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A thorny issue: woody plant defence and growth in an East African savanna

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Abstract

1. Recent work suggests that savanna woody plant species utilise two different strategies based on their defences against herbivory; a low nutrient/high chemical defence strategy and a nutrition paired with mostly architectural defences strategy. The concept that chemical and structural defences can augment each other and do not necessarily trade-off has emanated from this work. In this study we examine woody plant defence strategies, how these respond to herbivore removal and how they affect plant growth in an East African savanna.

2. At three paired long-term exclosure sites with high browser and mixed feeder densities at Mpala Ranch, Kenya we investigated: a) whether defences employed by the dominant fine- and broad-leaved woody savanna species form defence strategies and if these align with previously proposed strategies, b) how nine key plant defence traits respond to herbivore removal and c) how effective the different defence strategies are at protecting against intense herbivory (by measuring plant growth with and without herbivores present).

3. We identified three defence strategies. We found a group (1) with high N, short spines and high N-free secondary metabolites, a group (2) with high N, long spines and low N-free secondary metabolites and a group (3) with moderate N, no spines and low N-free secondary metabolites (most likely defended by unmeasured chemical defences). Structural defences (spine length, branching) were generally found to be induced by herbivory, leaf available N increased or did not respond, and N-free secondary metabolites decreased or did not respond to herbivory. Species with long spines combined with increased ‘caginess’ (dense canopy architecture arising from complex arrangement of numerous woody and spiny axis categories) of branches, maintained the highest growth under intense browsing, compared to species with short spines and high N-free secondary metabolites and species with no spines and low N-free secondary metabolites.

Synthesis. At our study site, structural traits (i.e. spines, increased caginess) were the most inducible and effective defences against intense mammalian herbivory. We propose that high levels of variability in the way that nutrient and defence traits combine may contribute to the coexistence of closely related species comprising savanna woody communities.

Key-words: herbivore exclosures, induced defences, mammal browsers, N-free secondary metabolites, plant defence strategies, plant–herbivore interactions, structural defences

Introduction

A longstanding conundrum in ecology is how plants persist in the face of intense herbivory when resources are limited. Plants growing in African savannas have a long history of co-evolution with a diverse array of mammalian herbivores, and have evolved a range of different strategies to deal with herbivory (Charles-Dominique et al., 2016). Classical defence theory suggests that plants can respond to herbivory in two ways, i.e. either tolerate or resist it (Herms & Mattson, 1992; Strauss & Agrawal, 1999; Núñez-Farfán, Fornoni, & Valverde, 2007). Defence theories make predictions as to how plants allocate resources to traits that confer tolerance *vs.* resistance depending on the environmental conditions and available resources. While much work has focussed on generating theories that predict allocation to defence-related plant secondary metabolites (e.g. see Stamp, 2003 for a review of the different theories), it is less clear under what conditions (i.e. resource and herbivory levels) plants should invest in structural defences and more specifically in spines as none of the existing defence theories are able to clearly predict the incidence of spinescence (Grubb, 1992; Herms & Mattson, 1992; Hanley, Lamont, Fairbanks, & Rafferty, 2007; Tomlinson et al., 2016).

More recently, studies of plant investments in traits related to herbivore tolerance or resistance have increasingly recognized that defence must be considered in terms of co-adapted trait complexes, i.e. ‘syndromes’, or ‘strategies’, rather than simple trade-offs between allocation to growth *vs.* defence (Steward & Keeler, 1988; Twigg & Socha, 1996; Koricheva, Nykänen, & Gianoli, 2004; Agrawal & Fishbein, 2006; Read et al., 2008; Cornelissen et al., 2009; Moles et al., 2013; Barton, 2016). For example, Da Silva & Batalha, (2011) categorised plants growing in South American savannas into two defence syndromes; a low nutrient/high chemical defence syndrome (low nitrogen, low specific leaf area and presence of secondary metabolites) and a nutrition and defence syndrome (high nitrogen, thicker leaves and higher density of trichomes). Tomlinson et al., (2016) found that rather than trade-offs between traits, defences of savanna juvenile tree species could be grouped into two strategies: a low nutrient/high chemical defence strategy that may include low nutrient content, physical toughness and leaf chemicals (either digestion retardants such as tannins or poisons such as alkaloids) that make plants unattractive to herbivores, and a structural or architectural defence strategy which may include spinescence, branching and pubescence. Likewise, in a study of southern African savannas spanning a range of resource levels, Wigley, Fritz, & Coetsee, (2018) found a low nutrient/high chemical defence strategy with low N and high N-free secondary metabolites levels as well as a nutrition and defence strategy which included variable combinations of chemical and structural defences.

While the classification of savanna trees into these two categories - ‘low nutrient/high chemical defence’ and ‘nutrition combined with architectural defence’ - provides a first approximation of their overall defence strategies, defence strategies in savanna trees are likely to be more nuanced than these broad categories suggest. Previous studies have often found several ‘clusters’ or ‘groups’ within overarching strategies, particularly for the nutrition and defence strategy (Agrawal & Fishbein, 2006; Da Silva & Batalha, 2011; Wigley et al., 2018). For example, Wigley et al., (2018) proposed that the nutrition and defence strategy in southern African savanna trees can be subdivided into two groups; a strategy that includes high leaf N and high structural defence investment, but low investment in N-free secondary metabolites, as well as an additional strategy that includes plants with high leaf N and a high incidence of both structural defences and N-free secondary metabolites. Agrawal & Fishbein, (2006) similarly found two clusters within the nutrition and defence syndrome; in one, species were defended through a combination of latex and trichomes, and in the other, through cardenolides.

There are good reasons why multiple traits should evolve as strategies or syndromes, including the need for plants to defend themselves against a wide range of herbivores (Agrawal, 2011). However, there are only a few studies that have investigated how plant physical and chemical defences that comprise these strategies or syndromes are induced or relaxed in response to herbivore presence or exclusion (i.e. do plants induce different defence traits similarly in response to herbivory or are changes greater for one *vs.* the other?), ultimately limiting our understanding of the ‘syndrome’ concept (Barton, 2016). Furthermore, it is very difficult to evaluate how different defence strategies affect plant performance as this typically requires long-term herbivore exclusion experiments.

For syndromes to manifest, plants should be able to employ different combinations of physical and chemical defences (Barton, 2016). Plant defence theories predict that slow-growing plants (which are usually associated with limited resources) should invest heavily in defences and more specifically in low maintenance defences such as tannins and lignin (Bryant, Chapin III, & Klein, 1983; Coley, Bryant, & Chapin III, 1985; Coley, 1988; Herms & Mattson, 1992; Craine et al., 2003). Depending on the theory, growth-dominated plants are predicted to either have low investments in defences (Bryant et al., 1983; Herms & Mattson, 1992) or invest in qualitative defences (Coley et al., 1985; Coley, 1988). Architectural defences which include spines are generally predicted to be more prevalent on nutrient-rich soils, especially in savanna ecosystems (Grubb, 1992; Craine et al., 2003; Scholes et al., 2002; Hanley et al., 2007). Wigley et al., (2018) found some support for this with significantly higher branching and spine densities at nutrient-rich compared to nutrient poor sites in Southern African savannas. Architectural defences (spines and branching density or 'caginess') are generally induced by herbivory in savannas (e.g., Milewski et al., 1991; Wigley et al., 2015). Chemical defences on the other have been found to be down-regulated by herbivory in savannas, most likely due to C-limitation imposed by the repeated removal of photosynthesising leaf material by browsing herbivores (e.g., Scogings, Hjältén, & Skarpe, 2011, 2013; Scogings, Mamashela & Zobolo, 2013; Wigley et al., 2015). It is, however, still not clear if similar plant functional types (e.g., fine- *vs.* broad-leaved) or closely related species within a community growing on the same soils (i.e. same soil fertility status) employ similar suites or combinations of defences (defence syndromes).

We note that 'syndrome' or 'strategy' and 'cluster' or 'group' have often been used interchangeably in the plant defence literature (Agrawal & Fishbein, 2006; Da Silva & Batalha, 2011; Tomlinson et al., 2016). For the sake of expediency, we consistently use 'strategy' and 'group' hereafter to encompass the definitions of syndrome and cluster respectively. We examine defence strategies in savanna trees by quantifying the expression of plant physical and chemical defences as well as plant growth in the presence *vs.* absence of intense browsing by mammalian herbivores, using a set of long-term herbivore exclosures in an East African savanna in Laikipia County, Kenya. Specifically, we asked the following questions: 1) Are the defence strategies observed in the dominant fine- and broad-leaved woody species of this East African savanna similar to those documented in other savanna ecosystems – e.g., low nutrient/high chemical defence strategy (*sensu* Tomlinson et al., 2016) *vs.* nutrition and defence strategy (Tomlinson et al., 2016; Wigley et al., 2018)? 2) Are there differences in the extent to which alternative defence strategies are successful at resisting herbivory, i.e. as indexed by differences in growth in the presence and absence of herbivory? 3) Are there differences in how physical and chemical traits associated with different defence strategies respond to herbivory, i.e. which traits are more inducible? Based on previous work (Da Silva & Batalha, 2011; Tomlinson et al., 2016; Wigley et al., 2018), we expect to find different defence strategies being employed by woody plants in our study system with various combinations of chemical and structural defences. We expect that these different strategies will determine how successful plants are at resisting herbivory, i.e. we expect to find differences in growth between strategies. Finally, based on previous findings, we predict that structural defences will be higher (induced) with herbivores present, but chemical defences will not be up-regulated at high browsing intensities (e.g., Scogings, Hjältén, & Skarpe, 2013; Scogings, Mamashela & Zobolo, 2013; Wigley et al., 2015).

Methods

Study Sites

Our study was carried out at the Mpala Research Centre (MRC) and Mpala Ranch (190 km^2) in Laikipia County in central Kenya (37°53' E, O°17' N). We used three sets of herbivore exclosures constructed at MRC in 1999. The exclosures consisted of an 11-strand, 3-m tall electrified fence with additional mesh and electrified wires from 0 – 0.5 m height and excluded herbivores larger than 2 kg for seventeen years (Augustine & McNaughton, 2004; Sankaran, Augustine, & Ratnam, 2013). The savannas at these sites occur on red, sandy loam soils developed from basement, metamorphic parent materials (Augustine, 2003; Pringle, Prior, Palmer, Young, & Goheen, 2016). Topography consists of gently, rolling hills, interspersed with occasional granitic inselbergs (Augustine & McNaughton, 2006). The long-term mean annual rainfall (1972–2009) is 514 mm (Sankaran et al., 2013). Vegetation is characterized by an Acacia-dominated tree and shrub community and a discontinuous layer of perennial grasses (Augustine, 2003). The most common native browsers and mixed feeders found include impala *Aepyceros melampus* (c. 20 km^{-2}), Günther's dik-dik *Madoqua guentheri* (c. 140 km^{-2}) and elephant *Loxodonta africana* (c. 1.7 km^{-2}; Augustine, 2010). Impala and dik-dik are present throughout the year, while elephants tend to migrate into the area during the wet season and are less abundant during dry seasons (Augustine, 2010; Thouless, 1995). Eland *Taurotragus oryx*, giraffe *Giraffa camelopardalis reticulata* and greater kudu *Tragelaphus strepsiceros* also occur at Mpala at lower densities (see Augustine 2010). Previous studies have shown that the high densities of browsers and mixed-feeders at the study sites have major effects on the dynamics, population demography and structure of the woody vegetation (Augustine & McNaughton, 2004; Sankaran et al., 2013).

Trait sampling

In 2016, we sampled the six most abundant woody species for key plant traits constituting the architectural defence and low nutrient/high chemical defence strategies. Three of the six species were fine-leaved species (*Acacia mellifera, Acacia etbaica* and *Acacia brevispica*), and three were broad-leaved (*Balanites pedicellaris, Grewia tenax* and *Croton dichogamus*). Both *Balanites pedicellaris* and *Croton dichogamus* are evergreen species. Species nomenclature is based on Noad & Birnie (1990).

Measured traits that influences leaf quality included concentrations of leaf condensed tannins and total polyphenolics, leaf total and available N, specific leaf area and fibre. Architectural-related traits included spine length, spine density, bite size index and a branching index. For trait measurements, we randomly selected five individuals of each species at each site in the sapling size class (typically between 1 and 2 m in height) inside exclosures (h-) and in adjacent control plots with herbivores present (h+). We collected fully expanded, sun-exposed leaf material from each plant during the peak of the growing season for nutrient and chemical analyses. All leaf material was air-dried until samples reached constant weight at Mpala Research Station. Samples were milled using a MF10 basic IKA grinder fitted with a 1 mm sieve.

We analysed leaf material for nitrogen (N) using a Leco TruSpec CN Analyser (LECO Corporation, St. Joseph, MI). Dried leaf digestibility and leaf available N were determined as outlined in DeGabriel et al. (2008), but with some improvements. Briefly, 800 ± 10 mg of dry leaf material was transferred into pre-weighed filter bags (ANKOM F57, ANKOM Technology) and heat-sealed to lock in plant material. A maximum of 20 bags were placed in a 1 litre polypropylene screw cap container to which 35 ml of pepsin solution per bag was added. Bags were incubated at 37°C for 24 h with circular shaking in an orbital rotator (GFL 3040, Gesellschaft für Labortechnik mbH, Germany) at 14 rpm. The addition of rotational shaking far better simulates ruminal contraction or gut motility, which is an important physiological adaptation, which ensures constant mixing of ingested food and probably aids in digestion (Clauss et al., 2005). After incubation, bags were washed five times with distilled water, after which 35 ml of cellulose solution was added to each bag and incubated as outlined above for 48 h. After incubation, bags were again washed 10 times with distilled water and dried at 60°C to constant weight. To account for any loss to the bag in the incubation process, a blank bag was included. Dry matter digestibility was calculated from the amount of material lost in the incubation process and leaf available N was calculated by subtracting N remaining in the residue from the initial total leaf N. We then calculated how much of the total leaf N was available to herbivores on a dry weight basis and named this total available N (i.e. leaf N (mg g^{-1}) x proportion available N). We measured the concentration of total condensed tannins (CT) in each leaf sample following Hattas & Julkunen-Tiitto, (2012) and the concentration of total polyphenolic compounds (TP) following Hattas et al., (2005). We used an Ankom fibre analyser to measure acid detergent fibre (ADF) content of all leaf samples which provides an index of the unpalatable fibre component of leaf material and includes the cell-wall components of cellulose and lignin (Cooper, Owen-Smith, & Bryant, 1988).

To measure specific leaf area, we collected 10 - 20 healthy, fully expanded, sun-exposed leaves from each plant and immediately scanned them at a resolution of 300 dpi in the field using a Canon CanoScan LiDE 100 flatbed scanner. After scanning, the leaves were oven dried and weighed. We measured the area of the leaf scans using Black Spot (Varma & Osuri, 2013). We calculated specific leaf area (SLA) by dividing leaf area by dry weight for each plant.

We measured average spine lengths and average diameter at the spine base of ten mature spines on each of 3 branches per plant using digital Vernier callipers. We determined spine density by counting the total number of spines on a measured length of each of the same three branches and dividing by the branch length. We calculated branch density on three branches per tree by dividing the number of lateral branches on each branch by the length of the terminal branch (see Perez-Harguindeguy et al., 2013). The bite size index (BSI) for a given plant was estimated as the total dry weight of leaves removed from ten human bites taken from each plant (see Wigley et al., 2014; Charles-Dominique et al., 2015). An attempt was made to remove the maximum amount of leaf material with each bite. The same person conducted all BSI measurements to control for potential differences between individual recorders.

Plant growth measurements

At the time of fence construction in 1999, all individual trees and shrubs >0.5 m tall within a 50 x 50 m area in each exclosure and paired control site were mapped, tagged and their basal area (at 15 cm above-ground level, including all stems on multi-stemmed individuals), canopy dimensions (maximum length and width in the cardinal directions) and plant height were measured. All plots were fully censused again in 2002, 2009 and 2016. During each census, the height, basal area and canopy dimensions of all previously tagged plants were remeasured and all new recruits (>0.5 m) were also measured and recorded and all mortalities were noted. We calculated the mean change in plant height and basal area for each of the six species using all individuals that were mapped and measured in 1999 and that were still present in 2009 in each treatment at the three sites (*Acacia brevispica* n = 359, *Acacia etbaica* n = 349, *Acacia mellifera* n = 326, *Balanites pedicellaris* n = 24, *Grewia tenax* n = 205, *Croton dichogamus* n = 54). By 2009, plant densities inside the exclosures had significantly increased to a level where competition between plants was likely to have started affecting growth. We therefore only used growth data up until 2009 to avoid the effects of interspecific and intraspecific competition.

Statistical analyses

All analyses were performed using R version 3.3.1 (R Development Core Team, 2016). To test whether species adopted different defence strategies and how these strategies responded to herbivore exclusion, we ran a principal component analysis (PCA) of the nine measured defence traits using the function dudi.pca (ade4 package for R, Dray & Dufour 2007). We ran Horn's Parallel Analysis using the function paran (paran package for R) to evaluate the number of components to be retained in the principal components analysis. We first used the Fligner-Killeen test of homogeneity of variance (fligner.test in the *stats* package for R) to test if the data used for treatment (h- vs. h+) comparisons were normally distributed. When the assumption of normality was met, we used paired t-tests to evaluate effects of browser exclusion on the measured plant traits, change in plant height and woody basal area for each of the six dominant woody species. When the assumption of normality was violated, we used the nonparametric Wilcoxon rank sum test. We pooled trait data from the three exclosure sites for each species, giving 15 individuals sampled for each treatment for four of the species. *Acacia brevispica* and *Croton dichogamus* only occurred at two of the three sites, and consequently we only had 10 individuals sampled for each trait in each treatment.

Results

Defence strategies

Results from Horn's Parallel Analysis for factor retention based on 5000 iterations, using the mean estimate, showed that the first six components of the PCA should be retained. The PCA based on the measured plant traits showed that the six dominant species at the study site separated into three broad groups along the first two PC axes, which together, accounted for almost 60% of the total variance (Fig. 1). This separation was most strongly driven by differences in spine length and fibre on PC1, separating the two species with long spines and lower fibre (*Acacia etbaica* and *Balanites pedicellaris*) from the species with higher fibre and short spines (*Acacia brevispica* and *Acacia mellifera*) or no spines (*Grewia tenax* and *Croton dichogamus* (Table 1). On PC2 the groups were most strongly separated by differences in N-free secondary metabolites and spine density. Two of the broad-leaved species, *Grewia tenax* and *Croton dichogamus* were similar in terms of having no spines and lower concentrations of N-free secondary metabolites and were most strongly separated from *Acacia brevispica* and *Acacia mellifera,* which had high N-free secondary metabolites and spine densities (Fig.1, Table1).

Effects of herbivore removal on plant defence traits and strategies

The PCA (Fig. 1) illustrates the effects of herbivore removal and control treatments on the plant defence traits (and strategies), with herbivore removal treatments diverging from control treatments along both PC axes to varying degrees (Fig. 1). However, none of the species changed defence strategies as a result of herbivore removal; both structural and chemical defences responded to variable degrees both within and between strategies. The long-term removal of large mammalian browsers from this savanna did not affect leaf total N and acid detergent fibre for any of the six of the woody species (Table 2). Specific leaf area declined (i.e. leaves became smaller and thicker) with herbivore exclusion for one fine-leaved species (*A. mellifera*; $P = 0.05$) and increased (leaves became larger and thinner) with herbivore exclusion for two broad-leaved species *Balanites pedicellaris* ($P = 0.03$) and *Grewia tenax* ($P = 0.09$; Fig. 2). Herbivore removal resulted in higher total polyphenols in *A. etbaica* and *A. mellifera* ($P = 0.07$ and $P = 0.01$ respectively) and significantly higher condensed tannins in *A. brevispica* ($P = 0.003$; Fig. 2) and lower available N in *Acacia mellifera* ($P < 0.1$) and *Croton dichogamus* ($P < 0.01$). Plant architectural defences (spines and branching densities) were found to relax with herbivore removal, i.e. became less structurally defended. Herbivore removal decreased the branching index and branches per volume ($P < 0.001$) and increased the bite size index (i.e. more leaf biomass per bite; $P < 0.0001$) for all species except *Croton dichogamus* (Figs. 3 & 4). Herbivore removal resulted in significantly shorter spines in *A. etbaica* ($P < 0.001$) and *A. mellifera* ($P = 0.02$) and a significantly lower density of spines in *A. etbaica* ($P = 0.01$) (Fig. 3).

Growth, defence strategies and responses to herbivore removal

Over the 10-year period between 1999 and 2009, we documented substantial variation in growth which we present in relation to the three groups. When growing in the presence of herbivores, group 1 (*A. brevispica* and *A. mellifera*) decreased in height (-0.42 ± 0.07 m and -0.10 ± 0.09 m respectively) but increased in basal area (2.69 ± 1.16 cm^2 and 11.0 ± 6.56 cm^2). In this group, herbivore removal resulted in significantly taller plants with higher basal area than with herbivores present ($P < 0.001$, Table 3, Fig. 5). Group 2 (*Acacia etbaica* and *Balanites pedicellaris*) increased in height (0.31 ± 0.06 m and 0.24 ± 0.44

m respectively) and basal area (20.9 ± 3.14 cm^2 and 34.6 ± 12.4 cm^2) in the presence of herbivores. In this group, herbivore removal increased plant height ($P < 0.01$) but not basal area (Table 3, Fig. 5). Plants in group 3 (*Grewia tenax* and *Croton dichogamus*) generally decreased in height but no consistent trend was evident for basal area when herbivores were present. For *Grewia tenax*, herbivore removal resulted in significantly taller plants with higher basal areas ($P < 0.001$). *Croton dichogamus* plants showed a slight decrease in height and increase in basal area during the study period with herbivores present; however, neither of the measures of growth differed between the herbivore removal and herbivores present treatments ($P > 0.05$). Based on the measured changes in plant height and basal area in the presence *vs.* absence of browsers over the course of a decade, we ranked the three groups from low to high in terms of their capacity to persist in the face of intense browsing pressure, where group 1 < group 3 < group 2 (Table 3, Fig. 5).

Discussion

Our overarching goal was to examine how woody species in this East African savanna use suites of traits to contend with a relatively intense browsing regime imposed by a diverse and abundant assemblage of large, mammalian herbivores. The hypotheses which underpin classic plant defence theory, rely heavily on resource availability to predict whether plants invest in growth or defence (Herms & Mattson, 1992) and hence, do not predict relative investments in structural *vs.* chemical defences (Hanley et al., 2007) or which defences will be most effective under fixed resource levels but varying herbivory pressure. Our results indicate that 1) woody species in this system can be classified into three different groups or strategies based on their differential investment in structural and chemical defences, 2) structural and chemical defences responded to varying degrees to the removal of herbivores, both within and across groups, but plant defence strategies remained qualitatively unchanged even after nearly two decades of herbivore exclusion, 3) structural and chemical defences did not respond in the same way to herbivore removal, with structural traits (especially branching) typically responding more positively (i.e. increased), and 4) the species that were most resilient to intense browsing (i.e. achieved the highest growth) were those that maintained high spine length, spine density and branching in the presence of herbivores.

None of the species in our study site appear to be adopting the 'low nutrient/high chemical defence' strategy, with most species having moderate to high leaf N compared to species previously categorised as low nutrient/high chemical defence strategists in other savanna systems (Wigley et al., 2018, Fig. 1a). The three strategies we identified thus all fall within the broad remit of 'nutrition and defence', but species in these different groups appear to invest variably in structural and N-free chemical defences. Fine-leaved species such as *Acacia brevispica* and *Acacia mellifera* (group 1) have moderate to high leaf N, low investment in structural defences (short spines) and high investment in N-free chemical defences (high CT and TP). *Acacia etbaica* and *Balanites pedicellaris* (group 2) similarly had moderate to high leaf N and both species invested more in physical defences (dense long spines/thorns) than chemical defences (low N-free secondary metabolites). Finally, species such as *Grewia tenax* and *Croton dichogamus* (group 3; both broad-leaved) had moderate to high leaf N, no spines and low N-free secondary metabolites.

Based on our measured changes in plant height and basal area in the presence compared to absence of browsers over the course of one decade, we ranked the six woody species from low to high in terms of their capacity to coexist with intense browsing pressure, where *G. tenax* < *A. brevispica* < *A. mellifera* < *C. dichogamus* < *B. pedicellaris* < *A. etbaica* (Fig. 5). Our results for the two broad-leaved species that lack investment in spines (group 3; *G. tenax* and *C. dichogamus*) provide several key insights regarding strategies for "living with browsers". Based on the framework developed from southern African savannas (Wigley et al., 2018), we expected both species to invest heavily in N-free secondary metabolites, as a trade-off to the lack of armament. Contrary to this prediction, neither species invested in N-free secondary metabolites, either in the presence or absence of large herbivores.

Lower leaf N (both total and available) was one conspicuous trait distinguishing *Croton* from the other species in this study, and may contribute to the fact that this species is generally unpalatable and uneaten by large browsers (Kartzinel et al., 2014). However, leaf N (~2.2%) was not so low as to compromise ruminant digestion efficiency (which typically occurs closer to 1% N, Van Soest, 1994), suggesting that some other potentially costly, and as yet unmeasured aspect of Croton leaf chemistry is a key trait allowing this broad-leaved species to coexist with large browsers. *Croton spp*. in general are highly aromatic and known to contain multiple unique secondary chemicals including cembranoids, halimanes, crotofolanes, sesquiterpenoids, flavonoids, and cyclohexanol derivatives (Langat et al., 2016), some of which are very successful in limiting vertebrate and invertebrate herbivory (Levin, 1976; Kaplan, Halitschke, Kessler, Sardanelli, & Denno, 2008). Including these types of chemical defences in a generalized trait framework will be challenging as they are not ubiquitous across species and their expression can depend on specific herbivore elicitors (Moreira et al., 2013).

The lack of investment in N-free secondary metabolites by the second broad-leaved species, *Grewia tenax*, was also surprising, but consistent with our finding that growth of this species is severely negatively affected by large browsers, and with previous work showing substantial declines in all size classes of *G. tenax* in the presence of browsers in this savanna (Augustine & McNaughton, 2004; Sankaran et al., 2013). Rather than employing any form of costly chemical defence, this species appears to coexist (uneasily) with browsers by increasing the complexity of its branching architecture (BI), and growing in close association with other thorny species that create structural refugia where *G. tenax* saplings are protected from browsers (personal observation by all authors, see also Coverdale, Goheen, Palmer, & Pringle, 2018). One value of this strategy is that when browsing pressure is removed or low, the lack of investment in costly defences, combined with high leaf N, allows for rapid growth.

The remaining four species belonging to the other two groups all have some form of investment in spines. They can be arrayed along a gradient of increasing spine investment from group 1 to group 2, with species in group 1 consisting of those with short, straight spines (*A. brevispica*) or short, recurved spines (*A. mellifera*), and those in group 2 having either long, straight spines (*B. pedicellaris*), or both short, recurved and long, straight spines (*A. etbaica*). Species with the longest and most dense spines (group 2) showed little to no investment in CT or TP, while the species with low spine investment (group 1) showed the greatest concentrations of both CT and TP (Fig. 2). The latter strategy experiences greater consumption by browsers in this landscape relative to the former (Ford et al. 2014), consistent with previous studies which have reported that species with higher investment in structural defences (group 2) are most successful at coexisting with browsers in this savanna system (Goheen, Keesing, Allan, Ogada, & Ostfeld, 2004; Sankaran et al., 2013). In the presence of browsers, species in both groups 1 and 2 maintained an increased complexity of their branching architecture combined with smaller leaves (reflected in substantial reductions in bite size index). Increased branching, even in the absence of changes in thorn length and density, which occurred in some species but not others, can result in dramatic increases in the number of spines per unit volume of canopy (Archibald & Bond, 2003; Staver, Bond, Cramer, & Wakeling, 2012), serving as an effective deterrent for browsers.

To summarise, the strategies expressed by spinescent species in terms of “living with browsers” can be characterized as (1) very high and inducible investment in spines but not N-free secondary metabolites and low available N, leading to greatest success under intense browsing (*A. etbaica*), (2) moderate and inducible investment in straight spines, combined with thicker, more fibrous leaves, but high available N, leading to sufficient success under intense browsing (*B. pedicellaris*), (3) low and non-inducible investment in recurved spines combined with high N-free secondary metabolites and low available N, leading to reduced success under browsing (*A. mellifera*), and (4) low and non-inducible investment in short spines combined with moderate investment in N-free secondary metabolites and high available N, leading to low success under browsing (*A. brevispica*). Fornara and Du Toit (2008) suggested that physical defences together with mass compensatory growth abilities are key adaptations to living with high browsing pressure. Our findings lend strong support to this notion as the most heavily structurally defended species (*A. etbaica* and *B. pedicellaris*) performed the best in the presence of herbivores despite of low investments in N-free secondary metabolites. However, Scogings et al. (2011) predicted that the concentrations of N-free secondary compounds would depend on browsing intensity with the highest concentrations occurring at intermediate levels of browsing for certain functional groups. For example, Scogings et al. (2011), predict a linear increase in N-free secondary compounds with browsing intensity, with a potentially sudden decrease at very high levels of browsing pressure in deciduous savanna species. As our experimental design lacked an intermediate browsing pressure treatment, we were unable to test these predictions.

Our analyses of available N provided some interesting and valuable insights. Firstly, interpretations of browse quality differ depending on whether total N (also commonly converted to crude protein by multiplying by 6.25, e.g. Cooper et al. 1998) or available N is evaluated. For example, the three Acacia species in this study; *A. brevispica*, *A. mellifera and A. etbaica* all had similar and relatively high concentrations of leaf total N (~3.8, 3.5 and 3.3% respectively) but the proportion of the total N available to herbivores in *A. brevispica* was *ca*. 20% higher than in *A. mellifera* and *A. etbaica* (~2.4, 1.7 and 1.5% respectively, see Table 2). If we only looked at total N they would all appear to be of high quality, however if we look at available N it appears as if *A. brevispica* is of much higher quality than the other two species. Secondly, available N appears to be more responsive to herbivore removal than total leaf N. No differences in total N were evident between herbivory treatments, while available N was significantly higher in *A. mellifera* and *C. dichogamus* when herbivores were present. Thirdly, differences in available N do not seem to be linked to phylogeny (e.g., high in some Acacia species, low in others), functional type (e.g., fine-leaved *vs*. broad-leaved) nor concentrations of N-free secondary metabolites (available N found to be low in species with both high and low N-free secondary metabolites). Finally, these results raise some important questions regarding the commonly held view (especially in African savannas) that fine-leaved species (e.g. *Acacia*) have higher quality leaves and are structurally defended, while broad-leaved species are less nutritious and chemically defended. For example, *G. tenax* and *B. pedicellaris*, both broad-leaved species, had higher available N than the two fine-leaved species, *A.*

mellifera and A. etbaica. These findings highlight the need for further studies to elucidate the determinants of leaf available N and that caution needs to be taken when using total N as a measure of browse quality. Furthermore, our findings suggest that neither total N nor available N are sufficient by themselves to predict browser utilisation, highlighting the need to consider all traits collectively (i.e. defence strategies).

Conclusion

All of the dominant species at our study site had moderate to high leaf N concentrations but differed in available N, and in the way they combined chemical and structural defences, thus fitting under the broader 'nutrition and defence' syndrome previously described for savannas. Structural defences had more pronounced responses to herbivory through increased spine length and density and/or increased branching, while N-free secondary metabolites did not increase in response to herbivory. High investment in structural defences was the most successful defence strategy at our study sites with moderate resource levels (relative to other African savannas) and high herbivory pressure, resulting in the highest growth in this savanna system. This work has shown that within one overarching defence syndrome (nutrition and defence), species within a community can widely diverge in their adopted defence strategies. Endara et al., (2015) report high levels of dissimilarity in the defences of closely related co-occurring forest species and suggest that this would be necessary for the coexistence of closely related neighbours and could account for the high local diversity of tropical forests. Similarly, we suggest that diverse defence strategies enable savanna species (which are often closely related) to occupy different niches and defend against different types of herbivores resulting in more resilient and species rich woody communities.

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Author contributions

BJW, CC, DA, and MS co-wrote the manuscript. BJW and MS collected the data. DA, MS, and JR implemented the exclosure experiment. DH conceptualised and performed the analytical approaches with respect to fibre and available N determination and performed condensed tannin and total polyphenol analyses. All authors commented and added to earlier versions of the manuscript.

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Tables

Table 1. Eigenvector scores for the principal component analysis (PCA) based on nine defence traits. SLA = specific leaf area, TP = total polyphenols, CT = condensed tannins, ADF = acid detergent fibre, BI = branching index and BSI = bite size index.

	PC1 (33%)	PC2 (26%)	PC3 (15%)	PC4 (10%)	PC5 (8%)	PC6 (4%)
Spine length (mm)	-0.53	-0.05	0.19	-0.07	0.10	0.25
Spine density (spines cm^{-1})	-0.30	-0.46	0.13	0.04	0.43	0.16
Total available N (mg g^{-1})	0.08	0.38	-0.29	0.26	0.78	-0.26
SLA (cm^{2} g^{-1})	0.32	-0.04	0.48	-0.56	0.15	-0.39
TP (%)	0.28	-0.49	-0.28	0.09	-0.11	-0.40
CT (%)	0.33	-0.42	-0.36	0.09	0.04	0.37
ADF (%)	0.48	0.13	0.07	-0.30	0.24	0.61
BI (branches cm^{-1})	-0.14	0.37	-0.53	-0.40	-0.24	0.05
BSI (g)	0.28	0.28	0.37	0.59	-0.23	0.12

Table 2. Mean ± se for leaf total available nitrogen (mg g^{-1}), leaf total nitrogen (mg g^{-1}), specific leaf area (SLA, cm^2 g^{-1}), leaf total polyphenolics (TP, %), leaf condensed tannins (CT, %), leaf acid detergent fibre (ADF, %), branching index (BI, branches cm^{-1}), bite size index (BSI, g) and average spine length (ASL, mm). Treatments are herbivores excluded (h-) and herbivores present (h+). Group membership is indicated in parentheses next to each species. Significance levels are indicated by: $^{\cdot}$ = $p < 0.1$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ and are highlighted in bold.

Plant trait	treatment	*Aca bre (1)*	*Aca mel (1)*	*Aca etb (2)*	*Bal ped (2)*	*Gre ten (3)*	*Cro dic (3)*
Total available N	In (h-)	23.4 ± 1.42	**15.6 ± 1.06**	15.6 ± 0.86	21.5 ± 2.4	22.6 ± 0.99	**12.6 ± 0.24**
	Out (h+)	24.8 ± 1.11	**18.5 ± 1.05$^{\cdot}$**	15.2 ± 1.14	23.5 ± 1.71	22.0 ± 0.93	**16.7 ± 0.82****
Leaf total N	In (h-)	36.1 ± 2.20	34.4 ± 0.88	32.9 ± 0.90	28.3 ± 1.90	34.0 ± 0.90	21.4 ± 0.30
	Out (h+)	39.4 ± 1.30	35.2 ± 0.60	32.0 ± 0.80	31.2 ± 1.50	32.2 ± 0.90	23.4 ± 0.70
SLA	In (h-)	102 ± 7.34	**107 ± 5.44**	141 ± 7.86	**54.6 ± 5.07**	**150 ±5.14**	147 ± 6.00
	Out (h+)	119 ± 8.09	**118 ± 3.02***	139 ± 12.0	**40.7 ± 2.52***	**136 ± 7.12$^{\cdot}$**	146 ± 6.00
TP	In (h-)	3.31 ± 0.46	**6.26 ± 0.25**	**1.55 ± 0.09**	0.55 ± 0.03	1.09 ± 0.14	1.26 ± 0.06
	Out (h+)	2.78 ± 0.30	**5.35 ± 0.22***	**1.36 ± 0.07$^{\cdot}$**	0.55 ± 0.05	1.29 ± 0.13	1.42 ± 0.05
CT	In (h-)	**4.72 ± 0.57**	6.58 ± 1.04	0.38 ± 0.04	0.08 ± 0.007	1.42 ± 0.22	0.33 ± 0.03
	Out (h+)	**2.48 ± 0.37****	7.15 ± 0.51	0.41 ± 0.04	0.09 ± 0.01	1.54 ± 0.22	0.37 ± 0.01
ADF	In (h-)	32.9 ± 2.39	37.1 ± 1.98	30.4 ± 1.29	24.6 ± 0.89	43.0 ± 0.84	33.8 ± 0.45
	Out (h+)	35.0 ± 2.16	33.6 ± 1.83	28.8 ± 1.58	24.1 ± 0.60	42.0 ± 1.05	32.4 ± 1.16
BI	In (h-)	**0.07 ± 0.03**	**0.09 ± 0.03**	**0.04 ± 0.007**	**0.25 ± 0.03**	**0.16 ± 0.02**	0.67 ± 0.16
	Out (h+)	**0.45 ± 0.05*****	**0.38 ± 0.04*****	**0.32 ± 0.04*****	**1.25 ± 0.08*****	**1.19 ± 0.18*****	0.56 ± 0.07
BSI	In (h-)	**3.00 ± 0.23**	**2.01 ±0.15**	**0.81 ± 0.06**	**2.49 ± 0.23**	**5.57 ± 0.48**	5.34 ± 0.5
	Out (h+)	**0.87 ± 0.08*****	**0.37 ± 0.03*****	**0.11 ± 0.01*****	**0.44 ± 0.04*****	**1.08 ± 0.08*****	4.27 ± 0.49
ASL	In (h-)	3.46 ± 0.06	**3.73 ± 0.04**	**52.9 ± 1.01**	54.7 ± 1.25	-	-
	Out (h+)	3.47 ± 0.07	**3.85 ± 0.03***	**58. 6 ± 0.90*****	55.0 ± 0.86	-	-

Table 3 Mean ± se for plant height and basal area in 1999 and 2009 for the six species growing with and without herbivores present, net changes in plant height and basal area between the two years are also shown. All measurements are based on the same individual plants of each species that were present in the plots in 1999 and that were still alive in 2009.

Species	Treatment	Height 1999 (m)	Height 2009 (m)	Basal area 1999 (cm^2)	Basal area 2009 (cm^2)	Δ height	Δ basal area
Acacia brevispica	Out (h+)	1.95±0.07	1.52±0.05	13.79±1.74	16.49±2.09	-0.43±0.07	2.69±1.17
Acacia brevispica	In (h-)	2.01±0.06	2.97±0.08	9.91±0.96	16.4±1.47	0.95±0.06	6.49±0.88
Acacia mellifera	Out (h+)	2.28±0.11	2.18±0.1	83.68±15.98	94.65±13.4	-0.10±0.09	10.97±6.57
Acacia mellifera	In (h-)	2.19±0.09	3.49±0.1	80.48±13.31	122.36±14.92	1.3±0.05	41.88±6.11
Acacia etbaica	Out (h+)	1.68±0.08	1.99±0.1	36.99±6.16	57.86±7.15	0.31±0.06	20.87±3.14
Acacia etbaica	In (h-)	1.57±0.07	3.05±0.07	30.39±7.54	51.83±7.7	1.48±0.06	21.17±3.23
Balanaites pedicellaris	Out (h+)	2.5±0.7	2.86±0.5	88.23±46.82	122.82±50.08	0.36±0.47	34.59±12.42
Balanaites pedicellaris	In (h-)	1.96±0.28	4.01±0.26	25.42±8.29	71.93±16.45	2.05±0.23	46.50±12.83
Grewia tenax	Out (h+)	1.31±0.09	0.95±0.24	3.36±0.44	2.96±0.48	-0.36±0.24	-0.41±0.35
Grewia tenax	In (h-)	1.16±0.05	2.4±0.08	1.94±0.23	5.78±0.5	1.24±0.07	3.84±0.44
Croton dichogamus	Out (h+)	2.16±0.22	2.1±0.24	23.93±8.31	33.23±8.29	-0.07±0.14	9.29±2.17
Croton dichogamus	In (h-)	1.59±0.08	1.82±0.12	9.32±2.67	13.46±3.17	0.23±0.11	4.14±1.16

Figure legends

Figure 1. a) Plot of a linear discriminant analysis of the measured defence traits showing the grouping of the six Mpala woody species relative to Wigley et al's. (2018) dominant species from southern African savannas. b) PCA based on nine measured plant defence traits of the six woody plant species growing in the presence (black) and long-term absence (grey) of large mammalian herbivores in central Laikipia County, Kenya. Axis 1 explained 33% of the variance and was primarily associated with variation in spine length and ADF. Axis 2 explained 26% of the variance and was primarily associated with variation in CT, TP and spine density (see Table 1 for eigenvector scores of the PCA). Points represent the mean location of each species on PC axes 1 and 2 at each of the three paired exclosure and control sites. Ovals show the overall means (centre) for each species and treatment and dispersion (ellipses).

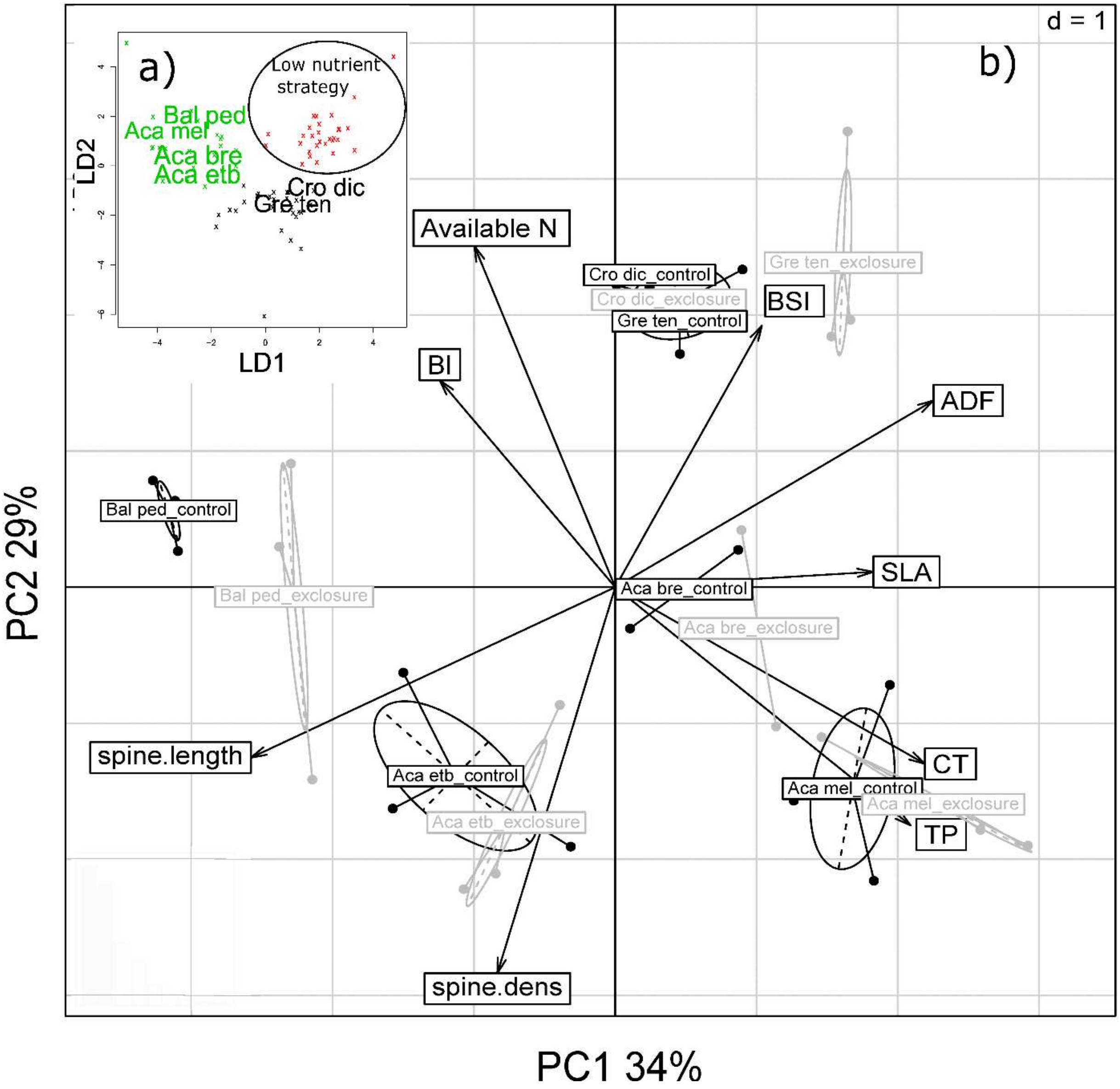
Figure 2. Mean ± se total available leaf nitrogen (total N x available N proportion a), specific leaf area b), total polyphenols c) and condensed tannins d) inside and outside of the exclosures. Aca bre = *Acacia brevispica*, Aca mel = *Acacia mellifera*, Aca etb = *Acacia etbaica*, Bal ped = *Balanites pedicellaris*, Gre ten = *Grewia tenax*, Cro dic = *Croton dichogamus*. Significance levels are: **.** = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$.

Figure 3. Mean ± se spine length a), spine diameter b), bite size index c) and branch density d) inside and outside the exclosures. Aca bre = *Acacia brevispica*, Aca mel = *Acacia mellifera,* Aca etb = *Acacia etbaica*, Bal ped = *Balanites pedicellaris*, Gre ten = *Grewia tenax*, Cro dic = *Croton dichogamus*. Significance levels are * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Figure 4. Examples of *Acacia brevispica* and *Balanites pedicellaris* growing in the absence of large mammalian herbivores (a & c) and the same species growing in the presence of large mammalian herbivores (b & d) in central Laikipia County, Kenya. Note the clear differences in architecture, branching densities, and browser damage between the treatments.

Figure 5. a) Mean ± se change in plant height between 1999 and 2009 for the six dominant species growing inside and outside the exclosures. b) Mean ± se change in stem basal area between 1999 and 2009 for the same six species. Significance levels are ** = $P < 0.01$, *** = $P < 0.001$.

Figure 1



a)
LD1
LD2
Low nutrient strategy
Bal ped
Aca mel
Aca bre
Aca etb
Cro dic
Gre ten
b)
d = 1
Available N
BI
BSI
ADF
SLA
CT
TP
spine.length
spine.dens
Cro dic_control
Cro dic_exclosure
Gre ten_control
Gre ten_exclosure
Bal ped_control
Bal ped_exclosure
Aca bre_control
Aca bre_exclosure
Aca etb_control
Aca etb_exclosure
Aca mel_control
Aca mel_exclosure
PC1 34%
PC2 29%

Figure 2

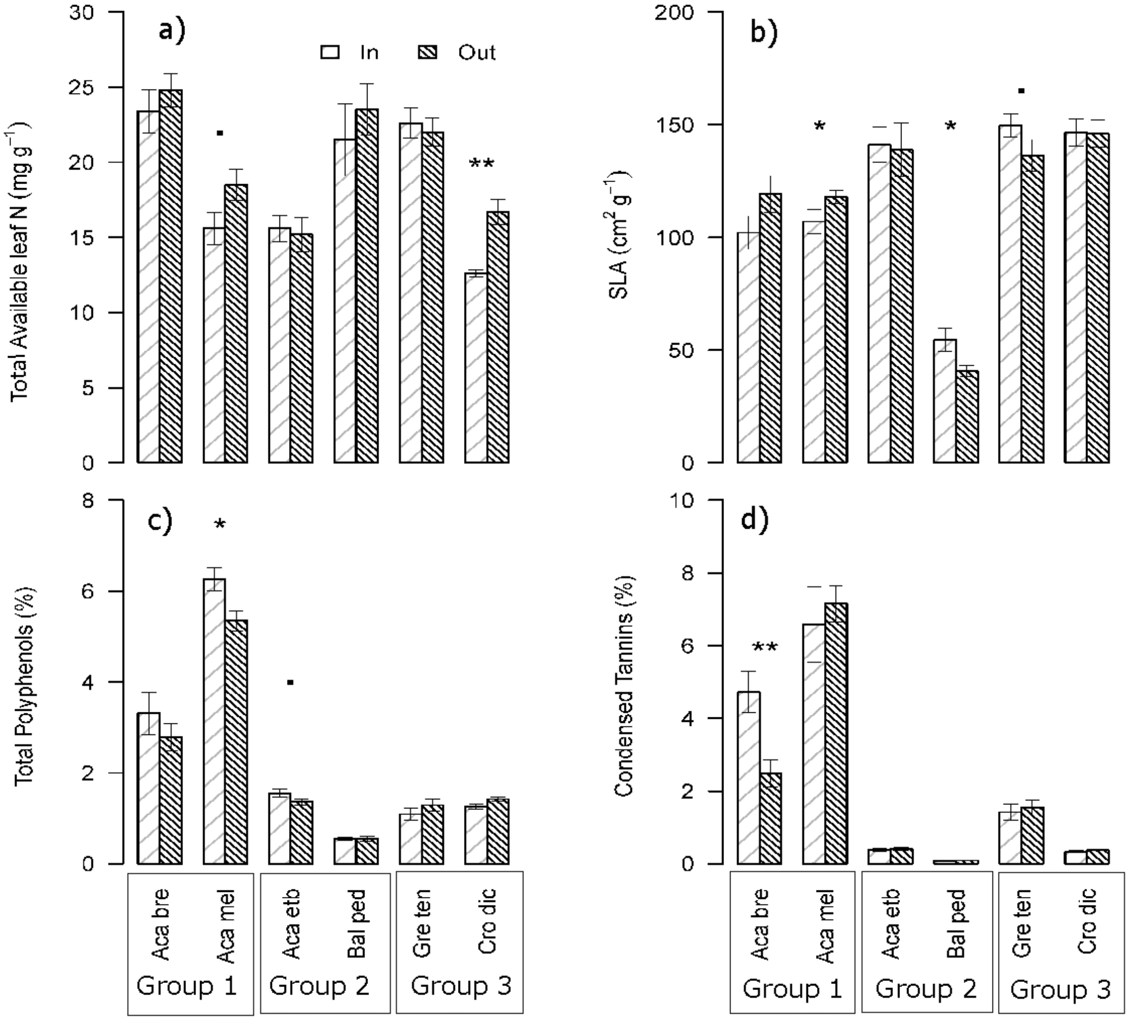
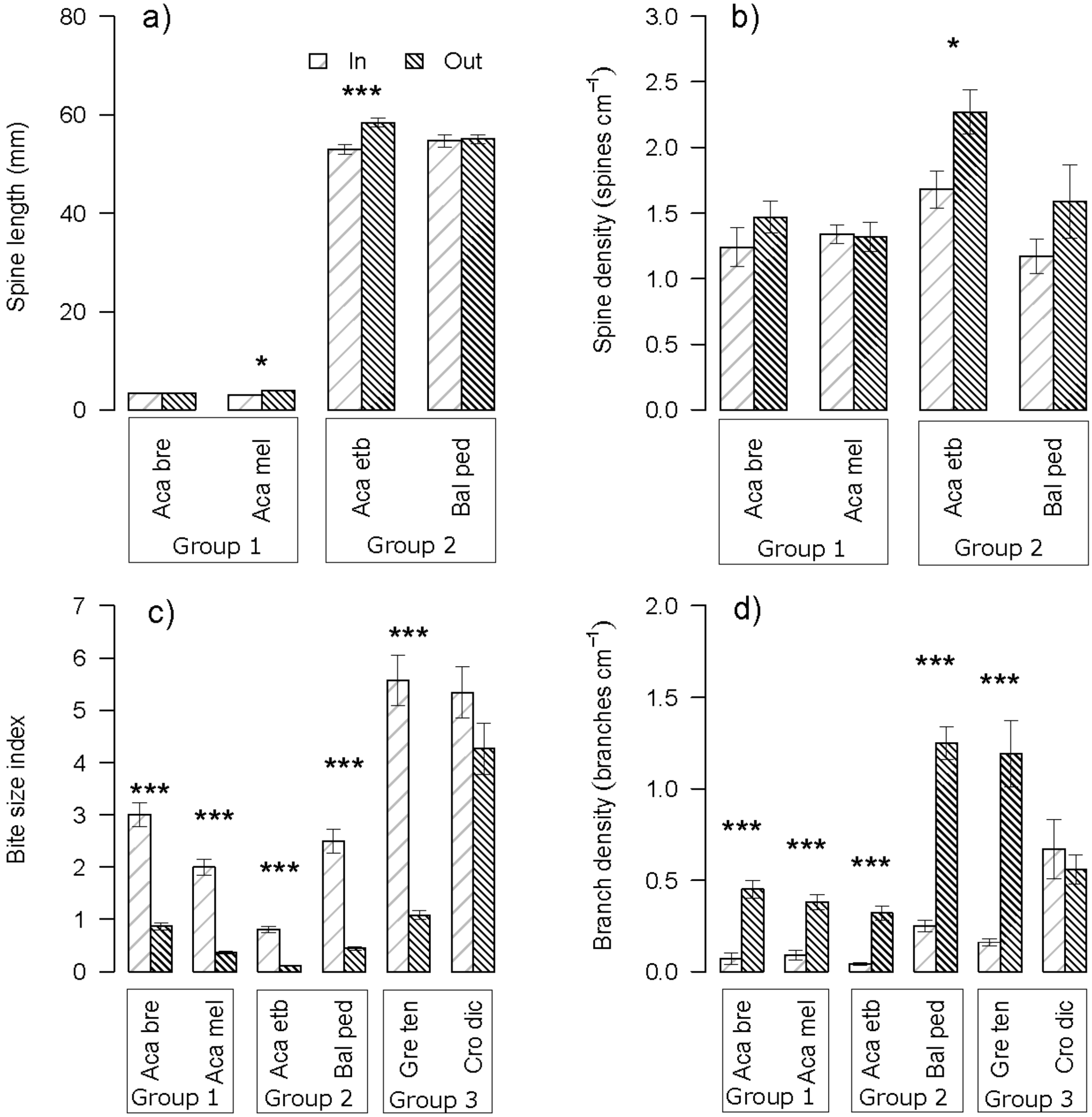

a)
b)
c)
d)
In
Out
Total Available leaf N (mg g^{-1})
SLA (cm^2 g^{-1})
Total Polyphenols (%)
Condensed Tannins (%)
30
25
20
15
10
5
0
200
150
100
50
0
8
6
4
2
0
10
8
6
4
2
0
.
**
*
*
.
*
.
**
Aca bre
Aca mel
Aca etb
Bal ped
Gre ten
Cro dic
Group 1
Group 2
Group 3

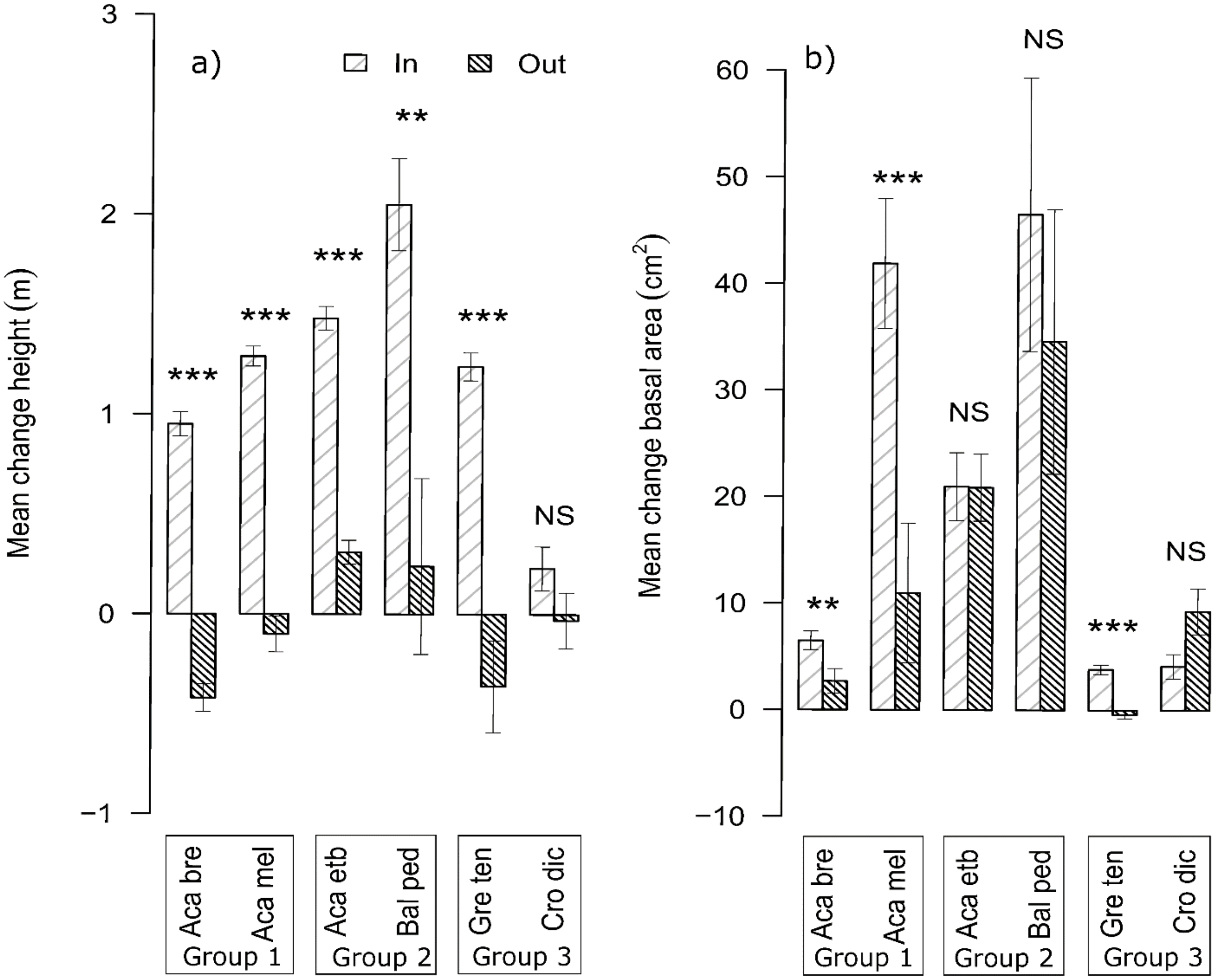
Figure 3



a)
Spine length (mm)
0
20
40
60
80
In
Out
*
***
Aca bre
Aca mel
Aca etb
Bal ped
Group 1
Group 2
b)
Spine density (spines cm^{-1})
0.0
0.5
1.0
1.5
2.0
2.5
3.0
*
Aca bre
Aca mel
Aca etb
Bal ped
Group 1
Group 2
c)
Bite size index
0
1
2
3
4
5
6
7
***
***
***
***
***
Aca bre
Aca mel
Aca etb
Bal ped
Gre ten
Cro dic
Group 1
Group 2
Group 3
d)
Branch density (branches cm^{-1})
0.0
0.5
1.0
1.5
2.0
***
***
***
***
***
Aca bre
Aca mel
Aca etb
Bal ped
Gre ten
Cro dic
Group 1
Group 2
Group 3

Figure 4

Figure 5


a)
In
Out
3
2
1
0
−1
Mean change height (m)
***
***
***
**
***
NS
Aca bre
Aca mel
Group 1
Aca etb
Bal ped
Group 2
Gre ten
Cro dic
Group 3
b)
60
50
40
30
20
10
0
−10
Mean change basal area (cm^2)
**
***
NS
NS
***
NS
Aca bre
Aca mel
Group 1
Aca etb
Bal ped
Group 2
Gre ten
Cro dic
Group 3