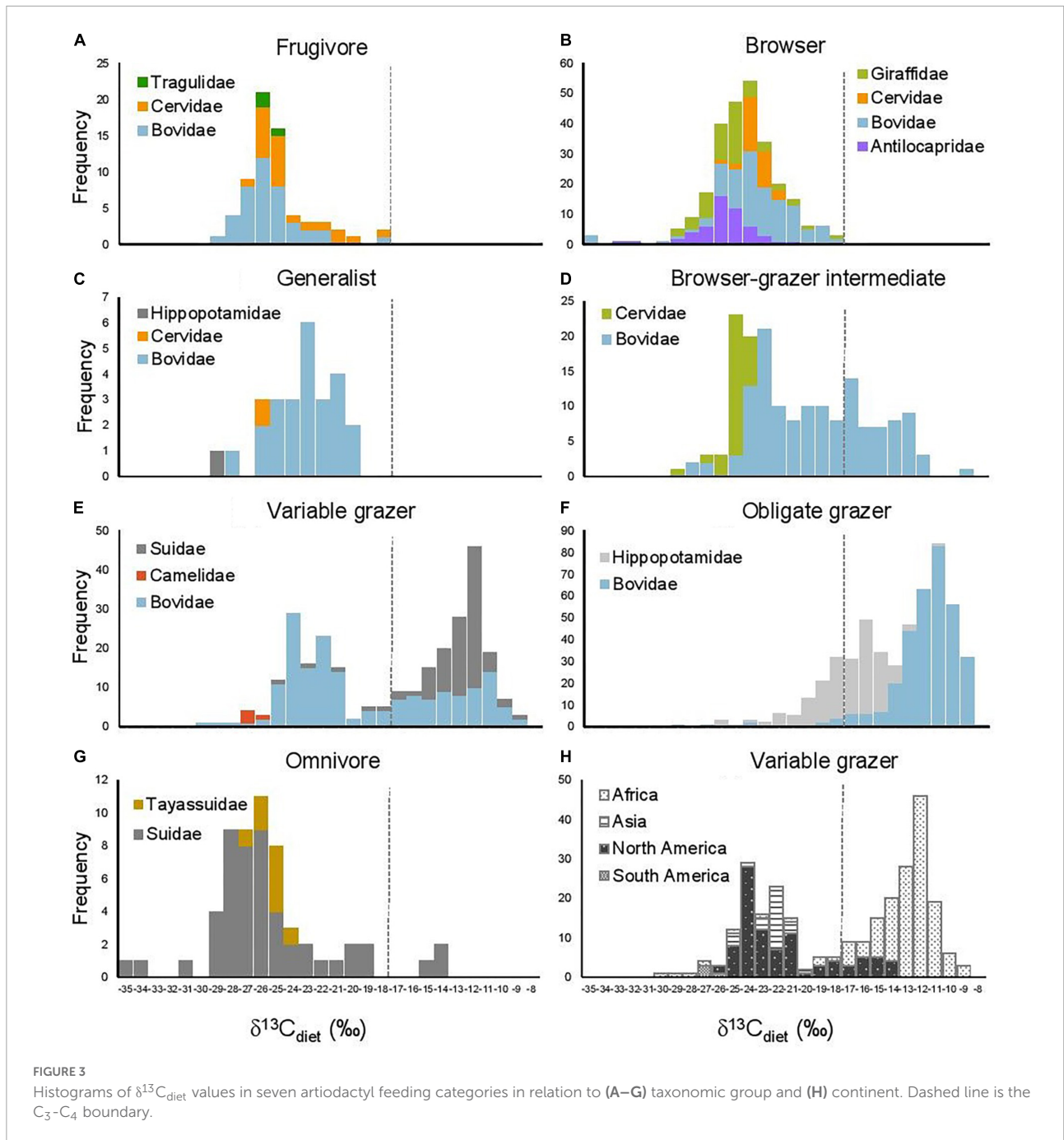
The total dataset includes representatives from nine out of ten families of terrestrial artiodactyls, missing only the Moschidae (**Table 2**).

Variation in $\delta^{13}C_{diet}$ among feeding categories

As expected, the seven artiodactyl feeding categories exhibit different ranges, means, and medians of $\delta^{13}C_{diet}$ values.

Group means differ significantly from each other, using both parametric and non-parametric tests ($p < 0.001$). *Post hoc* tests show that group means differ significantly for most pairwise comparisons ($p < 0.05$), except for that between frugivores and browsers and a few comparisons that involve omnivores or generalists (**Table 3B**). When using mean $\delta^{13}C_{diet}$ values of species to reduce the bias introduced by sampling, we found that pairwise comparisons between obligate grazers and other feeding categories remain significantly different both with and without controlling for phylogeny (**Tables 3C,D**). Mean $\delta^{13}C_{diet}$

FIGURE 4
Comparison of $\delta^{13}C_{diet}$ values among 11 terrestrial ecoregions. **(A)** Boxplots showing the (non-outlier) range (whiskers), first and third quartiles (box), median (vertical line), and outliers (circles) of $\delta^{13}C_{diet}$ values in each ecoregion. **(B)** Histogram of $\delta^{13}C_{diet}$ values of the ecoregions. Numbers in legend correspond to ecoregions in **(A)**, and the color scheme corresponds to that of **Figure 1**. Dashed vertical line is the C_3-C_4 boundary.

values of variable grazer species also differ from those of most other groups, although the differences are not significant when controlling for phylogeny. Among the six herbivorous feeding categories, mean $\delta^{13}C_{diet}$ values increase along the dietary continuum from frugivores, through browsers, the mixed feeders (generalists and browser–grazer intermediates), variable grazers, to obligate grazers (**Figure 2A** and **Table 3A**). Generalists and omnivores have smaller sample sizes than other feeding categories, but omnivores still exhibit a wide range of $\delta^{13}C_{diet}$ values, reflecting their wide dietary niche breadth (**Table 3A**).

The entire dataset exhibits a generally bimodal distribution in $\delta^{13}C_{diet}$ values, with a saddle around $-19‰$ (**Figure 2B**). Grazers dominate the C_4 (enriched) mode of the distribution but extend considerably into the C_3 realm. Mixed feeders occupy the intermediate range of values. Frugivores, browsers, and omnivores are prevalent in the C_3 range. The most depleted and most enriched $\delta^{13}C_{diet}$ values are found in a browser

(*Neotragus batesi*, the dwarf antelope) and an obligate grazer (*Redunca redunca*, the bohor reedbuck), respectively (**Figure 2** and **Table 2**).

The bimodal distribution of $\delta^{13}C_{diet}$ values in the total dataset is comprised of several different patterns among artiodactyl feeding groups (**Figure 3**). Taxonomic composition also differs among feeding categories. Most feeding categories exhibit a unimodal distribution but vary in mean, median, mode, and peak frequency (**Figure 3** and **Table 3A**). Obligate grazers and variable grazers exhibit patterns that differ from those of the other feeding categories. Obligate grazers, which all occur in Africa, exhibit a left-skewed bimodal distribution, with the higher peak driven primarily by the high frequency of enriched $\delta^{13}C_{diet}$ values in bovids (**Figure 3F**). All but one sample of Hippopotamidae are from *Hippopotamus amphibius*; this species makes up roughly an eighth of the total sample size (**Table 2**), and their $\delta^{13}C_{diet}$ values contribute to a second, lower mode in the obligate-grazer data. Variable grazers
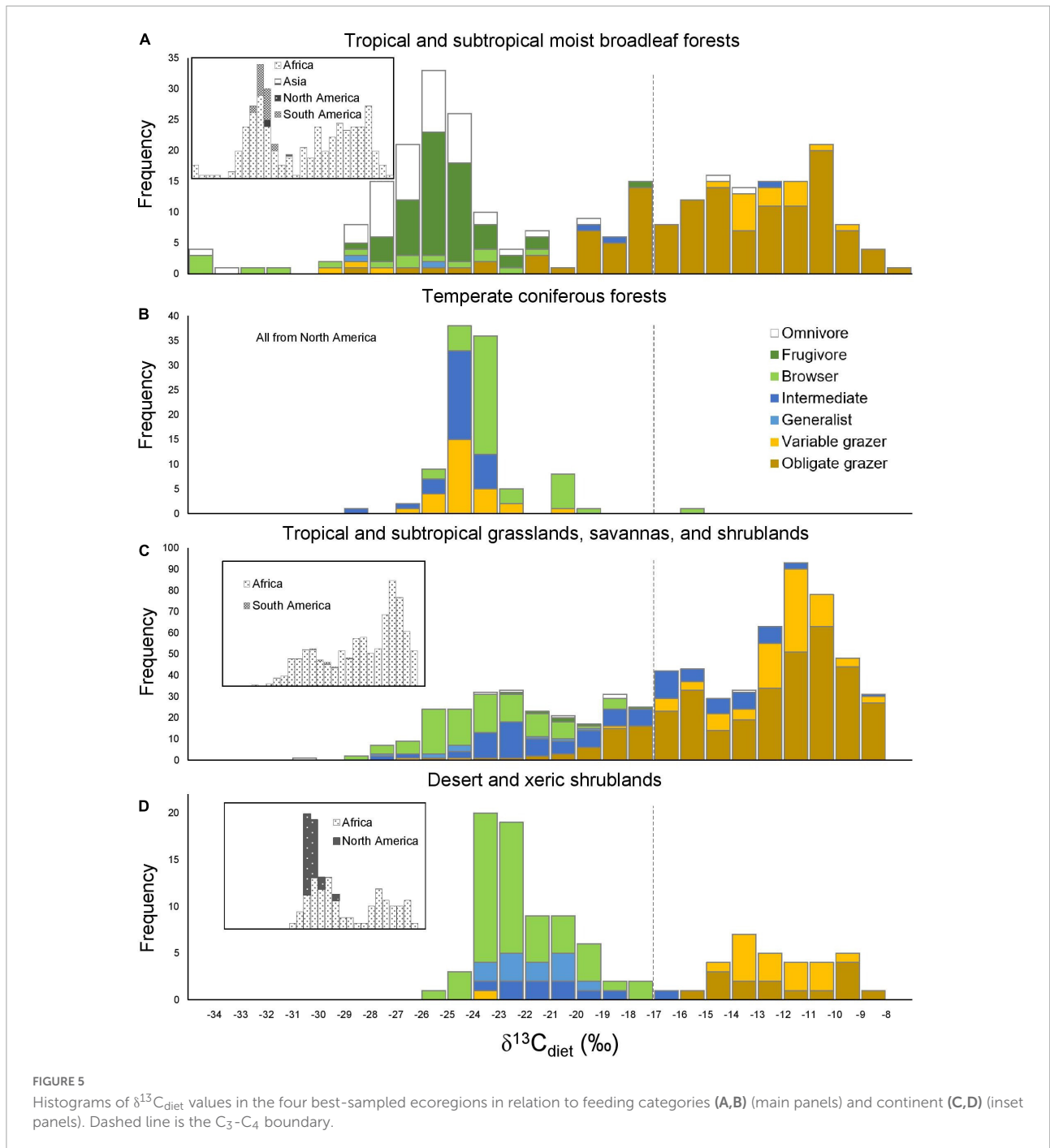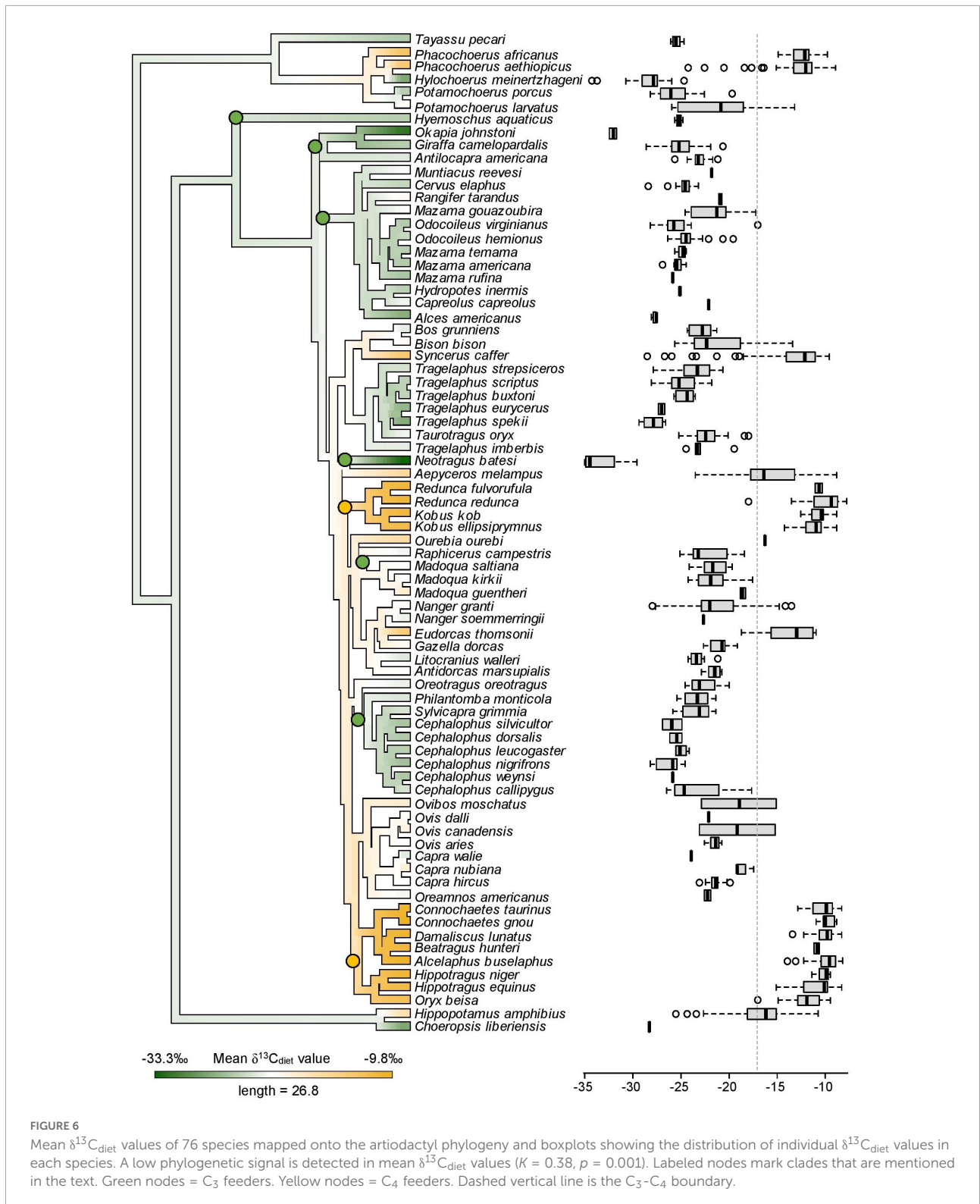
FIGURE 5
Histograms of $\delta^{13}C_{diet}$ values in the four best-sampled ecoregions in relation to feeding categories **(A,B)** (main panels) and continent **(C,D)** (inset panels). Dashed line is the C_3-C_4 boundary.

exhibit a bimodal distribution of $\delta^{13}C_{diet}$ values (**Figure 3E**). Within this group, bovids are the most numerous (as is the case for all herbivorous feeding categories) and are the main contributor to the bimodal pattern. Other families in this feeding category are well separated between C_3-feeding camelids (llamas) and primarily C_4-feeding suids (warthogs). Associated with taxonomic differentiation, the C_3-C_4 separation in variable grazers is strongly influenced by geography, with samples from North America being mostly in the C_3 range while

samples from Africa are mostly in the C_4 range (**Figure 3H**). Variable grazers in Asia and South America are also C_3-feeders.

Variation in $\delta^{13}C_{diet}$ among ecoregions

Sample localities in the dataset are distributed among 11 terrestrial ecoregions of the world (**Figure 4** and

FIGURE 6

Mean $\delta^{13}C_{diet}$ values of 76 species mapped onto the artiodactyl phylogeny and boxplots showing the distribution of individual $\delta^{13}C_{diet}$ values in each species. A low phylogenetic signal is detected in mean $\delta^{13}C_{diet}$ values (K = 0.38, p = 0.001). Labeled nodes mark clades that are mentioned in the text. Green nodes = C_3 feeders. Yellow nodes = C_4 feeders. Dashed vertical line is the C_3-C_4 boundary.

Table 4). The $\delta^{13}C_{diet}$ values of species in different ecoregions differ significantly from each other, using both parametric and non-parametric tests ($p < 0.001$). Pairwise comparisons yielded significant differences ($p < 0.05$) among forest ecoregions, between grassland ecoregions and forest ecoregions, as well as between desert and xeric

shrublands and most other ecoregions (Table 4B). Mesic environments (forests and woodlands from a range of temperature conditions) are more prevalent in the lower values of the $\delta^{13}C_{diet}$ spectrum while intermediate to semiarid environments (grasslands) occupy the middle and higher values (Figure 4B).

Because sample size is highly variable among ecoregions (Table 3A), histograms were only generated for the four ecoregions with the largest sample sizes. Tropical and

subtropical moist broadleaf forests, mostly occurring in Africa, exhibit a generally bimodal distribution of $\delta^{13}C_{diet}$ values (Figure 5A). The peak in the C_3 range is well-established and consists mostly of frugivores and omnivores, although other feeding categories are also present. The broad low peak in the C_4 range is primarily from obligate grazers, specifically those from East Africa (Supplementary Table 1). Samples from the tropical and subtropical moist broadleaf forests of South America, North America, and

TABLE 3 Comparison of $\delta^{13}C_{diet}$ values among seven feeding categories of artiodactyls.

(A) Summary statistics of all $\delta^{13}C_{diet}$ values.

Feeding category	N	Min.	Max.	Mean	Median	S.D.
1) Frugivore	66	−28.1	−17.2	−24.7	−25.3	2.17
2) Browser	262	−35.0	−17.0	−23.9	−23.9	2.57
3) Generalist	26	−28.3	−19.1	−22.9	−22.6	2.36
4) Browser–grazer intermediate	168	−28.3	−8.9	−19.9	−20.9	4.28
5) Variable grazer	273	−29.3	−8.8	−16.7	−14.9	5.31
6) Obligate grazer	513	−28.5	−7.7	−13.4	−12.3	3.62
7) Omnivore	58	−34.2	−13.2	−25.0	−25.7	3.96

(B) P-values from *post hoc* pairwise tests of all $\delta^{13}C_{diet}$ values.

	1	2	3	4	5	6	7
1)		0.756	0.458	**0.000**	**0.000**	**0.000**	0.999
2)	**0.000**		0.900	**0.000**	**0.000**	**0.000**	0.414
3)	**0.000**	**0.032**		**0.005**	**0.000**	**0.000**	0.263
4)	**0.000**	**0.000**	**0.002**		**0.000**	**0.000**	**0.000**
5)	**0.000**	**0.000**	**0.000**	**0.000**		**0.000**	**0.000**
6)	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		**0.000**
7)	0.107	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	

(C) P-values from *post hoc* pairwise tests of species' mean $\delta^{13}C_{diet}$ values.

	1	2	3	4	5	6	7
1)		0.995	0.999	0.292	**0.001**	**0.000**	1.000
2)	0.079		1.000	0.465	**0.000**	**0.000**	0.998
3)	0.712	0.719		0.721	**0.050**	**0.000**	0.999
4)	**0.003**	0.056	0.194		0.772	**0.000**	0.504
5)	**0.002**	**0.000**	**0.045**	0.360		**0.000**	**0.018**
6)	**0.000**	**0.000**	**0.002**	**0.001**	**0.001**		**0.000**
7)	0.958	0.308	0.835	0.074	**0.045**	**0.002**	

(D) Results from phylogenetic ANOVA of species' mean $\delta^{13}C_{diet}$ values.

	1	2	3	4	5	6	7
1)		−0.569	−0.272	−2.197	−4.705	−9.360	0.161
2)	1.000		0.126	−1.977	−4.976	−10.386	0.559
3)	1.000	1.000		−1.564	−3.357	−7.065	0.359
4)	1.000	0.060	1.000		−1.800	−5.982	1.845
5)	0.090	**0.021**	0.090	1.000		−5.113	3.509
6)	**0.021**	**0.021**	**0.021**	**0.021**	**0.021**		6.930
7)	1.000	1.000	1.000	1.000	1.000	0.429	

Above diagonal line: Tukey's HSD *post hoc* test. Below diagonal line: Mann–Whitney U test. Both ANOVA and Kruskal–Wallis tests yielded $p < 0.001$ of significant differences among feeding categories.
The test yielded $p = 0.001$. Above diagonal line: pairwise t-values. Below diagonal line: adjusted *P*-values.
P-values < 0.05 are in bold.

Asia fall in the mid-range of the C_3 realm (**Figure 5A**). Samples from temperate coniferous forests are all from North America (**Figure 5B**). There, variable grazers, browser–grazer intermediates, and browsers form a unimodal distribution of $\delta^{13}C_{diet}$ values, primarily in the C_3 range. Tropical and subtropical grasslands, savannas, and shrublands are represented almost exclusively by samples from Africa (**Figure 5C**), showing a grazer-dominated (especially obligate grazer) distribution of $\delta^{13}C_{diet}$ values in the C_4 range. Some browsers and mixed feeders are also present, forming a small mode of $\delta^{13}C_{diet}$ values in the C_3 range. Desert and xeric shrublands also have a bimodal distribution of $\delta^{13}C_{diet}$ values (**Figure 5D**). There is a clear division between non-grazers (browsers and browser–grazer intermediates), which comprise $\delta^{13}C_{diet}$ values in the C_3 range, and the grazers, which contribute to $\delta^{13}C_{diet}$ values in the C_4 range. Within this ecoregion, samples from Africa include both C_3- and C_4-feeders, while those from North America are all C_3-feeders.

Variation in $\delta^{13}C_{diet}$ among phylogenetic groups

The mean and range of $\delta^{13}C_{diet}$ values are more constrained in some artiodactyl clades than in others. Within the suborder Ruminantia, lineages with the oldest node ages (Tragulidae, Antilocapridae, Giraffidae, Cervidae) are exclusively C_3 feeders, while C_4 feeders appear in relatively young lineages (<13 million years old; **Figure 6**). Species with a pure C_4 diet, corresponding to their assignment in the obligate grazer category, belong in two bovid tribes, the Reduncini and the Hippotragini. Some groups within Bovidae maintained C_3 feeding, such as the Cephalophini, the Neotragini, and subgroups of the Antilopini. Other clades have a mixture of C_3 feeders, C_4 feeders, and mixed C_3-C_4 feeders. There is low phylogenetic signal in the $\delta^{13}C_{diet}$ values of artiodactyls (K = 0.38, p = 0.001). Similar $\delta^{13}C_{diet}$ values occur in multiple artiodactyl clades and

TABLE 4 Comparison of $\delta^{13}C_{diet}$ values of artiodactyls among 11 ecoregions, each found on multiple continents.

(A) Summary statistics.

Ecoregion	N	Min.	Max.	Mean	Median	S.D.
1) Tropical and subtropical moist broadleaf forests	278	−35.0	−7.7	−19.6	−19.4	6.67
2) Temperate broadleaf and mixed forests	25	−28.2	−17.0	−25.4	−25.7	2.35
3) Temperate coniferous forests	101	−28.3	−15.2	−23.7	−24.0	1.66
4) Boreal forests/taiga	5	−26.4	−20.7	−22.4	−21.9	2.30
5) Tropical and subtropical grasslands, savannas, and shrublands	709	−30.7	−8.2	−15.6	−14.3	5.29
6) Temperate grasslands, savannas, and shrublands	58	−26.7	−13.4	−20.1	−21.3	3.94
7) Flooded grasslands and shrublands	9	−21.1	−9.8	−15.9	−15.2	3.36
8) Montane grasslands and shrublands	51	−29.0	−12.0	−22.1	−21.9	2.77
9) Tundra	2	−22.8	−15.1	−19.0	−19.0	5.47
10) Mediterranean forests, woodlands, and scrub	15	−25.9	−18.1	−22.5	−22.9	2.16
11) Deserts and xeric shrublands	103	−25.4	−8.3	−19.0	−21.0	4.83

(B) *P*-values from *post hoc* pairwise tests.

	1	2	3	4	5	6	7	8	9	10	11
1)		**0.000**	**0.000**	0.983	**0.000**	1.000	0.577	0.067	1.000	0.585	0.994
2)	**0.000**		0.934	0.985	**0.000**	**0.001**	**0.000**	0.237	0.839	0.824	**0.000**
3)	**0.001**	**0.000**		1.000	**0.000**	**0.001**	**0.001**	0.757	0.971	0.999	**0.000**
4)	0.458	0.019	0.095		0.124	0.997	0.476	1.000	0.999	1.000	0.938
5)	**0.000**	**0.000**	**0.000**	0.010		**0.000**	1.000	**0.000**	0.998	**0.000**	**0.000**
6)	0.980	**0.000**	**0.000**	0.430	**0.000**		0.484	0.632	1.000	0.877	0.974
7)	0.138	**0.000**	**0.000**	**0.008**	0.579	**0.011**		0.042	1.000	0.095	0.837
8)	0.130	**0.000**	**0.000**	0.852	**0.000**	**0.023**	**0.000**		0.999	1.000	**0.022**
9)	0.820	**0.037**	**0.037**	0.847	0.332	0.918	0.554	0.469		0.998	1.000
10)	0.304	**0.000**	**0.012**	0.631	**0.000**	**0.030**	**0.000**	0.506	0.264		0.340
11)	0.054	**0.000**	**0.000**	0.342	**0.000**	0.267	**0.048**	**0.001**	0.963	**0.008**	

Above diagonal line: Tukey's HSD *post hoc* test. Below diagonal line: Mann–Whitney U test. Both ANOVA and Kruskal–Wallis tests yielded p < 0.001 of significant differences among ecoregions.
P-values < 0.05 are in bold.

individual clades show broad to narrow variation in $\delta^{13}C_{diet}$ values.

Discussion

We compiled and evaluated a global dataset of $\delta^{13}C_{diet}$ (isotope values of ingested forage) of extant artiodactyls, which documents variation among feeding categories, ecoregions, and clades. Our data compilation shows that research on artiodactyl $\delta^{13}C_E$ has covered just over 30% of species richness of extant artiodactyls. In addition, sampling is highly uneven both phylogenetically and geographically (**Figure 1** and **Table 2**). Only one species of tragulid has been sampled, and no data are available for moschids. Although both species of extant hippopotamid have been sampled, the hippopotamus (*Hippopotamus amphibious*) is represented by 182 samples, making up 13% of the total number of samples, while the pygmy hippopotamus (*Choeropsis liberiensis*) is only represented by one sample. Sampling is dense in sub-Saharan African ecosystems (**Figures 1**, **5**), where C_4 grasslands and closed-canopy C_3 environments are both extensive and artiodactyl species-richness is high. In contrast, sampling is sparse across the Eurasian continent. In East Africa, where sampling is the densest, savanna ecosystems lie adjacent to expansive tropical rainforest. Riverine and montane forest habitats and xeric shrublands also occur in patchy areas surrounded by tropical and subtropical grasslands there. Samples from this region largely contribute to the bimodal distributions in **Figure 5**, indicating consumption of both C_3 and C_4 plants by resident artiodactyls.

The herbivore dietary spectrum is expressed through a wide range of $\delta^{13}C_{diet}$ values, with the most depleted mean and median values in frugivores and the most enriched in obligate grazers. In general, grazing taxa have a broader range of dietary isotope values than browsing taxa. Notably, variable grazers exhibit a bimodal distribution of $\delta^{13}C_{diet}$ values, with North American taxa consuming C_3 vegetation and African taxa consuming C_4 vegetation, reflecting the different amounts of C_4 biomass available in temperate versus tropical environments. Variation in $\delta^{13}C_{diet}$ values also occurs among ecoregions, taxonomic groups, and geographic regions. Grassland ecoregions differ significantly from forest ecoregions in $\delta^{13}C_{diet}$ values. Some of the oldest ruminant lineages have maintained C_3 feeding, and pure C_4 dietary signals are restricted to two bovid clades. The $\delta^{13}C_{diet}$ values of species and faunas also vary across geographic regions and may be related to amount of C_4 vegetation in the environment. Most of the observed patterns correspond broadly with existing knowledge about stable isotope ecology, but deviations from the general trend can be identified in well-sampled taxa and regions. Additionally, we detected low phylogenetic signal (K = 0.38, p = 0.001) in the mean $\delta^{13}C_{diet}$ values of artiodactyl species. It is important to note, however, that ecology, phylogeny, biogeography, and environmental settings are often correlated, and the combination of available vegetation, ecological interactions, and physiological processes affects the $\delta^{13}C$ values recorded in artiodactyl tooth enamel.

The herbivore dietary spectrum

The frugivore–browser–grazer dietary spectrum corresponds to an increase in group-mean $\delta^{13}C_{diet}$ values over a range of 10‰ from frugivores (–24.7‰) to obligate grazers (–13.4‰) (**Figure 2** and **Table 3**). Feeding groups have different dietary preferences as well as niche breadths. The two herbivore dietary extremes (frugivores and obligate grazers) have the narrowest range of species-mean $\delta^{13}C_{diet}$ values, and obligate grazers can be most readily distinguished from other feeding categories (**Table 3**). The intermediate feeding categories (i.e., generalist and browser–grazer intermediate), by their defining criteria, have inherently wider dietary variation than the dietary extremes. Considerable variation also exists in browsers and variable grazers. The lowest $\delta^{13}C_{diet}$ values occur in two subcanopy browsers, the dwarf antelope (*Neotragus batesi*) and the okapi (*Okapia johnstoni*) (**Figure 6** and **Table 2**). Both species inhabit closed-canopy forests in equatorial Africa. Okapis are endemic to the forests in northeastern Congo Basin. The dwarf antelope has a discontinuous range in central and western equatorial Africa. The two specimens of *N. batesi* from Cameroon have more variable $\delta^{13}C_{diet}$ values (–29.5‰ and –34.7‰) than do the two specimens from the Ituri Forest (–34.2‰ and –35.0‰), and more variation may be present in this group across its geographic range than previously recognized. Variable grazers exhibit a bimodal distribution of $\delta^{13}C_{diet}$ values that is distinct from the other feeding categories, and the pattern can be best explained by a distinction between the North American species feeding primarily on C_3 vegetation and the African species feeding primarily on C_4 vegetation (**Figure 3H**). In North America, variable grazers include the bison (*Bison bison*), muskox (*Oreamnos americanus*), bighorn sheep (*Ovis canadensis*), and the Dall sheep (*Ovis dalli*). Bison are sampled from a range of latitudes in the western United States; their $\delta^{13}C_E$ values vary considerably and are correlated with mean annual temperature (Hoppe et al., 2006). In Africa, warthog (*Phacochoerus africanus*) makes up most of the variable-grazer sample; the rest are from oryx (*Oryx beisa*), oribi (*Ourebia ourebia*), and the marshbuck (*Tragelaphus spekii*). Species in both continental faunas consume over 60% monocots in their average diets. Their contrasting $\delta^{13}C_{diet}$ signatures likely reflect variation in the amount of C_4 biomass in the vegetation (Still et al., 2003), which is affected by both temperature and seasonality of precipitation (Boutton et al., 1980; Winslow et al., 2003; Kohn, 2010). Differentiating C_3 graze from C_3 browse in the fossil record would require incorporation of other kinds of data, such as morphological and use-wear traits.

Dietary breadth and flexibility

While sampled individuals may be categorized as a browser, a mixed feeder, or a grazer solely by their $\delta^{13}C_{diet}$ values (e.g., Cerling et al., 2015), it is evident from **Figure 2B** that there are no clear-cut boundaries among the three broad feeding types. Individuals belonging to the same phylogenetic group or feeding category can have a range of $\delta^{13}C_{diet}$ values. Consequently, the C_3-C_4 cutoff is frequently crossed by clades and feeding groups (**Figures 2**, **6**). Artiodactyls have been documented to shift their diets due to geographic variation in vegetation structure, seasonal climatic and environmental changes, and ecological interactions with sympatric species (Sponheimer et al., 2003; Djagoun et al., 2013; Radloff et al., 2013; Miranda et al., 2014). Such dietary changes increase the abundance of savanna herbivore species (Staver and Hempson, 2020).

Grazing species have wider dietary niche breadths than browsing species do, as represented by their $\delta^{13}C_{diet}$ values (**Figure 2** and **Table 3**). The difference between maximum and minimum $\delta^{13}C_{diet}$ values in browsing species (including frugivores and browsers) is ~18‰ (~12‰ if excluding the outliers from understory browsers), and no sample from browsing species plots in the C_4 range. Grazing species (including variable grazers and obligate grazers) have a total range of over 22‰, with many samples extending well into the C_3 range. Variable grazers have the widest range of mean $\delta^{13}C_{diet}$ values, ranging from –27.9‰ (*Tragelaphus spekii*) to –9.8‰ (*Connochaetes gnou*). Corresponding to this difference in extant artiodactyls, findings in the North American fossil record show that species with grazing-adapted morphology have broader diets than browsing-adapted ones and consume more browse than previously anticipated, thus morphological specialization may result in ecological generalization (Feranec, 2003; Pardi and DeSantis, 2021). Herbivores with grazing adaptations, such as hypsodont teeth, are able to consume grass but can also eat other foods when they are available. This dietary flexibility would have implications for species duration over evolutionary time scales, especially during times of climate change. For example, in the Miocene Siwalik record of Pakistan, ungulate species that were able to alter their diet (from browsing to mixed feeding or grazing) in response to vegetation change persisted substantially longer than those that were not (Badgley et al., 2008).

Future research

More isotopic data are needed for currently understudied regions and taxa to further explore the research questions posed in this study. In the current data compilation, tragulids are represented by only one species and moschids are absent. Both families are important forest dwellers in Asia and have substantial fossil records, and their $\delta^{13}C_{diet}$ values can help us investigate the resource use and partitioning in artiodactyl faunas in tropical, subtropical, and temperate forests, which may be important analogs for some paleo-ecosystems. Better sampling records from Eurasia in general are also needed. Although some isotope data from other body tissues (e.g., hair) have been reported, tooth enamel data are lacking for many regions of the continent. Due to variability in the carbon-isotope spacing between different body tissues, converting hair or bone collagen data to enamel equivalent values can confound paleodietary reconstructions (Codron et al., 2011, 2012, 2018; Bocherens et al., 2014). Therefore, $\delta^{13}C_E$ data are ideal for making direct comparisons between ungulate diets in modern and paleo ecosystems.

Incorporating different data types has proven valuable to studying paleoecology, and when reconstructing the diet of fossil species, more proxies are better than one. In addition to mandibular morphology and tooth-enamel isotopes, tooth-wear proxies should be incorporated (e.g., Codron et al., 2008; Luyt and Faith, 2014; Fillion et al., 2022). The microscopic wear patterns on the occlusal surface of teeth (microwear), for example, record feeding habits with the finest temporal resolution (daily or weekly) and can be used to detect subtle variations in broadly similar diets (e.g., Louail et al., 2021) as well as ecological responses to short-term environmental changes that would not be reflected in tooth-wear measurements (such as mesowear) or dental morphology (such as hypsodonty) (Mihlbachler et al., 2018). While carbon isotope analysis is limited in its ability to distinguish dicot fruits from leaves in a species'diet, microwear and mesowear analyses as well as mandibular morphology have proven useful for differentiating frugivores from browsers (e.g., Scott, 2012; Kaiser et al., 2013; Wang et al., 2022).

Our dataset is potentially useful for addressing many questions beyond this study. For example, how are the mean and range of $\delta^{13}C_{diet}$ values related to vegetation heterogeneity, topographic complexity, and climatic conditions? Do some species track the variation in environmental $\delta^{13}C$ values better than other species with similar geographic ranges? If so, what aspects of their ecology can explain the difference? How do species' $\delta^{13}C_{diet}$ values contract or expand from ecological interactions, such as co-occurrence with competitors for forage? What combination of isotopic signature and osteological characters can be used to improve paleoecological reconstructions? Some of these questions will need better sampling of targeted taxa, regions, or environmental settings, others will require more comparative data from plants.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

BW and CB developed the research plan and wrote the manuscript. BW gathered and analyzed the data and produced the figures. Both authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.958859/full#supplementary-material

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