



HAL
open science

Savanna woody plant community and trait responses to bottom-up and top-down controls, with a specific focus on the role of mammalian herbivory

Benjamin Joseph Wigley

► **To cite this version:**

Benjamin Joseph Wigley. Savanna woody plant community and trait responses to bottom-up and top-down controls, with a specific focus on the role of mammalian herbivory. Ecology, environment. Université Claude Bernard - Lyon I, 2013. English. NNT : 2013LYO10133 . tel-01860360

HAL Id: tel-01860360

<https://theses.hal.science/tel-01860360>

Submitted on 23 Aug 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Année 2013

THESE

Présentée devant l'Université Claude Bernard–Lyon 1 Pour l'obtention du

Diplôme de Doctorat

Dépôt initial remis le 7 Mai 2013 par Benjamin Joseph WIGLEY

Savanna woody plant community and trait responses to bottom-up and top-down controls, with a specific focus on the role of mammalian herbivory.



devant le jury composé de:

M. Hervé Fritz (CNRS Université Lyon 1)	Directeur de thèse
M. William Bond (University of Cape Town, South Africa)	Co-directeur de thèse
M. Jacques Gignoux (CNRS BioEmco, Paris)	Rapporteur
Mme. Christina Skarpe (Hedmark University College, Norway)	Rapporteur
Mme. Sandra Diaz (Universidad Nacional de Córdoba, Argentina)	Rapporteur
Mme. Gudrun Bornett (CNRS Université Lyon 1)	Examinatrice
Mme. Sonia Said (ONCFS)	Examinatrice
M. Emmanuel Desouhant (CNRS Université Lyon 1)	Président du Jury

UMR CNRS 5558
Laboratoire de Biométrie et Biologie Evolutive
Université Claude Bernard – Lyon I – Bâtiment G. Mendel
43, boulevard du 11 novembre 1918
69622 Villeurbanne

Acknowledgements

This PhD thesis was made possible thanks to funding from a BDI-PED grant from the CNRS as well as the Andrew W Mellon Foundation. First and foremost, thanks to my two supervisors, Herve Fritz and William Bond, without whom this thesis would never have been made possible. Corli, thank you too for your considerable input and help over these past years, what a journey it has been and I am so grateful that you have been part of it. Sandra Diaz thank you for your invaluable input and comments. The initial idea for this thesis came about after discussions with Sandra Diaz on Bond's (2005) paper on black, brown or green worlds. Sandra Diaz was also indirectly responsible for coming up with the 'bite size index'. Thanks Sandra for this informative index! Then to everyone that spent time with me in the field, a big thank you to Paul, David, Theresa, Lucas, Nicola, Steven and Siphon to name a few. Jasper thanks for your patience and help with all things statistical. Sandy Smuts, thank you your understanding and patience with the ongoing battle against bureaucracy. Dawood Hattas thanks for your advice and help with the chemical analyses. Edward Chirwa thank you for your time spent processing and analysing thousands of samples. Then to everyone else who played a role in helping me achieve this study, thank you and sorry for not mentioning you by name. Mom, a big thank you for your time spent proofreading the manuscript. I am hugely grateful to SANParks, Ezemvelo KZN-Wildlife and Zimbabwean National Parks for allowing me to work in their parks and for all of the logistical support and help they provided. Thanks to all of the game guards who ensured our safety during fieldwork. Then to everyone in the LBBE lab in Lyon, thank you for welcoming me and making me feel at home in France. Simon and Marion, thanks for your helpful comments and for your hospitality. Anne Dufour, a very big thank you for all of the time you spent with me trying to teach me how to best perform and understand multivariate statistical analyses.



Eric Khumalo, one of the many game guards who kept us safe while undertaking fieldwork.

Titre de la thèse

Réponses des traits spécifiques et des communautés ligneuses de savane aux processus de contrôle ascendant et descendant (*bottom-up/top-down*), avec une emphase sur le rôle des mammifères herbivores

Resume

Les savanes sont des écosystèmes complexes pilotées par plusieurs mécanismes ascendant (ex: les nutriments du sol ou pluviométrie) ou descendant (ex: feu ou herbivorie), mais l'importance relative de ces mécanismes reste largement débattue. En particulier, le rôle des herbivores brouteurs (browsers) reste mal compris en tant que source de perturbation, et donc de force de pression descendante influant sur la dynamique des savanes. Dans cette étude, deux approches ont été développées pour aborder le rôle des perturbations dans la dynamique des savanes.

Dans un première partie, j'ai utilisé une approche comparative inter-site pour explorer les réponses des communautés de plantes, et des principaux traits de ces plantes associés aux feuilles, branches, architecture et défense, aux variations de quatre facteurs : les nutriments dans le sol, la pluviométrie, la pression d'herbivorie et l'intensité du feu. Seize sites de savane, en Afrique du Sud et au Zimbabwe, ont été sélectionnés sur des gradients de chacun de ces facteurs. Les espèces ligneuses dominantes (>80 % de la biomasse) sur chaque site ont été identifiées et échantillonnées, afin de mesurer les traits des feuilles et des branches associés à l'appétence, architecture, ainsi qu'aux défenses physiques et chimiques de ces plantes. Des mesures ont également été faites pour estimer les effets des meso-brouteurs et mega-brouteurs. Des transects ont permis d'estimer la fréquence et l'intensité du feu sur chaque site, et l'effet sur les plantes. En préambule à l'analyse, et devant le manque de protocole standard pour estimer la fertilité des sols dans la littérature écologique, je propose une méthode et un échantillonnage afin de définir de manière robuste la fertilité des sols sur chaque site.

Dans cette partie inter-site, huit traits principaux ont été comparés sur le gradient de qualité de sol et de pluviométrie, et bien que quelques relations statistiques existent entre les traits des feuilles, le sol et la pluviométrie, ces relations sont très faibles comparées à celle trouvées dans les méta-analyses inter-biomes publiées dans la littérature. Cependant, ces approches inter-biome sont dominées par des sites tempérés qui ont des niveaux de perturbations bien inférieurs à

ceux des savanes africaines. L'évaluation des effets des meso-brouteurs et mega-brouteurs le long des gradients de sol et de pluviométrie sur vingt traits associés aux défenses structurelles et chimiques des plantes montre que les défenses structurelles sont plus corrélées aux caractéristiques du sol que les défenses chimiques, mais que seules les défenses structurelles sont fortement corrélées à l'impact par les brouteurs. Le niveau d'utilisation des plantes par les meso-brouteurs apparaît plus prévisible en fonction des traits des plantes que celui par les méga-brouteurs.

Dans une deuxième partie présentent des résultats de deux études basées sur des expériences d'exclos. Dans le parc national de Kruger, la composition de la communauté, l'abondance et la démographie des ligneux dominants ont été estimées à l'intérieur et à l'extérieur de trois exclos de 40 ans, et les brouteurs apparaissent comme ayant un impact significatif sur la distribution, la densité et la structure des populations des espèces arbustives et arborées ayant des traits préférés : forte concentration en azote foliaire et faible teneur en défenses chimiques. L'interaction entre les effets des brouteurs et du feu semble aussi affecter le recrutement des juvéniles ligneux dans les grandes classes de taille. Dans le parc de Hluhluwe-iMfolozi, cinq exclos ont été utilisés pour tester l'effet des brouteurs sur l'architecture, la croissance, les défenses chimiques et structurelles des jeunes individus de sept espèces d'acacia. Des différences nettes apparaissent entre les espèces d'acacia de savane semi-aride et plus humide dans les traits associés à l'appétence, l'architecture et les défenses. L'effet des brouteurs sur la concentration en nutriment dans les feuilles semble plus prononcé pour les espèces de savane semi-aride. Les espèces de savane plus humide investissent plus dans les défenses chimiques, probablement en raison d'une architecture fortement sélectionnée par le feu, alors que les espèces de milieux semi-arides, où le feu est absent, investissent plus dans des réponses architecturales et les défenses structurelles.

Ce travail de thèse, basé sur la combinaison entre approches comparatives entre sites contrastés et des expériences d'exclos à long-terme a mis en évidence l'effet primordial des perturbations (feu, herbivores brouteurs et leurs interactions), et donc des processus descendant (top-down) sur les traits fonctionnels, la structure et la dynamique des communautés de ligneux des savanes. Les nutriments semblent principalement influencer l'investissement dans les défenses, et notamment les défenses structurelles plus présentes chez les espèces des sols riches.

Abstract

Savannas are complex ecosystems affected by several bottom-up (e.g. soil nutrient availability and rainfall) and top-down (e.g. fire and herbivory) drivers. However, the relative importance of bottom-up vs. top-down drivers in influencing savanna dynamics is still widely debated. Within the top-down (disturbance) category of drivers, the role of mammal browsers in particular in driving savanna functioning is still not well understood. Two approaches were adopted to determine the role of disturbance in savannas.

Firstly, by using a comparative approach, I attempted to address the so-called ‘savanna problem’ by investigating how savanna woody plant community compositions and key plant traits relating to the leaves, stems, architecture, and defence are influenced by soil nutrient status, rainfall, fire and browsing. Sixteen sites were selected along gradients of these four drivers from savanna parks throughout South Africa and Zimbabwe. The dominant woody species (species that accounted for >80% of standing biomass) at each site were identified and sampled for the key leaf and stem traits relating to plant functioning, palatability, architecture, physical and chemical defences. Measurements were undertaken for each species in order to determine both meso-browser and mega-browser impact. Transects were undertaken in order to determine the relative abundance and the effects of fire on each species at each site. Due to the current lack of standardized soil sampling protocols in the ecological literature, and uncertainty around the definition of what denotes a fertile or infertile soil, I propose a number of standardized protocols and sampled according to these established protocols in order to accurately determine the soil nutrient status at each site.

Following this, the relationships between climatic variables and soil nutrients with both species means and community weighted means for eight key leaf traits were explored. Although some significant relationships were found between savanna leaf traits of woody plants, climate, soil nutrients and their interactions, these tended to be weaker than those found in meta-analyses. These broad-scale studies usually include sites from many biome types, many of which are from temperate regions where inherent levels of disturbance are typically much lower than in African savannas. The high levels of disturbance typically found in African savannas are thought to partially account for the high within site variability found in leaf traits and the weak relationships found between leaf traits, soil nutrients and rainfall.

To assess the importance of resources vs. disturbance in savannas functioning, the effects of soil nutrients, rainfall, fire and both meso-browser and mega-browser impact on twenty savanna woody plant traits relating to plant palatability, chemical and structural defences were explored. Structural defences were found to be more strongly correlated with soil characteristics than chemical defences, while browser impact was found to be strongly correlated with structural defences but not with chemical defences. Actual browser utilisation tended to be more predictable for meso-browsers than mega-browsers.

Secondly using an experimental approach, two sets of herbivore exclosures were utilized to directly test how mammal browsers influenced woody species distributions, abundance, population structure and plant traits relating to palatability and defence. The effects of three long-term herbivore exclosures in the Kruger National Park on savanna woody plant community compositions, population demographics and densities were determined. Browsers were found to have significant impacts on species distributions, densities and population structures by actively selecting for species with favourable traits, particularly higher leaf N. An interaction between browsers and fire which limited the recruitment of seedlings and saplings into larger size classes was also demonstrated.

In the final study, five herbivore exclosures in Hluhluwe iMfolozi Park were used to explore the effect of browsers on *Acacia* sapling architecture, growth rate, chemical and structural defences. Clear differences were evident in palatability, architecture and defence related traits between mesic and semi-arid *Acacia* species. Browser effects on leaf nutrient content were mostly negligible for the mesic species but more pronounced in the semi-arid species. A trade-off in defence strategy was evident, where mesic species invested more in chemical defences, possibly because their architectures were predetermined by fire, while semi-arid species invested more in architectural and structural defences in the absence of fire. This study also highlighted the importance of structural defences in defending plants against mammal browsers, while chemical defences appear to be unimportant as a defence against mammal browsers but could be more important in defending plants against insects.

This thesis has highlighted the importance of mammal browsers (also fire and fire-browser interactions) as a top-down control on savanna woody plant distributions, with strong effects on plant traits. Soil nutrient availability was found to have a strong effect on defence type with higher investments in structural defences typically found at higher nutrient availability.

Contents

Chapter 1: General introduction and motivation for the study.	10
Chapter 2: Description of the study sites, climate, herbivory and fire intensity.	24
Chapter 3: What do ecologists miss by not digging deep enough? Insights and methodological guidelines for assessing soil fertility status in ecological studies	37
Chapter 4: Savanna woody leaf traits across climate and soil fertility gradients.	75
Chapter 5: Chemical and structural defences along gradients of soil fertility, rainfall and mammalian browsing pressure in southern African savannas.	100
Chapter 6: Herbivores shape woody plant communities in the Kruger National Park: lessons from three long-term exclosures.	129
Chapter 7: Evidence for a trade-off in anti-mammal defences in mesic vs. semi-arid savanna <i>Acacia</i> species	162
Chapter 8: Synthesis and conclusions	199
References	206
Appendices	237



Elephant bull, Kruger National Park.

Chapter 1 General introduction and motivation for the study

Savanna dynamics

Savannas form one of the major biomes of the world. However current estimates of the global extent of savannas vary considerably. Grasslands and savannas are estimated to occupy more than 40% of the global terrestrial landscape by Chapin et al., (2001) while Scholes & Hall (1996) estimate that savannas occupy approximately 12% of the global land surface. Ramankutty & Foley (1999) estimated that savanna covers 33 million km² of a total of 150 million km². This equates to approximately 22% of the earth's land surface.

Savanna is the dominant vegetation type in Africa (Scholes 1997) and covers 54% of Southern Africa (Rutherford 1997). In Southern African savannas, the main functional distinction lies between the broad- and fine-leaved savannas (Huntley 1982; Scholes 1990). The major underlying ecological difference is related to soil fertility and moisture with fine-leaved savanna usually occurring on nutrient-rich soils in lower rainfall areas and broad-leaved savanna usually occurring on nutrient-poor soils in wetter areas (Scholes 1997).

The determinants of tree cover in savannas are still widely debated, controversial and complex (Bond 2005, 2008). The debate over the importance of bottom up (soil nutrients and climate) vs. top-down (disturbance e.g. fire and herbivory) controls on savanna functioning continues. Our understanding of savanna functioning is limited by the complex suite of interactions and feedbacks between potential drivers. Much work has been done on the determinants of woody cover in savannas often with a focus on tree-grass interactions (e.g. Belsky, 1994; Scholes & Archer 2003; Sankaran et al. 2004; Bucini & Hanan 2007; Lehmann et al. 2011; Hirota et al. 2011). However few studies have investigated the determinants of species assemblages within savanna woody plant communities. In this study I therefore set out to investigate how climate, soil fertility, fire and herbivory influence woody plant communities and their functional attributes within Southern African savannas. The grassy layer is largely ignored in this study due to logistical constraints; furthermore grass dynamics in savannas are better understood as a result of numerous studies on grazers and fire (e.g. see Higgins et al. 2003; van Langevelde et al. 2003; Midgley et al. 2010).

This study aimed at improving our understanding of the determinants of savanna woody plant structure and functioning with a particular focus on the role of bottom-up drivers (soil fertility and rainfall) on the one hand and top-down drivers (mammalian herbivory and fire) on the other. Owing to the magnitude of this question it was necessary to break the potential drivers down into components and then attempt to test the role of each separately. My intention was to contribute to the debate regarding the importance of top-down vs. bottom-up controls on vegetation by addressing some of the uncertainties surrounding this debate (e.g. quantifying the concept of soil ‘nutrient-richness’, exploring which plant traits correlate with climate and soils and determining when fire or herbivory might play an important role in determining woody plant traits, cover and community composition). Turkington, in his 2009 review of top-down and bottom-up forces in mammalian herbivore-vegetation systems commented that it was puzzling that mammals, especially large mammals have not been more central in the trophic control literature. He further expressed the need to test spatially explicit changes in top-down and bottom-up forces along a gradient where there is known variation in the resource levels of the soils and the abundance of herbivores.

What to study, communities, species, or functional groups?

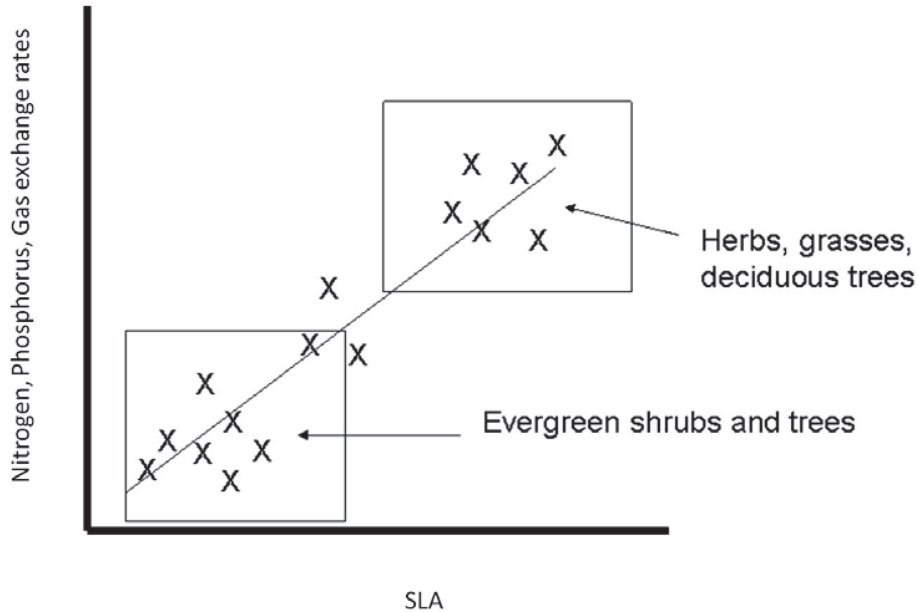
Turkington (2009) noted that although whole-community effects are important and interesting, focusing on these may mask many effects that happen at the species level. He further proposed that what is of more interest is how increases in particular herbivores give rise to decreases in particular plant species or functional groups. I explore this question further and ask how different densities of different guilds of browsers affect the plant functional types and plant traits of the dominant woody species along gradients of rainfall, soil fertility and mammalian herbivory.

Why study plant functional types and traits?

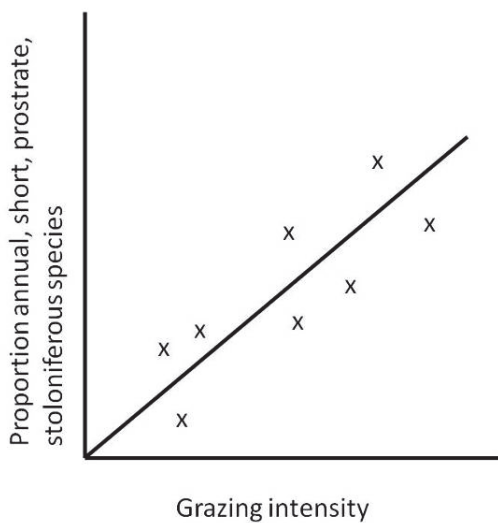
Westoby & Wright (2006) emphasized the importance of functional traits in terrestrial plant ecology. To date a number of trait dimensions have been recognized as important at a global scale, these include:

- I. Conservative vs. acquisitive plant types (e.g. Diaz et al. 2004); also referred to as the leaf economic spectrum by Wright et al. (2004). This spectrum runs from species with high specific leaf area (SLA) or low leaf mass per area (LMA), which are usually short lived,

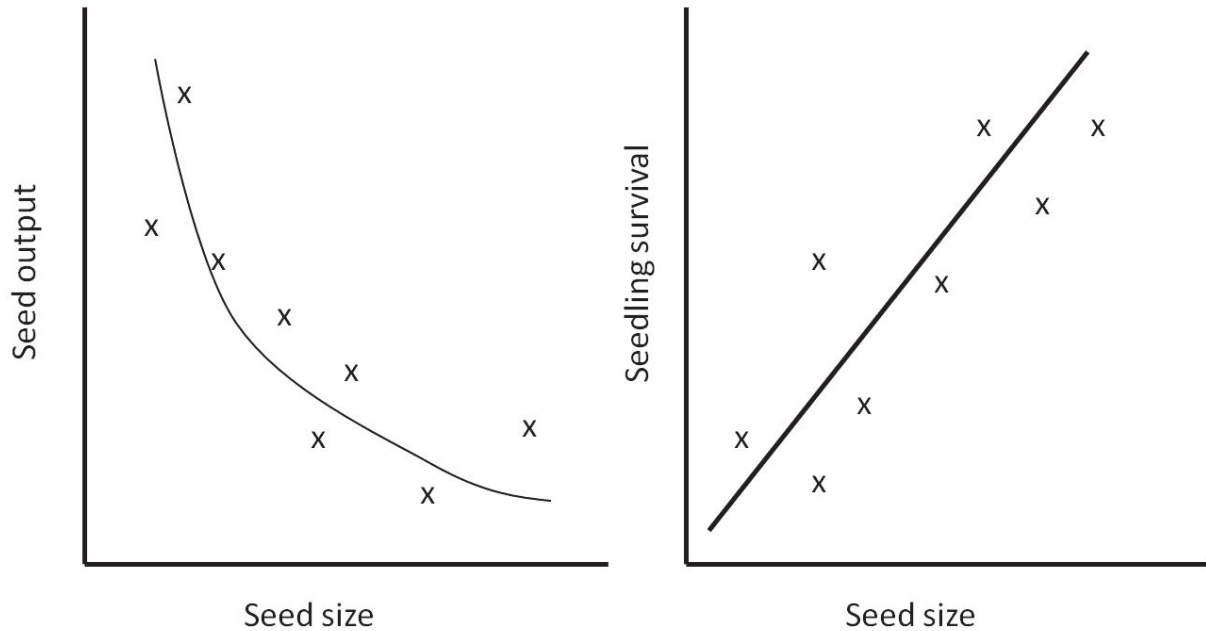
to species with low SLA or high LMA which have a long life span. Species with high SLA generally have high N and P concentrations and faster gas exchange rates. They usually include herbs, grasses and deciduous trees. Species with lower SLA usually have lower leaf N and P concentrations and slower gas exchange rates and are typical of evergreen shrubs and trees.



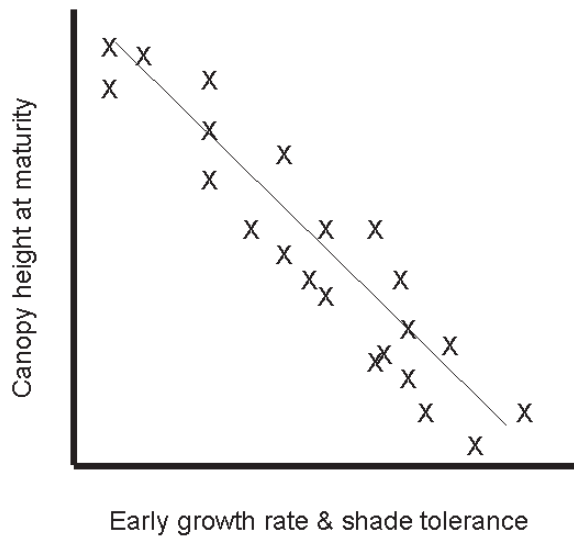
- II. There is a spectrum of plant responses to grazing with grazing favouring annual over perennial plants, short plants over tall plants, prostrate over erect plants and stoloniferous plants over tussock architecture (e.g. Diaz et al. 2001; Diaz et al. 2007).



- III. There is a trade-off where species with large seeds have lower seed output but better seedling survival than species with smaller but higher output (e.g. Westoby et al. 2002)



- IV. Canopy height at maturity also forms a trade-off with taller final height trading off with rapid early height growth or tolerance of low light (e.g. King et al. 2005; Poorter et al. 2003). Thus low shade tolerance would result in tall trees, while high shade tolerance would result in short trees.



- V. Chave et al. (2009) have suggested that a wood economics spectrum also exists where wood density appears to be negatively related to soil fertility and positively related to temperature, while no relationship between wood density and rainfall is evident.

Most of the above patterns have generally been found to hold true at the global scale and have helped improve our understanding of community ecology. Furthermore rapid progress is being made on a number of other traits as demonstrated by Westoby & Wright (2006). These include xylem hydraulics, wood density and leaf size, root to shoot ratios; N:P ratios in leaves, nutrient limitation and growth strategy (Westoby & Wright 2006). Progress has also been made on some of the trade-offs between leaf traits and decomposition (e.g. see Hattenschwiler et al. 2011).

Despite the rapid progress in the general trait literature, it is still not clear how trait responses to fire or herbivory gradients map onto gradients of site productivity, which is the axis typically used to explore global patterns and trade-offs with plant traits such as the conservative vs. acquisitive axis of Diaz et al. (2004), or the leaf economic spectrum of Wright et al. (2004).

In this study, I explore trait variation across productivity gradients while also considering trait responses to disturbance in the form of fire and herbivory. Furthermore, this is done in savannas where fire and herbivory are both important in different places but vary along a productivity gradient. It has been convenient to collapse climate and soil fertility variation into a

single gradient of ecosystem productivity. In this thesis, I explicitly separate rainfall and soil nutrient gradients to explore their effects on plant traits.

Linking plant traits and savannas

Savanna dynamics are known to be influenced by a number of bottom-up processes including climate, rainfall and soil nutrients and by two major top-down disturbances in the form of herbivory and fire (Frost & Robertson 1985; Scholes & Archer 1997). The roles of the different drivers in savanna dynamics are broadly understood at the landscape scale, for example, Bond (2005) suggested that large parts of the world, especially savannas, are green (controlled by climate), brown (controlled by herbivory) or black (controlled by fire). However at the finer scale savanna dynamics are still poorly understood. There have been a number of studies suggesting that different drivers play different roles in savannas at a number of scales in space and time (patch dynamics), for example (Gillson 2004; Wiegand et al. 2006).

Our understanding of how savanna woody plant communities respond to browsing and fire is still somewhat limited. African savannas typically occur in areas with summer rainfall and winter drought which has important consequences for both fire and herbivores. These savannas can be broadly divided into two categories, those dominated by fire and those dominated by herbivory (see Staver et al. 2012).

Fire dominated savannas and grasslands are usually found in higher rainfall or mesic areas where the soils are often leached resulting in higher biomass of lower quality (high C:N ratios). In South Africa this type of vegetation has been referred to as sourveld (Ellery et al. 1995). The dominant grass species found to grow in these areas usually belong to the Andropogoneae, a highly flammable tribe of grasses (Bond et al. 2003; Bond & Archibald 2003; Visser et al. 2011). To survive in this environment savanna woody species need a number of adaptations. Most importantly, they need to be able to tolerate frequent fire. A number of traits have evolved in savanna woody species allowing them to tolerate frequent fires. Firstly they need to be able to resprout in the case of top-kill, to achieve this they usually have substantial underground starch reserves, and the ability to resprout from the base, stem or crown (Bond & van Wilgen 1996; Gignoux et al. 1997; Bond & Midgley 2001). Trees growing in frequently burnt areas may also use underground starch reserves to achieve rapid growth rates in the main stem between fire events, facilitating escape from the fire-trap (Bond & van Wilgen 1996; Wigley et al. 2009;

Schutz et al. 2009). Despite high plant biomass in high productivity, frequently burnt areas, it is usually of very low quality, resulting in low herbivore diversity comprised mainly of the megaherbivores (elephant and buffalo), which are able to feed on low quality forage (Bell 1982; Fritz et al. 2002).

Savannas dominated by herbivory typically occur on soils derived from nutrient-rich parent materials, (e.g. basalts, Ecca shales or gabbro intrusions in Southern Africa), in lower rainfall areas (semi-arid to arid), and have been referred to as sweetveld in South Africa (e.g. Ellery et al. 1995). The lower rainfall in these areas helps to maintain the fertile soil status as a result of lower nutrient leaching in these soils (e.g. see Austin & Vitousek 1998). Herbivory has important effects on savanna soil nitrogen cycling (McNaughton et al. 1998), which effects in turn strongly influence the plant responses to grazing (Leriche et al. 2003). A number of studies have found herbivory to increase nitrogen availability to plants in grazed sites (e.g. McNaughton et al. 1997; Hamilton & Frank 2001; Le Roux et al. 2003; Stock et al. 2010; Coetsee et al. 2011). McNaughton (1985) proposed a direct feedback between grazers and grasses as herbivores are attracted to areas of high nutrients due to better quality forage, which in turn increases nutrient availability to the plants.

Another level of complexity usually exists in savannas in the form of the woody component and the herbivores that feed on these trees and shrubs. Woody plants also have direct effects on nutrient cycling. Woody plants can alter the soils and the microclimate in their immediate vicinity, thereby affecting both nutrient pool sizes and flux rates (Georgiadis 1989; Frost & Edinger 1991; Isichei & Muoghalu 1992; Mordélet et al. 1993; Belsky 1994; Archer et al. 2001; Ludwig et al. 2004). The proposed mechanisms for this phenomenon include: 1) the trees may draw nutrients from deep soil layers and from areas beyond the canopy and deposit them under the canopy in the form of litter or canopy leaching (Kellman 1979; Belsky 1994; Scholes & Hall 1996), 2) the tree may act as an atmospheric dust trap (Escudero et al. 1985; Bernhard-Reversat 1988; Mills et al. 2012), and 3) mammals and roosting birds may be attracted by the shade and refuge supplied by the trees and add nutrients in droppings (Georgiadis 1989, Belsky 1994). Animals may enrich the soil via defecation and burrowing (Archer et al. 2001; Treydte et al. 2007).

Trees in savannas therefore provide nutrient hotspots in the landscape which are attractive to both grazers (attracted by nutrient-rich grass species underneath them, e.g. *Panicum maximum*,

and shade) and browsers (food source). As a result of high herbivore numbers in these lower rainfall savannas the grassy layer does not accumulate high biomass as it does in the high rainfall areas. Many of the grass species found here have evolved ways of tolerating high grazing pressure and have growth forms found at the high end of the grazing spectrum as found by Diaz et al. (2007). These species often belong to the grass subfamily Chloridoideae, and some are capable of vegetatively spreading by stolons or rhizomes, especially under high grazing pressure (Bond et al. 2003; see Visser et al. 2012 for phylogenetic analysis).

The low rainfall, nutrient-rich savannas do not burn as frequently as the high rainfall mesic savannas; however they are faced with a different challenge in the form of high herbivore densities. To survive high herbivore pressure, plants require attributes to help them tolerate or deter herbivory. There are a number of ways of achieving this; plants can use chemical and physical defences to protect themselves, or, if conditions allow it, they might tolerate high levels of herbivory by achieving rapid growth rates. A vast literature exists on the two main strategies (to grow or to defend) that plant species adopt to cope with herbivory. The traditional school of thought was that plants either evolved mechanisms and traits that allowed them to tolerate herbivory or they evolved traits that improved their defences against herbivory (e.g. Herms & Mattson 1992; Skarpe & Hester 2008). Recent empirical evidence suggests that plants simultaneously allocate resources to both strategies, thereby exhibiting a mixed defence strategy (Nunez-Farfan et al. 2007). General herbivore defence theory is surprisingly poorly developed in the context of savannas. For example, there is very little literature on structural vs. chemical defences and little or no theory to explain the common pattern of structural defences where mammal herbivory is high, and lack of structural defences where herbivore pressure is low (see e.g. Craine et al. 2003).

Climate also plays an important role in determining savanna dynamics. Using data from 845 savanna sites across Africa, Sankaran et al. (2005) showed that maximum tree cover is constrained by rainfall. They found that below *ca.* 650 mm mean annual precipitation (MAP), maximum tree cover increases linearly with increasing MAP. According to these authors, these savannas can be thought of as ‘stable’ systems where tree cover is constrained by water thereby permitting grasses to co-exist. Furthermore, the interactions of fire, herbivory and soil properties further determine tree cover. Above 650 mm MAP, savannas can be thought of as ‘unstable’ systems where water availability is sufficient to allow canopy closure, but the interactions of

disturbances in the form of fire and herbivory maintain these systems as savannas instead of turning into closed woody vegetation (forests).

The occurrence of dry forest or closed canopy thicket in semi-arid to arid areas is not accounted for in the Sankaran et al. (2005) data set. Vast areas of northern Limpopo, South Africa, occurring in low rainfall (< 500 mm MAP) areas are in fact covered by dense dry thicket. A possible hypothesis is that this has resulted from the replacement of large browsing herbivores with grazers in these systems (see van Vegten 1984), thereby allowing the woody component to increase to levels forming a closed canopy, a negative feedback resulting in the loss of grass from the system thereby excluding fire which results in further woody thickening. This is a further example of how complex the interaction between fire and herbivory is in savanna systems and points to the need for a better understanding of these drivers in savanna functioning. The challenge is to come up with innovative ways of achieving this. This study will therefore attempt to determine if the plant functional type (PFT) and plant trait approach will be helpful in this regard.

Possible predictions from a PFT perspective

To better understand savanna structure and the underlying dynamics, a major challenge for ecologists is to unravel the layers of complexity demonstrated above. To achieve this we need a better understanding of the three main sets of factors determining savanna dynamics:

- I. The abiotic template (climate and measures of soil fertility)
- II. Herbivory (grazing and browsing)
- III. Fire (frequency and intensity).

The effects of climate are commonly described by mean annual precipitation (MAP), mean annual temperature (MAT) and potential evapotranspiration (PET) which all relate to water availability and the direct and indirect effects these have on soil properties. Soil depth and texture, in turn, modify soil moisture availability to plants. Rainfall seasonality has also been implicated; both as a direct influence on trees and indirectly via fire (see Fensham et al. 2008; Lehmann et al. 2011.)

Vertebrate herbivory can be broken down into grazing and browsing, while fire can be characterised by its frequency and intensity. It is usually difficult to study any of these factors in isolation in the field because of their interconnectedness resulting in a number of feedback loops.

However a number of long-term experiments (e.g. experimental burn plots and herbivore exclosures) exist which can be examined to determine the role of the different controls on savanna function and structure.

As response variables, and indicators of the relative importance of different drivers, I used plant functional types (PFTs) and traits to determine the importance of each axis in shaping and influencing savanna structure and function. The dominant plant community occurring at any one site is a direct result of past interactions between top-down disturbances and bottom-up influences relating to climate and soil properties. The plants that have prevailed under these past circumstances should therefore possess mechanisms or adaptations that have allowed them to survive and dominate the present landscape. The combination of the ecophysiological and physical characteristics, (i.e. plant traits), evident in different plant communities is what is of interest to this study. The dominant traits present in any plant community should therefore provide clues as to the relative importance of fire, herbivory, and climate in shaping that community.

In theory major differences in the types of traits needed to survive or tolerate the different forms of disturbance or to grow under levels of different water and nutrient availabilities are expected. Predictions can be made, often using empirical evidence, about what types of traits a plant might need to survive under a specific set of conditions. A plant species might need to grow, survive and reproduce despite frequent fires or high herbivory pressure or low rainfall or low nutrient availability or any combination of these. For example, to survive frequent fire a plant within the flame zone would need to be able to resprout after fire damage. To accomplish this it would need buds and starch reserves for initial bud and shoot growth (Hoffmann 2000; Bond & Midgley 2001) and a stem architecture enabling rapid growth to escape the flame zone (e.g. Bond & van Wilgen 1996; Archibald and Bond 2003; Wigley et al. 2009). Large trees taller than the flame zone might need thick bark as insulation against frequent fires (Gignoux et al. 1997; Midgley et al. 2010).

Mammal herbivory poses a different set of challenges. To survive heavy browsing pressure a plant can either invest in physical defences such as thorns or spines or in chemical defences such as condensed tannins, polyphenols and other chemical compounds (Cooper & Owen Smith 1986). If nutrients and water availability are not limiting the plant may compensate

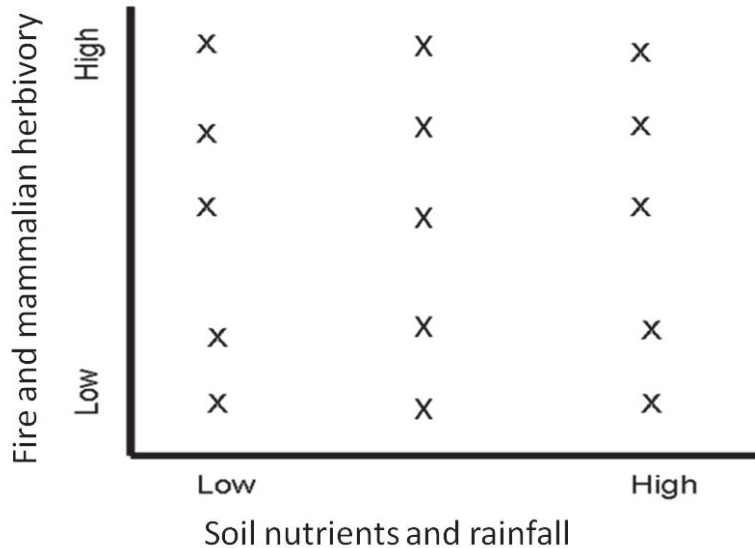
for high browser off-take by the rapid production of leaf material (high SLA) at a low cost to the plant.

In savannas (especially African savannas which often still possess the full suite of herbivores, including mega-herbivores) the situation is confounded by interactions between herbivores, nutrients and fire. For example, in Hluhluwe Game Reserve, South Africa, a form of *Acacia Karroo*, occurs with thin tall stems. It has been proposed (e.g. Bond & van Wilgen 1996; Archibald & Bond 2003; Wigley et al. 2009) that these narrow stems are selected for in frequently burnt savannas to promote rapid height growth and higher escape rates from the flame zone. Once the plants have escaped out of the flame zone they grow into reproductive adults. The major cost of this strategy is these plants are extremely vulnerable to herbivores such as elephants which are easily able to snap these thin stems (*Pers. Obs.*). In areas of high browsing pressure, acacias typically have cage-like architectures protecting the main stem as it grows above the browse height of common antelope (Archibald & Bond 2003). Staver et al. (2012) have shown that different architectures, ‘cage’ or ‘pole’, occur in acacia species along a gradient of high to low herbivory or low to high fire frequency. Because of these tradeoffs between traits relating to different disturbances we predict that the dominant traits of savanna communities will reflect the most influential drivers in that system. This has important implications for conservation areas in savannas around the world, where concerns have been raised about the future trajectory of plant communities in the face of widespread uncertainty around land use and global change.

Study Design

It is possible to predict ‘*a priori*’ where each of the major savanna drivers are likely to be important and then go to these areas and measure the traits of the dominant vegetation. I planned to determine the effects of top-down controls (fire and herbivory) on plant traits along a gradient of bottom-up controls (soil nutrient availability and rainfall) as shown in the simplified figure below. I planned to compare traits across existing long-term exclosures (with and without mammal herbivory) and in long term burning experiments (with varying fire frequencies) across rainfall and soil nutrient gradients. Unfortunately, existing experiments were not well placed for testing rainfall or soil nutrient effects. Instead, due to both logistical reasons and a serious dearth of studies investigating the importance of browsers in shaping savanna landscapes, I focussed on

this form of top-down disturbance in this study. Where possible, I also considered the effects of varying fire activity.



Ultimately, data needs to be gathered along precipitation and nutrient gradients from sites spanning the world's savannas and grasslands. A number of joint collaborations will be needed to achieve this. Data from my study will contribute to a global database envisioned and called for by initiatives such as TRY (Kattge et al. 2011).

Structure of thesis

The relative influence of bottom-up vs. top-down drivers in savanna functioning is still widely debated. This thesis aims to address some of this uncertainty by improving our understanding of how different savanna woody plant species or communities and their corresponding traits respond to the two different categories of drivers. The influence of mammal browsers in particular as a driver of savanna functioning is currently still not completely understood. Two different approaches have been adopted in order to improve our understanding of the role of mammal browsers in savanna dynamics. A comparative approach is firstly adopted in order to explore how plant traits are influenced by bottom-up factors (soil nutrients and rainfall) on the one hand and top-down factors (fire and herbivory) on the other. This is followed by an experimental approach

which uses two sets of herbivore exclosures to empirically test the role of mammal browsers in influencing woody plant distributions, population structures and traits relating to palatability and both structural and chemical defences.

Part I of the thesis constitutes the site descriptions and methodology used. A detailed description of the study sites are given in Chapter 2. Due to the lack of a clear ecological definition of what denotes a fertile or infertile soil, I address this issue in Chapter 3 as it is of major importance for this study. I therefore first attempt to provide some guidelines on how and what to sample in order for ecologists to be able to make meaningful soil measurements and comparisons of the intrinsic soil nutrient status at a study site (this chapter has been published in *Acta Oecologica* and is formatted according to this journal with references included).

In Part II, I have adopted the comparative approach where first I investigate how climate and soil fertility affect savanna woody leaf traits at both the species and community levels, (Chapter 4). This is followed by an exploration of how these same two bottom-up controls as well as browsing pressure by two different mammalian herbivore guilds influence chemical and structural defences in woody savanna plants (Chapter 5).

In part III, I have used the experimental approach to: a) investigate what the effects of forty years of herbivore exclusion have been on woody plant community traits and compositions and species abundances using three sets of herbivore exclosures in the Kruger National Park (Chapter 6, accepted in *Koedoe - African Protected Area Conservation and Science* and is formatted accordingly with references included); and b) investigate how herbivore exclusion has affected plant architecture, chemical and structural defences and growth rates in seven *Acacia* species growing in mesic vs. semi-arid savannas in Hluhluwe-iMfolozi Game Reserve (Chapter 7, submitted to *Biotropica*, formatted accordingly with references included). The overall findings and a synthesis of the thesis are provided in Chapter 8. A list of the sampled species at each site is provided in Appendix 1, while a list of abbreviations commonly used in the thesis with their explanations and units is provided in Appendix 2.



One of the many challenges facing woody plants growing in savannas.

Chapter 2 Description of the study sites, climate, herbivory and fire intensity

Study site selection

Sixteen study sites were selected along climatic and soil fertility gradients in Southern African savannas (Table 1).

Table 1. Site names, average daily potential evapotranspiration in January (ADPETJ, mm), mean annual precipitation (MAP, mm), mean annual temperature (MAT, °C), mean daily solar radiation in January (SRJ, MJ.m⁻².day⁻¹) and GPS co-ordinates. KNP = Kruger National Park.

Site	ADPETJ	MAP	MAT	SRJ	CO-ORD E	CO-ORD S
KNP Nhangwini	5.17	678	20	27.4	31.292633	-25.19907
KNP Ship Mountain	5.17	676	20	27.1	31.372687	-25.21342
Makhohlolo Exclosure	5.36	550	20	27.5	31.913737	-25.262083
KNP Satara Basalt	5.7	525	21	27.9	31.81545	-24.27660
KNP Satara Granite	5.71	576	22	27.6	31.643196	-24.52574
KNP N'waxitshumbe	5.67	495	22	28.3	31.25825	-22.77818
Mapungubwe Sands	6.1	314	22	29.4	29.377933	-22.24107
Mapungubwe Gabbro	6.1	334	22	29.2	29.350367	-22.21167
Marakele Sands	5.87	661	17	30.3	27.57363	-24.46010
Enseleni	4.87	1200	22	25.5	32.00747	-28.69054
Hluhluwe Isivivaneni	4.97	900	20	26.1	32.04439	-28.10670
Pongola	4.92	660	21	29.3	31.72011	-27.47445
Hwange Khatshana	4.83	600	22	27.1	26.9009	-18.63221
Hwange Dopi Pan	4.71	600	22	27.1	26.89354	-18.64268
Hwange Baikiaea	4.71	600	22	27.1	26.89347	-18.64278
Addo Elephant Camp	5.63	440	19	28.4	25.73626	-33.46466

Study site descriptions

Kruger National Park (KNP)

The Kruger National Park occurs in the north eastern Lowveld of South Africa, spanning the Mpumalanga and Limpopo provinces. The Lowveld consists mainly of plains with low to moderate relief and on average occurs at 300m above sea level with a gentle slope to the east (Venter et al. 2003). The strike of the lithology of Kruger is usually north-south resulting in the

geological succession changing from west to east, subdividing Kruger into roughly north-south bands of different geology. Granitic rocks predominate in the west while basaltic rocks predominate in the east (Venter et al. 2003). The savannas of Kruger can be broadly divided into clay-poor or broad-leaved savanna in the west and clay rich, fine-leaved savanna in the east according to the geological pattern described above. The climate is characterised by high mean temperatures in summer and mild, generally frost-free, winters. Rainfall is strongly concentrated in the summer months between October and April. There is a general trend of decreasing mean annual rainfall from south to north.

The Kruger National Park's game population supports 147 mammal species (Michel et al. 2006). The main browsing species include black rhinoceros (*Diceros bicornis*), giraffe (*Giraffa camelopardalis*), steenbok (*Raphicerus campestris*), gray duiker (*Sylvicapra grimmia*), eland (*Taurotragus oryx*), bushbuck (*Tragelaphus scriptus*) and greater kudu (*Tragelaphus strepsiceros*). While the main mixed feeders include impala (*Aepyceros melampus*), nyala (*Tragelaphus angasii*) and the African elephant (*Loxodonta africana*) (Codron et al. 2007). In general, animal densities tend to be higher on the nutrient-rich basaltic soils found in the east of the park compared to the nutrient-poor granitic soils found in the west of the park (Smit et al. 2007).

Nhlangwini Exclosure

The Nhlangwini exclosure occurs in the south western portion of the Kruger National Park near Pretoriuskop rest camp. This is the wettest part of KNP with MAP close to 700 mm per annum. The vegetation type is classified as broad-leaved bushveld occurring on sandy soils derived from granitic rocks. Two sites were sampled here, one inside the exclosure which was established in 1973 and one outside the exclosure. Common mammalian browsers in the area include the African elephant, giraffe, greater kudu and impala (Levick et al. 2010).

Ship Mountain

The Ship Mountain site is found in close proximity to the Nhlangwini site and is therefore similar in climate and rainfall. However it occurs on a Gabbro intrusion which has resulted in soils of basaltic origin. The vegetation is described as fine-leaved tree savanna which would result in higher browser and mixed feeder densities.

Makhohlolo enclosure

This 4 ha enclosure was constructed in the early 1970s. In the south-eastern corner of the KNP just north of Crocodile Bridge. The enclosure occurs in the *Sclerocarya birrea/Acacia nigrescens* savanna type. The site occurs on soils derived from rocks of basaltic origin with mean annual rainfall of *ca.* 600 mm. Two sites were also sampled here, one inside the enclosure and one outside the enclosure. High densities of the African elephant, giraffe, greater kudu and impala occur at this site as a result of the nutrient-rich basaltic soils and moderately high rainfall.

Satara Basalt

The Satara Basalt site occurs in the eastern part of central Kruger National Park. MAP is slightly lower here than in the south west at approximately 525 mm per annum. The site occurs on soils derived from rocks of basaltic origin and the vegetation type at this site is also described as fine-leaved tree savanna. High densities of the African elephant, giraffe, greater kudu and impala also occur at this site as a result of the nutrient-rich basaltic soils and moderate rainfall.

Satara Granite

The Satara Granite site occurs in the western part of central Kruger National Park. It is similar in rainfall and climate to the Satara Basalt site; however it occurs on sandy soils derived from granite. The vegetation at this site is described as broad-leaved bushveld. Low herbivore densities occur at this site due to the nutrient-poor granitic soils.

N'waxitshumbe

The N'waxitshumbe enclosure occurs in the arid north eastern section of Kruger National Park and was established in 1967. MAP is less than 500 mm per annum and the soils here are derived from rocks of basaltic origin. The vegetation type is classified as *Colophospermum mopane* shrubveld growing in broad-leaved bushveld. Two sites were sampled here, one inside the enclosure and one outside the enclosure in close proximity. The most common browser and mixed feeder species in this area include the African elephant, giraffe, greater kudu and impala (Levick & Rogers 2008).

Mapungubwe National Park

Mapungubwe National Park is situated at the confluence of the Limpopo and Shashi Rivers in the Limpopo Valley in the Limpopo Province of South Africa (Gotze et al. 2008). The climate in the area is semi arid with a long term MAP of 350 mm (Robinson 1996). Rainfall tends to be highly variable usually falling in the summer months of October to March. Acocks (1988) placed the vegetation of Mapungubwe in Veld type 15 (Mopane Veld), while Van Rooyen & Bredenkamp (1996) named the vegetation type Mopane Bushveld. Mucina & Rutherford (2006) place the vegetation of Mapungubwe in the Limpopo Ridge Bushveld type in the Mopane Bioregion of the Savanna Biome. The main herbivores in the park include eland, gemsbok (*Oryx g. gazella*), impala, kudu, waterbuck (*Kobus ellipsiprymnus*), wildebeest (*Connochaetes taurinus*) and zebra (*Equus burchelli*), while two mega-herbivores species; elephant and white rhino (*Ceratotherium simum*) are also present (SANParks 2012).

Mapungubwe Sands

The Mapungubwe Sands site occurred on deep, freely drained sandy soils. The vegetation forms a moderately open savanna dominated by *Colophospermum mopane*, *Terminalia sericea*, *Grewia flava* and *Combretum apiculatum*. Due to the far distance from water and infertile sandy soils, herbivore densities at this site were low.

Mapungubwe Gabbro

The Mapungubwe Gabbro site occurred adjacent to the alluvial plain of the Limpopo River. The underlying geology is formed from rocks of the Beit Bridge Complex resulting in a calcareous clayey soil and shallow gravels (Mucina & Rutherford (2006). The vegetation is dominated by *Adansonia digitata*, *Commiphora* spp., *Terminalia prunoides* and *Acacia senegal* var. *leiorhachis*. The nutrient-rich soils and closer proximity to water at this site has resulted in higher densities of the main herbivore species found in the park, particularly during the dry season.

Marakele National Park

Marakele National Park occurs in the south western part of the Limpopo Province, South Africa. MAP in the park varies from 560-630 mm and falls mainly during the summer months (Van Staden & Bredenkamp 2005). Summers are warm and wet with average daily temperatures of 32

°C, while winters are cool and dry with frost occurring in the low lying areas (Van Staden 2003). The underlying parent rock is predominantly sandstone of the Kransberg Subgroup, Sandriviersberg Formation (Van Staden & Bredenkamp 2005). Soils derived from these sandstones vary from shallow to deep sandy soils (Van Staden 2003). Marakele National park is situated mainly in the Waterberg moist Mountain Bushveld classified by Low & Rebelo (1996). Vegetation in the park includes Acocks' (1988) Sour Bushveld (Veld Type 20), Mixed Bushveld (Veld Type 18), Sourish Mixed Bushveld (Veld Type 19) and North-Eastern Mountain Sourveld (Veld Type 8). Mucina & Rutherford (2006) classified the vegetation here as Waterberg Mountain Bushveld. Large mammal species found in this park include the African elephant, black rhino, white rhino, while common antelope species include, kudu, eland, bushbuck and impala.

Marakele Sands

The Marakele Sands site is situated in the sour bushveld vegetation type on slopes of a moderately steep gradient. The soils at the site were sandy, shallow and rocky typical of the midslopes of the sandstone mountains occurring in the area. Rainfall is estimated at between 700 and 800 mm per annum due to the higher altitude at the site. The vegetation at the site was dominated by *Faurea saligna* and *Protea caffra* typical of the higher mountain slopes of this vegetation type (Mucina & Rutherford 2006). The location of this site in the sour bushveld type has resulted in low densities of all herbivore species.

Enseleni Game Reserve

Enseleni is a 293 Ha game reserve in Zululand, close to Richards Bay in the KwaZulu-Natal Province of South Africa. The vegetation consists of a mosaic of coastal grasslands and forests. MAP is around 1200 mm and the mean summer temperature is 30 °C with a mean minimum winter temperature of approximately 13 °C. The geology of the area consists of 18 000 year old Quaternary sediments of marine origin, resulting in well leached and nutritionally poor soils (Mucina & Rutherford 2006). The main herbivore species found in this game reserve include wildebeest, zebra, impala, reedbuck (*Redunca arundinum*), waterbuck, bushbuck, bushpig, (*Potamochoerus porcus*) reed (*Cephalophus natalensis*), blue (*Cephalophus monlicola*) and gray duiker and hippopotamus (*Hippopotamus amphibius*).

Hluhluwe-iMfolozi Park (HiP)

Hluhluwe-iMfolozi Park occurs in northern Zululand, also in the Kwa-Zulu Natal Province of South Africa. A strong altitudinal gradient exists between Hluhluwe Game Reserve in the north and iMfolozi Game Reserve in the south (40 -750 m asl). Balfour & Howison (2001) demonstrated how the altitudinal gradient has resulted in a strong rainfall gradient from north to south with MAP in the north of the park of around 990 mm, while in iMfolozi MAP is less than 600 mm (Bond & Archibald 2003). The temperatures in the region are moderate to hot with temperatures of between 14°C and 40°C in summer and between 6°C and 34°C in winter (Staver et al. 2009). Frost seldom occurs due to the mild winter temperatures and low altitudes at the study sites. Thunderstorms are a common feature of the summer rainfall season with lightning strikes occurring at densities of *ca.* five to six ground flashes per square kilometre per year (Anon 1994). The geology of the area usually results in clay rich soils derived from shales, mudstones and dolerite outcrops of the Karoo Supergroup. Acocks (1953) defined two major veld types in the Hluhluwe-iMfolozi Park; these were Zululand Thornveld (type 6) and the Lowveld Tropical Bush Savanna (type 10). These were later changed to three broad vegetation types for the area (Acocks 1975). These included grasslands and forested hilltops, which are usually found at higher altitudes (above 450 m asl). Riverine forest usually dissects the park along its watercourses. The remainder of the park is a mixture of fine-leaved Acacia savannas and broad-leaved woodland. The Acacia savanna ranges from open to closed canopy patches. The main fine-leaved species in Hluhluwe Game Reserve include *Acacia karroo*, *Acacia nilotica* and *Dichrostachys cinerea*.

Hluhluwe Isivivaneni

This site was chosen in the northern higher altitude high rainfall part of Hluhluwe Game Reserve. The vegetation at the site formed a mixture of fine leaved savanna and broadleaved woodland interspersed with clumps of thicket. Impala are the most numerous of the herbivores in Hluhluwe (24 per km², Staver et al. 2012). African elephant numbers have also steadily increased over the past decades, while other browser species include giraffe, kudu, nyala, bushbuck, red and gray duiker and black rhino (Wigley et al. 2010).

Pongola Private Game Reserve

This private game reserve is located approximately 10 km south of the Pongola town in Northern KwaZulu-Natal. The climate and geology is similar to that of iMfolozi Game Reserve with low altitudes resulting in MAP of approximately 600 mm. Soils are deep and clayey derived from a distinct variety of clastic sediments of the Dwyka, Ecca, Beaufort and igneous rocks of the Lebombo Group (Mucina & Rutherford 2006). The vegetation at the site is dominated by fine-leaved *Acacia* spp. and *Spirostachys africana* woodland. High densities of elephant, impala, giraffe, nyala, kudu, bushbuck and gray duiker occurred at this site.

Hwange National Park

Hwange National Park is situated on the North western border of Zimbabwe. The long term MAP is approximately 600 mm with the majority of rain falling between October and April (Chamaille'-Jammes et al. 2007a). The vegetation type is typical of Southern African dystrophic wooded savannas interspersed with patches of grasslands (Rogers 1993). There are two main vegetation types in the park. Mopane *Colophospermum mopane* (Kirk ex Benth.) Kirk ex J. Léonard dominates areas with clay-rich soils, while mixed woodland and bushlands (*Combretum* spp., *Acacia* spp., *Terminalia sericea* Burch ex DC., *Baikiaea plurijuga* Harms) develop on the deep Kalahari sands (Chamaille'-Jammes et al. 2007b). Common browser and mixed feeder mammal species found in Hwange include elephant, giraffe, impala, kudu, steenbok, gray duiker and bushbuck (Chamaille'-Jammes et al. 2009).

Hwange Khatshana

This site occurs on a private concession just north of Main Camp in Hwange National Park. The vegetation type is Miombo woodland dominated by *Brachystegia* and *Terminalia* spp. Nutrient-poor sandy soils at this site result in low herbivore densities.

Hwange Dopu Pan

This site is situated inside Hwange National Park, approximately 20 km south of Main Camp. The site occurs on deeper clayey soils typically found in the low lying areas surrounding pans in the park. The vegetation is comprised mainly of fine-leaved *Acacia* spp. and *Dichrostachys cinerea* forming open savanna. The higher quality of browse material and close proximity to

water at this site result in high herbivore densities, especially during the dry season when seasonal water pans dry up.

Hwange Baikiaea

This site is found in close proximity to the Dopu Pan site, approximately half way between Main Camp and Dopu Pan. The vegetation at the site is representative of the extensive *Baikiaea plurijuga-Terminalia sericea* Kalahari sand woodlands that occur widely throughout southern Africa. Low quality forage resulting from the nutrient-poor sandy soils results in low herbivore densities at this site.

Addo Elephant National Park

Addo Elephant National Park is located in the Eastern Cape Province, South Africa in the heart of the Albany Thicket Biome. Albany Thicket is found in semi-arid areas of the Eastern Cape and Western Cape Provinces. Rainfall in the biome varies from 200-900 mm per annum (Vlok and Euston-Brown 2002). Two prevailing climate systems converge in the region which results in all year rainfall with spring and autumn maxima (Aucamp & Tainton 1984). Rainfall is unreliable and summer temperatures are high, while winter temperatures are low with a low to frequent occurrence of frost along the altitudinal gradient (Mucina & Rutherford 2006). The vegetation of the region has been described as Sundays Thicket by Mucina & Rutherford (2006). The landscape is composed of undulating plains and low mountains and foothills covered with tall, dense thicket, where trees, shrubs and succulents are common with numerous succulent species.

Addo Elephant Camp

This site occurs within the original boundaries of the Addo Elephant National Park. A section of the park was fenced off with an elephant proof fence in 1954 to prevent elephants from moving to adjacent farms. The fenced off area was originally called the elephant camp and has subsequently been enlarged on a number of occasions as elephant numbers increased and more land became available. The vegetation within the elephant camp is characteristic of Sundays Thicket with a high prevalence of the succulent woody tree *Portulacaria afra*. MAP is around 400 mm with high summer temperatures and cold winter temperatures. The park environs form a series of low undulating hills with altitudes ranging from 76 to 341 m asl. The soils are predominantly light-

red clay-loams derived from sandstones and mudstones of the Sundays River Stage, Uitenhage Series, Cretaceous System (Toerien 1972; Stuart-Hill 1992). The main herbivore species found in the original elephant camp section of Addo Elephant National Park include buffalo (*Syncerus caffer*), zebra, red hartebeest (*Aelaphus buselaphus*), grysbok (*Raphicerus melanotis*), bushpig, warthog (*Phacochoerus aethiopicus*), eland, elephant, ostrich (*Struthio camelus*), black rhino, kudu, bushbuck, gray duiker and steenbok. Census data for the period between 1991 and 2004 showed increased populations for elephant, kudu, eland and bushbuck.

Climatic variables measured

Mean annual precipitation (MAP), potential evapotranspiration (PET), mean annual temperature (MAT), and solar radiation (SR) were determined for each site using nearby long-term weather stations when available, alternatively these were taken from the South African Atlas of Agrohydrology and Climatology (Schultze et al. 1997). Evapotranspiration rates at the sites were taken from the global MODIS evapotranspiration dataset explained by Mu et al. (2011) available at <ftp://ftp.ntsg.umd.edu/pub/MODIS/Mirror/MOD16/>.

Measures of soil fertility

The methods used to determine the soil fertility status at each site will be discussed in detail in Chapter 3. In summary, at each site five replicate samples were taken at least five meters apart from three depths (0-10 cm, 10-20 cm and 40-50 cm) using a soil auger. Additionally at each site a soil pit was excavated in order to sample bulk density at the same depths. Soil samples were analysed for nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K), as well as carbon (C) and pH.

Principal component analysis on measures of soil fertility

A principal component analysis (PCA) on the measures of soil fertility showed a clear separation of the sites along PC1 and PC2 (Figure 1). PC1, which accounted for 63% of the variability in soils summarised sites according to variation in soil N, C:N and sum of bases (SB). Sites were clearly segregated along this axis with the predefined nutrient-rich sites showing higher and more variable values for all three measures of soil fertility. PC2 which accounted for a further 25% of

the variability in soils was primarily driven by differences in soil P. Sites defined as nutrient-poor showed very little variability along this P axis.

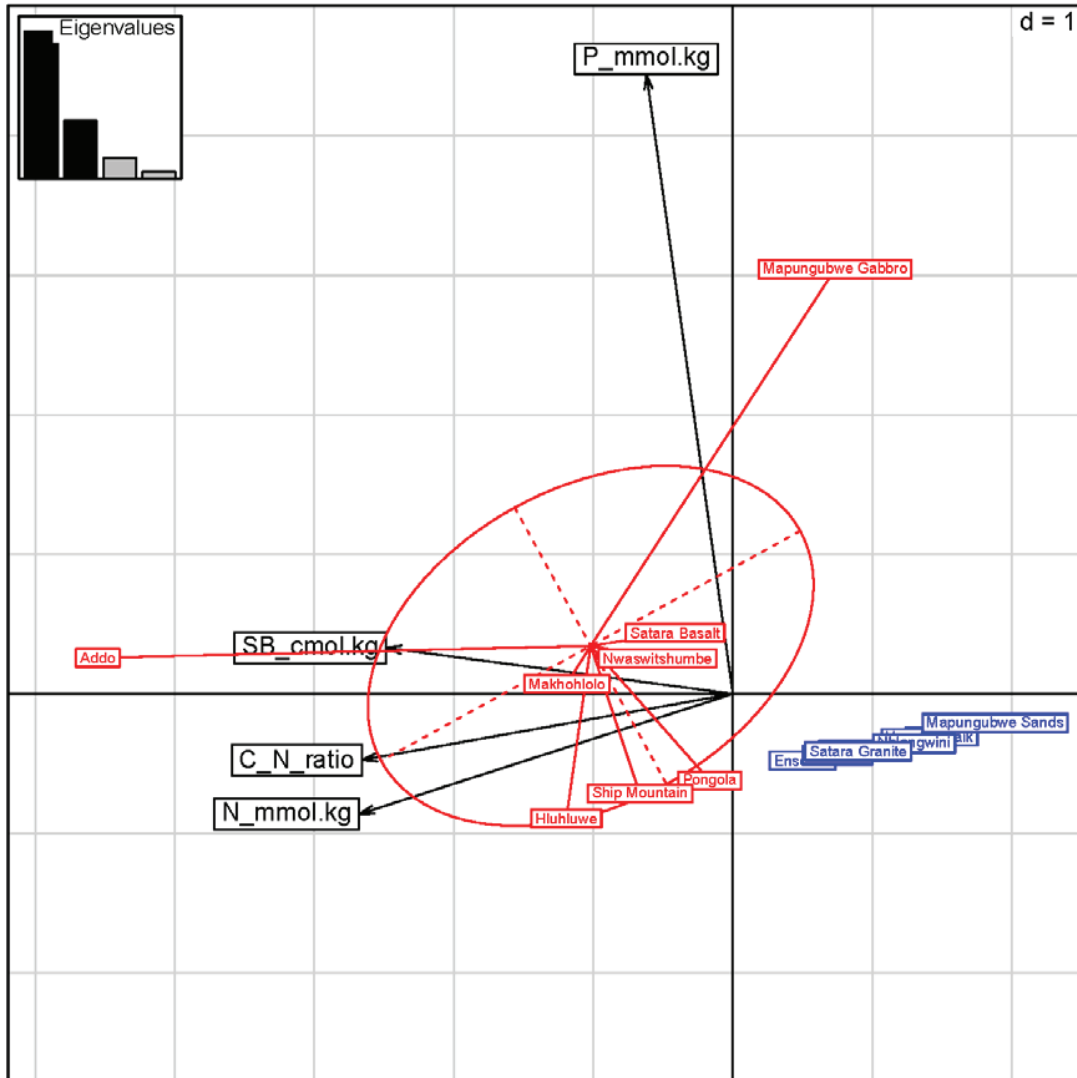


Figure 1. PCA based on measures of soil fertility, PC1 (x-axis, significant) explains 63% of the variance in soils with negative loadings for soil N, soil C:N and soil SB. PC2 (y-axis, non-significant) explains 25% of the variance with positive loadings for soil P. For this and subsequent figures the sites allocated to the nutrient-rich category are shown in red, while sites assigned to the nutrient-poor category are shown in blue.

Principal component analysis on climatic variables

A PCA on climatic variables showed a more or less even distribution of sites along both PC1 and PC2 (Figure 2). Variation along PC1 which accounted for 61% of the variability in climate was driven by differences in mean potential evapo-transpiration in January (PETJ), solar radiation in January (SRJ) and mean annual precipitation (MAP). PC2 which accounted for a further 26% of the variance was primarily driven by mean annual temperature (MAT).

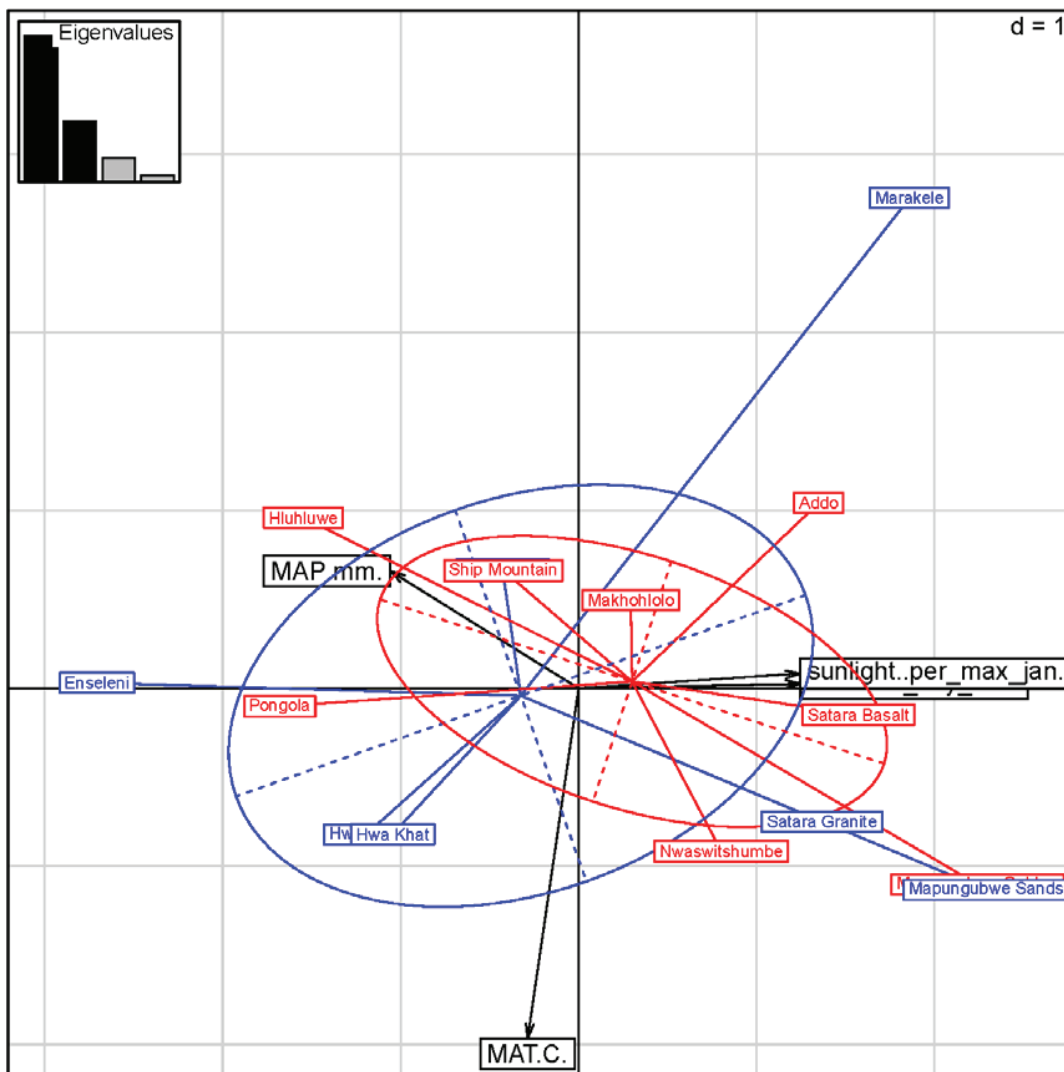


Figure 2. PCA based on climatic variables, PC1 (61% of variance, significant) with positive loadings for maximum sunlight (%) in January and PET in January and negative loadings for MAP, PC2 (26% of variance, non-significant) with negative loadings for MAT.

Browsing index

Due to the nonexistence of animal census data for many of the sites and different methodology used for those sites with census data, I decided to rather use direct measures of herbivore impact on the woody vegetation at each site. The methods used to establish the degree of browser utilisation for each woody species at each site by meso-browsers and mega-browsers are explained in detail in Chapter 6.

Fire intensity

Two methods were used to get an estimate of fire intensity at each study site. Firstly, for each of the selected species at each site, ten individuals were randomly selected to determine the degree of browser utilisation and fire damage. For each plant the maximum height at which signs of fire damage were visible was recorded as well as the maximum diameter of scorched twigs. The degree of stem damage and resprouting height was also recorded. The second approach was to walk four 100 x 4 m transects at each site. The identity, height, canopy diameter, number of stems and life history stage were recorded for each woody plant encountered along each transect. The types of fire damage (maximum scorch height and maximum diameter of scorched twigs) were recorded for each individual.



Miombo woodland at the Hwange Baikaiea site, Hwange National Park, Zimbabwe.

Chapter 3 What do ecologists miss by not digging deep enough? Insights and methodological guidelines for assessing soil fertility status in ecological studies

Published in *Acta Oecologica* 51 (2013) 17-27.

Benjamin J. Wigley, Corli Coetsee, Anthony S. Hartshorn & William J. Bond

Wigley, B.J. (corresponding author, benwigley@gmail.com): UMR CNRS 5558 – LBBE, Université Claude Bernard Lyon 1, Bât. Grégor Mendel 43 bd du 11 novembre 1918, 69622 Villeurbanne cedex.

Coetsee, C. (Corli.Coetsee@nmmu.ac.za): School of Natural Resource Management, Nelson Mandela Metropolitan University, P/Bag 6531, George, 6530, South Africa.

Hartshorn, A.S. (soildoc@gmail.com): Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717, United States.

Bond W.J. (William.Bond@uct.ac.za): Department of Botany, University of Cape Town, P/Bag, Rondebosch, 7701, South Africa.

Abstract

Soil fertility is one of the major drivers of ecological processes and is therefore frequently investigated in ecological research. Although often referred to in studies, soil fertility is not well quantified. Consequently some studies have resorted to classifying site soil fertility according to the potential fertility associated with underlying geology, ignoring the soil nutrient status of the rootzone. A common protocol is for ecologists to sample the upper soil layers only (<20 cm). Unfortunately these surface layers are those most likely to be altered by the vegetation itself and may not necessarily reflect the influence of the geological substrate. Using examples, we attempt to provide some practical guidelines on how to determine the intrinsic nutrient status of soils. Soil data from five sites in southern African savannas were used to demonstrate: a) when deeper soil

sampling may not be needed, b) how to determine which nutrients may be limiting at a site, c) the importance of bulk density measurements and d) the effect of three different sampling methods. Our data illustrate that the effects of fine scale landscape variability on soil nutrients were evident to variable soil depths. Frequent fires affected soils only to depths of <5 cm, the presence of tree canopies affected soils up to 50 cm, while topographic position affected soil nutrients to a depth of 90 cm. Bulk density did not differ between depths nor between treatments within sites, but differed amongst sites. None of the alternative methods used to collect soil samples (i.e. augering vs. digging soil pits and sampling by depth or horizon) resulted in significant differences in nutrient measures. Standardised sampling from at least three depths together with bulk density measurements allow for calculation of nutrient stocks as a measure of intrinsic soil nutrient status, while also providing insights into nutrient distributions with depth, thereby allowing meaningful cross-site comparisons.

Keywords: bulk density; nutrient cycling; plants; sampling methods; soil depth; soil nutrients

1. Introduction

The quantification of soil fertility is fundamental to gaining insights into many ecological processes. Soil fertility is measured for a variety of reasons that include how climate, land-use change, vegetation change, and disturbances such as fire or herbivory affect soil properties and vice versa (Fritz and Duncan, 1994; Ritchie et al., 1998; Fritz et al., 2002; Augustine, 2003; Lechmere-Oertel et al., 2005; Augustine and McNaughton, 2006; Grange and Duncan, 2006; Homann et al., 2007; Yang et al., 2008; Diocion et al., 2009; Sandoval-Perez et al., 2009; Anderson et al., 2010). Measures of soil fertility are also used to ascertain how soil nutrients

drive plant distributions and traits (Jobbágy and Jackson, 2001; Wright et al., 2001; Ruggiero et al., 2002; Dezzeo et al., 2004; Ordonez et al., 2009; He et al., 2010). Soil fertility has also been considered important in determining vegetation patterns at continental and regional scales. In African savannas, for example, Scholes and Walker (1993) noted two major soil types based on nutrient status: nutrient-rich savannas dominated by acacias and other Mimosaceae and nutrient-poor savannas dominated by members of the Caesalpiniaceae (e.g., miombo woodlands). These two types also differ in their grass species and other ecological attributes. Nutrient-rich savannas support more mammal biomass distributed in different body mass categories than nutrient-poor savannas (Fritz and Duncan, 1994; Fritz et al., 2002).

Although soil fertility is often referred to in the literature, it is not always clear what denotes a “fertile” or “infertile” site. For example, Fritz and Duncan (1994) used geological substrate as indicators of relative fertility in their analysis of the effects of soil fertility on mammal biomass reflecting "field experience" because no quantifiable descriptors of soil fertility were available. In Kruger National Park (KNP), Grant and Scholes (2006) highlight that these broad classifications ignore the finer scale variability in the landscape which gives rise to fertile patches in otherwise ‘infertile’ landscapes. A further example highlighting this problem is provided by Bowman and Prior (2005) in their review on why evergreen trees dominate the Australian seasonal tropics. They stress the lack of comparative studies that determine how infertile Australian soils are by world standards, with this being particularly true for soils from northern Australia relative to other tropical regions.

A potential explanation for the continuing lack of quantifiable limits to what ecologists consider fertile or infertile is that ecologists often simply do not know enough about the soils they

are referring to and are therefore unable to adequately answer this important question. Ecologists frequently sample the soil at their study sites because that is deemed the correct thing to do.

When ecologists are faced with the plethora of literature on soil sampling methodology which is mostly biased towards agronomy, it is often easier to follow the example of other ecologists.

Thus the cycle continues and ecologists may continue to follow their predecessors without really clarifying or quantifying the soil nutrient status.

Jobbágy and Jackson (2001) in their meta-analysis of the global distribution of soil nutrients with depth and the imprint that plants have on these distribution patterns clearly show that plants are instrumental in driving nutrient distributions, particularly in shallow soil layers. Thus nutrients that are limiting to plants are typically cycled in the upper soil layers, while those that are not limiting to plants may be leached from the surface layers and show a more homogenous distribution or even increase with depth. Relatively few studies justify the reason for the depth of soil sampled. Previous work has cautioned against under-estimating soil carbon (C) or drawing wrongful conclusions regarding changes in soil C over time when only shallow soil layers are sampled (Harrison et al., 2011; Johnson et al., 2011; Zabowski et al., 2011). Similarly, often the standard protocol for establishing soil fertility is to sample surface soil layers typically ranging from *ca.* 5 to 20 cm, e.g., 5 cm (Garnier et al., 2007), 7.5 cm (Bullock et al., 2001; Lauber et al., 2008), 10 cm (Dupouey et al., 2002; Carney et al., 2004; Garnier et al., 2004), and 15 cm (Verheyen et al., 1999; Fraterrigo et al., 2005). However by sampling surface, soils ecologists are biasing their results to those soil properties which are most influenced by the vegetation itself and not by the intrinsic geological substrate (Jobbágy and Jackson, 2001). In other words, by only sampling the top layer we cannot infer the inherent soil fertility status of that site as is frequently assumed. For example, He et al. (2010) looking at taxonomic identity, phylogeny, climate and

soil fertility as drivers of leaf traits across grassland biomes in China, refer to soil fertility yet their measure of soil fertility was based on measurements from the top 20 cm only. Similarly, Fritz and Duncan (1994) used geological substrate as their measure of soil fertility in analysing determinants of mammal biomass in African savannas and ignored soils data completely. However, intrinsically nutrient-poor soils can be enriched locally by high mammal populations (Blackmore et al., 1990).

Very few ecological studies that measure soil fertility measure bulk density. In a review of soil fertility decline in the tropics, Hartemink (2006) noticed that one of the major errors in soil sampling was that soil data was mostly expressed on a concentration (or weight) rather than an areal (or volume) basis. Boone et al. (1999) suggested that the adoption of a standard of expressing soils data on an areal basis should facilitate cross-site comparisons and synthesis and better ensure the comparability of long-term data sets. Reporting soil nutrients as concentrations (e.g., percentage by weight) limits direct comparisons between sites as soil bulk densities vary.

Ecological studies are often limited by time constraints and resources. How do we find the balance between useful information relating to soil fertility and the effort spent on acquiring it? In this paper we attempt to provide some practical guidelines on how to determine the nutrient status of soils with modest sampling effort. These guidelines toward standardising soil sampling methods for ecologists could be used in conjunction with handbooks such as Cornelissen et al. (2003) and Perez-Harguindeguy et al. (2013).

Our guidelines are motivated by the following questions: 1) Should we sample deep soils and in which situations is it not important to sample to depths of greater than 20 cm? 2) How do we determine which nutrients are likely to be limiting to plant growth at a site from standard soil

analyses? 3) How similar is bulk density with increasing depth at the local and landscape scales?
4) How do different sampling methods used to collect soil samples (use of soil auger, sampling soil pit by fixed interval, sampling soil pit by horizon) affect study results and interpretations?

We sampled soils in three different ways and analyzed samples from multiple depths for nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), carbon (C), bulk density, and pH from five sites across southern Africa. Each of the five sites included a pair of locations intended to help assess the effects of one of three different ecological drivers (fire, tree canopies, or topographic position) known to cause heterogeneity in soil nutrients (e.g., Jones, 1973; Belsky, 1994; Coetsee et al., 2010). The effects of these treatments were included to investigate to what extent they may alter the intrinsic nutrient status of the soil.

2. Materials and Methods

2.1 Study Sites

Three of the five sites were in KNP, South Africa, and two sites were in or near Hwange National Park, Zimbabwe. We sampled for P, Ca, Mg, Na, K and C at all sites, but used an existing dataset from Shabeni in the KNP to look at effects of canopies and burning on N (see Coetsee et al., 2010 for comprehensive methods). Mean annual rainfall for the Kruger sites is ~650-750 mm and the Hwange sites receive ~600 mm of rainfall (Childes and Walker 1987). Fire effects were assessed at the Shabeni Burn Plots (25° 8' 2.04" S; 31° 14' 4.4154" E) in KNP, at a long-term (~60 y) on-going fire experiment (van Wilgen et al., 2007; Venter and Govender 2012). Here the soils overlie granite and can be classified as Alfisols and Luvisols according to classification systems, respectively, of the United States Soil Taxonomy and World Reference Base (Deckers et al.,

2011). We contrasted soil nutrient (not N) and C levels from the two most dissimilar experimental treatments: a plot that had not been intentionally burnt since the beginning of the experiment and a plot in the adjacent firebreak that is burnt annually ('burn' and 'no burn' hereafter). For N, samples were taken from an annually burnt treatment and the plot that has not been burnt since the start of the experiment at the same Shabeni fire experiment.

Tree canopy effects were assessed at two sites in KNP, and at one site near Hwange National Park by using paired tree canopy and away from a canopy sampling locations, 'canopy' and 'no canopy' hereafter. All no canopy samples were taken approximately 20 m away from the outside edge of the canopy. For N, the effect of a tree canopy was tested using the triennial burn plot at Shabeni. Four replicate samples were taken from below adult *Sclerocarya birrea* (A.Rich.) Hochst. trees, and four paired replicate samples away from canopies. For the other nutrients, the first canopy site (the Nhangwine enclosure; 25° 12' 14.04" S; 31° 17' 47.796" E) near Pretoriuskop overlies granite, where soils have been classified as Inceptisols, and Ferralsols. Here we sampled paired locations under and away from a 12-m tall *Diospyros mespiliformis* Hochst. tree. The second canopy site (the Makhohlolo enclosure; 25° 15' 43.4988" S; 31° 54' 49.4532" E) overlies basalt near Crocodile Bridge, and soils have been classified as Vertisols in both systems; here we sampled under and away from a 12-m *Sclerocarya birrea* tree. The third canopy site was located in an area called Khatshana which is on the outskirts of Hwange National Park (18° 37' 55.956" S; 26° 54' 3.24" E). The site is on Kalahari sands which are classified as Entisols and Regosols. Here we sampled under and away from an 18-m *Brachystegia spiciformis* Benth. tree.

Topographic (catenary) effects on soil fertility were assessed inside Hwange National Park at Dopu Pan (18° 38' 33.6474" S; 26° 53' 36.744" E), where we sampled a vlei or 'dambo', a seasonally water-logged depression or pan (Matiza 1992), and away from the dambo ('pan' and

‘non-pan’ hereafter) in the surrounding fine-leaved *Acacia* woodland. Non-pan soils here can be classified as Entisols, and Regosols; pan soils would best be classified as Alfisols, and Gleysols.

2.2 Soil sampling procedures

At each of the five pairs of sampling locations, soil samples were taken to determine soil C and nutrients, excluding N, from multiple depths. These were collected by auger, at fixed intervals, or by soil horizon. Auger samples were collected from three depths (0-10, 10-20, and 40-50 cm) with a 72-mm diameter auger from five points, at least 5 m apart. For fixed-interval and horizon sampling, a single rectangular soil pit (1.5 x 0.5 m) was excavated to a depth of 1 m or until rock was encountered. Depth-specific samples were then collected from the four sides of the pit and pooled for analysis. For N, we used data from an existing dataset and the following soil depths were sampled: 0-2, 2-5, 5-10, 10-20, 20-40, 40-60, 60-80 and 80-100 cm depending on soil depth. For C and the other nutrients, fixed-interval samples were collected at: (1) every 1 cm for the first 5 cm with an additional bulked sample from 5-10 cm, (2) from 0-5 and 5-10 cm, (3) from 0-10 and 10-20 cm, and finally, (4) 0-20 cm, 20 cm to just above (10 cm) the bottom of the pit (i.e. the C horizon), and from the bottom 10 cm of the pit. For horizon sampling, the A horizon extended from 0-20 cm while the B horizon extended from *ca.* 20 cm to approximately 90-100 cm. At the Makhohlolo canopy site the B horizon ended at 50 cm, where rock was encountered.

To calculate stocks of C and nutrients, for all sampling methods, we converted each depth interval i into a volume by using the bulk density (ρ) and summed these over the maximum depth z :

$$S_z = \sum(N_i \rho_i D_i (1 - R_f)_i), \quad \text{equation 1}$$

where S_z is soil nutrient stock ($\text{g or mg cm}^{-2} \text{ z}^{-1}$), N is nutrient concentration ($\text{g [kg oven dry soil]}^{-1}$), ρ is bulk density ($\text{g oven dry soil [cm field-moist soil]}^{-3}$), D is soil depth (cm), and R_f is the rock (particles >2 mm) fraction.

2.3 Bulk Density

We calculated bulk density from samples collected from 0-10, 10-20, and 40-50 cm intervals. Four replicates were taken for each interval from each side of a soil pit. Samples were collected by vertically knocking a sharpened 48-mm diameter steel pipe into the soil, between 5 and 10 cm from the edge of the pit. A spade was then used to dig out the side of the pit until the pipe was exposed, and the spade placed underneath the rim of the pipe to ensure no soil was lost while the core was retrieved. The soil cores were then carefully emptied into a labelled brown paper bag which was dried to constant weight in a 60°C drying oven. The bulk density was calculated as

$$\rho = M_s / V_s, \quad \text{equation 2}$$

where ρ is bulk density (g cm^{-3}), M_s is mass of oven-dried soil (g), and V_s is the field-moist soil volume (cm^3) (Boone et al., 1999).

2.4 Sample analyses

We chose a suite of soil properties that are often measured in soil fertility studies. Samples were analysed for C, P, Ca, Mg, Na and K, as well as pH at the Elsenburg Laboratory, Institute for Plant Production, Stellenbosch, South Africa. Carbon was analysed by a rapid dichromate oxidation method using the Walkley-Black procedure (Walkley 1947). Samples were not pre-treated for carbonates as the pH was well below 7.4, which suggests that carbonates are not a large component in our samples (Nelson and Sommers 1996). Extractable P, Ca, Mg, Na and K were extracted with 1% citric acid and analysed by using a Thermo ICP iCAP 6000 Series

Spectrometer (ThermoFisher Scientific, Surrey, UK). Total soil N was analysed at the Archaeometry Laboratory, University of Cape Town, Cape Town using a Carlo Erba NCS 2500 elemental analyser (Carlo Erba Instruments, Milan, Italy). The precision for N on the Carlo Erba was 0.002% of standard error. Duplicate samples analysed for C (%), Ca/ Mg (both $\text{cmol}_c \text{kg}^{-1}$), Na, K and P (all three mg kg^{-1} converted to $\text{cmol}_c \text{kg}^{-1}$) had coefficients of variation of 0.08 (C), 0.10 (Ca), 0.07 (Mg), 0.20 (Na), 0.05 (K) and 0.13 (P) respectively. The pH was determined in a 1:10 ratio of 0.01 M KCl (McLean 1982). Sum of bases was calculated as the sum of Ca, Na, Mg, and K and is reported as $\text{cmol}_c (\text{kg oven dry soil})^{-1}$.

2.5 Concentration factors

The vertical distributions of nutrients were described on a relative basis within soil profiles according to the methods described by Jobbagy and Jackson (2001). Here, the content of C or a nutrient in the top 20 cm was compared to the full soil profile to 1 m ('concentration factor' hereafter). We then used the concentration factors to compute ' Δ ', which is the difference in nutrients or C between paired treatments (i.e. burn-no burn, can-no canopy, pan-non-pan). We calculated normalized deltas (ΔN) which is the delta compared to the average nutrient or carbon control values (no burn, no canopy, non-pan) across all sites, except for P, where Makhohlolo was excluded from the average value, due to very high (outlier) values. Normalized deltas for P at Makhohlolo are estimated as the fractional change relative to the no canopy site. A positive (+) ΔN indicates that the effect of the treatment (burn, canopy, pan) was greater than the average control mean (no burn, no canopy, non-pan) and negative (-) that the effect of the treatment was lower than the average control mean.

2.6 Statistical analyses

All analyses were performed using R (R Development Core Team 2011). We tested for differences in nutrient depth profiles (by weight first, by volume second) within each of three categories of drivers (fire, canopy, drainage). We tested if nutrient levels in the different treatments were significantly different at the depth intervals that were sampled. Analysis of variance (ANOVA) was used to test for differences in bulk density among depths within each treatment, treatments (annual burn *vs.* no burn, canopy *vs.* no canopy and pan *vs.* non-pan) and sites, as the conditions for homoscedasticity were not violated (Fligner Test: $P > 0.05$, Conover et al., 1981). Nonparametric (Kruskal-Wallis and Wilcoxon) tests were used to analyse the relationships between treatments, soil nutrients and sampling method (by weight *vs.* by volume) as the conditions for homoscedasticity were violated (Fligner Test: $P < 0.05$). The KW.multi.comp (asbio) function in R, which uses the Bonferroni correction, was used to make pairwise comparisons between treatments and methods. Lastly, we tested how different methods of soil sampling and different methods of soil data reporting affected study results and interpretations.

3. Results

Differences in soil nutrient and C due to fire, topography, or the presence of tree canopies depended on the depth interval. For example, burning increased soil nutrients and C at shallow depths (<5 cm for K, Ca, Mg and P; <20 cm for C), but not at greater depths (Fig. 1).

Surprisingly, total N was higher in the annually burn treatment compared to the no burn treatment; however this difference was not statistically significant ($P > 0.05$, Fig. 3a). On the other hand, pan soils contained more soil nutrients than non-pan soils throughout the soil profile

to 90 cm (Fig. 1). The effect of tree canopies depended on the site and type of soil characteristic measured. Overall, soils under canopies were higher in N, K, Ca, Mg, Na, P and C (Fig. 2 and 3b; Table 1). The higher nutrients and C under tree canopies were most noticeable in the upper 10 to 20 cm and was not obvious by 50 cm (Fig. 2 and 3). Nutrients and C were the highest under tree canopies at the Makhohlola site which is situated on clay soil derived from basalt (Fig. 2).

Total nutrient stocks (g m^{-2}) for the full profile at each site (Table 1) showed that soils derived from basalt (Makhohlola) are relatively nutrient-rich, soil derived from granites (Nhlangwini and Shabeni) are relatively poor, while Kalahari sands (Dopi and Khatshana) are the most nutrient-poor. Total stocks of the five nutrients and C at Makhohlola (basalt) were consistently higher than all other sites despite the soil profile being half as deep.

The upper 20 cm of nearly all of the soil profiles sampled contained a disproportionate fraction (>35%), of N, K, Ca, P and C compared to the total soil profile, while the proportions of Mg and Na were more evenly distributed throughout the soil profile (Table 1 and Fig. 3c), although there were lower proportions associated with the Dopi and Khatshana sites (Table 1). A greater fraction of soil nutrients and C was found in the top 20 cm under tree canopies compared to no canopies (Fig. 3c and 4, Table 1). Soil nutrient concentrations were relatively uniform between 30 and 100 cm (Fig. 4). Significant differences were found between canopy vs. no canopy treatments for sum of bases and C (Table 2).

Overall, normalized deltas ranged from +3.6 (P in the full profile below canopies at Makhohlolo) to -1 (Ca in the full profile below canopies at Nhlangwini). Nevertheless, only 22 of the 60 combinations (six nutrients or C \times partial or full profiles \times five sites) showed normalized deltas greater than 50%, whether positive or negative. Of the 60 combinations, only six were strongly negative (Ca and Mg from Nhlangwini [both depth intervals], as well as K and

P from Shabeni [0-20 cm]). By contrast, 26 of the combinations showed strongly positive shifts, but this is relative to 28 of the combinations showing shifts smaller than or equal to +/-30%.

Together, these data suggest that factors such as fire, canopy, or drainage can have variable and subtle influences on soil properties. None of the 30 nutrients or C × site combinations revealed opposite signs ('+' vs. '-') between the 20 cm and full profile intervals (Table 1).

Bulk densities differed little with depth within treatments, except for the Nhlangwini (granite) canopy treatment, where the 0-10 cm bulk density was significantly lower than for the 40-50 cm interval ($P = 0.02$). Bulk density differed ($P < 0.05$) between treatments with a tree canopy present versus no canopy at two of the five sites (Nhlangwini [granite] Makhohlolo [basalt]). Bulk density was significantly different between all but two of the sites, Dopi and Khatshana (Kalahari sand, Table 3). No significant differences ($P > 0.05$) were found for pH (not shown), sum of bases (SB), P or C values whether reported as total nutrient stocks (e.g., g m^{-2}) or simply as mean concentrations (e.g., mg kg^{-1}) amongst the three methods used to collect soil samples (Fig. 5).

4. Discussion

Our data highlight how different ecological drivers can affect soil nutrients to varying depths and how this has implications for sampling. Fire, for example, principally affected only the uppermost 10 cm of the soil profiles, consistent with previous work (e.g., Augustine, 2003; Coetsee et al., 2008; Da Silva and Batalha, 2008). The effects associated with tree canopy, by contrast, were evident to greater depths. Although total N has been found to be higher under canopies up to depths of 50 cm (e.g., Weltzin and Coughenour, 1990; Mordelet et al., 1993), our results suggest tree canopy effects on soils may be nutrient-specific. Furthermore the canopy

effect was much more pronounced at the site with clay soils derived from basalt compared to the more sandy soils. The increased soil nutrients and C associated with the pan persisted to 50 cm, where clays and nutrients accumulate (Matiza, 1992). Restricting soil sampling to the upper 5, 10, or 20 cm of a soil profile, therefore, could underestimate tree canopy effects on soil nutrients or soil fertility status as it relates to ecosystem dynamics.

Ecologists have used soil fertility measurements to evaluate the potential of a site to support vegetation cover and structure (e.g., Sankaran et al., 2008), or species diversity and biomass (e.g., Buschbacher et al., 1988; Sander and Wardell-Johnson, 2011). However these evaluations are often based on samples collected from the upper layers or horizons of a profile. Bond (2010) has explored the importance of nutrient stocks in limiting the development of forests: Ca and K stocks were most limiting for woody stem biomass. In fact, stocks of these nutrients in surface soils were insufficient to supply enough Ca and K for the woody biomass needed to build a forest at many sites. However, if nutrient stocks in deeper soil layers were considered, these nutrients were seldom limiting.

Rooting depths are an important consideration in sampling approach. Sampling of shallow soil layers is often justified by the argument that most roots are in the surface layers. If roots do not explore deeper soil layers for nutrients, then sampling of soils to deeper depths might not be justified in explaining soil correlates with existing vegetation but would be necessary for predicting nutrient constraints on potential vegetation with different rooting depths. Meta-analyses of root biomass distribution have found that approximately 50% of total root biomass is in the top 30 cm of the soil with most of the remaining root biomass between 30 and 100 cm (Jackson et al., 1996; Schenk and Jackson, 2002). Mean maximum rooting depths were more than double this for grasses (2.7 m) and far deeper for trees (>7 m). February and Higgins (2010)

found both tree and grass roots to depths of up to 140 cm in Southern African savanna soils. As roots appear to be sufficiently deeply distributed to access deeper nutrients in most natural ecosystems, we recommend that at least one soil sample be collected at a depth of greater than 20 cm, and preferably two (e.g., 50 and 100 cm) consistent with the sampling approach adopted by the United States Geological Survey as part of a continent-wide characterization effort (e.g., Smith et al., 2009). In our study, the average proportions of total nutrient stocks for the five measured nutrients in the top 20 cm ranged from 0.19 to 0.46, while for C the average proportion found in the top 20 cm was 0.45 (Table 1). Therefore if only the top 20 cm is sampled, between 54 and 81% of nutrients accessible by plant roots in the top 1 m of soil would not be accounted for.

Jobbagy and Jackson (2001) showed how the relative concentration of a nutrient in the upper soil layers (typically <20 cm) can be used to indicate if that nutrient is limiting in that particular system. From a theoretical initial condition of equal concentrations with increasing soil depth, nutrients would tend to be leached to deeper soil layers or brought to the soil surface by the plants growing in the soil. High relative concentrations in the upper soil layer therefore suggest that a nutrient is limiting to plant growth and tightly recycled in the upper soil layer. Nutrients least in demand would be lost through leaching and have low relative concentrations in the topsoil. The ranking of soil nutrients according to their vertical distributions in our study was comparable to that of Jobbagy and Jackson (2001). Mean topsoil concentration factors for all sites were similar to those of Jobbagy and Jackson (2001) for C and K, while N and P were generally lower at our sites (Fig. 6). Our sites however had higher concentration factors for Ca and Mg, suggesting that according to Jobbagy and Jackson (2001), these nutrients are more limiting (Fig. 3 and 4). The calculation of topsoil concentration factors is relatively easy and highly informative. It also

facilitates direct comparisons of nutrient limitations among sites and studies using standard single-measure soil nutrient analyses.

Bulk density in our study was affected little by ecological drivers such as fire, drainage, and tree canopies. Previous work has found bulk density to be lower under canopies (Mordelet et al., 1993; Hibbard et al., 2001; Tate et al., 2004). This discrepancy could be due to our relatively coarse sampling intervals, which were 0-10, 10-20 and at 40-50 cm. Bulk density did however differ significantly between sites (Table 2). We therefore suggest that unless study sites are within close proximity of one another on similar soil types (e.g., our Khatshana and Dopi sites on Kalahari Sands showed no significant differences), bulk density measurements should be taken at each site. Although rocks (>2 mm particles) were absent from the soils sampled in this study, rocky soils can be problematic for bulk density determinations. For example, a corer cannot easily be used where the rock fragment content is high; alternative density methods such as compliant cavity (Bradford and Grossman 1982) are likely necessary. Harrison et al. (2011) caution against ignoring the C content of rock (>2 mm fraction) as it may offset differences in C concentration among sites. However, most ecologists are likely to ignore the C concentration (and nutrients) in rock as these nutrients are not available to plants. The inclusion of this fraction would therefore depend on the research question being addressed as ecologists are usually interested in the fraction available to plants or animals in the short-term.

As noted previously, presentation of results in volumetric units requires measurement of bulk density (or at a minimum, an estimation) to convert from weight to volume basis. We emphasize, however, the importance of explicitly noting all dimensions associated with volumes, including the depth. Although our different sampling methods yielded similar results, our study demonstrated the importance of drivers (e.g., fire, drainage and canopy) for defining an

appropriate sampling depth. Researchers should therefore choose the appropriate method for sampling according to their available tools and resources.

There is a large literature on comparative analyses of plant traits, including an increasing number on global patterns of trait distribution (e.g., Lavorel and Garnier, 2002; Diaz et al., 2004; Ordonez et al., 2009). Climate data is readily accessible in a format suitable for analysing the importance of climate variables on trait and vegetation distribution. However hypotheses on the importance of soil nutrients in determining plant traits and the properties of vegetation (e.g., Scholes and Walker, 1993; Hopper, 2009; Lloyd et al., 2008, see Bond (2010) on savanna *vs.* forest) and the herbivore communities they support (e.g., Fritz and Duncan, 1994; Fritz et al., 2002) will, we believe, be greatly advanced by a quantification of soil nutrients that better reflect the intrinsic properties of the substrate and is less confounded by the properties of the vegetation growing there.

5. Conclusions

For general ecological studies where the vegetation potential, expressed in terms of biomass or productivity, or where the influence of soil fertility on plant traits is being investigated, we urge ecologists to sample to deeper depths. This is necessary to avoid the circularity implicit in attributing vegetation properties to soil nutrient properties which themselves are strongly influenced by the vegetation. If ecologists follow the guidelines for sampling soils set out in this paper, the quantification of ‘nutrient-rich’ *vs.* ‘nutrient-poor’ conditions for local to global studies will be significantly advanced, which in turn will facilitate comparisons across sites, studies and disciplines.

Acknowledgements

The authors thank Hervé Fritz, Edmund February, David Smith and anonymous reviewers for helpful comments and suggestions on a previous draft of the manuscript, the staff of scientific services, KNP (South Africa) and Zimbabwean Parks. The work forms part of the PhD of BJW and was funded by the Mellon Foundation and CNRS/France.

References

- Anderson, T.M., Hopcraft, J.G.C., Eby, S., Ritchie, M., Grace, J.B. & Olf, H. 2010. Landscape-scale analyses suggest both nutrient and anti-predator advantages to Serengeti herbivore hotspots. *Ecology* 91: 1519-1529.
- Augustine, D.J. 2003. Long-term, livestock-mediated redistribution of nitrogen and phosphorus in an East African savanna. *J. Appl. Ecol.* 40: 137-149.
- Augustine, D.J. & McNaughton, S. 2006. Interactive effects of ungulate herbivores, soil fertility and variable rainfall on ecosystem processes in a semi-arid savanna. *Ecosystems* 9: 1242-1256.
- Belsky, A.J. 1994. Influences of trees on savanna productivity: tests of shade, nutrients, and tree-grass competition. *Ecology* 75: 922-934.
- Blackmore, A.C., Mentis, M.T. & Scholes, R.J. 1990. The origin and extent of nutrient-enriched patches within a nutrient-poor savanna in South Africa. *J. Biogr.* 17: 463-470.
- Bond, W.J. 2010. Do nutrient-poor soils inhibit development of forests? A nutrient stock analysis. *Plant Soil* 334: 47-60.
- Boone, R.D., Grigal, D.F., Sollins, P., Ahrens, R.J. & Armstrong, D.E. 1999. Soil sampling, preparation, archiving and quality control. In Robertson, G.P., Coleman, D.C., Bledsoe,

- C.S., Sollins, P. eds. *Standard soil methods for long-term ecological research*. Oxford University Press, Oxford USA.
- Bowman, D.M.J.S. & Prior, L.D. 2005. TURNER REVIEW No. 10 Why do evergreen trees dominate the Australian seasonal tropics? *Austr. J. Bot.* 53: 379–399.
- Bradford, J. M. & Grossman, R. B. 1982. In-situ Measurement of Near-surface Soil Strength by the Fall-cone Device. *Soil Sci. Soc. Am. J.* 46: 685–688.
- Bullock, J.M., Franklin, J., Stevenson, M.J., Silvertown, J., Coulson, S.J., Gregory, S.J. & Tofts, R. 2001. A plant traits analysis of responses to grazing in a long-term experiment. *J. Appl. Ecol.* 38: 253–267.
- Buschbacher, R., Uhl, C. & Serrao, E.A.S. 1988. Abandoned pastures in Eastern Amazonia. II. Nutrient stocks in the soil and vegetation. *J. Ecol.* 76: 682-699.
- Carney, K.M., Matson, P.A. & Bohannan, B.J.M. 2004. Diversity and composition of tropical soil nitrifiers across a plant diversity gradient and among land-use types. *Ecol. Lett.* 7: 684-694.
- Childes, S.L. & Walker, B.H. 1987. Ecology and dynamics of the woody vegetation on the Kalahari Sands in Hwange National Park, Zimbabwe. *Vegetatio* 72: 111-128.
- Coetsee, C., February, E.C. & Bond, W.J. 2008. Nitrogen availability is not affected by frequent fire in a South African savanna. *J. Trop. Ecol.* 24: 647-654.
- Coetsee, C., Bond, W.J. & February, E.C. 2010. Frequent fire affects soil nitrogen and carbon in an African savanna by changing woody cover. *Oecologia* 162: 1027-1034.
- Conover, W.J., Johnson, M.E. & Johnson, M.M. 1981. A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data. *Technometrics* 23: 351-361.

- Cornelissen, J.H.C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G. & Poorter, H. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51: 335–380.
- Da Silva, D.M. & Batalha, M.A. 2008. Soil-vegetation relationships in cerrados under different fire frequencies. *Plant Soil* 311: 87-96.
- Deckers, J., Driessen, P., Nachtergaele, F., & Spaargaren, O. 2001. World reference base for soil resources—in a nutshell. European Soil Bureau – Report 7: 173-181.
- Dezzeo, N., Chacón, N., Sanoja, E. & Picón, G. 2004. Changes in soil properties and vegetation characteristics along a forest-savanna gradient in southern Venezuela. *Forest Ecol. Manag.* 200: 183-193.
- Díaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J.H.C. et al., 2004. The plant traits that drive ecosystems: evidence from three continents. *J. Veg. Sci.* 15: 295-304.
- Diochon, A., Kellman, L. & Beltrami, H. 2009. Looking deeper: An investigation of soil carbon losses following harvesting from a managed northeastern red spruce *Picea rubens* Sarg. forest chronosequence. *Forest Ecol. Manag.* 257: 413-420.
- Dupouey, J.L., Dambrine, E., Laffite, J.D., Moares, C. 2002. Irreversible impact of past land use on forest soils and biodiversity. *Ecology* 83: 2978-2984.
- February, E.C. & Higgins, S.I. 2010. The distribution of tree and grass roots in savannas in relation to soil nitrogen and water. *S. Afr. J. Bot.* 76: 517-523.
- Fraterrigo, J., Turner, M.G., Pearson, S.M. & Dixon, P. 2005. Effects of past land use on spatial heterogeneity of soil nutrients in Southern Appalachian forests. *Ecol. Monogr.* 75: 215-230.

- Fritz, H. & Duncan, P. 1994. On the carrying capacity for large ungulates of African savanna ecosystems. *Proceedings of the Royal Society, London, Biol. Sci.* 256: 77-82.
- Fritz, H., Duncan, P., Gordon, I.J. & Illius, A.W. 2002. Megaherbivores influence trophic guilds structure in African ungulate communities. *Oecologia* 131: 620-625.
- Garnier, E., Cortez, J., Billès, G., Navas, M-L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C. & Toussaint, J-P. 2004. Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85: 2630–2637.
- Garnier, E., Lavorel, S., Ansquer, P., Castro, H., Cruz, P., Dolezal, J., Eriksson, O., Fortunel, C., Freitas, H., Golodets, C., Grigulis, K., Jouany, C., Kazakou, E., Kigel, J., Kleyer, M., Lehsten, V., Leps, J., Meier, T., Pakeman, R., Papadimitriou, M., Papanastasis, V.P., Quedsted, H., Quetier, F., Robson, M., Roumet, C., Rusch, G., Skarpe, C, Sternberg, M., Theau, J.P., Thebault, A., Vile, D. & Zarovali, M.P. 2007. Assessing the effects of land-use change on plant traits, communities and ecosystem functioning in grasslands: a standardized methodology and lessons from an application to 11 European sites. *Ann. Bot.-London* 99: 967–985.
- Grange, S. & Duncan, P. 2006. Bottom-up and top-down processes in African ungulate communities: resources and predation acting on the relative abundance of zebra and grazing bovids. *Ecography* 29: 899-907.
- Grant, C. C. & Scholes, M. C. 2006. The importance of nutrient hot-spots in the conservation and management of large wild mammalian herbivores in semi-arid savannas. *Biol. Cons.* 130: 426-437.

- Johnson, D. W., Murphy, J. D., Rau, B. M. & Miller, W. W. 2011. Subsurface carbon contents: Some case studies in forest soils. *Forest Sci.* 57: 3-10.
- Harrison, R. B., Footen, P. W. & Strahm, B. D. 2011. Deep soil horizons: contribution and importance to soil carbon pools and in assessing whole-ecosystem response to management and global change. *Forest Sci.* 57: 67-76.
- Hartemink, A.E. 2006. Assessing soil fertility decline in the tropics using soil chemical data. *Adv. Agron.* 89: 180-225.
- He, J.S., Wang, X., Schmid, B., Flynn, D.F.B., Li, X., Reich, P.B. & Fang, J. 2010. Taxonomic identity, phylogeny, climate and soil fertility as drivers of leaf traits across Chinese grassland biomes. *J. Plant Res.* 123: 551-561.
- Hibbard, K.A., Archer, S., Schimel, D.S. & Valentine, D.W. 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. *Ecology* 82: 1999-2011.
- Homann, P.S., Kapchinske, J.S. & Boyce, A. 2007. Relations of mineral soil-C and N to climate and texture: regional differences within the conterminous USA. *Biogeochem.* 85: 303-316.
- Hopper, S.D. 2009. OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant Soil* 322: 49- 86.
- Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E. & Schulze, E.D. 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* 108: 389-411.
- Jobbágy, E.G. & Jackson, R.B. 2001. The distribution of soil nutrients with depth: Global patterns and the imprint of plants. *Biogeochemistry* 53: 51-77.

- Jones, M.J. 1973. The organic matter content of the savanna soils of West Africa. *J. Soil Sci.* 24: 42-53.
- Lauber, C.L., Strickland, M.S., Bradford, M.A. & Fierer, N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol. Biochem.* 40: 2407–2415.
- Lavorel, S., & Garnier, E. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct. Ecol.* 16: 545–556.
- Lloyd J., Bird, M.I., Vellen, L., Miranda, A.C., Veenendaal, E.M., Djagbletey, G., Miranda, H.S., Cook, G., Farquhar, G.D. 2008. Contributions of woody and herbaceous vegetation to tropical savanna ecosystem productivity: a quasi-global estimate. *Tree Physiol.* 28: 451–468.
- Lechmere-Oertel, R.G., Kerley, G.I.H. & Cowling, R.M. 2005. Patterns and implications of transformation in semi-arid succulent thicket, South Africa. *J. Arid Environ.* 62: 459-474.
- Nelson, D.W. & Sommers, L.E. 1996. Total carbon, organic carbon and organic matter. In: *Methods of soil analyses*, Part 2, 2nd Edition. Page, A.L. *Agronomy*, 9, 961-1010. American Society of Agronomists Inc., Madison, WI, USA.
- Matiza, T. 1992. The utilization and status of dambos in southern Africa: a Zimbabwe case study. In Matiza, T., Chabwela, H.N. eds. *Wetlands Conservation Conference for Southern Africa. Proceedings of the Southern African Development Coordination Conference held in Gaborone, Botswana, 3-5 June 1991*. IUCN, Gland, Switzerland.
- McLean, E.O. 1982. Soil pH and lime requirements. In: *Methods of soil analysis*, Part 2. Page, A.L., 199. American Society of Agronomists Inc., Madison, WI, USA.

- Mordelet, P., Abbadie, L., & Menaut, J.-C. 1993. Effects of tree clumps on soil characteristics in a humid savanna of West Africa Lamto, Côte d'Ivoire. *Plant Soil* 153: 103-111.
- Ordoñez, J.C., Van Bodegom, P.M., Witte, J.-P.M., Wright, I.J., Reich, P.B. & Aerts, R. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecol. Biogeogr.* 18: 137-149.
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G. Thompson, K., Morgan, H.D., ter Steege, H., van der Heijden, M.G.A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, J., Staver, A.C., Aquino, S. & Cornelissen, J.H.C. 2013. New handbook for standardise measurement of plant functional traits worldwide. *Aus. J. Bot.* 61: 1-234.
- R Development Core Team 2011. R: *A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org> [accessed 1 October 2011]
- Ritchie, M.E., Tilman, D. & Knops, J.M.H. 1998. Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology* 79: 165-177.
- Ruggiero, P.G.C., Batalha, M.A., Pivello, V.R. & Meirelles, S.T. 2002. Soil-vegetation relationships in cerrado Brazilian savanna. and semideciduous forest, Southeastern Brazil. *Plant Ecol.* 160: 1-16.
- Sander, J., & Wardell-Johnson, G. 2011. Impacts of soil fertility on species and phylogenetic turnover in the high – rainfall zone of the Southwest Australian global biodiversity hotspot. *Plant Soil*: DOI 10.1007/s11104-011-0763-5.

- Sandoval-Pérez, A.L., Gavito, M.E., Garcia-Oliva, F. & Jaramillo, V.J. 2009. Carbon, nitrogen, phosphorus and enzymatic activity under different land uses in a tropical, dry ecosystem. *Soil Use Manage.* 25: 419-426.
- Sankaran, M., Ratnam, J. & Hanan, N. 2008. Woody cover in African savannas: the role of resources, fire and herbivory. *Global Ecol. Biogeogr.* 17: 236–245.
- Schenk, H.J. & Jackson, R.B. 2002. The global biogeography of roots. *Ecol. Monogr.* 72: 311-328.
- Scholes, R.J. & Walker, B.H. 1993. An African savanna: synthesis of the Nylsvley study. *Cambridge studies in applied ecology and resource management.* Cambridge University Press, Cambridge, England.
- Smith, D.B., Woodruff, L.G., O'Leary, R.M., Cannon, W.F., Garrett, R.G., Kilburn, J.E. & Goldhaber, M.B. 2009. Pilot studies for the North American Soil Geochemical Landscapes Project – Site selection, sampling protocols, analytical methods, and quality control protocols: *Appl. Geochem.* 24: 1357- 1368.
<http://dx.doi.org/10.1016/j.apgeochem.2009.04.008>
- Tate, K.W., Dudley, D.M., McDougald, N.K. & George, M.R. 2004. Effect of canopy and grazing on soil bulk density. *J. Range Manage.* 57: 414-417.
- Van Wilgen, B.W., Govender, N. & Biggs, H.C. 2007. The contribution of fire research to fire management: a critical review of a long-term experiment in the Kruger National Park, South Africa. *Int. J. Wildland Fire* 16: 519-530.
- Venter, F.J. & Govender, N. 2012. A geomorphic and soil description of the longterm fire experiment in the Kruger National Park, South Africa. *Koedoe* 54(1), Art. #1037, 10 pages.
<http://dx.doi.org/10.4102/koedoe.v54i1.1037>.

- Verheyen, K., Bossuyt, B., Hermy, M. & Tack, G. 1999. The land use history 1278-1990. of a mixed hardwood forest in western Belgium and its relationship with chemical soil characteristics. *J. Biogeogr.* 26: 1115-1128.
- Walkley, A. 1947. A critical examination of a rapid method for determining organic carbon in soils: Effect of variations in digestion conditions and inorganic soil constituents. *Soil Sci.* 63: 251-263.
- Weltzin, J.F. & Coughenour, M.B. 1990. Savanna tree influence on understory vegetation and soil in North-West Kenya. *J. Veg. Sci.* 1: 325-332.
- Wright, I.J., Reich, P.B. & Westoby, M. 2001. Strategy-shifts in leaf physiology, structure and nutrient content between species of high and low rainfall, and high and low nutrient habitats. *Funct. Ecol.* 15: 423-434.
- Yang, Y., Fang, J., Tang, Y., Ji, C., Zheng, C., He, J. & Zhu, B. 2008. Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. *Global Change Biol.* 14: 1592-1599.
- Zabowski, D., Whitney, N., Gurung, J. & Hatten, J. 2011. Total soil carbon in the coarse fraction and at depth. *Forest Sci.* 57: 11-18.

Tables

Table 1. A comparison of soil nutrient stocks for K, Ca, Mg, Na, P and C in the topsoil (20 cm) and full profile (100 cm except at Makhohlolo, 50 cm); No can = no canopy. 'Δ' denotes the difference in nutrients or C between treatments. 'ΔN' denotes the calculated delta normalized to the average nutrient or carbon control value (**bolded**) across all sites, except for P, where Makhohlolo was excluded from the average value; due to outlier values, Makhohlolo's P ΔN is estimated as the fractional change relative to the no canopy site. Shifts in normalized deltas greater than 50% are highlighted. 'Prop.' is the proportion of the full profile nutrient or C stock represented by the uppermost 20 cm; values >0.20 (>0.40 for Makhohlolo) imply surface enrichment. Average proportions across all sites and treatments are contained in the last line of the table.

Site	Treatment	K (g m ⁻²)			Ca (g m ⁻²)			Mg (g m ⁻²)		
		20 cm	Full	Prop.	20 cm	Full	Prop.	20 cm	Full	Prop.
Shabeni	AB	50	110	0.45	60	111	0.54	20	50	0.40
	NB	80	130	0.62	120	222	0.54	50	90	0.56
Δ (Burn-No Burn)		-30	-20		-60	-111		-30	-40	
	ΔN	-0.6	-0.2		-0.3	-0.2		-0.6	-0.3	
Dopi	Pan	40	210	0.19	80	510	0.16	20	110	0.18
	Away	10	40	0.25	30	170	0.18	7	37	0.19
Δ (Pan-Non-pan)		30	170		50	340		13	73	
	ΔN	0.6	1.4		0.3	0.7		0.3	0.5	
Nhlangwini	No can	40	110	0.36	240	610	0.39	50	160	0.31
	Canopy	40	90	0.44	60	130	0.46	18	60	0.30
Δ (Canopy-No can)		0	-20		-180	-480		-32	-100	
	ΔN	0.0	-0.2		-1.0	-1.0		-0.7	-0.7	
Makhohlolo	No can	100	300	0.33	410	1050	0.39	110	320	0.34
	Canopy	200	600	0.33	660	1360	0.49	150	360	0.42
Δ (Canopy-No can)		100	300		250	310		40	40	
	ΔN	2.1	2.5		1.4	0.6		0.9	0.3	
Khatshana	No can	10	30	0.33	80	350	0.23	14	73	0.19
	Canopy	20	50	0.40	160	580	0.28	20	80	0.25
Δ (Canopy-No can)		10	20		80	230		6	7	
	ΔN	-0.2	-0.1		0.5	0.5		0.1	0.1	
Average				0.37			0.36			0.31

Site	Treatment	Na (g m ⁻²)			P (mg m ⁻²)			C (g m ⁻²)		
		20 cm	Full	Prop.	20 cm	Full	Prop.	20 cm	Full	Prop.
Shabeni	AB	2.5	15	0.17	1091	2225	0.49	1838	4446	0.41
	NB	2.8	15	0.19	947	2025	0.47	2079	5467	0.38
Δ (Burn-No Burn)		-0.3	0		144	200		-241	-1021	
ΔN		-0.1	0.0		0.0	0.0		-0.1	-0.2	
Dopi	Pan	1	9	0.11	2188	9928	0.22	2010	8958	0.22
	Away	0.6	5	0.12	684	2124	0.32	812	2864	0.28
Δ (Pan-Non-pan)		0.4	4		1504	7804		1198	6094	
ΔN		0.1	0.2		0.4	1.5		0.5	1.2	
Nhlangwini	No can	3	17	0.18	878	2336	0.38	1550	3623	0.43
	Canopy	3	15	0.20	1979	4665	0.42	4542	8792	0.52
Δ (Canopy-No can)		0	-2		1101	2329		2992	5169	
ΔN		0.0	-0.1		0.3	0.5		1.3	1.0	
Makhohlolo	No can	10	80	0.13	14715	17145	0.86	6507	11043	0.59
	Canopy	20	110	0.18	34446	41106	0.84	13458	18143	0.74
Δ (Canopy-No can)		10	30		19731	23961		6951	7100	
ΔN		3.0	1.3		5.5	4.6		2.9	1.4	
Khatshana	No can	0.0009	3	0.00	576	2196	0.26	920	1892	0.49
	Canopy	0.0009	4	0.00	936	2376	0.39	1492	3040	0.49
Δ (Canopy-No can)		0	1		360	180		572	1148	
ΔN		0.0	0.0		0.1	0.0		0.2	0.2	
Average		0.13			0.47			0.46		

Table 2. Chi-squared values, degrees of freedom and *P* values for Kruskal-Wallis tests comparing canopy with no canopy treatments; sum of bases (SB), nitrogen (N), phosphorus (P), and carbon (C). All depths sampled were used as replicates (total samples for SB, P and C = 45, total samples for N = 40).

	Volumetric			Areal		
	χ^2	DF	<i>p</i> -value	χ^2	DF	<i>p</i> -value
SB						
($\text{cmol}_c \text{ kg}^{-1}$, $\text{cmol}_c \text{ m}^{-2}$)	13.6	1	0.0002	3.96	1	0.05
N (% , g m^{-2})	8.08	1	0.005	3.56	1	0.06
P (mg kg^{-1} , mg m^{-2})	3.21	1	0.07	1.88	1	0.17
C (% , g m^{-2})	7.13	1	0.008	5.25	1	0.02

Table 3. Results of ANOVA testing for differences in bulk density among sites (N = 116, F = 17.97, *P* = 0.0001; NS = not significant, ***P* < 0.01, ****P* < 0.001).

	Khatshana	Nhlangwini	Shabeni	Makhohlolo
Dopi	NS	***	**	***
Khatshana		***	**	***
Nhlangwini			**	***
Shabeni				***

Figure captions

Figure 1. Soil profiles showing changes in concentrations of K (a), Ca (b), Mg (c), Na (d), P (e) and C (f) with depth for an annual burn (□) vs. no burn (■) comparison and for the seasonally flooded pan, known locally as a dambo (○) vs. non-pan (●) comparison.

Figure 2. Soil profiles showing changes in concentrations of K (a), Ca (b), Mg (c), Na (d), Ln P (e) and C (f) with depth for three canopy (open symbols) vs. no canopy (closed symbols) comparisons. NC = Nhlangwini (granite) canopy, NCA = Nhlangwini no canopy, KC = Khatshana (Kalahari sand) canopy, KCA = Khatshana no canopy, MC = Makhohlolo (basalt) canopy, and MCA = Makhohlolo no canopy.

Figure 3. Soil profiles showing changes in concentrations of N with depth for four annual burn (○) vs. no burn comparisons (●) (a), four canopy (□) vs. no canopy (■) comparisons (b), and a comparison of the proportions of total soil N in the top 1 m of soil for canopy vs. away from canopy (c).

Figure 4. Proportions of total soil K, Ca, Mg, Na, P and C in the top 1 m of soil for the three canopy (open symbols) vs. no canopy (closed symbols) comparisons. The proportions of all nutrients for the three depth classes between 20 and 80 cm were calculated from an aggregate soil sample taken from that depth, bulk density did also not vary between these depth classes. This explains the similar values depicted for these depths. NC = Nhlangwini (granite) canopy, NCA = Nhlangwini no canopy, KC = Khatshana (Kalahari sand) canopy, KCA = Khatshana no canopy, MC = Makhohlolo (basalt) canopy, and MCA = Makhohlolo no canopy.

Figure 5. Mean (a) sum of bases (SB; calculated as the sum of Ca, Na, Mg, and K), (b) P and (c) C values calculated according to the three sampling methods; auger (▣), horizon (▤), and depth (▥). There were no statistically significant differences ($P > 0.05$) between the three methods for all three comparisons.

Figure 6. Mean topsoil concentration factors for N, P, C, K, Ca, Mg and Na (▧) compared with Jobbagy and Jackson (2001) values (▨) which are mean values calculated from >20000 profiles taken mostly from the US. The dashed line indicates a topsoil concentration factor of 0.2, corresponding with a homogeneous or random vertical distribution.

Figures

Figure 1

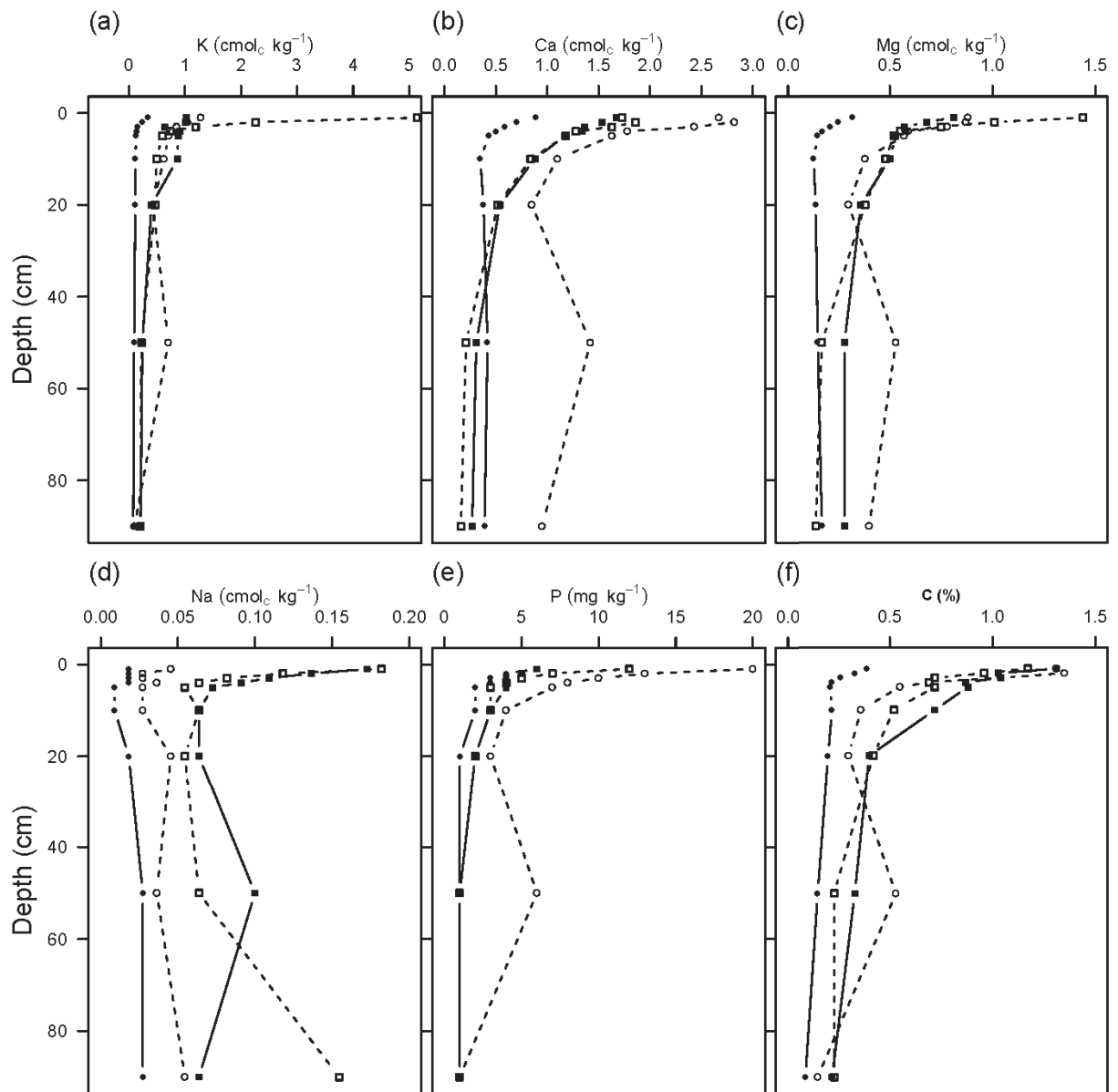


Figure 2

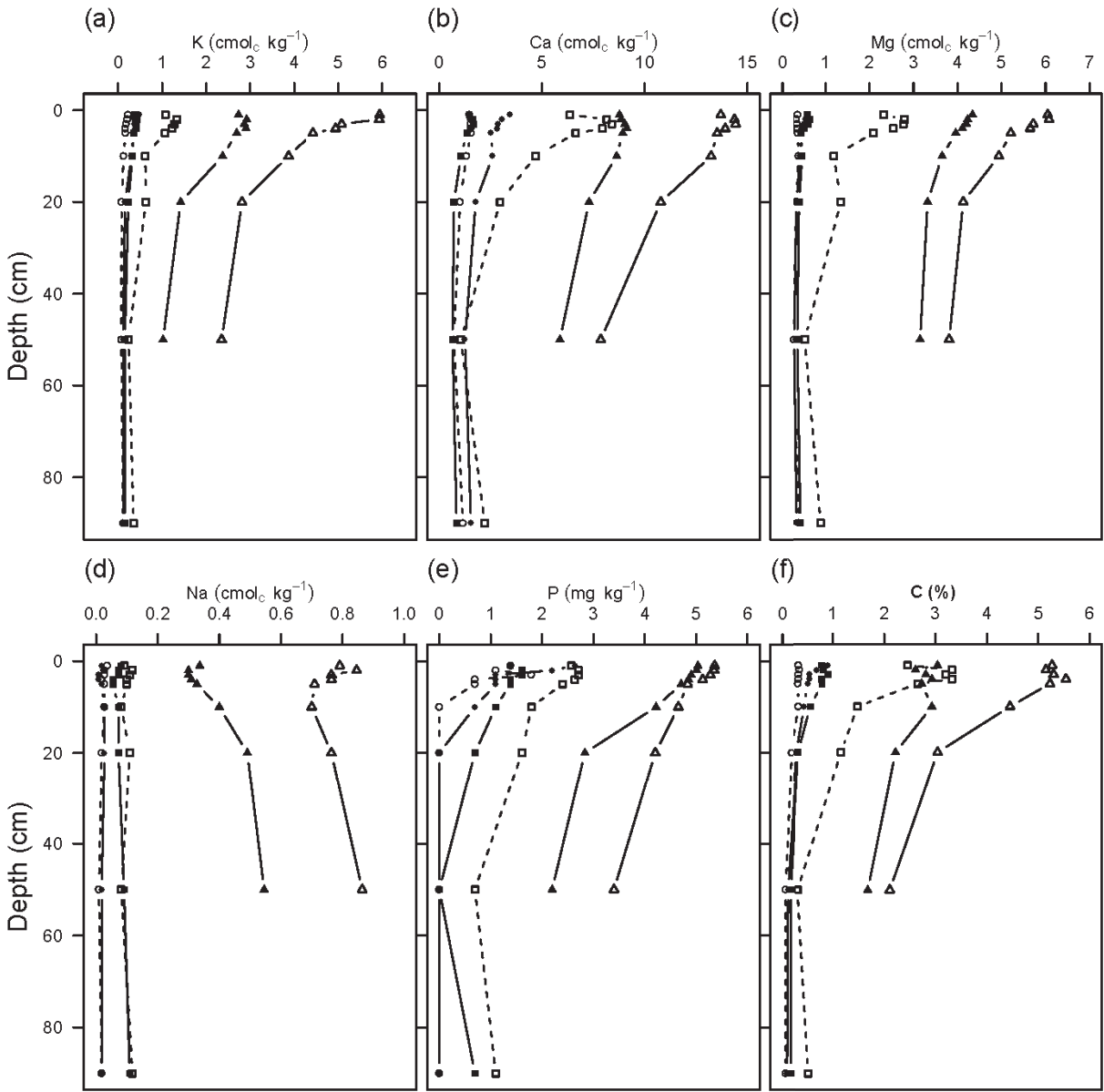


Figure 3

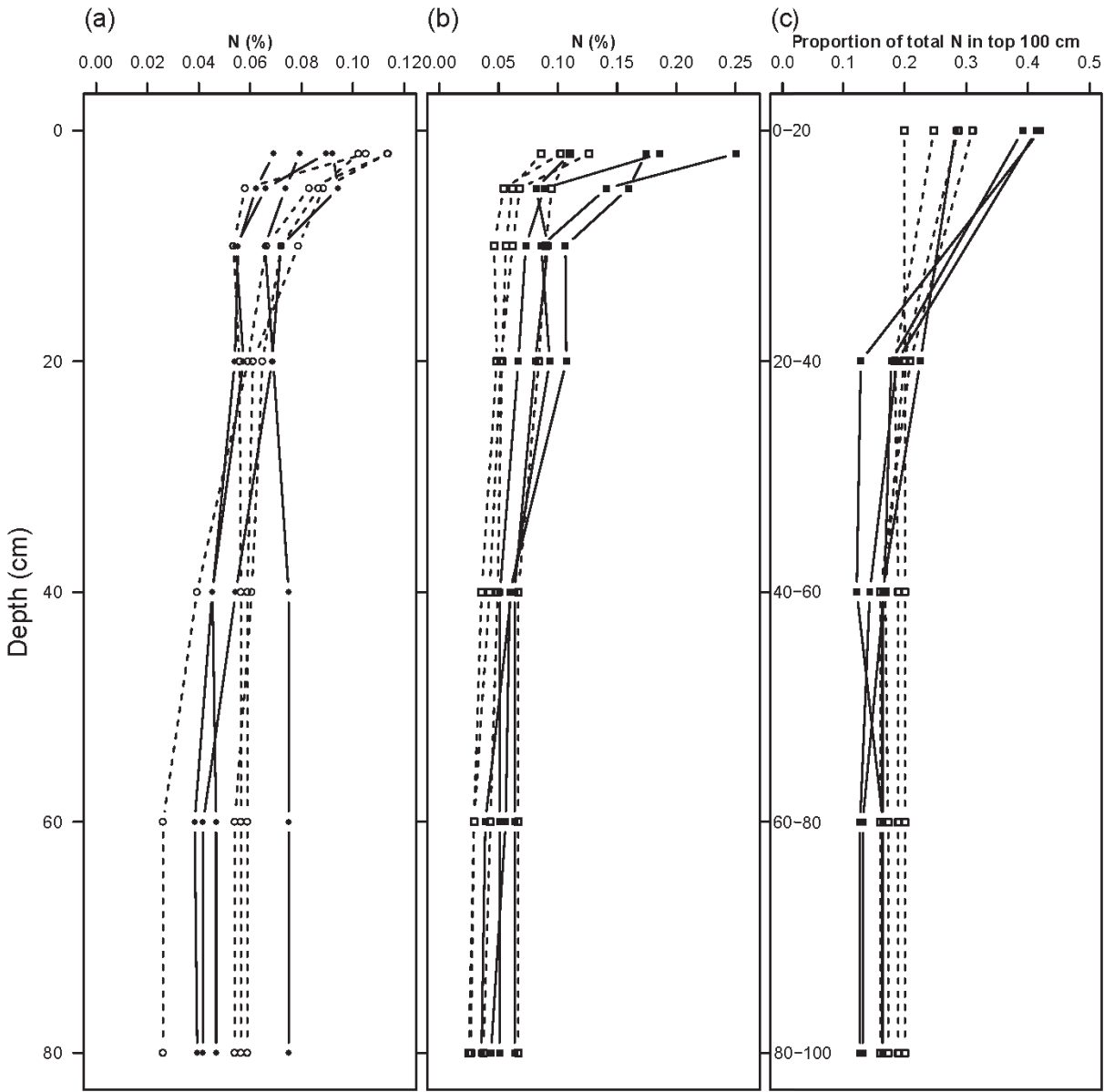


Figure 4

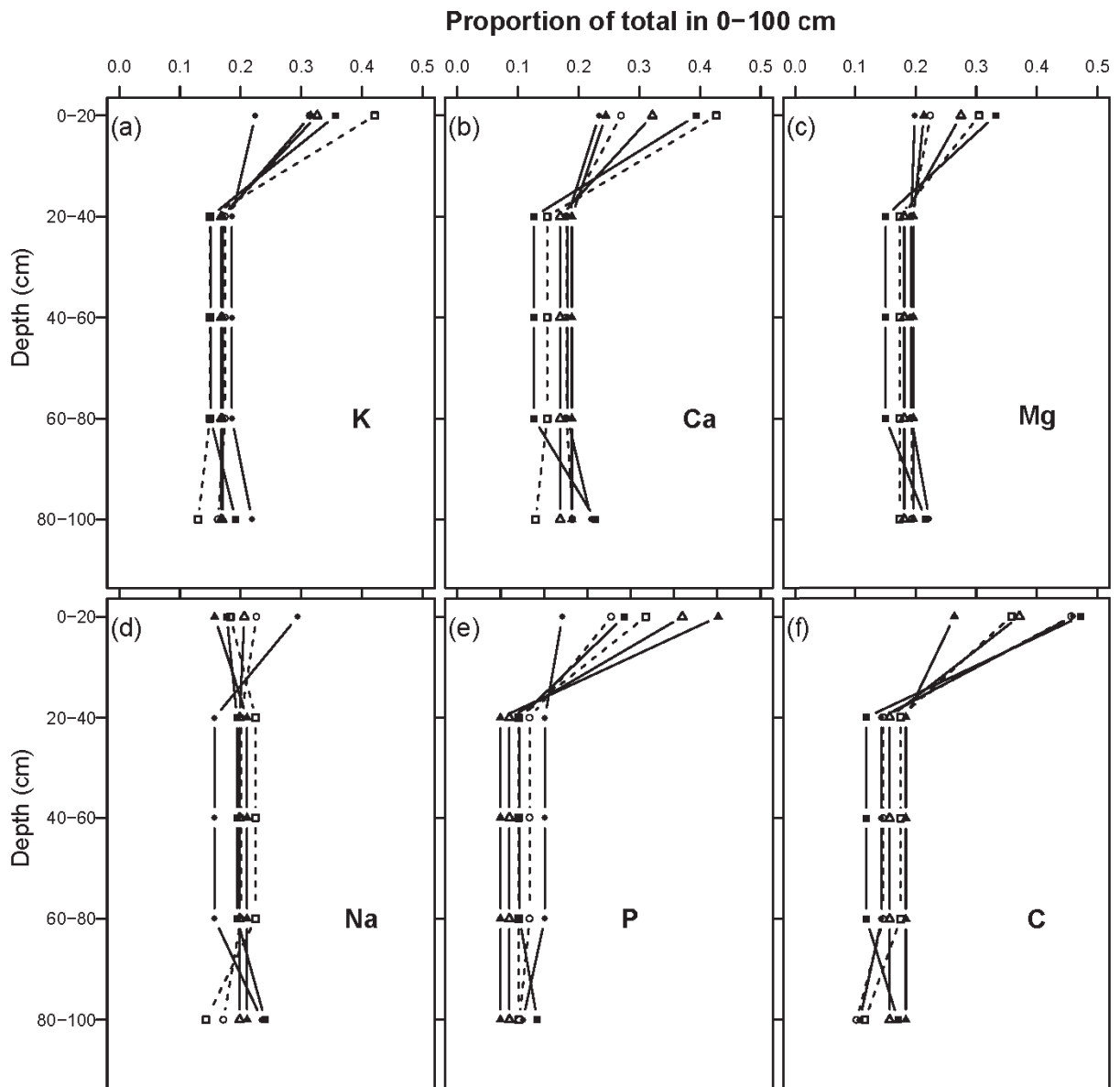


Figure 5

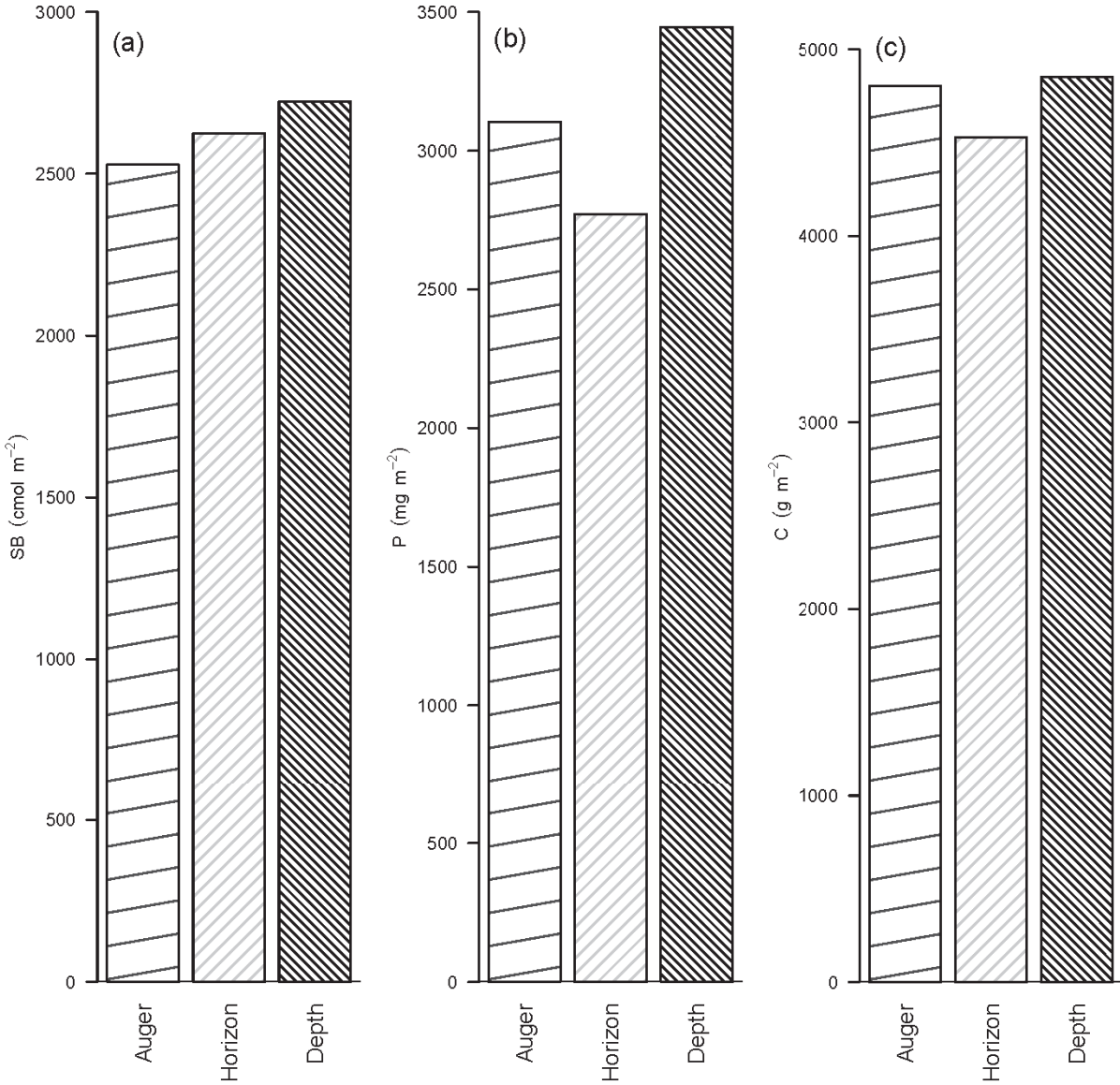
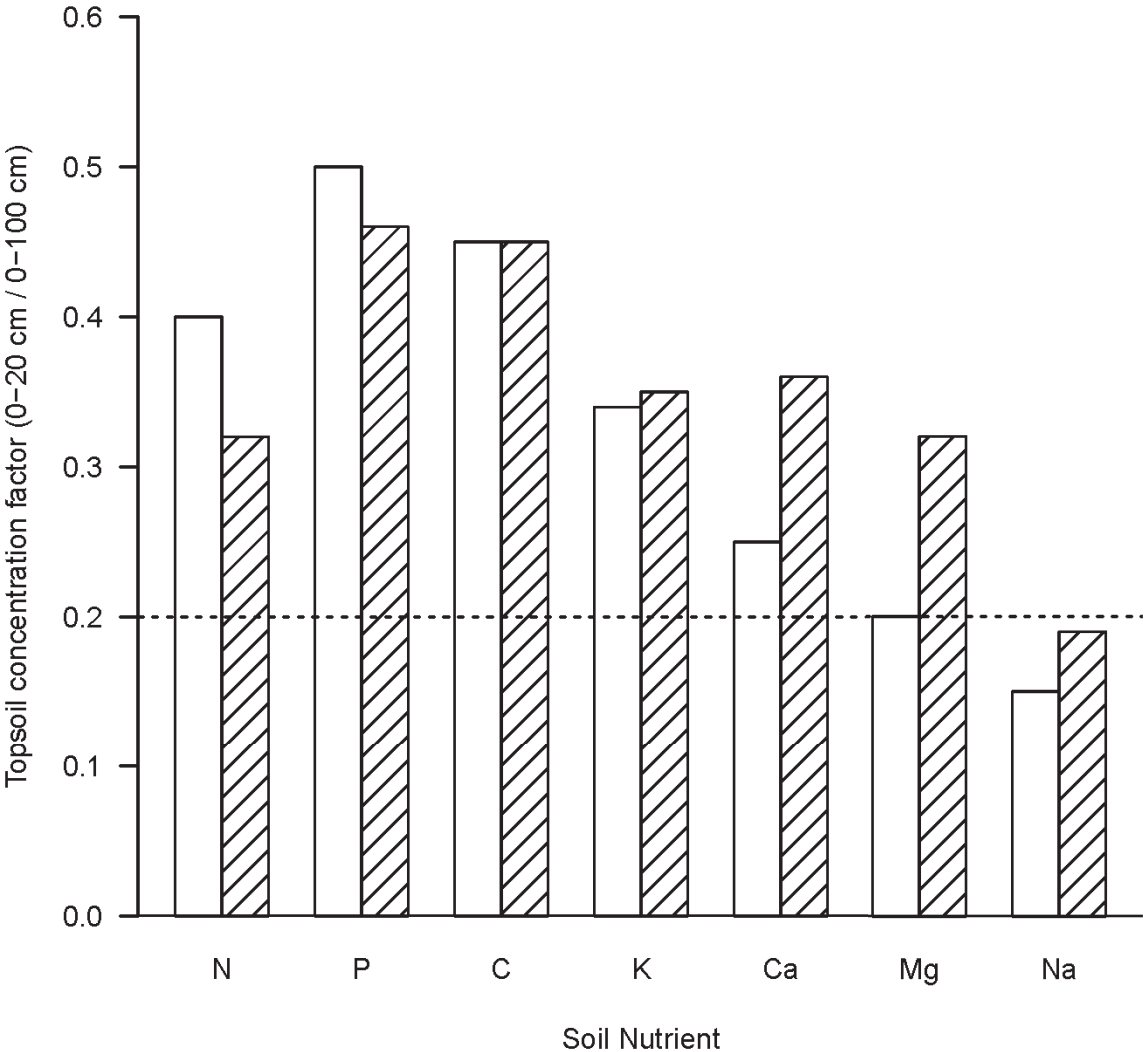


Figure 6





Soil sampling, Marakele National Park.

Chapter 4 Savanna woody leaf traits across climate and soil fertility gradients

Abstract

In this study, relationships between climatic variables and soil nutrients with both species means and community weighted means of eight key leaf traits were explored. Leaf nitrogen concentration, leaf carbon to nitrogen ratio, leaf phosphorus concentration, leaf nitrogen to phosphorus ratio, specific leaf area, average leaf area, leaf dry matter content and leaf tensile strength was measured for the dominant woody species at sixteen sites from across southern Africa. A number of significant relationships were found between savanna woody leaf traits, climate, soil nutrients and their interactions. The community weighted means of the leaf traits did not significantly increase the strength of the relationships between climate, soil nutrients and leaf traits when compared to species means. In general, relationships tended to be weaker than those found in meta-analyses which usually include plant species from many biome types. The weak correlations between leaf traits, climate and soil nutrients found in this study could possibly be explained by the high levels of disturbance typically found in African savannas.

Introduction

Abiotic filters are known to constrain the species and traits from a regionally available pool which can persist at a site (Díaz et al. 1999; García-Palacios 2012). Environmental factors impose selective pressures on species traits thereby filtering the composition and structure of local communities; this has led to the concept of community assembly by environmental filters (Keddy 1992; Weiher & Keddy 1995; 1999; Grime 2001; 2006; Shipley et al. 2006; Castro-Díaz et al. 2012). Global patterns of species traits have often been related to climatic variability (e.g. Wright et al. 2004; 2005). More recently there has been an increase in the number of studies investigating the relationships between plant traits and edaphic factors (e.g. Ordonez et al. 2010; Holdaway et al. 2011; Dominguez et al. 2012; Katabuchi et al. 2012; Laliberte et al. 2012) as well as plant traits and climate (e.g. Ordonez et al. 2009).

The availability of soil resources is a major determinant of plant productivity and is known to strongly influence plant community assembly (Tilman 1982; Grime 2006; García-Palacios 2012). Two functional and opposing strategies have been distinguished as end points

along the axis of trait variation that defines the ‘leaf economic spectrum’ (Diaz et al. 2004; Wright et al. 2004). At one end of the spectrum in resource-rich or wetter environments are species with resource acquisition strategies that allow for rapid resource capture and rapid growth rates usually characterised by thin short-lived leaves of high quality (Poorter & Garnier 1999; Wright & Westoby 2001; Diaz et al. 2004; Ruíz-Robledo & Villar 2005). At the other end of the spectrum in resource poor and dry environments are species with a conservative resource-use strategy, typically with long lived high density leaves with low nitrogen concentrations (Coley 1988; Reich et al. 1991, 1998; Villar et al. 2006).

The availability of soil nutrients is thought to be one of the main factors determining plant community composition (Ordonez et al. 2009). However it has also been recognized that there are strong feedbacks between vegetation and soil properties. Plant species/communities often play an important role in determining soil fertility through positive feedbacks to nutrient cycling (Hobbie 1992). Plants are thought to reinforce patterns of nutrient availability in natural systems through their uptake and use of nutrients. In low nutrient ecosystems, slow growth rates and higher nutrient use efficiencies are typical. This minimizes the demand for, and loss of, nutrients resulting in plant traits which promote slow nutrient cycling. In high nutrient ecosystems plants are able to achieve rapid growth rates with low nutrient use efficiency, thereby promoting rapid nutrient cycling (Hobbie 1992).

The interactions between plant leaf traits and climate or soil fertility are fairly well documented (see Chapin 1980; Aerts & Chapin 2000 for reviews), particularly at the broad scale (e.g. the leaf economic spectrum of Wright et al. (2004)). However, the quantification of trait responses at differing spatial scales is still missing (Lavorel & Garnier 2002; Ordonez et al. 2009). The need for quantitative data to improve our understanding of ecosystem functioning has been stressed by a number of authors (e.g. Chapin 2003; McGill et al. 2006; Ordonez et al. 2009). Although there has been a recent increase in the number of studies investigating the relationships between plant traits and climatic and edaphic factors, there have been very few studies in savanna ecosystems that have attempted to quantify the effects of soil fertility and climate on plant traits. This study investigates the relationship between a number of leaf traits (see Table 1 for a list of the measured traits) with soil fertility and climate in southern African savannas. I expected to find a suite of ‘conservative’ or ‘retentive’ species with associated leaf traits resulting in ‘slow and tight’ nutrient dynamics on the resource limited side of the spectrum and a suite of ‘acquisitive’

species with associated leaf traits on the opposite resource-rich end of the spectrum resulting in ‘fast and leaky’ nutrient dynamics, with intermediate trait values in-between, in accordance with the findings and predictions of Diaz et al. (2004), Wright et al. (2004) and Grime (2006).

The following questions were addressed in this study: 1) How do savanna woody plant leaf traits compare across the climatic and soil fertility gradients and does the leaf economic spectrum, or conservative vs. acquisitive strategies hold true across our gradients? 2) Do we find similar relationships at the species level compared to the community level (using community weighted means)?

Materials and Methods

Site selection

Sixteen sites (Table 1), spanning semi-arid (<300 mm mean annual precipitation, MAP) and mesic savannas (>1200 mm MAP) were selected in conservation areas in South Africa and Zimbabwe. Potential evapo-transpiration, mean temperature and solar radiation also varied between sites. Sites were chosen to include a range of underlying geologies and soil fertility status. Mean annual precipitation and mean annual temperature (MAT) were taken from the closest weather stations to each site, mean potential evapo-transpiration in January (PETJ) and solar radiation in January (SRJ) were taken from the International Water Management Institute’s (IWMI) World Water and Climate Atlas (<http://www.iwmi.org>).

Soil sampling

At each of the sites five replicate soil samples were taken at least five meters apart at depths of 0-10 cm, 10-20 cm and at 50-60 cm using a soil auger with a diameter of 72 mm. At each site a soil pit was also excavated in order to determine bulk density and depth of soil horizons. For bulk density measurements four replicate samples were taken at the same depth intervals that soils were sampled at. Samples were collected by vertically knocking a sharpened 48 mm diameter steel pipe into the soil, between 5 and 10 cm from the edge of the pit. A spade was then used to dig out the side of the pit until the pipe was exposed, and the spade placed underneath the rim of the pipe to ensure no soil was lost while the core was retrieved. The soil cores were then carefully emptied into a labelled brown paper bag which was dried to constant weight in a 60°C drying

oven. The bulk density was calculated as $\rho = M_s / V_s$ (Boone et al. 1999), where ρ is bulk density (g.cm^{-3}), M_s is mass of oven-dried soil (g), and V_s is the field-moist soil volume (cm^3).

Sample analyses

We chose a suite of soil properties that are often measured in soil fertility studies. All samples were analysed for carbon (C), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K), as well as pH at the Elsenburg Laboratory, Institute for Plant Production, Stellenbosch, South Africa. Carbon was analysed by a rapid dichromate oxidation method using the Walkley-Black procedure (Walkley 1947). Samples were not pre-treated for carbonates as the pH was well below 7.4, which suggests that carbonates are not significant in our samples (Nelson & Sommers 1996). Extractable P, Ca, Mg, Na and K were extracted with 1 % citric acid and analysed by using a Thermo ICP iCAP 6000 Series Spectrometer (ThermoFisher Scientific, Surrey, UK). Duplicate samples analysed for C (%), Ca, Mg (both cmol.kg^{-1}), Na, K and P (all three mg.kg^{-1}) had CVs of 0.08 (C), 0.10 (Ca), 0.07 (Mg), 0.20 (Na), 0.05 (K) and 0.13 (P) respectively. The pH was determined in a 1:10 ratio of 0.01 M KCl (McLean 1982). Sum of bases (SB) was calculated as the sum of Ca, Na, Mg, and K and is reported as cmol^+ ($\text{kg oven dry soil}^{-1}$) (Da Silva & Batalha 2008). Total soil nitrogen (N) was analysed by total combustion at Bemlab (Pty) Ltd., Somerset West, South Africa, using a Leco Nitrogen Analyser FP 528 (LECO Corporation, St. Joseph, MI). Bulk density measurements allowed for soil nutrients to be expressed as total nutrient stocks for each site, taking the depth of the soil into account. N, P and C are expressed as g.m^{-2} , while SB is expressed as cmol.m^{-2} . Soil nutrient stocks were calculated to 1 m of depth for all sites except Makhohlolo, where the soil profile was 50 cm in depth. Soil total N, and soil total P are commonly used as proxies of the size of nutrient pools while soil C:N is a proxy for the quality of organic matter (Heal et al. 1997). Soil SB is the sum of four important plant nutrients.

Leaf trait measurements

At each site the species that formed the dominant woody community (> 80%) of standing biomass were identified and sampled for eight leaf traits which are generally known to be related to soil nutrient supply and climate (Table 1). Leaf nitrogen concentration (LNC), leaf phosphorus concentration (LPC), SLA and ALA are known to be positively related to plant relative growth

rates, leaf carbon assimilation rates and energy supply (e.g. Lambers & Poorter 1992; Lavorel & Garnier 2002; Niklas et al. 2005). Leaf C:N can be used as a proxy of nutrient limitation (e.g. Norby & Cotrufo 1998; Oren et al. 2001). Leaf N:P is a common proxy for the type of nutrient limitation (e.g. Koerselman & Meuleman 1996; Gusewell 2004). LDMC has been shown to be negatively correlated with potential growth rates and positively related to leaf life span. Species with low LDMC tend to be associated with productive environments (Cornelissen et al. 2003; Hodgson et al. 2011). Leaf TS is strongly related to leaf life span and litter quality (Cornelissen et al. 2003), thereby influencing nutrient cycling and availability.

A total of 104 species were sampled from the 16 sites. For each species the leaves were analysed for nitrogen (N) and carbon (C) 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). Specific leaf area (SLA), average leaf area (ALA), leaf tensile strength (TS), leaf dry matter content (LDMC) were measured according to the methods described in Cornelissen et al. (2003).

Community weighted means (CWM) were calculated for each leaf trait at each site. CWMs were calculated as $\sum P_i \times \text{Trait}_i$ where P_i is the relative abundance of the species “i” in the community, and Trait_i is the average trait value obtained for species “i” (Violle et al. 2007; Domínguez et al. 2012). The relative abundances of each species at each site were determined by walking four (100 m x 4 m) transects at each site and recording the number and identity of all encountered plants.

Data analyses

All statistical analyses in this study were performed using R (R Development Core Team 2011). Many of the leaf traits and measures of soil fertility were approximately log-normally distributed and were therefore \log_{10} transformed in order to attain approximate normality and homogeneity of residuals. Sites were assigned *a priori* into two broad categories (nutrient-poor and nutrient-rich) according to the soil parent material identity at each site. Thus sites with soils derived from granite and sandstones were assigned to the nutrient-poor category, while sites with soils derived from basalts, gabbros and mudstones were assigned to the nutrient-rich category.

Table 1. List of measured traits, abbreviations and units used.

Trait	Abbreviation	Unit
Leaf nitrogen concentration	LNC	mg.g ⁻¹
Leaf carbon to nitrogen ratio	Leaf C:N	NA
Leaf phosphorus concentration	LPC	mg.g ⁻¹
Leaf nitrogen to phosphorus ratio	Leaf N:P	NA
Specific leaf area	SLA	cm ² g ⁻¹
Average leaf area	ALA	cm ²
Leaf dry matter content	LDMC	mg g
Leaf tensile strength	TS	N mm ⁻¹

Principle components analyses (PCA) were used to explore the relationships between sites and measures of soil fertility and sites and climatic variables. PCAs were performed using firstly, the mean values of species leaf traits measured at each site and secondly, using the CWM values for each trait at each site. The function `PCAsignificance` available in the `BiodiversityR` package (Kindt & Coe 2005) in R was used to test for significant PCA axes using the broken-stick distribution. For all figures showing PCAs the plotted PCs are represented by the eigenvalues in black (shown as an inset figure). The first bar always represents PC1 and subsequent bars represent further PCs. The first PC shown in black is always plotted on the x-axis and the second PC shown in black is always on the y-axis. Thus for example in figure 3 PC1 is shown on the x-axis while PC2 is shown on the y-axis.

Co-inertia analysis (COIA) is a multivariate method for coupling or linking two ecological tables (Dray et al. 2003). COIA was used to analyse the response of plant traits to environmental conditions at both the species and community level using species mean trait values and CWMs. The co-inertia function in the `ade4` package (Dray & Dufour 2007) was used to run the co-inertia analyses. RV coefficients (coefficient of correlation between the two tables, which varies between 0 and 1, with higher values indicating a stronger correlation between the tables) were calculated and tested for significance for each COIA.

Distance matrices (euclidean distance) were calculated for the sites using the CWM trait values and the measures of soil fertility. These were then used to construct hierarchical cluster

diagrams for the sites based on traits and soils. The `mantel.rtest` in the `ade4` package was then used to test the strength of the correlation between the trait and soil matrices.

Bivariate analyses of trait-soil and trait-climate relationships and multivariate analysis of the combined effects of soils and climate on leaf traits were analysed using linear mixed models with maximum likelihood (ML) methods, available in the `nlme` (Pinheiro et al. 2012) and `lme4` (Bates & Sarkar 2007) packages in R. Soil variables, rainfall and their interactions were treated as fixed effects while site was treated as a random effect to account for the effects created by the non-independence of leaf trait measurements at each site. Random effects are expressed as variances and can be compared with the error variance; however there is no equivalent to r^2 , a known limitation of the ML method (Ordonez et al. 2009). Bivariate relationships were explored using the pooled (104) observations from 16 sites, as well as by using the CWMs for each site. The first two principle components (PCs) for both soils and climate were then plotted against the CWMs of the eight leaf traits to test for relationships between leaf traits with soil nutrient supply and climate.

To avoid problems of collinearity among the independents used in the bivariate and multivariate analyses, only variables with a Pearson coefficient lower than 0.5 were included in the models. All possible combinations of independents that fitted this constraint were then analysed. The dredge function in the `MuMIn` package (Barton 2012) was used for model selection based on the corrected Akaike information criterion (AICc). The ratio of the variance among sites explained by a model that included the fixed effects of soil and rainfall to a null model with no fixed effects was calculated as an estimate of the variance explained by soils and/or rainfall and their interactions. Model assumptions were checked by plotting: a) the standardised residuals against fitted values (test for non constant variance), b) the response against fitted values (linearity), and c) residuals against quantiles of standard normal distribution by site (errors normally distributed in all sites).

Results

Bivariate relationships of leaf traits with soil fertility and climate

The two *a priori* defined classes of soil fertility status (Figure 1, Chapter 2) were shown to be justified according to actual measures of soil fertility. The two classes of ‘nutrient poor’ or

'nutrient rich' are therefore used throughout the thesis. There were several significant correlations between the leaf traits of all species pooled together with measures of soil fertility (Figure 1). Leaf C:N was found to be negatively related to soil P and soil SB. LPC was positively correlated to soil N, P and SB. SLA and ALA were negatively correlated with soil P and soil C:N, while ALA was also negatively correlated to soil SB. Soil N and C:N were not good predictors of leaf traits. LNC, leaf N:P, LDMC, and TS showed no significant correlations with any of the measures of soil fertility. LPC showed a negative correlation with MAP, while SLA and ALA showed positive correlations with MAP (Figure 2).

Multivariate relationships of leaf traits with soil fertility and climate

The results from the linear mixed models (Table 2) showed that, for the most part, the leaf traits were poorly predicted by soils, rainfall and their interactions. For both LNC and leaf C:N all multivariate models were worse than models which only included the single predictor, soil P. Both of these models however were very poor at predicting LNC and leaf C:N, with 88% of the variance in LNC and 86% of the variance in leaf C:N accounted for by amongst site variability. For LPC and Leaf N:P none of the models had significant interaction terms. The best model for predicting LPC included soil N and MAP, both of which were significant. For leaf N:P the best model also included soil N and MAP, however these were only significant at $p < 0.1$ level. For LPC and leaf N:P, 47% and 61% of the variability was accounted for by amongst site variance respectively. For SLA and ALA none of the models had significant interaction terms. The best models for predicting SLA and ALA included soil C:N and MAP, both of which were non-significant for SLA at $p < 0.05$ level, for ALA soil C:N was significant. For SLA and ALA, 58% and 63% of the variability was accounted for by amongst site variance respectively. The best model for the prediction of variation in LDMC included soil N, soil C:N, soil P, the three possible two-way interactions and the three-way interaction. All interaction terms except soil N \times Soil C:N were significant with positive signs for the two way interactions and a negative sign for the three-way interaction indicating that as soil C:N or soil P increases, the slopes of LDMC on soil N or soil P become less negative. This model also showed that less than 1% of the variance in LDMC was accounted for by amongst site variance. For TS all multivariate models were worse than the single model using soil N only. This model still performed very badly at predicting TS with 93% of the variability in TS explained by amongst site variance.

Multivariate analyses

The first three axes of the PCA based on species means of the eight leaf traits (Figure 3) explained almost 67% of the variance. PC1 (Figure 3 A-B) accounted for 31% of the variance and described mostly LNC, leaf C:N and to a lesser extent SLA variation, with positive loadings for LNC and SLA and negative loadings for C:N. PC2 explained 20% of the variance and described mostly LPC and leaf N:P variation with positive loadings for leaf N:P and negative loadings for LPC. PC3 explained a further 15% of the variance and described LDMC, ALA and SLA variation with positive loadings for LDMC and negative loadings for ALA and SLA. Sites were only slightly segregated along PC1 with slightly higher LNC values at nutrient-rich sites and PC2 with slightly higher LPC values at nutrient-rich sites. PC3 showed a more pronounced segregation of nutrient-rich from nutrient-poor sites, with higher SLA and ALA values at nutrient-poor sites and higher LDMC values at nutrient-rich sites (Figure 3 C-D).

The first three axes of the PCA based on the community weighted means (CWM) of the eight leaf traits (Figure 4) improved the explained total variance from 67% for the species means to over 75% for the CWMs. PC1 (Figure 4 A-B) which accounted for 35% of the variance still described LNC, but now had higher loadings for LDMC and ALA and lower loadings for LPC than the PCA based on species means. PC2 which accounted for 26% of the variance mostly described leaf N:P and LPC variation, with positive loadings for N:P and negative loadings for LPC. PC3 (Figure 4 C-D) explained a further 14% of the variance. This axis described SLA and ALA variation, with negative loadings for both.

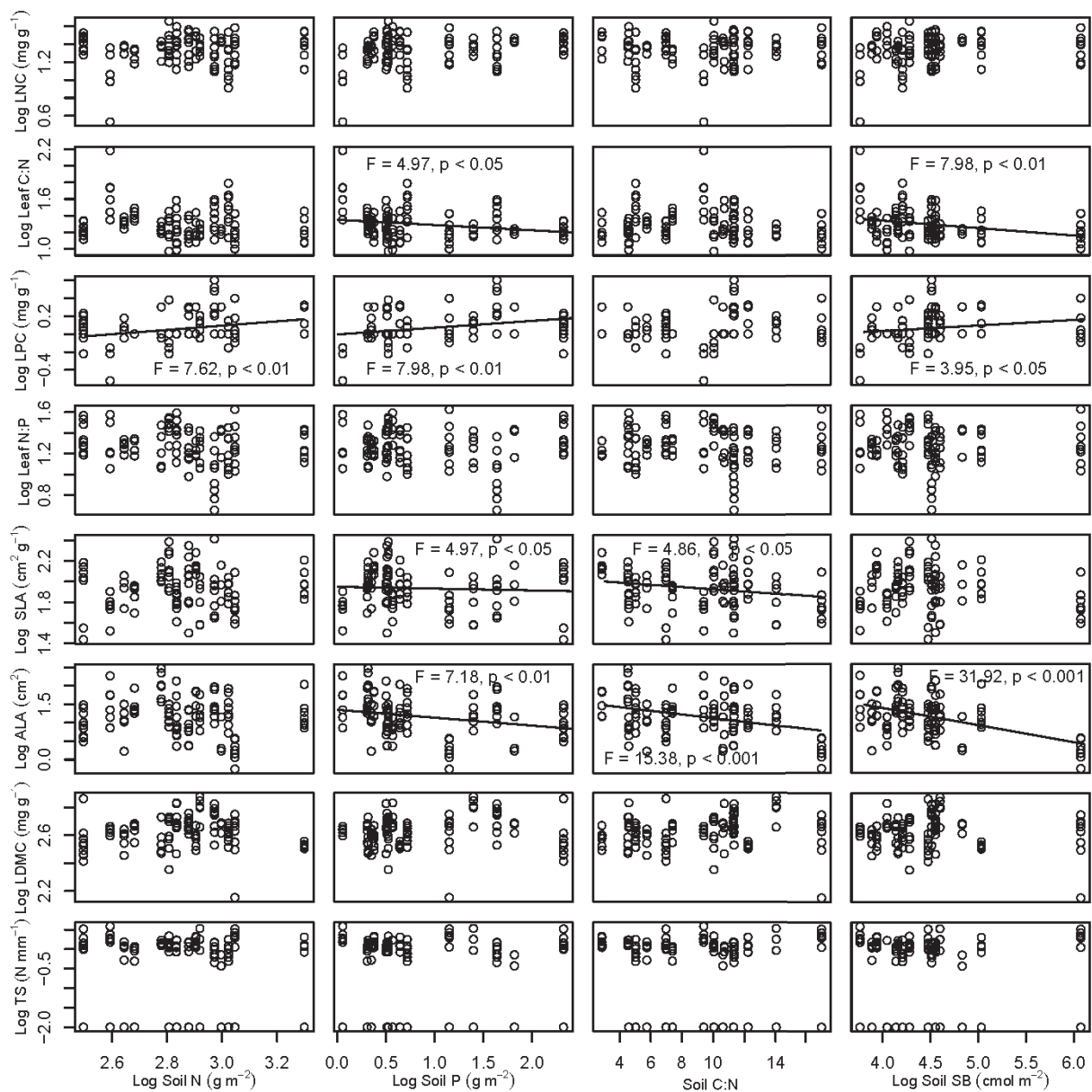


Figure 1. Relationships between the leaf traits and measures of soil fertility for all species. Lines were plotted for relationships with $p < 0.05$. All leaf traits and measures of soil fertility, except soil C:N were \log_{10} transformed, $n=104$, sites = 16.

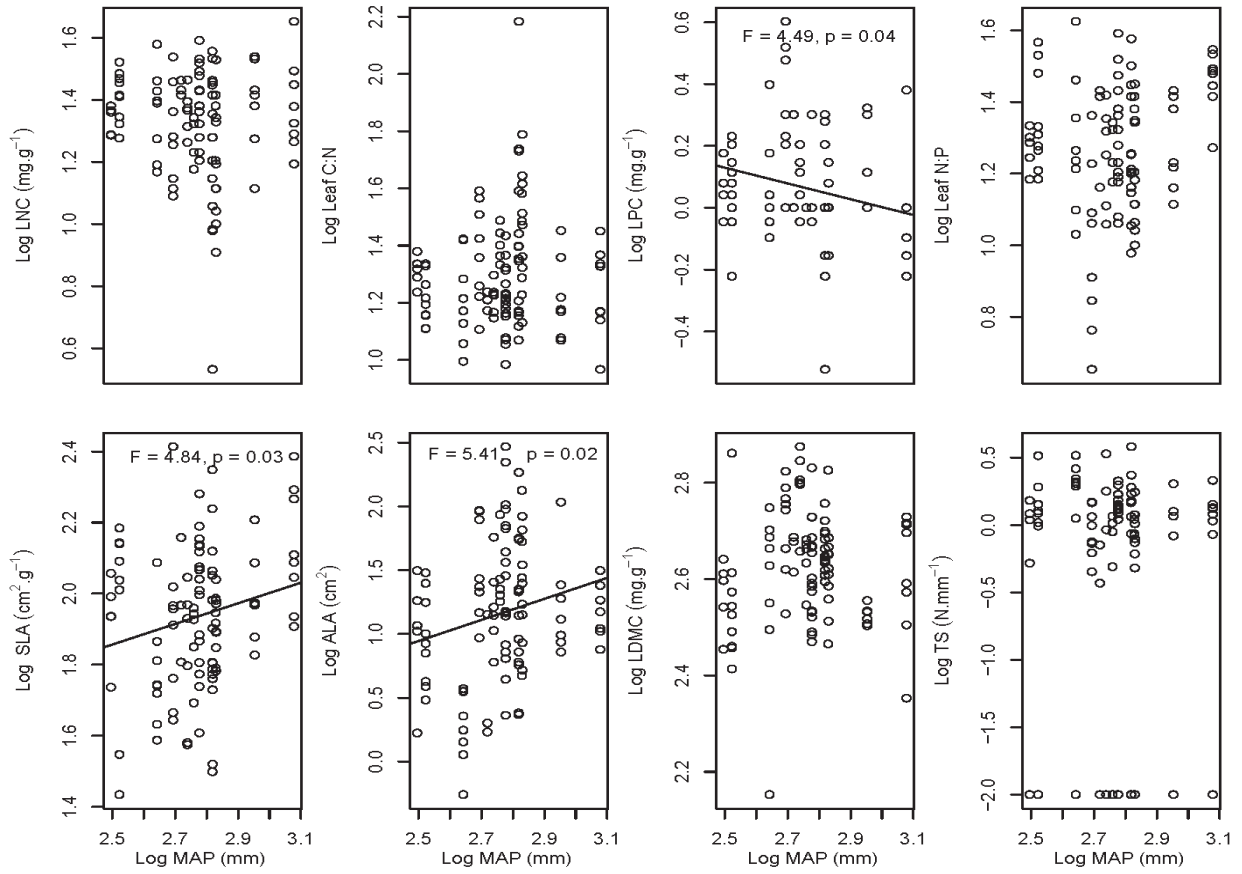


Figure 2. Relationships between the leaf traits and MAP for all species. Lines were plotted for relationships with $p < 0.05$. All leaf traits and MAP were \log_{10} transformed, $n=104$, sites = 16.

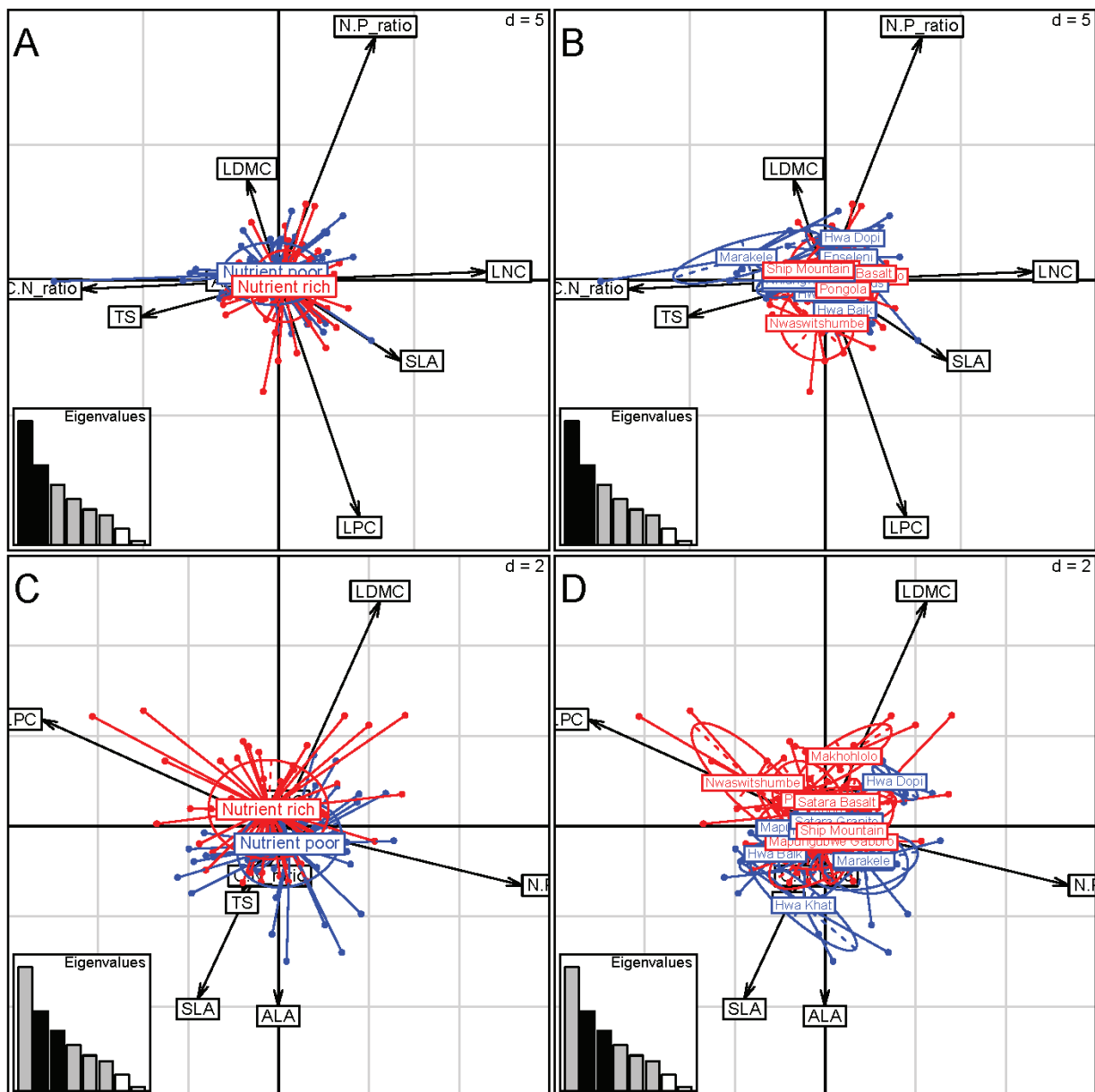


Figure 3. PCA based on species means of the eight leaf traits. PC1 (31% of variance) with positive loadings for leaf LNC and SLA and negative loadings for leaf C:N and TS. PC2 (20% of variance) with positive loadings for leaf N:P and negative loadings for LPC. PC3 (C&D y-axis; 15% of variance) with positive loadings for LDMC and negative loadings for SLA and ALA. A and C show the sites according to the predefined classes of nutrient-rich vs. nutrient-poor, B and D show the positions of each site on each PCA.

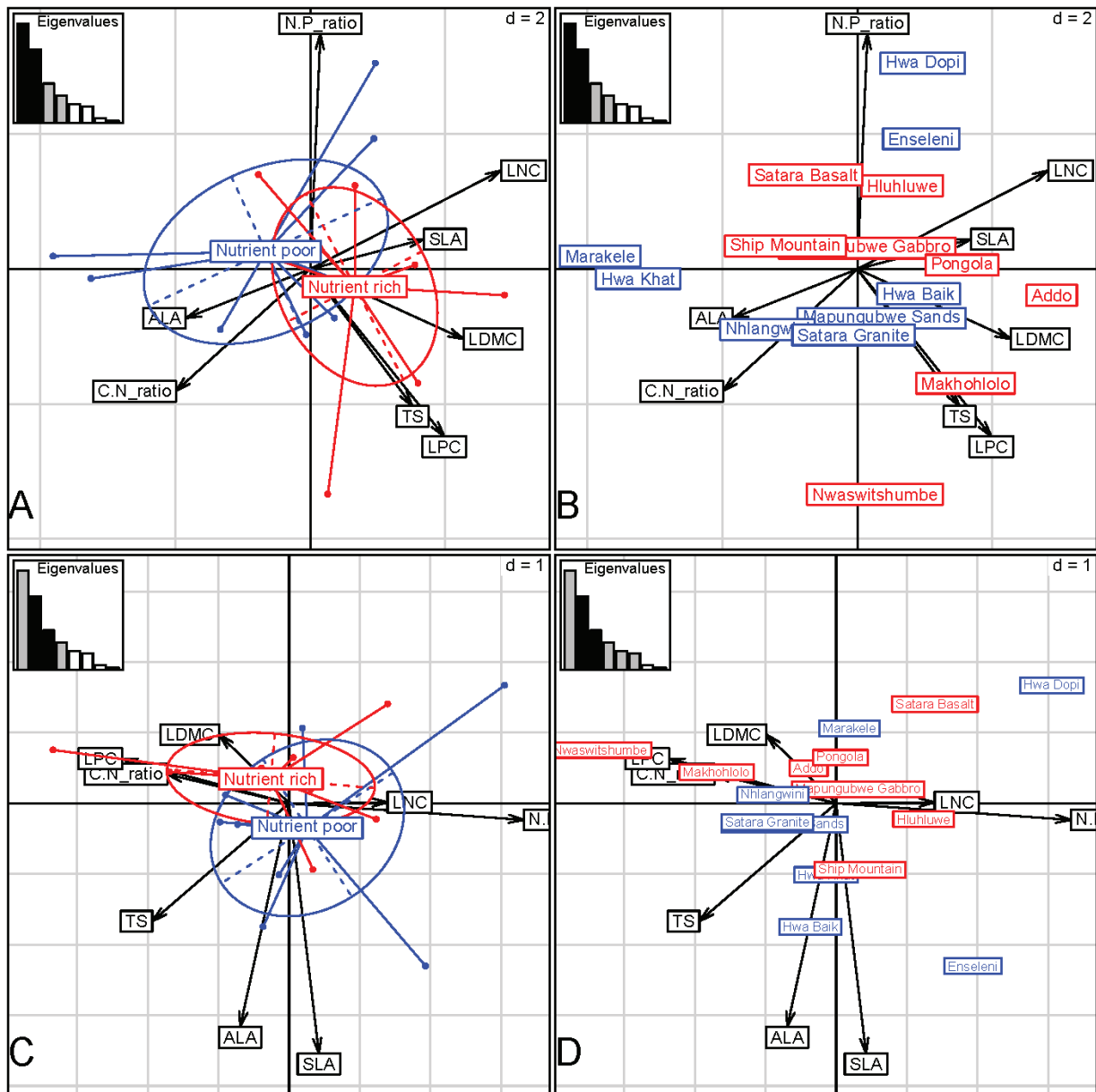


Figure 4. PCA based on CWMs of the eight leaf traits. PC1 (35% of variance) with positive loadings for leaf LNC and SLA and negative loadings for ALA. PC2 (26% of variance) with positive loadings for leaf N:P and negative loadings for LPC and TS . PC3 (C&D y-axis; 14% of variance) with negative loadings for SLA and ALA. A and C show the sites according to the predefined classes of nutrient-rich vs. nutrient-poor, B and D show the positions of each site on each PCA.

The PCA based on CWMs of the leaf traits resulted in a higher degree of segregation along PC1 whereby nutrient-rich sites were found to have higher LNC and LDMC values. Segregation along PC2 was less pronounced with slightly higher LPC values for some of the nutrient-rich sites. PC3 also showed some segregation of sites with higher SLA and ALA values for the nutrient-poor sites.

The co-inertia analysis (COIA) based on species mean trait values (Figure 5) showed poor correlation between soils and traits (Monte Carlo Test, RV value of 0.07; simulated p value of 0.012; 999 repetitions). Species within individual sites differed quite widely in their positions along both axes of the COIA and that there was no strong pattern between sites that indicated a relationship between soil fertility and trait values. A COIA based on CWMs (Figure 6) showed a slight but non-significant improvement in the correlation between the two datasets (Monte Carlo Test, RV value of 0.26; simulated p value of 0.22; 999 repetitions). This COIA showed some convergence in leaf traits, especially among the nutrient-rich sites (e.g. Satara Basalt, Makhohlolo, Nwaswitshumbe and Mapungubwe Gabbro). The overall pattern however was not strong, as sites with similar soil characteristics (e.g. Hwange Baikiaea, Hwange Dopi and Hwange Khatshana) tended to have very different positions according to their trait values.

The hierarchical cluster analysis based on soil nutrients (Figure 7A) showed two main clusters of sites. The composition of the two groups was mostly as expected according to the *a priori* division of sites into nutrient-rich and nutrient-poor classes. However, the cluster analysis showed that two of the nutrient-rich sites (Ship Mountain and Pongola) were in fact more similar to the nutrient-poor sites than the rest of the nutrient-rich sites. The hierarchical cluster analysis based on the eight leaf traits (Figure 7B) showed a somewhat different pattern of clustering. The clustering of sites according to the similarity of leaf traits showed nutrient-rich and nutrient-poor sites to be more interspersed among clusters. There was a reasonable degree of correlation between the two distance matrices (Monte Carlo test; $r_M = 0.27$; simulated p value = 0.07; 9999 simulations).

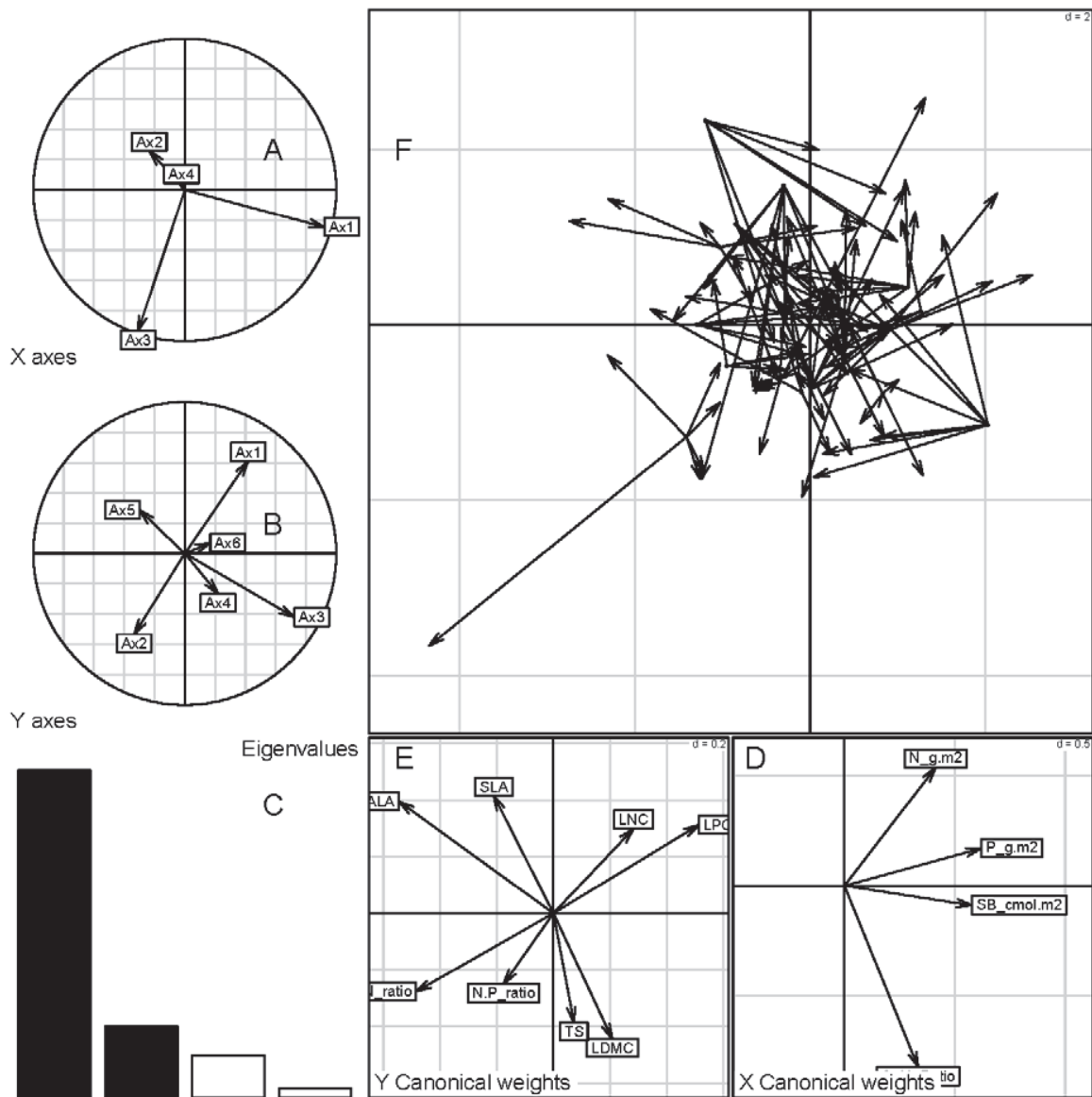


Figure 5. Co-inertia analysis linking the species means of the eight leaf traits with the measures of soil fertility. A) Correlation circle showing the projections of the first two axes of the PCA based on soils onto the first two axes of the co-inertia analysis. B) Correlation circle showing the projections of the axes of the PCA based on leaf traits onto the axes of the co-inertia analysis. These two circles represent a view of the rotation needed to associate the two datasets. C) Screeplot showing the eigenvalues of the co-inertia analysis. D-E) Scatter plots representing the co-efficients of the combinations of the variables for each table used to define the co-inertia axes. F) Scatter plot of the co-inertia analysis. The beginning of each arrow is a species position

described by the soil data set while the end of each arrow is the position of the species described by the eight leaf traits.

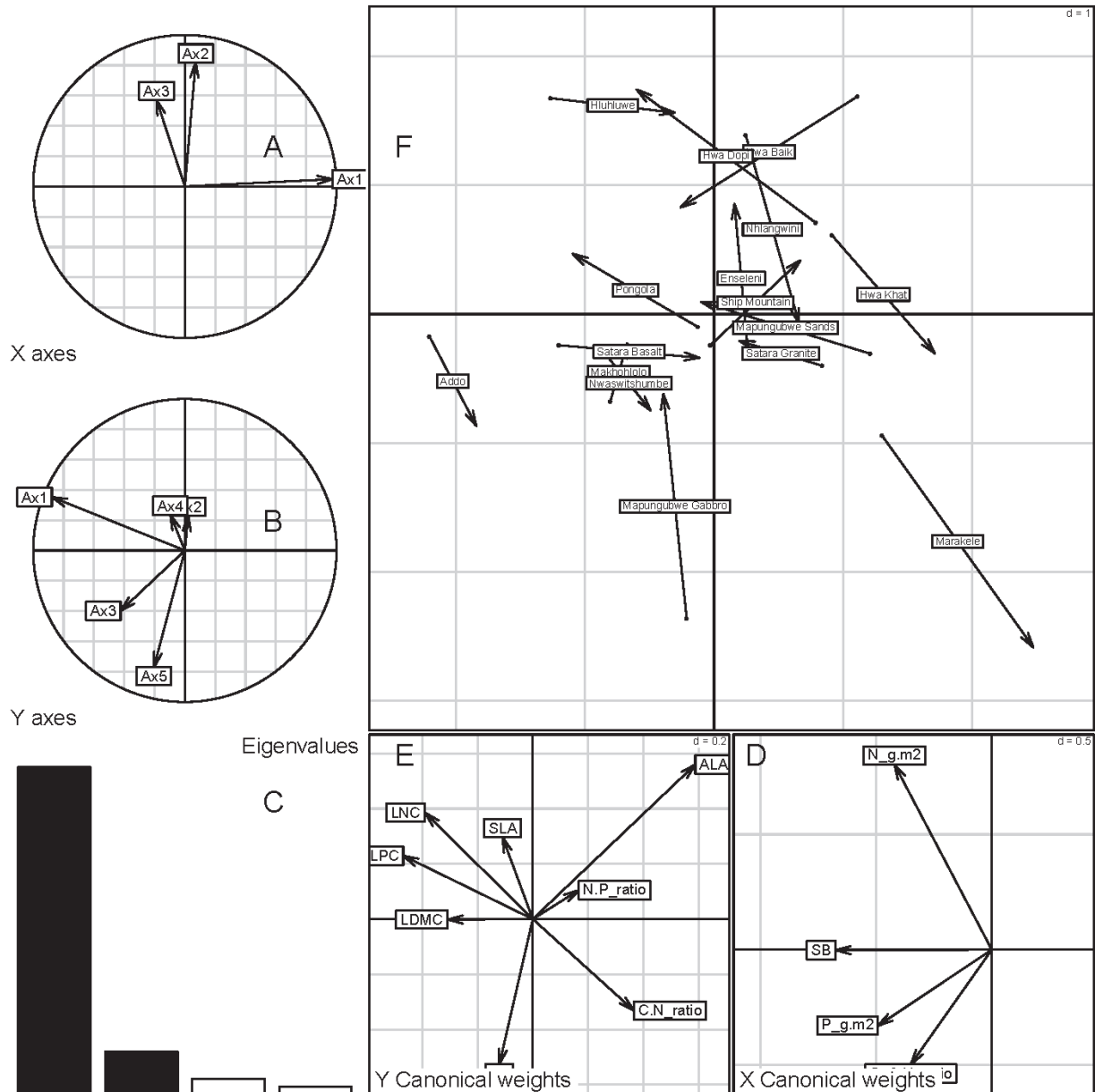


Figure 6. Co-inertia analysis linking the CWMs of the eight leaf traits with the measures of soil fertility. See figure 7 for descriptions of the individual graphs, with each arrow representing a site not a species.

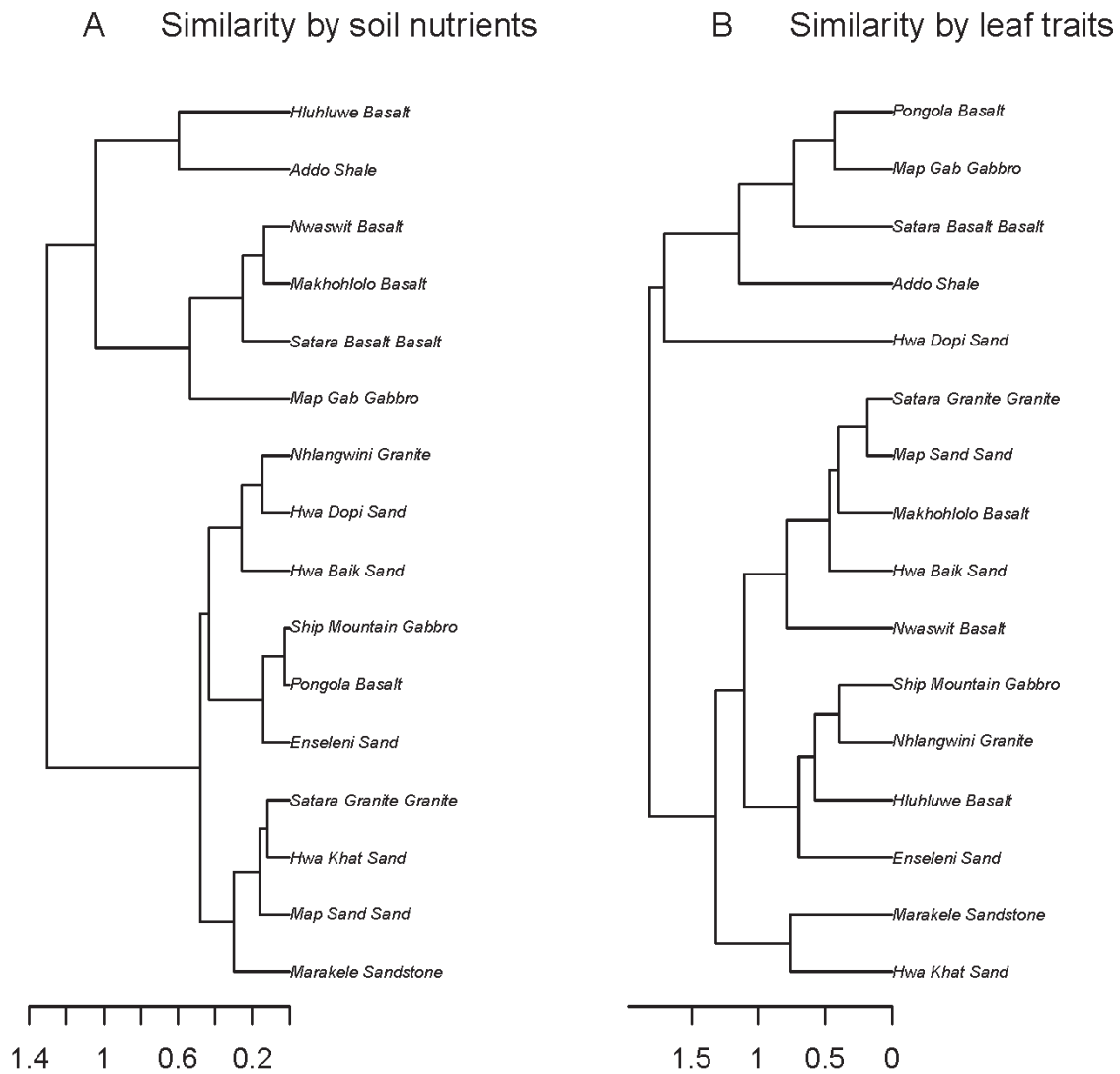


Figure 7. Groupings of sites based on the hierarchical cluster analyses of (A) Measures of soil fertility and (B) CWMs of the eight leaf traits. The underlying geology at each site is shown after the site name.

Table 2. Summary of the best mixed effects models used to predict the eight leaf traits for the fixed effects of soils and rainfall and their interactions with the sign of the relationship, (d), F-values (F) and probabilities (p). The ratio of the variance among sites explained by a model including fixed effects of climate and soils to a null model with no fixed effects is included as an estimate of the remaining variance explained by soil, rainfall and their interactions.

Variables in the model		LNC			Leaf C:N		
		d	F	p	d	F	p
Soils	Log soil P	+	1.54	0.23	-	2.12	0.17
Variance explained			(%)			(%)	
Among-sites variance			12			14	
Variables in the model		LPC			Leaf N:P		
		d	F	p	d	F	p
Soils	Log soil N	+	9.1	0.01	-	3.46	0.09
Climate	Log MAP	-	6.9	0.02	+	3.53	0.08
Variance explained			(%)			(%)	
Among-sites variance			53			39	
Variables in the model		SLA			ALA		
		d	F	p	d	F	p
Soils	Soil C:N	-	3.24	0.09	-	5.02	0.04
Climate	Log MAP	+	2.86	0.11	+	2.6	0.13
Variance explained			(%)			(%)	
Among-sites variance			42			37	
Variables in the model		LDMC			TS		
		d	F	p	d	F	p
Soils	Log soil N	-	0.43	0.54	-	0.62	0.44
	Soil C:N	-	0.32	0.58			
	Log soil P	-	6.35	0.04			
Interactions	Log soil N x Soil C:N	+	0.33	0.58			
	Log soil N x Log soil P	+	6.03	0.04			
	Log soil P x Soil C:N	+	5.89	0.04			
	Log soil N x Soil C:N x Log soil P	-	5.65	0.04			
Variance explained			(%)			(%)	
Among-sites variance			> 99			7	

Discussion

The clear separation of the sites into two broad classes evident in the PCA based on measures of soil fertility (Figure 1, Chapter 2) confirms that the two predefined classes of nutrient-poor and nutrient-rich were justified. An interesting observation was that the nutrient-poor sites were all fairly similar in terms of the four measures of soil fertility despite the variability in underlying geologies. The nutrient-rich sites on the other hand tended to be more variable in soil N, P, C:N and SB. No separation of nutrient-poor vs. nutrient-rich sites was evident in the PCA based on climatic variables (Figure 2, Chapter 2). Thus the selection of sites to cover gradients of soil fertility and climatic variability appear to have been highly successful, leaving us in a good position to tackle the questions we set out to address in this study.

Savanna species leaf traits related to soil fertility and rainfall

Although we found several significant interactions in this attempt at quantifying relationships between savanna leaf traits and soil fertility and rainfall for multiple sites within southern African savannas, these relationships appeared to be far weaker and less consistent than many broader scale studies that have compared sites across biomes and continents (e.g. Diaz et al. 2004; Wright et al. 2004; Ordonez et al. 2009). Evidence for a fundamental trade-off in plant functioning as defined by the acquisitive vs. conservative strategies described by Diaz et al. (2004), or the leaf economic spectrum of Wright et al. (2004), was very weak. Of the eight leaf traits, LNC, leaf N:P, TDMC, and TS showed no significant correlations with any of the measures of soil fertility. Although correlations were found between measures of soil fertility, rainfall and LPC, SLA and ALA which are known to be positively related to plant relative growth rates, leaf carbon assimilation rates and energy supply (e.g. Lambers & Poorter 1992; Lavorel & Garnier 2002; Niklas et al. 2005), these relationships tended to be weak with high intra-site variability typically evident. Furthermore the directions of the relationship between SLA and ALA with soil P were in the opposite direction to that which was expected. Leaf C:N was found to be negatively related to soil P and soil SB, which is in accordance with the expected trade-offs where, at low soil fertility, species would be limited by soil nutrients resulting in low quality foliage (higher C:N).

There was only a weak separation of the nutrient-rich from the nutrient-poor sites along both of the first two axes of the PCA based on the eight leaf traits of the 104 species. The sites were most clearly separated by differences in LDMC, ALA and SLA. However the patterns

evident for ALA, SLA and LDMC are all in the opposite directions to the expected relationships. ALA and SLA which are expected to be highly correlated were found to be higher at the nutrient-poor sites; the opposite was expected where nutrient-rich sites should have larger leaves together with higher SLA and faster relative growth rates (Cornellisen et al. 2003). LDMC was also expected to show the opposite trend to the observed one where species growing at higher soil fertility were expected to have lower rather than higher LDMC values. This unexpected pattern is most likely explained by the fact that nutrient rich sites are dominated by fine-leaved species. Levels of herbivory are also highest at this nutrient rich sites, thus plants cannot afford to grow large thin leaves as they would be easily removed by herbivores. Thus leaves on nutrient rich soils instead tend to be smaller and denser than might have been expected from other studies (e.g. Rusch et al. 2009). Leaf TS which is known to be tightly coupled with leaf lifespan and therefore nutrient cycling (Cornellisen et al. 2003) showed no clear patterns between sites. TS was expected to be lower at nutrient-rich sites as a result of higher quality leaf material, which in turn would improve the litter quality and nutrient availability.

Of the eight measured leaf traits, only LPC and leaf C:N exhibited significant relationships with soil fertility and rainfall in accordance with the expected patterns from studies which have related plant traits to environmental variables. LNC and TS showed no relationships between soil fertility or rainfall and no major differences were evident between nutrient-poor and nutrient-rich sites. Leaf N:P showed that soils often tended to be limited by P as species from many of the sites had N:P ratios of above 20 while very few species showed N:P of below 10, which would suggest N to be more limiting (Gusewell 2004). Further evidence for the lack of correlation between measures of soil fertility and the leaf traits measured at the species level was provided by the co-inertia analysis (Figures 7). The COIA clearly showed that at any given soil fertility status at a site (beginning points of each cluster of arrows) species differed widely in their mean trait values (end points of the arrows). Furthermore a poor degree of correlation was evident between sites, i.e. the end points of the arrows all end in different places due to the low degree of convergence in trait values in the COIA. Thus it is evident that the relationships between species mean trait values and measures of soil fertility and rainfall within the savanna biome are much weaker and in different directions to those found in studies that covered multiple biomes at larger scales.

Trait community weighted means related to soil fertility and rainfall

Castro-Diaz (2012) reported that studies comparing species and community trait trends along environmental gradients often revealed similar trends, but that these trends were often much more scattered and less significant at the species level (e.g. Ackerly et al. 2002; Cingolani et al. 2005; Vile et al. 2006; Garnier et al. 2004). We therefore analyzed the data from the same eight leaf traits, using the community weighted mean for each trait at each site, instead of species means, to determine if it improved the relationships between leaf traits, measures of soil fertility and rainfall.

On the whole, the strength of the correlations between the CWMs of the eight leaf traits and the measures of soil fertility and rainfall were not improved when compared to the correlations based on species means. The only significant relationships found using CWMs were between SLA and MAP (positive), ALA and P (negative), and TS and soil SB (positive).

Furthermore almost no significant correlations were found between trait CWMs and the first two axes of both the PCAs based on soil and climate, which in both cases accounted for high proportions of the variance in soil fertility and climate. The PCA based on the CWMs of the eight leaf traits did lead to an improvement in the amount of explained variance, the first three axes of the new PCA explained 75% compared to 67% in the species based PCA. Nutrient-rich sites appeared to be more separated from nutrient-poor sites along PC1 which separated sites mostly according to differences in LNC and LDMC, and PC3 which separated sites according to differences in SLA and ALA. LNC tended to be higher at nutrient-rich sites which would be expected. On average LDMC was still higher at nutrient-rich sites, while SLA and ALA also tended to be higher at nutrient-poor sites, contrary to expectations.

The COIA based on CWMs was still not statistically significant and did not improve the degree of correlation between the measures of soil fertility and leaf traits. As evident from the COIA (Figure 8), sites which were fairly similar in soil characteristics (beginning points of arrows) usually did not result in similar traits (end points of arrows). This is also evident from the hierarchical cluster analysis (Figure 9) which showed a somewhat higher degree of correlation between the distance matrices used to construct the cluster diagrams (mantel test, $r_m = 0.27$) than the COIA. The hierarchical cluster analysis based on soil nutrients (Figure 9A) more or less divided the sites as expected, however the hierarchical cluster analysis based on the CWMS of the eight leaf traits (Figure 9B) showed nutrient-rich and nutrient-poor sites to be more

interspersed among clusters. The use of CWMs therefore did not appear to significantly improve the strength of relationships between leaf traits, soils and rainfall when compared to species means.

Predicting savanna woody leaf traits from measures of soil fertility and rainfall

As already shown, bivariate relationships between leaf traits and soil nutrient status and rainfall were mostly found to be weak or nonexistent. The multivariate analyses failed to improve the predictability of leaf traits using the measures of soil fertility and rainfall for most of the leaf traits (Table 2).

After Ordonez et al. (2009) the relative filtering capacity of soils and climate was estimated by comparing the proportion of variance among sites explained by a model including the effects of soils and rainfall with a null model which did not include these effects. For LNC, leaf C:N and leaf TS all multivariate models were worse than single models using only soil P for LNC and leaf C:N, and only soil N for leaf TS. Moreover these three models were not statistically significant. LPC was significantly related to indicators of both soil N and rainfall, an improvement on the two models that only included soil N or MAP alone. For leaf N:P, although not statistically significant, the multivariate model that included both soil N and MAP improved the amount of explained variance compared to models that only included soil N or MAP. It was a somewhat surprising result that leaf N:P was not more tightly coupled to either soil N or soil P, as it is thought to be a reliable indicator of soil nutrient limitations (e.g. see Gusewell 2004). For SLA and ALA the two predictors; soil C:N and MAP combined improved the amount of variability explained, compared to models only including soil C:N or MAP, however for both models interactions between the two were not included. This in contrast to other studies (e.g. Ordonez et al. 2009) which found that the response of SLA to soil N availability (C:N) was modified by MAP. For LDMC which was the only leaf trait found to have significant interactions, these interactions were only between soil nutrients and did not include MAP.

The explanatory power of this model was extremely high with significant interactions between soil N and soil P, between soil P with soil C:N, as well as the three way interaction, thus the response of LDMC to each of the three soil nutrients was modified by the other soil nutrients included in the model.

High variability in savanna woody leaf traits

High levels of variability were evident for most of the soil/rainfall-trait relationships. Within-site variability tended to be the highest source of variability for all of the leaf traits (clearly evident in Figures 1&2). The linear mixed models showed that high levels of the total variability in each trait were not captured by soil and rainfall. This was especially true for LNC, leaf C:N, and leaf TS for which within-site variability accounted for over 85% of the variance of each trait. Within-site variability for LPC, leaf N:P, SLA and ALA also accounted for much of the variability in these four traits (between 47 and 63%). Leaf TS was the only trait with high levels of the variance accounted for by differences among sites.

High levels of variability in relationships between leaf traits, soils and rainfall have been found in a number of previous studies (e.g. Poorter & De Jong 1999; Fonseca et al. 2000; Wright et al. 2004; Wright et al. 2005; Ordonez et al. 2009). Causes for such variability have been attributed to disturbance (Grime 2006), microsite variability and alternative evolutionary solutions to similar environmental challenges and frequency dependent processes (Westoby & Wright 2006). Another possible explanation for the lack of strong relationships in savannas could be that particular groups of species could be influencing patterns. For example fine-leaved acacias are typically found on nutrient rich soils, these species tend to have lower than expected SLA and leaf size possibly because large thin leaves would be easily removed by herbivores. The converse is true for broad-leaved savanna species which are typically found on nutrient poor soils or in high rainfall areas. These species tend to have larger leaves, which are often also sclerophyllous making them unattractive to mammal herbivores. These findings could partially explain some of the unexpected patterns evident in leaf traits. Further analyses looking at traits within specific groups (e.g Mimosoideae and Combretaceae) across the gradients might result in better relationships between leaf traits and resources than those found using the full set of species.

The exceptionally high levels of disturbance in African savannas, which still contain a diverse array of mammalian herbivores and are frequently burnt, are likely to affect plant leaf traits as plants might be forced to evolve counter-adaptations to these disturbances (e.g. they may evolve smaller leaves and invest more resources in structural defences in order to reduce herbivory). This could partially account for the high levels of within-site variability and the poor predictive power of soils and rainfall at our sites for predicting savanna leaf traits.

Conclusion

Previous studies have found strong relationships between leaf traits, soils and climate and interactions between soils and climate. These studies have typically been undertaken at the broad scale usually including sites taken from multiple biomes across the globe. At such scales the patterns tend to be fairly robust. There is a recognised need for studies to investigate these relationships at finer scales. This study therefore set out to investigate these general patterns at the regional scale within the savanna biome in southern Africa. The results showed that many of the patterns which hold true at the broad scale no longer applied when tested within the savanna biome. Although some significant relationships were found between the measured leaf traits, the measures of soil fertility and rainfall, these tended to be weak and often in the opposite direction to that expected from the literature. The use of trait community weighted means which has often been found to improve the strength of the relationships between traits and soils or rainfall did not significantly improve the strength of the relationships found in this study. The low levels of variance explained by among-site variability, and high levels of variance typically explained by within-site variability in each trait is thought to reflect the high levels of disturbance inherent in southern African savannas. Measures of disturbance from the same sites should thus be collected and included in analyses to determine if they improve our understanding of the relationships between savanna traits and the environment.



Measurements of leaf traits, Shingwedzi Research Camp, Kruger National Park.

Chapter 5 Chemical and structural defences along gradients of soil fertility, rainfall and mammalian browsing pressure in southern African savannas

Abstract

In this study the effects of soil nutrients, rainfall and both meso-browser and mega-browser impacts on twenty savanna woody plant traits relating to palatability and both chemical and structural defences were explored. On nutrient poor soils, woody plants were found to grow larger and to have lower quality browse material with high fibre content and higher total phenolic concentrations. On more fertile soils, plants tended to have higher quality browse material but invested more in structural defences, particularly in higher levels of branching and spinescence. Structural defences tended to be more strongly correlated with soil characteristics than chemical defences, while browser impact was found to be strongly correlated with structural defences but not with chemical defences. Actual browser utilisation tended to be more predictable for meso-browsers which tended to target plants of higher nutritional value with lower tensile strength and fibre (less tough). Mega-browser impact on the other hand was best predicted by soil nitrogen and rainfall but completely unpredictable based on both chemical and structural defences.

Introduction

Large mammalian herbivores are known to generate strong direct and indirect feedbacks on plant community composition, structure (Augustine & McNaughton 1998) and associated plant functional traits. Herbivory has been shown to act as an environmental filter, thereby restricting plant distribution (Harley 2003; Da Silva & Batalha 2011). Environmental filters are thought to select for species with similar traits which would allow them to survive in the face of specific abiotic and biotic pressures (Fukami et al. 2005). African savannas are generally exposed to high levels of disturbance deriving from both frequent burning in mesic savannas (Bond et al. 2003) and high levels of herbivory (van Langeveld et al. 2003). Unlike many parts of the world, African savannas often still contain their full complement of indigenous mammalian herbivores with high species diversity of grazers, mixed feeders and browsers, ranging in size from a few kilograms to over 4000 kilograms (e.g. see Coe, Cumming, & Phillipson 1976; East 1984; Fritz & Duncan 1994). In areas with high levels of mammalian herbivory, savanna plant species with low investments in resistance or defence traits are likely to be excluded by herbivory. I therefore

expect mammalian herbivory in many African savannas to act as a strong environmental filter selecting for species with high investments in defence related traits. The contributing role of mammalian herbivores in determining woody plant community composition and total woody cover in African savannas has been demonstrated by Augustine & McNaughton (1998) and Sankaran et al. (2008).

The palatability, growth rate, and decomposition rate of plant species are often linked (Hobbs 1996). It therefore follows that there should be strong links between plant traits relating to palatability with many of the traits relating to resource capture. These links could partially account for the findings of chapter 4 which did not find strong relationships between leaf traits, soils and climate along gradients of soil fertility and rainfall at the same study sites. These results contrast with those of a number of broad scale studies (e.g. Grime et al. 1997; Díaz et al. 2004; Wright et al. 2004) which found traits that could be characterised as resource-acquisitive in resource-rich environments to resource-retentive in more resource limited environments.

It has been suggested that studies of trait evolution in grasslands and savannas are hampered by our lack of understanding of the key traits needed to survive in grass-fuelled fire regimes (Bond & Keeley 2005). By the same token, this could be extended to include our lack of understanding of the traits needed to survive high levels of mammalian herbivory and how these traits relate to nutrient and water availability within African savannas. While there is a plethora of studies that have examined the interactions and responses of plant traits to invertebrate herbivory (e.g. see review by Ohgushi 2005), fewer studies have focused on how plant traits respond to mammalian herbivory. Of the studies on mammalian herbivory, many have tended to be species specific, often with a focus on differences in defence-related traits between browsed *vs.* unbrowsed plants with the use of exclosures and/or induced herbivory through clipping (e.g. Pellew 1983; Stuart-Hill & Tainton 1989; Rooke et al. 2004a; Hraber et al. 2009) or on differences in defence during different life history stages (e.g. Rooke et al. 2004b).

There has been a considerable amount of work done on the role of plant defences and how these deter both insect and mammalian herbivory (e.g. Palo & Robins 1991), however many of these studies have focused on chemical defences (e.g. see reviews by Lindroth 1988; Bryant et al. 1991; Herms & Mattson 1992). Less attention has been paid to the types, development and deterrent role that structural defences play in plant-mammal interactions (Grubb 1992; Schoonhoven et al. 2005). In a recent review, Hanley et al. (2007) have summarized the main

types of structural defences such as spinescence, pubescence, sclerophylly and raphides, and described how these contribute to plant defences against herbivory.

The growth-defence trade-off is well established (see Coley et al. 1985, Fine et al. 2004, 2006), where resource availability in the environment is thought to be a major determinant of both the amount and type of defence a plant invests in. In resource-poor environments, where plants generally tend to have slow growth rates, it would therefore benefit plants to invest in more anti-herbivore defences in order to preserve the maximum amount of plant tissue. At the other extreme in resource-rich environments, plants tend to be fast growing and therefore can afford to lose some of their tissues to herbivores. As highlighted by Hanley et al. (2007), both structural and chemical defences rely on the allocation of nitrogen (N) and carbohydrate resources by the plant. Classical plant defence theory therefore predicts an allocation 'dilemma' with regard to which type of defence to invest in (Rhoades 1979; Coley et al. 1985; Herms and Mattson 1992). Hanley et al. (2007) suggest a trade-off where species that invest in chemical defences would be expected to possess more limited structural defences, and vice versa. However a recent comprehensive study by Moles et al. (2013) found weak support for a trade-off in defence syndromes. Instead they suggest that plants rather display a range of combinations of defence traits.

The debate over the relative costs of chemical vs. structural defences continues (e.g. see Skogsmyr & Fagerstrom 1992; Choong, 1996; Hanley & Lamont 2002; Craine et al. 2003; Gowda 2003; Hanley et al. 2007). According to classical plant defence theory, structural defences should be most common in resource-poor environments (Hanley et al. 2007). While some support has been found for this (e.g. Grubb 1992; Koricheva 2002; Wright et al. 2004), studies have also found the opposite trend. Grubb (1992), for example, draws attention to the fact that spinescence is also often common in nutrient-rich environments. It is clear however that availability of mineral nutrients, light and water play a pivotal role in dictating the allocation of plant resources to growth, reproduction and the different types of plant defence (Owen-Smith 2002; Gowda 2003; Hanley et al. 2007). Hanley et al. (2007) emphasize the fact that despite a comprehensive literature documenting experimental tests of plant defence theory; very few attempts have been made to determine how resource availability influences the expression of structural defences. For chemical defences, Scogings et al. (2011) highlight the fact that very few studies have

compared more than two levels of browsing intensity, which is typically inferred from browser abundance or distance from water.

Herbivores can play a major role in determining which species of plants dominate in a community, as well as in which habitats a species will be successful (Fine et al. 2006). However, resource availability is also important in determining the relative investment by plants in defensive traits which can explain how unrelated species may converge on a similar suite of traits to minimize herbivore damage (Agrawal 2007; Tanentzap et al. 2011). This study sets out to explore how plant traits relating to palatability, chemical and structural defences vary across sites with varying nutrient availability, rainfall and browser utilisation. It further seeks to test for convergence in suites of defence traits in savanna woody plant communities within and among sites. Rather than inferring herbivory from site location or general browser density, I followed Fine et al. (2006) in using indices of actual herbivory to quantify relative effects of plant traits on browser choices. This approach takes into account the entire arsenal of plant defences as experienced by the herbivores themselves.

Measured traits were divided into two broad categories, of which nine traits related to nutritional and chemical defence, and eleven traits related to architectural and structural defence, as listed in Table 1. Nutritional quality can be thought of as a defence, as low nutritional quality makes herbivory less likely (Mattson 1980; Da Silva & Batalha 2011). N is a limiting nutrient for herbivores, furthermore low levels of N increases feeding time and, consequently, the exposure to natural enemies and energy expenditure on consuming and processing food (Lavoie & Oberhauser 2004; Owen-Smith 2005; Craine et al. 2009; Da Silva & Batalha 2011). Plants with high N content and high specific leaf area (SLA) are more susceptible to herbivory (Weiher et al. 1999; Coley et al. 2006), while plants with higher fibre or lignin and therefore higher cell wall toughness, have more mass per leaf area and consequently low SLA (Weiher et al. 1999; Cornelissen et al. 2003). Low SLA usually indicates high investment in nutritional or chemical defences, whereas high specific leaf areas indicate greater palatability (Weiher et al. 1999; Cornelissen et al. 2003). The SLA of a species is usually strongly correlated to its potential growth rate. Lower SLA values tend to correspond with relatively high investments in defences, both structural and chemical, and long leaf life span. Species growing in resource-rich environments usually have higher SLA values than nutrient-poor environments (Cornelissen et al. 2003).

Leaf tensile strength is a good indicator of the relative carbon (C) investment in structural protection of the photosynthetic tissue. Physically stronger leaves are better protected from abiotic (winds, storms) and biotic mechanical damage (insect and mammal herbivory). Higher physical strength usually results in longer leaf lifespan; however this is usually combined with other physical and chemical defences such as leaf spines and tannins. Leaves with high tensile strength also tend to form low quality litter with slow decomposition rates (Cornelissen et al. 2003). Tougher leaves should reduce the consumption of leaves by herbivores and herbivore (typically insects) growth rates (Perez-Harguindeguy et al. 2003; Agrawal & Fishbein 2006). Condensed tannins and polyphenols are organic N-free chemical defences that bind with protein, reducing N availability to herbivores, thereby decreasing preference by mammal herbivores (Haslam 1988; Bergvall and Leimar 2005; Owen-Smith 2005; Craine 2009). Plant height is often associated with competitive vigour, whole plant fecundity (Westoby 2002) and with the time intervals between disturbances (fire, herbivory and drought). There are a number of important trade-offs between plant height and tolerance or avoidance of environmental stress. Alternatively, tall plants may avoid fire damage to the green parts and meristems in the canopy (Cornelissen et al. 2003). Height has also been found to correlate allometrically with other size traits in broad comparisons (e.g. aboveground biomass, rooting depth and leaf size) (Cornelissen et al. 2003). Plant height also has important implications for the different suites of mammal browsers that feed on it. The canopy diameter and height of first ramification are also important from an herbivores perspective as they determine the total available area on which feeding may be possible. The degree of multi-stemmedness or resprouting capacity of a species is highly related to the prevalent disturbance regime (Bond & Midgley 2001). Thus in areas of higher disturbance we expect a higher degree of multistemmedness than in areas of low disturbance. A dense stem provides structural strength and durability for long lived plants (Cornelissen et al. 2003). Stem specific density broadly trades off against relative growth rate, and stem defences against pathogens, herbivores and mechanical damage (Cornelissen et al. 2003).

The efficacy of spines is influenced by their arrangement on adjacent branches. Densely ramified shoots produce cage-like defences whereas sparsely branched shoots can offer easier animal access. As an index of the efficacy of the structural defences, we developed a 'bite size index' (BSI) where an observer attempts to remove as much foliage from a shoot as possible

using only the mouth to remove the tissue (see Figure 1). The index is simply the dry weight of total leaf material recovered from ten human bites and is expected to show a negative relationship with degree of ramification and physical defences. Plants with high levels of physical defences, (spines, thorns and prickles or highly branched) will therefore be harder to browse, resulting in lower BSI values. The degree of branching in a woody plant affects the ease with which a browsing herbivore can reach its leaves (Staver et al. 2012). Spinescence is a relatively common trait in African savannas and is found to occur in a number of dominant savanna families and genera. A spine is usually a pointed modified leaf, leaf part or stipule while a thorn is a hard, pointy modified twig or branch, while a prickle is modified epidermis (Cornelissen et al. 2003). The different types, sizes and densities of spines, thorns and prickles are thought to act against different herbivores. Thorns, spines and prickles are thought to play an important anti-herbivory role for woody species found in African savannas which are home to a wide range of herbivore browsers.

The main questions addressed in this study are: 1) Do we find different defence syndromes along gradients of soil fertility and rainfall within southern African savannas? 2) How predictable are the chemical and physical defence related traits based on measures of soil fertility and rainfall? 3) How do defence syndromes relate to actual herbivore utilisation at the study sites? 4) Does utilisation vary depending on the browser, focussing on differences in the impacts of meso-browsers and mega-browsers. In other words, how effectual are plant chemical and structural defences against small to medium sized browsers (meso-browsers) and large browsers (mega-browsers)?

Methods

Descriptions of the study sites, species selection and soil sampling methods have been explained in detail in the previous chapter.

Trait measurements.

A total of 20 traits related to plant palatability and defence were measured for 82 woody species from 15 sites. Each trait was measured on five plants for each of the dominant species (the species that made up > 80% of the standing biomass) at each site. Leaf C and N concentrations were determined using a Leco TruSpec CN Analyser (LECO Corporation, St. Joseph, MI). The

C:N ratio was then calculated as an indicator of plant nutritional quality. Specific leaf area (SLA) is the one sided area of a leaf divided by its dry mass. Leaf dry matter content (LDMC) is the dry mass of a leaf (mg) divided by its water saturated fresh mass (g), expressed as mg.g^{-1} . Leaf tensile strength is the force needed to tear a leaf (or part of a leaf) divided by its width expressed in N.mm^{-1} . Total condensed tannins (CT) were calculated according to the methods described by Hattas & Julkunen-Tiitto (2012) while total polyphenols (TP) were calculated according to the methods described by Hattas et al. (2005). Cell wall constituents, i.e. neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined in an Ankom fibre analyzer, whereas acid detergent lignin (ADL) was determined in accordance with the acid detergent lignin in beakers method. Ash was determined by incinerating the filter bag containing plant residue in a muffle furnace at $525\text{ }^{\circ}\text{C}$ for three hours. All cell wall constituents were determined consecutively as outlined in the Ankom technology procedures (Ankom technology, http://www.ankom.com/09_procedures/procedures.shtml).



Figure 1. A demonstration of how the bite size index (BSI) was measured in order to simulate browsing by meso-herbivores.

Table 1. Measured traits relating to palatability and defence, abbreviations used, and units.

Trait	Abbreviation	Unit
<i>Palatability traits/chemical defences</i>		
Leaf N	N	%
Leaf carbon to N ratio	Leaf C:N	NA
Specific leaf area	SLA	cm ² g ⁻¹
Leaf tensile strength	TS	N mm ⁻¹
Total condensed tannins	CT	%
Total polyphenols	TP	%
Neutral detergent fibre	NDF	%
Acid detergent fibre	ADF	%
Acid detergent lignin	ADL	%
<i>Architectural/ structural defences</i>		
Maximum canopy height	MCH	m
Canopy diameter	CD	m
Height of first ramification	HFR	m
Average number of stems	NS	NA
Stem specific density	SSD	g cm ⁻³
Bite size index	BSI	g 10 bites ⁻¹
Branching index 1	BI1	no. branches cm terminal branch ⁻¹
Branching index 2	BI2	no. branches 0.125 m ⁻³
Average spine length	ASL	mm
Average spine thickness	AST	mm
Average spine density	ASD	spines cm

Maximum canopy height was taken as the highest point of the canopy. Canopy diameter was taken as the average of two perpendicular measurements of the maximum canopy width. The height of first ramification was taken as the lowest height above ground level at which a primary branch branches off the main stem. The average number of stems is simply the average number of stems per plant measured on five individuals at each site. Stem specific density (SSD) is a measure of the oven dried mass of a stem section divided by its fresh volume and is expressed as g.cm⁻³. The BSI of a species is the total dry weight of the leaves removed from two human bites taken from five individuals (total of ten bites). An attempt was made to remove the maximum amount of leaf material with each bite. The BSI for all species was measured by the same person in order to control for potential differences between individual recorders. Two different indices were calculated in order to assess the degree of branching for each species. The first branching index is simply the number of branches found on a terminal branch divided by its length. The

second browsing index is the average total number of terminal branchlets measured in 0.125 m³ (50 x 50 x 50 cm cube). This index is the average from four measurements taken between zero and two meters in height from four different sides of the canopy of each plant. This was repeated on ten individuals of each species at each site. Thorns, spines and prickles were collectively called spines in this study. Average spine length and thickness are given as the average length or thickness in mm measured on a total of 50 spines (10 spines on 5 branches) for each species. Average spine density is the total number of spines on a terminal branchlet divided by its length.

Measures of browser utilisation

Although procedures for estimating the grazing intensity of herbaceous plants are relatively well established (McNaughton, Milchunas & Frank 1996), estimating the browsing intensity of woody plants can be far more problematic. Because trees and shrubs often have complex growth forms and only a proportion of the plant is available to browsers, researchers often estimate the utilization rate by counting browsed vs. unbrowsed twigs (Maccracken & Viereck 1990; Bergstrom & Guillet 2002; Edenius, Ericsson & Naslund 2002) or quantifying the proportion of mass removed from individual, linear shoots of the current year (Ferguson & Marsden 1977; Jensen & Urness 1981; Mahgoub, Pieper & Ortiz 1988).

The alternative has been to use animal stocking rates to infer browser utilisation. Because animal census data were not available for many of the study sites, measurements were undertaken to estimate actual browser utilisation at each of the study sites. To achieve this, two different approaches were adopted. All measurements were undertaken towards the end of the growing season (March-May), which allowed the accumulated effect of browsing to be determined. The first approach was to record actual utilisation on the same dominant species at each site upon which trait measurements were performed. For each of these species ten individuals were selected. Each individual was selected to ensure that at least part of its canopy was accessible to a wide range of herbivores (between 0 and 2 m above ground level). For each plant, the height, canopy diameter, numbers of stems and height of first ramification were first recorded. An open wire cube measuring 0.5 m x 0.5 m x 0.5 m was then inserted into the canopy at a randomly chosen height between 0 and 2 m on four different sides of the canopy. Within each cube the total number of twigs or branch endings, the number of browsed twigs and an estimate of the total proportion of removed foliage were measured and recorded. The total number of branches and

damaged number of branches within the branch diameter size classes < 10 mm, 10 – 20 mm, 20 – 50 mm and > 50 mm were then estimated for each individual. The proportion of bark damage was also estimated when evident.

The second approach adopted was to walk four 100 x 4 m transects at each site. The identity, height, canopy diameter, number of stems and life history stage were recorded for each woody plant encountered along the transects. The types of browser utilisation (absence/presence of leaf, twig or branch damage) and fire damage (stem and/or canopy) were also recorded. These measurements allowed for the relative abundance, size class distribution and the level of mammal utilisation to be calculated for each species at each site. Two browsing indices were then calculated for each species according to the type and degree of herbivore utilisation. A meso-browser index was calculated to include utilization by small to meso browsers (Impala, Bushbuck, Nyala, Kudu). The meso-browser index was calculated according to the following formula:

$$\text{Meso-BI} = ((L_d + T_d + \text{Br}10_d) / 3) * \text{RA},$$

where L_d is the proportion of leaves removed, T_d is the proportion of twig damage, $\text{Br}10_d$ is the proportion of damaged branches < 10 mm diameter and RA is the relative abundance of a species at a site. The mega-browser index which mostly included utilisation by elephant was calculated according to the following formula:

$$\text{Mega-BI} = ((\text{Br}20_d + \text{Br}50_d + \text{Br}>50_d) / 3) * \text{RA},$$

where $\text{Br}20_d$ is the proportion of damaged branches between 10 – 20 mm in diameter, $\text{Br}50_d$ is the proportion of damaged branches between 30 – 50 mm in diameter and $\text{Br}>50_d$ is the proportion of damaged branches greater than 50 mm in diameter and as before RA is the relative abundance of a species at a site. Thus each index falls between zero and one, with a value close to zero indicating low utilization and higher values indicating heavy utilisation.

Statistical analyses

All statistical analyses in this study were performed using R (R Development Core Team 2011). For the analyses, the fifteen sites were divided into two broad classes according to their soil fertility status. Principle components analyses (PCA) were used to explore the relationships between species, soil fertility and site with palatability, physical and architectural defences (Table 1) and browser utilisation. The function `PCAsignificance` available in the BiodiversityR package

(Kindt & Coe 2005) in R was used to test for significant PCA axes using the broken-stick distribution. ANOVAs were used to test for differences in the plant traits between the two soil fertility classes when the requirements for homogeneity of variance were met, failing which Kruskal-Wallis tests were used.

Distance matrices (euclidean distance) were calculated for the sites using species mean values for the nine traits relating to palatability and eleven traits relating to physical and architectural defences, the measures of meso-browser and mega-browser impact, as well as for the measures of soil fertility. These were then used to construct hierarchical cluster diagrams for the sites based on the soils, defence traits and browser utilisation at each site. The `mantel.rtest` in the `ade4` package was then used to test the strength of the correlations between each of the distance matrices.

Bivariate and multivariate analyses of all palatability and physical defence related traits with the measures of soil fertility and rainfall were analysed using linear mixed models with maximum likelihood (ML) methods, available in the `nlme` (Pinheiro et al. 2012) package in R. For these analyses, soil variables and rainfall and their interactions were treated as fixed effects while site was treated as a random effect to account for the effects created by the non-independence of soil fertility measurements at each site.

Bivariate analyses of meso-browser and mega-browser impacts with the measures of soil fertility and rainfall, palatability and structural defences as well as multivariate analysis of the combined effects of each group were also performed. Soil variables and rainfall, palatability related traits, and physical and architectural defences and their interactions were treated as fixed effects while site was treated as a random effect to account for the effects created by the non-independence of browser utilisation measurements at each site. Random effects are expressed as variances and can be compared with the error variance; however there is no equivalent to r^2 , a known limitation of the ML method (Ordóñez et al. 2009). Bivariate relationships were explored using the pooled (82) observations from 15 sites. The dredge function in the `MuMIn` package (Barton 2012) was used for model selection based on the corrected Akaike information criterion (AICc). The ratio of the variance among sites explained by a model that included the fixed effects to a null model with no fixed effects was calculated as an estimate of the variance explained by each model. Model assumptions were checked by plotting: a) the standardised residuals against fitted values (test for nonconstant variance), b) the response against fitted values (linearity) and c)

residuals against quantiles of standard normal distribution by site (errors normally distributed in all sites).

Results

The principal components analysis (PCA) of the nine traits related to leaf palatability (Figure 2) showed little separation of nutrient-rich from nutrient-poor sites along PC1 (27% of variance; insignificant compared to broken-stick distribution), which was driven primarily by ADL, NDF, leaf N (positive loadings) and leaf C:N and TP (negative loadings). A slight degree of separation was evident along PC2 (25% of variance, significant) which was most influenced by leaf N, leaf C:N and ADF. The sites were more strongly separated along PC3 (12% of variance, insignificant) which was most strongly driven by differences in TP. The PCA of the eleven traits relating to physical and architectural defences (Figure 3) showed a clear separation of nutrient-rich from nutrient-poor sites along PC1 (33% of variance, significant) which was most strongly influenced by plant height, canopy diameter, height of first ramification and bite size index, all with negative loadings and spine length, spine diameter and spine density all with positive loadings. Sites were more or less evenly distributed along PC2 (23% of variance, significant), and slightly separated along PC3 (13% of variance, insignificant) where nutrient-rich sites had a higher average number of stems and higher branching indices.

The results of the ANOVA and Kruskal-Wallis analyses confirmed these patterns with leaf N higher at the nutrient-rich sites while leaf C:N, TP, NDF and ADF were all significantly higher at the nutrient-poor sites (Table 2). For the traits relating to physical and architectural defences, canopy height and diameter, the average number of stems and the bite size index were all higher at nutrient-poor sites, while the two branching indices and spine length, diameter and density were all significantly higher at the nutrient-rich sites.

The linear mixed models used to explore the relationships between the palatability related traits and soil fertility (Table 3) showed a number of significant patterns. SLA was best predicted by the single predictor soil C:N. The best model for predicting total phenolic content included soil P only and accounted for 76% of the variability. The best model for predicting total condensed tannins was highly significant and included both soil N and soil P. NDF was best predicted by the single predictor soil C:N accounting for most of the among site variance.

The linear mixed models used to explore relationships between physical and architectural defence related traits and measures of soil fertility and rainfall (Table 4) also showed a number of significant relationships. Soil N and MAP together accounted for a high degree of the among site variability in plant height and the height of first ramification to a lesser degree, while canopy diameter was best explained by a model that included both soil C:N and MAP. Both the average number of stems and the bite size index were best predicted by soil N and MAP accounting for 47 and 40% of the variance respectively. Spine length and diameter were best predicted by soil N and soil P accounting for between 70 and 80% of the variance. The first branching index (no. of branches per cm of terminal branch) was the only model with significant interactions where the three-way interaction between soil N, soil C:N and MAP was statistically significant and accounted for nearly all of the among site variance in this branching index. The soil N x C:N, N x MAP and C:N x MAP interactions were positive while the three way N x C:N x MAP interaction was negative showing that as N or C:N increased the slope of soil C:N or MAP became less negative, while for the three-way interaction as C:N and MAP increased the slope of the branching index on soil N became flatter.

The results of the hierarchical cluster analyses (Figure 4; Table 5) showed no evidence of correlations between soil fertility as well as chemical defence and browser impact. There were also no significant correlations between chemical defences and both structural defences and browser impact. There were however statistically significant correlations between soil fertility and structural defences and between structural defences and browser impact.

The PCA based on the measures of browser impact showed a clear separation of nutrient-rich from nutrient-poor sites along both PC1 (46% of variation, significant) which was driven primarily by measures of browser impact relating to meso-browser utilisation (leaf, twig and small branch damage) and PC2 (21% of variance, significant) which was mainly driven by measures of browser impact relating to mega-browser utilisation (branches of > 20 mm diameter; Figure 5A&B). Small to meso-browser impact was found to be significantly higher at nutrient-rich sites compared to nutrient-poor sites (Figure 5C), while no significant differences in large or mega-browser impacts were evident between nutrient-poor and nutrient-rich sites (Figure 5D).

Table 2. ANOVA or Kruskal-Wallis analyses testing for differences in the traits relating to leaf palatability on the left and structural defences on the right. F-values are shown, $p < 0.1$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, n = 82 for all analyses. The column titled 'higher' shows which soil nutrient status had the higher mean or median (rich = sites on basalt or gabbro, poor = sites on granite or sand).

Leaf palatability	ANOVA			Structural defences	ANOVA			Kruskal-Wallis		
	F	p	higher		F	p	higher	chi-squared	p	higher
Leaf N	2.89	.	rich	Canopy height	4.2	*	poor			
Leaf C:N	6.9	**	poor	Canopy diameter	3.0		poor			
SLA	0.69	NS		HFR	1.17	NS				
TS	0.36	NS		Ave. number stems	3.3	.	poor			
TP	4.48	*	poor	BSI	14.		poor			
CT	0.02	NS		SD	1	NS				
NDF	12.82	***	poor	Branching index 1	7.17	**	rich			
ADF	9.3	**	poor	Branching index 2	7.3	**	rich			
ADL	0.89	NS		Spine length	2		rich	11.31	**	rich
				Spine diameter			rich	6.65	**	rich
				Spine density			rich	8.73	**	rich

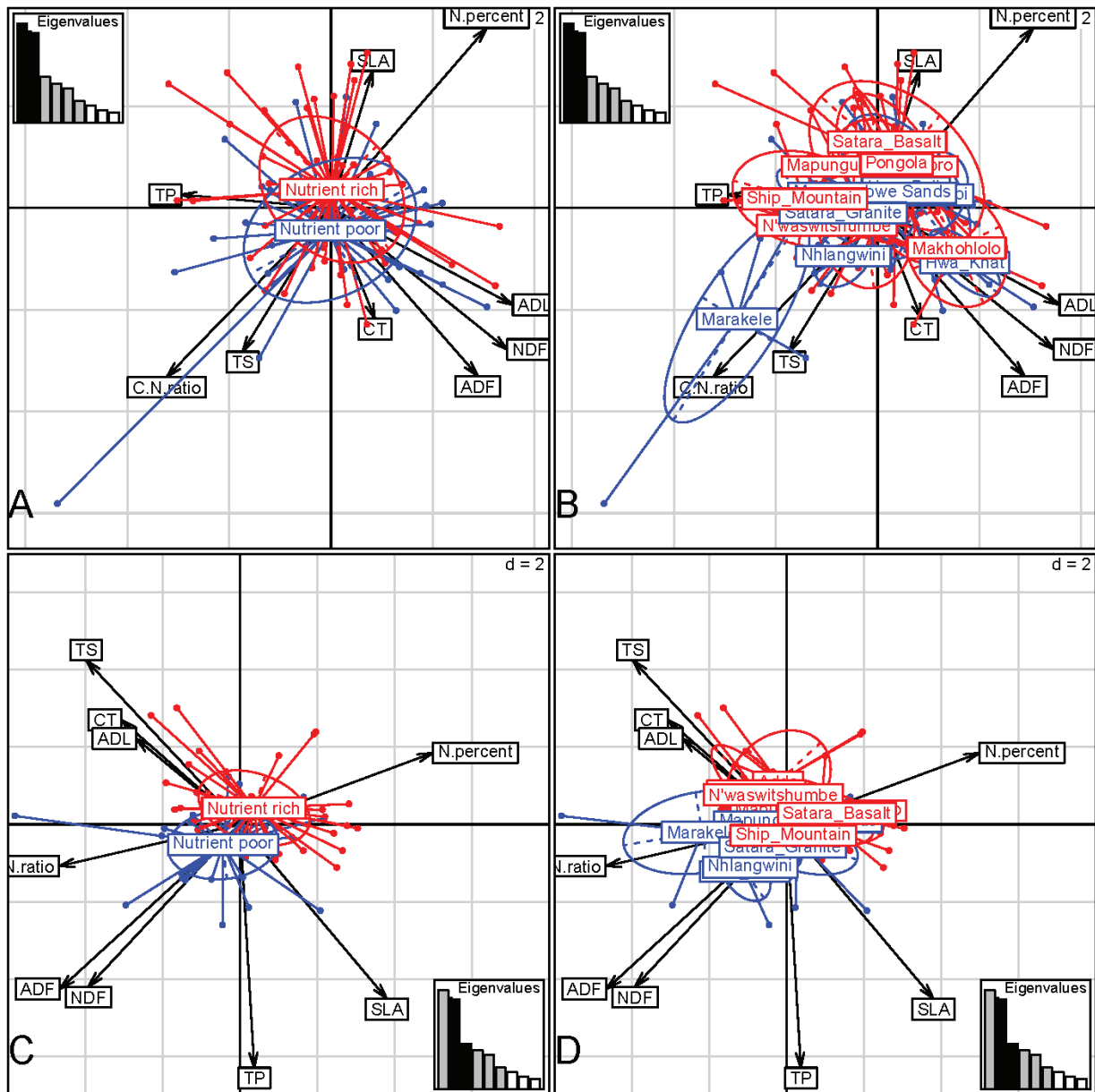


Figure 2. PCA based on species means of nine plant traits related to palatability. PC1 (A&B x-axis, 27% of variance) with positive loadings for ADL, NDF, leaf N and ADF and negative loadings for leaf C:N and TP. PC2 (25% of variance) with positive loadings for leaf N and SLA and negative loading for leaf C:N. PC3 (C&D y-axis; 12% of variance) with negative loadings for TP. A and C show the sites according to the predefined classes of nutrient-rich vs. nutrient-poor, B and D show the positions of each site along the principal components.

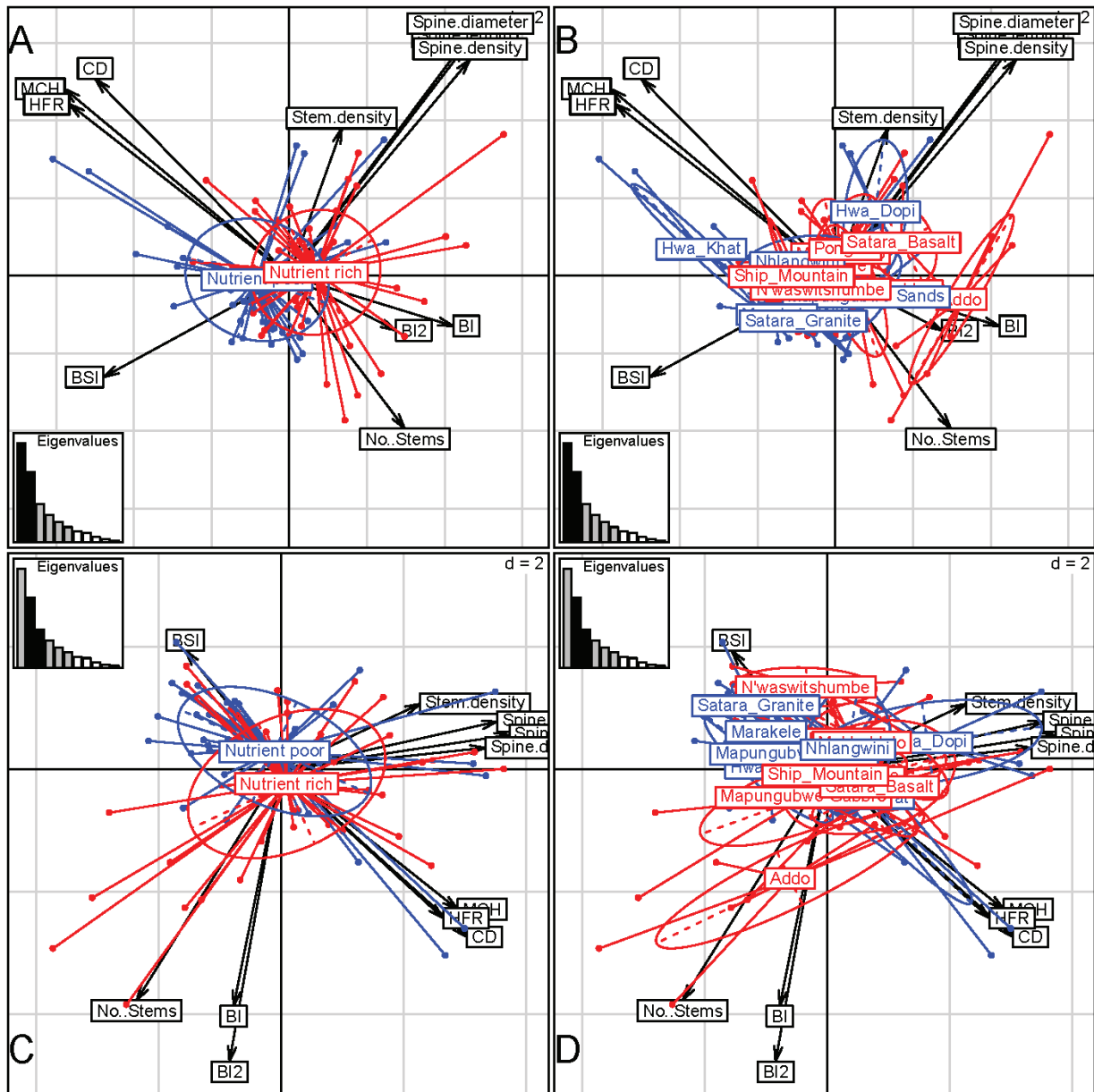


Figure 3. PCA based on species means of the eleven traits related to physical and architectural defences. PC1 (33% of variance) with positive loadings for spine length, diameter and density and negative loadings for plant height, canopy diameter and height of first ramification. PC2 (23% of variance) with positive loadings for spine length, diameter and density. PC3 (13% of variance) with negative loadings for both of the branching indices and average number of stems. A and C show the sites according to the predefined classes of nutrient-rich vs. nutrient-poor, B and D show the positions of each site along the principal components.

Table 3. Summary of the best mixed effects models used to predict the traits relating to leaf palatability for the fixed effects of soils and rainfall. The ratio of the variance among sites explained by a model including the fixed effects to a null model with no fixed effects is included as an estimate of the remaining variance explained by soil and rainfall.

Variables in the model	Leaf N			Leaf C:N			Leaf TS		
	d	F	p	d	F	p	d	F	p
Log Soil N	+	1.07	0.32	-	4.49	0.06	-	1.23	0.29
Log MAP				+	3.61	0.08			
Variance explained		(%)			(%)			(%)	
Among-sites variance		10			32			12	
<hr/>									
Variables in the model	SLA			TP			CT		
	d	F	p	d	F	p	d	F	p
Log Soil N							+	8.18	0.01
Log Soil C:N	-	5.06	0.04						
Log Soil P				-	10.3	0.007	-	9.12	0.01
Variance explained		(%)			(%)			(%)	
Among-sites variance		38			76			>99	
<hr/>									
Variables in the model	NDF			ADF			ADL		
	d	F	p	d	F	p	d	F	p
Log Soil N							+	2.59	0.13
Log Soil C:N	-	13.47	0.003	-	3.9	0.07			
Variance explained		(%)			(%)			(%)	
Among-sites variance		>99			41			43	

Table 4. Summary of the best mixed effects models to predict the traits relating to physical and architectural defence for the fixed effects of soils and rainfall.

Variables in the model	Canopy height			Canopy diameter			HFR			Average no. stems		
	d	F	p	d	F	p	d	F	p	d	F	p
Log Soil N	-	11.97	0.005	-	-	-	-	2.52	0.14	+	2.55	0.13
Log Soil C:N				-	4.88	0.05						
Log MAP	+	26.01	0.0003	-	4.45	0.06	+	5.76	0.03	-	7.56	0.02
Variance explained		(%)			(%)			(%)			(%)	
Among-sites variance		98			53			50			47	

Variables in the model	BSI			Stem density			Spine diameter			Spine density		
	d	F	p	d	F	p	d	F	p	d	F	p
Log Soil N	-	5.62	0.04	-	6.58	0.02	+	6.58	0.02			
Log MAP	+	6.15	0.03	+	1.58	0.23						
Log Soil P				-			-	7.02	0.02	+	2.54	0.13
Variance explained		(%)			(%)			(%)			(%)	
Among-sites variance		40			29			74			22	

Variables in the model	Spine length			Branching index 1			Branching index 2		
	d	F	p	d	F	p	d	F	p
Log Soil N	+	2.92	0.11	-	11.49	0.01			
Log Soil C:N				-	11.45	0.01			
Log Soil P	+	4.75	0.05						
Log MAP				-	10.36	0.01	-	5.59	0.03
N*C:N				+	12.27	0.01			
N*MAP				+	10.94	0.01			
C:N*MAP				+	10.89	0.01			
N*C:N*MAP				-	11.69	0.01			
Variance explained		(%)			(%)			(%)	
Among-sites variance		80			97			37	

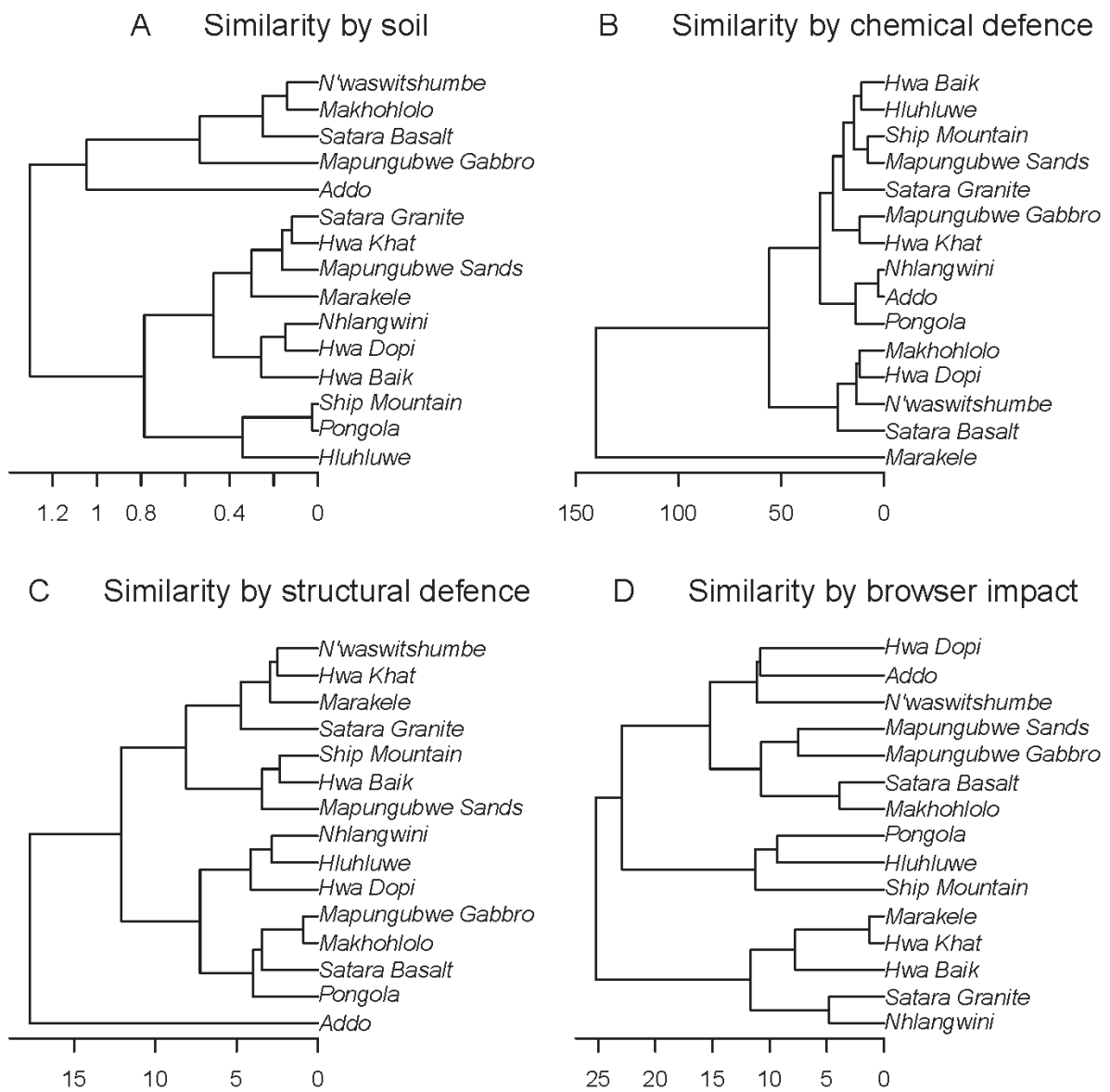


Figure 4. Cluster diagrams based on hierarchical cluster analyses of soil characteristics (A), chemical defence (B), structural defence (C) and browser impact (D).

Table 5. Mantel correlations between the distance matrices used to construct the cluster diagrams shown in Figure 4. The lower diagonal shows the Monte-Carlo test values and the upper diagonal shows simulated p-values based on 9999 permutations.

	Soils	Chemical defence	Structural defence	Browser impact	
Soils			<i>0.22</i>	<i>0.05</i>	<i>0.42</i>
Chemical defence	0.08			<i>0.44</i>	<i>0.32</i>
Structural defence	0.35	-0.03			<i>0.01</i>
Browser impact	0.02	0.07	0.3		

Significant correlations were found between meso-browser impact and leaf N (positive) and leaf C:N (negative) and leaf TS (negative). No other significant correlations were found between both meso-browser and mega-browser impact and the other traits relating to palatability and physical and architectural defences (Figures 6 & 7). Many of these relationships appeared to be triangular or non-linear. Low values on the x-axis often showed high browser impact (and low impact) while high values showed low impact. This could suggest that plants are either edible or not.

The results from the linear mixed models (Table 6) showed that meso-browser impacts were reasonably well predicted (44% of variance explained) by the measures of soil fertility. For the meso-browser index all multivariate models fitted worse than the model which only included the single predictor soil C:N. The mega-browser index on the other hand was very well predicted by a model which included soil N and rainfall but not the interaction. The model predicting meso-browser impact based on the traits relating to palatability included the significant effect of leaf TS ($p < 0.01$), and the effects of CT and ADF ($p < 0.1$ for both). For the models predicting the mega-browser index based on the traits relating to palatability all multivariate models fitted worse than the model which only included the single predictor ADL which performed very poorly ($< 1\%$) at predicting mega-browser impact. The model predicting the meso-browser index based on the plant traits relating to physical and architectural defences included the highly significant effect of BSI ($p = 0.001$) and the height of the first ramification ($p = 0.01$). The mega-browser index was again poorly predicted ($p = 0.24$) by physical and architectural defences with the best model including only the single predictor of average spine length.

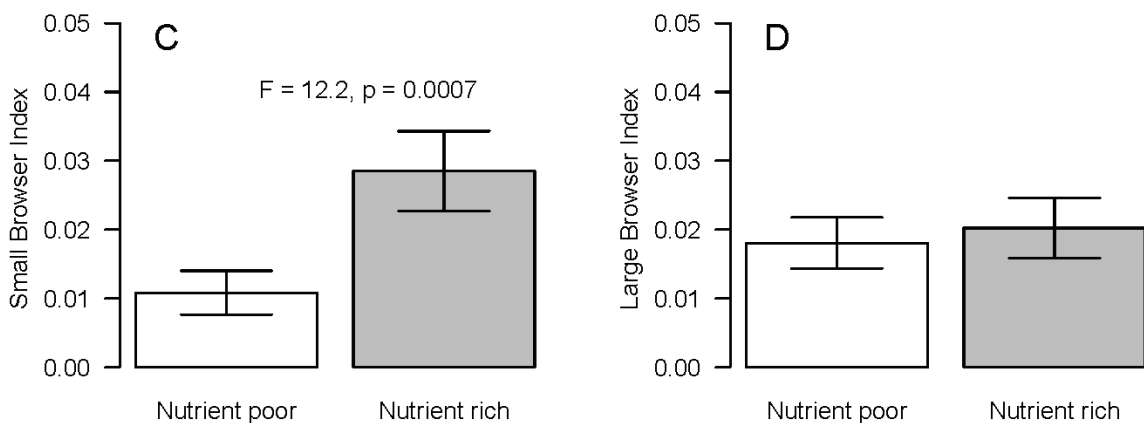
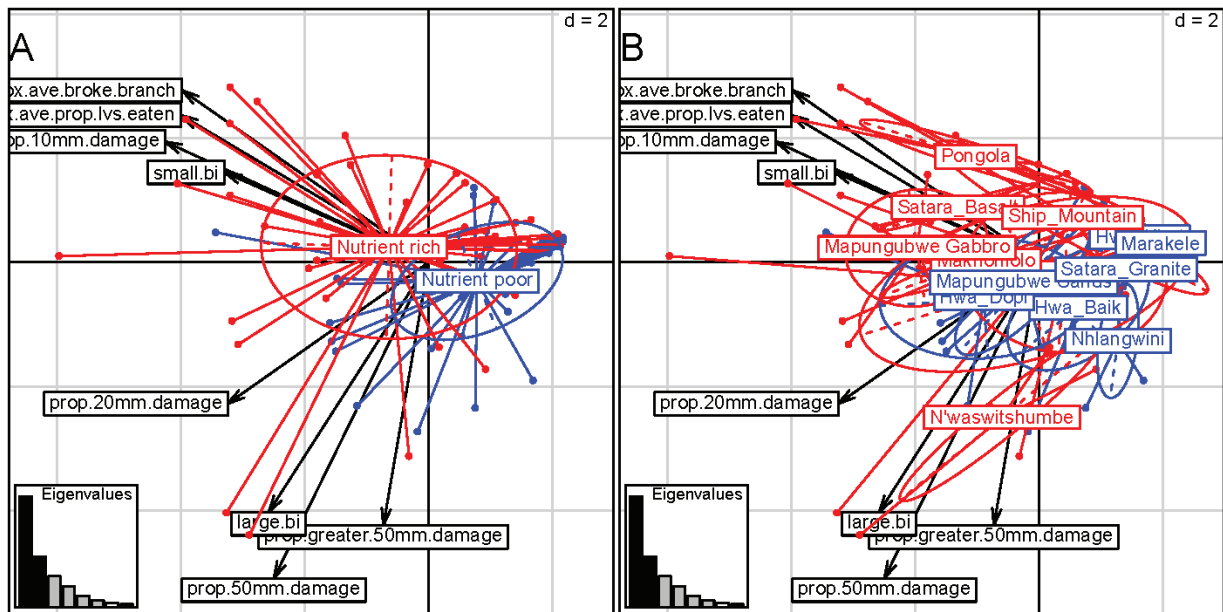


Figure 5. PCA based on small to meso-browser and large or mega-browser impacts on the woody species at the study sites, grouped according to soil fertility status (A) and site (B). PC1 (46% of variance) had positive loadings for leaf, twig and 10 mm diameter branch damage, while PC2 (21% of variance) had negative loadings for 10-20 mm, 30-50 mm and > 50 mm diameter branch damage. Mean \pm se browsing indices for small to meso-browsers (C) and large or mega-browsers (D) at nutrient-poor and nutrient-rich sites.

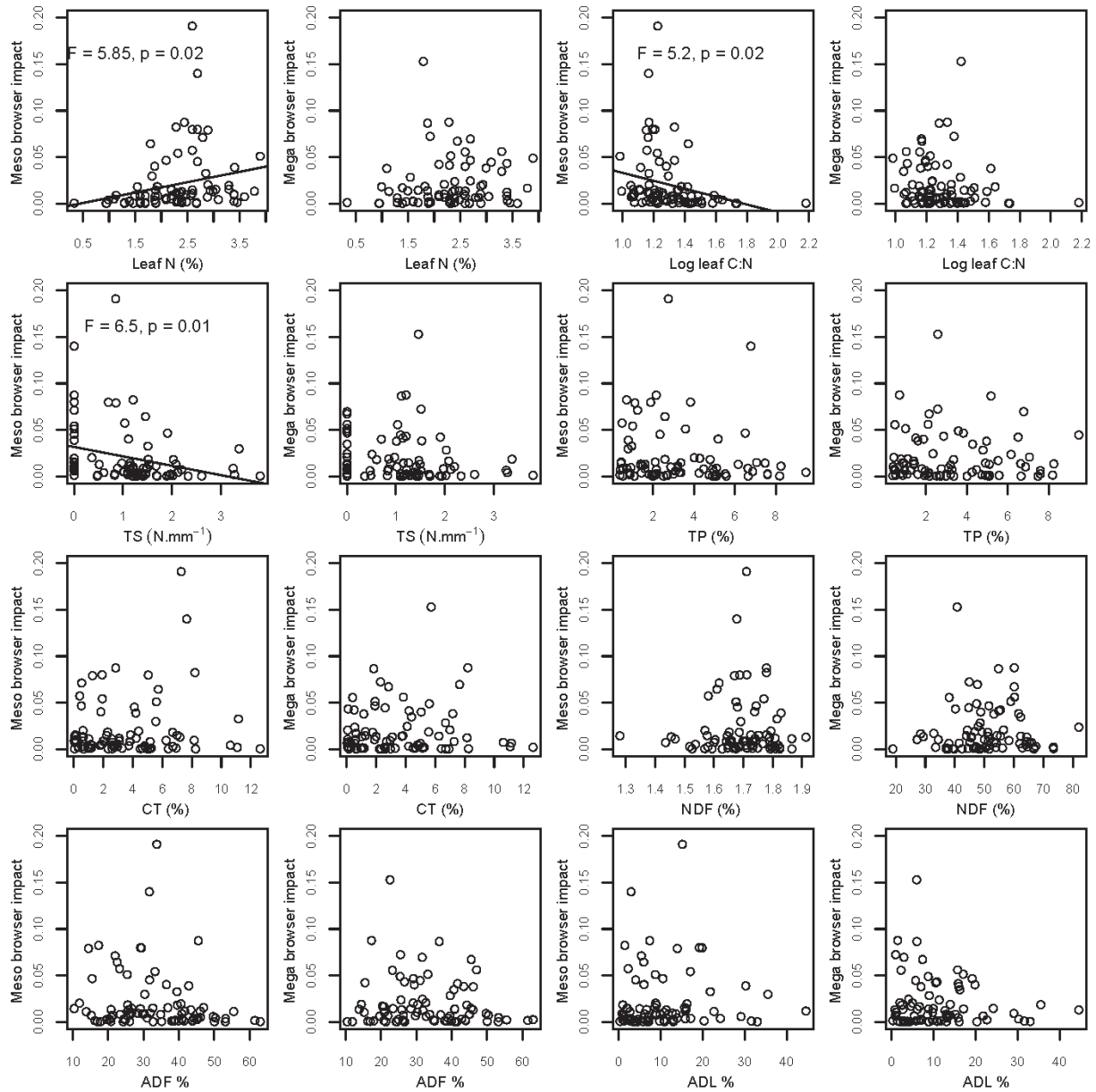


Figure 6. Relationships between the indices of meso-browser and mega-browser impact and the nine plant traits relating to palatability for each woody species.

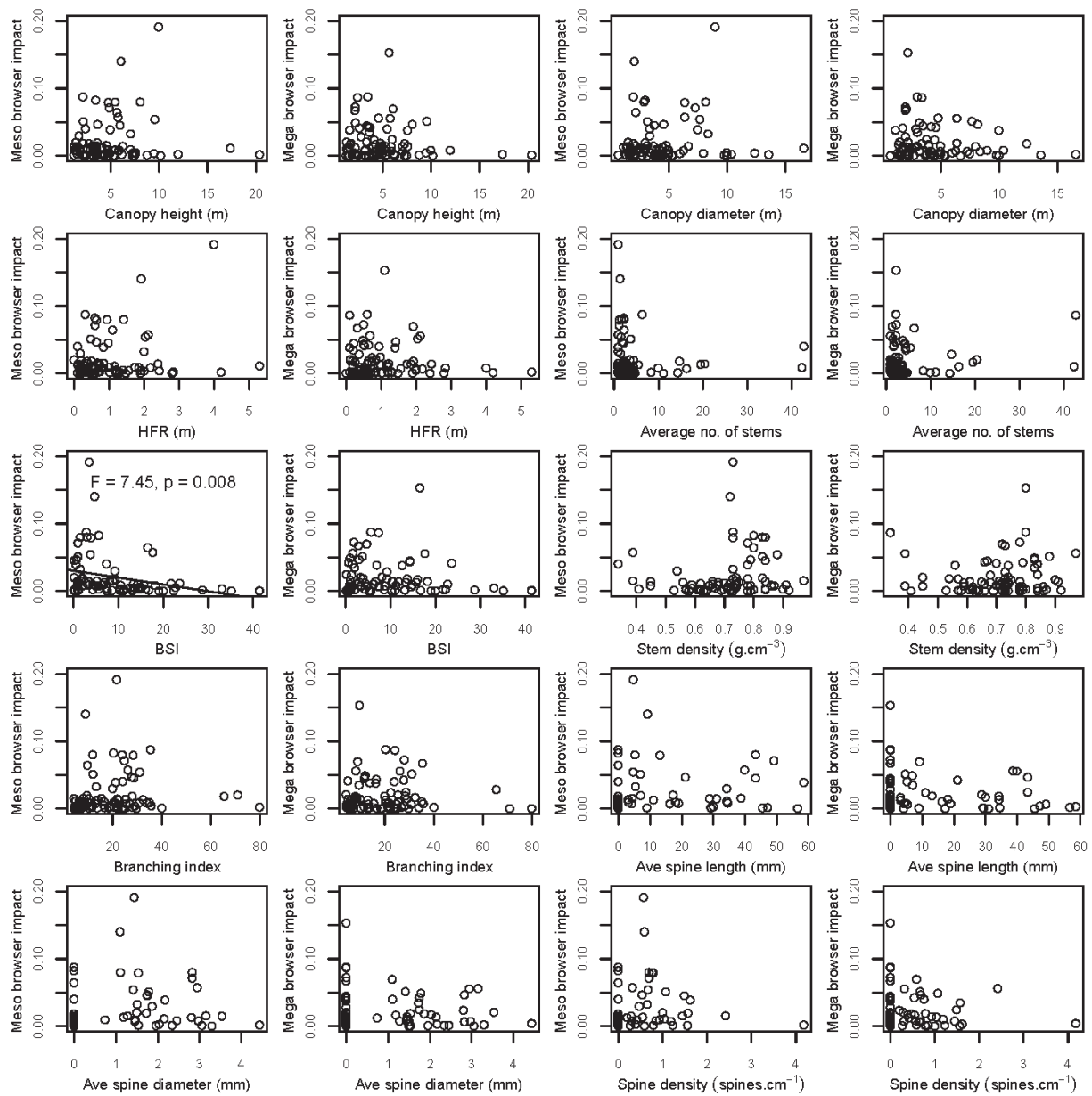


Figure 7. Relationships between the indices of meso-browser and mega-browser impact and the plant traits relating to physical and architectural defences of the woody species.

Table 6. Summary of the best mixed effects models to predict the indices of meso-browser and mega-browser impacts for the fixed effects of soils and rainfall, traits relating to palatability and traits relating to physical and architectural defences. The sign of the relationship, (d), F-values (F) and probabilities (p) are shown. The ratio of the variance among sites explained by a model including the fixed effects to a null model with no fixed effects is included as an estimate of the remaining variance explained by soil, rainfall, palatability and physical defences.

Variables in the model		Meso-BI			Mega-BI		
		d	F	p	d	F	p
Soils & MAP	Log Soil N				+	6.78	0.02
	Soil C:N	+	0.99	0.15			
	Log MAP				-	12.39	0.004
Variance explained			(%)			(%)	
Among-sites variance			44			>99	

Variables in the model		Meso-BI			Mega-BI		
		d	F	p	d	F	p
Chemical defence	TS	-	7.72	0.007			
	CT	+	3.76	0.06			
	ADF	-	2.92	0.09			
	ADL				+	1.35	0.25
Variance explained			(%)			(%)	
Among-sites variance			72			< 1	

Variables in the model		Meso-BI			Mega-BI		
		d	F	p	d	F	p
Structural defence	BSI	-	11.32	0.001			
	HFR	+	6.96	0.01			
	ASL				-	1.36	0.24
Variance explained			(%)			(%)	
Among-sites variance			> 99			<1	

Discussion

Comprehensive soil sampling at the study sites (reported in chapter 4) showed a clear separation of 'nutrient-rich' from 'nutrient-poor' sites determined primarily by the underlying geologies. Furthermore nutrient-rich and nutrient-poor sites were equally distributed along a rainfall gradient. This set-up provided a good framework from which to address the first question of this study which was whether different defence syndromes were apparent on sites differing in soil fertility and rainfall. According to classical defence theory (e.g. see Hanley et al. 2007) the expectation would be to find higher investment in defences at the sites where nutrients may be limiting while at sites where nutrients are less limiting plants should invest less in defences and more in faster growth rates. However the nature of the defences, whether structural or chemical, and the type of herbivore eating the plant (vertebrate or invertebrate) are not specified in classical theory. Indeed very few papers explore theoretical predictions of optimum defences, structural or chemical, in different environmental settings in the African context where large mammals are major herbivores.

Leaf N and C:N responded as might have been expected (e.g. Ordonez et al. 2009) with higher quality leaf material at the nutrient-rich sites. The only other chemical traits to show significant differences between the two soil fertility classes were total phenolic content and fibre content (both NDF and ADF) which were all higher at nutrient-poor sites. There is therefore some evidence for the existence of different syndromes of leaf palatability at the study sites with nutrient-rich sites tending to have foliage of higher quality, while nutrient-poor sites showed some evidence for higher investment in phenolic compounds and tended to have higher fibre content. Surprisingly there was no evidence for differences in SLA, tensile strength, condensed tannins or lignin content between the high and low soil fertility.

Structural defences showed much stronger variation than chemical defences in relation to soil nutrients. Species growing at nutrient-poor sites tended to be significantly taller with larger canopy diameters. Species growing at nutrient-rich sites were generally more branched with a higher density of thorns than those growing at nutrient-poor sites. The 'cage-like' architecture of highly ramified species reduced leaf loss from simulated browsing as reflected in the bite size index. This was lower at nutrient-rich sites consistent with more effective structural defence against leaf loss to browsers.

These results provide strong evidence for the existence of two different syndromes or strategies regarding physical and architectural defences. The observed pattern of taller plants with wider canopies at nutrient-poor sites may reflect selection to escape fire damage at higher rainfall sites with frequent fires (Bond & van Wilgen 1996; Staver et al. 2012). Species growing at nutrient-rich sites are exposed to high levels of herbivory (Olf et al. 2002) where increased branching and higher levels of spinescence afford greater protection for leaves. The effectiveness of this strategy is evident from the lower BSI at the nutrient-rich sites which is a direct measure of how easily an herbivore can remove foliage from a plant.

The defence syndromes evident at the study sites in southern African savannas may offer some light on the so called 'allocation dilemma'. The findings of this study suggest that savanna species tend to be of lower nutritional value when soil nutrients are limiting with higher fibre content and more investment in polyphenolic compounds as a chemical defence. Furthermore species growing in nutrient limited environments tended to be bigger (height and canopy diameter) with much lower levels of branching and low investment in physical defences. Species growing in more fertile environments on the other hand tended to be much more branched and to invest more heavily in spines. Thus these findings seem to be in agreement with the predictions of Coley et al. (1985) but in direct contrast to other studies such as that of Grubb (1992) who observed that the distribution of spinescence followed a general pattern of being more common but not confined to drier less fertile parts of the world. The findings are also in strong contrast to Hanley et al. (2007) who predicted that plants should invest more in structural defences when soil nutrients are limiting. Their predictions might work in some parts of the world; however they do not seem to hold for African savannas which usually contain a rich diversity of mammalian herbivores of many different size classes.

Soil P was found to be a good predictor of total polyphenol content showing that as soil P decreased, polyphenol concentrations increased. Soil N together with soil P explained most of the variability in total condensed tannins, with a positive relationship with soil N but negative relationship with soil P. This could suggest that if N is not limiting, the plant can afford to invest in costly tannins in order to conserve P. Soil C:N was found to be significantly related to the cell wall constituent - neutral detergent fibre - accounting for most of the among site variability in NDF. Surprisingly, this relationship had a negative slope suggesting that as soil C:N increased NDF decreased.

The mixed effect models provided further support for the existence of different defence syndromes depending on soil fertility. The most important findings suggest that plants invest more in the chemical defences, polyphenolics and condensed tannins when soil P is limiting. The final aim of this study was to determine how predictable the two types of browser impact were, based firstly on the measures of soil fertility, secondly on the suite of palatability related traits and thirdly on the suite of physical and architectural defences. Meso-browser impact was poorly predicted by environmental variables, while mega-browser impact was found to be positively related to soil N and negatively related to rainfall, with the highest mega-browser impact at sites with high soil N but low rainfall. The best model at predicting meso-browser impact from the palatability related traits included tensile strength, condensed tannins and ADF, which together accounted for 72% of the among site variance. There appeared to be no evident relationships between mega-browser impact and chemical defences, with the best model accounting for less than one percent of the among site variance. Of the structural defences, meso-browser impact was best predicted by the bite size index and the height of first ramification, once again showing how effective BSI is at predicting meso-browser impact. Mega-browser impact was again found to be completely unrelated to any of the structural defence traits.

Thus clear differences in the efficacy of defences according to mammal body size, even within mammals as a class of herbivores, were evident. For example, elephants seem to be able to ignore both chemical and structural defences and are therefore relatively more important browsers in higher rainfall or nutrient-poor sites while smaller browsers are most important in nutrient-rich sites. These findings are not entirely consistent with those of Fritz et al. (2002), who investigated patterns of mega- vs. meso-herbivores in African savannas and found mega-herbivore biomass to increase with rainfall but was not related to soil nutrients. The findings from this study showed mega-herbivore impact to be positively related to soil N but negatively related to rainfall. It also emphasises the need to develop plant defence theory that specifies the herbivore type, unlike previous reviews which often fail to do so (e.g. Coley et al. 1985; Herms and Mattson 1992).

Conclusion

Two distinct defence syndromes were evident from the study sites which were situated along gradients of soil fertility and rainfall within southern African savannas. Under low soil fertility

conditions savanna species tended to grow larger and to have low quality browse material with high fibre content and higher total phenolic concentrations. In contrast, under higher soil fertility conditions savanna species tended to have higher quality browse material but invested more in structural defences, particularly in higher levels of branching and spinescence. The suite of structural defences tended to be more strongly correlated with soil characteristics than chemical defences while browser impact was also found to be strongly correlated with structural defences but not with chemical defences. Actual browser utilisation tended to be more predictable for meso-browsers which targeted plants of higher nutritional value with lower tensile strength (less tough) and lower concentrations of polyphenols, condensed tannins and fibre. Meso-browsers therefore tend to browse most on the most heavily structurally defended plants which are also the least chemically defended. It could therefore be asked if this suggests that chemical defences are in fact more efficient in deterring meso-browsers? This however is unlikely as the meso-browsers are most likely targeting the more heavily structurally defended plants due to their higher nutritional quality.

The bite size index was found to be a very good proxy and predictor of meso-browser impact. Thus if a plant species growing in a savanna was not chemically defended and poorly structurally defended it would be heavily utilised, probably to the point of extinction, explaining the non-existence of such species at the study sites. In this study, the plants most impacted were those with smallest BSI, so meso-browsers keep targeting them despite a reduced intake rate, suggesting that these species are highly beneficial to meso-browsers. There may however be a threshold of branching and BSI for which it becomes non-profitable to continue browsing on these plants. Such species were not found at the study sites, thus it would be interesting to sample more intensely at more sites from around the globe. Mega-browser impact on the other hand was well predicted by soil N and rainfall but completely unpredictable based on both chemical and structural defences. Thus it would appear that plant defences simply do not matter to mega-browsers such as elephant.



Elephant cow with her calf browsing on *Acacia tortilis*, Mapungubwe National Park.

Chapter 6 Herbivores shape woody plant communities in the Kruger National Park: lessons from three long-term exclosures

Accepted for publication in *Koedoe - African Protected Area Conservation and Science*

B.J. Wigley^{1*}, H. Fritz¹, C. Coetsee² & W.J. Bond³

¹ UMR CNRS 5558 – LBBE, Université Claude Bernard Lyon 1, Bât. Grégor Mendel 43 bd du 11 novembre 1918, 69622 Villeurbanne cedex

² School of Natural Resource Management, Nelson Mandela Metropolitan University, George, 6530

³ Department of Botany, University of Cape Town, P/Bag, Rondebosch, 7701, South Africa

*Corresponding author: benjamin.wigley@nmmu.ac.za

ABSTRACT

The role of grazers in determining vegetation community compositions and structuring plant communities is well recognized in grassy systems. The role of browsers in affecting savanna woody plant communities is less clear. We used three long-term exclosures in the Kruger National Park (KNP) to determine the effect of browsers on species compositions and population structures of woody communities. Species assemblages, plant traits relating to browsing and soil nutrients were compared inside and outside of the exclosures. Our results show that browsers directly impact plant species distributions, densities and population structures by actively selecting for species with traits which make them desirable to browsers. Species with high leaf N, low total phenolic content and low acid detergent lignin appear to be favoured by herbivores and therefore tend to be rare outside of the exclosures. This study also suggests that browsers have important indirect effects on savanna functioning, as the reduction of woody cover can result in less litter of lower quality, which in turn can result in lower soil fertility. However, the magnitude of browser effects appears to depend on inherent soil fertility and climate.

Keywords: browsers, herbivores exclosures, plant defences, plant traits, soil nutrients, woody plant communities

Conservation implication

Browsers are shown to have significant impacts on plant communities. They have noticeable effects on local species diversity and population structure, as well as soil nutrients. These impacts are shown to be related to the underlying geology and climate. The effects of browsers on woody communities are shown to be greater in low rainfall, fertile areas compared to high rainfall, infertile soils.

INTRODUCTION

The role of herbivory in structuring plant communities and determining community compositions is well recognized (see reviews by Huntly 1991; Augustine & McNaughton 1998). For example, native ungulate browsers have been found to have major impacts on shrub dynamics in East African savannas (Augustine & McNaughton 2004). Different feeding behaviours and food preferences of browser species have major impacts on South African (Owen-Smith & Cooper 1987) and West African savanna woody communities (Jachmann & Croes 1991). These impacts are often attributed to selective feeding related to food quality (Owen-Smith & Cooper 1987). As in savannas (e.g. Staver *et al.* 2009), herbivores have been found to regulate recruitment rate and species composition of trees in temperate forests (Ammer 1996; Van Hees, Kuiters & Slim 1996; Kriebitzsch *et al.* 2000), and grassland (Anderson & Briske 1995).

Many of the studies on browser-plant interactions in savannas have tended to focus on interactions between browsers and specific woody species. For example, Coetzee *et al.* (2008), Gadd (2002) and Helm & Witkowski (2012) looked at browser interactions with *Sclerocarya birrea*, while Fornara & Du Toit (2007) documented the response of *Acacia nigrescens* to ungulate browsing. These two species are important browse species in southern African savannas. As savanna woody species tend to be highly variable in form and function, these studies usually do not provide a better understanding of savanna dynamics at the community level. Augustine & McNaughton (2004) emphasized the need for replicated experiments that assess the effects of native browsers on shrub dynamics in African savannas as our understanding of the importance

of browser effects on woody plant dynamics remains unclear. A better understanding of how browsers and woody plants interact at the community level and how these interactions affect savanna dynamics will be invaluable in managing browser populations.

The existence of three long-term herbivore exclosures in the Kruger National Park, South Africa, provides a good opportunity to empirically determine the role of browsers in determining woody species composition and population structures of woody communities growing at the three study sites. Previous studies have shown evidence for taller canopies and higher tree and shrub densities inside exclosures at Nhlanguwini (Trollope *et al.* 1998; Asner *et al.* 2009; Levick *et al.* 2010), Makhohlola (Trollope *et al.* 1998; Levick *et al.* 2009) and N'waxitshumbe (Trollope *et al.* 1998; Levick & Rogers 2008; Asner *et al.* 2009). In these studies the analyses were generally based on remote sensing and ignored species identity. Differences in woody community compositions and plant traits between exclosure treatments are therefore still largely undocumented.

Differences in species compositions between treatments would be highly informative as the species found inside but not outside, are likely to be vulnerable to herbivory and therefore no longer found outside of the exclosures or found at reduced densities. The traits of these species can be used to provide us with clues as to what makes them vulnerable to herbivory. For example, Diaz *et al.* (2007) found that at the global scale there is a spectrum of plant responses to herbivory with high levels of herbivory favouring annual over perennial plants, short plants over tall plants, prostrate over erect plants and stoloniferous plants over those with tussock architecture.

This paper aims to determine how browsers and plant traits interact and how these interactions affect woody plant population dynamics and community assemblages. We aim to determine if there are differences in species compositions and key plant functional traits between the woody communities found growing inside and outside of three long-term exclosures in Kruger National Park (KNP). Plant traits are important in determining both the type of and degree of vertebrate herbivory while they also can reflect plant responses to herbivory. We measured a number of plant traits that are likely to impact and respond to browsing; these included leaf nitrogen and

phosphorus, leaf C:N ratio, specific leaf area, leaf size, leaf dry matter content, leaf tensile strength, bite size index, and levels of secondary compounds. High leaf nitrogen (N) and leaf phosphorus are associated with higher nutritional quality for herbivores (Cornelissen *et al.* 2003). Leaves with lower C:N ratios are much more attractive to herbivores as these leaves have higher N content (Cornelissen *et al.* 2003). The specific leaf area (SLA) of a species is usually a good correlate of its potential growth rate. Lower SLA values tend to correspond with relatively high investments in defences, both structural and chemical, and long leaf life span, all of which affect nutritional value (Cornelissen *et al.* 2003). Environmental nutrient stress and disturbance as well as phylogenetic factors play a role in determining leaf size (Cornelissen *et al.* 2003). Smaller leaves with higher SLA are typically more attractive to herbivores. Leaves with high leaf dry matter content (LDMC) values tend to be tough and therefore more resistant to disturbances such as herbivory or wind than leaves with low LDMC. Species with low LDMC are usually associated with productive and highly disturbed areas (Cornelissen *et al.* 2003). Leaf tensile strength is a good indicator of the relative carbon investment in structural protection of the photosynthetic tissue. Physically stronger leaves are better protected from abiotic (winds, storms) and biotic mechanical damage (herbivory). Higher physical strength usually results in longer leaf span, however this is usually combined with other physical and chemical defences such as spines and tannins (Cornelissen *et al.* 2003). Bite size index (BSI) is a measure of how easily leaves can be browsed by a simulated browser (a human), while stem specific density broadly trades off against relative growth rate and stem defences against pathogens, herbivores and mechanical damage (Cornelissen *et al.* 2003). Condensed tannins and polyphenols are organic N-free chemical defences that bind with protein, reducing N availability to herbivores, thereby decreasing preference by mammal herbivores (Haslam 1988; Bergvall & Leimar 2005; Owen-Smith 2005). The concentrations of the cell wall constituents (neutral detergent fibre [NDF], acid detergent fibre [ADF], and acid detergent lignin [ADL]) determine the digestibility of leaf material for herbivores. Detergent analysis has been used to compare fibre digestibility across different plant types (e.g. see Codron *et al.* (2007) and references therein).

The following questions are specifically addressed using the three exclosures. 1) Has the long-term exclusion (> 40 years) of herbivores led to differences in woody plant species compositions at the three exclosures? 2) Do the structures, densities and abundances of the woody species

populations differ inside and outside of the exclosures? 3) Are there differences in plant leaf and stem traits between the species only found inside vs. common species found both inside and outside? 4) Has the long-term removal of herbivores from the exclosures had any effects on soil nutrient cycling? 5) Do the exclosures help us to predict under what conditions herbivores play an important role in determining the structure and composition of woody plant communities in savannas?

METHODS

Study sites

Three long term herbivore exclosures (> 40 years old) exist in the Kruger National Park (KNP). These include the Nhlanguini exclosure near Pretoriuskop, the Makhohlola exclosure near Crocodile Bridge and the N'waxitshumbe exclosure on the northern plains (Table 1). At each exclosure two treatments were sampled, one inside the exclosure and one outside the exclosure at close proximity.

The 220 ha Nhlanguini exclosure was established in 1973 and occurs in the south western portion of the Kruger National Park near Pretoriuskop rest camp. This is the wettest part of KNP with MAP close to 750 mm. The exclosure is situated within the broad-leaved bushveld vegetation type that occurs on sandy soils derived from granitic rocks, common species include *Terminalia sericea* and *Sclerocarya birrea* (Venter *et al.* 2003). Mean fire return interval is similar inside and out, with fire occurring on average every 3.6 years inside (Asner *et al.* 2009) and less than every 4 years outside (Smit *et al.* 2012). The Makhohlola exclosure is a 2.4 ha exclosure constructed in the early 1970s. It is located in the south-eastern corner of the KNP, just north of Crocodile Bridge. It occurs in the *Sclerocarya birrea* / *Acacia nigrescens* savanna type (Venter *et al.* 2003). The site occurs on soils derived from rocks of basaltic origin with mean annual rainfall of *ca.* 600 mm. The mean fire return interval in the Makhohlola exclosure (4.5 years) was slightly longer than outside (3.2 years) until 2004 when a new fire experiment was set up (see Levick *et al.* 2009). Since 2004, half of the exclosure has been protected from fire while the other half is burnt in conjunction with the surrounding landscape (Levick *et al.* 2009). From 2004 to the time of measurements in March 2011, the burn treatment and surrounding areas were burnt twice, in 2006 and 2010. The N'waxitshumbe exclosure is situated in the arid north eastern

section of Kruger National Park and was established in 1967. The original N'waxitshumbe enclosure was 230 ha with an additional 72 ha added in 1986 (Asner *et al.* 2009). Mean annual precipitation is close to 400 mm and the soils here are derived from rocks of basaltic origin. The vegetation type is classified as *Colophospermum mopane* shrubveld growing in broad-leaved bushveld (Venter *et al.* 2003). The mean fire return interval for the N'waxitshumbe enclosure is approximately 4 years (Asner *et al.* 2009), while Smit *et al.* (2012) showed that the area surrounding the N'waxitshumbe enclosure has a mean fire return interval of less than 4 years.

The faunal assemblage in the park includes all the major mammal species typical of the region. Common browser species include kudu *Tragelaphus strepsiceros* (Pallas 1766), giraffe *Giraffa camelopardalis* (Linnaeus 1758), black rhinoceros *Diceros bicornis* (Linnaeus 1758), steenbok *Raphicerus campestris* (Thunberg 1811), grey duiker *Sylvicapra grimmia* (Linnaeus, 1758) and bushbuck *Tragelaphus scriptus* (Pallas 1766). Common mixed-feeders include impala *Aepyceros melampus* (Lichtenstein, 1812), and the African elephant (*Loxodonta africana*) (Linnaeus 1758).

Community composition

The distribution, abundance and density of the species growing both inside and outside of each of the enclosures was determined by walking four 100 m long by 4 m wide transects for each treatment at each site (1600 m²) in March 2011. The four transects in each treatment were taken parallel to each other and 50 m apart. Inside and outside pairs were situated in as close a proximity as fences and firebreaks permitted. Care was also taken to ensure that the paired transects were on similar positions along catenal gradients. At Nhlanguwini the paired transects were taken perpendicular to the northern fence of the enclosure and parallel to the drainage in the area, outside transects were taken to the north west of the enclosure. At Makhohlola the paired transects were taken perpendicular to the western fence and perpendicular to the drainage at the site, outside transects were taken to the north of the enclosure. Due to the limited size of the Makhohlola enclosure (100 x 240 m) two of the four transects were taken in the no-burn part of the fire experiment (since 2004) and two were taken in the part that burns in conjunction with the surrounding landscape. At N'waxitshumbe the paired transects were taken perpendicular to the southern fence in the south western portion of the enclosure and parallel to the drainage in that area.

Species identity was recorded for each woody plant encountered in each transect using a PDA with CyberTracker software. The abundance data was used to create an inside abundance index whereby the proportion of a species found in the inside compared to the outside treatment was calculated. This index was calculated by dividing the number of individuals of each species found inside by the total number of individuals found both inside and outside. Thus a value of 1 indicates the species was only found inside while a value of 0 indicates the species was only found outside. The inside abundance index was then plotted against a suite of leaf traits to test whether species growing on the inside had high values of these traits. This index can be used as a proxy to determine which plant species are preferentially selected for or 'favoured' by browsers relative to their abundance.

Trait sampling

The dominant woody plant species making up 80 - 90 % of the woody biomass inside and outside the three exclosures in KNP were determined (Table 2). Fourteen different plant traits relating to the leaves and stems were sampled for each species (Table 3). All trait data (except bite size index) were measured and recorded according to the methods explained in Cornelissen *et al.* (2003). For each species leaf material was collected from five individuals and analysed for nitrogen (N), phosphorus (P) and carbon (C) content using a Leco TruSpec CN Analyser (LECO Corporation, St. Joseph, MI). SLA and average leaf area (ALA) were measured using four healthy and complete sun-exposed leaves from five individuals (20 leaves per species). The bite size index (BSI) of a species is the total dry weight of the leaves removed from two human bites taken from five individuals (total of ten bites). An attempt was made to remove the maximum amount of leaf material with each bite. The BSI for all species was measured by the same person in order to control for potential differences between individual recorders. Total condensed tannins (CT) were calculated according to the methods described by Hattas & Julkunen-Tiitto (2012) while total polyphenols (TP) were calculated according to the methods described by Hattas *et al.* (2005). Cell wall constituents, i.e. neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined in an Ankom fibre analyzer, whereas acid detergent lignin (ADL) was determined in accordance with the acid detergent lignin in beakers method. Ash was determined by incinerating the filter bag containing plant residue in a muffle furnace at 525 °C for three

hours. All cell wall constituents were determined consecutively as outlined in the Ankom technology procedures (Ankom technologies).

Soil sampling

Five replicate soil samples were collected both inside and outside of each enclosure at three depths (0-10 cm, 10-20 cm and at 40-50 cm) using a soil auger. Care was taken to sample away from the canopies of trees. Soil bulk density measurements were taken at these same depths. The methods used for soil sampling and analyses are described in detail by Wigley *et al.* (2013).

Statistical analyses

All analyses were performed using R (R Development Core Team 2012). Chi square tests were used to test for differences in tree densities between treatments. Non-parametric Wilcoxon tests were used to test for differences in trait measurements as the low replication resulting from the low species diversity at each site precluded the use of statistical tests founded on the normal distribution. ANOVAs were used to test for differences in soil nutrients between treatments using pooled data from all three depths, as the conditions for homoscedasticity were not violated (fligner.test: $P > 0.05$, Conover *et al.* 1981). Principle components analyses (PCA) were used to explore the relationships between treatments, sites and measured plant traits. The function PCAsignificance available in the BiodiversityR package (Kindt & Coe 2005) in R was used to test for significant PCA axes using the broken-stick distribution.

RESULTS

Community composition

At the Nhlanguini enclosure, there were a number of differences between the two communities. The four most common species for both treatments were *Terminalia sericea*, *Dichrostachys cinerea*, *Sclerocarya birrea* and *Acacia gerrardii*. However *Terminalia sericea* was found to be much more abundant (nearly 80% of all trees) inside compared to outside (*ca.* 35%) of the enclosure (Figure 1). *Dichrostachys cinerea* showed the opposite pattern with a higher relative abundance outside (30%) compared to inside (15%) the enclosure (Figure 1). *Sclerocarya birrea*

and *Acacia gerrardii* showed similar abundances between treatments (Figure 1). Both treatments had a few unique species, with *Strychnos madagascariensis*, *Catunaregam spinosa* and *Searsia leptodictya* only found inside while *Antidesma venosum*, *Combretum hereroense*, *Albizia harveyi*, *Philenoptera violacea*, and *Piliostigma thonningii* were only found outside. Species diversity was found to be higher on the outside of the enclosure with a total of 16 species compared to 12 species inside.

There were distinct differences in woody communities growing inside and outside of the Makhohlola enclosure (Figure 1). *Acacia nigrescens* was found to be more abundant inside the enclosure (32 %) but was still relatively abundant outside (22 %). *Flueggea virosa* was common inside (15%) but rare outside (4%). *Gymnosporia senegalensis* was found to have a much higher abundance outside (33%) compared to inside (12%). *Lannea schweinfurthii* and *Sclerocarya birrea* had lower abundances outside (7 and 1%) compared to inside (12 & 7%). *Dichrostachys cinerea* and *Albizia harveyi* were relatively common outside (13 and 9%) but rare inside (3% for both). *Dalbergia melanoxylon*, *Ehretia rigida* and *Euclea undulata* were only found inside the enclosure, making species diversity higher on the inside (13 species) than outside (10 species).

There were also distinct differences in the woody communities growing inside and outside of the N'waxitshumbe enclosure. At this site *Colophospermum mopane* was found to be the most common species both inside (43%) and outside (40%, Figure 1). The second most common species inside the enclosure (37%), *Dalbergia melanoxylon*, was not found outside of the enclosure. *Combretum imberbe* and *Philenoptera violacea* were more common outside (14% for both) compared to inside (7 and 1%). *Sclerocarya birrea* and *Gymnosporia senegalensis* were relatively rare both inside and outside of the enclosures. *Dalbergia melanoxylon*, *Grewia monticola* and *Ozoroa obovata* were unique to inside while *Dichrostachys cinerea*, *Albizia harveyi* and *Acacia nigrescens* were only found outside of the enclosure. Thus species diversity was the same inside and outside of the enclosure but species composition differed.

Population structure

Total woody plant densities were significantly higher for the inside treatments at all three sites (Figure 2). These large differences were determined by the most common species growing at each

site. The size class distributions for the two most common species growing at the Nhlangwini enclosure (*Terminalia sericea* and *Dichrostachys cinerea*) show very different patterns for each treatment. The size class distribution of *Terminalia sericea* inside the enclosure shows an inverse J-shaped curve, with many plants in the smaller size classes less than 3 m and fewer plants in the large size classes. The size class distribution for outside the enclosure shows much lower numbers of plants in the smaller size classes and fewer plants in the very large size classes (Figure 3). A different pattern was evident for *Dichrostachys cinerea* with most plants falling into size classes between 2 and 4 m inside the enclosure while plants on the outside were more evenly distributed in a greater range of size classes (Figure 3).

At Makhohlola major differences were evident between treatments for each of the four most common species (Figure 4). The size class distributions of *Lannea schweinfurthii* and *Acacia nigrescens* showed an approximately inverse J-shaped curve for the populations growing inside the enclosures but not for the outside populations. The inside populations of these two species both had a much higher number of individuals in the smaller (< 2 m) size classes than the outside populations (Figure 4). The outside population of *Gymnosporia senegalensis* was more uniform in height (1-2 m) than the inside population (0-3 m). There were many more and taller *Flueggea virosa* plants growing on the inside of the enclosure compared to the outside (Figure 4).

At N'waxitshumbe, the two most common species *Colophospermum mopane* and *Combretum imberbe* had similar size class distributions in each treatment (Figure 5). However, there were higher densities in each size class for the inside treatment for *Colophospermum mopane*, while *Combretum imberbe* had similar numbers in each size class for both populations. The most striking difference was found for *Dalbergia melanoxylon*, where a population spanning the full range of size classes was found inside the enclosure while not one individual was found outside of the enclosure in the sampling area (Figure 5).

Leaf traits of the species unique to inside vs. species common outside

There were no significant differences ($p > 0.05$; Wilcoxon test) in leaf nutrient concentrations (N, carbon (C), C:N, P, Ca, Mg, Na and K) between each of the inside and outside treatments (Table 4). There were however some consistent trends, the lack of statistically significant differences

was likely a result of the small sample sizes resulting from low woody species diversity at the sites. Mean leaf N (%) was noticeably higher for the inside treatment at all three sites (Table 4). The higher N and similar C concentrations inside of the Nhlanguwini enclosure resulted in a lower mean C:N ratio, while the other two sites had similar C:N ratios (Table 4). Leaf P (%) was similar between treatments at all three sites (Table 4).

There were no significant differences ($p > 0.05$; Wilcoxon test) in SLA, leaf size, BSI and tensile strength between treatments. Mean SLA was consistently higher for species restricted to the inside treatments (Table 4). Average leaf area, which was highly correlated to bite size index (BSI), were both higher inside the enclosures at the two nutrient rich sites on basalts (Makhohlola and N'waxitshumbe), while the opposite pattern was true for the Nhlanguwini site which is situated on nutrient poor granite derived soils (Table 4). Mean leaf tensile strength was higher outside enclosures at the two basalt sites but no difference was evident between treatments at the granite site (Table 4).

The pooled data showing the inside abundance index of each species plotted against leaf traits and BSI showed a weak positive relationship between the inside abundance index and leaf N and P (Figure 6 A and B). Almost no relationship was evident between the inside abundance index and SLA and BSI (Figure 6 C and D, $p > 0.05$ for all correlations).

Chemical defence

No significant differences ($p > 0.05$; Wilcoxon test) in total phenolics, condensed tannins and fibre were evident between treatments. However, an interesting pattern emerged; mean leaf phenolic concentrations were consistently lower for species restricted to the inside treatments compared to those in the outside treatment, with the biggest difference evident at the Nhlanguwini site (Table 4). Mean condensed tannin concentrations showed no consistent patterns between treatments and sites (Table 4). The three types of fibre analyses showed different patterns. Neutral detergent fibre (NDF, Table 4), which includes hemicellulose, cellulose and lignin was slightly lower for the outside treatments at Makhohlola and N'waxitshumbe, while the opposite pattern was found at Nhlanguwini. Acid detergent fibre (ADF, Table 4), which includes cellulose

and lignin showed no consistent patterns. Acid detergent lignin (ADL, Table 4), which only includes lignin was consistently lower for the inside treatments at all three sites.

Soil nutrients

Although the pooled data for all depths of soil N and P showed a pattern of higher values for the three inside treatments, the only statistically significant difference were evident at N'waxitshumbe ($p < 0.05$, Figure 7A and C). Soil C and K also showed a pattern of being consistently higher for the inside treatments at Makhohlola and N'waxitshumbe, with no differences evident at Nhlangwini (Figure 7 B-D). Similar patterns were evident for N, C and P when total nutrient stocks were calculated using soil bulk density data (data not shown).

Multivariate analyses

The principal components analysis performed on the data using 13 traits relating to the leaves and stem (Figure 8) showed that the first two principal components together explained 44% of the total variation amongst species. The most important traits separating species along PCA1 were NDF, ADL and TP. The most important traits separating species along PCA2 were leaf N, leaf C:N, and stem density (Figure 8). The PCA showed that the species from the inside tended to more variable along Axis 2 (higher leaf N and stem density) while the species from the outside tend to be more variable along Axis 1 (higher fibre and TP values). The variance explained by the first PCA axis was found to be insignificant as the percentage of explained variance (24.3%) was not larger than the corresponding percentage of variance of the broken-stick distribution (24.5%), while the second PCA axis was significant as the percentage of explained variance (20.3%) was larger than the corresponding percentage of variance of the broken-stick (16.8%) distribution (Kindt & Coe 2005).

DISCUSSION

Clear differences were found in both the species composition and population structure of the woody plant communities growing inside and outside of three herbivore exclosures in KNP. Although the three exclosures were situated in very different vegetation types, some similarities exist in species' responses to low herbivory. Some species were frequently encountered both

inside and outside of the exclosures (e.g., *Terminalia sericea* and *Dichrostachys cinerea* at Nhlangwini, *Acacia nigrescens* and *Gymnosporia senegalensis* at Makhohlola, *Colophospermum mopane* at N'waxitshumbe). Smaller size classes tended to be better represented inside, and larger size classes outside for species which were found in large numbers both inside and outside of the exclosures. There were some exceptions though; *Gymnosporia senegalensis* for instance, had similar population structures both inside and out. This could be due to the fact that this species is not a preferred browsing species as a result of the high condensed tannin and ADL concentrations in the leaves (see Shrader *et al.* 2011). We argue that savanna woody species are either well adapted to browsing (such as *Dichrostachys cinerea*), well defended (such as *Gymnosporia senegalensis*) or both (such as *Acacia nigrescens*). For example, Fornara & du Toit (2007) showed that heavily browsed *Acacia nigrescens* trees develop tolerance traits such as high regrowth rates and extensive branching as well as resistance traits such as close thorn spacing, suggesting that it is both well adapted and well defended. In fact, a meta-analysis by Carmona *et al.* (2011) has shown that secondary metabolites are less important in plant defence when compared to other defensive plant traits such as physical resistance, gross morphology and growth rates.

Some species were found more frequently outside of the exclosures, such an example is *Dichrostachys cinerea* which was found in higher numbers outside of the exclosure at Nhlangwini and *Albizia harveyi* at all exclosures. These species are most likely stimulated by disturbance, for example, *Dichrostachys cinerea* shrubs rootsucker and expand significantly when stimulated by fire and browsing (Wakeling & Bond 2007). More frequently, species were only found inside the exclosures (e.g. *Dalbergia melanoxylon*, *Grewia monticola*, and *Ozoroa obovata* at N'waxitshumbe). *Dalbergia melanoxylon* had the highest leaf N of all the species in this study and we assume that the species found only on the inside of the exclosures are mostly very palatable and targeted by browser species outside of the exclosures. *Dalbergia melanoxylon* was found outside of the N'waxitshumbe exclosure in a previous study, but in lower lying areas than at our study area (Levick & Rogers 2008). It is uncertain whether *Dalbergia melanoxylon* has since disappeared outside the exclosure or whether this species may be preferentially targeted by browsers at specific positions along the catena.

Why do species become locally extinct outside of exclosures? Impala and kudu are highly selective when foraging in nutrient-poor broadleaf savannas (Cooper & Owen-Smith 1985; Owen-Smith & Cooper 1987). This selectivity is attributed to differences among species in the level of defensive chemical compounds. Alternatively, these species may also be predisposed to pollarding, uprooting and ringbarking and coppice poorly in response to elephant damage (O'Connor *et al.* 2007). A number of studies have documented the local extinction of plant species resulting from elephant impact (e.g. Penzhorn *et al.* 1974; O'Connor *et al.* 2007 and references therein; Boundja & Midgley 2010). Several studies have shown that other herbivores can lead to the local extirpation of plant species. Bond & Loffel (2001) found that *Acacia davyi* was no longer found in areas accessible to giraffe in Ithala Game Reserve.

Total tree densities were significantly higher for the inside treatments at all three sites, usually with more individuals falling into the smaller size classes inside of the exclosures. These findings are in strong agreement with previous studies from the same study sites. Trollope *et al.* (1998), Levick & Rogers (2008) and Asner *et al.* (2009) reported higher tree densities and different population structures between treatments. Moncrieff *et al.* (2011) reported different tree allometries between trees growing inside and outside of the same herbivore exclosures in KNP with heavily browsed trees shorter for a given stem diameter. Our results are therefore in agreement with previous studies and provide strong evidence that herbivory has a major impact on savanna woody plant diversity, species population structure and overall woody plant densities.

The next step was to determine if there were differences between treatments for some of the key plant functional traits of each woody community. There was a trend in leaf N concentration with higher mean N for the species unique to the inside treatments, compared to the species found growing outside. No obvious patterns or trends were evident for leaf C:N or P. Leaf structural traits, SLA, average leaf size, and BSI which are all correlated, showed similar trends with higher values inside the exclosures at the two fertile sites, and lower values inside compared to outside at the infertile site. The opposite pattern was evident for tensile strength where the leaves were generally tougher on the outside at the two fertile sites with no difference at the infertile site. Apart from leaf N, total phenolic content and lignin content were the only traits that showed consistent patterns between treatments with higher values for species outside of the exclosures.

Increases of carbon-based structural metabolites with decreases in leaf N have been shown with high browsing intensity in previous work (Bryant *et al.* 1991; Wessels *et al.* 2007). An alternative theory for increases in lignin and leaf N inside the exclosures may be that herbivores preferentially select species with higher leaf N, and that species with lower total phenolic and lignin content are also targeted by herbivores.

In general, the removal of browsers led to higher soil nutrients, the response was the greatest at the two basalt sites. At N'waxitshumbe differences in soil N and P were higher with browsers absent while soil C and K were also higher inside of the exclosures. The same pattern was evident at Makhohlola, while the nutrient-poor site (Nhlangwini) showed almost no differences between treatments for all measured soil nutrients. Although herbivores have been found to increase soil nutrients (e.g. Augustine & McNaughton 2006; Frank 2008), Ritchie *et al.* (1998) found that herbivores can actually depress soil N by indirectly decelerating N cycling by decreasing the abundance of plant species with N-rich tissues. The increased soil fertility inside of the exclosures could possibly be explained by differing litter dynamics. The expectation would be that leaf litter with higher N and lower fibre content would result in faster cycling and improved soil fertility (e.g. Reich *et al.* 2001). Furthermore the higher density of woody plants would increase the amount of leaf litter reaching the soil. Thus the higher soil nutrients found inside of the exclosures could possibly be due to the indirect effect of herbivores, as their removal resulted in higher tree densities. Similarly, fire indirectly affects nitrogen cycling in the Kruger National Park by decreasing woody densities (Coetsee *et al.* 2010). The similar soil nutrient concentrations found inside and outside of the Nhlangwini exclosure can most likely be attributed to the overall low litter quality and lower densities of herbivores resulting from the nutrient poor granitic soils at this site.

CONCLUSION

This study has demonstrated the important role browsers play in savanna dynamics. Browsers directly impact species distributions, densities and population structures by actively selecting for species with favourable traits; this study suggests that species with high leaf N, low total phenolic content and low acid detergent lignin are favoured. Forty years of no browsing impact was insufficient to allow the recruitment of the dominant species into larger size classes. This supports

the idea that cohorts of trees recruit simultaneously, they would however need a break from both herbivory and fire. This study also suggests that browsers have important indirect effects on savanna functioning through their impact on soil nutrient cycling. The magnitude of these indirect effects on soil nutrient cycling appears to depend on inherent soil fertility and climate. The effect of browsers on both vegetation and soils was highest at the low rainfall site with high soil fertility, with a somewhat lesser effect at the higher rainfall basalt site, with almost no effect at the infertile high rainfall granite site. This work highlights the importance of herbivore exclosures in protected areas and some of the challenges faced by researchers when studying browser effects on savanna ecosystems due to the paucity of replicated long-term herbivory exclosures. Future work should identify other possible sites in savannas from around the globe that could be used to corroborate the findings from this study.

ACKNOWLEDGEMENTS

This work forms part of the PhD thesis of BJ Wigley and was made possible thanks to funding from a BDI-PED grant from the CNRS as well as the Andrew W Mellon Foundation. I am hugely grateful to: SANParks, Kruger National Park Scientific Services for allowing the study and logistical support, and Scientific Services game guards for protection in the field. Thanks to two anonymous reviewers for their helpful comments which helped improve this manuscript.

Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

Author contributions

BJW is a PHD student and was responsible for the project design, data collection, analyses and reporting of data, CC assisted in collection of data and reporting, WJB and HF are both supervisors of BJW and made valuable contributions to the refinement of the analyses and the MS.

REFERENCES

- Ammer, C. 1996, 'Impact of ungulates on structure and dynamics of natural regeneration of mixed mountain forests in the Bavarian Alps', *Forest Ecology and Management* 88, 43-53.
- Anderson, V. J., & Briske, D. D., 1995, 'Herbivore-induced species replacement in grasslands: is it driven by herbivory tolerance or avoidance?' *Ecological Applications* 5, 1014-1024.
- Ankom technology, <http://www.ankom.com/09procedures/procedures.shtml>
- Asner, G. P., Levick, S. R., Kennedy-Bowdoin, T., Knapp, D. E., Emerson, R., Jacobson, J. & Martin, R. E., 2009, 'Large-scale impacts of herbivores on the structural diversity of African savannas', *Proceedings of the National Academy of Sciences* 106, 4947-4952.
- Augustine, D. J., & McNaughton, S. J., 1998, 'Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance', *The Journal of Wildlife Management* 62, 1165-1183.
- Augustine, D. J., & Mcnaughton, S. J., 2004, 'Regulation of shrub dynamics by native browsing ungulates on East African rangeland', *Journal of Applied Ecology* 41, 45-58.
- Augustine, D. J., & McNaughton, S. J., 2006, 'Interactive effects of ungulate herbivores, soil fertility, and variable rainfall on ecosystem processes in a semi-arid savanna', *Ecosystems* 9, 1242-1256.
- Bergvall, U. A., & Leimar, O. 2005. 'Plant secondary compounds and the frequency of food types affect food choice by mammalian herbivores' *Ecology* 86, 2450-2460.
- Bond, W. J., & Loffell, D., 2001, 'Introduction of giraffe changes acacia distribution in a South African savanna', *African Journal of Ecology* 39, 286-294.
- Boundja, R. P., & Midgley, J. J., 2010, 'Patterns of elephant impact on woody plants in the Hluhluwe-Imfolozi park, Kwazulu-Natal, South Africa', *African Journal of Ecology* 48, 206-214.

- Bryant, J. P., Provenza, F. D., Pastor, J., Reichardt, P. B., Clausen, T. P., & du Toit, J. T., 1991, 'Interactions between woody plants and browsing mammals mediated by secondary metabolites', *Annual Review of Ecology and Systematics* 22, 431-446.
- Carmona, D., Lajeunesse, M. J., & Johnson, M. T., 2011, 'Plant traits that predict resistance to herbivores', *Functional Ecology* 25, 358-367.
- Codron, D., Lee-Thorp, J. A., Sponheimer, M., Codron, J., De Ruiter, D., & Brink, J. S. 2007, 'Significance of diet type and diet quality for ecological diversity of African ungulates' *Journal of Animal Ecology* 76, 526-537.
- Coetsee, C., Bond, W.J. & February, E.C. 2010, 'Frequent fire affects soil nitrogen and carbon in an African savannaby changing woody cover' *Oecologia* 162, 1027-1034.
- Coetzee, B. J., Engelbrecht, A. H., Joubert, S. C. J., & Retief, P. F., 2008, 'Elephant impact on *Sclerocarya caffra* trees in *Acacia nigrescens* tropical plains thornveld of the Kruger National Park', *Koedoe-African Protected Area Conservation and Science* 22, 39-60.
- Conover, W.J., Johnson, M.E. & Johnson, M.M., 1981, 'A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data', *Technometrics* 23: 351-361.
- Cooper, S.M. & Owen-Smith, N., 1985, 'Condensed tannins deter feeding by browsing ruminants in a South African savanna', *Oecologia* 67, 142-146.
- Cornelissen, J. H. C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D. E., & Poorter, H., 2003, 'A handbook of protocols for standardised and easy measurement of plant functional traits worldwide', *Australian Journal of Botany* 51, 335-380.
- Díaz, S., Lavorel, S., McIntyre, S. U. E., Falczuk, V., Casanoves, F., Milchunas, D. G., & Cambell, B. D., 2007, 'Plant trait responses to grazing—a global synthesis', *Global Change Biology* 13, 313-341.
- Fornara, D. A., & Toit, J. D., 2007, 'Browsing lawns? Responses of *Acacia nigrescens* to ungulate browsing in an African savanna', *Ecology* 88, 200-209.

- Gadd, M. E., 2002, 'The impact of elephants on the marula tree *Sclerocarya birrea*', *African Journal of Ecology* 40, 328-336.
- Haslam, E. 1988. 'Plant polyphenols (syn. vegetable tannins) and chemical defense- a reappraisal' *Journal of Chemical Ecology* 14, 1789-1805.
- Hattas, D., Stock, W. D., Mabusela, W. T., & Green, I. R., 2005, 'Phytochemical changes in leaves of subtropical grasses and fynbos shrubs at elevated atmospheric CO₂ concentrations', *Global and Planetary Change* 47, 181-192.
- Hattas, D., & Julkunen-Tiitto, R., 2012, 'The quantification of condensed tannins in African savanna tree species', *Phytochemistry Letters* 5, 329-334.
- Helm, C. V., & Witkowski, E. T., 2012, 'Continuing decline of a keystone tree species in the Kruger National Park, South Africa', *African Journal of Ecology*, DOI: 10.1111/aje.12032.
- Huntly, N., 1991, 'Herbivores and the dynamics of communities and ecosystems'. *Annual Review of Ecology and Systematics* 22, 477-503.
- Jachmann, H., & Croes, T., 1991, 'Effects of browsing by elephants on the *Combretum/Terminalia* woodland at the Nazinga Game Ranch, Burkina Faso, West Africa', *Biological Conservation* 57, 13-24.
- Kindt, R., & Coe, R. 2005, 'Tree diversity analysis: A manual and software for common statistical methods for ecological and biodiversity studies' World Agroforestry Centre, Eastern and Central Africa Program.
- Kriebitzsch, W. U., Oheimb, G. V., Ellenberg, H., Engelschall, B., & Heuveldop, J., 2000, 'Development of woody plant species in fenced and unfenced plots in deciduous forests on glacial moraine soils in northern Germany', *Allgemeine Forst-und Jagdzeitung* 171, 1-10.
- Levick, S., & Rogers, K., 2008, 'Patch and species specific responses of savanna woody vegetation to browser exclusion', *Biological Conservation* 141, 489-498.

- Levick, S. R., Asner, G. P., Kennedy-Bowdoin, T., & Knapp, D. E., 2009, 'The relative influence of fire and herbivory on savanna three-dimensional vegetation structure', *Biological Conservation* 142, 1693-1700.
- Levick, S. R., Asner, G. P., Kennedy-Bowdoin, T., & Knapp, D. E., 2010, 'The spatial extent of termite influences on herbivore browsing in an African savanna', *Biological Conservation* 143, 2462-2467.
- Lewis, D. M., 1991, 'Observations of tree growth, woodland structure and elephant damage on *Colophospermum mopane* in Luangwa Valley, Zambia', *African Journal of Ecology* 29, 207-221.
- Moncrieff, G. R., Chamaille-Jammes, S., Higgins, S. I., O'Hara, R. B., & Bond, W. J., 2011, 'Tree allometries reflect a lifetime of herbivory in an African savanna', *Ecology* 92, 2310-2315.
- O'Connor, T. G., Goodman, P. S., & Clegg, B., 2007, 'A functional hypothesis of the threat of local extirpation of woody plant species by elephant in Africa', *Biological Conservation* 136, 329-345.
- Owen-Smith, N. 2005. 'Incorporating fundamental laws of biology and physics into population ecology: the metaphysiological approach' *Oikos*, 111, 611-615.
- Owen-Smith, N., & Cooper, S. M., 1987, 'Palatability of woody plants to browsing ruminants in a South African savanna', *Ecology* 68, 319-331.
- Penzhorn, B. L., Robbertse, P. J., & Olivier, M. C., 1974, 'The influence of the African elephant on the vegetation of the Addo Elephant National Park', *Koedoe-African Protected Area Conservation and Science* 17, 137-158.
- R Core Team, 2012, 'R: A language and environment for statistical computing', R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Shrader, A. M., Bell, C., Bertolli, L., & Ward, D., 2011, 'Forest or the trees: At what scale do elephants make foraging decisions?', *Acta Oecologica* 42, 3-10.

- Smallie, J. J., and T. G. O'Connor., 2000, 'Elephant utilization of *Colophospermum mopane*: possible benefits of hedging', *African Journal of Ecology* 38, 1–9.
- Smit, I. P., Smit, C. F., Govender, N., Linde, M. V. D., & MacFadyen, S., 2012, 'Rainfall, geology and landscape position generate large-scale spatiotemporal fire pattern heterogeneity in an African savanna', *Ecography* 35, 1-13.
- Staver, A. C., Bond, W. J., Stock, W. D., Van Rensburg, S. J., & Waldram, M. S., 2009, 'Browsing and fire interact to suppress tree density in an African savanna', *Ecological Applications* 19, 1909-1919.
- Trollope, W.S.W., Trollope, L.A., Biggs, H.C., Pienaar, D. & Potgieter, A.L.F., 1998, 'Long-term changes in the woody vegetation of the Kruger National Park, with special reference to the effects of elephants and fire', *Koedoe-African Protected Area Conservation and Science* 41, 103–112.
- Van Hees, A. F. M., Kuiters, A. T., & Slim, P. A., 1996, 'Growth and development of silver birch, pedunculate oak and beech as affected by deer browsing', *Forest Ecology and Management* 88, 55-63.
- Venter, F. J., Scholes, R. J., & Eckhardt, H. C., 2003, 'The abiotic template and its associated vegetation pattern', *The Kruger experience: Ecology and management of savanna heterogeneity*, 83-129.
- Wakeling, J. L., & Bond, W. J., 2007, 'Disturbance and the frequency of root suckering in an invasive savanna shrub, *Dichrostachys cinerea*', *African Journal of Range and Forage Science* 24, 73-76.
- Wessels, D.C.J., van der Waal, C., de Boer, W.F., 2007, 'Induced chemical defences in *Colophospermum mopane* trees', *African Journal of Range and Forage Science* 24, 141–147.
- Wigley, B.J., Coetsee, C., Hartshorn, A.S., Bond, W.J., 2013, 'What do ecologists miss by not digging deep enough? Insights and methodological guidelines for assessing soil fertility status in ecological studies', *Acta Oecologica* 51, 17-27.

TABLES

Table 1. Site names, locations and descriptions of the exclosures in the Kruger National Park where sampling took place.

Site	Location	Rainfall, geology	Co-ords E	Co-ords S
Nhlangwini exclosure	Pretoriuskop	750 mm, granites	31.292633°	-25.19907°
Makhohlola exclosure	Crocodile Bridge	600 mm, basalts	31.913737°	-25.262083°
N'waxitshumbe exclosure	Northern Plains	400 mm, basalts	31.25825°	-22.77818°

Table 2. Dominant woody species accounting for >80% of standing biomass for inside and outside treatments at the three exclosures.

Site	Treatment	Species
Makhohlola	IN	<i>Acacia gerrardii</i>
Makhohlola	IN	<i>Acacia nigrescens</i>
Makhohlola	IN	<i>Dalbergia melanoxylon</i>
Makhohlola	IN	<i>Lannea schweinfurthii</i>
Makhohlola	OUT	<i>Albizia harveyi</i>
Makhohlola	OUT	<i>Gymnosporia senegalensis</i>
Makhohlola	OUT	<i>Acacia gerrardii</i>
Makhohlola	OUT	<i>Acacia nigrescens</i>
Nhlangwini	IN	<i>Dalbergia melanoxylon</i>
Nhlangwini	IN	<i>Euclea divinorum</i>
Nhlangwini	IN	<i>Grewia monticola</i>
Nhlangwini	IN	<i>Strychnos madagascariensis</i>
Nhlangwini	OUT	<i>Antidesma venosum</i>
Nhlangwini	OUT	<i>Dichrostachys cinerea subsp. africana</i>
Nhlangwini	OUT	<i>Sclerocarya birrea</i>
Nhlangwini	OUT	<i>Terminalia sericea</i>
Nwaswitshumbe	IN	<i>Dalbergia melanoxylon</i>
Nwaswitshumbe	IN	<i>Grewia monticola</i>
Nwaswitshumbe	IN	<i>Ozoroa paniculosa</i>
Nwaswitshumbe	IN	<i>Pterocarpus rotundifolius</i>
Nwaswitshumbe	IN	<i>Sclerocarya birrea</i>
Nwaswitshumbe	OUT	<i>Colophospermum mopane</i>
Nwaswitshumbe	OUT	<i>Combretum imberbe</i>
Nwaswitshumbe	OUT	<i>Philenoptera violacea</i>

Table 3. Traits measured for each species at three exclosures in the Kruger National Park.

Trait	Abbreviation	Unit
Leaf N content	Leaf N	%
Leaf carbon:nitrogen	Leaf C:N	ratio
Leaf phosphorus content	Leaf P	%
Specific leaf area	SLA	cm ² g ⁻¹
Average leaf area	ALA	cm ²
Leaf dry matter content	LDMC	mg g ⁻¹
Leaf tensile strength	TS	N mm ⁻¹
Bite size index	BSI	NA
Stem specific density	Stem density	g cm ⁻³
Total polyphenols	TP	%
Condensed tannins	CT	%
Neutral detergent fibre	NDF	%
Acid detergent fibre	ADF	%
Acid detergent lignin	ADL	%

Table 4. Mean (\pm se) values of the measured leaf traits for the inside and outside treatments of three exclosures in the Kruger National Park.

Trait	Makhohlola		N'waxitshumbe		Nhlangwini	
	IN	OUT	IN	OUT	IN	OUT
Leaf N (%)	2.48 \pm 0.25	2.16 \pm 0.16	2.15 \pm 0.43	1.83 \pm 0.26	2.35 \pm 0.53	1.50 \pm 0.33
Leaf C (%)	39.4 \pm 2.00	36.7 \pm 0.88	46.3 \pm 1.33	44.9 \pm 1.76	45.5 \pm 0.4	46.1 \pm 2.39
Leaf C:N	16.1 \pm 1.03	16.9 \pm 1.5	25.6 \pm 5.27	25.6 \pm 4.09	21.4 \pm 4.65	32.2 \pm 5.28
Leaf P (%)	0.13 \pm 0.01	0.12 \pm 0.02	0.21 \pm 0.04	0.23 \pm 0.08	0.11 \pm 0.02	0.09 \pm 0.001
SLA cm ² g ⁻¹	80.2 \pm 9.08	57.3 \pm 19.5	82.6 \pm 10.3	120 \pm 69.1	93.6 \pm 9.16	81.4 \pm 7.45
Leaf size cm ²	28.0 \pm 14.7	12.7 \pm 3.57	46.9 \pm 16.1	41.3 \pm 25.3	15.7 \pm 5.73	38.0 \pm 14.8
BSI	11.0 \pm 5.73	8.06 \pm 3.56	19.8 \pm 4.52	18.8 \pm 2.45	4.12 \pm 0.82	9.50 \pm 1.60
TS N mm ⁻¹	1.35 \pm 0.43	2.44 \pm 0.93	0.71 \pm 0.09	1.14 \pm 0.20	0.91 \pm 0.14	0.75 \pm 0.27
TP (%)	0.95 \pm 0.08	1.24 \pm 0.22	2.62 \pm 0.41	3.21 \pm 1.20	2.23 \pm 0.46	4.68 \pm 1.47
CT (%)	5.11 \pm 1.30	3.99 \pm 0.79	2.68 \pm 0.73	3.56 \pm 1.50	4.03 \pm 2.61	4.41 \pm 1.71
NDF (%)	60.8 \pm 2.65	58.0 \pm 4.53	49.7 \pm 3.62	42.9 \pm 6.44	52.3 \pm 4.34	57.3 \pm 2.57
ADF (%)	36.2 \pm 6.33	37.5 \pm 4.59	33.9 \pm 4.07	28.3 \pm 6.76	32.9 \pm 2.02	37.2 \pm 4.47
ADL (%)	15.4 \pm 3.67	18.9 \pm 3.32	4.45 \pm 1.50	8.89 \pm 3.55	5.61 \pm 1.46	7.43 \pm 3.19

FIGURE CAPTIONS

Figure 1. The relative abundance of the species found inside and outside the three exclosures in the Kruger National Park. Species names have been abbreviated using the first three letters of both the genus and species names.

Figure 2. Total tree densities for all woody plants greater than 0.5 m in height for inside and outside the three long-term herbivore exclosures in the Kruger National Park.

Figure 3. Size class distributions of *Terminalia sericea* and *Dichrostachys cinerea* populations growing inside and outside of the Nhlangwini exclosure in the Kruger National Park.

Figure 4. Size class distributions of *Lannea schweinfurthii*, *Gymnosporia senegalensis*, *Flueggea virosa* and *Acacia nigrescens* populations growing inside and outside of the Makhohlola exclosure in the Kruger National Park.

Figure 5. Size class distributions of *Colophospermum mopane*, *Combretum imberbe* and *Dalbergia melanoxylon* populations growing inside and outside of the N'waxitshumbe exclosure. No *Dalbergia melanoxylon* plants were found growing on the outside of the exclosure in the Kruger National Park.

Figure 6. Leaf nitrogen (A), leaf phosphorus (B), specific leaf area (C) and bite size index (D) plotted against the proportion of species found on the inside compared to the outside of three exclosures in the Kruger National Park. A value of 1 indicates that the species was only found inside while a value of 0 indicates the species was only found outside of the exclosures.

Figure 7. Mean (\pm se) soil N, C, P and K concentrations inside and outside of three exclosures in the Kruger National Park. Plotted means are calculated from five replicates taken at three depths, N = 15 for each treatment.

Figure 8. Principal components analysis of trait measurements from 20 species sampled inside and outside of three exclosures in the Kruger National Park. NDF, ADL and TP were most

influential along PCA1 (24 % of total variation) while leaf N, leaf C:N and stem density were most influential along PCA2 (20 % of total variation). The locations of each species on the first two PCs are shown as well as the groupings by treatment (inside *vs.* outside).

FIGURES

Figure 1

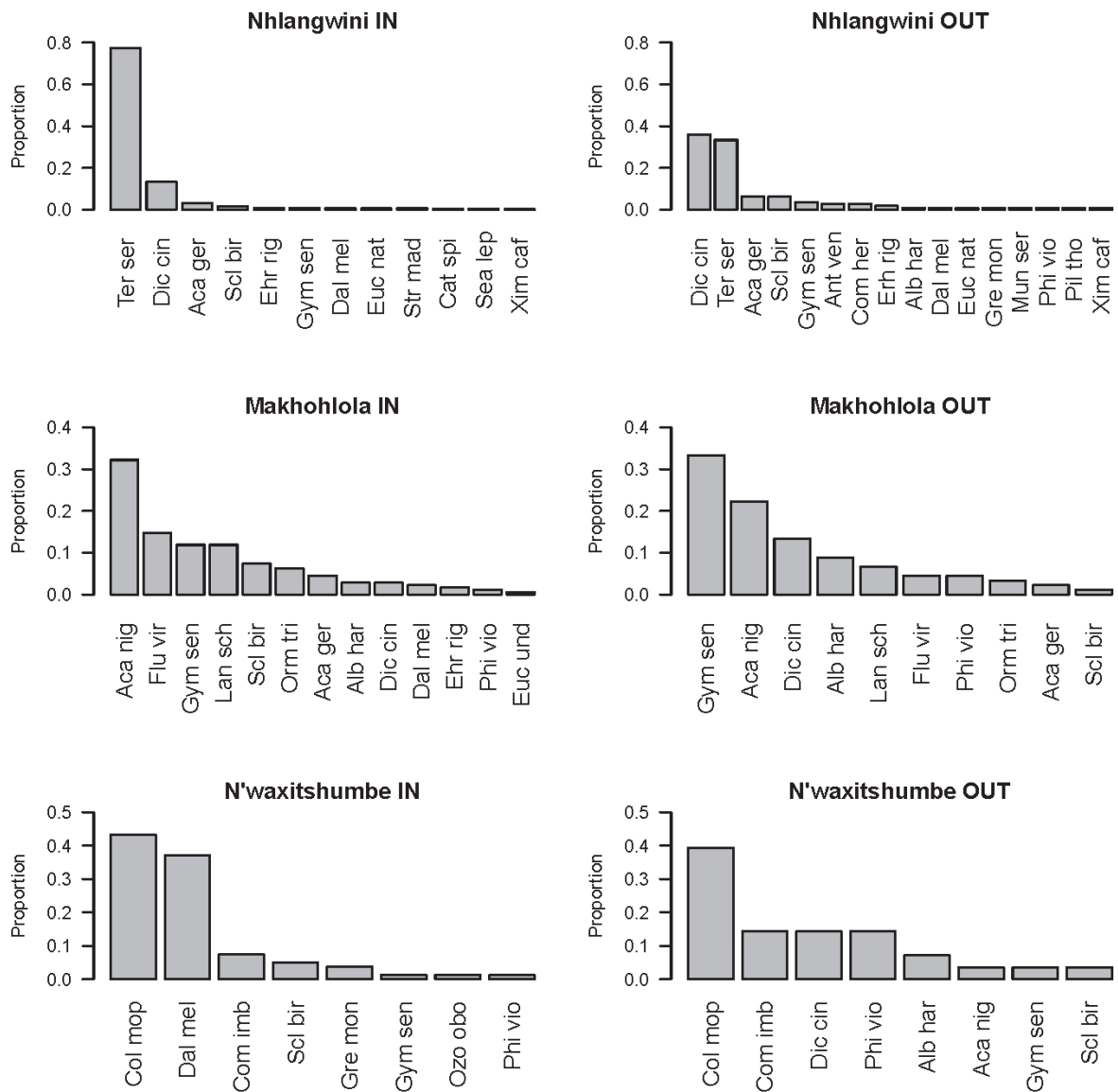


Figure 2

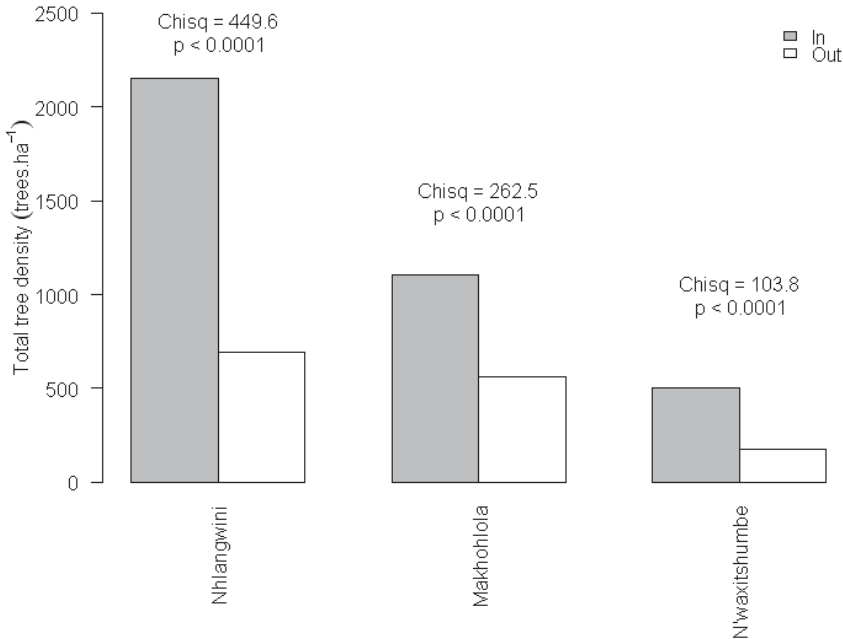


Figure 3

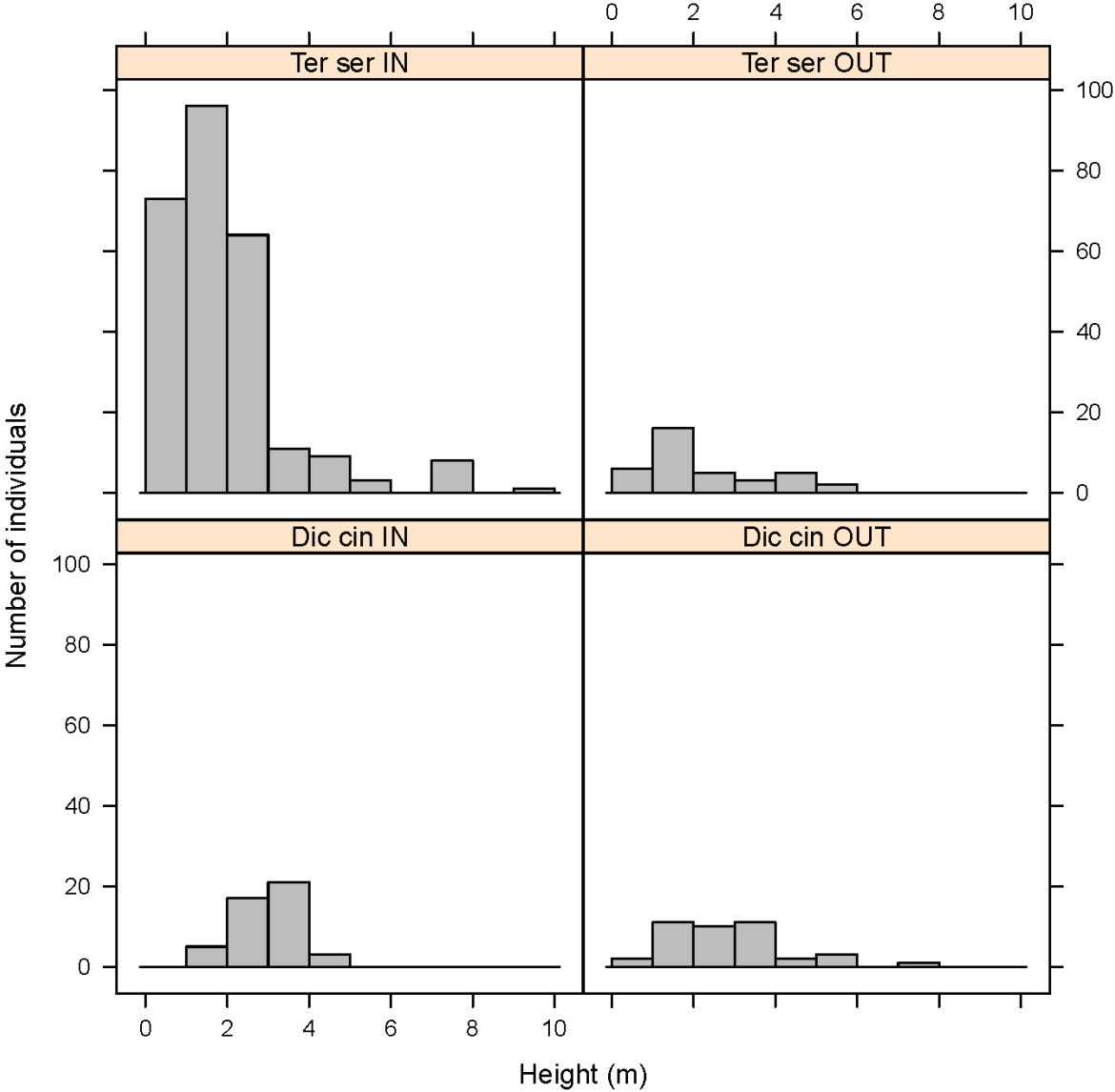


Figure 4

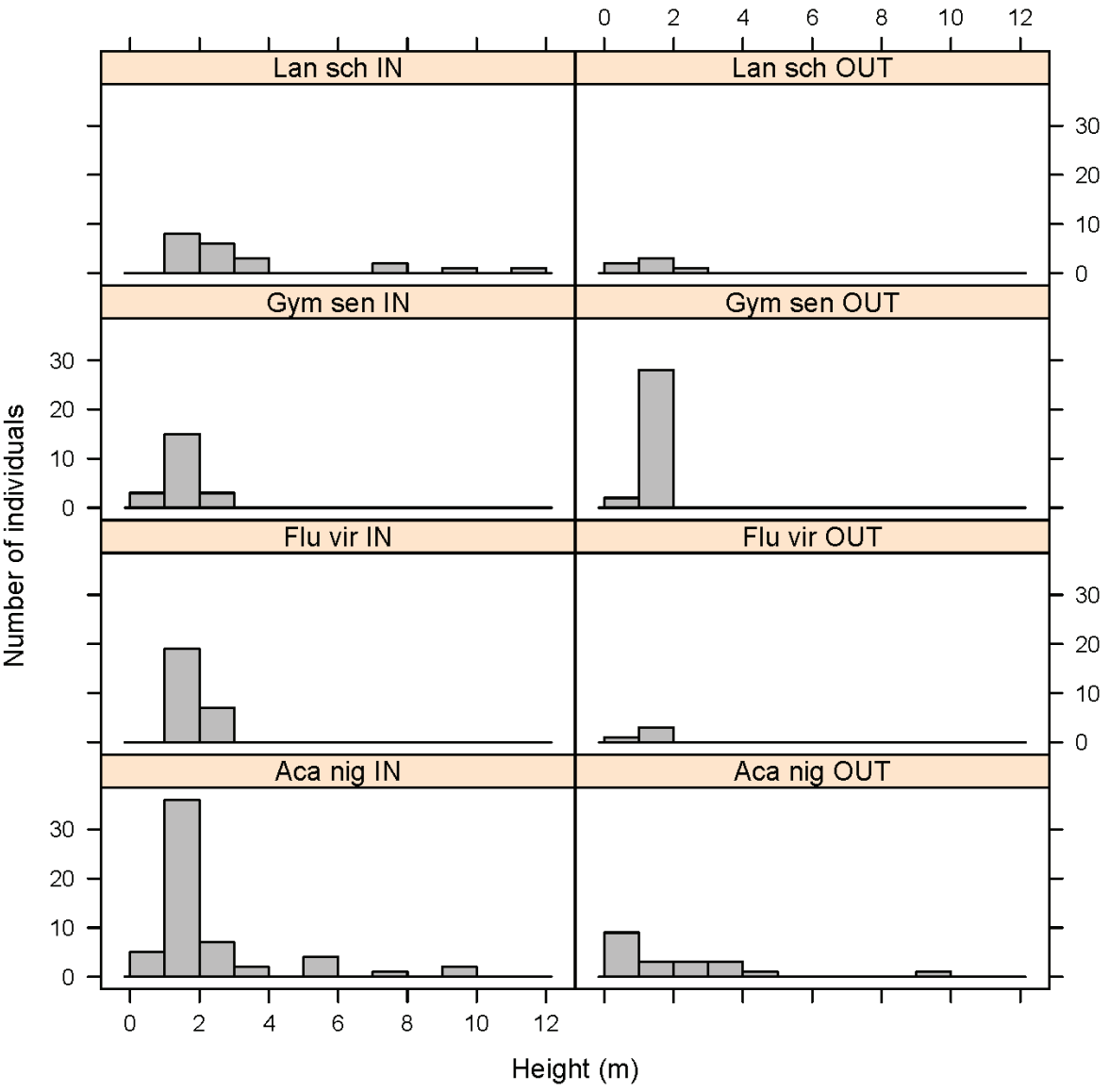


Figure 5

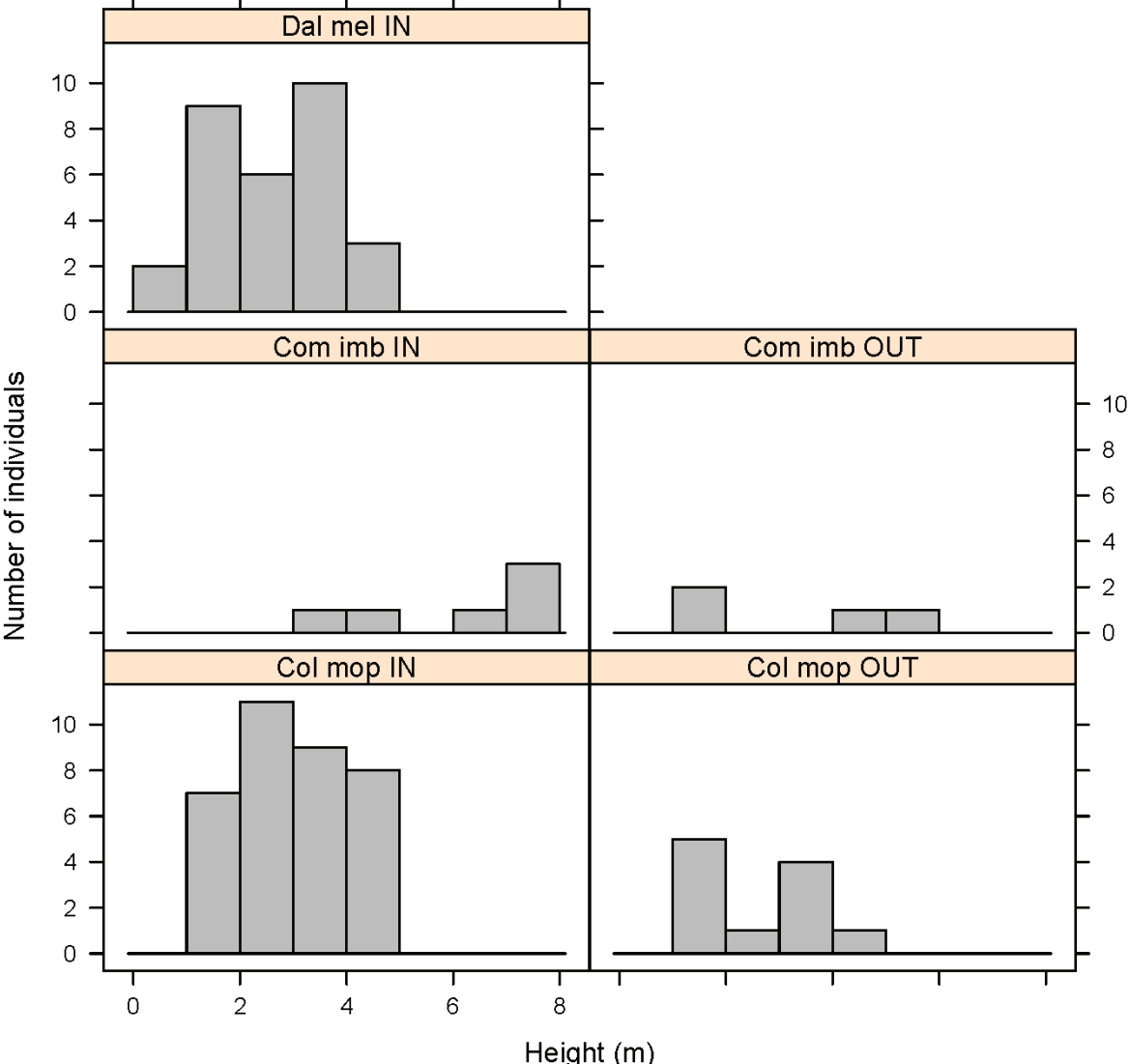


Figure 6

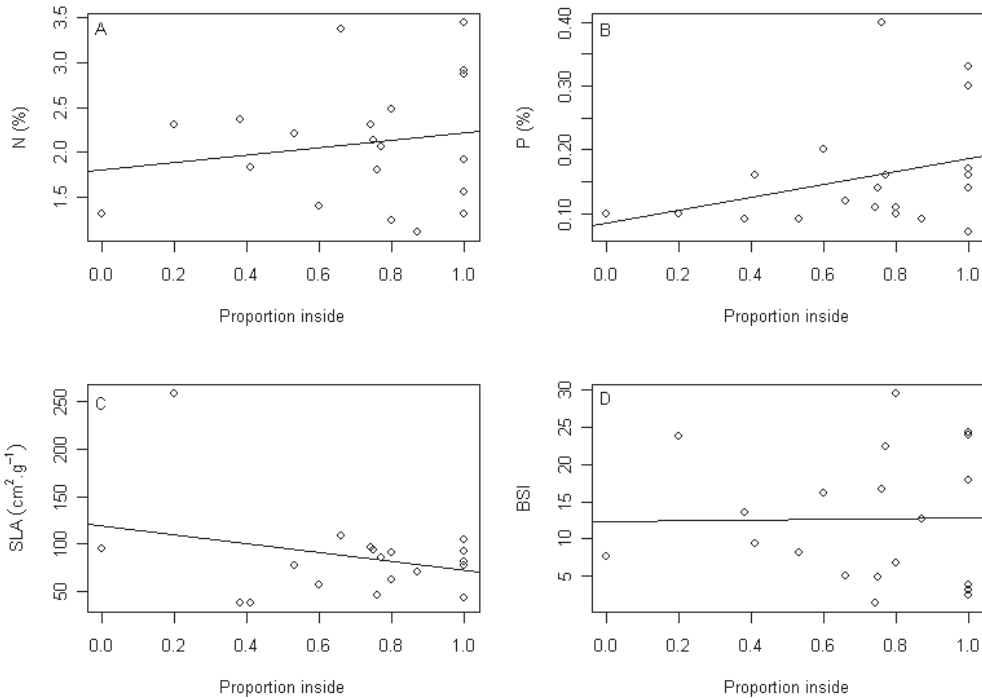


Figure 7

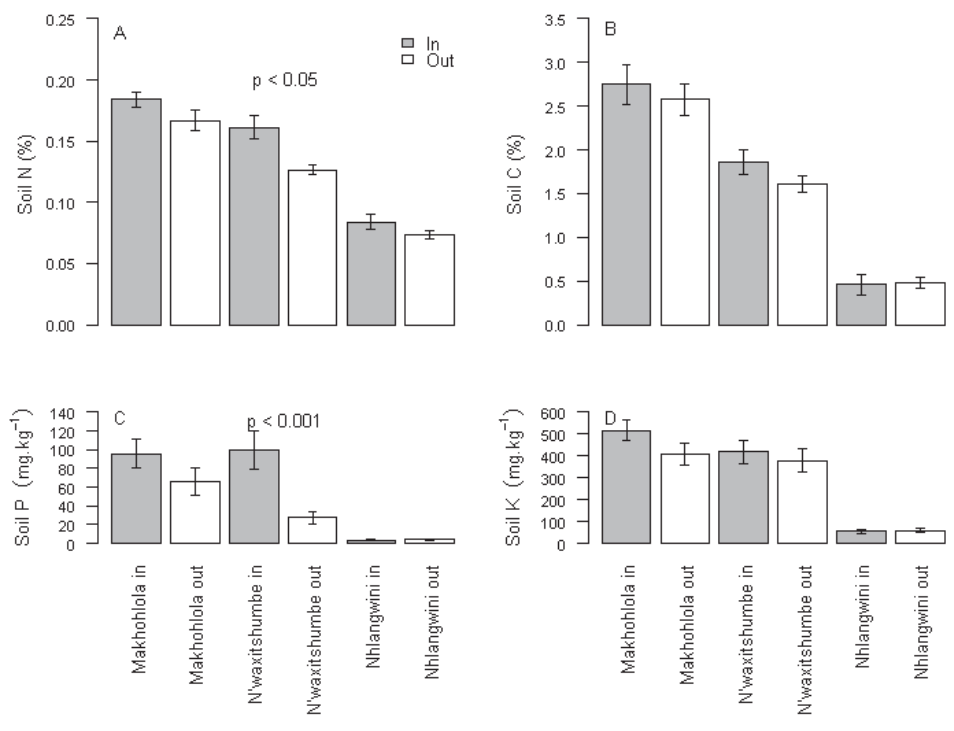
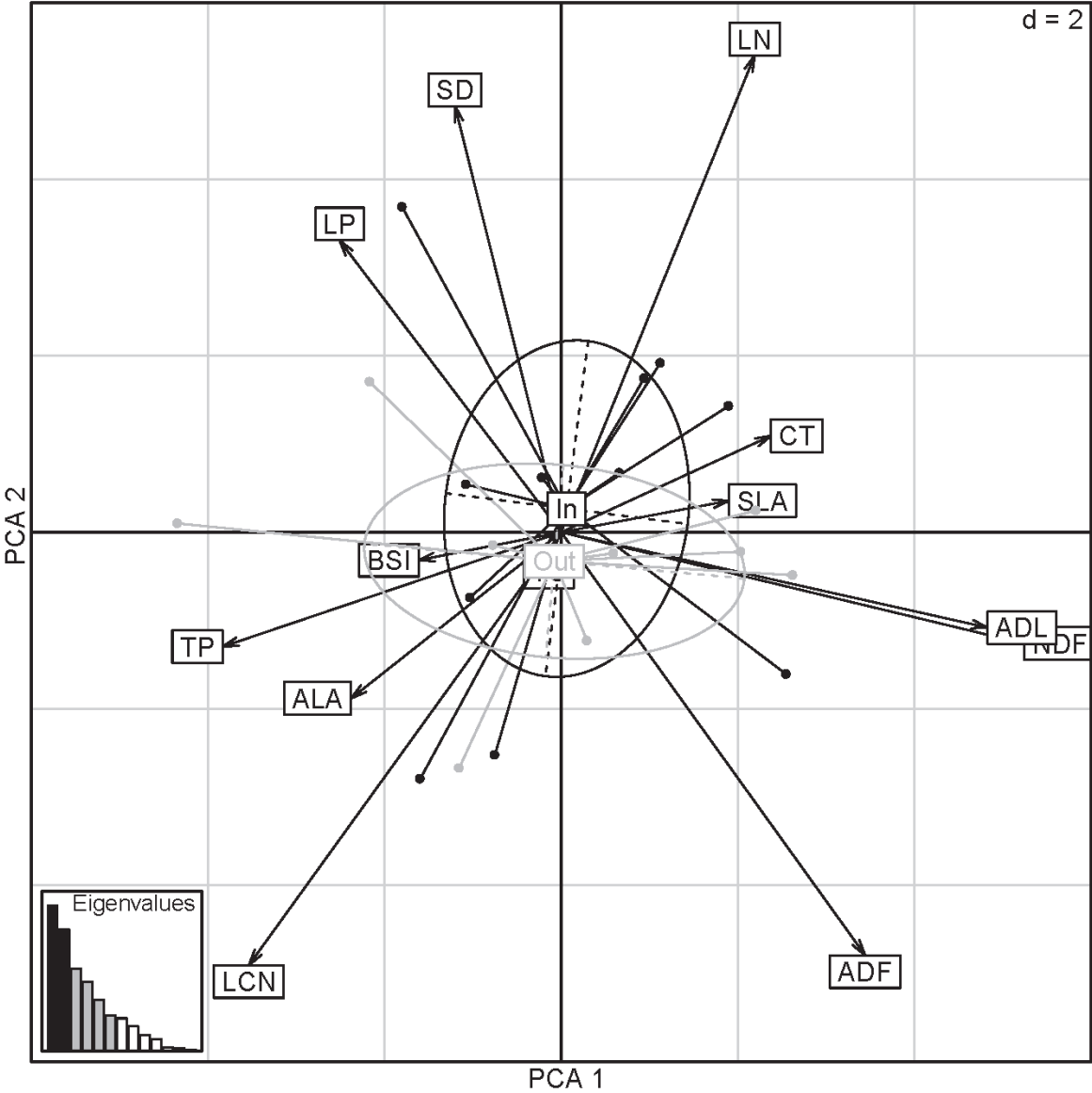


Figure 8





Higher tree densities evident in the Makhohlolo exclosure, Kruger National Park.

Chapter 7 Evidence for a Trade-off in Acacia Anti-mammal Defences in South African Savannas

Submitted to *Biotropica*

Benjamin J. Wigley^{1,2*}, William J. Bond³ & Hervé Fritz²

¹ School of Natural Resource Management, Nelson Mandela Metropolitan University, George campus, South Africa

² UMR CNRS 5558 – LBBE, Université Claude Bernard Lyon 1, Bât. Grégor Mendel 43 bd du 11 novembre 1918, 69622 Villeurbanne cedex

³ Department of Biological Sciences, University of Cape Town, P/Bag, Rondebosch, 7701, South Africa

* Corresponding author: email: benjamin.wigley@nmmu.ac.za, telephone: +27 44 8015018, fax: +27 44 8056625

Abstract

Previous studies have shown a major dichotomy in savanna woody plant strategies and architectures. Mesic species often tend to be fast growing and have pole-like architectures allowing for rapid escape from fire, while semi-arid species often tend to have cage-like architectures which protect the growing parts from browsers. We investigate whether this ecological trade-off can be extended to defence traits. Five sets of herbivore exclosures situated in mesic and semi-arid savannas in Hluhluwe-iMfolozi Park, South Africa were used to explore

and contrast the effects of mammal browsers on savanna *Acacia* sapling growth rates, palatability, structural and chemical defences. Browser effects on leaf nutrient content were mostly negligible for mesic species but tended to increase the quality of leaf material in semi-arid species. A clear trade-off in defence strategy was evident; fire adapted mesic species invested more heavily in growth and chemical defences, while herbivore adapted semi-arid species invested more in architectural and structural defences. Mammal browsers resulted in significantly lower levels of total polyphenols in two of three semi-arid species, while their effect was mostly negligible in mesic species. The effect of browsers on architecture and structural defences was most pronounced in semi-arid species resulting in cage-like architectures and longer and thicker spines. The findings of this study suggest that chemical defences are not important in deterring browsers in semi-arid species that are exposed to high levels of herbivory but may more important in mesic species, possibly as a defence against insect herbivory.

Keywords: herbivore exclosures; mesic; plant defences; semi-arid

HERBIVORE DAMAGE TO PLANTS can often lead to changes in their nutrient status, the production of defensive chemicals and volatile substances, physical defence structures of thorns, spines, and trichomes, plant architecture, and phenology of plants (Karban & Baldwin 1997). These changes in plant traits following herbivory are important in determining food and habitat suitability for herbivores that subsequently utilize the same plant (Ohgushi 2005). While many studies have examined the interactions and responses of plant traits to invertebrate herbivory (*e.g.* see review by Ohgushi 2005), fewer studies have focused on how plant defence traits respond to mammalian herbivory. Hanley *et al.* (2007) in their review of the types of structural defences and

their role in anti-herbivore defence highlight how poorly our understanding of plant structural defence fits into modern plant defence theory.

In recent years a number of studies investigating the role of browsers in influencing savanna vegetation have taken advantage of the fact that African savannas still have a rich diversity of native mammalian browsers. Herbivore exclosures have effectively been used to elucidate the effect of these mammalian herbivores on savanna vegetation and dynamics. For example, studies by Staver *et al.* (2009, 2012) demonstrated the importance of the interaction between fire and browsers in controlling woody densities and growth rates. Sankaran *et al.* (2013) found that browsers, in the absence of fire, significantly affect recruitment, growth rates and mortality rates of woody species in a semi-arid east African savanna. Young *et al.* (2013) showed that the response of east African savanna vegetation to herbivore exclusion, or replacement of wildlife species by domestic species, is strongly modulated by soil properties and rainfall.

More commonly, studies investigating the responses of savanna woody species to herbivory have simulated herbivory in clipping experiments or observed the response of specific species to natural herbivory by browsers (*e.g.* Du Toit *et al.* 1990, Rooke *et al.* 2004). Studies on plant responses to herbivory typically measure foliar nutrients and secondary metabolites relating to chemical defence, while some also include the response of structural defences to herbivory. The response of foliar nutrients and carbon based secondary metabolites (CBSMs) to browsing intensity is inconsistent among studies in savannas (*e.g.* see Scogings *et al.* 2011). Increased resource allocation to structural defences after induced herbivory has been reported (*e.g.* Rohner & Ward 1997, Hean & Ward 2011), while no response was found by Rooke *et al.* (2004).

The variability in plant response to herbivory highlights the uncertainty regarding the role of chemical vs. structural defences in deterring mammalian browsers in savannas. The

importance of plant chemical defences in deterring insect herbivory is well documented (*e.g.* see reviews by Herms and Mattson 1992, Ogushi 2005). Surprisingly few studies have investigated the importance of insect herbivory in savannas (Anderson *et al.* 1991). Manual defoliation has been found to increase chemical defences in the regrowth of fast growing but not slow growing savanna species in South Africa (Bryant *et al.* 1991). Evidence showing the effectiveness of chemical defences in deterring mammal herbivory is limited and may vary seasonally (Cooper & Owen-Smith 1985, Cooper *et al.* 1988). Owen-Smith (2002) showed very little difference in the palatability of South African savanna woody species related to leaf chemistry (polyphenols and tannins) except for species with extreme values. On the other hand structural defences have been shown to effectively slow down rates of herbivory in mammalian herbivores (Cooper & Owen-Smith 1986, Illius *et al.* 2002, Wilson & Kerley 2003). It is clear that the relative importance of each type of defence in protecting plants from mammalian herbivory in African savannas remains far from being well understood.

It is therefore interesting to explore whether mammalian herbivory induces changes in both structural and chemical defences. African savannas have been shown to differ in the intensity of herbivory versus damage from fire, with evidence for a trade-off such that architectures of acacias growing in mesic frequently burnt savannas are more vulnerable to browsing but more resistant to fire, while the reverse is true for semi-arid acacias (Archibald & Bond 2003, Staver *et al.* 2012). Can this trade-off be extended to induced responses to browsing where browsers are the major factor limiting sapling release in semi-arid savannas vs. fire in mesic savannas?

The results of previous studies suggest that the effect of simulated or observed browsing often results in increased regrowth or overcompensation, while the response of foliar nutrients and defences tends to be more variable. This study aims to determine how a suite of plant traits

relating to architecture, growth rate, structural and chemical defences in seven African savanna *Acacia* (four mesic and three semi-arid) species are affected by mammal herbivory. This was determined by comparing traits on saplings that either had never been exposed to herbivory (h-) or that had always been exposed to herbivory (h+). The following specific questions are addressed: (1) Do traits relating to plant palatability, architecture, structural and chemical defences differ between saplings of the same species exposed to (h+) or protected from (h-) mammal herbivory in mesic vs. semi-arid savannas? (2a) Are there differences in growth rates between plants growing inside and outside of the exclosures? and (2b) Are there differences in growth rates between mesic vs. semi-arid species? (3) Does simulated herbivory (clipping) affect growth rate, architecture, chemical and structural defences of *Acacia nilotica* saplings that had not previously been exposed to herbivory?

We predict lower investments in structural defences in fire-adapted mesic *Acacia* species compared to herbivore-adapted semi-arid species. If chemical defences are effective at deterring mammal herbivores, the expectation would be that semi-arid species which experience high levels of herbivory would invest in chemical defences. We expect higher chemical and structural defences in plants exposed to herbivory compared to protected plants. Mesic species are predicted to have faster growth rates than semi-arid species. Clipping is expected to result in increased growth rates and structural defences in *Acacia nilotica*.

METHODS

STUDY SITES — Hluhluwe-iMfolozi Park (HiP) is a regional game reserve in northern Zululand, KwaZulu-Natal, South Africa. A strong altitudinal gradient exists between Hluhluwe Game Reserve in the north and iMfolozi Game Reserve in the south (40 -750 m asl). Balfour &

Howison (2001) demonstrated how the altitudinal gradient has resulted in a strong rainfall gradient from north to south with mean annual precipitation (MAP) in the north of the park of around 990 mm, while in iMfolozi MAP is less than 600 mm. The temperatures in the region are moderate to hot with temperatures of between 14°C and 40°C in summer and between 6°C and 34°C in winter (Staver *et al.* 2009). Frost seldom occurs due to the mild winter temperatures and low altitudes at the study sites. Thunderstorms are a common feature of the summer rainfall season with lightning strikes occurring at densities of *ca.* five to six ground flashes per square kilometre per year. The geology of the area usually results in clay rich soils derived from shales, mudstones and dolerite outcrops of the Karoo Supergroup.

Three broad vegetation types have been described in HiP. These include grasslands and forested hilltops, which are usually found at higher altitudes (above 450 m asl). Riverine forest usually dissects the park along its watercourses. The remainder of the park is a mixture of fine-leaved Acacia savannas and broad-leaved woodland. The Acacia savanna ranges from open to closed canopy patches (Acocks 1988).

A number of herbivore exclosures were erected in Hluhluwe-iMfolozi Park (HiP) in the late 1990s as part of a study investigating the role of fire and browsers on tree recruitment (see Staver *et al.* 2009 for details). The ‘total exclosure’ (excludes hares and all larger herbivores) treatment was chosen from three of the sites in the mesic Hluhluwe part of the reserve, and two of the sites in the semi-arid iMfolozi part of the reserve. All sites have been burned on a two-year fire return interval for the majority of the experiment, a frequency similar to the management burns of surrounding areas. A number of recruits of mostly Acacia species have subsequently grown into the sapling size class within these exclosures (see Table 1 for exclosure names and sampled species). The above-ground stems of these individuals have therefore never been exposed to browsers.

GROWTH MEASUREMENTS — During the early growing season of 2009 (October), five saplings of *A. nilotica*, *A. caffra* and *A. burkei* were selected both inside and outside of the full enclosure at the Klassana enclosures and five saplings of *A. karroo* were selected inside and outside the full enclosure at Le Dube in the Hluhluwe section of HiP. In the iMfolozi section five saplings of *A. grandicornuta* were selected inside and outside of the full enclosure at the Mona enclosures and five individuals of *A. nigrescens* and *A. tortilis* were selected inside and outside of the full enclosure at Thobothi. Each selected sapling was labelled, and the stem diameter was measured at 10 cm above ground level. The point where the measurement was taken was marked using waterproof red paint. On each individual, three branches were selected and marked from the lower, mid and upper canopy as done by Rooke *et al.* (2004). The length and diameter of each branch was recorded and the points where diameters were measured were also marked with red paint. The total number and length of all branchlets growing on each selected branch were counted, measured and recorded.

For the clipping experiment, five *A. nilotica* saplings were selected and tagged inside and outside of the Seme enclosure in the Hluhluwe section of the park. Inside the enclosure a further five saplings were selected for clipping. For all three treatments, the five individuals were measured and marked as described above. All branchlet tips (3-5 cm) were then clipped off every auxiliary branch of each of the five individuals chosen for clipping. These plants were then re-clipped in the same fashion, two and four months later. If growth is all about escaping the fire-trap in mesic savannas (Bond & van Wilgen 1996), we would expect few shoots after clipping and for these to extend in length a lot, while in herbivore dominated semi-arid species we would expect the plant to grow new shoots in order to become more cagey and therefore to have lots of little branches, which would form a cage around the branch that was cut.

At the end of the growing season (April 2010 and again in April 2011) all tagged plants were re-measured in order to determine growth rates. For the clipped plants the length of new shoots and the length and diameter of thorns occurring on the new growth were measured and recorded. At the Mona enclosure where *A. grandicornuta* was monitored, the enclosure was removed after the first year of measurements, the same individuals were re-measured again after a year of exposure to herbivores.

PLANT TRAIT MEASUREMENTS — Plant traits relating to leaf chemistry, plant defence and plant architecture from individuals of the same species growing inside and outside of herbivore enclosures (> 12 years of herbivore exclusion) were measured. Saplings (< 3m height) were chosen for this experiment as it ensured that the sampled plants inside of the enclosures had never been exposed to herbivory (i.e. were younger than the enclosures). Furthermore, selective browsing on seedlings and saplings has a strong effect on woody vegetation and may determine the composition of the mature stand (Vourc'h *et al.* 2002, Danell *et al.* 2003). Bond (2008) and Wakeling *et al.* (2011) have also shown that selective pressure by fire and herbivory is most pronounced in the sapling stage in savannas.

Average spine length and diameter (at thorn base) was determined for each plant by measuring ten mature spines from each of three branches, taken from the upper, middle and lower canopy of each plant using a set of digital vernier callipers. Spines were measured starting from the outer edge moving inwards on each branch. Spine density was determined by dividing the total number of spines on each of the three branches and by branch total length. All spine measurements were repeated twice, at the same time as the growth measurements. The efficacy of spines is influenced by their arrangement on adjacent branches. Densely ramified shoots produce cage-like defences whereas sparsely branched shoots can offer easier animal access. As

an index of the efficacy of the structural defences, we developed a ‘bite size index’ (BSI) where an observer attempts to remove as much foliage from a shoot as possible using only the mouth to remove the tissue (see Wigley *et al.* 2013 for further details). The BSI (dry weight of leaves recovered from ten human bites) for each individual of each species in both treatments was determined. Leaf material was taken from each individual in order to determine carbon (C) and nutrient concentrations [nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), and calcium (Ca)]. For each species the leaves were analysed for N and C using a Leco TruSpec CN Analyser (LECO Corporation, St. Joseph, MI). Leaf P, K, Mg, Na and Ca were analysed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Varian Vista MPX, Palo Alto, CA, USA). Total condensed tannins (CT) were calculated according to the methods described by Hattas & Julkunen-Tiitto (2012) while total polyphenols (TP) were calculated according to the methods described by Hattas *et al.* (2005). Cell wall constituents, *i.e.* neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined in an Ankom fibre analyzer, whereas acid detergent lignin (ADL) was determined in accordance with the acid detergent lignin in beakers method. Ash was determined by incinerating the filter bag containing plant residue in a muffle furnace at 525 °C for three hours. All cell wall constituents were determined consecutively as outlined in the Ankom technology procedures (Ankom technologies).

STATISTICAL ANALYSES — In order to explore the data, principal component analyses (PCA) were performed using the `dudi.pca` function in the `ade4` package (Dray *et al.* 2007) in R (R Development Core Team 2012). The function `PCAsignificance` available in the `BiodiversityR` package (Kindt & Coe 2005) in R was used to test for significant PCA axes by comparing the proportion of explained variance of each PC with randomly selected bits of variance from the

broken-stick distribution. Due to the small sample sizes (< 6), non-parametric Wilcoxon tests (`wilcox.test` in the stats package for R) were used to test for significant differences in the measured traits between the inside (h-) and outside (h+) treatments for the seven *Acacia* species. For mesic vs. semi-arid comparisons, data was normally distributed allowing for ANOVAs to be performed using the function `aov` in the stats package for R.

RESULTS

The PCA of leaf traits (Fig. 1) showed a clear separation of sites and species along the first two axes. PC1 was related to leaf quality, showing the Hluhluwe sites and species to have higher leaf C and TP content than the iMfolozi sites and species which had higher leaf nutrients. PC2 was most closely related to CT and fibre content. The plot of species \times treatment showed the inside and outside treatments to separate along both axes, however the pattern tended to differ according to species. A PCA of architectural traits also separated mesic from semi-arid sites and species (not shown). This separation was most strongly related to the bite size index (BSI) and structural defences along PC1 and branching pattern along PC2. The effect of exclosures was most pronounced in the iMfolozi species where plants exposed to herbivores tended to be better defended, resulting in lower BSIs. Both PC1 and PC2 for both PCAs were found to explain a significant proportion of variance when compared to the proportion of variance explained using the broken stick distribution.

Leaf N differed significantly between h+ and h- treatments for *A. grandicornuta*, *A. nigrescens* and *A. nilotica* (Wilcoxon test, $p < 0.05$) with higher N concentrations in plants exposed to herbivores (Fig. 2a). Leaf C:N was significantly higher in the h- treatment of *A.*

nilotica and higher but not significantly so ($p > 0.05$) for the h- treatments of *A. grandicornuta* and *A. nigrescens* (Fig. 2b). The h+ treatment of *A. nigrescens* had a higher concentration of leaf P than the h- treatment (Wilcoxon test, $p < 0.05$). No differences were evident for all other species (Fig. 2c). There were no treatment effects on leaf tissue concentrations of K (Fig. 2d) and Ca and leaf Na was significantly higher for the h- treatment of *A. karroo*.

TP concentrations were highest in *A. burkei*, *A. karroo* and *A. nilotica*. There were no significant enclosure effects on TPs for these species. In the semi-arid savannas, however, *A. grandicornuta* and *A. nigrescens* had significantly higher (Wilcoxon test, $p < 0.05$) concentrations of TPs when protected from herbivores (Fig. 3a). Total CT concentrations were highest for *A. burkei*, *A. caffra* and *A. karroo*, with very low levels evident in *A. grandicornuta*, *A. nigrescens*, *A. nilotica* and *A. tortilis*. There were no significant differences between treatments (Wilcoxon test, $p < 0.05$) for all species (Fig. 3b). There were no obvious trends for the fibre analyses with mostly non-significant differences between treatments for NDF, ADF and ADL.

The increase in average number of branchlets and total branchlet length measured on the three marked branches of each species were both significantly higher in the semi-arid species compared to the mesic species (ANOVA, $p < 0.05$, Figs. 4a&b). In contrast to this, mean stem diameter increase and increase in plant height were both significantly higher in the mesic species when compared to the semi-arid species (ANOVA, $p < 0.05$, Figs. 4c&d). The bite size index (BSI; Fig. 5a) tended to be higher (i.e. more leaf per bite) for all species where plants were protected from herbivores except for *A. caffra*. Differences were most pronounced for the three species from semi-arid iMfolozi, with three of these (*A. grandicornuta*, *A. nigrescens* and *A. tortilis*) showing significantly higher values for the inside treatments (Wilcoxon test, $p < 0.05$). Spines were significantly longer (Wilcoxon test, $p < 0.05$) in *A. grandicornuta*, *A. nilotica* and *A.*

tortilis growing outside of the exclosures (Fig. 5b), while *A. karroo* exhibited the same pattern. Average spine diameter showed a similar pattern with significantly thicker spines for *A. grandicornuta*, *A. nilotica* and *A. tortilis* exposed to herbivores (Fig. 5c). Spine density was also found to be higher for the outside treatments of *A. grandicornuta*, *A. nigrescens* and *A. tortilis*, differences were significant in *A. grandicornuta* and *A. tortilis* (Wilcoxon test, $p < 0.05$; Fig. 5d).

For *A. grandicornuta*, total branch number and length, number and total length of all branchlets occurring on three main branches from the lower, middle and upper canopy of each tree, increased when herbivores were excluded (evident in Fig. 6). Mean main stem (trunk) diameter increase appeared higher for the inside treatment in *A. nigrescens* and higher for the outside treatment in *A. nilotica* and *A. tortilis*, however none of these differences were significant (Table 2). Mean height increase was higher for the outside treatment of *A. burkei* and higher for the inside treatments of *A. grandicornuta*, *A. karroo* and *A. nigrescens* (Table 2). There was an increase in the number of branchlets on the three measured branches in the outside treatment of *A. burkei* and *A. tortilis*, while for *A. nigrescens* and *A. nilotica* there was a trend of increased branchlets in the inside treatments (Table 2). *A. burkei* and *A. grandicornuta* showed a much higher increase in the length of branchlets for trees in the outside treatment, while *A. nigrescens* and *A. nilotica* showed the opposite trend with a higher increase in branchlet length in the inside treatment (Table 2). Due to the small sample sizes and large variance in these growth measurements, differences tended to be non-significant at the $p < 0.05$ level.

Repeated clipping of *A. nilotica* did not result in an increase in branch number or length. In fact it resulted in decreased growth compared to the unclipped plants (Table 3). One year of exposure to browsers of previously protected *A. grandicornuta* plants did not result in significant increases in growth compared to plants that were always exposed to herbivory, however the number of branches and total length of branches was much higher in previously exposed plants

(Table 3). Clipping did however result in significant (Wilcoxon test, $p < 0.001$) increases in both thorn length and diameter compared to the unclipped *A. nilotica* plants (Fig. 7). Exposure to natural browsers in *A. grandicornuta* also resulted in highly significant (Wilcoxon test, $p < 0.001$) increases in both thorn length and diameter (Fig. 7).

DISCUSSION

Dantas & Pausas (2013) have recently reviewed the importance of the two major agents of top-down disturbances (fire and herbivory) in shaping the structure of savannas. Previous work has shown a major dichotomy in savanna woody plant strategies whereby species growing in fire-prone mesic savannas tend to be fast growing with pole-like architectures which allow escape from the so called ‘fire-trap’ (e.g. Gignoux *et al.* 1997, Archibald & Bond 2003, Wigley *et al.* 2009). Species growing in arid to semi-arid savannas tend to have cage-like architectures which are thought to protect the growing parts from browsing herbivores (Marquis 1996, Archibald & Bond 2003). Staver *et al.* (2012) stressed that this ecological trade-off axis has been poorly explored and warrants more attention. The results from these authors’ study found a clear single trade-off axis whereby fire-adapted species had pole-like architectures and higher starch concentrations in roots while herbivore-adapted species tended to have cage-like architectures and lower root starch concentrations. The findings from our study are in strong agreement with those of Staver *et al.* (2012), where *Acacia* architectures were clearly found to form a similar trade-off axis.

By comparing palatability and defence related traits between mesic (fire adapted) and semi-arid (herbivore adapted) *Acacia* species, this study was able to determine if the trade-off in architecture can be extended to plant palatability, defences (chemical and structural) and growth

rates. Furthermore, by controlling for the effect of herbivory through the use of herbivore exclosures we were able to determine if the *Acacia* species exhibited any induced responses to herbivory and how these responses differed in fire adapted vs. herbivore adapted species.

A comparison of the palatability related traits showed that leaf nutrient concentrations tended to be higher in the semi-arid species compared to the mesic species. A potential ecophysiological mechanism for this is provided by Wright *et al.* (2002) who showed that leaf mass per area (LMA) and leaf N are higher in arid species. This is thought to enhance water conservation during photosynthesis (Mooney *et al.* 1978, Wright *et al.* 2001). Leaf N, one of the most important nutrients for browsers as it strongly correlates to protein content (see Owen-Smith 2005), was similar between h- and h+ treatments in three of the four mesic species. Interestingly, the three species (*A. grandicornuta*, *A. nigrescens* and *A. nilotica*) which were found to have differences in leaf N all had higher N in the h+ treatments, suggesting that herbivory led to increased leaf N. Scogings & Macanda (2005) suggested that with intensive foliage removal, the root:shoot ratio increases, resulting in more nutrients being available for plant growth. An alternative explanation could be that the presence of browsers led to higher input of nutrients from urine and dung, possibly also explaining the higher levels of P and Mg in browsed plants (*e.g.* Augustine & McNaughton 2006, Fornara & du Toit 2008).

Total phenolic content and condensed tannin content were found to be much lower in the three semi-arid species compared to the mesic species. *A. nilotica* showed an interesting pattern with the highest TP content but low CT concentrations (see Fig. 3). This species (*A. nilotica*) has previously been shown to be a browse tolerant mesic savannas species (Bond *et al.* 2001). Herbivory appeared to have variable effects on these measures of chemical defence. Three of the seven species showed almost no response in leaf TP with browsing, while the trend in the herbivore adapted species showed higher TP when herbivory was excluded, suggesting that

herbivory resulted in lower TP content. Surprisingly, browsing appeared to have almost no effect on CT, with no obvious trends evident between treatments. This could suggest that condensed tannins are more important for deterring insect herbivory in the mesic species. Condensed tannin concentration is known to be an inducible defence (see Peters & Constable 2002) thus if CTs were effective at deterring mammals we would have expected a higher concentration of tannins in the plants exposed to herbivory.

Scogings *et al.* (2011) hypothesised that polyphenols and condensed tannins would decrease with increased browsing intensity as more C is required for plant growth in response to foliar removal by herbivores. Our findings support this hypothesis as the semi-arid species exposed to higher levels of herbivory in iMfolozi (*e.g.* Staver *et al.* 2009; 2012) had lower levels of both polyphenols and condensed tannins than the mesic species. Another interesting finding was that all three measures of fibre generally did not show clear differences between mesic and semi-arid species, nor with browsing, suggesting that these cell wall constituents are not important in deterring herbivory, or they are not inducible.

The traits relating to structural defences showed much stronger and more consistent differences between mesic and semi-arid species and between herbivory treatments. The degree of ramification differed radically between mesic and semi-arid species, with a significantly higher number and length of branches in semi-arid species. A similar pattern was true for spines, where spine length, diameter and densities were all higher at semi-arid sites. BSI was found to be a very informative trait regarding structural defences as it incorporates both architectural and physical defences. BSI tended to be higher (more leaf per bite) in the mesic species, however the differences in BSI between herbivory treatments was most striking (see Fig. 5). BSI was lower with browsing in all but one of the species. These differences were also much more pronounced in the semi-arid species. Spine length and thickness generally tended to be longer on trees

growing outside of the exclosures, with the most pronounced differences evident in *A. grandicornuta* and *A. tortilis*. Spine density on the other hand tended to be fairly similar between treatments, with only *A. grandicornuta* and *A. tortilis* showing higher densities for the outside treatment.

These results strongly support the idea that structural defences are more important and/or more effective as a defence mechanism against vertebrate browsers than chemical defences, especially at high browsing pressures such as those found at iMfolozi. They also support previous findings that spine length and thickness are fairly plastic traits which plants are able to manipulate according to browsing pressure (e.g. Gómez & Zamora 2002). Spine density however appears to be less plastic, thus plants seem to be able to control spine length and thickness while spine density generally appears to be more of a genetically constrained trait.

The effect of browsers on growth and branching patterns which determine tree architecture was mostly negligible for the mesic species growing in Hluhluwe where fire can be thought of as the main herbivore. There were however much stronger effects on the three semi-arid species growing in iMfolozi. The effect was strongest in *A. grandicornuta* where browsing appears to have stimulated lateral growth and branching (see photographs in Fig. 6). Archibald & Bond (2003) found that branching patterns in *A. karroo* varied according to the disturbance agents affecting populations with cage-like structure developing with high browsing intensities.

There were clear differences in growth rates between mesic vs. semi-arid species with faster growth in the mesic species compared to the semi-arid species. This was not surprising because the mesic species with their pole-like architectures were predicted to grow faster in attempting to escape the fire-trap. This was previously shown for *A. karroo* which has a fundamental pole-like architecture and extremely fast growth rates (e.g. see Schutz *et al.* 2009, Wigley *et al.* 2009).

The clipping experiment in *A. nilotica* and exclosure removal in *A. grandicornuta* allowed for controlled testing of the results from the longer-term exclosure experiments. The results from both of these experiments supported the findings from the herbivore exclosures, showing induced responses in structural defences as a response to browsing.

CONCLUSIONS — This study found clear differences in the palatability, architecture and defence related traits between mesic and semi-arid *Acacia* species. The findings were in line with previous studies which found a trade-off axis in plant architecture and resprouting ability between fire-adapted mesic species and herbivore adapted semi-arid species. The findings of this study suggest that this trade-off axis can be extended to leaf palatability as well as investment in both chemical and structural defences. The effect of browsers on leaf nutrient content was almost negligible for the mesic species but increased browse quality in the semi-arid species. Another surprising result given the extensive focus on chemical defences in the literature was that browsing had very little effect on condensed tannins and fibre content of leaves. For TPs, browsing also resulted in better quality browse, i.e. lower TP with browsing. These findings provide strong support for the browsing lawn concept proposed by Fornara & Du Toit (2007). The effects of browsers on structural defences were much stronger than for chemical defences, particularly for the semi-arid species. These species tended to have more cage-like architectures resulting from high levels of branching and longer and thicker spines in the h+ treatment.

In conclusion two distinct strategies were evident in this study. Plant architecture, chemical and structural defences and growth rates of the mesic species were mostly unaffected by browsers. These species are likely to be more adapted to frequent fires resulting from higher fuel loads in mesic savannas. The mesic species did however appear to invest more in both polyphenol and condensed tannin defences, possibly suggesting insect herbivory to be more

important in mesic savannas. The architecture, defence and growth rates of the three semi-arid species and herbivore-adapted *A. nilotica* were much more affected by browsing, with structural defences most influenced by mammal herbivory. Thus a clear trade-off in defence strategy appeared to be in place whereby fire-adapted mesic species invested more in chemical defences while herbivore adapted semi-arid species invested more in structural defences.

ACKNOWLEDGMENTS

This work forms part of the PhD thesis of BJ Wigley and was made possible thanks to funding from a BDI-PED fellowship from the CNRS as well as the Andrew W Mellon Foundation. I am hugely grateful to Ezemvelo KZN Wildlife for allowing the study in their reserve and for all of the logistical support provided. C Coetsee provided valuable comments on an earlier draft of the MS.

LITERATURE CITED

- ACOCKS, J. P. H. 1988. Veld types of South Africa, Memoirs of the Botanical Survey of South Africa (No. 57, Ed. 3), National Botanical Institute, Kirstenbosch, South Africa.
- ANDERSEN, A. N. AND LONSDALE, W. M. 1990. Herbivory by insects in Australian tropical savannas: a review. *J. Biog.*, 17: 433-444.
- ARCHIBALD, S. AND BOND, W. J. 2003. Growing tall vs growing wide: tree architecture and allometry of *Acacia karroo* in forest, savanna, and arid environments. *Oikos*, 102: 3-14.

- AUGUSTINE, D. J. AND MCNAUGHTON, S. J. 2006. Interactive effects of ungulate herbivores, soil fertility, and variable rainfall on ecosystem processes in a semi-arid savanna. *Ecosystems*, 9: 1242-1256.
- BALFOUR, D. A. AND HOWISON, O. E. 2001. Spatial and temporal variation in a mesic savanna fire regime: responses to variation in annual rainfall. *Afr. J. Range Forage Sci.*, 19: 43-51.
- BOND, W. J. 2008. What limits trees in C 4 grasslands and savannas? *Annu. Rev. Ecol. Evol. Syst.*, 39: 641–659.
- BOND, W. J. AND VAN WILGEN, B.W. 1996. *Fire and Plants*, Springer.
- BOND, W.J., SMYTHE, K. A. AND BALFOUR, D. A. 2001. Acacia species turnover in space and time in an African savanna. *J. Biog.*, 28: 117-128.
- COOPER, S. M. AND OWEN-SMITH, N. 1985. Condensed tannins deter feeding by browsing ruminants in a South African savanna. *Oecologia*, 67: 142-146 .
- COOPER, S. M. AND OWEN-SMITH, N. 1986. Effects of plant spinescence on large mammalian herbivores. *Oecologia*, 68: 446-455.
- COOPER, S. M., OWEN-SMITH, N. AND BRYANT, J. P. 1988. Foliage acceptability to browsing ruminants in relation to seasonal changes in the leaf chemistry of woody plants in a South African savanna. *Oecologia*, 75: 336-342.
- DANELL, K. BERGSTRÖM, R., EDENIUS, L. AND ERICSSON, G. 2003. Ungulates as drivers of tree population dynamics at module and genet levels. *For. Ecol. Manage.*, 181: 67-76.

- DANTAS, V.D L. AND PAUSAS, J. G. 2013. The lanky and the corky: fire-escape strategies in savanna woody species. *J. Ecol.*, doi: 10.1111/1365-2745.12118.
- DRAY, S. AND DUFOUR, A. B. 2007. The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Softw.*, 22: 1-20.
- DU TOIT, J. T., BRYANT, J. P. AND FRISBY, K. 1990. Regrowth and palatability of *Acacia* shoots following pruning by African savanna browsers. *Ecology*, 71: 149-154.
- FORNARA, D. AND DU TOIT, J. T. 2007. Browsing lawns? Responses of *Acacia nigrescens* to ungulate browsing in an African savanna. *Ecology*, 88: 200-209.
- FORNARA, D. AND DU TOIT, J. T. 2008. Responses of woody saplings exposed to chronic mammalian herbivory in an African savanna. *Ecoscience*, 15: 129-135.
- GIGNOUX, J. CLOBERT, J. AND MENAUT, J.C. 1997. Alternative fire resistance strategies in savanna trees. *Oecologia*, 110: 576-583.
- GÓMEZ, J. M. AND ZAMORA, R. 2002. Thorns as induced mechanical defense in a long-lived shrub (*Hormathophylla spinosa*, Cruciferae). *Ecology*, 83: 885-890.
- HANLEY, M. E. LAMONT, B. B., FAIRBANKS, M. M. AND RAFFERTY, C. M. 2007. Plant structural traits and their role in anti-herbivore defence. *Perspect. Plant Ecol.*, 8: 157-178.
- HATTAS, D. STOCK, W. D., MABUSELA, W. T. AND GREEN, I.R. 2005. Phytochemical changes in leaves of subtropical grasses and fynbos shrubs at elevated atmospheric CO₂ concentrations. *Global Planet. Change*, 47: 181-192.

- HATTAS, D. AND JULKUNEN-TIITTO, R. 2012. The quantification of condensed tannins in African savanna tree species. *Phytochem., Lett.* 5: 329-334.
- HEAN, J. W. AND WARD, D. 2011. Fire and herbivory are not substitutable: evidence from regrowth patterns and changes in physical and chemical defences in *Acacia* seedlings. *J. Veg. Sci.* 23: 13-23.
- HERMS, D. A. AND MATTSON, W. J. 1992. The dilemma of plants: to grow or defend. *Quart. Rev. Biol.*, 67: 283-335.
- ILLIUS, A.W. DUNCAN, P., RICHARD, C. AND MESOCHINA, P. 2002. Mechanisms of functional response and resource exploitation in browsing roe deer. *J. An. Ecol.*, 71: 723-734.
- KARBAN, R. AND BALDWIN, I. T. 1997. *Induced responses to herbivory.* University of Chicago Press
- KINDT, R. AND COE, R. 2005. *Tree diversity analysis: A manual and software for common statistical methods for ecological and biodiversity studies.* World Agroforestry Centre, Eastern and Central Africa Program
- MARQUIS, R. J. 1996. Plant architecture, sectoriality and plant tolerance to herbivores. *Plant. Ecol.*, 127: 85-97.
- MOONEY, H. A., FERRAR, P. J., SLATYER, R. O. 1978. Photosynthetic capacity and carbon allocation patterns in diverse growth forms of *Eucalyptus*. *Oecologia*, 36: 103-111.
- OHGUSHI, T. 2005. Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annu. Rev. Ecol. Evol. Syst.*, 36: 81-105.

- OWEN-SMITH, N. 2002. Adaptive herbivore ecology: from resources to populations in variable environments. Cambridge University Press.
- OWEN-SMITH, N. 2005. Incorporating fundamental laws of biology and physics into population ecology: the metaphysiological approach. *Oikos*, 111: 611-615.
- PETERS, D. J. AND CONSTABEL, C. P. 2002. Molecular analysis of herbivore-induced condensed tannin synthesis: cloning and expression of dihydroflavonol reductase from trembling aspen (*Populus tremuloides*). *The Plant Journal*, 32: 701–712.
- R CORE TEAM 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>
- ROHNER, C. AND WARD, D. 1997. Chemical and mechanical defense against herbivory in two sympatric species of desert Acacia. *J. Veg. Sci.*, 8: 717-726.
- ROOKE, T. BERGSTRÖM, R., SKARPE, C. AND DANELL, K. 2004. Morphological responses of woody species to simulated twig-browsing in Botswana. *J. Trop. Ecol.*, 20: 281-289.
- SANKARAN, M. AUGUSTINE, D. J. AND RATNAM, J. 2013. Native ungulates of diverse body sizes collectively regulate long-term woody plant demography and structure of a semi-arid savanna. *J. Ecol.*, doi: 10.1111/1365-2745.12147.
- SCHUTZ, A. E. N., BOND, W. J. AND CRAMER, M. D. 2009. Juggling carbon: allocation patterns of a dominant tree in a fire-prone savanna. *Oecologia*, 160: 235-246.

- SCOGINGS, P. AND MACANDA, M. 2005. Acacia karroo responses to early dormant season defoliation and debarking by goats in a semi-arid subtropical savanna. *Plant Ecol.*, 179: 193-206.
- SCOGINGS, P. F., HJÄLTÉN, J., SKARPE, C. 2011. Secondary metabolites and nutrients of woody plants in relation to browsing intensity in African savannas. *Oecologia*, 167: 1063-1073.
- STAVER, A. C., BOND, W. J., STOCK W. D., VAN RENSBURG, S. J. AND WALDRAM, M. S. 2009. Browsing and fire interact to suppress tree density in an African savanna. *Ecol. Appl.*, 19: 1909-1919.
- STAVER, A. C., BOND, W. J., CRAMER, M. D. AND WAKELING, J. L. 2012. Top down determinants of niche structure and adaptation among African Acacias. *Ecol. Lett.*, 15: 673-679.
- VOURC'H, G., DE GARINE-WICHATITSKY, M., LABBÉ, A., ROSOLOWSKI, D., MARTIN, J. L. AND FRITZ, H. 2002. Monoterpene effect on feeding choice by deer. *J. Chem. Ecol.*, 28: 2411-2427.
- WAKELING, J. L. STAVER, A. C. AND BOND, W. J. 2011. Simply the best: the transition of savanna saplings to trees. *Oikos* 120: 1448-1451.
- WIGLEY, B. J., CRAMER, M. D. AND BOND, W. J. 2009. Sapling survival in a frequently burnt savanna: mobilisation of carbon reserves in *Acacia karroo*. *Plant. Ecol.*, 203: 1-11.
- WIGLEY, B. J., FRITZ, H., COETSEE, C. AND BOND, W. J. 2013. Herbivores shape woody plant communities in the Kruger National Park: lessons from three long-term exclosures. Koedoe, in press.

- WILSON, S. L. AND KERLEY, G. I. H. 2003. The effect of plant spinescence on the foraging efficiency of bushbuck and boergoats: browsers of similar body size. *J. Arid. Env.*, 55: 150-158.
- WRIGHT, I. J., REICH, P. B. AND WESTOBY, M. 2001. Strategy shifts in leaf physiology, structure and nutrient content between species of high and low rainfall and high and low nutrient habitats. *Funct. Ecol.*, 15: 423-434.
- WRIGHT, I. J., WESTOBY, M. AND REICH, P. B. 2002. Convergence towards higher leaf mass per area in dry and nutrient poor habitats has different consequences for leaf life span. *J. Ecol.*, 90: 534-543.
- YOUNG, H. S., MCCAULEY, D. J., HELGEN, K. M., GOHEEN, J. R., OTÁROLA-CASTILLO, E., PALMER, T. M., PRINGLE, R. M., YOUNG, T. P. AND DIRZO, R. 2013. Effects of mammalian herbivore declines on plant communities: observations and experiments in an African savanna. *J. Ecol.*, 101: 1030-1041.

Tables

Table 1. Study site locations in the Hluhluwe-iMfolozi Park.

Site	Location	<i>Acacia</i> species	Savanna type	Co-ords E	Co-ords S
Klassana	Hluhluwe	<i>burkei, caffra</i>	mesic	31.975046°	-28.152036°
Le Dube	Hluhluwe	<i>karroo</i>	mesic	32.016764°	-28.234241°
Seme	Hluhluwe	<i>nilotica</i>	mesic	31.968602°	-28.168240°
Thobothi	iMfolozi	<i>nigrescens, tortilis</i>	semi-arid	31.771126°	-28.224621°
Mona	iMfolozi	<i>grandicornuta</i>	semi-arid	31.799335°	-28.220977°

Table 2 Mean (\pm se) annual growth of main stems, increase in plant height, increase in branchlet number and branchlet length for three branches from the lower, middle and upper canopy and mean neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) concentrations for saplings growing inside (h-) vs. outside (h+) the exclosures. N = 5 for each treatment of each species. The missing values for *A. caffra* could not be obtained as these plants were destroyed by elephants during the experiment.

Plant trait	<i>A. burkei</i>	<i>A. caffra</i>	<i>A. grandicornuta</i>	<i>A. karroo</i>	<i>A. nigrescens</i>	<i>A. nilotica</i>	<i>A. tortilis</i>
Stem diameter increase (mm)	h-	11.7 \pm 1.31	2.40 \pm 0.17	8.48 \pm 2.52	7.64 \pm 0.37	8.01 \pm 1.64	9.82 \pm 2.26
	h+	11.8 \pm 1.62	2.98 \pm 0.60	8.22 \pm 1.06	5.16 \pm 1.12	12.5 \pm 2.59	12 \pm 3.83
Height increase (cm)	h-	30.0 \pm 12.64	20.0 \pm 5.47	76.8 \pm 10.25	50.0 \pm 5.70	43.1 \pm 9.54	38.0 \pm 12.4
	h+	41.0 \pm 4.58	14.0 \pm 5.33	61.4 \pm 4.16	20.4 \pm 11.7	48.8 \pm 11.3	45.0 \pm 11.6
Δ in branch number	h-	2.80 \pm 1.69	2.80 \pm 1.02	5.60 \pm 2.87	27.8 \pm 5.56	9.33 \pm 7.99	24.8 \pm 7.89
	h+	12.6 \pm 4.84	0.40 \pm 9.41	6.60 \pm 3.70	13.6 \pm 3.18	4.37 \pm 1.91	28.8 \pm 5.33
Δ in branch length	h-	85.0 \pm 45.1	81.0 \pm 17.1	137 \pm 78.1	288 \pm 49.1	313 \pm 90.5	456 \pm 134
	h+	270 \pm 107	365 \pm 115	167 \pm 97.7	64.8 \pm 17.9	183 \pm 56.2	460 \pm 126
NDF (%)	h-	59.7 \pm 1.93	53.5 \pm 1.35	51.7 \pm 3.31	59.7 \pm 1.14	61.4 \pm 1.93	32.2 \pm 1.42
	h+	62.9 \pm 0.93	54.8 \pm 1.62	55.9 \pm 2.29	57.9 \pm 0.86	64.9 \pm 1.68	36.9 \pm 1.44
ADF (%)	h-	35.9 \pm 0.77	32.5 \pm 1.60	25.4 \pm 4.52	38.1 \pm 0.37	37.3 \pm 1.94	12.7 \pm 0.48
	h+	37.9 \pm 0.85	34.2 \pm 2.02	20.2 \pm 0.80	37.1 \pm 1.74	39.2 \pm 1.52	14.1 \pm 0.48
ADL (%)	h-	15.8 \pm 0.41	19.1 \pm 1.94	4.87 \pm 1.75	21.6 \pm 2.19	18.6 \pm 1.78	2.69 \pm 0.36
	h+	18.5 \pm 0.71	22.7 \pm 3.43	2.34 \pm 0.38	20.7 \pm 2.88	20.3 \pm 1.93	3.73 \pm 0.57

Table 3 Mean (\pm se) changes in branchlet number and branchlet length (mm) of saplings of *A. grandicornuta* one year after exclosure removal (ER) compared to plants always growing outside of the exclosure (OUT) and *A. nilotica* after one year of repeated clipping (CLIP) compared to non-clipped plants (NCLIP). N = 5 for each treatment of each species.

Effect	Treatment	<i>A. grandicornuta</i>	<i>A. nilotica</i>
Δ in branch number	ER	3 \pm 3.2	
	OUT	17 \pm 9.7	
	CLIP		9.8 \pm 5.3
	NCLIP		24 \pm 11.7
Δ in branch length	ER	59 \pm 79	
	OUT	490 \pm 241	
	CLIP		245 \pm 102
	NCLIP		446 \pm 186

Figure legends

Figure 1. PCA of the twelve leaf traits relating to leaf chemical composition, fibre content and chemical defence. Leaf C, Ca and total phenolics (TP) had the highest loadings along PC1 (x-axis), while ADF, ADL and condensed tannins had the highest loadings along PC2 (y-axis), (a). The positions of sites (b), species (c) and species x treatment (d) on PC1 (40% of total variation) and PC2 (29% of total variation) are shown. Mesic or fire-adapted species and sites are shown in blue, while semi-arid or herbivore-adapted species and sites are shown in red. The length of each cell in the grid represents two units for both axes ($d=2$).

Figure 2. Mean (\pm se) percentages of leaf N, C:N, P and K for saplings of the seven species growing inside vs. outside of the exclosures. Full species names are provided in Table 1. * $p < 0.01$, ** $p < 0.01$, *** $p < 0.001$, $n = 5$ for each treatment of each species.

Figure 3. Mean (\pm se) percentages of leaf total polyphenols and condensed tannins for the inside and outside treatments of the seven *Acacia* species. * $p < 0.01$, ** $p < 0.01$, *** $p < 0.001$, $n = 5$ for each treatment of each species.

Figure 4. Average change in number of branchlets (a) and change in sum of branchlet length (b) on three monitored branches taken from the upper middle and lower canopy of each plant for mesic vs. semi-arid species. Mean annual stem diameter increase, measured at 10 cm above ground level (c) and mean annual total plant height increase (d) are also shown for mesic vs. semi-arid species. $N = 30$ for each savanna type, * $p < 0.01$, ** $p < 0.01$, *** $p < 0.001$.

Figure 5. Mean (\pm se) bite size index (BSI, large value = more leaf), spine length, spine diameter and spine density for saplings of the seven species growing inside (h-) and outside (h+) the exclosures. * $p < 0.01$, ** $p < 0.01$, *** $p < 0.001$, $n = 5$ for each treatment of each species.

Figure 6. Photographs showing the different growth forms of *A. grandicornuta* growing (a) outside (h+) and (b) inside (h-) of the Mona exclosure.

Figure 7. Mean (\pm se) thorn length (a) and diameter (b) in clipped vs. non-clipped (NCLIP) treatments of *A. nilotica* before clipping (AN CLIP begin) and after clipping (AN CLIP end). Also mean (\pm se) thorn length (c) and diameter (d) of *A. grandicornuta* before exclosure removal (AG ER begin) and after exclosure removal (AG ER end) compared to *A. grandicornuta* saplings that had always grown outside of the exclosure (AG OUT). * $p < 0.01$, ** $p < 0.01$, *** $p < 0.001$, $n = 5$ for each treatment of each species.

Figures

Figure 1

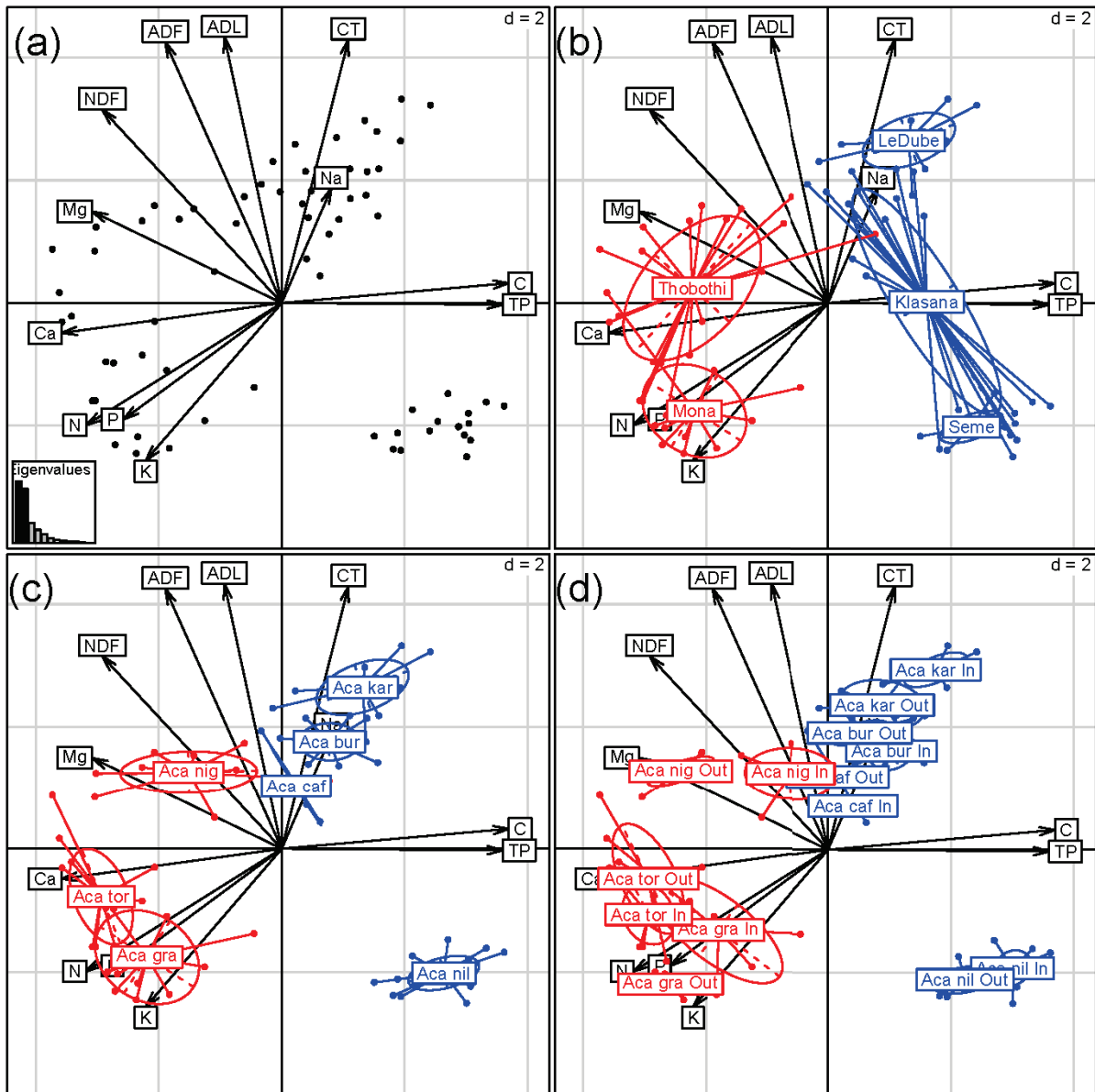


Figure 2

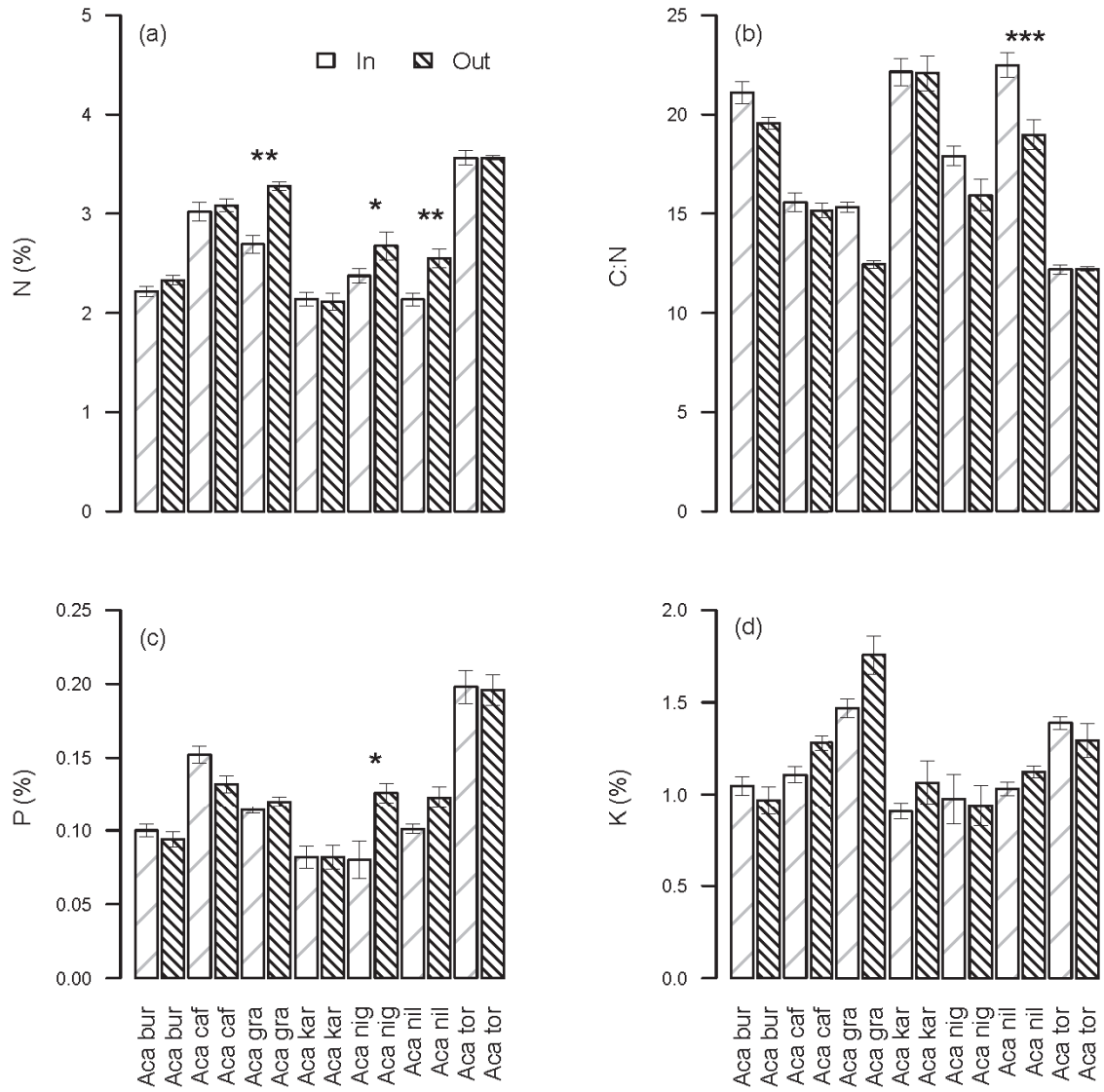


Figure 3

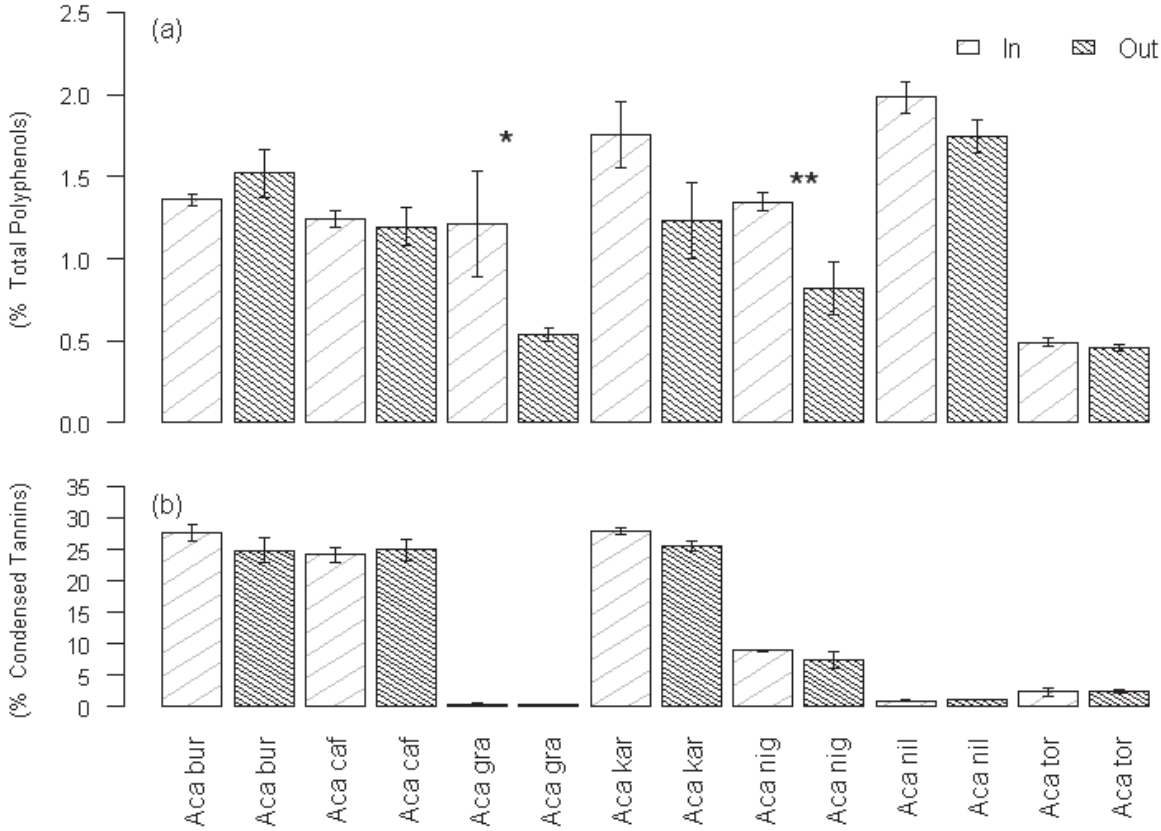


Figure 4

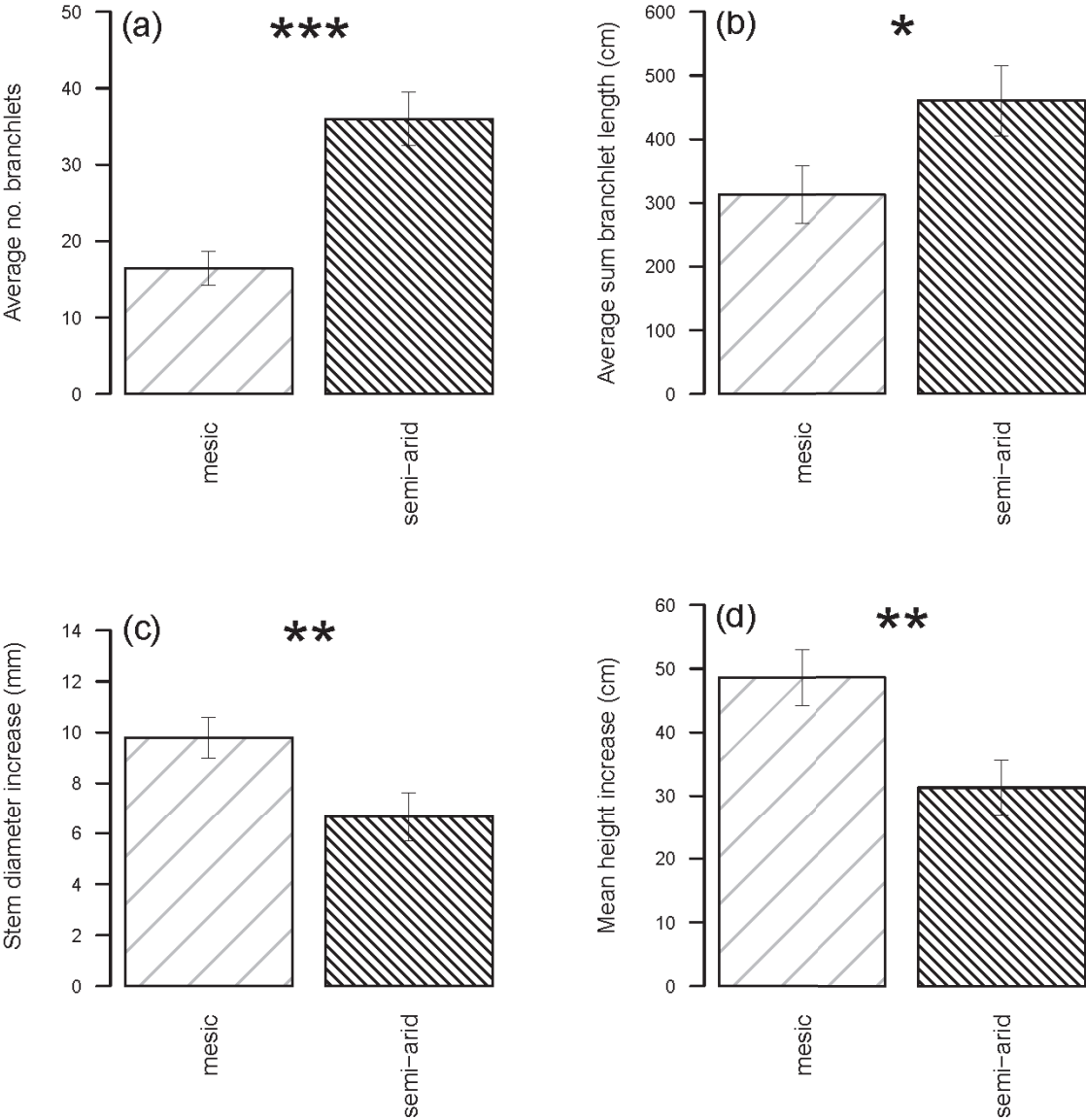


Figure 5

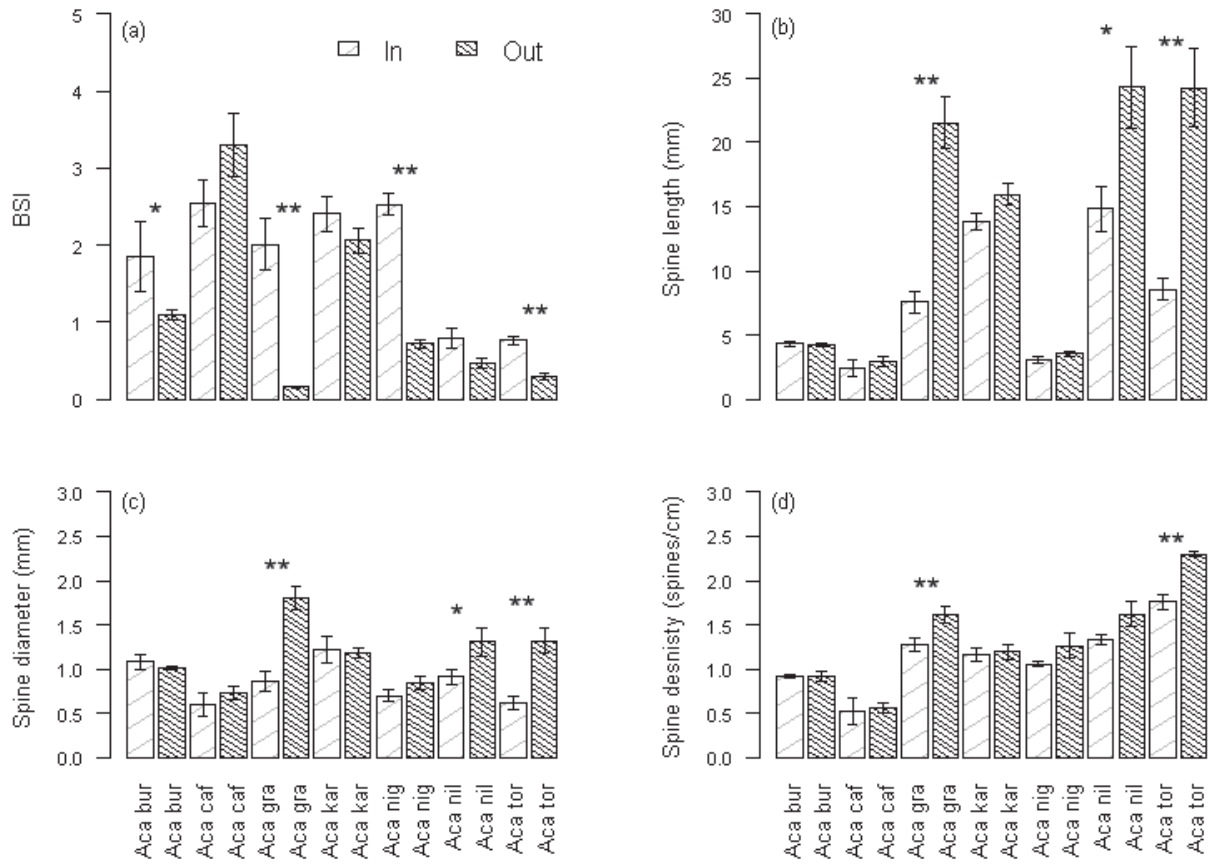
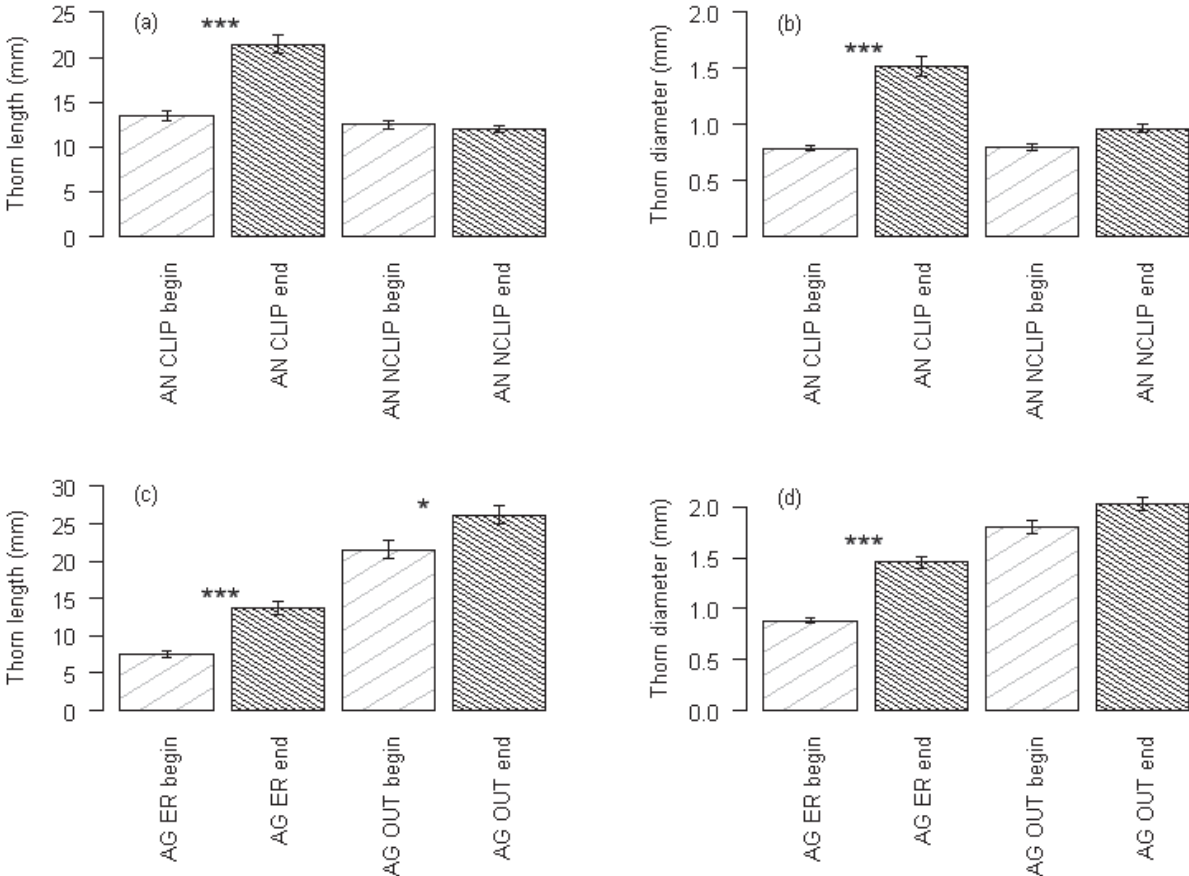


Figure 6



Figure 7





Field measurements on an *Acacia nilotica* sapling in the Seme exclosure, Hluhluwe-iMfolozi Park.

Chapter 8 Synthesis and conclusions

Savannas are known to be dynamic and highly complex systems affected by a suite of ecological drivers. These drivers have been polarised into two broad classes usually referred to as either bottom-up or top-down controls or drivers (e.g. see Bond 2005; 2008). Bottom-up controls include the abiotic template thereby incorporating soil nutrient availability and climate. Top-down controls include any form of disturbance, which in savannas typically include fire and herbivory. The relative importance of each of the different drivers is fairly well understood at the local scale with a number of well established feedback loops between different drivers. For example at higher rainfall sites, high levels of leaching may lead to infertile soils, especially on soils derived from granites. This typically results in grass species of lower nutritional value which results in lower grazer densities which in turn results in a higher amount of biomass of fuel, winter drought then results in the drying out of this fuel. An ignition source is all that is needed in order for a fire to consume this biomass.

However, as demonstrated by Bond (2008), bottom up controls fail to accurately account for the realised global distribution of savanna ecosystems. Bond (2005) suggests that much of the mismatch between actual and realised vegetation could be explained by fire. Dynamic global vegetation models (DGVMs) aided by ever improving satellite imagery have added support to this hypothesis (Bond et al. 2003; 2005).

The other key top-down control on savanna functioning comes in the form of herbivory. Although there have been a number of studies investigating the impacts of herbivores on plant assemblages (e.g. Huntly 1991; Paine 2000), fewer studies have identified the key plant functional traits needed to survive or withstand mammalian herbivory or fire (e.g. Pausas & Lavorel 2003; Diaz et al. 2007). Very few studies have compared plant functional traits and trade-offs between communities growing under different resource (soil nutrients and rainfall) availability and exposed to different levels of disturbance from both fire and herbivory (Bond 2005). This study is therefore novel in that it attempts to explore and compare key plant functional traits of savanna woody communities at the regional scale along both resource and disturbance gradients with a particular focus on mammalian herbivory.

Although soil fertility is well recognized as a bottom-up control on savanna functioning, it remains unclear in the ecological literature as to what exactly denotes a fertile or infertile soil,

and what and how should soils be sampled in order to determine the inherent soil fertility status at any given site? This posed a serious problem for this study, as one of the main objectives was aimed at determining how the bottom-up drivers, specifically soil fertility and rainfall affect or determine woody plant traits in savannas. In Chapter 3 my co-authors and I therefore attempted to address this problem using empirical data from a number of my study sites. We attempted to provide ecologists with basic protocols for soil sampling that would allow for meaningful comparisons between sites and studies. This was by no means an easy task and we acknowledge that there are still problems with our recommendations, however it is a start and will hopefully stimulate further input and debate from fellow ecologists on this subject.

Upon establishing a set of suitable protocols for measuring the inherent soil nutrient status at each site, I was in a position to tackle one of the main research questions addressed in this thesis which was how savanna woody plant leaf traits respond to soil nutrient availability and rainfall (Chapter 4). The results from this study were surprising. The expectation was that the leaf traits at the study sites would largely follow the patterns found in the leaf economic spectrum of Wright et al. (2004) or vary according to the acquisitive *vs.* conservative strategies outlined by Diaz et al. (2004). I therefore expected to find a suite of ‘conservative’ species with associated leaf traits on the resource limited side of the spectrum, and a suite of ‘acquisitive’ species with associated traits on the opposite resource-rich end of the spectrum, and intermediate trait values in-between. The majority of measured leaf traits analysed at the species level, however, failed to show these expected patterns. The use of community weighted means did result in a slightly higher degree of separation along the spectrum.

These findings are puzzling as, according to previous studies (e.g. Huntley 1982; Scholes 1990) African savannas can generally be divided into broad-leaved savannas on nutrient-poor soils and fine-leaved savannas on nutrient-rich soils. Why then are these patterns not evident in this study? A look at the community compositions at each site (Appendix 1) shows that although there is some evidence for this pattern at the study sites, the overall pattern is not so clear cut. Many of the ‘fine-leaved’ savannas still contain a substantial proportion of broad-leaved species and vice versa suggesting that a polarised classification is an oversimplification for the sites I studied. This phenomenon could also account for the lack of a clear division in plant strategies found in this study at the species level and the improvement found when using CWMs which took the relative abundance of each species into account. The lack of pattern in separating

species along the conservative-acquisitive axis found in this study could also be due to the high levels of variability evident for most of the soil/rainfall-trait relationships. The analyses showed that within site variability tended to be the highest source of variability for all of the measured leaf traits. Such high levels of variability in relationships between leaf traits, soils and rainfall have also been found in a number of other studies and have generally been attributed to disturbance, micro-site variability and alternative evolutionary solutions to similar environmental conditions (see Grime 2006; Westoby & Wright 2006). The high levels of disturbance found in African savannas could therefore account for the high levels of within-site variability of the measured leaf traits observed at the study sites. These findings suggest that bottom-up controls alone cannot account for the observed patterns of leaf traits at the study sites and that southern African savannas do not necessarily follow the general patterns found at larger scales such as the leaf economic spectrum of Wright et al. (2004). The majority of sites included in studies based on meta-analyses (e.g. Wright et al. (2004) often tend to be from temperate systems. The lower local faunal and floral diversity together with a degree of lower disturbance (e.g. lower diversity of mammal herbivores, loss of mega-herbivores, less frequent/intense fires or no fires) in these temperate systems would partially account for the neatly observed patterns in leaf traits described by Wright et al. (2004), for example. Bottom-up controls in temperate systems are therefore likely to play a more important role in shaping leaf traits than in tropical or sub-tropical systems where top-down controls also have a strong influence on plant traits.

After establishing that bottom-up drivers related to resource capture did not explain a large proportion of the variability in the measured leaf traits, I shifted the focus of the study to determine the role of top-down drivers or disturbance in shaping savanna woody plant leaf traits. Due to logistical constraints, I focused on the role of mammalian herbivory in shaping communities and their effects on plant traits as this aspect of savanna ecology has not been well established.

Owing to the large overlap of resource-capture related traits with a number of defence related traits, I first explored and compared the effects of soil nutrients, rainfall and herbivory on both chemical and physical or structural plant traits. The findings of this study can significantly contribute to the debate around defence syndromes. The existence of two different defence syndromes was confirmed at the study sites. Under nutrient limited conditions plants tended to invest more in chemical defences, and/or attempted to avoid herbivory by growing larger and

taller and by having poor quality browse material. On the other hand, when nutrients were not limiting, plants tended to have higher quality browse material but this material was well defended with physical or structural defences. Interestingly, the suite of structural defences was more closely related to soil nutrient availability than the chemical defences. These findings support the predictions of Craine et al. (2003) who predicted that at low N supply, C-based defences should have the highest net C gain for the plant, while at high N supply, N-based and structural defences should have the highest net C gain. Another important finding evident from this study was the clear divide in the effectiveness of plant defences against the two different guilds of herbivores. While meso-browser impact was strongly positively correlated with structural defences and negatively correlated with chemical defences, mega-browser impact was poorly correlated with both chemical and structural defences, suggesting that defences are mostly ineffectual against mega-herbivores such as elephant. In other words, meso-browsers preferably browse on structurally, compared to chemically, defended plants.

I then went on to directly test the role of mammalian herbivores in determining and structuring savanna woody plant communities. This was achieved by comparing the composition, structure and abundance of the woody species in three different woody communities in savannas growing in high, moderate and low rainfall areas with different soil nutrient availabilities. Despite the unfortunate unavailability of additional sites, the findings from the three available sites were telling. The results from this study showed that mammalian browsers play a very important role in savanna dynamics as they directly affect species distributions, densities, population structures and plant functional traits. This was found to be true at all sites, however the overall effect was highest at the low rainfall site with high nutrient availability, with a somewhat lower effect at the moderate rainfall site high with high nutrient availability while the high rainfall nutrient-poor site showed the least effect. This study also demonstrated the importance of browser-fire interactions at moderate to higher rainfall sites where herbivore removal allowed for a significant increase in seedling and sapling densities; however these seedlings and saplings were prevented from recruiting into larger size classes by regular fires.

The final study undertaken in this thesis also made use of herbivore exclosures to directly test the role of herbivores in determining plant architectures and growth rates and the effect that herbivores have on chemical and structural defences within seven *Acacia* species growing in mesic vs. semi-arid savannas. Two distinct strategies were evident here where, in mesic sites, the

architecture, chemical and structural defences and growth rates of *Acacia* spp. were mostly unaffected by browsers. These species were therefore thought to invest their available resources in fire-related traits such as thicker bark and belowground reserves. The strategy adopted by the semi-arid species was completely different where these species tended to have higher investments in structural defences resulting in distinct cage-like architectures and longer and thicker spines. The results from the analyses of the measured chemical defences showed some surprising patterns. Firstly, condensed tannins and total polyphenolics were found to be higher in general in the mesic species with almost no herbivore effect evident, while in the semi-arid species both chemical defences tended to be lower than the mesic species but a much stronger herbivore effect was evident in total polyphenolic content. Surprisingly total polyphenolic content tended to be higher in the plants that were not exposed to herbivores. This could again lend support to the predictions of Craine et al. (2003) in that in the absence of herbivores these plants had extra carbon available to invest in C-rich polyphenolic defences.

The overall findings of this work have a number of important implications for conservation managers working in savanna ecosystems. A meso-browser vs. mega-browser dominated herbivore community would have very different impacts on the woody plant communities at any given site. This should be taken into consideration when deciding on which suite of herbivore species and at what densities to stock. Mega-herbivores are often introduced into reserves as they are very effective at attracting tourists who are the life-blood of these conservation areas. In the past this was often done without taking the implications of such introductions for the local plant communities into account. This could partially account for the fact that many southern African savannas are currently faced with woody plant proliferation or bush encroachment in some areas (e.g. see Wigley et al. (2010) and references therein), while in other areas, managers are concerned about the loss of big trees (e.g. Eckhardt et al. 2000). The findings of this study clearly demonstrate the importance of mammal herbivory in determining woody plant community composition, structure and population densities within savannas. Managers could potentially make use of different herbivore guilds to control encroaching woody species. Meso-browsers would be more effective at nutrient-rich sites where fine-leaved species tend to be the main encroaching species while mega-herbivores would be more effective at nutrient-poor sites where broad-leaved species are often problematic encroaching species.

The finding that mega-herbivore impact was only marginally influenced by soil fertility status and that neither chemical nor structural defences were efficient at deterring mega-herbivores could account for the high fecundity rates of elephants that are often evident when other resources such as water are not limiting. This is highly relevant in light of the ongoing elephant culling debate in the Kruger National Park, as it suggests that elephant numbers are not limited by food quality but rather by quantity and other key resources other than food. Thus if the elephant population in the Kruger National Park is left unchecked their impacts on the woody component are likely to continue increasing.

In conclusion, this study has highlighted the importance of top-down controls on savanna dynamics. Although soil nutrient status and rainfall did explain some of the patterns evident in savanna woody leaf traits, these patterns were generally not as strong as might have been expected from previous studies undertaken at larger scales. Mammalian herbivores were found to have a strong influence on the distribution, population structure, abundance and both chemical and structural defences of savanna woody species. This study has also highlighted the need for an African wide collaborative effort among ecologists in order to extend the range of soil/rainfall gradients and to capture, where possible, fire and herbivore effects. It would also be useful to determine different ecosystem or biome responses to both indigenous herbivores and domestic livestock depending on their suite of plant traits. It might be expected that the vegetation in parts of Africa is designed for heavy herbivory and would therefore be more resilient to 'overutilisation' by livestock compared to most of the other continents because of the long history of co-evolution with larger herds of recently extirpated grazers and browsers. Similar trait analyses would also be highly beneficial for conservation planners in order to identify which parts of the world are most suitable for re-wilding.



An eland browsing on a rocky ridge, Mapungubwe National Park.

References

- Ackerly, D. D., Knight, C. A., Weiss, S. B., Barton, K. & Starmer, K. P. (2002). Leaf size, specific leaf area and microhabitat distribution of woody plants in a California chaparral: contrasting patterns in species level and community level analyses. *Oecologia*, 130, 449-457.
- Acocks, J. P. H. (1953). Veld types of South Africa, *Memoirs of the Botanical Survey of South Africa*, No. 28.
- Acocks, J. P. H. (1975). Veld types of South Africa, 3rd edn. *Memoirs of the Botanical Survey of South Africa*, No. 57. Botanical Research Institute, Pretoria.
- Acocks, J. P. H. (1988). Veld types of South Africa (No. 57, Ed. 3).
- Aerts, R. & Chapin, F. S. III. (2000). The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*, 30, 1-67.
- Agrawal, A. A., & Fishbein, M. (2006). Plant defense syndromes. *Ecology*, 87(sp7), 132-149.
- Agrawal, A. A. (2007). Macroevolution of plant defense strategies. *Trends in Ecology & Evolution*, 22(2), 103-109.
- Ågren, G. I. (2008). Stoichiometry and nutrition of plant growth in natural communities. *Annual Review of Ecology, Evolution, and Systematics*, 39, 153-170.
- Ammer, C. (1996). Impact of ungulates on structure and dynamics of natural regeneration of mixed mountain forests in the Bavarian Alps. *Forest Ecology and Management*, 88, 43-53.
- Anderson, T.M., Hopcraft, J. G. C., Eby, S., Ritchie, M., Grace, J. B. & Olf, H. (2010). Landscape-scale analyses suggest both nutrient and anti-predator advantages to Serengeti herbivore hotspots. *Ecology*, 91, 1519-1529.
- Anderson, V. J., & Briske, D. D. (1995). Herbivore-induced species replacement in grasslands: is it driven by herbivory tolerance or avoidance? *Ecological Applications*, 5(4), 1014-1024.
- Archer S., Boutton, T.W., Hibbard, K.A. (2001). Trees in grasslands: biogeochemical consequences of woody plant expansion. In: *Global Biogeochemical Cycles in the Climate System*, pp. 115-138. Academic Press, Durham, NC

- Archibald, S., & Bond, W. J. (2003). Growing tall vs growing wide: tree architecture and allometry of *Acacia karroo* in forest, savanna, and arid environments. *Oikos*, 102(1), 3-14.
- Asner, G. P., Levick, S. R., Kennedy-Bowdoin, T., Knapp, D. E., Emerson, R., Jacobson, J. & Martin, R. E. (2009). Large-scale impacts of herbivores on the structural diversity of African savannas. *Proceedings of the National Academy of Sciences*, 106(12), 4947-4952.
- Aucamp, A. J., & Tainton, N. M. (1984). Veld management in the Valley Bushveld of the Eastern Cape [South Africa]. Department of Agriculture.
- Augustine, D. J., & McNaughton, S. J. (1998). Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. *The Journal of Wildlife Management*, 62(4), 1165-1183.
- Augustine, D.J. (2003). Long-term, livestock-mediated redistribution of nitrogen and phosphorus in an East African savanna. *Journal of Applied Ecology*, 40, 137-149.
- Augustine, D. J., & Mcnaughton, S. J. (2004). Regulation of shrub dynamics by native browsing ungulates on East African rangeland. *Journal of Applied Ecology*, 41(1), 45-58.
- Augustine, D. J., & McNaughton, S. J. (2006). Interactive effects of ungulate herbivores, soil fertility, and variable rainfall on ecosystem processes in a semi-arid savanna. *Ecosystems*, 9(8), 1242-1256.
- Austin, A. T., & Vitousek, P. M. (1998). Nutrient dynamics on a precipitation gradient in Hawai'i. *Oecologia*, 113(4), 519-529.
- Baas, P., Ewers, F. W., Davis, S. D., & Wheeler, E. A. (2004). Evolution of xylem physiology. *The Evolution of Plant Physiology*, (21), 273.
- Balfour, D.A., & Howison O.E. (2001). Spatial and temporal variation in a mesic savanna fire regime: responses to variation in annual rainfall. *African Journal of Range and Forage Science*, 19, 43-51.
- Barton, K. (2012). MuMIn: Multi-Model Inference. R package version 1.6. 6.Barton 2012
- Bates, D., & Sarkar, D. (2007). lme4: Linear mixed-effects models using S4 classes.

- Bell, R.H.V. (1982). The effect of soil nutrient availability on the community structure in African ecosystems. In: Huntley BJ, Walker BH (eds) *Ecology of tropical savannas*. Springer, Berlin Heidelberg New York, pp 193-216.
- Belsky, A. J. (1986). Does herbivory benefit plants? A review of the evidence. *The American Naturalist*, 127(6), 870-892.
- Belsky, A. J. (1994). Influences of trees on savanna productivity: tests of shade, nutrients, and tree-grass competition. *Ecology*, 75(4), 922-932.
- Bergström, R., & Guillet, C. (2002). Summer browsing by large herbivores in short-rotation willow plantations. *Biomass and Bioenergy*, 23(1), 27-32.
- Bergvall, U. A., & Leimar, O. (2005). Plant secondary compounds and the frequency of food types affect food choice by mammalian herbivores. *Ecology*, 86(9), 2450-2460.
- Bernhard-Reversat, F. (1988). Soil nitrogen mineralization under a Eucalyptus plantation and a natural Acacia forest in Senegal. *Forest Ecology and Management*, 23(4), 233-244.
- Blackmore, A. C., Mentis, M. T. & Scholes, R. J. (1990). The origin and extent of nutrient-enriched patches within a nutrient-poor savanna in South Africa. *Journal of Biogeography*, 17, 463-470.
- Bond, W. J. (2005). Large parts of the world are brown or black: a different view on the 'Green World' hypothesis. *Journal of Vegetation Science*, 16(3), 261-266.
- Bond, W. J. (2008). What limits trees in C4 grasslands and savannas? *Annual Review of Ecology, Evolution, and Systematics*, 39, 641-659.
- Bond, W. J., & van Wilgen, B. V. (1996). *Fire and plants*. Chapman & Hall Ltd.
- Bond, W. J., & Loffell, D. (2001). Introduction of giraffe changes acacia distribution in a South African savanna, *African Journal of Ecology*, 39, 286-294.
- Bond, W. J., & Midgley, J. J. (2001). Ecology of sprouting in woody plants: the persistence niche. *Trends in Ecology & Evolution*, 16(1), 45-51.

- Bond, W. J., Midgley, G. F., & Woodward, F. I. (2003). What controls South African vegetation - climate or fire? *South African Journal of Botany*, 69, 79-91.
- Bond, W. J., & Keeley, J. E. (2005). Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology & Evolution*, 20(7), 387-394.
- Bond, W. J., Woodward, F. I. & Midgley, G. F. (2005). The global distribution of ecosystems in a world without fire. *New Phytologist*, 165, 525-538.
- Bond, W.J. (2010). Do nutrient-poor soils inhibit development of forests? A nutrient stock analysis. *Plant and Soil*, 334, 47-60.
- Boone, R. D., Grigal, D. F., Sollins, P., Ahrens, R. J., & Armstrong, D. E. (1999). Soil sampling, preparation, archiving and quality control. In Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (eds). *Standard soil methods for long-term ecological research*. Oxford University Press, Oxford USA.
- Boundja, R. P., & Midgley, J. J. (2010). Patterns of elephant impact on woody plants in the Hluhluwe-Imfolozi Park, Kwazulu Natal, South Africa, *African Journal of Ecology*, 48, 206-214.
- Bowman, D. M. J. S., & Prior, L.D. (2005). TURNER REVIEW No. 10 Why do evergreen trees dominate the Australian seasonal tropics? *Australian Journal of Botany*, 53, 379-399.
- Bradford, J. M. & Grossman, R. B. (1982). In-situ Measurement of Near-surface Soil Strength by the Fall-cone Device. *Soil Science Society of America Journal*, 46, 685-688.
- Bryant, J. P., Provenza, F. D., Pastor, J., Reichardt, P. B., Clausen, T. P., & du Toit, J. T. (1991). Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Annual Review of Ecology and Systematics*, 22, 431-446.
- Bucini, G., & Hanan, N. P. (2007). A continental scale analysis of tree cover in African savannas. *Global Ecology and Biogeography*, 16(5), 593-605.
- Bullock, J. M., Franklin, J., Stevenson, M. J., Silvertown, J., Coulson, S. J., Gregory, S. J. & Tofts, R. (2001). A plant traits analysis of responses to grazing in a long-term experiment. *Journal of Applied Ecology*, 38, 253-267.

- Buschbacher, R., Uhl, C., & Serrao, E. A. S. (1988). Abandoned pastures in Eastern Amazonia. II. Nutrient stocks in the soil and vegetation. *Journal of Ecology*, 76, 682-699.
- Carmona, D., Lajeunesse, M. J., & Johnson, M. T. (2011). Plant traits that predict resistance to herbivores. *Functional Ecology* 25, 358-367.
- Carney, K. M., Matson, P. A. & Bohannon, B. J. M. (2004). Diversity and composition of tropical soil nitrifiers across a plant diversity gradient and among land-use types. *Ecological Letters*, 7, 684-694.
- Castro-Díez, P. (2012). Functional traits analyses: Scaling-up from species to community level. *Plant and Soil*, 357, 9-12.
- Chamaillé-Jammes, S., Valeix, M., & Fritz, H. (2007a). Managing heterogeneity in elephant distribution: interactions between elephant population density and surface water availability. *Journal of Applied Ecology*, 44(3), 625-633.
- Chamaillé-Jammes, S., Fritz, H., Valeix, M., Murindagomo, F., & Clobert, J. (2007b). Resource variability, aggregation and direct density dependence in an open context: the local regulation of an African elephant population. *Journal of Animal Ecology*, 77(1), 135-144.
- Chamaillé-Jammes, S., Valeix, M., Bourgarel, M., Murindagomo, F., & Fritz, H. (2009). Seasonal density estimates of common large herbivores in Hwange National Park, Zimbabwe. *African Journal of Ecology*, 47(4), 804-808.
- Chapin, F. S. III. (1980). The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, 11, 233-260.
- Chapin F. S., Sala O. E., & Huber-Sannwald E. (2001). *Global Biodiversity in a Changing Environment: Scenarios for the 21st Century*. New York. Springer-Verlag.
- Chapin, F. S. (2003). Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. *Annals of Botany*, 91(4), 455-463.
- Chave, J., Coomes, D., Jansen, S., Lewis, S. L., Swenson, N. G., & Zanne, A. E. (2009). Towards a worldwide wood economics spectrum. *Ecology Letters*, 12(4), 351-366.

- Childes, S. L. & Walker, B. H. 1987. Ecology and dynamics of the woody vegetation on the Kalahari Sands in Hwange National Park, Zimbabwe. *Vegetatio*, 72, 111-128.
- Choong, M. F. (1996). What makes a leaf tough and how this affects the pattern of *Castanopsis fissa* leaf consumption by caterpillars. *Functional Ecology*, 10(5), 668-674.
- Cingolani, A. M., Noy-Meir, I., & Díaz, S. (2005). Grazing effects on rangeland diversity: a synthesis of contemporary models. *Ecological Applications*, 15(2), 757-773.
- Codron, D., Lee-Thorp, J. A., Sponheimer, M., Codron, J., De Ruiter, D., & Brink, J. S. (2007). Significance of diet type and diet quality for ecological diversity of African ungulates. *Journal of Animal Ecology*, 76(3), 526-537.
- Coe, M. J., Cumming, D. H., & Phillipson, J. (1976). Biomass and production of large African herbivores in relation to rainfall and primary production. *Oecologia*, 22(4), 341-354.
- Coetsee, C., February, E.C., & Bond, W.J. (2008). Nitrogen availability is not affected by frequent fire in a South African savanna. *Journal of Tropical Ecology*, 24, 647-654.
- Coetsee, C., Bond, W.J., & February, E.C. (2010). Frequent fire affects soil nitrogen and carbon in an African savanna by changing woody cover. *Oecologia*, 162, 1027-1034.
- Coetsee, C., Stock, W. D., & Craine, J. M. (2011). Do grazers alter nitrogen dynamics on grazing lawns in a South African savannah? *African Journal of Ecology*, 49(1), 62-69.
- Coetzee, B. J., Engelbrecht, A. H., Joubert, S. C. J., & Retief, P. F. (2008). Elephant impact on *Sclerocarya caffra* trees in *Acacia nigrescens* tropical plains thornveld of the Kruger National Park. *Koedoe-African Protected Area Conservation and Science*, 22(1), 39-60.
- Coley, P. D., Bryant, J. P., & Chapin III, F. S. (1985). Resource availability and plant antiherbivore defense. *Science*, 230(4728), 895-899.
- Coley, P. D. (1988). Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia*, 74(4), 531-536.

- Conover, W. J., Johnson, M. E., & Johnson, M. M. (1981). A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data. *Technometrics*, 23, 351-361.
- Cooper, S.M. & Owen-Smith, N., (1985). Condensed tannins deter feeding by browsing ruminants in a South African savanna, *Oecologia*, 67, 142-146.
- Cooper, S. M., & Owen-Smith, N. (1986). Effects of plant spinescence on large mammalian herbivores. *Oecologia*, 68(3), 446-455.
- Cornelissen, J. H. C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D. E., & Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, 51(4), 335-380.
- Cornwell, W. K., Cornelissen, J. H., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., et al., & Westoby, M. (2008). Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters*, 11(10), 1065-1071.
- Craine, J., Bond, W., Lee, W., Reich, P., & Ollinger, S. (2003). The resource economics of chemical and structural defenses across nitrogen supply gradients. *Oecologia*, 137(4), 547-556.
- Craine, J. M., Ballantyne, F., Peel, M., Zambatis, N., Morrow, C., & Stock, W. D. (2009). Grazing and landscape controls on nitrogen availability across 330 South African savanna sites. *Austral Ecology*, 34(7), 731-740.
- da Silva, D. M., & Batalha, M. A. (2008). Soil-vegetation relationships in cerrados under different fire frequencies. *Plant and Soil*, 311(1), 87-96.
- da Silva, D. M., & Batalha, M. A. (2011). Defense syndromes against herbivory in a cerrado plant community. *Plant Ecology*, 212(2), 181-193.
- Danell, K., Bergström, R., & Edenius, L. (1994). Effects of large mammalian browsers on architecture, biomass, and nutrients of woody plants. *Journal of Mammalogy*, 75(4), 833-844.

- Danell, K., Bergström, R., Edenius, L., & Ericsson, G. (2003). Ungulates as drivers of tree population dynamics at module and genet levels. *Forest Ecology and Management*, 181(1), 67-76.
- Dangerfield, J. M., & Modukanele, B. (1996). Over compensation by *Acacia erubescens* in response to simulated browsing. *Journal of Tropical Ecology*, 12(6), 905-908.
- Dezzeo, N., Chacón, N., Sanoja, E. & Picón, G. (2004). Changes in soil properties and vegetation characteristics along a forest-savanna gradient in southern Venezuela. *Forest Ecology and Management*, 200, 183-193.
- Díaz, S., Cabido, M., Zak, M., Martínez Carretero, E., & Aranibar, J. (1999). Plant functional traits, ecosystem structure and land use history along a climatic gradient in central western Argentina. *Journal of Vegetation Science*, 10(5), 651-660.
- Díaz, S., Noy-Meir, I., & Cabido, M. (2001). Can grazing response of herbaceous plants be predicted from simple vegetative traits? *Journal of Applied Ecology*, 38(3), 497-508.
- Díaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J.H.C. et al., (2004). The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science*, 15, 295-304.
- Díaz, S., Lavorel, S., McIntyre, S. U. E., Falczuk, V., Casanoves, F., Milchunas, D. G., & Cambell, B. D. (2007). Plant trait responses to grazing-a global synthesis. *Global Change Biology*, 13(2), 313-341.
- Diochon, A., Kellman, L., & Beltrami, H. (2009). Looking deeper: An investigation of soil carbon losses following harvesting from a managed northeastern red spruce *Picea rubens* Sarg. forest chronosequence. *Forest Ecology and Management*, 257, 413-420.
- Domínguez, M. T., Aponte, C., Pérez-Ramos, I. M., García, L. V., Villar, R., & Marañón, T. (2012). Relationships between leaf morphological traits, nutrient concentrations and isotopic signatures for Mediterranean woody plant species and communities. *Plant and Soil*, 357, 407-424.

- Dray, S., Chessel, D., & Thioulouse, J. (2003). Co-inertia analysis and the linking of ecological data tables. *Ecology*, 84(11), 3078-3089.
- Dray, S., & Dufour, A. B. (2007). The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22(4), 1-20.
- Dray, S., Dufour, A. B., & Chessel, D. (2007). The ade4 package II: Two-table and K-table methods. *R news*, 7(2), 47-52.
- Du Toit, J. T., Bryant, J. P., & Frisby, K. (1990). Regrowth and palatability of Acacia shoots following pruning by African savanna browsers. *Ecology*, 71(1), 149-154.
- Dupouey, J.L., Dambrine, E., Laffite, J.D., Moares, C. (2002). Irreversible impact of past land use on forest soils and biodiversity. *Ecology*, 83, 2978-2984.
- East, R. (1984). Rainfall, soil nutrient status and biomass of large African savanna mammals. *African Journal of Ecology*, 22(4), 245-270.
- Eckhardt, H. C., Wilgen, B. V., & Biggs, H. C. (2000). Trends in woody vegetation cover in the Kruger National Park, South Africa, between 1940 and 1998. *African Journal of Ecology*, 38(2), 108-115.
- Edenius, L., Ericsson, G., & Näslund, P. (2002). Selectivity by moose vs the spatial distribution of aspen: a natural experiment. *Ecography*, 25(3), 289-294.
- El Fatih Mahgoub, Pieper, R. D., & Ortiz, M. (1988). Use of leader lengths and diameters to estimate production and utilization of *Cercocarpus breviflorus*. *Journal of Range Management*, 41(2), 153-155.
- Ellery, W. N., Scholes, R. J., & Scholes, M. C. (1995). The distribution of sweetveld and sourveld in South Africa's grassland biome in relation to environmental factors. *African Journal of Range and Forage Science*, 12(1), 38-45.
- Escudero, A., Garcia, B., Gomez, J. M., & Luis, E. (1985). The nutrient cycling in *Quercus rotundifolia* and *Quercus pyrenaica* ecosystems ("dehesas") of Spain. *Acta Oecologia*, 6(1), 73-86.

- February, E.C., & Higgins, S.I. (2010). The distribution of tree and grass roots in savannas in relation to soil nitrogen and water. *South African Journal of Botany*, 76, 517-523.
- Fensham, R. J., Fairfax, R. J., & Ward, D. P. (2008). Drought induced tree death in savanna. *Global Change Biology*, 15(2), 380-387.
- Ferguson, R. B., & Marsden, M. A. (1977). Estimating overwinter bitterbrush utilization from twig diameter-length-weight relations. *Journal of Range Management*, 30(3), 231-236.
- Field, C., Merino, J., & Mooney, H. A. (1983). Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. *Oecologia*, 60(3), 384-389.
- Fine, P. V., Mesones, I., & Coley, P. D. (2004). Herbivores promote habitat specialization by trees in Amazonian forests. *Science*, 305(5684), 663-665.
- Fine, P. V., Miller, Z. J., Mesones, I., Irazuzta, S., Appel, H. M., Stevens, M. H. H., et al. & Coley, P. D. (2006). The growth-defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology*, 87(7), 150-162.
- Fonseca, C. R., Overton, J. M., Collins, B., & Westoby, M. (2000) Shifts in trait-combinations along rainfall and phosphorous gradients. *Journal of Ecology*, 88, 964-977.
- Fornara, D. A., & Du Toit, J. D. (2007). Browsing lawns? Responses of *Acacia nigrescens* to ungulate browsing in an African savanna. *Ecology*, 88(1), 200-209.
- Fornara, D., & Du Toit, J. T. (2008). Responses of woody saplings exposed to chronic mammalian herbivory in an African savanna. *Ecoscience*, 15(1), 129-135.
- Fraterrigo, J., Turner, M.G., Pearson, S.M., & Dixon, P. (2005). Effects of past land use on spatial heterogeneity of soil nutrients in Southern Appalachian forests. *Ecological Monographs*, 75, 215-230.
- Fritz, H., & Duncan, P. (1994). On the carrying capacity for large ungulates of African savanna ecosystems. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 256(1345), 77-82.

Fritz, H., Duncan, P., Gordon, I. J., & Illius, A. W. (2002). Megaherbivores influence trophic guilds structure in African ungulate communities. *Oecologia*, 131(4), 620-625.

Frost, P. G. H., Robertson, F. (1985). The ecological effects of fire in savannas. In: Walker BH (eds) *Determinants of Tropical Savannas*. International Council of Scientific Unions, Miami, pp pp 93-140.

Frost, W. E., & Edinger, S. B. (1991). Effects of tree canopies on soil characteristics of annual rangeland. *Journal of Range Management*, 44(3), 286-288.

Fukami, T., Martijn Bezemer, T., Mortimer, S. R., & Putten, W. H. (2005). Species divergence and trait convergence in experimental plant community assembly. *Ecology Letters*, 8(12), 1283-1290.

Gadd, M. E. (2002). The impact of elephants on the marula tree *Sclerocarya birrea*. *African Journal of Ecology*, 40(4), 328-336.

García-Palacios, P., Maestre, F. T., & Milla, R. (2013). Community-aggregated plant traits interact with soil nutrient heterogeneity to determine ecosystem functioning. *Plant and Soil*, 364, 119-129.

Garnier, E., Cortez, J., Billès, G., Navas, M. L., Roumet, C., Debussche, M., et al., & Toussaint, J. P. (2004). Plant functional markers capture ecosystem properties during secondary succession. *Ecology*, 85(9), 2630-2637.

Garnier, E., Lavorel, S., Ansquer, P., Castro, H., Cruz, P., Dolezal, J., Eriksson, O., Fortunel, C., Freitas, H., Golodets, C., Grigulis, K., Jouany, C., Kazakou, E., Kigel, J., Kleyer, M., Lehsten, V., Leps, J., Meier, T., Pakeman, R., Papadimitriou, M., Papanastasis, V.P., Queded, H., Quetier, F., Robson, M., Roumet, C., Rusch, G., Skarpe, C, Sternberg, M., Theau, J.P., Thebault, A., Vile, D., & Zarovali, M.P. (2007). Assessing the effects of land-use change on plant traits, communities and ecosystem functioning in grasslands: a standardized methodology and lessons from an application to 11 European sites. *Annals of Botany*, 99, 967-985.

Georgiadis, N. J. (1989). Microhabitat variation in an African savanna: effects of woody cover and herbivores in Kenya. *Journal of Tropical Ecology*, 5(1), 93-108.

- Gertenbach, W.P.D. (1980). Rainfall patterns in the Kruger National Park. *Koedoe*, 23, 35-44.
- Gignoux, J., Clobert, J., & Menaut, J. C. (1997). Alternative fire resistance strategies in savanna trees. *Oecologia*, 110(4), 576-583.
- Gillson, L. (2004). Evidence of Hierarchical Patch Dynamics in an East African Savanna? *Landscape Ecology*, 19(8), 883-894.
- Gotsch, S. G., Geiger, E. L., Franco, A. C., Goldstein, G., Meinzer, F. C., & Hoffmann, W. A. (2010). Allocation to leaf area and sapwood area affects water relations of co-occurring savanna and forest trees. *Oecologia*, 163(2), 291-301.
- Götze, A. R., Cilliers, S. S., Bezuidenhout, H., & Kellner, K. (2008). Analysis of the vegetation of the sandstone ridges (Ib land type) of the north-eastern parts of the Mapungubwe National Park, Limpopo Province, South Africa. *Koedoe*, 50(1), 72-81.
- Gowda, J. H. (2003). Physical and chemical response of juvenile *Acacia tortilis* trees to browsing. Experimental evidence. *Functional Ecology*, 11(1), 106-111.
- Grange, S., & Duncan, P. (2006). Bottom-up and top-down processes in African ungulate communities: resources and predation acting on the relative abundance of zebra and grazing bovids. *Ecography* 29: 899-907.
- Greyling, T., & Huntley, B. J. (1984). Directory of southern African conservation areas. National Scientific Programmes Unit: CSIR.
- Grime, J. P. (2006). Trait convergence and trait divergence in herbaceous plant communities: mechanisms and consequences. *Journal of Vegetation Science*, 17(2), 255-260.
- Grime, J. P., Thompson, K., Hunt, R., Hodgson, J. G., Cornelissen, J. H. C., Rorison, I. H., et al., & Whitehouse, J. (1997). Integrated screening validates primary axes of specialisation in plants. *Oikos*, 79, 259-281.
- Grime, J.P. (2001). *Plant strategies, vegetation processes, and ecosystem properties*. Wiley, Chichester, UK

- Grubb, P. J. (1992). A positive distrust in simplicity--lessons from plant defences and from competition among plants and among animals. *Journal of Ecology*, 80(4), 585.
- Güsewell, S. (2004). N: P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, 164(2), 243-266.
- Hamilton III, E. W., & Frank, D. A. (2001). Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology*, 82(9), 2397-2402.
- Hanley, M. E., & Lamont, B. B. (2002). Relationships between physical and chemical attributes of congeneric seedlings: how important is seedling defence? *Functional Ecology*, 16(2), 216-222.
- Hanley, M. E., Lamont, B. B., Fairbanks, M. M., & Rafferty, C. M. (2007). Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, 8(4), 157-178.
- Harley, C. D. (2003). Abiotic stress and herbivory interact to set range limits across a two-dimensional stress gradient. *Ecology*, 84(6), 1477-1488.
- Hartemink, A.E. (2006). Assessing soil fertility decline in the tropics using soil chemical data. *Advanced Agronomy*, 89, 180-225.
- Haslam, E. (1988). Plant polyphenols (syn. vegetable tannins) and chemical defense- a reappraisal. *Journal of Chemical Ecology*, 14(10), 1789-1805.
- Hattas, D., Stock, W. D., Mabusela, W. T., & Green, I. R. (2005). Phytochemical changes in leaves of subtropical grasses and fynbos shrubs at elevated atmospheric CO₂ concentrations. *Global and Planetary Change*, 47(2), 181-192.
- Hattas, D., & Julkunen-Tiitto, R. (2012). The quantification of condensed tannins in African savanna tree species. *Phytochemistry Letters*, 5(2), 329-334.
- He, J.S., Wang, X., Schmid, B., Flynn, D.F.B., Li, X., Reich, P.B. & Fang, J. (2010). Taxonomic identity, phylogeny, climate and soil fertility as drivers of leaf traits across Chinese grassland biomes. *Journal of Plant Research*, 123, 551-561.

- Heal, O. W., Anderson, J. M., & Swift, M. J. (1997). Plant litter quality and decomposition: an historical overview. *Driven by nature: plant litter quality and decomposition*. CAB International, Wallingford, 3-30.
- Hean, J. W., & Ward, D. (2011). Fire and herbivory are not substitutable: evidence from regrowth patterns and changes in physical and chemical defences in *Acacia* seedlings. *Journal of Vegetation Science*, 23(1), 13-23.
- Helm, C. V., & Witkowski, E. T. (2012). Continuing decline of a keystone tree species in the Kruger National Park, South Africa. *African Journal of Ecology*, 51(2), 270-279.
- Herms, D. A., & Mattson, W. J. (1992). The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, 67(3), 283-335.
- Hibbard, K.A., Archer, S., Schimel, D.S., & Valentine, D.W. (2001). Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. *Ecology*, 82, 1999-2011.
- Higgins, S. I., Bond, W. J., & Trollope, W.S.W. (2000). Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. *Journal of Ecology*, 88, 213-229.
- Higgins, S. I., Bond, W. J., & Trollope, W. S. (2003). Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. *Journal of Ecology*, 88(2), 213-229.
- Hirota, M., Holmgren, M., Van Nes, E. H., & Scheffer, M. (2011). Global resilience of tropical forest and savanna to critical transitions. *Science*, 334(6053), 232-235.
- Hobbie, S. E. (1992). Effects of plant species on nutrient cycling. *Trends in Ecology & Evolution*, 7(10), 336-339.
- Hobbs, N. T. (1996). Modification of ecosystems by ungulates. *The Journal of Wildlife Management*, 60(4), 695-713.
- Hodgson, J. G., Montserrat-Martí, G., Charles, M., Jones, G., Wilson, P., Shipley, B., et al., & Pla, F. R. (2011). Is leaf dry matter content a better predictor of soil fertility than specific leaf area? *Annals of Botany*, 108(7), 1337-1345.

- Hoffmann, W. A. (2000). Post Establishment Seedling Success in the Brazilian Cerrado: A Comparison of Savanna and Forest Species I. *Biotropica*, 32(1), 62-69.
- Holdaway, R. J., Richardson, S. J., Dickie, I. A., Peltzer, D. A., & Coomes, D. A. (2011). Species and community level patterns in fine root traits along a 120 000 year soil chronosequence in temperate rain forest. *Journal of Ecology*, 99(4), 954-963.
- Homann, P. S., Kapchinske, J. S. & Boyce, A. (2007). Relations of mineral soil-C and N to climate and texture: regional differences within the conterminous USA. *Biogeochemistry*, 85, 303-316.
- Hopper, S. D. (2009). OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant and Soil*, 322, 49-86.
- Hrbar, H., Hattas, D., & Du Toit, J. T. (2009). Differential effects of defoliation by mopane caterpillars and pruning by African elephants on the regrowth of *Colophospermum mopane* foliage. *Journal of Tropical Ecology*, 25(3), 301-309.
- Huntley, B. J., & Walker, B. H. (1982). *Ecology of tropical savannas*. Springer-Verlag.
- Huntly, N. (1991). Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology and Systematics*, 22, 477-503.
- Isichei, A. O., & Muoghalu, J. I. (1992). The effects of tree canopy cover on soil fertility in a Nigerian savanna. *Journal of Tropical Ecology*, 8(3), 329-338.
- Jachmann, H., & Croes, T. (1991). Effects of browsing by elephants on the *Combretum/Terminalia* woodland at the Nazinga Game Ranch, Burkina Faso, West Africa. *Biological Conservation*, 57(1), 13-24.
- Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E., & Schulze, E.D. (1996). A global analysis of root distributions for terrestrial biomes. *Oecologia*, 108, 389-411.
- Jensen, C. H., & Urness, P. J. (1981). Establishing browse utilization from twig diameters. *Journal of Range Management*, 34(2), 113-116.

- Jobbágy, E.G. & Jackson, R.B. (2001). The distribution of soil nutrients with depth: Global patterns and the imprint of plants. *Biogeochemistry*, 53, 51-77.
- Jones, M.J. (1973). The organic matter content of the savanna soils of West Africa. *Journal of Soil Science*, 24, 42-53.
- Karban, R., & Baldwin, I. T. (1997). *Induced responses to herbivory*. University of Chicago Press.
- Katabuchi, M., Kurokawa, H., Davies, S. J., Tan, S., & Nakashizuka, T. (2012). Soil resource availability shapes community trait structure in a species rich dipterocarp forest. *Journal of Ecology*, 100(3), 643-651.
- Kattge, J., Diaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Bönisch, G., et al., & Ford, H. (2011). TRY- a global database of plant traits. *Global Change Biology*, 17(9), 2905-2935.
- Keddy, P. A. (1992). A pragmatic approach to functional ecology. *Functional Ecology*, 6(6), 621-626.
- Kellman, M. (1979). Soil enrichment by neotropical savanna trees. *The Journal of Ecology*, 67(2), 565-577.
- Kindt, R., & Coe, R. (2005). *Tree diversity analysis: A manual and software for common statistical methods for ecological and biodiversity studies*. World Agroforestry Centre Eastern and Central Africa Program.
- King, D. A., Davies, S. J., Supardi, M. N., & Tan, S. (2005). Tree growth is related to light interception and wood density in two mixed dipterocarp forests of Malaysia. *Functional Ecology*, 19(3), 445-453.
- Koerselman, W., & Meuleman, A. F. (1996). The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, 33, 1441-1450.
- Koricheva, J. (2002). Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. *Ecology*, 83(1), 176-190.

- Kriebitzsch, W. U., Oheimb, G. V., Ellenberg, H., Engelschall, B., & Heuveldop, J. (2000). Development of woody plant species in fenced and unfenced plots in deciduous forests on glacial moraine soils in northern Germany. *Allgemeine Forst-und Jagdzeitung*, 171(1), 1-10.
- Laliberté, E., Shipley, B., Norton, D. A., & Scott, D. (2012). Which plant traits determine abundance under long term shifts in soil resource availability and grazing intensity? *Journal of Ecology*, 100(3), 662-677.
- Lambers, H. A. N. S., & Poorter, H. (1992). Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Academic Press.
- Lauber, C. L., Strickland, M. S., Bradford, M. A., & Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry*, 40, 2407-2415.
- Lavoie, B., & Oberhauser, K. S. (2004). Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. *Environmental Entomology*, 33(4), 1062-1069.
- Lavorel, S., & Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 16(5), 545-556.
- Le Roux, X., Bardy, M., Loiseau, P., & Louault, F. (2003). Stimulation of soil nitrification and denitrification by grazing in grasslands: do changes in plant species composition matter? *Oecologia*, 137(3), 417-425.
- Lechmere-Oertel, R. G., Kerley, G. I. H., & Cowling, R. M. (2005). Patterns and implications of transformation in semi-arid succulent thicket, South Africa. *Journal of Arid Environments*, 62, 459-474.
- Lehmann, C. E., Archibald, S. A., Hoffmann, W. A., & Bond, W. J. (2011). Deciphering the distribution of the savanna biome. *New Phytologist*, 191(1), 197-209.
- Leriche, H., Le Roux, X., Desnoyers, F., Benest, D., Simioni, G., & Abbadie, L. (2003). Grass response to clipping in an African savanna: testing the grazing optimization hypothesis. *Ecological Applications*, 13(5), 1346-1354.

- Levick, S., & Rogers, K. (2008). Patch and species specific responses of savanna woody vegetation to browser exclusion. *Biological Conservation*, 141(2), 489-498.
- Levick, S. R., Asner, G. P., Kennedy-Bowdoin, T., & Knapp, D. E. (2009). The relative influence of fire and herbivory on savanna three-dimensional vegetation structure. *Biological Conservation*, 142, 1693-1700.
- Levick, S. R., Asner, G. P., Kennedy-Bowdoin, T., & Knapp, D. E. (2010). The spatial extent of termite influences on herbivore browsing in an African savanna. *Biological Conservation*, 143(11), 2462-2467.
- Lewis, D. M. (1991). Observations of tree growth, woodland structure and elephant damage on *Colophospermum mopane* in Luangwa Valley, Zambia. *African Journal of Ecology*, 29, 207-22
- Lindroth, R. L. (1989). Mammalian herbivore-plant interactions. *Plant-animal interactions*. McGraw-Hill, New York, 163-206.
- Lloyd J., Bird, M. I., Vellen, L., Miranda, A. C., Veenendaal, E. M., Djagbletey, G., Miranda, H. S., Cook, G., & Farquhar, G. D. (2008). Contributions of woody and herbaceous vegetation to tropical savanna ecosystem productivity: a quasi-global estimate. *Tree physiology*, 28(3), 451-468.
- Low, A. B., & Rebelo, A. G. (1996). *Vegetation of South Africa, Lesotho and Swaziland. A companion to the Vegetation Map of South Africa, Lesotho and Swaziland*. Department of Environmental Affairs and Tourism, Pretoria.
- Ludwig, F., de Kroon, H., Berendse, F., & Prins, H. H. (2004). The influence of savanna trees on nutrient, water and light availability and the understory vegetation. *Plant Ecology*, 170(1), 93-105.
- MacCracken, J. G., & Viereck, L. A. (1990). Browse regrowth and use by moose after fire in interior Alaska. *Northwest Science*, 64(1), 11-18.
- Marquis, R. J. (1996). Plant architecture, sectoriality and plant tolerance to herbivores. *Plant Ecology*, 127(1), 85-97.

- Matches, A.G. (1992). Plant response to grazing: a review. *Journal of Production Agriculture*, 5, 1-7.
- Matiza, T. 1992. The utilization and status of dambos in southern Africa: a Zimbabwe case study. In Matiza, T., Chabwela, H.N. (eds.), *Wetlands Conservation Conference for Southern Africa. Proceedings of the Southern African Development Coordination Conference held in Gaborone, Botswana, 3-5 June 1991*. IUCN, Gland, Switzerland.
- McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution*, 21(4), 178-185.
- McLean, E. O. (1982). Soil pH and lime requirement. *Methods of soil analysis. Part 2. Chemical and microbiological properties, (methodsofsoilan2)*, 199-224.
- McNaughton, S. J. (1985). Ecology of a grazing ecosystem: the Serengeti. *Ecological monographs*, 55(3), 259-294.
- McNaughton, S. J., Milchunas, D. G., & Frank, D. A. (1996). How can net primary productivity be measured in grazing ecosystems? *Ecology*, 77(3), 974-977.
- McNaughton, S. J., Banyikwa, F. F., & McNaughton, M. M. (1997). Promotion of the cycling of diet-enhancing nutrients by African grazers. *Science*, 278(5344), 1798-1800.
- McNaughton, S. J., Ruess, R. W., & Seagle, S. W. (1988). Large mammals and process dynamics in African ecosystems. *BioScience*, 38(11), 794-800.
- Michel, A. L., Bengis, R. G., Keet, D. F., Hofmeyr, M., Klerk, L. D., Cross, P. C., et al., & Godfroid, J. (2006). Wildlife tuberculosis in South African conservation areas: implications and challenges. *Veterinary Microbiology*, 112(2), 91-100.
- Midgley, J. J., Lawes, M. J., & Chamaille-Jammes, S. (2010). Turner Review No. 19. Savanna woody plant dynamics: the role of fire and herbivory, separately and synergistically. *Australian Journal of Botany*, 58(1), 1-11.

Mills, A.J., & Fey, M.V. 2003. Declining soil quality in South Africa: effects of land use on soil organic matter and surface crusting. *South African Journal of Science*, 99, 429-436.

Mills, A. J., Milewski, A. V., Sirami, C., Rogers, K. H., Witkowski, E. T., Stalmans, M., & Fey, M. V. (2012). Aerosol capture by small trees in savannas marginal to treeless grassland in South Africa. *Geoderma*, 189, 124-132.

Moles, A. T., Peco, B., Wallis, I. R., Foley, W. J., Poore, A. G. B., Seabloom, E. W., Vesk, P. A., Bisigato, A. J., Cella-Pizarro, L., Clark, C. J., Cohen, P. S., Cornwell, W. K., Edwards, W., Ejrnæs, R., Gonzales-Ojeda, T., Graae, B. J., Hay, G., Lumbwe, F. C., Magaña-Rodríguez, B., Moore, B. D., Peri, P. L., Poulsen, J. R., Stegen, J. C., Veldtman, R., von Zeipel, H., Andrew, N. R., Boulter, S. L., Borer, E. T., Cornelissen, J. H. C., Farji-Brener, A. G., DeGabriel, J. L., Jurado, E., Kyhn, L. A., Low, B., Mulder, C. P. H., Reardon-Smith, K., Rodríguez-Velázquez, J., De Fortier, A., Zheng, Z., Blendinger, P. G., Enquist, B. J., Facelli, J. M., Knight, T., Majer, J. D., Martínez-Ramos, M., McQuillan, P. and Hui, F. K. C. (2013). Correlations between physical and chemical defences in plants: tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? *New Phytologist*, 198: 252–263. doi: 10.1111/nph.12116.

Moncrieff, G. R., Chamaille-Jammes, S., Higgins, S. I., O'Hara, R. B., & Bond, W. J. (2011). Tree allometries reflect a lifetime of herbivory in an African savanna. *Ecology*, 92(12), 2310-2315.

Mooney, H. A., Ferrar, P. J., & Slatyer, R. O. (1978). Photosynthetic capacity and carbon allocation patterns in diverse growth forms of *Eucalyptus*. *Oecologia*, 36(1), 103-111.

Mordelet, P., Abbadie, L., & Menaut, J. C. (1993). Effects of tree clumps on soil characteristics in a humid savanna of West Africa (Lamto, Cote d'Ivoire). *Plant and Soil*, 153(1), 103-111.

Mu, Q., Zhao, M., & Running, S. W. (2011). Improvements to a MODIS global terrestrial evapotranspiration algorithm. *Remote Sensing of Environment*, 115(8), 1781-1800.

Mucina, L., & Rutherford, M. C. (2006). The vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute.

- Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. *Methods of Soil Analysis Part 3-Chemical Methods*, (methodsofsoilan3), 961-1010.
- Niklas, K. J., Owens, T., Reich, P. B., & Cobb, E. D. (2005). Nitrogen/phosphorus leaf stoichiometry and the scaling of plant growth. *Ecology Letters*, 8(6), 636-642.
- Norby, R. J., Cotrufo, M. F., Ineson, P., O'Neill, E. G., & Canadell, J. G. (2001). Elevated CO₂, litter chemistry, and decomposition: a synthesis. *Oecologia*, 127(2), 153-165.
- O'Connor, T. G., Goodman, P. S., & Clegg, B. (2007). A functional hypothesis of the threat of local extirpation of woody plant species by elephant in Africa. *Biological Conservation*, 136, 329-345.
- Ohgushi, T. (2005). Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology, Evolution, and Systematics*, 36, 81-105.
- Olf, H., Ritchie, M. E., & Prins, H. H. (2002). Global environmental controls of diversity in large herbivores. *Nature*, 415(6874), 901-904.
- Ordóñez, A., Wright, I. J., & Olf, H. (2010). Functional differences between native and alien species: a global scale comparison. *Functional Ecology*, 24(6), 1353-1361.
- Ordoñez, J. C., Van Bodegom, P. M., Witte, J. P. M., Wright, I. J., Reich, P. B., & Aerts, R. (2009). A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography*, 18(2), 137-149.
- Oren, R., Ellsworth, D. S., Johnsen, K. H., Phillips, N., Ewers, B. E., Maier, C., et al., & Katul, G. G. (2001). Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature*, 414, 469-472.
- Owen-Smith, N. (2005). Incorporating fundamental laws of biology and physics into population ecology: the metaphysiological approach. *Oikos*, 111(3), 611-615.
- Owen-Smith, N., & Cooper, S. M. (1987). Palatability of woody plants to browsing ruminants in a South African savanna. *Ecology*, 68(2), 319-331.

- Owen-Smith, R. N. (2002). Adaptive herbivore ecology: from resources to populations in variable environments. Cambridge University Press.
- Paine, R. T. (2000). Phycology for the mammalogist: marine rocky shores and mammal-dominated communities-how different are the structuring processes? *Journal of Mammalogy*, 81(3), 637-648.
- Palo, R. T., & Robbins, C. T. (1991). Plant defenses against mammalian herbivory. Boca Raton, CRC Press Llc.
- Pausas, J. G., & Lavorel, S. (2003). A hierarchical deductive approach for functional types in disturbed ecosystems. *Journal of Vegetation Science*, 14(3), 409-416.
- Pellew, R. A. (1983). The giraffe and its food resource in the Serengeti. I. Composition, biomass and production of available browse. *African Journal of Ecology*, 21(4), 241-267.
- Penzhorn, B. L., Robbertse, P. J., & Olivier, M. C. (1974). The influence of the African elephant on the vegetation of the Addo Elephant National Park, Koedoe-African Protected Area *Conservation and Science*, 17, 137-158.
- Pérez Harguindeguy, N., Díaz, S., Vendramini, F., Cornelissen, J. H., Gurvich, D. E., & Cabido, M. (2003). Leaf traits and herbivore selection in the field and in cafeteria experiments. *Austral Ecology*, 28(6), 642-650.
- Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. (2013). nlme: Linear and nonlinear mixed effects models. R package version 3.1-108.
- Poorter, H. & De Jong, R. (1999). A comparison of specific leaf area, chemical composition and leaf construction costs of veld plants from 15 habitats differing in productivity. *New Phytologist*, 143, 163-176.
- Poorter, H., & Garnier, E. (1999). Ecological significance of inherent variation in relative growth rate and its components. *Handbook of functional plant ecology*, pp 81-120.
- Poorter, L., Bongers, F., Sterck, F. J., & Wöll, H. (2003). Architecture of 53 rain forest tree species differing in adult stature and shade tolerance. *Ecology*, 84(3), 602-608.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.

Ramankutty, N., & Foley, J. A. (1999). Estimating historical changes in global land cover: Croplands from 1700 to 1992. *Global Biogeochemical Cycles*, 13(4), 997-1027.

Reich, P. B., Ellsworth, D. S., Walters, M. B., Vose, J. M., Gresham, C., Volin, J. C., & Bowman, W. D. (1999). Generality of leaf trait relationships: a test across six biomes. *Ecology*, 80(6), 1955-1969.

Reich, P. B., Walters, M. B., Ellsworth, D. S., Vose, J. M., Volin, J. C., Gresham, C., & Bowman, W. D. (1998). Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups. *Oecologia*, 114(4), 471-482.

Reich, P. B., Peterson, D. W., Wedin, D. A., & Wrage, K. (2001). Fire and vegetation effects on productivity and nitrogen cycling across a forest-grassland continuum. *Ecology*, 82(6), 1703-1719.

Rhoades, D. F. (1979). Evolution of plant chemical defense against herbivores. *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York, 3-54.

Ritchie, M. E., Tilman, D., & Knops, J. M. (1998). Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology*, 79(1), 165-177.

Robinson, G.A. (1996). Limpopo Valley National Park: towards trans-frontier conservation in South Africa. Unpublished report, South African National Parks.

Rogers, C.M.L. (1993). A Woody Vegetation Survey of Hwange National Park. Department of National Parks and Wildlife Management, Harare, Zimbabwe.

Rohner, C., & Ward, D. (1997). Chemical and mechanical defense against herbivory in two sympatric species of desert *Acacia*. *Journal of Vegetation Science*, 8(5), 717-726.

- Rooke, T. (2003). Growth responses of a woody species to clipping and goat saliva. *African Journal of Ecology*, 41(4), 324-328.
- Rooke, T., Bergström, R., Skarpe, C., & Danell, K. (2004a). Morphological responses of woody species to simulated twig-browsing in Botswana. *Journal of Tropical Ecology*. 20, 281-289.
- Rooke, T., Danell, K., Bergström, R., Skarpe, C., & Hjältén, J. (2004b). Defensive traits of savanna trees-the role of shoot exposure to browsers. *Oikos*, 107(1), 161-171.
- Rooke, T., & Bergström, R. (2007). Growth, chemical responses and herbivory after simulated leaf browsing in *Combretum apiculatum*. *Plant Ecology*, 189(2), 201-212.
- Ruggiero, P.G.C., Batalha, M.A., Pivello, V.R., & Meirelles, S.T. (2002). Soil-vegetation relationships in cerrado Brazilian savanna. and semideciduous forest, Southeastern Brazil. *Plant Ecology*, 160, 1-16.
- Ruiz-Robledo J, Villar, R. (2005). Relative growth rate and biomass allocation in ten woody species with different leaf longevity using phylogenetic independent contrasts PICs. *Plant Biology*, 7, 484-494.
- Rusch, G. M., Skarpe, C., & Halley, D. J. (2009). Plant traits link hypothesis about resource-use and response to herbivory. *Basic and Applied Ecology*, 10(5), 466-474.
- Rutherford, M.C. (1997) Categorization of biomes In: Cowling, R.M., Richardson, D.M., & Pierce, S.M. (eds). *Vegetation of Southern Africa*. Cambridge University Press, Cambridge, pp 91-98. ISBN 0-521-57142-1.
- Sander, J., & Wardell-Johnson, G. (2011). Impacts of soil fertility on species and phylogenetic turnover in the high - rainfall zone of the Southwest Australian global biodiversity hotspot. *Plant and Soil*, 22(5), 766-780.
- Sandoval-Pérez, A.L., Gavito, M.E., Garcia-Oliva, F., & Jaramillo, V.J. (2009). Carbon, nitrogen, phosphorus and enzymatic activity under different land uses in a tropical, dry ecosystem. *Soil Use Management*, 25, 419-426.

- Sankaran, M., Ratnam, J., & Hanan, N. P. (2004). Tree-grass coexistence in savannas revisited-insights from an examination of assumptions and mechanisms invoked in existing models. *Ecology Letters*, 7(6), 480-490.
- Sankaran, M., Hanan, N. P., Scholes, R. J., Ratnam, J., Augustine, D. J., Cade, B. S., et al., & Zambatis, N. (2005). Determinants of woody cover in African savannas. *Nature*, 438(7069), 846-849.
- Sankaran, M., Ratnam, J., & Hanan, N. (2008). Woody cover in African savannas: the role of resources, fire and herbivory. *Global Ecology and Biogeography*, 17(2), 236-245.
- SANParks (2012). Mapungubwe National Park and World Heritage Site Management Plan. http://sanparks.org.za/assets/docs/conservation/park_man/mapungubwe-draft-plan.pdf.
- Schenk, H.J., & Jackson, R.B. (2002). The global biogeography of roots. *Ecological Monographs*, 72, 311-328.
- Scholes, R. J. (1990). The influence of soil fertility on the ecology of southern African dry savannas. *Journal of Biogeography*, 17(4), 415-419.
- Scholes, R. J. (1997). Savanna. *Vegetation of Southern Africa*, 258-277.
- Scholes, R.J., & Walker, B.H. 1993. An African savanna: synthesis of the Nylsvley study. *Cambridge studies in applied ecology and resource management*. Cambridge University Press, Cambridge, UK.
- Scholes, R. J., & Hall, D. O. (1996). The carbon budget of tropical savannas, woodlands and grasslands. *Scope Scientific Committee on Problems of the Environment International Council of Scientific Unions*, 56, 69-100.
- Scholes, R. J., & Archer, S. R. (1997). Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics*, 28, 517-544.
- Schoonhoven, L. M., Loon, J. V., & Dicke, M. (2005). *Insect-plant biology* (No. Ed. 2). Oxford University Press.

- Schulze, R.E., Maharaj, M., Lynch, S.D., Howe, B.J., & Melvil Thomson, B. (1997) South African atlas of agrohydrology and climatology. Water Research Commission, Pretoria.
- Schutz, A. E. N., Bond, W. J., & Cramer, M. D. (2009). Juggling carbon: allocation patterns of a dominant tree in a fire-prone savanna. *Oecologia*, 160(2), 235-246.
- Scogings, P., & Macanda, M. (2005). Acacia karroo responses to early dormant season defoliation and debarking by goats in a semi-arid subtropical savanna. *Plant Ecology*, 179(2), 193-206.
- Scogings, P. F., Hjältén, J., & Skarpe, C. (2011). Secondary metabolites and nutrients of woody plants in relation to browsing intensity in African savannas. *Oecologia*, 167(4), 1063-1073.
- Shipley, B., Vile, D., & Garnier, É. (2006). From plant traits to plant communities: a statistical mechanistic approach to biodiversity. *Science*, 314(5800), 812-814.
- Shrader, A. M., Bell, C., Bertolli, L., & Ward, D. (2011). Forest or the trees: At what scale do elephants make foraging decisions? *Acta Oecologica*, 42, 3-10.
- Skarpe, C., & Hester, A. J. (2008). Plant traits, browsing and grazing herbivores, and vegetation dynamics. In *The ecology of browsing and grazing* (pp. 217-261). Springer Berlin Heidelberg.
- Skogsmyr, I., & Fagerström, T. (1992). The cost of anti-herbivory defence: an evaluation of some ecological and physiological factors. *Oikos*, 64, 451-457.
- Smallie, J. J., & T. G. O'Connor. (2000). Elephant utilization of *Colophospermum mopane*: possible benefits of hedging. *African Journal of Ecology*, 38, 1-9.
- Smit, I. P., Grant, C. C., & Devereux, B. J. (2007). Do artificial waterholes influence the way herbivores use the landscape? Herbivore distribution patterns around rivers and artificial surface water sources in a large African savanna park. *Biological Conservation*, 136(1), 85-99.
- Smith, D.B., Woodruff, L.G., O'Leary, R.M., Cannon, W.F., Garrett, R.G., Kilburn, J.E. & Goldhaber, M.B. (2009). Pilot studies for the North American Soil Geochemical Landscapes

Project - Site selection, sampling protocols, analytical methods, and quality control protocols: *Applied Geochemistry*, 24, 1357- 1368. <http://dx.doi.org/10.1016/j.apgeochem.2009.04.008>.

Soil Survey Staff. (1993). *Soil survey manual*. U.S. Department of Agriculture Handbook No. 18 U.S. Government Printing Office, Washington, DC, 437-1036.

Staver, A. C., Bond, W. J., Stock, W. D., Van Rensburg, S. J., & Waldram, M. S. (2009). Browsing and fire interact to suppress tree density in an African savanna. *Ecological Applications*, 19(7), 1909-1919.

Staver, A. C., Archibald, S., & Levin, S. A. (2011). The global extent and determinants of savanna and forest as alternative biome states. *Science*, 334(6053), 230-232.

Staver, A. C., Bond, W. J., Cramer, M. D., & Wakeling, J. L. (2012). Top down determinants of niche structure and adaptation among African Acacias. *Ecology Letters*, 15, 673-679.

Stock, W. D., Bond, W. J., & van de Vijver, C. A. (2010). Herbivore and nutrient control of lawn and bunch grass distributions in a southern African savanna. *Plant Ecology*, 206(1), 15-27.

Stuart-Hill, G. C. (1992). Effects of elephants and goats on the Kaffrarian succulent thicket of the Eastern Cape, South Africa. *Journal of Applied Ecology*, 29(3), 699-710.

Stuart-Hill, G. C., & Tainton, N. M. (1989). The competitive interaction between *Acacia karroo* and the herbaceous layer and how this is influenced by defoliation. *Journal of Applied Ecology*, 26(1), 285-298.

Tanentzap, A. J., Bazely, D. R., Koh, S., Timciska, M., Haggith, E. G., Carleton, T. J., & Coomes, D. A. (2011). Seeing the forest for the deer: Do reductions in deer-disturbance lead to forest recovery? *Biological Conservation*, 144(1), 376-382.

Tate, K.W., Dudley, D.M., McDougald, N.K., & George, M.R. (2004). Effect of canopy and grazing on soil bulk density. *Journal of Range Management*, 57, 414-417.

Teague, W. R. (1987). Response of *Acacia karroo* trees to intensity of defoliation at different phenophases in a South African savanna. In *Rangelands: a resource under siege*. Proceedings of the International Rangeland Congress, Cambridge University Press, Cambridge, UK, 394-397.

Tilman, D. (1982). *Resource Competition and Community Structure*. (MPB-17)(Vol. 17). Princeton University Press, Princeton, New Jersey, USA.

Toerien, D. K. (1972). Geologie van die Addo-Olifant Nasionale Park. *Koedoe: African Protected Area Conservation and Science*, 15, 67-75.

Treydte, A. C., Heitkönig, I., Prins, H. H., & Ludwig, F. (2007). Trees improve grass quality for herbivores in African savannas. *Perspectives in Plant Ecology, Evolution and Systematics*, 8(4), 197-205.

Trollope, W.S.W., Trollope, L.A., Biggs, H.C., Pienaar, D., & Potgieter, A.L.F. (1998) Long-term changes in the woody vegetation of the Kruger National Park, with special reference to the effects of elephants and fire. *Koedoe*, 41, 103-112.

Turkington, R. T. R. (2009). Top-down and bottom-up forces in mammalian herbivore-vegetation systems: an essay review. *Botany*, 87(8), 723-739.

Van Hees, A. F. M., Kuiters, A. T., & Slim, P. A. (1996). Growth and development of silver birch, pedunculate oak and beech as affected by deer browsing. *Forest Ecology and Management*, 88(1), 55-63.

Van Langevelde, F., Van De Vijver, C. A., Kumar, L., Van De Koppel, J., De Ridder, N., Van Andel, J., et al., & Rietkerk, M. (2003). Effects of fire and herbivory on the stability of savanna ecosystems. *Ecology*, 84(2), 337-350.

Van Rooyen, N., & Bredenkamp, G. J. (1996). Mopane Bushveld. *Vegetation of South Africa, Lesotho and Swaziland*. Pretoria: Department of Environmental Affairs and Tourism, 20-21.

Van Staden, P. J., & Bredenkamp, G. J. (2007). Major plant communities of the Marakele National Park. *Koedoe-African Protected Area Conservation and Science*, 48(2), 59-70.

Van Staden, P.J. (2002). An ecological study of the plant communities of Marakele National Park. MSc Thesis, University of Pretoria, Pretoria.

Van Vegten, J. A. (1984). Thornbush invasion in a savanna ecosystem in eastern Botswana. *Vegetatio*, 56(1), 3-7.

- Van Wilgen, B.W., Govender, N. & Biggs, H.C. (2007). The contribution of fire research to fire management: a critical review of a long-term experiment in the Kruger National Park, South Africa. *International Journal of Wildland Fire*, 16, 519-530.
- Venter, F. J., Scholes, R. J., & Eckhardt, H. C. (2003). The abiotic template and its associated vegetation pattern. *The Kruger experience: Ecology and management of savanna heterogeneity*, 83-129.
- Verheyen, K., Bossuyt, B., Hermy, M., & Tack, G. (1999). The land use history 1278-1990 of a mixed hardwood forest in western Belgium and its relationship with chemical soil characteristics. *Journal of Biogeography*, 26, 1115-1128.
- Vile, D., Shipley, B., & Garnier, E. (2006). A structural equation model to integrate changes in functional strategies during old-field succession. *Ecology*, 87(2), 504-517.
- Villar, R., Robledo, J. R., De Jong, Y., & Poorter, H. (2006). Differences in construction costs and chemical composition between deciduous and evergreen woody species are small as compared to differences among families. *Plant, Cell and Environment*, 29(8), 1629-1643.
- Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, 116(5), 882-892.
- Visser, V., Woodward, F. I., Freckleton, R. P., & Osborne, C. P. (2011). Environmental factors determining the phylogenetic structure of C4 grass communities. *Journal of Biogeography*, 39(2), 232-246.
- Vlok, J. H. J., & Euston-Brown, D. I. W. (2002). The patterns within, and the ecological processes that sustain, the Subtropical Thicket Vegetation in the planning domain for the Subtropical Thicket Ecosystem Planning (Step) Project. TERU report, 40, 142pp.
- Vourc'h, G., De Garine-Wichatitsky, M., Labbé, A., Rosolowski, D., Martin, J. L., & Fritz, H. (2002). Monoterpene effect on feeding choice by deer. *Journal of Chemical Ecology*, 28(12), 2411-2427.

- Wakeling, J. L., & Bond, W. J. (2007). Disturbance and the frequency of root suckering in an invasive savanna shrub, *Dichrostachys cinerea*. *African Journal of Range and Forage Science*, 24(2), 73-76.
- Waldram, M. S., Bond, W. J., & Stock, W. D. (2008). Ecological engineering by a mega-grazer: white rhino impacts on a South African savanna. *Ecosystems*, 11(1), 101-112.
- Walkley, A. (1947). A critical examination of a rapid method for determining organic carbon in soils-effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science*, 63(4), 251-264.
- Weiher, E., & Keddy, P. (Eds.). (2001). *Ecological assembly rules: perspectives, advances, retreats*. Cambridge University Press.
- Weltzin, J.F., & Coughenour, M. B. (1990). Savanna tree influence on understory vegetation and soil in North-West Kenya. *Journal of Vegetation Science*, 1: 325-332.
- Wessels, D. C. J., van der Waal, C., de Boer, W. F. (2007). Induced chemical defences in *Colophospermum mopane* trees. *African Journal of Range and Forage Science*, 24, 141-147.
- Westoby, M. (1998). A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, 199(2), 213-227.
- Westoby, M., & Wright, I. J. (2006). Land-plant ecology on the basis of functional traits. *Trends in Ecology & Evolution*, 21(5), 261-268.
- Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., & Wright, I. J. (2002). Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics*, 33,125-159.
- Wiegand, K., Saltz, D., & Ward, D. (2006). A patch-dynamics approach to savanna dynamics and woody plant encroachment-Insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics*, 7(4), 229-242.
- Wigley, B. J., Cramer, M. D., & Bond, W. J. (2009). Sapling survival in a frequently burnt savanna: mobilisation of carbon reserves in *Acacia karroo*. *Plant Ecology*, 203(1), 1-11.

Wigley, B. J., Bond, W. J., & Hoffman, M. (2010). Thicket expansion in a South African savanna under divergent land use: local vs. global drivers? *Global Change Biology*, 16(3), 964-976.

Wright, I. J., Reich, P. B., & Westoby, M. (2001). Strategy shifts in leaf physiology, structure and nutrient content between species of high and low rainfall and high and low nutrient habitats. *Functional Ecology*, 15(4), 423-434.

Wright, I. J., Reich, P. B., Cornelissen, J. H., Falster, D. S., Garnier, E., Hikosaka, K., et al., & Westoby, M. (2005). Assessing the generality of global leaf trait relationships. *New Phytologist*, 166(2), 485-496.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al., & Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821-827.

Wright, I. J., Westoby, M., & Reich, P. B. (2002). Convergence towards higher leaf mass per area in dry and nutrient poor habitats has different consequences for leaf life span. *Journal of Ecology*, 90(3), 534-543.

Wright, I.J., Reich, P.B., & Westoby, M. (2001). Strategy-shifts in leaf physiology, structure and nutrient content between species of high and low rainfall, and high and low nutrient habitats. *Functional Ecology*, 15, 423-434.

Yang, Y., Fang, J., Tang, Y., Ji, C., Zheng, C., He, J., & Zhu, B. (2008). Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. *Global Change Biology*, 14, 1592-1599.

Appendices

Appendix 1. Woody species identity, site names, broad soil nutrient status and leaf type.

Species	Site	Soil	Broad/Fine leaved
<i>Azima tetracantha</i>	Addo	Nutrient-rich	Broad-leaved
<i>Capparis sepiaria</i>	Addo	Nutrient-rich	Broad-leaved
<i>Carissa haematocarpa</i>	Addo	Nutrient-rich	Broad-leaved
<i>Euclea undulata</i>	Addo	Nutrient-rich	Broad-leaved
<i>Gymnosporia pyracantha</i>	Addo	Nutrient-rich	Broad-leaved
<i>Portulacaria afra</i>	Addo	Nutrient-rich	Broad-leaved
<i>Searsia (Rhus) pyroides</i>	Addo	Nutrient-rich	Broad-leaved
<i>Schotia afra</i>	Addo	Nutrient-rich	Broad-leaved
<i>Carissa bispinosa</i>	Enseleni	Nutrient-poor	Broad-leaved
<i>Chaetachme aristata</i>	Enseleni	Nutrient-poor	Broad-leaved
<i>Dalbergia armata</i>	Enseleni	Nutrient-poor	Fine-leaved
<i>Englerophytum natalense</i>	Enseleni	Nutrient-poor	Broad-leaved
<i>Maytenus mossambicensis</i>	Enseleni	Nutrient-poor	Broad-leaved
<i>Pisonia aculeata</i>	Enseleni	Nutrient-poor	Broad-leaved
<i>Rothmania globosa</i>	Enseleni	Nutrient-poor	Broad-leaved
<i>Uvaria caffra</i>	Enseleni	Nutrient-poor	Broad-leaved
<i>Acacia caffra</i>	Hluhluwe	Nutrient-rich	Fine-leaved
<i>Acacia karroo</i>	Hluhluwe	Nutrient-rich	Fine-leaved
<i>Acacia nilotica</i>	Hluhluwe	Nutrient-rich	Fine-leaved
<i>Gymnosporia senegalensis</i>	Hluhluwe	Nutrient-rich	Broad-leaved
<i>Searsia (Rhus) pentheri</i>	Hluhluwe	Nutrient-rich	Broad-leaved
<i>Sclerocarya birrea</i>	Hluhluwe	Nutrient-rich	Broad-leaved
<i>Ziziphus mucronata</i>	Hluhluwe	Nutrient-rich	Broad-leaved
<i>Baikiaea plurijuga</i>	Hwa Baik	Nutrient-poor	Broad-leaved
<i>Bauhinia petersiana</i>	Hwa Baik	Nutrient-poor	Broad-leaved
<i>Combretum adenogonium</i>	Hwa Baik	Nutrient-poor	Broad-leaved
<i>Guibourtia coleosperma</i>	Hwa Baik	Nutrient-poor	Broad-leaved
<i>Ochna pulchra</i>	Hwa Baik	Nutrient-poor	Broad-leaved
<i>Pseudolachnostylis maprouneifolia</i>	Hwa Baik	Nutrient-poor	Broad-leaved
<i>Acacia ataxacantha</i>	Hwa Dopi	Nutrient-poor	Fine-leaved
<i>Acacia erioloba</i>	Hwa Dopi	Nutrient-poor	Fine-leaved
<i>Acacia fleckii</i>	Hwa Dopi	Nutrient-poor	Fine-leaved
<i>Acacia luederitzii</i> var. <i>luederitzii</i>	Hwa Dopi	Nutrient-poor	Fine-leaved
<i>Dichrostachys cinerea</i> subsp. <i>africana</i>	Hwa Dopi	Nutrient-poor	Fine-leaved
<i>Diospyros lycioides</i> subsp. <i>lycioides</i>	Hwa Dopi	Nutrient-poor	Broad-leaved
<i>Brachystegia boehmii</i>	Hwa Khat	Nutrient-poor	Broad-leaved
<i>Brachystegia spiciformis</i>	Hwa Khat	Nutrient-poor	Broad-leaved
<i>Burkea africana</i>	Hwa Khat	Nutrient-poor	Broad-leaved
<i>Erythrophleum africanum</i>	Hwa Khat	Nutrient-poor	Broad-leaved

<i>Peltophorum africanum</i>	Hwa Khat	Nutrient-poor	Fine-leaved
<i>Terminalia brachystemma</i>	Hwa Khat	Nutrient-poor	Broad-leaved
<i>Acacia gerrardii</i>	Makhohlolo	Nutrient-rich	Fine-leaved
<i>Acacia nigrescens</i>	Makhohlolo	Nutrient-rich	Fine-leaved
<i>Dalbergia melanoxylon</i>	Makhohlolo	Nutrient-rich	Fine-leaved
<i>Lannea schweinfurthii</i>	Makhohlolo	Nutrient-rich	Broad-leaved
<i>Albizia harveyi</i>	Makhohlolo	Nutrient-rich	Fine-leaved
<i>Gymnosporia senegalensis</i>	Makhohlolo	Nutrient-rich	Broad-leaved
<i>Acacia senegal</i>	Mapungubwe Gabbro	Nutrient-rich	Fine-leaved
<i>Albizia harveyi</i>	Mapungubwe Gabbro	Nutrient-rich	Fine-leaved
<i>Boscia albitrunca</i>	Mapungubwe Gabbro	Nutrient-rich	Broad-leaved
<i>Commiphora neglecta</i>	Mapungubwe Gabbro	Nutrient-rich	Broad-leaved
<i>Commiphora schimperi</i>	Mapungubwe Gabbro	Nutrient-rich	Broad-leaved
<i>Cordia ovata</i>	Mapungubwe Gabbro	Nutrient-rich	Broad-leaved
<i>Grewia flavescens</i>	Mapungubwe Gabbro	Nutrient-rich	Broad-leaved
<i>Grewia hispida</i>	Mapungubwe Gabbro	Nutrient-rich	Broad-leaved
<i>Terminalia prunoides</i>	Mapungubwe Gabbro	Nutrient-rich	Broad-leaved
<i>Colophospermum mopane</i>	Mapungubwe Sands	Nutrient-poor	Broad-leaved
<i>Combretum apiculatum</i>	Mapungubwe Sands	Nutrient-poor	Broad-leaved
<i>Commiphora mollis</i>	Mapungubwe Sands	Nutrient-poor	Broad-leaved
<i>Grewia bicolor</i>	Mapungubwe Sands	Nutrient-poor	Broad-leaved
<i>Rhigozum zambesiacum</i>	Mapungubwe Sands	Nutrient-poor	Broad-leaved
<i>Acacia caffra</i>	Marakele	Nutrient-poor	Fine-leaved
<i>Burkea africana</i>	Marakele	Nutrient-poor	Broad-leaved
<i>Combretum molle</i>	Marakele	Nutrient-poor	Broad-leaved
<i>Faurea saligna</i>	Marakele	Nutrient-poor	Broad-leaved
<i>Ozoroa paniculosa</i>	Marakele	Nutrient-poor	Broad-leaved
<i>Protea caffra</i>	Marakele	Nutrient-poor	Broad-leaved
<i>Dalbergia melanoxylon</i>	Nhlangwini	Nutrient-poor	Fine-leaved
<i>Euclea divinorum</i>	Nhlangwini	Nutrient-poor	Broad-leaved
<i>Grewia monticola</i>	Nhlangwini	Nutrient-poor	Broad-leaved
<i>Strychnos madagascariensis</i>	Nhlangwini	Nutrient-poor	Broad-leaved
<i>Antidesma venosum</i>	Nhlangwini	Nutrient-poor	Broad-leaved
<i>Dichrostachys cinerea subsp. africana</i>	Nhlangwini	Nutrient-poor	Fine-leaved
<i>Sclerocarya birrea</i>	Nhlangwini	Nutrient-poor	Broad-leaved
<i>Terminalia sericea</i>	Nhlangwini	Nutrient-poor	Broad-leaved
<i>Dalbergia melanoxylon</i>	Nwaswitshumbe	Nutrient-rich	Fine-leaved
<i>Grewia monticola</i>	Nwaswitshumbe	Nutrient-rich	Broad-leaved
<i>Ozoroa paniculosa</i>	Nwaswitshumbe	Nutrient-rich	Broad-leaved
<i>Pterocarpus rotundifolius</i>	Nwaswitshumbe	Nutrient-rich	Broad-leaved
<i>Sclerocarya birrea</i>	Nwaswitshumbe	Nutrient-rich	Broad-leaved
<i>Colophospermum mopane</i>	Nwaswitshumbe	Nutrient-rich	Broad-leaved
<i>Combretum imberbe</i>	Nwaswitshumbe	Nutrient-rich	Broad-leaved

<i>Philenoptera violacea</i>	Nwaswitshumbe	Nutrient-rich	Broad-leaved
<i>Acacia borleae</i>	Pongola	Nutrient-rich	Fine-leaved
<i>Acacia brevispica</i>	Pongola	Nutrient-rich	Fine-leaved
<i>Acacia luederitzii</i> var. <i>luederitzii</i>	Pongola	Nutrient-rich	Fine-leaved
<i>Acacia nigrescens</i>	Pongola	Nutrient-rich	Fine-leaved
<i>Acacia tortilis</i>	Pongola	Nutrient-rich	Fine-leaved
<i>Euclea undulata</i>	Pongola	Nutrient-rich	Broad-leaved
<i>Pappea capensis</i>	Pongola	Nutrient-rich	Broad-leaved
<i>Spiriostachys africana</i>	Pongola	Nutrient-rich	Broad-leaved
<i>Ziziphus mucronata</i>	Pongola	Nutrient-rich	Broad-leaved
<i>Acacia nigrescens</i>	Satara Basalt	Nutrient-rich	Fine-leaved
<i>Acacia tortilis</i>	Satara Basalt	Nutrient-rich	Fine-leaved
<i>Fluggea virosa</i>	Satara Basalt	Nutrient-rich	Broad-leaved
<i>Albizia harveyi</i>	Satara Granite	Nutrient-poor	Fine-leaved
<i>Combretum apiculatum</i>	Satara Granite	Nutrient-poor	Broad-leaved
<i>Combretum collinum</i>	Satara Granite	Nutrient-poor	Broad-leaved
<i>Lannea schweinfurthii</i>	Satara Granite	Nutrient-poor	Broad-leaved
<i>Terminalia sericea</i>	Satara Granite	Nutrient-poor	Broad-leaved
<i>Acacia nigrescens</i>	Ship Mountain	Nutrient-rich	Fine-leaved
<i>Combretum apiculatum</i>	Ship Mountain	Nutrient-rich	Broad-leaved
<i>Combretum hereroense</i>	Ship Mountain	Nutrient-rich	Broad-leaved
<i>Combretum zeyheri</i>	Ship Mountain	Nutrient-rich	Broad-leaved

Appendix 2 Frequently used abbreviations with explanations and units.

Abbreviation	Explanation	Unit
ADF	Acid detergent fibre	%
ADL	Acid detergent lignin	%
ALA	Average leaf area	cm ²
ASD	Average spine density	spines cm ⁻¹
ASL	Average spine length	mm
AST	Average spine thickness	mm
BD	Bulk density	g cm ⁻³
BI	Browser index	NA
BI1	Branching index 1	no. branches cm terminal branch ⁻¹
BI2	Branching index 2	no. branches 0.125 m ⁻³
BSI	Bite size index	g 10 bites ⁻¹
CBSMs	Carbon based secondary metabolites	NA
CD	Canopy diameter	m
COIA	Co-inertia analysis	NA

CT	Total condensed tannins	%
CWM	Community weighted mean	NA
HFR	Height of first ramification	m
HiP	Hluhluwe iMfolozi Park	NA
KNP	Kruger National Park	NA
LDMC	Leaf dry matter content	mg g ⁻¹
Leaf C:N	Leaf carbon to nitrogen ratio	NA
Leaf N:P	Leaf nitrogen to phosphorus ratio	NA
LMA	Leaf mass per area	g m ⁻²
LNC	Leaf nitrogen concentration	mg g ⁻¹
LPC	Leaf phosphorus concentration	mg g ⁻¹
MAP	Mean annual precipitation	mm
MAT	Mean annual temperature	°C
MCH	Maximum canopy height	m
ML	Maximum likelihood	NA
NDF	Neutral detergent fibre	%
NS	Average number of stems	NA
PCA	Principal component analysis	NA
PC	Principal component	NA
PET	Potential evapotranspiration	mm yr ⁻¹
PFT	plant functional type	NA
RA	Relative abundance	NA
SB	Sum of bases	cmol kg ⁻¹
SLA	Specific leaf area	cm ² g ⁻¹
SSD	Stem specific density	g cm ⁻³
TP	Total polyphenols	%
TS	Leaf tensile strength	N mm ⁻¹
