

Taxonomic, anatomical, and spatio-temporal variations in the stable carbon and nitrogen isotopic compositions of plants from an African savanna

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Abstract

Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios are commonly used to reconstruct palaeodiets and palaeoenvironments. The method is based on our knowledge of isotopic patterns in plants, which are subject to taxonomic and environmental variability. While previous researchers have addressed isotopic variability amongst plants, no studies have looked extensively at a broad suite of taxa over multiple temporal scales from within the savanna biome so as to provide baseline data for palaeodietary and palaeoenvironmental studies. Here we document variations in the isotopic compositions of plants collected over two years from the Kruger National Park, South Africa, with respect to species and anatomical differences, and the influences of geological substrate and spatio-temporal shifts in climate. Results show that environmentally-induced carbon isotopic variations in plants within this region are generally smaller than 2‰, which is lower than what has been previously reported for plants compared across multiple habitat-types. These data suggest that $\delta^{13}\text{C}$ differences of $\sim 2\text{‰}$ or more (or $\sim 1\text{‰}$ if the diet is predominantly C_4) between animals from a given area reliably indicate real dietary differences. Plant $\delta^{15}\text{N}$ values vary greatly between different microhabitats (by up to 4‰), responding to a range of environmental influences that may, in turn, significantly influence variation in animal $\delta^{15}\text{N}$ values. © 2005 Elsevier Ltd. All rights reserved.

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1. Introduction

Stable carbon and nitrogen isotope analysis of animal remains are commonly used to reconstruct the palaeodiets and palaeoenvironments of ancient human

populations [3,7,9,26,32,33,50,52,53]. Stable isotopic ratios in mammals are dependent on the isotopic composition of plants at the base of the food chain. Accordingly, our ability to reconstruct ancient diets and environments is limited by our knowledge of ^{13}C and ^{15}N abundances in modern plants and associated fauna.

The well-documented distinction between $\delta^{13}\text{C}$ values of C_3 (trees, shrubs, forbs)- and C_4 (grasses)-photosynthesizing plants, persists in the tissues of animals feeding on these plants, thereby reflecting the relative proportions of C_3 to C_4 biomass intake [8,30,61]. $\delta^{13}\text{C}$

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values of fossil fauna have provided considerable insights into mammalian (including early hominin) diets and foodwebs, particularly in species that lived following the radiation of C_4 grasslands after ca. 7 Ma. [6,9,31,32,41,52,53].

The distinction between $\delta^{13}C$ values of C_3 and C_4 plants is due to different photosynthetic pathways to fix atmospheric CO_2 . C_3 plants discriminate more heavily against ^{13}C than do C_4 plants, and thus the former have consistently lower $\delta^{13}C$ values [15,39,62]. Today, the $\delta^{13}C$ value of atmospheric CO_2 is close to -8‰ [5]. C_3 plant $\delta^{13}C$ values range globally from ~ -22 to -37‰ , averaging around -27‰ , while C_4 plants have $\delta^{13}C$ values between ~ -9 and -15‰ , with a mean of $\sim -12.5\text{‰}$ [15,38,62,63]. In the past, $\delta^{13}C$ values of C_3 and C_4 plants would have been slightly higher than those of modern plants, because changes in the concentration of atmospheric CO_2 , due largely to the burning of fossil fuels, have resulted in a $\sim 1.5\text{‰}$ depletion in ^{13}C content of atmospheric CO_2 since the 1950s [5,18,19,34].

$\delta^{13}C$ values of C_3 plants vary between individuals, species, and populations, because environmental factors and different physiological adaptations and responses lead to further isotopic discrimination during photosynthesis, mainly by affecting CO_2 conductance (through the stomata) and carboxylation rates [15,17,38,39,57]. Solar radiation levels, temperature, water availability, and water-use efficiency are widely quoted as the principal factors underlying carbon isotopic variations within C_3 plants [16,17,25,35,39,57]. Similar environmentally-induced variations in C_4 plants are less pronounced, because CO_2 is fixed early during the photosynthetic process, limiting further fractionation or discrimination [38,62]. However, C_4 plants following alternative photosynthetic sub-pathways, i.e. nicotinamide adenine dinucleotide phosphate-malic enzyme (NADP-ME), nicotinamide adenine dinucleotide-malic enzyme (NAD-ME), and phosphoenolpyruvate carboxykinase (PCK), discriminate differently against ^{13}C , resulting in small ($\sim 1\text{‰}$) but significant variations in $\delta^{13}C$ between C_4 sub-types [23,44].

$\delta^{15}N$ values in plants are related to several environmental factors including soil type and $\delta^{15}N$ value, climate, nitrogen assimilation, and nitrogen availability [22,24,37,43,46]. Fractionation effects that occur during biological denitrification processes in soils may be as large as 30‰ , and even larger (60‰) in the case of N_2O production via nitrification [22]. Such extreme fractionations are nevertheless rare, at least within any one system, and $\delta^{15}N$ values in biological materials generally range between ~ -10 and $+20\text{‰}$.

Although stable nitrogen isotopic data are usually limited to studies of animals from the more recent past (within ~ 10 ka due to post-mortem denaturation of biological proteins) they have been used to investigate

the diets of ancient hominins, including Neandertals and anatomically modern humans [3,7,26,33,50]. While these studies have provided new insights, there is widespread agreement that the controlling mechanisms for ^{15}N abundances in terrestrial foodwebs are poorly understood [2,51,54]. Importantly, predicted patterns of ^{15}N abundances in plants at the base of the food chain are not always consistent. For example, in principle biological fixation of nitrogen tends to decrease plant and soil $\delta^{15}N$, but several studies have shown that nitrogen-fixing plants are not always ^{15}N -depleted relative to non-fixers [21,46].

Because plant $\delta^{13}C$ and $\delta^{15}N$ values (and hence those for animals) may vary under different environmental conditions, accurate dietary reconstructions require baseline information about environmentally-induced variations in the isotopic composition of plants [25,28,57]. Previous studies of isotopic variation amongst plants have mostly examined vegetation from a broad array of habitat types, often ranging across arid, semi-desert to evergreen rainforest regions, and for a limited number of taxa [15,24,25,55]. There have been few intensive studies of plants from within a savanna environment. This gap is a constraint to studies of palaeodiet, especially when investigating the diets of extinct mammals, including the hominins that inhabited savanna habitats of the Plio-Pleistocene.

In this study we examine variations in the $\delta^{13}C$ and $\delta^{15}N$ values of plants available to mammals foraging within a single savanna region, the Kruger National Park (KNP), South Africa. The relatively large area covered by the Park ($18\,992\text{ km}^2$) incorporates several microhabitat types that are distinct in climate, geological substrate, and vegetational composition [59,60]. At least some of these microhabitats are likely similar to those inhabited by early hominins and sympatric mammals during the southern African Plio-Pleistocene [42]. We aimed to quantify isotopic shifts in KNP plants associated with taxonomic, anatomical, geographical, and seasonal differences. Results of isotopic analysis of a total 1553 plant specimens, sampled biannually over a period of two years, are presented. These data are intended to provide an extensive modern frame of reference for future isotope-based dietary and environmental reconstructions of early savanna environments. Findings are discussed in terms of the magnitude by which isotopic variations in plants may influence palaeodiet studies.

2. Materials and methods

2.1. Study area

The KNP is situated in the northeast of South Africa and forms part of the ‘lowveld’ savanna. It lies at

~300 m above sea level, between the Drakensberg escarpment to the west and the Mozambique coastal plain in the east. The climate is temperate, with rainfall occurring in the austral summer between November and March, peaking in January and February.

Six geo-morphological substrates have been described for the Park, associated with 36 vegetational landscapes [60]. Seven major river systems traverse the area, namely (from South to North) the Crocodile, Sabie, Olifants, Letaba, Shingwedzi, Luvuvhu, and Limpopo Rivers. For the purpose of general ecological studies, the KNP can be divided into four main eco-zones, comprising northern and southern granites and basalts, respectively. The southern KNP (area south of the centrally-located Olifants River) lies within the 'lowveld bushveld' zone with an average annual rainfall of 500–700 mm, and the north forms part of the arid 'bushveld', where mean annual rainfall is 300–500 mm [60]. The geological succession changes from west to east, with granitic rocks in the west and basaltic rocks in the east. Granites tend to form nutrient-poor substrates, while basaltic soils are considered to be more nutrient rich [60]. Regional woody vegetation is dominated by broadleaved trees such as *Combretum* spp. on the southern granites, fine-leaved species like *Acacia* on southern basalts, and *Colophospermum mopane* (mopane) on the northern granites and basalts [60].

2.2. Methods

Plant samples were collected from both southern (South of the Sabie River) and northern (Shingwedzi area) regions of the KNP, on basaltic and granitic geological substrates within each region. Collections were made biannually during the dry (winter/June) and wet (summer/January/February) seasons, from June 2002 to February 2004. Thus, the data represent two distinct seasons repeated for two years (with the exception of Punda Maria in the far north, where collections were made for one year).

Collection sites were roughly circular transects < 10 m in diameter, established at various localities throughout KNP (Fig. 1). These were selected so as to represent a diversity of microhabitats ranging from open grassland to dense riparian woodland. A brief description of the microhabitat of each site follows, after Venter et al. [60], with a visual description provided in parentheses. Sites 1 (open grassland) and 2 (dense riverine thicket) are on southern basalts (Fig. 1), forming part of the "Satara Land System", which is characterized as fine-leaved savanna dominated by *Acacia nigrescens*, *Dichrostachys cinerea*, *Combretum hereroense*, *Philonoptera violacea*, and *Sclerocarya birrea*. Sites 3 (open shrubland), 4 (closed woodland), and 10 (riverbed dominated by reeds and sedges) represent southern granites (Fig. 1), located

within the broad-leaved "Skukuza Land System", dominated by *Acacia* spp., *A. nigrescens*, *Combretum apiculatum*, *C. zeyheri*, and *Terminalia sericea*. Site 5 (open grassland) on northern basalts (Fig. 1) forms part of the "Letaba Land System", described as broad-leaved *C. mopane* (mopane) "shrubveld". Sites 6 (dry riverbed), 7 (open woodland on granites), 8 (sodic soil patch on alluvial floodplains), and 9 (dense riparian woodland on alluvial plains) are all located on the "Phalaborwa Land System", comprising broad-leaved bushveld and broad-leaved mopane "veld", and dominated by *C. mopane*, *C. apiculatum*, and *T. sericea*. Sites 11 (open woodland) and 12 (dense woodland) are located near the Punda Maria restcamp in the far north (Fig. 1), forming part of the "Pafuri Land System", characterized by high densities of woody plants, including *Burkea africana*, *C. mopane*, *C. apiculatum*, *Euclea divinorum*, *Kirkia acuminata*, *Pseudolachnostylis maprouneifolia*, and *T. sericea*.

Annual rainfall varies between these Land Systems, but also within them, due largely to a ca. 18-year wet/dry cycle that occurs in the region [60]. A comparison of predicted rainfall for each region with actual rainfall, taken from data for the weather station nearest to our sampling sites, i.e. Punda Maria weather station for Pafuri Land System, Shingwedzi for Phalaborwa System, Lower Sabie for the Satara System, and Skukuza for Skukuza, showed that rainfall during our study period was lower than the predicted totals and the means for the past two decades (Table 1). While low rainfall may be expected to compromise some aspects of this study, the effects for interpreting isotopic variations amongst plants are minimized because rainfall was equally low in all regions of KNP. Moreover, partitioning of rainfall across the seasonal cycle for the study period was maintained, with the wet season occurring between October and March, while April to September were dry months (Fig. 2).

We collected a wide range of plant growth forms, species, and parts at each site so as to represent the maximum variation in local vegetation. Trees (including leaves, bark, and fruits), forbs (whole specimens, leaves, and roots), and grasses (whole specimens, leaves/stems, roots, and seeds) were collected from within each transect. Specimens were collected from three to five individuals of every tree, forb, and grass species present at each site. Smaller sample sets of reeds, sedges, geophytes, and succulents, were also collected. Even though reeds are members of the Poaceae, we treated these as separate from grasses because they are ecologically very different from other graminoids. Geophytes were defined as monocotyledonous annuals and perennials that bear underground storage organs, such as corms and tubers. A subset of *A. nigrescens* flowers ($n = 20$) was obtained from random localities throughout the Park when these trees were flowering at the end of the dry season (August and September).

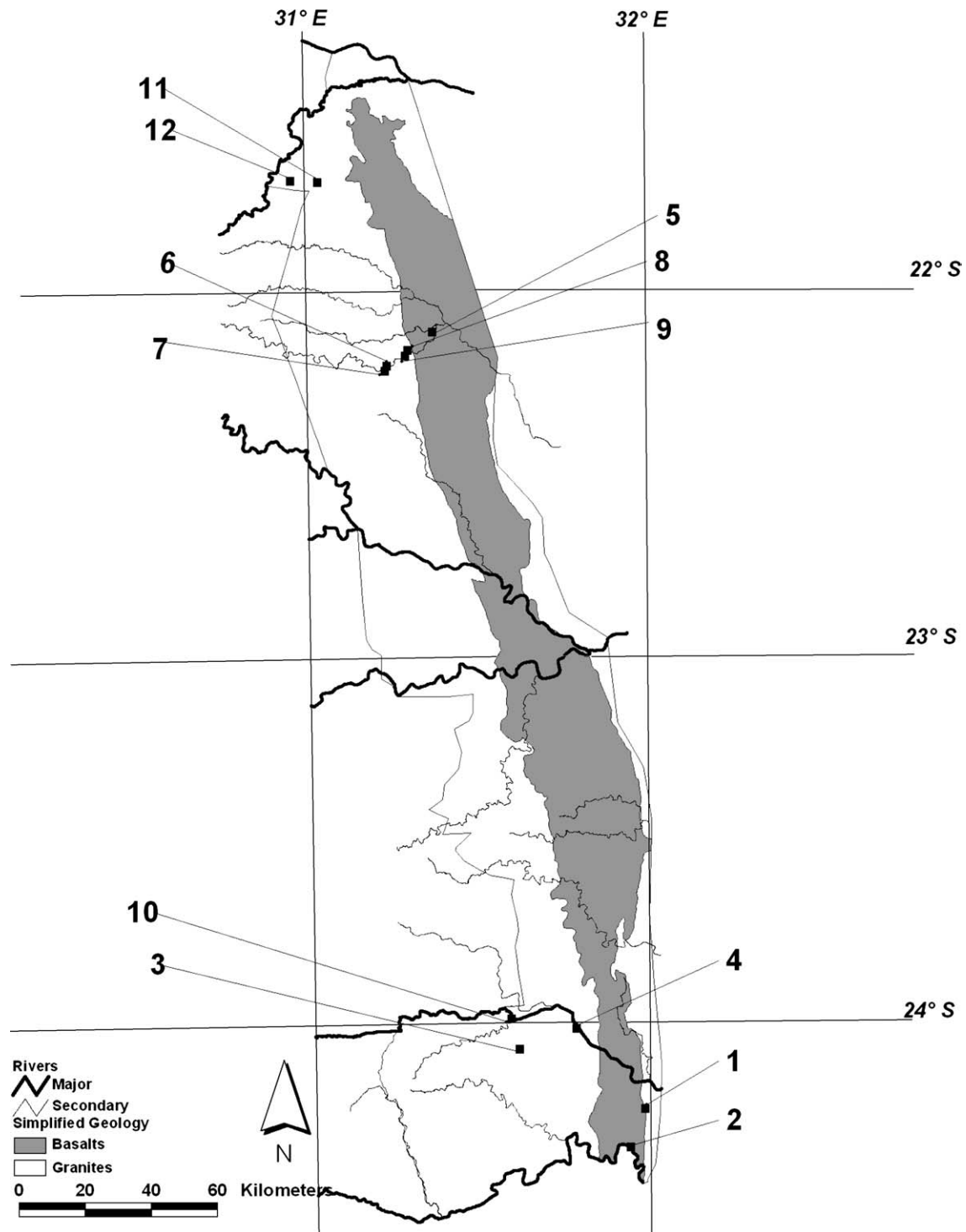


Fig. 1. Map of KNP indicating location of sampling sites (numbered 1–12) in relation to northern and southern granite/basalt geological subdivisions and the KNP river network.

Plant specimens were oven dried at 60 °C for 24 h and mill-ground through a 1 mm sieve into a homogenous powder. Powdered samples were combusted individually in an automated Elemental Analyzer (Carlo-Erba), and the resultant CO₂ and N₂ gases were introduced to a Mass Spectrometer (MAT 252 or DELTA XP) using a contin-

uous flow-through inlet system. ¹³C/¹²C and ¹⁵N/¹⁴N ratios are presented in delta (δ) notation, by convention, in per mil (‰) relative to the PDB and N₂ air standards, respectively. Standard deviations of repeated measurements of laboratory plant, protein, and chocolate standards were less than 0.1‰ for δ¹³C, and 0.3‰ for δ¹⁵N.

Table 1

Comparison of predicted annual rainfall for each Land System of KNP represented in this study (from Venter et al. [60]) with actual rainfall from weather stations nearest to plant sampling sites (including data from the past two decades, as well as over the study period)

Land system	Weather station	Rainfall (mm)		
		1985–2004	2002/3	2003/4
Pafuri (400–650 mm)	Punda Maria	524	342	393
Phalaborwa (450–600 mm)	Shingwedzi	454	362	300
Satara (500–650 mm)	Lower Sabie	596	495	466
Skukuza (500–750 mm)	Skukuza	599	297	489

3. Results

3.1. Distribution of C_3 and C_4 photosynthesis

All trees, and almost all forbs, were C_3 (6% of forbs sampled were C_4); all grass $\delta^{13}C$ values were consistent with C_4 vegetation (Fig. 3), conforming to predictions for savanna vegetation [8,61]. Reeds (mostly *Phragmites australis*) were predominantly C_3 (88%). Amongst KNP sedges, C_4 photosynthesis was only evident in 25% of individuals, while 75% were C_3 . Stock et al. [56] reported similar results from herbarium collections for sedges from this region (~30–40% C_4). Of the five

individual geophytes sampled, three were C_3 and two C_4 . Succulents had $\delta^{13}C$ values indistinguishable from that of C_4 plants, indicating use of the obligate Crassulacean Acid Metabolism (CAM) photosynthetic pathway common amongst xerophytes [27,45].

3.2. Species differences in plant $\delta^{13}C$ and $\delta^{15}N$

Differences in mean foliar $\delta^{13}C$ values between different tree species are displayed in Fig. 4. In order to accommodate meaningful statistical comparisons, the analysis included only those taxa for which sample sizes (n) were ≥ 20 (forbs were excluded as relatively few taxa were identified to genus or species level). Mean $\delta^{13}C$ values were highest in *C. mopane* ($-25.6 \pm 1.1\text{‰}$, $n = 34$), *Ziziphus mucronata* ($-25.8 \pm 1.2\text{‰}$, $n = 20$), *Combretum* spp. ($-26.1 \pm 0.9\text{‰}$, $n = 40$), and *Philonoptera violacea* ($-26.2 \pm 1.5\text{‰}$, $n = 30$), and lowest in *Diospyros mespiliformis* ($-28.3 \pm 1.3\text{‰}$, $n = 23$). However, all *D. mespiliformis* samples were found and collected at riverine sites, which may have contributed to the ^{13}C -depletion of this species [57]. Outside of *D. mespiliformis*, the lowest mean $\delta^{13}C$ values were observed for *Gymnosporia* spp. ($-27.4 \pm 1.4\text{‰}$, $n = 35$), *Euclea divinorum* ($-27.2 \pm 1.6\text{‰}$, $n = 26$), and *Acacia* spp.

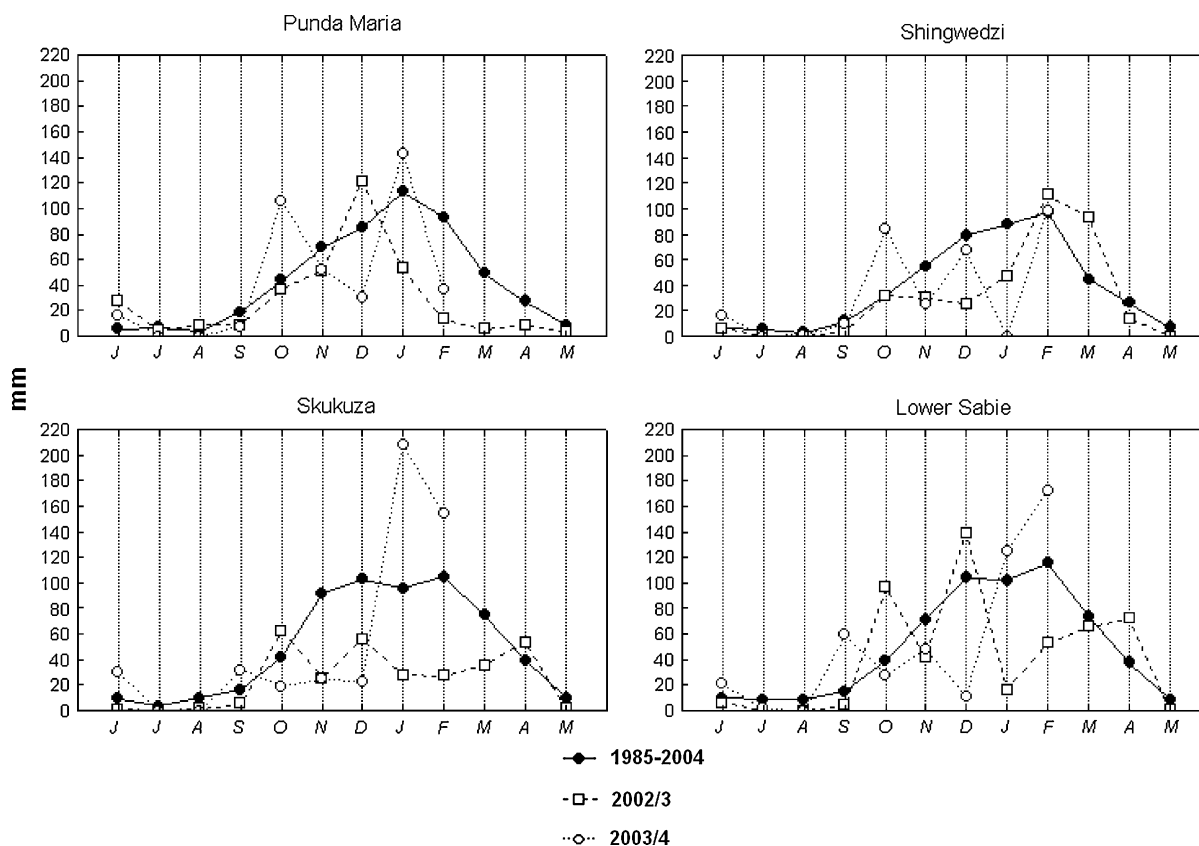


Fig. 2. Monthly rainfall (mm) for weather stations in KNP nearest to plant sampling sites (Punda Maria = sites 11 and 12; Shingwedzi = sites 5 through 9; Skukuza = sites 3, 4, and 10; and Lower Sabie = sites 1 and 2). Data presented as mean for the preceding two decades (1985–2004) compared with data for the study period (June 2002 to February 2004).

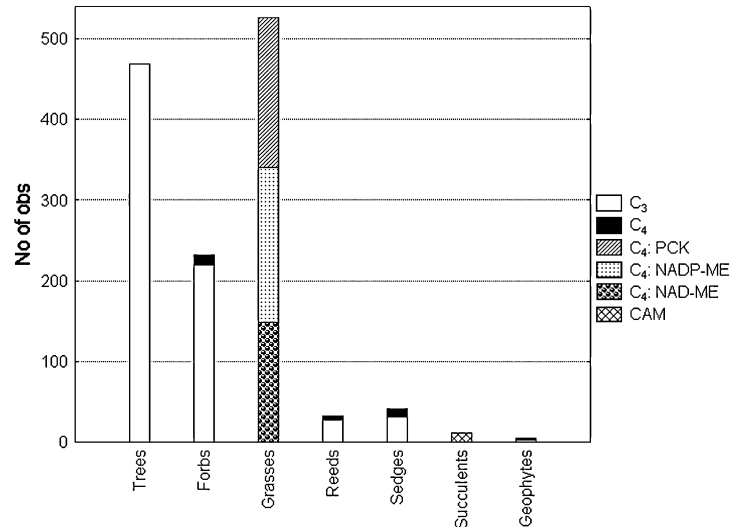


Fig. 3. Histogram indicating the distribution of C₃ and C₄ (or CAM) photosynthesis amongst various plant types (trees, forbs, grasses, reeds, sedges, succulents, and geophytes) from KNP.

($-27.1 \pm 1.2\text{‰}$, $n = 87$). Thus, excluding *D. mespiliformis*, carbon isotopic variation in trees due to species differences was 1.8‰ . Tieszen [57] suggested that genetic differences between species resulted in carbon isotopic differences of up to 3‰ , but this large variation is only evident amongst KNP trees if *D. mespiliformis* is included.

Mean $\delta^{15}\text{N}$ varied from $2.2 \pm 1.6\text{‰}$ ($n = 30$) in *P. violacea* to $5.1 \pm 2.3\text{‰}$ ($n = 44$) in *Grewia* spp. (Fig. 4). Nitrogen-fixing plants (mostly leguminous species) are often believed to be ^{15}N -depleted compared to non-nitrogen fixers, although this difference is not necessarily

consistent [21,37,46]. Leguminous forbs (superfamily Fabaceae; $2.8 \pm 3.5\text{‰}$, $n = 39$) were consistently depleted in ^{15}N compared to non-leguminous forbs ($4.8 \pm 2.5\text{‰}$, $n = 193$) ($p < 0.0001$). Similarly, leguminous trees had lower mean $\delta^{15}\text{N}$ values ($3.5 \pm 2.3\text{‰}$, $n = 211$) than non-legumes ($4.5 \pm 2.3\text{‰}$, $n = 256$) ($p < 0.001$). However, some common leguminous tree taxa such as *Acacia* spp. ($4.0 \pm 2.5\text{‰}$, $n = 87$) and *C. mopane* ($4.4 \pm 1.4\text{‰}$, $n = 40$) had a significantly higher ^{15}N content ($p < 0.0001$) in comparison with other members of the Fabaceae such as *P. violacea* ($2.2 \pm 1.6\text{‰}$, $n = 30$) and *Dichrostachys cinerea* ($2.7 \pm 1.7\text{‰}$, $n = 40$), thus the nitrogen isotopic distinction between leguminous and non-leguminous trees cannot be considered consistent.

Amongst C₄ grasses, mean $\delta^{13}\text{C}$ values of the various taxa ranged from $-12.8 \pm 0.8\text{‰}$ (*Chloris* spp., $n = 14$; and *Dactyloctenium australe*, $n = 23$) to $-10.5 \pm 1.4\text{‰}$ (*Setaria* spp., $n = 4$). Grass $\delta^{13}\text{C}$ values were very slightly, but significantly ($p < 0.001$), lower in taxa utilizing the NAD-ME ($-12.4 \pm 0.9\text{‰}$, $n = 146$) and PCK ($-12.3 \pm 1.0\text{‰}$, $n = 187$) sub-pathways compared to those using the NADP-ME ($-11.8 \pm 1.1\text{‰}$, $n = 192$) sub-pathway (see Fig. 5; assignment of taxa to sub-pathways from ref [44]; ref [1] for *Panicum* spp.). *Aristida* spp. had unusually low $\delta^{13}\text{C}$ values for an NADP-ME grass ($-12.5 \pm 0.9\text{‰}$, $n = 52$). Removal of *Aristida* from the analysis did not radically change the mean value for the NADP-ME group ($-11.6 \pm 1.1\text{‰}$, $n = 140$), although this strengthened the statistical difference between NADP-ME and NAD-ME/PCK grasses ($p < 0.0001$).

Grass $\delta^{15}\text{N}$ ranged between $0.8 \pm 1.3\text{‰}$ in *Heteropogon contortus* and $6.7 \pm 3.5\text{‰}$ in *Chloris* spp., but was not significantly different from trees and forbs ($p = 0.5978$). Interestingly, isotopic separation of C₄

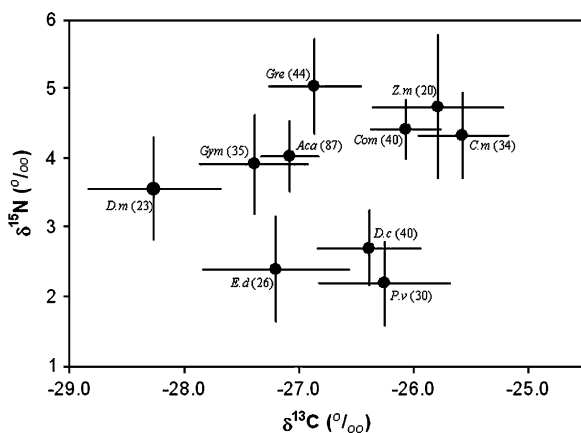


Fig. 4. Bivariate plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for tree species (leaves only) from KNP (only taxa with $n \geq 20$). Symbols represent the means, and the bars indicate $\pm 95\%$ confidence intervals. Number of samples for each taxon is displayed in parentheses after the abbreviations (*Aca* = *Acacia* spp., *C.m* = *Colophospermum mopane*, *Com* = *Combretum* spp., *D.c* = *Dichrostachys cinerea*, *D.m* = *Diospyros mespiliformis*, *E.d* = *Eualea divinorum*, *Gre* = *Grewia* spp., *Gym* = *Gymnosporia* spp., *P.v* = *Philonoptera violacea*, *Z.m* = *Ziziphus mucronata*).

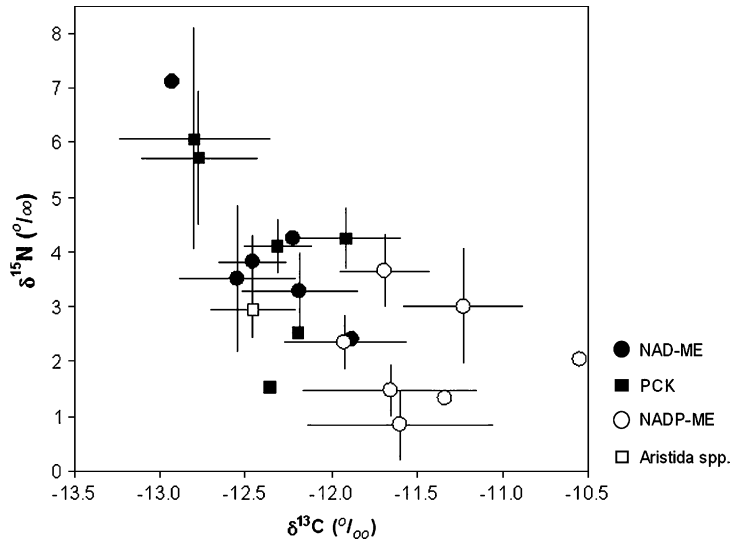


Fig. 5. Bivariate plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for grass species from KNP (leaves and stems only), showing the degree of separation between the NAD-ME, PCK, and NADP-ME photosynthetic subtypes. Symbols represent the mean for each taxon, and the bars indicate $\pm 95\%$ confidence intervals (bars omitted if $n < 10$). *Aristida* spp. had unusually low $\delta^{13}\text{C}$ values compared to other NADP-ME grasses, and is displayed separately in the graph.

sub-pathways was enhanced by bivariate analysis of $\delta^{13}\text{C}$ with $\delta^{15}\text{N}$, although the NAD-ME and PCK groups overlapped in several cases (Fig. 5). NADP-ME grasses had the lowest mean $\delta^{15}\text{N}$ values ($2.4 \pm 1.8\text{‰}$), followed by NAD-ME ($3.7 \pm 2.2\text{‰}$), while grasses of the PCK photosynthetic sub-type had the highest $\delta^{15}\text{N}$ values ($4.5 \pm 2.6\text{‰}$). Differences in mean $\delta^{15}\text{N}$ values of grass taxa following the three photosynthetic sub-pathways were significant among all groups ($p < 0.05$).

Reeds and sedges showed a bimodal $\delta^{13}\text{C}$ distribution, indicating that some were C_3 (combined mean = $-26.8 \pm 1.4\text{‰}$, $n = 59$) and others were C_4 (combined mean = $-11.7 \pm 1.7\text{‰}$, $n = 14$). $\delta^{15}\text{N}$ of reeds and sedges (mean = $6.1 \pm 1.3\text{‰}$, $n = 78$) was not different from that of trees, grasses, and forbs ($p = 0.9506$). Mean $\delta^{13}\text{C}$ of succulents (-13.1 ± 1.4 , $n = 11$) was similar to that of C_4 grasses, but succulents were significantly ^{15}N -enriched (mean = $7.6 \pm 3.7\text{‰}$, $n = 11$; $p < 0.0001$) compared to other plant types, a result consistent with previous findings from East African vegetation [29,37].

3.3. Isotopic differences between plant parts

Previously, authors have noted that different parts of a plant may vary in $\delta^{13}\text{C}$ by up to 2‰ [25,39]. Overall, fruit (mean $\delta^{13}\text{C} = -25.1 \pm 1.5\text{‰}$, $n = 24$) and bark ($-25.0 \pm 1.2\text{‰}$, $n = 31$) samples from all tree taxa were significantly ^{13}C -enriched compared to foliage ($-26.6 \pm 1.9\text{‰}$, $n = 312$) ($p < 0.0001$; Table 2). A relative ^{13}C -enrichment of C_3 fruits compared to foliage has also been reported for plants from the Ituri Forest, Democratic Republic of Congo [10]. In the KNP, the leaf–fruit enrichment factor varied from 0.9‰ in *Z.*

mucronata to 2.9‰ in *Grewia* spp. The most substantial ^{13}C -enrichment of tree bark compared to foliage was observed in *Gymnosporia* spp. (2.4‰), and the least in *S. africana* (0.6‰). Similarly, flowers of *A. nigrescens* (mean $\delta^{13}\text{C} = -25.1 \pm 1.0\text{‰}$, $n = 20$) were $\sim 2\text{‰}$ enriched relative to leaves of *Acacia* spp. (mean $\delta^{13}\text{C} = -27.1 \pm 1.2\text{‰}$, $n = 87$) ($p < 0.001$). It is unclear whether flowers would display a similar enrichment across a variety of taxa, although the *A. nigrescens* flowers were also 1.5‰ enriched compared to foliage of all tree taxa from KNP ($p < 0.01$).

Sample sizes of C_3 forbs, reeds, and sedges were too small for meaningful statistical anatomical comparisons to be made between parts such as leaves, stems, and roots (Table 2). Nevertheless, the data suggest similar patterns to that observed in trees. Mean $\delta^{13}\text{C}$ values of forb fruits ($-26.0 \pm 2.3\text{‰}$, $n = 11$) were slightly higher than those of forb leaves and stems (combined mean = $-26.8 \pm 1.2\text{‰}$, $n = 223$). Seeds of C_3 reeds and sedges were ^{13}C -enriched (mean = $-23.7 \pm 0.2\text{‰}$, $n = 2$) compared to leaves and stems (combined mean = $-26.8 \pm 1.5\text{‰}$, $n = 78$), and compared to sedge roots ($-26.4 \pm 1.5\text{‰}$, $n = 14$).

Anatomically, C_4 plants varied very little in carbon isotopic composition. Grass seeds were slightly, but not significantly ($p = 0.6838$) enriched in ^{13}C ($-11.9 \pm 0.5\text{‰}$, $n = 13$) compared to leaves and stems (combined mean = $-12.1 \pm 1.0\text{‰}$, $n = 510$) and grass roots ($-12.3 \pm 1.4\text{‰}$, $n = 83$; $p = 0.3935$).

The nitrogen isotopic composition of plants displayed few consistent anatomical variations (Table 2). Tree bark had a lower ^{15}N content (mean $\delta^{15}\text{N} = 1.7 \pm 2.2\text{‰}$, $n = 31$) than leaves ($3.9 \pm 2.2\text{‰}$, $n = 312$; $p < 0.0001$), fruits ($3.1 \pm 1.8\text{‰}$, $n = 24$; $p < 0.05$), and

Table 2
Anatomical differences in the carbon and nitrogen isotopic composition of KNP plants

Growth form/taxon	Plant Part	<i>n</i>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Trees				
<i>Acacia</i> sp.	Leaves	87	-27.1 ± 1.2	4.0 ± 2.5
	Bark	5	-25.2 ± 0.6	0.8 ± 1.6
	Flowers	20	-25.1 ± 1.0	4.4 ± 2.8
<i>Cassia abbreviata</i>	Leaves	1	-27.4	-0.8
	Fruit	1	-27.5	0.6
<i>Combretum</i> sp.	Leaves	41	-25.7 ± 2.7	4.4 ± 1.4
	Bark	5	-24.5 ± 1.5	2.4 ± 1.9
	Fruit	6	-24.2 ± 1.0	3.6 ± 2.3
<i>Dichrostachys cinerea</i>	Leaves	40	-26.4 ± 1.4	2.7 ± 1.7
	Bark	4	-24.4 ± 0.9	0.8 ± 0.6
	Fruit	5	-25.0 ± 1.8	3.7 ± 1.6
<i>Ficus sycomorus</i>	Leaves	2	-29.2 ± 0.4	5.1 ± 0.5
	Fruit	2	-26.6 ± 0.9	4.5 ± 0.6
<i>Grewia</i> sp.	Leaves	44	-26.9 ± 1.3	5.1 ± 2.3
	Bark	7	-25.9 ± 1.7	2.0 ± 3.6
	Fruit	2	-24.0 ± 0.7	3.8 ± 2.7
<i>Gymnosporia</i> sp.	Leaves	35	-27.4 ± 1.4	3.9 ± 2.1
	Bark	3	-25.0 ± 1.4	1.1 ± 0.8
<i>Philonoptera violacea</i>	Leaves	31	-25.7 ± 3.2	2.1 ± 1.7
	Bark	2	-25.0 ± 0.5	0.8 ± 0.2
<i>Spirostachys africana</i>	Leaves	9	-25.4 ± 0.8	3.7 ± 1.7
	Bark	4	-24.8 ± 0.6	2.9 ± 2.4
<i>Strychnos madagascariensis</i>	Leaves	1	-28.7	-0.2
	Fruit	1	-26	-0.8
<i>Ziziphus mucronata</i>	Leaves	20	-25.8 ± 1.2	4.8 ± 2.2
	Bark	1	-23.8	3.0
	Fruit	6	-24.9 ± 1.4	2.9 ± 0.9
<i>Azelia quanzensis</i>	Fruit	1	-26.9	1.9
	Leaves	1	-26.5	2.2
All trees	Leaves	312	-26.6 ± 1.9	3.9 ± 2.2
	Bark	31	-25.0 ± 1.2	1.7 ± 2.2
	Fruit	24	-25.1 ± 1.5	3.1 ± 1.8
	Flowers	20	-25.1 ± 1.0	4.4 ± 2.8
C₃ forbs	Leaves and stems	223	-26.8 ± 1.2	4.3 ± 2.7
	Fruit	11	-26.0 ± 2.3	5.1 ± 3.0
Grass				
<i>Aristida</i> sp.	Leaves and stems	52	-12.5 ± 0.9	3.0 ± 1.9
	Roots	12	-12.8 ± 1.0	2.8 ± 1.7
<i>Bothriochloa</i> sp.	Leaves and stems	38	-11.9 ± 1.1	2.4 ± 1.5
	Roots	5	-11.3 ± 1.6	1.6 ± 0.8
<i>Cenchrus ciliaris</i>	Leaves and stems	23	-11.7 ± 0.6	3.7 ± 1.5
	Roots	3	-10.9 ± 1.2	2.4 ± 1.7
	Seeds	1	-11.2	5.1
<i>Chloris</i> sp.	Leaves and stems	14	-12.8 ± 0.8	6.1 ± 3.5
	Roots	3	-12.5 ± 0.6	6.7 ± 3.0
<i>Dactyloctenium australe</i>	Leaves and stems	23	-12.8 ± 0.8	5.7 ± 2.8
	Roots	3	-12.4 ± 0.4	5.2 ± 1.7
<i>Digitaria eriantha</i>	Leaves and stems	20	-11.2 ± 0.7	3.0 ± 2.3
	Roots	2	-11.4 ± 0.5	1.9 ± 0.0
	Seeds	1	-10.8	2.7
<i>Enneapogon cenchroides</i>	Leaves and stems	16	-12.5 ± 0.6	3.5 ± 2.5
	Roots	3	-13.2 ± 1.0	1.9 ± 0.8

Table 2 (continued)

Growth form/taxon	Plant Part	<i>n</i>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
<i>Eragrostis</i> sp.	Leaves and stems	88	-12.5 ± 0.9	3.8 ± 2.2
	Roots	12	-13.0 ± 0.9	2.9 ± 1.7
	Seeds	1	-12	5
<i>Heteropogon contortus</i>	Leaves and stems	18	-11.6 ± 1.1	0.8 ± 1.3
	Roots	3	-11.3 ± 0.9	0.3 ± 1.7
<i>Panicum coloratum</i>	Leaves and stems	30	-12.2 ± 0.9	3.3 ± 1.9
	Roots	5	-12.1 ± 1.1	4.2 ± 0.9
	Seeds	3	-12.2 ± 0.3	5.8 ± 1.2
<i>Panicum maximum</i>	Leaves and stems	89	-12.3 ± 0.9	4.1 ± 2.5
	Roots	14	-12.6 ± 1.8	3.0 ± 2.3
	Seeds	7	-12.0 ± 0.5	4.4 ± 1.7
<i>Panicum repens</i>	Leaves and stems	7	-11.9 ± 0.7	2.4 ± 1.4
	Roots	2	-12.0 ± 0.3	2.8 ± 0.4
<i>Setaria</i> sp.	Leaves and stems	4	-10.5 ± 1.4	2.0 ± 0.6
	Roots	1	-11.6	2.6
<i>Themeda triandra</i>	Leaves and stems	31	-11.7 ± 1.4	1.5 ± 1.3
	Roots	7	-11.8 ± 2.4	2.3 ± 2.1
<i>Urochloa mossambicensis</i>	Leaves and stems	57	-11.9 ± 1.2	4.2 ± 2.2
	Roots	8	-12.3 ± 1.7	3.5 ± 2.2
All grass	Leaves and stems	510	-12.2 ± 1.0	3.5 ± 2.4
	Roots	83	-12.3 ± 1.4	2.9 ± 2.0
	Seeds	13	-11.9 ± 0.5	4.7 ± 1.6
C₃ reeds and sedges	Leaves and stems	78	-26.8 ± 1.5	6.6 ± 1.9
	Roots	14	-26.4 ± 1.5	5.0 ± 0.7
	Seeds	2	-23.7 ± 0.2	4.9 ± 1.6
C₄ reeds and sedges	Leaves and stems	15	-11.6 ± 1.7	5.0 ± 1.1
	Roots	8	-12.4 ± 1.3	4.1 ± 1.1
	Seeds	2	-11.8 ± 0.4	5.7 ± 0.3
C₃ geophytes	Leaves	2	-26.7 ± 0.2	5.5 ± 1.1
	Bulbs/corms	1	-26.1	3.9
C₄ geophytes	Leaves	2	-10.1 ± 0.3	5.1 ± 1.5
	Bulbs/corms	1	-9.8	4.7

Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are presented relative to the PDB and atmospheric N_2 standards, respectively, together with number of samples (*n*) and standard deviation (\pm).

flowers ($4.4 \pm 2.8\text{‰}$, $n = 20$; $p < 0.01$). Grass roots had significantly ($p < 0.05$) lower $\delta^{15}\text{N}$ values ($2.9 \pm 2.0\text{‰}$, $n = 83$) compared to seeds ($4.7 \pm 1.6\text{‰}$, $n = 13$). Grass leaves and stems had intermediate ^{15}N -content (combined mean = $3.5 \pm 2.4\text{‰}$, $n = 510$), but these overlapped considerably in $\delta^{15}\text{N}$ with grass roots and seeds.

3.4. Geographical and seasonal variations

To examine spatio-temporal variations in the isotopic composition of KNP plants, trees and forbs were combined into a single C₃-photosynthetic group, and

compared with C₄ grasses (Table 3). The carbon isotopic composition of C₄ grasses showed no significant ($p = 0.6573$) spatial variations within the KNP, with means varying from $-12.6 \pm 1.8\text{‰}$ (site 9) to $-12.0 \pm 1.2\text{‰}$ (sites 1 and 2). Mean $\delta^{13}\text{C}$ values of C₃ plants also did not vary significantly ($p = 0.3727$) across different geological substrates (basalts and granites), or between the higher and lower rainfall zones of the southern and northern KNP ($p = 0.5468$), respectively (Table 3; Fig. 6).

C₃ plants and plant parts growing in densely wooded areas, where exposure to solar radiation is lower, and atmospheric recycling of CO₂ is intensive, are expected to be relatively depleted in ¹³C (the “canopy effect”) [15,17,35]. In addition, lower plant $\delta^{13}\text{C}$ values are often associated with high rainfall, or at least with greater water availability [55,57]. Thus, one might have expected plants from the more arid northern KNP to be ¹³C-enriched compared to their southern counterparts. This was not the case. Furthermore, C₃ trees and forbs from the densely wooded areas around Punda Maria were

isotopically similar to plants collected elsewhere in the Park ($p = 0.1203$). It may be that canopy cover here, even though Punda Maria is one of the most heavily wooded regions of KNP, is insufficient for intensive recycling of CO₂. By contrast, C₃ plants obtained from the dry riverbed site (site 6; mean $\delta^{13}\text{C} = -27.4 \pm 1.4\text{‰}$, $n = 29$) and those from the densely wooded riparian site (site 9; $-28.2 \pm 1.3\text{‰}$, $n = 52$) were significantly ¹³C-depleted compared to plants from every other region of the KNP, where mean $\delta^{13}\text{C}$ was between -26.6 and -25.9‰ ($p < 0.01$ for site 6; $p < 0.0001$ for site 9; Fig. 6). Hence, while canopy cover and local precipitation did not influence C₃ plant $\delta^{13}\text{C}$, depleted values appeared to be associated with proximity to perennial water sources.

$\delta^{15}\text{N}$ values were similar in trees, forbs, and grasses, and geographical variations in plant $\delta^{15}\text{N}$ were similar for all plant types (Table 3; Fig. 6). Thus, for statistical comparison of plants from different regions of the KNP, trees, forbs, and grasses were treated as one combined group. High plant $\delta^{15}\text{N}$ values are expected to be

Table 3
Regional and seasonal differences in the carbon and nitrogen isotopic composition of KNP plants

Region	Plant group	Season	<i>n</i>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Southern granites	C ₃ trees and forbs	Dry	100	-26.1 ± 1.4	4.7 ± 2.7
		Wet	93	-26.7 ± 1.0	5.7 ± 2.5
	C ₄ grass	Dry	58	-11.8 ± 1.0	3.8 ± 1.9
		Wet	49	-12.5 ± 0.7	6.1 ± 2.4
Southern basalts	C ₃ trees and forbs	Dry	129	-26.6 ± 2.2	2.6 ± 2.2
		Wet	103	-26.6 ± 1.3	3.5 ± 1.5
	C ₄ grass	Dry	72	-11.2 ± 1.2	1.3 ± 1.5
		Wet	87	-12.6 ± 0.7	2.7 ± 1.3
Northern granites	C ₃ trees and forbs	Dry	27	-26.2 ± 0.8	2.8 ± 1.0
		Wet	32	-26.5 ± 1.2	3.2 ± 0.9
	C ₄ grass	Dry	35	-12.1 ± 1.4	1.6 ± 1.1
		Wet	37	-12.1 ± 0.8	3.1 ± 1.6
Northern basalts	C ₃ trees and forbs	Dry	12	-26.0 ± 0.8	6.0 ± 0.9
		Wet	18	-25.9 ± 1.1	5.5 ± 2.0
	C ₄ grass	Dry	12	-12.7 ± 0.6	4.9 ± 1.5
		Wet	15	-12.1 ± 0.9	5.8 ± 1.7
Punda Maria	C ₃ trees and forbs	Dry	15	-26.4 ± 0.9	3.2 ± 1.7
		Wet	27	-26.6 ± 3.4	3.1 ± 3.0
	C ₄ grass	Dry	19	-12.3 ± 0.4	1.9 ± 1.4
		Wet	20	-12.0 ± 0.9	2.5 ± 1.6
Dry riverbed	C ₃ trees and forbs	Dry	5	-27.3 ± 1.4	3.6 ± 1.3
		Wet	24	-27.6 ± 1.4	3.8 ± 2.6
	C ₄ grass	Dry	20	-12.0 ± 0.7	2.2 ± 1.6
		Wet	25	-12.8 ± 0.5	4.9 ± 1.8
Sodic site	C ₃ trees and forbs	Dry	20	-25.8 ± 1.1	6.9 ± 2.1
		Wet	22	-26.0 ± 1.7	6.6 ± 2.3
	C ₄ grass	Dry	22	-12.8 ± 0.7	5.9 ± 2.2
		Wet	22	-12.2 ± 0.7	6.4 ± 2.3
Dense riparian site	C ₃ trees and forbs	Dry	23	-28.7 ± 1.3	4.4 ± 1.6
		Wet	29	-27.8 ± 1.1	4.3 ± 2.3
	C ₄ grass	Dry	4	-13.7 ± 0.5	5.1 ± 0.5
		Wet	10	-12.2 ± 2.0	4.4 ± 1.0

The analysis is based on tree leaves, and leaves and stems of forbs and grass. The dry season is represented by June 2002 and 2003, and the wet season by January 2003 and February 2004. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are presented relative to the PDB and atmospheric N₂ standards, respectively, together with number of samples (*n*) and standard deviation (\pm).

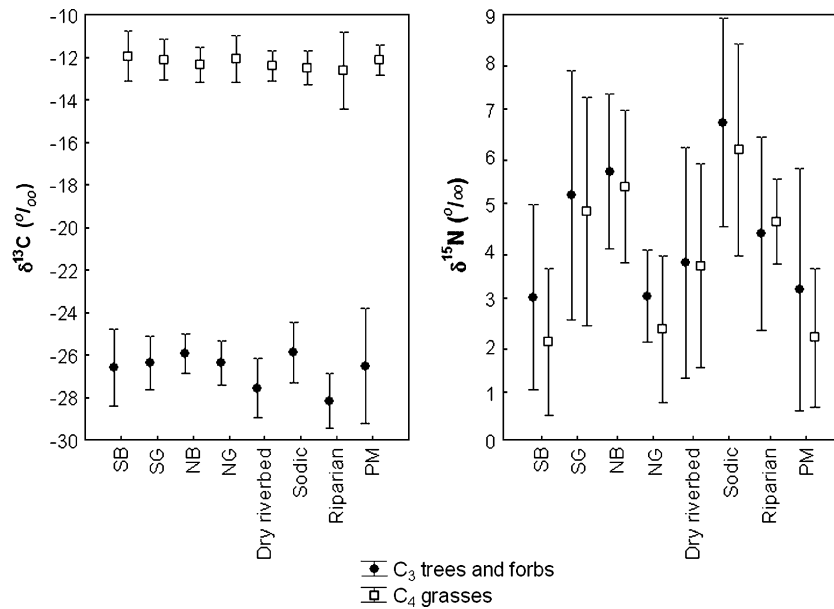


Fig. 6. Geographical differences in the isotopic composition of C₃ (trees and forbs) and C₄ (grasses) plants from sampling sites 1 through 9, 11, and 12, representing different microhabitats in KNP (SB = southern basalts, SG = southern granites, NB = northern basalts, NG = northern granites, Dry riverbed = Site 6, Sodic = Site 8, Riparian = Site 9, PM = Punda Maria). For the analysis, only tree leaves, forb leaves and stems, and grass leaves and stems were used. The symbols in the graph indicate the mean value and the bars represent the standard deviation.

associated with dry, sandy environments [24,37], as is the case for southern granites (mean $\delta^{15}\text{N} = 5.1 \pm 2.6\text{‰}$, $n = 300$) compared to the clay-based southern basalts ($2.6 \pm 1.9\text{‰}$, $n = 391$; $p < 0.0001$). However, plants from the dry, sandy northern granites ($2.7 \pm 1.4\text{‰}$, $n = 131$; $p < 0.0001$) had lower $\delta^{15}\text{N}$ compared to plants from northern basalts ($5.5 \pm 1.6\text{‰}$, $n = 57$). The highest $\delta^{15}\text{N}$ values were observed in plants collected from a sodic environment (site 8; $6.4 \pm 2.2\text{‰}$, $n = 86$; $p < 0.0001$ compared with other microhabitat types), as suggested previously by Heaton [24]. This complexity underscores the multi-factorial influences on nitrogen isotope balances in soils and plants.

Seasonal changes in plant isotopic composition were examined by comparing dry season (June) with wet season (January/February) data. Overall, C₃ tree and forb $\delta^{13}\text{C}$ values remained similar during both the seasons ($p = 0.0712$; dry season mean = $-26.5 \pm 1.8\text{‰}$, $n = 331$ and wet season mean = $-26.7 \pm 1.6\text{‰}$, $n = 348$; see Table 3). C₄ grasses were only slightly (but significantly, $p < 0.01$) ^{13}C -depleted during the wet season (dry season mean = $-11.9 \pm 1.2\text{‰}$, $n = 242$ and wet season mean = $-12.4 \pm 0.8\text{‰}$, $n = 265$). This does not mean that seasonal changes in plant $\delta^{13}\text{C}$ were always negligible. C₃ plants from the Skukuza Land System showed significantly lower ($p < 0.01$) $\delta^{13}\text{C}$ values during both wet seasons, being roughly 1.5‰ depleted compared to plants from the dry season (Fig. 7). C₄ grasses from both the Skukuza and Satara (Lower Sabie) Land Systems were between ~ 0.5 and

$\sim 1.5\text{‰}$ depleted in ^{13}C in the dry months compared to wet season plants ($p < 0.0001$). Interestingly, where significant temporal carbon isotopic shifts occurred in KNP plants, these were associated with rainfall, i.e. higher rainfall corresponded with lower $\delta^{13}\text{C}$ values (Fig. 7), as found by previous studies [55,57]. Nevertheless, overall KNP plant differences are smaller than previously reported seasonal and annual isotopic shifts in both trees and grasses ($\sim 1.0\text{‰}$) [20,21,25,36]. Our data incorporate a larger number of samples from a wider array of taxa than most of the earlier studies. The implication is that some plant species, growing under certain conditions, may indeed show significant seasonal shifts in isotopic composition, but comparisons of numerous taxa from a range of microhabitats minimizes the effects of seasonal changes within a single species or habitat type.

Overall, KNP vegetation increased significantly ($p < 0.0001$) in ^{15}N -content from the dry to the wet season (see Table 3). C₃ tree and forb mean $\delta^{15}\text{N}$ was $3.8 \pm 2.5\text{‰}$ ($n = 331$) during the dry season and $4.4 \pm 2.4\text{‰}$ ($n = 348$) during the wet season, while grass values were $2.7 \pm 2.2\text{‰}$ ($n = 242$) and $4.1 \pm 2.3\text{‰}$ ($n = 265$), respectively. At a regional scale, significant temporal shifts were observed for $\delta^{15}\text{N}$ of C₃ plants from the Skukuza and Satara/Lower Sabie Systems ($p < 0.001$), and of C₄ plants from the Skukuza, Lower Sabie, and Phalaborwa/Shingwedzi Systems ($p < 0.001$, 0.0001, and 0.0001, respectively) (Fig. 8). These shifts were in the order of ~ 1.5 – 2.0‰ for C₃ plants, and ~ 2.0 – 3.0‰ in the case of C₄ grass. As was the case with

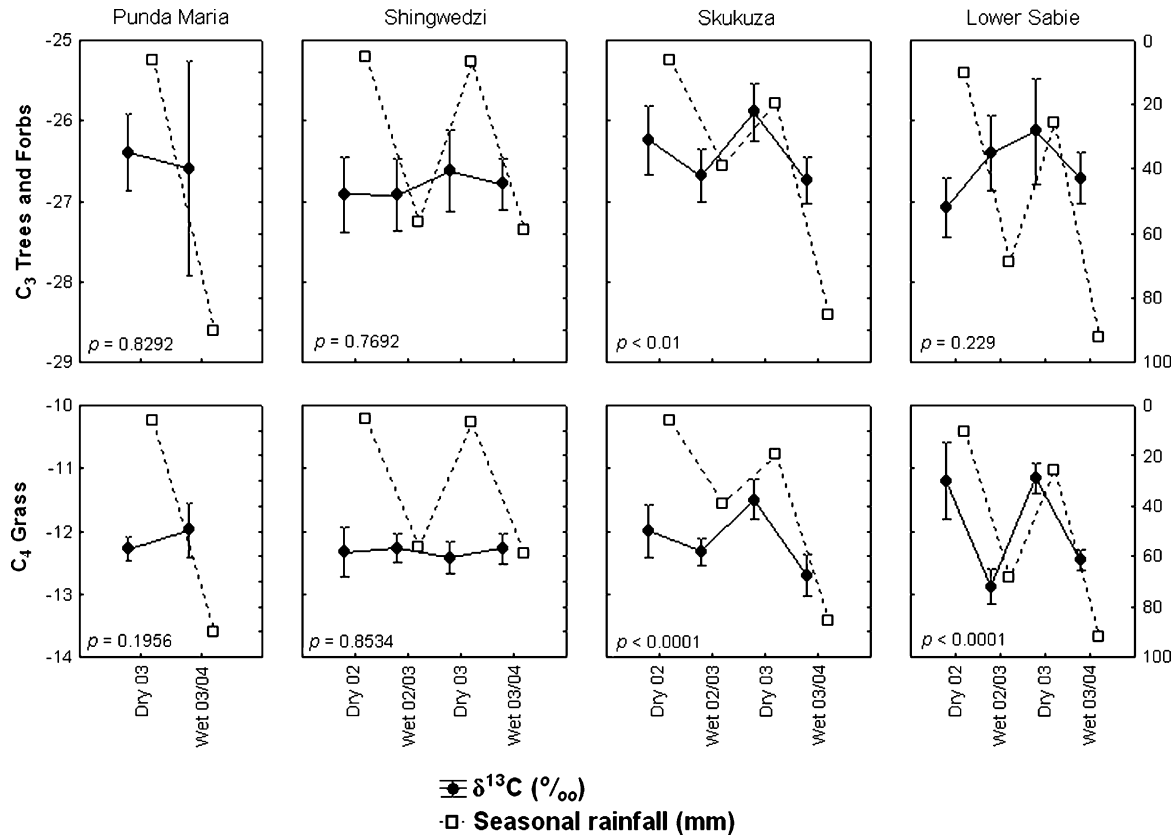


Fig. 7. Temporal patterns in the carbon isotopic composition of KNP plants in relation to seasonal rainfall, including ANOVA p -values (significance level 0.05) for differences between temporally separated $\delta^{13}\text{C}$ means. Monthly rainfall is treated in terms of season (dry = April to September, wet = October to March) for each year. For the analysis, only tree leaves, forb leaves and stems, and grass leaves and stems were used. Symbols indicate mean values, and the bars represent the $\pm 95\%$ confidence intervals. Note that the rainfall axis (right y -axis) is inverted to illustrate the pattern of decreasing $\delta^{13}\text{C}$ with decreasing rainfall in cases where seasonal shifts were significant.

$\delta^{13}\text{C}$, seasonal shifts in plant $\delta^{15}\text{N}$ corresponded with seasonal rainfall, although in this instance, higher rainfall corresponded with higher $\delta^{15}\text{N}$ values (Fig. 8; see also [24]). Plants from the Pafuri System (Punda Maria) showed no significant seasonal changes in $\delta^{15}\text{N}$, possibly because of relatively low rainfall in this region during the study period (Fig. 2).

4. Discussion

4.1. Variations in plant $\delta^{13}\text{C}$: implications for C_3 and C_4 diets

Typically, a C_3 diet is expected to have a carbon isotopic composition averaging around -27‰ , and a C_4 diet averages $\sim -12.5\text{‰}$ [8,30,62]. Plants from KNP have similar values, and deviations from these means were very small, given the range of species, plant parts, and microhabitats studied. Overall, carbon isotopic variations based on species, anatomical, geological, climatic, and seasonal differences were less than 2‰ for C_3 trees and forbs, and even smaller (less than 1‰) for C_4 grasses (Table 4).

Previous studies have reported somewhat larger variations in plant isotopic composition, especially in the case of C_3 plants growing in different habitats. In high rainfall areas, or those with very dense canopy cover, C_3 plant $\delta^{13}\text{C}$ values range between -34 (in dense forests) and -27‰ [20,35,55], while in very arid environments these values range between -26 and -20‰ [13,14]. Such large deviations in mean $\delta^{13}\text{C}$ are clearly not evident within this semi-arid savanna biome, where mean annual rainfall varies only between ~ 400 and 700 mm. The implication here is that the carbon isotopic composition of savanna animal diets are unlikely to be significantly influenced by climatic variation in the $\delta^{13}\text{C}$ values of local vegetation.

Overall, vegetation data from KNP suggest that caution should be exercised in interpreting carbon isotopic differences of less than 2‰ between animal groups (at least those with predominantly C_3 -based diets) as diets differing in C_3/C_4 content (see also [25]). For instance, two diets including similar proportions of C_3 and C_4 foods may differ by up to 2‰ in $\delta^{13}\text{C}$, which is equivalent to an error of $\sim 10\text{--}15\%$ in terms of percentage C_4 consumed. In reality, this variation is likely to be much reduced because animals tend to feed

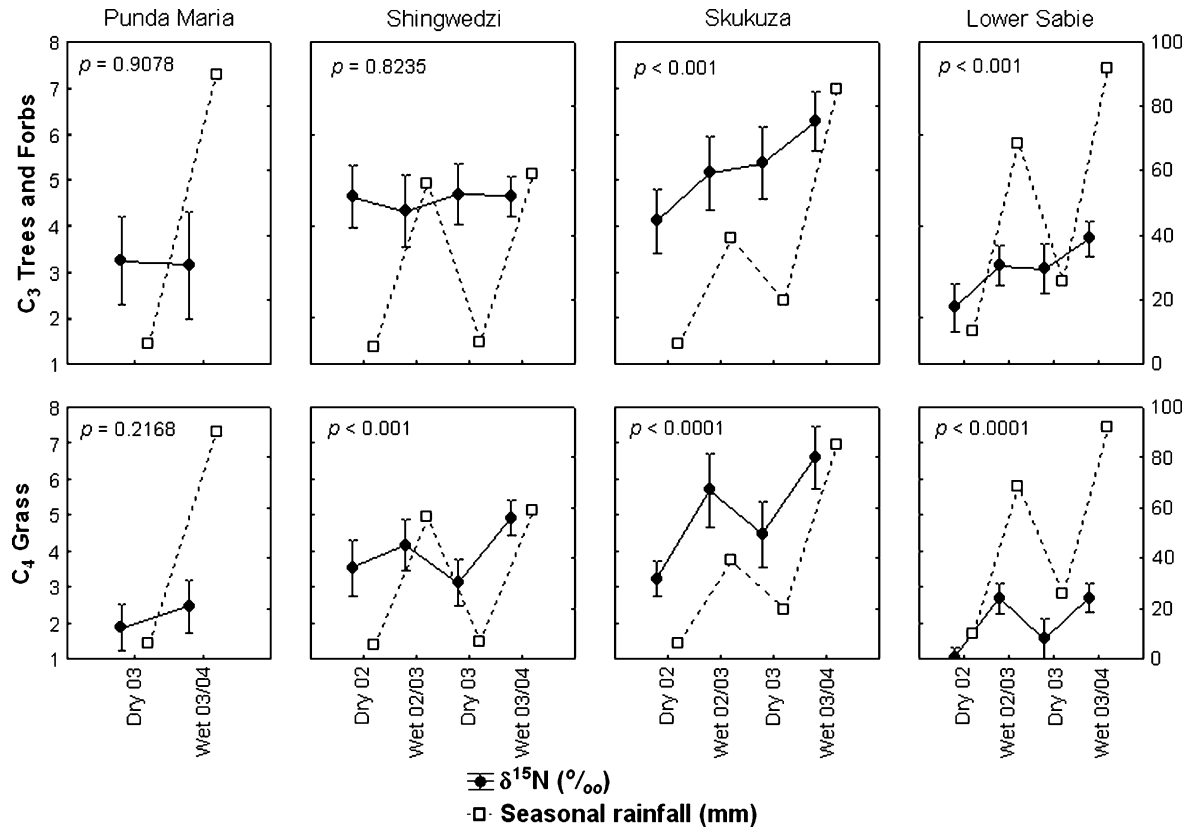


Fig. 8. Temporal patterns in the nitrogen isotopic composition of KNP plants in relation to seasonal rainfall, including ANOVA p -values (significance level 0.05) for differences between temporally separated $\delta^{15}\text{N}$ means. Monthly rainfall is treated in terms of season (dry = April to September, wet = October to March) for each year. For the analysis, only tree leaves, forb leaves and stems, and grass leaves and stems were used. Symbols indicate mean values, and the bars represent the $\pm 95\%$ confidence intervals.

on a variety of plant species in order to maximize nutrient uptake and overcome the physical and chemical defense mechanisms of plants [11,12,40]. Differentiation between real (but subtle) dietary differences and environmentally-induced variations in plants may then only present problems when dealing with taxa that forage intensively in riverine or wetland areas (which were ^{13}C -depleted compared to other regions of KNP) or whose diets vary substantially in plant parts consumed. For example, small variations in plant parts will probably not be discernible in animal $\delta^{13}\text{C}$ values, but major differences, such as pure frugivory versus pure folivory, may well be. This observation prompts other promising insights into diet. It may be possible, for example, to make carbon isotopic distinctions between folivores and frugivores, provided that the data were combined with other methods for assessing relative levels of leaf and fruit intake, such as dental morphology or dental microwear [58]. Furthermore, as browsers shift their diets throughout the seasonal cycle, say from leaves to fruits and flowers (as these become available), we might expect corresponding shifts in the animal's body $\delta^{13}\text{C}$ that could possibly be detected in high resolution studies of incrementally calcified tissues.

At the C_4 end of the scale, shifts in plant carbon isotopic composition are more subtle, and thus even less likely to have a major effect on dietary interpretations. Small differences ($\sim 0.6\%$ during both the wet and dry seasons) were observed between grasses following different C_4 sub-pathways; NAD-ME and PCK taxa were ^{13}C -enriched compared to NADP-ME grasses (Fig. 5, Table 4). In East Africa savannas, somewhat larger (~ 1.0 – 1.5%) differences have been reported between these grass types [8]. Cerling and Harris [8] suggested that this isotopic distinction would be observed in the tooth enamel of grazers feeding in different areas, i.e. grazers from mesic areas, where grasses were mostly NADP-ME, should show higher enamel $\delta^{13}\text{C}$ values compared to animals that grazed in xeric habitats, where NAD-ME and PCK grasses predominated. The smaller variation in KNP grass $\delta^{13}\text{C}$ for different subtypes shown in our study likely reflects our focus on large numbers of samples from a single area, and that all C_4 sub-types were represented at each sampling locality. It seems improbable that isotopic distinctions between differential utilization of NAD/PCK or NADP-ME grasses could be made within a single habitat, where the difference between these grass types is less than 1% . Thus, isotopic differences between grazing

Table 4
Principle environmental determinants of carbon and nitrogen isotopic variations in plants from KNP

Environmental influence	C ₃ trees and forbs	$\Delta_{\text{Mean}} (\text{‰})$		C ₄ grasses	$\Delta_{\text{Mean}} (\text{‰})$	
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Anatomical	Leaves	-0.2	+0.1	Leaves/stems	+0.0	+0.3
	Fruits	+1.5*	-0.5	Seeds	-0.1	+0.9
	Flowers	+1.5*	+0.4*			
	Bark	+1.4*	-1.9*	Roots	+0.3	-0.9*
Taxonomic	Species differences	-1.0 to +0.8*	-1.7 to +1.3*	NAD-ME	-0.2	-0.1
	Legumes		-0.8*	PCK	-0.1	+0.7*
	Non-legumes		+0.2	NADP-ME	+0.4*	-1.4*
Geographical	Basalt, south	+0.0	-1.5*	Basalt, south	+0.2	-1.7*
	Granite, south	+0.2	+1.0*	Granite, south	+0.1	+1.0*
	Basalt, north	+0.7	+1.4*	Basalt, north	-0.2	+2.0*
	Granite, north	+0.2	-1.4*	Granite, north	+0.1	-1.4*
	Punda Maria	+0.1	-1.4*	Punda Maria	+0.1	-1.6*
	Sodic substrate	+0.7	+2.3*	Sodic substrate	-0.3	+2.3*
	Dry riverbed	-1.0*	-0.4	Dry riverbed	-0.2	+0.8
	Riparian	-1.6*	+0.3	Riparian	-0.4	-0.1
Seasonal	Dry	+0.1	-0.3*	Dry	+0.3*	-0.9*
	Wet	-0.1	+0.3*	Wet	-0.2*	+0.3*

The magnitude of variation is represented by Δ_{mean} , denoting the difference between the mean $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ value of the respective plant growth form (given in brackets in the header row) and that of the particular variable examined. Asterisks (*) illustrate statistically significant differences between these respective means (Tukey's HSD, $p < 0.05$).

taxa more likely reflect differential habitat use (i.e. xeric versus mesic grasslands), or small shifts to incorporate some C₃ plants during certain seasons, rather than consumption of grasses with different photosynthetic sub-pathways.

The relatively small seasonal shifts (less than 1‰) observed in the carbon isotopic composition of KNP plants indicate minimal influence of seasonal variations on diet interpretation. Heaton [25] asserted that such small seasonal differences in $\delta^{13}\text{C}$ may be an artificial result arising from difficulties in obtaining plant samples containing only the carbon fixed during a particular season, since cell solubles presumably have faster turnover rates than fibrous cell walls. While this may be true, most herbivores are well-equipped to digest cellulose and other resistant plant tissues, at least to some extent, so it seems unlikely that herbivores would eat and digest only those plant parts with a seasonally-specific carbon isotopic signal. Hence our data on seasonal $\delta^{13}\text{C}$ variation in plants is likely a reasonable reflection of what would be digested by herbivores and ultimately reflected in their tissues.

A potential caveat to diet studies is that CAM plants mimic C₄ plant $\delta^{13}\text{C}$ values. However, these plants, such as succulents, are seldom deemed important food sources for savanna herbivores. Reeds and sedges can be either C₃ or C₄, and may thus complicate diet studies in areas where these form important food sources for certain taxa. Our data, and previously published data for herbarium specimens from the same region [56], indicate that reeds (88% C₃) and sedges (75% C₃)

should be regarded as principally C₃-based food sources, at least in a South African savanna such as the KNP.

4.2. Significance of plant $\delta^{15}\text{N}$ variation

Geographic separation, associated with soil type and climate, appear to be the principal mechanism controlling nitrogen isotopic composition of KNP plants, influencing mean $\delta^{15}\text{N}$ by up to 4‰ (Table 4). It is unclear what the implications of these trends might be on dietary studies, because animal $\delta^{15}\text{N}$ values do not simply reflect the patterns of the plants they eat; a 2–4‰ stepwise enrichment in ^{15}N appears to exist along different trophic levels of the food chain [2,47,51,54]. Variations, sometimes of a greater magnitude than the trophic level effect, occur within trophic levels, due to processes of nitrogen/protein metabolism that are affected by climate, digestive physiology, and protein intake [2,4,51,54]. Nevertheless, it is likely that the distribution of ^{15}N within foodwebs relies primarily on local vegetation [22]. For example, all animals, regardless of dietary preferences and digestive physiology, from arid and/or sandy environments might be expected to have higher (but not necessarily consistently higher) $\delta^{15}\text{N}$ values in comparison with animals living in wetter areas or on clay-based soils. Animal $\delta^{15}\text{N}$ values may even be different between individuals/species that forage intensively on sodic patches compared to animals feeding elsewhere.

Low animal $\delta^{15}\text{N}$ values have previously been ascribed to the consumption of leguminous plants, such as *Acacia* spp. by baboons (*Papio* spp.; [4]) and members

of the Caesalpinioideae by chimpanzees (*Pan troglodytes*; [48]). Our data for legumes suggest that such interpretations should be made with caution, since important dietary species like *Acacia* and *C. mopane* had relatively high $\delta^{15}\text{N}$ values compared to other legumes.

The most fruitful contributions of nitrogen isotopic data to ecological studies are made when $\delta^{15}\text{N}$ is analyzed in conjunction with carbon isotopic data [22,48,49]. This also appears to be the case amongst KNP savanna plants; in spite of the complexity of ^{15}N abundances, $\delta^{15}\text{N}$ with $\delta^{13}\text{C}$ in combination show taxonomic and anatomical patterning. For example, C_3 vegetation (trees and forbs) from KNP varied anatomically (by $\sim 2\text{‰}$) and taxonomically (by $\sim 3\text{‰}$), in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Table 4). These observations might enable distinctions between diets based on different plant parts, under certain circumscribed conditions. Similarly, combined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis of tissues of grazers may assist by distinguishing the subtle differences between animals utilizing grasses following different C_4 sub-pathways. A further scenario could be devised from the relative ^{15}N -enrichment of succulent plants. High animal $\delta^{15}\text{N}$ associated with enriched $\delta^{13}\text{C}$ values might be evidence of extensive utilization of CAM-photosynthesizing succulents, although this may be rare in savanna systems.

Overall, plant $\delta^{15}\text{N}$ values vary greatly depending on a number of environmental and physiological factors, which often have a combined effect on isotopic discriminations [22,43, this study]. The complexity shown in these trends means that future studies of $\delta^{15}\text{N}$ in both archaeological and modern contexts require improved understandings of ^{15}N abundances in plants from different kinds of environments.

Previous stable carbon and nitrogen isotopic comparisons of plants from a range of habitats have described larger variations, based on environmental factors such as local precipitation and canopy cover, than observed here. That predicted patterns are in many instances different (and of a smaller magnitude) within this savanna biome is a clear indication that isotopic studies of diet and palaeodiet need to be based on habitat-specific knowledge. In KNP, real isotopic differences do indeed exist between taxa, microhabitat, etc., but these are relatively small in this seasonal system with a significant rainfall gradient. Isotopic studies of diet should use general trends described from comparisons of rainforests and xeric environments with caution, as the trends are not necessarily the same at the habitat-specific scale.

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