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Investigating the impact of herbivory and nitrogen-fixation on savanna plant and soil nutrient dynamics

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A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science

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The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.



I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Wesley N. Hattingh
(Signature of candidate)

Submitted for the degree of Master of Science on this, the 3rd day of September 2015, In Braamfontein, Johannesburg

SUMMARY

Plant functional traits provide a means to investigate the diverse ecological strategies employed by plants and a tangible link to assess how the variability in these traits might influence ecosystem processes and functioning. The aim of this dissertation has been to determine how plant and soil nutrient dynamics in a savanna environment are affected by two primary drivers, one a top-down driver, being herbivory by large mammalian herbivores and the other a bottom-up driver, the variable N_2-fixation capacity of tree species. To the best of my knowledge this is the most comprehensive study to date to investigate the bioavailability of soil nutrients and the link between these availabilities and plant functional traits. Furthermore this study provides important insight into the use of a novel technology, ion exchange resin capsules in a South African savanna context.

By studying a selection of plant functional traits (nutrient concentrations, ratios as well as specific leaf area, relative chlorophyll content and leaf dry matter content) and soil nutrients (suite of macro- and micronutrients) associated with two species of savanna tree of contrasting N_2-fixation capacities, I went about investigating how herbivory differentially influences the nutrient dynamics of this system. Selecting individuals of the N_2-fixing *Acacia tortilis* and the non-N_2-fixing *Combretum hereroense* both inside an exclosure and on the adjacent land allowed me to determine the potential impacts by herbivores. These include both direct impacts from foraging and indirect impacts through the regulation of nutrient input pathways via deposition of dung and urine. The work compiled for this dissertation is based on the experimental work conducted in a mesic savanna system in the Marakele Park (PTY) Ltd. During the course of this dissertation, I investigated herbaceous and woody biomass in relation to protection from and exposure to herbivory, determining any differences in the functional leaf traits between individuals inside and outside the exclosure, if these differences were exhibited in the associated herbaceous biomass as well as a comprehensive assessment of the bioavailability of 15 important micro- and macronutrients using ion exchange resin capsules. These capsules were incubated in the soil over the entire summer rainfall period, providing a

cumulative view of nutrient bioavailability during the growing season. In this work I also demonstrated whether particular nutrients are associated with specific drivers (i.e. herbivory, canopy position or N_2-fixation). Furthermore, these results were then looked at together to suggest the mechanism by which herbivory and N_2-fixation drive nutrient dynamics and make recommendations on the use of these results in managing savanna systems in the future.

Between the two sites, aboveground herbaceous biomass was significantly greater when protected from herbivores than on the adjacent land. Both exposure to herbivory and N_2-fixation capacity were found to alter plant functional traits. Herbivore presence was associated with an increase in herbivore-resistant or structural traits such as C/N, C/P, foliar C and SLA as well as a reduction in N and P content. These less palatable leaves were accompanied by a significantly lower availability of a number of important soil elements, namely NO_3-N, inorganic N, P, K, Na, Cu, B, Mg, and S. This suggests a feedback loop between these two components of the ecosystem. N_2-fixation capacity is associated with greater concentrations of elements such as N and P and a reduction in traits that are illustrative of a greater structural investment into leaves. Soil nutrient bioavailability however, shows a reduction in certain nutrients when associated with *Acacia*. A number of nutrients which show a reduction in availability are those which are essential to N_2-fixation machinery, namely B and Fe but also lower bioavailabilities of Al and Mg. Finally, Ca, NO_3-N, B, Fe, Al and inorganic N were found in greater quantities below the tree canopy than beyond it.

In conclusion both herbivory by large mammalian herbivores and N_2-fixation have significant effects on tree health, through their regulation of limiting nutrients and alteration of leaf traits. Given the changes which these drivers are capable of exerting on plant and soil nutrient dynamics, this has important consequences for ecosystem processes and functioning and highlights potential considerations in the long-term sustainable management of savannas.

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"Have courage and be kind"- Chris Weitz

This dissertation is the result of a two year MSc project completed in the School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, as part of the Department of Science and Technology's Centre of Excellence in Tree Health Biotechnology (CTHB).

The last 2 years and five months have been the most challenging of my life. I have been through a great deal of personal obstacles and changes. At times I did not know how I was going to accomplish this mammoth task before me but somehow I have managed and have produced a piece of work I am both proud of and satisfied with. Not only have I grown a great deal in my ability to tackle life's challenges but I firmly believe that this process has shaped me into the scientist that I wish to be. I have learnt that when things do not work out, try, try and try again and that even if you stumble you must eventually get back up and conquer what stands before you. "Rage, rage against the dying of the light," is a line from a poem by Dylan Thomas which exemplifies this idea and is always in the forefront of my mind.

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-Wesley (June 2015)

GLOSSARY OF ABBREVIATIONS

N_2:	Dinitrogen
Al:	Aluminium
B:	Boron
BA:	Basal area
BNF:	Biological nitrogen fixation
C/N:	Carbon to nitrogen ratio
C/P:	Carbon to phosphorus ratio
C:	Carbon
Ca:	Calcium
CTHB:	Centre For Tree Health Biotechnology
Cu:	Copper
CV:	Coefficient of variation
DPM:	Disc pasture meter
DST:	Department of Science and Technology
Fe:	Iron
FIA:	Flow injection analysis
HSD:	Honest significant difference
ICP:	Inductively coupled plasma mass spectrometry
IERs:	Ion exchange resin capsules
K:	Potassium
KNP:	Kruger National Park
LA:	Leaf area
L-DFA:	Linear Discriminant Function Analysis
LDM:	Leaf dry mass
LDMC:	Leaf dry matter content
LFT's:	Leaf functional traits
MAP:	Mean annual precipitation
Mg:	Magnesium
Mn:	Manganese
MNP:	Marakele National Park
N/P:	Nitrogen to phosphorus ratio
N:	Nitrogen
Na:	Sodium
NRF:	National Research Foundation
OC:	Outside canopy
P:	Phosphorus
RCC:	Relative chlorophyll content
RCs:	Resin capsules

S:	Sulphur
SDFC:	Standardised discriminant function coefficient
SLA:	Specific leaf area
VAM:	Vesicular arbuscular mycorrhiza
WC:	Within canopy
WSB:	Western sandy bushveld
Zn:	Zinc

TABLE OF CONTENTS

CHAPTER 1

1.1. Rationale and research development 1

1.2. The impact of large herbivores on nutrient dynamics 6

1.2.1. How do herbivores influence nutrient dynamics? 7

1.2.2. Is this effect positive or negative? 9

1.3. Plant functional traits 10

1.3.1. What are plant functional traits? 10

1.3.2. How do functional traits effect ecosystems? 11

1.3.3. Variation in plant functional traits and the consequent effects on nutrient cycling 13

1.4. Ion exchange resin capsules (RCs) 14

1.4.1. Ion exchange resin capsules: their suitability for use in measuring nutrient bioavailability 14

1.5. The plant-soil system 16

1.5.1. What is the plant-soil system? 16

1.5.2. Mycorrhizal relationships 18

1.6. Research aims and objectives 19

1.7. Literature cited 20

CHAPTER 2 31

2.1. ABSTRACT 32

2.2. INTRODUCTION 33

2.3. METHODS AND MATERIALS 38

2.3.1. Study site description 38

2.3.2. Study species 42

2.3.3. Experimental Design and Approach 44

2.3.4. Field and laboratory Methods 46

2.3.5. Quantitative analyses 51

2.4. RESULTS 52

2.4.1. Aboveground herbaceous and woody biomass 53

2.4.2 Functional leaf traits 55

2.5. DISCUSSION 65

H1: Both aboveground woody and herbaceous biomass will be greater and trees taller in the exclosure 65

H2: The functional traits of plants subject to herbivory will differ from those which are not. 67

H3: Leaf traits of the N_2-fixing *A. tortilis* will differ from the non-N_2-fixing *C. hereroense*. 69

H4: I expect SLA to scale positively with relative chlorophyll values 71

H5: There will be a positive association between leaf traits and the dominant grass species within the extent of influence of the tree 72

2.6. CONCLUSION 73

2.7. ACKNOWLEDGEMENTS 74

2.8. APPENDICES 74
2.9. LITERATURE CITED 75
CHAPTER 3
3.1. ABSTRACT 84
3.2. INTRODUCTION 84
3.3. METHODS AND MATERIALS 89
3.3.1. Study site description 89
3.3.2. Study species 93
3.3.3. Experimental Design and Approach 95
3.3.4. Field and laboratory Methods 99
3.3.5. Quantitative analysis 103
3.4. RESULTS 105
3.4.1. The effect of herbivory on standing stocks 105
3.4.2. Bioavailable soil nutrients 107
3.4.3. Predicting group membership 112
3.4.4. Total soil nutrients and texture 114
3.5. DISCUSSION 118
3.5.1. Herbaceous and woody plant biomass responses to herbivory or lack thereof 118
3.5.2. Soil nutrient availability and exposure to herbivores 119
3.5.3 Nutrient availability and tree species association 121
3.5.4. Nutrient availability and canopy position 122
3.5.5. Interactions among factors 123
3.5.6. Total N and P versus bioavailable N and P 124
3.5.7. Predicting group membership 125
3.6. CONCLUSION 126
3.7. ACKNOWLEDGEMENTS 127
3.8. LITERATURE CITED 127
CHAPTER 4
4.1. Linking leaf functional traits and soil nutrient bioavailability 136
4.2. Implications for tree and greater ecosystem "health" 140
4.3. Contributions towards the management of savanna systems 141
4.4. LITERATURE CITED 141

CHAPTER 1

GENERAL INTRODUCTION

1.1. Rationale and research development

Savannas are ecosystems characteristically defined by the co-existence of trees and grasses with both of these components concurrently influencing ecosystem structure and function (Scholes and Archer 1997; Bond 2008; Hill and Hanan 2011). Savannas constitute approximately 20% of the terrestrial land surface area and as much as 50% of the African continent (Scholes and Walker 1993; Scholes and Hall 1996). This highly productive biome supports a large number of people who are socially and economically disadvantaged, making this habitat particularly vulnerable to exploitation and land-use change (Vitousek et al. 1997b; Augustine et al. 2003). In South Africa, 76% of the countries rural population inhabit savannas (Shackleton et al. 2002). Globally, human alteration of biomes has resulted in a number of rapid changes to the structure, composition and function of many ecosystems (Vitousek et al. 1997b) -often overwhelming or eroding their capacity to provide essential services such as drought mitigation, climate stability, **soil fertility** (Palmer et al. 2004), fuel wood, agriculture and pastoral land among others (Kremen 2005). The population of Africa is predicted to increase four-fold (>4 billion) by 2100 (United Nations 2013). This will only result in an enhanced dependence of this burgeoning population on the savanna ecosystem. Increases in human population are expected to be accompanied by alterations to a number of process, all which have a bearing on ecosystem structure and function. These include changes to fire regimes, in terms of alterations to the frequency and intensity of fires (Archibald et al. 2009; van Wilgen et al. 2014; Levick et al. 2015; Dantas et al. 2015), intensified livestock production (maximisation of stocking rates) and global warming all of which have the capacity to mediate the availability of nutrients (Cech et al. 2008; Bai et al. 2013). For example a recent meta-analysis by Bai et al. (2013) suggested that global warming will on average increase rates of N mineralization and nitrification by 52.2% and 32.2% respectively. The question of how these

temperature-mediated increases will interact with the likes of intensified grazing, as well as woody encroachment (possible increase in woody legumes, see Buitenwerf et al. (2012)) is one of great importance.

In light of the predicted impacts of global change, it is important to understand the key biotic and abiotic drivers which shape and govern the structure, function and provisioning of services in savannas (Kremen 2005). Recently savannas have been implicated as the biome responsible for regulating interannual variability in the global terrestrial CO_2 sink (Ahlstrom et al. 2015), thus an understanding of factors which mediate this process are of of particular importance. This is also important given that recently there has been a renewed focus on conducting research that investigates fundamental or basic ecology (Courchamp et al. 2015). These types of studies are essential as they elucidate the many complex interactions between organisms and their biotic and abiotic environments (Begon 2006; Lindenmayer et al. 2015).

There are a number of drivers known to regulate structure and function in savannas, primary among these include top down controls/processes such as fire (Parr and Chown 2003; Bond et al. 2005; Archibald et al. 2005; Orians and Milewski 2007; Cech et al. 2008; van Wilgen et al. 2014; Levick et al. 2015; Pierre 2015) and herbivory by micro- and macro-fauna (McNaughton 1983; McNaughton 1985; Warren et al. 1986; Scholes and Walker 1993; Sankaran et al. 2008) as well as bottom up controls such as water and nutrient availability (Medina and Silva 1990; Knapp et al. 2001; Austin et al. 2004; Cech et al. 2008; Cech et al. 2010; Kupper et al. 2012).

Water availability is usually cited as the primary limitation to plant growth and overall productivity in savanna environments (Lohse et al. 2009). This biome is only located in areas which experience strongly seasonal rainfall (Lehmann et al. 2011). Water availability directly influences the rate of primary productivity, the relative allocation of aboveground-belowground biomass, phenology, decomposition and therefore nutrient cycling (Skarpe 1992; Chapin et al. 2011). In relatively arid environments the availability of water can directly limit carbon (C) and nitrogen (N) transformations (Noy-Meir 1973; Reynolds et al. 2004). However, the availability of nutrients is another one of the primary mechanisms controlling plant production

in the terrestrial ecosystem. The limitation of certain nutrients that are essential to plant growth will thus also have a pronounced effect on vegetation and indeed many aspects of species composition, structure and physiology. Nutrient limitation is regulated by diverse external inputs and processes occurring *in situ* which control the conversion of recalcitrant nutrients into forms that are bioavailable to plants (Vitousek 2004). The majority of terrestrial ecosystems tend to be limited by N and/or phosphorus (P) (Vitousek 1986; Sterner and Elser 2002; Werner 2005; Elser 2012), however there are a suite of macro and micronutrients that influence plant productivity (Chapin et al. 2011). African savannas are generally accepted as being P-limited ecosystems -due in large part to the fact that they tend to be situated in relatively old soils; 0.5- 3.1 billion years (Scholes and Walker 1993; Raghothama 1999). These soils have experienced a considerable amount of weathering and leaching and are therefore frequently P-limited. This is based on the premise that newly formed soils contain all the P they are likely to have in a natural environment while soil available N increases at the onset of biological process (Vitousek and Walker 1989; Karasov and Martínez del Rio 2007). There are however, multiple complex relationships in savanna systems that confound clear cut nutrient limitation (Cech 2008; Menge et al. 2012).

In this research I focus on the effects of two factors responsible for regulating the conversion, provision and availability of nutrients in a mesic savanna system. These two factors are herbivory by meso- (4- 450 kg) and mega-herbivores (>450 kg) (*sensu* Fritz et al. 2002) and the differential input of nutrients by two species of savanna tree with contrasting N_2-fixation capacities. Herbivory and N_2-fixation have not been extensively studied in conjunction to elucidate potential interactions. Some studies have looked at the effects of tree species on the vegetation (grasses) (Treydte et al. 2007; Treydte et al. 2008) and soils (Ludwig et al. 2001; Ludwig et al. 2003; Ludwig et al. 2004) beneath their canopies and how this influences selection by herbivores (Treydte et al. 2010; Treydte et al. 2011; Treydte et al. 2013); others have looked at how the density of N_2-fixing tree species influence nutrient dynamics (Sitters et al. 2013; Sitters et al. 2015) while still others have investigated the effects

of herbivores on the soil system (Augustine 2003; Augustine et al. 2003; Augustine and McNaughton 2006; Augustine et al. 2013) and plant characteristics (Cargill and Jefferies 1984; Hobbs 1996; Goheen et al. 2007; Fornara and Du Toit 2007; Riginos and Grace 2008; Goheen et al. 2010; Aarrestad et al. 2011; Tessema et al. 2011; Asner and Levick 2012; Holdo et al. 2013). However, relatively few studies have looked exclusively at the combined effects of both N_2-fixation and herbivory- with this research only having been done in an east African savanna, which is comparatively far more wet (MAP of ~1050 mm) in comparison to the more arid savannas of South Africa (MAP~550 mm) (Cech et al. 2008; Cech et al. 2010). The knowledge of how N_2-fixation and herbivory concurrently influence savanna systems will aid in predicting and assessing the impacts of widespread environmental and land-use change as well as the potential consequences of management by humans on this habitat (Marchant 2010).

Herbivory by large mammalian herbivores is a top down control which can both directly and indirectly effect nutrient availability and savanna structure and function. African savannas are well known for their diverse assemblage of large mammalian fauna (Cumming 1982; Toit and Cumming 1999) and consequently the high levels of herbivory associated with this (Owen-Smith 1992; Arsenault and Owen-Smith 2002). Chronic herbivory can have significant effects on nutritional budgets as they are able to regulate the quantity and rate of flow of nutrients through ecosystems (McNaughton et al. 1988; Frank 1998; Augustine et al. 2003; Augustine and McNaughton 2006; Cherif and Loreau 2013). Herbivores consume vegetation in one location after which they may travel to a new location (or remain in the same locale) where they defecate and urinate, thus altering the spatial distribution of nutrients (Augustine et al. 2003; Bakker et al. 2004). In addition to nutrient displacement, intense herbivory by large mammals also increases the rate at which mineralization occurs (Ruess and McNaughton 1984; Ruess and McNaughton 1988) as well as mediating the availability of certain nutrients (van der Waal et al. 2011; Waal et al. 2011). In habitats occupied by large populations of ungulate herbivores, the quantity of bioavailable N in soils tends to be greater, relative to the N from the decomposition of plant litter alone (Frank et al. 1998; Schowalter et al. 2011).

Individual trees can substantially influence their microenvironment in terms of soil physico-chemical properties, such as nutrients, temperature and moisture (Belsky 1994; Treydte et al. 2007; Treydte et al. 2008; Reis et al. 2010). Savannas are characterised ecologically as consisting of a continuous grass layer with scattered trees (Scholes and Archer 1997). This co-existence has important consequences for any research in this system as both components must be studied to obtain a holistic understanding of the entire system. Savanna trees are able to influence herbaceous production, biomass allocation (root vs. shoot) phenology and species composition through a combination of direct and indirect effects (Scholes and Archer 1997). Biological N_2-fixation by legumes can contribute as much as 97% of bioavailable N in natural, un-fertilised ecosystems (Vitousek and Howarth 1991; Vitousek et al. 2002; Vitousek et al. 2013). A conspicuous component of African savannas is the abundance of woody legumes, especially of the genus *Acacia*, the majority of which have the capacity to fix N_2 (Cramer et al. 2007; Houlton et al. 2008). Although most *Acacia* appear to have the ability to nodulate (i.e. fix N_2) not all actively do as the costs of N_2-fixation may exceed the returns. Cramer et al. (2010) demonstrated that in the absence of water limitation in savannas *Acacia* seedlings facultatively fix N_2 as this facilitates successful competition with grass species for access to N.

The mechanisms by which trees can directly or indirectly influence the soil system include; alteration of localised nutrient availability (N addition from legumes), as well as shading or competition for access to water. Soils surrounding N_2-fixing woody legumes accumulate up to 60% more N than surrounding soils under non-N_2-fixers under certain conditions (Ludwig et al. 2001; Ludwig et al. 2004). Because ecosystems are highly heterogeneous in terms of local edaphic conditions, intensity of herbivory, plant assemblages and diversity, water availability and nutrient cycling in general; many questions remain about how N_2-fixing species influence greater plant and soil nutrient dynamics and availabilities, especially with respect to P. Of particular interest is also the interaction between herbivory and local changes in soil nutrient availability as a result of tree-mediated inhibition or facilitation. For example, the N/P values of vegetation within the Komati region of South Africa suggested

P limitation in these soils (Hattingh 2012). Some plants have the ability to fix N but may then be limited by the availabilities of other elements, particularly phosphorus but also molybdenum and iron (Vitousek et al. 2002; Vitousek et al. 2013). Other factors which have the potential to limit N_2-fixation include temperature (Gundale et al. 2010), soil-water availability (Marino et al. 2007), salinity (Faghire et al. 2011), mineral N availability (Hartwig 1998) and soil oxygen supply (Schulze 2004). Furthermore the interaction between N_2-fixation and herbivory as drivers of nutrient cycling is of particular interest in *Acacia*-dominated savannas and under conditions where global change and land-use intensification is expected to have profound impacts on traditional savanna ecosystem function.

1.2. The impact of large herbivores on nutrient dynamics

After fire large herbivores are the biggest consumers of biomass in African savannas (McNaughton 1985; McNaughton and Georgiadis 1986; Asner et al. 2004). Plant species interact with herbivores to influence nutrient cycling through both direct and indirect mechanisms. Herbivores are capable of affecting ecosystem function and structure, through the modification of feedbacks between plant species and biogeochemical cycles (McNaughton et al. 1988; Huntly 1991; Pastor and Naiman 1992; Pastor et al. 1993; Pastor and Cohen 1997; Ritchie et al. 1998; Holdo et al. 2007; Treydte et al. 2013). Modification can be direct when herbivores excrete rapidly decomposable organic matter, containing essential elements that are immediately accessible to plants- this is achieved through the addition of faeces and urine, which increases the rate at which these chemical elements are returned to soil nutrient pools (Wardle 2002; Wardle et al. 2002; Bardgett and Wardle 2003; Lagerström et al. 2011; Van der Putten et al. 2013). This is because they return nutrients to the soil in a form that is more rapidly mineralised (Bakker et al. 2004). However, this pathway may also be indirect where selective foraging has the capability to alter community composition and therefore the characteristics of the senesced plant material entering the soil system (i.e. herbivores may alter the functional traits of plants as a result of foraging) (Huntly 1991; Pastor et al. 1993; Wardle 2002; Hättenschwiler et al. 2005; Hättenschwiler et al. 2011; Staver and Koerner 2015;

Baumgartner et al. 2015). Both grazing and browsing have important effects on savanna ecosystem functioning (Pachzelt et al. 2015), although grazing is more pronounced, representing ~80% of the meso-herbivore (4-450 kg) metabolic biomass in both eastern and southern Africa (Fritz et al. 2002).

1.2.1. How do herbivores influence nutrient dynamics?

The ways in which herbivores influence nutrient cycling can be fast (within a single growing season) or slow (over multiple seasons) and depends on the ways in which they interact with the vegetation and soil systems (Schmitz 2008). One mechanism by which herbivores can accelerate biogeochemical cycles is through the consumption of fully functioning leaf material which has not yet senesced. This facilitates the transport of a greater quantity of nutrients than would be gained from leaf litter alone. Leaves which constitute leaf litter are senesced and resorption of mineral nutrients has already taken place, lowering the concentration of elements within their tissue.

Different herbivores will also have different effects on nutrients, given their diet preferences and movement patterns (McNaughton and Georgiadis 1986; Waal et al. 2011). Domestic and wild herbivores are known to re-distribute nutrients by feeding in one area and then moving to another area, excreting as they do so. Redistribution of nutrients by herbivores may result in either a local depletion or enrichment of bioavailable nutrients (Augustine 2003; Peco et al. 2006; Stritar et al. 2009; Cech et al. 2010). This may result in the maintenance of nutrient-rich patches in an otherwise nutrient-poor region, with significant effects on the vegetation within these patches. Thus herbivores promote the heterogeneity of nutrients in the landscape (Cech et al. 2008; Augustine et al. 2013).

Beyond simply the redistribution of nutrients, herbivores alter N dynamics as ammonia volatilises more easily from dung and urine than other sources (Ruess and McNaughton 1984; Frank et al. 1994; Frank et al. 2004). This causes a loss of N from the system (Ruess and McNaughton 1988; Seagle et al. 1992; Frank et al. 2004). Thus the excretion of N in dung and urine offers an accelerated (direct), alternative pathway to decomposition of litter for N

turnover. Furthermore excretion by herbivores often accelerates the decomposition of organic matter, up-regulating rates of mineralisation and increasing the overall quantity of bioavailable nutrients (Hobbs 1996; Frank and Groffman 1998; Fornara and du Toit 2008). This same process results in an increased P availability relative to N in areas where animals deposit their excreta (Frank et al. 1998; Augustine et al. 2003). Ungulate faeces contain more N relative to C and are therefore less resistant to microbial breakdown, providing a suitable substrate that is more readily decomposed while at the same time enhancing conditions for decomposition by increasing the availability of N in the soil pool relative to C. Thus available C/N ratios in the soil give an indication of nutrient turnover as this determines whether microbes immobilise or mineralize N (Chapin et al. 2011; Smith et al. 2015). As discussed previously ungulates are able to alter nutrient dynamics by providing a pathway in which N is more rapidly mineralised, but they can also influence nutrient availability by decreasing competition with microbes, allowing a greater quantity of nutrients to be available to the plant (Wardle 2002).

The affect herbivores can have on ecosystem processes are important because in most natural, undisturbed systems, external inputs account for an almost negligible portion of the required nutrient supply, with the majority of nutrients originating from internal cycling (Hobbs 1996). These effects are mediated by the *in-situ* conditions of a specific environment (e.g. soil type). In general, resource-poor (low nutrient availability) environments tend to have plants which invest heavily in terpenes and polyphenols, carbon based secondary metabolites that are used as a defence mechanism to deter herbivory (Hobbie 1992; Hobbie et al. 2012; Hobbie 2015). These secondary metabolites are, however, antimicrobial and therefore litter tends to decompose more slowly relative to that of litter from resource-rich environments where plants invest less in defence mechanisms and produce litter of higher quality. Low-quality, or unpalatable vegetation is usually distinguished by those with high C/N ratios and/or a high C:P ratio (Robbins 1983; Karasov and Martínez del Rio 2007), given the importance of N and P (Vitousek 1986; Vitousek et al. 1997a; Elser et al. 2007). A higher proportion of C is usually associated with an increase in the concentration of tannins, lignins and secondary compounds that deter herbivory (Chapin et al. 2011).

1.2.2. Do herbivores exert a positive or negative effect?

There is debate as to the net effect of herbivory on soil N turnover- with increases in N mineralisation observed in some areas such as in Yellowstone National Park where bison are common (Frank and Groffman 1998) while other regions have observed decreases such as the semi-arid rangelands of Kenya which supports a large cattle population (Augustine and McNaughton 2006). In seminal work by Ritchie et al. (1998) in Minnesotan oak savannas, the effect of large herbivores was found to indirectly decrease the rate of N cycling, through a reduction in the biomass of N-rich tissues. This calls for more extensive research into the net effects of herbivory on savanna nutrient dynamics. Through the consumption of biomass, herbivores may also reduce fuel load for fires, decreasing the intensity and frequency of fire events (Waal et al. 2011; Staver and Koerner 2015; Levick et al. 2015; Pachzelt et al. 2015). Finally herbivores are known to enhance biomass production through grazing thereby functioning as ecosystem engineers (Eldridge et al. 2010; Sanders et al. 2014). Changes in species composition as well as structure are common outcomes of herbivory (Frank et al. 1998) - this may also result in the alteration of a number of plant functional traits. In low-nutrient ecosystems, browsing herbivores will preferentially select forage of higher quality and therefore high quality litter, promoting the growth of species with leaves of lower nutritional quality (greater structural investment) which then produce low-quality litter and consequently slows nutrient cycling (Hobbie 1992; Hobbie 2015). In some ecosystems however, particularly those that have a high nutrient availability, herbivores tend to enhance nutrient cycling (positive feedback) as grazing, and not nutrient limitation is the primary limiting mechanism where consumption promotes re-growth of vegetation of superior nutritional quality (Hobbie and Vitousek 2000). This mechanism is reinforced as a result of the deposition of faeces of a greater nutritional content. The overall effect of herbivores on the nutrient dynamics of savannas is largely unknown, especially in terms of whether herbivory facilitates or inhibits N and P cycling (Cech et al. 2008). Intense herbivory has the ability to convert a high-nutrient ecosystem to a low-nutrient ecosystem depending on whether the interaction between herbivores and nutrients is positive or negative.

1.3. Plant functional traits

1.3.1. What are plant functional traits?

Functional traits are defined as the phenotypic component of an organism that regulates its ability to influence ecosystem processes (Petchey and Gaston 2006). Green leaves are the primary photosynthetic organ of living plants and are responsible for the vast majority of terrestrial carbon assimilation. Nutrient availability and disturbances such as herbivory will be represented in leaf tissue as varying physico-chemical properties. When leaves are abscised this leaf litter undergoes decomposition- thereby influencing biogeochemical cycles (Onoda et al. 2011). Different species will have leaves of varying properties, or leaf traits based on the environment within which the plant occurs. Leaf traits incorporate the physiological, morphological and phenological characteristics of a plant species at the individual level and reflect the outcome of complex, dynamic evolutionary processes which are mediated by both biotic and abiotic limitations (Violle et al. 2007; Pérez-Harguindeguy et al. 2013). Plant functional traits are widely utilised in ecology as proxies for individual fitness and performance and often have an impact on ecosystem functioning based on the diversity and range of a specified trait (Díaz and Cabido 2001; Díaz et al. 2004; Wright et al. 2004; Lohbeck et al. 2012; Lohbeck et al. 2014). Substantial advancement has been made in the field of terrestrial ecology on the basis of leaf functional traits (Westoby and Wright 2006),based on two primary reasons-(1) functional traits have the ability to mediate and modify ecosystem process because of their given nutrient concentrations and (2) knowledge of the costs of certain leaf traits and how these traits change over the landscape can help to understand and possibly even predict vegetation properties which is particularly useful in terrestrial ecosystem modelling where leaf traits act as base-line input data (Wright et al. 2004). Multiple studies have shown that there are certain core functional traits such as growth rate, leaf lifespan, mineral nutrient concentrations, leaf toughness (SLA) and resistance to decomposition (C/N) that are related to ecosystem functioning in fundamental, unambiguous ways (Díaz et al. 2004; Garnier et al. 2004). Nutrient availability is closely linked to many

functional traits. For example, in ecosystems where nutrient availabilities are low; rates of mineralisation are low and plant growth is slower due to limited nutrient uptake. These resource-poor environments tend to be associated with plants species with leaf functional traits such as a longer leaf lifespan, lower nutrient concentrations and have a low specific leaf area (Hobbie 1992; Kattge et al. 2011; Pérez-Harguindeguy et al. 2013). In contrast those environments that are resource-rich tend to allocate more resources to above-ground parts, grow faster and can therefore "afford" to have shorter leaf lifespans, higher nutrient concentrations (given increased nutrient availability) and leaves with a high specific leaf area (Cornelissen et al. 1997). Functional traits are a particularly useful tool in that they allow one to link easily measureable leaf traits to broader ecosystem process such as decomposition and investigate the feedback between traits and soil nutrient pools. Disturbances such as intense herbivory will alter leaf traits, influencing ecosystem nutrient dynamics and potentially establishing trade-offs and feedback loops. In terrestrial ecosystems the below- and above-ground environments are closely related in terms of nutrient cycling with plant functional traits playing a vital role in reinforcing the feedback between them (Hobbie 1992; Wardle et al. 2002; Wardle et al. 2004). This system becomes more complex however when the effect on this system is considered in conjunction with herbivory which has the capability to mediate both above- and below-ground systems.

1.3.2. How do functional traits effect ecosystems?

A number of studies have shown that functional traits of leaves exert a greater influence on the rate of decomposition than do local environmental conditions (Xuluc-Tolosa et al. 2003; Cornwell et al. 2008; Bakker et al. 2011; Carreño-Rocabado 2013). Two such traits of importance are SLA and LDMC. SLA or its inverse leaf mass per unit area (LMA) are very important and indeed useful traits as they provide a great deal of information about many aspects of the species from which they are measured (Lamont et al. 2015). For example, SLA can be used to; (1) compare the leaf properties of different species of varying functional types or ages (Grubb et al. 2015); (2) make comparisons to other leaf traits such as nutrient content

(Lamont et al. 2002; Wright et al. 2004; Wright et al. 2005a; Dubey et al. 2011; Holdo 2013) and age or leaf lifespan (Witkowski et al. 1992; Wright and Westoby 2003; Wright et al. 2004); (3) act as a proxy by which to infer mechanical resistance to herbivory (Hanley et al. 2007) and (4) even represent the regulation of the abiotic environment (temperature and rainfall) on leaf structure (Groom and Lamont 1997). SLA is also useful as it incorporates leaf density and thickness (Witkowski and Lamont 1991) which are factors that influence the palatability of vegetation to herbivores (McNaughton and Georgiadis 1986; Asner et al. 2004). A means by which SLA can strongly effect ecosystem level processes is by influencing the rate at which leaf litter decomposes. This is because mechanical traits such as SLA and LDMC regulate how easily the nutrients which constitute them enter the soil system by determining the palatability and accessibility of the leaves to detritivores (Chapin et al. 2011). SLA positively influences the decomposition rate (Cornelissen et al. 1999; Vaieretti et al. 2005; Santiago 2007; Kurokawa and Nakashizuka 2008; Vaieretti et al. 2013) while LDMC has a negative effect (Kazakou et al. 2006; Cortez et al. 2007; Kurokawa and Nakashizuka 2008; Cornwell et al. 2008; Kurokawa et al. 2010; Castro et al. 2010) as it functions to decrease the rate at which the leaves can be decomposed due to higher structural toughness. Other important traits include, leaf thickness or toughness as well as C/N (carbon to nitrogen ratios) (Moore et al. 2006; Kurokawa et al. 2010; Eskelinen et al. 2012) and mineral nutrient concentrations particularly N and P (Santiago 2007; Ågren et al. 2012).

Plants have the capacity to exert strong controls over the cycling of organic matter in ecosystems (Cornelissen et al. 1999) through the functional traits that characterise their leaf tissue. As a result functional differences between species can have marked impacts on the dynamics of the soil system. These effects are far reaching and may be even more significant effects than previously imagined. If changes to functional traits occur, and if these plant traits are heritable, then evolution of plant populations may have ecosystem-level consequences (Fussmann et al. 2007). This is based on the premise that the ecosystem-level effects of plant

evolution could potentially feedback and result in the alteration of plant fitness if the functional traits subject to natural selection result in changes to the ecosystem (Fitzpatrick et al. 2015).

Often fresh, green leaves are better predictors of potential decomposition rates than are the traits of leaf litter (Bakker et al. 2011). In a study by Bakker et al. (2011) leaf nitrogen content (Cornwell et al. 2008), specific leaf area (Cornelissen and Thompson 1997; Cornelissen et al. 1999; Brovkin et al. 2012), and chlorophyll content were shown to be very good predictors of the decomposition of leaves- more so than phosphorus despite the study taking place in a region which is P-limited.

1.3.3. Variation in plant functional traits and the consequent effects on nutrient cycling

The leaves of different species can exhibit a great deal of plasticity in response to the characteristics of their growing conditions, and hence can vary in their anatomy, physiology and morphology (Witkowski and Lamont 1991). Two factors which have been shown to drive this plasticity are herbivory (Zanne and Falster 2010; Loiola et al. 2012; Cárdenas et al. 2014) and nutrient availability in the soil system (Mooney et al. 1978; Jurik et al. 1982; Shaver 1983; Chapin et al. 1990). At the most basic level, leaves are under natural selection to fulfil two primary criteria; (1) on a day-by-day basis they must intercept light and photosynthesise at a rate that is competitive, and (2) over the lifetime of the leaf it must provide a positive return on the resources invested in its construction (Wright et al. 2004; Reich 2014). Depending on the species and the conditions it is exposed to, leaf traits may vary substantially within a relatively localised area. Leaf litter chemistry and differences in functional leaf traits have a marked influence over how leaves decompose and indeed how plants influence the environment in which they occur (Norris et al. 2012). Although climate has a large impact on decomposition, plant species are able to influence nutrient cycling through differences in litter quality (Hobbie 1992; Grootemaat et al. 2015; Dale et al. 2015). Leaf litter quality and the decomposition of this litter for a single species are directly linked to the functional traits of the living organism (Quested et al. 2007). They effect the rate of nutrient cycling based on the following basic idea: leaves which are thin (high SLA), have a high nutrient content (low C/N-greater

proportion of N relative to the structural component, C) and high photosynthetic rates, decompose more easily and at a greater rate than recalcitrant, thicker leaves (low SLA) with lower nutrient concentrations (high C/N) and low photosynthetic rates (Wright et al. 2005b; Santiago 2007).

Functional traits are highly variable amongst species. Given this fact it has been suggested that they could be used to predict how different species may respond to climate change (Soudzilovskaia et al. 2013), one of the primary focuses of modern ecology. Many of the traits which vary considerably among species also display extraordinary plasticity. The fact that some traits are plastic is an important component that needs to be considered if we hope to predict the responses of species to a changing climate (Liancourt et al. 2015).

1.4. Ion exchange resin capsules (RCs)

1.4.1. Ion exchange resin capsules: their suitability for use in measuring nutrient bioavailability

Ion-exchange resin capsules, hereafter referred to as simply resin capsules-RCs, were first introduced almost four decades ago with the primary purpose of estimating plant-available nutrients in soils (Jones et al. 2013a). RCs provide a simple, time-efficient and easily repeatable method for assessing soil nutrient availability (Skogley et al. 1990; Skogley and Dobermann 1996). Traditionally, soil nutrient availability has been determined by extraction methods using several chemical solutions (Dobermann et al. 1994; Johnson et al. 2005; van Raij et al. 2009). This is time consuming, expensive and is inherently a static measure which does not account for the kinetics of nutrient release and transport (Dobermann et al. 1994). This makes RCs particularly useful in environments where nutrient availability varies temporally. The use of RCs in soil analysis also allows for the standardization and comparability of results, removing the bias associated with different testing methods and inter-laboratory variation (Skogley et al. 1990; Yang et al. 1991a; Yang et al. 1991b).

Resin capsules (RC) are small elliptical cylinders that are about 2 cm in diameter and contain resin beads which equilibrate with ions present in the soil solution (Cooperband and

Logan 1994; Sardi et al. 1996) (**Fig. 1.1**). In this way the capsules simulate uptake by plant roots, absorbing nutrients via diffusion (Skogley and Dobermann 1996). Although these resins adequately mimic diffusion, they are unable to mimic mass flow and interception, the two other mechanisms utilised by plants in nutrient uptake (Yang et al. 1991a). The vast majority of plant nutrients are, however, obtained through the process of diffusion (Chapin et al. 2011). Diffusion is also the primary mechanism by which plants gain access to potentially limiting nutrients (e.g. N and P).

Resin capsules are buried in the soil at a specified depth for a set period of time. In theory the quantity of nutrients absorbed by the resin capsule will be related to the quantity available to plant roots and therefore will provide a measure of diffusion-controlled soil-plant relations (Jones et al. 2013b; Jones et al. 2013a). These capsules were originally designed to be used in relatively fertile agricultural soils and much debate has arisen regarding the suitability of this method in semi-arid, low nutrient ecosystems (Jones et al. 2012). A recent study by Jones et al. (2012) showed that capsules provided a more accurate representation of ammonium (NH_4-N) than traditional KCL extraction, while nitrate extraction was less well correlated but still within acceptable bounds. P adsorbed by RCs was, however, poorly

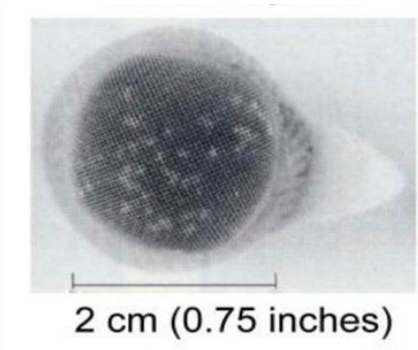


Fig. 1.1*:* Unibest PST-1 resin capsule (RC)

correlated in comparison with sodium-bicarbonate-extractable P. Jones et al. (2013a) concludes that estimation of N bioavailability is very promising while RCs are unable to effectively measure P bioavailability. However in a meta-analysis conducted on over 70 experiments reported in the literature, van Raij et al. (2009) found that resin capsules were a far more suitable method for estimating bioavailable P than other long-standing methods such as Mehlich 1 and Bray-1 which tend to extract non-labile forms of P. RCs have been used

previously to assess extractable P, however, this often involved the use of a single soil sample (Witkowski and Mitchell 1987) and in essence reflects P availability at a single point in time. The fundamental difference in this research is that I left RCs *in situ* over the entire summer rainy reason. Therefore, extracted nutrients in this case represent a holistic accumulation of nutrient bioavailability.

1.5. The plant-soil system

1.5.1. What is the plant-soil system?

The way in which plants interact with the soil system they inhabit is of primary importance to ecology, as it facilitates the understanding of the complex relationships between different biotic and abiotic components (Ehrenfeld et al. 2005). Plant roots are responsible for driving many diverse ecological interactions and processes (Casper and Jackson 1997; Wardle 2002; Casper et al. 2003) and may account for the majority of biomass in some communities (Jackson et al. 1996). As a result it is widely accepted that plant-soil interactions are the primary mechanisms which drive global ecosystem functioning (Bever 2003; Reynolds et al. 2003; Van der Putten 2003; Van der Putten et al. 2013), based on the diverse nutrient foraging strategies employed by different plant species (Cahill and McNickle 2011). Furthermore, the interaction between plants and soils is critically important as it is one of the factors that influence soil formation (Jenny 1994) and has even played a role in the evolution of terrestrial flora (Selosse and Le Tacon 1998). What is important is that plants have the ability to alter the biotic and abiotic properties of soils which then influence primary productivity, competitiveness and plant development (physiological, plant functional traits, etc.) (Ehrenfeld et al. 2005). Some of the ways in which plants influence soils involve the mechanisms controlling root placement. Nutrients have the ability to significantly affect plants in that they serve as one of the primary cues governing root placement and growth (Hodge 2004). This will ultimately influence the feedback between plants and the soil system in terms of both nutrient cycling and the variability in plant functional traits. Feedback describes a series of events whereby the outcomes of a process affect the conditions that initially generate the

process that is now being regulated (**Fig. 1.2**). In a scenario involving a positive feedback, the outcome of a process (e.g. nutrient availability) causes the process itself to become more effective, where the increase in magnitude facilitates yet a further increase. A negative feedback however, is one in which the outcomes of a process result in a decrease in the magnitude of the process itself. In simpler terms, positive feedbacks are directional and can alter the prevailing conditions, while negative feedback is generally stabilizing in nature (Pernilla Brinkman et al.

2010).

Other than nutrients, water is a key component which mediates the interaction between plants and the soil system as water is necessary for soil biogeochemical cycling- facilitating the delivery or transport of important minerals and nutrients to plant roots as well as the

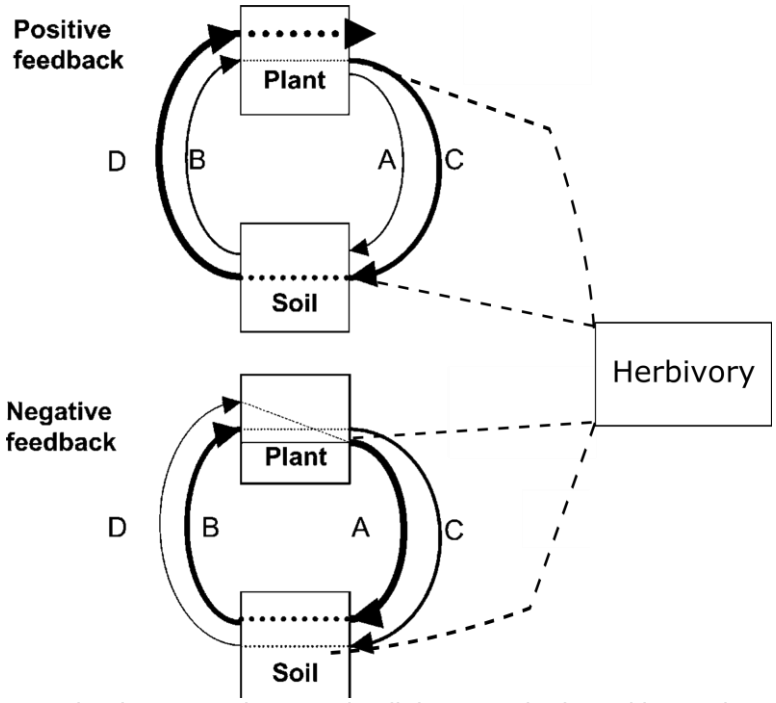


***Fig. 1.2*:** In the interaction between plants and soil there are both positive and negative feedbacks. In positive feedback, plants have an effect on the soil system (A) which causes a reciprocating effect of the soil on plants (B), e.g. resource availability; this may then amplify the effect of plants on soil (C) which in turn further amplifies the effect of the soil on the plants (D). In a negative feedback scenario, the plants affect the soil (A) which causes a reciprocating effect of soil on plants (B); this then attenuates the effect of plants on the soil (C) which then further attenuates the effect of soil on plants (D)- when some threshold level is reached by D it causes the onset of a decreasing effect (i.e. an increase in one component may result in a decrease in another at this threshold level). Adapted from Ehrenfeld et al. (2005). This mechanism is further complicated by the addition of herbivory which influences both the plant and soil components.

microbial populations responsible for breaking down plant-sourced organic matter (e.g.

leaves) (Lohse et al. 2009). For example Morillas et al (2015) shows that the frequency of drying-rewetting cycles has significant effects on the ability of the soil system to cycle certain nutrients such as C and N. Other edaphic characteristics such as soil structure (i.e. texture, aggregates etc.) (Oades 1993), temperature (Hillel 1998) and pH (Hinsinger et al. 2003; Lüttge 2008), may also have an effect on the interactions between plants and the soil system.

1.5.2. Mycorrhizal relationships

Mycorrhizal associations are one of the most common nutrient-acquiring mechanisms employed by plants (Stock et al. 2003). These are mutualistic relationships between plant roots and a group of fungi known collectively as the mycorrhizas. Mycorrhizae serve as an extension to the root system into the bulk soil, providing as much as an additional 1-15 m of hyphal growth per cm of root tissue (Chapin et al. 2011), reducing the diffusion limitation of absorption by plants. These associations are particularly important as they assist with the absorption of many nutrients, especially those that diffuse slowly through the soil system such as phosphorus and ammonium (Johansen et al. 1993; Frey and Schüepp 1993; Chapin et al. 2011) compared with nitrate and potassium which diffuse more quickly (Witkowski et al. 1990). Other nutrients which are taken up by mycorrhizas and made available to the plant include zinc, copper and potassium (Marschner and Dell 1994). Mycorrhizas are very important in soils of low fertility (Menge 1983), where nutrient additions are highly heterogeneous (Cavagnaro et al. 2005) and in regions where the primary input of nutrients is infrequent. Most terrestrial ecosystems are dominated by vesicular arbuscular mycorrhizas (VAM)- which is ~74% of angiosperms (Brundrett 2009). South Africa is no exception (Augé 2001; Stock et al. 2003). At a finer scale, 85.5% of savanna plants species exhibit VAM associations (Treseder and Cross 2006).

Herbivory in general has adverse effects on mycorrhizal associations as aboveground herbivores consume photosynthetic tissue, which in turn may impair the ability of the plant to support its normal complement of mycorrhizae (Gehring and Whitham 1994). Herbivores compete for access to the photosynthate produced by the plant and given the high energy

requirement of mycorrhizae, 3-20% of plant produced photosynthate (Stribley et al. 1980; Johnson et al. 2002), this competition may significantly reduce mycorrhizal associations.

N_2-fixing plant species have been shown to be highly dependent on mycorrhizae in order to effectively absorb nutrients that are required for N_2-fixation and plant growth (André et al. 2003). This is highlighted by the fact that there is a positive relationship between the size of rhizobial (*Rhizobia*, are responsible for the formation of N_2-fixing root nodules on capable species) populations and that of mycorrhizas (Ibijbijen et al. 1996; Bearden and Petersen 2000).

1.6. Research aims and objectives

The experimental work in this MSc has investigated the combined effects of exposure to chronic herbivory by large mammals and the differential nutrient input by tree species of varying N_2-fixation capacity on a mesic savanna system in the Waterberg region of Limpopo, South Africa. I compare the effects of herbivore presence and N_2-fixation capacity of the dominant tree species (*Acacia tortilis* and *Combretum hereroense*) on plant functional traits (Chapter 2) and soil nutrient dynamics (Chapter 3). Besides gaining a deeper understanding of nutrient cycling in savanna systems, this research will provide information useful for managing these ecosystems sustainably and for beginning to assess how changes in land-use, woody encroachment or an alteration in grazing intensity may influence soil nutrient availability, and consequently how this might interact with the functional characteristics of local species.

More specifically, the aims of each research chapter are:

Chapter 2: The differential impact of herbivory on the functional traits of two co-occurring savanna tree species with contrasting N_2-fixation capacity

To determine if there are any differences between the functional plant traits of species protected from herbivory versus those exposed to herbivory and whether these impacts are affected by the ability of a species to fix atmospheric N. Ultimately I wanted to see if herbivore

pressure results in any feedbacks between the soil system and the vegetation to display differential functional traits which may influence tree health.

Chapter 3: Large mammalian herbivory and N_2-fixation as drivers of soil nutrient dynamics in a southern African savanna

To determine if the presence of large mammalian herbivores have the ability to alter the availabilities of a range of macro and micronutrients and other edaphic characteristics. I also examined the ability of the different bioavailable nutrients and their utility in discriminating between different sites, tree species and canopy locations.

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CHAPTER 2

The differential impact of herbivory on the functional traits of two co-occurring savanna tree species with contrasting N_2-fixation capacities

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2.1. ABSTRACT

Studies investigating the differential effects of herbivory by large mammalian herbivores on tree species of varying N_2-fixation capacity are uncommon- with this relationship only recently receiving more attention. I studied how the functional leaf traits of two co-occurring savanna tree species, the potentially N_2-fixing *Acacia tortilis* and the non-N_2-fixing *Combretum hereroense* are influenced by exposure to chronic herbivory versus protection from herbivores in an exclosure (~10 years). I found that the functional traits; Leaf carbon (C), nitrogen (N), phosphorus (P) , C/N, C/P and N/P ratios as well as specific leaf area (SLA) and leaf dry matter content (LDMC) all differed significantly depending on whether the trees were exposed to herbivory by large mammalian herbivores or not. Similarly all traits, except leaf C were found to differ significantly between the two species. Trees exposed to herbivory exhibited higher C/N, C/P and lower leaf N and P concentration. *Acacia* display greater concentrations of N and P (and subsequently N/P) while *Combretum* tend to exhibit more herbivore-resistant traits such as greater C/N, C/P and LDMC. Relative chlorophyll content (RCC) did not differ significantly between sites, although *Acacia* exhibited lower RCC values relative to *Combretum*. The functional traits of herbaceous biomass beneath tree canopies differed only in terms of C concentration, with a lower C concentration in grasses exposed to herbivores and relative to *Acacia*. I suggest that exposure to herbivory and the N_2-fixation capacity of tree species both influence the functional leaf traits displayed by local vegetation and stress the importance of investigating both concurrently. I also discuss the potential effects this could have on ecosystem nutrient cycling given that savannas are an ecosystem currently experiencing substantial woody encroachment and an alteration in herbivore foraging intensity.

Keywords: functional plant traits, herbivory, leaf traits, nitrogen, nitrogen fixation, nutrients, nutrient dynamics, phosphorus, savanna.

2.2. INTRODUCTION

Savannas constitute approximately 20% of the terrestrial land surface area and occupy 50% of the African continent (Scholes and Hall, 1996; Scholes and Walker, 1993). The spatial extent of savannas and indeed the number of people this ecosystem supports highlights the need for understanding the factors that drive structure and function in this ecosystem, especially in the light of global climate change (Parr et al., 2014, Lindenmayer., 2015).

The primary factors regulating savannas include top-down process/controls such as fire (Archibald et al., 2005; Bond et al., 2005; Cech et al., 2008; Orians and Milewski, 2007; Parr and Chown, 2003; Pierre, 2015) and herbivory by micro- and macro-fauna (McNaughton, 1985, 1983; Sankaran et al., 2008; Scholes and Walker, 1993; Warren et al., 1986), as well as bottom-up process/controls such as water and nutrient availability (resource availability) (Austin et al., 2004; Cech et al., 2010, 2008; Knapp et al., 2001; Kupper et al., 2012; Medina and Silva, 1990). In this research I focus directly on herbivory by large mammals and indirectly on nutrient availability through the provision of bioavailable N (as a product of biological nitrogen fixation). Ecologists have only recently begun to explicitly consider the interaction among top-down and bottom-up process in shaping the structure of savannas (Koerner and Collins, 2014; Staver and Koerner, 2015). These processes may be synergistic, additive or antagonistic. However, there is conflicting evidence as to the mechanism by which the two aforementioned variables/processes influence savanna systems. Hence, the primary goal of this chapter was to investigate this interaction and specifically to elucidate the ways in which these variables influence plant functional traits- characteristics which can be unambiguously linked (albeit in complex ways) to ecosystem processes (Díaz and Cabido, 2001).

Africa and savannas in general are well known for the diversity and abundance of large mammalian carnivores and herbivores (Cumming, 1982; Keast et al., 1972; McNaughton and Georgiadis, 1986). This is attributed to two primary reasons, the first being the result of large-scale adaptive radiation driven by habitat fragmentation and the expansion of savanna

grasslands (deMenocal, 2004; Keast, 1968; Keast et al., 1972); The second and more modern of the two is that late quaternary hunting (and hence anthropogenic not climatic or environmental constraints) in other parts of the world resulted in a disproportionally large mammal diversity in Africa relative to other regions (Faurby and Svenning, 2015). These high levels of herbivory found in African Savannas (Arsenault and Owen-Smith, 2002; Owen-Smith, 1992) will however, have significant implications for nutritional budgets. This is because herbivores are able to regulate the rate of flow of nutrients through ecosystems as a consequence of their foraging and they are therefore a major factor determining ecosystem fertility and plant properties (Blackhall et al., 2015; Cherif and Loreau, 2013; Frank et al., 1998; Frank and Groffman, 1998; Koerner et al., 2014; McNaughton et al., 1997; Pastor et al., 1993; Ritchie et al., 1998; Sinclair, 1974). Thus top-down controls are considered to be especially important in the savanna ecosystem. Bond (2005) describes vegetation as being driven by a combination of resource-controlled and consumer-controlled states in what he refers to as a multi-coloured view of the world. He makes reference to vegetation as being characteristic of a “green” (resource-acquisition traits), “brown” (browser/grazer survival traits) or “black” (fire survival traits) world or a combination thereof. The abundance of large mammalian herbivores in African savannas aligns itself appropriately with this view.

The effect herbivores have on a system is regulated by their ecology, in terms of aspects such as forage selection, size, herding behaviour, habitat preference, and spatial movement patterns. Grazers for example have significant effects on the grass layer in savannas, where high grazing pressure can trigger the formation of grazing lawns (Cromsigt and Kuijper, 2011; Hempson et al., 2014; McNaughton, 1984). These grazing lawns then attract herbivores and become associated with high levels of primary production (Frank and Groffman, 1998) as herbivores facilitate increases in the availability of light, water as well as essential nutrients such as nitrogen. This is through mechanisms such as trampling (Warren et al., 1986), nutrient addition (and the spatial redistribution thereof) via defecation and urination (Augustine, 2003; Augustine et al., 2013, 2003; Bakker et al., 2004) and directly as a result of consumption of

woody and herbaceous browse and graze (Augustine and Mcnaughton, 2004; Fornara and Du Toit, 2008, 2007). A host of empirical studies have suggested that in many ecosystems herbivory will lead to a reduction in primary production and a reduced rate of nutrient cycling because the system becomes dominated by slower growing plants that are unpalatable (Pastor and Cohen, 1997; Ritchie, 1998). This is based on the premise that plants respond to herbivore pressure with an alteration in their leaf traits to deter consumption (Coley et al., 1985). In contrast there are also studies which show that in systems where herbivores are abundant, herbivory stimulates primary production and nutrient cycling (Cargill and Jefferies, 1984; McNaughton and Georgiadis, 1986; Shariff et al., 1994).

One method which has shown promise in elucidating interactions between top-down and bottom-up processes and indeed the impact of herbivores on plants and whole ecosystems is that of utilising plant functional traits. This is because in terrestrial environments, herbivory is regulated mainly by bottom-up processes (Stiling and Moon, 2005), and a large constituent of these processes are mediated by the underlying leaf traits which in turn are regulated by the basic ecological conditions (resource availability) (Curtis and Ackerly, 2008) or dominant plant functional groups (Pringle et al., 2011; Silva et al., 2015). This concept is strengthened by the idea that the response of plants to herbivory is expected to depend on resource availability (Bryant et al., 1983; Coley et al., 1985). This availability however, is often influenced by the presence of herbivores (See Chapter 3 for more detail) creating a feedback loop. Thus exposure to herbivory is likely to influence plant functional traits (Scogings et al., 2011; Wigley et al., 2015). During the last few years researchers have been searching for easily measurable and universally applicable predictors of the effects of global change on terrestrial ecosystems (Dubey et al., 2011). This has resulted in the focus on plant functional traits. These traits are characterised by demographic and life-history features, resource dynamics and physiology which regulate their responses to environmental factors and their greater role in overall ecosystem function (Wright et al., 2004). For example, at the level of individual species, the rate of ecosystem processes such as N mineralization and

decomposition is regulated by a small number of functional traits (e.g. leaf dry matter content - LDMC) and/or by functional types (i.e. species are grouped because they share similar functional traits) such as N_2-fixers (Díaz and Cabido, 2001). These plant traits have been proposed as proxies for predicting changes in ecosystem processes (Díaz et al., 2004). Furthermore, herbivory is capable of exerting strong selective pressures on leaf traits (Coley et al., 1985; Fine et al., 2004), as evidenced by co-evolutionary studies (Ehrlich and Raven, 1964). The effect of herbivore pressure on functional traits is thus directly (through consumption and consequently selection) and indirectly (through nutrient availability) capable of regulating the characteristics of the local vegetation. Thus herbivory pressure may differentially affect leaf traits of tree species based on the functional group to which the species belongs. Some studies show that under chronic herbivory leaf traits such as N content decrease (Bryant et al., 1991; Wessels et al., 2007) while others suggest an increase in leaf N (Anderson et al., 2007).

Whether a species is capable of fixing atmospheric nitrogen is one such functional type/group which is of particular importance in savanna systems. Biological N_2-fixation by legumes can contribute up to 97% of the bioavailable N in natural, un-fertilised ecosystems (Vitousek et al., 2002; Vitousek and Howarth, 1991). Legumes, a taxa in which many of the members are able to fix N_2, are a conspicuous component of African savannas. The most abundant genus of woody legumes is that of *Acacia*, the majority of which appear to be N_2-fixers (Cramer et al., 2007; Houlton et al., 2008). Not only are herbivores able to influence plant functional traits (top-down control) but so too are individual trees, as in the case of N_2-fixers. Individual trees can substantially influence their microenvironment by altering soil physico-chemical properties, such as nutrient availability, temperature and moisture (Belsky, 1994; Reis et al., 2010). For example, soils surrounding woody legumes which are able to undergo N_2-fixation may accumulate up to 60% more N under certain conditions than the soil below the canopy of non-N_2-fixers (Ludwig et al., 2004, 2001).

In this study my aim was to provide insight into the means by which herbivores influence the functional leaf traits and whole-plant traits of savanna vegetation and primarily of savanna tree species of varying N_2-fixation capacity. To this end, data for three mechanical (specific leaf area-SLA, LDMC, leaf area- LA) and seven chemical/physiological (N, P and C content; N/P, C/N and C/P as well as relative chlorophyll value) leaf traits from two co-occurring species of tree, one which has the capacity to fix N_2, *Acacia tortilis* (Forssk.) Hayne subsp. *Heteracantha* (Burch.), and another which does not, *Combretum hereroense* Schinz (1888), were collected over a period of two years (2013-2014). The effect of herbivores on leaf traits was elucidated by selecting focal trees from both species in an exclosure (exclosure-exclusion of animals >2 kg) and on land directly adjacent to this. Individual trees within the exclosure were thus protected from large mammalian herbivores while individuals on adjacent land were left exposed to the effects of herbivory. In effect I investigated the direct impact herbivores have on vegetation as well as whether these effects are in any way mediated by the ability of the tree species to differentially modify their local environment (via contributions from N_2-fixation). A knowledge of how N_2-fixation and herbivory concurrently influence savanna systems will aid in predicting and assessing the impacts of widespread environmental and land-use change as well as the potential consequences of management by humans on this ecosystem (Augustine et al., 2003; Marchant, 2010).

I tested several hypotheses:

(1) Both aboveground woody and herbaceous biomass will be greater and consequently trees taller within the exclosure where herbivores are excluded than on adjacent land.
(2) The leaf traits of plants subject to herbivory will differ from those which are not, e.g. I expect leaves exposed to intense herbivory to exhibit greater structural investment (low SLA, high C/N ratio, and high C/P ratio) and lower concentrations of important nutrients such as N and P.
(3) Leaf traits of N_2-fixers will differ relative to those of non-N_2-fixers, specifically, N content will be greater and P content lower in *A. tortilis*.

(4) I expect SLA to scale positively with relative chlorophyll values

(5) There will be a positive association between tree leaf traits and the dominant grass species within the extent of influence of the tree.

2.3. METHODS AND MATERIALS

2.3.1. Study site description

The study was conducted within the Greater Marakele National Park (MNP) and more specifically in the Marataba region of Marakele Park (PTY) Ltd, which lies within the Greater MNP (**Fig. 2.1**). The research was undertaken at two sites within the reserve, an exclosure as well as the land adjacent to it. The exclosure, measuring 0.5 ha (24. 2992733° E; 27, 4944182° S, 1.07% slope) and adjacent land measuring 0.8 ha (24.0011795° E; 27, 4942729° S, 1.54% slope) both at an elevation of 992 m with east facing slopes. The exclosure is made up of a standard electrified game fence of ~2.5 m high. The reserve is situated in the south-western corner of the Waterberg in Limpopo Province, South Africa. MNP is one of the more recently proclaimed parks, with its development only having been initiated in 1986, following which it was declared as Kransberg National Park in 1994 (Suich et al., 2012). In November 2000 an agreement between South African National Parks (SANParks) and Marakele Park (PTY) Ltd saw the establishment of the Marakele National Park through the consolidation of land, with plans to continually expand the reserve given adequate resources. Currently MNP covers approximately 63 926 ha in total with 15 753 ha making up the Marataba section of the reserve (Novellie and Andre, 2014; Van Staden and Bredenkamp, 2005). Under this agreement Marakele Park (PTY) Ltd is tasked with all infrastructure and developmental costs in the Marataba region while SANParks is responsible for the introduction and stocking of wildlife (population re-establishment has taken place since 1991) (Suich et al., 2012). This co-management agreement is governed by a single management plan (see, Novellie and Andre, 2014) with SANParks and Marakele Park (PTY) Ltd responsible for the management of their respective land parcels.

Greater Marakele National Park lies within the Savanna Biome, the biome comprising the greatest percentage of land cover within the country. The reserve is composed of three vegetation units, namely, Waterberg-Magaliesburg Summit Sourveld, Western Sandy Bushveld as well as Waterberg Mountain Bushveld (Mucina and Rutherford, 2006). The area within which this study took place was representative of Western Sandy Bushveld (WSB; all subsequent descriptions of vegetation will be in reference to this vegetation type). This vegetation type comprises a variable mixture of both fine- and broad-leaved species. Approximately 50% of the measured range of WSB is formally conserved within the Marakele National Park (Van Staden, 2002). Consequently the reserve is an important focal point in the conservation of this vegetation. The soil is described as being plinthic (i.e. clear concentration of iron oxides in a defined layer), eutrophic and as being freely drained with a high base status (high cation exchange capacity) (Fey, 2010). The underlying geology is composed of mafic volcanics of the Letaba formation (Mucina and Rutherford, 2006). The region receives summer rainfall and experiences particularly dry winters with a mean annual precipitation (MAP) ranging from 450 mm in the north to 650 mm in the south (Mucina and Rutherford, 2006). Mean annual temperature is high at 19.3 °C with a mean monthly maximum and minimum of 36.0 °C and -3.7 °C, respectively (Schulze, 1997). The annual precipitation coefficient of variation (CV) for this vegetation type is relatively high at 29% (Mucina and Rutherford, 2006).

The Waterberg serves as an important ecotone between the greater savanna and grassland biomes and is consequently capable of maintaining a diverse assemblage of both fauna and flora (for a list of re-introduced mammals see Novellie and Andre, 2014; for plant communities see Van Staden and Bredenkamp, 2005). Dominant tree species include many species belonging to the genus *Acacia* (*Vachellia or Senegalia*[1]) such as *A. erioloba*, *A. nigrescens*, *A. erubescens*, and *A. nilotica* as well as *A. tortilis* and *Combretums* such as *C.*

[1] For more information on the taxonomic debate with reference to African *Acacia* refer to the following literature for viewpoints from both sides of the argument: Brummitt (2010); Carruthers (2010); Kull and Rangan (2012); Luckow et al. (2005) and Thiele et al. (2011). For the purposes of this research I will refer to the genus as *Acacia*.

apiculatum, *C. zeyheri*, and *C. imberbe*. Other common species include *Lannea discolor, Peltophorum africanum, Combretum hereroense, Grewia bicolor* and *G. flava* (Mucina and Rutherford, 2006). MNP has a rich assemblage of large mammalian herbivores and includes Elephant (*Loxodonta africana*), Giraffe (*Giraffa camelopardalis*), Blue Wildebeest (*Connochaetes taurinus*), Burchells Zebra (*Equus quagga burchellii*), African Buffalo (*Syncerus caffer*), Impala (*Aepyceros melampus*), Greater Kudu (*Tragelaphus strepsiceros*), Waterbuck (*Kobus ellipsiprymnus*), Black (*Diceros bicornis*) and White (*Ceratotherium simum*) Rhinoceros, among others. It is important to note that mega-herbivores such as elephant, rhinoceros, and giraffe as well as insect herbivores such as termites are common in the reserve. These organisms serve as ecosystem engineers, given their ability to indirectly and directly modulate the availability of resources (e.g. nutrients, habitat availability) for other species (Jones et al., 1994a, 1994b; Wright and Jones, 2006). Historically the land was utilised as farm-land, primarily for cattle grazing, however crops such as watermelon and maize were also grown in certain areas but this practice was abandoned in the 1980's (Dr A. Uys, *pers. comm.*). Intensive rehabilitation of these land fragments has been ongoing, as is expected given the herbivory and biodiversity mandate laid out in the 2014 MNP Management plan (Novellie and Andre, 2014). Despite these prior land uses the majority of this region has historically been unaltered. Fire is managed by means of a '*laissez faire'* approach, with natural lightning induced fires occurring approximately every 2 years (Daphne and Soundy, 2006). The fire season is April-June with point ignitions set by management early in the fire season with the goal of creating a patch mosaic in order to maximise biodiversity (Bird et al., 2008; Brockett et al., 2001; Daphne and Soundy, 2006). Fire frequency is >3 years, with a fire not having occurred for the duration of this research at the study site.

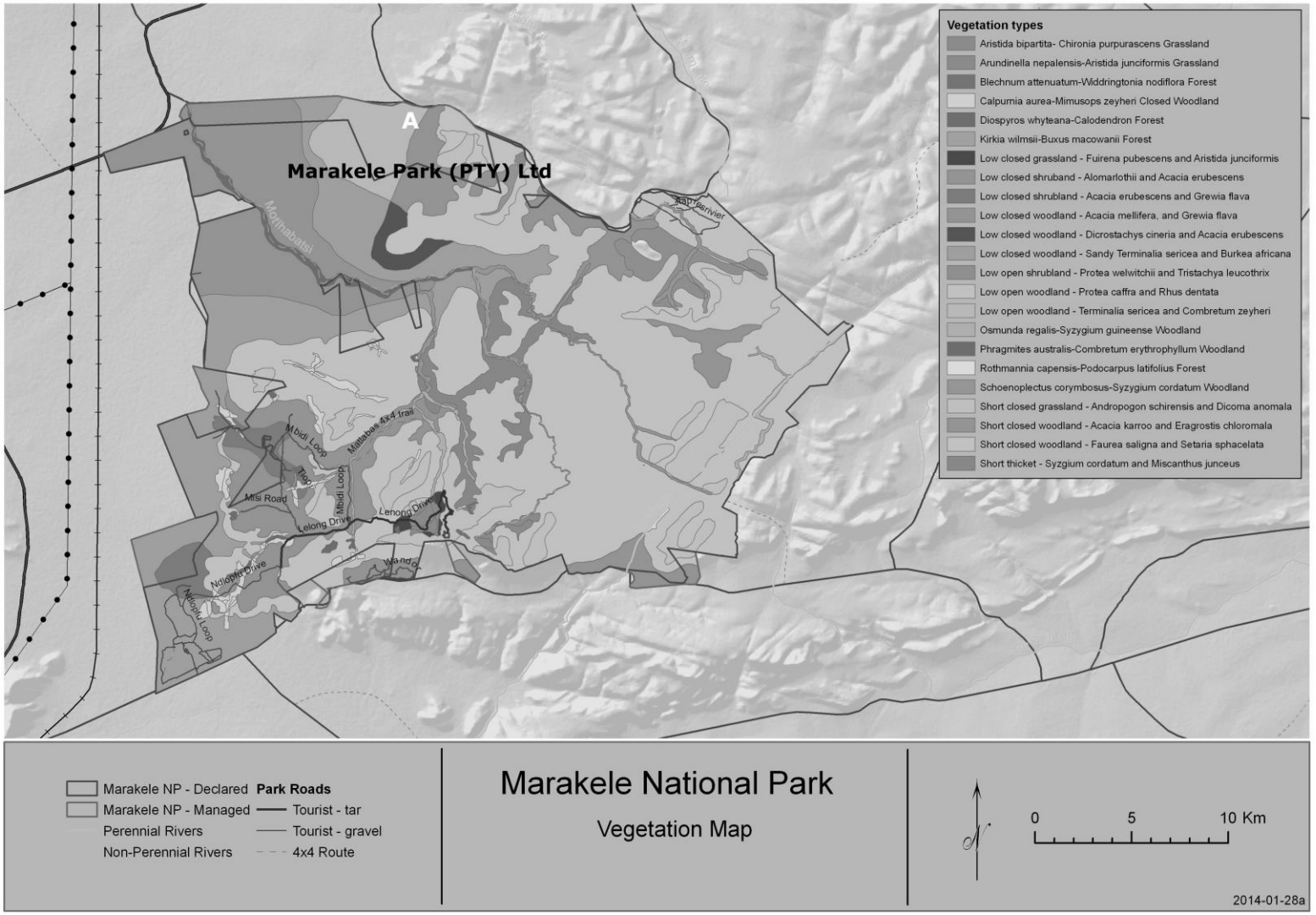


Fig. 2.1: The vegetation types located within Marakele National Park and surrounds. A=location of the research site in the Marataba region (15 753 ha) of the Marakele Park (PTY) Ltd. The study site is located within the northern region, operated by Marakele Park (PTY) Ltd while the southern region constitutes the National Park (Source: adapted from the Marakele National Park Management Plan 2014-2024).

2.3.2. Study species

2.3.2.1. *Acacia tortilis*

Acacia tortilis (Forssk.) Hayne subsp. *heteracantha* Burch. (Family Fabaceae), common name- Umbrella thorn, is a medium to large tree (may occur as a wiry bush in extremely arid environments) that varies in size from 5 to 20 m and is most commonly single-stemmed (Coates Palgrave et al., 2002). *A. tortilis* occurs abundantly throughout northern and eastern Africa, extending into the Arabian peninsula as well as southward into much of southern Africa (Dharani, 2006; Kennenni and Maarel, 1990; Ross, 1981). It is a widespread species in low-altitude, arid habitats (Brenan, 1983; Coates Palgrave et al., 2002). Smaller trees often have rounded crowns while larger individuals exhibit a characteristic flat-topped or umbrella-shaped canopy (Smit, 1999). The leaves are particularly short (<2 cm) and closely arranged along the stem, resulting in a dense, leafy canopy (Carr, 1976; Coates Palgrave et al., 2002). Each leaf has on average 2-10 pinnae, each with 6-19 pairs of leaflets (0.5-3 mm long x 0.1-1 mm wide) (van Wyk and van Wyk, 2013). Flowers are white to cream and sometimes yellow in colour occurring in globose heads, with the flowering season falling between Nov- Jan. The fruit, develop as pods which are twisted and contorted into a characteristic spiral from Mar-Jun (van Wyk and van Wyk, 2013)- usually 5-9 mm wide (Coates Palgrave et al., 2002).

The leaves and pods of *A. tortilis* are highly nutritious and are selectively foraged by many common browsers in savannas (Fornara and Du Toit, 2008; van Wyk and van Wyk, 2013). Leaves and shoots are consumed by numerous antelope while black rhinoceros (Rooke et al., 2004) and elephant forage additionally on the branches (MacGregor and O'Connor, 2004). While *A. tortilis* does not actively disperse its seeds, Loth et al. (2005) show that there is considerable regeneration outside of tree canopies. Germination of seedlings tends to be poor below tree canopies but increases in grass dominated or bare soil patches (Loth et al., 2005). This species exhibits a high level of palatability in all types of savanna vegetation (Carr, 1976; Palmer and Pitman, 1972). *A. tortilis* shows an increase in resource allocation when exposed to simulated herbivory displaying greater shoot length, diameter, number of lateral

shoots as well as shoot biomass (Rooke et al., 2004). Furthermore Fornara and Du Toit (2008) illustrated that seedlings exposed to chronic herbivory have greater regrowth abilities, twice the number of stems in the crown and double the root diameter at shallow soil depths. Frequent fire and browsing by large herbivores (specifically giraffe) limits the development of mature tree canopy (Pellew, 1983), significantly reducing the proportion of tall, mature trees over time (Strauss and Packer, 2015). This is compounded by the fact that the growth rate of the species is particularly slow in comparison to other common savanna tree species (Loth et al., 2005). *A. tortilis* is believed to be capable of facultative (if not obligate) N_2-fixation and is not considered a member of the non-nodulating Monacantha sect of African *Acacia* described by Harrier et al. (1997). Furthermore Schulze et al. (1991) showed that *A. tortilis* does indeed fix N, albeit in smaller amounts in comparison to other N_2-fixing species. This finding is supported by other studies (Assefa and Kleiner, 1998) and thus illustrates that *A. tortilis* has the capacity to nodluate and fix atmospheric N.

2.3.2.1. *Combretum hereroense*

Combretum hereroense Schinz (1888) (Family Combretaceae), common name- Russet Bushwillow, is a small (3-5 m) deciduous tree that frequents low altitude dense or sparse bushveld and wooded grassland, often occurring on termitaria (van Wyk and van Wyk, 2013). The species is found from Uganda and Kenya, southwards towards Namibia and Botswana, extending into northern South Africa (Carr, 1988). *C. hereroense* tolerates a wide variety of soils and is particularly resilient to a range of soil pH and nutrient levels (Carr, 1988). Usually single-stemmed, however may be multi-stemmed with a number of defunct stems emerging from the base- likely the result of fire (Carr, 1988).The leaves occur on short shoots and range from being elliptic to obovate (20-70 x 10-45 mm) with noticeably raised tertiary veins and are densely covered below in brown silky hairs (Coates Palgrave et al., 2002). Flowers bloom between Sept-Nov and are white, occurring in dense axillary and terminal spikes up to 6 cm long (Carr, 1988; van Wyk and van Wyk, 2013). The fruit develop as 4-winged samaras between Jan-Jun. They are conspicuous with a dark reddish-brown colour and a size of ~2 x

2 cm (Coates Palgrave et al., 2002). This species is generally highly palatable and is browsed by both cattle and game (van Wyk and van Wyk, 2013). The leaves are favoured by kudu, impala, steenbok, elephant and giraffe. The large fruiting structure of *C. hereroense* provides a means of protection from fire (Shackleton, 2007).

2.3.3. Experimental Design and Approach

This study was conducted in the form of a factorial experiment in the Marataba region of the Marakele Park (PTY) Ltd (April 2013-Dec 2014). It compared the leaf traits within an exclosure from which large mammalian herbivores (>2 kg) have been excluded for approximately ten years to adjacent land that experiences intense herbivory. The exclosure utilised in this research served originally as a temporary predator enclosure but has not been inhabited for a number of years (~10 years). Two tree species, abundant both inside and outside the exclosure were used. *Acacia tortilis* an N_2-fixer (Cramer et al., 2010) and *Combretum hereroense* a non-N_2-fixer were the dominant species at the site. Individuals were selected in October 2013 and marked using steel identification tags to allow for repeated visitation. Only reproductively mature plants (i.e. observed to have pods in the case of *A. tortilis* or samaras in the case of *C. hereroense*) were considered for selection. Ten individuals of each species were then selected, maintaining a minimum distance (>5 m) from the edge of one trees' canopy to the next (to minimise spatial autocorrelation) as well as avoiding the selection of any individuals on termitaria. Thus selection in this manner resulted in the automatic exclusion of specific trees in both sites. GPS coordinates for each individual were also recorded (**Appendix A**). Leaf and fruit (pods/samaras) samples were collected and the species identifications were confirmed at the C.E. Moss Herbarium at the University of the Witwatersrand, Johannesburg, with the aid of the herbarium curator (D. McCallum).

In order to compare the effect of herbivory and N_2-fixation on leaf functional traits ten individuals were selected within the exclosure, and ten outside the exclosure on adjacent land-resulting in 5 *Acacias* and 5 *Combretums* in each treatment. Furthermore, the soil surrounding each tree was divided into two regions, 1) within the canopy (<1.5 m from the stem) and 2)

outside the canopy (>2 m from the stem). Soil texture was measured for each tree in order to determine the similarities amongst trees in both sites. A range of functional leaf and whole-plant traits were also measured.

The basic design of this study is thus factorial in nature, with three factors: (1) N_2 fixation capacity of species; 2) canopy position; and 3) exposure to herbivory (inside or outside exclosure), each with two levels (**Table 2.1**). Comparisons were made both within the exclosure and adjacent land as well as between them (note: the exclosure and adjacent land will henceforth be referred to as sites where appropriate).

Table 2.1*:* The factorial design of this study and the corresponding category descriptors. A total of 20 focal trees (10 *Acacia tortilis* and 10 *Combretum hereroense*) were utilised in this study, with n=10 both within the exclosure and on the adjacent land.

Treatment combinations	N_2 fixation capacity of tree species (yes or no)	Canopy position (inside or outside)	Exposure to herbivory (inside or outside exclosure)
1	Yes	Inside	Exclosure
2	No		
3	Yes	Outside	
4	No		
5	Yes	Inside	Adjacent land
6	No		
7	Yes	Outside	
8	No		

2.3.4. Field and laboratory Methods

2.3.4.1. Field methods

Plant height

Height of focal trees was determined by measuring the distance from the base of the tree to the maximum height of the foliage. Measurements were made using a Haglöf CI clinometer (Haglöf Instruments, Sweden) with three consecutive readings taken (facing the tree from different directions), following which an average was computed for any further calculations. Measured plant heights were then compared with data from Shackleton (1997) (330 and 80 data points for *A. tortilis* and *C. hereroense* respectively).

Woody biomass

In order to calculate woody biomass, the tree height, basal diameter (10 cm from the ground) and diameter at breast height (DBH) for each focal tree within the study (N=20) were measured. Biomass was then estimated using the widely used, combined species equation from Rutherford (1979) (**Equation 1 ; Equation 2**) in the case where species specific equations were unavailable. Leaf biomass was then estimated separately to illustrate the differential impact of herbivores inside and outside the exclosure (**Equation 3**).

$$Estimated\ total\ biomass = -8.5997 + 1.0472\ x \qquad \textbf{Equation 1}$$

$$x = \ln(stem\ diameter\ at\ breast\ height)^2\ .tree\ height \qquad \textbf{Equation 2}$$

$$Leaf\ biomass = -8.2511 + 0.7782\ x \qquad \textbf{Equation 3}$$

Basal area of trees was determined by recording the DBH of each focal tree within the exclosure as well as the comparable area of land adjacent to this exclosure. DBH was then converted to basal area (BA) (**Equation 4**) to account for multi-stemmed trees to acquire a more accurate estimate of biomass. This was achieved by summing the BA's of each measured stem of a multi-stemmed individual and then summing these together. A new,

"single-stemmed" diameter for this individual was then computed from this combined BA value. This new compound diameter is the value which was used to estimate woody biomass utilising Rutherfords equation.

$$BA = \pi(\frac{DBH}{2})^2$$ ***Equation 4***

Herbaceous biomass

The standing stocks of herbaceous material were quantified, relative to each sampled tree. A north-facing line was demarcated from the base of the tree stem, indicating the area within the canopy (<1.5m) and outside the canopy (>2m). Eight disc pasture readings per focal tree (Dörgeloh, 2002; Zambatis et al., 2006) were taken perpendicularly to one another along the four compass points to determine peak summer (January 2014) standing stocks of herbaceous vegetation. This resulted in two readings for within canopy and two for outside the canopy for each focal tree, which was then combined to provide a mean standing stock for each species, outside and inside the canopy and for each site. Field recorded DPM readings were converted to biomass (kg/ha) based on the allometric equation developed by Trollope and Potgieter (1986) (**Equation 5**). "*x"* refers to the height of the disc (in cm) when placed atop the vegetation being measured.

$$B = -3019 + 2260x$$ ***Equation 5***

Collection of leaf tissue

All tissue utilised in the quantification of functional leaf and whole-plant traits were collected during the summer season (November 2013- February 2014). To ensure data were statistically sound and to facilitate comparisons with other studies, all traits were measured in accordance with the "New handbook for standardised measurement of plant functional traits worldwide" as described by Pérez-Harguindeguy *et al.* (2013). Leaf tissue samples were collected exclusively from mature individuals of both species. Furthermore collection of samples was restricted to only terminal (lowest reaching primary branch), fully-expanded, hardened and sun drenched

leaves[2] which were visibly healthy, free from observable necrosis, visible fungal or bacterial pathogens as well as any signs of excessive insect foraging. Whole twig sections (~10-15 cm), with attached leaves were collected, with leaf tissue only removed during processing. Any arthropods were removed before the sample was placed into storage to ensure no contamination of the specimen. This served to standardise samples among species and categories. To account for canopy-wide variation within an individual tree, samples were collected from all four compass points and then composited to yield a single sample. This was necessary to avoid sample bias and also facilitated accurate comparisons. In the case of the compound *Acacia* leaf, a leaf was defined as the entire compound leaf, including the woody rachis [this is consistent with the methodology set out in Pérez-Harguindeguy *et al.* (2013)]. From each focal tree (N=20), five bulk (whole twig sections) leaf tissue samples were collected between 10-20th January 2014 for each trait under investigation. From this bulk sample, 15 single leaves were selected for measurement of SLA, LDMC and leaf area as these require immediate, *in-situ* processing to avoid closure of leaflets and to obtain water-saturated fresh mass (refer to 2.3.4.2 for detailed methodology). Nutrient ratios and elemental concentrations were likewise processed from a separate (but simultaneous) collection of five bulk (whole twig sections) leaf tissue collections per focal tree and then analysed on a per mass basis. This technique was used as analysis of an individual leaf was not possible as it did not meet the minimum tissue-mass requirements (>1 g) for processing through the elemental analyser. Overall this yielded a sample size of 25 leaves per category (four primary treatment combinations; N_2 and non-N_2-fixer within exclosure and adjacent land) per functional leaf trait.

The number of collected samples exceeds (SLA, LDMC, LA) or meets (elemental concentrations and ratios) the minimum number of replicates suggested to ensure statistical rigor. Pérez-Harguindeguy et al. (2013) recommends a minimum sampling intensity of five replicates from five different individuals. The water-saturated fresh mass of the tissue was

[2] A leaf in this case refers to a compound leaf and all of its components, including the woody rachis as well as the individual leaflets.

determined *in situ* using a Sartorius field scale (model number: PMA600H; Sartorius Balances, Germany) correct to two decimal places immediately after the sample was collected from that tree. Following removal and weighing, each leaf was photographed against a scaled background and placed in an individually labelled paper packet. The one-sided fresh area of the leaf was then computed using the pixel-counting software package ImageJ (Rasband, 2012). The samples were subsequently left to air-dry in the field in brown paper bags.

Collection of herbaceous biomass

Elemental analysis (%C, %N and %P) was also undertaken for herbaceous biomass, specifically grass. Samples were collected concurrently with tree leaf tissue (10-20th January 2014). A representative sample of the dominant grass species (*Panicum maximum*) both within and outside the canopy of each focal tree was collected by clipping one grass tuft to ~1 cm. Clipped grass was stored in brown paper bags and allowed to air-dry in the field subsequent to collection. This resulted in a total of 40 samples for elemental analysis (replicates of n=5 per category, see **Table 2.1**).

2.3.4.2. Laboratory and Quantitative Methods

Mechanical functional leaf traits

Mechanical leaf traits included specific leaf area (SLA), leaf dry matter content (LDMC) and leaf area (LA). The mechanical leaf traits investigated are those fundamental to all functional ecological studies as they provide a means to quantify the investment by the plant in the construction of a leaf per unit of light-intercepting area (Poorter et al., 2009). SLA was reported for each of the 15 representative leaves from the bulk sample for a single tree. These same leaves were then marked and oven-dried at 60°C for 72 hours. Oven-dried leaves were then weighed to the nearest 0.0001g using a Sartorius 2007 MP mass balance. The SLA for each leaf was then calculated (**Equation 6**).

$$SLA\ (mm^2.mg^{-1}) = \frac{one{-}sided\ area\ of\ fresh\ leaf(m^2)}{oven{-}dried\ mass\ (g)} \qquad \textbf{\textit{Equation 6}}$$

Leaf dry matter content (LDMC) was calculated using the same leaves as used in the determination of the SLA. However, in order to produce this value, the water-saturated fresh mass of the leaf tissue was measured prior to oven drying and then the LDMC calculated (**Equation 7**).

$$LDMC(mg.g^{-1}) = \frac{oven\ dried\ mass\ of\ a\ leaf(mg)}{water\ saturated\ fresh\ mass\ (g)} \qquad \textbf{\textit{Equation 7}}$$

Nutrient concentrations

Functional traits that are nutrient based were only sampled once, during the summer season of 2013/2014 (10-20th January 2014) given the costs involved in obtaining these data. All samples (both leaf and grass tissue) were oven dried at 60°C for 72 hours or until change in mass was negligible. Plant tissue was then prepared for digestion by being ground in a MRC SW-2 rotary mill with a mesh size of 0.25mm. All processed samples were then dispensed into 2 ml sterile cuvettes. The mill was thoroughly cleaned between samples to avoid cross-sample contamination. Samples were then stored in a dessicator (< 10% relative humidity) until being sent for analyses.

Prepared samples were sent for elemental analysis at the Institute for Commercial Forestry Research (ICFR, internationally accredited) in Pietermaritzburg, Kwazulu-Natal, South Africa. All samples were analysed for % nitrogen, % carbon and % phosphorus. Carbon and nitrogen concentrations were determined by means of a LECO CHN628 elemental analyser (LECO Corporation, Michigan) while phosphorus was quantified by an inductively coupled plasma mass spectrometer. Nutrient ratios were then computed from these data and presented as mass ratios as this is the convention in terrestrial ecology (Güsewell, 2004).

Relative chlorophyll content

The simple, inexpensive and time efficient sampling of relative chlorophyll content could reduce reliance on N analyses which are far more costly. As such the RCC of the leaves for each of the 20 focal trees was measured. Prior research has demonstrated that there is a strong correlation between SPAD readings and foliar % nitrogen (Wu Feibo, 1998). A Konica

Minolta, SPAD-502 chlorophyll meter (Spectrum Technologies, Plainfield) was used. This device determined the RCC by measuring transmittance through the leaf in the red and infrared spectrums (Ling et al., 2011). Each reading was taken from the centre of a fully-expanded, sun drenched leaf near, but not including, the mid-rib. This was repeated until 15 replicates for each tree were obtained (independent to leaves sampled for other analyses) and then averaged to obtain measures of mean leaf RCC for each species in both sites (n=75, N=300). The RCC facilitated the comparison between *C. hereroense* and *A. tortilis* based on their exposure to herbivory and variable N_2-fixation capacity. Furthermore the viability of this tool in assessing foliar nitrogen concentrations of the species under investigation was assessed by determining the degree of correlation between values.

2.3.5. Quantitative analyses

Data were pooled by species based on locality (i.e. focal trees were not compared to one another). Firstly basic descriptive statistics such as category means, variance and standard deviation were computed to provide an overall picture of the variability in the traits under contrasting conditions of herbivory and N_2-fixation capacity. For the purposes of the analyses, categorical explanatory variables are N_2-fixation capacity (N_2-fixer, non-N_2-fixer), and herbivore exposure (within exclosure and outside exclosure). These were coupled with a number of different continuous “response” variables (i.e. leaf traits) which are considered independent from one another. Leaf and whole-plant traits, particularly nutrient concentrations were tested for normality using Shapiro-Wilks tests and where necessary transformed to achieve a normal distribution. Multiple data transformations were attempted to acquire normally distributed data, including log, box-cox and power transformations (Sakia, 1992). If the data were normally distributed, multiple nested ANOVA’s were then computed (Krzywinski et al., 2014), comparing explanatory variables for each trait. A Tukey HOD (honest significant difference) *Post Hoc*, multiple comparisons test was then run to determine which factor or level was responsible for any significant difference observed. In the case of non-normal response variables (residuals), the non-parametric alternative to a one-way analysis of variance, a

Kruskal-Wallis rank sum (H) test was used (Kruskal, 1952). All data was checked for significant outliers using Grubbs test for outliers (Grubbs, 1969), with analyses subsequently rerun to determine any changes in statistical inferences. All analyses were carried out using the statistical computing software, R (R Development Core Team, 2015). The R graphics package "ggplot2" contributed by Wickham and Chang (2014) was used to create a number of the plots presented in this chapter. Furthermore post-hoc analyses for non-parametric data were computed with the use of the package "pgirmess" contributed by Giraudoux (2014).

2.4. RESULTS

The height of *A. tortilis* individuals was significantly different inside and outside the exclosure, with trees inside on average 1.45 ± 0.4 m taller (*t*-test, d.f.(8), *t*=2.13, *P*=0.038) (**Table 2.3**). *C. hereroense* individuals were also taller in the exclosure (by 0.62 ± 0.35 m) albeit not significantly so. Not only were the trees found to be taller when protected from herbivores but exhibited a greater mean basal area, 213 ± 129 cm^2 and 84 ± 67 cm^2 for *Acacia* and *Combretum* respectively. Similarly, mean leaf dry mass (LDM) in *Acacias* was significantly greater within the exclosure than on adjacent land, by 7.3 mg or 22.3% (*t*-test, d.f.(148), *t*=4.03, *P*<0.001). Leaf dry mass in *Combretums* was however consistent between sites. Interestingly, despite differences in the leaf dry mass of *Acacias,* average leaf area between sites was very similar, while in comparison *Combretums* that exhibited a similar dry leaf mass were shown to have a greater leaf area (by 24 mm^2) in the exclosure (**Table 2.2**). 80% of *Acacias* sampled were multi-stemmed, while only 20% of *Combretums* exhibited a multi-stemmed habit (**Table**

Table 2.2: Leaf dry mass and leaf area (mean ± SE) of *Acacia tortilis* and *Combretum hereroense* inside the exclosure and on adjacent land. Significance determined by *t*-tests with d.f. =148 throughout.

	Exclosure	Adjacent land	*t*	*P*
Leaf dry mass (mg)				
Acacia tortilis	32.8 ± 1.6	25.6 ± 0.9	4.03	<0.001**
Combretum hereroense	42.3 ±1.7	43.4 ± 1.4	0.51	0.304
Leaf area (mm^2)				
Acacia tortilis	274 ± 14	277 ± 16	0.158	0.438
Combretum hereroense	395 ± 14	371 ± 12	1.32	0.093

Significant values: *=P<0.05, **=P<0.001

Table 2.3: A suite of general plant traits, including tree height, basal area, total biomass, leaf biomass (mean ± SE) as well as the number of multi-stemmed individuals of *Acacia tortilis* and *Combretum hereroense* inside the exclosure and on adjacent land. Significance determined by *t*-tests with d.f. =8 throughout.

	Exclosure	Adjacent land	*t*	*P*
Tree height (m)				
Acacia tortilis	6.04 ± 0.60	4.59 ± 0.30	2.13	0.038*
Combretum hereroense	5.61 ± 0.45	1.99 ± 0.25	1.14	0.147
Basal area (cm^2)				
Acacia tortilis	434 ± 183	221 ± 74	1.13	0.149
Combretum hereroense	187 ± 87	103 ± 47	0.62	0.278
Total biomass (kg $tree^{-1}$)				
Acacia tortilis	146 ± 71	43 ± 16	1.56	0.085
Combretum hereroense	48 ± 24	23 ± 12	0.70	0.252
Leaf Biomass (kg leaves $tree^{-1}$)				
Acacia tortilis	5.8 ± 2	2.4 ± 0.6	1.49	0.089
Combretum hereroense	2.5 ± 1	1.4 ± 0.6	0.66	0.263
Number of mutli-stemmed trees				
Acacia tortilis	3	5	-	-
Combretum hereroense	1	1	-	-

Significant values: *=P<0.05, **=P<0.001

2.3).

2.4.1. Aboveground herbaceous and woody biomass

Aboveground herbaceous biomass was significantly different (Kruskal-Wallis test: $H_{(7, 152)}$ =96.231, *P*< 0.001) between exclosure and adjacent land, with a greater biomass measured within the exclosure both inside and outside the tree canopy for both species under investigation (**Fig. 2.2**). Herbaceous biomass was greater (albeit not statistically significant, Kruskal Wallis multiple comparisons test: $H_{(7, 152)}$, *P*=0.3404) within the tree canopy for both species at both sites by 49%, 18%, and 64% for *Combretum* on adjacent land (*Combretum*-AL), *Acacia* in the exclosure (*Acacia*-E) and *Acacia* on the adjacent land (*Acacia*-AL), respectively. Only *Combretum* within the exclosure (*Combretum*-E) exhibited less herbaceous biomass within the canopy than outside (by 4.2%). Biomass within the exclosure was on average 90% and 95% greater for *Combretum* and *Acacia* respectively than outside the exclosure (**Fig. 2.2**).

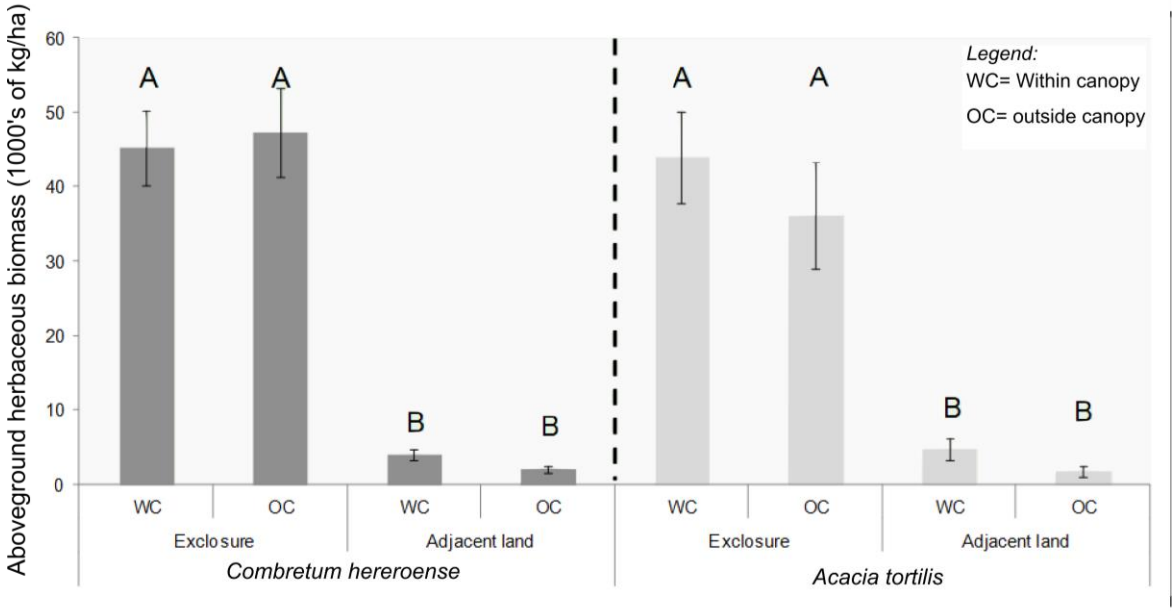


Fig. 2.2: A comparison of the (mean ± standard errors) aboveground biomass using a disc pasture meter (kg/ha) of herbaceous vegetation of each *Combretum hereroense* and *Acacia tortilis* focal tree, both within the tree canopy (WC) and outside the tree canopy (OC). *Letters* denote significant differences (Post hoc, multiple comparisons tests after a Kruskal-Wallis test: $H_{(7, 152)}$ =96.231, P< 0.001).

While herbaceous biomass was significantly different inside and outside of the exclosure, the total aboveground woody biomass of focal trees did not differ significantly (*t*-test, d.f.(8), *t*=1.56, *P*=0.085 and *t*-test, d.f.(8), *t*=0.70, *P*=0.252 for *Acacia* and *Combretum,*

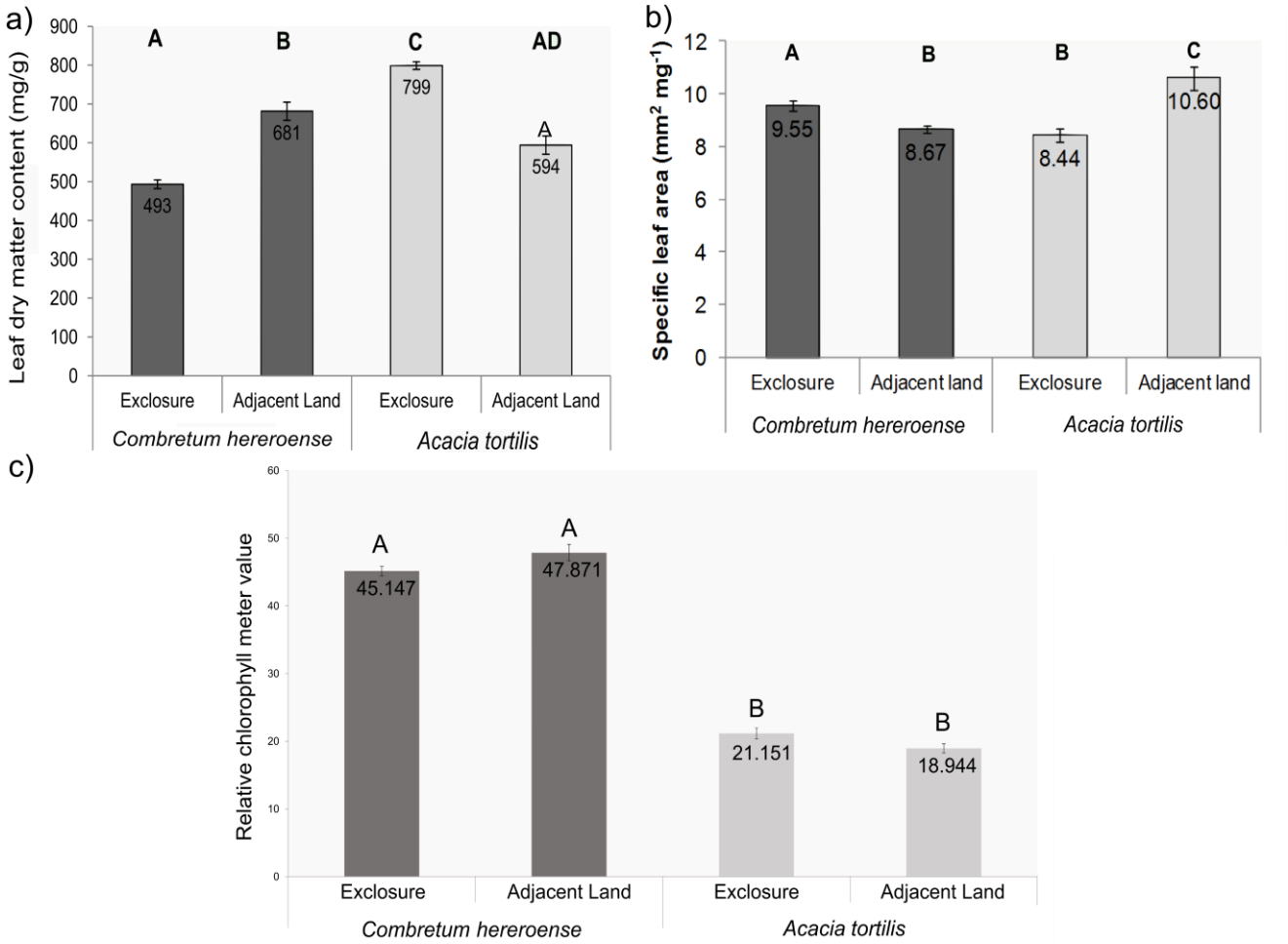


Fig. 2.3: Measured functional leaf traits (mean ± SE) in relation to protection from (exclosure) and exposure to herbivory (adjacent land) for two African savanna trees, the broad-leaved *Combretum hereroense* and the N_2-fixing fine-leaved *Acacia tortilis*. (a) Leaf dry matter content, (b) Specific leaf area and (c) Relative chlorophyll content of the leaves. *Letters* denote significant differences (following multiple comparisons tests).

respectively), nor did the leaf biomass. It is however, important to note that both total biomass and leaf biomass were greater for trees within the exclosure than for the same species in the adjacent land (**Table 2.3**).

2.4.2 Functional leaf traits

Specific leaf area was significantly different between the exclosure and the adjacent land for both *Acacia* and *Combretum* (Kruskal-Wallis test: $H_{(1, 298)}$ =29.94, P <0.001). Mean SLA was 9% greater in *Combretum* trees inside the exclosure than those on adjacent land (9.5 ± 1.6 $mm^2\ mg^{-1}$ vs. 8.67 ± 1.3 $mm^2\ mg^{-1}$). In *Acacia* however, the opposite relationship was exhibited, with individuals from the adjacent land having mean SLA values of 20% greater than their conspecifics in the exclosure (10.6 ± 4.04 $mm^2\ mg^{-1}$ vs. 8.44 ± 1.97 $mm^2\ mg^{-1}$) (**Fig. 2.3**b).

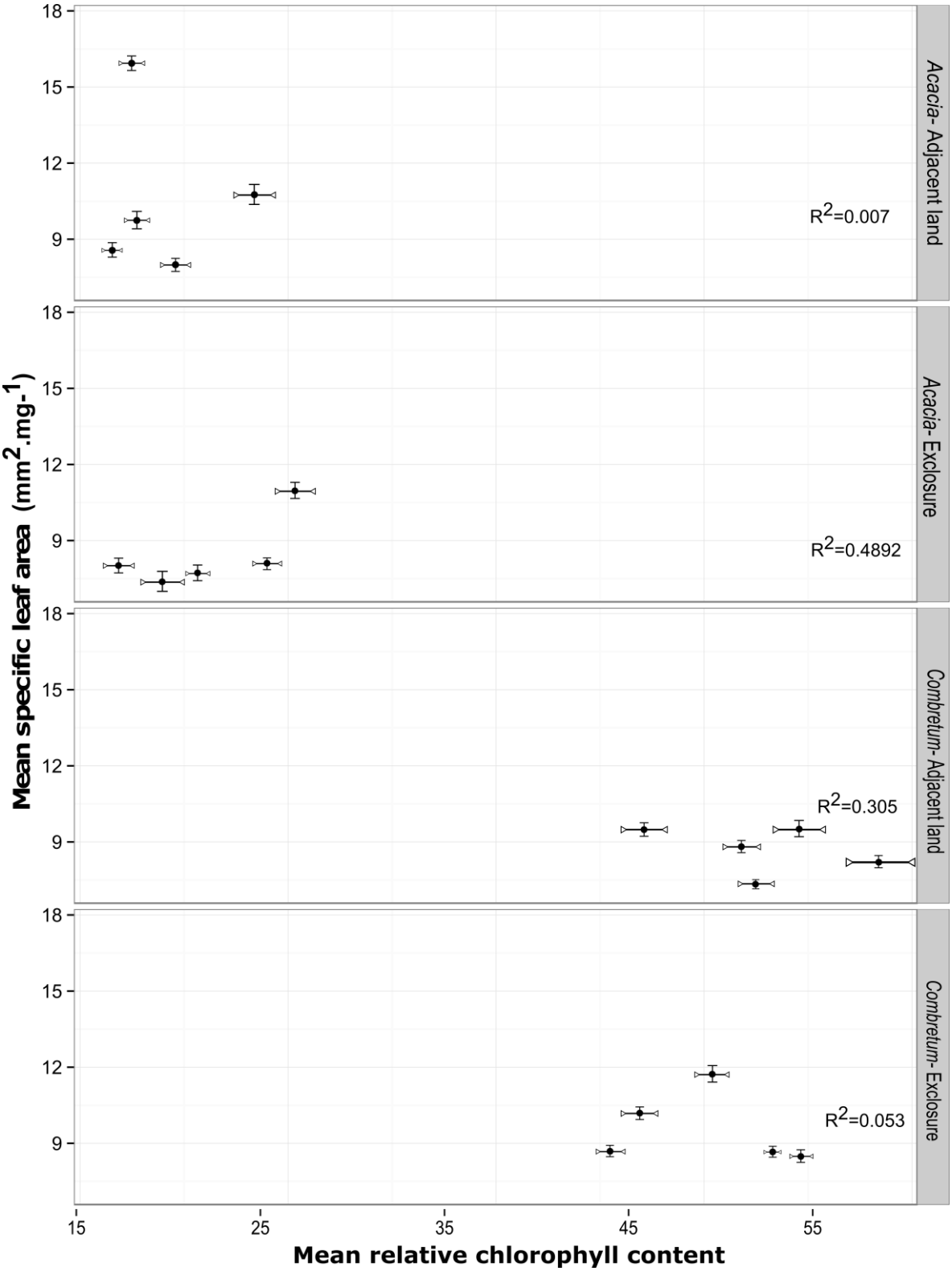


Fig. 2.4: Relationship between the mean relative chlorophyll content (mean ± SE) and mean specific leaf area (mean ± SE) for *Acacia tortilis* and *Combretum hereroense* within an exclosure and on adjacent land experiencing chronic herbivory. R^2 values denote the strength of the relationship (regression analyses) between the two variables for each category (see legend).

Leaf dry matter content (LDMC), as with SLA differed significantly between exclosure and adjacent land for both *Acacia* and *Combretum* (Kruskal-Wallis test: $H_{(1, 298)}$ =84.387, P< 0.001) (**Fig. 2.3**a). Mean LDMC was 28% greater for leaves from *Combretum* individuals (681 ± 204 mg/g vs. 493 ± 97 mg/g). Conversely, *Acacia* individuals in the exclosure exhibited leaves with a 26% greater LDMC than conspecifics on adjacent land.

Table 2.4: Coefficients of variation (CV) for specific leaf area, leaf dry matter content as well as relative chlorophyll content for *Acacia tortilis* and *Combretum hereroense* inside an exclosure and on the adjacent land.

	Exclosure	***Adjacent land***
Specific leaf area (mm^2 mg^{-1})		
Acacia tortilis	23%	38%
Combretum hereroense	17%	15%
Leaf dry matter content (mg g^{-1})		
Acacia tortilis	11%	35%
Combretum hereroense	20%	30%
Relative chlorophyll meter value (SPAD units)		
Acacia tortilis	33%	31%
Combretum hereroense	14%	22%

Relative chlorophyll content did not differ significantly between sites by species. However, there was a significant difference between species (Kruskal-Wallis test: $H_{(1,298)}$ =216.63, P< 0.001). *Combretum* individuals exposed to herbivory exhibited a mean SPAD unit value of 5.7% greater than their conspecifics in the exclosure (47.87 ± 10.62 vs. 45.15 ± 6.24) while the mean SPAD unit value of *Acacia* trees in the exclosure was 10.4 % greater than the trees on adjacent land (21.15 ± 6.9 vs. 18.94 ± 5.78) (**Fig. 2.3c**).

With regards to SLA, LDMC and relative chlorophyll meter values, within-species variation was fairly high (**Table 2.4**). A regression of relative chlorophyll meter values against specific leaf area (**Fig. 2.4**), indicated no detectable relationships for *Acacia*-AL and *Combretum*-E (R^2=0.007 and R^2=0.053 respectively). However the same regression registered detectable, albeit weak relationships between SLA and chlorophyll content for *Acacia*-E and *Combretum*-AL. *Acacia* individuals in the exclosure exhibited a positive correlation between SPAD units and mean SLA (R^2=0.489, P=0.186) while *Combretums* on the adjacent land

demonstrated a slightly weaker, but negative correlation between these two variables (R^2=0.305, *P*=0.552).

2.4.2.1. Elemental traits

Nutritional traits in relation to tree leaves

One hundred leaf tissue samples were analysed to determine their elemental concentrations (N=100, n=25). Nitrogen content of leaf tissue differed significantly between *Acacia* and *Combretum* (Kruskal-Wallis test: $H_{(1, 98)}$ =58.801, *P*< 0.001). N content was not different between *Acacia* exposed to large mammalian herbivory and those protected from it (28.74 ± 1.66 mg/g vs. 28.13 ± 3.29 mg/g) (**Fig. 2.5***a*). This same relationship was found in *Combretums* with no difference in N content in individuals exposed to or protected from herbivory (24.24 ± 2.60 mg/g vs. 22.06 ± 1.51 mg/g). N content was, however, significantly different in trees exposed to versus protected from herbivory when species were pooled (Kruskal-Wallis test: $H_{(1, 98)}$ =4.39, *P*=0.036). A fairly large amount of variation was exhibited in *Acacia* exposed to herbivores (*CV*=12%) in comparison to those protected from herbivores (*CV*=6%).

Leaf phosphorus content differed significantly between *Acacia* exposed to herbivores and those protected from herbivory (Kruskal-Wallis test: $H_{(1, 98)}$ =77.63, *P*< 0.001). *Combretum,* however did not differ between the treatments (Kruskal-Wallis test: $H_{(1, 98)}$ =3.96, *P*=0.79) (**Fig. 2.5***b*). Both *Acacia* (significant) and *Combretum* (non-significant) have greater mean P content, 20.4% and 6.1% respectively, in individuals within the exclosure (0.98 ± 0.079 vs. 0.78 ± 0.097 mg/g and 0.53 ± 0.102 vs. 0.50 ± 0.107 mg/g respectively) than those on adjacent land.

Carbon content exhibited significant differences intraspecifically (exclosure versus adjacent land) with respect to *Combretum*s (nested ANOVA $F_{(1, 98)}$=6.108, *P*=0.029) but not with regards to *Acacia* (nested ANOVA $F_{(1, 98)}$=3.66, *P*=0.489) (**Fig. 2.5***c*). Mean C content of leaf tissue was greater for both *Acacia* and *Combretum* in the adjacent land by 0.72% and 1.2% (505.34 ± 5.47 vs. 501.68 ± 11.34 mg/g and 503.31 ± 8.61 vs. 497.20 ± 9.98 mg/g,

respectively). Carbon content differed significantly between sites (nested ANOVA $F_{(1, 98)}$=7.419, *P*=0.008).

With respect to elemental ratios (by mass) N/P and C/P exhibited similar relationships with significant differences between *Acacia* and *Combretum* (nested ANOVA $F_{(1, 98)}$=80.4, *P*<0.001 and Kruskal-Wallis test: $H_{(1, 98)}$ =78.262, *P*<0.001, respectively) as well as a significant difference between *Acacias* protected from herbivory and conspecifics exposed to herbivory on the adjacent land (nested ANOVA $F_{(1, 98)}$=9.831, *P*=0.002 and Kruskal-Wallis test: $H_{(1, 98)}$ =7.604, *P*=0.006, for N/P and C/P respectively). *Combretum* individuals however, did not exhibit significant differences in N/P and C/P ratios between the sites (**Fig. 2.5***d* and *f*).

Mean N/P ratios were 18.9% greater for *Acacias* found on adjacent land (36.44 ± 5.62 vs. 29.57 ± 2.92), while values were 1.9% greater (negligible) for *Combretums* within the exclosure (46.79 ± 6.19 vs. 45.89 ± 8.19). C/N ratios differed interspecifically (i.e. between species) (Kruskal-Wallis test: $H_{(1, 98)}$ =61.35, *P*<0.001), but not intraspecifically (i.e. between conspecifics) (Kruskal-Wallis test: $H_{(1, 98)}$ =8.56 *P*>0.05). Despite the lack of significant differences among conspecifics, mean C/N ratios for both species were greater on the adjacent land by 3.8% in *Acacias* (18.2 ± 2.1 vs. 17.5 ±0.81) and 9.5% in *Combretums* (22.89 ± 1.30 vs. 20.72 ± 2.12) (**Fig. 2.5***e*).

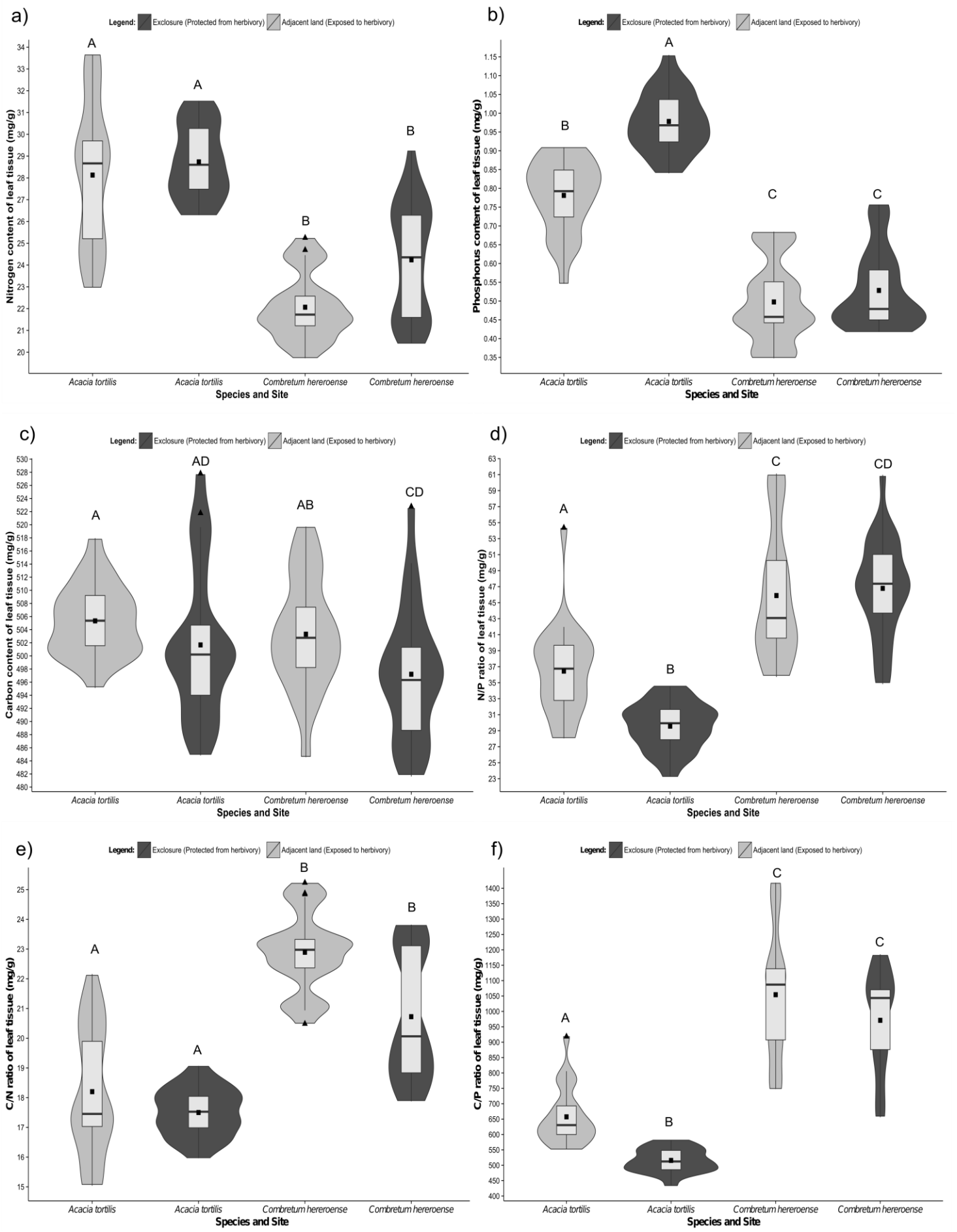


Fig. 2.5: Nutritional leaf traits in relation to protection from (exclosure) and exposure to (adjacent land) large mammalian herbivory for two African savanna tree species, the broad-leaved *Combretum hereroense* and the N_2-fixing fine-leaved *Acacia tortilis.* Functional traits include: (a) Nitrogen content by mass, (b) Phosphorus content by mass, (c) Carbon content by mass, (d) N/P ratio, (e) C/N ratio and (f) C/P ratio. *Letters* denote significant differences (following appropriate multiple comparisons tests –refer to text). Violin plots illustrate the distribution and density of data points. Squares indicate mean values and dark grey horizontal bars the median value, while triangles illustrate outliers.

In summary, globally[3] leaf **N** (Kruskal-Wallis test: $H_{(1, 98)}$ =4.4, P=0.04), **C** (nested ANOVA $F_{(1, 98)}$=7.4, P=0.008), **P** (Kruskal-Wallis test: $H_{(1, 98)}$ =6.4, P=0.01), **C/N** (Kruskal-Wallis test: $H_{(1, 98)}$ =5.6, P=0.02), **N/P** (nested ANOVA $F_{(1, 98)}$=9.8, P=0.002) and **C/P** (Kruskal-Wallis test: $H_{(1, 98)}$ =7.6, P=0.006) differ significantly depending on whether they are exposed to herbivory (adjacent land) or protected from it (exclosure) while leaf **N** (Kruskal-Wallis test: $H_{(1, 98)}$ =53.702, P<0.001), **P** (Kruskal-Wallis test: $H_{(1, 98)}$ =67.6, P<0.001), **C/N** (Kruskal-Wallis test: $H_{(1, 98)}$ =54.9, P<0.001), **N/P** (nested ANOVA $F_{(1, 98)}$=80.4, P<0.001) and **C/P** (Kruskal-Wallis test: $H_{(1, 98)}$ =67.7, P<0.001) differ significantly (all but leaf C) between species (i.e. *Acacia* or *Combretum*).

Correlations amongst traits

Pair-wise comparisons between leaf traits of the focal tree species, *A. tortilis* and *C. hereroense* yielded important distinctions between *Acacia* and *Combretum* (**Fig. 2.6**). In both *Combretum* exposed to and protected from herbivory (**Fig. 2.6**, *c* and *d*) there is a moderate to strong positive correlation between leaf N and P content (Spearman rank correlation coefficient, r_S=0.64, and r_S=0.59, respectively). Furthermore in *Combretum*-E and *Combretum*-AL there is a moderate to strong positive correlation between leaf N and C content (Spearman rank correlation coefficient, r_S=0.44, and r_S=0.64, respectively). Finally there is a strong negative correlation between leaf P and C/N ratio for *Combretum* in both the exclosure and on the adjacent land (r_S=-0.70, and r_S=-0.59, respectively).

In *Acacia* protected from herbivores (**Fig. 2.6**a) there is a strong correlation between between leaf N and C content (r_S=0.62) while there is no correlation between the same two variables in *Acacia* subject to herbivory (r_S=0.07) (**Fig. 2.6**b). Interestingly the variables that were strongly correlated in *Combretum* (N and P contents, N and C as well as P with C/N) do not exhibit the same relationships in *Acacia.* There is little to no correlation between these

[3] Globally refers to the entire measured dataset

same variables in *Acacia* individuals at either site (r_s=0,07 and r_s=0.25 for N and P,r_s=0.24 and r_s=0.21 for P and C/N, for *Acacia*-E and *Acacia*-AL respectively).

Nutritional traits in relation to herbaceous biomass

Elemental analysis of the functional traits for the herbaceous biomass surrounding each tree was done in order to compare the potential infuence of the tree on the local vegetation. Despite the differences exhibited among conspecifics in different sites in relation to tree—leaf traits, five of the six traits measured in the herbaceous vegetation were not significantly different between *Acacia* and *Combretum* protected from and exposed to large mammalian herbivores. This included grass N and P content, C/N, C/P and N/P ratios (ANOVA $F_{(7,\ 32)}$=2.19,*P*=0.061; Kruskal Wallis test: $H_{(7,\ 32)}$ =10.90, *P*=0.143; ANOVA $F_{(7,\ 32)}$=1.46, *P*=0.218; Kruskal Wallis test: $H_{(7,\ 32)}$ =10.60, *P*=0.157; ANOVA $F_{(7,\ 32)}$=1.46, *P*=0.217, respectively) (**Fig. 2.7**). Only grass C content was significantly different, with differences in the C content between *Acacia* and *Combretum* (ANOVA $_{(7,\ 32)}$, *P*<0.001) and between sites and varying canopy position (within tree canopy and outside tree canopy).

The only significant intraspecific difference was exhibited between the C content of *Combretum*-E-WC (within canopy of the exclosure) and *Combretum*-AL-OC, i.e. the within canopy C content of herbaceous vegetation for individuals in the exclosure was different to the C content of the vegetation outside the canopy for individuals from the adjacent land (**Fig. 2.7***c*).

Despite the lack of significant difference there were some consistent trends with grasses in the adjacent land showing lower N, P and C content and greater C/N and C/P ratios

(**Fig. 2.7**) than in the exclosure, however there were no discernible trends in relation to the effect of N_2-fixation capacity (i.e. species) or canopy position.

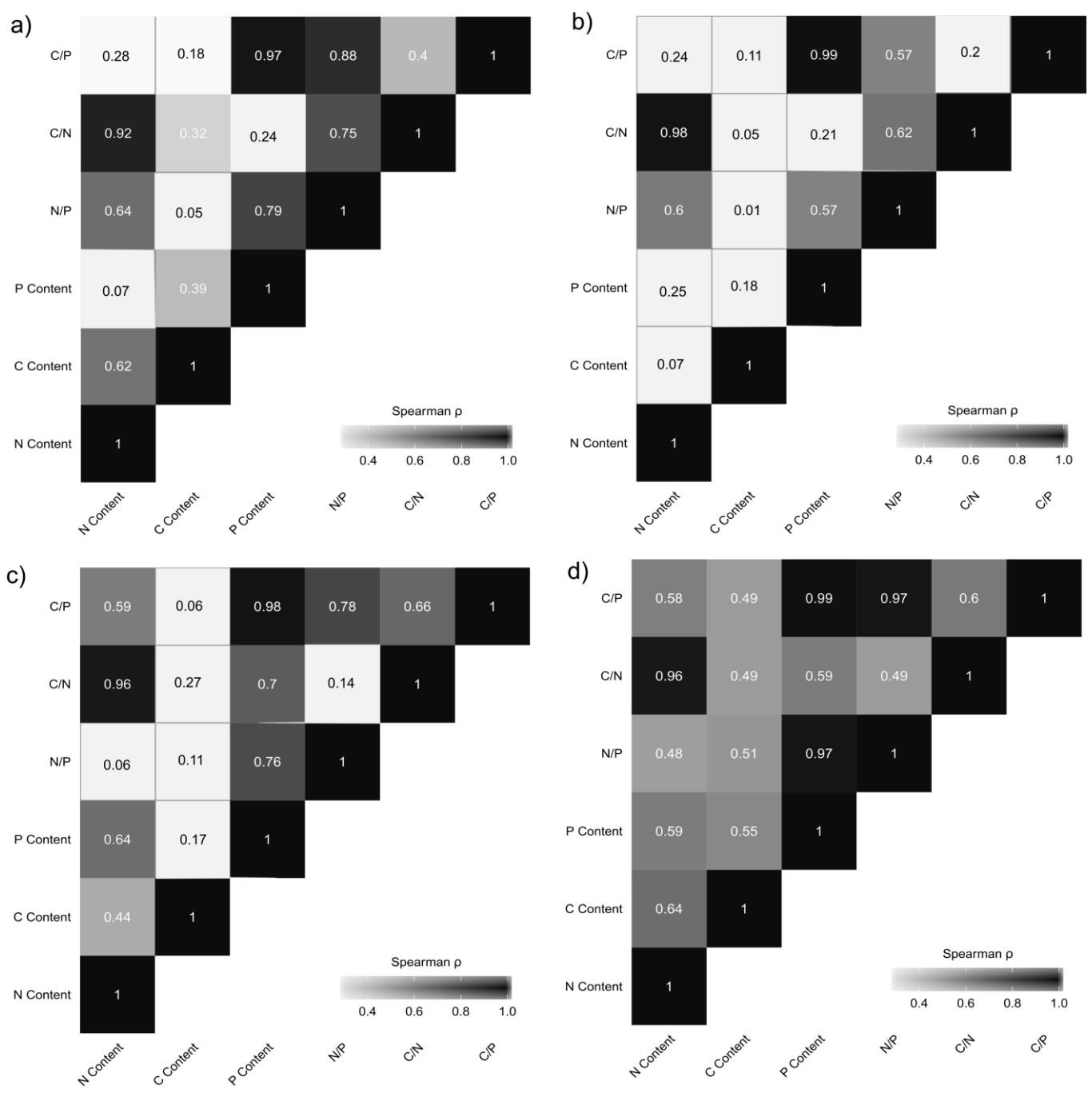


Fig. 2.6: Correlation between leaf traits of focal tree species, *Acacia tortilis* and *Combretum hereroense*. Data presented are the absolute values of Spearman rank correlation coefficients, where the shade of grey indicates the strength of the correlation- darker shades indicate strong correlations while the lightest shade of grey indicates weak correlations of <0.3. (a) *A. tortilis* within the exclosure (protected from mammalian herbivores), (b) *A. tortilis* on the adjacent land (exposed to mammalian herbivores), (c) *C. hereroense* within the exclosure and (d) *C. hereroense* on the adjacent land.

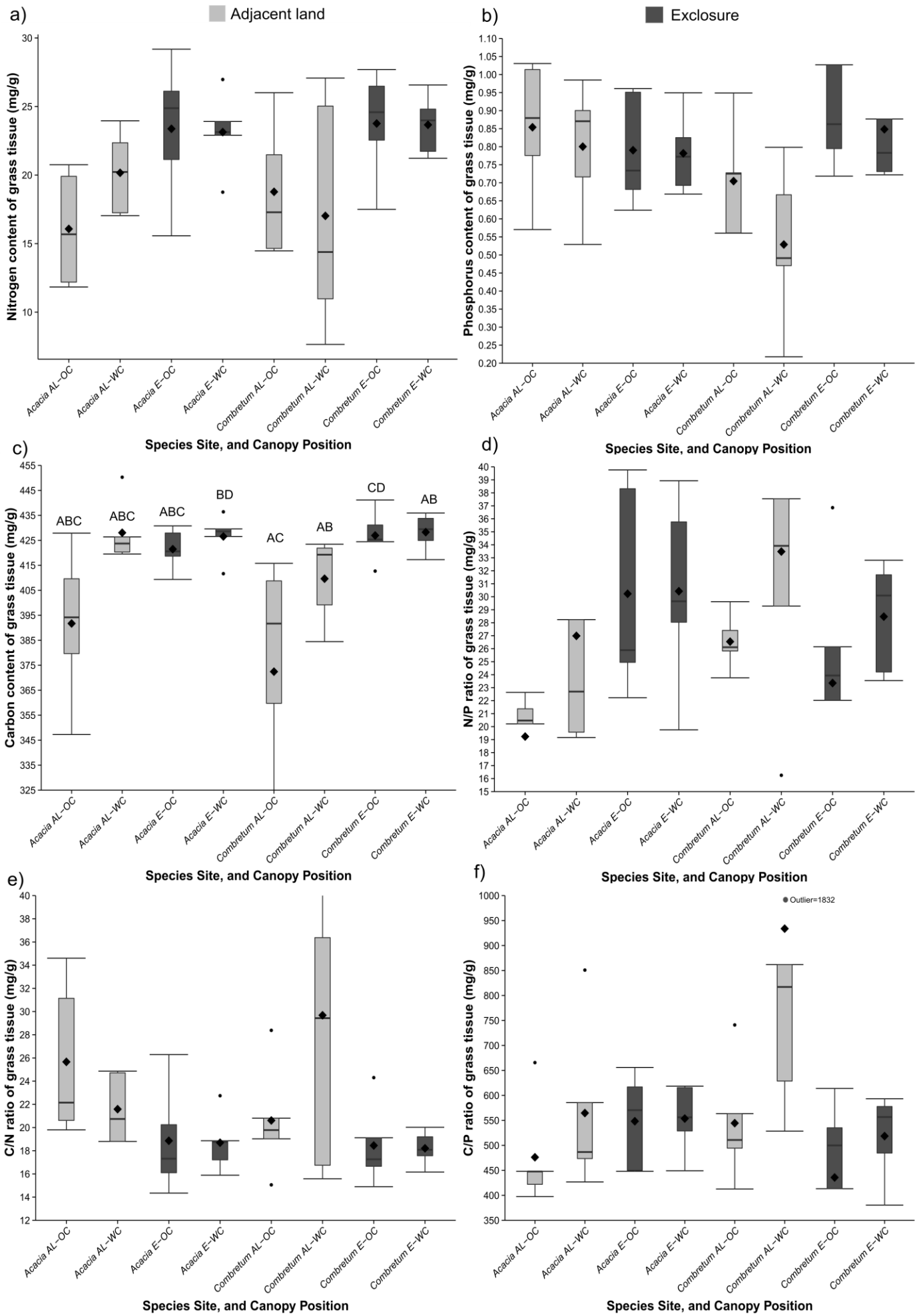


Fig. 2.7: Nutritional traits of the herbaceous biomass (*Panicum maximum*) associated with each individual tree, within the tree canopy (WC) and outside the influence of the tree canopy (OC). (a) N content of grass tissue, (b) P content, (c) C content, (d) N/P ratio, (e) C/N ratio and (f) C/P ratio. *Letters* denote significant differences (following appropriate multiple comparisons tests –refer to text). Letters are absent in cases of no significant differences. Black diamonds indicate mean values and dark grey horizontal bars the median value. Black dots show the presence of outliers while boxplots show the spread of data along with whiskers indicating minimum and maximum values.

2.5. DISCUSSION

This study shows that herbivory by large mammalian herbivores does indeed differentially affect the leaf functional traits of the species in question. It also suggests that the ways in which herbivores influence these traits differs significantly among species of contrasting N_2-fixation capacity. The combination of exposure to, or protection from herbivory in conjunction with the N_2-fixation capacity of the species, results in interactions between these two variables which bring about specific responses in the leaf tissue.

H1: Both aboveground woody and herbaceous biomass will be greater and trees taller in the exclosure

In the experimental plots herbaceous biomass per unit area was significantly greater in the exclosure protected from herbivory. The fact that herbaceous biomass in the exclosure, relative to either species was 95% and 90% greater for *Acacia* and *Combretum* respectively was expected. However, the finding that mean woody biomass per tree did not differ between sites nor species is unexpected. I must acknowledge that the relatively small sample (n=5 *Acacia* in the exclosure and 5 on adjacent land, similarly for *Combretum*) size of trees in this study may bias average measures of biomass, however I was restricted by the number of trees within the exclosure. In certain circumstances plants may respond to increased herbivore pressure by displaying varying levels of compensatory growth (Belsky, 1986; Gadd et al., 2001; Liu et al., 2012). The ability of savanna trees to tolerate herbivory is reflected in traits such as high regrowth capacity in response to chronic herbivory. This has been demonstrated in *A. tortilis* (Fornara and Du Toit, 2008) and *A. nigrescens* (Fornara and Du Toit, 2007) where saplings exhibited 100% greater capacity to regrow clipped shoots. Consequently I suggest that the continuous herbivory to which species are subject to on the adjacent land has resulted in compensatory regrowth, and therefore individuals exhibit similar amounts of foliage despite continuous defoliation. However it must be acknowledged that I did not account for a systematic difference in the ages of the trees. In contrast the ability of herbivores to significantly

affect herbaceous biomass is well documented (Hempson et al., 2014; McNaughton, 1984). It is important to note that herbaceous standing stocks in the exclosure (>30 t/ha) are far greater than is typical for a savanna environment where 4 t/ha is sufficient for most management burns (van Wilgen et al., 2014).

Acacia individuals were significantly taller on average within the exclosure which is in agreement with my hypothesis. *Combretum* however, displayed similar sizes in both sites. Given that herbivores generally select for higher quality forage (Treydte et al., 2013), and the fact that N and P content was higher in *Acacia* than *Combretum* (see *H2* for more details) I suggest that herbivores may be directly responsible for the smaller *Acacia* trees on adjacent land by selectively foraging on these species. Moncrieff et al. (2011) show that heavily browsed trees may be shorter than conspecifics in an exclosure for a given stem diameter. Basal area did not differ significantly between sites, and therefore may suggest trees of similar ages (Niklas et al., 2003). Although tree growth rate is highly variable even within the same species. If tree age is not a confounding effect then we can assume that herbivory is responsible for the differential tree height observed. Other factors that affect tree height-diameter relationships include neighbour density as well as local light conditions (Henry and Aarssen, 1999). To single out herbivory as the dominant factor these two variables would need to be investigated. Furthermore tree-cores and a dendrochronological approach, as well as direct observation and quantification of mammalian foragers, could help elucidate this issue. Based on in-field, *in situ* observations, *Acacia* from outside the exclosure appeared to have been subject to far greater levels of mammalian herbivory than *Combretum*. Furthermore, when comparing focal trees with other literature (Shackleton, 1997) the mean height of *C. hereroense* concurs well, while *A. tortilis* individuals appear to be taller than average for the data available (mean of 2.3 m and 4.0 m for *Acacia* and *Combretum,* respectively).

H2: The functional traits of plants subject to herbivory will differ from those which are not.

In this study I predicted that leaves from plants exposed to intense herbivory would exhibit greater structural investment in the production of leaf tissue (i.e. low SLA, high C/N, and high C/P ratios). Mechano-structural leaf traits differed based on exposure to mammalian herbivory with SLA, LDMC and LDM all significantly different between the exclosure and adjacent land. LDM as well as LDMC were found to be greater while SLA was lower in the exclosure, protected from herbivores. This finding was unexpected as this suggests that plants in the exclosure select for leaves with a lower nutrient content and consequently greater structural investment. However, structural investment to deter herbivory would be costly and in a site protected from herbivory it would be advantageous to forgo these expenses (Wright et al., 2004). This would have been expected on the adjacent land where exposure to herbivory may drive trait selection towards herbivore-resistant traits such as high LDMC and SLA. For example, in a study conducted by Wigley et al. (2014) in the Kruger National Park, mean SLA was consistently higher within sampled exclosures relative to the surrounding vegetation for a variety of species (including *A. gerrardii, A. nigrescens* and *C. imberbe*).

When considering nutritional traits, only RCC did not differ significantly inside and out of the exclosure, while leaf N, C, and P as well as C/N, N/P and C/P were different inside and out. Both leaf N and P content were greater within the exclosure while leaf C, C/N, N/P and C/P were higher in vegetation from the adjacent land. These data are intriguing as it has different implications to those implied by the mechano-structural traits. N and P content were greater in the exclosure despite low SLA values while plants from the adjacent land exhibited traits suggestive of exposure to continuous herbivory (i.e. high C/N and C/P). This is more in line with what was expected. The finding that N content of leaves was lower in the presence of herbivory is consistent with other studies which have shown a reduction in N under conditions of chronic browsing (Bryant et al., 1983, 1991; Wessels et al., 2007). However, there is evidence that post-defoliation regrowth exhibits higher concentrations of nutrients

(Anderson et al., 2007). This finding was attributed to the physiological increase in specific enzymes from defoliation by herbivores (Anderson et al., 2006). This process occurs in the short term immediately following herbivory and as such is unlikely to be represented in the case of this research which investigates long-term herbivore exclusion. Inconsistent with prior studies, is the fact that I show significantly greater P in the exclosure as well as trends in elemental ratios such as C/N (Wigley et al., 2014). Logically you should expect a change in C/N, given that if C is increasing or decreasing in the plant tissue, another element must be increasing or decreasing, such as N. The high leaf C content of leaf tissue in the adjacent land may further indicate a response to herbivory as C is associated with the concentration of tannins, lignin and other carbon based secondary compounds that deter herbivory (Chapin et al., 2011).

N/P ratios far exceeding the threshold level for classifying nutrient limitation outlined by Güsewell (2004), indicate that the system is severely P-limited (Ågren, 2008, 2004; Ågren et al., 2012), and in this case the adjacent land more so than the exclosure. Which is consistent with the fact that the vegetation of the exclosure consists of leaves containing a greater concentration of P. African soils and savannas in general are most commonly P-limited as they are ancient, well weathered soils which have undergone a great deal of leaching (Menge et al., 2012; Zechmeister-Boltenstern et al., 2015). Assessing nutrient limitation on the basis of foliar traits should however, be regarded as only a coarse measure of potential nutrient limitation. Work by Craine et al. (2008) in the Kruger National Park show that N/P ratios have limited ability in predicting actual limitation in soil nutrients. As such factorial-based aboveground experiments are needed to confirm these findings.

In order to better understand these trends it is important to take into account other factors which may be influencing leaf traits. These data show that herbivory does indeed have a differential impact on plant functional traits but given the contradictory relationships exhibited there is clearly another factor at play (see *H3*).

H3: Leaf traits of the N_2-fixing *A. tortilis* will differ from the non-N_2-fixing *C. hereroense*.

I found that the N_2-fixation capacity of a species is indeed associated with the differential expression of certain key functional leaf traits. All traits with the exception of leaf C content were found to differ significantly between species. Leaf N and P content were higher in *Acacia* while C/N, N/P and C/P (two fold) were greater in *Combretum.* As expected I did indeed find *Acacia* to have tissue with greater N content given the ability to fix atmospherically derived N. However inconsistent with my expectations was the fact that *Acacia* leaves also exhibited a greater P content. This is surprising given the fact that N_2-fixation machinery is P dependent and would utilise a large proportion of available P in fixing N (Cramer et al., 2010; Vitousek et al., 2010). Higher leaf N content in N_2-fixing species has also been demonstrated In other studies (Cornelissen et al., 1997; Peltzer et al., 2009) while a recent meta-analysis by Werner et al. (2015) showed that legumes in a state of "high symbiotic persistence" (i.e. continued symbiosis) demonstrate a strong, positive correlation to leaf N and P content. This is consistent with the data collected from Marakele. Foliar N and P content (n=5 for *Acacia;* n=5 for *Combretum*) were also found to be higher in *A. tortilis* (23.9 mg/g and 1.05 mg/g, for N and P respectively) relative to *C. hereroense* (18.9 mg/g and 0.096 mg/g for N and P respectively) sampled from the Kruger National Park (KNP) (Colgan et al., 2015). Foliar samples in this data, although illustrating the same trend yielded greater N and lower P content when comparing like species from Kruger. Interestingly leaf P in *C. hereroense* from the KNP yielded a higher P content even in comparison to *A. tortilis* from my study. This could suggest that the KNP sites are less phosphorous limited than Marakele. However, in this dataset by Colgan et al. (2015) both species were sampled from lowland/riparian habitat which we know tends to accumulate certain nutrients because of the position in the catenal sequence (Ben-Shahar, 1990; Scholes and Walker, 1993).

Herbivore-resistant leaf traits such as high C/N, C/P, and LDMC and lower SLA, N and P content were prevalent in *Combretum* individuals suggesting that this species is less palatable to herbivores. Interestingly, higher N/P ratios in *Combretum* suggest that the litter of

this species would contribute more so to nutrient cycling as higher N/P is linked to quicker turnover times. However, at the same time high C/N and C/P ratios may also indicate recalcitrant or resistant litter which is not so easily decomposed (Bakker et al., 2011; Inagaki et al., 2011; Karasov and Martínez del Rio, 2007; Wardle, 2002). Bakker et al. (2011) show that functional traits of fresh leaves are often better predictors of potential plant litter decomposition than are senesced leaves, even after resorption of nutrients by the tree. This supports the idea that it is indeed possible to infer potential decomposition from fully functional leaves.

Despite the higher N and P content of the leaves in *Acacia*, this species also exhibited a higher LDMC when protected from herbivores which is inconsistent with what would have been expected and may represent a confounding factor not yet considered. Kurokawa et al. (2010) showed that N_2-fixers had higher leaf N content and N/P ratios and lower C/N ratios than non-N_2-fixing species. The greater N content and lower C/N ratios is consistent with my findings. This study could also not detect a significant difference between species with regards to any of the other traits common between studies.

The RCC was significantly greater in *Combretum* which is surprising given the lower leaf N content measured in the species. I suggest that the SPAD-502 chlorophyll meter does not cope well with compound leaves, given the many small leaflets which fail to adequately cover the stage in order to obtain a reading. This is corroborated by the fact that I was not able to obtain any meaningful correlation between RCC and leaf N content. It is thus evident that RCC on an area basis (as measured by the SPAD) is not related to the concentration of N measured on a dry mass basis. This finding provides an important insight into the use of this technique and one which has potentially been overlooked in the literature.

Combretum individuals yielded a strong, positive correlation between N and P content, while *Acacia* individuals did not. This is likely due to the capacity of *Acacias* to fix N_2,

uncoupling the relationship between foliar N and P, even though N and P content is higher in this species.

A very important outcome of this data was the fact that both *Acacia* and *Combretum* vegetation exposed to herbivory exhibited a far greater variability in all leaf traits (**Fig. 2.5**), highlighting the extreme phenotypic plasticity in these species. The exclosure is only ~10 years old and given that in this short time leaf traits have already experienced a great deal of variation is very important and bears consideration in predicting the effects of herbivory and N_2-fixation on savanna ecosystems in the future. Future studies should investigate what happens to functional leaf traits of trees historically protected from herbivores and then re-exposed to this disturbance. How long do the traits take to again resemble those of species which have continuously been exposed to herbivory, or indeed do they? If plasticity is shown to not be reversible then this could have significant impacts to how we view human mediated disturbance such as erecting exclosures as well as regulating the herbivore carrying capacity and consequently controlling herbivore foraging intensity.

H4: I expect SLA to scale positively with relative chlorophyll values

In this research I could elucidate no meaningful relationships between SLA and relative chlorophyll content (RCC- measured in SPAD units). Even in cases where a moderately strong correlation between SLA and RCC was achieved, these were not statistically significant. This finding is unexpected as it has been widely shown that there is a strong association between SLA and N content (Shipley et al., 2006; Wright et al., 2004) - where the majority of chlorophyll is rich in nitrogenous compounds and should display a similar relationship. An increase in the relative chlorophyll content (RCC) would generally be associated with an increase in N content (Coste et al., 2010; Ling et al., 2011; Loh et al., 2002). However, the opposite was found, with a greater N content in *Acacias* relative to *Combretums* which is accompanied by a significantly greater RCC in the latter. This may align with the findings of some studies which have shown a negative relationship between SLA and RCC (Marenco et al., 2009). The fact that the

measured RCC values are not associated with measured N content (see H3) suggests that utilising SPAD values as proxy data for nitrogen content (on a dry mass basis) may not yield data that is easy to interpret as the relationship is complex and multi-dimensional and often not linear. With respect to linearity- Uddling et al. (2007) demonstrated that the relationship between measured SPAD values and leaf chlorophyll content of a number of commercially important crop species were non-linear or what they described as curvi-linear. This non-linearity of SPAD values as chlorophyll proxy data thus also introduces inherent issues in assessing the correlation between SPAD and foliar N. On this basis It is evident that the use of SPAD data as a proxy for N content is highly questionable and unless thorough, species specific calibrations are undertaken, this method should perhaps be avoided. However, elucidation of relationships could potentially have been more resolved in my research if SLA and RCC values were measured concurrently for a single leaf instead of computing correlations on the basis of mean values.

H5: There will be a positive association between leaf traits and the dominant grass species within the extent of influence of the tree

The functional traits of the grasses were highly variable and therefore yielded no significant differences between sites, canopy position or species for grass N, P, C/N, C/P and N/P. Grass C content was found to differ between sites, as well as beneath the two species. Grass C content was greater in the exclosure protected from herbivores, and beneath *Combretum* trees. This is inconsistent with leaf traits where C content was greater in the adjacent land. I suggest that this lack of statistical significance in the majority of traits may in part be due to the small sample sizes per category. However, despite this there were some consistent trends, with grasses exposed to herbivores tending towards herbivore-resistant (unpalatable) traits such as high C/N and C/P ratios and lower N and P content. This differs with the finding by Smith et al (2015) that grass tissue N decreases with increasing exclosure duration. Smith and colleagues attribute this finding to the removal of herbivores which subsequently decreases the rate of N cycling in their study system (Upland UK).

Trees do have the ability to affect the herbaceous biomass beneath their canopies with 25% greater N and P concentrations recorded in the sub-canopy grasses (*P. maximum*) of trees in Timbavati Private Game Reserve, South Africa (Treydte et al., 2007). Treydte et al. (2007) also suggest that the N_2-fixation capacity of the tree (their study included the N_2-fixers, *A. nigrescens*, *A. zanzibarica, A. nilotica, A. mellifera* and *Dichrostachys cinerea*) has a pronounced effect on functional traits of grasses. While I could discern no trends in grasses beneath *Acacia* versus those beneath *Combretum* it has been shown that N_2-fixation capacity of tree species significantly elevates grass leaf P contents and N/P ratios (Treydte et al., 2007). The null effect of trees on the grasses may be a result of the size of the trees. Grasses growing beneath large trees (~9m) can have as much as 40% greater N and P content in comparison to grasses not growing under a tree canopy (Treydte et al., 2009a). Treydte et al. (2009) found that small trees (<2.3 m in height) had no impact on the grasses beneath their canopies. This suggests that there may in fact be a threshold tree size, below which trees do not influence the soil system to a great enough extent, whether this be a result of root biomass, litter input in relation to tree size (i.e. larger trees result in more litter input) or hydrological aspects. In my study tree heights fell between 3.8-6.2 m for *Acacia* and 4.2-6.6 m for *Combretum* and were perhaps not large enough to significantly influence the vegetation beneath their canopies.

2.6. CONCLUSION

Overall this study showed that herbivory by large mammals and N_2-fixation capacity of trees, both have an influence on key functional leaf traits. Trees protected from herbivores invested less in structural defence and displayed greater concentrations of important elements. Further, *Acacia* displayed greater concentrations of important elements given the ability to access other sources of N while *Combretum* instead shows more herbivore-resistant traits. By understanding the ways in which herbivores differentially effect functional plant traits in savanna ecosystems we can begin to predict how woody encroachment, alterations to community composition (e.g. greater proportion of N_2-fixing species) and changes in both domestic and wild stocking densities of herbivores may influence the landscape. For example,

if N_2-fixing species protected from herbivores (as a result of reduced stocking densities, extinction etc) are allowed to proliferate this may have dramatic consequences to N cycling due to the variable decomposition of plant litter. Decomposition will be strongly influenced by the variability in key functional leaf traits involved in this study and form an unambiguous link between functional traits and this key ecosystem process (Díaz et al., 2004; Díaz and Cabido, 2001; Garnier et al., 2004).

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2.8. APPENDICES

Appendix A: location of the individuals used in this research within Marakele Park (PTY) Ltd.

Tree Number	Species	Site	GPS Coordinates	
			S	E
1	*A. tortilis*	Exclosure	24.29927	27.49458
2	*A. tortilis*	Exclosure	24.29918	27.49432
3	*A. tortilis*	Exclosure	24.29944	27.49439
4	*A. tortilis*	Exclosure	24.2993	27.494
5	*A. tortilis*	Exclosure	24.29906	27.49441
6	*C. hereroense*	Exclosure	24.29915	27.49451
7	*C. hereroense*	Exclosure	24.29946	27.49461
8	*C. hereroense*	Exclosure	24.29939	27.49424
9	*C. hereroense*	Exclosure	24.29922	27.49434
10	*C. hereroense*	Exclosure	24.29939	27.49431
11	*A. tortilis*	Adjacent land	24.29966	27.49447
12	*A. tortilis*	Adjacent land	24.2999	27.49428
13	*A. tortilis*	Adjacent land	24.30002	27.49405
14	*A. tortilis*	Adjacent land	24.29981	27.49454
15	*A. tortilis*	Adjacent land	24.29984	27.49437
16	*C. hereroense*	Adjacent land	24.29977	27.49455
17	*C. hereroense*	Adjacent land	24.30067	27.49408
18	*C. hereroense*	Adjacent land	24.30026	27.49446

19	*C. hereroense*	Adjacent land	24.30036	27.49419
20	*C. hereroense*	Adjacent land	24.30038	27.49438

2.9. LITERATURE CITED

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CHAPTER 3

Large mammalian herbivory and N_2-fixation as drivers of soil nutrient dynamics in a southern African savanna

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3.1. ABSTRACT

Studies investigating the impacts of herbivory by large mammalian herbivores and the effect of mature trees of varying N_2-fixation capacity on soil nutrient dynamics of a mesic savanna system are uncommon and have not to my knowledge been undertaken as extensively as in this study. I studied how the bioavailability of 15 key macro (NO_3-N, NH_4-N, inorganic N, P, K, Ca, Mg and S) and micro soil nutrients (Na, Fe, B, Cu, Zn, Al, and Mn) differ based on association with the N_2-fixing *Acacia tortilis* and the non-N_2-fixing *Combretum hereroense* as well as exposure to or protection from herbivory in an exclosure (~10 years) compared with the adjacent land. To do this I made use of ion exchange resin capsules which act as plant root simulators, placed in the soil from October 2013 to March 2014, i.e. across the whole summer rainy season. I found that nutrient availability was greater in the exclosure protected from herbivores (Zn>P>Na>K>NO_3-N>Cu>B>S>Mg>inorganic N). Further, bioavailable nutrients occurred in higher quantities (Mg, Al, B and Fe) below the canopy of the non-N_2-fixing *Combretum* relative to *Acacia.* Likewise, bioavailable nutrients (Ca, NO_3-N, B, Fe, Al and inorganic N) were greater sub-canopy versus beyond the canopy. I suggest that disturbance by herbivory primarily alters the bioavailability of macronutrients while species differences influence the availability of micronutrients in the soil. A possible explanation for a reduction in nutrient availability on the adjacent land is whereby herbivory decreases the clay content by increasing exposure to weathering/erosion. This then results in a net loss of mineral elements from the system. I go on to discuss the implications of these findings in the management of savanna systems and the effect of such disturbances on the cycling of nutrients.

Keywords: soil traits, herbivory, soil nutrients, nitrogen, nitrogen fixation, nutrients, nutrient dynamics, phosphorus, savanna.

3.2. INTRODUCTION

There are a number of primary drivers known to regulate savannas, including top-down process such as fire (Parr and Chown 2003; Bond et al. 2005; Archibald et al. 2005; Orians

and Milewski 2007; Cech et al. 2008; Pierre 2015) and herbivory by micro- and macro-fauna (McNaughton 1983; McNaughton 1985; Warren et al. 1986; Scholes and Walker 1993; Sankaran et al. 2008), as well as bottom-up controls such as water and nutrient availability (resource availability) (Medina and Silva 1990; Knapp et al. 2001; Austin et al. 2004; Cech et al. 2008; Cech et al. 2010; Kupper et al. 2012). The availability of nutrients is one of the primary mechanisms controlling primary production in terrestrial ecosystems. The limitation of particular nutrients will thus have a pronounced effect on vegetation and indeed many aspects of vegetation composition, structure and physiology. Nutrient limitation is regulated by external inputs and diverse processes occurring *in situ* which control the conversion of unavailable/recalcitrant nutrients into forms that are bioavailable (Vitousek 2004). The majority of terrestrial ecosystems tend to be limited by soil N and/or soil P (Vitousek 1986; Sterner and Elser 2002; Werner 2005; Elser 2012). In this research on soil nutrient dynamics, we will focus exclusively on the effects of two of the drivers mentioned above, namely herbivory by large mammals and the differential nutrient input of tree species of varying N_2-fixation capacity, both of which influence nutrient availability.

Large mammalian herbivores are a major agent of disturbance and can have significant effects on the ecosystems they inhabit (Shannon et al. 2008). Grazing and browsing mediates the structure and function of savanna ecosystems by regulating the flow of energy between the vegetation and by altering the availability of nutrients. Herbivores are thus a major determinant of ecosystem fertility and plant properties (Augustine et al. 2003; Augustine and Mcnaughton 2004; Augustine and McNaughton 2006; Cherif and Loreau 2013). This is especially prominent given that African savannas have one of the most diverse and abundant assemblages of large mammals (Cumming 1982; Toit and Cumming 1999).The mechanisms by which herbivores influence ecosystems include; trampling (Warren et al. 1986), nutrient addition through the deposition of faeces and urine (Augustine 2003; Augustine et al. 2003; Bakker et al. 2004; Augustine et al. 2013) as well as through the consumption of woody and herbaceous browse and graze (Augustine and Mcnaughton 2004; Fornara and Du Toit 2007; Fornara and Du Toit 2008). Herbivores consume vegetation in one location after which they

may travel to a new location where they defecate and urinate, thus facilitating the lateral diffusion of nutrients across a landscape (Augustine et al. 2003; Allington and Valone 2013). Using a model-based approach, Wolf et al. (2013) suggest that this lateral diffusion of nutrients is a previously unrecognized ecosystem service. Large mammalian herbivores have a high capacity to transport nutrients and given the fact that megaherbivores have experienced proportionally higher extinction rates, the lateral flow of nutrients by wild animals is expected to decline globally (Wolf et al. 2013). In addition to nutrient displacement, areas exposed to chronic herbivory experience increased rates in specific ecosystem processes such as soil N mineralization (Ruess and McNaughton 1984; Ruess and McNaughton 1988; Seagle et al. 1992) as well as an alteration in the availability of certain key nutrients (Waal et al. 2011). The quantity of bioavailable N in soils tends to be greater relative to the N from decomposition of plant litter alone (Frank 1998; Schowalter et al. 2011). This suggests that herbivores result in a net gain of soil N in areas they inhabit.

There has been much debate over the years as to whether the presence of herbivores accelerates or decelerates the rate of N cycling (and other nutrients) - factors such as plant species composition and the quantity and quality of leaf litter production have been considered to regulate this outcome (Pastor and Cohen 1997; Ritchie et al. 1998; Frank and Groffman 1998). The quality of leaf litter is dependent on the concentration of nutrients in leaf tissue, which in turn is partially controlled by the availability of particular soil nutrients, which may be altered via herbivore mediated nutrient inputs. Thus, studies concurrently investigating both of these factors are vital. Herbivores are capable of such pronounced effects given that they return nutrients to the soil in a form (urine and faeces) that is more rapidly mineralised than the nutrients of leaf litter (Bakker et al. 2004). Furthermore, herbivores consume photosynthesising leaf material, which has not yet senesced or undergone resorption of nutrients, and in this way mediate the transport of a greater quantity of nutrients than would be gained from just leaf litter (Chapin et al. 2011). By increasing the availability of nutrients such as N, this stimulates primary production and leaf litter production, which has a direct impact on the accumulation of organic matter in soils (Carroll et al. 2003; Tipping et al. 2012)

and by proxy the cycling of nutrients. Herbivores can also have dramatic effects on vegetation composition, for example Asner et al. (2009) showed that there was a marked increase in aboveground biomass (38%-80%) even in short-term exclosure experiments (~ 6 years), while in the longer-term (~22 years) there was as much as an 11-fold increase in woody biomass in areas protected from mammalian herbivores.

Not only do herbivores have marked impacts on ecosystem nutrient dynamics but so too do trees. They do this by modifying the inputs and outputs of nutrients. The degree of alteration and the type of nutrients affected are dependent on species traits as well as interactions with top-down process such as fire and herbivory (Schowalter et al. 2011; Sitters et al. 2015). Tree species which have the ability to symbiotically fix N, making it bioavailable, are uniquely capable of dramatically altering nutrient dynamics in savanna systems. Legumes, a taxa in which many of the members are able to fix N_2, are a conspicuous component of African savannas (Mattick 1969 *in* Schulze et al. 1991) and also the largest source of N in these environments (Cleveland et al. 1999; Vitousek et al. 2013). Up to 97% of the bioavailable N in natural, un-fertilised ecosystems can be contributed by species capable of biological nitrogen fixation (BNF) (Vitousek and Howarth 1991; Vitousek et al. 2002). As a result, the presence of N_2-fixing species can significantly alter nutrient dynamics, particularly in terms of soil N. There are, however two primary mechanisms by which plant species are able to impact N-cycling. Firstly differences in nitrogen use efficiency of the species can create feedbacks between the tree and soil system, altering rates of cycling. Secondly, plant species also have differential capacities to control the inputs and losses of N from ecosystems (Knops et al. 2002). Species capable of BNF add additional N to the system (Ludwig et al. 2004) but also have a high phosphorus requirement (Vitousek et al. 2002; Binkley et al. 2003) and therefore may result in a decrease in the availability of P (Cech et al. 2008). However, in a study investigating the N_2-fixing shrub, *Dichrostachys cinerea,* Blaser et al. (2014) found no decline in available soil P, despite increasing N availability.

Vegetation in the sub-canopy region of individual trees has been shown to differ from that outside the canopy, as large trees are able to strongly influence their immediate surroundings, in terms of soil (Belsky 1994; Ludwig et al. 2004) and local vegetation and fauna (Skarpe 1991; Treydte et al. 2008; Treydte et al. 2009; Treydte et al. 2010; Treydte et al. 2011; Haftay et al. 2013). As a result, trees are able to alter the nutrient conditions in their local environments- potentially creating a heterogeneous patch-mosaic of nutrient availability across the landscape (Weltzin and Coughenour 1990; Vetaas 1992; Belsky 1994; Ludwig et al. 2001). In many systems, this is further exacerbated by the fact that herbivores modify their local habitat in much the same way (Augustine et al. 2003).Recent research by Sitters et al. (2013) and Sitters et al. (2015) illustrated that both N and P dynamics were significantly affected by an increase in the density of N_2-fixing trees. In their biogeochemical approach to the study they were able to show that N inputs increased with an increase in the density of N_2-fixing trees.

In this study, my aim was to investigate the means by which herbivores and tree species of varying N_2-fixation capacity (functional type) differentially influence the edaphic characteristics of a mesic savanna system. To this end, I investigated the influence of these factors on soil nutrient availability, total soil nutrients and other edaphic characteristics such as soil texture. Soil nutrient bioavailability was measured using ion exchange resin capsules (RCs). This technique was chosen as it is well-suited to determining available nutrients in regions that experience highly variable or intermittent rainfall (Dobermann et al. 1994) such as is prevalent in southern African savannas. Soil from both the sub-canopy and beyond-canopy of two co-occurring species of tree, one an N_2-fixer, *Acacia tortilis* and another a non-N_2-fixer, *Combretum hereroense,* were sampled over a period of two years (2013-2014). The effect of large mammalian herbivores was investigated by selecting focal trees from both species in two separate sites- an exclosure protected from herbivores and the land adjacent to this which is subject to chronic herbivory. I tested the following hypotheses:

(1) Herbaceous and woody plant biomass will be greater in the site protected from herbivores than on the adjacent land subject to chronic herbivory.

(2) Soil N and P availabilities will be greater sub-canopy than beyond-canopy regardless of N_2-fixation and incidence of herbivory.

(3) Exposure to herbivores will increase the overall availability of nutrients such as N, P and Na.

(4) There will be more bioavailable soil N and less soil P within the canopy of N_2-fixing species compared with non N_2-fixers, and hence the N/P ratios will differ.

(5) Total soil N and P will be greater in soil within the exclosure, (i.e. there will be a negative correlation between total nutrient load and what is bioavailable) where there is greater biomass.

3.3. METHODS AND MATERIALS

3.3.1. Study site description

This research took place within the Greater Marakele National Park (MNP) and more precisely in the Marataba region of Marakele Park (PTY) Ltd, northwards of Marakele proper (**Fig. 3.1**). The study was carried out at two localities within the reserve, a herbivore exclosure as well as the land directly adjacent to it. The Exclosure, with an area of 0.5 ha (24.2992733° E; 27. 4944182° S, 1.07% slope) and adjacent land with an area of 0.8 ha (24.0011795° E; 27. 4942729° S, 1.54% slope) were both found at an altitude of 992 m with east facing slopes. The reserve is found in the south-western corner of the Waterberg region in Limpopo, South Africa. MNP is a relatively recently proclaimed park, with its development only having been initiated in 1986, following which it was named Kransberg National Park in 1994 (Suich et al. 2012). In November 2000 an agreement between SANParks and Marakele Park (PTY) Ltd saw the establishment of the Marakele National Park through the consolidation of land, with plans to continually expand the reserve given adequate resources- currently MNP has an area

of 63 926 ha in total with 15 753 ha making up the Marataba section of the reserve (Van Staden and Bredenkamp 2005; Novellie and Andre 2014).

Greater Marakele National Park is located within the Savanna Biome- the largest in the country. The reserve is composed of three vegetation units, namely, Waterberg-Magaliesburg Summit Sourveld, Western Sandy Bushveld as well as Waterberg Mountain Bushveld (Mucina and Rutherford 2006). This study took place in an area dominated by Western Sandy Bushveld (WSB; all subsequent descriptions of vegetation will be in reference to this vegetation type). This vegetation unit comprises a variable mixture of both fine- and broad-leaved species. Approximately 50% of the measured range of WSB is formally conserved within the Marakele National Park (Van Staden 2002). Consequently, the reserve is an important focal point in the conservation of this vegetation type. The soil is described as being plinthic (i.e. clear concentration of iron oxides in a defined layer), eutrophic and as being freely drained with a high base status (high cation exchange capacity) (Fey 2010). The region receives summer rainfall and experiences particularly dry winters with a MAP ranging from 450 mm in the north to 650 mm in the south (Mucina and Rutherford 2006). Mean annual temperature is high at 19.3 °C with a mean monthly maximum and minimum of 36.0 °C and -3.7 °C, respectively (Schulze 1997).The annual precipitation coefficient of variation is 29% (Mucina and Rutherford 2006).

The Waterberg is an important ecotone in the greater savanna biome and is capable of maintaining a diverse assemblage of both fauna and flora (for a list of re-introduced mammals see Novellie and Andre, 2014; for plant communities see Van Staden and Bredenkamp, 2005). Dominant tree species include members of the genus *Acacia* (*Vachellia*[4]) such as *A. erioloba*, *A. nigrescens*, *A. erubescens*, and *A. nilotica* as well as *A. tortilis* and *Combretums* such as *C. apiculatum*, *C. zeyheri*, and *C. imberbe*. Other common species

[4] For more information on the taxonomic debate with reference to African *Acacia* refer to the following literature for viewpoints from both sides of the argument: Brummitt (2010); Carruthers (2010); Kull and Rangan (2012); Luckow et al. (2005) and Thiele et al. (2011). For the purposes of this research I will refer to the genus as *Acacia*.

include *Lannea discolor, Peltophorum africanum,Combretum hereroense, Grewia bicolor* and *G. flava* (Mucina and Rutherford 2006). MNP has a rich assemblage of large mammalian herbivores and includes species such as Elephant (*Loxodonta africana*), Giraffe (*Giraffa camelopardalis*), Blue Wildebeest (*Connochaetes taurinus*), Burchells Zebra (*Equus quagga burchellii*), African Buffalo (*Syncerus caffer*), Impala (*Aepyceros melampus*), Greater Kudu (*Tragelaphus strepsiceros*), Waterbuck (*Kobus ellipsiprymnus*), Black (*Diceros bicornis*) and White (*Ceratotherium simum*) Rhinoceros, among others. It is important to note that mega-herbivores such as elephant and rhinoceros, as well as insect herbivores such as termites are common in the reserve, although breeding herds of elephant are not common in this site, only large males. These organisms act as ecosystem engineers, due to their ability to directly and indirectly modulate the availability of resources (e.g. nutrients, habitat availability) for other species (Jones et al. 1994a; Jones et al. 1994b; Wright and Jones 2006). Historically the region was managed as farm-land, primarily for cattle grazing, however crops such as watermelon and maize were also grown in certain areas but this practice was abandoned in the early 1980's (Dr A. Uys, *pers. comm.*). Intensive rehabilitation of these land fragments has been ongoing. Despite these prior land uses the majority of this region has historically been unaltered. Fire is managed by means of a '*laissez faire'* approach, with natural lightning induced fires occurring approximately every 2 years (Daphne and Soundy 2006). The fire season occurs in April-June with point ignitions set by management personal early in the fire

season. This is done with the goal of creating a patch mosaic as this maximises biodiversity (Brockett et al. 2001; Daphne and Soundy 2006; Bird et al. 2008).

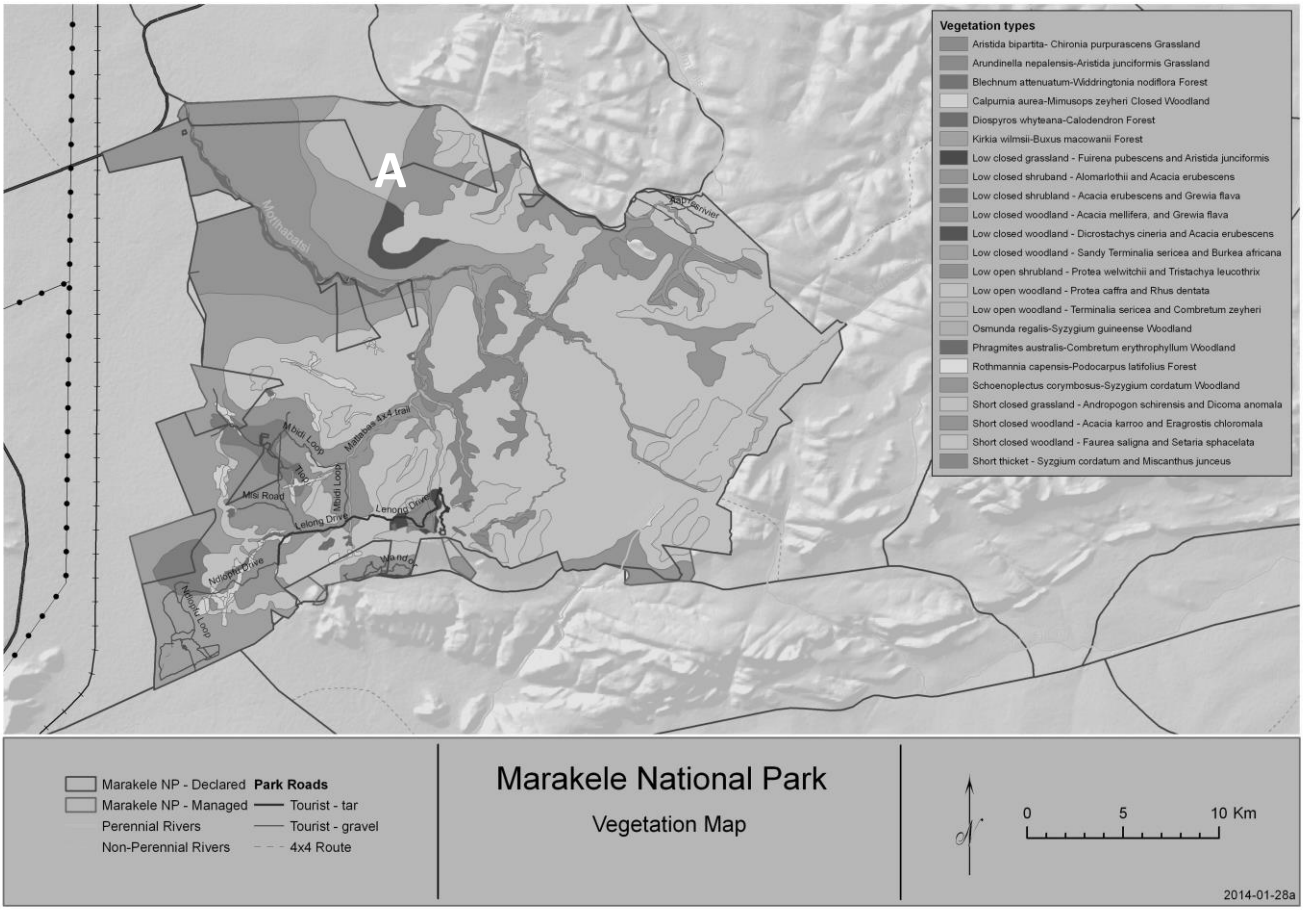


Fig. 3.1: The vegetation types located within Marakele National Park and surrounds. A=location of the research site in the Marataba region (15 753 ha) of the Marakele Park (PTY) Ltd. The study site is located within the northern region, operated by Marakele Park (PTY) Ltd while the southern region constitutes the National Park (source: adapted from the Marakele National Park Management Plan 2014-2024).

3.3.2. Study species

3.3.2.1. *Acacia tortilis*

Acacia tortilis (Forssk.) Hayne subsp. *heteracantha* Burch. common name- Umbrella thorn, Is a deciduous, medium to large tree (may occur as a wiry bush in extremely arid environments) that varies in height from 5 to 20 m (Coates Palgrave et al. 2002). *A. tortilis* occurs in abundance throughout northern and eastern Africa, extending into the Arabian peninsula as well as southward into most of Southern Africa (Ross 1981; Kennenni and Maarel 1990; Dharani 2006). It is widespread in low-altitude, arid habitats (Brenan 1983; Coates Palgrave et al. 2002). Smaller trees often have rounded crowns while larger individuals exhibit the characteristic flat-topped or umbrella-shaped canopy (Smit 1999). The leaves are particularly short (<2 cm) and closely arranged along the stem, forming a dense, leafy canopy (Carr 1976; Coates Palgrave et al. 2002). Each leaf has on average 2-10 pinnae, along with 6-19 pairs of leaflets (0.5-3 mm long x 0.1-1 mm wide) (Wyk and Wyk 2013). Flowers are white to cream or even yellow in colour and occur in round heads. The flowering season falls between November-January. The fruit develop as pods which are twisted and contorted into a characteristic spiral from March-June (Wyk and Wyk 2013) and are usually 5-9 mm wide (Coates Palgrave et al. 2002).

The leaves and pods of this species are highly nutritious and are selectively foraged by many common browsers in savanna grasslands (Fornara and Du Toit 2008; Wyk and Wyk 2013). Leaves and shoots are consumed by numerous antelope while black rhino (Rooke et al. 2004) and elephant forage additionally on the branches (MacGregor and O'Connor 2004). While *A. tortilis* does not actively disperse its seeds, Loth et al. (2005) shows that there is considerable regeneration outside of tree canopies. Germination of seedlings tends to be poor below tree canopies but increases in patches of bare soil or those dominated by grasses (Loth et al. 2005). This species exhibits a high level of palatability in all types of savanna vegetation (Palmer and Pitman 1972; Carr 1976). Some populations of *A. tortilis* increase the amount of resources allocated to certain regions when exposed to simulated herbivory, displaying

greater shoot length, diameter, number of lateral shoots as well as shoot biomass (Rooke et al. 2004). Furthermore Fornara and Du Toit (2008) illustrated that seedlings exposed to chronic herbivory have greater regrowth abilities with twice the number of stems in the crown and double the root diameter at shallow soil depths. Frequent fire and browsing by large herbivores (specifically giraffe) limits the development of mature tree canopy (Pellew 1983), significantly reducing the proportion of tall, mature trees over time (Strauss and Packer 2015). This is compounded by the fact that the growth rate of *A. tortilis* is slow in comparison to other common savanna tree species (Loth et al. 2005).

3.3.2.2. *Combretum hereroense*

Combretum hereroense Schinz (1888), common name Russet Bushwillow, is a small (3-5 m) deciduous or semi-deciduous tree that is abundant in low-altitude dense or sparse bushveld and wooded grassland, often occurring on termitaria (van Wyk and van Wyk 2013). The species is found from Uganda and Kenya, southwards towards Namibia and Botswana, extending into northern South Africa (Carr 1988). *C. hereroense* is capable of tolerating a wide variety of soils and is particularly resilient to a range of soil pH and nutrient levels (Carr 1988). It is usually single-stemmed, but may be multi-stemmed, with a number of defunct stems emerging from the base, which is likely the result of fire (Carr 1988). The leaves occur on short shoots and range from being elliptic to obovate (20-70 x 10-45 mm) with noticeably raised tertiary veins and the underside is densely covered in brown silky hairs (Coates Palgrave et al. 2002). Flowers bloom between September-November and are white, and occur in dense axillary and terminal spikes that can be up to 6 cm long (Carr 1988; Wyk and Wyk 2013). The fruit develop as 4-winged samaras between January-June. These fruit are conspicuous with a dark reddish-brown colour and a size of ~2 x 2 cm (Coates Palgrave et al. 2002). This species is generally highly palatable and is browsed by both cattle and game (Wyk and Wyk 2013). The leaves are highly favoured by kudu, impala, steenbok, elephant and giraffe. The fruiting structure of *C. hereroense* provides a concentrated fuel-load around the seeds inside,

ultimately reducing post-fire germination (Shackleton 2007). *C. hereroense*, however has larger fruit than certain other species and is thus more resilient to fire.

3.3.3. Experimental Design and Approach

This research was conducted in the form of a factorial experiment in the Marataba region of the Marakele Park (PTY) Ltd (from April 2013-December 2014). This research compared the nutritional and physical characteristics of soil beneath and beyond the tree canopy of two tree species belonging to different functional groups. This experiment was carried out within an exclosure from which large mammalian herbivores (>2 kg) have been excluded and the adjacent land that is exposed to chronic herbivory (**Fig. 3.2**). The herbivore exclosure utilised in this research has not been subject to large mammalian herbivory for ~10 years. Two tree

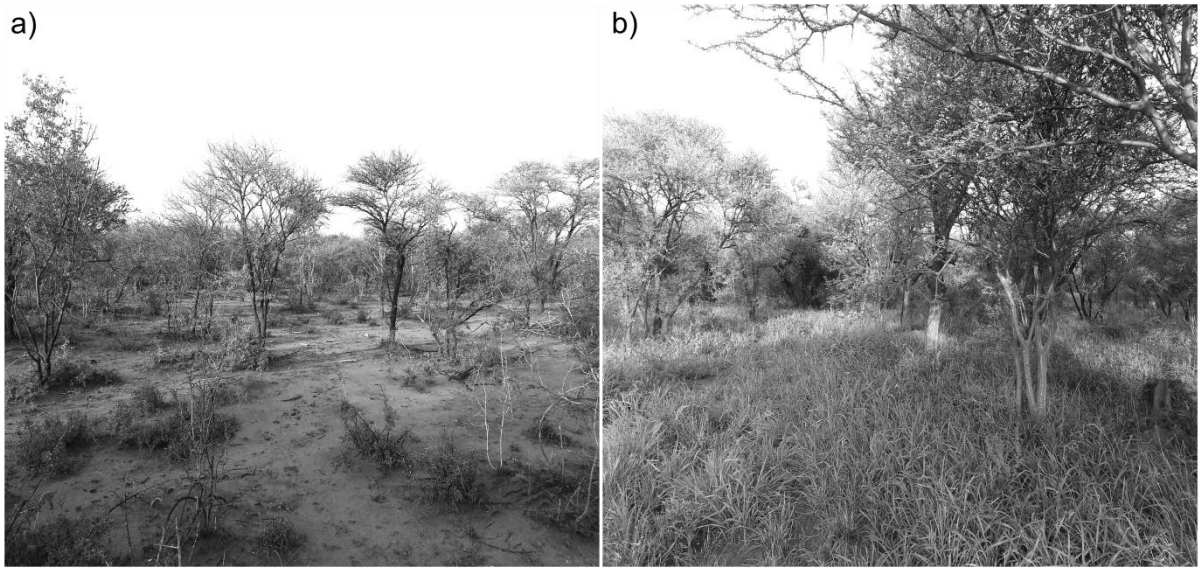


Fig. 3.2: Representative images of the two sites utilised in this research, (a) Land adjacent to the exclosure (exposed to herbivory) and (b) within the exclosure (protected from herbivory). Both of these sites occur in the Marataba region (15 753 ha) of the Marakele Park (PTY) Ltd. Notice the stark contrast in terms of the herbaceous cover. January 2014, ©Wesley Hattingh.

species, abundant both inside and outside the exclosure were used. *Acacia tortilis* (Forssk.) Galasso & Banfi (2008) an N_2-fixer (Cramer et al. 2010) and *Combretum hereroense* Schinz (1888) a non-N_2-fixer. Individuals were selected in October 2013 and marked using steel identification tags to allow for repeated visitation. Only reproductively mature, adult plants were considered for selection. Ten individuals of each species were then chosen, maintaining a

minimum distance (>5 m) from the edge of one trees canopy to the next while avoiding the selection of any individuals on termitaria. GPS coordinates for each individual were also recorded. Leaf and fruit (pods/samaras) samples were collected and the species identifications were confirmed at the C.E. Moss Herbarium situated in the University of the Witwatersrand, Johannesburg, with the aid of the herbarium curator (D. McCallum).

In order to compare the effect of herbivory and N_2-fixation on relative soil nutrient dynamics, ten individuals were selected within the exclosure, and ten outside the exclosure on adjacent land- resulting in 5 *Acacias* and 5 *Combretums* in each treatment. Furthermore, the soil surrounding each tree was divided into two regions, (1) within the canopy (<1.5 m from the stem) and (2) outside the canopy (>2 m from the stem). This was done in order to quantify the spatial distribution of nutrients and determine if proximity to the tree influences nutrient quantities (both available and total). Ion exchange resin capsules (Unibest PST-1; U.S. Patent 5,355,736) were then deployed 5 cm below the soil surface to measure bioavailable soil nutrients (mobile ions) (see 3.3.3.1. for a detailed description) as well as collecting soil samples. The rainfall experienced at the sites was monitored throughout the deployment period as water is essential to allow for the transport of nutrient ions in the soil solution.

The basic design of this study was thus factorial in nature, with three factors: (1) N_2 fixation capacity of species; 2) canopy position; and 3) exposure to herbivory (inside or outside exclosure), each with two levels (**Table 2.1**; **Fig. 3.3**). Comparisons were made both within the exclosure and adjacent land as well as between them (note: the exclosure and adjacent land will henceforth be referred to as sites where appropriate).

Table 3.3*:* The factorial design of this study and the corresponding category descriptors. A total of 20 focal trees (10 *Acacia tortilis* and 10 *Combretum hereroense*) were utilised in this study, with n=10 both within the exclosure and on the adjacent land.

Treatment combinations	N_2 fixation capacity of tree	Canopy position (inside or outside)	Exposure to herbivory (inside or outside exclosure)

	species (yes or no)		
1	Yes	Inside	Exclosure
2	No		
3	Yes	Outside	
4	No		
5	Yes	Inside	Adjacent land
6	No		
7	Yes	Outside	
8	No		

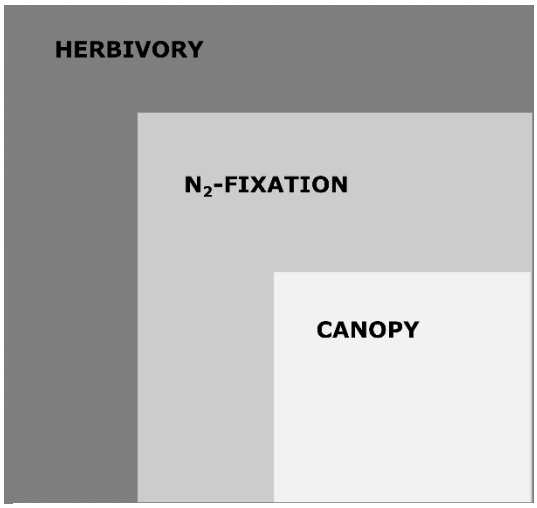


Fig. 3.3: Experimental design indicating the nested nature of the factors investigated-herbivory (exposed to or protected from herbivory), N_2-fixation (N_2-fixer vs. non-N_2-fixer and Canopy (sub-canopy vs. beyond-canopy).

3.3.3.1. *Ion exchange resin capsules*

The relative availability of nutrient ions was quantified using mixed-bed ion exchange resin capsules (RCs, Unibest PST-1; U.S. Patent 5,355,736) as these act as plant root simulators (Binkley and Matson 1983; Dobermann et al. 1994; Skogley and Dobermann 1996). RCs contain an equal mixture (50/50) of cation to anion resin beads (AmberliteIR-150, Rohm &

Haas Company). Ion affinities remain unchanged even as capsules reach saturation levels unless Ca exceeds 500 ppm in which case laboratory testing has indicated desorption of NH_4 albeit only by 3-4 ppm. This resin is then encased within a porous polyester membrane which allows water to pass through and the mixed-bead resins to equilibrate with ions in the soil (Jones et al. 2012). The capsules (diameter of 2 cm) function by acting as a strong sink for mobile ions, thereby undergoing exchange reactions with the soil solution and only accumulating those nutrients which are bioavailable-much the same way in which the roots of a plant would (Yang et al. 1991a; Yang et al. 1991b; Qian et al. 1992). Pampolino and Hatano (2000) showed that P, K and N absorbed by resin capsules were comparable to that of plant roots under conditions of unsaturated soils (arid environments). Savanna systems are fairly arid, making this a suitable technology to use in order to determine the quantity of nutrients that are available to plants. A thorough review of the published literature revealed little prior usage of these capsules in African savanna systems (Cech et al. 2010; Sitters et al. 2013; Blaser et al. 2014), however a number of studies have made use of this technology in north American arid systems (Johnson et al. 2010; Woodward et al. 2013), food crops such as rice (Dobermann et al. 1996; Srivastava et al. 2000; Kavoosi et al. 2003) and onion (Pampolino et al. 2000), in old growth forests (Choromanska and DeLuca 2001; Berglund et al. 2004; MacKenzie and DeLuca 2006; MacKenzie et al. 2006), grasslands (Frank et al. 1994; Giese et al. 2011), free air carbon enrichment experiments- or FACE (Johnson et al. 2004; Iversen et al. 2011; Garten et al. 2011) as well as in the fynbos region of southern African (Witkowski 1987; Witkowski and Mitchell 1987; Witkowski et al. 1990).

RCs provide a simple, time-efficient and easily repeatable method for assessing soil nutrient availability (Skogley et al. 1990; Skogley and Dobermann 1996; Sardi et al. 1996). Traditionally, bioavailable soil nutrients were determined by extraction methods using several chemical solutions (Dobermann et al. 1994). This is time consuming, expensive and provides an inherently static measure which does not account for the kinetics of nutrient release and transport (Dobermann et al. 1994).

3.3.4. Field and laboratory Methods

3.3.4.1. Field methods

Herbaceous and woody plant biomass

Plant height and woody biomass were determined for *A. tortilis* and *C. hereroense,* both for individuals exposed to herbivory (adjacent land) and those protected from it (exclosure). In conjunction to this, herbaceous biomass both within the canopy and outside the canopy of both species was determined using a disc pasture metre (for detailed methodologies see Chapter 2, 2.3.4.1-*Field Methods*).

Soil nutrients

Plant available soil nutrients:

Following the selecting of individual focal trees, the within canopy and outside canopy areas were demarcated (**Fig. 3.4**). This was done by attaching a tape to the base of the tree and then rotating about this point at specified distances from the trunk (within canopy- 1.5 m and outside canopy- >2 m). The relative nutrient availability of primary macro (NO_3-N, NH_4-N, P, K, Ca, Mg and S) and micro soil nutrients (Na, Fe, B, Cu, Zn, Al, and Mn) was determined by placing ion exchange resin capsules (UNIBEST PST-1) *in*-situ during the summer rainfall season of 2013/2014 (October 2013- March 2014). The soil nutrient status relative to each focal tree in the study was analysed by means of resin capsules (RC), with each tree receiving 10 (n=25 per category, N=200). Five capsules were placed within the tree canopy and five outside of the tree canopy (**Fig. 3.4**). Capsule site selections were chosen randomly within each microsite (canopy position) by means of random scattering of capsules in the designated area. A minimum distance of 10 cm was maintained between each implanted capsule and a grass tuft. Each RC was attached to a piece of fishing gut (to facilitate easy removal) and then inserted 5 cm below the soil surface by means of a soil corer. Prior to implantation soil humus and leaf litter debris was brushed aside and then replaced following insertion. This depth was chosen as this constitutes the most biogeochemically active soil horizon. A sharpened stainless steel soil-corer (10 cm x 2.5 cm) was hammered 5cm into the ground and the

resulting soil core removed. The RC was then inserted into this hole and the soil core replaced, packing it tightly to ensure contact between the RC and the soil surface. Painted pebbles (Green-within canopy; Blue-outside canopy) were placed atop the soil directly above the capsule to facilitate retrieval. GPS coordinates for each RC were also recorded.

Capsules were deployed in early October 2013, prior to the onset of the wet season (first rainfall on 20th October) and left to incubate *in-situ* for 145 days (~5 months, i.e. capsules represent cumulative wet season nutrient availability). For the entire wet season. RCs were then retrieved in March 2014. Cumulative rainfall (in mm of water per rainfall event) at the two sites was recorded throughout the incubation period by means of PVC rain gauges (garden variety). Cumulative rainfall over the summer season amounted to 550 mm. The volume of water following each rainfall event was recorded by reserve staff. Upon retrieval of the RCs, any foreign soil particles were carefully rinsed off the membranes surface with deionised water. Cleaned capsules were then packaged into individual, labelled plastic containers and then stored under refrigeration until shipped to UNIBEST for analysis.

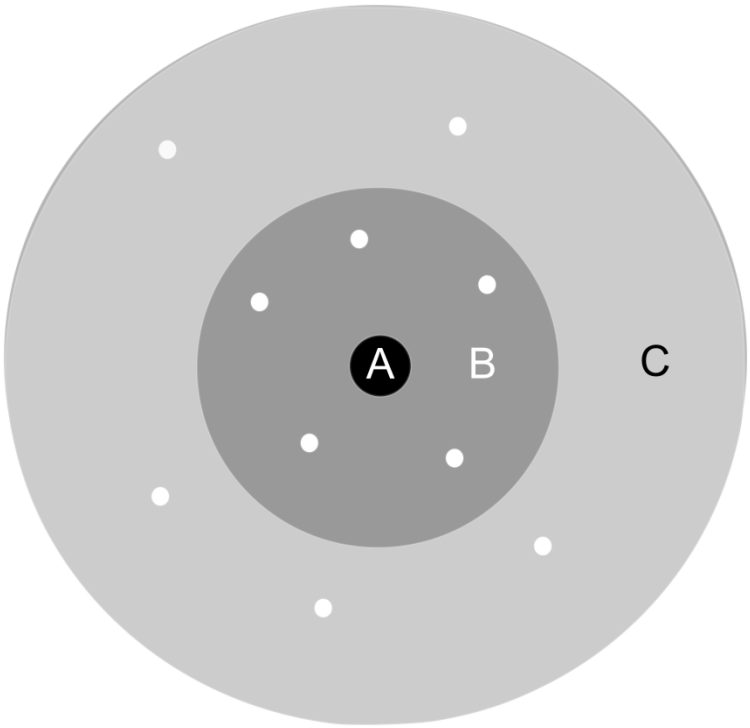


Fig. 3.4: Diagrammatic (birds-eye-view) representation of the experimental set up relative to each focal tree-A. This includes a simplified representation of the within canopy area (from the base up to <1.5 m) of the tree-B, as well as the region outside of the tree canopy-C (>2 m from the stem).

Total soil nutrients and physical properties:

While RCs were utilised to measure plant available nutrients- to obtain a holistic view of nutrient dynamics, total soil nutrients were also quantified. This analysis was restricted to the two primary elements, namely total N and total P. A total of 160 soil cores, for two time periods (80 at each collection period) were collected, namely; prior to the onset of the wet season (in conjunction with RC placement) as well as the end of the wet season (upon retrieval of RCs). A sharpened stainless steel soil-corer (10 cm deep x 2.5 cm diameter) was used to obtain representative soil cores. This steel tube was hammered into the soil surface to a depth of 10 cm. The resultant volume of soil was then placed into plastic Ziploc bags and refrigerated at 4 °C until further processing in the laboratory. Soil samples were collected from locations adjacent to the location of a RC. Two samples from within the canopy and two from outside the canopy were collected for each focal tree at each sampling interval (beginning and end of the wet season). In conjunction with these soil nutrients, soil texture was also determined, as % clay, % silt, as well as % fine, medium and course sand.

3.3.4.2. Laboratory and quantitative methods

Ion exchange resin capsules

Subsequent to field collections, the resin capsules underwent further cleaning and preparation in the laboratory. The RCs were air dried at room temperature prior to being stored in individual containers. These were then packaged and returned to Unibest laboratories (Walla Walla, Washington, USA) for extraction and subsequent quantification of nutrients. Fifteen different nutrients were measured, including “total N” i.e. inorganic N (only nitrates and ammonium and not all N species) nitrate (NO_3-N), ammonium (NH_4-N), labile phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), sodium (Na), iron (Fe), boron (B), copper (Cu), zinc (Zn), aluminium (Al), and manganese (Mn). Although this list is extensive,

special attention will be given to N and P as these are the nutrients which most often limit primary production.

The absorption capacity of each capsule is ~800 *meq* (~400 *meq* cations and ~400 *meq* anions), while the order of cation affinity of each capsule is $Ca^{+2} > Fe^{+2/+3} > Mg^{+2} > K^{+} > Mn^{+2} > NH_4^{+}$ while relative anion affinity is $NO_3^{-} > HSO_4^{-} > NO_2^{-2} > HSO_3^{-2} > SO_4^{-2} > HPO_4^{-2}$ (K.J. Borgman, Unibest Inc, *pers. comm.*). Nutrient extraction was achieved through the addition of hydrochloric acid (K.J. Borgman, Unibest Inc, *pers. comm*). This was done by subjecting each capsule to a 1 ml/minute drip of 50 ml 2M HCl. This ensured that once capsule elution was complete, 98-99% of absorbed nutrients had been removed from the capsule (K.J. Borgman *pers. comm*). Twelve of the 14 nutrients; P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B, Al and Na were analysed by means of inductively coupled plasma mass spectrometry (ICP) while NO_3-N and NH_4-N were analysed by making use of a flow injection machine (flow injection analysis-FIA) and quantities determined by colour metrics. Data are presented in parts per million of the element in the extracted solution (i.e. per capsule).

Soil sample processing

Soil samples collected in the field were oven-dried at 50 °C for 48 hours. Like samples were then pooled by focal tree and canopy position for both sampling periods (i.e. four samples of equal volume were pooled to create one composite sample). This resulted in a sample size of 40 with n=5 per treatment combination (**Table 3.1**). Soil was then pulverised in a mortar and pestle, following which each sample was sieved through a #10 mesh (2.00-mm throughput), USA standard testing sieve to result in homogeneity. Equipment was thoroughly cleaned between each sample to avoid cross contamination. Each sieved sample was then packaged in brown paper bags and couriered to Bemlab (Somerset West, Western Cape, SA) for further analysis of N, P and soil texture.

Soil sample analyses

Nutrients and soil texture (Bemlab)

Total N was determined by combustion analysis in a LECO analytical element analyser (LECO-Nitrogen/protein). Data output for total N was in the form of % nitrogen per soil sample. Total phosphorus present in the sampled soil was measured by means of digestion (Bray and Kurtz 1944) and subsequent elemental quantification in an inductively coupled plasma mass spectrometer (ICP). Data for soil phosphorus content was presented as mg of phosphorus per kg of soil (mg kg^{-1}).

Prepared soil (sieved) was analysed for texture using the particle size/hydrometer analysis method (Bouyoucos 1962; Mckenzie et al. 2002). Prepared soil was placed into a measuring cylinder along with sodium hexametaphosphate- this acts as a deflocculant. This soil solution was then measured at specific time intervals in order to determine % clay, sand and silt according to the methodology laid out in Bouyoucos (1962).

3.3.5. Quantitative analysis

To address the first hypothesis basic descriptive statistics (mean, median etc.) were calculated for the aboveground biomass per treatment combination (**Table 3.1**). Differences among treatments were assessed by means of non-parametric Kruskal-Wallis tests following failure of the data to meet the necessary prerequisites of normality. The normality of all response variables in this research was determined by using a Shapiro-Wilks test and/or a one-way Kolmogorov-Smirnov test. If data were not normal, a number of transformations were attempted, however, non-normal transformed data resulted in the selection of non-parametric alternatives in all cases (Krzywinski and Altman 2014a). Differences in the mean woody biomass (whole tree and leaf) between sites was assessed using *T*-tests. Herbaceous biomass was assessed using non-parametric Kruskal-Wallis tests followed by Kruskal Wallis multiple comparisons tests in the R package “pgirmess” (Giraudoux 2015).

The second, third, fourth and fifth hypotheses were addressed by using a number of 3-way analyses of variance (ANOVA) followed by multiple comparisons tests, Tukey HSD

(honest significant difference). These 3-way ANOVAs were used to describe any differences among treatment combinations and the interactions between individual factors. Subsequently Linear Discriminant Function Analysis (L-DFA) was used to predict group membership (I.e. treatment combination) (Manly 2010) given the range of bioavailable nutrients measured. Basic descriptive statistics including means, medians, standard deviations and coefficients of variation (CVs) were also calculated for each treatment combination. To determine if there were significant differences in soil nutrient availability among the eight treatment combinations, multiple 3-way-ANOVAs were performed, one for each nutrient under investigation. The influence of all explanatory variables (site, canopy position and N_2-fixation capacity) on measured response variables was considered independently and also in terms of all possible interactions. For the L-DFA, data were prepared by standardizing (the difference between a value and the group mean/the standard deviation) each value of the response variable and then calculating a new Z score. Following this the data were then processed using a multivariate analysis of variance (MANOVA) to determine the equality of means and variances (Wilks Lambda, Pillai's trace and P-values) among all treatment combinations (i.e. groups) and the measures of nutrient availabilities as determined by the resin capsules. The data were then run through the L-DFA to determine the standardized canonical discriminant function coefficients which illustrate which predictor variables exhibit the highest factor loadings. Higher loadings represent a greater capability of a variable being able to predict group membership. The accuracy of the model in predicting group membership was assessed by comparing actual measurements to predicted values for each group. The higher the percentage accuracy the greater the hit rate or prediction capability of the model.

I then assessed the sixth hypothesis by performing non-parametric Kruskal-Wallis tests to determine any differences among the treatments. These tests were chosen based not on the normality of the response variables but rather due to small sample sizes ($n<5$ per treatment combination) (Gibbons and Chakraborti 2011; Weissgerber et al. 2015).

All analyses were carried out using the statistical computing software, R (R Development Core Team 2015). The majority of graphics were created using a combination of the open-source package “ggplot2” contributed by Wickham and Chang (2012), “BoxplotR” by Spitzer et al. (2014) and “plotrix” by Lemon et al. (2015). Box plots were chosen as this allows the reader to evaluate the distribution of the entire continuous dataset in order to adequately assess my conclusions (Streit and Gehlenborg 2014; Krzywinski and Altman 2014b; Weissgerber et al. 2015). L-DFA was carried out with functions provided in the R package “MASS” (Ripley et al. 2015).

3.4. RESULTS

3.4.1. The effect of herbivory on standing stocks

Herbaceous biomass within the exclosure was on average 90% (*Acacia)* and 95% (*Combretum*) higher than for conspecifics exposed to herbivory on adjacent land. Aboveground herbaceous biomass was significantly different (Kruskal-Wallis test: $H_{(7,\ 152)}$ =96.231, $P< 0.001$) between the exclosure, protected from herbivores and the adjacent land, which was exposed to chronic herbivory (**Fig. 3.5**). It did not differ significantly between canopy positions (sub canopy and beyond canopy) nor between species. While herbaceous biomass was significantly different inside and outside of the exclosure, the total aboveground woody biomass of focal trees did not differ significantly (*t*- test, d.f. (8), t=1.56, P=0.085 and *t*-test, d.f. (8), t=0.70, P=0.252 for *Acacia* and *Combretum,* respectively), nor did the leaf biomass per tree (**Fig. 3.6**).

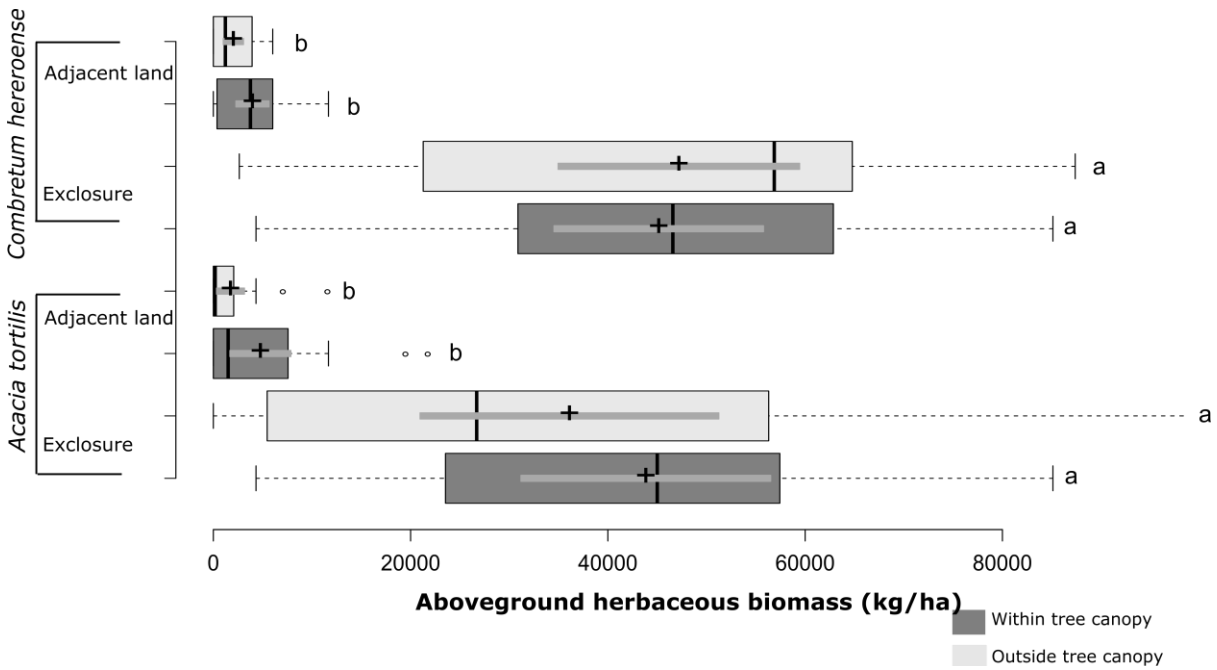


Fig. 3.5: A comparison of the aboveground herbaceous biomass using a disc pasture meter (kg/ha) for each individual of *Combretum hereroense* and *Acacia tortilis*, both within the tree canopy and outside the tree canopy. *Centre lines* show the medians; *box limits* indicate the 25th and 75th percentiles as determined by R software; *whiskers* extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by *dots; crosses* represent sample means and bars indicate 95% confidence intervals of the means. N= 20 samples per category. *Letters* denote significant differences (Post hoc, multiple comparisons tests after a Kruskal-Wallis test: $H_{(7,\ 152)}$ =96.231, P< 0.001).

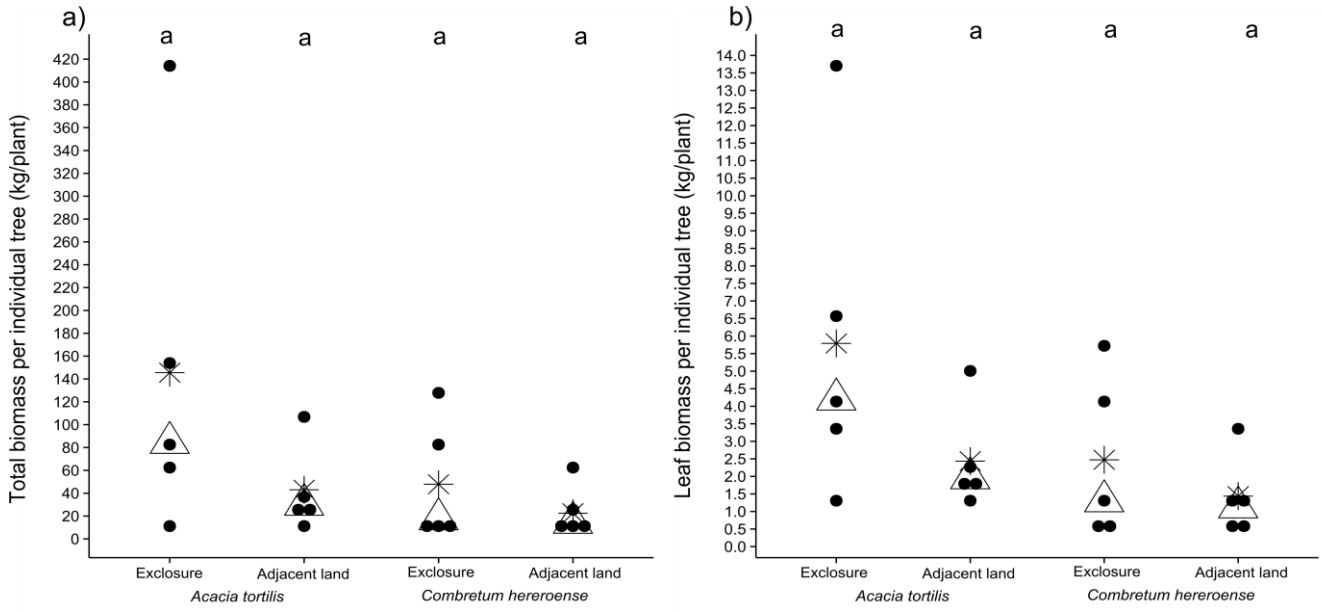


Fig. 3.6*:* A comparison of the (a) total aboveground biomass (kg tree^{-1}) and (b) leaf biomass (kg tree^{-1}) of two species of savanna trees, *Acacia tortilis* and *Combretum hereroense*. *Circles* represent each data point; *Triangles* show the median values and *stars* represent mean values. *Letters* denote significant differences (*t*-test,df (8)=1.56, P=0.085 and *t*-test, df (8)=0.70, P=0.252, for *A. tortilis and C. hereroense*, respectively).

The height of *A. tortilis* individuals was significantly different inside and outside the exclosure, with trees inside on average 1.45 ± 0.4 m taller (*t*-test, d.f.(8), *t*=2.13, *P*=0.038) (**Table 3.2**). *C. hereroense* individuals tended to be taller in the exclosure (by 0.62 ± 0.35 m) albeit not significantly so. The basal area of trees inside and outside the exclosure did not differ significantly, although those in the exclosure tended to exhibit larger basal areas (213 ± 129 cm^2 and 84 ± 67 cm^2 for *Acacia* and *Combretum* respectively).

Table 3.2: A suite of general plant traits, including tree height, basal area, total biomass, leaf biomass (mean ± SE) as well as the number of multi-stemmed individuals of *Acacia tortilis* and *Combretum hereroense* inside the exclosure and on adjacent land. Significance determined by *t*-tests with d.f. =8 throughout.

	Exclosure	Adjacent land	*t*	*P*
Tree height (m)				
Acacia tortilis	6.04 ± 0.60	4.59 ± 0.30	2.13	0.038*
Combretum hereroense	5.61 ± 0.45	1.99 ± 0.25	1.14	0.147
Basal area (cm^2)				
Acacia tortilis	434 ± 183	221 ± 74	1.13	0.149
Combretum hereroense	187 ± 87	103 ± 47	0.62	0.278
Total biomass (kg $tree^{-1}$)				
Acacia tortilis	146 ± 71	43 ± 16	1.56	0.085
Combretum hereroense	48 ± 24	23 ± 12	0.70	0.252
Leaf Biomass (kg leaves $tree^{-1}$)				
Acacia tortilis	5.8 ± 2	2.4 ± 0.6	1.49	0.089
Combretum hereroense	2.5 ± 1	1.4 ± 0.6	0.66	0.263
Number of mutli-stemmed trees				
Acacia tortilis	3	5	-	-
Combretum hereroense	1	1	-	-

Significant values: *=P<0.05, **=P<0.001

3.4.2. Bioavailable soil nutrients

Nutrient accumulation in resin capsules was 9-158% higher inside of the exclosure compared to outside (Zn>P>Na>K>NO_3-N>Cu>B>S>Mg>inorganic N) (**Table 3.3**)- Inorganic N (34.12 ppm ± 3.6 vs. 31.12 ppm ± 7.0), nitrates (28.48 ppm ± 3.47 vs. 16.76 ppm ± 2.39), boron (0.030 ppm ± 0.0009 vs. 0.023 ppm ± 0.0008), sulphur (11.99 ppm ± 1.24 vs. 9.22 ppm

± 0.81), zinc (0.111 ppm ± 0.06 vs. 0.013 ppm ± 0.0008), magnesium (18.62 ppm ± 0.88 vs. 16.87 ppm ± 1.44), potassium (113.45 ppm ± 7.62 vs. 62.07 ppm ± 6.11), sodium (8.87 ppm ± 0.87 vs. 3.64 ppm ± 0.45), copper (0.032 ppm ± 0.004 vs. 0.021 ppm ± 0.002) and phosphorus (2.107 ppm ± 0.58 vs. 0.349 ppm ± 0.09). None of the capsules became fully saturated during the time they were left *in situ*. Bioavailable soil nutrient availability was far less variable beneath the canopy of *C. hereroense* than beneath *A. tortilis* for both macronutrients (**Fig. 3.7 and 3.8**) and micronutrients (**Fig. 3.9 and 3.10**). The only nutrient that did differ significantly among any of the treatment combinations nor between species, site or canopy position was ammonium (NH_4) (**Table 3.3**). 67% (10 of 15) of resin capsule measured available nutrients were found to differ significantly based on site location.

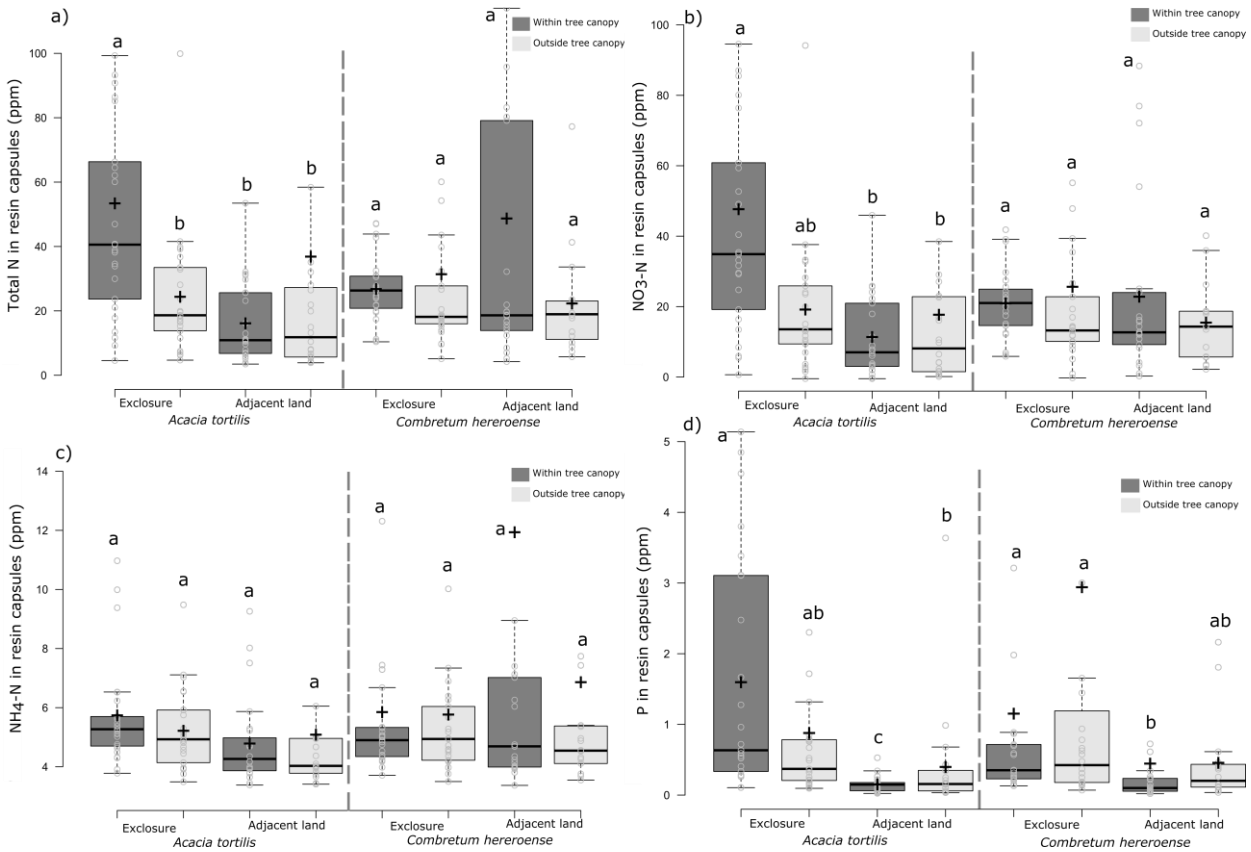


Fig. 3.7: Bioavailable soil macronutrients in relation to protection from (exclosure) and exposure to (adjacent land) large mammalian herbivores for two African savanna tree species, the non-N_2-fixing *Combretum hereroense* and the N_2-fixing *Acacia tortilis*. Macronutrients include available; (a) Total nitrogen (i.e. inorganic N), (b) nitrate-nitrogen (NO_3-N), (c) ammonium-nitrogen (NH_4-N) and (d) phosphorus (P). Centre lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots and black crosses represent sample means. Data points are plotted as open circles. n=25 sample points per category unless all capsules could not be retrieved. Letters denote significant differences (Tukey HSD, multiple comparisons tests after a 3-way-ANOVA).

In contrast only four of the measured nutrients showed any significant differences depending on whether the resin capsules were placed relative to an *Acacia* or *Combretum* individual. These nutrients were magnesium, aluminium, boron and iron (**Table 3.3**). Resin capsules associated with *Combretum* trees all absorbed a greater quantity of nutrients than those associated with *Acacias* (**Fig. 3.9**)- magnesium (19.96 ppm ± 1.32 vs. 15.86 ppm ± 0.99), aluminium (2.29 ppm ± 0.23 vs. 1.67 ppm ± 0.13), boron (0.028 ppm ± 0.0009 vs. 0.025 ppm ± 0.0009) and iron (0.87 ppm ± 0.07 vs. 0.67 ppm ± 0.052).

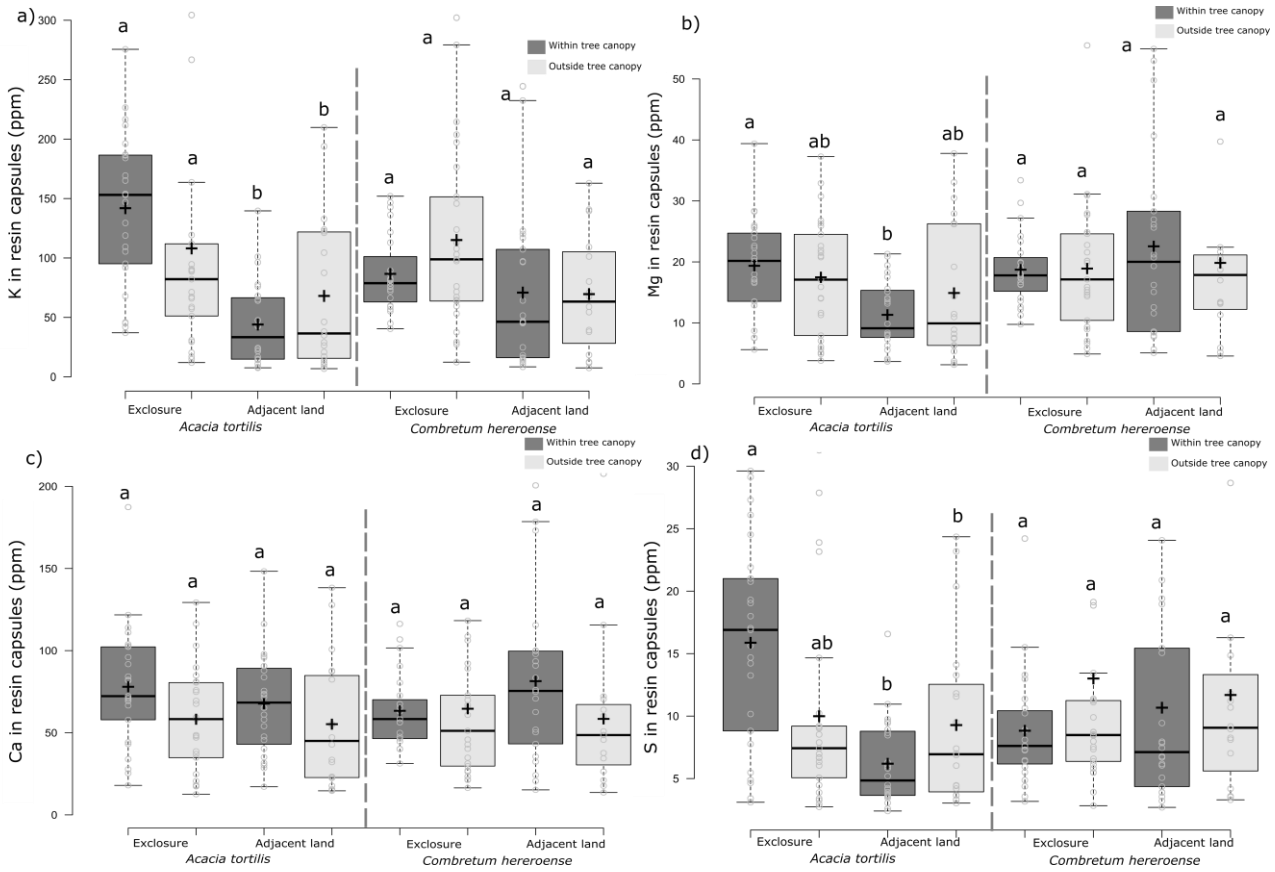


Fig. 3.8*:* Bioavailable soil macronutrients in relation to protection from (exclosure) and exposure to (adjacent land) large mammalian herbivores for two African savanna tree species, the non-N_2-fixing *Combretum hereroense* and the N_2-fixing *Acacia tortilis*. Macronutrients include available; (a) potassium (K) (b) magnesium (Mg), (c) calcium (Ca) and (d) Sulphur (S). Centre lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots and black crosses represent sample means. Data points are plotted as open circles. n=25 sample points per category unless all capsules could not be retrieved. Letters denote significant differences (Tukey HSD, multiple comparisons tests after a 3-way-ANOVA).

A similar pattern was observed upon investigation of canopy position on nutrient bioavailability. Calcium, aluminium, boron, iron, inorganic N and nitrate-N were found to be

significantly different between sub canopy and beyond canopy regions (**Table 3.3**). The bioavailability was greater within the canopy region for all nutrients showing significant differences- calcium (72.58 ppm ± 3.8 vs. 59.42 ppm ± 4.7), aluminium (2.11 ppm ± 0.17 vs. 1.83 ppm ± 0.20), boron (0.03 ppm ± 0.0009 vs. 0.02 ppm ± 0.0007) , iron (0.85 ppm ± 0.07 vs. 0.67 ppm ± 0.05) and NO_3 (26.02 ppm ± 3.11 vs. 20.00 ppm ± 3.15) .

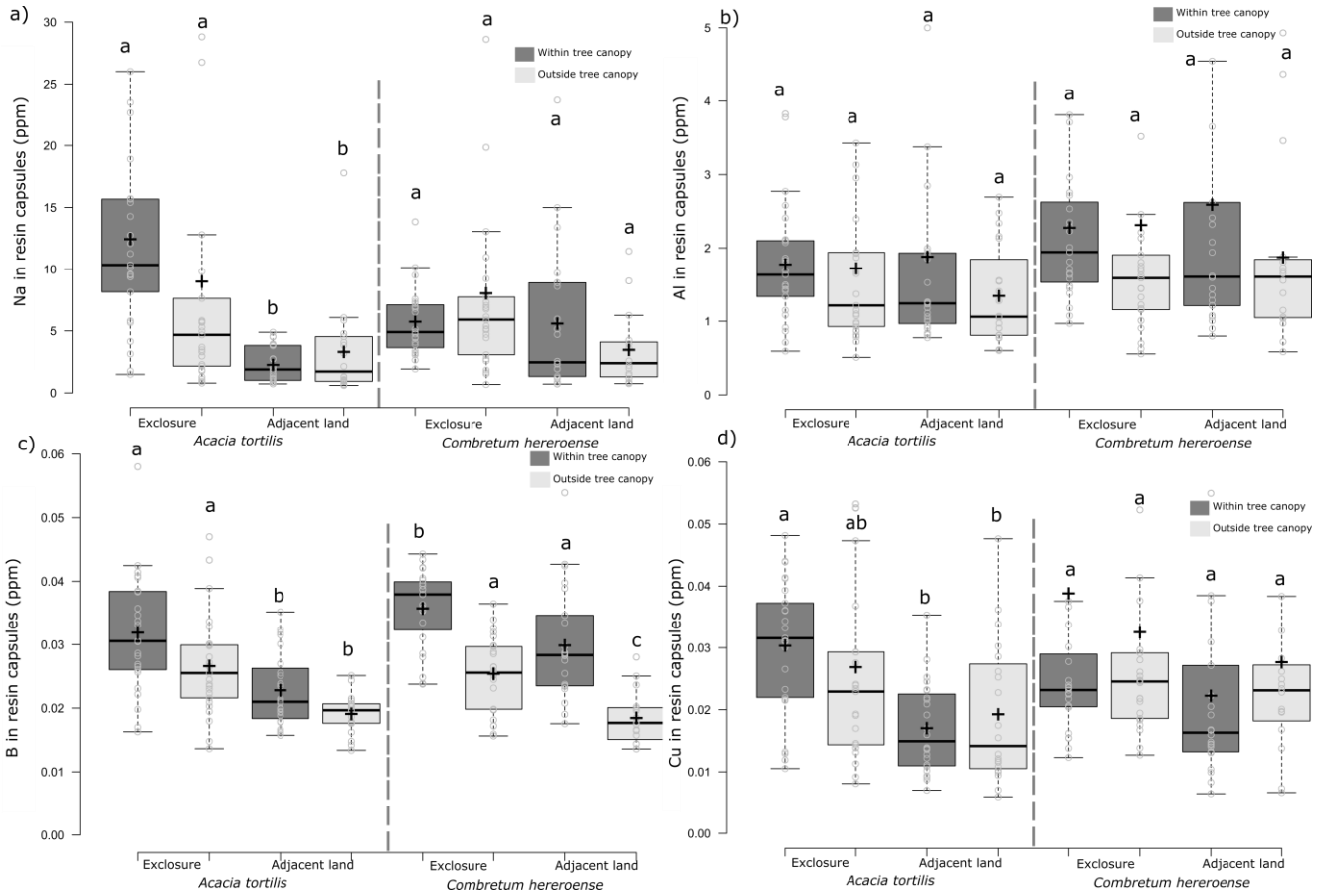


Fig. 3.9*:* Bioavailable soil micronutrients in relation to protection from (exclosure) and exposure to (adjacent land) large mammalian herbivores for two African savanna tree species, the non-N_2-fixing *Combretum hereroense* and the N_2-fixing *Acacia tortilis*. Micronutrients include available; (a) sodium (Na) (b) aluminium (Al), (c) Boron (B) and (d) copper (Cu). Centre lines *show the* medians; *box limits* indicate the 25th and 75th percentiles as determined by R software*; whiskers* extend 1.5 times the interquartile range from the 25th and 75th percentiles, *outliers* are represented by dots and black crosses represent sample means. Data points are plotted as open circles. n=25 sample points per category unless all capsules could not be retrieved. *Letters* denote significant differences (Tukey HSD, multiple comparisons tests after a 3-way-ANOVA).

There were significant 3-way interactions (**Table 3.3**) between site, canopy position and species for inorganic N, Sulphur and Sodium. Significant two way interactions for canopy position: site were found for sulphur (ANOVA $F_{(1, 173)}$=4.79, *P*=0.030). Only boron was found to have a significant interaction for canopy position: tree species (ANOVA $F_{(1, 173)}$=9.426,

P=0.0024). Finally a host of elements were found to have a significant two way interaction between the site: tree species, including inorganic N (ANOVA $F_{(1, 173)}$=6.87, *P*=0.010), nitrate-nitrogen (ANOVA $F_{(1, 173)}$=6.096, *P*=0.015), magnesium (ANOVA $F_{(1, 173)}$=4.95, *P*=0.027), sulphur (ANOVA $F_{(1, 173)}$=7.24, *P*=0.008) and sodium (ANOVA $F_{(1, 173)}$=6.503, *P*=0.012).

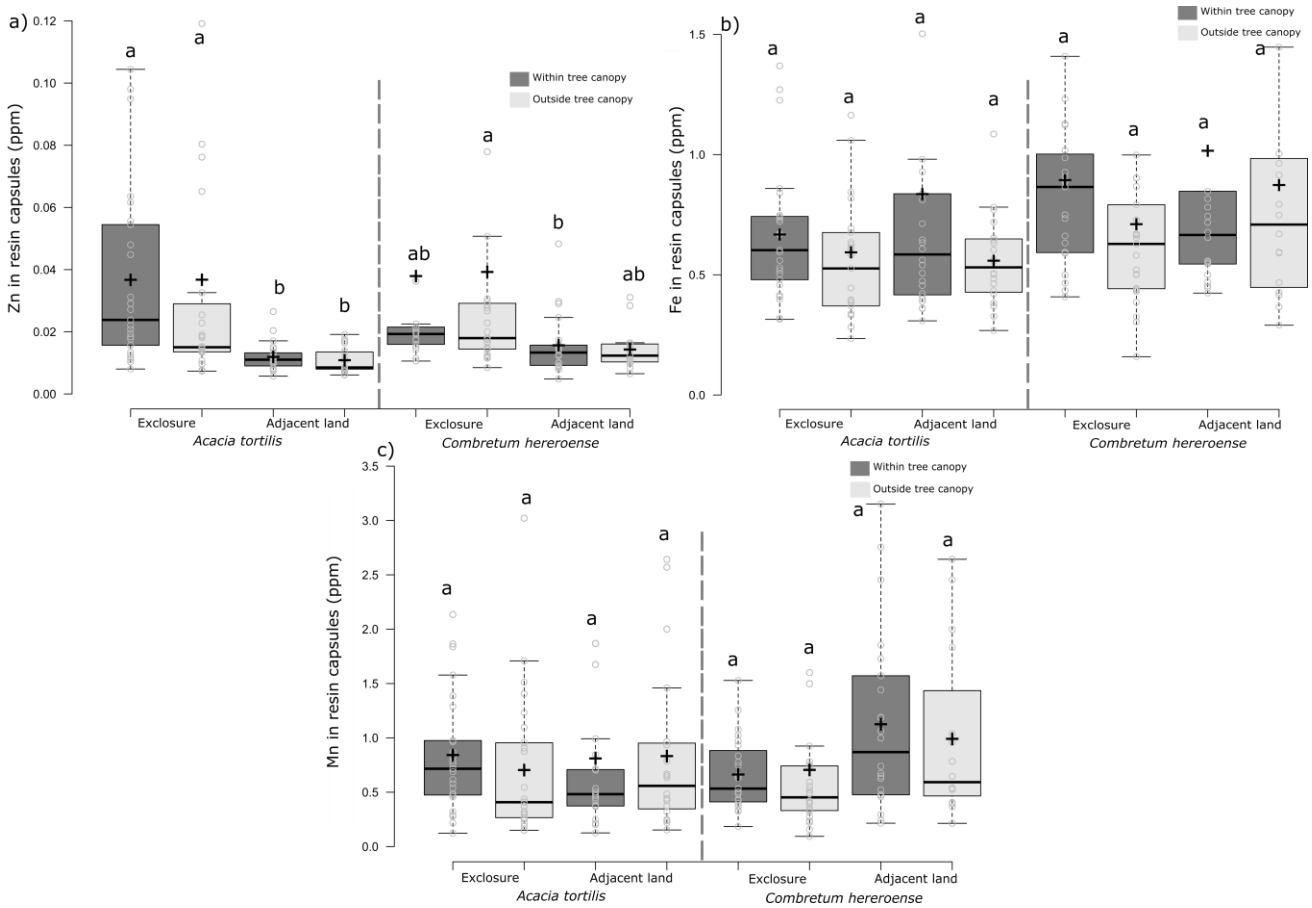


Fig. 3.10*:* Bioavailable soil micronutrients in relation to protection from (exclosure) and exposure to (adjacent land) large mammalian herbivores for two African savanna tree species, the non-N_2-fixing *Combretum hereroense* and the N_2-fixing *Acacia tortilis*. Micronutrients include available; (a) zinc (Zn) (b) Iron (Fe) and (c) Manganese (Mn). Centre lines *show the* medians; *box limits* indicate the 25th and 75th percentiles as determined by R software*; whiskers* extend 1.5 times the interquartile range from the 25th and 75th percentiles, *outliers* are represented by dots and black crosses represent sample means. Data points are plotted as open circles. n=25 sample points per category unless all capsules could not be retrieved. *Letters* denote significant differences (Tukey HSD, multiple comparisons tests after a 3-way-ANOVA).

Table 3.3: Statistical values for partial three-way-ANOVA models of soil nutrient availability as measured by resin capsules. Factors presented include; Canopy position (sub- and outside canopy), Site (exclosure and adjacent land), tree species (N_2-fixing *A. tortilis* or non-N_2-fixing *C. hereroense*) and the interaction between these three factors. d.f.=$_{1,173}$, n=181 throughout.

	Canopy Position		Site		Tree species		Interaction	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Inorganic N	4.50	0.036*	12.40	*P*<0.001**	1.30	0.262	4.20	0.042*
Ammonium (NH_4)	0.30	0.584	0.46	0.494	1.89	0.170	2.81	0.096

Nitrates (NO_3)	4.30	0.040*	17.26	P<0.001**	1.64	0.201	0.53	0.467
Sulphur	0.01	0.908	6.67	0.011*	0.28	0.598	4.76	0.030*
Zinc	0.20	0.658	41.53	P<0.001**	1.66	0.200	0.05	0.823
Calcium	10.51	0.001*	0.44	0.509	0.03	0.861	0.32	0.571
Magnesium	0.83	0.363	6.28	0.013*	7.60	0.007*	0.85	0.359
Aluminium	4.70	0.031*	0.90	0.353	6.90	0.01*	0.83	0.835
Boron	55.85	P<0.001**	57.13	P<0.001**	5.27	0.022*	0.75	0.387
Iron	6.41	0.012*	0.62	0.431	8.88	0.003*	0.60	0.441
Manganese	0.58	0.448	1.40	0.238	1.97	0.162	1.19	0.278
Potassium	0.11	0.739	40.00	P<0.001**	0.37	0.546	2.51	0.115
Sodium	2.92	0.088	52.40	P<0.001**	0.23	0.633	5.40	0.021*
Copper	0.00	0.946	19.64	P<0.001**	2.35	0.127	0.08	0.773
Phosphorus	0.21	0.644	56.27	P<0.001**	0.39	0.531	0.84	0.360

*=*P*<0.05 **=*P*<0.001

3.4.3. Predicting group membership

***Table 3.4*:** Linear Discriminant Function Analysis (L-DFA) aimed to predict group (treatment combinations, see Table 3.1) membership based on bioavailable soil macro and micronutrients. Fourteen predictor variables were used in the L-DFA model. The equality and significance of group means is presented by MANOVA associated P- and F-values. Standardised discriminant function coefficients provide an indication of which predictor variables have the greatest prediction capability of group membership for each component of the L-DFA model (bolded and weighted by highest number, followed by the next and so on-significance was taken into account and the factor loading ignored if non-significant).

Predictor variable (ppm)	F-value	P-value	*Standardised discriminant function coefficient*
Iron	2.43	<0.001	**-3.000**
Boron	17.50	<0.001	**2.200**
Potassium	7.05	<0.001	**1.100**
Sulphur	3.59	<0.001	**-1.100**
Sodium	9.76	<0.001	**0.750**
Aluminium	1.85	0.080	3.260
Calcium	1.75	0.101	-2.700
Manganese	1.15	0.333	-0.720
Magnesium	3.06	0.005	0.680
Copper	3.76	<0.001	-0.550
Phosphorus	3.59	<0.001	-0.500
Zinc	6.33	<0.001	0.370
Nitrate-nitrogen	4.57	<0.001	-0.005
Ammonium-nitrogen	1.23	0.290	0.004

The linear discriminant function analysis was carried out in order to determine if the availabilities of certain nutrients could predict group membership. Groups in this case referring to the treatment combinations. The predictor variables were able to discriminate between the different groups at a statistically significant level (Wilks Lambda=0.052, $F_{(7,173)}$ =6.17; *P*<0.001, Pillai's trace=2.001; $F_{(7,173)}$ =4.72; *P*<0.001).

The individual contributions of the predictor variables to group membership vary from those that lean to group membership and those that have little effect (**Table 3.4**). However, despite being statistically significant (P<0.05) the factor loadings are what reveal actual relationships. The discriminant function model indicated that the strongest bioavailable nutrient predicting group discrimination was Iron (standardized discriminant function coefficient (SDFC= -3.00), followed by Boron (SDFC=2.200), Potassium (SDFC=1.100) and sulphur (SDFC=-1.100). Other bioavailable nutrients which have a bearing on group membership (SDFC>0.5) in decreasing order of effect, include sodium, magnesium, aluminium, copper and phosphorus (**Table 3.4**). Absolute values are considered when examining SDFC's with the signs simply suggesting the way in which these factor loadings interact with one another. Classification of the results provided a fair model accuracy rate in predicting group membership (66.1%) (**Fig. 3.11**) (Quinn and Keough, 2002).

Following the primary L-DFA model created to try predict group membership across the eight treatment combinations, I created another three models assessing the capability of bioavailable nutrients in the prediction of membership to; (1) herbivore present (adjacent land) or herbivore absent (exclosure) sites, (2) species association (N_2-fixing *Acacia*, or non-N_2-fixing *Combretum*) and (3) canopy position (sub canopy or beyond canopy). All three models yielded greater accuracy rates in their prediction of group membership than non-nested groups (i.e. treatment combinations) with accuracy scores of 75.6%, 97.2% and 80% for group membership to species, site, and canopy position, respectively. The highest factor loadings and therefore greatest SDFC's in predicting actual group membership to either species association were magnesium (4.83), followed by aluminium (1.33), manganese (-0.96) and

finally boron (0.84). The model that illustrated the greatest prediction accuracy was that in attempting to predict group membership to either herbivore occupied or herbivore absent sites. This suggests that herbivore absence or presence has a marked impact on nutrient availabilities. Predictor variables best capable of highlighting site discrimination include boron (1.88), followed by potassium (1.35), sulphur (-1.16) and finally sodium (0.77). Again neither of the nitrogen based nutrients featured. Finally the L-DFA model discriminating membership to either within canopy or beyond canopy regions was most strongly affected by iron (2.5), aluminium (-2.3), boron (2.3) and finally magnesium (-1.83).

3.4.4. Total soil nutrients and texture

In addition to available soil nutrients, total soil N and P were also measured. Total soil N was greater in soil samples taken from the sub-canopy region of both *Acacia* (mean=0.080% ± 0.013 vs. 0.039% ± 0.010) and *Combretum* (mean=0.043% ± 0.007 vs. 0.036% ± 0.007) individuals albeit not at a statistically significant level when explicitly considering the variation among treatment combinations (**Fig. 3.12**). However, inorganic N was greater within the canopy than beyond the canopy when samples were pooled based solely on this factor, ignoring species and site effects (Kruskal-Wallis test: $H_{(1, 38)}$ =17.64, P< 0.001). Total soil N did not differ significantly between species or across sites (Kruskal-Wallis test: $H_{(1, 38)}$ =0.281, P= 0.569 and Kruskal-Wallis test: $H_{(1, 38)}$ =0.125, P= 0.723, respectively).

Total soil P did not differ significantly among treatment combinations (**Fig. 3.12**), although when pooled soil P was found to be significantly greater (46%) in soil protected from the influences of large mammalian herbivory with values of 134 mg/kg ± 5.5 in the exclosure versus 80 mg/kg ± 2.2 in soil on the adjacent land exposed to herbivory (Kruskal-Wallis test: $H_{(1, 38)}$ =25.861, P<0.001). There was however no difference in total soil P between canopy positions nor in relation to the species of tree from below which the soil was collected (Kruskal-Wallis test: $H_{(1, 38)}$ =2.72, P=0.099 and Kruskal-Wallis test: $H_{(1, 38)}$ =0.036, P=0.850, for canopy position and species respectively).

The percentage of both clay and coarse sand were found to differ significantly between the exclosure and the adjacent land, with a higher clay component (~10%) and lower coarse sand component (~15%) in the site protected from herbivory (**Fig. 3.13**) (Kruskal-Wallis test: $H_{(1, 38)}$ =5.80, P=0.016 and Kruskal-Wallis test: $H_{(1, 38)}$ =15.077, P<0.001 for clay and coarse respectively). Furthermore the percentage of medium sand in soil cores was significantly different depending on where the sample was taken from in relation to the two species

(Kruskal-Wallis test: $H_{(1, 38)}$ =5.30, P=0.021).

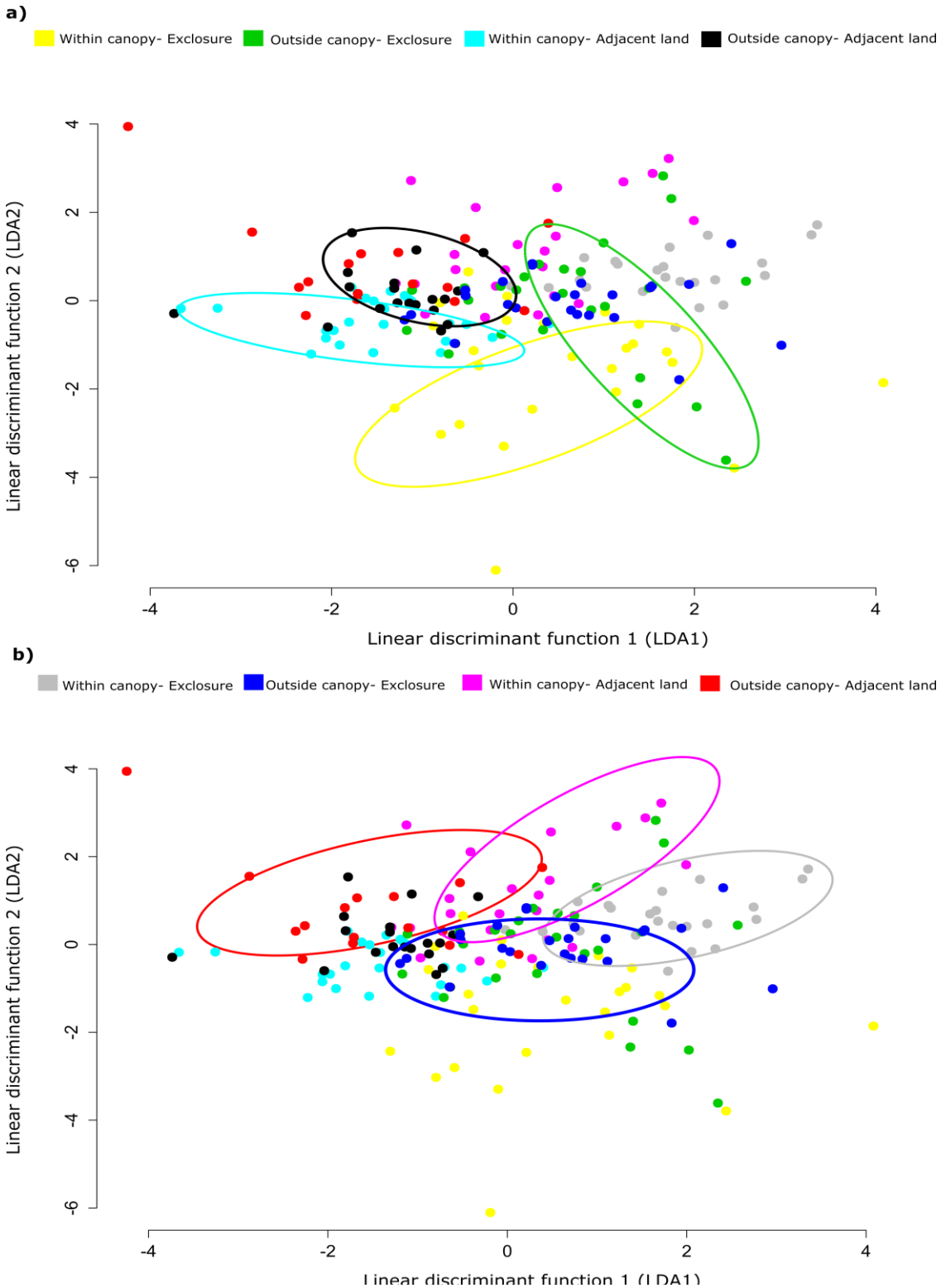


Fig. 3.11*:* Linear discriminant function analysis (L-DFA) was used to predict group membership (eight treatment combinations) based on a number of continuous response variables (bioavailable soil nutrients as measured by resin capsules). Data shown represent the two discriminant functions (of 7; K-1) or axes that together account for the majority of the predictive capabilities of the L-DFA model (L-DFA1=57.3% and L-DFA2=19%). (a) L-DFA1 and L-DFA2 illustrate the capability of the model in predicting group membership in reference to *Acacia tortilis* and (b) in reference to *Combretum hereroense*. Coloured points and circles correspond to the different groups.

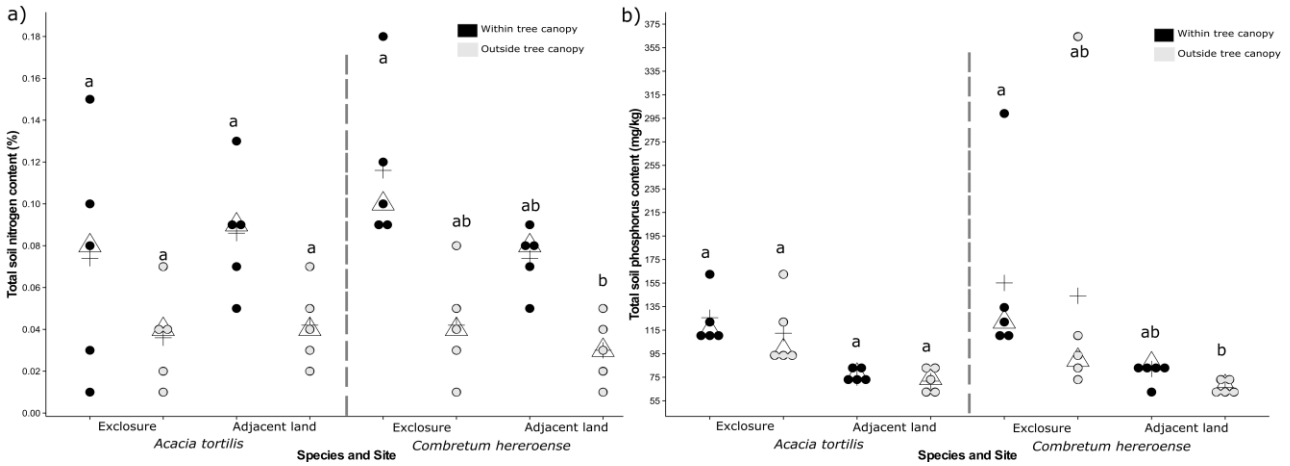


Fig. 3.12: A comparison of the (a) total soil nitrogen content (%) and (b) total soil phosphorus content (mg/kg) both below the tree canopy and beyond the tree canopy of two savanna trees, *Acacia tortilis* and *Combretum hereroense*. *Circles* represent each data point; *Triangles* show the median values and *crosses* represent mean values. *Letters* denote significant differences (Kruskal-Wallis multiple comparisons Post hoc tests following Kruskal-Wallis).

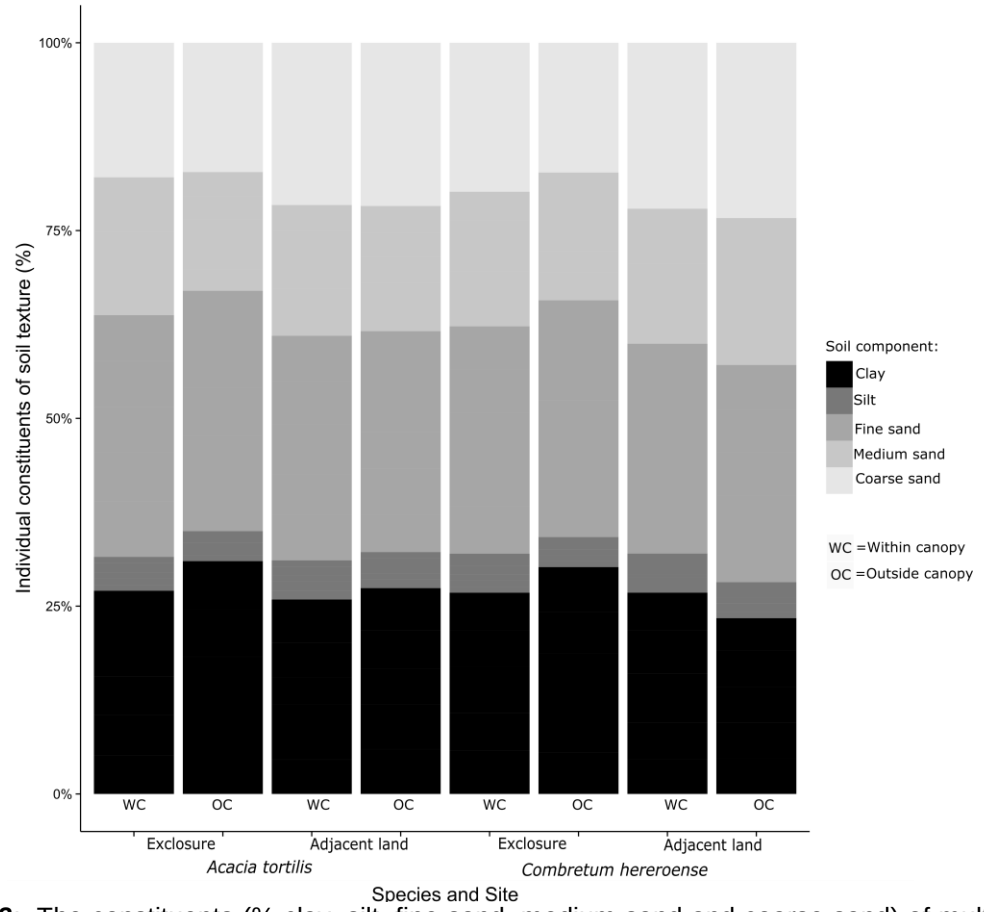


Fig. 3.13: The constituents (% clay, silt, fine sand, medium sand and coarse sand) of multiple soil samples taken from two sites, under varying conditions of exposure to large mammalian herbivory, canopy position (within canopy an outside canopy) and local exposure to N_2-fixing tree species. *Letters* denote significant differences (Kruskal-Wallis followed by Kruskal-Wallis multiple comparisons tests).

3.5. DISCUSSION

The results reveal a high degree of difference in soil bioavailable nutrients between two co-occurring savanna tree species with contrasting N_2-fixation capacities, either exposed to or protected from herbivory. This suggests that the way in which trees interact with the soil system is dependent on both the functional group (N_2-fixation capacity) within which the species falls and external disturbances such as herbivory.

3.5.1. Herbaceous and woody plant biomass responses to herbivory or lack thereof

I found that standing stocks of herbaceous biomass were 1.9-1.95x higher within the exclosure than outside (*H1*). These data serve as a proxy illustrating exclosure success and insure assumptions of exposure to versus protection from herbivores are founded. Further, herbaceous biomass did not differ based on where in the canopy it was located or with which species it was co-existing. This was expected given the absence of large mammalian herbivores in the exclosure and their accompanying forage preferences. These preferences have the potential to alter vegetation standing stocks if herbaceous biomass exhibits greater palatability (McNaughton and Georgiadis 1986) in either the sub-canopy or beyond-canopy regions. Given that herbaceous biomass was no different sub canopy versus beyond the canopy outside the exclosure, suggests that grasses did not display increased palatability and that all herbaceous biomass was equally utilised. This is consistent with other data from this site (see Chapter 2, 2.4.2.1). While herbaceous biomass differed between the two sites, total aboveground woody and leaf biomass per individual tree did not. However, I suggest that this is not due to a lack of herbivory outside the exclosure but rather due to the capability of trees experiencing chronic herbivory to exhibit compensatory regrowth. This has been shown in *A. tortilis* (Fornara and du Toit 2008) and *A. nigrescens* (Fornara and Du Toit 2007) (for a more detailed discussion see Chapter 2, 2.5: *H1*).

Finally, the finding that mean woody biomass per tress did not differ between sites has important implications for mycorrhizal associations. Compensatory regrowth could feasibly

reduce competition for photosynthate between aboveground herbivores and the vesicular arbuscular mycorrhizae (VAM) that colonise the plant roots of the two species. As such, it is unlikely that VAM would differentially influence soil nutrient availabilities inside and outside the exclosure.

3.5.2. Soil nutrient availability and exposure to herbivores

The largest differences in soil nutrient availabilities inside and outside the exclosure occurred for zinc, phosphorus, sodium, potassium, nitrate-nitrogen, copper, boron, sulphur, magnesium and inorganic N (nitrate + ammonium). Ten of the 15 bioavailable nutrients differed significantly based on herbivore presence or absence. What is very interesting is that all of these availabilities were greater in the absence (exclosure) of large mammalian herbivores. Thus my hypothesis (*H4*) is not supported.

There has been much debate over whether the presence of large mammalian herbivores have positive or negative effects on nutrient cycling and specifically soil N availability within an ecosystem (Augustine and McNaughton 2006; Cech et al. 2008), especially with regards to N. Given that the availability of N was greater within the exclosure, this suggests that herbivore presence may in fact slow N cycling. In addition, the extra herbaceous vegetation in the exclosure should also be holding a higher proportion of N than the grasses outside. This is reinforced by the fact that total soil N (see *H6*) was equal between sites, suggesting that N mineralisation and decomposition of organic material occurs at a greater rate within the exclosure. This finding is unexpected, as I hypothesised that there would be a greater concentration of bioavailable nutrients in the site exposed to herbivores and consequently the deposition of nutrients in forms that are immediately accessible to plants. A number of studies however, have shown that this input by herbivores may in fact result in a loss of N from the system as ammonia volatilises more readily from dung and urine than plant sources (Ruess and McNaughton 1988; Seagle et al. 1992; Frank et al. 2004). I suspect that this may be the case in this study system, especially with regards to N (i.e.net

loss of N). Albeit unexpected, these results agree with those found in studies investigating the soil characteristics of >40 year old exclosures in a boreal forest (Pastor et al. 1993). Here exclosures also displayed greater nutrient availabilities, N mineralization rates as well as higher total N. A similar finding was reported by Wigley et al. (2014), with higher N, P and K within exclosures in the Kruger National Park (KNP) compared to outside as well as by Cech et al. (2010) who measured greater N and P bioavailability in herbivore exclosures than in the surrounding land of a Tanzanian savanna. Lu et al. (2014) studied the effects of long-term herbivore exclusion in a Mongolian grassland (2-30 years), and found an increasing availability of inorganic N with exclosure duration, which is consistent with my data. Tessema et al. (2011) showed greater concentrations of total N and available Ca, K, Na and Mg under conditions of light grazing when compared to intense grazing. This suggests that sites which experience chronic herbivory may display a reduction in nutrient availability and that exposure to herbivory may result in the deterioration of a natural ecosystem. A possible explanation for the findings in my data may be attributed to the higher basal cover and greater standing biomass within the exclosure. This increased cover results in an increase in the aboveground litter which then enhances soil organic matter content. This could be unpacked if soil organic matter content was analysed in this study. Aboveground leaf litter was noted to be virtually non-existent outside of the exclosure and limited to woody, more recalcitrant material.

In Marakele the data seems to suggest that herbivory inhibits both N and P cycling given greater bioavailable quantities of both within the exclosure. Indeed, this serves as important evidence which points to a slowing down of nutrient cycling within this system and hence a “negative” impact by herbivores (Cech et al. 2010). Further, these results have important consequences for the management of savanna ecosystems in general. Hence, if stocking densities are increased, the resultant slowing down of nutrient cycles may ultimately cause a reduction in net primary productivity. This is alarming given that CO_2 concentrations are continuing to climb (Corlett 2015) and that the terrestrial environment plays an important role in sequestering some of this excess carbon. Particularly as savanna systems have been

implicated as the biome responsible for regulating interannual variability in the terrestrial C sink (Ahlstrom et al. 2015).

3.5.3 Nutrient availability and tree species association

A much smaller number, only four of the 15 elements differed in concentration depending on whether they were found in association with to *Acacia* or *Combretum*, these included magnesium, and three micronutrients, aluminium, boron and iron. All of which occurred in greater quantities when associated with *Combretum* (**Fig. 3.8-3.10**). Interestingly a recent study by Colgan et al. (2015) in the KNP savanna showed that the greatest differences in foliar chemistry between 12 savanna trees species occurred for magnesium, boron, zinc and manganese. My data is consistent with those findings (albeit in soils), at least for magnesium and boron, given the differential availabilities of these elements in the soils associated with the two species.

Two of these, iron and aluminium did not differ between sites and therefore this suggests a species specific effect or perhaps that one species absorbs a greater quantity of these elements than the other. That is to say that perhaps the ability of *Acacia* to undergo N_2-fixation results in the differential absorption of nutrients. N_2-fixation is heavily reliant on P availability in the soil (Vitousek et al. 2002) and given the fact that African savannas are considered P-limited (Elser et al. 2007; Elser 2012) (see Chapter 2 for supporting evidence) I would have expected bioavailable P to be lower when associated with *Acacia.* However, a number of other studies have actually reported an increase in bioavailable P beneath the canopy of N_2-fixing savanna trees (Ludwig et al. 2004; Sitters et al. 2013; Blaser et al. 2014; Sitters et al. 2015). This alleviation of P limitation is believed to be linked to the release of more N-rich phosphatases as a result of symbiotic N_2-fixation (Houlton et al. 2008). Furthermore in a recent meta-analysis, Blaser et al. (2013) concluded that globally, N_2-fixing plants increase soil available N, and this is supported by a growing number of studies (Ludwig et al. 2001; Power et al. 2003; Ludwig et al. 2004; Cech et al. 2010). This is inconsistent with

my findings, suggesting that perhaps *A. tortilis* in this study is not deriving its N from the atmosphere. Another possible explanation may be that this species has the capacity to function as a facultative N_2-fixer. Given the high soil N content and low soil P content at the site, species capable of fixing N_2 would gain no competitive advantage over non-N_2-fixers and therefore undergo selection in favour of a down-regulation in their N_2-fixation machinery in response to these environmental conditions (Hartwig 1998; Menge et al. 2009). Indeed it has been observed that many legumes in Africa display a facultative, rather than an obligate N_2-fixation strategy (Barron et al. 2011). This down-regulation would also account for the lack of changes to soil P availability. Despite these findings, the lower plant available iron in soils associated with *Acacia* may indeed point to N_2-fixation, albeit perhaps to a limited degree, as N_2-fixation is dependent on iron availability (Chapin et al. 2011). N_2-fixation activity is further supported by the fact that the availability of boron was found to be lower in the presence of *Acacia*. Boron is an essential component of plant growth (Brown et al. 2002) and particularly in N_2-fixation where it aids in the formation of nodule walls (Broadley et al. 2007). As such I suggest that *Acacia* individuals are fixing N, and that if they are accumulating a higher N content in their leaf tissue that this is not feeding back (possibly due to mineralisation or the rate of decomposition) into the soil-system resulting in a negligible effect on soil N availability.

3.5.4. Nutrient availability and canopy position

Similar to species specific effects, ~50% of the measured nutrients were found to differ in their availabilities depending on whether they were measured below the canopy or beyond the canopy. These included calcium, inorganic N, nitrate-N, calcium, aluminium, boron and iron. All of these were found to have greater availabilities within the tree canopy than outside it. This suggests that tree species in some may mediate the availability of these nutrients. According to the data, herbaceous biomass was no different between the two canopy positions, which therefore suggests that the effects being seen are indeed due to the tree species themselves. Given that three of the same nutrients also differ based on species, this

further reinforces the idea that mechanisms specific to either species may result in the differential nutrient availabilities observed. Consistent with my hypothesis, soil N was greater sub-canopy than beyond-canopy regardless of N_2-fixation capacity or incidence of herbivory, however the same was not true for P availability.

3.5.5. Interactions among factors

The significant three-way interaction among all experimental factors (i.e. site, species and canopy position) for inorganic N, sulphur and sodium illustrates a complex dynamic. A possible explanation for these may be the establishment of feedback loops which alter the relative availability of these nutrients. In summary it appears that those nutrients which differ by site are mediated primarily by herbivore effects while those that differ by tree species and canopy position illustrate the capability of species to function as ecosystem engineers. However, this is not entirely so simple as inorganic N, nitrate-nitrogen, sulphur, magnesium and sodium also exhibit a significant interaction between site and tree species suggesting either negative/inhibitory interactions. When considering the effect of individual factors, the majority of differences among treatment combinations come from micronutrients, while significant interactions occurred primarily for macronutrients. A possible explanation for this trend could be that macronutrients are required in larger quantities by plants as they are responsible for regulating growth and cellular structure (Vitousek 1982). Micronutrients on the other hand primarily mediate metabolic processes. This is to say that fine-scale differences between species are fed back into the soil system, with differences in soil nutrient availabilities a reflection of differential metabolism between species. We know that given the two different functional groups that *Acacia* and *Combretum* fall into based on their N_2-fixation capacity, it is indeed conceivable, even expected that they would have different metabolic requirements. Factors operating at a larger scale, such as whether the soil is exposed to or protected from herbivory differ primarily in their micronutrients, which regulates growth and ultimately primary productivity. I suggest that herbivory primarily alters the bioavailability of macronutrients and hence regulates growth and cellular structure while different species alter micronutrient

bioavailability, reflecting differences in the biochemistry and metabolic requirements of individual species.

3.5.6. Total N and P versus bioavailable N and P

Total soil N (soil core) did not differ significantly between the sites while total soil P (soil core) was found to be significantly higher within the exclosure. This is partially consistent with my hypothesis (*H6*). As total N did not differ between sites, this illustrates that I am exclusively comparing the effects of herbivore exclusion and N_2-fixation capacity and not the inherent differences in N between sites. This finding also demonstrates the fact that resin capsule data is far more useful and indeed biologically meaningful than the measurement of total nutrient pools. This is because a great deal of total N and P exist in organic, bound, microbial or recalcitrant forms and are not available to the plant. Thus investigating bioavailable mineral elements provides greater resolution and is more indicative of ecological process such as decomposition, mineralization etc. The application of resin capsules in this research is evidenced by the fact that bioavailable N (specifically nitrate derived N) differs based on exposure to herbivory and canopy position. Total N however did not. This has significant implications for predicting relative growth as nitrates are one of the key elements limiting growth (Chapin et al. 2011).

The percentage of clay and coarse sand in soils was found to differ significantly, with higher clay and lower coarse sand content in the exclosure. The original composition of the soil in both sites was likely to have been identical prior to the establishment of the exclosure given the close proximity of the two sites. The higher clay content and lower coarse soil content in the exclosure may account for the higher nutrient availabilities within this site as clay is able to absorb a greater quantity of nutrients. This is however still indirectly due to herbivore presence as increased trampling by animals on the adjacent land as well as lower herbaceous cover would lead to the blowing away of clay particles in the absence of rain or their washing away during the rainy season. Clay soils have a greater cation exchange capacity (CEC) (Hillel

1998), reducing the amount of leaching that can occur. An increase in the herbaceous cover/biomass is correlated with reduced soil erosion as well as a reduction in nutrient losses from systems (Owino et al. 2006). Thus on the adjacent land, the presence of herbivores leads to a reduction in herbaceous biomass relative to inside the exclosure, which consequently results in the loss of clay particles to erosion, weathering and trampling by herbivores. This ultimately reduces the clay content relative to the sand, increasing the rate at which ions can be lost from the soil, and thereby reducing nutrient availability, especially of the more mobile elements (e.g. phosphorus, which moves particularly slowly through the soil, which did not differ between sites). Most forms of P are fairly immobile (Witkowski et al. 1990), while nitrates and potassium can easily be leached from the soil (Schachtman et al. 1998).

3.5.7. Predicting group membership

L-DFA showed that iron, boron, potassium and sulphur were the elements best able to predict group membership across treatments combinations. This suggests that nutrient availabilities provide the appropriate degree of variability in order to infer exposure to herbivory, species association as well as canopy position and their interactions. Interestingly all but two of the five best factors in predicting group membership are micronutrients, namely Iron, Boron and Sodium. Model accuracy was 66.1% and while not very high, it suggests that nutrient availabilities can distinguish among treatment combinations. This was especially evident when the two discriminant functions describing the majority of model variation were analysed individually- creating distinct groupings in the axis space (**Fig. 3.11**). Model accuracy and predictor variables changed as factors were considered independently, with an increase in model accuracy and the ability to predict group membership for site, canopy position and species (97.2%, 80% and 75.6%, respectively). Magnesium, aluminium, manganese and boron were the best predictors of species association which for the most part is also consistent with the nutrients which were found to differ significantly between species (manganese is the exception). This reinforces the fact that *Acacia* and *Combretum* individuals are differentially influencing soil bioavailability of these elements. Discrimination between herbivore absence

or presence was best predicted by the bioavailability of boron, potassium, sulphur and sodium. Again boron features as being a strong predictor. A similar pattern is revealed in predicting canopy position. Given the data, I suggest that the significance of boron is underrated and that future studies need to look more closely at the biological significance and importance of boron in ecology, given the differences of this element between factors. As such it would be beneficial to understand the cycling of boron through the savanna system.

Group membership to soil location was best predicted by magnesium, boron and manganese which correlates well with a recent study by Colgan et al. (2015) where L-DFA and multivariate regression tree analysis revealed that the concentrations of these three elements in leaf tissue in addition to Zn (significantly different between sites in my data), lignin and hemi-cellulose predict the species membership. This highlights the potential application of developing threshold levels of these nutrients to identify species, particularly for use in spectranomics to identify species based on their chemical composition (Asner et al. 2015).

3.6. CONCLUSION

While I do not investigate the effect of tree density on nutrient dynamics as has been done recently (Sitters et al. 2013; Sitters et al. 2015), I reduce the scale to investigate more localised trends. I focus on the small-scale “single-tree” impacts on multiple macro and micronutrients in conjunction with the influence of herbivore presence and foraging. I found that herbivore presence decreases the bioavailability of certain key nutrients. Furthermore I suggest that herbivory primarily alters the bioavailability of macronutrients and hence regulates growth and cellular structure while different species alter micronutrient bioavailability, reflecting differences in the biochemistry and metabolic requirements of individual species in the soil. A possible mechanism by which herbivores decrease nutrient availabilities is through the reduction in clay content and an increase in sandy soil content, which reduces the CEC of the adjacent land and results in the leaching of nutrients and a net loss in the long term. Furthermore I suggest that herbivore presence slows down nutrient cycling. Ultimately this has important implications for managers of savanna systems in terms

of changing grazing pressures and woody encroachment. I also illustrate the value of the cumulative nature of resin capsules over more traditional estimates of total soil nutrients and suggest that they are biologically more meaningful. Finally, I suggest that predictions of group membership of both soil and foliar traits could have potential applications in the field of spectranomics.

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CHAPTER 4

GENERAL DISCUSSION AND SYNTHESIS

This research has its basis in the fact that the distribution and indeed the variability in different plant functional traits present within a given system has significant implications for the functioning and dynamics of this same ecosystem (Enquist et al. 2015). Functional traits provide a means to investigate the diverse ecological strategies employed by different species within a community (Violle et al. 2014), and ultimately predict how a species with a particular set of traits will interact with co-occurring individuals (Grime 1998). In the short-term, biomass inputs from the species within a given system will have immediate effects on ecosystem functioning (Grime 1998). Trait values characterising this biomass are the outcome of complex biotic and abiotic interactions. It is therefore crucial to investigate how different factors might influence these traits and interactions and in turn this will facilitate a greater comprehension of impacts on ecosystem processes. Before synthesising the data presented in this dissertation it is necessary to acknowledge that I am aware of the fact that the results are suggestive of trends rather than reflection of fact, especially given that the data presented illustrate a "snapshot" of a single season and year. Long-term data over many years would be needed to determine the validity of the conclusions presented in this research.

4.1. Linking leaf functional traits and soil nutrient bioavailability

I went about investigating how two such drivers, herbivory (top-down control) and symbiotic N_2-fixation (bottom-up control) drive savanna plant and soil nutrient dynamics. I did this by means of a systematic approach, where I independently examined the effects of these drivers on leaf functional traits (LFT's) and soil nutrient characteristics. In general, under the influence of herbivory, low bioavailability of soil nutrients was associated with an increase in foliar C concentration, C/N, C/P and SLA, and a decrease in foliar N and P concentrations, all indicative of a reduction in leaf palatability. This suggests that vegetation and soil nutrient dynamics respond in similar ways to the factors under investigation. At the very least it is important to note that the effects of herbivory and N_2-fixation on leaf traits is corroborated by the influence of these same two factors on soil nutrient bioavailabilities. What I mean by this is that exposure to herbivory ultimately reduces nutrient concentrations in the leaf tissue, and

likewise in terms of soil nutrients (**Fig. 4.1**). Despite this, the mechanism by which each is influenced differs. When N_2-fixation is considered independently of herbivory it can be seen that the functional characteristics of

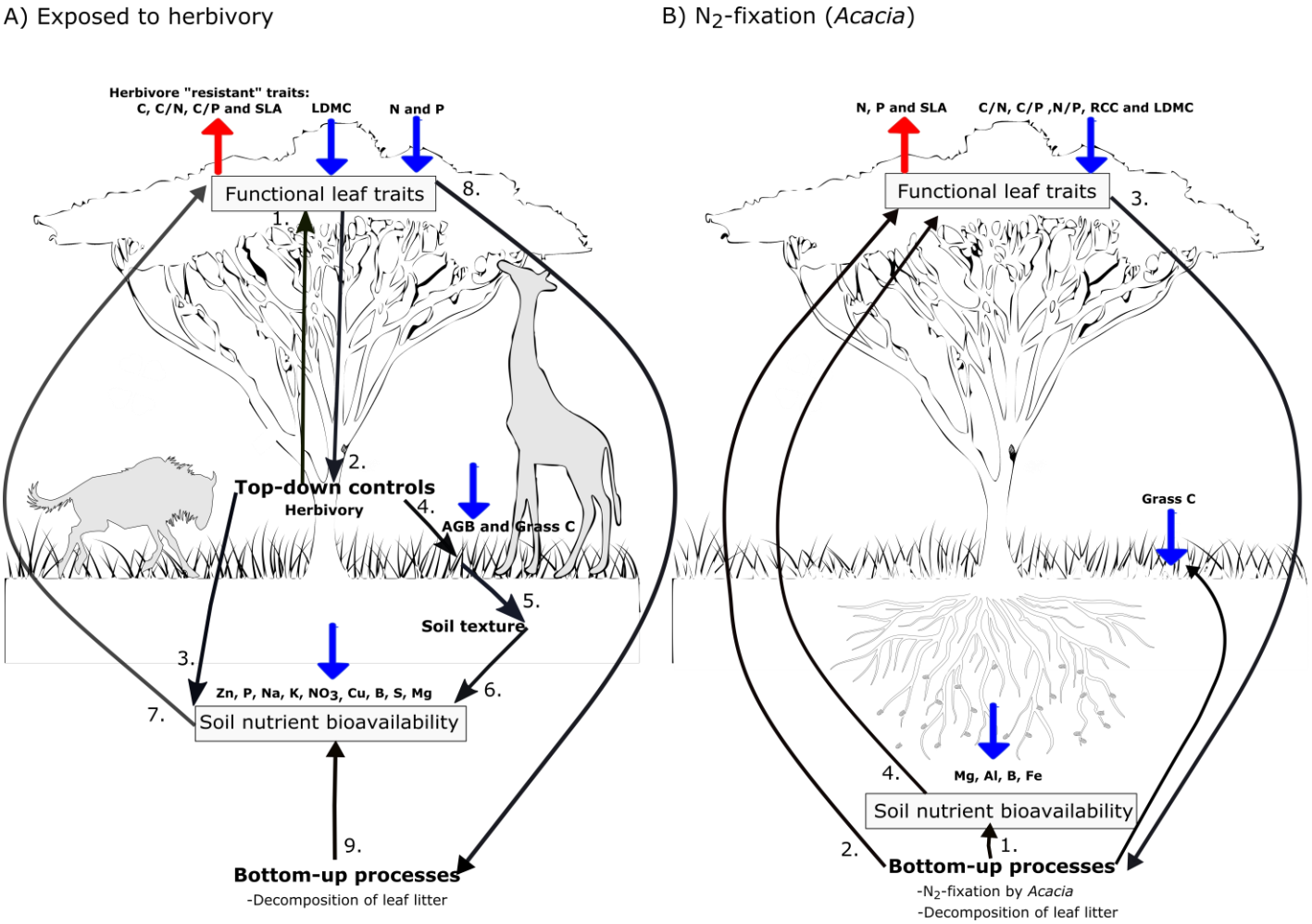


Fig. 4.1: Schematic diagram representing the relationships/associations between functional leaf traits and soil nutrient bioavailability under the influence of; (A) exposure to herbivory (adjacent land) and (B) Symbiotic N_2-fixation capacity (*Acacia tortilis*).
A: Under the influence of herbivory (top-down control) both grazers and browsers consume plant biomass, influencing the selection of functional traits which deter herbivores by decreasing palatability (1), changes in these functional traits in turn alter herbivore forage selection in the long-term as they will preferentially select for high quality forage (2), furthermore deposition of faeces and urination by herbivores directly effects soil nutrient bioavailability, ultimately causing a net loss of certain nutrients from the system (3). The mechanism through which this occurs is via the reduction of aboveground herbaceous biomass (AGB) (4), the loss of biomass, in conjunction with trampling by herbivores causes a reduction in the soil clay content and an increase in the sandy soil component (5), this ultimately reduces the cation exchange capacity, increasing the degree of leaching, which then results in the reduced bioavailability of a number of mineral elements (6). Reduced soil bioavailability of key elements will then feedback into the system and influence leaf functional traits as these are dependent on soil nutrient availabilities (7) - The reduced bioavailability likely accounts for the increase in herbivore resistant traits as plants are limited in their uptake. The variability in the functional traits will in turn also influence nutrient availability when leaves decompose (8 and 9) creating a negative feedback loop.
B: The process of symbiotic N_2-fixation directly results in the reduction of particular bioavailable nutrients due to the inherent requirements of this mutualism (1). N_2-fixation also directly influences particular functional leaf traits, generally

increasing the palatability and increasing the overall nutrient concentration of the leaves (N and P) (2), the variability of these traits will then feedback into the soil system when leaves are decomposed (3) altering nutrient availabilities which will then reinforce the functional leaf traits displayed (4).

a species does not influence LFT's in the same way as does herbivory. Under the influence of N_2-fixation (a bottom-up process) LFT's display increased foliar N and P concentrations and a reduction in C, C/N, RCC and C/P. Thus while herbivory reduces nutrient concentrations of primary limiting nutrients, N_2-fixation functions to increase them. However, this pattern is not extended to soil nutrient availabilities. Under conditions of N_2-fixation (*Acacia*) a number of elements show reduced bioavailability relative to non-N_2-fixation (*Combretum*), namely Mg, Al, B and Fe. It would be interesting to measure these same nutrients in leaf tissue and this is a potential future avenue of research. I predict that Mg, Al, B and Fe would all show increased concentrations in the leaves, given their uptake during the N_2-fixation process which is what I attributed the decrease in soil nutrients to. Magnesium and B seem to be highly sensitive to differences among species (Colgan et al. 2015), most likely due to different metabolic strategies employed by different species. It would be interesting to conduct a large-scale study investigating the coupling between these elements in both leaves and soil to determine their degree of correlation. Which is likely to be negative. Thus overall herbivory seems to function to reduce nutrient availability while N_2-fixation partially improves it (**Fig. 4.1**).

Overall I found that there was a marked difference in the standing herbaceous biomass inside and outside of the exclosure. Herbaceous biomass inside the exclosure was as much as 90% greater than the adjacent land, which has significant implications to both functional leaf traits and soil nutrient bioavailabilities given that these factors are inherently coupled (**Fig. 4.1**). Woody biomass however exhibited no significant differences between sites. It is likely that compensatory regrowth following defoliation accounts for this finding on the adjacent land. Regrowth is comprised of leaves with a higher investment in C relative to N and P, which would result in these leaves becoming less palatable to herbivores. If herbivory on the adjacent land functions to reduce the concentrations of key limiting elements in the leaf tissue, then

over time, through continued selection of high quality forage there may be a change in community abundance outside of the exclosure relative to inside. This would serve as an interesting starting point for future research in this system and would enable greater elucidation of the long-term impacts of herbivore exclusion. Not only do trees exposed to herbivory exhibit traits suggestive of herbivore defence (tougher, leathery and less palatable leaves-on the basis of SLA, LDMC and foliar nutrient concentrations) but also a reduction In LDMC. Low LDMC has been associated with highly productive, often disturbed environments (Poorter et al. 2009). Interestingly the site exposed to herbivory demonstrated significantly lower nutrient bioavailabilities for 67% of measured nutrients. I suggested that exposure to herbivory in fact slows down the cycling of nutrients in the ecosystem. Future research involving the quantification of mineralization rates will provide evidence to corroborate or disprove this claim. However, reduced cycling rates is supported by the finding in these data that nutrient bioavailability is greatly reduced on the adjacent land (**Fig. 4.1**). This reduction in soil nutrient availability will subsequently feed back into the system, further regulating plant functional traits. Given that soil nutrient availabilities are reduced, it is expected that herbivore-resistant functional leaf traits will undergo reinforcement, maintaining their herbivore-induced status.

Previously I mentioned that given equivalent woody biomass across sites, that VAM is unlikely to influence nutrient availabilities due to a lack of competition between herbivores and mycorrhiza for access to photosynthate. This however, is not to say that the intensity of root colonization by VAM could differ inside relative to outside the exclosure. A recent global meta-analysis conducted by Soudzilovskaia et al. (2015) showed that root colonization intensity (biomass) by VAM is greatest at sites with continental temperatures, mild summers and a high availability of soil nitrogen. This suggests that root colonization by VAM could potentially be higher in trees within the exclosure, given the higher soil N bioavailability. If this is indeed the case it could provide further evidence to support the higher foliar N and P concentrations and generally greater foliar N and P content relative to C, given that mycorrhizal associations

improve access to nutrients. This addition further highlights the complex dynamics at play within a single ecosystem where top-down controls such as herbivory, influence functional leaf traits and soil nutrient availability, with these factors then feeding back into one another and then undergoing further modification at the hands of associations such as VAM. Future research should investigate this by quantifying VAM colonization intensity in both sites as well as in soils relative to both species.

4.2. Implications for tree and greater ecosystem "health"

Tree health can be broadly defined as the biotic and abiotic factors which effect plant vigour and productivity (Boa 2003). Thus tree health refers to the state of well-being and ability of an individual to undergo normal growth and development. I found that herbivory and N_2-fixation both have significant effects on tree health, through their regulation of limiting nutrients and in turn their facilitation of effective metabolism and growth. These effects have the ability to significantly impair tree health (Boxman et al. 1994). Similarly an increase in nutrient availability can improve tree health (Huettl 1988). By altering the availability of nutrients, some nutrients may become limiting, especially in reference to N and P. For example in the adjacent land the availability of both N and P was lower relative to a site excluded from herbivores. Likewise, this reduction in nutrient availability was associated with LFT's indicative of lower nutrient concentrations. This suggest that in this case, exposure to herbivory adversely effects tree health relative to vegetation that is protected from herbivory. What future research needs to address is both the quantification of the intensity or degree of herbivory in order to determine if varying degrees of intensity differentially effect LFT's and soil nutrient availabilities as well as experimentally determine relative growth rates. Especially considering the idea of intermediate disturbance (i.e. intermediate disturbance hypothesis) (Roxburgh et al. 2004), whereby a condition intermediate to chronic herbivory and complete herbivore exclusion may function to improve tree health. It is difficult to speculate as such only having explored the two extremes.

4.3. Contributions towards the management of savanna systems

Through the course of this dissertation I have illustrated the complex ways in which herbivory and N_2-fixation capacity influence savanna plant and soil nutrient dynamics. Changes to grazing and browsing intensity will significantly alter the nutrient dynamics of both the vegetation and the soil. This will in turn influence a number of ecosystem processes, including decomposition (dependent on both foliar traits and soil nutrient availabilities) (Hobbie 2015), nutrient cycling (both the rate and flux of elements), ecosystem productivity (variable density of different species characterised by different traits and their resultant influences on the ecosystem) and ultimately the services which are provisioned by savannas (e.g. fuel wood and soil fertility for crop growing). Based on this research it is recommended that reserve managers avoid dramatically increasing their stocking densities as this may hamper the ability of the ecosystem to provide resources to sustain these large populations. Furthermore, if an increasing African population results in the intensification of herbivory to support both domestic and wild mammalian herbivore populations this could have significant consequences to the sequestration of C in the terrestrial carbon sink. If a decrease in soil and foliar nutrient availability is indeed linked to reduced growth rates, which is generally the case (Chapin et al. 2011), this will decrease the amount of CO_2 that the vegetation is able to take up.

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