Dietary variation in impala *Aepyceros melampus* recorded by carbon isotope composition of feces *

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Abstract　Stable carbon isotope data（$\delta^{13}C$）from feces of mixed-feeding impala *Aepyceros melampus* living in South Africa's Kruger National Park are used to study dietary variations across a variety of spatio-temporal scales. We test hypotheses that ungulate dietary variations are related to relative proportions of woody plants：grass in surrounding landscapes，and that rainfall regulates ungulate ecology. Results show that impala diets traverse the browser/grazer spectrum，varying widely in proportions of browse：grass consumed at monthly，seasonal，annual，and regional scales. Impala living in open savanna and grassland landscapes generally eat more grass than their counterparts in savanna woodlands，but this differentiation is not consistent at all temporal scales. In one densely wooded region，Punda Maria in the far north of Kruger，impala consume more grass than elsewhere in the Park. Impala in riparian areas eat lower quantities of grass than in other habitat types，especially during the dry periods when spatial differences in diet are more pronounced. Hence assumptions that ungulate feeding ecology reflects the woody plant：grass composition of their habitats are not supported by our data，nor was a relationship between diet and rainfall detected. The results do support a model of increased grass consumption with increasing protein content of available grasses. Fecal％N data show minimal variations across space and time，suggesting that impala are selective feeders that choose foods，whether browse or grass，in order to maintain optimal levels of diet quality. Given these results，it is more likely that detailed models of food selection，available through optimal foraging theory，can better describe ecological variation［*Acta Zoologica Sinica* 52（6）：1015 – 1025，2006］.

Key words　Impala，*Aepyceros melampus*，Browse，Grass，Herbivore，Kruger Park

黑斑羚粪便中碳同位素揭示的食性变化 *

摘　要　利用稳定碳同位素数据（δ¹³C）分析了南非克鲁格国家公园混食性黑斑羚（*Aepyceros melampus*）时间和空间尺度上的食性变化，验证了两个假说，即有蹄类食性变化是由生境中木本植物与草本植物的相对配比导致；降雨控制有蹄类生态。结果表明：黑斑羚的食性涵盖了精食者－粗食者采食谱系，且食性中木本与草本比例在不同月间、季节、年度和区域间存在很大变化。栖息于开放性热带稀树草原和草原中的黑斑羚通常采食比生境中更高比例的草本，但在时间尺度上并不恒定。在克鲁格北部的一个区域（Punda Maria），黑斑羚采食的草本比克鲁格国家公园中其它任何区域都多。与其它生境相比，在河边的黑斑羚采食草本数量更少，尤其是在食性空间变化更为明显的旱季。因此，我们的数据不支持有蹄类食性组成变化是由生境中木本与草本比例不同造成的假说，食性与降雨间也无明显的关系。我们的结果支持草本中蛋白含量增加引起黑斑羚采食比例的增加这一模型。粪便中氮含量在时间和空间上的变化很小，揭示在可利用食物中，无论木本还是草本，黑斑羚进行选择采食以保证最好的食物质量。基于这些结果，我们认为更具体的食物选择和可利用性最适采食理论能够更好地解释这种生态学变化［动物学报 52（6）：1015－1025，2006］。

关键词　黑斑羚　木本　草本　食草动物　克鲁格公园

The evolution and ecological diversity of ungulate herbivores in the African savanna biome is functionally related to environmental heterogeneity at various patch，habitat and temporal scales（du Toit and Cumming，1999；du Toit，2003）. Dietary variations across these scales reflect the complexity of plant-mammal interactions，and are thus of primary importance for understanding patterns of population and community ecology. From a conservation perspective，spatio-temporal variations in diet can reveal local trends about animal performance，impacts of herbivory on vegetation，and coupled with long-term records，also provide insight into animal responses to global change（see Dalerum and Angerbjorn，2005）.

Two hypotheses prevail in the literature as explanations for ecological variation amongst ungulates. The first is based on an assumption that dietary variations reflect proportions of woody plants：grass in the landscape（Dunham，1980；Monro，1980；Attwell and Bhika，1985；Scholes et al.，2003）. The second widely-accepted model is that rainfall controls ungulate population demographics and other aspects of their ecology（Coe et al.，1976）. This hypothesis has been criticized for the small data set on which it is based，and because the authors only accounted for rainfall as a predictor of primary production（Fritz et al.，2002）. Testing of these two hypotheses has hitherto been difficult because of constraints to our knowledge of ecological variation amongst ungulates. For example，a number of studies have examined dietary variations of several species throughout the seasonal cycle（e.g.，Dunham，1980；Monro，1980；Owen-Smith and Cooper，1989；and see du Toit，2003），but few have successfully quantified variations across a variety of habitat types，since studies isolated in time are seldom comparable. Stable carbon isotope ecology is a powerful tool that can help address this gap. This is because the stable carbon isotope ratios of savanna herbivore tissues and excreta faithfully record relative contributions of C_3（trees，shrubs，and forbs）

to C_4（grass）plant biomass in the diet（Vogel，1978；Tieszen et al.，1979；Lee-Thorp，1987；Cerling and Harris，1999）. Analysis of materials with relatively short turnover times，such as feces，can be used to depict variations in the proportions of browse（C_3 plants）to grass（C_4 plants）consumed across numerous spatial and temporal scales（Tieszen et al.，1979；Sponheimer et al.，2003a，b；Codron et al.，2005a）.

Stable carbon isotope studies of ungulate diets are useful because the majority of classification schemes express diet in terms of the presumed dichotomous trophic differentiation of browsers and grazers（Hofmann and Stewart，1972；Hofmann，1989；du Toit，2003）. Impala *Aepyceros melampus* are notoriously enigmatic mixed-feeders，with diets ranging from almost pure browse to nearly exclusive grass intake，depending on local habitat conditions，season，and even the sex of the animal（Dunham，1980；Monro，1980；van Rooyen and Skinner，1989；Skinner and Smithers，1990；Meissner et al.，1996；Wronski，2002）. Impala are therefore an ideal species for studying dietary variations in relation to landscape and habitat heterogeneity across the savanna.

Our preliminary carbon isotope data from feces of impala living in South Africa's Kruger National Park suggested that populations in the southern regions of the Park，in areas of fine-leaved *Acacia* and *Combretum* savanna，eat more grass than their counterparts inhabiting the northern regions，where broad-leaved *Colophospermum mopane* savanna abound（Sponheimer et al.，2003a）. However，the spatial mosaic of ecotypes found within Kruger Park incorporate an even wider array of habitat types（Venter et al.，2003）. Data presented in Sponheimer et al.（2003a）also represent only one season of one year（dry season/June 2002）. The extreme seasonality of the rainfall cycle of the southern African interior adds a temporal dimension to the heterogeneity of the landscape. Thus，given the diet breadth expected for impala，

broad-scale comparisons between southern and northern Kruger populations, over only one season, probably oversimplify the full range of variation that exists in this heterogeneous savanna environment.

Here, we present results of a carbon isotope study of impala dietary variations based on feces collected over three years from numerous landscape and habitat types in Kruger Park. We first examine the degree of variations in proportions of C_3 (browse)：C_4 (grass) consumed through space and time to determine the scale (s) at which the magnitude of variation is greatest. We then assess dietary switches in relation to environmental factors predicted to regulate dynamics of ungulate ecology, including seasonality, rainfall, plant nutritional quality, and structural composition of the vegetation (woody plant：grass cover).

1　Materials and methods

Kruger National Park is a large (~ 2 000 000 ha) semi-arid savanna, 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 (Fig.1). The Park forms part of the "lowveld" savanna, lying at ~300 m above sea level on average, although this varies from 900 m in the southwest to 200 m in the east. Mean annual rainfall varies between 500 and 700 mm in the south to between 300 and 500 mm in the north (Venter et al., 2003). Rainfall is highly seasonal, falling mainly during the austral summer between October and March, while April to November is dry.

The spatial mosaic of landscapes in Kruger Park offer resident herbivores a wide range of contrasting habitats differing in available resources. The physical characteristics of the Park's various ecotypes have been well-studied and are reviewed in Venter et al. (2003). The geological succession and associated vegetational landscapes can be simplistically reduced along a longitudinal gradient, with relatively nutrient-poor granites associated with woodland savannas in the west while nutrient-rich basalts give rise to open grasslands in the east (Venter et al., 2003; Fig.1). There is a marked difference in the vegetation of the northern regions compared to the south; the region to the north of the centrally-located Olifants River is dominated by broadleaved *Colophospermum mopane* savanna, while the south is dominated by fine-leaved *Acacia* and *Combretum* savanna (Venter et al., 2003). Based on these geological and vegetational differences, the landscapes of Kruger Park can be broadly categorized as northern and southern granites and basalts, respectively. The densely wooded, mountainous region of Punda Maria in the far northern sector is treated here as a fifth habitat type.

Impala feces were collected from Kruger Park from June 2002 to May 2005, biannually during the first two years, and at monthly intervals during the final year. Only fresh feces were collected, i.e. damp or wet specimens, to ensure that these represented the appropriate season, and that they were free of contaminants such as fungi, soil, or insects (see Wrench et al., 1996). Each dung pile encountered was assumed to represent a different individual. Latitude and longitude co-ordinates were recorded for each fecal sample using a Global Positioning System (GPS; Fig.1), and visual descriptions of surroundings were noted (e. g., open grassland, woodland, sodic patch, riparian zone, waterhole). Collection sites that often yielded an abundance of fecal samples and/or latrines were revisited during consecutive field trips.

Feces were oven-dried at 60℃ for 24 hours, and mill-ground into a homogenous powder through a 1mm sieve. Powdered samples were individually combusted in an automated Elemental Analyzer (Carlo Erba, Milan), and the resultant CO_2 gas introduced to a Finnigan MAT 252 or DELTA XP Mass Spectrometer via a continuous flow-through inlet system (Conflo). $^{13}C/^{12}C$ ratios are expressed in the delta ($\delta^{13}C$) notation in parts per mil (‰) relative to the VPDB standard. Standard deviations of repeated measurements of laboratory plant, protein, and chocolate standards were less than 0.1‰. $^{15}N/^{14}N$ ratios were determined from simultaneous N_2 gas yields. For completeness we present a summary of fecal $\delta^{15}N$ variations (relative to the atmospheric N_2 standard) in Table 1, but because controlling mechanisms for fecal ^{15}N-abundances are poorly understood, we do not consider these data when interpreting the results. This procedure does, however, provide percent nitrogen (%N) for each sample, offering some indication of diet quality because these data roughly reflect crude protein content of the diet (Holecheck et al., 1982; Grant et al., 2000).

Fecal $\delta^{13}C$ values were converted to estimates of %C_4 grass intake using a dual-mixing model (e.g., Post, 2002; Sponheimer et al., 2003a)：

$$(\delta^{13}C_{C3\ plant} + \Delta\delta^{13}C - \delta^{13}C_{animal}) / (\delta^{13}C_{C3\ plant} - \delta^{13}C_{C4\ plant}),$$

Where $\Delta\delta^{13}C$ is the magnitude of isotopic discrimination from diet to animal material, assumed here to be -0.9‰ for feces based on controlled-feeding experiments using ungulates fed a variety of C_3- and C_4-based diets (Sponheimer et al., 2003b; Codron et al., 2005a). C_3 and C_4 endpoint values used in the model are based on seasonal and regional means for local vegetation (data from Codron et al., 2005b and Codron, 2006). Use of regionally and temporally specific baseline data has been shown to enhance the accuracy of calculations of %C_4-intake from fecal
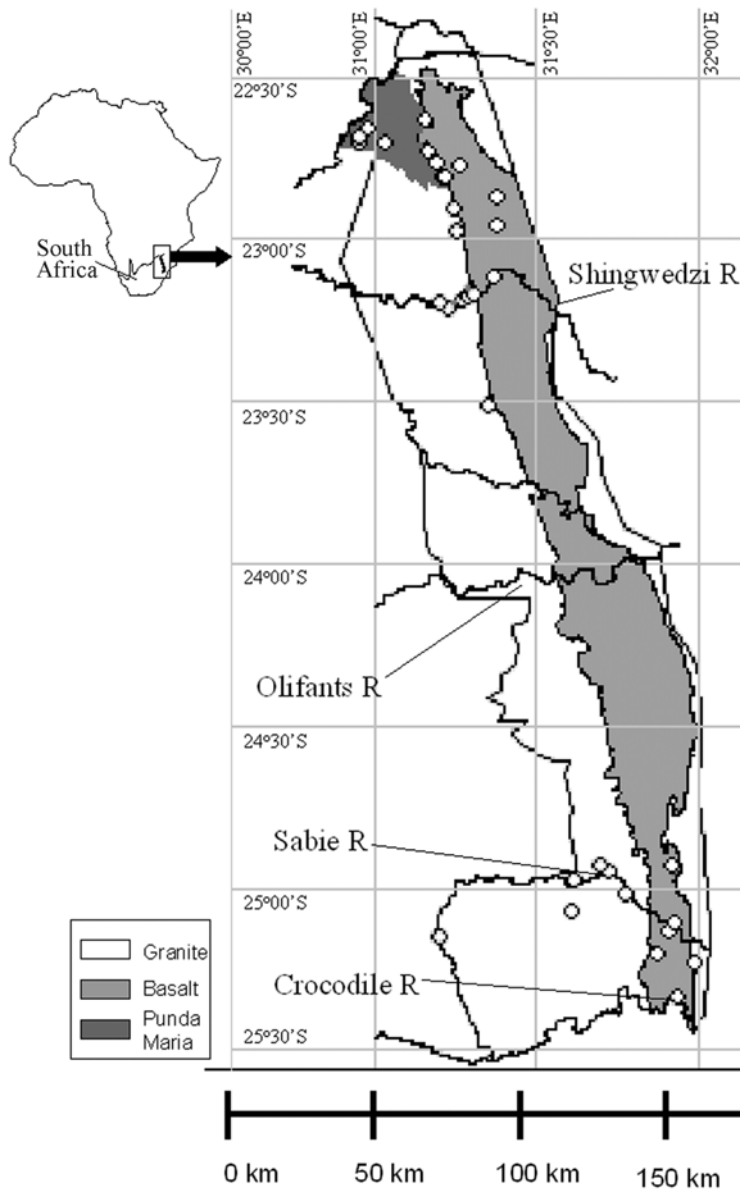
Fig. 1　Map showing geographical positioning of Kruger National Park in Africa，major geological zones and river systems，and fecal sampling localities（white circles）

The Olifants River separates northern *Colophospermum mopane*-dominated from southern *Acacia* and *Combretum* landscapes.

δ^{13}C（S.D. $\pm 5\%$；Codron et al.，2005a）.

Except for month-to-month comparisons，distinction was made between wet（October to March）and dry（April to September）season data，within each of the five landscapes studied here（northern and southern granites and basalts，respectively，and Punda Maria）. Significant differences between groups were tested using analysis of variance（ANOVA）and Tukey's HSD *post hoc* test（P-level 0.05），after normal distributions were confirmed using the Shapiro-Wilks test（δ^{13}C SW-W = 0.98，$P <$ 0.0001；%N SW-W=0.94，$P<0.0001$）.

While useful for ecological studies at a broad scale，the granite-basalt distinction of Kruger Park landscapes oversimplifies the geological and associated vegetational complexity of the region（see Venter et al.，2003）. For further resolution of spatial heterogeneity in diet，%C_4 intake was assessed along a west-to-east gradient based on GPS locality converted to decimal degree points. To control for animal movement between feeding and defecation sites，samples collected within 10 km^2 were treated as having the same GPS point，based on home range areas of ～5 km^2 in this species（Skinner and Smithers，1990；Owen-Smith pers.comm.）. Although territorial males may have much larger home ranges（up to 80 km^2），the vast majority of individual specimens were collected from defecation sites of breeding herds，

hence a 10 km^2 spatial separation is considered sufficient for these analyses.

To test the effects of environmental variation and diet quality on diet，we used a multiple linear regression model（MLRM）based on mean％C$_4$ intake for each habitat per season per year as a dependant variable. Diet quality（fecal％N），seasonal rainfall（data from the South African Weather Bureau），decimal degrees longitude，and mean％N of local C$_3$（tree and forb）and C$_4$（grasses）vegetation（unpublished data from plant specimens presented in Codron et al.，2005a and Codron，2006）were used as continuous independent predictors of dietary variation. The MLRM was repeated with removal of residual outliers（O′-level＝±3）to confirm findings.

2　Results

2.1　Carbon isotope evidence for dietary variation

Impala diets ranged from ～37％ to 74％ in C$_4$ content between the five major landscapes（changing by ～20％ to 30％ between wet and dry seasons；Table 1）. Variation in C$_4$ consumption at the annual scale was between ～27％ and 86％（Table 1）. At the landscape scale，C$_4$ intake was generally higher on basalts than on granites（$P<0.0001$），but maximum C$_4$ intake（～72％ of bulk）occurred at Punda Maria in the far north（$P<0.0001$；Table 1）. Landscape differences were much larger during the dry season（～30％）than the wet（～15％），due to dry-to-wet season increases in C$_4$ consumption in three regions（$P<0.001$）［no seasonal shifts occurred on southern basalts（$P=0.99$）or at Punda Maria（$P=1.00$）］. Moreover，year-to-year differences（up to ～40％）often exceeded the magnitude of overall regional and seasonal differences（Fig.2）. Significant annual diet changes are noted for southern granites and basalts，northern basalts，and Punda Maria across dry seasons，and for southern basalts and Punda Maria between consecutive wet seasons（$P<0.01$）.

Table 1　Mean δ^{13}C and calculated％C$_4$ grass intake，δ^{15}N，and％N of impala feces from Kruger Park showing regional（northern and southern granites and basalts，respectively，and Punda Maria），seasonal（dry，April to September and wet，October to March），and annual dietary changes

| Region	Season/year	δ^{13}C（‰）				δ^{15}N（‰）		％N	
		n	Mean	SD	％C$_4$ in Diet	Mean	SD	Mean	SD
Southern basalts	Dry ′02	9	−19.5	3.0	52	3.6	0.7	1.9	0.5
	Dry ′03	21	−19.4	2.4	53	2.7	1.0	1.5	0.2
	Dry ′04	31	−16.6	1.8	71	5.4	1.3	1.9	0.6
	Wet ′02/03	11	−20.2	2.9	52	4.3	1.6	2.2	0.7
	Wet ′03/′04	14	−16.4	2.0	80	6.4	0.7	2.0	0.6
	Wet ′04/05	20	−17.1	1.9	74	6.4	2.1	2.0	0.4
Southern granites	Dry ′02	12	−18.9	1.5	57	6.6	0.4	2.2	0.2
	Dry ′03	19	−22.0	2.2	35	5.5	1.1	1.7	0.2
	Dry ′04	36	−21.7	2.5	37	7.4	1.9	2.1	0.4
	Wet ′02/03	12	−19.4	3.1	58	8.0	1.7	2.3	0.5
	Wet ′03/′04	18	−18.0	1.9	67	9.2	1.9	2.4	0.8
	Wet ′04/05	20	−20.6	2.7	49	7.3	2.3	2.1	0.3
Punda Maria	Dry ′03	4	−15.2	2.6	86	4.6	0.8	1.4	0.1
	Dry ′04	41	−18.0	2.2	66	3.7	1.5	1.7	0.5
	Dry ′05	20	−16.1	0.7	79	5.9	0.5	2.2	0.4
	Wet ′04/05	60	−16.7	1.6	74	5.2	1.3	1.9	0.3
Northern basalts	Dry ′02	11	−22.5	1.8	33	4.9	0.9	1.9	0.2
	Dry ′03	5	−22.6	1.6	32	5.5	0.9	2.2	0.6
	Dry ′04	52	−19.7	2.1	54	4.0	1.8	1.7	0.3
	Dry ′05	20	−17.9	2.5	68	6.9	1.3	2.5	0.6
	Wet ′02/03	2	−19.0	4.9	57	6.9	0.8	1.6	0.1
	Wet ′03/′04	16	−15.0	1.9	85	6.3	2.2	1.8	0.4
	Wet ′04/05	60	−16.9	1.5	72	7.4	2.0	1.9	0.2
Northern granites	Dry ′02	7	−21.4	3.4	40	6.0	1.0	2.7	0.8
	Dry ′03	8	−23.3	2.1	27	5.1	0.6	2.2	0.4
	Dry ′04	29	−21.6	2.2	39	5.6	1.9	2.0	0.4
	Wet ′02/03	12	−21.5	2.6	41	6.1	0.5	1.6	0.3
	Wet ′03/′04	16	−18.2	2.2	64	7.8	1.1	2.1	0.6
	Wet ′04/05	20	−20.0	1.5	51	6.3	1.8	2.1	0.2

n＝number of samples；SD＝standard deviation.
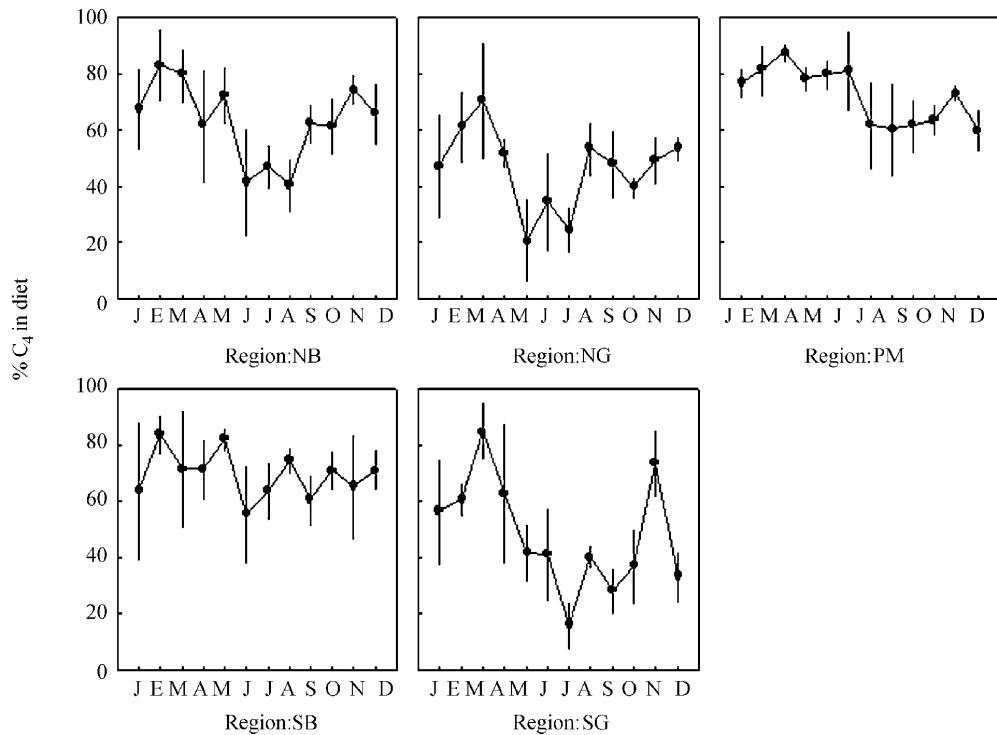
Fig. 2 **Monthly changes in% C$_4$ grass consumed by impala for the period February 2004 to January 2005（June 2004 to May 2005 for Punda Maria），as reflected by fecal δ^{13}C（means presented with standard deviation）**

Even larger variations in proportions of C$_3$ and C$_4$ foods consumed are evinced from month-to-month comparisons（Fig. 2）. At this scale，impala diets fluctuated between ～16% and 89% C$_4$ throughout the seasonal cycle. Monthly shifts in diet are significant for northern granites and basalts，and southern granites，with impala diets in these landscapes changing from more than 60% C$_4$ during the wet season to less than 40% in the dry（$P < 0.0001$）. The shift away from a C$_4$-rich diet to include more C$_3$ occurred at the onset of the dry season（April），and continued through the dry season reaching a minimum during the driest months（May，June，July）. Sharp C$_4$ peaks（diets comprised of 80% to 90% grass）were noted during March and December on southern granites and February on northern basalts.

Monthly data also portray more detailed seasonal patterns for southern basalts and Punda Maria that are not discernible through broader dry/wet season comparisons（Fig. 2）. On southern basalts，impala diets generally comprised between ～60% and 70% C$_4$，but during the wet season months of January and February，and the dry season month of May，C$_4$ intake was greater than 80%. Punda Maria data evince significantly lower C$_4$ intake for the period of June to December 2004 compared to the period of January to May 2005（$P < 0.05$）. In this case，however，the difference can be attributed to the sudden switch to increased C$_4$ consumption that occurred between De-

cember 2004 and January 2005（$P < 0.001$；Fig. 2），rather than to changes along a seasonal gradient.

2.2 Changes beyond the granite-basalt gradient

Linear regression analyses revealed that impala indeed varied their diets along a latitudinal gradient（Fig. 3），at least during the dry seasons when spatial differences in diet were more pronounced than in wet seasons（see Table 1）. The effect differed，however，between impala from the northern and southern regions of Kruger Park，respectively. Impala from the south increased the amount of C$_4$ in their diets from west-to-east（$r^2 = 0.26$，$P < 0.0001$），consistent with the finding that basalt-based grassland landscapes in the east generally support a more grass-dominated diet than do granites in the west. By contrast，impala from northern regions decreased their C$_4$ intake from west-to-east（$r^2 = 0.17$，$P < 0.0001$），although this result is apparently skewed by the unusually high C$_4$ intake by impala at Punda Maria. The latitudinal effect for the north disappears when Punda Maria is excluded from the analysis（$r^2 = 0.00$，$P = 0.46$）.

These analyses also show changes in diet at habitat scales beyond broader differences between granitic and basaltic landscapes（Fig. 3）. While samples representing closed woodland，open grassland，and sodic habitats within each landscape do not differ significantly from the west-to-east regression slope（$P > 0.05$ in all cases），samples representing riparian
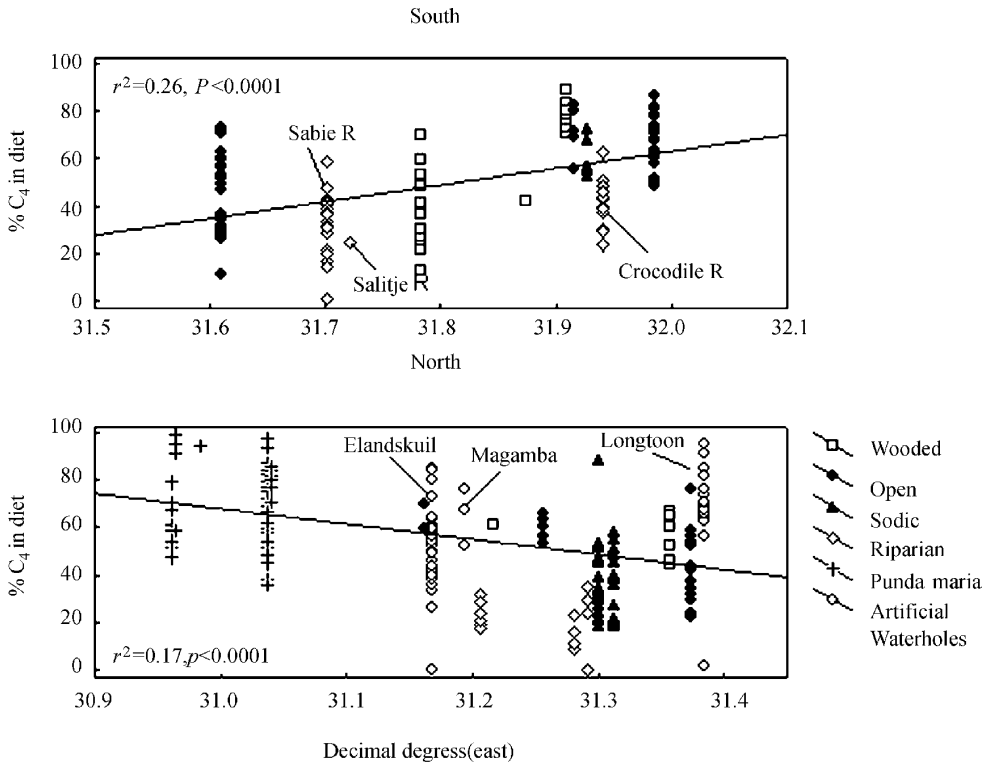
Fig. 3   Spatial differences in dry season% C$_4$ grass intake by impala along a west-to-east gradient within southern and northern Kruger Park landscapes

Sabie R，Salitje R，and Crocodile R are riparian sampling sites.

zones and artificial watering points do. Riparian samples consistently fall below the regression lines，depicting significantly lower C$_4$ intake than regional means in both northern （~21% C$_4$；$P<0.001$） and southern landscapes （~35% C$_4$；$P<0.0001$）. This influence of riparian habitats on diet is exemplified by the fact that samples from two separate southern riparian sites，one along the densely wooded areas on granites above the Sabie River and the other on open basaltic plains above the Crocodile River （see Fig. 1），reflect similar diets （~32% and 40% C$_4$，respectively，$P=0.99$），despite the large difference between southern granite and basalt diets during the dry season （~ 40% and 62% C$_4$，respectively，$P<0.0001$）. In the case of waterholes，impala feces collected around the Longtoon Dam （22°52′18.4″S，31°23′5.5″E） and Magamba waterhole （22°46′3.4″S，31°11′38.1″E） reflected C$_4$-rich diets relative to other northern habitats （~68% and 65%，respectively，$P<0.05$）. By contrast，feces from the Elandskuil waterhole （22°44′9.1″S，31°10′6.0″E） do not portray particularly high C$_4$ intake （~52%；$P=0.99$）.

2.3   Diet quality and correlates of food selection

Diet quality，as evinced from fecal N content，seems to vary minimally in this species，means varying between 1.8% ± 0.4% and 2.3% ± 0.5% （Table 1）. No differences are evident between

mean% N of impala feces from different landscapes of Kruger Park，or between dry and wet seasons within each landscape （$P=0.15$ to 0.99）. The only exception is feces from southern granites，which during the wet season have slightly higher values than feces from northern landscapes （$F_{9,\ 596}=5.173$；$P=0.04$）.

The MLRM shows that grass nutritional content （based on% N），rather than diet quality （fecal% N） and longitude，has the greatest influence on% C$_4$ intake （Table 2；Beta = 0.37，$P=0.00$）. The same result was found after residual outliers （O′-level = ±3） were removed from the dataset （Beta = 0.57 and − 0.09，$P=0.00$ and 0.01，respectively）. Fig. 4 shows the results of linear regression analyses comparing variations in C$_4$ intake across space and time with grass% N. C$_4$ intake increases linearly with grass% N （$r^2=0.23$，$P<0.001$），which must be considered a relatively strong correlation given that our data do not account for more subtle variations in diet such as plant species or plant parts eaten. The MLRM offers no support for a relationship between diet and rainfall （Beta = 0.04，$P=0.30$，and Beta = − 0.01，$P=0.79$ after removal of outliers）.

3   Discussion

Carbon isotope evidence for dietary variations amongst impala are not congruent with either of the

Table 2 Results of a Multiple Linear Regression Model correlating%C₄ grass intake by impala in different habitats，seasons，and years with various environmental parameters expected to influence food choice

Variable	Initial model			Model repeat-No residuals		
	Beta	S.E. of Beta	P	Beta	S.E. of Beta	P
Fecal%N	−0.24	0.04	0.00	−0.33	0.04	0.00
C₄ Grass%N	0.37	0.05	0.00	0.57	0.05	0.00
C₃ Browse%N	−0.08	0.05	0.07	−0.07	0.04	0.07
Seasonal rainfall（mm）	0.04	0.04	0.30	−0.01	0.04	0.79
Decimal degrees East	−0.09	0.04	0.02	−0.09	0.04	0.01
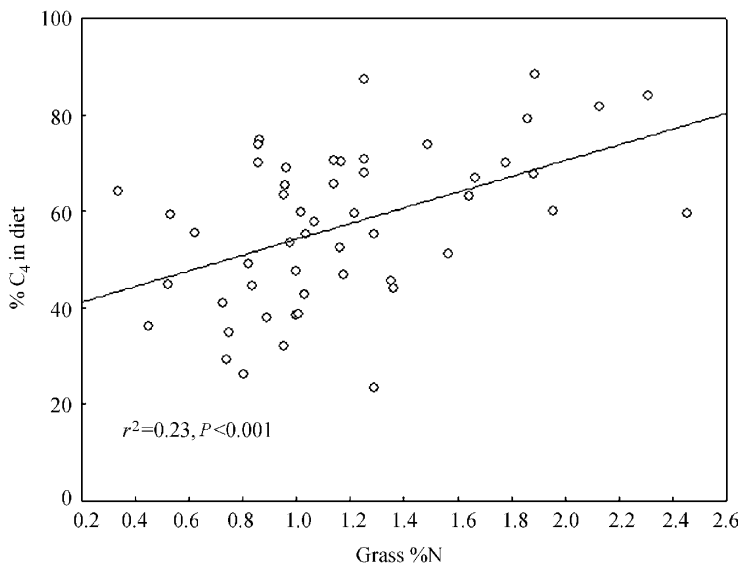
Fig.4 Linear correlation between%C₄ grass intake and%N of available grass by impala revealed by a multiple linear regression model，after removal of residual outliers（O′-level = ±3）
Symbols depict means for dry and wet seasons of each year across all habitat types included in the study.

two hypotheses commonly assumed for ungulate ecological variation，i.e. that diet reflects proportions of woody plants：grass in the landscape，and that rainfall is the ultimate mechanism regulating ecology. Fecal δ^{13}C of Kruger Park impala for the three-year study period indicate that northern and southern populations have similar diets（~56% C₄-intake in both regions；$F_{1,479} = 0.087$，$P = 0.77$）. Thus，the north-south dietary difference reported by Sponheimer et al.（2003a）for feces collected during a single dry season（June 2002）did not persist. Given that northern habitats offer a greater proportion of woody plants：grass for herbivores than do southern regions（Scholes et al.，2003；Venter et al.，2003），one might have expected northern impala to consistently eat less C₄（grass）than their counterparts in the south. Several studies of impala elsewhere in Africa have indicated that they will eat more grass when this resource is available in abundance，notably during the wet season when fresh grasses become available（Monro，1980；Dunham，1980；Meissner

et al.，1996；Wronski，2002）. By contrast，Gaerdes（1965）reported that impala in Namibia feed mainly on browse plants，and one might assume that this reflects a low preponderance of grasses in the semi-desert Namibian environments.

While Kruger Park impala on sandy，granitic substrates that support wooded savanna habitats consumed less C₄ grass overall compared with their counterparts living in clay-based open savanna and grassland habitats on basalts，this difference was not consistent throughout the study. For example，impala on southern basalts had similar diets to those on southern granites during 2002 and 2003（~50% C₄ intake）. Impala in the densely wooded region of Punda Maria ate more C₄ grass than impala in all other（more open）landscapes. Further spatial analysis of diet shifts along a west-to-east gradient revealed other apparent anomalies at the habitat scale，even when the effects of geological substrate were accounted for. The graphs in Fig.3 show that impala foraging in riparian zones prefer a C₃ browse-rich diet（only

~20% to 35% C_4), despite the fact that water-courses generally support an abundance of both browse-foods and grass (Dunham，1994). Moreover，impala showed dietary similarities in all riparian sites within Kruger，including the Shingwedzi River flood-plains in the north，and more importantly between our densely wooded Sabie River and open Crocodile River sampling sites in the south. In addition，dry-to-wet season increases in C_4 grass consumption a-mongst Kruger Park impala were only apparent for three out of the five principal landscapes included.

A similar discordance between diet and habitat composition has also been documented for the African elephant *Loxodonta africana*. Scholes et al. (2003) proposed that browse intake，and hence impact on woody vegetation by this megaherbivore，would be higher in northern mopane-dominated landscapes of Kruger Park where tree： grass leaf ratios are higher than in the fine-leaved savannas of the southern re-gions of the Park. Carbon isotope evidence for ele-phant diets in Kruger Park have shown that they eat more C_4 grass in the broad-leaved，more wooded mopane landscapes of the north than in the less-wood-ed southern regions (Codron et al.，2006).

We also found no evidence for a relationship be-tween rainfall and dietary variations amongst Kruger Park impala，even after the effects of landscape and geological heterogeneity and diet quality were con-trolled for. However，impala did appear to increase C_4 grass intake under conditions of elevated grass nu-tritional (%N) content. Thus，their diets do appear to be related to primary production of the grass layer. Rainfall therefore only affected diet in as far as this variable influenced primary production，but it cannot be regarded as the primary ultimate driver of the dy-namics of impala feeding ecology. Indeed，factors be-sides rainfall such as temperature，soil physical and chemical properties，etc. may have an equal or greater influence on primary production (Ellery et al.，1995).

Optimal foraging models provide an alternative approach，taking into account food availability as well as other factors that regulate food use，such as travel-ing distance，encounter rate，time-risk/benefit as-sessment，plant palatability and nutritional value (Owen-Smith and Novellie，1982; Owen-Smith and Cooper，1989; Wronski，2002). Impala are widely regarded as selective feeders，able to choose foods from the surrounding environment to optimize nutri-tional uptake (Dunham，1980; Monro，1980; At-twell and Bhika，1985; Meissner et al.，1996; Wronski，2002). In our study，C_4 grass intake was higher in regions/periods where the nutritional quali-ty of grasses (%N) was relatively high. This result suggests that impala prefer grasses and will select for

this food type when palatable forms are available in abundance，as predicted by Monro (1980) and Dun-ham (1980) based on results of field studies，and by Hofmann (1989) based on stomach morphophysi-ology.

The majority of landscape and habitat variations in diet appear to be controlled by grass quality. For example，basaltic substrates in Kruger support more nitrogen rich habitats than do granites (Grant et al.，2000; Venter et al.，2003)，which seems to account for the general (though not consistent) trend of high-er grass intake in the former. Within northern basalt habitats，impala consumed more grass at waterholes (as high as 68% at Longtoon Dam even during the dry season)，and artificial watering points are expect-ed to promote growth of nutritious grass species (Grant et al.，2002). The Elandskuil waterhole，however，did not appear to promote grazing，likely because this waterhole was closed during the course of this study as part of implementation of the Park's management policy for promoting habitat heterogene-ity (see Gaylard et al.，2003). Similarly，dry season grass%N at the riparian sites was lower than the oth-er sites within these landscapes ($P < 0.05$ in the north，$P < 0.01$ in the south)，and this likely ex-plains the low grass intake by impala using riparian habitats. The exceptionally high C_4 grass intake by impala at Punda Maria，however，can not be ex-plained by patterns in grass%N，since this variable was no different for Punda Maria grasses compared with grasses from other landscapes ($P = 0.16$).

Although impala feed anywhere along the C_3/C_4 spectrum，their fecal%N remained intermediate be-tween reported fecal N content of grazers (1.2‰ to 1.8‰) and browsers (2.3‰ to 2.9‰) from Kruger Park (Sponheimer et al.，2003a; Codron et al.，2006)，and there is a lack of significant correlation between C_4 intake and fecal%N. This low variation in fecal%N across habitats and seasons，suggests op-timal foraging enables impala to maintain consistent quality diets despite considerable regional and tempo-ral differences in proportions of browse： grass eaten. This occurred despite that in African savannas，woody plants have consistently higher N content than grasses. Previously，both Meissner et al. (1996) and Sponheimer et al. (2003a) concluded that impala diet quality is maintained in all environments，regardless of the type of food they eat. Fecal%N data may also reflect avoidance of plant secondary compounds such as condensed tannins that act as antifeedants to herbi-vores (Cooper and Owen-Smith，1985; du Toit，2003). Fecal%N of herbivores feeding on woody plants are often elevated by condensed tannins in the diet that reduce protein digestibility，resulting in pre-cipitation of nitrogen in feces (Robbins et al.，

1987). The absence of this phenomenon amongst impala feces reflecting high browse consumption suggests that they do limit tannin intake by selecting a variety of browse species, and possibly explains why impala prefer grass to browse in the densely wooded regions around Punda Maria. An alternative explanation might be that impala extract more nitrogen (and other nutrients) from browse foods than grass, thereby excreting less nitrogen so that fecal%N levels are similar on browse- and grass-rich diets. This is unlikely, however, since impala increased their grass intake when grasses had higher N content, and hence probably obtained similar protein intake on all diets.

In conclusion, accounts of spatial variation in diet have significant influences on perceptions of ecological patterns in ungulates. Predicted responses to rainfall and seasonality were not consistent across all habitat types, or from year-to-year, and neither were proportions of woody plant to grass cover necessarily reflected in impala diets. These findings imply alternate mechanisms that regulate the dynamics of ungulate feeding ecology. Such mechanisms can be resolved by optimal foraging models that describe multivariate influences on food selection under different environmental conditions. For impala there appears to be a preference for grass when high quality grasses are available. These differences appear to enable impala to maintain optimal nutrient balance under all conditions. These patterns of optimal foraging are easily detected through carbon isotope analysis. Future research across a wide array of spatial dimensions will be invaluable for improving our understanding of the mechanistic links between habitat heterogeneity, ecological variation, and ultimately evolutionary diversification of the ungulate group.

References

Attwell CAM, Bhika M, 1985. Feeding ecology of impala on Starvation Island, Lake Kariba. S. Afr. J. Wildl. Res. 15: 41 – 48.

Cerling TE, Harris JM, 1999. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. Oecologia 120: 347 – 363.

Codron D, 2006. The Ecological and Evolutionary Significance of Browsing and Grazing in Savanna Ungulates. PhD Thesis. University of Cape Town, RSA.

Codron D, Codron J, Sponheimer M, Lee-Thorp JA, Robinson T, Grant CC, de Ruiter D, 2005a. Assessing diet in savanna herbivores using stable carbon isotope ratios of feces. Koedoe 48: 115 – 124.

Codron J, Codron D, Lee-Thorp JA, Sponheimer M, Bond WJ, de Ruiter D, Grant R, 2005b. Taxonomic, anatomical, and spatiotemporal variations in the stable carbon and nitrogen isotopic compositions of plants from an African savanna. J. Archaeol. Sci. 32: 1 757 – 1 772.

Codron J, Lee-Thorp JA, Sponheimer M, Grant CC, Codron D, de Ruiter D, 2006. Elephant Loxodonta africana diets in the Kruger National Park, South Africa: spatial and landscape differences. J. Mammal. 87: 27 – 34.

Coe MJ, Cumming DH, Phillipson J, 1976. Biomass and production of large African herbivores in relation to rainfall and primary production. Oecologia 22: 341 – 354.

Cooper SM, Owen-Smith N, 1985. Condensed tannins deter feeding by browsing ruminants in a South African Savanna. Oecologia 67: 142 – 146.

Dalerum F, Angerbjorn A, 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144: 647 – 658.

Dunham KM, 1980. The diet of impala Aepyceros melampus in the Sengwa Wildlife Research Area, Rhodesia. J. Zool. Lond. 192: 41 – 57.

Dunham KM, 1994. The effect of drought on the larger mammal populations of Zambezi riverine woodlands. J. Zool. Lond. 234: 489 – 526.

Ellery WN, Scholes RJ, Scholes MC, 1995. The distribution of sweetveld and sourveld in South Africa's grassland biome in relation to environmental factors. Afr. J. Range For. Sci. 12: 38 – 45.

Fritz H, Duncan P, Gordon IJ, Illius AW, 2002. Megaherbivores influence trophic guilds structure in African ungulate communities. Oecologia 131: 620 – 625.

Gaerdes JH, 1965. The impala of Southwest Africa. Afr. Wildl. 19: 108 – 113.

Gaylard A, Owen-Smith N, Redfern J, 2003. Surface water availability: implications for heterogeneity and ecosystem processes. In: du Toit JT, Rogers KH, Biggs HC ed. The Kruger Experience. Washington DC: Island Press, 171 – 188.

Grant CC, Peel MJS, Zambatis N, van Ryssen JBJ, 2000. Nitrogen and phosphorus concentration in feces: an indicator of range quality as a practical adjunct to existing range evaluation methods. Afr. J. Range For. Sci. 17: 81 – 92.

Grant CC, Davidson T, Funston PJ, Pienaar DJ, 2002. Challenges faced in the conservation of rare antelope: a case study on the northern basalt plains of the Kruger National Park. Koedoe 45: 45 – 66.

Hofmann RR, 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78: 443 – 457.

Hofmann RR, Stewart DRM, 1972. Grazer or browser: a classification based on the stomach structure and feeding habits of East African ruminants. Mammalia 36: 226 – 240.

Holecheck JL, Vavre M, Pieper RD, 1982. Methods for determining the nutritive quality of range ruminant diets: a review. J. Anim. Sci. 54: 363 – 376.

Lee-Thorp JA, van der Merwe NJ, 1987. Carbon isotope analysis of fossil bone apatite. S. Afr. J. Sci. 83: 712 – 715.

Meissner HH, Pieterse E, Potgieter JHJ, 1996. Seasonal food selection by male impala Aepyceros melampus in two habitats. S. Afr. J. Wildl. Res. 26: 56 – 63.

Monro RH, 1980. Observations on the feeding ecology of impala. S. Afr. J. Zool. 15: 107 – 110.

Owen-Smith N, Cooper SM, 1989. Nutritional ecology of a browsing ruminant, the kudu Tragelaphus strepsiceros, through the seasonal cycle. J. Zool. Lond. 219: 29 – 43.

Owen-Smith N, Novellie P, 1982. What should a clever ungulate eat? Am. Nat. 119: 151 – 178.

Post DM, 2002. Using stable isotopes to estimate trophic position:

Models，methods，and assumptions. Ecology 83：703 − 718.

Robbins CT，Hanley TA，Hagerman AE，Hjejord O，Baker DL，Schwartz CC，Mautz WW，1987. Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68：98 − 107.

van Rooyen RJ，Skinner JD，1989. Dietary differences between the sexes in impala *Aepyceros melampus*. Trans. Royal Soc. S. Afr. 47：181 − 185.

Scholes RJ，Bond WJ，Eckhardt HC，2003. Vegetation dynamics in the Kruger ecosystem. In: du Toit JT，Rogers KH，Biggs HC ed. The Kruger Experience. Washington DC: Island Press，242 − 262.

Skinner JD，Smithers RHN，1990. The Mammals of the Southern African Subregion, 2nd edn. Pretoria: University of Pretoria.

Sponheimer M，Grant CC，de Ruiter D，Lee-Thorp J，Codron D，Codron J，2003a. Diets of impala from Kruger National Park: evidence from stable carbon isotopes. Koedoe 46：101 − 106.

Sponheimer M，Robinson T，Ayliffe L，Passey B，Roeder B，Shipley L，Lopez E，Cerling T，Dearing D，Ehleringer J，2003b. An experimental study of carbon-isotope fractionation between diet，hair，and feces of mammalian herbivores. Can. J. Zool. 81：871 − 876.

Tieszen L，Hein D，Qvortrup D，Troughton J，Imbamba S，1979. Use of $\delta^{13}C$ values to determine vegetation selectivity in east African herbivores. Oecologia 37：351 − 359.

du Toit JT，2003. Large herbivores and savanna heterogeneity. In: du Toit JT，Rogers KH，Biggs HC ed. The Kruger Experience. Washington DC: Island Press，292 − 309.

du Toit JT，Cumming DHM，1999. Functional significance of ungulate diversity in African savannas and the ecological implications of the spread of pastoralism. Biodivers. Cons. 8：1 643 − 1 661.

Venter FJ，Scholes RJ，Eckhardt HC，2003. The abiotic template and its associated vegetation pattern. In: du Toit JT，Rogers KH，Biggs HC ed. The Kruger Experience. Washington DC: Island Press，83 − 129.

Vogel JC，1978. Isotopic assessment of the dietary habits of ungulates. S. Afr. J. Sci. 74：298 − 301.

Wrench JM，Meissner HH，Grant CC，Casey NH，1996. Environmental factors that affect the concentration of P and N in faecal samples collected for the determination of nutritional status. Koedoe 39：1 − 6.

Wronski T，2002. Feeding ecology and foraging behaviour of impala *Aepyceros melampus* in Lake Mburo National Park，Uganda. Afr. J. Ecol. 40：205 − 211.