

# UNIVERSITY OF ZULULAND



## RESEARCH MASTER'S DISSERTATION

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## Contents

Acknowledgements.....	iii
Abstract.....	iv
Declaration by the candidate.....	vi
CHAPTER ONE .....	1
1. INTRODUCTION.....	1
1.1. Problem statement .....	4
1.2. Aim .....	5
1.3. Objective .....	5
1.4. Hypothesis.....	5
CHAPTER TWO .....	6
2. LITERATURE REVIEW .....	6
2.1. Effect of browsing on physical properties of trees .....	6
2.2. Effect of browsing on chemical properties of trees.....	8
2.3. The role of flavonoids in plants.....	10
2.4. The role of condensed tannins in plants.....	11
2.5. Role of nitrogen in plants.....	12
CHAPTER THREE .....	13
3. MATERIALS AND METHODS.....	13
3.1. Study area .....	13
3.2. Study species.....	13
3.3. Experimental setup .....	14
3.4. Data collection .....	15
3.4.1. Treatments.....	15
3.4.2. Quantification of physical traits.....	16
3.4.3. Quantification of chemical traits .....	18
3.5. Statistical analysis .....	20
CHAPTER FOUR .....	22
4. RESULTS.....	22
4.1. Effect of fertiliser on chemical tree traits in October 2013 .....	22
4.2. Effect of the interaction between fertiliser and clipping treatment .....	25
4.2.1. Aboveground tree traits.....	25
4.2.2. Chemical tree traits.....	33
CHAPTER FIVE .....	38

5. DISCUSSION AND CONCLUSION.....	38
5.1. General conclusion.....	49
5.2. Recommendations .....	50
5.3. Contribution to body of knowledge.....	51
REFERENCES.....	52
APPENDIXES .....	60
Appendix A.....	60

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# Response of marula to simulated browsing on a productivity gradient

## Abstract

Marula (*Sclerocarya birrea*) is a heavily utilised savannah tree species and is widely used by people and animals for its leaves, bark, fruits and roots. Because of its importance in terms of economy, ecology, cultural values and dominance in some low veld areas of South Africa, it is therefore of importance to understand responses of marula trees to environmental factors. Two key factors in regulating savannah functioning are browsing and soil fertility. The aim of this research was to assess the effects of simulated browsing on the physical and chemical properties of marula trees growing on a productivity gradient in a controlled environment. The hypothesis to be tested was that, marula saplings respond to browsing intensity by showing a trend of increasing tree height, stem diameter, old shoot extension, number of new shoots, new shoot length, leaf biomass, and foliar nitrogen along a productivity gradient. Conversely, marula saplings showed a trend of decreasing flavonoid content, condensed tannin concentration, Neutral-detergent fibre, acid-detergent fibre, and acid-detergent lignin in response to increasing browsing intensity along a productivity gradient. Browsing was simulated by clipping the saplings at a range of intensities and a productivity gradient was achieved by applying fertiliser at a range of levels. The interaction between clipping and fertiliser treatments had a significant effect in explaining final tree height ( $P < 0.001$ ), stem diameter ( $P < 0.001$ ), old shoot extension ( $P = 0.028$ ), and number of new shoots ( $P = 0.040$ ) but the significance of the interaction for final tree height and stem diameter was influenced by both initial tree height and initial stem diameter as covariates. New shoot length and leaf biomass were significantly influenced by fertiliser and clipping separately not by their interaction ( $P = 0.005$ ,  $P = 0.001$ ;  $P = 0.043$ ,  $P < 0.001$ ) respectively. Clipping treatment

significantly influenced ADL at the end of the experimental period ( $P=0.048$ ). None of the studied fixed factors significantly influenced N, flavonoids, CT, NDF, and ADF at the end of the experiment. Marula saplings growing along a productivity gradient tend to compensate by new shoot productivity (number and length) in response to intermediate clipping but this response did not reflect the compensatory ability of total aboveground biomass, because tree height and stem diameter were not increased. Severe clipping and defoliation treatment induced defence responses regardless of increased fertiliser rates, thus suggesting carbon-limitations. The results from this study also indicated that physical responses to a single clipping event persist for longer than chemical responses do.

**Key words:** Carbon-based secondary metabolites, *Sclerocarya birrea*, condensed tannins, growth, herbivory, plant defences

### **Declaration by the candidate**

I declare that I have complied with the University's policies and rules for dissertation

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compilation. I also certify that this dissertation is unique and is free of plagiarism.

<b>Candidate's signature</b>	
<b>Date</b>	

# CHAPTER ONE

## 1. INTRODUCTION

Marula (*Sclerocarya birrea subsp. caffra*) is a dioecious tree species belonging to the family *Anacardiaceae*, and is the dominant tree species in some low veld areas of South Africa, such as the Southern landscapes of the Kruger National Park and elsewhere. *S. birrea* is important in terms of economy, ecology, cultural value and its dominance in many African landscapes (Wynberg, 2002). In African savannahs *S. birrea* is a heavily consumed tree species and is widely utilised by people and wild-life for its leaves, bark, fruits and roots (Shackleton, 2002). The canopy provides habitat for insects and bird species (Bergström, 1992). Humans in ancient times used the bark of this tree to treat dysentery and diarrhoea and also for its prophylactic effects against malaria (Shackleton, 2002). Traditional healers also collect material for medicinal purposes.

### Plant defence strategies against herbivory

African savannahs are characterised by co-dominance of trees and grasses which provide feed for herbivores (Scogings and Mopipi, 2008). Large herbivores and African savannahs coevolved, with woody plants developing defence mechanisms against herbivory (Du Toit *et al.*, 1990). Woody plants show a wide variety of defence traits against herbivory. Chemical defences may include the production of secondary metabolites or reduced nutrient content (e.g. protein, measured as nitrogen concentration [N]). Mechanical defence comprises the growth of thorns and spines while growth response involves compensatory shoot and stem growth (Ward and Young, 2002).

Herbivore induced defences may also be categorized as: (1) plant resistance traits that diminish herbivory: (2) tolerance traits (i.e., re-growth such as mass compensatory growth

abilities) that reduce adverse effects following herbivory; and (3) phenological escape, that moderates plant accessibility when herbivores are most active and affective (Fornara and Toit, 2007). Defensive strategies vary; among plant species, within a plant, over time, and also vary in response to herbivory (Stevens *et al.*, 2007).

Models that explain defensive strategies by variation in carbon-based secondary metabolites (CBSMs) predict that CBSMs are inversely proportional to nutrient concentration and growth rate. This is because there is a physiological trade-off between cellular growth and differentiation (Herms and Mattson, 1992; Le Bot *et al.*, 2009). A trade-off between different defensive strategies may occur, depending on the interactive effects of different key environmental factors on plant growth (Fornara and Toit, 2007).

#### Adaptation of herbivores to plant defence traits

Herbivores have physiologically adapted to reduce the effect of plant defence traits, such as tannins, against herbivory by having a tannin-resistant rumen flora, improved liver capacity to detoxify tannins, and by the secretion of tannin-binding salivary protein (Robbins *et al.*, 1991). Tannin-binding salivary protein minimises faecal-nitrogen loss in animals consuming tannins by increasing the amount of tannin bound per unit of protein, thus minimising tannin absorption and toxicity. Tannin-binding salivary proteins also minimise reduction in plant-fibre digestion in animals with an extensive fermentation capacity (Robbins *et al.*, 1991).

Dietary fibre helps for normal functioning of rumen in ruminants. Dietary fibre, through microbial degradation and synthesis, supplies energy to support maintenance, growth, lactation and production (Lu *et al.*, 2005). For e.g., in lactating goats, fibre is required to maintain normal milk content (Lu *et al.*, 2005). Herbivores have also morphologically adapted to tannins by having a mouth adapted to eat a certain type of species that is low in

condensed tannin concentration [CT] , e.g., thorny species, thus selective feeding (Borchard *et al.*, 2011).

#### Plant traits affecting feed quality

Plant traits such as secondary metabolites (e.g., tannins and flavonoids) and nutrient concentrations affect herbivore food choice by affecting feed digestibility and palatability (Skarpe and Hester, 2008). Feed digestibility is determined by quantifying different types of feed properties such as: (1) metabolisable nutrients (e.g., protein) measured as N: (2) digestibility-reducing substances (e.g., cellulose and hemi-cellulose) measured as neutral-detergent fibre (NDF), acid-detergent fibre (ADF), and acid-detergent lignin (ADL): and (3) secondary metabolites (e.g., condensed tannins) which have been shown to decrease digestibility (Getachew *et al.*, 2000) and fermentation (Makkar *et al.*, 1995; Getachew *et al.*, 2000), then affect availability of nutrients in browse species. Plant palatability is influenced by the concentration of plant secondary metabolites and N content (Getachew *et al.*, 2000). Chemical composition and the physical form of plant fibre components influence productive performance of browsers and therefore quantifying leaf fibre components chemically and physically can be useful as nutritional indicators (Lu *et al.*, 2005).

Forage quality is calculated by determining relative feed value (RFV) or relative forage quality (RFQ). Relative forage quality is preferred over RFV because it involves dry matter digestibility (DMD) and total digestible nitrogen (TDN) which measures the total energy consumed by animals, not just fibre (Moore and Undersander, 2002). On the other hand RFV is determined by two factors namely, NDF and ADF. Neutral detergent fibre indicates fibre components while ADF indicates digestibility (Moore and Undersander, 2002).

In addition, foliar digestibility is affected by foliar nitrogen concentration [N] and condensed tannin concentration [CT], which are influenced by increasing browsing intensity (Jachmann

and Bell, 1985; Bergström, 1992; Wessels *et al.*, 2007). Low foliar [N] and high [CT] reduce foliar digestibility and vice-versa. Reduced foliar digestibility is due to the binding of microbial enzymes which then inhibit the fermentation process and also inhibit the breakdown of fibre (Jachmann and Bell, 1985; Getachew, 2008). Available foliar N gives an indication of N that is available to animals rather than the total N of the plant material (Wallis *et al.*, 2010). Available N is important for knowing forage quality which helps to determine animal performance (Wallis *et al.*, 2010). Available foliar nitrogen is also assumed to be the appropriate measure in studies of biological stoichiometry (Wallis *et al.*, 2010).

Concern over loss of mature trees in African savannahs, due to damage caused by heavy browsing and environmental factors that are responsible for regulating savannah functioning, has resulted in a multitude of studies on keystone tree species (O'Connor *et al.*, 2007; Helm *et al.*, 2009). The abundance of mammalian herbivores in Africa is high compared to other continents (Fritz and Loison, 2006), but the extent to which mammalian herbivores directly influence the physical and chemical traits of woody plants in African savannahs is unknown. The effects of browsing on woody plants in African savannahs are a critical component of research, with the ultimate objective of developing improved management policies (Scogings *et al.*, 2014).

### **1.1. Problem statement**

In order to better understand and predict the environmental variables that affect browse-browser interaction and browse quality of African savannah tree species, it is important to better understand changes in plant nutrient concentration, chemical plant defence traits, and compensatory growth with changes in environmental factors simultaneously. Better understanding of African savannah tree species, such as marula trees, will help in providing basic knowledge for conserving these keystone species.

## **1.2. Aim**

The aim of this research was to assess the effects of simulated browsing on the physical and chemical properties of marula saplings growing on a productivity gradient in a controlled environment.

## **1.3. Objective**

The objective is to simulate browsing by clipping the saplings at a range of intensities and create a productivity gradient by applying fertiliser at a range of levels. Furthermore, assessment of tree height, stem diameter, old shoot extension, number of new shoots, new shoot length, leaf biomass, foliar nitrogen, flavonoid content, condensed tannin concentration, NDF, ADF, and ADL was conducted at the end of the experimental period.

## **1.4. Hypothesis**

It is hypothesised that marula saplings respond to browsing intensity by showing a trend of increasing tree height, stem diameter, old shoot extension, number of new shoots, new shoot length, leaf biomass, and foliar nitrogen along a productivity gradient. Conversely, marula saplings show a trend of decreasing flavonoid content, condensed tannin concentration, NDF, ADF, and ADL in response to increasing browsing intensity along a productivity gradient.

# CHAPTER TWO

## 2. LITERATURE REVIEW

### 2.1. Effect of browsing on physical properties of trees

Browsing is one of the most important environmental factors affecting the ecology of a tree and the browse-browser interaction by influencing the physical and chemical properties of trees in savannahs (Bergström, 1992). Browsing is categorised into light browsing and severe browsing. Light browsing involves leaf harvesting without branch breakage, while severe browsing includes branch breakage and affects shoot growth (Makhabu *et al.*, 2006). Woody plant species respond to browsing by having either a well-developed re-growth capacity and a poor defence mechanism or visa-versa (Meijden and Marijke, 1988).

Re-growth capacity implies the saving and storing of energy and nutrients in organs that are free from herbivory, which are then reallocated following browsing (Meijden and Marijke, 1988). This suggests that the frequency and intensity of browsing may influence a plant's response to browsing. Light browsing is assumed to increase carbon (C) exudation in the rhizosphere, which stimulates microbial activity and nutrient mineralisation, resulting in positive effects on shoot length (Hamilton and Frank, 2001; Hamilton *et al.*, 2008; Landhäusser and Lieffers, 2012). On the other hand, severe browsing intensity has been found to result in an increase in growth rate of the re-sprouting shoots (Messina *et al.*, 2002), and the production of fewer but long shoots (Herms and Mattson, 1992).

In addition, browsing speeds up energy flow by increasing the recycling of nutrients bound in standing plant matter (Teague, 1988a), but this is tolerated within limits of environmental conditions. When environmental conditions are critical, as in intense competition or inadequate nutrients, then compensatory growth would fail because the plant cannot get enough energy resource replacement to compensate for the reduction in photosynthesis

caused by the loss of foliage (Mopipi *et al.*, 2009). When environmental conditions are favourable (light browsing or no carbon limitations) the induction of meristematic activity, photosynthesis, and allocation of resources may increase plant tolerance and growth even after heavy browsing (Tiffin, 2000), allowing them to cope with the severe browsing experienced in nature.

It is assumed that defence is costly because investments in defence come at the expense of investments in growth and reproduction (Ward and Young, 2002). When the cost of defence is high and levels of herbivory are variable, plants are assumed to have inducible defences (Herms and Mattson, 1992). Plants growing in conditions where nutrients are limited produce less leaf biomass. The costs of producing a unit of leaf biomass is similar to that of any other plant tissue therefore stem biomass or leaf biomass indicates plant performance (Chapin *et al.*, 1987). Defoliation has also been reported to result in re-growth with smaller shoots and leaves (Rooke and Bergström, 2007).

Most studies of tree response to browsing in savannahs have only focused on shoot growth, which is assumed to be positively correlated to long-term severe browsing (Makhabu *et al.*, 2006; Riginos and Young, 2007; Hrabar *et al.*, 2009). Branch breakage as a result of browsing reduces the number of meristems, often causing reduced number of shoots and increased shoot size; sometimes with high [N] and low [CBSMs] (Makhabu *et al.*, 2006; Bergström and Danell, 1987). But shoot response alone does not indicate total plant growth. According to Bowman *et al.* (2013), whole-plant performance is determined from dimensions closely related to plant mass such as plant height or stem diameter. Helm *et al.* (2009) also support that growth rate in terms of plant height and stem diameter of woody plants are important parameters to consider in understanding savannah ecosystems. This is because intense browsing suppresses plant height by allocating carbon to growth of new shoots (Wessels *et al.*, 2007), and the significance of stem size is that it is closely related to plant

mass and therefore is likely to reflect the whole plant's response (Hester *et al.*, 2006). Stem diameter has been found to either increase or decrease under long-term severe browsing. This phenomenon is species specific and also depends on the intensity and frequency of browsing (Scogings *et al.*, 2013). Plant response to browsing also depends on mass compensatory growth ability (Penderis and Kirkman, 2014). If the compensatory growth of a plant exceeds its biomass losses following clipping, it will increase in total biomass.

Conversely, if biomass losses exceed growth, the total biomass of a plant will decrease. Plant performance also depends on its ability to capture resources such as nutrients and light. Plants may become accustomed to nutrient-poor environments either by increasing their ability to compete for nutrients or by reducing losses of nutrients by minimising their biomass turnover (Penderis and Kirkman, 2014). It is important to assess total plant growth because whole-plant under-compensation indicates progressive carbon limitations and reduced life expectancy (Hester *et al.*, 2006; Palacio *et al.*, 2014. ). Because soil nutrient availability is among the most important factors limiting plant growth (Hirose, 2012), assessment of plants growing along a productivity gradient is of importance in understanding African savannahs because it would indicate plant life expectancy.

## **2.2. Effect of browsing on chemical properties of trees**

Browsing can often lead to changes in the nutrient status of plants and changes in the production of defensive chemical traits such as CBSMs (Wigley *et al.*, 2015). In woody plant species from tropical savannahs, light browsing increases resistance traits to herbivory by increasing CBSMs and reducing N in the leaves (Gadd *et al.*, 2001). On the other hand, severe browsing intensity has been found to have opposite effects resulting in reduced chemical defences by decreasing CBSMs and increasing N content in the leaves (Du Toit *et al.*, 1990; Persson *et al.*, 2005). Defoliation has also been reported to increase a plant's defence mechanism (Du Toit *et al.*, 1990; Persson *et al.*, 2005). According to Bryant *et al.*

(1991) defoliation results in variation in foliar chemical composition. Growth of new shoots following severe browsing increases the demand for carbon which then results in decreased synthesis of CBSMs (Du Toit *et al.*, 1990; Herms and Mattson, 1992). Therefore, heavily browsed plants tend to be rich in nitrogen (N) and have carbon-poor tissues which implies that they are more palatable since they have less CBSMs e.g., condensed tannins (CT) to function as anti-herbivore defence. This puts these palatable plants at risk of being browsed repeatedly and can eventually lead to plant death (Fornara and Toit, 2007; Skarpe and Hester, 2008). Recent studies also indicate that “Plant secondary metabolites tend to increase with decreasing plant nutrient concentration following intermediate browsing or less” (Scogings *et al.*, 2013). According to Scogings *et al.* (2011) the response of CBSMs to severe browsing intensity is inconsistent among studies in savannahs.

A growing body of knowledge indicates that plants might respond to actual and simulated browsing by increasing the remobilisation of resources from damaged and undamaged tissues to stem and roots. This process is called “herbivore-induced resource sequestration” (Gadd *et al.*, 2001; Ward and Young, 2002; Holland, 2012). Plants activate a defence mechanism when herbivory is initiated so that the resources are not wasted before there is a need for defence (Ward and Young, 2002). According to the Resource Availability Hypothesis of Coley *et al.* (1985), plants growing in highly fertilised conditions should invest heavily in growth as resources are not limited. Conversely, plants from nutrient-poor conditions should invest more in defence traits because they cannot replace lost tissues easily as a result of limited available resources. While most studies have measured browser-induced changes in either growth or CBSMs in savannahs, few have considered the responses of above ground plant-growth, CBSMs, nutrient concentration and plant-fibre components simultaneously (Scogings *et al.*, 2013). This study is designed to address this gap in the literature.

### 2.3. The role of flavonoids in plants

Flavonoids are the building blocks of condensed tannins and are one of the most widely studied groups of phenolics (Weston, 2013). These phenolic compounds have a variety of functions in plants: (1) Combine to create flower pigmentation which serve as signals for pollinators. (2) Block UV radiation destructive to nucleic acids. (3) Allow selective admittance of Blue-Green and Red light for photosynthesis. (4) Reduce the palatability of plants or cause herbivores to avoid the plants altogether. (5) Affect interaction of plants with other organisms: Inhibiting or encouraging bacteria and mychorizae associations (Weston, 2013). Flavonoids are a diverse group of secondary metabolites that are divided into subgroups including: Anthocyanins, Flavonols, Flavones, IsoFlavones (Weston, 2013). All these subgroups possess a wide range of biological activities. The defence-related flavonoids are divided into two groups: preformed and induced flavonoids (Treutter, 2006). The preformed flavonoids are synthesised during the normal development of plant tissues while the induced flavonoids are synthesised by plants following physical injury or stress (Gadd, 2002; Gallet *et al.*, 2004). The preformed flavonoids play a signalling role or defence role in plants (Treutter, 2006). The induced compounds occur as phytoalexins only after infection or several types of stress (Treutter, 2006).

Most studies assume that plant secondary metabolites (and among them flavonoids), have evolved for defence against herbivores (Treutter, 2006). In non-adapted herbivores, flavonoids may reduce the nutritive value of their feed by behaving as antifeedants, digestibility reducing agents, and as toxins (Chen *et al.*, 2004; Thoison *et al.*, 2004). In addition to the ecological factors that induce flavonoids, nutrient supply can modify secondary metabolism and hence flavonoids (Treutter, 2006) by controlling growth and differentiation processes (Herms and Mattson, 1992). Therefore, the stimulation of both flavonoid's biosynthesis and accumulation with a defence-promoting goal may be limited by

substrate availability, e.g., energy and browsing intensity alters root/shoot ratio and thus carbon supply (Treutter, 2006). The Growth-differentiation-balance hypothesis (Herms and Mattson, 1992), Carbon-Nutrient-balance hypothesis (Bryant *et al.*, 1983), and Resource-Availability hypothesis (Coley *et al.*, 1985) all generally assume that the production of CBSMs is limited by the availability of photosynthates and that growth processes dominate over differentiation or production of condensed tannins (CT) as long as conditions are favourable for growth. Conversely, when growth is more limited than photosynthesis then allocation towards CT will increase (Herms and Mattson, 1992). Furthermore, there are several reports of an increase in flavonoids with simultaneous reduction in growth in N-deficient plants (Stewart, 2001; Mattson, 2004; Penderis and Kirkman, 2014).

#### **2.4. The role of condensed tannins in plants**

Tannins are high molecular weight polyphenols defined by their capacity to bind proteins which help plants to defend themselves against herbivory through the effects on protein digestion (Harinder, 2003). There are two main classes of tannins with differing biological functions; namely hydrolysable tannins and condensed tannins. Jachmann and Bell (1985) proposed that hydrolysable tannins (ellagi and gallo-tannins) serve to deactivate the gastrointestinal enzymes of herbivores, while condensed tannins (proanthocyanidins) are attached to the cellulose and fibre-bound proteins of cell walls, thereby protecting plants against microbial and fungal attack. Furthermore, condensed tannins minimise methane and nitrous oxide (N<sub>2</sub>O) production of ruminants (Hassanat and Benchaar, 2013). In leaf litter, condensed tannins are a main source of carbon for soils and influence litter breakdown and nutrient cycling (Tharayil, 2011). Therefore, condensed tannins are important components of ecosystems because of their roles in facilitating important ecological processes (Iason *et al.*, 2012).

## 2.5. Role of nitrogen in plants

Either increasing N levels in the soil or decreasing browsing intensity reduces carbon limitation in plants (Herms and Mattson, 1992). Nitrogen is positively correlated to shoot growth since high levels of soil N favours shoot growth (Scogings, 1998; Rooke and Bergström, 2007). Nitrogen is essential in plants for making amino-acids, enzymes, and leaf chlorophyll (the concentration of which is correlated with plant N content) (Daughtry *et al.*, 2000). Organic N is required by plants to allow carbohydrates to be utilised for growth (Theodoridou, 2010). However the relationship between foliar N and leaf chlorophyll differ in species and was found not to be strong enough to eliminate the need for foliar analysis for foliar N content (Klooster *et al.*, 2012). Nitrogen concentration in the leaves influences leaf nutritional value and hence forage quality.

The concentration of nutrients in plants is said to be mainly related to growth dynamics and nutrient supply in the growing season (Mengel and Kirby, 1987). Mineral nutrient concentration increases due to the high rate of nutrient allocation as compared to growth rate (Mengel, 1987). Mineral nutrient concentration in the leaf and stem tissues decreases due to rapid growth, therefore plant chemical composition or plant quality as feed for herbivores changes during the growing season (Hanley and McKendrick, 1983). Plant nitrogen is assumed to be positively related to plant protein content and dry matter digestibility (DMD) therefore it is used as an index of plant quality and hence an index of digestible energy (Robbins, 1993). Digestible energy and protein are the nutritional factors most likely to restrict herbivores (Robbins, 1993). Associated with browsing and nutrient content are other major environmental factors such as soil moisture content, temperature and length of the growing season (Schimel *et al.*, 2004).

# CHAPTER THREE

## 3. MATERIALS AND METHODS

### 3.1. Study area

This research was conducted at the Mondi Tree Improvement Nursery near kwaMbonambi, KwaZulu-Natal, South Africa (28° 36' S; 32° 05' E). The average midday temperatures range from 23°C in June to 29°C in January (Climates, 2014). Average annual rainfall at this area is high; approximately 1400mm per annum (Pillay and Ward, 2012), which eliminates the need for irrigation in this study. The soil at the site is sandy and acidic (Table 2.1), which limits nutrient availability. The type of land use or agricultural practices in kwaMbonambi is growth of timber and sugarcane.

**Table 2.1:** Results from soil analysis conducted prior to the start of the experiment at the study site (unpublished data). The analyses were done by Soil Fertility & Analytical Services, Cedara. Results are concentrations of key plant nutrients, pH, and clay content.

Soil nutrients	N	Mean	Std. Deviation
P (mg/L)	6	72.17	12.922
K (mg/L)	6	76.50	12.406
pH (KCl)	6	4.1800	0.21270
Total Cations (cmol/L)	6	2.1983	0.32896
MIR N (%)*	6	0.1017	0.05419
MIR Organic C (%)*	6	2.1667	0.96885
MIR Clay content (%)*	6	10.6667	3.72380

\*Total nitrogen, organic carbon, and clay content were estimated by mid infrared (MIR).

### 3.2. Study species

This research was performed using young marula (*Sclerocarya birrea subsp. caffra*) trees that had not yet reached reproductive age by the time the experiment was terminated. This was to avoid fruit production interrupting carbon allocation to shoot growth and other tissues during the experiment. Marula saplings were chosen for this experiment for various reasons, namely: (1) they have a fast growth rate which is ideal for a short term study (Van Wyk *et al.*, 1997), (2) leaf size is suitable for the use of the Dualex series4 instrument in determining flavonoid

content, (3) the species is adapted to growing in sandy soils (Van Wyk *et al.*, 1997), like those at the study site, and (4) it is an iconic species in savannahs (Van Wyk *et al.*, 1997), but its responses to factors such as browsing have been poorly studied.

### **3.3. Experimental setup**

Fertiliser and clippings (Appendix A) were used to manipulate plant productivity and simulate browsing respectively. Thirty factorial treatments involving five levels of inorganic fertiliser and six levels of clipping, each with 10 randomly selected replicates, were applied to the marula saplings. A soil productivity gradient was achieved by applying fertiliser as topdressing around each plant in September 2013 and every two months thereafter to maintain constant soil nutritional value throughout the experimental period. The ground around the base of each plant was cleared every two months to eliminate grass competition for water and nutrients and trees were sprayed monthly with pyrethroid insecticides to control insect herbivores. A gradient of browsing intensity was created by manually clipping the saplings to simulate browsing in October 2013. Clipping at this time allows for compensatory growth to take place during the wet season, since plant physiological responses are far more pronounced in the wet season than the dry season, (Scogings *et al.*, 2012) and thus far easier to detect. Physical study variables were quantified prior to fertiliser application at the start of the experiment (i.e. early August 2013) and just before the trees were defoliated at the end of the experimental period (April 2014). Chemical variables were quantified at the time of clipping (October 2013) and at the end of the experimental period (April 2014) using the leaf material that was clipped and defoliated respectively. This design allows quantification of: (1) initial conditions prior to application of the two treatment factors and (2) responses of measured variables to the treatments. The experiment was concluded in April 2014 before the start of the dry season.

### 3.4. Data collection

#### 3.4.1. Treatments

A composite fertiliser N:P:K (3:1:5)(26) (Wonder™) containing slow release nitrate was used for this experiment. The fertiliser treatment was applied by topdressing at a rate of: 0, 30, 60, 120 and 180g per plant in September 2013 and every two months thereafter, according to manufacturer recommendation. Fertiliser was applied when rain was expected to fall the following day, to facilitate infiltration of nutrients into the soil while eliminating the need for irrigation. Fertiliser application rates were determined in a pilot study in 2012/2013 and N:P:K (3:1:5)(26) fertiliser was superior in terms of stimulating growth as compared to other studied fertilisers (unpublished data). Contamination between treated saplings and controls was minimised: The trees were planted approximately 1.5m apart; the gradient of the study site was flat, and the soil had good infiltration so drift of fertiliser nutrients amongst trees was unlikely.

Clipping (Appendix A) was performed to simulate browsing during the start of the wet season (late October 2013) since plant physiological responses are far more pronounced in the wet season than in the dry season, (Scogings *et al.*, 2012). For clipping, branches were counted and classified into small, medium and large branches, e.g., a branch that was 12cm long and 1cm in diameter was described as medium. Small and medium-sized branches were entirely consumed (clipped near its base) while larger branches were addressed by classifying the sub-branches into small, medium or large size classes and then clipped accordingly. Clipping started at the highest branch, working downwards and was performed by the same person throughout to maintain consistency. The following clipping intensities were performed: 0% (control), 25% (where every 4th branch in each class was clipped), 50% (where every 2nd branch in each class was clipped), 75% (where three out of every four branches in each class were clipped), 100% (clipping 90-100% of available big, then medium

and lastly small branches) and 100% defoliation (stripping of 90-100% of leaflets; the way in which the leaves were stripped resulted in many of the midribs being left behind, which were not removed). Branches were classified because browser bite-size differs in relation to branch size. Clipping at the start of the wet season allows post-browsing growth of new shoots to occur during the growth season (Scogings *et al.*, 2012).

### **3.4.2. Quantification of physical traits**

#### Tree height

The height of each tree was measured (in cm; to the tip of highest shoot) using a measuring rod, in early August 2013, before the fertiliser and clipping treatments were applied and in early February 2014 before the experiment was terminated. August 2013 tree height was used in the statistical analyses to control for the variation in initial tree size. Tree height in early February 2014 was measured in response to the effect of clipping and fertiliser.

#### Stem diameter

The stem diameter of each tree was consistently measured (in mm) at 10cm above ground level using digital callipers before the time of fertiliser and clipping treatment application in early August 2013, and in early February 2014 before the experiment was terminated. As with initial tree height, the first stem diameter measures were used to control for variation in initial stem diameter size between replicates, and the data collected just before the end of the experimental period were used to assess the effect of clipping and fertiliser.

#### Old shoot extension

In August 2013 (before fertiliser application) all available shoots (>1cm in length) per tree were measured (in cm) with a tape measure from the base of the shoot to the base of the apical bud and marked at the base of the shoot with correction fluid. In February 2014 (after clipping and regrowth during the wet season), shoots with correction fluid were re-measured

from the base of the shoot to the base of the newly formed apical bud. The shoot lengths were averaged per tree. The average initial old shoot lengths per tree were subtracted from the average final old shoot extension per tree to assess the effect of fertiliser and clipping treatments on old shoot extension at the end of the experimental period.

#### Number of new shoots

The number of new shoots per tree was determined by counting shoots with no correction fluid marker in February 2014 to assess the effect of clipping on the number of new shoots (as form of compensatory growth) along the productivity gradient from October 2013 to February 2014.

#### New shoot length

The length of each new shoot (without correction fluid marker) per tree was measured (in cm) from its base to the base of the apical bud using a measuring tape in early February 2014. This was done to quantify the effect of the interaction between clipping and fertiliser on new shoot length production during the experimental period. New shoot lengths were averaged per tree.

#### Biomass

The leaf and shoot material resulting from the clipping treatments applied in October 2013 was separated, oven dried at 30°C, and weighed (in g): thus quantifying the biomass removed in each of the clipping treatments to confirm that applied clipping intensities were precise. At the end of the experimental period, in early April 2014, all trees were 100% defoliated by hand. The leaf material was used to quantify the effect of the interaction between clipping and fertiliser on leaf biomass production during the experimental period.

### 3.4.3. Quantification of chemical traits

Ten random samples per fertiliser treatment were taken from the clipped material in October 2013 to determine the initial effect of fertiliser on studied leaf chemical traits.

#### Nitrogen

Leaf nitrogen content was determined using a LECO TruSpec (FP628) Nitrogen Analyser. Leaf material collected in October 2013 and April 2014 was oven-dried at 30°C and then weighed to 0.1g in foils used to encapsulate samples and then placed into the nitrogen analyser. Nitrogen concentration was expressed as a percentage (%). Results from leaf material collected in October 2013 were used to assess the effect of fertiliser treatment at the start of the experiment. The results from the leaf material collected in April 2014 were used to assess the effect of the interaction between fertiliser and clipping treatments at the end of the experimental period.

#### Flavonoid content

Thirty to thirty-five mature leaves per tree (green stage) with pink midrib and green leaflets were analysed for flavonoid content using a Dualex series 4 instrument. Values were averaged per tree. Flavonoid content was measured in mid-November 2013, mid-December 2013, mid-January 2014 and late March 2014. The November 2013 flavonoid measures from unclipped trees were used to verify the effect of fertiliser on marula saplings in the early stages of the experiment. The March 2014 flavonoid was quantified for all trees to monitor the effect of fertiliser and clipping at the end of the experimental period. When measuring flavonoid content, the Dualex series 4 instrument also measures air temperature and because air temperature is an important factor in determining flavonoids (A *et al.*, 2010), it was included in the statistical analysis of flavonoids as a covariate.

#### Condensed tannins

Dried leaves were milled to a particle size of 0.5mm, after which condensed tannin concentrations were determined using the Acid-butanol assay (Porter *et al.*, 1985). To convert absorbance to condensed tannins, the initial sample mass inverse was determined and then multiplied by the product of absorbance (obtained by the Acid-butanol assay) and slope (from regression line for purified *Sorghum*;  $y = 7.017x + 0.146$ ) (Hattas and Julkunen-Tiitto, 2012), and then again multiplied by 30 which is the standard factor for *Sorghum* tannin equivalent (Porter *et al.*, 1985). Condensed tannin concentrations were expressed as *Sorghum* tannin equivalents (STE) (Porter *et al.*, 1985).

#### Neutral-detergent fibre (NDF) and acid-detergent fibre (ADF)

Sample masses of 0.45-0.55g were weighed into filter bags. Neutral detergent (ND) solution for NDF and acid detergent (AD) solution for ADF was added into the bag suspender. The samples were then agitated and heated for 75 minutes using a fibre analyser. After the extraction, samples were rinsed with water and alpha-amylase twice for 5 minutes (Note: no alpha amylase for ADF). The samples were then soaked in acetone for 3-5 minutes. After soaking, the samples were air dried and oven-dried at 102°C for 2-4 hours. Thereafter the samples were cooled to ambient temperature in desiccators and weighed. The following formula was used to determine both NDF and ADF:

$$\%NDF \text{ or } \%ADF = \frac{W3 - (W1 \times C1)}{W2} \times 100$$

Where W1=bag tarred weight, W2=sample weight, W3=dried weight of bag with fibre after extraction process and C1= blank bag correction (final oven-dried weight divided by the original blank bag weight).

#### Acid-detergent lignin (ADL)

Sulphuric acid (72%; 25ml at room temperature) was added to the filter bags with residues from the ADF determination and extraction was carried out for three hours while stirring every hour. The samples were then rinsed three times with boiling water and oven dried at 105°C. The samples were then cooled in desiccators and weighed. To determine acid detergent lignin:

$$\%ADL = \frac{W3 - W1}{W2} \times 100$$

Where W1=bag tarred weight, W2=sample weight, W3=dried weight of bag with fibre after extraction process.

### 3.5. Statistical analysis

All the data for studied variables were first checked for normality using IBM SPSS® statistics version 23. The data that were not normally distributed were  $\log_{10}$  transformed to meet the assumptions of parametric statistical testing. The initial effect of fertiliser on flavonoids (November 2013), nitrogen, condensed tannins, NDF, ADF and ADL in October 2013 was tested using a one-way analysis of variance (ANOVA). The effect of the interaction of clipping and fertiliser on tree heights, stem diameter, old shoot extension, number of new shoots, new shoot lengths, leaf biomass, N, flavonoids, CT, NDF, ADF, and ADL with trees as replicates was analysed using a two-factor analysis of covariance (ANCOVA). Since not all trees were the same size at the start of the experiment, and because trees of different sizes are not expected to respond uniformly, initial tree height and initial stem diameter were included in the ANCOVA model as covariates. For flavonoid data, air temperature measured at the time of each recording was included as an extra covariate in the ANCOVA model, because it is known that air temperature may affect flavonoid concentration (Beatriz *et al.*, 2005). All studied variables were dependent variables in the model. If either of the covariates interacted significantly with either of the two fixed factors then the ANCOVA was

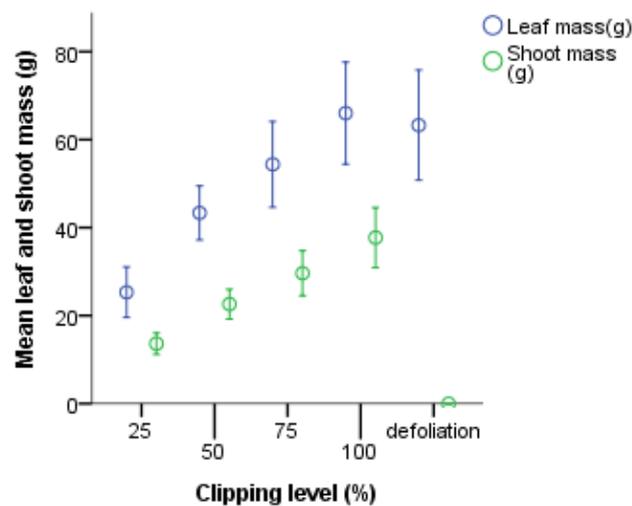
abandoned and linear regression analyses between the covariate and the response variable in each level of the factor with which the covariate interacts were conducted instead. Tukey's post-hoc test was used to separate the means. Type III sum of squares was used for unequal sample sizes. The level of significance was confirmed when  $P < 0.05$ . Possible trends were not ignored when  $0.10 > P > 0.05$ .

# CHAPTER FOUR

## 4. RESULTS

### 4.1. Effect of fertiliser on chemical tree traits in October 2013

The clipping treatment that was applied in October 2013 indicated that the amount of tree material that was initially removed was representative of each clipping level performed because an increase in clipping level resulted in a consistent increase in leaf and shoot material (dry mass) removed (Figure 4.1.1). Leaf mass removed from defoliated plants was similar to that of 100% clipped plants (60-70g), and the other clipping treatments were roughly proportionally less (e.g., ~20g shoot from the 50% and ~40g shoot from the 100% clipped plants; Figure 4.1.1).



**Figure 4.1.1:** Mean ( $\pm$  SE) leaf and shoot material (dry mass) removed in each clipping treatment in October 2013.

### Nitrogen

Fertiliser application was positively significant in explaining [N] before clipping took place in October 2013 ( $P=0.001$ ). In general [N] increased with fertiliser application rates (Figure 4.1.2a;  $P=0.001$ ), however statistically significant differences existed for [N] only between 180g/tree of fertiliser and control plants [difference between the means (MD) = 0.1414,

P<0.001], and 30g/tree (MD=0.912, P=0.031) and 60g/tree (MD=0.969, P=0.019) respectively. Statistically insignificant results are not reported.

### Flavonoids

Flavonoids for unclipped trees at the start of the experiment were not significantly influenced by fertiliser application but were significantly influenced by air temperature, as a covariate (P=0.042). There was no significant interaction between air temperature and flavonoids therefore the figure indicating the interaction between air temperature and flavonoids was not included. Though not significant, the trend of decreasing flavonoids after 60g/tree of fertiliser application was not ignored because of the similarity with that of tannins following increased fertiliser rates (Figure 4.1.2b and 4.1.2c).

### Condensed tannins

Condensed tannin concentrations of trees treated with different fertiliser application rates differed significantly at the start of the experiment (Figure 4.1.2c; P=0.020). There was a negative significant effect of increase in fertiliser application (P=0.020). Statistically significant differences for tannins were found between control plants and 30g/tree (MD= -9.63, P=0.020), and between 30g/tree of fertiliser and 120 (MD=11.55, P=0.007) and 180g/tree (MD=13.25, P=0.002) respectively. Insignificant results are not reported.

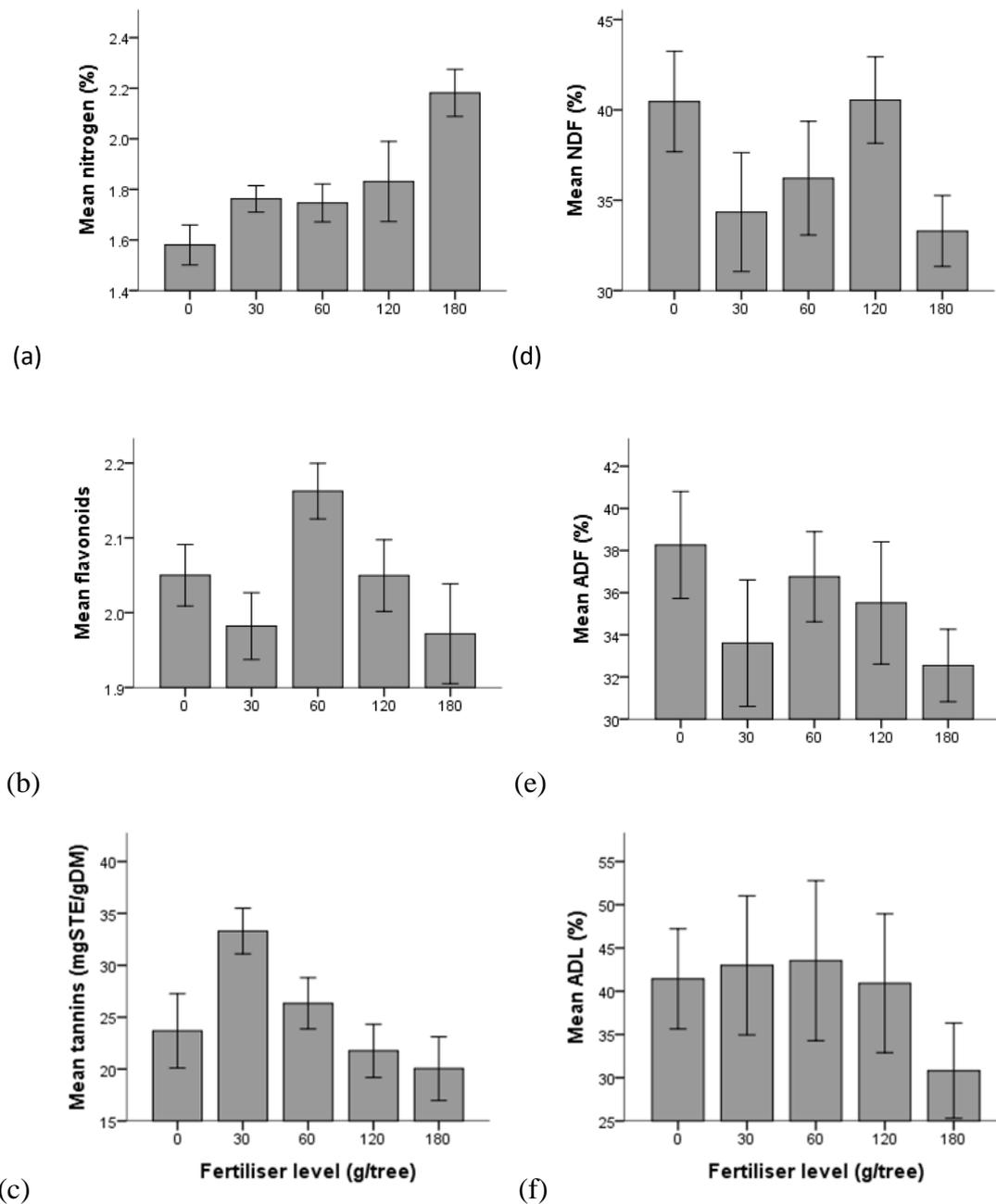
### Neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL)

Fertiliser application had no significant effect on NDF%, ADF, and ADL at the start of the experiment (Table 4.1.1; Figure 4.1.2d, e, and f). Even though the means for NDF were initially less than the control plants, there was an initial increase of NDF when fertiliser application was increased to 120g/plant but further increasing fertiliser rates reduced NDF (Figure 4.1.2d). For ADF, fertiliser application resulted in lower values than the control

plants for all levels, although not significant (Figure 4.1.2e). Percentage ADL values were somewhat lower at 180g/tree fertiliser application rate than for all other treatments although again, no significant differences were found (Figure 4.1.2f).

**Table 4.1.1:** ANOVA statistics for leaf chemical traits assessed from the October 2013 sample per tree in relation to fertiliser application on Marula saplings. Flavonoids were measured from unclipped saplings in November 2013 with air temperature as a covariate.

Dependent variable	Source	Type III				
		Sum of Squares	df	Mean Square	F	P
Nitrogen (%)	Fertiliser	0.106	4	0.026	5.860	0.001
	Error	0.203	45	0.005		
Flavonoids	Fertiliser	0.146	4	0.036	1.791	0.149
	Temperature	0.090	1	0.090	4.409	0.042
	Error	0.833	41	0.020		
	Fertiliser	1076.684	4	269.171	3.228	0.020
Condensed tannins (mgSTE/gDM)	Error	3836.041	46	83.392		
	Fertiliser	459.644	4	114.911	1.509	0.216
NDF (%)	Error	3426.206	45	76.138		
	Fertiliser	214.121	4	53.530	0.851	0.500
ADF (%)	Error	2829.590	45	62.880		
	Fertiliser	1088.771	4	272.193	0.489	0.744
ADL (%)	Error	25035.847	45	556.352		



**Figure 4.1.2:** Means ( $\pm$  SE) of leaf chemical traits assessed in response to fertiliser application on Marula saplings (n=10). Flavonoids were measured from unclipped saplings in November 2013.

## 4.2. Effect of the interaction between fertiliser and clipping treatment

### 4.2.1. Aboveground tree traits

#### Tree height

Both studied fixed factors significantly interacted with the initial tree height (as a covariate) in explaining final tree height (Figure 4.2.1a and d; Table 4.2.1). Clipping significantly

interacted with the initial stem diameter in explaining final tree height ( $P < 0.001$ ) but the figure indicating the interaction was not included because it was not effective. With the exception of 50% clipping intensity (grey line), there was a clear pattern of progressively increasing negative effects of increasing clipping intensity in initial tall trees (ranging between 100-120cm; Figure 4.2.1a). Conversely, in initially short trees (<60cm), defoliation resulted in final tree height similar to that of control trees and 100% clipped trees resulted in lowest final tree height, indicating a mixed pattern (Figure 4.2.1a). There were increasingly positive effects of high fertiliser rates in initial tall trees, but not in initial short trees, e.g., in Figure 4.2.1d, when initial tree height was greater than 100cm then high fertiliser rates (the purple 120 and yellow 180g/tree lines) had the greatest positive effect on final tree height, but when initial tree height was less than 80cm, the response to fertiliser rates was less strong (the purple 120 and yellow 180g/tree lines are similar to that of control plants; Figure 4.2.1d).

**Table 4.2.1:** ANCOVA statistics for tree height and stem diameter according to treatment groups (fertiliser and clipping) and their interaction with initial tree height and stem diameter as covariates.

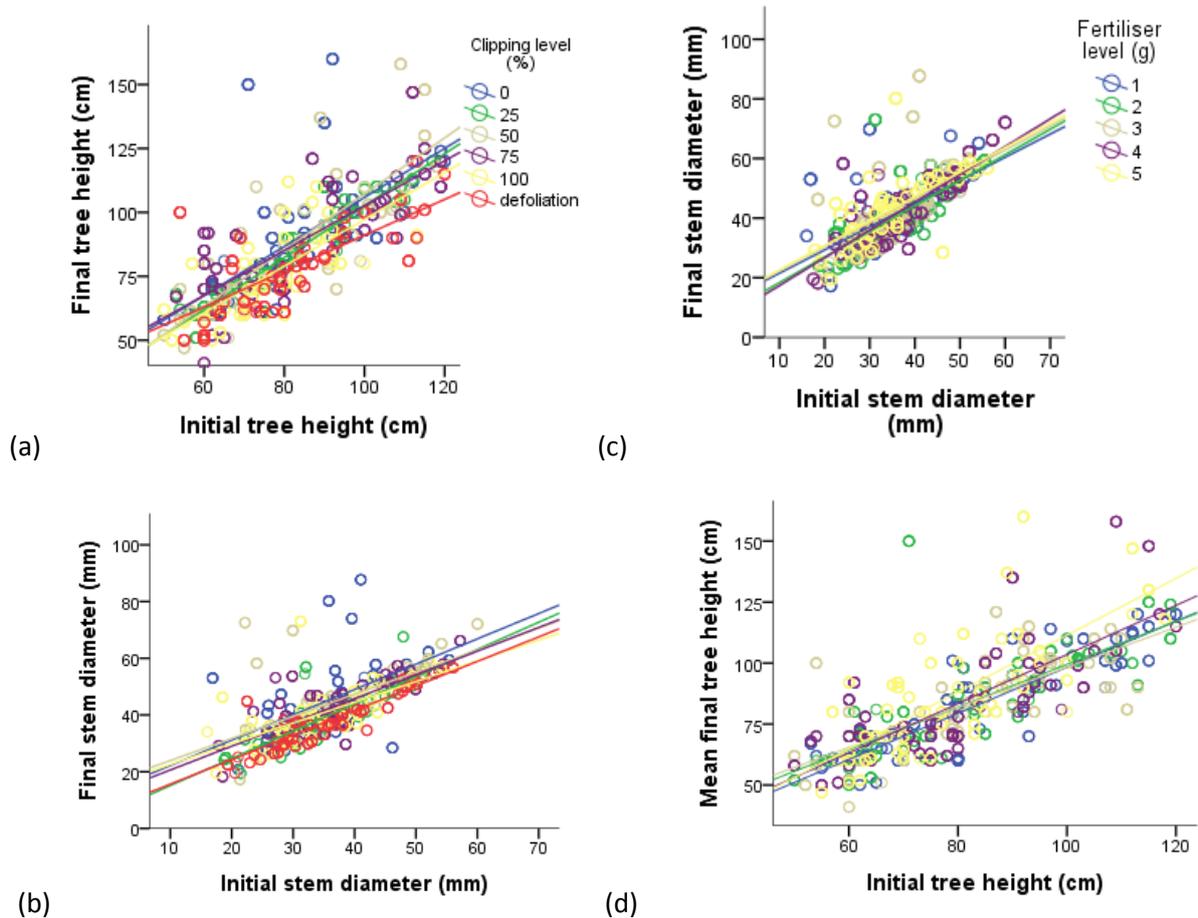
Dependent variable	Source	Type III					
		Sum of Squares	Df	Mean Square	F	P	
Tree height	Fertiliser	0.006	4	0.002	0.503	0.733	
	Clipping	0.101	5	0.020	6.649	<0.001	
	Fertiliser * Clipping	0.346	20	0.017	5.679	<0.001	
	Initial tree height	0.139	1	0.139	45.600	<0.001	
	Clipping * Initial tree height	0.131	5	0.026	8.608	<0.001	
	Fertiliser * Initial tree height	0.343	5	0.017	5.645	<0.001	
	Clipping * Initial stem diameter	0.081	6	0.013	4.429	0<.001	
	Error	2.860	939	0.003			
	Stem diameter	Fertiliser	0.158	4	0.039	7.736	<0.001
		Clipping	0.089	5	0.018	3.491	0.004
Fertiliser * Clipping		0.536	20	0.027	5.248	<0.001	
Initial tree height		0.044	1	0.044	8.590	0.003	
Initial stem diameter		0.031	1	0.031	6.005	0.014	
Clipping * Initial tree height		0.074	5	0.015	2.914	0.013	
Fertiliser * Initial tree height		0.185	4	0.046	9.079	<0.001	
Clipping * Initial stem diameter		0.058	5	0.012	2.266	0.046	
Fertiliser * Initial stem diameter		0.127	4	0.032	6.229	<0.001	
Error		4.752	931	0.005			

Non-significant interactions between studied fixed factors and the corresponding covariates in each analysis were excluded from the final models.

### Stem diameter

Both studied fixed factors significantly interacted with the initial tree height and stem diameter (as covariates) in explaining final stem diameter (Figure 4.2.1b and c; Table 4.2.1). Figures indicating the interaction between studied fixed factors and initial tree height in explaining final stem diameter were not included because they were not effective. There was a clear pattern on increasing negative effects of increasing clipping intensity in initially broad stemmed trees, but not in initially narrow stemmed trees (Figure 4.2.1b). When initial stem diameter was ranging between 50-60mm then severe clipping intensity (the shallower slopes of the red defoliation line or yellow 100% line) had the greatest negative effect on final stem diameter (Figure 4.2.1b). When initial stem diameter was close to 20mm then the response to

clipping intensity was mixed (the green 25% line and the red defoliation line are lower than the others, and the grey 50% line had the greatest increase in final stem diameter; Figure 4.2.1b). There was an increasing positive effect of increasing fertiliser application in initial broad stemmed trees (Figure 4.2.1c). It can be seen that when initial stem diameter was ranging between 60-70mm then high fertiliser rates (the purple 120g line and yellow 180g line) had the greatest positive effect in final stem diameter, but with a 120g line resulting in the greatest increase compared to others (Figure 4.2.1c). In initial narrow stemmed trees, there was no clear pattern of final stem diameter relative to increase in fertiliser rates (Figure 4.2.1c).



**Figure 4.2.1:** Mean final tree height (cm) and stem diameter (mm) per tree ( $\pm$ SE) at the end of the experimental period in February 2014 for *Sclerocarya birrea* saplings as a result of the

interaction between fixed factors (clipping and fertiliser) and covariates (initial tree height and stem diameter) (n≈200). Linear models were fitted for each level of the fixed factor.

**Table 4.2.2:** Refers to figure 4.2.1; significant linear models within each treatment interacting with a covariate for tree height and stem diameter at the end of the experiment.

		r <sup>2</sup>	F	P	Equation
Tree height	Clipping*Initial tree height				
	Clipping level =0	0.456617	F <sub>1,222</sub> =185.711	0.001	Y=0.959178x+9.95164
	Clipping level =25	0.360237	F <sub>1,139</sub> =77.7049	0.001	Y=0.649218x+30.2655
	Clipping level =50	0.676281	F <sub>1,157</sub> =328.989	0.001	Y=1.09765x-2.55455
	Clipping level =75	0.604486	F <sub>1,184</sub> =279.689	0.001	Y=0.877525x+14.8825
	Clipping level =100	0.496593	F <sub>1,168</sub> =164.739	0.001	Y=0.9146602x+5.8256
Tree height	Fertiliser*Initial tree height				
	Fertiliser Level=0	0.769576	F <sub>1,198</sub> =657.944	<0.001	Y=0.944466x+3.85775
	Fertiliser Level=30	0.54439	F <sub>1,186</sub> =221.049	<0.001	Y=0.877193x+11.7946
	Fertiliser Level=60	0.550462	F <sub>1,198</sub> =241.228	<0.001	Y=0.821332x+16.1797
	Fertiliser Level=120	0.596872	F <sub>1,217</sub> =319.81	<0.001	Y=1.0074x=2.80182
	Fertiliser Level=180	0.560656	F <sub>1,207</sub> =262.881	<0.001	Y=1.16554x-5.08569
Stem diameter	Clipping*Initial stem diameter				
	Clipping level =0	0.283391	F <sub>1,222</sub> =87.397	0.001	Y=0.89305x+13.268
	Clipping level =25	0.725852	F <sub>1,139</sub> =365.377	0.001	Y=0.959956x+5.52993
	Clipping level =50	0.360874	F <sub>1,157</sub> =88.0832	0.001	Y=0.778603x+161778
	Clipping level =75	0.623543	F <sub>1,184</sub> =303.112	0.001	Y=0.837721x+12.2447
	Clipping level =100	0.421918	F <sub>1,168</sub> =121.886	0.001	Y=0.730807x+15.2753
Stem diameter	Fertiliser*Initial stem diameter				
	Fertiliser Level=0	0.43983	F <sub>1,198</sub> =154.679	0.001	Y=0.777549x+13.7563
	Fertiliser Level=30	0.494548	F <sub>1,186</sub> =181.009	0.001	Y=0.858159x+9.75315
	Fertiliser Level=60	0.258833	F <sub>1,198</sub> =68.7971	0.001	Y=0.808689x+14.2954
	Fertiliser Level=120	0.662689	F <sub>1,217</sub> =424.359	0.001	Y=0.932028x+8.05938
	Fertiliser Level=180	0.444687	F <sub>1,207</sub> =164.962	0.001	Y=0.822237x+14.3288
November flavonoids	Fertiliser*Temperature				
	Fertiliser Level=0	0.058643	F <sub>1,9</sub> =0.498	0.500	Y=-0.019139x+2.6165
	Fertiliser Level=30	0.047512	F <sub>1,8</sub> =0.349	0.573	Y=0.020493x+1.3969
	Fertiliser Level=60	0.506743	F <sub>1,8</sub> =7.191	0.032	Y=0.0915x-0.582556
	Fertiliser Level=120	0.300806	F <sub>1,9</sub> =3.442	0.101	Y=0.041490x+0.81734
Fertiliser Level=180	0.217772	F <sub>1,8</sub> =1.949	0.205	Y=0.065644x+0.03893	

### Old shoot extension

Fixed studied factors and their interaction had a significant effect in explaining old shoot extension (Table 4.2.3). Except for defoliation treatment, clipping significantly reduced old

shoot extension but fertiliser treatment increased old shoot extension in unclipped trees (Figure 4.2.2c). The interaction between clipping and fertiliser was due to trees clipped at the highest intensity not responding to fertiliser in the same way as other trees (Figure 4.2.2c). Trees that were 75% clipped or less tended to increase old shoot extension with increasing fertiliser rates but the results were not consistent (Figure 4.2.2c). The most severe (100%) clipping treatments significantly reduced old shoot extension at all rates of fertiliser application as compared to control plants (unclipped and unfertilised plants; Figure 4.2.2c). Defoliation treatment tended not to reduce old shoot extension (Figure 4.2.2c). There was a slight increment in old shoot extension when fertiliser was applied even though the means were still less than that of control plants (Figure 4.2.2c).

#### Number of new shoots

The interaction between clipping and fertiliser application had a positive significant effect in explaining the number of new shoots ( $P=0.040$ ). The interaction was due to trees clipped at the highest intensity (100% and defoliation) not responding to fertiliser in the same way as other trees. Plants treated with 75% or less tended to increase in the number of new shoots with the increase in fertiliser rates but the results were not consistent. The most severe (100%) clipping treatments significantly increased the number of new shoots as compared to others, but the number of new shoots decreased with increasing fertiliser application rates within this clipping level (Figure 4.2.2d). The increase in the number of new shoots may have been a direct result of the clipping treatment (25-100% clipping), rather than fertiliser treatment. Defoliation treatment also reduced the number of new shoots regardless of fertilisation rates (Figure 4.2.2d).

**Table 4.2.3:** ANCOVA statistics for old shoot extension and number of new shoots according to treatment groups (fertiliser and clipping) and their interaction.

Dependent variable	Source	Type III				
		Sum of Squares	Df	Mean Square	F	Sig.
Old shoot extension	Fertiliser	1.239	4	0.310	3.697	0.007
	Clipping	5.104	5	1.021	12.186	<0.001
	Fertiliser * Clipping	2.839	19	0.149	1.784	0.028
	Error	13.737	164	0.084		
Number of new shoots	Fertiliser	0.164	4	0.041	0.658	0.622
	Clipping	12.761	5	2.552	40.879	<0.001
	Fertiliser * Clipping	2.108	20	0.105	1.688	0.040
	Error	9.865	158	0.062		

Non-significant interactions between studied fixed factors and the corresponding covariates in each analysis were excluded from the final models.

#### New shoot length

The increase in new shoot lengths was positively significantly explained by clipping and fertiliser treatments separately but not their interaction (Table 4.2.4). The lack of significant interaction between studied fixed factors was explained by trees in low, medium and high clipping intensity responding the same way by increasing new shoot length at all rates of fertiliser application but the increase was not consistent (Figure 4.2.2e). Unclipped trees significantly increased new shoot length at all fertiliser rates as compared to control plants (Table 4.2.4 and Figure 4.2.2e). The longest mean new shoot length in unclipped trees was obtained when fertiliser application was maximised (Figure 4.2.2e). With the exception of defoliation treatment, clipping treatment significantly increased mean new shoot length in all clipping levels but the results were not consistent (Table 4.2.4 and Figure 4.2.2e). Severe

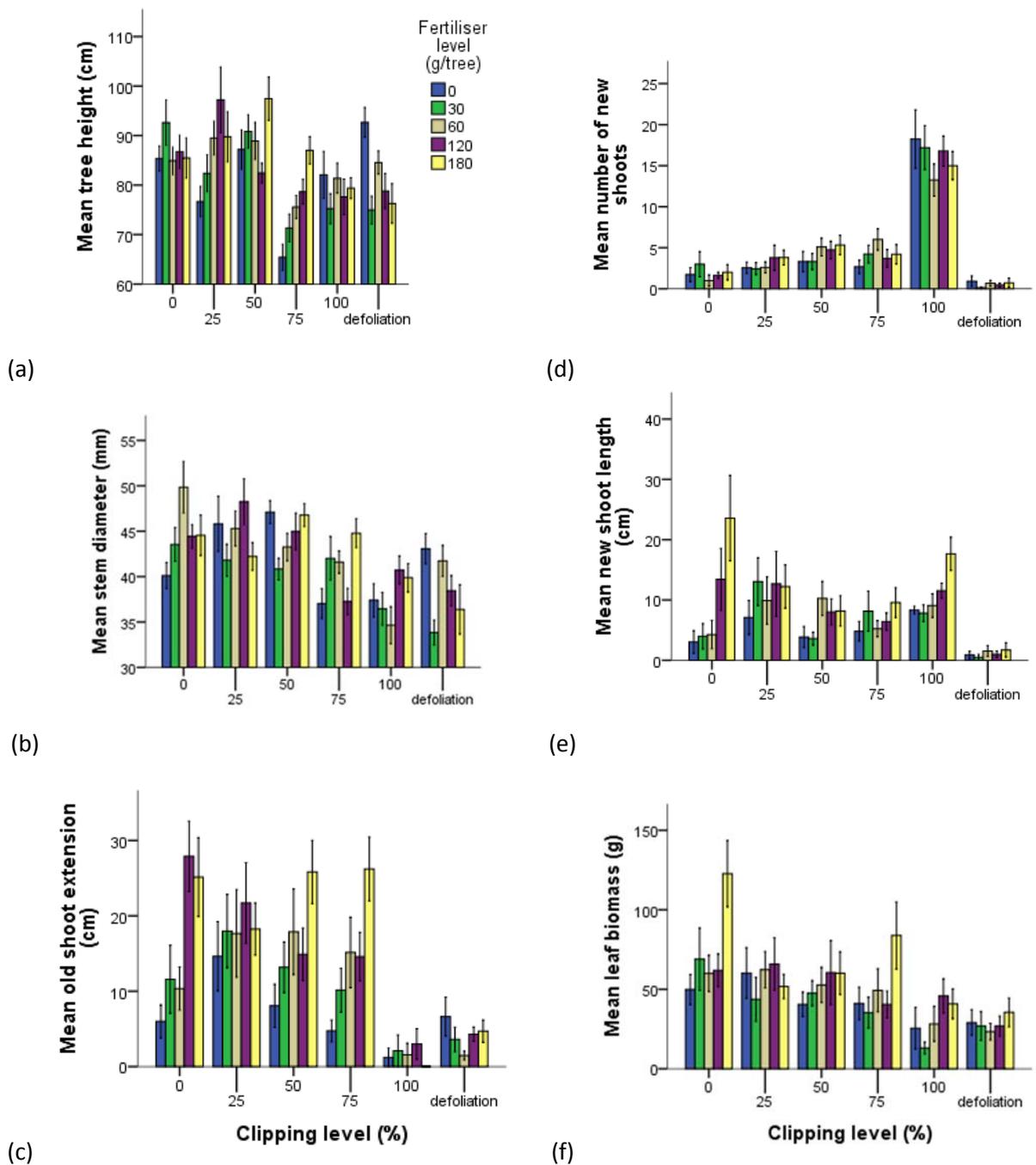
clipping (defoliation) reduced new shoot length relative to unclipped trees and other clipping treatments, but new shoot length increased with fertiliser application even though the means were less than that of the control plants (Figure 4.2.2e).

### Leaf biomass

Clipping and fertiliser treatments separately had a significant effect in explaining leaf biomass but the interaction of these two treatments had no significant effect in explaining leaf biomass (Table 4.2.4). An increase in clipping intensity significantly reduced leaf biomass (Table 4.2.4 and Figure 4.2.2f). In unclipped trees, mean leaf biomass significantly increased with the increase in fertiliser application where 180g/tree of fertiliser resulted in maximum mean leaf biomass (Table 4.2.4 and Figure 4.2.2f). In lightly (25%) clipped trees, medium (60-120g/tree) fertiliser rates increased mean leaf biomass, but not low (30g) or high (180g) fertiliser rates (Figure 4.2.2f). In moderately (50%) clipped trees, the increase in fertiliser application resulted in consistently increased mean leaf biomass as compared to others (Figure 4.2.2f). In high or severe clipping treatments, high fertilisation rates increased leaf biomass, but the results were not consistent (Figure 4.2.2f). This explains the lack of significant interaction between studied fixed factors.

**Table 4.2.4:** ANCOVA statistics for new shoot length and leaf biomass according to treatment groups (fertiliser and clipping) and their interaction.

Dependent Variable	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
New shoot length	Fertiliser	1.364	4	0.341	3.859	0.005
	Clipping	1.991	5	0.398	4.507	0.001
	Fertiliser * Clipping	2.410	20	0.121	1.364	0.148
	Error	13.737	164	0.084		
Leaf biomass	Fertiliser	1.960	4	0.490	2.495	0.043
	Clipping	7.820	5	1.564	7.967	<0.001
	Fertiliser * Clipping	1.898	20	0.095	0.483	0.971
	Error	48.494	247	0.196		



**Figure 4.2.2:** Indicate means of the above-ground tree traits per tree ( $\pm$ SE) assessed in relation to the interaction between fertiliser and clipping treatments on Marula saplings during the fast growth phase in the wet season ( $n \approx 200$ ).

#### 4.2.2. Chemical tree traits

##### Nitrogen

None of the studied fixed factors significantly explained leaf nitrogen content at the end of the experimental period (Table 4.2.5). However, fertiliser alone and the interaction between clipping and fertiliser had marginally significant effects in explaining N at the end of the experimental period, therefore their p-values was not ignored (Table 4.2.5). Clipping tended to reduce [N] in plants that received low amounts of fertiliser but the results were not consistent (Figure 4.2.3a). Clipped trees growing in highly fertilised conditions did not respond the same in terms of [N] when low and high clipping levels were compared (Figure 4.2.3a). Plants that were 50% clipped or less tended to increase [N] when highly fertilised. Conversely, plants that were 75 or 100% clipped tended to reduce [N] regardless of high amounts of fertiliser application, but the results were not consistent (Figure 4.2.3a). Defoliation treatment resulted in plants responding the same way as the plants that received 50% clipping or less (increasing nitrogen following high levels of fertiliser application).

### Flavonoids

Studied fixed factors and temperature (as a covariate) had no significant effect on flavonoids at the end of the experimental period (Table 4.2.5). Clipping treatment alone had a marginally significant effect in explaining flavonoids at the end of the experimental period (Figure 4.2.3b). Clipping tended to reduce flavonoids in plants that received intermediate amounts of fertiliser, increase flavonoids in heavily fertilised plants, but caused a hump-shaped response in unfertilised plants (Figure 4.2.3b).

### Condensed tannins

Both studied fixed factors had no significant effect on [CT] but clipping treatment may have had a marginal effect (Table 4.2.5). Clipping increased [CT] in all clipping levels as compared to control plants (Figure 4.2.3c). When growth is more limited than photosynthesis due to simulated browsing, then allocation towards CT will increase (Herms and Mattson,

1992). With the exception of defoliation treatment, as the clipping intensity was increased it caused a hump-shaped response in [CT] (Figure 4.2.3c). Defoliation treatment tended to result in highest [CT] in plants that were highly fertilised (Figure 4.2.3c).

**Table 4.2.5:** ANCOVA statistics for nitrogen, flavonoids and, condensed tannins according to treatment groups (fertiliser and clipping) and their interaction with initial tree height and stem diameter as covariates.

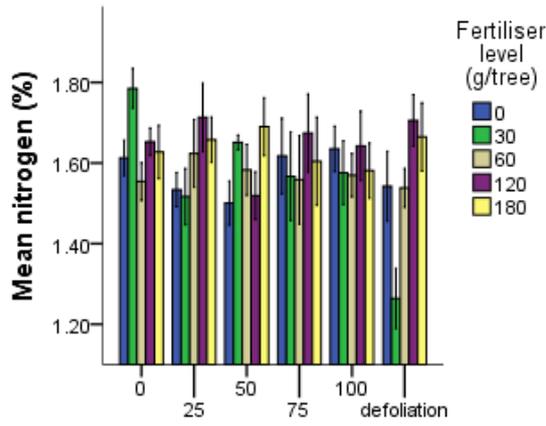
Dependent Variable	Source	Type III Sum of Squares	df	Mean Square	F	P
Nitrogen	Fertiliser	0.404	4	0.101	2.339	0.057
	Clipping	0.195	5	0.039	0.901	0.482
	Fertiliser * Clipping	1.295	20	0.065	1.498	0.085
	Error	8.472	196	0.043		
Flavonoids	Fertiliser	.057	4	0.014	1.428	0.224
	Clipping	.067	5	0.013	1.345	0.244
	Fertiliser * Clipping	.312	20	0.016	1.561	0.059
	Error	3.900	390	0.010		
Condensed tannins	Fertiliser	415.522	4	103.881	1.638	0.166
	Clipping	661.660	5	132.332	2.086	0.069
	Fertiliser * Clipping	1446.824	20	72.341	1.141	0.311
	Error	12939.107	204	63.427		

Neutral-detergent fibre (NDF), acid-detergent fibre (ADF), and acid-detergent lignin (ADL)

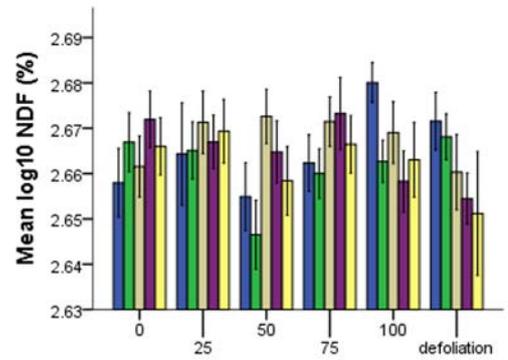
None of the fixed studied factors were significant in explaining NDF and ADF (Table 4.2.6). Acid detergent lignin was positively significantly explained by clipping treatment (Table 4.2.6 and Figure 4.2.3f). Clipping tended to increase ADL of unfertilised plants in all clipping levels and both severe clipping and defoliation treatment resulted in plants with highest ADL ( $F_{5, 241}=2.277$ ;  $P=0.048$  and Figure 4.2.3f) respectively.

**Table 4.2.6:** ANCOVA statistics for NDF, ADF and ADL according to treatment groups (fertiliser and clipping) and their interaction with initial tree height and stem diameter as covariates.

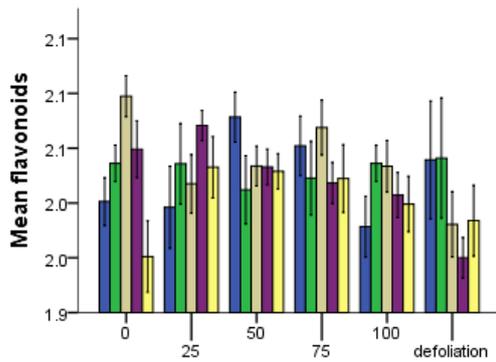
Dependent variable	Source	Type III				
		Sum of Squares	df	Mean Square	F	P
NDF	Fertiliser	1413.070	4	353.268	0.842	0.500
	Clipping	2091.707	5	418.341	0.997	0.421
	Fertiliser * Clipping	9715.672	20	485.784	1.158	0.294
	Error	84315.211	201	419.479		
ADF	Fertiliser	169.100	4	42.275	0.779	0.540
	Clipping	232.724	5	46.545	0.857	0.511
	Fertiliser * Clipping	1036.097	20	51.805	0.954	0.520
	Error	11023.152	203	54.301		
ADL	Fertiliser	0.143	4	0.036	0.640	0.635
	Clipping	0.636	5	0.127	2.277	0.048
	Fertiliser * Clipping	1.153	20	0.058	1.032	0.426
	Error	11.620	208	0.056		



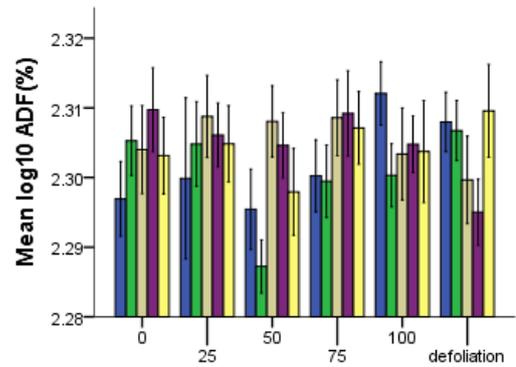
(a)



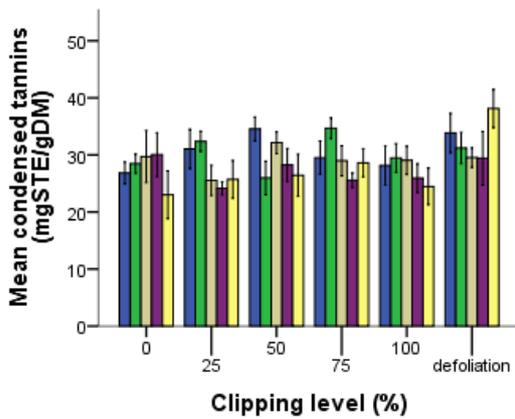
(d)



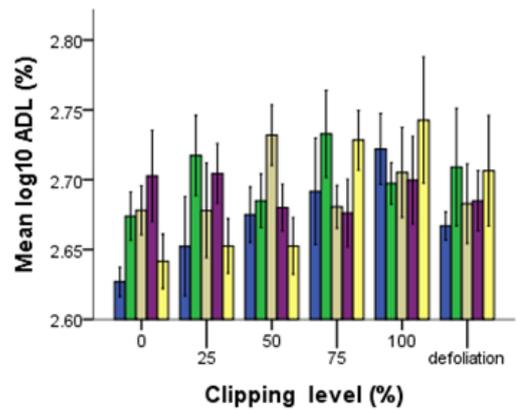
(b)



(e)



(c)



(f)

**Figure 4.2.3:** Represent mean leaf chemical traits assessed per tree ( $\pm$ SE) in relation to interaction between fertiliser and clipping treatment on Marula saplings during the experimental period ( $n \approx 200$ ). To transform  $\log_{10}$  values back to normal, 10 is raised to the power  $\log_{10}$  times value of a variable [i.e.,  $10^{\log_{10}(\text{variable value})}$ ].

# CHAPTER FIVE

## 5. DISCUSSION AND CONCLUSION

### Tree height

Against expectations, initially tall saplings that were intensively clipped (100% clipping and defoliation, Figure 4.2.1a) and growing along a productivity gradient did not increase in height (Figure 4.2.2a), because highly clipped saplings had no leaves for photosynthesis therefore compensatory growth failed to compensate for reduction in photosynthesis caused by loss of foliage (Renton *et al.*, 2007; Mopipi *et al.*, 2009). The failure to compensate after severe clipping can occur because of either carbon-limitation or bud limitation. Carbon-limitation happens if either stored C or photosynthesised C in new leaves that grow after clipping are insufficient for growth in height or stem diameter (Landhäusser and Lieffers, 2012). These results support observations of related studies of species elsewhere (Teague and Walker, 1988b). It is suggested that plant height or stem diameter maybe unaffected following browsing, while shoot biomass can be affected either positively or negatively, suggesting that the responses of shoots do not necessarily reflect the whole aboveground plant performance.

Given that marula is deciduous, it is likely that C reserves were depleted by the first flush of growth at the start of the growth season. Therefore stored C was already limited when clipping was applied. In addition to this, severe clipping did not only delay the availability of C from photosynthesis because all leaves had been removed and had to be replaced using stored C, but also severely depleted the availability of buds for regrowth. However, the bigger plants (either taller or with thicker stems) did not compensate, whereas smaller ones did seem to compensate. The most intuitive explanation for this difference is that the smaller ones may have had relatively larger roots for some unknown reason. Because roots were not

studied, it can only be assumed that some difference in the way the plants were initially raised at the nursery from which they were bought affected their aboveground growth relative to their belowground growth. On this issue, all the plants were growing in nursery bags of similar volume when they were transplanted into the experimental plot. Thus, it is possible that the bigger plants had proportionally lower root-mass than the smaller plants. Why this may have been the case is not immediately clear because it could be related to various factors, such as differences in the age of the plants, composition of the growing medium in the pots, or genetic differences.

Marula is dioecious, so the differences between bigger and smaller plants could also be related to differences between male and female plants (Gadd, 2002). The trees were of the same age and were similar in size and appearance when planted two years prior the start of the experiment, yet some of them were much taller than others when measured at the start of the experiment, suggesting that even though the plants were all the same age, similar in appearance, and had been raised in the same conditions in the nursery, some unknown factor or factors (possibly site-related or genotypic) led to very different growth rates after transplanting. It could be that genetically related differences in inherent growth rate were only fully expressed when the plants were removed from the nursery bags and put into the acidic soil at the study site.

When saplings were 50% clipped or less, final tree height was increased with an increase in fertiliser application (Figure 4.2.2a), therefore these trees have tolerance traits such as mass compensatory growth abilities when environmental nutrient conditions are favourable (Mopipi *et al.*, 2009). The absence of the negative effect of clipping intensity in saplings that were 50% clipped or less also suggests an absence of carbon-limitations when growing along a productivity gradient (Figure 4.2.2a). Marula saplings are therefore more tolerant to simulated browsing (clipping) once they have reached a certain size and the environmental

nutrient conditions are favourable and thus have a greater chance of survival. The final mean tree height of control trees was similar to trees that were highly fertilised but not clipped (Figure 4.2.2a), this supports previous studies which indicated that, it is browsing not soil fertility that result in an increase in growth rate of re-sprouting shoots (Messina *et al.*, 2002).

### Stem diameter

The hypothesis which states that marula saplings respond to browsing intensity by showing a trend of increasing stem diameter along a productivity gradient was not supported because initially tall and broad-stemmed saplings had smaller final stem diameters following severe clipping, while short and narrow-stemmed trees had no clear pattern following clipping treatment (Figure 4.2.1a and Figure 4.2.1b) respectively. These findings also support findings by Bryant *et al.* (1983) that, saplings are selected for resistance (decreased growth traits and increased CBSMs) to browsers during the juvenile growth phase. In low and intermediate clipping intensity, mean stem diameter was greater than that of control trees and the interaction with fertiliser application also resulted in mean stem diameter greater than that of control trees (Figure 4.2.2b). This indicates that the degree of damage was insufficient to reduce stem diameter because the plant is not experiencing carbon-limitations as a result of fertiliser application.

According to Zhu *et al.* (2014), browsing alters plant water relations and patterns of carbon assimilation and allocation therefore this phenomenon explains how thicker stems may occur under browsing because of the thickening of xylem tissues resulting from these physiological changes. Conversely, severe browsing intensity reduced growth rate of stem diameter regardless of fertiliser application, this indicates that marula saplings failed to compensate in stem diameter and switched their strategies from growth to defence traits in response to severe clipping regardless of fertile environmental conditions.

### Old shoot extension

The hypothesis which states that marula saplings respond to browsing intensity by showing a trend of increasing old shoot extension along a productivity gradient was not fully supported because saplings growing along a productivity gradient did not increase old shoot extension when treated with 100% clipping level and defoliation. This phenomenon is supported by other previous studies which indicated that the extension of shoot length is not permissible when environmental conditions are unfavourable, e.g., in severe browsing and in poor-nutrient conditions (Mopipi *et al.*, 2009). Mean old shoot extension for unclipped saplings treated with 120 and 180g/tree of fertiliser were similar to 50 and 75% clipped saplings treated with 180g/tree of fertiliser (Figure 4.2.2c), therefore this indicates that old shoot extension was due to fertiliser treatment rather than just the effect of clipping.

### Number of new shoots

Consistent with expectations, the number of new shoots increased following an increase in clipping intensity in saplings growing along a productivity gradient compared to control trees except for the defoliation treatment (Figure 4.2.2d). These results support results from previous studies which indicated that shoot growth is stimulated following clipping and this stimulation occurs because clipping accelerates energy flow by increasing recycling of nutrients bound in standing plant matter, but this is permissible when saplings are highly clipped and nutrients are inadequate (Gadd *et al.*, 2001; Hamilton *et al.*, 2008; Mopipi *et al.*, 2009). Regardless of fertiliser application, mean number of new shoots and new shoot length was elevated in relation to clipping intensity (Rooke and Bergström, 2007; Fornara and du Toit, 2008). Therefore it can be said that marula saplings are able to compensate for browsing by browsers and this species performs even better when environmental nutrient conditions are favourable, this is consistent with observations of other species browsed by elephants

(Makhabu *et al.*, 2006; Young *et al.*, 2009). Increased clipping intensity reduces apical buds' dominance and stimulated the growth of new shoots rich in nitrogen. This may favour repeated browsing by herbivores and cause C exhaustion and eventually result in reduced life expectancy of a tree (Palacio *et al.*, 2014).

### New shoot length

Consistent with expectations, new shoot length was increased following an increase in clipping intensity in marula saplings growing along a productivity gradient compared to control trees except for defoliation treatment (Figure 4.2.2e). These results support other explanations by Fornara and du Toit (2007) and Hrabar *et al.* (2009) that a positive feedback is due to allocation of carbon to the growth of new shoots rather than CBSMs, which can result from alteration of the root: shoot ratio such that shoot growth is favoured to restore the ratio (Herms and Mattson, 1992; Renton *et al.*, 2007). Conversely, in the defoliation treatment, new shoot length was not increased because all leaves were removed and root: shoot ratio was not altered therefore plants switched their defence strategy to allocate carbon to synthesis of CBSMs and new leaves rather than to increased new shoot length.

### Biomass

Mean leaf biomass (at the end of experimental period) was reduced following an increase in clipping intensity but when interacting with fertiliser, mean leaf biomass was increased (Figure 4.2.2f). In 25 and 50% clipping levels, an increase in fertiliser application resulted in mean leaf biomass similar to that of control trees (Figure 4.2.2f). These results may be as a result of a short term increase in biomass production of the remaining crown of clipped saplings growing in fertilised conditions (Pinkard and Beadle, 2000). When clipping exceeded 50%, mean leaf biomass was reduced as compared to control trees regardless of an increase in fertiliser rates (Figure 4.2.2f). These results may be as a result of depletion of

carbohydrates reserved in storage organs such as the stem and root of marula saplings after clipping treatment as these reserves are used for the initial regrowth or chemical leaf traits (Chandrashekara, 2007). These results also support the model theory confirmed by Sachs (1981), which states that, it is reasonable to predict that clipped saplings would increase the partitioning of newly produced aboveground biomass to wood and decrease the partitioning to leaves.

The response of leaf biomass to studied fixed factors differed compared to that of new shoot productivity (number and length). Fertiliser generally increased the number and length of new shoots and the biomass of leaves, while clipping generally increased new shoot productivity but reduced leaf biomass (Figure 4.2.2d, e and f). It is not quite clear why shoot and leaf responses were different from each other, but the most likely reason is that most of the leaf biomass was not produced on the new shoots, but on old shoots (which personal observations of the plants would suggest was the case), and leaf biomass on new shoots and old shoots may respond differently. In other words, even if leaf biomass on the new shoots increased, total leaf biomass could have decreased if the leaf mass on old shoots had decreased substantially. The likelihood that unknown factors may have confounded the total aboveground responses means that it is not immediately clear whether or why the compensatory ability of shoots and leaves in response to clipping did not reflect the compensatory ability of total aboveground biomass as represented by plant height and stem diameter.

### Nitrogen

The results of this study indicated that, leaf chemical properties of marula saplings that were clipped early in the wet season and growing along a productivity gradient varied at the end of the wet season and the cause of variability remains unclear. Nitrogen concentration was

significantly increased following an increase in fertiliser application rates early in the wet season (Table 4.1.1 and Figure 4.1.2a). The statistically significant differences found between 180g/tree of fertiliser and control plants, 30, and 60g/tree indicated that soil N was a limiting factor for foliar N content of marula saplings in the study site and thus limited growth at the start of the experiment. The increase in foliar nitrogen early in the wet season can be explained by raised soil water content which drives soil and fertiliser nutrient mineralisation (Scholes *et al.*, 2003; Scogings *et al.*, 2015). In general, marula saplings growing along a productivity gradient increased foliar nutrient status early in the wet season when soil moisture content was high.

Clipping treatment tended to reduce N in plants that received low amounts of fertiliser, but the decrease was not consistent (Figure 4.2.3a). Consistent with expectations, clipped trees growing in highly fertilised conditions increased foliar nitrogen but the increase was also not consistent (Figure 4.2.3a). The trend of decreasing N from clipped trees that received low amounts of fertiliser can also be explained by shoot re-growth which occurred towards the end of the wet season when there was less rainfall thus less nutrient mineralisation (Mårell *et al.*, 2006), and thus increased carbon-limitations (Makhabu *et al.*, 2006). Conversely, highly fertilised plants maintained foliar nitrogen because they did not experience carbon-limitations (Makhabu *et al.*, 2006). The increasing trend of [N] following defoliation treatment in highly fertilised plants suggests that the saplings were efficient at balancing their nutrient budgets (Ratnam *et al.*, 2008) because, bud removal was minimal therefore root/shoot ratio was not altered and, plants did not experience carbon-limitations since soil nutrient-status was improved by high fertilisation.

### Flavonoids

Fertiliser treatment had no significant effect in explaining November flavonoids but air temperature as a covariate had a significant effect in explaining November flavonoids. The trend of these flavonoids was not ignored due to its similarities with that of CT after fertiliser application. Leaf flavonoid content in November 2013 was highest at 60g/tree of fertiliser application resulting in a hump-shaped pattern when fertiliser rates were further increased (Figure 4.1.2b). The hump-shaped relationship in flavonoids indicated a reduction in carbon-limitations beyond a certain threshold level (60g/tree) of fertiliser application rates (Herms and Mattson, 1992).

The increase in clipping intensity initially increased leaf flavonoid content in unfertilised plants (at the end of the experimental period) up to a certain maximum level, with the exception of defoliation, further increasing clipping intensity ultimately decreased flavonoids as clipping intensities were severe resulting in a hump-shaped relationship between flavonoid content and clipping intensity (Figure 4.2.3b). The hump-shaped relationship might be explained by high costs of producing CBSMs resulting in a point at which the plant can no longer afford to invest in secondary compounds. This is because the plant has insufficient reserves to make the investment caused by foliar loss, and they need to rapidly restore their photosynthetic tissues instead (Kohi *et al.*, 2010). The plants receiving the most fertiliser grew faster (indicated by the shoot and leaf results, but is not so clear in the tree height and stem diameter results). The increased production of flavonoids represent an induced defence response (faster growing plants might be less C limited than slower growing plants and so can shunt C into secondary metabolites). On the other hand, a reduction in flavonoids (in unfertilised plants) with clipping suggests carbon-limitation, which may have been caused by allocation of C to growth when fertiliser was not applied. The responses to clipping in fertilised plants could probably be explained in terms of the relative availability of C and N.

These results support findings by Herms and Mattson (1992) that either increasing N levels in the soil (fertilisation) or decreasing browsing intensity reduces carbon-limitations. The findings from this experiment corroborate other studies and support the hypotheses which state that, beyond a certain threshold level of physical damage, plants do not further increase their investment in anti-herbivore defence (Kohi *et al.*, 2010). The flavonoid response (at the end of the experimental period) indicated that responses that are linear at one level of resource availability may be non-linear at another level of resource availability, but the mechanisms are complex and unclear.

### Tannins

Tannins differed significantly between control plants and fertiliser rates in October suggest that [CT] is inversely proportional to [N] (fertilisation) and also indicate poor soil-nutrient conditions (i.e., carbon-limitations) in unfertilised plants. The soil-nutrient status was improved as a result of increased fertilisation rates in the study site. The initially significant effect of fertiliser treatment on condensed tannins and foliar nitrogen content in October 2013 favoured further assessment of the interaction between fertiliser and clipping treatment during the wet season. When sufficient leaves were available for photosynthetic carbohydrate production, i.e., at low carbon stress (50% clipping or less, when tree height and stem diameter growth were not reduced compared to control plants), marula saplings invested relatively more in the synthesis of secondary metabolites than in compensation for leaf loss (Figure 4.2.3c).

Clipping causes carbon stress in plants (Rooke and Bergström, 2007) and also stimulates the growth of new shoots and leaves (Bryant *et al.*, 1992). The growth of new shoots and leaves increases the carbohydrate demand and hence limits the production of CBSMs (Danell and Bergstrom, 1985). Such a response was not found in this study (Figure 4.2.3c). Because

plants were fertilised every two months throughout the experiment (until 2 months before termination), defoliated trees resulted in increased [CT] when heavily fertilised, i.e., plants did not experience carbon stress. However, the final application of fertiliser (in January 2014) did not coincide well with a substantial rainfall event (the expected rain did not fall) and therefore the effectiveness of the fertiliser treatment may have been reduced. If the timing had been better, then the outcome may have been very different. The increase in clipping intensity increased mean [CT] in all clipping levels (not significant) but there was a hump-shaped relationship in [CT] between 25 and 100% clipping intensity (Figure 4.2.3c), these results support observations of related studies of species elsewhere (du Toit *et al.*, 1990; Scogings and Macanda, 2005; Scogings *et al.*, 2011). It is supposed that at low browsing intensity, carbon demand for growth of new shoots was not enough to cause carbon-limitations, resulting in increased [CT]. In severe browsing, carbon-limitations are induced due to increased carbon demands for growth of new shoots, resulting in decreased [CT] such that lightly browsed plants are similar to heavily browsed plants.

These results are also explained by findings found by Bryant *et al.* (1983) who proposed that, because browsing of saplings reduces tree height and thus competitive ability, these saplings are selected for resistance to browsers during the juvenile growth phase, therefore marula saplings have responded to browsing by evolving strong defences during juvenile stages. It appears that, marula saplings do increase chemical defences in the form of flavonoids and tannins in response to defoliation treatment regardless of high fertiliser application (Figure 4.2.3b and c) respectively. This is because in defoliation treatment apical bud removal was minimal therefore carbon demands were underestimated, which allowed CBSMs to increase because photosynthesis was not allocated to growth of new shoots (Scogings and Macanda, 2005).

Neutral-detergent fibre (NDF), acid-detergent fibre (ADF), and acid-detergent lignin (ADL)

The lack of significant difference in NDF, ADF, and ADL following fertiliser application indicated that fertiliser rates were underestimated by the mentioned leaf fibre components even though 180g/tree of fertiliser slightly reduced the mentioned fibre components in October (Table 4.1.1; Figure 4.1.2d, e and f) respectively. Neither of the fixed factors had significant effect in explaining NDF and ADF but clipping had a marginally significant effect on ADL. Both severe clipping and defoliation treatments had highest ADL when highly fertilised. Clipped trees growing in highly fertilised conditions were expected to have higher N and lower CT, flavonoids, NDF, ADF, and ADL concentrations than trees growing in the opposite conditions. The hypothesis was not fully supported because severe clipping (100% clipping and defoliation treatment) reduced [N] and increased [CT] and ADL at the end of the experiment. This, together with the observed increase in ADL, indicated reduced nutritional feed value of marula trees growing in the conditions of the study.

The mechanism normally hypothesised for increased N relative to CT of savannah trees is that it reduces inter-shoot competition for N (Scogings and Mopipi, 2008), presumably because browsing increases root:shoot ratio and overall availability of N influences the response. This mechanism explains the CT or ADL:N ratio obtained due to clipping in highly fertilised conditions of this study, that the results may have resulted from increased inter-shoot competition for N (Scogings and Mopipi, 2008), and the leaf age was old by the time of final defoliation therefore the leaves were highly lignified by the end of the experimental period (Scholes *et al.*, 2003). Marula saplings growing along productivity gradient early at the start of the experiment contained below 45% NDF, ADF, and ADL on dry matter (DM) basis and this qualifies them as good quality browse since fibrous feed with NDF content of less than 45% of DM are classified as high quality feeds (Melaku *et al.*, 2010). The increase in fibre components can be explained by re-growth of new shoots which occurred at the end of the wet season (Scholes *et al.*, 2003; Scogings *et al.*, 2015).

In summary, marula saplings that were 50% clipped or less, early in the wet season (October 2013) did not experience carbon-limitations when growing along a productivity gradient and resulted in increased physical properties at the end of the wet season when harvested in April the following year. Conversely, removing more than 50% of leaf biomass early in the wet season resulted in plants experiencing carbon-limitations at the end of the wet season because compensatory growth failed to compensate for the reduction in photosynthesis caused by loss of foliage regardless of the increase in soil-nutrient availability, with the exception of defoliation treatment at the start of the experiment. The results were not consistent. Marula saplings growing in nutrient-poor conditions (unfertilised) responded to clipping by having reduced foliar N content, increased CBSMs (flavonoids and tannins) and fibre components (NDF, ADF, and ADL) at the end of the wet season (April 2014) thus reduced feed digestibility towards the end of the fast growth season (Ortega *et al.*, 2013). On the other hand, Marula saplings growing along a productivity gradient responded to clipping treatment early in the wet season by having increased foliar N content extending to early stages of the dry season (April 2014) when harvested in April the following year. Conversely, CBSMs and fibre components of marula leaves growing along a productivity gradient during the wet season were decreased following clipping early in the wet season (October 2013) when harvested in April the following year. Therefore marula saplings growing in nutrient-rich conditions, when browsed early in the wet season, extend their nutritive value to early months of the dry season. Results were not consistent.

### **5.1. General conclusion**

Marula saplings responded to increased browsing intensity by showing a trend of increasing old shoot extension, number of new shoots, new shoot length along a productivity gradient. The failure of marula saplings to compensate for above-ground growth (in terms of tree height, stem diameter, and leaf biomass) indicated carbon-limitations when plants are

growing along a productivity gradient following increased clipping intensity. However, studied leaf chemical properties of marula saplings growing along a productivity gradient (following increased browsing intensity) varied at the end of the experimental period and the cause of variability remains unclear. Severe clipping and defoliation treatment indicated induced defence responses regardless of increased fertiliser rates, thus suggesting a lack of carbon-limitations. The results from this study also indicated that physical responses to a single clipping event persist for longer than chemical responses do. In order to better management policies in game ranches, there must be not more than 50% of plant folia mass removed by browsers regardless of soil nutrient status because the plant will fail to compensate for growth and face carbon-limitations then eventually die.

## **5.2. Recommendations**

Plant response to browsing may occur by changes in tissue chemistry, growth rate or morphology (Bryant *et al.*, 1992). A number of factors influence these responses, such as the browsing intensity or frequency, soil nutrient status, time of browsing, and water availability (Guillet and Bergstrom, 2006). In this experiment average rain-fall during the growth season was not recorded or monitored. For future purposes of similar studies, it is recommended that:

- Irrigation must be included to mimic increased expected rainfall during the growth season.
- A frequent clipping treatment must be applied to mimic frequent browsing during the growth season.
- Different dates of clipping early in the wet season and during leaf harvesting at the end of the wet season must also be monitored since time of browsing is assumed to influence plant traits at the end of the wet season (Guillet and Bergstrom, 2006).

### 5.3. Contribution to body of knowledge

- This research will help in improving management policies in game ranch systems where the stocking density of herbivores is limited by the availability of their forage resources, thus, if conservation of both intensive browsers and *S. birrea* is an important objective of a particular game ranch system.
- This research will also help in assessing the extent to which herbivores directly influence physiological and chemical traits of woody plants in patches of African savannahs with nutrient-poor or nutrient-rich conditions, since the sustainability of herbivore population in game ranch systems is influenced by the sustainability of forage quality (Scogings *et al.*, 2015).

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## APPENDIXES

### Appendix A

#### Marula clipping protocol

#### Clipping intensity

Intensity can be defined relative to either (i) total canopy “volume” (defined as “productive, shoot/leaf bearing branches”), or (ii) total number of available “bites” (defined as branches of certain minimum length and diameter). The problem with relating intensity to bites of pre-determined size might be that not all the canopy comprises such bites, so the intensity relative to bites might be less than that relative to total canopy. The way to overcome this constraint is to assume that a variety of browsers eat the same individual plant, such that the largest browsers take the largest bites (mainly from the upper canopy), medium browsers take the next largest bites (mainly from the middle of the canopy), while the smallest browsers take the smallest bites that are left over. Then the approach that defines clipping intensity relative to total productive canopy converges with an approach that defines clipping intensity relative to available bites for browsers of different sizes.

#### 6 levels

1=control (0%)

2=100% defoliation (stripping of 90-100% of leaflets, usually leaving the midrib behind and not damaging buds)

3=25% clipping (clipping 15-35% of available big, then medium and lastly small “bites”)

4=50% clipping (clipping 40-60% of available big, then medium and lastly small “bites”)

5=75% clipping (clipping 65-85% of available big, then medium and lastly small “bites”)

6=95% clipping (clipping 90-100% of available big, then medium and lastly small “bites”)

#### Available “bites”

Defining available bites of different sizes essentially requires defining different size (diameter and length) classes of branches, recognizing that larger plants will also have larger branches than smaller plants. First, a maximum length must be defined for each bite size class, eg, large could be up to 20cm from the branch tip, medium up to 12cm, and small up to 4cm. Second, a maximum diameter must be defined, eg, up to 15mm for large bites, up to 10mm for medium bites, and up to 5mm for small bites. Assume that smaller plants are only browsed by small-medium browsers and larger plants are browsed by all browsers, ie, if the plant is small and has no large “bites” available, then only the medium and small bites are clipped.

Bite size class	Metric	
	Max length (cm)	Max diam. (mm)
Large	20	15
Medium	12	10
Small	4	5

## Procedure

Level 1: No treatment

Level 2: Strip off all the leaflets on each branch by hand. Midribs are strong and most of them remain attached. Avoid damaging apical/axillary buds.

Levels 3-6: Roughly count the number of big, medium and small branches. Classify branches according to length first, then diameter, eg, a branch that is 10cm long with 4mm diameter would be “medium” and entirely consumable. Clip the required proportion of shoots in each bite size class. Clip according to length or diameter depending in which removes the most material, eg, a branch that is 10cm long with 4mm diameter would be clipped near its base.

## Considerations

Bigger plants have bigger branches

Tree height (cm)	Max diameter to clip (mm)
51-75 (n=130)	6-12?
76-100 (n=130)	10-15?
101-135 (n=60)	13-17?

Plants with no/few branches can't be used for some treatments

Number “main” branches	Treatment possibilities		Number branches to clip				
	1 (0%)	2 (100% defoliation)	3 (20-25-33%)	4 (40-50-60%)	5 (66-75-80%)	6 (100%)	
0	0	Y				1	
1	0	Y		1		2	
2	0	Y	1		2	3	
3	0	Y	1	2	3	4	
4	0	Y	1	2-3	4	5	
5	0	Y	2	3	4	6	
6	0	Y	2	3-4	5	7	
7	0	Y	2	4	6	8	
8	0	Y	3	4-5	6	9	
9	0	Y	3	5	7	10	
10	0	Y	3	5-6	8	11	
11	0	Y	3	6	9	12	
12	0	Y	3	6-7	10	13	
13	0	Y	4	7	10	14	
14	0	Y	4	7-8	11	15	
15	0	Y	4	8	12	16	