No evidence for the simultaneous induction of structural and chemical defences in spiny southern African savanna trees

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Abstract   It is still not well established whether plant chemical and physical defence traits can be simultaneously induced in savanna trees and how this would affect plant nutritional quality. Here, we use a long-term mammalian herbivore exclosure experiment in a dystrophic semi-arid African savanna to test how chemical (condensed tannins [CTs] and total polyphenols [TPs]) and structural (spines and thorns) defences are affected by herbivore exclusion in five common savanna woody species. We also tested whether nutritional quality interacted with either physical or chemical defences after protected trees were reintroduced to herbivores. Trees that remained available to herbivores did not increase CTs and TPs, rather these were found to be higher without mammalian herbivory in some species. In contrast, herbivory resulted in a significant induction of structural defences (i.e. longer and thicker spines) and at the same time, improved nutritional quality (i.e. higher available crude protein and lower C:N ratios) for some species. We found that the plant defence traits of the treatment which previously excluded herbivores were mostly similar to the control, which both differed from the exclosure, suggesting that legacies of herbivore exclusion on plant defence traits are mostly short-lived.

Key words: condensed tannins, exclosures, induction syndromes, mammalian herbivory, nitrogen-free secondary compounds, plant nutritional quality, spines, total polyphenols.

INTRODUCTION

The iconic thorny acacias are the poster trees of African savannas. The thorns are in fact spines and prickles which together form an important defence against the diverse suite of large mammalian herbivores (LMHs) present in many African savannas (Scholes 1990; Scholes & Walker 2004). Spines are not the only defence; however, African savanna trees have been found to typically use one of three defence syndromes (Wigley *et al.* 2018, 2019). These include (i) species high in leaf nitrogen (N) and defended by both spines and chemicals, (ii) species high in leaf N and highly structurally defended (i.e. spiny and highly branched architectures) and (iii) species low in leaf N but high in chemical defences. Whether these defence strategies are always present in any particular plant species (i.e. constitutive) or whether they are

induced by herbivory is not always clear (Agrawal & Fishbein 2006; Barton 2016; Armani *et al.* 2019).

Condensed tannins (CTs) and total polyphenols (TPs) are commonly measured secondary metabolites in studies that investigate plant–herbivore interactions and plant defence (Cooper & Owen-Smith 1985; Ward & Young 2002; DeGabriel *et al.* 2009; Scogings *et al.* 2011, 2014, 2015; Wigley *et al.* 2015, 2018). Plants high in CTs or TPs deter feeding by herbivores due to their capacity to bind to proteins resulting in reduced protein digestion as well as reducing cell wall digestion via the inhibition of rumen bacteria (Cooper & Owen-Smith 1985; Bernays *et al.* 1989; Barbehenn & Constabel 2011). Although several studies have suggested that high concentrations of tannins have little impact on herbivores (Bernays *et al.* 1989; Schultz 1989; Close & McArthur 2002), others have found that higher tannin levels are associated with lower rates of mammal (Furstenburg & Van Hoven 1994) and insect

herbivory and reproductive fitness (Forkner *et al.* 2004; Pearse 2011). In addition to defence, tannins also play a role in protection against fungal and microbial decay, ultraviolet radiation, plant growth regulation, wound sealing and structural support (Scalbert 1991; Kraus *et al.* 2003).

Previous studies have proposed that the levels or concentrations of defences present in plants will depend on resource availability, such that different levels of soil fertility result in different levels of defences. For instance Janzen (1974) and Fine *et al.* (2006) have argued that in resource-poor tropical environments, plants cannot afford to lose tissue and invest heavily in *chemical defences* (e.g. phenolics) to curb losses. Although the resource-based hypotheses (i.e. carbon-nutrient balance hypothesis (Bryant *et al.* 1983) and growth differentiation hypothesis (Herms & Mattson 1992)) have been challenged (Koricheva 2002a; Hahn & Maron 2016), Hamilton *et al.* (2001) and Koricheva (2002b) predict that nitrogen-free secondary compounds (e.g. CTs) will be preferentially produced (i.e. induced) over nitrogen-rich secondary compounds or other N-rich compounds (e.g. proteins) on nutrient-poor soils. Furthermore, Sampedro *et al.* (2011) showed in pine (*Pinus pinaster*) that investment in constitutive N-free secondary compounds were higher under low phosphorus availability. Apart from soil fertility, edaphic stresses that specific soils (e.g. those high in clay) place on trees may also necessitate high levels of defence (Pringle *et al.* 2016). Similarly, whether the presence and levels of *physical defences* are related to the availability of resources such as nutrients have been questioned (Hanley *et al.* 2007). However, the induction of physical defence traits is common and widespread (Barton 2016). For instance, allocation to spines in African savanna trees is induced at a wide range of soil fertilities when accessible to herbivores or rather, as many of these studies have used exclosures, relaxed with the exclusion of herbivores (Young & Okello 1998; Young *et al.* 2003; Wigley *et al.* 2015, 2019).

In a review of the induction of plant physical defence traits, Barton (2016) found that in studies where physical and chemical defence traits were measured in the same experiment, the magnitude of increase was typically found to be similar between physical and chemical defence traits, which should result in particularly unpalatable plants. Based on previous studies, we did not expect this to be the case in savannas, as heavily browsed savanna trees have often been found to decrease N-free secondary compounds in response to intense herbivory (Rohner & Ward 1997; Scogings *et al.* 2011, 2014; Wigley *et al.* 2014, 2015, 2019). One explanation for this phenomenon is that frequent and intense browsing can result in a carbon deficit in plants as carbon is

preferably allocated to building new shoots and leaves in order to increase the plant's photosynthetic capacity (Du Toit *et al.* 1990; Skogsmyr & Fagerström 1992; Scogings *et al.* 2011).

The experimental set-up at our study site on nutrient-poor Kalahari sands provided a unique opportunity to measure herbivory effects on the inducibility (after relaxation) of a suite of defence traits (i.e. tannins, polyphenols and spines) and plant nutritional quality. We compared traits for the dominant tree species in a treatment where LMHs have been excluded for 10 years (exclosure), a treatment that remained open to herbivory (control), and a treatment that excluded herbivores for 7 years but then was accessible to herbivores for 3 years pre-sampling (previous). We used this exclosure experiment to address the following questions: (i) Do we find evidence for the simultaneous induction of physical and chemical defence traits? (ii) In association with question 1, did we find any changes in plant nutritional quality in response to herbivory? For (i) and (ii) we predicted low levels of induction of N-free secondary compounds by herbivores, but high levels of induction for physical defences. We further predicted that leaf quality would either remain unchanged or improve with herbivory. (iii) Did mid to long-term herbivore removal (7 years) have any long-lasting effects on plant nutritional quality, chemical defences and structural defences after sustained herbivore reintroduction (3 years)? We predicted that the reintroduction of herbivores would result in the induction of physical defences such that spines would be significantly increased after a relatively short period of re-exposure (3 years) to herbivory and that leaf quality would return to levels that were similar to plants that remained exposed to herbivores.

METHODS

Study sites

This study was undertaken in Hwange National Park (HNP), situated on the western border of Zimbabwe. The study site is situated near the old airstrip in the vicinity of the Main Camp in HNP (−18.726869°S, 26.962251°E). The vegetation at the study site is typical of Southern African dystrophic wooded savannas and has been classified as bushed grassland (Arraut *et al.* 2018), which is dominated by a mix of Acacia and broadleaved species. The study site is situated on nutrient-poor soils of the eastern Kalahari sands (Rogers 1993). The long-term mean annual rainfall is approximately 600 mm with most of this falling between October and April (Chamaillé-Jammes *et al.* 2007). Common browser and mixed feeder mammal species found in HNP include elephant *Loxodonta africana*, giraffe *Giraffa camelopardalis*, impala *Aepyceros melampus*, kudu *Tragelaphus strepsiceros*, steenbok *Raphicerus campestris*, grey duiker *Sylvicapra grimmia* and bushbuck *Tragelaphus sylvaticus*

(Chamaillé-Jammes *et al*. 2009). Densities of elephants reached above 2 per km^2 on average over the year in the Main Camp area but were slightly lower in the immediate vicinity of the experimental site. Giraffe were around 0.5 per km^2, impala and kudu slightly over 1.5 per km^2 and steenbok about 1 per km^2 (Valeix M. Game Census Summary 2015, unpubl. report). Plant species nomenclature in this study is based on Coates-Palgrave (2002).

Sampling methodology

The exclosure experiment

This study makes use of an herbivore exclosure experiment that was established in 2007 as part of the ANR funded BIOFUN project within the Hwange Environmental Research Development (HERD) program. Two full exclosures (exclude all large mammals greater than 2 kg) of 120 × 120 m were constructed using wooden poles and 2.4 m high welded mesh (20 × 20 cm) with additional finer mesh (10 × 10 cm) around the bottom meter to exclude smaller herbivores. The exclosures were close enough to Main Camp headquarters to be checked daily, allowing for any detected damage to be quickly repaired to ensure the continued exclusion of herbivores. An adjacent area of the same size as the exclosures remained open to all herbivores and was demarcated and used as a control treatment (hereafter referred to as control). The two exclosure plots and the control plot were all within 1 km of each other in an open savanna patch of approximately 50 ha, dominated by a mixture of fine-leaved and broad-leaved woody species. The three plots were placed in homogenous vegetation of similar woody densities (see Google Earth imagery from 2007 when the experiment was set up (Appendix S1). Google Earth imagery from 2019, 2 years after sampling, shows a slight increase in woody cover which was not limited to the fenced treatment, but is evident in all three plots (Appendix S1). At the time of sampling in 2017, the three plots were fairly open and interspersed with bush clumps typically composed of multiple species (see Appendix S2). Preliminary analyses at the start of the experiment found no differences in soil nutrients between the exclosures and control, J.C. Lata, S. Barot unpubl. data, 2008). All three plots were at a similar distance to the main camp and other possible disturbances, which included a seldom used landing strip. We, therefore, assumed that herbivore movements and utilization would have been similar for all treatments before the exclosures were erected. After 7 years (November 2014) one of the exclosures was removed (hereafter referred to as previous) and herbivores could thereafter access the previously fenced area until plant trait sampling at the end of the 2016/17 growing season (May 2017). The remaining exclosure (hereafter referred to as exclosure) continued to be maintained until sampling was completed.

Plant trait selection and measurement

We determined the dominant woody species that represented the woody community at the study site and that were commonly found in all three treatments. These included three fine-leaved species (*Acacia erioloba*, *Acacia tortilis* and *Dicrostachys cinerea*) and two broad-leaved species (*Z. mucronata* and *Rhus tenuinervis*). For each of these species five individuals were chosen in each of the plots for trait sampling. To address the lack of replicate exclosures per 'treatment', and the related issues relating to pseudoreplication (Hurlbert 1984), we ensured that the five sampled individuals of each species were as far apart as possible within each of the three treatments, that is, we avoided sampling individuals of the same species that were closely spaced (<20 m) or in the same clump (e.g. see Appendix S2). We further ensured that all individuals of each species were of similar size, both within and between treatments (2–3 m, see Appendix S2). Of the five species, *D. cinerea* was the only clonal (root suckering) species with fruit that is not animal dispersed (Wakeling & Bond 2007), we were therefore extra careful in ensuring that the sampled individuals of *D. cinerea* were far apart. We sampled three traits related to browse nutritional quality. These included leaf N:P, leaf C:N and available crude protein (ACP), calculated as 6.25 × [leaf nitrogen] minus half CT concentrations expressed in %, following Cooper *et al*. (1988) and Owen-Smith and Cooper (1989). Two chemical defence traits (leaf TP concentrations and CT concentrations) and three structural defence traits (spine length, spine diameter and spine density) were also measured.

For CT and TP analyses, we collected sun exposed leaves from the outer edge of the canopy at a height of 1–2 m (i.e. in reach of the dominant browsers and mixed feeders at the site). All leaf material was air dried at HNP then ground to pass through a 1-mm sieve. Ground leaf samples were analysed for C and N using a Leco TruSpec CN Analyser (LECO Corporation, St. Joseph, MI). Leaf phosphorus (P) was analysed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Varian Vista MPX, Palo Alto, CA, USA). CTs were calculated according to the methods described by Hattas and Julkunen-Tiitto (2012) using *Sorghum* tannins as a standard. Extractable CTs from leaf material of each species was quantified in triplicate using the acid-butanol assay for proanthocyanidins described by Porter *et al*. (1985) and modified by Hagerman (2002). TPs were calculated according to the methods described by Hattas *et al*. (2005), which involved analysing leaf material in supernatants using the redox general phenolic assay of Price and Butler (1977), as modified by Hagerman (1995) and using Gallic acid as a standard.

We acknowledge recent work by Marsh *et al*. (2020), who proposed that the reason why total tannins rarely correlate with animal feeding is that total tannins and polyphenols mask the classes of N-free secondary compounds that do act as defences. However, information on the methods and the specific groups of tannins that influence mammal herbivore feeding is lacking (most studies have focused on insects) and would probably depend on both the mammal species and the mix of plant species being fed on (Hattas *et al*. 2011; Salminen & Karonen 2011; Marsh *et al*. 2020). We propose that until such a time as CTs are indisputably shown to not be important for vertebrate feeding or until another group of tannins has been shown to be more important, we can continue to assume that we have

measured the most appropriate classes of N-free secondary compounds.

We have not differentiated between thorns, spines and prickles in this study and refer to all types as spines hereafter (all species had spines except *R. tenuinervis* and *D. cinerea*, which had thorns). Average spine length and spine diameter (measured at the spine base) were measured using digital vernier callipers. On each plant, we selected three branches taken from the lower, middle and upper parts of the canopy then measured 10 mature (hardened) spines on the outer ends of each of these branches to ensure that the current season's spines were being measured. Spine density (spines cm^{-1}) was measured on these same three branches, then averaged for each plant.

Statistical analyses

All analyses were performed using R version 4.0.1 (R Development Core Team 2020). For each of the measured traits, we ran the Levene test for homogeneity of variance (leveneTest in the *car* package for R) at both the species and treatment levels (i.e. trait ~ species×treatment). When the underlying assumption of normality was met ([TP], [CT], ACP, leaf N:P and leaf C:N), we used ANOVA to test for differences in the measured plant traits at the species×treatment levels using Tukey honestly significant differences (in the *stats* package for R) for multiple comparisons. Because of the design of the experiment, we treated each individual within a species as an independent replicate, although we acknowledge that our design is inherently pseudoreplicated. When the assumption of normality was violated (spine length, spine diameter and spine density), we used the nonparametric Dunn test (dunn.test in the Dunn.test package V1.3.5 for R, Dinno 2017) with a Bonferroni correction for multiple comparisons. To test whether species adopted different induction strategies, we ran a principal component analysis (PCA) of the eight measured defence traits using the function dudi.pca (ade4 package for R, Dray & Dufour 2007) with scale set as true, to allow for the column vectors to be normed for the row weightings.

RESULTS

For the two traits related to chemical defence, [TP] was significantly higher inside the exclosure (herbivore exclusion) for *A. tortilis* compared with the previous exclosure ($F = 3.98$, df = 8, $P < 0.001$) but did not significantly differ between treatments in the other species (Fig. 1a). Across all species [TP] did not differ between the previous and control treatments but was significantly higher in the exclosure compared with both the control ($P = 0.025$) and previous ($P = 0.001$) treatments (Fig. 1b). Total CTs were not affected by the different treatments at the species level (Fig. 1c) but across all species, CT showed a slight increase (not statistically significant, $F = 2.07$, df = 2, $P = 0.12$) with the exclusion of herbivores (Fig. 1d).

Structural defences in general were induced by the presence of herbivores. We found a significant positive influence of presence of herbivores on both spine length ($\chi^2 = 149.3$, df = 2, $P < 0.001$, Fig. 2a,b) and spine diameter ($\chi^2 = 181.3$, df = 2, $P < 0.001$, Fig. 2c,d) for all species except *Z. mucronata*. Spine density was only slightly lower when protected from herbivores compared with the control and previous treatments (Fig. 2e,f).

For the three traits related to browse nutritional quality, ACP was significantly lower when herbivores were excluded compared with both the control ($F = 11.04$, df = 2, $P = 0.001$) and previous ($P < 0.001$) treatments (Fig. 3b), leaf N:P was significantly higher in the control treatment compared with the exclosure ($F = 22.85$, df = 2, $P < 0.001$) and previous ($P < 0.001$) treatments (Fig. 3d), and leaf C:N was significantly higher when protected from herbivores but similar in the open, control and previous treatments and ($F = 23.51$, df = 8, $P < 0.001$, Fig. 3f). Across all species, leaf C:N was significantly ($P = 0.03$) correlated with dry leaf matter content (LDMC) and showed very similar patterns according to species and treatment, LDMC was, therefore, removed from further analysis. When considering specific species, *A. tortilis* had significantly higher ($F = 2.88$, df = 8, $P < 0.001$) ACP in the previous exclosure treatment compared with the exclosure treatment, while *Z. mucronata* had significantly lower ACP in the exclosure treatment compared with the control ($P < 0.001$) and previous ($P = 0.04$) treatments (Fig. 3a). For *Z. mucronata*, leaf N:P was significantly higher in the control treatment compared with the exclosure ($F = 2.73$, df = 8, $P = 0.003$) and previous ($P = 0.0002$) treatments (Fig. 3c). Leaf C:N was similar for the control and previous treatments and significantly higher when herbivores were excluded for *A. tortilis*, *R. tenuinervis* and *Z. mucronata* ($F = 3.21$, df = 8, $P < 0.05$, Fig. 3e).

The PCA based on the eight measured traits in this study showed that the traits of the five dominant species were mostly similar for the three treatments on PC1 (37%) which was most strongly influenced by TP, CT and ACP (Fig. 4, Table 1). However, on PC2 (30%), which was most strongly influenced by leaf C:N and spine length, the trait values of the five species were mostly similar for the control and previous treatments but clearly separated from the exclosure treatment (Fig. 4).

DISCUSSION

The main findings from this long-term exclosure experiment in a semi-arid dystrophic savanna site in HNP, Zimbabwe include (i) little to no response in the measured chemical defences, (ii) induction of
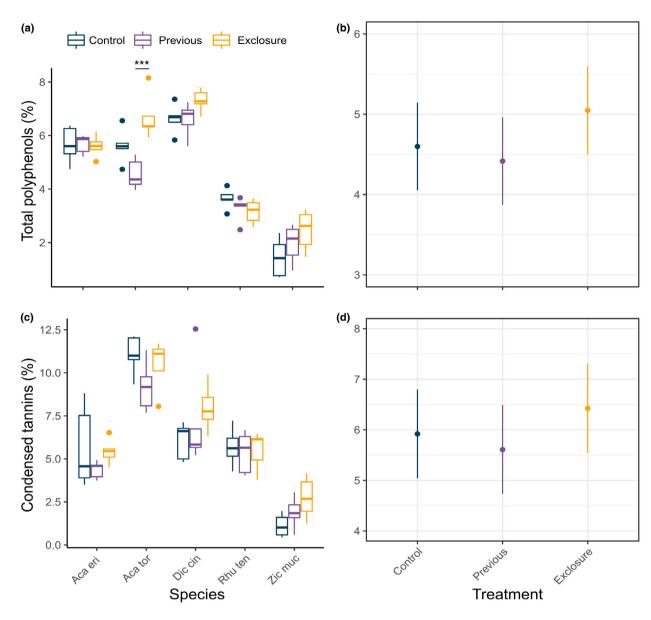
Fig. 1. (a) Boxplots showing median values and spread of total polyphenols. (b) Mean ± LSD total polyphenols. (c) Box-plots showing median values and spread of condensed tannins. (d) Mean ± LSD condensed tannins. Treatment effects are shown at the species level in a and c with significant differences between treatments within species indicated. We have not shown differences between species as this was not an objective of this study. Treatment effects are shown across all species in b and d for which overlapping intervals show no significant differences ($P > 0.05$).

structural responses in most species (all species were spinescent), (iii) no evidence for simultaneous induction of chemical and structural defences, (iv) the presence of herbivores resulted in increased plant nutritional quality (↑ACP and ↓C:N ratios) of browse and (v) the plant defence traits of the treatment where herbivores were previously excluded were similar to the treatment that remained open to LMHs – which both differed from the exclosure treatment, indicating that defence traits such as spinescence can be induced (and relaxed) over a relatively short time period.

Although spinescence increased with herbivory in all species, there was no corresponding increase in CTs and TPs. The presence of LMH herbivory, if anything, decreased the concentrations of TPs. These results are in agreement with previous work that found increased CTs and TPs in savanna trees with herbivore exclusion (Rohner & Ward 1997; Scogings et al. 2011, 2014; Wigley et al. 2014, 2015, 2019). Here, we explore three potential mechanisms that might explain the observed pattern of limited responses of the specific N-free, large weight
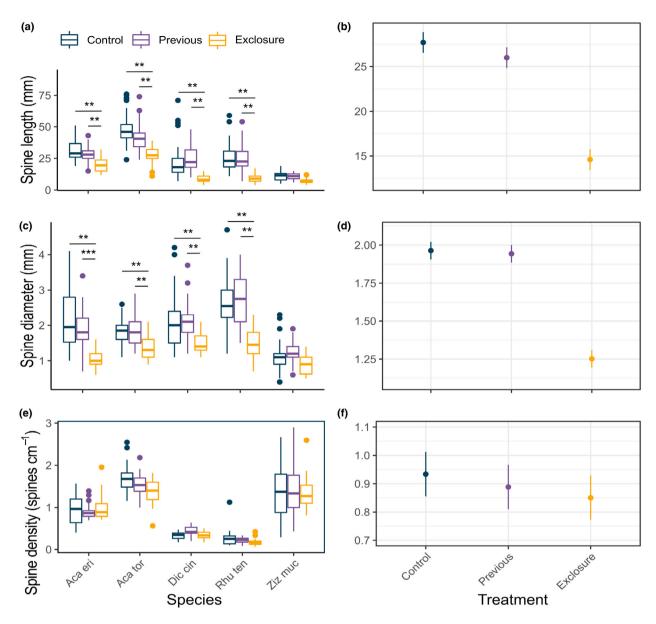
Fig. 2. (a) Boxplots showing median values and spread of spine length. (b) Mean ± LSD spine length. (c) Boxplots showing median values and spread of spine diameter. (d) Mean ± LSD spine diameter. (e) Boxplots showing median values and spread of spine density. (f) Mean ± LSD spine density. Treatment effects are shown at the species level in a, c and e with significant differences indicated and across all species in b, d and f for which overlapping intervals show no significant differences ($P > 0.05$).

metabolites measured, together with a significant induction of structural defences in response to mammalian herbivory.

1. Increased N-free secondary chemical concentrations do not offer increased defences against vertebrate herbivory. Some work has proposed that secondary compounds may 'incidentally' (i.e. 'neutral resistance' of Edwards 1989) act as defences (Carmona *et al.* 2011). Some studies propose that N-free compounds have non-defence functions including anti-fungal actions

(Scalbert 1991), while others show that tannins can be beneficial to animal health (Cooper & Owen-Smith 1985; Cooper *et al.* 1988; Bernays *et al.* 1989; Close & McArthur 2002; Hoste *et al.* 2006; Alonso-Díaz *et al.* 2010). Additionally, it is possible that herbivores and consumers other than mammals (e.g. insects) increased in the exclosures as has been found elsewhere (Goheen *et al.* 2004, 2018; Long *et al.* 2017) and that the increases in TPs happen in response to increased herbivory by groups other than LHMs.
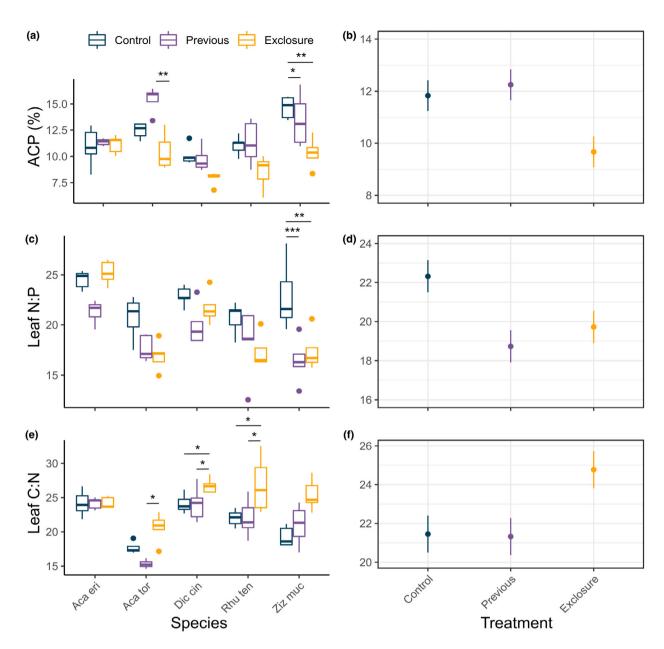
Fig. 3. (a) Boxplots showing median values and spread of available crude protein. (b) Mean ± LSD available crude protein. (c) Boxplots showing median values and spread of leaf N:P. (d) Mean ± LSD leaf N:P. (e) Boxplots showing median values and spread of leaf C:N. (f) Mean ± LSD leaf C:N. Treatment effects are shown at the species level in a, c and e with significant differences indicated and across all species in b, d and f for which overlapping intervals show no significant differences ($P > 0.05$). Aca eri = *Acacia erioloba*, Aca tor = *Acacia tortilis*, Dic cin = *Dicrostachys cinerea*, Rhu ten = *Rhus tenuinervis* and Ziz muc = *Ziziphus mucronata*.

2. There is a temporal mismatch between the expression of N-free secondary metabolites and physical responses such as spines. Physical defences can only be produced in the growth that happens after the plant has been exposed to herbivory. Chemical defences, on the other hand, may be produced in the tissues already present when the plant is browsed. However, with chronic browsing, both of these would be expected to be produced in new growth and once N-free secondary metabolites are produced, they should persist (Scogings 2005; Scogings *et al.* 2011). Large-molecular-weight N-free secondary metabolites, such as CTs are immobile because they are metabolically inactive (Coley *et al.* 1985; Zinn *et al.* 2007; Read *et al.* 2008). They are usually produced in young tissue and remain in the leaves until senescence, and in this
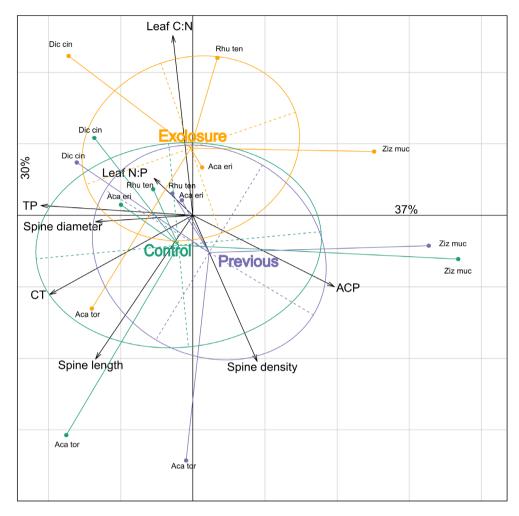
Fig. 4. PCA based on the eight measured plant traits of the five dominant woody plant species growing in the control (green), previous (purple) and exclosure (yellow) treatments in Hwange National Park, Zimbabwe. Axis 1 explained 37% of the variance and was most strongly associated with variation in total polyphenols and condensed tannin and available crude protein. Axis 2 explained 30% of the variance and was most strongly associated with variation in leaf C:N and spine length (see Table 1 for eigenvector scores of the principal component analysis). Points represent the mean location of each species on principal component axes 1 and 2 for each of the three treatments. Ovals show the overall means (centre) for each treatment and dispersion (ellipses).

Table 1. Eigenvector scores for the principal component analysis (PCA) based on the eight measured traits

	PC1 (37%)	PC2 (30%)	PC3 (15%)	PC4 (13%)	PC5 (2%)	PC6 (2%)
ACP (%)	0.47	−0.24	0.33	0.18	0.02	0.41
Leaf N:P	−0.13	0.13	0.23	0.88	−0.25	−0.27
Leaf C:N	−0.07	0.61	−0.13	0.05	0.59	−0.21
TP (%)	−0.51	0.03	−0.24	0.26	0.16	0.76
CT (%)	−0.48	−0.27	−0.26	−0.14	−0.39	−0.21
Spine length (mm)	−0.33	−0.49	0.21	0.08	0.43	−0.26
Spine diameter (mm)	−0.33	−0.02	0.71	−0.22	0.20	0.06
Spine density (spines cm^{-1})	0.22	−0.49	−0.38	0.23	0.44	−0.12

ACP, available crude protein; CT, condensed tannin; TP, total polyphenol.

way may affect decomposition (Kraus *et al.* 2003; Valachovic *et al.* 2004).

3. N-free secondary compounds are more costly to produce than physical defences such as spines and branching in browsed savanna trees. There is a trade-off in the allocation of resources to spines *vs.* allocation to N-free secondary compounds that depends on a plants palatability,

growth rate and available resources. Accordingly, as intensely browsed plants become C-starved on nutrient-poor soils, a trade-off occurs in favour of spines as they are less costly to produce in terms of photosynthates than N-free secondary compounds. Although there is some debate in the literature as to whether physical defences are indeed 'cheap' to produce, especially in the presence of herbivores (Gómez & Zamora 2002; Craine *et al.* 2003; Armani *et al.* 2019, 2020), this mechanism (i.e. allocation to new shoots and structural defences) has been proposed before to explain the observed lower concentrations of N-free secondary compounds with browsing (Fornara & Du Toit 2007; Hrabar *et al.* 2009) as these compounds are expensive to produce when a plant is carbon stressed (Skogsmyr & Fagerström 1992).

The second main objective of this study was to test if a reduction in plant nutritional quality was evident in response to herbivory (i.e. did plants become more unpalatable with herbivory?) Barton (2016) suggests that if both physical and chemical defences are induced by herbivory but where nutritional quality stays unchanged, then this will give rise to particularly unpalatable plants. Our results, which agree with previous studies (e.g. Scogings *et al.* 2013; Wigley *et al.* 2014, 2015, 2019), showed the opposite pattern, where browsed plants generally had higher nutritional quality (higher ACP and lower C:N ratios) than plants protected from herbivory. Fornara and Du Toit (2007, 2008) showed that African *Acacia* species can be maintained in a high N, hedged state by chronic browsing analogous to the positive feedback between grazing and regrowth that gives rise to 'grazing lawns'. A reduction in intershoot competition as a result of repeated browsing is expected to result in increased browse quality and to maintain a browsing-regrowth feedback loop (Du Toit 1990; Fornara & Du Toit 2007).

Our final objective was to determine if the reintroduction of herbivory after long-term herbivore removal (i.e. previous treatment) unravelled any long-lasting effects of herbivory suppression on plant chemical defences, structural defences and plant nutritional quality. Our findings suggest that the defence traits of the plants in this treatment had all returned to levels that were similar (evident in the PCA; Fig. 4) to the control treatment (i.e. continuously exposed to herbivores) for the measured secondary chemicals (i.e. tannins and polyphenols), structural defences and traits related to plant nutritional quality. One notable exception was evident in the N:P ratios of the previous treatment, which were found to be lower than the control treatment but

similar to the exclosure treatment. The differences in N:P ratios were primarily driven by higher leaf P concentrations in the previous treatment compared with both control and exclosure treatments (data not shown), a surprising finding for which we do not have any explanatory mechanism.

In conclusion, this is the first study that we are aware of that has used mammal herbivore exclusion to explicitly test for the simultaneous induction of multiple defence traits and furthermore, to test for the induction of defences after herbivory was excluded and defences were relaxed by using a herbivore reintroduction treatment. We found no evidence to suggest that both chemical (tannins and TPs) and structural defences are simultaneously induced by herbivory in our study. Instead, we suggest that mammalian herbivory results in the induction of structural defences, little change in total polyphenolics and CTs and an increase in plant nutritional quality. This is a syndrome widely encountered in savanna species; that is, cluster B of Wigley *et al.* (2018). We propose that variable costs of chemical *vs.* physical defences and how these interact with growth may be responsible for the patterns in defence responses that we observed. It may be that plants that are highly structurally defended, lose less resources, resulting in higher availability of resources for growth and the production of nutrient-rich foliage. At the same time, the costs of producing physical *vs.* chemical defences and how this trades off with growth, especially for savanna trees that have long evolutionary histories of being browsed, remain poorly understood. Whether plants that have high levels of structural defences have a higher availability of resources, for example, N which can be allocated to the leaves, may be an interesting future study direction.

AUTHOR CONTRIBUTIONS

Benjamin Joseph Wigley: Conceptualization (equal); formal analysis (equal); methodology (equal); writing – original draft (lead). **Corli Coetsee:** Formal analysis (equal); visualization (equal); writing – original draft (equal). **Kuzivakwashe Adolf Mawoyo:** Data curation (equal); investigation (equal); methodology (equal). **Hervé Fritz:** Conceptualization (equal); funding acquisition (equal); methodology (equal); project administration (equal); resources (lead); writing – original draft (supporting).

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data used in this study will be archived at the Dryad Digital Repository

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SUPPORTING INFORMATION

Additional supporting information may/can be found online in the supporting information tab for this article.

Appendix S1. Google Earth imagery of the exclosure, control and previous exclosure plots in 2007 (top) and 2019 (bottom). Each treatment measured 120 m × 120 m (1.44 ha).

Appendix S2. The study sites were grass-dominated open savanna interspersed with trees and bush clumps (left). *Acacia erioloba, Rhus tenuinervis* and *Dicrostachys cinerea* growing next to each other in a bush clump in the exclosure treatment (right).