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Natural variation of magnesium isotopes in mammal bones and teeth from two South African trophic chains

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Abstract

Isotopic fractionations accompanying element transfer through terrestrial ecosystems have the potential to shed light on ecological interactions between primary producers and consumers, but with the exception of carbon and nitrogen this potential has barely been exploited. Here, the magnesium stable isotope composition of bones and teeth of extant mammals from Kruger National Park (KNP) and Western Cape (WC), South Africa was measured for the first time. The nature of the geological substrate proves to be a major determinant of the ecosystem isotope baseline, as indicated by the lighter magnesium isotope ratios measured in WC mammals (ranging from −1.58‰ to −0.79‰) compared to those from KNP mammals (ranging from −1.01‰ to −0.04‰). Therefore, comparisons between the isotope signatures of taxa must be restricted to a predefined geographic area with a homogeneous substrate. In both parks, Mg shows slight enrichment in heavier isotopes from herbivores to carnivores. Plant remains trapped in the dentition of herbivores provide direct evidence of dietary source and, when available, were measured. In KNP only, δ^{26}Mg of plant remains is systematically lighter than the values for herbivore teeth. These results invite further exploration of the variability of Mg isotopes in vertebrate ecosystems in order to test whether magnesium, a bio-essential element present in relatively large proportions in bone and teeth apatite, may serve as an additional trophic tracer to nitrogen, which is a constituent of collagen that rapidly degrades after burial.

1. INTRODUCTION

Magnesium possesses three stable isotopes, ^{24}Mg, ^{25}Mg and ^{26}Mg with relative abundances of around 78.99%, 10.00% and 11.01% respectively. Earth materials range in δ^{26}Mg from −4.81‰ to 0‰, including rocks (Galy et al., 2002; Young and Galy, 2004; Tipper et al., 2006a,b; Teng et al., 2007 for speleothems; Tipper et al., 2006b for limestones and dolostones), soils (Tipper et al., 2006a; Teng et al., 2010; Bolou-Bi et al., 2012), seawater (Carder et al., 2004; Young and Galy, 2004; de Villiers et al., 2005), precipitation (Bolou-Bi et al., 2012; Tipper et al., 2012), rivers (Tipper et al., 2006a,b; Brenot et al., 2008) and plants (Bolou-Bi et al., 2010 for roots, stem, shoots; Bolou-Bi et al., 2012). It has been shown that fractionation occurs during weathering, with heavier isotopes concentrated in soils while lighter isotopes are carried away in the dissolved phase of river systems (e.g. Tipper et al., 2006a; Teng et al., 2010). In living organisms, magnesium isotopes have been measured in the calcite skeletons of marine invertebrates (e.g. Hippler et al., 2009) and in marine biogenic carbonate sediment (e.g. Wombacher et al., 2011). Variations in magnesium isotope compositions in vertebrate tissue are virtually unquantified.

The role of magnesium in growth has been recognized for a long time (Leroy, 1926). Magnesium, as a bio-essential element for metabolism, is ingested in large quantities (Coudray et al., 2005) and its deficiency leads to severe

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disorders (e.g. Nadler and Rude, 1995). In plants, magnesium also plays an important role as it represents the metal centre of chlorophyll (Black et al., 2006). These observations suggest a link between metabolic processes and magnesium isotope fractionation. Exploration of the isotope variability of this bio-essential element between and within vertebrate communities may provide some insight into biological processes, and may ultimately reveal trophic level effects.

Thus, Mg, like other new isotope systems, could provide evidence for reconstructing food webs among extinct animals, and an opportunity to understand ecological interactions in deep time. In bone apatite ($(Ca,Mg)_{10}(CO_3,PO_4)_6(OH)_2$) magnesium is the second most abundant metal (up to 1.4 wt.%) after calcium, for which it substitutes. Magnesium's occurrence in the calcium site of apatite suggests that it might be resistant to alteration. Thus, potentially, magnesium isotopes might remain unaltered by post-mortem processes in fossil bones, and Mg isotope data could complement other approaches for reconstructing trophic level, for which diagenesis hampers further interpretation (Trueman and Tuross, 2002).

Here, we present the first database on the variability of magnesium isotope composition in extant mammalian communities and associated plant remains at two locations in South Africa. Although the precise links between metabolic and fractionation processes within a single organism still need to be characterized, we discuss evidence for the suggestion that natural variation of magnesium isotopes in mineralized tissues may be indicative of diet.

2. MATERIALS AND METHODS

2.1. Sample collection

Samples consisted of bones of herbivores and carnivores from the Kruger National Park (KNP) and Western Cape (WC), South Africa. The samples were collected at the Ditsong National Museum of Natural History (Pretoria, South Africa). In addition to bone and teeth, plant residue trapped in the selenodont dentition of herbivorous mammals was extracted with a scalpel and analyzed.

2.2. Analytical techniques

Powdered samples of bone and teeth apatite were completely dissolved in 1 ml double-distilled 15.3 N HNO_3 overnight, then evaporated and re-dissolved in 350 µl 2.0 N HNO_3. Plant samples were dissolved in 1 ml 15.3 N HNO_3 using microwave digestion. An aliquot of all solutions was taken for concentration analysis of magnesium and other elements. This was done on an Element 2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) by peak-height comparison with an in-house standard. Mg, Ca and Sr concentrations on the river standard SLRS-5 reproduce to 7–8% (2SD), and are accurate relative to certified values to 3%.

Magnesium was separated from the remaining solution using cation-exchange resin (AG-50WX-12, 200–400 mesh) with ultrapure 2.0 N HNO_3 as the elution agent (see Pogge von Strandmann, 2008 for details). In order to avoid isotopic fractionation during column separation, over 99% of the magnesium was collected. This was verified by collecting and measuring the Mg content of a separate fraction of the eluant before and after the magnesium elution peak.

The purified Mg fraction was measured for Mg isotopes on a Thermo Neptune Multicollector ICP-MS using an Apex-Q for sample introduction (Foster et al., 2010). Purified samples were diluted in 2% HNO_3 to a concentration of 100 ppb. Measurements were conducted at low resolution and each analysis consisted of 20 four-second integrations of the ^{24}Mg, ^{25}Mg and ^{26}Mg signals in static mode. Each analysis was preceded by a measurement of blank 2% nitric, and the signals for this background were subtracted from analyte signal before calculation of isotope ratios. Delta values were obtained via bracketing analyses of DSM-3 (Galy et al., 2003), with CAM-1 used as a secondary standard. In each analytical session, samples (and occasional CAM-1) bracketed by DSM-3 were measured in turn. This sequence was then repeated three more times so that four separate analyses of the same sample solution were obtained. The uncertainties reported in Table 1 represent 2 standard deviations of these four analyses.

$\delta^{26}Mg$ and $\delta^{25}Mg$ values presented for samples in Table 1 are defined as:

$$\delta^{26}Mg = (((^{26}Mg/^{24}Mg_{sample})/(^{26}Mg/^{24}Mg_{DSM3})) - 1) * 1000 \quad (1)$$

$$\delta^{25}Mg = (((^{25}Mg/^{24}Mg_{sample})/(^{25}Mg/^{24}Mg_{DSM3})) - 1) * 1000 \quad (2)$$

For $\delta^{26}Mg$ the maximum uncertainty is 0.30‰ and for $\delta^{25}Mg$ it is 0.18‰. The CAM-1 standard ($n = 74$ over a period of around 18 months) yielded a mean $\delta^{26}Mg$ of $-2.57‰ \pm 0.16$ (2SD) and a mean $\delta^{25}Mg$ of $-1.33‰ \pm 0.10$. These are close to published values, e.g. $\delta^{26}Mg = -2.58‰ \pm 0.14$ (Galy et al., 2003). The $\delta^{26}Mg$ and $\delta^{25}Mg$ values obtained for all samples and standards measured in this study lie on a line ($R^2 = 0.996$) with a slope of 0.517 ± 0.007 (using a weighted regression, MSWD −0.61), within error of both the value (Young et al., 2002) for pure kinetic processes (0.510) and that for pure equilibrium processes (0.520) (Fig. 1).

3. RESULTS

Magnesium isotope data for samples are reported in Table 1 and in Fig. 1. The total range of measured values for $\delta^{26}Mg$ is 1.54‰, from −1.58‰ to −0.04‰. When available ($n = 6$), tooth enamel and bone were both measured from the same individual. The very slightly more positive values for tooth enamel are not significantly different from the values obtained for bone. A positive offset has been reported for mineral carbon isotopes, with enamel apatite more ^{13}C-enriched than bone apatite by more than 2‰ (Warinner and Tuross, 2009). In contrast, a negative offset of about 1‰ is observed for calcium isotopes between enamel and bone (Heuser et al., 2011).

The KNP offers the biggest sample of mammal species in this study ($n = 12$; Fig. 2a). The lower number of mammal species measured for WC ($n = 4$; Fig. 2b) precludes further discussion on trophic level effect at this site, but the data from WC will be discussed below with regard to the importance of the geological substrate or other sources. In KNP,

Table 1
Magnesium-isotope ratios and associated elemental concentrations of magnesium, calcium and strontium measured in extant vertebrate tissues and plant remains recovered from the dentition of mammals from Kruger National Park and Western Cape (South Africa).

Sample number	Origin	Taxon	Tissue	δ^{26}Mg average (‰)	2σ	δ^{25}Mg average (‰)	2σ	Log (Sr/Ca)	Log (Ba/Ca)	Log (Mg/Ca)	Replicates
Carnivores											
TM13273	KNP	*Crocuta crocuta*	Enamel	−0.04	0.08	−0.03	0.02	−3.9	−4.1	−1.8	4
TM13273J			Bone	−0.29	0.04	−0.14	0.02	−3.9	−4.2	−1.6	3
TM19370e	KNP	*Crocuta crocuta*	Enamel	−0.24	0.08	−0.13	0.01	−4.1	−5.0	−1.7	4
TM19370J			Bone	−0.48	0.11	−0.26	0.08	−4.2	−5.1	−1.6	2
TM4403bone	KNP	*Panthera leo*	Bone	−0.27	0.01	−0.15	0.03	−4.3	−5.3	−1.7	3
TM4403bone			Bone	−0.30	0.03	−0.17	0.03	−4.3	−5.2	−1.6	3
TM13924	KNP	*Panthera leo*	Bone	−0.46	0.09	−0.25	0.04	−4.4	−5.0	−1.6	4
TM4404D	KNP	*Hyaena brunnea*	Bone	−0.21	0.07	−0.08	0.01	−4.3	−5.0	−1.2	3
TM4404J1			Bone	−0.25	0.07	−0.16	0.04	−4.3	−5.0	−1.6	3
TM38252e	WC	*Panthera leo*	Enamel	−0.84	0.01	−0.43	0.03	−4.1	−5.2	−1.8	4
TM38253dm1g	WC	*Panthera leo*	Enamel	−1.17	0.06	−0.61	0.04	−4.1	−4.9	−1.5	4
TM38253bone(2)			Bone	−1.19	0.09	−0.61	0.07	–	–	–	2
TM38246	WC	*Panthera leo*	Enamel	−0.91	0.08	−0.45	0.06	−4.0	−4.9	−1.3	4
TM38246D			Bone	−0.79	0.15	−0.40	0.09	−4.2	−5.1	−1.1	4
TM38253bone	WC	*Panthera leo*	Bone	−1.18	0.10	−0.61	0.07	–	–	–	4
Herbivores											
TM17009	KNP	*Syncerus caffer*	Bone	−0.09	0.06	−0.05	0.07	−4.3	−5.0	−1.6	4
TM17006e	KNP	*Damaliscus lunatus*	Enamel	−0.43	0.06	−0.22	0.02	−4.3	−4.8	−1.8	4
TM17006J			Bone	−0.59	0.01	−0.31	0.02	−4.2	−4.4	−1.5	2
TM17007	KNP	*Damaliscus lunatus*	Bone	−0.26	0.12	−0.14	0.09	−4.0	−4.4	−1.5	2
TM16999	KNP	*Hippotragus niger*	Enamel	−0.59	0.01	−0.32	0.03	−4.2	−4.0	−1.4	3
TM16999e			Enamel	−0.33	0.10	−0.17	0.07	−4.2	−4.5	−1.7	4
TM16998e	KNP	*Hippotragus niger*	Enamel	−0.22	0.10	−0.14	0.05	−4.3	−4.5	−1.8	4
TM17003J	KNP	*Hippotragus niger*	Bone	−0.20	0.03	−0.08		−4.2	−4.4	−1.4	2
TM16994	KNP	*Taurotragus oryx*	Enamel	−0.56	0.06	−0.28	0.02	−4.1	−4.6	−1.9	3
TM16763J1	KNP	*Raphicerus campestris*	Bone	−0.82	0.13	−0.41	0.12	−3.8	−4.6	−1.4	2
AZ1132	KNP	*Equus burchellii*	Enamel	−0.80	0.07	−0.42	0.04	−3.8	−5.0	−1.8	4
TM16690p2g	KNP	*Equus burchellii*	Enamel	−0.48	0.06	−0.28	0.06	−3.7	−4.7	−1.4	3
AZ2137ml3	KNP	*Aepyceros melampus*	Enamel	−0.62	0.02	−0.32	0.02	−4.2	−4.6	−1.5	3
TM17657e	KNP	*Aepyceros melampus*	Enamel	−0.70	0.08	−0.37	0.02	−4.0	−4.9	−1.9	4
TM17657J			Bone	−0.94	0.07	−0.51	0.03	−3.8	−4.4	−1.5	3
AZ2124LH	KNP	*Aepyceros melampus*	Bone	−0.71	0.04	−0.38	0.05	−3.1	−3.7	−1.5	3
AZ2131RH	KNP	*Aepyceros melampus*	Bone	−0.86	0.07	−0.44	0.03	−3.9	−4.3	−1.5	4
AZ2131	KNP	*Aepyceros melampus*	Bone	−1.01	0.04	−0.50	0.02	−3.9	−4.3	−1.5	3
AZ2135RH	KNP	*Aepyceros melampus*	Bone	−0.41	0.10	−0.20	0.02	−4.2	−4.8	−1.5	3
AZ2126MC	KNP	*Aepyceros melampus*	Bone	−0.67	0.10	−0.32	0.02	−3.9	−4.3	−1.5	3
AZ2133RH	KNP	*Aepyceros melampus*	Bone	−0.76	0.03	−0.39	0.03	−3.2	−3.7	−1.5	3
TM3059J	WC	*Philamtomba monticola*	Bone	−1.41	0.06	−0.73	0.02	−4.3	−5.5	−1.4	4
TM3061J	WC	*Philamtomba monticola*	Bone	−0.92	0.08	−0.49	0.02	−4.5	−4.6	−1.4	4
TM9333J	WC	*Raphicerus melanotis*	Bone	−1.45	0.06	−0.74	0.02	−3.9	−5.1	−1.5	3
TM3220e	WC	*Tragelaphus scriptus*	Enamel	−1.21	0.14	−0.65	0.07	−4.1	−5.5	−1.9	3
TM3220J		*Tragelaphus scriptus*	Bone	−1.58	0.09	−0.79	0.07	−3.9	−4.6	−1.5	3
Plant remains taken from teeth											
TM16690PR	KNP	Of *Equus burchellii*	Plant remains	−0.82	0.13	−0.42	0.18	−3.5	−3.6	−0.4	3
TM16998PR	KNP	Of *Hippotragus niger*	Plant remains	−0.67	0.10	−0.40	0.11	−3.9	−3.9	−1.2	3
TM16661PR	KNP	Of *Sylvicapra grimmia*	Plant remains	−0.72	0.08	−0.42	0.07	−3.2	−3.0	−0.8	3
TM16994PR	KNP	Of *Taurotragus oryx*	Plant remains	−0.92	0.02	−0.48	0.01	−3.3	−3.5	−0.7	3
TM3220PR	WC	From *Tragelaphus scriptus*	Plant remains	−1.15	0.05	−0.65	0.15	−3.3	−3.5	−0.9	3
TM13461PR	WC	From *Tragelaphus scriptus*	Plant remains	−1.04	0.12	−0.61	0.10	−3.3	−3.0	−0.5	3
TM3164PR	WC	From *Raphicerus melanotis*	Plant remains	−0.97	0.06	−0.46	0.03	−3.5	−3.9	−0.7	3

δ^{26}Mg values reported for carnivore taxa range from −0.48‰ to −0.04‰. Herbivorous taxa ($n = 21$) outnumber the carnivorous taxa ($n = 9$) in the dataset, and although an overlap is obvious between the carnivorous taxa and the heaviest isotope values of some herbivores (notably for *Hippotragus niger*, *Damaliscus lunatus* and *Syncerus caffer*), the total range of values for the herbivores spreads beyond this overlap toward lighter values (the lowest value being

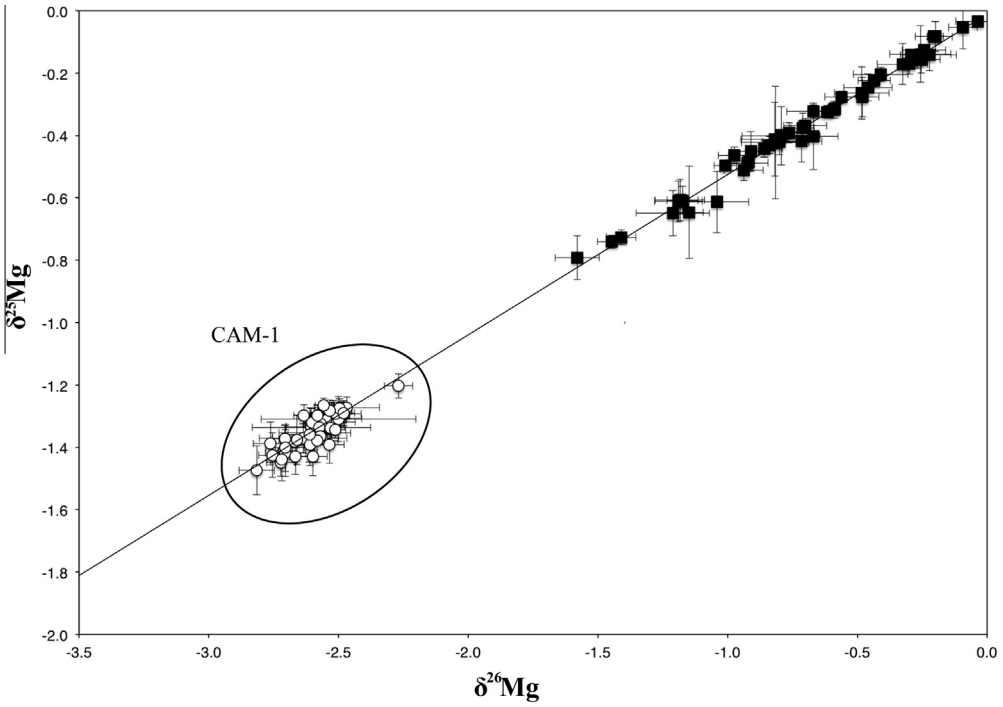

Fig. 1. Mass-dependent fractionation line for all the samples analyzed for magnesium isotope ratios (in ‰) at KNP and WC. Uncertainties plotted for samples (filled squares) are 2SD of the four separate analyses of the purified Mg fraction (done within a single analytical session). Uncertainties plotted for the CAM-1 analyses (open circles) are 2SD of the 20 integrations in a single analysis.

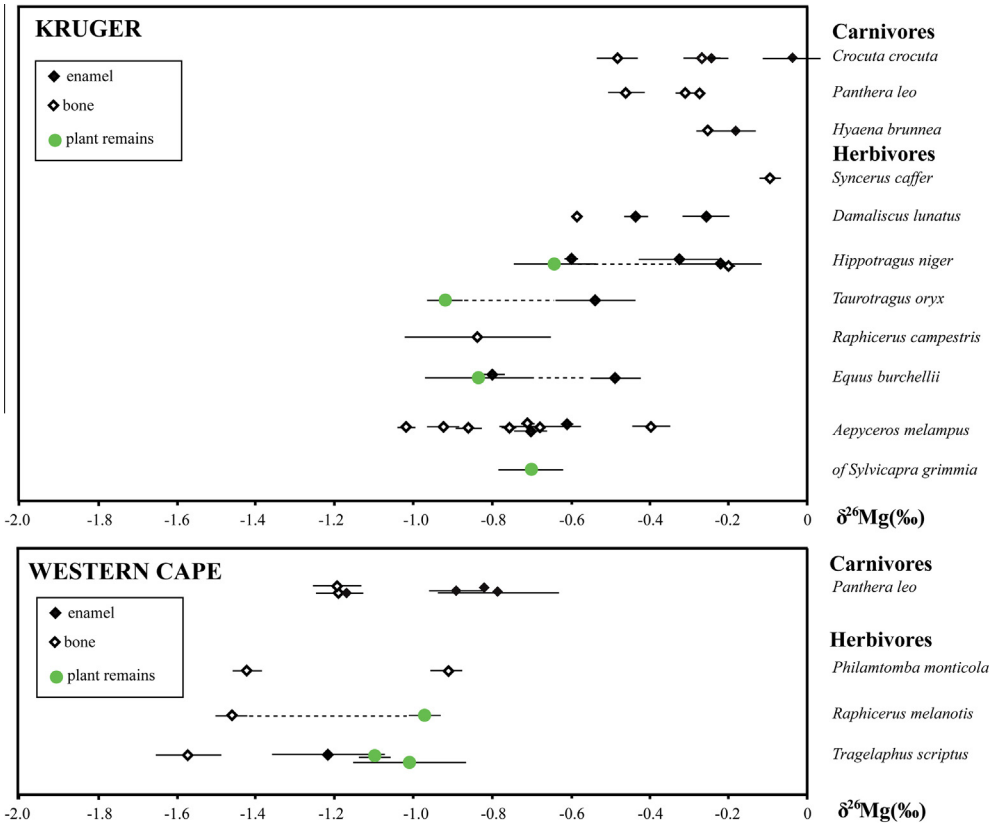

Fig. 2. Compilation of δ^{26}Mg values (in ‰) measured in this study for extant mammals and plants from KNP and WC. Data are presented in Table 1.

−1.01‰). δ^{26}Mg isotope values for plant residues from KNP range from −0.92‰ to −0.67‰ and are the most depleted in ^{26}Mg among all measured tissues of the dataset.

The δ^{26}Mg isotope values obtained from the WC fauna range between −1.58‰ and −0.79‰ whereas those of the KNP fauna range between −1.01‰ and −0.04‰. On average, WC data are 0.55‰ lower than KNP data. In WC, plant residues obtained from the dentition of *Raphicerus melanotis* ($n = 1$) and *Tragelaphus scriptus* ($n = 2$) are all enriched in ^{26}Mg by about 0.4‰ relative to bone.

Elemental ratios for Sr/Ca, Ba/Ca and Mg/Ca are presented in Table 1 for samples from both KNP and WC. The relation of Sr/Ca and Ba/Ca recalls previous studies (e.g. Balter, 2004) but it should be noted that some subtleties arise when considering teeth or bone. Nevertheless, whether it is appropriate to use teeth or bone for exploring biopurification processes is beyond the scope of the present study.

4. DISCUSSION

4.1. Magnesium as a trophic tracer

In KNP, the δ^{26}Mg values are normally distributed within carnivores and herbivores, and Student's t test results ($p < 10^{-3}$) show that the δ^{26}Mg values are significantly different between carnivores and herbivores (Fig. 3). It is to be expected that carnivore magnesium isotope values cannot be clearly differentiated from the heaviest herbivore values because carnivores such as those presented in the dataset (*Panthera leo*, *Hyaena brunnea*, *Crocuta crocuta*) feed on any available animals. This opportunistic dietary behavior may involve consumption of herbivores with both light and heavy δ^{26}Mg signatures, as well as other carnivores, which possess the heaviest signatures among mammals. The precise diet of an individual carnivore will depend on the availability of herbivores as carcasses, or animals prone to easy predation (old, young or sick individuals). For example, those individual carnivores from which teeth and bone samples derive that have fed exclusively on "heavy" herbivores such as *H. niger*, *D. lunatus*, *S. caffer*, will display δ^{26}Mg values close to 0‰. On the other hand, carnivores that have fed on a mixed diet of both "heavy" and "light" herbivores, will display relatively lighter δ^{26}Mg values.

Although based on a limited dataset ($n = 4$), further differences are observed between herbivores from KNP and the plants they consume (Fig. 3). Here, δ^{26}Mg values for plants directly sampled from the dentition of herbivores are systematically ^{26}Mg-depleted relative to enamel, with an isotopic fractionation ranging from −0.92‰ to −0.67‰. The non-parametric Wilcoxon's test must be applied due to the small number of plant residues, but these show ($p = 0.013$) that the δ^{26}Mg values are different between plant residues and herbivores. Whatever the physiological location of the magnesium isotopic fractionation between diet and mineralized tissues, i.e. during intestinal absorption and/or apatite mineralization, this calls for experimental studies with mammals reared on food of known δ^{26}Mg value (Skulan and DePaolo, 1999; Balter et al., 2010, 2013; Hotz et al., 2011).

Non-essential elements, such as strontium, tend to be discriminated in the bodies of consumers, a process referred to as biopurification (e.g. Balter, 2004; Balter et al., 2012), which is indicative of trophic level position. It is possible that this process is also partly observed here, in Sr/Ca versus Ba/Ca in the dataset from KNP, although values from teeth are responsible for some overlap (Fig. 4a). The dataset also shows a less obvious relationship between Sr/Ca and Mg/Ca ratios (Fig. 4b). Finally, the whole dataset for δ^{26}Mg from KNP was plotted against Sr/Ca elemental ratio to test for trophic discrimination. The Sr/Ca ratio is equivalent to the δ^{26}Mg in discriminating trophic levels, again with some overlap due to the small dataset available and partly caused by the inclusion of teeth (Fig. 4c).

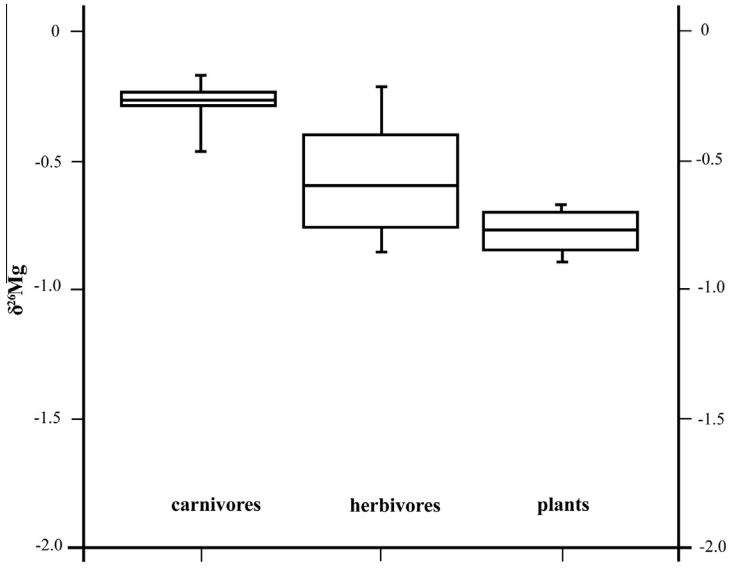

Fig. 3. δ^{26}Mg values (in ‰) for carnivorous and herbivorous mammals and plants from KNP. The boxes represent the lower and upper quartiles with the medians as horizontal lines. The whiskers represent the 10th and 90th percentiles.

4.2. The provenance effect

Comparing the dataset with published δ^{26}Mg values of rock substrates, waters draining those rocks and plants growing on those substrates, raises the possibility that natural variability of magnesium isotopes in vertebrate tissues does not originate exclusively from fractionation, but that part of it might be a result of mixing. This hypothesis is supported by the fact that δ^{26}Mg isotope values measured in the WC fauna are lighter than those measured in the KNP fauna by about 0.55‰. Moreover, unlike in KNP where the δ^{26}Mg value of plant residues is lighter than tooth enamel, three plant residues from WC obtained from the dentition of *R. melanotis* ($n = 1$) and *T. scriptus* ($n = 2$) are all enriched in ^{26}Mg by about 0.4‰ relative to bone.

At the bottom of the food chain, sources of magnesium in herbivores are plants and water, two reservoirs of magnesium readily available to mammals. The present study, together with initial reports, suggests that the magnesium isotope variability in plants is non-negligible (from −0.93‰ to 0.73‰ in the sample of Bolou-Bi et al., 2012; from −0.92‰ to −0.6‰ in the present study).

Besides plants, an important source of magnesium for mammals is drinking water. River waters draining sedimentary rocks, notably limestones, tend to be depleted in δ^{26}Mg (less than −2‰) whereas water draining silicate rocks have higher values (over −1‰) (Tipper et al., 2006a,b). It is noteworthy that the geological substrate in WC consists of Ordovician-Carboniferous sedimentary rocks – including conglomerates, sandstones and shales (Shone and Booth, 2005), whereas KNP substrate consists of granites, basalts and gabbro (Schutte, 1986). But a direct assessment of δ^{26}Mg values of river waters in KNP and WC is lacking and whether the observed differences between the more positive values of mammals from KNP and the relatively more negative values for mammals from WC may be interpreted as a substrate effect superimposed on fractionation patterns, with theoretical river water δ^{26}Mg values always more negative than the values recovered from vertebrate tissues, needs to be confirmed with future work.

Skulan and DePaolo (1999) reported a trophic level effect of about 1‰ for calcium isotopes in various animals. More recently, Heuser et al. (2011) analyzed dinosaur bones from several localities and ages and could not detect any trophic level effect when considering all the data together. Heuser et al. (2011) recognized that a variability of 1‰ could be easily dismissed when comparing taxa from different ecosystems, implying that DePaolo (2004) was probably too optimistic in suggesting that isotope values could be compared across different ecosystems. The preliminary interpretation for magnesium from this study is that comparing isotope ratios in taxa from different ecosystems may provide non-interpretable results, because the substrate defines a primary magnesium signature that will be communicated up the trophic chain. Fractionation differences between plants, herbivores and carnivores may be fixed because they are linked to biological processes (which still need to be assessed) but the baseline (or substrate isotope value) might differ from one area to another and will imprint the initial isotope composition at the base of the food chain. Mixing might further blur trophic-level discrimination, for example, if drinking water represents a larger source of magnesium than food in herbivores and carnivores. As an example, the average δ^{26}Mg value of the top carnivore *P. leo* bones and teeth from KNP is −0.34‰ whereas it is −1.02‰ in WC. This difference spans the whole range of values for carnivores and herbivores in a single ecosystem, and thus overprints any evidence for a trophic level effect if one compares taxa from WC and KNP. It is therefore recommended to compare isotope values of taxa living on a similar substrate and from close geographic provenance.

4.3. Variability of δ^{26}Mg values among herbivores

The different species of herbivorous mammals from KNP sampled here cover a wide spectrum of magnesium isotope values. These herbivores have different feeding

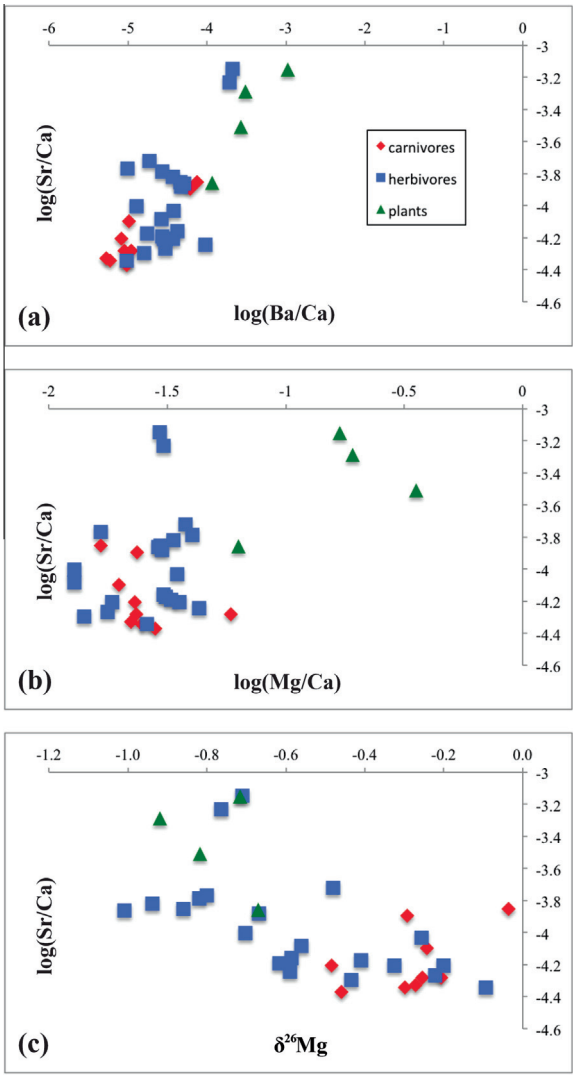

Fig. 4. Comparison of Sr/Ca, Ba/Ca and Mg/Ca ratios among mammals and plants from KNP. (A) log(Sr/Ca) versus log(Ba/Ca); (B) log(Mg/Ca) versus log(Sr/Ca); (C) log(Sr/Ca) plotted against δ^{26}Mg (in ‰).

ecologies according to the type of vegetation ingested. In subtropical climates, vegetation can follow two main photosynthetic pathways called C3 and C4. Here, the herbivore sample is dominated by Bovidae ($n = 7$ species), with the exception of the zebra *Equus burchellii*. Codron et al. (2007) refined the distinction between browsing, grazing and mixed feeding niches, i.e. mammals feeding on C3, C4 or a mix of C3-C4 plants respectively, for several species of herbivorous mammals from KNP. A detailed analysis of plants sampled according to their physiology, and a comparison of magnesium results with associated $\delta^{13}C$ values, would help evaluate whether magnesium isotope ratios can trace photosynthetic pathways. In the present mammal dataset, restricting comparisons to bovids only reveals that inter-species variations in $\delta^{26}Mg$ values are at least consistent with different feeding ecologies.

Aepyceros melampus is the taxon for which most measurements are available in this study ($n = 8$), with $\delta^{26}Mg$ values ranging from $-0.94‰$ to $-0.41‰$. Codron et al. (2007) classify *A. melampus* as a mixed feeder, incorporating both C3 and C4 plants. The $\delta^{26}Mg$ values of *Raphicerus campestris* ($n = 1$) and *Taurotragus oryx* ($n = 1$) are in the same range as those of *A. melampus* ($-0.55‰$ to $-0.82‰$). These two taxa are classified as browsers by Codron et al. (2007), and therefore rely primarily on C3 plants. Other bovids (*H. niger*, *D. lunatus* and *S. caffer*) known as exclusive C4 feeders (Codron et al., 2007) and analyzed for $\delta^{26}Mg$ in this study show values ranging from $-0.59‰$ to $-0.09‰$. This range of values is more positive than the range of values of *A. melampus*, *R. campestris* and *T. oryx* (Student's *t* test for 'browsers' including *E. burchellii* versus grazers with *p* value $< 10^{-3}$). Therefore, as concerns bovids only, grazers can be discriminated from a group formed by mixed-feeders and browsers (Fig. 5). Seasonality plays a major role in the availability of food resources, and mixing of C3 and C4 plants in the diet might explain the scatter observed within each feeding niche, indicating a distribution of magnesium values along a continuum according to the available food ingested. Codron et al. (2007) compiled behavioral observations, measured $\delta^{13}C$ in feces, estimated the percentage of C4 plant intake into the diet of herbivorous mammals from KNP and reported that some mixed-feeder species increased C4 grass intake during the wet season. The same authors also reported that a pure C3 feeder, the bovid *Sylvicapra grimmia* could switch to a more enriched C4 diet during the wet season, a possibility highlighted here by the relatively positive $\delta^{26}Mg$ value of the plant residue found in the dentition of *S. grimmia* ($-0.72‰$; Fig. 2).

The distinction between feeding niches among herbivores certainly involves more subtleties when non-bovid mammals are considered, which will require collection of additional samples. For example, *E. burchellii* ($n = 2$), reputedly an exclusive grazer, falls in the same range of values ($-0.48‰$ to $-0.8‰$) as the C3 and mixed feeder bovids *A. melampus*, *R. campestris* and *T. oryx*. Based on the results obtained for *E. burchellii*, it is therefore not possible to distinguish a grazing feeder from a browsing feeder on the basis of $\delta^{26}Mg$. Explaining this contradictory result is however impossible with the present limited dataset. At least two areas of research will have to be explored focusing on seasonality of available food resources and herbivore digestive physiology. As a comparison for the question on seasonality, it has previously been reported based on $\delta^{13}C$ values that some Mio-Pliocene horses from Florida reverted to browsing (MacFadden et al., 1999). Testing the second question requires assessment of whether digestive tissues induce fractionation of the ingested magnesium from plant fibers and how this differs in herbivores using different digestive strategies: bovids are foregut fermenters, repeatedly chewing small amounts of pre-digested plant material whereas equids are hindgut fermenters that ingest

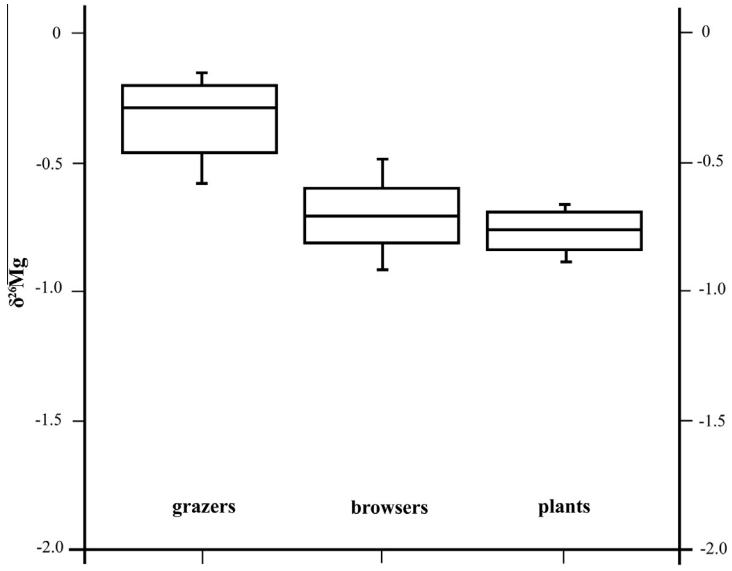

Fig. 5. $\delta^{26}Mg$ values (in ‰) for grazers and browsers Bovidae and plants from KNP. The boxes represent the lower and upper quartiles with the medians as horizontal lines. The whiskers represent the 10th and 90th percentiles.

a large quantity of plant material. Certainly, different mammal taxa present different magnesium isotope values in their osseous tissues. The mechanisms explaining the magnesium isotopic variability in mammals will have to be characterized.

4.4. Future directions of research

The dataset presented here is the first to document δ^{26}Mg variability in osseous tissues, highlighting a noticeable scatter in magnesium isotopic values. The novel nature of the dataset makes difficult the interpretation of the mechanisms involved behind the observed variability of magnesium values in herbivore and carnivore osseous tissues. Whether such scatter is due to fractionation, mixing or both remains largely speculative, but does invite further exploration of various aspects of the magnesium cycle in terrestrial ecosystems. Future avenues of research might follow two main complementary avenues: further documentation of natural variation in δ^{26}Mg in selected ecosystems, or an experimental approach that investigates fractionations associated with physiological processes. In the first case, sampling will have to be focused on small geographic areas, augmenting taxon samples and considering their substrate including bedrock, soil but also fluids such as local precipitation and drainage. Experimental approaches might, for example, consider δ^{26}Mg measurements in a variety of tropical plants according to their photosynthetic pathway; or at the scale of animal digestive tissues, measure δ^{26}Mg in foregut and hindgut herbivores under controlled diets. The recognition that δ^{26}Mg may represent a new isotopic tracer for reconstructing diet launches new research perspectives to understand the mechanisms behind magnesium biological fractionation in terrestrial ecosystems. Ultimately, δ^{26}Mg, along with other isotopes may help investigate deep-time ecosystems, allowing substantial refinement of our knowledge of the diet of extinct organisms.

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