Trace element analyses indicative of paleodiets in Middle Miocene mammals from the Somosaguas site (Madrid, Spain)

L. DOMINGO [1, 2, 3]   N. LÓPEZ-MARTÍNEZ [2, 3, †]   S.T. GRIMES [4]

[1] **Earth and Planetary Sciences Department, University of California**
Santa Cruz, CA 95064 (USA). E-mail: lauradomingo@geo.ucm.es; Fax: +34 913944849

[2] **Departamento de Paleontología, Facultad CC. Geológicas, Universidad Complutense de Madrid-Instituto de Geología Económica (CSIC)**
28040 Madrid, Spain

[3] **Unidad de Investigación de Paleontología, Instituto de Geología Económica (CSIC)**
28040 Madrid, Spain

[4] **School of Geography, Earth and Environmental Sciences, University of Plymouth**
Drake Circus, Plymouth, Devon, PL4 8AA, United Kingdom. E-mail: stephen.grimes@plymouth.ac.uk; Fax: +44 (0)1752 584776

† Deceased, December 2010

─┤ A B S T R A C T ├─

Trace element analysis of fossil bone and enamel constitutes a useful tool to characterize the paleoecological behavior of mammals. Up to now, most trace element studies have focused on Plio-Pleistocene fossils. Here, we show that paleodietary inferences based on trace element analyses can be also obtained from ~14Ma old Miocene mammals, in a period of time when important paleoclimatic changes took place due to the development of the East Antarctic ice sheet. Trace element ratio (Ba/Ca, Sr/Ca) analyses have been performed on herbivore tooth enamel (gomphothere *Gomphotherium angustidens,* equid *Anchitherium* cf. *A. cursor*, suid *Conohyus simorrensis* and ruminants) across three stratigraphic levels from the Somosaguas site (Middle Miocene, Madrid Basin, Spain). Previous scanning electron microscope, rare earth element and stable isotope analyses suggested minimal diagenetic alteration of the tooth enamel samples. Trace element analyses reported here show different paleoecological behavior among the studied fossil taxa. *Anchitherium* cf. *A. cursor* shows higher Ba/Ca and Sr/Ca ratios than *Gomphotherium angustidens*, indicating the equid was a mixed-feeder, while the gomphothere was a browser. The enrichment in Ba/Ca and Sr/Ca ratios in the ruminants is attributed to differences in their gastrointestinal tracts and to a more grazing diet. A high variability in trace element values characterizes the suid *Conohyus simorrensis*, which is believed to be connected to some degree of omnivory.

KEYWORDS | Geochemistry. Tooth enamel. Diagenesis. Paleoecology. Herbivores.

INTRODUCTION

The last couple of decades have witnessed an increase in the use of trace elements as a tool to characterize mam-

malian paleoecological behavior (Sillen, 1986, 1992; Nedin, 1991; Gilbert *et al.*, 1994; Safont *et al.*, 1998; Sponheimer *et al.*, 2005; Sponheimer and Lee-Thorp, 2006). In particular, strontium and barium have been used to infer

paleodiets (Gilbert *et al.*, 1994; Safont *et al.*, 1998) as mammals discriminate against these elements (biopurification of calcium) in such a way that a decrease in Sr/Ca and Ba/Ca ratios up the food chain has been detected (Elias *et al.*, 1982; Sillen and Kavanagh, 1982; Burton *et al.*, 1999; Blum *et al.*, 2000) with carnivores providing the lowest values (Fig. 1). Therefore, trace element analyses in recent and fossil material allow the distinction between different types of diet; herbivory (grazing *vs.* browsing), carnivory and omnivory (Balter *et al.*, 2002; Palmqvist *et al.*, 2003; Sponheimer *et al.*, 2005; Sponheimer and Lee-Thorp, 2006). The concentration of trace elements in fossil teeth and bones may also provide an indication of the proportion of these elements in the soil and groundwater which in turn will control the trace element content in plants (Bowen and Dymond, 1955; Wallace and Romney, 1971). Furthermore, different plant parts may show different trace element concentrations (Runia, 1987; Burton *et al.*, 1999). Caution is recommended when making comparisons between trace element concentrations from fossil taxa from different paleontological sites situated far away from each other as different lithologies may exist at each site ultimately affecting the tooth enamel geochemical signal (Sillen and Kavanagh, 1982; Sponheimer and Lee-Thorp, 2006). Therefore, when making paleoecological comparisons among taxa, it is desirable to select trace element data from taxa coming from a single site (Nedin, 1991; Sponheimer and Lee-Thorp, 2006).

Previous studies have concentrated on Plio-Pleistocene and Holocene fossil and sub-fossil material (Sillen, 1986, 1992; Gilbert *et al.*, 1994; Safont *et al.*, 1998; Palmqvist *et al.*, 2003; Sponheimer *et al.*, 2005; Sponheimer and Lee-Thorp, 2006). Older fossils were thought to be unsuitable for such paleoecological studies because of diagenesis giving rise to the loss of the original geochemical signals due to intensive chemical interactions within the burial environment. However, time is not directly related to the degree of geochemical alteration, as the signals of pristine preservation can be detected even in very old fossil material (Kolodny and Luz, 1991; Botha *et al.*, 2005; Fricke *et al.*, 2008). In this sense, Eberle *et al.* (2009) have demonstrated that trace element analyses can shed light on the dietary behavior of different Early Oligocene mammalian taxa from North America.

Following Reynard *et al.* (1999) and Lécuyer *et al.* (2003), rare earth element (REE) patterns can be used to verify the degree of diagenetic alteration in fossil bones and teeth and thus, determine if original paleoecological information is preserved in their chemical composition. Previous scanning electron microscopy (SEM), REE, and stable isotope analyses supported the notion that the Somosaguas mammalian tooth enamel, which is as old as

~14Ma, is well preserved and not significantly altered by diagenetic processes (Domingo *et al.*, 2009).

Here, we apply a suite of trace element analyses for the first time to Miocene mammalian paleoecology. We show that differences in trace element patterns are consistent in different mammalian species and are congruent with their expected paleodiets across the Middle Miocene stratigraphic succession from the Somosaguas site (Madrid Basin, Spain), during a critical span of time when the reestablishment of the East Antarctic ice cap brought about a sharp decrease in temperature and strengthening of aridity.

GEOLOGICAL SETTING

Somosaguas is a Middle Miocene vertebrate paleontological site situated in the Universidad Complutense de Madrid Campus in the locality of Pozuelo de Alarcón (Madrid Basin, Spain, Fig. 2A). It has been systematically excavated since 1998 and up to now, two different sites have been discovered: North Somosaguas (recording mainly macro-mammal remains) and South Somosaguas (recording micro-mammal remains) separated by a distance of 60m. To date, a total of 29 vertebrate species, 24 of which are mammals, have been found (Hernández Fernández *et al.*, 2006; Perales *et al.*, 2009). An analysis of the vertebrate assemblage suggests a tropical or subtropical savannah paleoenvironment around a temporal/seasonal lake, with isotopic analyses of bioapatite and inorganic carbonates across the ~5meters-thick section suggesting a paleoclimatic shift towards a cooler and more arid environment (López-Martínez *et al.*, 2000; Hernández Fernández *et al.*, 2006; Carrasco *et al.*, 2008; Domingo *et al.*, 2009) as a consequence of the reestablishment of the ice cap in Antarctica.

The majority of the paleontological mammal record of the Madrid Basin is placed in the Aragonian continental
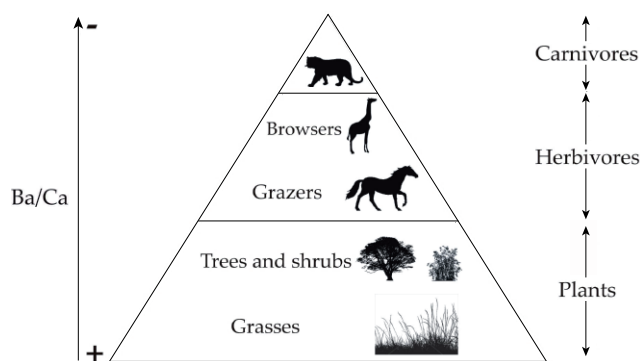

FIGURE 1 | Trophic pyramid showing that Ba/Ca ratio decreases up the food chain with grasses having the highest values and carnivores the lowest ones.

stage (~17-11Ma) as defined by Daams *et al.* (1977). Luis and Hernando (2000) re-evaluated the Somosaguas site on the basis of the micromammals and proposed an age range from 14.1 to 13.8Ma (Biozone E, MN 5, Late Middle Aragonian, Middle Miocene).

Three different stratigraphic units (T1, T2, T3) made up of arkosic and micaceous sands and clays have been recognized in Somosaguas (Fig. 2B): T1, T2 and T3 have all been identified as middle to distal facies of alluvial fans arriving northwards from the Sistema Central Range. Only T1 and T3 have provided vertebrate remains. T2 pinches out to the North, so that T3 lies directly over T1 at North Somosaguas (Mínguez-Gandú, 2000).

After a 3 dimensional reconstruction of about 2000 positioned fossils, three distinct levels have been defined in the apparently massive arkosic level of the T3 unit, namely T3-1, T3-2 and T3-3 from the bottom to the top. These three levels have provided the tooth enamel samples analyzed in this study (Elez, 2005; Cuevas-González and Elez, 2006).

MATERIAL AND METHODS

In the past, most trace element analyses have focused on fossil bone due to the abundance of this material at paleontological sites (Toots and Voorhies, 1965; Sillen, 1986; Gilbert *et al.*, 1994; Palmqvist *et al.*, 2003). However, it is well known that bone is more susceptible to undergo diagenetical alteration compared to the more resistant dental enamel (Lee-Thorp and van der Merwe, 1987, 1991; Ayliffe *et al.*, 1994; Wang and Cerling, 1994; Sponheimer and Lee-Thorp, 1999; Hoppe *et al.*, 2003; Lee-Thorp and Sponheimer, 2003; Tütken *et al.*, 2008). Due to this issue, Sponheimer *et al.* (2005) and Sponheimer and Lee-Thorp (2006) began to perform trace element analyses on the enamel of different mammalian taxa.

Due to the scarcity and value of the carnivore remains at the Somosaguas site, only herbivore tooth enamel was considered in this study. The gomphothere *Gomphotherium angustidens* (n=24)*,* the equid *Anchitherium* cf. *A. cursor* (n=17), the suid *Conohyus simorrensis* (n=8) and ruminants (n=7) were analyzed as part of this study. Both *Gomphotherium* and *Anchitherium* are considered browsing species, although the latter is represented at Somosaguas by a species (*Anchitherium* cf. *A. cursor*) which is better adapted to more open environments (Salesa and Sánchez, 2000). *Conohyus simorrensis* is a suid that may have had an omnivorous diet, as deduced from its teeth (hypertrophid premolars, bunodont molars with well-developed pyramidal cuspids and hyaenoid-type teeth, Sánchez, 2000). In Somosaguas, three different ruminant taxa have been found, a cervid (aff. *Heteroprox* sp.), a
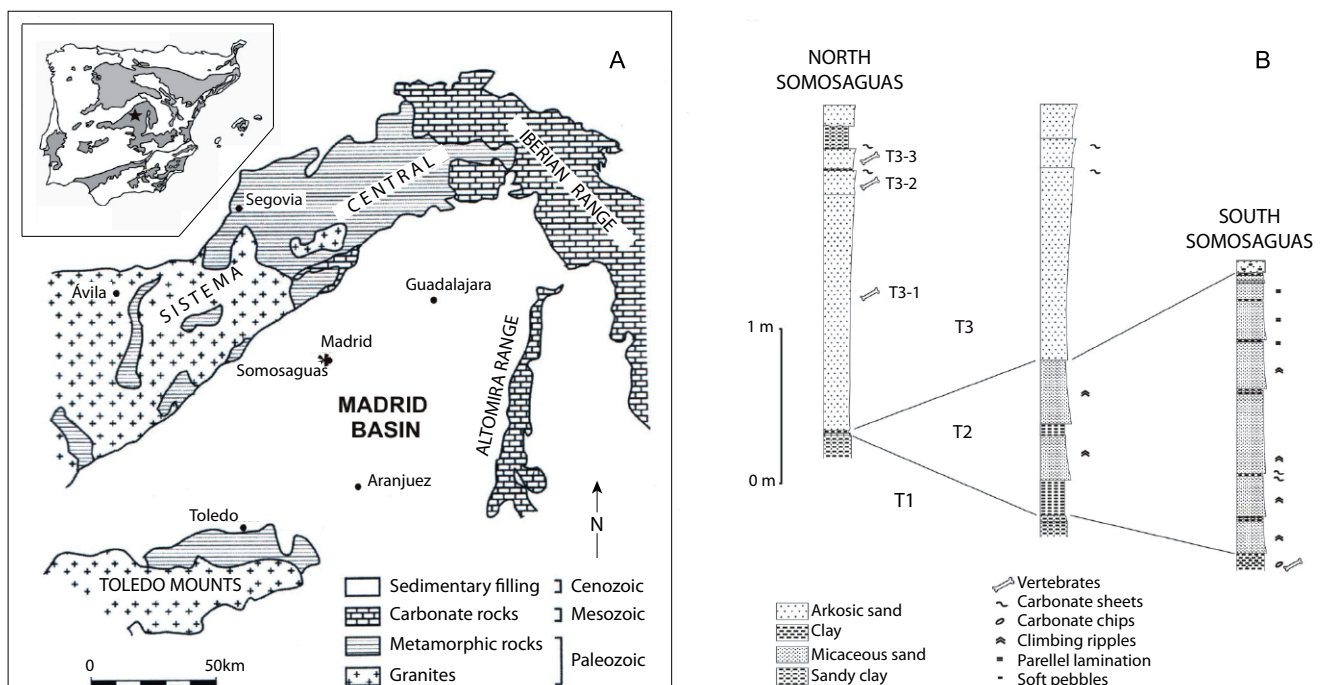

FIGURE 2 | A) Geological setting of the Madrid Basin. The Somosaguas site is marked by an asterisk near the city of Madrid. On the upper left hand corner, the Tertiary basins of the Iberian Peninsula are shown with the black star indicating the position of the Madrid Basin; B) Stratigraphic columns from North and South Somosaguas. The main fossiliferous sites (T1 and T3) can be observed. Modified from Calvo (1989) and Cuevas-González (2006).

bovid (*Tethytragus* sp.) and a moschid (*Micromeryx* sp., Sánchez and Morales, 2006), even though analyses were only performed on the first two taxa.

Powdered tooth enamel was recovered by using a rotary drill with a diamond tipped dental burr. Between 1.5mg and 2.0mg of powder was enough to carry out the analysis. Following Sponheimer and Lee-Thorp (2006), the dental enamel was removed from as large an area of the tooth enamel surface as possible since our purpose was to obtain an average dietary signal, not influenced by seasonal variations. The fragmentary nature of the Somosaguas site fossils, where almost all the excavated material is composed by splinters, made it impossible to fully identify the type of tooth selected for geochemical analysis. Tooth enamel samples were treated with 1M acetic acid and they were ultrasonically bathed at the Universidad Complutense de Madrid. Subsequently, they were acid digested by adding 1ml of 4M nitric acid for ~1h, which was then diluted in ultrapure water before analysis. Trace elements (Ba, Sr) were analyzed by using a PlasmaQuad PQ2+ Turbo inductively coupled plasma-mass spectrometer (ICP-MS) at the University of Plymouth. The precision of the ICP-MS instrument is better than ±2-4%. Each standard and sample was analyzed with three replicate scans. The average value of these three measurements was calculated to determine a final mean value. The standard used was the Bone Ash SRM1400. For the determination of Ba, the standard value proposed by Balter and Lécuyer (2004) was selected (245±3μg/g). Sr and Ca concentrations in the Bone Ash SRM1400 standard are 249±7μg/g and 38.18±0.3 (wt%), respectively. Elemental data are presented as ratios multiplied by 1000 (e.g., (Ba/Ca) x 1000) (Sillen, 1992; Balter *et al.*, 2002; Palmqvist *et al.*, 2003; Sponheimer and Lee-Thorp, 2006). The Ca content was analyzed by using atomic absorption spectrometry (AAS) on a Varian Spectr AA at the University of Plymouth. The precision of the AAS is ~±2%.

DIAGENESIS

Domingo *et al.* (2009) carried out different tests to check the degree of diagenesis that affected fossil mammalian tooth enamel from the Somosaguas site. Three different lines of evidence are indicative of a low degree of alteration of these samples:

F/P *versus* Ca/P analyses performed on the Somosaguas dental enamel by using SEM show that the mineralogy of this tissue is closer to the carbonate hydroxyapatite end-member which is regarded as the pristine mineralogy of bioapatite, rather than the fluorapatite end-member. Furthermore, SEM images do not show any evidence of enamel surface alteration caused by the action of micro-organisms (Fig. 3A).

The majority of the dental enamel samples from Somosaguas have a flat REE pattern. According to Reynard *et al.* (1999) and Lécuyer *et al.* (2003) a "bell shaped" REE pattern (enrichment of the middle REEs) in enamel is the result of extensive recrystallization of the apatite in the presence of REE-bearing fluids, since Ca^{2+} is substituted by middle REE as they show similar ionic sizes (Trueman
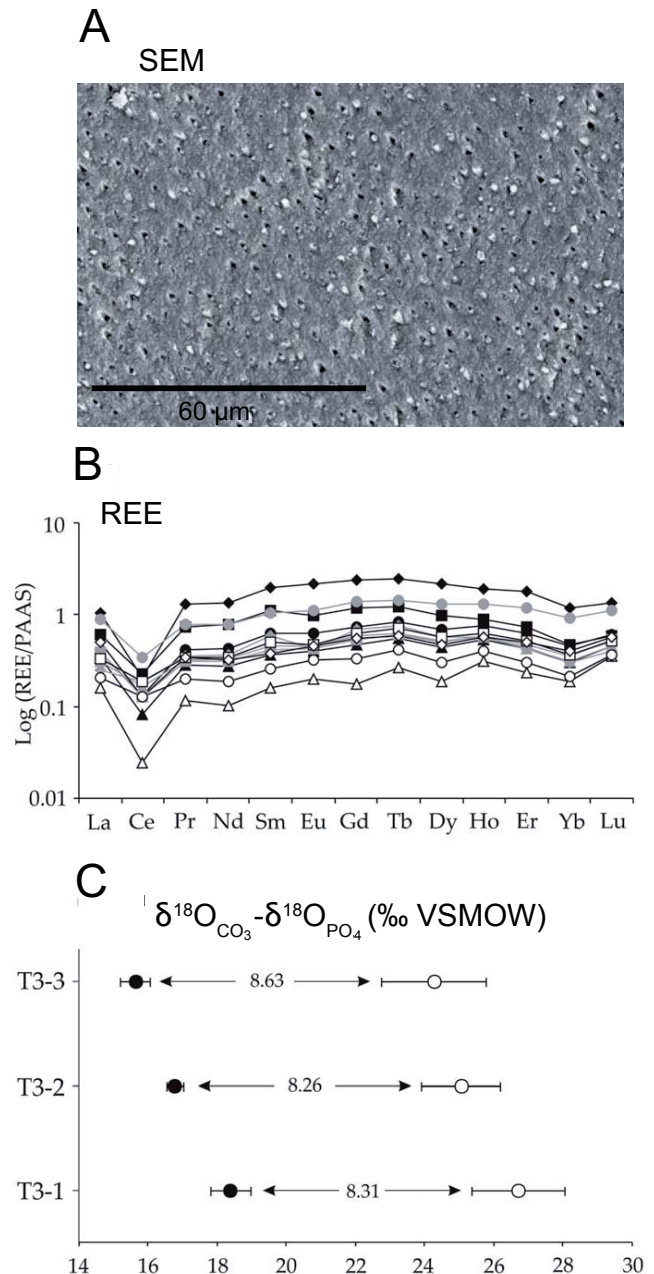

FIGURE 3 | A) Scanning electron microscope image of Somosaguas ruminant dental enamel; B) Rare earth element patterns of Somosaguas mammalian dental enamel. Black symbols: T3-3; grey symbols: T3-2; white symbols: T3-1. Square: *Gomphotherium angustidens*; triangle: Anchitherium cf. *A. cursor*; diamond: ruminants; circle: *Conohyus simorrensis*; C) Mean $\delta^{18}O_{CO_3}$ values (white circles) *versus* $^{18}O_{PO_4}$ values (black circles) (‰ VSMOW) of the Somosaguas mammalian dental enamel. After Domingo *et al.* (2009).

and Benton, 1997). In contrast, a flat REE profile is more indicative of the absence of a strong late stage diagenesis and recrystallization (Fig. 3B). A strong negative anomaly was pinpointed in the case of Ce which may be due to oxidizing conditions in the depositional environment (Metzger *et al.*, 2004; Martin *et al.*, 2005).

The difference between $\delta^{18}O_{CO_3}$ and $\delta^{18}O_{PO_4}$ values found in the Somosaguas mammalian tooth enamel is close to the ideal isotopic equilibrium fractionation (8.6-9.1‰) that occurs between these two phases when modern bioapatites precipitate (Bryant *et al.*, 1996; Iacumin *et al.*, 1996) (Fig. 3C).

RESULTS

The results of the trace element analysis of fossil mammalian enamel from the Somosaguas site are given in Figure 4 (and Table I; Fig. I, Electronic appendix available at www.geologica-acta,com). Somosaguas tooth enamel Ba/Ca and Sr/Ca ratios are compatible with those shown by modern mammals (Fig. II Electronic appendix; Elias *et al.*, 1982; Gilbert *et al.*, 1994; Burton *et al.*, 1999; Balter *et al.*, 2002) which might be indicative, once more, of a low degree of diagenesis in the Somosaguas dental enamel.

The following information can be deduced from each individual level (Fig. 4; Table I Electronic appendix):

Level T3-1: The equid *Anchitherium* cf. *A. cursor* shows higher Ba/Ca and Sr/Ca ratios in comparison with the proboscidean *Gomphotherium angustidens*, which is statistically significant in the case of Sr/Ca (p=0.024, t-Student=-2.377). Ruminants, on the other hand, have the highest Ba/Ca (0.88±0.59) and Sr/Ca (1.75±0.34) ratios when compared to the rest of the taxa. The single *Conohyus simorrensis* sample has the lowest Sr/Ca ratio of the assemblage and the second lowest Ba/Ca ratio at T3-1.

Level T3-2: Once more, *Anchitherium* cf. *A. cursor* shows an enrichment compared to *Gomphotherium angustidens* for the two ratios and again the difference is significant for the Sr/Ca ratio (p=0.023, t-Student=-2.323). The only ruminant sample available from this level on the other hand does not show any enrichment in the trace element content compared to the rest of the taxa. *Conohyus simorrensis* shows a high variability in trace element ratios. This suid has the highest Ba/Ca ratio among the mammalians from this level and an intermediate Sr/Ca ratio.

Level T3-3: As in the previous levels, a consistent enrichment in the Ba/Ca and Sr/Ca ratios exists in the equid *Anchitherium* cf. *A. cursor* compared to *Gomphotherium angustidens*, which is statistically significant for the

Sr/Ca ratio (p=0.004, t-Student=-3.455). Ruminants show the highest values in the two ratios compared to the rest of the taxa. The suid *Conohyus simorrensis* shows no defined pattern, as observed in previous levels.

When considering the variation of Ba/Ca and Sr/Ca ratios across successive levels T3-1, T3-2 and T3-3, no defined trends have been pinpointed (Fig. III Electronic appendix).

DISCUSSION

Sponheimer *et al.* (2005) and Sponheimer and Lee-Thorp (2006) indicated a higher Sr/Ca and Ba/Ca ratio in grazers than in browsers, since grasses show higher levels of these ratios compared to other plants (Runia, 1987; Burton *et al.*, 1999). The highest trace element values consistently shown by the equid *Anchitherium* cf. *A. cursor* compared to the gomphothere *Gomphotherium angustidens* are indicative of a higher grass content in the equid diet. This result is congruent with the paleoecological requirements inferred for this particular equid species. Although the genus *Anchitherium* has been traditionally considered a browser, it has been proposed that *Anchitherium cursor* occupied a more open landscape compared to other species of the subfamily Anchitheriinae, as deduced by a bigger dentition and some adaptations of the postcranial skeleton (Salesa and Sánchez, 2000). Our trace element results support these morphofunctional inferences, demonstrating that *Anchitherium cursor* was a mixed-feeder herbivore. Kaiser (2009) and Tütken and Vennemann (2009) also proposed this type of dietary behavior for the species *Anchitherium aurelianense* from Sandelzhausen (Early/Middle Miocene, Germany) by means of mesowear analysis and stable isotope analysis, respectively.

At levels T3-1 and T3-3, ruminants (n=4 and n=2, respectively) show an enrichment in trace element ratios compared to the rest of taxa. This is consistent with a more grazing behavior, but other interpretations have been also proposed. Balter *et al.* (2002) stated that the enrichment of Ba in ruminants when compared to horses, mammoths and rhinoceros could be due to differences in their gastrointestinal tracts, as ruminants have foregut microbial fermentation whereas horses, mammoths and rhinoceros are monogastric herbivores. As far as we know, the behavior of Sr in different gastrointestinal tracts has not been studied, so no conclusive remarks can be drawn on this issue. However, it is possible, that a longer persistence of the gastric content in ruminant bodies result in a larger concentration of trace elements, like Ba and possibly Sr, in their skeletons, compared to other herbivores (Balter *et al.*, 2002).

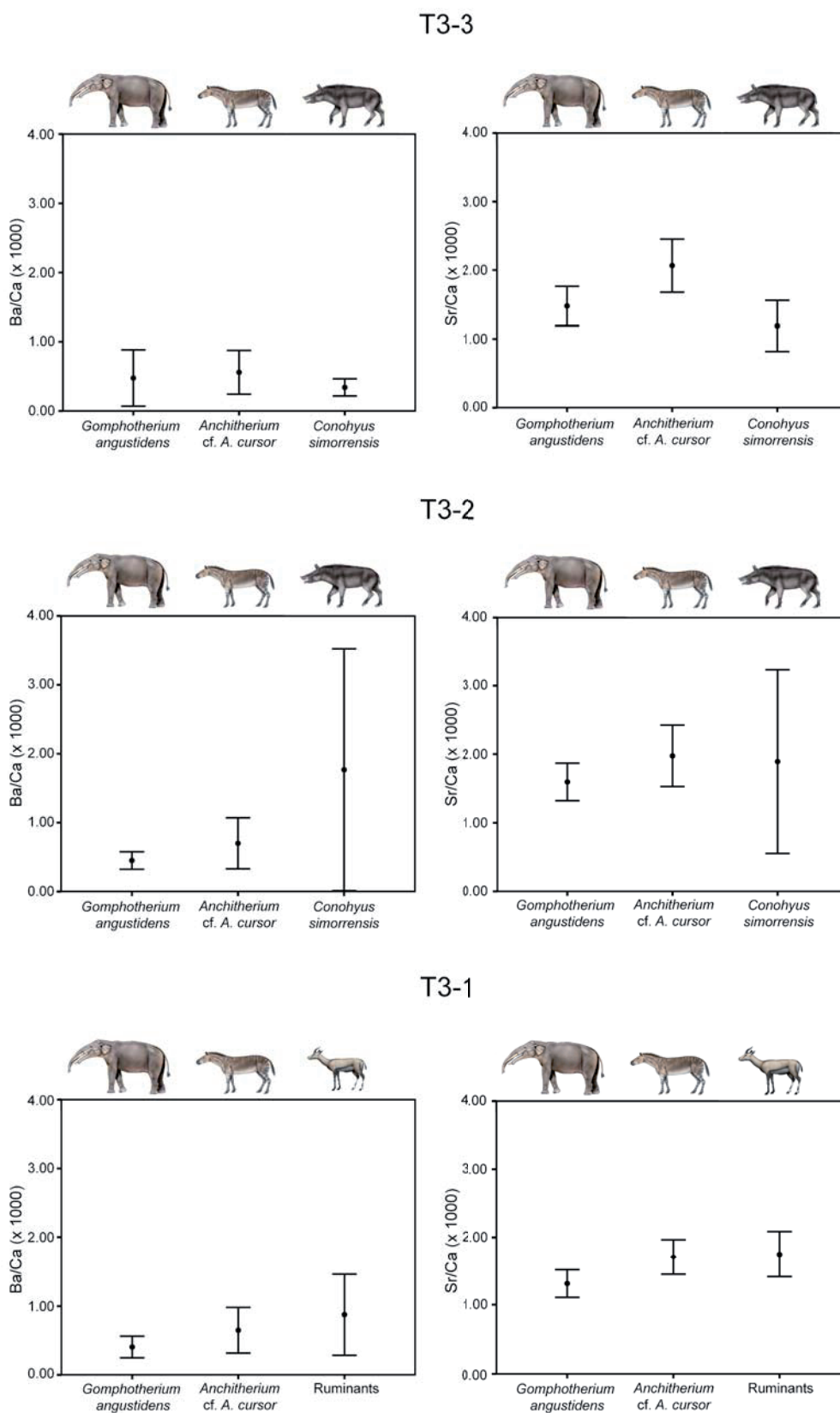In the case of the suid *Conohyus simorrensis* (n=8), trace element analyses show a high variability, which may

FIGURE 4 | Mean value ± 1 standard deviation plots for enamel Ba/Ca and Sr/Ca ratios of *Gomphotherium angustidens, Anchitherium* cf. *A. cursor, Conohyus simorrensis* and ruminants at levels T3-1, T3-2 and T3-3 from the Somosaguas site. Mammal reconstructions by Sergio Pérez, not to scale.

support an omnivorous diet. These heterogeneous values may also be explained by a herbivorous diet based on different plant parts. As Burton *et al.* (1999) stated, Ba and Sr are preferentially accumulated in the roots and stems and therefore, they will show higher Ba/Ca and Sr/Ca ratios, whereas these ratios are lower in the leaves and fruits due to a lower accumulation of the heavier alkaline-earth elements. However, due to the dental features shown by *Conohyus simorrensis* (Sánchez, 2000), the high variability in trace element ratios observed in this suid is more likely related to an omnivorous behavior.

The absence of well defined trends across the stratigraphic succession (Fig. III Electronic appendix) is in contrast with the paleoclimatic signal demonstrated by oxygen and carbon isotopic analyses (Domingo *et al.*, 2009), which were indicative of a trend towards cooler and more arid climate over the time span represented by the entire Somosaguas section. In spite of these paleoclimatic changes, trace element analyses presented here demonstrate that the paleoecological requirements of the studied mammalian taxa were maintained. Nowadays, it has been observed that herbivore taxa inhabiting arid environments show a wider range of versatility in their diet in order to cope with extreme climatic conditions (Sinclair, 2000; Hernández Fernández *et al.*, 2009). Somosaguas taxa may have adopted this same strategy when faced with a dry habitat by broadening their dietary requirements.

CONCLUSIONS

Trace elements analyses have been performed for the first time on Middle Miocene mammals. Fiftysix tooth enamel samples have been analyzed from different herbivore mammalian taxa (*Gomphotherium angustidens*, *Anchitherium* cf. *A. cursor*, *Conohyus simorrensis*, aff. *Heteroprox* sp. and *Tethytragus* sp.) from three successive stratigraphic levels at the ~14Ma Somosaguas site (Pozuelo de Alarcón, Madrid Basin, Spain).

The use of trace element analysis on tooth enamel has supported paleoecological determinations initially proposed by morphofunctional studies. In this sense, the equid *Anchitherium* represented by the species *A. cursor* shows higher Ba/Ca and Sr/Ca ratios when compared to the more specialized browser gomphothere *Gomphotherium angustidens*, demonstrating a mixed-feeding behavior for this particular equid species. Trace element analysis has shown it is possible to detect differences in the elemental ratio between ruminants and the rest of the herbivores (higher Ba/Ca and Sr/Ca ratios), due to differences in diet and/or gastrointestinal tracts. Finally, trace element analyses of the suid *Conohyus simorrensis* show a high variability, supporting a more omnivorous diet. These well defined paleoecological patterns based on trace element analyses of Miocene herbivore tooth enamel are consistently observed among taxa across the three stratigraphic levels analyzed in this study, in spite of the paleoclimatic shift recorded by isotopic analyses. The preservation of different and specific paleoecological patterns among taxa indicates that dental enamel trace element ratios may be useful to infer paleodiets of extinct mammals as far back as the Middle Miocene.

ACKNOWLEDGMENTS

Dr. Nieves López-Martínez passed away while completing this work. Her wide knowledge and her enthusiasm were a constant inspiration for colleagues and students. We would like to dedicate this study to her memory.

REFERENCES

Ayliffe, L.K., Chivas, A.R., Leakey, M.G., 1994. The retention of primary oxygen isotope compositions of fossil elephant skeletal phosphate. Geochimica et Cosmochimica Acta, 58, 5291-5298.

Balter, V., Lécuyer, C., 2004. Determination of Sr and Ba partition coefficients between apatite and water from 5ºC to 60ºC: A potential new thermomether for aquatic paleoenvironments. Geochimica et Cosmochimica Acta, 68, 423-432.

Balter ,V., Bocherens, H., Person, A., Labourdette, N., Renard, M., Vandermeersch, B., 2002. Ecological and physiological variability of Sr/Ca and Ba/Ca in mammals of West European mid-Würmian food webs. Palaeogeography, Palaeoclimatology, Palaeoecology, 186, 127-143.

Blum, J.D., Taliaferro, H., Weisse, M.T., Holmes, R.T., 2000. Changes in Sr/Ca, Ba/Ca and ^{87}Sr/^{86}Sr ratios between trophic levels in two forest ecosystems in the northeastern USA. Biogeochemistry, 49, 87-101.

Botha, J., Lee-Thorp, J., Chinsamy, A., 2005. The palaeoecology of the non-mammalian cynodonts *Diademodon* and *Cynognathus* from the Karoo Basin of South Africa, using stable light isotope analysis. Palaeogeography, Palaeoclimatology, Palaeoecology, 223, 303-316.

Bowen, H.J.M., Dymond, J.A., 1955. Strontium and barium in plants and soils. Proceedings of the Royal Society of London, 144(B), 355-368.

Bryant, J.D., Koch, P.L., Froelich, P.N., Showers, W.J., Genna, B.J., 1996. Oxygen isotope partitioning between phosphate and carbonate in mammalian apatite. Geochimica et Cosmochimica Acta, 60, 5145-5148.

Burton, J.H., Price, T.D., Middleton, W.D., 1999. Correlation of bone Ba/Ca and Sr/Ca due to biological purification of calcium. Journal of Archaeological Science, 26, 609-616.

Calvo, J.P., 1989. Terciario. In: Pérez-González, A., Calvo, J.P. (eds.). Memoria de la Hoja Geológica 1:50.000, Madrid, No 559. Madrid, Instituto Geológico y Minero de España (IGME), 9-45.

Carrasco, A., Sacristán, S., Benítez-López, G., Romero-Nieto, D., Fesharaki, O., López-Martínez, N., 2008. Estudio mineralógico del yacimiento de vertebrados miocenos de Somosaguas: aplicaciones paleoclimáticas y paleoambientales. Paleontologica Nova. Seminarios de Paleontología de Zaragoza, 8, 135-149.

Cuevas-González, J., 2006. Estudio isotópico de δ^{13}C y δ^{18}O en sedimentos y fósiles de los yacimientos de Somosaguas del Mioceno Medio (Cuenca de Madrid). Madrid, Trabajo de investigación para la obtención del Diploma de Estudios Avanzados (DEA), Universidad Complutense de Madrid, 31pp.

Cuevas-González, J., Elez, J., 2006. Arquitectura deposicional en un depósito aluvial basada en la distribución de fósiles de vertebrados del Mioceno de Somosaguas. Madrid, XXII Jornadas de la Sociedad Española de Paleontología, Libro de Resúmenes, 110-111.

Daams, R., Freudenthal, M., Van De Weerd, A., 1977. Aragonian, a new stage for continental deposits of Miocene age. Newsletters on Stratigraphy, 6, 42-55.

Domingo, L., Cuevas-González, J., Grimes, S.T., Hernández Fernández, M., López-Martínez, N., 2009. Multiproxy reconstruction of the palaeoclimate and palaeoenvironment of the Middle Miocene Somosaguas site (Madrid, Spain) using herbivore dental enamel. Palaeogeography, Palaeoclimatology, Palaeoecology, 272, 53-68.

Eberle, J., Sponheimer, M., Marchitto, T., 2009. Biogenic patterning of Sr and Ba in Early Oligocene (Orellan) mammalian tooth enamel. Journal of Vertebrate Paleontology, 29, 91A.

Elez, J., 2005. Aplicación GIS 3D a los yacimientos paleontológicos de Somosaguas. Trabajo de investigación para la obtención del Diploma de Estudios Avanzados (DEA), Madrid, Universidad Complutense de Madrid, 39pp.

Elias, R.W., Hirao, Y., Patterson, C.C., 1982. The circumvention of the natural biopurification of calcium along nutrient pathways by atmospheric inputs of industrial lead. Geochimica et Cosmochimica Acta, 46, 2561-2580.

Fricke, H.C., Rogers, R.R., Backlund, R., Dwyer, C.N., Echt, S., 2008. Preservation of primary stable isotope signals in dinosaur remains, and environmental gradients of the Late Cretaceous of Montana and Alberta. Palaeogeography, Palaeoclimatology, Palaeoecology, 266, 13-27.

Gilbert, C., Sealy, J., Sillen, A., 1994. An investigation of barium, calcium and strontium as palaeodietary indicators in the Southwestern Cape, South Africa. Journal of Archaeological Science, 21, 173-184.

Hernández Fernández, M., Cárdaba, J.A., Cuevas-González, J., Fesharaki, O., Salesa, M.J., Corrales, B., Domingo, L., Elez, J., López Guerrero, P., Sala-Burgos, N., Morales, J., López Martínez, N., 2006. Los yacimientos de vertebrados del Mioceno medio de Somosaguas (Pozuelo de Alarcón, Madrid): implicaciones paleoambientales y paleoclimáticas. Estudios Geológicos, 62, 263-294.

Hernández Fernández, M., Alcalde, G., DeMiguel, D., García Yelo, B., Azanza, B., 2009. Functional groups in ruminants as environmental proxies. Journal of Vertebrate Paleontology, 29(3), 113A.

Hoppe, K.A., Koch, P.L., Furutani, T.T., 2003. Assessing the preservation of biogenic strontium in fossil bones and tooth enamel. International Journal of Osteoarchaeology, 13, 20-28.

Iacumin, P., Bocherens, H., Mariotti, A., Longinelli, A., 1996. Oxygen isotope analyses of co-existing carbonate and phosphate in biogenic apatite: a way to monitor diagenetic alteration of bone phosphate? Earth and Planetary Science Letters, 142, 1-6.

Kaiser, T.M., 2009. *Anchitherium aurelianense* (Equidae, Mammalia): a brachydont "dirty browser" in the community of herbivorous large mammals from Sandelzhausen (Miocene, Germany). Paläontologische Zeitschrift, 83, 131-140.

Kolodny, Y., Luz, B., 1991. Oxygen isotopes in phosphates of fossil fish- Devonian to Recent. In: Taylor, H.P., O'Neil, J.R., Kaplan, I.R. (eds.). Stable Isotope Geochemistry: A Tribute to Samuel Epstein. The Geochemical Society, 3 (Special Publications), 105-119.

Lécuyer, C., Bogey, C., Garcia, J.P., Grandjean, P., Barrat, J.A., Floquet, M., Bardet, N., Pereda-Suberbiola, X., 2003. Stable isotope composition and rare earth element content of vertebrate remains from the Late Cretaceous of northern Spain (Laño): did the environmental record survive? Palaeogeography, Palaeoclimatology, Palaeoecology, 193, 457-471.

Lee-Thorp, J.A., Sponheimer, M., 2003. Three case studies used to reassess the reliability of fossil bone and enamel isotope signals for paleodietary studies. Journal of Anthropological Archaeology, 22, 208-216.

Lee-Thorp, J.A., Van der Merwe, N.J., 1987. Carbon isotope analysis of fossil bone apatite. South African Journal of Science, 83, 712-715.

Lee-Thorp, J.A., Van der Merwe, N.J., 1991. Aspects of the chemistry of modern and fossil biological apatites. Journal of Archaeological Science, 18, 343-354.

López Martínez, N., Elez Villar, J., Hernando Hernando, J.M., Luis Cavia, A., Mazo, A., Minguéz Gandú, D., Morales, J., Polonio Martín, I., Salesa, M.J., Sánchez, I.M., 2000. Los fósiles de vertebrados de Somosaguas (Pozuelo, Madrid). Coloquios de Paleontología, 51, 69-85.

Luís, A., Hernando, J.M., 2000. Los microvertebrados fósiles del Mioceno Medio de Somosaguas Sur (Pozuelo de Alarcón, Madrid, España). Coloquios de Paleontología, 51, 87-136.

Martin, J.E., Patrick, D., Kihm, A.J., Foit, F.F., Grandstaff, D.E., 2005. Lithostratigraphy, tephrochronology, and rare earth element geochemistry of fossils at the classical Pleistocene fossil lake area, South Central Oregon. Journal of Geology, 113, 139-155.

Metzger, C.A., Terry, Jr.D.O., Grandstaff, D.E., 2004. Effect of paleosol formation on rare earth element signatures in fossil bone. Geology, 32, 497-500.

Mínguez-Gandú, D., 2000. Marco estratigráfico y sedimentológico de los yacimientos paleontológicos miocenos de Somosaguas (Madrid, España). Coloquios de Paleontología, 51, 183-195.

Nedin, C., 1991. The dietary niche of the extinct Australian marsupial lion: *Thylacoleo carnifex* Owen. Lethaia, 24, 115-118.

Palmqvist, P., Gröcke, D.R., Arribas, A., Fariña, R.A., 2003. Paleoecological reconstruction of a lower Pleistocene large mammal community using biogeochemical (δ^{13}C, δ^{15}N, δ^{18}O, Sr:Zn) and ecomorphological approaches. Paleobiology, 29, 205-229.

Perales, R., Serrano, H., García Yelo, B.A., Hernández Fernández, M., 2009. Inferencias paleoambientales del Mioceno Medio de Somosaguas (Pozuelo de Alarcón, Madrid) basadas en la estructura de tamaños corporales de su fauna de mamíferos. Paleolusitana, 1, 317-325.

Reynard, B., Lécuyer, C., Grandjean, P., 1999. Crystal-chemical controls on rare earth element concentrations in fossil biogenic apatites and implications for paleoenvironmental reconstructions. Chemical Geology, 155, 233-241.

Runia, L.T., 1987. Strontium and calcium distribution in plants: Effect on palaeodietary studies. Journal of Archaeological Science, 14, 599-608.

Safont, S., Malgosa, A., Subirà, M.E., Gibert, J., 1998. Can trace elements in fossils provide information about palaeodiet? International Journal of Osteoarchaeology, 8, 23-37.

Salesa, M.J., Sánchez, I.M., 2000. Estudio de los restos de *Anchitherium* Meyer, 1834 (Equidae; Perissodactyla) del yacimiento de Somosaguas (Pozuelo de Alarcón, Madrid). Coloquios de Paleontología, 51, 197-212.

Sánchez, I.M., 2000. Rumiantes Mammalia, Artiodactyla del yacimiento de Somosaguas Aragoniense Medio, Madrid, España. Coloquios de Paleontología, 51, 223-234.

Sánchez, I.M., Morales, J., 2006. Distribución biocronológica de los Moschidae (Mammalia, Ruminantia) en España. Estudios Geológicos, 62, 533-546.

Sillen, A., 1986. Biogenic and diagenetic Sr/Ca in Plio-Pleistocene fossils of the Omo Shungura Formation. Paleobiology, 12, 311-323.

Sillen, A., 1992. Strontium-calcium ratios (Sr/Ca) of *Australopithecus robustus* and associated fauna from Swartkrans. Journal of Human Evolution, 23, 495-516.

Sillen, A., Kavanagh, M., 1982. Strontium and paleodietary research: A review. Yearbook of Physical Anthropology, 25, 67-90.

Sinclair, A.R.E., 2000. Adaptation, niche partitioning, and coexistence of African bovidae: clues to the past. In: Vrba, E.S., Schaller, G.B. (eds.). Antelopes, deer, and relatives. New Haven, Yale University Press, 247-260.

Sponheimer, M., Lee-Thorp, J.A., 1999. Alteration of enamel carbonate environments during fossilization. Journal of Archaeological Science, 26, 143-150.

Sponheimer, M., Lee-Thorp, J.A., 2006. Enamel diagenesis at South African Australopith sites: Implications for paleoecological reconstruction with trace elements. Geochimica et Cosmochimica Acta, 70, 1644-1654.

Sponheimer, M., de Ruiter, D., Lee-Thorp, J., Späth, A., 2005. Sr/Ca and early hominin diets revisited: new data from modern and fossil tooth enamel. Journal of Human Evolution, 48, 147-156.

Toots, H., Voorhies, M.R., 1965. Strontium in fossil bones and the reconstruction of food chains. Science, 149, 854-855.

Trueman, C.N., Benton, M.J., 1997. A geochemical method to trace the taphonomic history of reworked bones in sedimentary settings. Geology, 25, 263-266.

Tütken, T., Vennemann, T., 2009. Stable isotope ecology of Miocene large mammals from Sandelzhausen, southern Germany. Paläontologische Zeitschrift, 83, 207-226.

Tütken, T., Vennemann, T.W., Pfretzschner, H.U., 2008. Early diagenesis of bone and tooth apatite in fluvial and marine settings: Constraints from combined oxygen isotope, nitrogen and REE analysis. Palaeogeography, Palaeoclimatology, Palaeoecology, 266, 254-268.

Wallace, A., Romney, E.M., 1971. Some interactions of Ca, Sr, and Ba in plants. Agronomy Journal, 63, 245-248.

Wang, Y., Cerling, T.E., 1994. A model of fossil tooth and bone diagenesis: implications for paleodiet reconstruction from stable isotopes. Palaeogeography, Palaeoclimatology, Palaeoecology, 107, 281-289.

ELECTRONIC APPENDIX

TABLE I | Raw Ba, Sr and Ca data and Ba/Ca and Sr/Ca ratios of the mammalian dental enamel samples from the Somosaguas site

Signature	Taxa	Level	Ba (ppm)	Sr (ppm)	Ca (ppm)	Ba/Ca	Sr/Ca
3015	*Gomphotherium angustidens*	T3-3	156.89	610.78	350299.40	0.45	1.74
2431	*Gomphotherium angustidens*	T3-3	555.39	744.08	385687.72	1.44	1.93
2377	*Gomphotherium angustidens*	T3-3	222.41	640.10	393283.33	0.57	1.63
2214	*Gomphotherium angustidens*	T3-3	97.41	463.79	372844.83	0.26	1.24
1562	*Gomphotherium angustidens*	T3-3	116.78	611.41	377516.78	0.31	1.62
914	*Gomphotherium angustidens*	T3-3	104.19	516.28	411627.91	0.25	1.25
598	*Gomphotherium angustidens*	T3-3	94.12	450.98	372549.02	0.25	1.21
170	*Gomphotherium angustidens*	T3-3	104.67	462.00	386666.67	0.27	1.19
2890	*Anchitherium* cf. *A. cursor*	T3-3	89.09	824.24	384848.48	0.23	2.14
2821	*Anchitherium* cf. *A. cursor*	T3-3	164.44	811.11	361111.11	0.46	2.25
2474	*Anchitherium* cf. *A. cursor*	T3-3	99.11	601.75	412966.67	0.24	1.46
2348	*Anchitherium* cf. *A. cursor*	T3-3	161.38	972.41	360344.83	0.45	2.70
2323	*Anchitherium* cf. *A. cursor*	T3-3	217.66	888.16	382746.58	0.57	2.32
2015	*Anchitherium* cf. *A. cursor*	T3-3	173.19	808.92	386311.11	0.45	2.09
701	*Anchitherium* cf. *A. cursor*	T3-3	359.23	596.92	350000.00	1.03	1.71
154	*Anchitherium* cf. *A. cursor*	T3-3	390.68	695.04	373528.42	1.05	1.86
1077	Ruminant	T3-3	236.93	904.56	383817.43	0.62	2.36
623	Ruminant	T3-3	355.67	930.73	394729.25	0.90	2.36
2951	*Conohyus simorrensis*	T3-3	94.40	430.40	378000.00	0.25	1.14
2579	*Conohyus simorrensis*	T3-3	129.24	401.99	445182.72	0.29	0.90
2357	*Conohyus simorrensis*	T3-3	217.61	565.64	393553.19	0.55	1.44
635	*Conohyus simorrensis*	T3-3	149.49	730.64	434343.43	0.34	1.68
146	*Conohyus simorrensis*	T3-3	115.54	344.22	438247.01	0.26	0.79
3090	*Gomphotherium angustidens*	T3-2	156.10	585.37	423780.49	0.37	1.38
2912	*Gomphotherium angustidens*	T3-2	124.81	572.18	360902.26	0.35	1.59
2895	*Gomphotherium angustidens*	T3-2	257.02	877.19	392543.86	0.65	2.23
2867	*Gomphotherium angustidens*	T3-2	134.63	532.47	350649.35	0.38	1.52
1747	*Gomphotherium angustidens*	T3-2	242.74	607.26	375000.00	0.65	1.62
1621	*Gomphotherium angustidens*	T3-2	126.14	545.75	352941.18	0.36	1.55
1611 bis	*Gomphotherium angustidens*	T3-2	177.45	542.22	395636.36	0.45	1.37
655	*Gomphotherium angustidens*	T3-2	145.22	545.22	361464.97	0.40	1.51
2848	*Anchitherium* cf. *A. cursor*	T3-2	493.97	832.47	373924.64	1.32	2.23
2157	*Anchitherium* cf. *A. cursor*	T3-2	358.77	1073.95	413058.52	0.87	2.60
1714	*Anchitherium* cf. *A. cursor*	T3-2	149.59	933.88	446280.99	0.34	2.09
1611	*Anchitherium* cf. *A. cursor*	T3-2	229.14	701.99	362582.78	0.63	1.94
718	*Anchitherium* cf. *A. cursor*	T3-2	252.34	461.68	355140.19	0.71	1.30
713	*Anchitherium* cf. *A. cursor*	T3-2	147.22	742.59	437500.00	0.34	1.70
2116	Ruminant	T3-2	209.64	450.60	385542.17	0.54	1.17
3030	*Conohyus simorrensis*	T3-2	207.83	372.67	394165.87	0.53	0.95
2681	*Conohyus simorrensis*	T3-2	1152.82	1087.16	383058.46	3.01	2.84
3001	*Gomphotherium angustidens*	T3-1	201.05	584.21	360526.32	0.56	1.62
2969	*Gomphotherium angustidens*	T3-1	188.13	547.95	374429.22	0.50	1.46
2444	*Gomphotherium angustidens*	T3-1	93.75	413.39	395089.29	0.24	1.05
2183	*Gomphotherium angustidens*	T3-1	252.55	426.49	389772.42	0.65	1.09
1744	*Gomphotherium angustidens*	T3-1	169.38	548.01	393568.98	0.43	1.39
1189	*Gomphotherium angustidens*	T3-1	143.50	515.70	372197.31	0.39	1.39
1186	*Gomphotherium angustidens*	T3-1	85.48	402.42	360887.10	0.24	1.12
1071	*Gomphotherium angustidens*	T3-1	92.17	507.76	375624.79	0.25	1.35
3120	*Anchitherium* cf. *A. cursor*	T3-1	346.34	508.13	361788.62	0.96	1.40
2952	*Anchitherium* cf. *A. cursor*	T3-1	110.30	696.97	372727.27	0.30	1.87
2816	*Anchitherium* cf. *A. cursor*	T3-1	271.56	723.07	392618.68	0.69	1.84
2819	Ruminant	T3-1	357.29	574.58	415625.53	0.86	1.38
2813	Ruminant	T3-1	212.87	539.60	349009.90	0.61	1.55
2586	Ruminant	T3-1	113.64	681.82	342532.47	0.33	1.99
2453	Ruminant	T3-1	658.30	805.15	387384.26	1.70	2.08
3109	*Conohyus simorrensis*	T3-1	164.04	403.51	361842.11	0.45	1.12

TABLE I (continuation). n: number of analyzed samples

Mean and standard deviation of Ba/Ca and Sr/Ca in every level from the Somosaguas site.

Level	Taxa	n	Mean Ba/Ca	S.D. Ba/Ca	Mean Sr/Ca	S.D. Sr/Ca
T3-3	*Gomphotherium angustidens*	8	0.48	0.41	1.48	0.29
	Anchitherium cf. *A. cursor*	8	0.56	0.32	2.07	0.39
	Ruminant	2	0.76	0.20	2.36	0.00
	Conohyus simorrensis	5	0.34	0.12	1.19	0.37
T3-2	*Gomphotherium angustidens*	8	0.45	0.13	1.60	0.27
	Anchitherium cf. *A. cursor*	6	0.70	0.37	1.98	0.45
	Ruminant	1	0.54		1.17	
	Conohyus simorrensis	2	1.77	1.76	1.89	1.34
T3-1	*Gomphotherium angustidens*	8	0.41	0.16	1.31	0.20
	Anchitherium cf. *A. cursor*	3	0.65	0.33	1.71	0.26
	Ruminant	4	0.88	0.59	1.75	0.34
	Conohyus simorrensis	1	0.45		1.12	

Mean and standard deviation of Ba/Ca and Sr/Ca in all the levels from the Somosaguas site.

Level	Taxa	n	Mean Ba/Ca	S.D. Ba/Ca	Mean Sr/Ca	S.D. Sr/Ca
TOTAL	*Gomphotherium angustidens*	24	0.44	0.25	1.46	0.27
	Anchitherium cf. *A. cursor*	17	0.62	0.39	1.97	0.39
	Ruminant	7	0.79	0.44	1.84	0.48
	Conohyus simorrensis	8	0.71	0.94	1.36	0.67

Rare earth element (REE) data of the mammalian tooth enamel from the Somosaguas site.

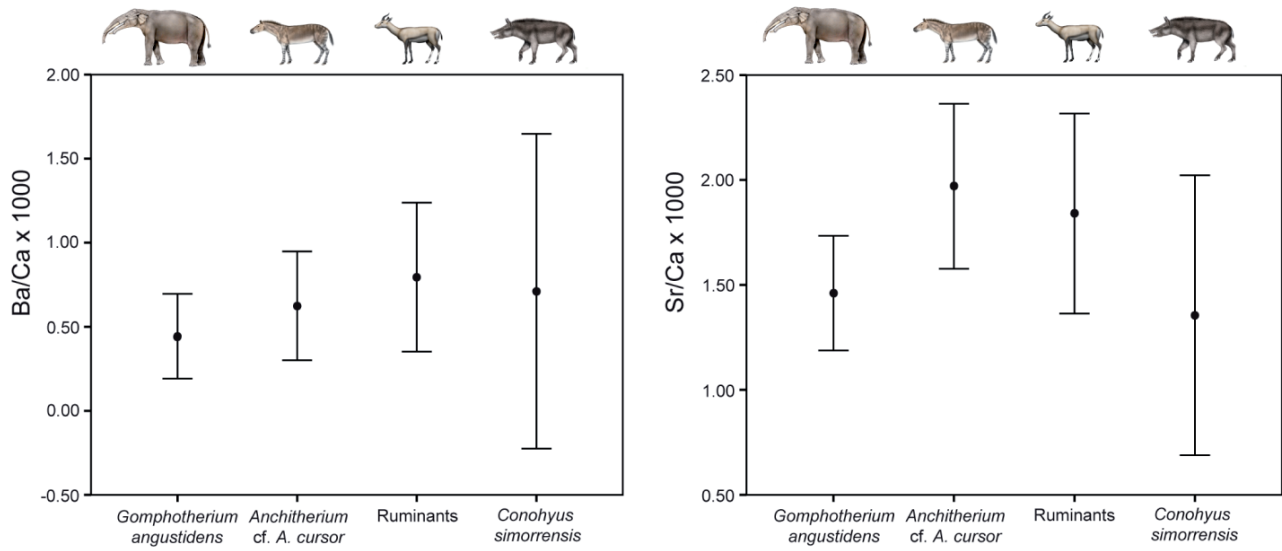| Level	Taxa	n	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Yb	Lu	ΣREE (ppm)
T3-3	*Gomphotherium angustidens*	8	20.86	17.76	6.52	26.43	6.26	1.04	5.59	0.95	4.53	0.88	2.07	1.33	0.26	94.49
	Anchitherium cf. *A. cursor*	8	12.90	6.42	2.48	9.38	2.04	0.43	2.16	0.42	2.04	0.54	1.23	0.85	0.20	41.09
	Ruminant	2	12.70	14.40	11.36	13.32	10.80	2.31	11.04	1.80	10.08	1.88	5.03	3.35	0.57	98.65
	Conohyus simorrensis	5	6.71	10.61	2.08	14.49	3.52	0.69	3.45	0.64	3.22	0.74	1.78	1.30	0.26	49.49
T3-2	*Gomphotherium angustidens*	8	14.67	10.03	2.78	10.43	2.27	0.46	2.49	0.47	2.32	0.58	1.34	1.00	0.23	49.07
	Anchitherium cf. *A. cursor*	6	12.00	12.98	2.86	11.09	2.54	0.49	2.71	0.49	2.41	0.56	1.23	0.87	0.20	50.42
	Ruminant	1	9.44	14.96	3.16	12.40	3.22	0.46	3.19	0.60	2.68	0.57	1.26	0.84	0.18	52.96
	Conohyus simorrensis	2	5.12	5.37	0.96	26.34	5.78	1.19	6.52	1.12	6.07	1.28	3.39	2.61	0.48	66.22
T3-1	*Gomphotherium angustidens*	8	12.51	15.00	3.01	11.74	2.77	0.51	2.94	0.55	2.69	0.62	1.47	1.04	0.22	55.06
	Anchitherium cf. *A. cursor*	3	6.13	1.91	1.03	3.41	0.88	0.22	0.81	0.21	0.88	0.31	0.66	0.53	0.15	17.11
	Ruminant	4	7.42	3.10	3.04	10.91	2.11	0.49	2.54	0.45	2.20	0.57	1.45	1.14	0.25	35.66

FIGURE I | Mean value ± 1 standard deviation plots for enamel Ba/Ca and Sr/Ca ratios of *Gomphotherium angustidens, Anchitherium* cf. *A. cursor, Conohyus simorrensis* and ruminants of all levels from the Somosaguas site. Mammal reconstructions by Sergio Pérez, not to scale.
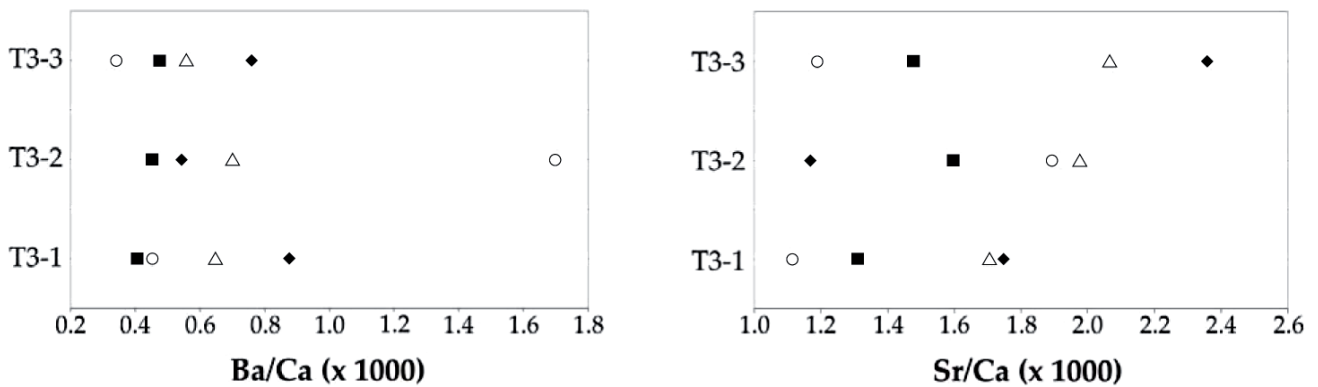

FIGURE III | Variation of Ba/Ca and Sr/Ca ratios across levels T3-1, T3-2 and T3-3 from the Somosaguas site. No well defined trends can be observed. Black square: *Gomphotherium angustidens*, white triangle: *Anchitherium* cf. *A. cursor*, black diamond: ruminant, white circle: *Conohyus simorrensis*.
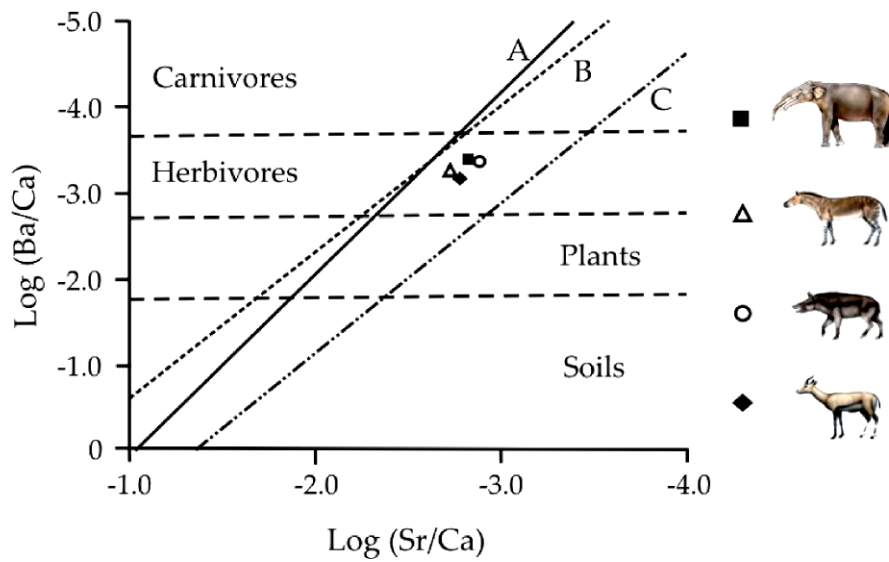
FIGURE II | Log (Ba/Ca) vs. Log (Sr/Ca) diagram. Somosaguas mammalian taxa show typical herbivore values. Line A represents the Southwestern Cape ecosystem (South Africa; Gilbert et al., 1994); line B corresponds to the Yosemite Park ecosystem (USA; Elias et al., 1982); line C represents Michigan/Wisconsin border ecosystem (USA; Burton et al., 1999). Modified from Balter et al. (2002). Mammal reconstructions by Sergio Pérez, not to scale.