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Does large herbivore removal affect secondary metabolites, nutrients
and shoot length in woody species in semi-arid savannas?

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Abstract

We hypothesised that exclusion of long-term browsing leads to decreased nutrient concentrations, increased carbon-based secondary metabolites (CBSMs) and decreased shoot length in woody plants in semi-arid savannas. To test this, we sampled four deciduous woody species in a large-scale, long-term exclusion experiment. We found a tendency for large herbivore exclusion to either decrease nitrogen or increase tannin in two tree species. Phosphorus and total polyphenol responses indicated complex interactions with herbivore assemblage. Shoot length decreased under exclusion in two species, while a similar tendency existed in the remaining species. Therefore, we found limited support for our predictions that exclusion of browsing results in reduced nitrogen or increased CBSMs, but moderate support for decreased shoot length in semi-arid savannas. *Dichrostachys cinerea*, a fast-growing, aggressive invader in savannas, displayed C-limitation with removal of large herbivores because both shoot length and CBSMs decreased. In conclusion, effects of long-term browsing on deciduous trees in semi-arid savannas depend on the plant species, specific nutrients or CBSMs measured and the composition of the herbivore assemblages. Because elephant densities in the study area were high and most responses were associated with their exclusion, we conclude that elephants are important drivers of browse quality in the study area.

Keywords: Elephant, Exclusion experiment, Nitrogen, Phenol, Plant defence, Tannin
1. Introduction

Plant species display various combinations of physical and chemical traits that have evolved in response to selection by environmental conditions. Some of these traits enhance the fitness of plants in response to herbivores because the traits either reduce the rate of consumption, or increase the rate of regrowth (Briske, 1996). These traits are regarded as constituting broad strategies of avoidance and tolerance, which are not mutually exclusive and are collectively known as herbivore resistance (Herms and Mattson, 1992; Stevens et al. 2007; Skarpe and Hester, 2008). Plant communities comprise mixtures of species, each displaying a range of strategies contingent on fluctuations in herbivore impacts and environmental factors (Hjältén et al., 1996; Hester et al., 2006). Understanding how individual plant species respond to herbivores in semi-arid savannas is critical for understanding how plant assemblages respond to changing environments (Hester et al., 2006).

Carbon-based secondary metabolites (CBSMs), such as tannins, are assumed to function as chemical defences that contribute to herbivore-avoidance strategies (Bryant et al., 1985; Skarpe and Hester, 2008). The concentration of CBSMs in individual plants depends on resource availability in the environment (Koricheva et al., 1998; Stevens et al., 2007) and the effect that herbivores have on resource allocation and availability in plants (Nykanen and Koricheva, 2004; Ballare, 2011; Orians et al., 2011). Plant responses to herbivores may also depend on the severity of damage (Hjältén et al., 1993, 1994; Nykanen and Koricheva, 2004).

A major component of research in semi-arid savannas concerns the effects of large herbivores (especially elephants) on woody vegetation, with the ultimate objective being the development of improved management policies (O’Keefe and Alard, 2002).
While defoliation may induce defences in woody plants in semi-arid savannas (Ward and Young, 2002), severe browsing by large herbivores in savannas has been reported to maintain elevated shoot growth associated with increased N concentrations or reduced tannin concentrations in individual plants (du Toit et al., 1990). Heavily browsed plants that have switched to a vegetative state characterised by N-rich/C-poor tissues are in positive feedback loops because the switch increases the probability of browsing (Fornara and du Toit, 2007; Skarpe and Hester, 2008). One possible explanation for reduced CBSMs in browsed plants is a relative increase in allocation of C to growth of new shoots rather than CBSMs (Fornara and du Toit, 2007; Hrabar et al., 2009), which can occur if the root:shoot ratio is altered such that shoot growth increases (Herms and Mattson, 1992; Renton et al., 2007). Evidence for the responses of nutrients and CBSMs to long-term browsing in semi-arid savannas is inconsistent, but shoot growth rates consistently increase (Ward and Young, 2002; Fornara and du Toit, 2007; Hrabar et al., 2009).

Our objective was to study the effects of large herbivore exclusion on woody species in terms of (i) condensed tannin (CT), total polyphenol (TP), N and P concentrations in mature leaves and (ii) shoot length. The premise of this approach is that exclusion of herbivores allows their effects to be determined (O’Keefe and Alard, 2002). Mature leaves comprise the main component of foliage in all browsers’ diets for most of the growing season. Therefore, browsers would potentially be affected by changes in mature leaves rather than young leaves that are only briefly available early in the wet season. Although the response of shoot length to browsing is unlikely to be directly related to total shoot biomass because the number of shoots is often reduced by browsing (Bergström et al., 2000), the response of shoot length in Acacia trees has been found to be comparable to that of total shoot productivity (Milton, 1988;
Dangerfield and Modukanele, 1996; Fornara and du Toit, 2007). In addition, neither shoot length nor total shoot growth necessarily reflect total plant growth because they are the result of resources being allocated to adjust the root:shoot ratio (Teague, 1985; Teague & Walker, 1988). Consistent with responses commonly reported in the literature (e.g., du Toit et al., 1990, Ward & Young, 2002; Fornara & du Toit, 2007) we postulated that concentrations of CBSMs are increased, but nutrient concentrations decreased, in plants under herbivore exclusion compared to plants under long-term browsing. We also expected shoot length to be shorter in plants under herbivore exclusion compared to browsed plants (Hrabar et al., 2009). We tested the above hypotheses by sampling four woody species within a large-scale, long-term exclusion experiment established in 2002 in a semi-arid savanna in Kruger National Park, South Africa.

2. Material and methods

2.1. Study area

The Nkuhlu Large-scale Long-term Exclusion Experiment (24° 58′ S, 31° 46′ E) is adjacent to the Sabie River, Kruger National Park, South Africa (O’Keefe and Alard, 2002). The climate is semi-arid subtropical with two broadly distinct seasons: a hot, occasionally wet, growth season (October-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 is 200-230 m above mean sea level. Topography comprises undulating landscape derived from granite. Crests and middle slopes are characterized by shallow, sandy, coarse soil, while footslopes are characterized by deep, sodic, duplex soil (Khomo and Rogers, 2005; Grant and Scholes, 2006). Sodic soils are regarded as stressful environments for vegetation, which is sparse, but regarded as more nutritious than crest vegetation to large herbivores (Tarasoff et al., 2007; Levick and Rogers, 2008).

Abundant woody species (Schmidt et al., 2007) include *Acacia grandicornuta* and *Euclea divinorum* on the footslopes, and *Dichrostachys cinerea*, *Acacia exuvialis*, *Combretum apiculatum* and *Grewia flavesens* on the middle slopes and crests (Siebert and Eckhardt, 2008). Most of the woody species are deciduous and shorter than 10 m. Common mammal herbivores (Skinner and Chimimba, 2005) include impala (*Aepyceros melampus*), African elephant (*Loxodonta africana*), hippo (*Hippopotamus amphibius*), black rhino (*Diceros bicornis*), white rhino (*Ceratotherium simum*), greater kudu (*Tragelaphus strepsiceros*), giraffe (*Giraffa camelopardalis*), steenbok (*Raphicerus campestris*), scrub hare (*Lepus saxatilis*), blue wildebeest (*Connochaetes taurinus*), Cape buffalo (*Syncerus caffer*) and plains zebra (*Equus quagga*). Elephant, impala, black rhino, kudu, giraffe and steenbok are the main consumers of woody vegetation in the study area. Elephant density fluctuates around 0.5-2.0 individuals/km² (Grant et al., 2008). A breeding herd of 30-40 impala, several impala bachelors, two black rhino and an unknown number of steenbok are resident in the study area, while low numbers of giraffe and kudu are occasionally present.
The Nkuhlu Large-scale Long-term Exclusion Experiment comprises one exclusion plot (70 ha) that excludes all herbivores the size of hares and larger (total exclusion) and one exclusion plot (44 ha) that excludes elephants, but not other herbivores (partial exclusion). The two exclusion plots, established in 2002, are separated by a control area (25 ha) open to all large herbivores (no exclusion).

Treatments could not be replicated because of the large scale of the experimental plots (Levick and Rogers, 2008; Jonsson et al., 2010). If excluding either elephants or all large herbivores shows similar responses then it can be inferred that the main source of the effect is elephants (Jonsson et al., 2010). If excluding elephants shows a different response to excluding all large herbivores then it can be inferred that the main source of the effect is not elephants.

The plant species selected were among the most abundant and evenly distributed in the study area, allowing for the desired sampling strategy to be implemented. The species were *Acacia grandicornuta* Gerstner, *Dichrostachys cinerea* (L.) Wright and Arn. subsp. *africana* Brennan and Brummitt (hereafter simply *D. cinerea*), *Combretum apiculatum* Sond. and *Grewia flavescens* Juss. *Acacia grandicornuta* is a deciduous legume with fine, bi-compound leaves and long straight spines in pairs at the nodes. *Dichrostachys cinerea* is a multi-stemmed, deciduous legume with fine, bi-compound leaves and stem-spines (short shoots that have sharp tips). Both *C. apiculatum* and *G. flavescens* are deciduous and broad-leaved, but *C. apiculatum* is a tree, while *G. flavescens* is a many-stemmed large shrub. Sampling was scheduled according to preliminary observations in two previous years at the site, which indicated that effects of browsing on CTs and TPs of mature leaves were most readily detected in the middle of the wet season (Zululand/Sweden Kruger Browse Project -...
ZSKBP, unpublished data). Sampling leaves for chemical analysis in January was appropriate because CTs and TPs are thought to accumulate as the season progresses (Barton and Koricheva, 2010). Our preliminary data also indicated that sampling in the middle of the wet season was suitable for detecting effects of browsing on nutrients. Sampling only mature leaves at one time ensured that leaf phenology would not confound our results. A 30 x 30 m grid was used to randomly locate 18 sites on the crest and 18 on the footslope in each plot (54 sites altogether per catena position). One tree of each study species was sampled in each grid cell on condition that it was (i) closest to the grid-cell centre, (ii) taller than 2 m, and (iii) 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 grew taller than 2 m. If any condition was not met for the closest tree, then the next closest tree meeting the conditions was sampled. No tree was sampled if the closest suitable tree was > 15 m from the grid-cell centre. The total number of sampled trees was 196, comprising 53 *A. grandicornuta*, 49 *C. apiculatum*, 53 *D. cinerea* and 41 *G. flavescens*.

Samples were collected on 28-30 January 2008 by clipping branches 3-8 mm in diameter from the northern, sunlit half of the canopy 1.5-2.2 m above ground, which was within reach of most browsers in the study area. For *G. flavescens*, the lowest permissible height for collection was 1.0 m because of its low stature. Branches were collected 1-4 hours after sunrise (05:25). Mature leaves that did not show signs of severe insect herbivory were removed for drying at 60 °C for 24 hours. Each dried sample of leaves was milled (1.0 mm) and then analysed for concentrations of condensed tannins ([CT]) (Porter et al., 1986), 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 dry mass. [CT] and [TP] were expressed as sorghum tannin equivalents (STE) and gallic acid equivalents (GAE), respectively. Differences in concentrations were assumed to reflect differences in absolute amounts because the limited growth of mature leaves would not lead to dilution.

Preliminary observations in the study area in previous years indicated that effects of browsing on current season shoot growth were most readily detected early in the wet season (ZSKBP, unpublished data). The shoots on all the species started growing a week after the first rainfall and continued for up to 6 weeks, so measuring shoot length at one time early in the season reflected maximum growth rate. Shoot growth could not be measured later in the wet season because most current season shoots were no longer intact. Therefore, the lengths of 10 randomly selected current season shoots were measured on each of five randomly selected trees per species on 5-6 November 2007. The length of each new shoot was measured from its base to the base of the apical bud.

2.3. Statistical analyses

Data were analysed separately for each species. To fulfil the requirement of normality and equal variances, all chemical data were log$_{10}$ transformed, while average shoot length per tree was square-root transformed for *A. grandicorne* and log$_{10}$ transformed for *C. apiculatum, G. flavescens* and *D. cinerea*. The effect of treatment on each chemical and growth variable was analysed as a single-factor ANOVA, assuming trees were replicates within each treatment. Therefore, pseudo-replication constrained the analysis and the estimates of model parameters should be
viewed with caution (Underwood, 1997). During the analysis of *G. flavescens*, it appeared that there were two populations that differed in [CT], such that one population had higher [CT] than the other. The two populations were subsequently assumed to represent two varieties, namely var. *flavescens* and var. *olukondae* (Schinz.), of which the latter was not expected in the study area (Schmidt et al., 2007).

Close examination of sampled plants in the field with the benefit of hindsight indicated that the more abundant, low-CT (< 37 mg/g) plants had smoother, softer leaves characteristic of var. *olukondae*, while the less abundant, high-CT (> 42 mg/g) plants had rougher, harder leaves characteristic of var. *flavescens*. Effects of exclusion on [CT] were therefore analysed for the two varieties separately. Significance was declared when $P < 0.05$, while possible trends were not ignored when $0.10 > P > 0.05$.

From an environmental management perspective, it is better to relax $P$ and make a Type I error when the statistical power of the experiment is not strong, than to make a Type II error in accepting the null hypothesis when it is false (Underhill, 1997).

3. Results

Each species that we studied expressed at least one chemical response to exclusion of large herbivores. For *A. grandicornuta*, exclusion treatments negatively affected [N] ($F_{2,47} = 3.89; P = 0.027$), but had no effect on other chemical variables ($P > 0.05$) (Fig. 1). For *C. apiculatum*, exclusion increased [CT] ($F_{2,46} = 8.76; P = 0.001$), but did not significantly affect other chemical variables ($P > 0.05$) (Fig. 1). In *D. cinerea*, [TP] tended to be lowest in the partial exclusion plot and highest in the control plot.
(\(F_{2,49} = 3.10; P = 0.054\)), while there were no significant effects on the other chemical variables (\(P > 0.05\)) (Fig 1).

In G. flavescens, partial exclusion of large herbivores increased [P] (\(F_{2,38} = 4.57; P = 0.017\)), but did not affect other chemical variables (\(P > 0.05\)), except that a marginally significant negative effect was noted for [TP] (\(F_{2,37} = 2.67; P = 0.083\)) (Fig. 1). Analysis of var. olukondae produced a significant positive effect of exclusion (\(F_{2,26} = 3.32; P = 0.052\)), where mean [CT] was 31.5 mg/g (SEM: 1.79) in the partial exclusion plot, 32.0 mg/g (SEM: 1.13) in the total exclusion plot and 27.4 mg/g (SEM: 1.34) in the control plot.

Notwithstanding the effects of large herbivores, tree species varied substantially in terms of measured chemical variables (Fig. 1) as well as in shoot length. Acacia grandicornuta had 4-8 times longer shoots than other species. Shoot length of D. cinerea (\(F_{2,11} = 12.23; P = 0.002\)) and A. grandicornuta (\(F_{2,11} = 6.60; P = 0.013\)) were shorter under total exclusion, but C. apiculatum (\(F_{2,12} = 2.02; P = 0.175\)) and G. flavescens (\(F_{2,12} = 1.68; P = 0.235\)) were not affected (Fig. 2). Total exclusion reduced shoot length by 50% and 46% for D. cinerea and A. grandicornuta respectively.

4. Discussion

The hypothesis that concentrations of CBSMs increase, but nutrient concentrations decrease, in plants after removal of large herbivores in semi-arid savannas received limited support. In no species did concentrations of both CBSMs and nutrients change together. Either one CBSM increased or one nutrient decreased in two of the four study species when herbivores were excluded, suggesting the presence of positive
feedback loops between large herbivores and trees of some species. While responses of [N] and [CT] suggested elephant browsing has the potential to initiate positive feedback in semi-arid savannas, responses of [P] and [TP] indicated that large herbivores other than elephants have similar potential. Although our results provided limited support for our hypothesis, the general trends in the results were aligned with predictions and corroborate observations of other studies (du Toit et al., 1990; Ward and Young, 2002).

The hypothesis that current season’s shoots are shorter in plants after exclusion of large herbivores than in browsed plants in semi-arid savannas was supported for two species we studied. The two fine-leaf, spiny species, *D. cinerea* and *A. grandicornuta*, had shorter shoots after total exclusion compared to either no exclusion or exclusion of elephants. Mean shoot lengths on the two broad-leaf, spineless species were not significantly affected by exclusion, but showed a similar trend. Nevertheless, our results indicated that the study species were able to increase shoot growth rate in response to browsing (Renton et al., 2007; Mopipi et al., 2009) and suggests that spines or N₂-fixation may have a role in compensatory shoot growth, although the mechanism is unclear. The observations for *A. grandicornuta* and *D. cinerea* confirmed preliminary observations of shoot lengths in the previous wet season (ZSKBP, unpublished data). The observations for *C. apiculatum* and *G. flavescens*, however, contrasted with our preliminary observations and other studies that demonstrated shorter shoots without browsing (Bergström et al., 2000) and may have been affected by the specific time of sampling (we suspect that significant effects may have been detected in December when there was a second flush of growth that was more vigorous than the first flush). Unlike the clustered arrangement of leaves at nodes on short-shoots (spurs) in *A. grandicornuta* and *D. cinerea*, leaves on *C.*
apiculatum and G. flavescens are produced on long-shoots with one or two leaves per node (Schmidt et al., 2007). Species with the latter growth pattern cannot produce new photosynthetic tissues without first producing new somatic tissues, which requires plenty of water and N (Ganqa and Scogings, 2007). We postulate that required resources were not sufficient at the time of sampling to meet the requirements of long-shoot production, leading to the lack of exclusion effect on shoot length in C. apiculatum and G. flavescens. Nevertheless, our evidence altogether indicates that shoot growth rate increases under browsing pressure, which supports other studies (Fornara and du Toit, 2007; Hrabar et al., 2009).

The decrease in both shoot length and [TP] in D. cinerea following exclusion of large herbivores (the latter contradicting our predictions) suggests that herbivory does not necessarily result in C limitation in deciduous trees, which supports studies among boreal species (Nykanen and Koricheva, 2004; Palacio et al., 2008). However, responses to browsing may be modified by the frequency of browsing, nutrient availability, the timing of damage and the plant tissue sampled (Hjältén et al., 1993, 1994; Lindroth et al., 2007). Plants accumulate C under nutrient stress (Herms and Mattson, 1992), which could result in increased levels of CBSMs in browsed plants (Rooke and Bergstrom, 2007). Alternatively, browsed plants may accumulate C from compensatory photosynthesis and allocate some of it to increased CBSMs (Medhurst et al., 2006; Olesen et al., 2008). If sufficient leaves remain after browsing, photosynthesis contributes more than storage to the allocation of C to growth or defence (ibid.). However, the relative contributions of compensatory photosynthesis and storage to the increase in both [TP] and shoot growth in D. cinerea are unclear. Dichrostachys cinerea is a fast-growing, aggressive invader that readily produces root suckers (Tobler et al., 2003; Wakeling and Bond, 2007). In the study area, D. cinerea
stems grow faster than stems of other species (Scogings, 2011). Carbon allocation patterns in browsed *D. cinerea* are possibly linked to high photosynthetic capacity and extensive storage associated with its growth habit. Given that our plants were not obviously nutrient stressed, compensatory photosynthesis is a likely source of C for allocation to CBSMs in *D. cinerea*, but storage cannot be ignored as a potential source as well. Studies of C allocation in deciduous species in semi-arid savannas would improve the understanding of responses to browsing.

5. Conclusions

In conclusion, effects of browser exclusion on woody plants were species-specific. We found only limited support among the four study species for our prediction that removal of large herbivores results in decreased plant quality (reduced N and increased CBSM) in semi-arid savannas. Furthermore, there was moderate support for our prediction that removal of large herbivores results in decreased shoot length. Therefore, manifestation of positive browse-browser feedback loops in savannas depends on the plant species observed, the specific nutrient or CBSM measured, and the density and composition of the associated herbivore assemblage. Since elephant densities in the study area were high and most positive responses were detected in the control plot, we conclude that elephants probably are important drivers of feedback loops among woody plants taller than 2 m in the study area.

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Figure legends

Fig. 1. Mean concentrations of N and P (mg/g), and total polyphenols (TP, mg gallic acid equivalents/g) and condensed tannins (CT, mg sorghum tannin equivalents/g) in mature leaves from four species (Ag = A. grandicornuta, Ca = C. apiculatum, Dc = D. cinerea, Gf = G. flavescens) at Nkuhlu, 28-30 January 2008. Letters above columns indicate significant differences among means within species, according to Bonferroni means comparison ($P < 0.05$). Error bars are standard errors of means ($n = 15-18, 14-18, 16-18$ and $10-16$ for Ag, Ca, Dc and Gf, respectively).

Fig. 2. Mean length (cm) of unbrowsed new shoots at 1.5-2.5 m on A. grandicornuta, C. apiculatum and D. cinerea, and above 1 m on G. flavescens, at Nkuhlu (November 2007). Ten shoots were averaged per tree. Error bars are standard errors of means. Letters above columns indicate significant differences among means within species according to Bonferroni means comparison ($P < 0.05; n = 5$).
Figure 2

The bar chart shows the comparison of new shoot length (cm) among different species under three exclusion treatments: No exclusion, Partial exclusion, and Total exclusion. The species include Acacia grandicornuta, Combretum apiculatum, Grewia flavescens, and Dichrostachys cinerea. The chart indicates statistical differences among the treatments, with lowercase letters (a, b) highlighting significant differences.