



Seasonal variations in nutrients and secondary metabolites in semi-arid savannas depend on year and species



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ABSTRACT

Semi-arid savannas are characterised by alternating wet and dry seasons and large inter-annual rainfall fluctuations that affect plant growth. Carbon-based secondary metabolites (CBSMs) vary inversely with growth and nutrients because of the physiological trade-off between cellular growth and differentiation. We predicted that (1) nutrient concentrations decrease during the wet season, (2) CBSM concentrations increase during the wet season and (3) nutrient concentrations are lowest and CBSM concentrations are highest in the dry season. We measured nitrogen, phosphorus, total polyphenols and condensed tannins in six woody species (including one evergreen) seasonally at the Nkuhlu enclosure, Kruger National Park, South Africa, for three consecutive years, including one 'wet' year (above-average rainfall) and two 'dry' years (below-average rainfall). Neither N nor P consistently decreased during wet seasons, while CBSMs did not consistently increase. Neither N nor P in the evergreen species was consistently lowest in dry seasons, while CBSMs were not consistently highest in dry seasons. We discuss the inconsistent responses in the context of species-specificity and high inter-annual rainfall variation. We conclude that seasonal variations in N, P and CBSMs in semi-arid savannas cannot be easily generalised because they depend on species and year.

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1. Introduction

Savannas are characterised by alternating wet and dry seasons that each last several months (Kutsch et al., 2008). Most subtropical savannas in Africa are semi-arid and water is the main limiting resource for plant growth for most of the year (Scholes et al., 2003; Jacobs et al., 2007). Nitrogen is most available early in the wet season when elevated soil water drives mineralization (Scholes et al., 2003). Plant growth is therefore intermittent and occurs for only brief periods, mainly early in the wet season (Williams et al., 2009). However, semi-arid savannas also experience large, inter-annual fluctuations in the timing and amount of rainfall that

affect plant growth (Wiegand et al., 2006). When water and nutrients are abundant, e.g., early in the growing season, then woody species may be expected to have fast growth rates, high nutrient concentrations and low secondary metabolite concentrations (Herms and Mattson, 1992; Martz et al., 2010). Nutrients, such as N and P, contribute to a plant's overall value for mammal herbivores (Mårell et al., 2006) and their concentrations generally decrease during the growing season (Lesage et al., 2000; Nahm et al., 2007; Cernusak et al., 2011) and are generally lower in years with below-average rainfall (Nahm et al., 2007).

Carbon-based secondary metabolites (CBSMs), such as tannins and polyphenols, are ecologically important compounds in plants for various reasons, e.g., as protection against herbivores, pathogens or UV radiation (Skarpe and Hester, 2008; Tharayil et al., 2011). Their roles in plant–herbivore interactions include

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reducing plant palatability and intake or digestion by herbivores (Basha et al., 2013) and reducing internal parasite loads in herbivores (Copani et al., 2013). In leaf litter, CBSMs are a major source of carbon for soils and influence litter decomposition and nutrient cycling (Tharayil et al., 2011). Therefore, CBSMs are important components of ecosystems because of their roles in mediating vital ecological processes (Iason et al., 2012). Increased knowledge on how nutrient and CBSM concentrations vary seasonally and annually could contribute to understanding variations in herbivore populations and nutrient cycling (Covelo and Gallardo, 2001; Tharayil et al., 2011). High nutrient or low CBSM concentrations early in the wet season could compromise plant fitness, but may benefit herbivores.

Our aim was to determine the variations in nutrient and CBSM concentrations in foliage of abundant woody species in a semi-arid savanna, as affected by season and year. The abundance of mammal herbivores in Africa is high compared to other continents (Fritz and Loison, 2006), but the extent to which mammal herbivores directly influence nutrient and CBSM concentrations in woody plants in African savannas is unknown. Hence, our scale of interest was the foliage available for large, mammal herbivores, rather than individual leaves, and our framework was a basic mechanistic one as follows. Concentrations of CBSMs are assumed to be inversely related to growth and therefore are expected to increase when growth rate is relaxed late in the growing season. Surplus C accumulates and becomes available for production of CBSMs (Covelo and Gallardo, 2001; Gayler et al., 2007; Tharayil et al., 2011) because they are biochemical end-products (Riipi et al., 2002; Martz et al., 2010). However, the magnitude and timing of fluctuations in both nutrient and CBSM concentrations is species-specific and some species express minimal changes (Mårell et al., 2006; Cella-Pizarro and Bisigato, 2010; Narvaez et al., 2010; Basha et al., 2012).

We predicted that (1) nutrient (N and P) concentrations decrease during the course of the wet season, (2) CBSMs, measured as total polyphenol (TP) and condensed tannin (CT) concentrations, increase during the course of the wet season, and (3) nutrient concentrations are lower and CBSM concentrations are higher in the dry season, when nutrients are less available and the risk of herbivory by mammals is greater because of forage depletion, than in the wet season. We also expected that seasonal changes would be influenced by the year in which sampling occurred, because of inherently high inter-annual variability. We tested our hypothesis by sampling five deciduous and one evergreen species in Kruger National Park, South Africa, for three consecutive years. Including several species in the study allowed species-specific patterns to be explored. Sampling over three years allowed inter-annual variability to be considered. In particular, the study duration provided an opportunity to interpret our results in terms of annual variation in one of the strongest drivers of semi-arid vegetation, namely, rainfall. Our approach was at a scale that is useful for the management of extensive areas used for either biodiversity conservation or livestock production (Levick and Rogers, 2008).

2. Materials and methods

2.1. Study area and species

The study area comprised 30 ha at Nkuhlu (24° 58' S, 31° 46' E) directly east of the Sabie River in Kruger National Park, South Africa. The vegetation in the area was protected from fire and large herbivores by a firebreak and a fence since 2002, which allowed the study of intra- and inter-annual changes to be unaffected by either browsing or fire. The fence was designed to exclude mammal herbivores such as impala (*Aepyceros melampus*), African elephant

(*Loxodonta africana*), hippo (*Hippopotamus amphibius*), black rhino (*Diceros bicornis*), white rhino (*Ceratotherium simum*), blue wildebeest (*Connochaetes taurinus*), Cape buffalo (*Syncerus caffer*), plains zebra (*Equus quagga*), greater kudu (*Tragelaphus strepsiceros*), steenbok (*Raphicerus campestris*), giraffe (*Giraffa camelopardalis*) and scrub hare (*Lepus saxatilis*). Elephant, impala, black rhino, kudu, steenbok, giraffe and hares include substantial woody plant material in their diets.

The climate of the study area is semi-arid subtropical with two broadly distinct seasons: a hot, occasionally wet, growth season (from October/November to April), and a warm, dry, non-growing season (Williams et al., 2009). Mean annual rainfall at Skukuza, 30 km west of Nkuhlu, is ~550 mm. Average daily temperatures at Skukuza are 15.7 °C in June and 26.6 °C in January. Average minimum temperature in June is 5.7 °C and average maximum temperature in January is 32.6 °C. Altitude ranges from 200 to 230 m above mean sea level.

The study area included the complete sequence of terrain morphology from footslope to crest in undulating landscape derived from granite. The crests and middle slopes were characterised by shallow, sandy, coarse soil overlying rock and the footslopes below the seepline were characterized by deep, sodic, duplex soil, which is a typical pattern on catenas in granite-derived, semi-arid landscapes (Khomu and Rogers, 2005). Vegetation on sodic soils is sparse and regarded as more nutritious than crest vegetation for large herbivores, especially grazers and mixed feeders (Levick and Rogers, 2008). Abundant woody species included *Acacia grandicornuta* and *Euclea divinorum* on the footslopes, and *Dichrostachys cinerea*, *Acacia exuvialis*, *Combretum apiculatum* and *Grewia flavescens* on the middle slopes and crests (Siebert and Eckhardt, 2008).

A. exuvialis Verdoorn and *A. grandicornuta* Gerstner (both Mimocaceae) are deciduous, leguminous trees with bi-pinnate microphyllous leaves and long straight spines in pairs at the nodes. *D. cinerea* (L.) Wright and Arn. subsp. *africana* Brennan and Brummitt (hereafter referred to as *D. cinerea*) (Mimocaceae) is a deciduous, leguminous, multi-stemmed large shrub with bi-pinnate microphyllous leaves and stem-spines (short shoots that have sharp tips). *C. apiculatum* Sond. (Combretaceae) and *G. flavescens* Juss. (Tiliaceae) are deciduous and simple-leaved, but *C. apiculatum* is a tree, while *G. flavescens* is a many-stemmed large shrub. *E. divinorum* Hiern (Ebenaceae) is an evergreen, simple-leaved, many-stemmed, large shrub.

2.2. Sampling

Unlike woody plant growth in temperate and boreal forests, growth in semi-arid subtropical savannas occurs in recurrent and unpredictable flushes. Therefore, the start, middle and end of the wet season can vary by several weeks between one year and another. Most leaf growth of woody species in the study area occurred at the start of the wet season, although additional, smaller flushes occurred at other times (Scogings et al., 2013a,b). We sampled *A. grandicornuta*, *D. cinerea*, *C. apiculatum* and *G. flavescens* during three stages of each wet season (start of the wet season in late November/early December, middle of the wet season in late January/early February and end of the wet season in late March/early April) for three successive wet seasons (2005/2006, 2006/2007 and 2007/2008). We also sampled *A. exuvialis* and *E. divinorum* at each stage of two wet seasons (2005/2006, 2006/2007), but not in the third wet season because of practical constraints. Furthermore, *E. divinorum* was sampled in two dry seasons (2005 and 2007). In the 2005/2006 wet season, rainfall was consistently above average, but rainfall was below average in the latter half of the 2006/2007 and 2007/2008 wet seasons (Fig. 1).

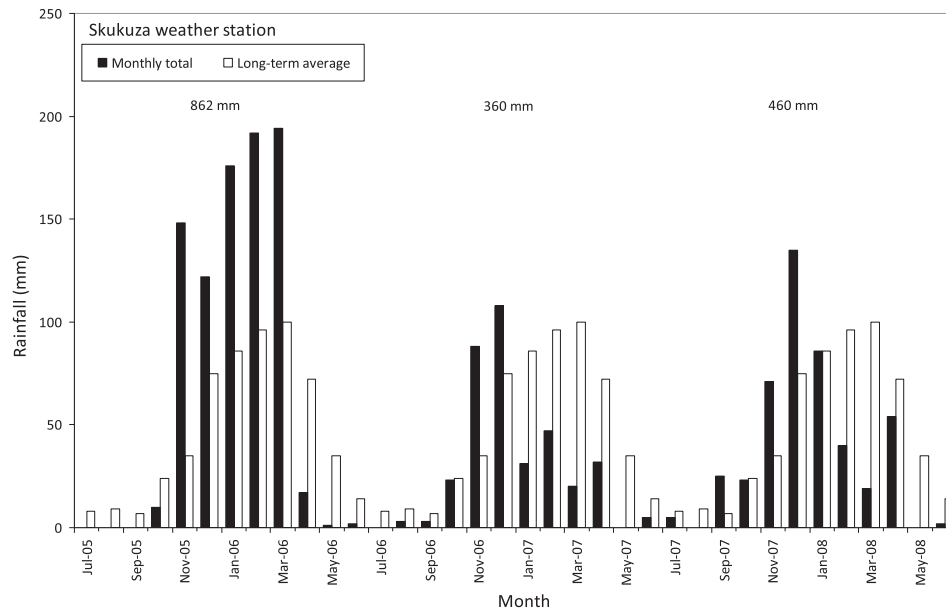


Fig. 1. Monthly rainfall at Skukuza during the study. Long-term mean annual rainfall at Skukuza is ~550 mm. For comparison, long-term average monthly rainfall is also shown. Total annual rainfall for each 'climatic' year (July–June) is indicated above the bars.

A 30×30 m grid superimposed on a LIDAR image of the study area was used to randomly locate 12 sites on each sampling date. We sampled at different sites within the study area to avoid re-sampling individual trees, thus avoiding confounding effects of destructive sampling. While the effect of sample site could confound the effects of sampling date, the scale of the study was the average responses of abundant woody species in the landscape. One plant of each study species was sampled in each grid cell (six for species inhabiting crests/midslopes and six for species on footslopes) on condition that the plant was (1) closest to the grid-cell centre, (2) taller than 2 m (1.5 m for *G. flavescens*), and (3) neither obviously stressed by disease, disturbance or neighbours nor obviously growing in a nutrient enriched patch. The minimum height of selected *G. flavescens* plants was 1.5 m because it seldom grows above 2 m. If any of the conditions failed to be met for the closest tree, then the next closest tree that met the conditions was sampled. No tree was sampled if the closest suitable tree was further than 15 m from the grid-cell centre to avoid infringement of neighbouring cells.

Leaf samples were collected from each plant by clipping branches 3–8 mm in diameter from the northern, sunlit half of the canopy between 1.5 and 2.2 m above ground, which was within the range of dominant browsers in the study area. For *G. flavescens*, the lowest permissible height for collection was reduced to 1.0 m because of its low stature. Branches were collected 1–4 h after sunrise. Mature (fully expanded) leaves were removed from the branches and leaves that were clearly damaged by insects were discarded to reduce any confounding effects of insect herbivory. A fresh mass of 30–50 g was thus obtained for drying in an oven for 24 h at 60 °C. Dried leaf samples were milled to pass a sieve with 1.0 mm apertures and were analysed for concentrations of condensed tannins according to the acid-butanol assay (Porter et al., 1986), which is widely used to quantify extractable condensed tannins (Hagerman, 2002). *Sorghum* tannin (ST) was used as the standard and results were expressed as mg ST equivalents g^{-1} dry matter. We also determined total polyphenols ([TP]) (Price and Butler, 1977), Kjeldahl-N ([N]) (AOAC, 1990) and phosphorus ([P]) (Murphy and Riley, 1962). All concentrations were expressed in mg g^{-1} dry mass. [CT] and [TP] were expressed as

Sorghum tannin equivalents (STE) and gallic acid equivalents (GAE), respectively. The use of *Sorghum* tannin as a standard may underestimate CT concentrations compared with other standards, but *Sorghum* tannin seems to be generally better than other commercially available tannins (Hattas and Julkunen-Tiitto, 2012). We note that changes in specific CBSM compounds that may have bioactive properties relevant to mammal browsers in savannas may have been obscured by the use of general assays.

2.3. Data analysis

Data were analysed separately for each species because we expected responses to be species-specific (Scogings et al., 2011). Plants were replicates. Data were transformed to $\log_{10}(x)$ when this normalised the data. The ANOVA model containing the interaction between season and year was tested for [N], [P], [TP] and [CT] of each species, assuming a completely randomised design and type III sums of squares for unequal sample sizes. Tukey's HSD test was used to separate the means. Significance was declared when $P < 0.05$, while possible trends were not ignored when $0.10 > P > 0.05$.

3. Results

Notwithstanding effects of time, species varied substantially in terms of measured chemical variables (Figs. 2–5). Regarding prediction 1, there was a significant interaction between the effects of season and year on [N] in *A. exuvialis* ($F_{2,32} = 4.57$; $P = 0.018$), *A. grandicornuta* ($F_{4,59} = 4.60$; $P = 0.003$), *C. apiculatum* ($F_{4,62} = 14.79$; $P < 0.001$), *D. cinerea* ($F_{4,64} = 6.33$; $P < 0.001$) and *G. flavescens* ($F_{4,63} = 3.92$; $P = 0.007$) (Fig. 2). Decreasing [N] during the wet season was observed in *A. exuvialis* in only one year. In *A. grandicornuta* [N] did not significantly decrease from start to end of the wet season. In *C. apiculatum* and *G. flavescens* [N] decreased during only two of the three wet seasons studied. In both *C. apiculatum* and *D. cinerea*, [N] reached its lowest levels in 'dry' years. Season significantly affected [N] in *E. divinorum* ($F_{3,49} = 5.30$; $P = 0.003$), which generally reached high levels at the end of the wet season.

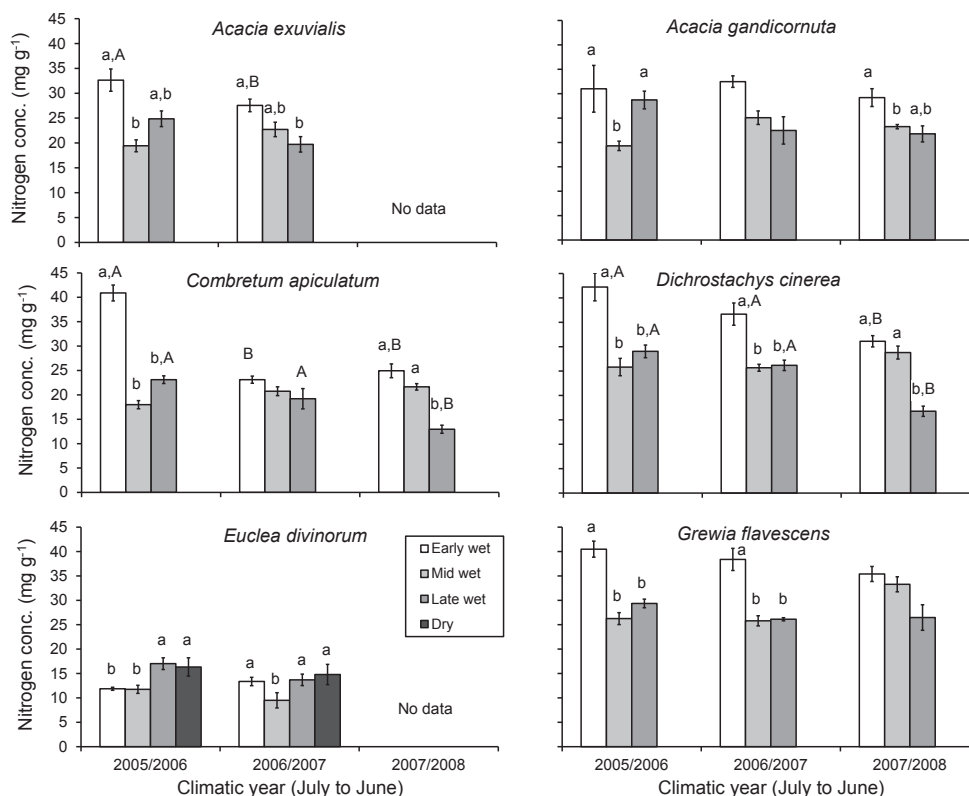


Fig. 2. Mean nitrogen concentration (mg g^{-1}) in leaves of *Acacia exuvialis* ($n = 3, 6, 6, 12, 6, 5$), *Acacia grandicornuta* ($n = 3, 6, 6, 12, 5, 2, 12, 18, 4$), *Dichrostachys cinerea* ($n = 3, 6, 6, 12, 5, 6, 12, 17, 6$), *Combretum apiculatum* ($n = 3, 6, 6, 12, 6, 6, 12, 17, 3$), *Grewia flavescens* ($n = 3, 6, 6, 12, 6, 6, 12, 16, 5$) and *Euclea divinatorum* ($n = 6, 6, 6, 9, 12, 6, 6, 6$) at Nkuhlu, Kruger National Park, during different seasons. Leaves were sampled between 1.5 and 2.2 m (1.0–1.5 m on *G. flavescens*). Letters indicate significant differences ($P < 0.05$) among seasons within year (lower case) and among years within season (upper case). The same letters, or none, indicate no significant differences. Error bars are standard errors of the means.

The effects of season and year on [P] interacted significantly in *A. grandicornuta* ($F_{4,59} = 22.45$; $P < 0.001$), *D. cinerea* ($F_{4,65} = 9.46$; $P < 0.001$), *C. apiculatum* ($F_{4,61} = 8.48$; $P < 0.001$), *G. flavescens* ($F_{4,63} = 24.68$; $P < 0.001$) and *E. divinatorum* ($F_{3,49} = 8.77$; $P < 0.001$) (Fig. 3). In *A. exuvialis* [P] was significantly affected by year ($F_{1,32} = 61.71$; $P < 0.001$). In all species [P] generally reached its lowest levels in 'dry' years. In *D. cinerea*, *C. apiculatum* and *G. flavescens* [P] did not increase during the wet season, but generally decreased or remained unchanged, while *E. divinatorum* [P] increased in the wet season in one year.

Giving support to prediction 2, *A. exuvialis* [TP] tended to decrease during the wet season ($F_{2,32} = 3.12$; $P = 0.058$) (Fig. 4). In *G. flavescens* [TP] was significantly affected by season ($F_{2,63} = 12.59$; $P < 0.001$) and year ($F_{2,63} = 21.59$; $P < 0.001$), such that [TP] increased during the wet season in the 'wet' year, but did not consistently reach its lowest levels in 'dry' years. A significant interaction between season and year was observed for [TP] in *A. grandicornuta* ($F_{4,59} = 3.39$; $P = 0.015$), *D. cinerea* ($F_{4,64} = 6.33$; $P < 0.001$), *C. apiculatum* ($F_{4,62} = 18.84$; $P < 0.001$) and *E. divinatorum* ($F_{3,49} = 24.55$; $P < 0.001$). In *A. grandicornuta* and *C. apiculatum* [TP] did not increase during the wet season. In *D. cinerea* [TP] increased during the wet season in a 'dry' year. In *E. divinatorum* [TP] did not change during the wet season, but changed between the wet and dry seasons in one year (see details below).

In *A. exuvialis* [CT] did not change during the wet season ($P > 0.05$) (Fig. 5). In *A. grandicornuta* [CT] tended to decrease during the wet season ($F_{4,58} = 2.97$; $P = 0.059$). The effects of season and year on [CT] interacted significantly in *D. cinerea* ($F_{4,65} = 4.19$; $P = 0.004$), *C. apiculatum* ($F_{4,64} = 5.87$; $P < 0.001$), *G. flavescens* ($F_{4,61} = 9.55$; $P < 0.001$) and *E. divinatorum* ($F_{3,49} = 4.41$; $P = 0.008$). In

D. cinerea [CT] increased during the wet season in the 'wet' year only. In *C. apiculatum* and *G. flavescens* [CT] increased during the wet season in two years (one 'wet' and one 'dry'). In *E. divinatorum* [CT] decreased during the wet season and tended to be lower in the 'dry' year than the 'wet' year ($F_{1,49} = 4.00$; $P = 0.051$).

With regards to prediction 3, which could only be tested for the evergreen *E. divinatorum*, [N] generally reached high levels in the dry season ($F_{3,49} = 5.30$; $P = 0.003$), while [P] was lowest in the dry season in one year, which was a 'dry' year ($F_{3,49} = 9.94$; $P < 0.001$). In *E. divinatorum*, [TP] was highest in the dry season in only one year, which was a 'wet' year ($F_{3,49} = 24.55$; $P < 0.001$), while [CT] generally increased in the dry season ($F_{3,49} = 4.41$; $P = 0.008$).

4. Discussion

Notwithstanding any possible confounding of our results by unmeasured factors such as soil nutrient and water availability at each sampling site, two of our predictions were partially supported and the third prediction was not supported. Therefore, while the results of the study showed that nutrient and CBSM concentrations varied through the growing season, they are highly variable and the causes of variability remain unclear. The results are discussed in the context of catenal position and variation in annual rainfall during the study period.

4.1. Nutrients during the wet season

Our first prediction that nutrients decrease during the wet season was partially supported. There was a general trend of decreasing [N] during the wet season, but it was not consistent. In

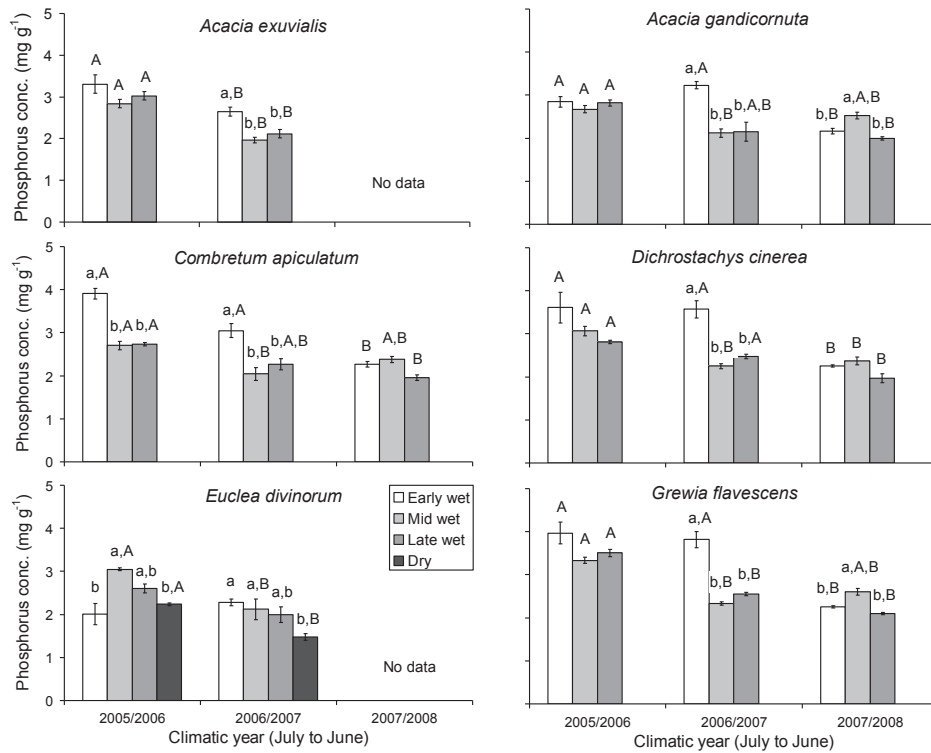


Fig. 3. Mean phosphorus concentration (mg g^{-1}) in leaves of *Acacia exuvialis* ($n = 3, 6, 6, 12, 6, 5$), *Acacia grandicornuta* ($n = 3, 6, 6, 12, 5, 2, 12, 18, 4$), *Dichrostachys cinerea* ($n = 3, 6, 6, 12, 5, 6, 12, 17, 6$), *Combretum apiculatum* ($n = 3, 6, 6, 12, 6, 6, 12, 6, 12, 17, 3$), *Grewia flavescens* ($n = 3, 6, 6, 12, 6, 12, 16, 5$) and *Euclea divinorum* ($n = 6, 6, 6, 9, 12, 6, 6, 6$) at Nkuhlu, Kruger National Park, during different seasons. Leaves were sampled between 1.5 and 2.2 m (1.0–1.5 m on *G. flavescens*). Letters indicate significant differences ($P < 0.05$) among seasons within year (lower case) and among years within season (upper case). The same letters, or none, indicate no significant differences. Error bars are standard errors of the means.

addition, phosphorus generally decreased during the wet season in 'dry' years only. Nitrogen reached its lowest levels in 'dry' years in three species, while phosphorus did so in all studied species. Several studies of seasonal changes in [N] in subtropical and temperate systems have demonstrated anomalous or species-specific changes (Khazaal et al., 1993; Woodward and Coppock, 1995; Styles and Skinner, 1997; Basha et al., 2012). For two of our study species [N] did consistently decrease with time during the wet season (*A. exuvialis* and *D. cinerea*), while [N] in the evergreen species increased late in the wet season (*E. divinorum*). The two deciduous species that conformed to our hypothesis are both relatively fast growing compared to other species in the study area (Scogings, 2011), which may explain why their [N] consistently decreased during the wet season. Fast-growing species are adapted to conditions of high resource availability, but rapidly re-allocate N to storage or reproduction when resources decline (Mårell et al., 2006; Pollastrini et al., 2010).

Both [N] and [P] have been observed to decrease during the growing season (Mårell et al., 2006; Cernusak et al., 2011). However, our results show that both [N] and [P] can increase in some species. The observation of increasing [N] and [P] during the wet season in *E. divinorum* is likely associated with its particular growth phenology of growing new shoots late in the wet season (Zululand/Sweden Kruger Browse Project – ZSKBP, unpubl. data). High nutrient concentrations associated with delayed onset of growth have also been observed for various deciduous woody species in northern temperate forests, but for reasons related to latitude (Lesage et al., 2000). The ability of *E. divinorum* to flush late in the wet season, as well as the ability of *A. grandicornuta* to maintain high foliar [N] during wet seasons, may be related to their occurrence on footslopes in semi-arid landscapes. A substantial

proportion of rainfall infiltrates the coarse sandy soil on crests and eventually accumulates in the deep clay-rich subsoil of footslopes late in the wet season (Jacobs et al., 2007). The difference in the timing of water availability across the catena is evident from observations that both *E. divinorum* and *A. grandicornuta* maintain positive radial stem growth in dry seasons, while species on sandy crests do not (Scogings, 2011). In contrast to the species on footslopes, those on crests decreased [N] and [P] more often than not during the wet season.

One explanation for unexpected seasonal fluctuations is the degree of synchronisation between fixed sampling events and stochastic rainfall events that affect some species more than others. For example, large differences between the beginning and middle of the first wet season of our study compared to other years were clear in *C. apiculatum*, which may have been caused by slightly different stages of leaf development at the start of the wet season in different years. Ideally, timing of sampling should be tied to species-specific phenology because sampling all species at one time may lead to changes being detected in some species but not all.

Both [N] and [P] in three species (*A. exuvialis*, *C. apiculatum* and *D. cinerea*) were affected by year and were generally lowest in 'dry' years. In contrast, [N] in the remaining species (*A. grandicornuta*, *E. divinorum* and *G. flavescens*) was unaffected by year, while [P] was affected by year and was lowest in 'dry' years. The latter species are thus important and reliable sources of N for herbivores. In particular, evergreen species are important in browser diets in the dry season (Basha et al., 2012). Generally, the observed effect of year on P indicates that P is an important limiting nutrient in savannas (Ratnam et al., 2008). The contrasting effects of year on crest and footslope species suggest that N is generally used more efficiently by species on crests than on footslopes in savannas (Hanan et al.,

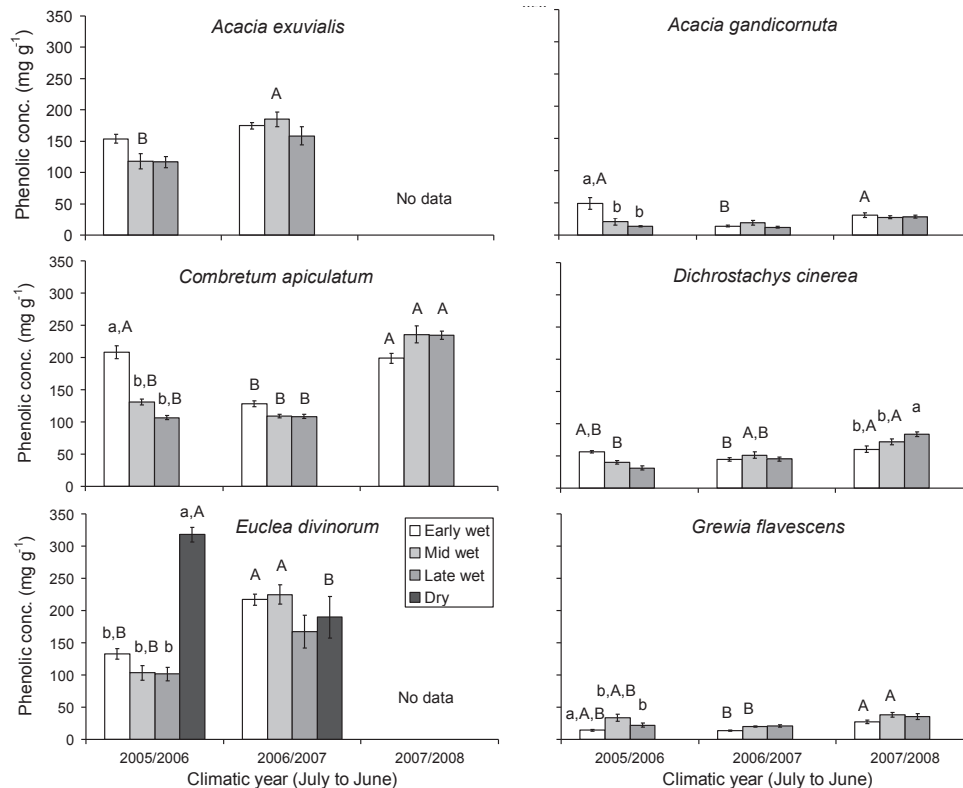


Fig. 4. Mean concentrations of total phenolics ($\text{mg gallic acid equivalents g}^{-1}$) in leaves of *Acacia exuvialis* ($n = 3, 6, 6, 12, 6, 5$), *Acacia grandicornuta* ($n = 3, 6, 6, 12, 5, 2, 12, 18, 4$), *Dichrostachys cinerea* ($n = 3, 6, 6, 12, 5, 6, 12, 17, 6$), *Combretum apiculatum* ($n = 3, 6, 6, 12, 6, 6, 12, 17, 3$), *Grewia flavescens* ($n = 3, 6, 6, 12, 6, 6, 12, 16, 5$) and *Euclea divinorum* ($n = 6, 6, 6, 9, 12, 6, 6, 6$) at Nkuhlu, Kruger National Park, during different seasons. Leaves were sampled between 1.5 and 2.2 m (1.0–1.5 m on *G. flavescens*). Letters indicate significant differences ($P < 0.05$) among seasons within year (lower case) and among years within season (upper case). The same letters, or none, indicate no significant differences. Error bars are standard errors of the means.

2011) and that footslope species are particularly prone to periods of P-limitation, while crest species are able to maintain balanced nutritional status most of the time.

4.2. CBSMs during the wet season

Our second prediction that CBSMs increase with time during the wet season was not consistently supported. Both [TP] and [CT] showed limited variation during the wet season in 'dry' years and did not consistently reach high levels in 'dry' years. In the 'wet' year [TP] decreased in three species (*A. grandicornuta*, *C. apiculatum* and *D. cinerea*) and increased in one (*G. flavescens*), but [CT] increased in three species (*C. apiculatum*, *D. cinerea* and *G. flavescens*) and decreased in one (*E. divinorum*). Concentrations of CBSMs in woody species have been observed to increase, decrease or not change seasonally in both subtropical and temperate woody species (Woodward and Coppock, 1995; Styles and Skinner, 1997; Khazaal et al., 1993; Riipi et al., 2005; Basha et al., 2012). CBSMs have also shown unimodal, hump-shaped responses during the growing season (Covelo and Gallardo, 2001). Decreasing or unchanging CBSMs have been ascribed to translocation, leaching, or turnover (Kleiner et al., 1999; Covelo and Gallardo, 2001). The role of these processes in regulating CBSMs in woody species during the wet season in semi-arid subtropical savannas remains unknown.

Among our limited set of species, the one that was able to increase both [TP] and [CT] during the wet season (*G. flavescens*) has also been reported to increase CBSM concentrations in response to browsing (Scogings et al., 2011). In the remaining species, for which CBSM concentrations either did not change or decreased during the wet season, the same responses have been observed under long-

term browsing (Scogings et al., 2011). The point here is that species that increase CBSMs during the wet season also increase them under long-term browsing, but species that decrease CBSMs in the wet season decrease them when browsed. Therefore, we suggest that the responses of CBSMs to resource availability (especially N) emulate responses to browsing. Our results also suggested that most woody species in conditions such as our study are not prone to accumulating C during the wet season because they either already have high proportional allocation of C to CBSMs, or are relatively slow growing (Scogings, 2011; Scogings et al., 2011). That [CT] increased while [N] decreased in three species (*C. apiculatum*, *D. cinerea*, *G. flavescens*) in the 'wet' year suggest that [N] and [CT] may be negatively correlated when resources are abundant (Herms and Mattson, 1992; Scogings and Macanda, 2005), but the relationship is clearly not general.

Differences in quality between species, and species-specific variation within and between years, means that herbivores are frequently faced with a changing array of plants from which to extract the nutrients and energy needed to meet their requirements (Mårell et al., 2006). Our results suggested that seasonal variations in potential palatability (in terms of [N] relative to [CT]) changed less in some species (e.g., *A. exuvialis*, *A. grandicornuta* and *E. divinorum*) than others (e.g., *C. apiculatum*, *D. cinerea*, *G. flavescens*). The latter species also showed significant inter-annual variation in [N] and [CT] early in the wet season, which could have important implications for browser populations that have high demands for nutrients at that time of year, e.g., for lactation (Lesage et al., 2000; Mårell et al., 2006). Our results indicated that browsers can be reasonably sure of the nutritional value of some species (straight-thorned acacias and spineless

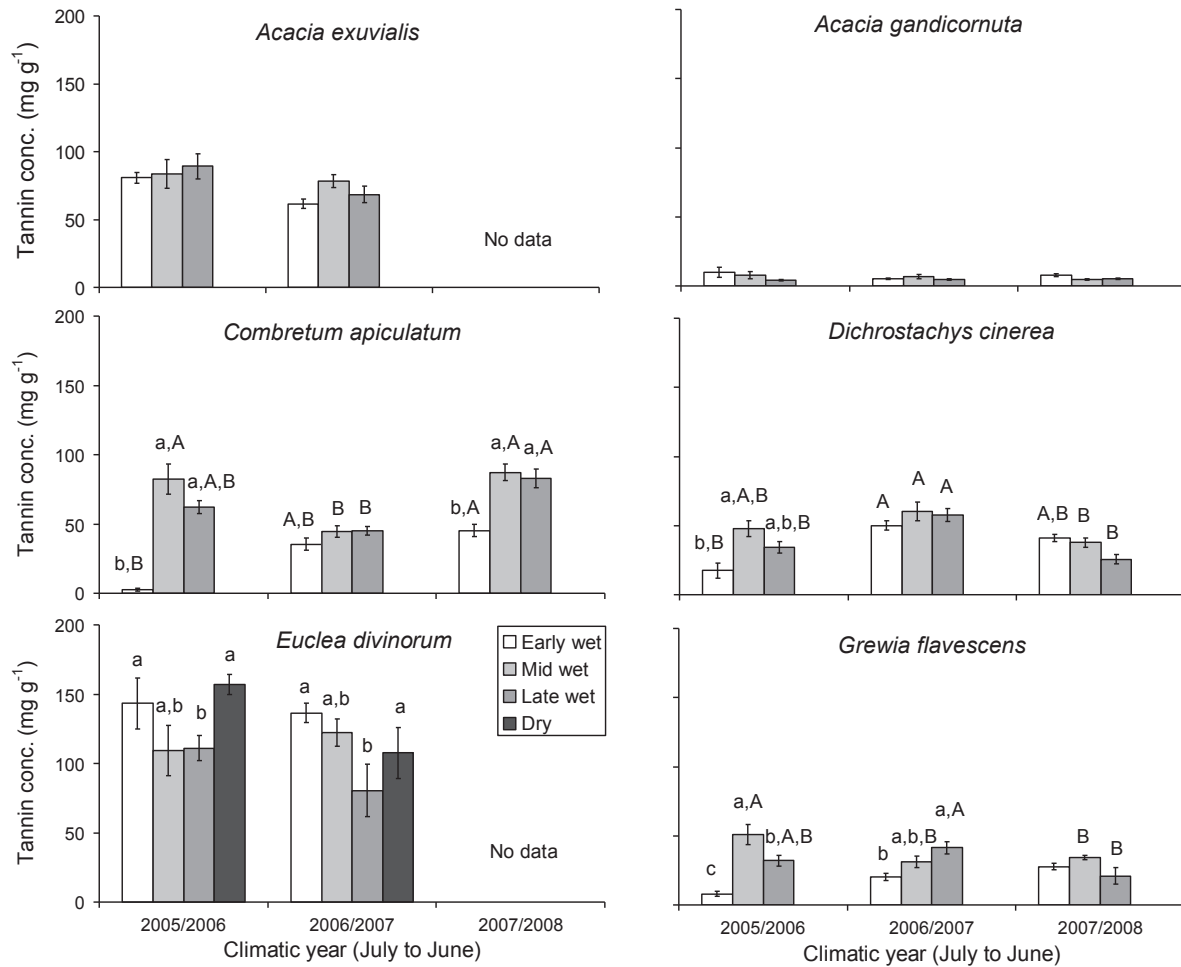


Fig. 5. Mean concentrations of condensed tannins (mg sorghum tannin equivalents g^{-1}) in leaves of *Acacia exuvialis* ($n = 3, 6, 6, 12, 6, 5$), *Acacia grandicornuta* ($n = 3, 6, 6, 12, 5, 2, 12, 18, 4$), *Dichrostachys cinerea* ($n = 3, 6, 6, 12, 5, 6, 12, 17, 6$), *Combretum apiculatum* ($n = 3, 6, 6, 12, 6, 6, 12, 17, 3$), *Grewia flavescens* ($n = 3, 6, 6, 12, 6, 6, 12, 16, 5$) and *Euclea divinorum* ($n = 6, 6, 6, 9, 12, 6, 6, 6$) at Nkuhlu, Kruger National Park, during different seasons. Leaves were sampled between 1.5 and 2.2 m (1.0–1.5 m on *G. flavescens*). Letters indicate significant differences ($P < 0.05$) among seasons within year (lower case) and among years within season (upper case). The same letters, or none, indicate no significant differences. Error bars are standard errors of the means.

evergreens in this study), but not others. Except for those species that are CBSM-rich and N-poor, which we assume to be minor components of most browsers' diets (Cooper and Owen Smith, 1985; Sebata and Ndlovu, 2012), species that are likely to be major parts of the diet could be unpredictable in nutritional value, making inter-annual variations very important for browser populations in semi-arid subtropical savannas. Inter-annual variations in plant growth and resource allocation may be a strategy for woody species to avoid herbivores, but the mechanisms are complex and demand research. The consequence for browsers is that they must be able to find good food in a highly variable landscape, which could become more variable as a result of climate change. Thus, diet selection patterns and associated impacts on vegetation will change.

4.3. Nutrients and CBSMs in the dry season

We predicted that nutrients are lowest, while CBSMs are highest in the dry season when resource availability is lowest and the probability of browsing is highest. This was not supported by our observations of *E. divinorum*. Nitrogen concentration was high in the dry season, but [P] was low and CBSMs were not consistently high in the dry season. In the study area, *E. divinorum* grows on

footslopes where water accumulates late in the wet season and growth only starts at the end of the wet season. Even though *E. divinorum* may have young green leaves at the start of the dry season, and leaf N:CT is likely to be at its highest, *E. divinorum* clearly remains unpalatable, compared to other species. While *A. grandicornuta* was not included in the analysis of wet and dry season differences because it is deciduous, we observed in one dry season when *A. grandicornuta* retained green leaves that [N] and [CT] stayed the same as wet season values, but [TP] increased 4-fold (ZSKBP, unpubl. data). While the hypothesis that CBSMs increase in the dry season was not consistently supported by *E. divinorum*, further exploration of the hypothesis could benefit from inclusion of facultative deciduous species such as *A. grandicornuta*.

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