Stable carbon isotope reconstruction of ungulate diet changes through the seasonal cycle

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We analysed stable carbon isotope ratios ($\delta^{13}C$) in faeces of 11 African ungulate species from three South African savanna environments to determine whether this approach is sufficiently sensitive to record short-term seasonal diet changes in browsers (BR), mixed-feeders (IM), and grazers (GR). At monthly intervals, faecal $\delta^{13}C$ revealed variations in proportions of C_3 (browse) to C_4 (grass) biomass consumed that were not detected by broader dry *versus* wet season comparisons, including subtle diet shifts amongst BR and GR. However, trends in faeces were influenced by changes in C_3 and C_4 plant isotope composition of up to 3‰. Nonetheless, faeces and plants showed strongly similar patterns of variation through the seasonal cycle, so that small diet shifts can be reliably inferred, provided that the variations in plants are controlled for. Faecal $\delta^{13}C$ of BR may be further influenced by consumption of isotopically different plant parts such as foliage *versus* fruit and flowers, and GR faeces may reflect differential utilization of grass following different photosynthetic sub-pathways. Future studies will need to incorporate data that capture isotopic variations in herbivore food sources, and if this is achieved, the approach may well become adopted as a routine addition to traditional methods for assessing diet, habitat use, and habitat condition.

Key words: browser, faeces, grazer, kruger park, mixed-feeder.

INTRODUCTION

Stable carbon isotope ecology is a powerful method for studying African savanna herbivore diets because the $^{13}C/^{12}C$ ratios (denoted as $\delta^{13}C$ in units per mil, ‰) of their tissues faithfully record proportions of C_3 (browse) to C_4 (grass) biomass intake (Vogel 1978; Tieszen *et al.* 1979; Lee-Thorp & van der Merwe 1987; Cerling & Harris 1999). This approach has yielded important insights into the diets of extinct ungulates (*e.g.* Lee-Thorp *et al.* 1989; Cerling *et al.* 1997), but is under-exploited in modern savanna contexts, largely because of perceptions that it can do little more than distinguish between the diets of recognized browsers, grazers and intermediate (mixed) feeders. However, field studies have shown that most grazers feed on some browse, and some browsers feed on some grass, at varying stages of the seasonal cycle (reviewed in Van Wieren 1996; Owen-Smith 1997; Gagnon & Chew 2000). If carbon isotope tools are sensitive enough to record these changes, their utility in African savanna ecology will be greatly improved.

Carbon isotope studies of African ungulate body tissues including hair, bone collagen, and tooth enamel carbonate, have revealed little evidence for dietary variability amongst browsers and grazers (Cerling *et al.* 2003; Sponheimer *et al.* 2003a). Indeed, these studies have demonstrated that most of these taxa have more monotonous diets than often predicted, in line with the fact that while intermediate feeders are common in more temperate regions, they represent the minority amongst African ungulates (Hofmann 1989; Owen-Smith 1997; du Toit 2003). But, diet changes in browsers and grazers are likely to be small (often <20% in terms of proportions of browse:grass consumed), short-term (restricted to a single season, month, or even shorter periods), and would therefore be dampened in signals from body tissues that integrate information over several months or even

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years due to their extended growth and turnover rates (Tieszen *et al.* 1983; Ambrose & Norr 1993; Sponheimer *et al.* 2003b; Ayliffe *et al.* 2004).

Faeces are short-lived materials that turn over within a few days (Coates *et al.* 1991; Sponheimer et al. 2003b). Controlled-feeding and field-based experiments have shown that faecal $\delta^{13}C$ is consistent with that of the diet, despite representing undigested food residue (Tieszen *et al.* 1979; Jones *et al.* 1981; Sponheimer *et al.* 2003b; Codron *et al.* 2005a,b). Analysis of faecal $\delta^{13}C$ should therefore detect subtle diet shifts across short time frames, as has been demonstrated for intermediate feeders (Botha & Stock 2005; Codron *et al.* 2006a,b) and possibly two grazers: blue wildebeest (*Connochaetes taurinus*) and warthog (*Phacochoerus africanus*) (Botha & Stock 2005). The aim of this study was to determine whether changes in faecal $\delta^{13}C$ are sufficient to record small changes in the diets of these three feeding types at various intervals through the seasonal cycle. The results are based on the faeces of 11 African ungulate species, collected at monthly intervals over one year, from three savanna habitats of South Africa, *i.e.* the Northern Basalt Plains and Punda Maria regions of the Kruger National Park, and the Hans Merensky Nature Reserve. These are augmented with data for changes in local vegetation, because small (usually 1.0 to 2.0‰) variations in C_3 and C_4 plant $\delta^{13}C$ through space and time may have important implications for interpreting patterns in consumer tissues (Heaton 1999; Cerling *et al.* 2003; Codron *et al.* 2005c).

METHODS

Kruger Park is a large (~2 000 000 ha) reserve situated in the northeast of South Africa between the latitudes 22°20′ and 25°32′S and the longitudes 30°53' and 32°02'E. The Northern Basalt Plains (Northern Plains) form a distinctive vegetational corridor in the northeast of Kruger Park, underlain by basalt-derived clay soils supporting mopane (*Colophospermum mopane*) 'shrubveld' on grassland (Venter *et al.* 2003). Punda Maria is situated on the sandstone-derived 'sandveld' regions of the far northern Kruger Park. Punda Maria represents one of the most densely wooded regions of the Park and is characterized by broad-leaved savanna woodland on undulating plains and hillslopes (Venter *et al.* 2003). Hans Merensky is a reserve of ~ 5000 ha situated ~100 km to the west of Kruger Park in the Tzaneen District of the Limpopo Province (23°39′S, 30°40′E). Vegetation on Hans Merensky is predominantly *C. mopane* woodland savanna on a relatively flat topography (428 to 542 m above sea level), underlain by granite-based sandy soils interspersed with dolerite intrusions. The three study areas form part of the temperate, summer rainfall region of South Africa, with mean annual rain varying between 400 and 600 mm, falling mainly during the austral summer between October and March (Venter *et al.* 2003). Rainfall during the study period (June 2004 to May 2005: Northern Plains 229 mm, Punda Maria 268 mm, Hans Merensky 272 mm), however, was more than 200 mm lower than the long-term annual mean for all three regions (Northern Plains 458 mm, Punda Maria 557 mm, Hans Merensky 558 mm – data from the South African Weather Bureau).

Plant and faecal samples were collected during field trips carried out monthly from June 2004 to May 2005 (see Codron 2006). Three vegetation sampling transects were established within each of the three habitats (see Codron *et al.* 2007). Plant collections included local diversity of C_3 (tree foliage and forbs) and C_4 (grass) plants within each transect, collecting between three and five specimens per species. Faecal collections were focused on the prominent browser (BR), grazer (GR) and intermediate feeder (IM) species within each region, with the aim of obtaining samples for 10 individuals (where possible) per species, region, and month. BR species included were giraffe (*Giraffa camelopardalis*) (Northern Plains and Hans Merensky) and kudu (*Tragelaphus strepsiceros*) (Punda Maria); IM were impala (*Aepyceros melampus*) (all regions) and nyala (*T. angasii*) (Punda Maria), and GR were buffalo (*Syncerus caffer*) (Northern Plains and Punda Maria), blue wildebeest (Northern Plains and Hans Merensky), Burchell's zebra (*Equus burchellii*), warthog, tsessebe (*Damaliscus lunatus*), roan antelope (*Hippotragus equinus*), and waterbuck (*Kobus ellipsiprymnus*) (Northern Plains). Each dung pile encountered was taken to represent a separate individual of the relevant species, and only fresh (wet or damp) samples were taken to ensure they represented the appropriate month.

Samples were oven-dried at 60°C for 24 hours, and mill-ground into a homogenous powder through a 1-mm sieve. Powdered samples were individually combusted in an automated Elemental Analyser (Carlo Erba, Milan), and the resultant CO_2 gas introduced to a Finnigan MAT 252 or

DELTA XP Mass Spectrometer (Finnigan, Bremen) via a continuous flow-through inlet system (Conflo). $^{13}C/^{12}C$ ratios are expressed in the delta (δ) notation in parts per mil (‰) relative to the Vienna PeeDee Belemnite (VPDB) standard. Standard deviations of repeated measurements of laboratory plant, protein, and a chocolate standard were less than 0.1‰. Faecal $\delta^{13}C$ values were converted to estimates of %C_4 grass intake using a dual-mixing model (Cerling *et al.* 2003; Sponheimer *et al.* 2003a; Codron *et al.* 2005a). For the model, diet-faeces ^{13}C-discrimination was assumed to be –0.9‰ (Sponheimer *et al.* 2003b; Codron *et al.* 2005a), and C_3 and C_4 endpoints were regional and monthly means of local vegetation. Results are presented as means ± standard error, and tests for significant changes are based on nested one-way ANOVA models for each species per region.

RESULTS

As expected, faecal $\delta^{13}C$ data distinguish between C_3-BR (–26.3 ± 0.0‰, n = 322; ~9% C_4 grass in diet), C_4-GR (–14.6 ± 0.0‰, n = 720; ~95% C_4), and C_3/C_4-IM (–19.4 ± 0.2‰, n = 462; ~62% C_4) (ANOVA $F_{2,1501}$ = 3272.319, $P < 0.0001$). Faecal $\delta^{13}C$ of most taxa changed significantly ($P < 0.05$) from the dry (April to September) to the wet season (October to March), with the exception of zebra, warthog, roan antelope, and waterbuck from the Northern Plains, buffalo at Punda Maria, and wildebeest at Hans Merensky (Tables 1 & 2). At the monthly scale, variations in faecal $\delta^{13}C$ exceed 1.0‰ in all cases, and changes were significant ($P < 0.001$) with the exception of only roan antelope (which approached significance, $P = 0.06$) and warthog on the Northern Plains ($P = 0.37$) (Table 2).

Thus, variations in faecal $\delta^{13}C$ of BR and GR seemed to reflect subtle diet switches within the C_3 and C_4 ends of the spectrum, respectively, and IM switched almost entirely across the two extremes (Table 1; Figs 1–3). However, the extent to which temporal (and regional) variations in faecal $\delta^{13}C$ reflected actual changes in dietary C_3/C_4 to some extent depends on corresponding changes in $\delta^{13}C$ of available plants. For example, despite significant dry-to-wet season differences in mean faecal $\delta^{13}C$ of buffalo on the Northern Plains, kudu and impala at Punda Maria, and giraffe at Hans Merensky, %C_4 intake did not change seasonally in these instances (Table 2). Changes in faecal $\delta^{13}C$ in these cases merely reflected changes in plant $\delta^{13}C$ through time, rather than true diet shifts. Indeed, at the monthly scale, changes in faecal $\delta^{13}C$ of BR and GR closely followed changes in C_3 and C_4 plant $\delta^{13}C$, respectively (Spearman's $R = 0.58$, $P < 0.0001$ and $R = 0.40$, $P < 0.0001$, respectively). The mixing model employed here for estimating %C_4 contributions to diet controls for variations in plant $\delta^{13}C$. Nevertheless, significant ($P < 0.05$) levels of variation in %C_4 intake were recorded for all cases at the monthly scale (with the exception of warthog on the Northern Plains; Table 2).

DISCUSSION

The consistency in patterns of monthly variation in $\delta^{13}C$ of C_3 plants with BR faeces, and C_4 grass with GR faeces, exemplifies the fidelity with which faecal $\delta^{13}C$ records the carbon isotope composition of herbivore diets. However, these patterns re-iterate the need for baseline isotopic control for accurate determinations of diet (see also Post 2002; Codron *et al.* 2005a), a feature absent from the majority of previous carbon isotope studies. For example, increases in BR faecal $\delta^{13}C$ of, say, 1.5‰, which might otherwise be interpreted as reflecting a decrease in C_3 foliage intake, may simply track isotopic changes in plants rather than changes in diet. The magnitude of variation in plant carbon isotope composition was even greater at the monthly scale (1.7 to 3.4‰ amongst C_3 and 1.2 to 2.6‰ amongst C_4 plants) than the difference observed at broader dry-to-wet season scales (<1.0‰; see also Codron *et al.* 2005c). The continual increase in C_3 plant $\delta^{13}C$ through the wet season was probably due to particularly low rainfall for this seasonal cycle, leading to reduced discrimination against ^{13}C during photosynthesis (*e.g.* Ehleringer & Cooper 1988; Tieszen 1991), and is therefore unlikely to persist for wetter years. Reliable baseline (plant) data can therefore be defined not only as data for diet sources from the same geographic origin as consumer tissues, but also from the same time period (as included here).

For these reasons, herbivore diets are more accurately reconstructed using mixing models that control for variations in vegetation, as opposed to *a priori* investigation of trends in raw $\delta^{13}C$ values. Fewer dry-to-wet season diet switches were recorded from estimates of %C_4 intake as opposed to changes in faecal $\delta^{13}C$ values, although significant changes in %C_4 intake were recorded for all taxa except warthog. However, while our mixing model captured most of the variation in plant $\delta^{13}C$ through space and time, there are other potential

Table 1. Monthly changes in faecal $\delta^{13}C$ for ungulate species included in this study. Values are presented as means ± 1 standard error; number of samples is given in brackets.

Month	**Species**				
	Browsers				
	Giraffe – NBP	Giraffe – HM	Kudu – PM		
Jun '04	–26.8 ± 0.2 (9)	–26.6 ± 0.2 (10)	–27.3 ± 0.1 (6)		
Jul '04	–26.8 ± 0.1 (5)	–26.6 ± 0.2 (9)	–27.2 ± 0.2 (7)		
Aug '04	–26.6 ± 0.1 (10)	–26.5 ± 0.2 (10)	–27.1 ± 0.1 (6)		
Sep '04	–26.7 ± 0.1 (9)	–26.4 ± 0.1 (10)	–26.8 ± 0.1 (9)		
Oct '04	–26.2 ± 0.1 (10)	–26.3 ± 0.2 (10)	–26.6 ± 0.2 (10)		
Nov '04	–26.1 ± 0.1 (8)		–25.7 ± 0.1 (10)		
Dec '04	–26.2 ± 0.3 (9)	–25.3 ± 0.2 (10)	–25.7 ± 0.1 (10)		
Jan '05	–26.1 ± 0.2 (9)	–26.7 ± 0.1 (10)	–25.2 ± 0.2 (9)		
Feb '05	–25.8 ± 0.2 (10)	–26.5 ± 0.4 (10)	–26.5 ± 0.2 (10)		
Mar '05	–25.5 ± 0.3 (8)	–26.5 ± 0.2 (10)	–25.9 ± 0.1 (10)		
Apr '05	–25.6 ± 0.2 (10)	–26.6 ± 0.1 (10)	–26.1 ± 0.2 (10)		
May '05	–25.8 ± 0.0 (10)	–26.6 ± 0.1 (9)	–26.2 ± 0.1 (10)		
	Intermediate feeders				
	Impala – NBP	Impala – HM	Impala – PM	Nyala – PM	
Jun '04	–20.2 ± 0.9 (10)	–19.9 ± 1.0 (10)	–16.1 ± 0.6 (10)	–21.5 ± 0.3 (2)	
Jul '04	–20.6 ± 0.3 (10)	–20.3 ± 0.7 (10)	–19.0 ± 0.5 (10)	–27.2 ± 0.1 (5)	
Aug '04	–21.5 ± 0.4 (10)	–19.1 ± 0.6 (10)	–18.8 ± 0.7 (10)	–27.8 ± 0.2 (6)	
Sep '04	–18.6 ± 0.3 (10)	–19.0 ± 0.2 (10)	–18.6 ± 0.4 (10)	–26.9 ± 0.1 (10)	
Oct '04	–18.3 ± 0.4 (10)	–18.1 ± 0.2 (10)	–18.2 ± 0.2 (10)	–17.8 ± 0.9 (10)	
Nov '04	–16.5 ± 0.2 (10)	–17.4 ± 0.3 (10)	–16.8 ± 0.1 (10)	–24.7 ± 1.0 (9)	
Dec '04	–17.7 ± 0.5 (10)	–16.5 ± 0.4 (10)	–18.7 ± 0.3 (10)	–24.6 ± 1.0 (10)	
Jan '05	–17.2 ± 0.4 (10)	–17.2 ± 0.2 (10)	–16.3 ± 0.2 (10)	–26.7 ± 0.3 (10)	
Feb '05	–15.9 ± 0.5 (10)	–18.3 ± 0.3 (10)	–15.6 ± 0.4 (10)	–21.8 ± 1.1 (10)	
Mar '05	–15.9 ± 0.3 (10)	–18.9 ± 0.3 (10)	–14.7 ± 0.2 (10)	–24.1 ± 0.4 (10)	
Apr '05	–18.6 ± 1.0 (10)	–21.5 ± 0.9 (10)	–16.2 ± 0.2 (10)	–21.8 ± 1.4 (10)	
May '05	–17.3 ± 0.4 (10)	–20.6 ± 0.3 (10)	–16.1 ± 0.2 (10)	–26.0 ± 0.3 (10)	
	Grazers				
	Zebra – NBP	Buffalo – NBP	Buffalo – PM	Warthog – NBP	
Jun '04	–15.4 ± 0.5 (10)	–15.3 ± 0.3 (9)	–15.0 ± 0.3 (10)	–14.6 ± 0.1 (10)	
Jul '04	–14.0 ± 0.3 (10)	–13.2 ± 0.3 (10)	–13.5 ± 0.3 (10)	–13.0 ± 0.8 (2)	
Aug '04	–14.2 ± 0.2 (10)	–13.7 ± 0.1 (10)	–13.6 ± 0.2 (10)	–14.4 ± 0.2 (10)	
Sep '04	–14.5 ± 0.3 (9)	–15.7 ± 0.4 (9)	–14.2 ± 0.3 (10)	–15.0 ± 0.4 (10)	
Oct '04	–14.9 ± 0.1 (10)	–15.2 ± 0.1 (10)	–15.3 ± 0.1 (10)	–14.4 (1)	
Nov '04	–13.6 ± 0.1 (10)	–14.2 ± 0.1 (10)	–14.8 ± 0.1 (10)		
Dec '04	–14.2 (1)	–15.0 ± 0.2 (10)	–15.9 ± 0.3 (10)		
Jan '05	–14.3 ± 0.2 (10)	–14.7 ± 0.1 (10)	–14.9 ± 0.1 (10)		
Feb '05	–15.0 ± 0.1 (8)	–15.1 ± 0.2 (10)	–15.6 ± 0.2 (10)		
Mar '05	–14.5 ± 0.1 (10)	–15.5 ± 0.1 (10)	–15.8 ± 0.1 (10)		
Apr '05	–13.8 ± 0.1 (10)	–15.7 ± 0.1 (10)	–15.6 ± 0.1 (10)		
May '05	–14.5 ± 0.2 (10)	–14.3 ± 0.1 (10)	–15.8 ± 0.1 (10)	–14.7 ± 0.7 (7)	
	Wildebeest – NBP	Wildebeest – HM	Tsessebe – NBP	Roan antelope – NBP	Waterbuck – NBP
Jun '04	–13.8 ± 0.1 (10)	–13.7 ± 0.2 (10)	–13.0 ± 0.2 (3)	–13.7 ± 0.1 (3)	
Jul '04	–13.9 ± 0.1 (8)	–13.8 ± 0.2 (10)	–13.2 ± 0.1 (3)	–13.7 ± 0.2 (3)	
Aug '04	–15.3 ± 0.1 (10)	–14.2 ± 0.1 (10)	–14.2 (1)	–13.8 (1)	
Sep '04	–15.0 ± 0.3 (2)	–15.1 ± 0.3 (10)	–14.2 ± 0.1 (4)	–14.0 ± 0.1 (3)	
Oct '04	–15.5 ± 0.1 (10)	–14.8 ± 0.1 (10)	–14.4 ± 0.2 (9)	–15.4 ± 0.9 (3)	
Nov '04	–14.9 ± 0.3 (10)	–13.8 ± 0.1 (10)	–14.6 ± 0.5 (4)	–14.3 ± 0.1 (3)	
Dec '04	–15.4 ± 0.3 (6)	–14.8 ± 0.2 (10)	–13.9 ± 0.4 (5)	–13.8 ± 0.7 (3)	–15.1 ± 0.1 (10)
Jan '05	–15.2 ± 0.3 (8)		–14.8 ± 0.2 (5)	–13.8 ± 0.1 (3)	–15.4 ± 0.2 (10)
Feb '05	–14.4 ± 0.3 (2)	–13.4 ± 0.2 (10)	–14.3 ± 0.1 (3)	–13.1 ± 0.3 (5)	–13.1 ± 0.3 (10)
Mar '05	–14.7 ± 0.2 (9)	–13.6 ± 0.2 (10)	–15.0 ± 0.1 (3)	–16.0 ± 1.5 (3)	–14.6 ± 0.2 (9)
Apr '05	–14.6 ± 0.6 (10)	–13.7 ± 0.2 (10)			–14.6 ± 0.5 (10)
May '05	–14.4 ± 0.1 (10)	–15.1 ± 0.1 (10)			–15.3 ± 0.3 (10)

NBP = Northern Basalt Plains, Kruger Park; PM = Punda Maria, Kruger Park; HM = Hans Merensky Nature Reserve.

Table 2. Comparison of diet variation recorded by carbon isotope analysis of faeces between seasonal (dry to wet) and month-to-month scales. Significant changes are shown in bold for *P*-level <0.05 (ANOVA), and if the temporal change in estimated %C_4 intake is ≥10%.

		Dry to wet season			Monthly		
Species	*n*	δ^{13}C (‰)	%C_4 in diet	%C_4 range	δ^{13}C (‰)	%C_4 in diet	%C_4 range
Browsers							
Giraffe – NBP	107	**<0.01**	**<0.0001**	4–9	**<0.0001**	**<0.0001**	**0–11**
Giraffe – HM	108	**<0.05**	**0.08**	10–12	**<0.001**	**<0.01**	8–16
Kudu – PM	107	**<0.0001**	0.37	10–11	**<0.0001**	**<0.0001**	**4–17**
Mixed-feeders							
Impala – NBP	120	**<0.0001**	**<0.0001**	**62–76**	**<0.0001**	**<0.0001**	**45–84**
Impala – HM	120	**<0.0001**	**<0.0001**	**59–73**	**<0.0001**	**<0.0001**	**50–82**
Impala – PM	120	**<0.05**	0.47	79–81	**<0.0001**	**<0.0001**	**64–92**
Nyala – PM	102	**<0.01**	**<0.05**	**20–11**	**<0.0001**	**<0.0001**	**3–78**
Grazers							
Zebra – NBP	108	0.86	**<0.01**	95–98	**<0.0001**	**<0.0001**	**90–100**
Buffalo – NBP	118	**<0.05**	0.12	94–95	**<0.0001**	**<0.0001**	**89–100**
Buffalo – PM	120	0.28	0.43	95–95	**<0.0001**	**<0.0001**	**85–100**
Warthog – NBP	40	0.21	0.26	93–97	0.37	0.25	**88–100**
Wildebeest – NBP	95	**<0.001**	**<0.0001**	91–97	**<0.0001**	**<0.0001**	86–100
Wildebeest – HM	110	0.22	0.51	98–98	**<0.0001**	**<0.05**	96–100
Tsessebe – NBP	40	**<0.0001**	**<0.01**	95–100	**<0.01**	**<0.0001**	**83–100**
Roan – NBP	30	0.34	0.14	94–100	0.06	**<0.05**	**75–100**
Waterbuck – NBP	59	0.18	0.06	93–96	**<0.001**	**<0.001**	**87–98**

n = number of samples; dry season = samples collected from April to September; wet season = October to March.
NBP = Northern Basalt Plains, Kruger Park; PM = Punda Maria, Kruger Park; HM = Hans Merensky Nature Reserve.

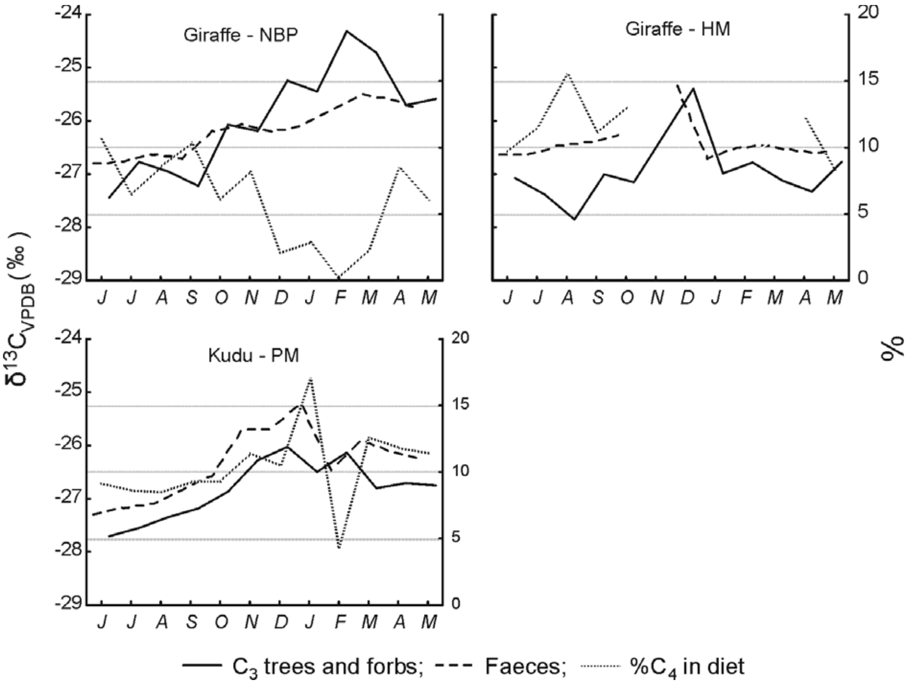

Fig. 1. Corresponding shifts in mean δ^{13}C of C_3 tree foliage and forbs and browser faeces (left *y*-axis) in comparison with monthly changes in %C_4 grass intake (right *y*-axis). Spearman's Rank correlation coefficient revealed significantly similar patterns of variation between δ^{13}C of C_3 plants and browser faeces ($R = 0.58$, $P < 0.0001$). NBP = Northern Basalt Plains, Kruger Park; PM = Punda Maria, Kruger Park; HM = Hans Merensky Nature Reserve.

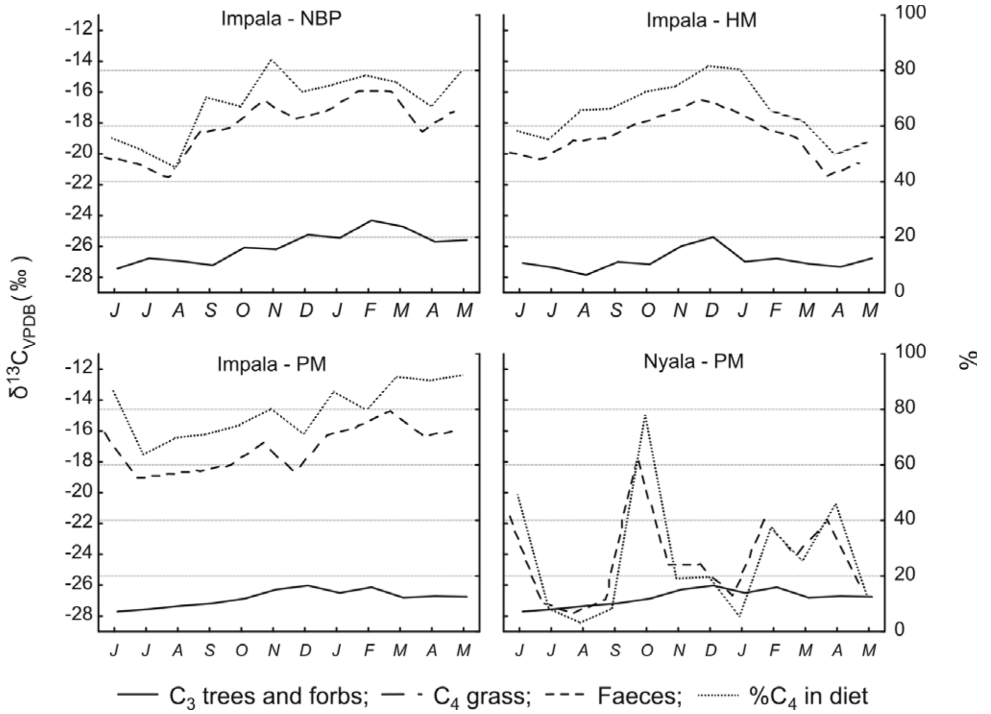

Fig. 2. Corresponding shifts in mean $\delta^{13}C$ of C_3 tree foliage and forbs, C_4 grass, and mixed-feeder faeces (left *y*-axis) in comparison with monthly changes in $\%C_4$ grass intake (right *y*-axis). NBP = Northern Basalt Plains, Kruger Park; PM = Punda Maria, Kruger Park; HM = Hans Merensky Nature Reserve.

sources of error, such as differences between plant species, plant parts, and small differences in diet-consumer (faeces) ^{13}C-discrimination between species and environments that may influence results (*e.g.* Gannes *et al.* 1997; Heaton 1999; Sponheimer *et al.* 2003b; Codron *et al.* 2005c). It has been shown that alternative endpoint values used in mixing models can lead to variations in inferred $\%C_4$ intake of about ± 5% (Codron *et al.* 2005a). In other words, significant diet shifts should only be inferred when the magnitude of change is $\geq$10% (see also Cerling *et al.* 2003; Sponheimer *et al.* 2003a). On this basis, only IM species in this study can be said to have changed their diets from the dry to the wet season (Table 2); C_4 consumption by impala on the Northern Plains changed from ~62 to 76%, at Hans Merensky from ~59 to 73%, and nyala from ~20 to 31%. By contrast, changes in $\%C_4$ intake from month-to-month were $\geq$10% for all taxa, including BR and GR, except for giraffe and wildebeest at Hans Merensky. Thus, carbon isotope data from faeces seem to record subtle diet changes faithfully, although shifts generally only become evident at higher temporal resolution (monthly).

The reliability of this approach is supported by the fact that, despite significant ($P < 0.05$) changes in faecal $\delta^{13}C$ and estimated $\%C_4$ grass intake recorded at monthly scales, the diets of BR generally remained almost entirely C_3-based (<10% C_4), and those of GR C_4-based ($\geq$90% C_4) throughout. However, some exceptions occurred. Amongst BR, giraffe diets are portrayed as ~10% C_4 in September on the Northern Plains, ~16% in August at Hans Merensky, and kudu appeared to consume $\geq$ 10% C_4 in January and March. While kudu diets may comprise 10% or more grass in the wet season (Owen-Smith & Cooper 1989), giraffe diets in the 'lowveld' savanna are expected to be almost entirely browse-based (Hall-Martin 1974). The most likely explanation for this apparent discrepancy is that these data reflect slight, but significant, increases in consumption of fruit and/or flowers during these late dry season months. Fruits and flowers of *Acacia nigrescens* flowers are ~1.0–1.5‰ enriched in ^{13}C compared to leaves of C_3 plants, at least in some South African savannas including this study region (Codron *et al.* 2005b,c). Fruits and flowers may form an important component of giraffe diets,

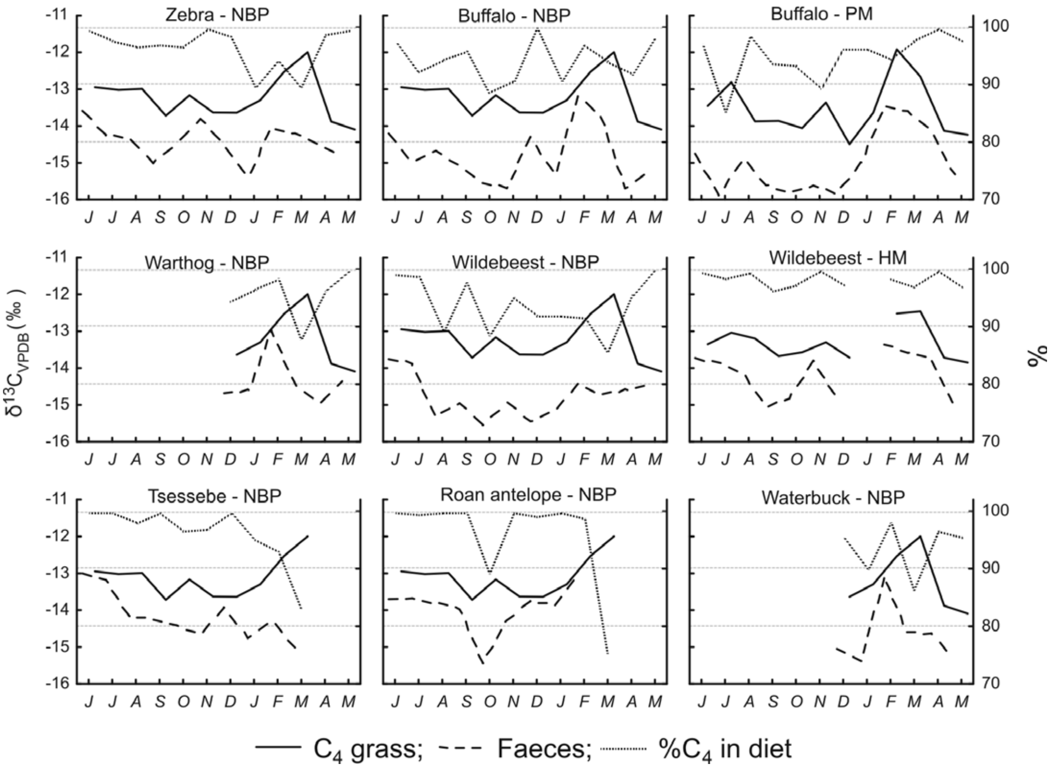

Fig. 3. Corresponding shifts in mean $\delta^{13}C$ of C_4 grass and grazer faeces (left *y*-axis) in comparison with monthly changes in %C_4 (grass) intake (right *y*-axis). Spearman's Rank correlation coefficient revealed significantly similar patterns of variation between $\delta^{13}C$ of C_4 grass and grazer faeces ($R = 0.40$, $P < 0.0001$). NBP = Northern Basalt Plains, Kruger Park; PM = Punda Maria, Kruger Park; HM = Hans Merensky Nature Reserve.

especially flowers of *A. nigrescens* that appear in these environments during August and September (Hall-Martin 1974; Owen-Smith 2004, pers. comm.).

Fluctuations in GR diets occurred at the end of the wet season (March), when all species on the Northern Plains (except buffalo) appeared to switch from almost pure C_4 grass-based diets to include between 10% and 20% C_3 foods. However, while these taxa are known to supplement their diets with browse items under certain conditions, this is expected to occur mainly during the dry season (*e.g.* Van Wieren 1996; Owen-Smith 1997). In the current study, only buffalo at Punda Maria appeared to eat some C_3 foods in the dry season (~15% in July). The increased consumption of C_3 foods by Northern Plains GR in March was likely because grass productivity declined towards the end of this dry seasonal cycle, and possibly also because much of the Northern Plains grass layer was burned in September and October 2004 so that by March 2005 insufficient rain had fallen to allow recovery of the grass layer. An additional caveat may be that $\delta^{13}C$ differences exist between C_4 grasses following different photosynthetic sub-pathways, *i.e.* NADP-ME, NAD-ME, and PCK. NAD and PCK grasses are ~1.0–1.5‰ depleted in ^{13}C relative to NADP species (Hattersley 1982; Cerling & Harris 1999). However, the difference is smaller (~0.5‰) within the current study areas (Codron *et al.* 2005c), hence differential NAD/PCK *versus* NADP grazing is unlikely to have affected the carbon isotope compositions of most GR species in this study. Whatever the case, it is clear that some form of diet shift did occur in GR, and that these shifts are recorded in the carbon isotope composition of their faeces.

Enhanced insights gained from $\delta^{13}C$ variations at monthly resolution are also evident amongst IM. Patterns of variation in these taxa revealed different responses to environmental change between impala and nyala. Impala generally consumed more C_4 grass during wet season months, as expected from field studies of this species (Meissner *et al.* 1996; du Toit 2003). For nyala, punctuated diet switches were recorded; for most of the year they ate mainly C_3 browse, but they changed to consume ~80% C_4 grass at the start of the wet season (October), switched back to C_3 browsing until January, and then ate between ~40 and 50% C_4 grass in the latter stages of the wet season. These results are consistent with predictions for increased grass intake by nyala in the wet season, including instances in which this species has been observed to consume almost only grass in conditions of fresh grass growth after the onset of the rainy season (Tello & van Gelder 1975).

CONCLUSION

Stable carbon isotope analysis of short-term materials, such as faeces, reveal subtle changes in diet through space and time, even amongst browsers and grazers. This approach offers a means to obtain much sought-after information about dietary variations in ungulate herbivores, which are pivotal to our knowledge of adaptation, behaviour, and evolution at species levels, as well as competition and coexistence at community levels. However, accurate reconstruction of diet requires rigorous baseline data to capture the isotopic variation that occurs in the food base. Further studies adopting this approach may establish stable carbon isotope ecology as a routine technique to be employed alongside traditional methods for diet, habitat utilization, and rapid assessment of habitat condition.

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