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Stable isotope analysis evidence dietary changes in toxodontids (Mammalia, Notoungulata) across the Neogene–Quaternary from Argentina

El análisis de isótopos estables evidencia cambios en la dieta de toxodóntidos (Mammalia, Notoungulata) a través del Neógeno–Cuaternario de Argentina

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Abstract: Toxodontidae was an extinct family of South American notoungulates, widely studied systematically, but not so much from an ecological perspective. This work aims to improve the knowledge about variations in diet and habitats of different representatives of this group in the context of biotic and abiotic events throughout the last 20 My. The tooth enamel carbon isotope composition ($\delta^{13}C$) of 7 genera recorded in 11 localities of Central, West and Northeastern Argentina, from the Early Miocene to the Late Pleistocene, was analyzed. The diet of the toxodontids studied in this work ranges from mainly C_3 resources during the Miocene, mixed C_3-C_4 in the Pliocene, to a significant consumption of C_4 plants during the Pleistocene. Differences in diet during the Late Pleistocene were also recorded, associated with geographic (*i.e.*, Northeastern vs. Central) and temporal (*i.e.*, glacial vs. interglacial periods) variations, evidencing the adaptive capacity of the group under ecological and environmental pressures.

Resumen: Toxodontidae constituye una familia extinta de notoungulados nativos de Sudamérica, ampliamente estudiada sistemáticamente, pero no tanto desde una perspectiva ecológica. Este trabajo busca mejorar el conocimiento sobre las variaciones en la dieta y hábitats de representantes del grupo en relación con eventos bióticos y abióticos ocurridos a lo largo de los últimos 20 Ma. Para ello se ha analizado la composición isotópica del carbono ($\delta^{13}C$) en el esmalte dental de 7 géneros registrados en 11 localidades del Centro, Oeste y Noreste de Argentina, desde el Mioceno Temprano hasta el Pleistoceno Tardío. La dieta de los toxodóntidos estudiados en este trabajo varía desde vegetación principalmente de tipo C_3 durante el Mioceno, mixta C_3-C_4 en el Plioceno, hasta plantas mayoritariamente C_4 durante el Pleistoceno. También se han registrado diferencias en la dieta durante el Pleistoceno tardío asociadas a variaciones geográficas (*i.e.,* Argentina Noreste vs. Central) y temporales (*i.e.*, fases glaciares vs. interglaciares), mostrando la capacidad adaptativa del grupo bajo presiones ecológicas y ambientales.

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INTRODUCTION

During most of the Cenozoic, the fauna of South America evolved independently, since this continent was isolated from other land masses ("splendid isolation" *sensu* Simpson, 1980). One of the most emblematic and successful mammalian native groups is the extinct order Notoungulata (included in the so-called South American native ungulates -**SANUs**-; see Croft *et al.*, 2020), characterized by their high abundance and diversity in the fossil record, as well as by their morphological disparity and wide variety of ecological niches occupied (Croft *et al.*, 2020; and references therein). Notoungulates are recorded from the Early-Middle Paleocene to the Late Pleistocene–Early Holocene boundary, being represented by two suborders (Typotheria and Toxodontia), 14 families, and more than 150 genera (Simpson, 1945; McKenna & Bell, 1997; Billet, 2011; Defler, 2019; Croft *et al.*, 2020; Zimicz *et al.*, 2020).

Particularly Toxodontidae (Toxodontia) includes medium to large-sized terrestrial representatives (103–3797 kg; Elissamburu, 2012), characterized by a somewhat early development of high-crowned (hypsodont) teeth (Middle Miocene) in their evolutionary history (Forasiepi *et al.*, 2014). They had a specialized anterior dentition, with a hypselodont incisor tusk (tusk-like i3) and hypsodont molars, which was related to increased herbivory (MacFadden, 1997; Bond *et al.*, 2006; Forasiepi *et al.*, 2014; Giannini & García-López,

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2014; Armella *et al.*, 2018; Hernández Del Pino, 2018). Toxodontidae (including the subfamilies Nosodontinae and Toxodontinae) has a stratigraphic distribution that ranges from the Late Oligocene (around 31–25 My) to the Late Pleistocene–Early Holocene boundary (*ca.* 11 ky) and a wide geographical distribution in South America (Bond *et al.*, 1995); they became very abundant and diverse particularly during the Miocene (Nasif *et al.*, 2000; Bond *et al.*, 2006). The record of toxodontid genera in North America is limited, being *Mixotodon* the only genus that participated in the Great American Biotic Interchange (**GABI**) and arrived in North America during the Pleistocene (Polaco *et al.*, 2004; Lundelius *et al.*, 2013). The relevance and interest on toxodontids dates to the first half of the 19th century, when Charles Darwin collected several specimens in Quaternary levels of Argentina and Uruguay during his 1831–1836 voyage on the H. M. S. Beagle (Darwin, 1845). These materials were studied by Richard Owen, who defined the emblematic species *Toxodon platensis* Owen, 1837, considered as the first South American native ungulate to be described in the scientific literature (Bond *et al.*, 2006; Pasquali *et al.*, 2011). This taxon was very relevant in favor of Darwin´s evolutionary thinking and their ideas on the transmutation of species (Fernicola *et al.*, 2009; Brinkman, 2010).

Ever since the 19th century, the representatives of Toxodontidae have been widely studied from systematic and phylogenetic perspectives (*e.g.*, Van Frank & Simpson, 1957; Bond *et al.*, 2006; Bonini *et al.*, 2017; Ferrero *et al.*, 2022); however, paleoecological analysis of these herbivorous mammals is still scarce and mostly based on anatomical features and tooth enamel microwear (*e.g.*, Cassini *et al.*, 2011; Braunn *et al.*, 2021; Varela & Fariña, 2015). Concerning this, dental evidence (*i.e.*, hypsodontia, which has traditionally been related to grazing habits in open grasslands, and tooth enamel microstructures that demonstrate increased resistance) suggests consumption of abrasive vegetation (Bond *et al.*, 1995; Townsend & Croft, 2008; Cassini *et al.*, 2011; Braunn *et al.*, 2021); although some authors proposed that hypsodonty is not always associated with a grazing habit (*e.g.*, Feranec, 2003; DeMiguel *et al.*, 2008, 2015; Townsend & Croft, 2008).

In recent decades, isotopic studies focused on the late Cenozoic fauna of South America have become increasingly frequent (*e.g.*, Domingo *et al.*, 2012, 2020; Dantas *et al.*, 2013, 2020, 2022; Bocherens *et al.*, 2016; Pansani *et al.*, 2019; Sanz-Pérez *et al.*, 2022, 2024; Varela *et al.*, 2023), although still, many questions remain. In this context, the number of works that include representatives of Toxodontidae is low, and most of them are limited to the Pleistocene genus *Toxodon* (*e.g.*, Lopes *et al.*, 2013; da Silva *et al.*, 2019; Pansani *et al.*, 2019; Gomes *et al.*, 2023; Varela *et al.*, 2023). It is particularly interesting the study of MacFadden (2005), who analyzed the ecology of Pleistocene *Toxodon* and *Mixotoxodon* by comparing values from different regions of South America. These studies point to a dietary plasticity of these genera, consuming both C_3 and C_4 plants depending on availability, for which they were traditionally considered as generalists (Defler, 2018; Pansani *et al.*, 2019; Dantas *et al.*, 2020). Additionally, Croft *et al.* (2020) highlighted that sometimes dietary interpretations derived from microwear analysis and morphological analyses do not agree. In this frame, therefore, analyses of stable isotopes constitute a very useful tool that can complement and shed light on dietary reconstructions.

According to all this, the goals of this work are: 1) to evaluate the paleoecology and paleodiet of Early Miocene-Late Pleistocene toxodontids from Argentina based on tooth enamel stable isotopes ($\delta^{13}C$); 2) to relate the recorded changes to abiotic (climatic/environmental) and biotic (ecological) events occurred over the last *ca.* 20 My (*e.g.*, Andean uplift, expansion of C_4 plants, establishment of the isthmus of Panama and the Great American Biotic Interchange, and alternating glacial/interglacial periods); and 3) to compare for the same genus (*Toxodon*), isotopic values obtained in different regions of Argentina and at different lapses of a specific period (Late Pleistocene), in order to detect possible similarities, differences, and trends linked to paleoenvironmental and paleoclimatic changes. In consequence, this contribution represents the first isotopic work that evaluates changes in the diet of Toxodontidae representatives over such a protracted temporal lapse (Early Miocene–Late Pleistocene). Additionally, the study of a continuous temporal sequence from the same geographic context allows us to analyze the temporal variation of vegetal cover and parameters such as temperature or precipitation, eliminating biases linked to the location of the fossiliferous sites, and vice versa.

GEOGRAPHICAL AND GEOLOGICAL SETTING

The material sampled for this work comes from fossiliferous sites located in Central, Northeastern, and Western regions of Argentina (Fig. 1, Tab. 1). The temporal interval covered by the studied sample spans *ca.* 20 My: the Early Miocene (Aisol Fm.), the Late Miocene (Cerro Azul Fm.), Early Pliocene (Monte Hermoso Fm.), Early Pleistocene (Ensenada Fm.), and Late Pleistocene (Cascada de Paleolama site, Santa Rosa Fm., Playa del Barco site, Toropí/Yupoí Fm. and Río Bermejo Fm.) (see Tab. 1 and Supp. Material 1).

MATERIAL AND METHODS

Fossil samples

We evaluated stable isotope values of 65 tooth enamel samples corresponding to different Neogene and Quaternary toxodontids from Argentina. This study focuses on the carbon isotope composition ($\delta^{13}C$) since our main aim is to explore variability in dietary behavior

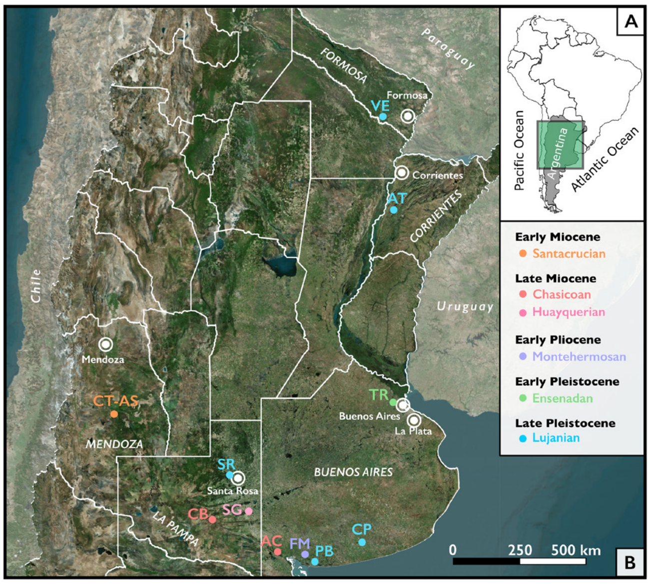

Figure 1. Geographical location of the studied paleontological localities. **A,** General map of South America with the study area indicated in a green box; **B,** Distribution of the localities in five provinces: Buenos Aires, Corrientes, Formosa, La Pampa, and Mendoza. Abbreviations: **AC**, Arroyo Chasicó; **AS,** Arroyo Seco de la Frazada; **AT**, Arroyo Toropí; **CB**, Cerro La Bota; **CP**, Cascada de Paleolama; **CT**, Cuesta de los Terneros; **FM**, Farola Monte Hermoso; **PB**, Playa del Barco; **SG**, Salinas Grandes de Hidalgo; **SR**, Santa Rosa; **TR**, Toscas del Río de la Plata; **VE**, Villa Escolar.

and in turn, to assess vegetation cover and habitat type in relation to biotic and abiotic changes. The oxygen isotope composition ($\delta^{18}O$) is used as a means to evaluate diagenetic alteration (see next section) and will not be further discussed. The choice of enamel over bone or dentine is a guarantee of the preservation of the original isotopic signal. This tissue is more stable against diagenetic alteration because of its larger apatite crystal size, lower organic matter content, lower porosity, and higher mineralogical purity compared to the other tissues (Kohn & Cerling, 2002). The isotopic analysis included 48 samples previously studied by Domingo *et al.* (2012, 2020) and 17 new samples. The works of Domingo *et al.* (2012, 2020) were more general paleoecological studies that also included other taxa and did not focus only on toxodontids. The samples belong to the genera *Nesodon* (n = 4), *Palyeidodon* (n = 2), *Stenotephanos* (n = 3), *Pisanodon* (n = 3), *Hemixotodon* (n = 12), *Xotodon* (n = 3), and *Toxodon* (n = 40), while others were assigned to Toxodontidae indet. (n = 8) (Tab. 2).

The sampled fossils analyzed in this work and those taken from previous studies are housed in different Argentinean paleontological collections: Museo de Historia Natural de San Rafael (**MHNSR-PV**; Mendoza, Mendoza), Museo Provincial de Ciencias Naturales de Villa Escolar (**PVE-F**; Villa Escolar, Formosa),

Table 1. Summary of the fossil-bearing levels studied in this work, the estimated age of the associations, their geographic location, and the sampled taxa. Abbreviations: **BA**, Buenos Aires Province; **Ch**, Chasicoan; **Co**, Corrientes Province; **En**, Ensenadan; **Fo**, Formosa Province; **Hu**, Huayquerian; **LP**, La Pampa Province; **Lu**, Lujanian; **Me**, Mendoza Province; **Mo**, Montehermosan; **Sa**, Santacrucian. The geographic location presented includes general coordinates of the localities.

Location	Coordinates	Region	Epoch	Age	Formation	Taxon	References
Villa Escolar	26°37′ S 58°40′ W	Northeastern (Fo)	Late Pleistocene (Lu)	12,400 ± 2.2 years BP	Río Bermejo	*Toxodon* sp.	Kruck *et al*., 2011
Playa del Barco	39°00′09″ S 61°34′52″ W	Central (BA)	Late Pleistocene (Lu)	19,849 years Cal BP	Playa del Barco	*Toxodon platensis*	Prado *et al*. (2015) Tomassini *et al*. (2020)
Santa Rosa	36º37′20.29″ S 64º17′43.12″ W	Central (LP)	Late Pleistocene (Lu)	28,170 years Cal BP	Santa Rosa	*Toxodon* sp.	Montalvo *et al*. (2013)
Arroyo Toropí	28°36′ S 59°02′ W	Northeastern (Co)	Late Pleistocene (Lu)	52,000 years Cal BP 36,600 years Cal BP	Toropí/Yupoí	*Toxodon platensis* *Toxodon sp.*	Tonni *et al*. (2005) Zurita *et al*. (2014)
Cascada de Paleolama	38º50′04″ S 60º32′06″ W	Central (BA)	Late Pleistocene (Lu)	~128,000 - 116,000 years BP	Undetermined formation (unit D of Pardiñas *et al*., 1996)	*Toxodon platensis*	Pardiñas *et al*. (1996) Sanz-Pérez *et al*. (2022)
Toscas del Río de La Plata	34º30′ S 58º28′ W	Central (BA)	Early Pleistocene (En)	without dating	Ensenada	*Toxodon* sp.	Soibelzon *et al*. (2008, 2019)
Farola Monte Hermoso	38º58′01″ S 61º41′43″ W	Central (BA)	Early Pliocene (Mo)	4.5 ± 0.2 My	Monte Hermoso	*Xotodon* sp.	Sanz-Pérez *et al*. (2024)
Salinas Grandes de Hidalgo	37°13'04.83″ S 63°36'04.71″ W	Central (LP)	Late Miocene (Hu)	5.9 ± 0.4 My	Cerro Azul	*Stenotephanos* cf. *S. plicidens* Toxodontidae indet.	Sanz-Pérez *et al*. (2024)
Arroyo Chasicó	38°38′44″ S 63°01′33″ W	Central (BA)	Late Miocene (Ch)	9.23 ± 0.09 My 9.7 ± 0.3 My	Cerro Azul	*Hemixotodon chasicoensis* *Pisanodon nazari* Toxodontidae indet.	Zárate *et al*. (2007) Sanz-Pérez *et al*. (2024)
Cerro La Bota	37°25'28.06″ S 65°21'30.70″ W	Central (LP)	Late Miocene (Ch)	without dating	Cerro Azul	*Hemixotodon chasicoensis*	Montalvo *et al*. (2019)
Arroyo Seco de la Frazada	34º54′16.1″ S 68º29′19.4″ W	Western (Me)	Early Miocene (Sa)	19.480 ± 0.025 Ma (basal levels)	Aisol	*Nesodon taweretus* *Palyeidodon* cf. *P. obtusum*	Forasiepi *et al*. (2014, 2015)
Cuesta de los Terneros	without date (old collections MHNSR-PV)	Western (Me)	Early Miocene (Sa)	without dating	Aisol	*Nesodon taweretus* Toxodontidae indet.	Forasiepi *et al*. (2014, 2015)

Table 2. Summary of stable isotope values of toxodontids from all the fossil localities analyzed in this work. Locality, epoch, genus, number of samples (#), mean $\delta^{13}C$ (‰ VPDB) and its standard deviation (*SD*), mean ± standard deviation %C4 diet of each taxon (following the equation described in Koch *et al.*, 2004). Raw isotopic dataset is available in SM 1. Abbreviations as in Figure 1 and Table 1. [1]Domingo *et al.* (2020), [2]Domingo *et al.* (2012).

Locality	Epoch	Genus	#	Mean $\delta^{13}C$ (‰ VPDB)	*SD* $\delta^{13}C$ (‰ VPDB)	Mean ± *SD* %C_4-diet
VE	Late Pleistocene (Lu)	*Toxodon*	4	-2.5	2.7	65 ± 18
PB [1]	Late Pleistocene (Lu)	*Toxodon*	9	-5.0	1.7	50 ± 11
SR [1]	Late Pleistocene (Lu)	*Toxodon*	5	-5.5	1.3	47 ± 8
TY	Late Pleistocene (Lu)	*Toxodon*	7	-1.8	2.6	70 ± 17
CP [1]	Late Pleistocene (Lu)	*Toxodon*	4	-7.9	0.3	27 ± 2
TR [2]	Early Pleistocene (En)	*Toxodon*	2	-9.0	0.0	20 ± 0
FM [1]	Early Pliocene (Mo)	*Xotodon*	3	-6.0	2.2	38 ± 15
SG[1]	Late Miocene (Hu)	*Stenotephanos*	3	-10.2	0.3	10 ± 2
		Toxodontidae indet.	3	-10.4	0.2	9 ± 1
AC [1]	Late Miocene (Ch)	*Hemixotodon*	5	-7.7	1.6	26 ± 10
		Pisanodon	6	-9.7	0.6	14 ± 4
		Toxodontidae indet.	1	-10.5		8.32
CB [1]	Late Miocene (Ch)	*Hemixotodon*	7	-8.7	1.3	20 ± 8
AS	Early Miocene (Sa)	*Nesodon*	4	-7.4	1.1	29 ± 6
		Palyeidodon	2	-8.3	0.5	23 ± 4
CT	Early Miocene (Sa)	*Nesodon*	1	-8.0		25
		Toxodontidae indet.	1	-9.0		18

Colecciones Paleontológicas de la Universidad Nacional del Nordeste “Dr. Rafael Herbst” (**CTES-PZ**; Corrientes), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa (**GHUNLPam**; Santa Rosa, La Pampa), Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (**MACN**; Ciudad Autónoma de Buenos Aires, Buenos Aires), Museo Municipal de Ciencias Naturales “Carlos Darwin” (**MD-CH**, **MD-FM**, and **MD-PDB**; Punta Alta, Buenos Aires), Museo Municipal de Ciencias Naturales “Vicente Di Martino” (**MMH-CH** and **MMH-QEQ**; Monte Hermoso, Buenos Aires), and Museo de La Plata (**MLP**; La Plata, Buenos Aires).

Stable isotope analyses

Samples of approximately 5 to 6 mg of tooth enamel were collected using a Dremel® diamond-studded drill. Samples were taken along the entire length of the enamel, from crown to root, to avoid obtaining a biased isotopic signal due to seasonal variability in the timing of mineralization. The bulk samples were treated with 30% H_2O_2 for 24 hours to eliminate the possible contamination of organic matter. Samples were rinsed 5 times in ultrapure water Milli-Q and soaked for 24 hours in 1 M acetic acid buffered to ~pH 5 using Ca acetate solution. This step allows the elimination of the external carbonate potentially precipitated on the surface of the teeth during the fossilization process. After 5 rinses with Milli-Q water, the resulting solid was freeze-dried at -40ºC and at a pressure of 25 x 10^{-3} Mbar for 24 hours. Analyses were conducted at the Stable Isotope Laboratory of the University of California Santa Cruz, using a ThermoScientific MAT253 dual inlet isotope ratio mass spectrometer coupled to a ThermoScientific Kiel IV carbonate device. Samples were roasted at 65ºC under vacuum for one hour and a half before IRMS analysis.

Stable isotope results are reported in δ-notation $\delta^{H}X_{sample} = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000$, where *X* is the element, H is the mass of the heavy isotope of the element and *R* = $^{13}C/^{12}C$ or $^{18}O/^{16}O$. Vienna Pee Dee Belemnite (**VPDB**) is the standard for $\delta^{13}C$ values, whereas Vienna Standard Mean Ocean Water (**VSMOW**) is the standard for $\delta^{18}O$ values.

Laboratory standards were Carrara Marble (CM12, $\delta^{13}C$ = 2.05‰), NBS-18 ($\delta^{13}C$ = -5.014‰) and Atlantis II ($\delta^{13}C$ = 0.87‰). The standard deviations for repeated measurements of CM12 (n = 152), NBS-18 (n = 55) and Atlantis II (n = 27) were 0.08‰, 0.09‰ and 0.12‰ for $\delta^{13}C$, respectively. Replicates of NIST120c (n = 18) were also analyzed as a quality control standard after having followed the same chemical treatment as the bioapatite samples. Mean and standard deviations $\delta^{13}C$ value for NIST 120c is -6.04 ± 0.09‰ (VPDB). Duplicate analyses were carried out for ~11% samples of each batch (n = 37). The average absolute difference for $\delta^{13}C$ values was 0.05‰ and the standard deviations of this average difference was 0.23‰. In order to make paleoecological and paleoenvironmental interpretations, it is important to confirm that the samples are not altered and that they preserve pristine stable isotope data. The diagenetic alteration of bioapatite of the samples has been surveyed by comparing the oxygen isotope composition of the carbonate and phosphate fraction of bioapatite samples. According to Bryant *et al.* (1996) and Iacumin *et al.* (1996) if CO_3^{-2} and PO_4^{-3} in bioapatite are formed in isotopic equilibrium with body water at a relatively constant mammalian body temperature, there should exhibit a linear relationship with a consistent difference ranging from 8.6–9.1‰ between $\delta^{18}O_{CO3}$ and $\delta^{18}O_{PO4}$

values (expressed as $\delta^{18}O_{CO3}$ - $\delta^{18}O_{PO4}$ = $\Delta^{18}O_{CO3\text{-}PO4}$). This is a guide to establish if a sample has suffered diagenesis or not, obtaining $\Delta^{18}O_{CO3\text{-}PO4}$ values close to this range indicates that $\delta^{18}O_{CO3}$ and $\delta^{18}O_{PO4}$ phases retain pristine isotopic values. In this work, the mean $\Delta^{18}O_{CO3\text{-}PO4}$ value for all the samples is 8.8 ± 0.7‰ (SM 1), close to the expected value.

Carbon stable isotopes in mammalian diets and habitats

The carbon isotope ratios in the tooth enamel of herbivorous mammals are influenced by the photosynthetic pathway (C_3, C_4, and CAM) of the plants they consume. The prevalence of these plant pathways in ecosystems is, in turn, shaped by various environmental and ecological factors (*e.g.*, aridity, temperature, canopy density, and concentration of CO_2 in the atmosphere; Farquhar *et al.*, 1989; Koch, 1998, 2007; Hayes, 2001). Therefore, the $\delta^{13}C$ values of the bioapatite are indicative of the carbon isotopic composition of the plants consumed by the animal, being useful in paleontological studies for paleodiet characterization and reconstruction of ancient habitats, providing information on the proportion of C_3-C_4 plants (Koch, 1998). Due to metabolic processes and carbonate equilibrium, there is an enrichment in $\delta^{13}C$ values between plant resource carbon and tooth enamel ($\varepsilon^*_{\text{diet-enamel}}$) ranging from 12‰ to 14‰, depending on the herbivore digestive systems (Lee-Thorp *et al.*, 1989; Cerling & Harris, 1999; Passey *et al.*, 2005; Tejada-Lara *et al.*, 2018). In the case of toxodontids, we used the value calculated for current ungulates (Perissodactyla, Artiodactyla, and Proboscidea), $\varepsilon^*_{\text{diet-enamel}}$ = +14.1‰ (Cerling & Harris, 1999; Domingo *et al.*, 2020), since the Order Notoungulata has no living counterparts.

In order to evaluate the type of toxodontids diet (C_3-dominated, mixed C_3-C_4, and C_4-dominated), the $\delta^{13}C$ values of atmospheric CO_2 ($\delta^{13}C_{atmCO2}$), source of the vegetative carbon, must be considered. Taking into account the isotopic composition of atmospheric CO_2 has changed over time, the $\delta^{13}C_{atmCO2}$ value at the time of study must be determined to establish the accurate dietary $\delta^{13}C$ ranges. The $\delta^{13}C_{atmCO2}$ values obtained for each period considered in this work, from Early Miocene to Late Pleistocene, are summarized in Table 2 (~-6.2‰ during the Miocene-Early Pliocene, -6.5‰ in the Pleistocene before the Last Glacial Maximum [LGM], -7.1‰ in the LGM, and -6.8‰ post-LGM [Koch *et al.*, 2004; Tipple *et al.*, 2010]).

Also, we used the $\delta^{13}C_{atmCO2}$ values of each studied time period (Tab. 2) to establish the vegetation $\delta^{13}C$ cut-off ranges in each temporal interval. The cut-off between C_3-dominated diet and intermediate C_3-C_4 diet ranges from ~-9.9‰ to -8‰, while the boundary between intermediate C_3-C_4 diet and C_4-dominated diet ranges from ~-2.9‰ to -1‰ (Tab. 3). Additionally, we used the $\delta^{13}C$ values to estimate an approximate percentage of C_4 plants in the diet of each taxon following the mass balance equation proposed by Koch *et al.* (2004):

$$(100)\ \delta^{13}C_{sample}=(100-X)\ \delta^{13}C_{100\%C3enamel}+(X)\delta^{13}C_{100\%C4enamel}$$

(1) where $\delta^{13}C_{100\%C3enamel}$ and $\delta^{13}C_{100\%C4enamel}$ values are taken from Table 2 for each period.

In addition, to construct temporal trends in carbon isotopic values of toxodontids, we grouped the localities by periods and calculated the weighted mean of the isotopic values:

$$X_{period}=\frac{((x_a+n_a)+(x_b+n_b)+\cdots)}{(n_a+n_b+\cdots)}$$

(2) where X_{period} is the mean isotopic $\delta^{13}C$ value for each period, while x_a, x_b, n_a, and n_b are mean isotopic values and the number of analyzed samples for taxa/locality a and b, respectively.

As suggested by Domingo *et al.* (2013), it is more convenient to use the weighted mean, since the number of samples analyzed differs between taxa or localities and, therefore, they do not contribute equally to the final mean.

Statistical analysis

Statistical analyses were performed with the R software version 4.3.1 (R Core Team, 2023). The ggstatsplot package (Patil, 2021) was used to create graphs with details of the statistical tests. Due to the small sample size, the normal distribution of our data cannot be attested. Therefore, we performed non-parametric analyses (Kruskal-Wallis) to identify significant differences between taxa. Since Holm adjustment does not seem to be indicated if the degree freedom is equal or more than five (Kim, 2015), we used the Tukey-Kramer Honest Significant Difference post–hoc analysis. To observe temporal and spatial changes of $\delta^{13}C$ and detect trends, we grouped our isotopic data according to: 1) different SALMAs

Table 3. Atmospheric, plant and enamel $\delta^{13}C$ values used in mass balance calculations and estimation of %C_4 resources in diets. $\delta^{13}C_{atmCO2}$ values are from Tipple *et al.* (2010) and Koch *et al.* (2004). Past values of $\delta^{13}C$ for C_3 and C_4 plants were estimated from the isotopic values of modern plants and adding the difference between modern $\delta^{13}C_{atmCO2}$ (-8‰) and past $\delta^{13}C_{atmCO2}$. Enamel values for pure C_3 and C_4 diet are estimated by adding ~14‰ to the plant values (Cerling & Harris, 1999). The $\delta^{13}C$ 100% C_3 and C_4 diets are calculated following the work of Passey *et al.* (2002). All values are relative to VPDB and expressed in ‰. Table modified from Koch *et al.* (2004).

	Miocene–Pliocene	**Pleistocene**		
		Pre-LGM	**LGM**	**Post-LGM**
$\delta^{13}C_{atmCO2}$	-6.2	-6.5	-7.1	-6.8
$\delta^{13}C$ C_3 plants	-25.3	-25.6	-25.9	-26.2
$\delta^{13}C$ C_4 plants	-10.8	-11.1	-11.7	-11.4
$\delta^{13}C$ 100% C_3 enamel	-8.5	-8.8	-9.4	-9.1
$\delta^{13}C$ 100% C_4 enamel	3.3	3.0	2.4	2.7

(South American Land Mammal Ages) or stages/ages represented: Santacrucian, Chasicoan, Huayquerian, Montehermosan, Ensenadan, early Lujanian, and late Lujanian; 2) geographical regions (Northeastern and Central Argentina), specifically for Late Pleistocene specimens; and 3) Marine Isotope Stages (**MIS** 2, **MIS** 3, **MIS** 5), specifically for Late Pleistocene specimens. Non-parametric tests (Kruskal-Wallis and Mann-Whitney post-hoc analyses with Holm's correction or Mann-Whitney U) were performed to check for significant differences among/between groups. In all cases, the significance level was set at $p = 0.05$.

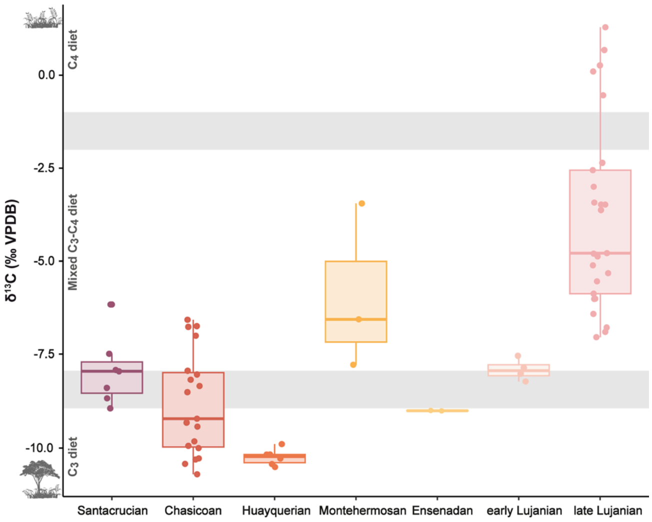

Figure 2. Boxplot distribution and median $\delta^{13}C$ values, grouped by stages, for Neogene-Quaternary toxodontids from Argentina. The grey horizontal bars depict the vegetation $\delta^{13}C$ cut–off approximate values between a C_3–dominated diet, intermediate C_3–C_4 diet and C_4–dominated diet.

RESULTS AND DISCUSSION

During the Miocene, the high-crowned dentition of toxodontids was a specialized adaptation to feed on grasses and abrasive diets, although later (Pliocene and Pleistocene) it allowed them to exploit a wide variety of diets depending on the local plant resources (MacFadden, 2005). We analyzed the tooth enamel $\delta^{13}C$ values of toxodontids from different Argentine localities spanning a time window of ~20 My to check potential dietary variability and flexibility across time and space.

$\delta^{13}C$ change through the late Cenozoic evolution of toxodontids

The $\delta^{13}C$ values of the toxodontids considered here ranged from -10.7‰ (*Hemixotodon*, Cerro La Bota) to 1.3‰ (*Toxodon*, Villa Escolar) (see Supp. Material 2), indicating a wide variety of plant resources spanning the whole vegetation spectrum from pure C_3 diets, mixed C_3-C_4 diets, and pure C_4 diets. First, tooth enamel $\delta^{13}C$ data were grouped by periods (Tab. 4, Fig. 2). The lowest mean $\delta^{13}C$ values belonged to the Huayquerian, Late Miocene ($\tilde{x} = -10.3 \pm 0.2$‰; n = 6), whereas the highest corresponded to the late Lujanian, Late Pleistocene ($\tilde{x} = -4.8 \pm 3.3$‰; n = 25) (Tab. 4). Non-parametric statistical tests confirmed significant differences among temporal groups ($\chi^2 = 47.534$, $p < 0.001$), which is mainly driven by the Lujanian increase in mean $\delta^{13}C$ values (Tab. 5).

In general, a significant change is observed from a low to moderate consumption of C_4 plants in all the Neogene toxodontids towards an important increase in its consumption in the Pleistocene *Toxodon* (Fig. 3). Statistical analysis evidences highly significant differences in $\delta^{13}C$ values when considering all the toxodontids from the selected time window ($\chi^2 = 48.364$, $p < 0.001$, Supp. Material 3), which supports the interpretation that they exploited a wide range of resources during the time involved.

Neogene: Miocene and Pliocene diets. Most of the Miocene toxodontids (*Palyeidodon*, *Stenotephanos*, *Pisanodon*) showed diets based primarily or exclusively on C_3 plants, although some others (*Nesodon* and *Hemixotodon*) also consumed a relatively low percentage of C_4 plants (Tab. 2, Fig. 3). Such low percentages of C_4 plants in diets might be due in part to the low abundance of this resource at this time; with respect to this, although there is evidence of the presence of C_4 plants in Argentina since 16.5 My ago, they did not become dominant on the South American continent until 7 My ago (Strömberg, 2011). According to Dantas *et al.* (2017), taxa can be attributed to the browser or grazer guilds if they feed on more than 80% of C_3 or C_4 plants, respectively; while they are considered mixed feeder if they do not reach those limits.

Table 4. Summary of stable isotope values of notoungulates grouped by land mammals ages or stages/ages in each case, number of samples (#), and Interquartile range (**IQR**). Locality abbreviations as in Figure 1.

Temporal interval	Locality	#	Mean $\delta^{13}C$ (‰ VPDB)	*SD* $\delta^{13}C$ (‰ VPDB)	Weighted mean	Median	IQR	Mode
late Lujanian	AT, SR, PB, VE	25	-3.8	2.5	-3.9	-4.8	3.3	-3
early Lujanian	CP	4	-7.9	0.3	-7.9	-7.9	7.3	-8
Ensenadan	TR	2	-9.0	0.0	-9.0	-9.0	0.0	-9
Huayquerian	SG	6	-10.3	0.2	-10.3	-10.3	0.2	-10
Montehermosan	FM	3	-5.9	2.2	-5.9	-6.6	2.2	-8
Chasicoan	CB, AC	19	-8.8	1.4	-8.9	-9.2	2.0	-10
Santacrucian	AS, CT	7	-8.0	0.9	-8.3	-8.0	0.8	-8

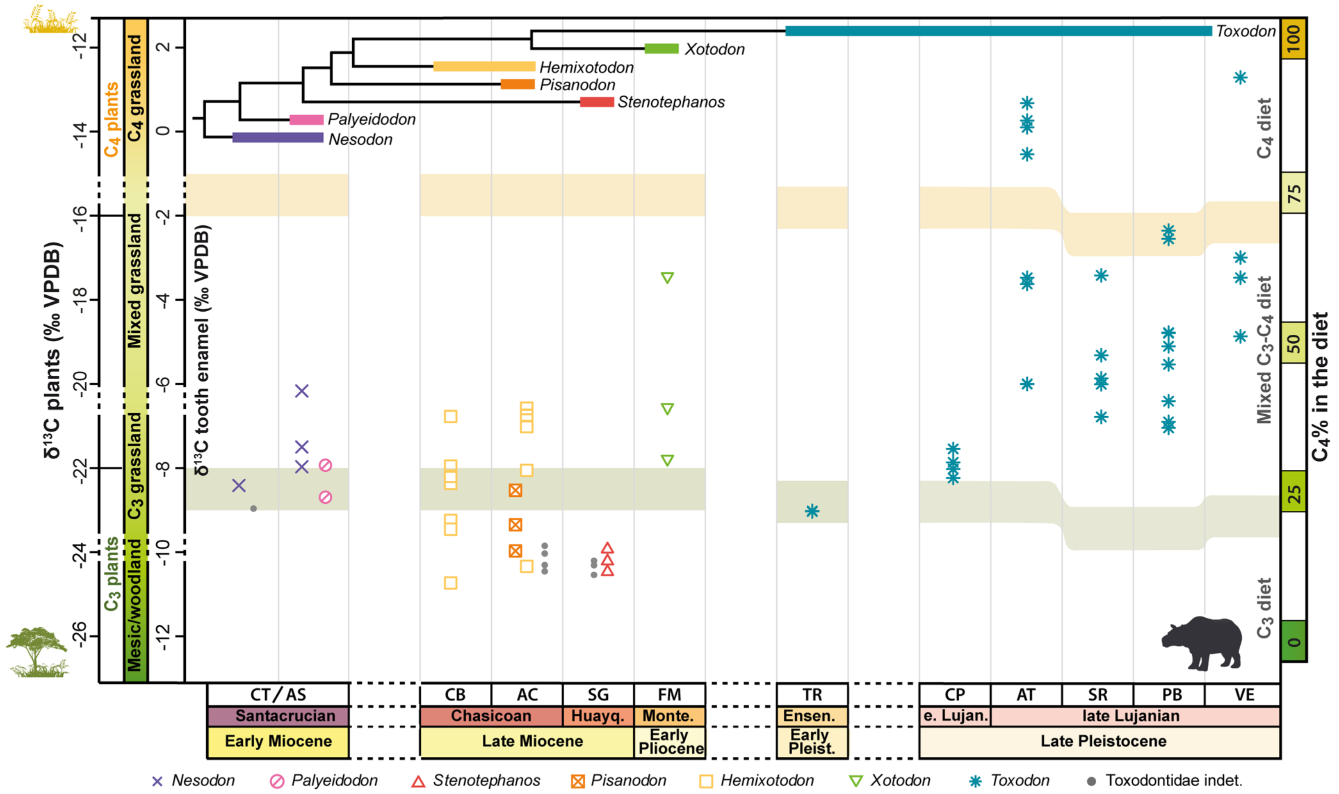

Figure 3. Temporal variability of tooth enamel δ^{13}C of Neogene-Quaternary toxodontids in Argentina. The different shape and color represent different genera and the gray dots identify Toxodontidae indet. samples. The horizontal bars depict the vegetation δ^{13}C cut–off values between a C$_3$–dominated diet, intermediate C$_3$–C$_4$ diet and C$_4$–dominated diet in each temporal interval (see Tab. 2). The percentage of C$_4$ plants is represented as ranges considering the variation of δ^{13}C$_{atmCO2}$ collected in Table 3. The x-axis represents the different localities analyzed in temporal order (abbreviations as in Fig. 1). The phylogeny of Toxodontidae has been elaborated following Nasif *et al.* (2000), Schmidt (2013), Bonini *et al.* (2017), Armella *et al.* (2018), and Ferrero *et al.* (2022); phylogeny reflects only the age of the samples. **Huayq**. = Huayquerian, **Monte.** = Montehermosan, **Ensen.** = Ensenadan, **e. Lujan.** = early Lujanian. Reconstruction of *Toxodon* modified from Mauro Muyano (Megafauna 3D Project).

In previous works, the environment of Salinas Grandes de Hidalgo has been identified as an open C$_3$ grassland, and all the herbivores analyzed present C$_3$-based diets (Domingo *et al.*, 2020; Sanz-Pérez *et al.*, 2024). In the case of some Late Miocene toxodontids, including Huayquerian *Stenotephanos* and Toxodontidae indet. from Salinas Grandes de Hidalgo, and Chasicoan *Pisanodon* and Toxodontidae indet. from Arroyo Chasicó, we infer that they were browsers living in C$_3$ grasslands (Tab. 2). Early Miocene (*Neosodon* and *Palyeidodon*) and the remaining Late Miocene (*Hemixotodon*) representatives showed preference for mixed C$_3$-C$_4$ resources from C$_3$ open grasslands.

Early Miocene toxodontid *Nesodon* has been previously considered to be a grazer or mixed feeder (Croft, 2016; Trayler *et al.*, 2020), but morphological and microwear studies indicate that it was probably a browser (*e.g.*, Townsend & Croft, 2008; Cassini & Vizcaíno, 2012; Cassini *et al.*, 2012). Cassini *et al.* (2011) concluded that representatives of this genus were mixed feeders that consumed a combination of resources, possibly based on the availability of vegetation depending on different factors (*e.g.*, seasonality, geographic or environmental conditions, among others). MacFadden *et al.* (1996) showed δ^{13}C values of *Nesodon* ranging from -14.2 to -11.5‰ from the Santacrucian of Santa Cruz and Chubut provinces (Patagonia, Argentina), which is in agreement with diets closer to the browser than the grazer end of the intake spectrum. Our results from Western Argentina point to a mixed-feeder habit, feeding on C$_3$ resources but with a slight proportion of C$_4$ plants (Fig. 3). The slight difference in diets may be due to geographic factors, but the number of samples in both regions needs to be expanded to allow clear conclusions.

MacFadden *et al.* (1996) also presented isotopic values for *Pisanodon* from the Chasicoan and Huayquerian of Arroyo Chasicó and Salinas Grandes de Hidalgo respectively whose δ^{13}C values ranged from -9.2 to -8.3‰. Our result agrees with the interpretation of C$_3$–based diets for this genus (Fig. 3).

Therefore, based on our results and information from previous work, we infer that during the Miocene, toxodontids showed a general trend towards feeding on C$_3$ grasslands.

The Mio–Pliocene transition witnessed the largest shift in mean δ^{13}C value for the Neogene taxa, from the Late Miocene C$_3$ browser *Stenotephanos* (-10.2 ± 0.3‰, Tab. 2) to the Early Pliocene mixed feeder *Xotodon* (-6.0 ± 2.2‰, Tab. 2). *Xotodon* tooth enamel from this study and other previous works (MacFadden *et al.*, 1996; Latorre *et al.*, 1997) from Monte Hermoso (Buenos Aires Province) and Corral Quemado (Catamarca Province) indicates that representatives of this genus fed on more or less percentage of C_3 or C_4 vegetation depending on the specimen analyzed. We noted that *Xotodon* exploited a wide range of resources (C_3 and C_4 plants), without the strong preference for C_3 vegetation displayed by Miocene toxodontids (Fig. 3). *Xotodon* explored the new resources available after the expansion of C_4 plants. Other contributions suggested this shift to C_4 plants in the diet of notoungulates occurred slightly earlier at ~7.6 My (Cerling *et al.*, 1997; Latorre *et al.*, 1997); however, works performed in the Central region of Argentina did not record a significant change in the diets of herbivorous mammals until the earliest Pliocene (Domingo *et al.*, 2020; Sanz-Pérez *et al.*, 2024). This ability to incorporate an increasing variety of dietary resources likely allowed toxodontids to colonize new regions during the GABI, reaching southern North America (MacFadden, 2005; Pérez-Crespo *et al.*, 2020).

Quaternary: Pleistocene diets. The distribution of *Toxodon* $\delta^{13}C$ values in the Quaternary samples shows a trend toward increasing values, from the Early (Ensenadan) ($\tilde{x} = -9.0 \pm 0.0$‰, Tab. 2; Fig. 3) to the Late Pleistocene (early Lujanian and late Lujanian) ($\tilde{x} = -7.9 \pm 0.3$‰ and -4.8 ± 3.3‰, respectively Tab. 2; Fig. 3). The latest Pleistocene (late Lujanian) showed significant differences in mean $\delta^{13}C$ value with respect to those of the Miocene and Pliocene (Tab. 5). Although Early Pleistocene samples could not be included in the statistical analysis due to the low sample number ($n < 3$), they were also very different from Late Pleistocene samples since they presented a pure C_3 vegetation diet (Fig. 2). The percentage of C_4 plants in the diet of the Early Pleistocene *Toxodon* is 20%, while in *Toxodon* representatives from the Late Pleistocene the values obtained exceed 45% of C_4 plants, even reaching ~70% in some localities (Tab. 2).

Soibelzon *et al.* (2008) interpreted that the Ensenadan vertebrate assemblage from Toscas del Río de La Plata reflects an open environment with arid/semiarid conditions; however, they also noted the existence of short warm pulses. In the isotopic study by Domingo *et al.* (2020), they show that the diet of the herbivores from this locality (Litopterna, Proboscidea and Perissodactyla) pointed to a C_3-based diet in all cases, with a low percentage of C_4 plants. Although it is necessary to expand the isotopic data and study other taxa from this locality, we can infer that the lower consumption of C_4 plants at this time could be due to the existence of warmer and wetter conditions than in later phases.

The samples of *Toxodon* from Cascada de Paleolama come from Late Pleistocene levels assigned to the early Lujanian, which were related to a warm event linked to MIS 5e (Pardiñas *et al.*, 1996; Beilinson *et al.*, 2017; Sanz-Pérez *et al.*, 2022). The values obtained are -8.2 to 7.5‰, closer to those of the samples from Toscas del Río de la Plata (Fig. 3), evidencing probably similar paleoenvironments and supporting the interpretation mentioned above on this site. On the contrary, values from other localities belonging to the latest Pleistocene, specifically to the late Lujanian (MIS 3 and MIS 2), showed more arid conditions and a higher percentage of C_4 plants in their diets. Although MIS 2 correspond to a glacial period with cool temperatures (Clark, 2009) where C_3 plants may have thrived, lower pCO_2atm values and the increase of continentality in the area due to the lowering of sea level during the glacial phase may have benefited the dominance of C_4 plants in the Central region of Argentina as recorded by toxodontid $\delta^{13}C$ values (see also Sanz-Pérez *et al.*, 2022).

Spatial variability in the *Toxodon* $\delta^{13}C$ through the Late Pleistocene

Within the South American Pleistocene megafauna, isotopic data indicate that *Toxodon* was a generalist with a high dietary plasticity (*e.g.*, MacFadden, 2005; Dantas *et al.*, 2013, 2017; Pansani *et al.*, 2019; Pérez-Crespo *et al.*, 2020; Gomes *et al.*, 2023; Varela *et al.*, 2023). Late Pleistocene *Toxodon* values span a range from -8.2 to 1.3‰, which may indicate a broad range of dietary adaptations. The ample sampling of one single genus, *Toxodon*, during the Late Pleistocene allowed us to evaluate potential spatial variability in the diets and vegetation cover between different regions (Northeastern and Central) of Argentina, ruling out that possible changes in values are related to differences in ecology or physiological traits.

The Northeastern region includes the localities of Villa Escolar (MIS 2; Last Glacial Maximum) and Arroyo Toropí (MIS 3; transitional interglacial stage), where *Toxodon* carbon isotopic composition ranged between -6.0 and 1.3‰ ($\tilde{x} = -3.0 \pm 3.7$‰, $n = 11$; Fig. 4). The Central region, including the localities of Cascada de Paleolama (MIS 5e; last interglacial stage before

Table 5. Non-parametric tests (Kruskal-Wallis and Mann-Whitney post-hoc analyses with Holm's correction) for toxodontid $\delta^{13}C$ values among the different SALMAs or stages/ages. Those pairs of taxa with significant differences ($p < 0.05$) are highlighted in bold.

	$\delta^{13}C$ (‰ VPDB) $X2 = 47.534$, df = 5, $p < 0.001$				
	Santacrucian	**Chasicoan**	**Huayquerian**	**Montehermosan**	**early Lujanian**
Chasicoan	1.000				
Huayquerian	0.051	0.255			
Montehermosan	1.000	0.325	0.423		
early Lujanian	1.000	1.000	0.213	1.000	
late Lujanian	**0.002**	**< 0.001**	**0.003**	1.000	**0.026**

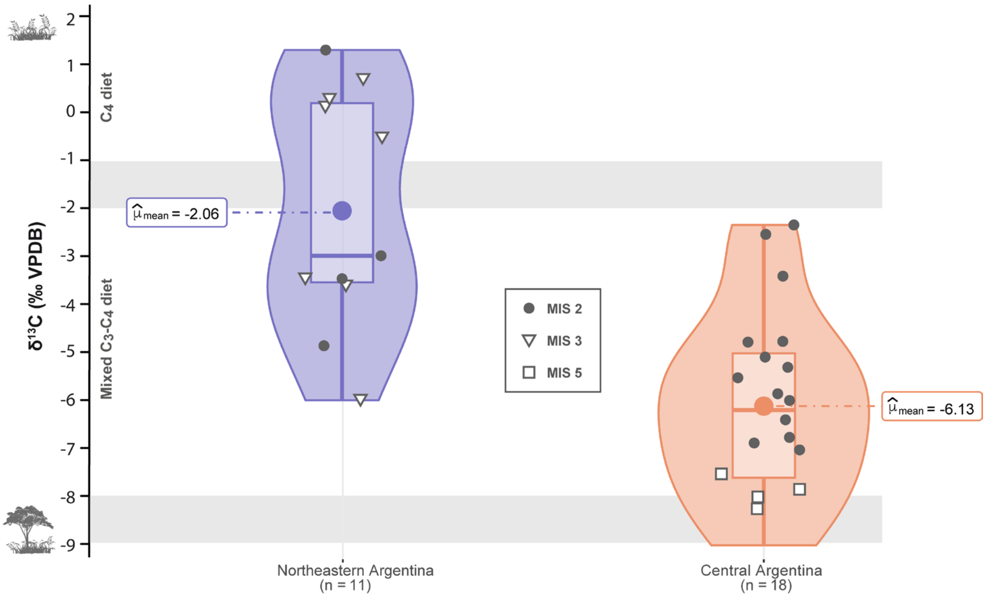

Figure 4. Violin plot of the *Toxodon* tooth enamel $\delta^{13}C$ values of the Northeastern region in purple and the Central region in orange. The rectangles inside the violin plots are the boxplots of the $\delta^{13}C$ values, where the solid horizontal line represents median $\delta^{13}C$ values. The grey horizontal bars depict the mean $\delta^{13}C$ cut-off values of vegetation between a C_3–dominated diet, intermediate C_3-C_4 diet and C_4-dominated diet. Symbols indicate the Marine Isotope Stage associated with the locality of each sample.

Holocene), Playa del Barco and Santa Rosa (MIS 2), shows $\delta^{13}C$ values that ranged between -8.2 and -2.4‰ ($\tilde{x}$ = -5.9 ± 2.1‰, n = 18; Fig. 4). There were statistically significant differences in the diet between specimens from both regions (Mann-Whitney U test: $W = 173$, $p < 0.001$). Therefore, although $\delta^{13}C$ values indicate mostly mixed-feeder habits in both regions, *Toxodon* individuals from the Northeastern region during the MIS 3 and MIS 2 point to diets characterized by a significantly greater consumption of C_4 plants, with some individuals having a diet based exclusively on vegetation from C_4 grasslands in both stages (Fig. 4).

Nevertheless, the MIS 2 is recorded in both regions (Northeastern and Central), which allows us to rule out the possibility that the differences between regions were due to temporal change. The $\delta^{13}C$ values of the toxodontids from MIS 2 pointed to mixed C_3-C_4 diet in both regions, but some individuals from the Northeastern preferred to feed on C_4 grasslands, while in Central all individuals fed in mixed grasslands (Fig. 4). Therefore, we infer a more grazing behavior in the Northeastern during the same glacial stage, which is in agreement with the phytolitic analysis performed by Contreras *et al.* (2019) in the Toropí/Yupoí Formation that shows woody savannas or open forests rich in herbaceous species.

We also compared the two interglacial stages, represented by the MIS 5 in the Central region and MIS 3 in the Northeastern region. The $\delta^{13}C$ values of both regions reported significant differences (W = 28, p = 0.006), with more abrasive diets recorded in Northeastern ($\tilde{x}$ = -0.5 ± 3.8‰) than in Central ($\tilde{x}$ = -7.9 ± 0.3‰). This situation could be related to the specific conditions of each stage, MIS 5 was characterized as a warm phase, during MIS5e global mean surface temperatures were recorded at least 2°C warmer than current temperatures (Rohling *et al.*, 2008). In turn, MIS 3 represents a transitional interglacial stage located within the last glacial period, which is characterized by a great climatic instability, with several Heinrich (colder) and Dansgaard-Oeschger (warmer) events (Agosta & Compagnucci, 2016).

This result is consistent with a latitudinal distribution of $\delta^{13}C$ values that has been previously identified in late Quaternary *Toxodon* samples from southern South America (MacFadden, 2005; França *et al.*, 2015; Asevedo *et al.*, 2021), which show a clear southward increase of C_3 vegetation in the diet (except for the specimens from the Amazon basin) and related to resources availability in the different areas. Different studies have shown the dietary flexibility of representatives of *Toxodon*:

- A browsing behavior feeding exclusively on C_3 forest in the Amazon region (*e.g.*, MacFadden, 2005; Lopes *et al.*, 2013).
- A generalist mixed-feeding diet and specialized C_4 grazers in the Brazilian Intertropical region (*e.g.*, Dantas *et al.*, 2013, 2017, 2020; França *et al.*, 2015; Pansani *et al.*, 2019; Omena *et al.*, 2021).
- A mixed-feeding behavior with high proportion of C_4 plants in Northern Argentina, Uruguay, and Southern Brazil (*e.g.*, MacFadden, 2005; Lopes *et al.*, 2013; Varela *et al.*, 2023), in agreement with our $\delta^{13}C$ values (Fig. 4).
- A mixed C_3 grazer in Central Argentina (*e.g.*, MacFadden, 2005; Domingo *et al.*, 2012; Bocherens *et al.*, 2016; Bellinzoni *et al.*, 2023), also in accordance with our $\delta^{13}C$ results (Fig. 4).

Differences in diet associated with temporal changes in Central Argentina were also observed. The samples from the MIS 5 ($\tilde{x}$ = -7.9 ± 0.3‰) and MIS 2 ($\tilde{x}$ = -5.4 ± 1.2‰) of this region separate in two well-delimited groups (Fig. 4), with significant differences (W = 56, p < 0.001). In both stages, *Toxodon* displayed a mixed-feeder habit, but in the MIS 2 samples indicated a greater intake (and potential availability) of C_4 plants in the environment with respect to the MIS 5. As explained above, conditions in the Central region during MIS 2, such as increased continentality and lower pCO_2atm values, may have favored the dominance of C_4 plants in comparison to the warmer MIS 5e (Sanz-Pérez *et al.*, 2022 and references therein).

On the contrary, in Northeastern Argentina differences linked with temporal changes are not recorded (Fig. 4), since MIS 3 and MIS 2 report no statistical differences ($W = 14$, $p = 1.000$). A possibility to explain this situation is that conditions between MIS 3 and MIS 2, at least in the lapses covered in this work, should not have been very different (see above); however, more detailed studies of the Northeastern faunas are necessary to reach firmer conclusions on this topic.

As MacFadden (2005) notes, it is reasonable to consider that the variability in $\delta^{13}C$ recorded in *Toxodon* is a good proxy that sheds light on the type of vegetation that existed in the study area. *Toxodon* would have had a truly opportunistic feeding strategy, varying its diet according to the availability of different resources and the degree of competition with other more specialized taxa (França *et al.*, 2015). Therefore, we can infer that conditions were more arid during glacial and interglacial stages in Northeastern Argentina than in Central Argentina.

CONCLUSIONS

This work highlights the importance of isotopic studies to infer the diet of South American endemic notoungulates, with no modern representatives. Our study presents stable isotope data of 7 Toxodontidae genera from 11 Neogene–Quaternary Argentine localities, spanning *ca*. 20 My, as opposed to previous studies that mainly focused on the Pleistocene genus *Toxodon*. Despite their high crowned teeth, *Pisanodon* and *Stenotephanos* tooth enamel $\delta^{13}C$ values point to a browsing behavior in Central Argentina during the Miocene. Neogene toxodontids record the change from C_3 diets (Miocene) to mixed C_3-C_4 (Early Pliocene) reflecting the expansion of C_4 plants. It is interesting to highlight that in our study, toxodontid $\delta^{13}C$ values did not point to a considerable percentage of C_4 plants in their diet until ~4.5 My ago, despite the C_4 expansion occurring ~7–8 My ago. In the future, it would be interesting to study Neogene toxodontid samples from other areas of South America to check whether this dietary change is synchronically or asynchronously registered.

During the Pleistocene, a trend of increasing consumption of C_4 plants was detected, reaching a maximum of 75%. This study evidences the high ecological adaptability of toxodontids that allowed them to use available resources and inhabit a wide variety of ecosystems.

During the Late Pleistocene, we recorded differences in the diet of *Toxodon* related to spatial changes. Although *Toxodon* showed mostly mixed-feeder habits in Northeastern and Central regions of Argentina, more abrasive diets were recorded in the Northeastern, with a significantly higher percentage of C_4 plants in the diet than in the Central. In the Central region, dietary changes were recorded between interglacial (MIS 5) and glacial (MIS 2) stages. During the Last Glacial Maximum (MIS 2) there was a greater dominance of C_4 plants in the area and *Toxodon* incorporated a higher percentage of C_4 plants in its diet. Therefore, it can be inferred that *Toxodon* efficiently exploited the dominant local resources. The results obtained for the Late Pleistocene provide novel information, from a different perspective, on different environmental factors that occurred in a context of extinction of one of the most emblematic endemic groups of South America.

Supplementary information. Supplementary Material 1, 2 and 3 are available at the Spanish Journal of Palaeontology website (https://sepaleontologia.es/spanish-journal-palaeontology/) linked to the corresponding contribution.

Supplementary Material 1. Details of the geological units and localities under study.

Supplementary Material 2. Tooth enamel stable isotope total dataset from all the localities analyzed in this study. Locality, province, epoch, SALMA or stage/age, collection number, order, family, genus/species, analyzed tissue, $\delta^{13}C$ (‰ VPDB), $\delta^{18}O_{CO3}$ (‰ VPDB), $\delta^{18}O_{CO3}$ (‰ VSMOW), $\delta^{18}O_{PO4}$ (‰ VSMOW), difference between $\delta^{18}O_{CO3}$ and $\delta^{18}O_{PO4}$ values ($\Delta\delta^{18}O_{CO3}$-$\delta^{18}O_{PO4}$) and references.

Supplementary Material 3. Tukey-Kramer Honest Significant Difference post–hoc analysis for $\delta^{13}C$ (‰ VPDB) values of all the taxa (n>3) of the fossil localities. Those pairs of taxa with significant differences ($p < 0.05$) are highlighted in bold.

Author contributions. DSP and MHF conceived the study. RT, CIM, and AEZ provided access to the material and LD sampled the material. DSP and LD treated the samples and analyzed them. All authors wrote the manuscript.

Competing interest. The authors declare no competing interests.

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