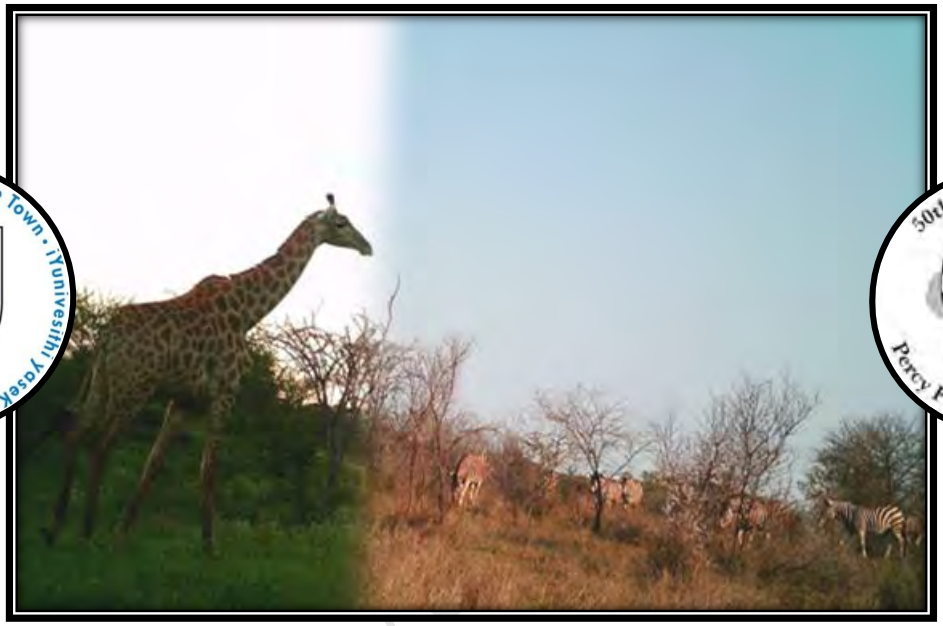
Is the grass really greener on the other side?

The potential effects of additional soil nitrogen, phosphorus and water on the feeding behavior and diet of the large herbivores within an African savanna



Wade Howard Lane

Supervisors: Dr. Edmund February, Dr. Leigh-Ann Woolley

8 February 2013

Submitted in partial fulfillment of the requirements for the degree of
Masters of Science in Conservation Biology

Percy FitzPatrick Institute of African Ornithology
University of Cape Town
Rondebosch
Cape Town
South Africa 7701

TABLE OF CONTENTS

PLAGIARISM DECLARATION ..... **iv**

ABSTRACT ..... **v**

ACKNOWLEDGEMENTS ..... **vi**

CHAPTER 1 - A LITERATURE REVIEW OF THE AFRICAN SAVANNAS WITH A FOCUS ON THE KRUGER NATIONAL PARK

1.1. Introduction **1**

1.2. Nutrient and Water Availability **2**
1.2.1. Nitrogen Availability 3
1.2.2. Phosphorus Availability 4
1.2.3. Water Availability 5
1.2.4. Movement Through the Trophic Levels 5

1.3. The Vegetation Structure **6**
1.3.1. Fire 8
1.3.2. Herbivory 8

1.4. The Dietary Decisions of Ungulates **10**
1.4.1. Morphological Mechanisms 10
1.4.1.1. Body Size 11
1.4.1.2. Digestive System 11
1.4.1.3. Rumino-Reticular Volume to Body Ratio 12
1.4.2. Behavioural Mechanisms 13
1.4.2.1. Creating and Adapting a Diet 13
1.4.2.2. An Element of Scale 14

1.5. The Study Outline **15**
1.5.1. The Study Site 15
1.5.1.1. Nitrogen and Atmospheric Pollution 16
1.5.1.2. Phosphorus and Aquatic Pollution 17
1.5.1.3 Water and Climate Change 17
1.5.2. The Aims and Objectives of the Study 18

CHAPTER 2 - THE POTENTIAL EFFECTS OF ADDITIONAL SOIL NITROGEN, PHOSPHORUS AND WATER ON THE FEEDING BEHAVIOUR AND DIET OF THE LARGE HERBIVORES WITHIN AN AFRICAN SAVANNA

2.1. Introduction 21

2.2. Methods 25
2.2.1. Study Area 25
2.2.2. Experimental Design 26
2.2.3. Sampling 28
2.2.4. Carbon, Nitrogen and Phosphorus Analysis 29
2.2.5. Determining the Composition of the Impala Diet 30
2.2.6. Determining Feeding Rate 31
2.2.7. Statistical Analyses 32

2.3. Results 34
2.3.1. Chemical Analyses 34
2.3.1.1. Grass Leaves 34
2.3.1.2. Tree Leaves 36
2.3.1.3. Ungulate Dung 38
2.3.2. Feeding Rates 41
2.3.3. Grass Height 43
2.3.4. Tree Assessments 47

2.4. Discussion 50
2.4.1. Vegetation Quality 50
2.4.2. Feeding Behaviour and Diet of Ungulates 52
2.4.3. Vegetation Quantity 54
2.4.4. Conclusion 56

CHAPTER 3 - STUDY SYNTHESIS AND REVIEW

3.1. Study Synthesis 58

3.2. Study Limitations 59
3.2.1. Pseudoreplication – A Necessary Evil? 59
3.2.2. The Leaf and Dung Samples 60
3.2.3. The Camera Traps 61
3.2.4. Why Use Water? 62

3.3. Future Research 63

REFERENCES 64

APPENDIX 73

PLAGIARISM DECLARATION

1. I know that plagiarism is wrong. Plagiarism is to use another's work and pretend it is your own.

2. Any statement(s) in this report derived from the work of others has been attributed to them and cited and referenced using the Harvard System of Referencing.

3. This report is my own work and I have not copied phrases, sentences or longer tracts word-for-word from other sources.

4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his/her own work.

Signature…….

ABSTRACT

While many studies focus on identifying pollutants within an ecosystem or how they affect primary producers, few look at how pollutants move through trophic levels or their influence on animal demography. The aim of this study was to determine whether additional nitrogen, phosphorus and water, to a savanna would alter the vegetation quality enough to influence the feeding behaviour and diet of the ungulate populations both of which would alter the vegetation quantity. The study was conducted in the Kruger National Park. One site was supplied with additional nutrients and compared to three control sites. The nitrogen and phosphorus content of grass and tree leaves collected at the enriched site were higher than the leaves collected at the control site, indicating the additional nutrients are improving the vegetation quality. Feeding rates (determined from photos captured by camera traps) indicated a higher degree of herbivory at the enriched site. However, there was no difference in the $\delta^{13}C$ value, nitrogen and phosphorus content in the ungulate dung collected amongst the study sites suggesting no change in the diet. The dominant grass was significantly shorter at the enriched site suggesting that increased grazing was diminishing grass biomass. Basic assessment of the trees indicated that the additional water at the enriched site seemed to be triggering an earlier start to the growing season for the trees. It was concluded that the additional nutrients have altered the vegetation structure enough to potentially influence animal demographics.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisors, Dr. Edmund February and Dr. Leigh-Ann Woolley, without which I would have been completely lost. I would also like to acknowledge Elliot Shilote, Happy Mangena and Ryan Taylor who assisted with the sampling and monitoring of the study sites; to Ian Newton, Nazlee Naudé and Shaamielah Davids who assisted with the processing of the leaf and dung samples; to Brittany Arendse, Dunja Basic, Eric Lane and Sheree Lane who reviewed and commented on the draft of this manuscript. I would also like to acknowledge the Mellon Foundation of New York and the South African National Research Foundation who assisted with funding of this study. Finally I would like to thank my family and colleagues (near and far) for their constant support.

CHAPTER 1

A LITERATURE REVIEW OF THE AFRICAN SAVANNAS WITH A FOCUS ON THE KRUGER NATIONAL PARK

1.1. INTRODUCTION

Savannas are one of the largest biomes on Earth, covering 20 % of the land surface (i.e. approximately 23 million km^2; Scholes and Archer 1997; Tews *et al*. 2004; Lehmann *et al.* 2011). The majority of savannas occur in Africa, with smaller amounts occurring in South America, India and Australia. Savannas are comprised of two structural components; a continuous herbaceous layer (which is dominated by members of Poaceae) with a dense to sparse woody layer (which, in the African savanna, is dominated by members of Fabaceae; Frost *et al.* 1985; Mucina and Rutherford 2006; Sankaran *et al.* 2004; February *et al*. 2013b). The grasses in African savannas have a C_4 photosynthetic pathway, while the trees have a C_3 photosynthetic pathway (Vogel *et al*. 1978; February and Higgins 2010). Savannas experience sub-tropical to tropical climates with approximately 60 to 90 % of the year's rainfall falling within a few months (Scholes 1990; Cowling *et al.* 1997; Mucina and Rutherford 2006; Higgins *et al*. 2007). This results in distinct wet seasons (seasonal floods in which vegetation experiences a period of rapid growth) alternating with dry seasons (seasonal droughts in which the vegetation enter a state of dormancy; Scholes 1990; Scholes and Archer 1997; Shorrocks 2007).

Anthropogenic pollution coupled with climate change pose a serious threat towards global ecosystem integrity and biodiversity (Tilman *et al*. 2001; Johns *et al*. 2003; Güsewell 2004;

Elser *et al*. 2007; Mahowald *et al*. 2008; Conley *et al*. 2009; Bobbink *et al*. 2010). While many studies focus on identifying and measuring anthropogenic pollution and climate change as well as their effect on primary producers, few studies consider the repercussions for the higher trophic levels. The aim of this study is to determine whether the additional nitrogen, phosphorus and water would alter the vegetation quality (i.e. the forage quality or concentration of nitrogen and phosphorus within the leaves) and quantity (i.e. biomass) enough to alter the feeding behaviour and diet of the large herbivore populations (i.e. ungulates). The following literature review will (in the context of African savannas) examine what factors naturally determine the availability of nitrogen, phosphorus and water within the soil and the vegetation, what factors naturally determine the vegetation structure and what factors naturally determine the feeding behavior and diet of ungulates. The literature review will end with a brief overview of the study area (i.e. the Kruger National Park) as well as the aims and objectives of this study.

1.2. NUTRIENT AND WATER AVAILABILITY

Nitrogen, phosphorous and water are the primary macronutrients required by all organisms. Nitrogen is needed for protein synthesis, phosphorous is needed for DNA, RNA and energy transfer, while water is needed to maintain cellular integrity, photosynthesis, metabolism and to transfer products and wastes into, out-of and within an organism (Güsewell 2004; Miller and Cramer 2004; Lambers *et al*. 2006; Elser *et al*. 2007; Conley *et al*. 2009). Production (i.e. the amount of resources devoted to growth and reproduction) at each trophic level is limited by the availability of nitrogen, phosphorus and water which are obtained from lower trophic levels or directly from the environment (Abrams 1993). The following section will examine

the factors responsible for the availability of nitrogen, phosphorus and water in the soil and how these nutrients are incorporated and conserved in the grasses and trees.

1.2.1. NITROGEN AVAILABILITY

The majority of soil nitrogen is the result of the decomposition of organic matter (via mineralization) and the fixing of atmospheric nitrogen (via biological nitrogen fixation; Frost *et al.* 1985; Miller and Cramer 2004; Cramer *et al*. 2010; Coetsee *et al*. 2012). In mineralization, bacteria and fungi decompose organic matter to form ammonium (NH_4^+; Miller and Cramer 2004). In biological nitrogen fixation, bacteria living within biological crusts or housed within root nodules (prevalent in leguminous plants) capture atmospheric nitrogen and convert it to ammonium (Miller and Cramer 2004). Ammonium can be converted to nitrate (NO_3^-) by nitrifying bacteria, however plants are able to utilize both forms of nitrogen (Miller and Cramer 2004).

Soil nitrogen is lost through several pathways or moved within a landscape creating regions with surplus or deficits of soil nitrogen. Bacteria can convert ammonium and nitrate into non-usable nitrogen compounds such as nitrite (NO_2^-; Miller and Cramer 2004). De-nitrifying bacteria can vaporize soil nitrogen (via volatilization) into various nitrogen containing gases (e.g. N_2, N_2O and NO) which are lost into the atmosphere (Miller and Cramer 2004; Coetsee *et al*. 2010). Leaching tends to lower soil nitrogen in high lying areas and raise soil nitrogen in low lying areas though this process depends on the topography of the landscape, the type of soil and the vegetative cover (Coetsee *et al*. 2012). Fire tends to lower soil nitrogen by destroying decaying matter and releasing the contained nitrogen into the atmosphere (Coetsee *et al*. 2012). Animal excretions (i.e. urine and dung) temporarily increase soil nitrogen and

can be a significant source in areas with high animal activity (Coetsee *et al*. 2010; Coetsee *et al*. 2012). Both fire and herbivory can directly influence the nitrogen content of the vegetation by removing mature leaves and branches and promoting new growth which have higher nitrogen content than mature tissue (Coetsee *et al*. 2010; Kambatuku *et al.* 2011; Coetsee *et al*. 2012).

1.2.2. PHOSPHORUS AVAILABILITY

Unlike nitrogen which is continually being recycled within the environment, phosphorus is a non-renewable resource (Lambers *et al*. 2006). The majority of the phosphorus content of the soil is due to the type and weathering of the parent bedrock; unlike ammonium and nitrate, phosphate (P_i) is highly immobile and can be rendered unusable if it becomes surrounded (i.e. occluded) by metal ions such as iron (Fe^{2+}, Fe^{3+}) and aluminium (Al^{3+}; Frost *et al.* 1985; Lambers *et al*. 2006). The majority of higher land plants are able to form mutualistic relationships with arbuscular mycorrhizal fungi in the roots to improve phosphorus uptake, however there is little evidence to suggest that this symbiosis takes place within the African savannas (du Toit *et al*. 2003; Lambers *et al*. 2006).

As with soil nitrogen, there are several pathways through which soil phosphorus is lost or moved within a landscape. Soil phosphorus is primarily lost through leaching; high lying areas tend to have lower soil phosphorus than low lying areas, again this is dependent on the topography of the landscape, the type of soil and the vegetative cover. Fire tends to raise soil phosphorus by volatilizing non-usable phosphorus compounds (Frost *et al.* 1985). Animal excretions (i.e. urine and dung) temporarily increase soil phosphorus (which can be a significant source in areas with high animal activity), while fire and herbivory can directly influence the phosphorus content of the vegetation by removing mature leaves and branches

promoting new growth which are allocated more of the assimilated phosphorus than mature tissue (Frost *et al.* 1985).

1.2.3. WATER AVAILABILITY

One of the characteristic features of savannas is the seasonal nature of the rainfall which (on the African continent) is brought about by the annual shifting of the Inter-Tropical Convergence Zone (ITCZ) and the Northern and Southern Hadley Cells (Cowling *et al.* 1997; Mucina and Rutherford 2006; Shorrocks 2007; February and Higgins 2010). The southward migration of these systems, during November – February, brings rain to the southern African savannas and suppresses rain in the northern African savannas and vice versa during June – August.

Apart from the seasonality of the rainfall, the water content of the soil depends on climatic conditions affecting evaporation (e.g. temperature and wind), topography of the landscape (i.e. low lying areas tend to be wetter than high lying areas), the soil type and vegetation cover which affects runoff and erosion.

1.2.4. MOVEMENT THROUGH THE TROPHIC LEVELS

The amount of nutrients and water present within the tissues of plants are derived from the soil in which they grow and the degree to which they conserve nutrients and water (Scholes 1990). The size and extent of the root system is the most important variable determining the access to nutrients and water; usually the size and degree of branching increases when nutrients and water are limited (Miller and Cramer 2004; Lambers *et al*. 2006). Since the soil solution tends to be more diluted than the cellular contents within the roots of plants, useable

nutrients are actively pumped into the root through specialized transporter proteins which are embedded in the plasma lemma of the individual cells (Miller and Cramer 2004; Lambers *et al*. 2006; Cramer *et al*. 2009). Water moves passively down a water potential gradient into the cells of the roots though aquaporins which are able to regulate water uptake (Cramer *et al*. 2009). Grasses limit the amount of water loss and conserve nutrients by engaging in C_4 photosynthesis (which reduces photorespiration) and by entering a state of dormancy during the dry season (Cowling *et al*. 1997; du Toit *et al.* 2003; Shorrocks 2007; Lehmann *et al.* 2011). Trees limit the amount of water loss and conserve nutrients through various means, usually a combination of: reduced leaves, dissected leaves, leaves with thick a cuticle (i.e. evergreen trees), leaves with sunken stomata, leaves with leaf hairs, folding the leaves in the height of the day to reduce exposure to sunlight (prominent in *Acacia*) and/or shedding leaves prior to the dry season (i.e. deciduous trees; Frost *et al.* 1985; Cowling *et al*. 1997; Shorrocks 2007; Cramer *et al*. 2009).

The nutrient content of the plants is derived from the soil in which they grow; the higher the nutrient content of plants, the more nutritional benefits can be derived for herbivores feeding on these plants (Scholes 1990; Abrams 1993). The factors that determine the feeding behavior and diet of the large herbivores (i.e. ungulates) will be discussed in a later section.

1.3. THE VEGETATION STRUCTURE

The relative abundance of grasses and trees is dictated by many complex and dynamic interactions among abiotic and biotic factors. According to the stress gradient hypothesis; as the abiotic conditions become more stressful (e.g. limited nutrient/water availability) tree-grass interactions become more facilitative and less competitive (Smit 2004; Staver *et al*.

2011a; Staver *et al*. 2011b; February *et al*. 2013b; Dohn *et al.* 2013). Grasses facilitate trees by creating a microclimate in which saplings can establish and mature (Kambatuku *et al.* 2011). Trees facilitate neighboring grasses by 1) creating microclimates as shade reduces air and soil temperatures which reduces evaporation from the soil and evapotranspiration from plants and 2) enhancing water and nutrient availability through the symbiosis with nitrogen fixing bacteria, the decomposition of the leaf litter, hydraulic lifting (i.e. the redistribution of water and soluble nutrients to the upper layers of the soil) and an increased abundance of animal dung due to the shade attracting animals seeking refuge from the heat (Frost *et al.* 1985; Weltzin and Coughenour 1990; Scholes and Archer 1997; Ludwig *et al*. 2004; Smit 2004; Shorrocks 2007; Kambatuku *et al.* 2011; Dohn *et al.* 2013; February *et al*. 2013a). However, grasses and trees acquire their nutrients and water from the same soil layers (< 20 cm) and to some degree will have to compete for nutrients and water (Weltzin and Coughenour 1990; Mordelet *et al*. 1997; Higgins *et al*. 2000; van Langevelde *et al*. 2003; February and Higgins 2010; Verweij *et al*. 2011; February *et al*. 2013a).

Alternatively, according to the stress gradient hypothesis, as the abiotic conditions become less stressful tree-grass interactions become more competitive (Sankaran *et al*. 2005; February *et al*. 2013b; Dohn *et al.* 2013). According to Sankaran and colleagues (2005) as well as Staver and colleagues (2011b) when the mean annual rainfall exceeds a specific threshold (more than 650 ± 134 mm reported in Sankaran *et al*. 2005; more than 1000 mm reported in Staver *et al*. 2011b) trees are able to outcompete grasses leading to a biome change with the formation of forest (Staver *et al*. 2011a). It is then the presence of disturbance regimes (i.e. fire and herbivory) which maintain the open canopies and, thus, the co-existence of trees and grasses (Sankaran *et al.* 2004; Sankaran *et al*. 2005; Staver *et al*.

2009; Kambatuku *et al.* 2011; Staver *et al*. 2011a; Staver *et al*. 2011b; Wakeling *et al*. 2011; February *et al*. 2013b).

1.3.1. FIRE

Fires range in intensity, duration and frequency depending on the climatic conditions and the fuel load (Scholes and Archer 1997; Higgins *et al*. 2000; Higgins *et al*. 2007). C_4 grasses are extremely productive, resulting in the rapid accumulation of biomass and secondary compounds (some of which are flammable) and thus form the majority of the fuel load (Higgins *et al*. 2000; van Langevelde *et al*. 2003; Lehmann *et al.* 2011). The herbaceous layer is able to recover quickly from fire through reseeding from a seed bank or resprouting from underground storage organs (Shorrocks 2007). Fire prevents the germination, establishment and maturation of tree saplings which adopt a pole-like architecture to maximize vertical growth (Higgins *et al*. 2000; Archibald and Bond 2003; Sankaran *et al.* 2004; Staver *et al*. 2011a; Staver *et al*. 2011b; Wakeling *et al*. 2011). Larger trees are able to compensate fire damage by resprouting from underground root storages or avoid fire damage by developing a thick bark (Higgins *et al*. 2000; Archibald and Bond 2003; Kambatuku *et al.* 2011; Wakeling *et al*. 2011).

1.3.2. HERBIVORY

In response to the presence of herbivory, savanna vegetation have developed an interesting suite of mechanical and chemical defenses. Grasses usually branch by producing new stems in the axils of older leaves and new growth takes place at the base of leaves (Shorrocks 2007). This allows grasses to continue growing despite apical appendages being removed and in some instances herbivory helps maintain an optimal growth rate (Shorrocks 2007). Trees

are usually equipped with spines and smaller trees adopt a cage-like architecture with smaller, sparser leaves to avoid herbivory (Skarpe *et al*. 2000; Archibald and Bond 2003). In both grasses and trees, secondary chemicals, such as tannins, are usually deposited in the leaves to deter herbivory (especially in the dry season when the vegetation enters a state of dormancy; Cowling *et al*. 1997; Skarpe *et al*. 2000; Scogings *et al*. 2004; Hattas *et al*. 2011; Scogings *et al*. 2011).

The distribution of large herbivores and the composition of their diets have long been the interest of range and wildlife ecologists (Smith 1940; Hanley 1982; Bailey *et al.* 1996). Large herbivores can roughly be divided into three feeding guilds; grazers which primarily feed on grasses, browsers which primarily feed on trees and shrubs and mixed-feeders which feed on both elements in various proportions. While some degree of herbivory helps to maintain the co-existence of trees and grasses as well as unique habitats (e.g. so-called grazing lawns which are maintained by intense rhino grazing), intense herbivory or a change in the prevalence of the feeding guilds can result in the exacerbation of destructive processes (e.g. soil erosion) and/or massive changes to the vegetation structure (e.g. proliferation of alien/undesirable species, woody encroachment; Smith 1940; Hanley 1982; Owen-Smith and Novellie 1982; Bailey *et al.* 1996; van Langevelde *et al*. 2003; Sankaran *et al.* 2004; Waldram *et al*. 2008; Coetsee *et al*. 2010). Intense grazing would deteriorate the herbaceous layer which would reduce the fuel load making fires less frequent and less intense allowing trees to become more dominant in the landscape (van de Koppel and Prins 1998; van Langevelde *et al*. 2003; Britz and Ward 2007; Bobbink *et al*. 2010; Goheen *et al.* 2010; Kambatuku *et al.* 2011). Alternatively, high animal activity (not only intense browsing) tends to modify the woody vegetation structure (e.g. breaking branches, stripping bark, trampling saplings). This disturbance causes the grass biomass to increase resulting in more intense

fires that top kill tree saplings (van de Koppel and Prins 1998; van Langevelde *et al*. 2003; Smit 2004; Britz and Ward 2007; Staver *et al*. 2009; Bobbink *et al*. 2010; Goheen *et al.* 2010; Kambatuku *et al.* 2011; Dohn *et al.* 2013).

1.4. THE DIETARY DECISIONS OF UNGULATES

The term 'ungulate' refers to hoofed mammals that are classified into two orders, Artiodactyls and Perissodactyls, depending on whether they have an even (Gr. *artio*-) or odd (Gr. *perisso*-) number of toes (Gr. *dactyla*; Estes 1992; Shorrocks 2007). Optimal forage theory was initially used to describe the distribution and dietary composition of ungulates; the theory proposes that natural selection confers the greatest genetic fitness on individuals which maximize their nutrient intake while minimizing the costs associated with acquiring and digesting food items (Hanley 1982; Owen-Smith and Novellie 1982; Bailey *et al.* 1996). However the optimal foraging theory has been criticized primarily because the functional hypotheses are untestable, but also because natural selection does not design animals to have an optimal diet (i.e. evolution is not purposeful), instead natural selection has resulted in the development of the organs and behaviour needed to survive and reproduce (Owen-Smith and Novellie 1982; Pierce and Ollason 1987; Senft 1987). The following sections will examine the morphological and behavioural mechanisms which ultimately determine the distribution and diet of ungulates.

1.4.1. MORPHOLOGICAL MECHANISMS

There are three morphological characteristics which dictate what type of food, and the amount thereof, an ungulate is most efficient at exploiting: body size, digestive system and

rumino-reticular volume to body weight ratio (Hanley 1982; Hanley and Hanley 1982; Senft 1987).

1.4.1.1. Body Size

The larger an animal is the more food is required to offset metabolic costs; log body weight (in kg) linearly relates to log basal metabolic rate (kcal/day) with a slope of ¾ and deviations attributable to sexual dimorphism and extreme external temperatures (Hanley 1982; du Toit and Owen-Smith 1989; Rooney *et al.* 2008; Parker *et al*. 2009). However, the relative requirements per unit body tissue are lower compared to smaller animals (Hanley 1982; Bailey *et al.* 1996; Rooney *et al.* 2008). Larger ungulates (e.g. Buffalo, Elephants and Giraffe) consume high quantities of low quality food, whereas smaller ungulates have more time available to selectively forage and consume small quantities of higher quality food (e.g. Duiker, Impala and Steenbok; Hanley 1982; Bailey *et al.* 1996).

1.4.1.2. Digestive System

Two types of digestive systems have evolved in ungulates to enable them to breakdown plant-cell walls into digestible carbohydrates: hindgut fermentation and rumination (Hanley 1982; Estes 1992; Shorrocks 2007). This breakdown is done by bacteria and protozoa through anaerobic fermentation (Hanley 1982; Estes 1992; Shorrocks 2007). Perissodactyls are hindgut fermenters, while Artiodactyls are ruminants (Hanley 1982; Estes 1992; Shorrocks 2007).

In hindgut fermentation, the content of the plant-cells is digested in the stomach while the cell walls are broken-down in the caecum and colon (Hanley 1982; Estes 1992; Shorrocks 2007). Ruminants have a more complex four-chambered stomach in order to accommodate

anaerobic fermentation prior to 'normal' digestion (Hanley 1982; Estes 1992; Shorrocks 2007). When the rumen is full, the coarsest food particles float to the top and are regurgitated and re-chewed in the mouth to reduce the size (Estes 1992; Shorrocks 2007). The re-chewed food is returned to the reticulum where rhythmic contractions sort the food particles according to size (Estes 1992; Shorrocks 2007). Food particles are then pumped into the omasum where they are filtered before entering the abomasum, or the true stomach, for digestion (Estes 1992; Shorrocks 2007). Ruminants are able to extract more nutrients from their food (cellulose utilization in a cow is about 80 %), but their digestive system is very slow (rate of passage in a cow is about 80 hours) which means that ruminants require high quality food (Hanley 1982; Estes 1992; Shorrocks 2007). Alternatively, hindgut fermenters are less efficient at extracting nutrients from their food (cellulose utilization in a horse is about 50 %), but their digestive system is much faster (rate of passage in a horse is about 48 hours) which means that hindgut fermenters can utilize food of a lower quality (Hanley 1982; Estes 1992; Shorrocks 2007).

1.4.1.3. Rumino-Reticular Volume to Body Ratio

The cell contents of plant cells consist of readily digestible carbohydrates, lipids, protein and amino-acids, while the cell walls consist of cellulose, hemicelluloses and lignin which restrict digestion (Hanley 1982; Owen-Smith and Novellie 1982). Cellulose is digestible through anaerobic fermentation; hemicellulose may or may not be digestible, depending on the type; and lignin is considered indigestible (Hanley 1982; Owen-Smith and Novellie 1982). The rumen-reticular volume to body weight ratio (RV:BW) of a ruminant determines what type of food is most efficient; RV:BW of North American ungulates range from 0.10 *l*/kg in deer (*Odocoileus* spp.) to 0.25 *l*/kg in domestic sheep (*Ovis aries*; Hanley 1982; Hanley and Hanley 1982).

To benefit from a high cellulose diet, food must be retained in the rumen for a sufficient time to allow the cellulose to be digested (Hanley 1982). Ruminants with a high RV:BW (slow rumen turnover rate) subsist on a high cellulose diet, that is to say, they graze on grasses, reeds and rushes (Hanley 1982; Shorrocks 2007). Lignin is not only indigestible, but also interferes with cellulose digestion (Hanley 1982). It would be disadvantageous for ruminants with a high RV:BW to consume a high lignin diet because a slow rumen turnover rate greatly reduces the efficiency of anaerobic fermentation. Ruminants with low RV:BW tend to consume a high lignin diet because cell contents are digested rapidly while little time is wasted trying to obtain nutrients from the lignified cell walls (Hanley 1982). However, in order for a ruminant to subsist on such a diet, food items must have relatively high amounts of cellular contents; such ruminants tend to be browsers feeding on tree foliage, fruit and herbs (Hanley 1982; Shorrocks 2007).

Ruminants with intermediate RV:BW tend to be mixed feeders, often switching from grazing in the wet season to browsing in the dry season (du Toit *et al*. 2003; Shorrocks 2007).

1.4.2. Behavioural Mechanisms

Animals exist within a spatially and temporally heterogeneous landscape with vegetation of varying nutrient quality and, therefore, must be able to alter their feeding behaviour in order to maintain homeostasis and to maximize dietary nutrient concentrations (Wiens 1989; Senft 1987; Day *et al.* 1998; Staver *et al*. 2009).

1.4.2.1. Creating and Adapting a Diet

At the genetic level, all animals possess some rudimentary feeding behaviour (e.g. pecking in birds) which initially results in the consumption of both food and non-food items (Kyriazakis

et al. 1999). The diet is refined through social learning (e.g. maternal nurturing and mimicking experienced conspecifics) and own experience (Day *et al.* 1998; Provenza *et al.* 1998). Feeding behaviour is driven by a series of motivational systems (e.g. feeding, mating, exploring) which are either positively reinforced (which initiates or maintains feeding bouts) or negatively reinforced (which arrests or terminates feeding bouts) through sensory or visceral information (Day *et al.* 1998; Provenza *et al.* 1998; Kyriazakis *et al*. 1999). This allows animals to associate food items with certain environmental (e.g. exposure to cold, danger of predation) and nutritional (e.g. concentration of nutrients, toxins and secondary chemicals) costs and benefits (Day *et al.* 1998; Provenza *et al.* 1998; Kyriazakis *et al*. 1999; du Toit *et al*. 2003). Animals also conduct exploratory behaviour in order to identify new food items (i.e. intrinsic exploration) or to monitor the costs and benefit of existing food items (i.e. extrinsic exploration; Bailey *et al.* 1996; Day *et al.* 1998). Intrinsic exploration usually only appears when a shortage of food stresses an animal to the point where novel food items are cautiously eaten, if the post-ingestive effects are positive, over time, more of the food item will be eaten and vice versa (Day *et al.* 1998). To a lesser or greater extent extrinsic exploration is always present as animals gather information while feeding (Day *et al.* 1998).

1.4.2.2. An Element of Scale

Ungulates, as with all animals, interact with food items/resources at several spatial and temporal resolutions (Senft 1987; Skarpe *et al*. 2000). Bailey and colleagues (1996) described six spatial and temporal scales within a foraging hierarchy: bite, feeding station, patch, feeding site, camp and home range. Ungulates display different foraging behaviours at the different scales; the decisions made at the higher levels have greater potential costs and benefits and constrain the potential decisions at lower levels (Wiens 1989; Senft 1987; Bailey

et al. 1996; Parker *et al*. 2009). Due to temporal and financial constraints, studies focusing on ungulate foraging behaviour usually occur at intermediate to lower levels (Wiens 1989). Therefore our understanding of higher level decisions such as dispersal and migration routes have been extrapolated from lower level decisions; however this is common practice in hierarchical theory (Wiens 1989; Senft 1987; Bailey *et al.* 1996; Parker *et al*. 2009).

1.5. THE STUDY OUTLINE

1.5.1. THE STUDY SITE

The Kruger National Park (KNP) was established in 1898 as the Sabie Game Reserve in order to control hunting and eliminate poaching within the area (Carruthers 1995; Saayman and Slabbert 2004; van der Merwe and Saayman 2008; Venter *et al*. 2008). Management has transitioned from a 'command and control' philosophy, adopted throughout the most of the 20th century in order to maximize productivity, to the idea that ecosystems are spatially and temporally heterogeneous (Carruthers 1995; Venter *et al*. 2008). The artificial waterholes and dams which were used to distribute water to the drier regions, particularly in the dry season, are being deconstructed (Pienaar 1963; Venter *et al*. 2008). The fire policy which aimed to exclude all fire, now aims to mimic the natural fire regime (Pienaar 1963; Venter *et al*. 2008). Culling which was used to control carnivore (to boost prey numbers) and herbivore (to prevent over-grazing/browsing) populations, has been discontinued on all mammals with known population control mechanisms (Pienaar 1963; Venter *et al*. 2008). And, fences which separated the KNP from private game parks, nature reserves and international game parks are being removed (Pienaar 1963; Venter *et al*. 2008).

The KNP covers an area of approximately 19 600 km^2 (making the KNP one of the largest game parks in southern hemisphere) with a summer rainfall regime (from October to March) and supports 147 species of mammals, 505 birds, 116 reptiles, 34 amphibians, 49 fishes, 1980 plants (including 404 trees and shrubs and 224 grasses) and a countless number of invertebrates within 11 distinct vegetation types and three drainage basins (the Luvuvhu/Letaba, the Olifants and the Inkomati; Pienaar 1963; Saayman and Slabbert 2004; Pienaar 1963; Carruthers 1995; Mucina and Rutherford 2006; Saayman and Saayman 2006; Venter *et al*. 2008). The KNP is one of the few national parks in the world that is financially self-sufficient because of its heavy reliance on the tourism industry (Turpie and Joubert 2001; Saayman and Slabbert 2004; Saayman and Saayman 2006; van der Merwe and Saayman 2008; Saayman and Saayman 2009). It is therefore financially sensible that managers invest into the conservation of the park in order to sustain the element of tourism (Turpie and Joubert 2001; Saayman and Saayman 2006; Saayman and Saayman 2009). However anthropogenic pollution coupled with climate change is becoming an increasing threat to the KNP.

1.5.1.1. Nitrogen and Atmospheric Pollution

Mphepya *et al*. (2006) analyzed the quality of rainwater, from 93 rainfall events at Skukuza (the main rest-camp in the KNP), and found that on average the pH was 4.72 with high concentrations of sulphate (16.3 μ $eq.l^{-1}$), ammonium (9.0 μ $eq.l^{-1}$) and nitrate (8.1 μ $eq.l^{-1}$) ions which were attributed to industrial emissions from Mpumalanga Highveld (Mphepya *et al*. 2004). In this study, nitrogen availability within the soil will be used as a surrogate for increasing atmospheric pollution as nitrogen compounds are common atmospheric pollutants. Venter and colleagues (2008) reported that the total atmosphere nitrogen deposition, within the KNP, has increased from 1-5 $kg.ha^{-1}.y^{-1}$ in the mid-20^{th} century to as much as 20

kg.(ha.y)$^{-1}$; this was also attributed to the industrial emission from Mpumalanga Highveld, especially the numerous coal-burning power stations (Spalding-Fecher and Matibe 2003; Galy-Lacaux *et al.* 2009; Scorgie and Kornelius 2009).

1.5.1.2. Phosphorus and Aquatic Pollution

de Villiers and Mkwelo (2009) discussed how the increasing effluents from agriculture, urban settlements, various industries (include numerous mines) in the upper and middle reaches of the Olifants River (one of the KNP primary water sources), have polluted the catchment to such an extent that the water quality exceeds the standards set by the South African Water Quality Guidelines and is therefore not suitable for either human or ecosystem consumption (Spalding-Fecher and Matibe 2003; Venter *et al*. 2008; Ashton 2010). Already fish and aquatic reptile populations, particularly the Nile Crocodile (*Crocodylus niloticus*), are declining as a result (Ashton 2010). Polluted water usually has a high phosphorus content derived from untreated sewage and industrial detergents which is then be redistributed onto land via flooding events and animals (i.e. urine derived from phosphorus enriched water sources and dung derived from phosphorus enriched riparian vegetation).

1.5.1.3 Water and Climate Change

The increasing concentration of greenhouse gasses in the atmosphere is affecting various aspects of weather, including the amount and distribution of precipitation (Johns *et al.* 2003; Zhang *et al.* 2007). Various scenarios of the HadCM3 model (as described in Johns *et al.* 2003) suggest that by 2100 ocean temperatures would have risen by 2 – 4 °C, while land temperatures would have risen by 4 – 8 °C resulting in a slight increase in the amount of rainfall at the ITCZ (Zhang *et al.* 2007). This increase in the amount of rainfall would result in longer and wetter growing seasons which could alter the vegetation structure within the

savannas (Johns *et al.* 2003; Zhang *et al.* 2007; Lehmann *et al.* 2011). Already the increasing levels of CO_2 and water availability are favoring the C_3 plants resulting in increasing bush encroachment (Smit 2004; Tews *et al*. 2004; Zhang *et al.* 2007; Venter *et al*. 2008).

1.5.2. THE AIMS AND OBJECTIVES OF THE STUDY

The question of this study relies on three premises. Firstly, the vegetation quality (i.e. the forage quality or concentration of nitrogen and phosphorus within the leaves) and quantity (i.e. biomass) is determined by complex interactions among the available nutrients and water as well as herbivory and fire (Frost *et al.* 1985). Secondly, ungulates are able to modify their feeding behavior more so than their diet in order to maximize nutrient gain while minimizing associated risks (Day *et al.* 1998; Provenza *et al.* 1998; Kyriazakis *et al*. 1999). Lastly, the Kruger National Park (KNP) is being threatened by anthropogenic pollution, coupled with climate change (Johns *et al.* 2003; Mphepya *et al*. 2006; Zhang *et al.* 2007; de Villiers and Mkwelo 2009; Ashton 2010). With this in mind, the question of this study is: if soil nutrient and water availability were to increase, due to increasing anthropogenic change, would there be enough change to the vegetation quality and quantity to have an effect on the feeding behavior and diet of the local ungulate populations?

In this study nitrogen, phosphorus and water additions are used as surrogates for increasing atmospheric pollution, aquatic pollution and precipitation (respectively). Prior to this study, nitrogen, phosphorus and water were already added in full permutation to 16 plots (i.e. there are eight possible combinations of these three nutrients, including one plot left untreated, each treatment is replicated once) in the central region of the KNP as part of Dr. Leigh-Ann Woolley's postdoctoral study. These plots are 30 m in diameter and spaced 10 m apart in a four-by-four block arrangement. The nutrient additions occurred in December of 2009, 2010

and 2011 with nitrogen added in the form of LAN 14%, phosphorus in the form of superphosphate and water added using an irrigation system. The data collected at the treatment site was compared with similar data collected at three control sites assuming these to be indicative of the normal environmental conditions. At each of these control sites three untreated plots (of 30 m in diameter and spaced 10 m apart) were set out.

I first determined whether the vegetation at the treatment site had more nutrients than the vegetation at the control sites. To do this I collected leaves from the grasses and trees to determine nitrogen and phosphorus content. Since the treatment site had been fertilized with nitrogen and phosphorus I expected the vegetation at this site to have higher nutrient content than the vegetation at the control sites.

Based on these results I then determined whether this change in the vegetation quality is enough for the ungulates to modify their feeding behavior and/or diet. To determine a change in the feeding behavior, I deployed several camera traps (ScoutGuard SG550, HCO Outdoor Products, Georgia, U.S.A) at each site and determined a rudimentary feeding rate. Since ungulates are able to modify their feeding behavior in order to exploit nutrient rich food, I expected the feeding rate to be higher at the treatment site than at the control sites. To determine a change in the diet, I then collected ungulate dung from each site and determined the nitrogen and phosphorus content as well as the $\delta^{13}C$ values. If ungulates were exploiting the enriched vegetation at the treatment site, I would expect the ungulate dung at the treatment site would have higher nitrogen and phosphorus content than the ungulate dung at the control sites. The different photosynthetic pathways of C_4 grasses and C_3 trees result in distinct, non-overlapping $\delta^{13}C$ values of -26.40 ± 0.24 ‰ for trees and -12.85 ± 0.29 ‰ for grasses (Vogel *et al*. 1978; Codron *et al.* 2005a; Codron *et al.* 2005b; February and Higgins

2010; February *et al*. 2013b). The $\delta^{13}C$ values of well identified ungulate dung should show the difference between pure grazers and pure browsers and (via the use of a mixing model; February and Higgins 2010) should show the proportion of grasses and trees in the diet of mixed feeders.

I concluded by determining the amount of change in the vegetation quantity at the treatment site compared to the control sites. Since the treatment site has been fertilized with nitrogen, phosphorus and water I expected the increase in productivity to result in taller grasses and larger trees with dense canopies than the vegetation at the control sites. Alternatively, if the feeding rate were higher at the treatment site the increased herbivory would result in shorter grasses and smaller trees with sparser canopies than the vegetation at the control sites. Fire would also result in diminished vegetation quantity and therefore was purposefully kept out of the system for the duration of the study. To determine a change in the vegetation quantity, in each plot, the height of every grass plant was measured along a transect spanning the diameter of the plots and specimens of the three dominant tree species were subjectively assessed. This tree assessment included estimating the height, basal diameter, canopy dimensions and composition of the canopy

If, at the treatment site, I am able to detect an increase in the vegetation quality as well as a change in the feeding behavior and diet of the ungulates and able to attribute this to a detectable change in the vegetation quantity at the treatment site, I can then conclude that nitrogen, phosphorus and water fertilization (i.e. anthropogenic pollution coupled with climate change) can initiate a cascading chain of events which could alter the vegetation structure and influence the animal demographics within the savannas of the KNP.

CHAPTER 2

THE POTENTIAL EFFECTS OF ADDITIONAL SOIL NITROGEN, PHOSPHORUS AND WATER ON THE FEEDING BEHAVIOUR AND DIET OF THE LARGE HERBIVORES WITHIN AN AFRICAN SAVANNA

2.1. INTRODUCTION

With the increase in the human population there has been an increase in agriculture, industrialization and urbanization to such an extent that anthropogenic change is threatening global ecosystem integrity and biodiversity (Tilman *et al*. 2001; Johns *et al*. 2003; Güsewell 2004; Elser *et al*. 2007; Mahowald *et al*. 2008; Conley *et al*. 2009; Bobbink *et al*. 2010). South Africa is fortunate enough to have one of the largest (19 600 km^2) and oldest (the original Sabie Game Reserve was established in 1989) game parks in the southern hemisphere, the Kruger National Park (KNP; Pienaar 1963; Carruthers 1995; van der Merwe and Saayman 2008). However, there is an increasing amount of evidence to suggest that the KNP is at risk of anthropogenic pollution coupled with climate change, even though the park is situated far from any urban or industrial centre. Mphepya *et al*. (2006) measured the quality of rainwater at Skukuza (the main rest-camp in the KNP) and found that it had high concentrations of ammonium and nitrate ions which were attributed to the increasing industrial emissions on the Mpumalanga Highveld (particularly the numerous coal-burning power stations; Galy-Lacaux *et al.* 2009; Scorgie and Kornelius 2009). Furthermore, the increasing concentration of greenhouse gasses in the atmosphere is affecting various aspects of weather, including precipitation distribution; various scenarios of the HadCM3 model (as

described in Johns *et al.* 2003) predict an increase in the amount of rainfall at the Inter-Tropical Convergence Zone (ITCZ) which would result in longer and wetter growing seasons for the KNP (Zhang *et al.* 2007). de Villiers and Mkwelo (2009) discussed how the increasing effluents from urban and rural settlements, irrigated agriculture and an array of industries in the upper and middle reaches of the Olifants River catchment (one of the primary water sources of the KNP), have polluted the water to such an extent that it exceeds the standards set by the South African water quality guidelines (Spalding-Fecher and Matibe 2003; Ashton 2010).

Savannas are comprised of a continuous grass layer and a discontinuous tree layer (Frost *et al.* 1985; Mucina and Rutherford 2006; Sankaran *et al.* 2004; February *et al*. 2013b). The relative abundance of grasses and trees is dictated by complex and dynamic interactions among nutrient and water availability, fire and herbivory (Frost *et al.* 1985; Scholes and Archer 1997; Higgins *et al*. 2000; van Langevelde *et al*. 2003; Smit 2004; Tews *et al*. 2004; Higgins *et al*. 2007; Lehmann *et al.* 2011; February *et al*. 2013b). The increasing anthropogenic changes to the KNP would result in the increasing availability of nitrogen (a common atmospheric pollutant), phosphorus (a common aquatic pollutant) and water (as a result of climate change within the area) in the soil. This will enrich the vegetation (i.e. higher nutrient concentrations within the leaves and stems) and stimulate productivity (Scholes 1990). In the absence of such nutrient and water limitations, trees (the superior competitor) are able to outcompete grasses leading to the formation of forests (Sankaran *et al*. 2005; Staver *et al*. 2011a; Staver *et al*. 2011b; Dohn *et al.* 2013). Disturbance regimes (i.e. fire and herbivory) intervene and prevent the germination, establishment and maturation of trees thus maintaining the co-existence of grasses and trees (Sankaran *et al.* 2004; Sankaran

et al. 2005; Staver *et al*. 2009; Kambatuku *et al.* 2011; Staver *et al*. 2011a; Staver *et al*. 2011b; Wakeling *et al*. 2011).

Pure grazers and browsers cannot change the components of their diet (grazers feed primarily on grasses while browsers primarily feed on trees) but mixed-feeders can (usually grazing in the wet season and browsing in the dry season; Grant *et al.* 1995; Day *et al.* 1998; du Toit *et al*. 2003; Sponheimer *et al.* 2003). The animals are equipped with a variety of senses that allow them to modify their diet in order to exploit the most nutritious source of food available (Day *et al.* 1998; Provenza *et al.* 1998; Kyriazakis *et al*. 1999). This was shown in Scholes (1990) where the abundance of ungulates increased at enriched sites. If anthropogenic changes in the KNP enrich the grasses more than trees there should be an increase in the grazing intensity (by grazers and mixed-feeders; Grant *et al.* 1995; Day *et al.* 1998; Sponheimer *et al.* 2003). Alternatively, if the anthropogenic changes enrich trees more than grasses there should be an increase in the browsing intensity (by browsers and mixed-feeders; Grant *et al.* 1995; Day *et al.* 1998; Sponheimer *et al.* 2003). The increase in grazing and/or browsing could alter the vegetation structure enough to cause a biome change. Perhaps the means to determine the affect of anthropogenic changes to the vegetation structure lies within the dung of the mixed-feeders. One can determine the proportion of grasses and trees in the diets in mixed-feeders through the determination of the $\delta^{13}C$ values of their dung, by using a simple mixing model in conjunction with known $\delta^{13}C$ values for the grasses and trees within the area (-12.85 ‰ for the grasses and -26.40 ‰ for the trees; Vogel *et al*. 1978; Codron *et al.* 2005a; Codron *et al.* 2005b; February and Higgins 2010).

In this study, I exclude fire while examining the potential (and unstudied) link between the increasing anthropogenic change and the effect it has on animal demographics. The aim is to determine whether additional nitrogen, phosphorus and water to a savanna ecosystem (within the KNP) would alter the vegetation quality (i.e. the forage quality or concentration of nitrogen and phosphorus within the leaves) and quantity (i.e. biomass) enough to influence the feeding behaviour and diet of the large herbivores (i.e. ungulates). I use nitrogen, phosphorus and water additions to represent an increase in atmospheric pollution, aquatic pollution and precipitation due to climate change (respectively). I predict an increase in vegetation quality and productivity (which would increase vegetation quantity). This would attract the attention of the local ungulate populations and the resulting increase in herbivory should diminish the vegetation quantity. Depending how severe these changes (to the factors maintaining the grass-tree co-existence) are, the savanna ecosystem could experience a biome change. The results of such preliminary studies could have significant impacts on conservation planning and implementation as well as place greater pressure on the government and industries to develop more effective means of cleaning agricultural, industrial and urban emissions and effluents.

2.2. METHODS

2.2.1. STUDY AREA

The study was conducted in the central region of the Kruger National Park (near the Satara rest-camp), Mpumalanga Province, South Africa (Fig. 1). The study area occurred on a narrow strip of sandy soil running north to south at the convergence of the, more dominate, Granite and Basalt soils (Fig. 1; Mucina and Rutherford 2006). The soil is derived from Karoo sedimentary rocks of the Ecca group and is rich in sodium and very susceptible to erosion (du Toit *et al*. 2003; Mucina and Rutherford 2006). The vegetation is described by Mucina and Rutherford (2006) as 'Delagoa Lowveld'; *Dichrostachys cinerea* is the dominant tree species, while *Urochloa mosambicensis* and *Chloris virgata* are the dominant grass species. The growing season starts with the first rains in late October, and ends with the last rainfalls in early April (Mucina and Rutherford 2006; February *et al*. 2013b). The mean annual rainfall is between 450 – 850 mm. Mean monthly maximum and minimum temperatures are 30 ˚C and 18 ˚C for December and 25 ˚C and 6 ˚C for June (Mucina and Rutherford 2006; February *et al*. 2013b).

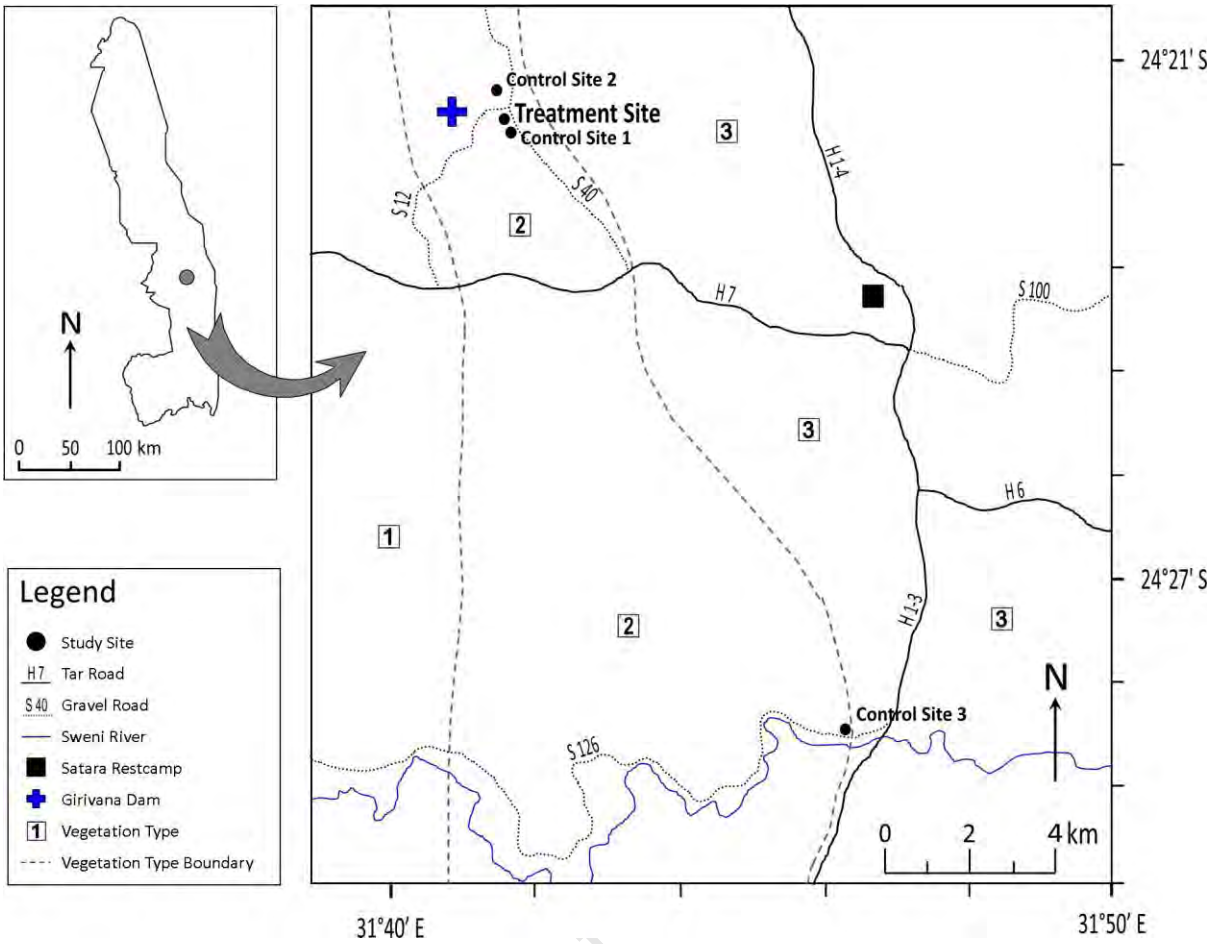


Figure 1: Map of the study area, showing the position of the four study sites (Google Earth 2011). Vegetation type 1 – Granite Lowveld derived from granite soil; Vegetation type 2 – Delagoa Lowveld derived from sandstone soil; Vegetation type 3 –Tshokwane-Hlane Basalt Lowveld derived from basalt soil (Mucina and Rutherford 2006).

2.2.2. EXPERIMENTAL DESIGN

Four sites were examined during the course of this study; the treatment site (24˚21'39"S, 31˚41'38"E), control site 1 (24˚21'43"S, 31˚41'38"E), control site 2 (24˚21'22"S, 31˚41'30"E) and control site 3 (24˚28'51"S; 31˚46'14"S) (Fig. 1; Google Earth 2011). Since fire has the capacity to alter the vegetation quality and quantity, fire was purposefully kept out of the system for the duration of the study (Higgins *et al*. 2000; Sankaran *et al.* 2004; Staver *et al*. 2011a; Staver *et al*. 2011b; Wakeling *et al*. 2011).

The treatment site consisted of sixteen plots of 30 m in diameter spaced 10 m apart in a crude 4x4 block arrangement (Fig. 2). Nutrient additions occurred in December of 2009, 2010 and 2011 following the application guidelines set by the Nutrient Network (NutNet; Lind *et al*. 2013). Two plots were treated with nitrogen (~12 $g.m^{-2}.yr^{-1}$ as limestone ammonium nitrate (LAN 14 %)), two with phosphorus (~5 $g.m^{-2}.yr^{-1}$ as superphosphate), two with both nitrogen and phosphorus, and two left untreated (LAN 14 % and superphosphate were used as they were readily available in bulk supply; Lind *et al*. 2013). One plot from each pair was irrigated (via an installed irrigation system linked to the Girivana dam reservoir) with the equivalent of 30 mm of rainfall per month for the growing season (October to March) to elevate the MAP beyond the threshold which limits the formation of forest (i.e. eliminate water as a limiting factor; Sankaran *et al*. 2005; Staver *et al*. 2011a). These eight treatments were replicated once.

Due to financial restrictions the treatment site could not be replicated, however three control sites were used to quantify the difference between the treatment and the controls (as was suggested in Oksanen 2001). The control sites were selected according to their close proximity and similarity to the treatment site. Here, similarity is meant in terms of expected soil and vegetation type (as described in du Toit *et al*. 2003 and Mucina and Rutherford 2006) and proximity to a water source (the treatment site and control sites 1 and 2 are roughly equidistant from the Girivana Dam, while control site 3 is next to the Sweni River). Each of the control sites consisted of three plots of 30 m in diameter spaced 10 m apart in a linear arrangement (Fig. 2). These plots were left un-treated.

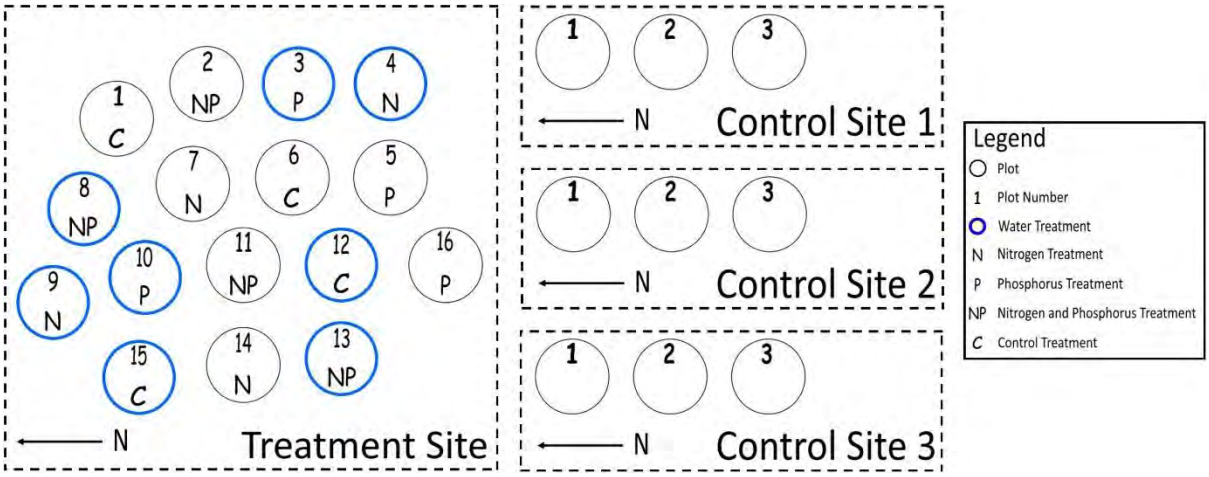


Figure 2: Schematic representation of the experimental design of the four study sites

2.2.3. SAMPLING

Sampling occurred in January (wet season) and June (dry season) of 2012. Sampling occurred at every plot; however the data were analyzed and presented at the site level. It is important to note that the treatment site was considered as a single enriched site which was then compared to three non-enriched sites.

In each plot, leaf samples were collected from the four dominant grass species (i.e. *Digitaria eriantha, Eragrostis rigidior*, *Panicum maximum* and *Urochloa mosambicensis*; 3 grams per species) and from the three dominant tree species (i.e. *Acacia nilotica*, *Acacia tortilis* and *Dichrostachys cinerea*; 3 grams per species) when present. Also in each plot, dung samples were collected from the dominant ungulate species (i.e. buffalo, duiker, elephant, giraffe, impala, steenbok, white rhino, wildebeest and zebra; at least 5 grams per species) when present. Sampling was done at random, but preference was given to new growth (young leaves of grasses and trees) and fresh dung (in accordance with Wrench *et al.* 1996). Samples were collected in paper packets, labeled, and dried for at least 48 hours in a 60˚C oven (in accordance with Wrench *et al.* 1996).

An infrared camera trap (ScoutGuard SG550, HCO Outdoor Products, Georgia, U.S.A) was deployed at the centre of each plot from August 2012 to November 2012. These were housed in metal casings, attached to a tree and orientated away from direct sunlight. The power was supplied by L91 15LF Eveready lithium batteries which last for approximately 600 still photos. The cameras have an effective range of 20 m which covers an area of 314 m^2. The cameras were set to have a minimum delay of 15 seconds between successive photos with the date and time of each photo recorded. The cameras were initially checked after a week and the photos were then downloaded on a monthly basis.

In each plot the maximum height of every grass plant was measured (using a meter ruler) along a transect spanning the diameter of each plot while taking note of the frequency of each species. Also, in each plot, three specimens of the dominant tree species (i.e. *A. nilotica*, *A. tortilis* and *D. cinerea*) were subjectively assessed according to an assessment devised by Leigh-Ann Woolley (2012 pers. comm.). The assessment required one to estimate the height of the tree (m), the basal stem diameter (mm), the area enclosing the canopy (m^2), the height of the canopy above the ground (m), how much of the canopy has been removed due to browsing and/or fire (%) and score the degree of damage (ranging from no damage (0) to severe (4)). The assessment also required one to estimate how much of the tree consisted of non-woody (i.e. herbaceous), new-wood and old-wood sections (totaling 100 %) as well as estimate how much of the canopy consisted of young leaves, mature leaves, senescent leaves, flowers and pods (totaling 100 %).

2.2.4. Carbon, Nitrogen and Phosphorus Analysis

Prior to carbon, nitrogen and phosphorous analysis the leaf and dung samples were ground to a fine powder using a Wiley Mill (Thomas Scientific, New Jersey, U.S.A; Codron *et al.*

2005a; February and Higgins 2010). Samples were sent to the Stable Light Isotope Laboratory (Department of Archeology, University of Cape Town, Cape Town) to determine the stable carbon isotope ratio as well as the nitrogen content. Samples were also sent to the Institute for Plant Production (Department of Agriculture, Elsenburg) to determine the phosphorus content.

The $\delta^{13}C$ values and nitrogen content (expressed as a percentage of the total weight) were determined using a Flash 2000 elemental analyzer (Thermo Scientific, Bremen, Germany) coupled with a Delta V Plus isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) via a Conflo IV gas control unit (Thermo Scientific, Bremen, Germany; February and Higgins 2010; I. Newton 2012 pers. comm.). Three internal standards were used to calibrate the results relative to the International Atomic Energy Agency standards; Atmospheric N_2 for nitrogen and Pee-Dee Belemnite for carbon (Codron *et al.* 2005a; February and Higgins 2010; I. Newton 2012 pers. comm.).

The phosphorus content (expressed as a percentage of the total weight) was determined by dry-ashing the ground samples at 480˚C for 8 hours and dissolving with a 1:1 (v/v) of Hydrochloric acid according to Kalra (1998) and Power *et al*. (2010). Assessment of the percentage Phosphorus concentration in solution was determined with an inductively coupled plasma atomic emission spectrometry (Varian Vista MPX, Mulgrave, Australia).

2.2.5. Determining the Composition of the Impala Diet

The proportion of grasses and trees in the diet of the Impala were determined using the mixing model described in February and Higgins (2010):

$$p = \frac{G - S}{G - W}$$

Where *p* the proportion of trees in the diet and 1 – *p* is the proportion of grasses in the diet. *G* is the $\delta^{13}C$ value of the grasses (which is -12.85 ‰) and *W* is the $\delta^{13}C$ value of the trees which is (which is -26.40 ‰; February and Higgins 2010). *S* is the mean (per study site per season) isotopic $\delta^{13}C$ value for the Impala dung samples (February and Higgins 2010).

2.2.6. DETERMINING FEEDING RATE

Usually, the camera trapping rate (i.e. the number of photos of a particular species per unit of time) is taken as a measure of animal abundance/density (Kuijper *et al.* 2009; Rovero and Marshall 2009). However, I modified the camera trapping rate by only using the photos depicting ungulates grazing/browsing to determine a feeding rate per 314 m^2 (i.e. the monitored area per plot) which will be used as a proxy for the degree of herbivory (which is determined by the feeding behavior) at each site. This feeding rate was calculated by adding the time (in seconds) ungulate species *x* spent grazing/browsing in front of camera trap *y*, divided by the number of photos camera trap *y* took of ungulate species *x* (in order to account for the difference in the number of photos between camera traps) and divided by the trapping effort of camera trap *y* (i.e. the duration, in days, the camera trap monitored the area; Yasuda 2004; Bowkett *et al.* 2007; Datta *et al.* 2008; Kuijper *et al.* 2009; Rovero and Marshall 2009). The time spent feeding was determined as the time between the first and last photo of a single individual or a herd seen grazing/browsing. In instances where there is a single photo of an individual or herd seen grazing/browsing, the time was assumed to be the same as the minimum time between successive photos (i.e. 15 seconds). Separate feeding rates were determined for the dry (late March to mid-October) and wet seasons (late October to mid-March) by dividing the trapping effort according to the rainfall records within the area.

2.2.7. STATISTICAL ANALYSES

All statistical analyses were conducted in R version 3.0.0 (R Development Core Team 2013). The dataset was separated according to the dry and wet season in order to account for the seasonal changes in the vegetation quality and quantity as well as the feeding behaviour and diet of the ungulates.

Kruskal-Wallis tests followed by post-hoc analyses (multiple comparisons tests, MCT, using the 'kruskalmc' function from the 'pgirmess' package; Giraudoux 2013) were used to determine whether there were any significant differences in the $\delta^{13}C$ values (only for the dung samples), the percentage nitrogen and the percentage phosphorus in the leaf and dung samples collected at the study sites (Stanton-Geddes *et al*. 2013). Kruskal-Wallis tests followed by multiple comparisons tests were also used to determine whether there were any significant differences in the feeding rates of the ungulates at the study sites (Giraudoux 2013; Stanton-Geddes *et al*. 2013).

Hierarchical Cluster Analyses, with the aid of the 'pvclust' package (Suzuki and Shimodaira 2011), were used to determine the degree of dis/similarity amongst the 25 plots based on the frequency of the grass species at the study sites (Crawley 2007). These were the only statistical tests conducted at the individual plot level; the remaining statistical tests were conducted at the site level. Euclidean distance was used as the distance measure, while the Ward's method was used as the clustering procedure. Dendrograms were constructed from 10 000 iterations with the approximate unbiased p-values and the bootstrap probabilities displayed at each branch.

Generalized Linear Models (GLM's), coupled with goodness-of-fit tests (Chi-square) and post-hoc analyses (Tukey HSD), were used to determine whether there were any significant differences in the height of the grasses among the study sites (Crawley 2007). These tests were conducted with the aid of the 'mvtnorm' (Genz *et al.* 2012) and the 'multcomp' (Hothorn *et al.* 2008) packages. A Quasi -Poisson distribution was assumed after examining the dispersion parameters and the data underwent Box-Cox transformations in order to normalize the residuals (Faraway 2005; Crawley 2007). The Box-Cox transformation is expressed as:

$$f_{transformed}(x) = \begin{cases} \frac{x^{\lambda} - 1}{\lambda} & \lambda \neq 0 \\ \log x & \lambda = 0 \end{cases}$$

Where lambda (λ) was determined by the 'boxcox' function from the 'MASS' package (Venables and Ripley 2002; Faraway 2005).

Principle Component Analyses (PCA's) were used to identify the combination of tree variables which best describe the variation within the tree assessments in order to determine whether there were morphological differences in the trees among the study sites (Crawley 2007). The variables were scaled to have a variance of one and shifted to be centered on zero. Irrespective of how much of the variation was explained, the results focused on the first two components which were presented as biplots. GLM's were used in the same manner as specified above, to test the significance of the tree variables identified by the PCA's as being indicative of the morphological differences in the trees among the study sites. However, a Gaussian distribution was assumed after examining the dispersion parameters and the data underwent an Arcsine transformation in order to normalize the data and residuals (Faraway 2005; Crawley 2007).

2.3. RESULTS

2.3.1. CHEMICAL ANALYSES

A total of 163 leaf samples of the four grass species (88 in the dry season and 75 in the wet season), 197 leaf samples of the three tree species (101 in the dry season and 96 in the wet season) and 140 dung samples of nine ungulate species (75 in the dry season and 65 in the wet season) were collected and analyzed (Appendix 1, 2 and 3). The aim was to maintain a standard sample size of three or more for each species present at the four study sites in both the wet and dry seasons, but in many instances it was not possible due to a lack of material *in situ* and/or after processing the samples (Appendix 1, 2 and 3).

2.3.1.1. Grass Leaves

The percentage nitrogen in the leaf samples collected at the treatment site were on average higher than the leaf samples collected at the control sites; the only exception being *P. maximum* (dry season) where the samples collected at control site 3 had higher percentage nitrogen than the samples collected at the treatment site (Table 1). There are two significant relationships to support this trend. The samples of *P. maximum* (wet season) had significantly higher percentage nitrogen (Kruskal-Wallis; $\chi^2 = 14.45$; df = 3; $p < 0.05$) at the treatment site than at control site 1 ($MCT_{0.05}$). The samples of *U. mosambicensis* (dry season) had significantly higher percentage nitrogen (Kruskal-Wallis; $\chi^2 = 9.93$; df = 3; $p < 0.05$) at the treatment site than at control site 1 ($MCT_{0.15}$).

The percentage phosphorus in the leaf samples collected at the treatment site were on average higher than the leaf samples collected at control sites 1 and 2; however, the leaf samples collected at control site 3 had the highest average percentage phosphorus (Table 1). There are

four significant relationships to support these trends. The samples of *P. maximum* (dry season) had significantly higher percentage phosphorus (Kruskal-Wallis; $\chi^2 = 4.50$; df = 1; $p < 0.05$) at control site 3 than at the treatment site ($MCT_{0.05}$). The samples of *P. maximum* (wet season) had significantly higher percentage phosphorus (Kruskal-Wallis; $\chi^2 = 8.06$; df = 3; $p < 0.05$) at control site 3 than at control site 1 ($MCT_{0.05}$). The samples of *U. mosambicensis* (dry season) had significantly higher percentage phosphorus (Kruskal-Wallis; $\chi^2 = 10.18$; df = 3; $p < 0.05$) at control site 3 than at the treatment site and control site 1 ($MCT_{0.10}$). The samples of *U. mosambicensis* (dry season) had significantly higher percentage phosphorus (Kruskal-Wallis; $\chi^2 = 10.25$; df = 3; $p < 0.05$) at control site 3 than at control site 1 ($MCT_{0.05}$).

Table 1: The average nitrogen and phosphorus content (%) of the leaf samples of *D. eriantha, E. rigidior, P. maximum* and *U. mosambicensis* collected at the study sites during the dry and wet season. Standard deviation is given as the measure of variation; n/a indicates where it is not possible to calculate the standard deviation (i.e. *n* = 1). The *p* value indicates the results of a Kruskal-Wallis test and (where $p < 0.05$) the letters in subscript indicate the results of a multiple comparisons test (MCT) conducted at the 0.05 level of significance; different letters indicate a significant difference.

	Season	Treatment Site	Control Site 1	Control Site 2	Control Site 3	*p* value
Percentage Nitrogen						
D. eriantha	Dry	1.28 ± 0.50	0.75 ± 0.17	0.90 ± 0.13	-	0.27
	Wet	1.48 ± 0.33	1.48 ± n/a	-	1.90 ± n/a	0.35
E. rigidior	Dry	1.28 ± 0.69	0.73 ± 0.24	1.04 ± n/a	-	0.33
	Wet	-	1.57 ± 0.11	-	-	-
P. maximum	Dry	1.18 ± 0.44	-	-	1.78 ± 0.44	0.16
	Wet	$2.80 \pm 0.37_{a}$	$1.81 \pm 0.06_{b}$	$2.02 \pm 0.10_{ab}$	$1.91 \pm 0.02_{ab}$	< 0.05
U. mosambicensis	Dry	$2.22 \pm 1.08_{a}$	$1.00 \pm 0.16_{a}$	$1.27 \pm 0.42_{a}$	$0.96 \pm 0.33_{a}$	< 0.05
	Wet	2.37 ± 0.65	1.89 ± 0.57	2.02 ± 0.33	2.00 ± 0.46	0.43
Percentage Phosphorus						
D. eriantha	Dry	0.17 ± 0.12	0.09 ± 0.04	0.15 ± 0.02	-	0.61
	Wet	0.25 ± 0.08	0.21 ± n/a	-	0.57 ± n/a	0.25
E. rigidior	Dry	0.14 ± 0.16	0.07 ± 0.02	0.11 ± n/a	-	0.38
	Wet	-	0.11 ± 0.01	-	-	-
P. maximum	Dry	$0.14 \pm 0.04_{a}$	-	-	$0.26 \pm 0.07_{b}$	< 0.05
	Wet	$0.38 \pm 0.50_{ab}$	$0.17 \pm 0.04_{a}$	$0.25 \pm 0.04_{ab}$	$0.48 \pm 0.02_{b}$	< 0.05
U. mosambicensis	Dry	$0.25 \pm 0.14_{a}$	$0.07 \pm 0.01_{a}$	$0.11 \pm 0.05_{a}$	$0.36 \pm 0.23_{a}$	< 0.05
	Wet	$0.25 \pm 0.08_{ab}$	$0.18 \pm 0.03_{a}$	$0.20 \pm 0.08_{ab}$	$0.66 \pm 0.12_{b}$	< 0.05

2.3.1.2. Tree Leaves

The percentage nitrogen in the leaf samples collected at the treatment site were on average higher than the leaf samples collected at the control sites; the only exception being *A. tortilis* (wet season) where the samples collected at the treatment site had the lowest percentage nitrogen in comparison to the samples collected at the control site (Table 2). There are two significant relationships to support this trend. The samples of *A. nilotica* (dry season) had significantly higher percentage nitrogen (Kruskal-Wallis; $\chi^2 = 10.02$; df = 3; $p < 0.05$) at the

treatment site than at control site 2 ($MCT_{0.05}$). The samples of *D. cinerea* (dry season) had significantly higher percentage nitrogen (Kruskal-Wallis; $\chi^2 = 12.54$; df = 3; $p < 0.05$) at the treatment site than at control site 2 ($MCT_{0.10}$).

The percentage phosphorus in the leaf samples collected at the treatment site were on average higher than the leaf samples collected at the control sites; with the exception of *A. tortilis* and *D. cinerea* (wet season) where the samples collected at control site 3 had higher percentage phosphorus than the samples collected at the treatment site (Table 2). There is only one significant relationship to support this trend. The samples of *A. nilotica* (wet season) had significantly higher percentage phosphorus (Kruskal-Wallis; $\chi^2 = 10.60$; df = 3; $p < 0.05$) at the treatment site than at control site 2 ($MCT_{0.05}$).

Table 2: The average nitrogen and phosphorus content (%) of the leaf samples of *A. nilotica, A. tortilis and D. cinerea* collected at the study sites during the dry and wet season. Standard deviation is given as the measure of variation; n/a indicates where it is not possible to calculate the standard deviation (i.e. *n* = 1). The *p* value indicates the results of a Kruskal-Wallis test and (where *p* < 0.05) the letters in subscript indicate the results of a multiple comparisons test (MCT) conducted at the 0.05 level of significance; different letters indicate a significant difference.

	Season	Treatment Site	Control Site 1	Control Site 2	Control Site 3	*p* value
Percentage Nitrogen						
A. nilotica	Dry	$2.29 \pm 0.51_{a}$	$2.05 \pm 0.47_{ab}$	$1.47 \pm 0.19_{b}$	$1.50 \pm n/a_{ab}$	< 0.05
	Wet	2.43 ± 0.48	2.40 ± 0.29	2.08 ± 0.23	2.10 ± n/a	0.47
A. tortilis	Dry	2.78 ± 0.45	2.12 ± 0.46	2.48 ± 0.31	2.71 ± 0.11	0.16
	Wet	2.54 ± 0.44	2.88 ± 0.43	2.82 ± 0.30	2.93 ± 0.26	0.32
D. cinerea	Dry	$2.57 \pm 0.49_{a}$	$2.07 \pm 0.51_{a}$	$1.83 \pm 0.17_{a}$	$1.90 \pm 0.18_{a}$	< 0.05
	Wet	2.64 ± 0.24	2.44 ± 0.13	2.59 ± 0.17	2.61 ± 0.11	0.34
Percentage Phosphorus						
A. nilotica	Dry	0.14 ± 0.04	0.13 ± 0.03	0.09 ± 0.02	0.08 ± n/a	0.08
	Wet	$0.21 \pm 0.30_{a}$	$0.13 \pm 0.02_{ab}$	$0.11 \pm 0.01_{b}$	$0.18 \pm n/a_{ab}$	< 0.05
A. tortilis	Dry	0.18 ± 0.06	0.12 ± 0.03	0.13 ± 0.03	0.16 ± 0.05	0.21
	Wet	0.16 ± 0.05	0.16 ± 0.02	0.15 ± 0.04	0.24 ± 0.03	0.18
D. cinerea	Dry	0.16 ± 0.04	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.08
	Wet	0.16 ± 0.04	0.14 ± 0.01	0.14 ± 0.01	0.18 ± 0.02	0.07

2.3.1.3. Ungulate Dung

The dung samples of White Rhino and Steenbok were excluded from the results because of the small sample sizes (Appendix 3).

The $\delta^{13}C$ values of the dung reflect the diet of the ungulates (Table 3; Vogel *et al*. 1978; Codron *et al.* 2005a; Codron *et al.* 2005b; February and Higgins 2010; February *et al*. 2013b). The dung of the grazers (i.e. Buffalo, Wildebeest and Zebra) had $\delta^{13}C$ values similar to the leaves of the grasses (-12.85 ‰; February and Higgins 2010). The dung of the browsers (i.e. Giraffe and Duiker) had $\delta^{13}C$ values similar to the leaves of the trees (-26.40 ‰; February and Higgins 2010). The dung of the mixed-feeders (i.e. Elephants and Impala) had $\delta^{13}C$ values in-between the $\delta^{13}C$ values of the grass and tree leaves. The only significant difference in the $\delta^{13}C$ values among the study site was seen in the dry season samples of Impala dung which had significantly lower $\delta^{13}C$ values (Kruskal-Wallis; $\chi^2 = 7.96$; df = 3; $p < 0.05$) in the samples collected at the treatment site than the samples collected at control site 1 ($MCT_{0.05}$).

According to the mixing model (presented by February and Higgins 2010) the Impala dung at the treatment site were composed of 62.66 % grass matter and 37.34 % tree matter in the dry season and 76.46 % grass matter and 23.54 % tree matter in the wet season. The Impala dung at control site 1 were composed of 15.65 % grass matter and 84.35 % tree matter in the dry season and 78.45 % grass matter and 21.55 % tree matter in the wet season. The impala dung at control site 2 and 3 were composed of roughly equal amounts (42 – 58 %) of grass and tree matter (except for the dung at control site 3 in the wet season which were composed of 73.72 % grass matter and 26.27 % tree matter).

Except for the dung samples of Impala (wet season) and Wildebeest (dry season), the percentage nitrogen in the dung samples collected at the treatment site was on average lower than the dung samples collected at one or more of the control sites (Table 3). There were no significant differences to support this trend. However multiple comparisons tests revealed that the wet season Elephant dung ($MCT_{0.10}$) and Zebra dung ($MCT_{0.15}$) samples collected at control site 3 had significantly higher percentage nitrogen than the samples collected at control site 2.

Except for the dry season dung samples of Impala and Wildebeest, the percentage phosphorus in the dung samples collected at the treatment site was on average lower than the dung samples collected at one or more of the control sites (Table 3). The samples of Impala dung (wet season) had significantly higher percentage phosphorus (Kruskal-Wallis; $\chi^2 = 8.89$; df = 3; $p < 0.05$) at control site 3 than at control site 2 ($MCT_{0.10}$). Though not significantly different at the site level, multiple comparisons tests revealed that the wet season Elephant dung samples collected at control site 3 had significantly higher percentage nitrogen than the dung samples collected at control site 2 ($MCT_{0.10}$).

Table 3: The average δ^{13} C values (‰) as well as the average nitrogen and phosphorus content (%) of the ungulate dung samples collected at the study sites during the dry and wet season. Standard deviation is given as the measure of variation; n/a indicates where it is not possible to calculate the standard deviation (i.e. *n* = 1). The *p* value indicates the results of a Kruskal-Wallis test and (where *p* < 0.05) the letters in subscript indicate the results of a multiple comparisons test (MCT) conducted at the 0.05 level of significance; different letters indicate a significant difference.

	Season	Treatment Site	Control Site 1	Control Site 2	Control Site 3	*p* value
δ13 values						
Buffalo	Dry	-14.58 ± 0.20	-15.72 ± 0.43	-14.16 ± n/a	-	0.08
	Wet	-14.88 ± 0.22	-	-	-14.58 ± 0.97	0.51
Duiker	Dry	-27.31 ± 0.59	-26.98 ± n/a	-26.48 ± 0.47	-	0.21
	Wet	-26.52 ± 0.52	-	-	-	-
Elephant	Dry	-24.88 ± 1.83	-24.09 ± n/a	-25.11 ± 0.53	-24.47 ± 1.23	0.70
	Wet	-23.97 ± 0.61	-22.47 ± 3.68	-23.81 ± 2.26	-19.09 ± 0.92	0.20
Giraffe	Dry	-26.93 ± 0.03	-26.34 ± 0.26	-26.99 ± 0.45	-27.37 ± 0.61	0.12
	Wet	-26.74 ± 0.88	-26.09 ± 0.09	-25.86 ± 0.27	-	0.25
Impala	Dry	$-17.91 \pm 2.50_{a}$	$-24.28 \pm 2.01_{b}$	$-19.95 \pm 1.64_{ab}$	$-20.69 \pm 1.49_{ab}$	< 0.05
	Wet	-16.04 ± 0.50	-15.77 ± 0.17	-20.45 ± 5.28	-16.41 ± 2.42	0.20
Wildebeest	Dry	-15.61 ± 2.23	-	-14.34 ± 0.21	-14.74 ± 0.22	0.14
	Wet	-14.59 ± 0.37	-14.37 ± 0.29	-14.62 ± 0.36	-	0.73
Zebra	Dry	-14.34 ± 0.28	-14.93 ± 0.32	-15.22 ± 0.48	-14.93 ± 0.46	0.11
	Wet	-13.65 ± 0.51	-14.27 ± 0.24	-13.98 ± 0.30	-13.94 ± 0.00	0.26
Percentage Nitrogen						
Buffalo	Dry	0.99 ± 0.09	1.30 ± 0.08	1.00 ± n/a	-	0.10
	Wet	1.18 ± 0.09	-	-	1.62 ± 0.68	0.51
Duiker	Dry	1.88 ± 0.41	2.12 ± n/a	1.72 ± 0.29	-	0.46
	Wet	1.68 ± 0.44	-	-	-	-
Elephant	Dry	0.83 ± 0.15	1.10 ± n/a	0.88 ± 0.13	1.05 ± 0.08	0.12
	Wet	0.87 ± 0.08	0.89 ± 0.31	0.68 ± 0.15	1.85 ± 0.08	0.06
Giraffe	Dry	2.34 ± 0.11	2.44 ± 0.15	2.29 ± 0.26	2.22 ± 0.38	0.74
	Wet	2.55 ± 0.37	2.45 ± 0.19	2.77 ± 0.25	-	0.39
Impala	Dry	1.64 ± 0.19	1.91 ± 0.10	1.78 ± 0.19	1.59 ± 0.06	0.12
	Wet	1.91 ± 0.20	1.72 ± 0.14	1.68 ± 0.12	1.71 ± 0.24	0.31
Wildebeest	Dry	1.24 ± 0.40	-	1.09 ± 0.07	1.21 ± 0.17	0.70
	Wet	0.89 ± 0.03	1.05 ± 0.24	0.97 ± 0.05	-	0.19
Zebra	Dry	0.86 ± 0.15	0.79 ± 0.06	0.87 ± 0.04	0.82 ± 0.04	0.29
	Wet	0.83 ± 0.15	0.62 ± 0.02	0.61 ± 0.11	1.05 ± 0.00	0.06

Continued on next page

	Season	Treatment Site	Control Site 1	Control Site 2	Control Site 3	*p* value
Percentage Phosphorus						
Buffalo	Dry	0.21 ± 0.03	0.27 ± 0.06	0.19 ± n/a	-	0.29
	Wet	0.40 ± 0.06	-	-	0.65 ± 0.35	0.51
Duiker	Dry	0.36 ± 0.08	0.25 ± n/a	0.37 ± 0.01	-	0.27
	Wet	0.38 ± 0.10	-	-	-	-
Elephant	Dry	0.13 ± 0.02	0.14 ± n/a	0.09 ± 0.02	0.13 ± 0.01	0.10
	Wet	0.11 ± 0.03	0.11 ± 0.07	0.07 ± 0.02	0.42 ± 0.07	0.07
Giraffe	Dry	0.33 ± 0.04	0.32 ± 0.10	0.49 ± 0.35	0.29 ± 0.05	0.70
	Wet	0.31 ± 0.08	0.39 ± 0.10	0.47 ± 0.12	-	0.15
Impala	Dry	0.51 ± 0.16	0.31 ± 0.08	0.39 ± 0.04	0.37 ± 0.07	0.13
	Wet	$0.54 \pm 0.07_a$	$0.70 \pm 0.18_a$	$0.34 \pm 0.21_a$	$0.77 \pm 0.13_a$	< 0.05
Wildebeest	Dry	0.34 ± 0.10	-	0.33 ± 0.16	0.33 ± 0.05	0.70
	Wet	0.16 ± 0.04	0.21 ± 0.01	0.23 ± 0.04	-	0.17
Zebra	Dry	0.26 ± 0.07	0.19 ± 0.04	0.23 ± 0.02	0.28 ± 0.02	0.16
	Wet	0.16 ± 0.06	0.10 ± 0.03	0.12 ± 0.02	0.38 ± 0.35	00.26

2.3.2. FEEDING RATES

Six camera traps (two from the treatment site, two from control site 1, one from control site 2 and one from control site 3) had to be excluded from the study due to the camera traps ceasing to function or due to the photos being corrupted. However, the dataset was increased by several monitoring periods, ranging from June 2011 to April 2012, in the camera traps were deployed at the treatment site and control site 1 in the same manner as specified in the methods.

In total the camera traps took 3368 photos (2107 in the dry season and 1261 in the wet season) of 17 animal species (twelve ungulates, four carnivores and one primate; Appendix 4). Using these photos, 130 separate feeding rates (77 in the dry season and 53 in the wet season) were calculated for the twelve ungulate species, however I excluded the feeding rates

of Duiker, Kudu, Steenbok, Warthog and White Rhino from the results because of small samples sizes and/or poor distribution among the study sites.

The feeding rates were on average higher at the treatment site than the control sites (Table 4). Kruskal-Wallis tests indicated no significant differences in the feeding rates among the study sites except for the feeding rates of Zebra in the dry season (Kruskal-Wallis; $\chi^2 = 8.47$; df = 2; $p < 0.05$) which were significantly higher at the treatment site than at control sites 2 and 3 ($MCT_{0.10}$; Table 4). There were four feeding rate outliers, all occurring at the treatment site: Buffalo (3.91 seconds per day per 314 m^2), Elephant (8.83 seconds per day per 314 m^2) and Impala (2.40 seconds per day per 314 m^2) in the dry season and Impala (5.54 seconds per day per 314 m^2) in the wet season.

Table 4: The average feeding rate (seconds per day per 314 m^2) for the ungulates grazing/browsing among the study sites during the dry and wet season. Standard deviation is given as the measure of variation; n/a indicates where it is not possible to calculate the standard deviation (i.e. $n = 1$). The p value indicates the results of a Kruskal-Wallis test and (where $p < 0.05$) the letters in subscript indicate the results of a multiple comparisons test (MCT) conducted at the 0.05 level of significance; different letters indicate a significant difference.

	Season	Treatment Site	Control Site 1	Control Site 2	Control Site 3	*p* value
Buffalo	Dry	1.22 ± 1.37	-	-	0.55 ± 0.46	0.51
	Wet	1.6 ± 0.21	-	-	-	-
Elephant	Dry	0.45 ± 0.67	-	0.15 ± 0.18	0.16 ± 0.21	0.51
	Wet	1.94 ± 2.68	-	0.34 ± n/a	0.62 ± n/a	0.34
Giraffe	Dry	0.63 ± 0.47	-	-	-	-
	Wet	0.80 ± 1.14	-	0.22 ± 0.08	-	-
Impala	Dry	1.43 ± 1.58	0.42 ± n/a	0.78 ± 0.24	-	0.65
	Wet	0.73 ± 0.22	0.03 ± n/a	0.39 ± 0.17	-	0.10
Waterbuck	Dry	0.36 ± 0.33	-	-	-	-
	Wet	1.21 ± 0.91	0.06 ± n/a	1.17 ± n/a	-	0.34
Wildebeest	Dry	1.7 ± 1.39	-	0.07 ± n/a	0.08 ± n/a	0.17
	Wet	0.23 ± 0.08	-	0.04 ± n/a	-	0.18
Zebra	Dry	$0.68 \pm 0.56_a$	-	$0.04 \pm 0.02_a$	$0.05 \pm 0.02_a$	< 0.05
	Wet	2.79 ± 4.08	-	0.28 ± 0.30	-	0.25

2.3.3. GRASS HEIGHT

In total 23 grass species were indentified in this study (13 spp. in the dry season, the remaining spp. are dormant during this period, and 21 spp. in the wet season) and 3180 grass plants were measured (1632 in the dry season and 1548 in the wet season; Appendix 5). Irrespective of season, the Hierarchical Cluster Analyses (based on the frequencies of the 23 grass species) grouped the 25 plots into significant clusters ($p < 0.05$) independent of study site, indicating that no study site had a unique composition of grasses (Fig. 3). There are, however, two significant clusters in the wet season which consisted of plots from a single study site; this was most likely the result of rare grass species occurring in high frequency on these plots (e.g. *Chloris gayana* at the treatment site and *Pogonarthria squarrosa* at control site 2; Fig. 3).

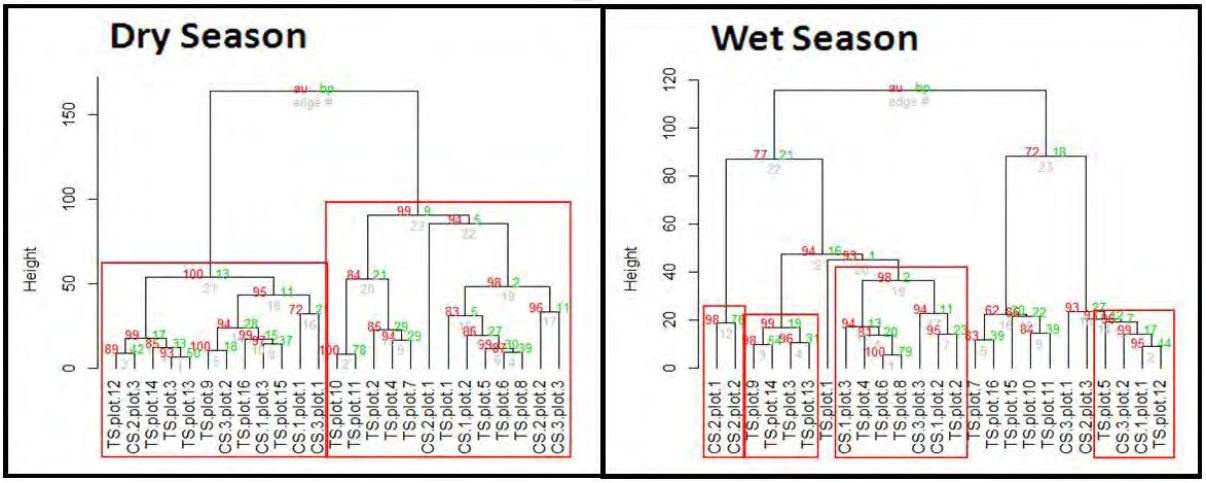


Figure 3: Hierarchical cluster analysis based on the frequencies of the grass species at the four study sites (here separated into the 25 individual plots) during the dry and wet season. TS stands for treatment site, CS 1 for control site 1, CS 2 for control site 2 and CS 3 for control site 3. The values at the branches are the approximate unbiased *p*-values (left), the bootstrap probabilities (right) and the cluster labels (bottom). Significant clusters (approximate unbiased *p*-values > 95) are indicated by a red rectangle.

Urochloa mosambicensis was the dominant grass species (46 % of the grass height measurements) followed by *Eragrostis rigidior* (14 %), *Digitaria eriantha* (8 %) and *Panicum maximum* (6 %; Appendix 5). Because of the large samples sizes ($n > 180$), these four grass species were used to test whether there were any differences in the grass heights among the study sites.

In the dry season, there was a significant difference in the height of *D. eriantha* among the study sites (GLM; $\lambda = 0.02$; $\chi^2 = 2.55$; df = 3; $p < 0.05$); *D. eriantha* was significantly taller at the treatment site than the grasses at control site 1 (Fig. 4) In the wet season, there were no significant differences in the height of *D. eriantha* among the four study sites (GLM; $\lambda = 0.85$; $\chi^2 = 18.01$; df = 3; $p = 0.09$), however *D. eriantha* at control site 3 was significantly taller than the grasses at control site 1 at the 0.1 level of significance (Fig. 4).

E. rigidior only occurred at the treatment site, control site 1 and (in the dry season) control site 2. In both the dry season (GLM; $\lambda = 0.21$; $\chi^2 = 0.69$; df = 2; $p = 0.44$) and the wet season (GLM; $\lambda = 0.89$; $\chi^2 = 4.50$; df = 1; $p = 0.28$) there were no significant differences in the height of *E. rigidior* among the study sites (Fig. 4).

In both the dry season (GLM; $\lambda = 0.50$; $\chi^2 = 13.07$; df = 3; $p < 0.05$) and wet season (GLM; $\lambda = 0.48$; $\chi^2 = 40.07$; df = 3; $p < 0.05$) there were significant differences in the height of *P. maximum* among the study sites (Fig. 4). In the dry season, *P. maximum* was significantly taller at the treatment site than the grasses at control site 1. *P. maximum* was also significantly taller at the treatment site than the grasses at control site 3 but only at the 0.1 level of significance. In the wet season, *P. maximum* was significantly taller at the treatment site than

the grasses at control sites 1 and 2 at the same time *P. maximum* was significantly taller at control site 3 than the grasses at the treatment site and control sites 1 and 2.

In both the dry season (GLM; $\lambda = 0.32$; $\chi^2 = 33.33$; df = 3; $p < 0.05$) and wet season (GLM; $\lambda = 0.57$; $\chi^2 = 182.12$; df = 3; $p < 0.05$) there were significant differences in the height of *U. mosambicensis* among the study sites (Fig. 4). In the dry season, *U. mosambicensis* was significantly shorter at the treatment site than the grasses at control site 1, 2 and 3 while *U. mosambicensis* was significantly taller at control site 2 than the grasses at control sites 1 and 3. In the wet season, *U. mosambicensis* was significantly shorter at the treatment site than the grasses at control sites 2 and 3 while *U. mosambicensis* was significantly taller at control site 3 than the grasses at control sites 1 and 2.

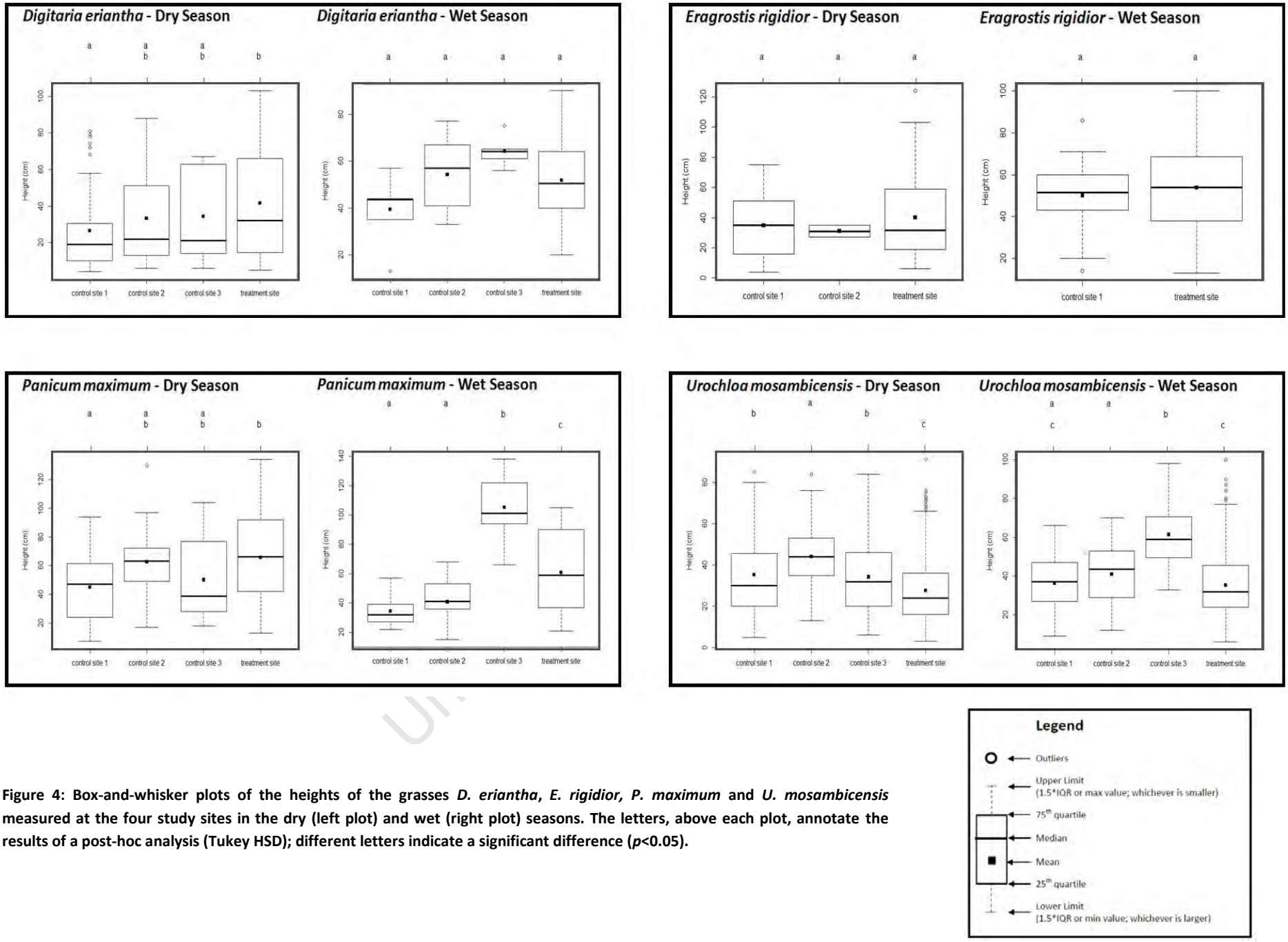


Figure 4: Box-and-whisker plots of the heights of the grasses *D. eriantha*, *E. rigidior, P. maximum* and *U. mosambicensis* measured at the four study sites in the dry (left plot) and wet (right plot) seasons. The letters, above each plot, annotate the results of a post-hoc analysis (Tukey HSD); different letters indicate a significant difference (p<0.05).

2.3.4. TREE ASSESSMENTS

In total 341 tree assessments were conducted; 155 in the dry season and 186 in the wet season (Appendix 6). *Acacia nilotica*, *Acacia tortilis* and *Dichrostachys cinerea* were present at all four study sites (Appendix 6). The following variables had to be removed, due to a low frequency or a constant zero value, in order to conduct the Principle Component Analyses (PCA): percentage of the canopy consisting of flowers, pods, young leaves (only in the dry season assessments) and senescent leaves (only in the wet season assessments).

The PCA based on the dry season assessments indicated that along the first two components there were no morphological differences in the trees among the four study sites (Fig. 5). However, the PCA based on the wet season assessments indicated several morphological differences based primarily on the percentage of non-woodiness (i.e. herbaceous stems) and the percentage of the canopy consisting of young and mature leaves (Fig. 5). Along the first component there was a negative relationship between the percentage of non-woodiness (eigenvalue of -0.36 in *A. nilotica*, -0.38 in *A. tortilis* and 0.38 in *D. cinerea*) and the remaining variables (eigenvalues ranging from 0.28 to 0.36 with *A. nilotica*, 0.16 to 0.36 with *A. tortilis* and -0.18 to -0.37 with *D. cinerea*) except for the percentage of the canopy consisting of mature leaves. Along the second component there was a negative relationship between the percentage of the canopy consisting of young leaves (eigenvalue of 0.54 in *A. nilotica*, -0.59 in *A. tortilis* and -0.67 in *D. cinerea*) and the percentage of the canopy consisting of mature leaves (eigenvalue of -0.53 in *A. nilotica*, 0.57 in *A. tortilis* and 0.67 in *D. cinerea*). Together the two components indicated that the trees at the treatment site and control site 3 (not seen with *A. nilotica*) tended to have a greater percentage of non-

woodiness and mature leaves within the canopy, whereas the trees at the control sites 1 and 2 tended to have a greater percentage of young leaves within the canopy.

The results of the generalized linear models and post-hoc analyses indicated that during the wet season these three tree variables (percentage of non-woodiness and the percentage of the canopy consisting of young and mature leaves) differed significantly among the study sites. *A. nilotica* (GLM; $\chi^2 = 0.39$; df = 3; $p < 0.05$) had significantly higher percentage non-woodiness at the treatment site than the trees at control site 1. *A. tortilis* (GLM; $\chi^2 = 0.81$; df = 3; $p < 0.05$) had significantly higher percentage non-woodiness at the treatment site than the trees at control sites 1 and 2. *D. cinerea* (GLM; $\chi^2 = 0.41$; df = 3; $p < 0.05$) had significantly higher percentage non-woodiness at the treatment site and control sites 2 and 3 than the trees at control site 1. *A. nilotica* (GLM; $\chi^2 = 1.95$; df = 3; $p < 0.05$), *A. tortilis* (GLM; $\chi^2 = 2.59$; df = 3; $p < 0.05$) and *D. cinerea* (GLM; $\chi^2 = 3.68$; df = 3; $p < 0.05$) had significantly higher percentage of young leaves in the canopy at control site 1and 2 than the trees at the treatment site and control site 3. *A. nilotica* (GLM; $\chi^2 = 2.43$; df = 3; $p < 0.05$), *A. tortilis* (GLM; $\chi^2 = 2.73$; df = 3; $p < 0.05$) and *D. cinerea* (GLM; $\chi^2 = 4.11$; df = 3; $p < 0.05$) had significantly higher percentage of mature leaves in the canopy at the treatment site and control site 3 than the trees at control sites 1 and 2.

There were two outliers at the treatment site: an *A. nilotica* and an *A. tortilis*, both of which had a high percentage of young leaves in the canopy (100 % and 80 % respectively) breaking the norm for the treatment site (< 50 % and < 40 % respectively; Fig. 5).

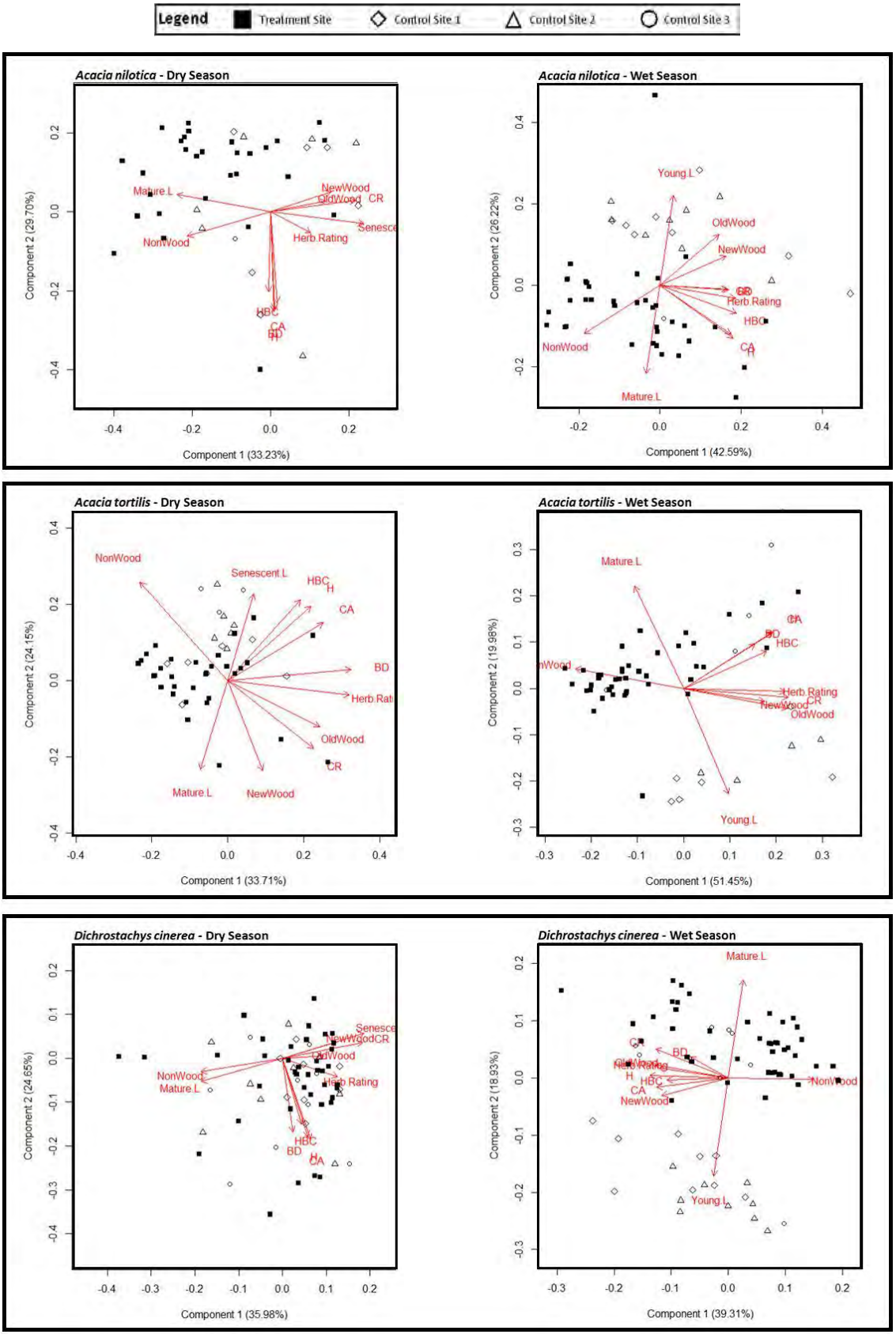


Figure 5: The first and second principle components identified based on the tree assessments conducted at the study sites separated according to the dry (left plot) and wet (right plot) season. The amount of variation explained by each component is indicated by the percentage in brackets. The original tree variables are represented by the red vectors, the direction and magnitude of which indicate the loadings onto the components. H = height; BD = basal stem diameter; HBC = height before branching; CA = canopy area; CR = % canopy cover removed; Herb.Rating = herbivory damage score; how much of the tree consists of old-wood (OldWood), new-wood (NewWood) and non-woody (NonWood) sections; how much of the canopy consists of young leaves (Young L), mature leaves (Mature L) and senescent leaves (Senescent L).

2.4. DISCUSSION

The use of multiple control sites in comparison with a single treatment site was dependant on the assumption that control sites mimicked the natural conditions at the treatment site. If this assumption is untrue, then any differences identified between the treatment site and a control site could be the result of a site effect and not a treatment effect (i.e. due to some spatial variation instead of the addition of nitrogen, phosphorus and water at the treatment site). The Hierarchical Cluster Analyses based on the frequencies of the grass species identified no unique composition of grasses among the four study sites (Fig. 3). Also the dominant tree species (i.e. *A. nilotica*, *A. tortilus* and *D. cinerea*) were present at all four study sites. These results seem to confirm the information given by du Toit *et al*. 2003 and Mucina and Rutherford 2006 that the four study sites share the same soil and vegetation type; therefore the assumption that the control sites mimicked the natural conditions at the treatment site holds true.

By separating the dataset according to season I also prevented any differences identified between the treatment site and a control site being the result of a season effect and not a treatment effect (i.e. due to seasonal variations in vegetation quantity/quality or herbivory instead of the addition of nitrogen, phosphorus and water as the treatment site).

2.4.1. VEGETATION QUALITY

The first step to answering the question posed in this study was to determine whether the additional nutrients at the treatment site were resulting in vegetation of higher quality (i.e. higher concentration of nitrogen and phosphorus within the leaves).

The leaf samples collected at the treatment site had higher nitrogen content than the leaf samples collected at the control sites (irrespective of season; Tables 1 and 2). Usually this was not a significant relationship, but it was for the leaf samples of *P. maximum* (wet season), *U. mosambicensis* (dry season), *A. nilotica* (dry season) and *D. cinerea* (dry season).

The leaf samples collected at the treatment site had higher phosphorus content than the leaf samples collected at the control sites (irrespective of season but excluding the grass samples collected from control site 3; Tables 1 and 2). Usually this was not a significant relationship, but it was for the leaf samples of *A. nilotica* (wet season). The grass samples collected at control site 3 had higher phosphorus content than the grass samples collected at the remaining study sites; this was a significant relationship for the samples of *P. maximum U. mosambicensis.* This is likely the result of high animal activity at control site 3, especially during the dry season (Appendix 4; Thrash *et al*. 1995). The accumulation of dung would have increased the phosphorus availability which would have resulted in phosphorus enriched grasses (the same phosphorus enrichment was not seen in the tree samples collected at control site 3; Dohn *et al.* 2013).

These results confirm that the additional nutrients at the treatment site increased the vegetation quality. Furthermore, the nitrogen addition seemed to be affecting the vegetation more than the phosphorus addition.

2.4.2. Feeding Behaviour and Diet of Ungulates

The next step to answering the question posed in this study was to determine whether the difference in vegetation quality was enough to influence the feeding behaviour and/or diet of the local ungulate populations.

A 'feeding rate' (seconds per day per 314 m^2) was developed to measure the feeding behaviour of the ungulate (i.e. the degree of herbivory) among the study sites. The resulting values were very small and seemly random (as evident in the relatively large standard deviation), however these values were determined systematically and did seem to be in proportion to the number of photos taken of feeding ungulates.

The feeding rates were highest at the treatment site (irrespective of season); however this was not a significant relationship for any of the ungulates seen grazing/browsing in the photos taken by the camera traps (Table 4). Ungulates are able to adapt their diet by adapting their feeding behaviour in order to maximize nutrient intake while minimizing costs associated with obtaining and digesting food items (Day *et al.* 1998; Provenza *et al.* 1998; Kyriazakis *et al*. 1999). Although the results were not significant the ungulates at the treatment site were feeding on the vegetation for longer periods of time (i.e. a greater degree of herbivory) than the ungulates feeding at the control sites. Perhaps significant results would be achieved if the camera traps were left to monitor the area for a longer period of time.

The chemical analyses of the ungulate dung were used to determine whether there was a difference in the diet of the ungulates among the study sites.

As expected, the $\delta^{13}C$ values for the dung samples of Buffalo, Wildebeest and Zebra (i.e. grazers) indicated a diet consisting of grasses, $\delta^{13}C$ values for the dung samples of Duiker and Giraffe (i.e. browsers) indicated a diet consisting of trees and the $\delta^{13}C$ values for the dung samples of Impala and Elephant (i.e. mixed-feeders) indicated a diet consisting of both grasses and trees (Table 3).

Since the nitrogen content of grasses lowers as they become mature or become moribund in the dry season, Impala switch from primarily grazing in the wet season to primarily browsing in the dry season in order to exploit the most abundant and most nutritious food source (Grant *et al.* 1995; Day *et al.* 1998; du Toit *et al*. 2003; Sponheimer *et al.* 2003). In the dry season, the $\delta^{13}C$ values of the Impala dung samples collected at the treatment site indicated a significantly higher proportion of grasses in the diet (62.22 % grasses and 37.34 % trees) than the dung samples collected at the neighboring control site 1 (15.65 % grasses and 84.35 % trees; Table 3). This suggests that the grasses at the treatment site are of sufficient quality to be maintained as a food source during the dry season (Sponheimer *et al.* 2003). If the mixed-feeders at the treatment site benefit more from the grasses than the trees, then the strict grazers would be at an advantage over the strict browsers. However a disparity in the feeding rates between the strict grazers and browsers were not seen at the treatment site (Table 4).

Elephants are also mixed-feeders but the composition of their diet was not determined because there was no significant difference in the $\delta^{13}C$ values of the dung samples among the study sites (hence no reason to determine and describe the composition of their diet). However, the $\delta^{13}C$ values of the dung samples do suggest that the elephants tend to predominantly browse (Table 3).

The nitrogen and phosphorus content of the dung samples did reflect dietary differences amongst the feeding guilds (i.e. the diet amongst the browsers, grazers and mixed-feeders) and between the seasons; however there is no significant difference amongst the study sites (Table 3). This suggests that, contrary to the initial predictions, the higher quality vegetation at the treatment site is not resulting in a change in the diet of the local ungulates. Either there is not enough of a change in the feeding behaviour (because the treatment site is not large enough or enriched enough to be noticed by the ungulates living in a naturally heterogeneous environment) or (because the diet of any animal is the result of the feeding behaviour over time) the minor differences in feeding behaviour have not persisted long enough for there to be a change in the diet of the ungulates among the study sites (Senft 1987; Bailey *et al.* 1996; Day *et al.* 1998; Provenza *et al.* 1998; Kyriazakis *et al*. 1999; Skarpe *et al*. 2000).

2.4.3. VEGETATION QUANTITY

The final step to answering the question posed in this study was to determine whether there was a change in the vegetation quantity at the treatment site due to an increase in productivity or an increase in herbivory. The additional nutrients at the treatment site should stimulate productivity, especially during the growing season (i.e. wet season), resulting in taller grasses and trees with thicker canopies comprised of younger leaves (Abrams 1993; Kambatuku *et al.* 2011). At the same time the higher quality and quantity of vegetation at the treatment site is increasing the degree of herbivory (indicated by the higher feeding rates at the treatment site) which should result in shorter grasses and trees with sparser canopies comprised of younger leaves (Hanley 1982; Owen-Smith and Novellie 1982; Grant *et al.* 1995; Bailey *et al.* 1996; Day *et al.* 1998; Bobbink *et al*. 2010).

Of the four grasses examined, *D. eriantha* and *P. maximum* were significantly taller at the treatment site than one or more of the control sites, while *U. mosambicensis* was significantly shorter at the treatment site than one or more of the control sites (Fig. 4). There was no difference in the height of *E. rigidior* among the study sites. The significantly taller *D. eriantha* and *P. maximum* at the treatment site could be attributed to the nutrient additions stimulating productivity, whereas the significantly shorter *U. mosambicensis* at the treatment site could be attributed to the increased grazing due to the presence of higher quality vegetation. The effect of increased grazing was not seen in is not seen in *E. rigidior*, *D. eriantha*, and *P. maximum* because these grasses were less abundant and are less palatable than *U. mosambicensis* (Chippindall and Crook 1976; Gibbs-Russell *et al.* 1990; Weltzin and Coughenour 1990).

Water stress is said to be the primary factor determining the phenology of savanna trees (Williams *et al.* 1997; Eamus 1999; Singh and Kushwaha 2005). Leaf-flushing (i.e. a period of new growth in leaves) and subsequent bud breaking (i.e. vegetative bud break initiates stem growth and reproductive bud break initiates flowering) is triggered in anticipation of the wet season or once the water potential of the soil reaches a threshold (due to the first rains of the wet season; Williams *et al.* 1997; Eamus 1999; Singh and Kushwaha 2005). The wet season tree assessments (conducted 13 January 2012) indicated that the trees at the treatment site had significantly higher percentages of non-woodiness (not seen in *D. cinerea*) and mature leaves within the canopy compared to the trees at control sites 1 and 2 which had a significantly higher percentages of young leaves within the canopy (Fig. 5). These results suggest that the trees at the treatment site had an earlier start to the growing season possibly due to the additional water affecting the phenology of the trees. This would have resulted in

the trees at the treatment site having dense canopies of young leaves earlier than the trees at the control sites.

The wet season tree assessments indicated that the trees at control site 3 also had significantly higher percentages of mature leaves within the canopy compared to the trees at control sites 1 and 2 (Fig. 5). This anomaly can be attributed to the trees at control site 3 being assessed more than a month later (23 January 2012) because of logistical issues surrounding tropical depression Dando. It is likely that earlier in the wet season the trees at control site 3 also had high percentages of young leaves within the canopy but during the course of the month the leaves matured. By the time the trees at control site 3 were assessed they resembled the trees at the treatment site.

2.4.4. CONCLUSION

Here we have shown that the addition of nutrients and water has improved the vegetation quality to the extent that herbivory has increased. These have contributed to a decreasing quantity of grass biomass (opposite trend is seen in the sub-dominant grass species) and a slight increasing quantity of tree biomass (the water addition is possibly resulting in an earlier and extended growing season). If these changes are left to persist the savanna could change to grassland as the additional nutrients would promote productivity and the resulting accumulation of grass biomass would outcompete tree saplings and fuel more intensive fires which would wear away the tree-layer (van de Koppel and Prins 1998; van Langevelde *et al*. 2003; Smit 2004; Britz and Ward 2007; Bobbink *et al*. 2010; Goheen *et al.* 2010; Kambatuku *et al.* 2011; Dohn *et al.* 2013). Alternatively the savanna could change to woodland as an extended growing season and increased grazing pressure (resulting in reduction of the fuel-

load making fires less frequent and less intense) would allow trees to outcompete grasses (van de Koppel and Prins 1998; van Langevelde *et al*. 2003; Smit 2004; Britz and Ward 2007; Venter *et al*. 2008; Bobbink *et al*. 2010; Goheen *et al.* 2010). In either scenario, various ungulate populations would be displaced (a reduction in the tree layer would result in the loss of browsers and an increase in the tree layer would result in the loss of grazers) which would then displace associated carnivore populations (e.g. Leopard, *Panthera pardus*, primary feed on smaller browsers such as Steenbok, Duiker and Impala while Lion, *Panthera leo*, primary feed on the larger, gregarious grazers such as Buffalo, Wildebeest and Zebra; Estes 1992; Smit 2004; Shorrocks 2007).

In conclusion, anthropogenic pollution coupled with climate change resulting in the increasing the nutrient and water availability within African savannas, has the potential to alter the quantity and quality of the vegetation as well as the feeding behavior and diets of the local ungulates.

In context of the Kruger National Park: the increasing agriculture, industrialization and urbanization of the Mpumalanga Highveld could potentially result in massive changes to the structure of the savanna vegetation as well as to the animal demographics which could disrupt conservation efforts and harm the park's tourism and prestige. There is a need to begin developing a management plan in order to detour and/or halt the current sources of anthropogenic pollution such as placing greater pressure on the government and industries to develop more effective means of cleaning urban and industrial, emissions and effluents in order to promote both sustainable development and conservation of ecological resources.

CHAPTER 3
STUDY SYNTHESIS AND REVIEW

3.1. STUDY SYNTHESIS

The aim of this study was to determine whether additional nitrogen, phosphorus and water, to a savanna within the Kruger National Park would alter the vegetation quality (i.e. concentration of nitrogen and phosphorus within the leaves) enough to influence the feeding behaviour and diet of the local ungulates resulting in a change in the quantity of vegetation (i.e. biomass). This study was conducted in the central part of the Kruger National Park, just outside the Satara rest-camp. The treatment site was supplied with additional nitrogen, phosphorus and water and compared to three control sites. The nitrogen and phosphorus content of grass and tree leaves collected at the treatment site were higher (though not always significantly so) than grass and tree leaves collected at the control sites illustrating that the additional nutrients are improving the vegetation quality. The feeding behavior was measured using a 'feeding rate' determined from photos taken by camera traps deployed at the study sites. The feeding rates were higher at the treatment site (though never significantly) than the control sites, suggesting that the higher quality vegetation is resulting in an increase in herbivory. The $\delta^{13}C$ value as well as the nitrogen and phosphorus content of ungulate dung collected at the study sites, indicated differences in the diet amongst the grazers, browsers and mixed-feeders but no difference among the study sites suggesting that the higher quality vegetation at the treatment site did not cause a change in the diet of the local ungulates. The only exception was the dung of Impala collected at the treatment site (during the dry season) indicated a diet with a high proportion of grasses which is interesting as Impala usually

switch to primarily browsing in the dry season. The dominant grass, *U. mosambicensis*, was significantly shorter at the treatment site, indicating that the increased herbivory is resulting in the decrease in the quantity of grasses at the Experimental Site. Principle component analyses based on assessments of the trees at the study sites (during the wet season) indicated that the trees at the treatment site had significantly higher percentages of non-woodiness (i.e. herbaceous branches) as well as significantly higher percentages of mature leaves within the canopy compared to the trees at the control sites which had significantly higher percentages of young leaves within the canopy. It was postulated that the additional water at the treatment site may have interfered with the phenology of the trees, resulting in an earlier and longer growing season.

In conclusion, the increasing agriculture, industrialization and urbanization of the Mpumalanga Highveld could potentially fertilize the savannas of the Kruger National Park to the extent that it will change the vegetation structure and animal demographics. There is a need to place greater pressure on the government and industries to develop more effective means of cleaning urban and industrial, emissions and effluents within this region.

3.2. STUDY LIMITATIONS

3.2.1. PSEUDOREPLICATION – A NECESSARY EVIL?

The most frequently criticized aspect of this study is the apparent 'pseudoreplication' resulting from not replicating the treatment site. Authors argue that without replication it's impossible to infer causal relationships (as any 'significant differences' could be the result of a site or a treatment effect) and that inferential statistics are meaningless (because 'replicates'

are not statistically independent; Hurlbert 1984; Heffner *et al*. 1996; Prosser 2010). However, Oksanen (2001) explains that we exist within a world that is spatially, temporally and financially restricted and in order to understand large-scale ecosystems often sacrifices, such as replication, need to be made.

The financial costs associated with establishing the treatment site (e.g. the LAN 14 % and superphosphate fertilizers, the installation and maintenance of the irrigation systems, hiring of game guards) was far too large for an M.Sc. study. Instead, three control sites were selected according to their similarity to the treatment site (in terms of proximity to a water source as well as soil and vegetation types) and were used to make multiple comparisons using the same treatment site. This setup follows one of Oksanen's (2001) solutions to studying large-scale ecosystems in light of the difficulties surrounding the replication of the treatment.

3.2.2. THE LEAF AND DUNG SAMPLES

The aim was to maintain a standard sample size of three samples (leaves of grasses and tress as well as ungulate dung) per species per site; usually this criterion was met. However, 1) the resulting sample sizes still proved too small, 2) the relative variation proved too large and 3) the effect sizes (in terms of the $\delta^{13}C$ values as well as the nitrogen and percentage content of the samples) proved too small for inferential statistics to yield a power exceeding 80 % (Bacchetti *et al.* 2005). The difference in the $\delta^{13}C$ values as well as the percentage nitrogen and percentage phosphorus of the samples among the study sites were tested using non-parametric tests; namely, Kruskal-Wallis couple with a multiple comparisons test which do not assume an underlying distribution. However, descriptive statistics (i.e. the mean and standard deviation) would have proved a more reliable means to present and discuss the data.

In this study I assumed that higher nitrogen and phosphorus content meant a higher forage quality or palatability, but this is not always true as both nitrogen and phosphorus are also used in the production of cell wall constituents and secondary compounds which lowers nutrient quality and palatability (Cooper and Owen-Smith 1985; Codron *et al.* 2007; Hattas *et al*. 2011). I also assumed that nitrogen and phosphorus content of the ungulate dung reflected the vegetation it consumed however, the nitrogen and phosphorus content of ungulate dung decreases when exposed to rain and sunlight and unfortunately it was not always possible to collect fresh dung (Wrench *et al.* 1996).

3.2.3. THE CAMERA TRAPS

Though many studies agree that camera trapping is a far better solution than observational studies (as it is a low labor, non-invasive means to gather an immense amount of data on species and their behaviour), there is much controversy as to what data are being collected (Silveira *et al.* 2003; Yasuda 2004; Datta *et al.* 2008; Marnewick *et al.* 2008; Rowcliffe *et al.* 2008; Stein *et al.* 2008). There are numerous complex models which use camera traps to determine animal abundance yet are not true reflection of animal movements and interactions (e.g. assuming animals behave as randomly as gases) and require parameters which are difficult to obtain (e.g. speed of movement or daily range; Silveira *et al.* 2003; Marnewick *et al.* 2008; Rowcliffe *et al.* 2008; Rovero and Marshall 2009). Rovero and Marshall (2009) discussed that it is intuitive that camera trapping rate (i.e. the number of photos of a particular species per unit of time) should be related to abundance because as animal density increases so would the chances of encounters between individuals and camera traps (Bowkett *et al.* 2007). To avoid wasting time, I used the camera trapping rate (Kuijper *et al.* 2009).

It was assumed that the encounter between ungulates and the cameras were random (Datta *et al.* 2008). However, the control sites were purposefully selected to be near waterholes in order to mimic the conditions of the treatment site; these are areas of high animal activity (especially in the dry season; Thrash *et al*. 1995). To resolve this issue, I modified the camera trapping rate by only using the photos depicting feeding ungulates and partitioning the dataset according to season.

3.2.4. WHY USE WATER?

Though water availability is a driving factor determining the structure of savanna vegetation and was added at the treatment site in order to eliminate any water limitations, I did not directly measure water properties of the vegetation. As such I can only speculate the influence the additional water (at the treatment site) had on the vegetation quality/quantity. The additional water is likely to not only influence the phenology of the trees but also the phenology of the grasses, though I have no evidence to say this.

Measuring the water properties of the vegetation (such as stomatal conductance or the water potential of the stems) would have had to be done *in situ* and would have required cumbersome, yet delicate instruments (porometer and Scholander pressure bomb respectively). Also, it is a time consuming process to obtain these measurements (porometers need to be brought back to atmospheric pressure before each measurement and Scholander pressure bombs require samples to be prepped, secured then subjected to high pressures before measurements are obtained) which would have drastically increased the sampling period.

3.3. FUTURE RESEARCH

Future research may need to begin mapping the extent of nitrogen and phosphorus deposition/pollution as well as a change in the precipitation within the Kruger National Park. This could then be modeled with changing vegetation types (such as the increasing bush encroachment and the collapsing of the savanna plains) and changing animal demographics (such as the change in herd size, home ranges and migration routes).

Should this study be repeated to confirm the results described, one would need to correct the limitations expressed in the previous sections. Firstly, one should only focus on nitrogen and water. The treatment site/s should be designed in the same manner as described in the methods but with larger plots (perhaps 50 m in diameter) and, along with a single control of similar dimensions, replicated once or twice several kilometers apart perhaps comparing different soils types. Leaf/dung sampling (and subsequent nutrient analyses) as well as camera trapping (and subsequent determination of feeding rate) should be conducted in the same manner as outlined in this study. The camera traps should be left out in the field for longer periods of time and should be frequently checked (once a week) to ensure that they are in working order and the photos are not blurry, overexposed or corrupted. Grass height measurements and tree assessments should be also be conducted and should be repeated every two months in order to pinpoint phenological variations Alternative variables such as stomatal conductance, stem water potential, photosynthetic rates and specific leave area could also be measured.

REFERENCES

Abrams, P.A. 1993. Effect of Increased Productivity on the Abundances of Trophic Levels. *The American Naturalist* **141**: 351-371

Archibald, S. and W.J. Bond. 2003. Growing tall vs. growing wide: tree architecture and allometry of *Acacia karroo* in forest, savanna, and arid environments. *Oikos* **102**: 3-14

Ashton, P.J. 2010. The demise of the Nile crocodile (*Crocodylus niloticus*) as a keystone species for aquatic ecosystem conservation in South Africa: The case of the Olifants River. *Aquatic Conservation Marine and Freshwater Ecosystems* **20**: 489-493

Bacchetti, P., L.E. Wolf, M.R. Segal and C.E. McCulloch. 2005. Ethics and Sample Size. *American Journal of Epidemiology* **161**: 105-110

Bailey, D.W., J.E. Gross, E.A. Laca, L.R. Rittenhouse, M.B. Coughenour, D.M. Swift and P.L. Sims. 1996. Mechanisms that result in large herbivore grazing distribution patterns. *Journal of Range Management* **49**: 386-400

Bobbink, R., K. Hicks, J. Galloway, T. Spranger, R. Alkemade, M. Ashmore, M. Bustamante, S. Cinderby, E. Davidson, F. Dentener, B. Emmett, J.-W. Erisman, M. Fenn, F. Gilliam, A. Nordin, L. Pardo and W. de Vries. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* **20**: 30-59

Bowkett, A.E., F. Rovero and A.R. Marshall. 2007. The use of camera-trap data to model habitat use by antelope species in the Udzungwa Mountain forests, Tanzania. *African Journal of Ecology* **46**: 479-487

Britz, M.-L. and D. Ward. 2007. Dynamics of woody vegetation in a semi-arid savanna, with a focus on bush encroachment. *African Journal of Range and Forage Science* **24**: 131-140

Carruthers, J. 1995. *The Kruger National Park: A Social and Political History*. University of Natal Press, Pietermaritzburg, South Africa

Chippindall, L.K.A. and A.O. Crook. 1976. *240 Grasses of Southern Africa*. M.O. Collins, Salisbury, U.K

Codron, D., J. Codron, J.A. Lee-Thorp, M. Sponheimer and D. de Ruiter. 2005a. Animal diets in the Waterberg based on stable isotopic composition of faeces. *South African Journal of Wildlife Research* **35**: 43-52

Codron, D., J. Codron, M. Sponheimer, J.A. Lee-Thorp, T. Robinson, C.C. Grant and D. de Ruiter. 2005b. Assessing diet in savanna herbivores using stable carbon isotopes ratios of faeces. *Koedoe* **48**: 115-124

Codron, D., J.A. Lee-Thorp, M. Sponheimer and J. Codron. 2007. Nutritional content of savanna plant foods: implications for browser/grazer models of ungulate diversification. *European Journal of Wildlife Research* **53**: 100-111

Coetsee, C., S. Jacobs and N. Govender. 2012. An Overview of Nitrogen Cycling in a Semiarid Savanna: Some Implications for Management and Conservation in a Large African Park. *Environmental Management* **49**: 387-402

Coetsee, C., W.D. Stock and J.M. Craine. 2010. Do grazers alter nitrogen dynamics on grazing lawns in a South African savannah? *African Journal of Ecology* **49**: 62-69

Conley, D.J., H.W. Paerl, R.W. Howarth, D.F. Boesch, S.P. Seitzinger, K.E. Havens, C. Lancelot and G.E. Likens. 2009. Controlling Eutrophication: Nitrogen and Phosphorus. *Science* **323**: 1014-1015

Cooper, S.M. and N. Owen-Smith. 1985. Condensed Tannins Deter Feeding by Browsing Ruminants in a South African Savanna. *Oecologia* **67**: 142-146

Cowling, R.M., D.M. Richardson and S.M. Pierce. 1997. *Vegetation of Southern Africa*. Cambridge University Press, Cambridge, U.K

Cramer, M.D., A van Cauter and W.J. Bond. 2010. Growth of N2-fixing African savanna *Acacia* species is constrained by below-ground competition with grass. *Journal of Ecology* **98**: 156-167

Cramer, M.D., H.-J. Hawkins and G.A. Verboom. 2009. The importance of nutritional regulation of plant water flux. *Oecologia* **161**: 15-24

Crawley, M.J. 2007. *The R Book*. John Wiley & Sons Ltd., England, U.K

Datta, A., M.O. Anand and R. Naniwadekar. 2008. Empty forest; Large carnivore and prey abundance in Namdapha National Park, north-east India. *Biological Conservation* **141**: 1429-1435

Day, J.E.L., I. Kyriazakis and P.J. Rogers. 1998. Food choice and intake: towards a unifying framework of learning and feeding motivation. *Nutrition Research Reviews* **11**: 25-43

de Villiers, S. and S.T. Mkwelo. 2009. Has monitoring failed the Olifants River, Mpumalanga? *Water SA* **35**: 671-676

Dohn, J., F. Dembélé, M. Karembé, A. Moustakas, K.A. Amévor and N.P. Hanan. 2013. Tree effects on grass growth in savannas: competition, facilitation and the stress-gradient hypothesis. *Journal of Ecology* **101**: 202-209

du Toit, J.T. and N. Owen-Smith. 1989. Body Size, Population Metabolism, and Habitat Specialization among Large Herbivores. *The American Naturalist* **133**; 736-740

du Toit, J.T., K.H. Rogers and H.C. Biggs (eds). 2003. *The Kruger Experience; Ecology and Management of Savanna Heterogeneity*. Island Press, Washington, U.S.A

Eamus, D. 1999. Ecophysiological traits of deciduous and evergreen woody species in the seasonally dry tropics. *Trends in Ecology and Evolution* **14**: 11-16

Elser, J.J., M.E.S. Bracken, E.E. Cleland, D.S. Gruner, W.S. Harpole, H. Hillebrand, J.T. Ngai, E.W. Seabloom, J.B. Shurin and J.E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**: 1135-1142

Estes, R.D. 1992. *The Behavior Guide to African Mammals*. University of California Press, Berkeley, California, U.S.A

Faraway, J.J. 2005. *Linear Models with R.* Chapman & Hall/CRC, Boca Raton, U.S.A

February, E.C. and S.I. Higgins. 2010. The distribution of tree and grass roots in savannas in relation to soil nitrogen and water. *South African Journal of Botany* **76**: 517-523

February, E.C., G.D. Cook and A.E. Richards. 2013a. Root dynamics influence tree-grass coexistence in an Australian savanna. *Austral Ecology* **38**: 66-75

February, E.C., S.I. Higgins, W.J. Bond and L. Swemmer. 2013b. Influence of competition and rainfall manipulation on the growth responses of savanna trees and grasses. *Ecology* **94**: 1155-1164

Frost, P., E. Medina, J.C. Menaut, O.T. Solberg, M. Swift and B. Walker (eds). 1985. *Responses of Savannas to Stress and Disturbance*. The International Union of Biological Sciences, Special Issue 10, Paris, France

Galy-Lacaux, C., D. Laouali, L. Descroix, N. Gobron and C. Liousse. 2009. Long term precipitation chemistry and wet deposition in a remote dry savanna site in Africa (Niger). *Atmospheric Chemistry and Physics* **9**: 1579-1595

Genz, A., F. Bretz, T. Miwa, X. Mi, F. Leisch, F. Scheipl, B. Bornkamp and T. Hothorn. 2012. *mvtnorm: Multivariate Normal and t Distributions*. R package version 0.9-9992. Available from http://cran.r-project.org/web/packages/mvtnorm [Software]

Gibbs-Russell, G.E., L. Watson, M. Koekemoer, L. Smook, N.P. Barker, H.M. Anderson and M.J. Dallwitz. 1990. Grasses of Southern Africa. *Memoirs of the Botanical Survey of South Africa* **58**: 1-437

Giraudoux, P. 2013. *pgirmess: Data analysis in* ecology. R package version 1.5.7. Available from http://cran http://cran.r-project.org/web/packages/pgirmess [Software]

Goheen, J.R., T.M. Palmer, F. Keesing, C. Riginos and T.P. Young. 2010. Large herbivores facilitate savanna tree establishment via diverse and indirect pathways. *Journal of Animal Ecology* **79**: 372-382

Google Earth. 2011. *Google Earth: Version 6.1.0.5001*. Accessed on 24 October 2012. Available from http://www.google.com/earth/download/ge/agree.html [Software]

Grant, C.C., H.H. Meissner and W.A. Schultheiss. 1995. The nutritive value as indicated by faecal phosphorous and nitrogen and its relation to the condition and movement of prominent ruminants during the 1992-1993 drought in the Kruger National Park. *Koedoe* **38**: 17-31

Güsewell, S. 2004. N:P ratios in terrestrial plants: variation and functional significance. *New Phytologist* **164**: 243-266

Hanley, T.A. 1982. The Nutritional Basis for Food Selection by Ungulates. *Journal of Range Management* **35**: 146-151

Hanley, T.A. and K.A. Hanley. 1982. Food Resource Partitioning by Sympatric Ungulates on Great Basin Rangeland. *Journal of Range Management* **35**: 152-158

Hattas, D., J. Hjältén, R. Julkunen-Tiitto, P.F. Scogings and T. Rooke. 2011. Differential phenolic profiles in six African savanna woody species in relation to antiherbivore defense. *Phytochemistry* **72**: 1796-1803

Heffner, R.A., M.J. Butler IV and C.K. Reilly. 1996. Pseudoreplication revisited. *Ecology* **77**: 2558-26562

Higgins, S.I., W.J. Bond and W.S.W. Trollope. 2000. Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. *Journal of Ecology* **88**: 213-229

Higgins, S.I., W.J. Bond, E.C. February, A. Bronn, D.I.W. Euston-Brown, B. Enslin, N. Govender, L. Rademan, S. O'Regan, A.L.F Potgieter, S. Scheiter, R. Sowry, L. Trollope and W.S.W Trollope. 2007. Effects of four decades of fire manipulation on woody vegetation structure in savanna. *Ecology* **88)**: 1119-1125

Hothorn, T., F. Bretz and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. *Biometrical Journal* **50**: 346-363

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54**: 187-211

Johns, T.C., J.M. Gregory, W.J. Ingram, C.E. Johnson, A. Jones, J.A. Lowe, J.F.B. Mitchell, D.L. Roberts, D.M.H Sexton, D.S. Stevenson, S.F.B. Tett and M.J. Woodage. 2003. Anthropogenic climate change for 1860 to 2100 simulated with the HadCM3 model under updated emissions scenarios. *Climate Change* **20**: 583-612

Kalra, Y.P. 1998. *Handbook of reference methods of plant analysis*. CRC Press, Boca Raton, U.S.A

Kambatuku, J.R., M.D. Cramer and D. Ward. 2011. Savanna tree-grass competition is modified by substrate type and herbivory. *Journal of Vegetation Science* **22**: 225-237

Kuijper, D.P.J., J.P.G.M. Cromsigt, M. Churski, B. Adam, B. Jędrzejewska and W. Jędrzejewska. 2009. Do ungulates preferentially feed in forest gaps in European temperate forest? *Forest Ecology and Management* **258**: 1528-1535

Kyriazakis, I., B.J. Tolkamp and G. Emmans. 1999. Diet selection and animal state: an integrative framework. *Proceedings of the Nutritional Society* **58**: 765-772

Lambers, H., M.W. Shane, M.D. Cramer, S.J. Pearse and E.J. Veneklaas. 2006. Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits. *Annals of Botany* **98**: 693-713

Lehmann, C.E.R., S.A. Archibald, W.A. Hoffmann and W.J. Bond. 2011. Deciphering the distribution of the savanna biome. *New Phytologist* **191**: 197-209

Lind, E.M., E. Borer, E. Seabloom, P. Adler, J.D. Bakker, D.M. Blumenthal, M. Crawley, K. Davies, J. Firn, D.S. Gruner, W.S. Harpole, Y. Hautier, H. Hillebrand, J. Knops, B. Melbourne, B. Mortensen, A.C. Risch, M. Schuetz, C. Stevens and P.D. Wragg. 2013. Life-history constraints in grassland plant species: a growth-defence trade-off is the norm. *Ecology Letters* **16**: 513-521

Ludwig, F., T.E. Dawson, H.H.T. Prins, F. Berendse and H. de Kroon. 2004. Below-ground competition between trees and grasses may overwhelm the facilitative effects of hydraulic lift. *Ecology Letters* **7**: 623-631

Mahowald, N., T.D. Jickells, A.R. Baker, P. Artaxo, C.R. Benitez-Nelson, G. Bergametti, T.C. Bond, Y. Chen, D.D. Cohen, B. Herut, N. Kubilay, R. Losno, C. Luo, W. Maenhaur, K.A. McGee, G.S. Okin, R.L. Siefert and S. Tsukuda. 2008. Global distribution of atmospheric phosphorus sources, concentration and deposition rates, and anthropogenic impacts. *Global Biogeochemical Cycles* **22**: GB4026

Marnewick, K., P.J. Funston and K.U. Karanth. 2008. Evaluating camera trapping as a method for estimating cheetah abundance in ranching areas. *South African Journal of Wildlife Research* **38**: 59-65

Miller, A.J. and M.D. Cramer. 2004. Root nitrogen acquisition and assimilation. *Plant and Soil* **274**: 1-36

Mordelet, P., J.-C. Menaut and A. Marietta. 1997. Tree and Grass Rooting Patterns in an African Humid Savanna. *Journal of Vegetation Science* **8**: 65-70

Mphepya, J.N., C. Galy-Lacaux, J.P. Lacaux, G. Held and J.J. Pienaar. 2006. Precipitation Chemistry and Wet Deposition in Kruger National Park, South Africa. *Journal of Atmospheric Chemistry* **53**: 169-183

Mphepya, J.N., J.J. Pienaar, C. Galy-Lacaux, G. Held and C.R. Turner. 2004. Precipitation Chemistry in Semi-Arid Areas of Southern Africa: A Case Study of a Rural and Industrial Site. *Journal of Atmospheric Chemistry* **47**: 1-24

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

Oksanen, L. 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue? *Oikos* **94**: 27-38

Owen-Smith, N. and P. Novellie. 1982. What Should a Clever Ungulate Eat? *The American Naturalist* **119:** 151-178

Parker, K.L., P.S. Barboza and M.P. Gillingham. 2009. Nutrition integrates environmental responses of ungulates. *Functional Ecology* **23:** 57-69

Pienaar, U.de V. 1963. The Large Mammals of the Kruger National Park. *Koedoe* **6**: 1-37

Pierce, G.J. and J.G. Ollason. 1987. Eight reasons why optimal foraging theory is a complete waste of time. *Oikos* **49**: 111-118

Power, S.C., M.D. Cramer, G.A. Verboom and S.B.M. Chimphango. 2010. Does phosphate acquisition constrain legume persistence in the fynbos of the Cape Floristic Region? *Plant and Soil* **334**: 33-46

Prosser, J.I. 2010. Replicate or lie. *Environmental Microbiology* **12**: 1806-1810

Provenza, F.D., J.J. Villalba, C.D. Cheney and S.J. Werner. 1998. Self-organization of foraging behaviour: From simplicity to complexity without goals. *Nutrition Research Reviews* **11**: 199-222

R Development Core Team. 2013. *R: a language and environment for statistical computing version 3.0.0*. The R Foundation for Statistical Computing, Vienna, Austria [Software]

Rooney, N., K.S. McCann and J.C. Moore. 2008. A landscape theory for food web structure. *Ecological Letters* **11**: 867-881

Rovero, F. and A.R. Marshall. 2009. Camera trapping photographic rate as an index of density in forest ungulates. *Journal of Applied Ecology* **46**: 1011-1017

Rowcliffe, J.M., J. Field, S.T. Turvey and C. Carbone. 2008. Estimating animal density using camera traps without the need for individual recognition. *Journal of Applied Ecology* **45**: 1228-1236

Saayman, M. and A. Saayman. 2006. Estimating the Economic Contribution of Visitor spending in the Kruger National Park to the Regional Economy. *Journal of Sustainable Tourism* **14**: 67-81

Saayman, M. and A. Saayman. 2009. Why travel motivation and socio-demographics matter in managing a national park. *Koedoe* **51**: 9 pages

Saayman, M. and E. Slabbert. 2004. A profile of Tourists visiting the Kruger National Park. *Koedoe* **47**: 1-8

Sankaran, M., J. Ratnam and N.P. Hanan. 2004. Tree-grass coexistence in savannas revisited – insights from an examination of assumptions and mechanisms invoked in existing models. *Ecology Letters* **7**: 480-490

Sankaran, M., N.P. Hanan, R.J. Scholes, J. Ratnam, D.J. Augustine, B.S. Cade, J. Gignoux, S.I. Higgins, X. Le Roux, F. Ludwig, J. Ardo, F. Banyikwa, A. Bronn, G. Bucini, K.K. Caylor, M.B. Coughenour, A. Diouf, W. Ekaya, C.J. Feral, E.C. February, P.G.H. Frost, P. Hiernaux, H. Hrabar, K.L. Metzger, H.H.T. Prins, S. Ringrose, W. Sea, J. Tews, J. Worden and N. Zambatis. 2005. Determinants of woody cover in African savannas. *Nature* **438**: 846-849

Schmidt, E., M. Lötter and W. McCleland. 2002. *Trees and Shrubs of Mpumalanga and Kruger National Park*. Jacana Media, Johannesburg, South Africa

Scholes, R.J. 1990. The Influence of Soil Fertility on the Ecology of Southern African Dry Savannas. *Journal of Biogeography* **17**: 415-419

Scholes, R.J. and S.R. Archer. 1997. Tree-Grass Interactions in Savannas. *Annual Review of Ecology, Evolution and Systematics* **28**: 517-544

Scogings, P.F., J. Hjältén and C. Skarpe. 2011. Secondary metabolites and nutrients of woody plants in relation to browsing intensity in African savannas. *Oecologia* **167**: 1063-1073

Scogings, P.F., L.E. Dziba and I.J. Gordon. 2004. Leaf chemistry of woody plants in relation to season, canopy retention and goat browsing in a semiarid subtropical savanna. *Austral Ecology* **29**: 278-286

Scorgie, Y. and G. Kornelius. 2009. Modelling of acid deposition over the South African Highveld. *Proceedings of the Clean Air Conference* 2009

Senft, R.L., M.B. Coughenour, D.W. Bailey, L.R. Rittenhouse, O.E. Sala and D.M. Swift. 1987. Large Herbivore Foraging and Ecological Hierarchies. *BioScience* **37**: 789-795 + 798-799

Shorrocks, B. 2007. *The Biology of African Savannahs*. Oxford University Press Inc., New York, U.S.A

Silveira, L., A.T.A. Jácomo, J.A.F. Diniz-Filho. 2003. Camera trap, line transect and track surveys: a comparative evaluation. *Biological Conservation* **114**: 351-355

Singh, K.P. and C.P. Kushwaha. 2005. Emerging paradigms of tree phenology in dry tropics. *Current Science* **89**: 964-975

Skarpe, C., R. Bergström, A.L. Bråten and K. Danell. 2000. Browsing in a heterogeneous savanna. *Ecography* **23**: 632-640

Skinner, J.D. and C.T. Chimimba. 2005. *The Mammals of the Southern African Subregion*. Cambridge University Press, Cambridge, U.K

Smit, G.N. 2004. An approach to tree thinning to structure southern African savannas for long-term restoration from bush encroachment. *Journal of Environmental Management* **71**: 179-191

Smith, C.C. 1940. The Effect of Overgrazing and Erosion Upon the Biota of the Mixed-Grass Prairie of Oklahoma. *Ecology* **21**; 381-397

Spalding-Fecher, R. and D.K. Matibe. 2003. Electricity and externalities in South Africa. *Energy Policy* **31**: 721-734

Sponheimer, M., C.C. Grant, D.J. de Ruiter, J.A. Lee-Thorp, D.M. Codron and J. Codron. 2003. Diets of Impala from Kruger National Park: evidence from stable carbon isotopes. *Koedoe* **46**: 101-106

Stanton-Geddes, J., R.G. Shaw and P. Tiffin. 2013. Insights from Population Genetics for Range Limits of a Widely Distributed Native Plant. *American Journal of Botany* **100**: 744-753

Staver, A.C., S. Archibald and S. Levin. 2011a. Tree cover in sub-Saharan Africa: Rainfall and fire constrain forest and savanna as alternative stable states. *Ecology* **92**: 1063-1072

Staver, A.C., S. Archibald and S.A. Levin. 2011b. The Global Extent and Determinants of Savanna and Forest as Alternative Biome States. *Science* **334**: 230-232

Staver, A.C., W.J. Bond, W.D. Stock, S.J. van Rensburg and M.S. Waldram. 2009. Browsing and Fire Interact to Suppress Tree Density in an African Savanna. *Ecological Applications* **19**: 1909-1919

Stein, A.B., T.K. Fuller and L.L. Marker. 2008. Opportunistic use of camera traps to assess habitat-specific mammal and bird diversity in northcentral Namibia. *Biodiversity and Conservation* **17**: 3579-3587

Suzuki, R. and H. Shimodaira. 2011. *pvclust: Hierarchical Clustering with P-Values via Multiscale Bootstrap Resampling.* R package version 1.2-2. Available from http://cran.r-project.org/web/packages/pvclust [Software]

Tews, J., N. Blaum and F. Jeltsch. 2004. Structural and Animal Species Diversity in Arid and Semi-arid Savannas of the Southern Kalahari. *Annals of Arid Zone* **43**: 413-425

Thrash, I., G.K. Theron and J. du P. Bothma. 1995. Dry season herbivore densities around drinking troughs in the Kruger National Park. *Journal of Arid Environments* **29**: 213-219.

Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W.H. Schlesinger, D. Simberloff, D. Swackhamer. 2001. Forecasting Agriculturally Driven Global Environmental Change. *Science* **292**: 281-284

Turpie, J. and A. Joubert. 2001. Estimating potential impacts of a change in river quality on the tourism value of Kruger National Park: An application of travel cost, contingent and conjoint valuation methods. *Water SA* **27**: 387-398

van de Koppel, J. and H.H. Prins. 1998. The importance of herbivore interactions for the dynamics of African savanna woodlands: an hypothesis. *Journal of Tropical Ecology* **14**: 565-576

van der Merwe, P. and M. Saayman. 2008. Travel motivations of tourists visiting Kruger National Park. *Koedoe* **50**: 154-159

van Langevelde, F., C.A.D.M. van de Vijver, L. Kumar, J. van de Koppel, N. de Ridder, J. van Andel, A.K. Skidmore, J.W. Hearne, L. Stroosnijder, W.J. Bond, H.H.T. Prins and M. Rietkerk. 2003. Effects of fire and herbivory in the stability of savanna ecosystems. *Ecology* **84**: 337-350

Venables, W.N. and B.D. Ripley. 2002. *Modern Applied Statistics with S; Fourth Edition*. Springer, New York, U.S.A

Venter, F.J., R.J. Naiman, H.C. Biggs and D.J. Pienaar. 2008. The Evolution of Conservation Management Philosophy: Science, Environmental Change and Social Adjustments in the Kruger National Park. *Ecosystems* **11**: 173-192

Verweij, R.J.T., S.I. Higgins, W.J. Bond and E.C. February. 2011. Water sourcing by trees in a mesic savanna: Responses to severing deep and shallow roots. *Environmental and Experimental Botany* **74**: 229-236

Vogel, J.C., A. Fuls and R.P. Ellis. 1978. The geographical distribution of Kranz grasses in South Africa. *South African Journal of Science* **74**: 209-215

Wakeling, J.L., A.C. Staver and W.J. Bond. 2011. Simply the best: the transition of savanna saplings to trees. *Oikos* **120**: 1448-1451

Waldram, M.S., W.J. Bond and W.D. Stock. 2008. Ecological Engineering by a Mega-Grazer: White Rhino Impacts on a South African Savanna. *Ecosystems* **11**: 101-112

Weltzin, J.F. and M.B. Coughenour. 1990. Savanna tree influence on understory vegetation and soil nutrients in northwestern Kenya. *Journal of Vegetation Science* **1**: 325-334

Wiens, J.A. 1989. Spatial Scaling in Ecology. *Functional Ecology* **3**: 385-397

Williams, R.J., B.A. Myers, W.J. Muller, G.A. Duff and D. Eamus. 1997. Leaf Phenology of Woody Species in a North Australian Tropical Savanna. *Ecology* **78**: 2542-2558

Wrench, J.M., H.H. Meissner, C.C. Grant and N.H. Casey. 1996. Environmental factors that affect the concentration of P and N in faecal samples collected for the determination of nutritional status. *Koedoe* **39**: 1-6

Yasuda, M. 2004. Monitoring diversity and abundance of mammals with camera traps: a case study on Mount Tsukuba, central Japan. *Mammal Study* **29**: 37-46

Zhang, X., F.W. Zwiers, G.C. Hegerl, F.H. Lambert, N.P. Gillett, S. Solomon, P.A. Scott and T. Nozawa. 2007. Detection of human influence on twentieth-century precipitation trends. *Nature* **448**: 461-466

APPENDIX

Appendix 1: The number of grass leaf samples, which underwent chemical analyses, collected at each site during the dry and wet season. Samples collected at the treatment site are separated according to the eight nutrient treatments; C refers to the control, N refers to nitrogen addition, P refers to phosphorus addition, NP refers to nitrogen and phosphorus addition and ( ) refers to water addition. Nomenclature follows Chippindall and Crook (1976) and Gibbs-Russell *et al.* (1990).

	Dry Season											Wet Season											
	Treatment Site								Control Site 1	Control Site 2	Control Site 3	Treatment Site								Control Site 1	Control Site 2	Control Site 3	Total:
	C	(C)	N	(N)	NP	(NP)	P	(P)				C	(C)	N	(N)	NP	(NP)	P	(P)				
Digitaria eriantha	3		1			2	2	1	2	2		2	2	2	2	2	1	2	2	1		1	30
Eragrostis rigidior	3	3	3	2	3	4	2	3	3	1										3			30
Panicum maximum			1		1		2				3	2	2	2	2	2	2	2	2	2	3	2	30
Urochloa mosambicensis	4	4	4	4	4	4	4	4	3	3	3	3	3	3	3	3	3	2	3	3	3	3	73
Total:	10	7	9	6	8	10	10	8	8	6	6	7	7	7	7	7	6	6	7	9	6	6	163

Appendix 2: The number of tree leaf samples, which underwent chemical analyses, collected at each site during the dry and wet season. Samples collected at the treatment site are separated according to the eight nutrient treatments; C refers to the control, N refers to nitrogen addition, P refers to phosphorus addition, NP refers to nitrogen and phosphorus addition and ( ) refers to water addition. Nomenclature follows Schmidt *et al.* 2002.

	Dry Season											Wet Season											
	Treatment Site								Control Site 1	Control Site 2	Control Site 3	Treatment Site								Control Site 1	Control Site 2	Control Site 3	Total:
	C	(C)	N	(N)	NP	(NP)	P	(P)				C	(C)	N	(N)	NP	(NP)	P	(P)				
Acacia nilotica	3	4	3	4	4	1	3	2	3	3	1	3	3	3	3	3	3	3	2	3	3	1	61
Acacia tortilis	2	2	3	3	2	4	3	3	3	3	3	3	3	3	4	2	3	3	4	3	3	2	64
Dichrostachys cinerea	4	4	3	3	4	4	4	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	72
Total:	10	10	8	6	7	9	9	9	9	9	8	8	9	9	9	8	10	10	8	6	7	9	197

Appendix 3: The number of ungulate dung samples, which underwent chemical analyses, collected at each site during the dry and wet season. Nomenclature follows Skinner and Chimimba (2005).

	Dry Season				Wet Season				
	Treatment Site	**Control Site 1**	**Control Site 2**	**Control Site 3**	**Treatment Site**	**Control Site 1**	**Control Site 2**	**Control Site 3**	**Total:**
Buffalo (*Syncerus caffer*)	3	3	1		3			3	13
Duiker (*Sylvicapra grimmia*)	3	1	3		3				10
Elephant (*Loxodonta africana*)	3	1	3	3	3	3	3	3	22
Giraffe (*Giraffa camelopardalis*)	3	3	3	3	3	3	3		21
Impala (*Aepyceros melampus*)	3	3	3	4	4	3	3	4	27
Steenbok (*Raphicerus campestris*)	3		1						4
White Rhino (*Ceratotherium simum*)								1	1
Wildebeest (*Connochaetes taurinus*)	4		3	3	3	3	3		19
Zebra (*Equus burchelli*)	3	3	3	3	3	3	3	2	23
Total:	25	14	20	16	22	15	15	13	140

Appendix 4: The number of photos taken by the camera traps at the four study sites during the dry and wet season. The number in brackets indicates the number of functioning camera traps at each site. Nomenclature follows Skinner and Chimimba (2005).

	Dry Season				Wet Season				Total:
	Treatment Site (*n* = 14)	Control Site 1 (*n* = 1)	Control Site 2 (*n* = 2)	Control Site 3 (*n* = 3)	Treatment Site (*n* = 14)	Control Site 1 (*n* = 1)	Control Site 2 (*n* = 2)	Control Site 3 (*n* = 1)	
Baboon (*Papio ursinus*)	1			2	3	2		4	12
Buffalo (*Syncerus caffer*)	46		1	374	150			2	573
Cheetah (*Acinonyx jubatus*)				1					1
Duiker (*Sylvicapra grimmia*)	8		1		5	7		1	22
Elephant (*Loxodonta africana*)	70		26	123	227		30	19	495
Giraffe (*Giraffa camelopardalis*)	122		4	28	39	5	68		266
Impala (*Aepyceros melampus*)	505	12	42	2	381	12	43	1	998
Kudu (Tragelaphus strepsiceros)	20	1	28		37	1	4	2	93
Leopard (*Panthera pardus*)					2				2
Lion (*Panthera leo*)	1			1					2
Spotted Hyena (*Crocuta crocuta*)	11			21	8	1	1	3	45
Steenbok (*Raphicerus campestris*)	2				1	4			7
Warthog (*Phacochoerus africanus*)	25		2	4	30	1			62
Waterbuck (*Kobus ellipsiprymnus*)	6			1	61	6	22	2	98
White Rhino (*Ceratotherium simum*)	8	2	18	6		1			35
Wildebeest (*Connochaetes taurinus*)	41		39	44	9		15		148
Zebra (*Equus burchelli*)	153	3	51	252	11	3	33	4	510
Total:	1019	18	212	859	964	43	216	38	3369

Appendix 5: The number of grass measurements recorded at each site during the dry and wet season. Measurements recorded at the treatment site are separated according to the eight nutrient treatments; C refers to the control, N refers to nitrogen addition, P refers to phosphorus addition, NP refers to nitrogen and phosphorus addition and ( ) refers to water addition. Nomenclature follows Chippindall and Crook (1976) and Gibbs-Russell *et al.* (1990).

	Dry Season											Wet Season											
	Treatment Site								Control Site 1	Control Site 2	Control Site 3	Treatment Site								Control Site 1	Control Site 2	Control Site 3	Total:
	C	(C)	N	(N)	NP	(NP)	P	(P)				C	(C)	N	(N)	NP	(NP)	P	(P)				
Aristida congesta subsp. *baricollis*													1										1
Aristida congesta subsp. *congesta*									1											2			3
Botriochloa radicans											2									2			4
Cenchrus ciliaris																						8	8
Chloris gayana												15	2	13	7	4	21	1	20	4	1	11	99
Chloris virgata		41		43	9	4		61															158
Dactyloctenium aegyptium			2		3								26		42	46	3		24				146
Dactyloctenium australe																						2	2
Digitaria eriantha	13	15	8		2	14	15		47	79	5	3	13		1		1	10	10	6	19	5	266
Eragrostis nindensis												1											1
Eragrostis rigidior	43	39	27	12	30	11	21	37	34	2		45	31	27	9	21	7	18	9	38			461
Eragrostis superba																					2	22	24
Heteropogon contortus										5											1	5	11
Panicum coloratum								8											4				12
Panicum maximum		9	10		13	1	16	1	16	21	30		2	7		7	1	7	13	10	9	16	189
Perotis patens											13												13
Pogonarthria squarrosa										35											20		55
Schimidia pappophoroides				1					3	18		11		3			2	4	1	14	81	2	140
Setaria sphaceleta																						13	13
Themeda triandra																						1	1
Tragus berteronianus												25	1	21	7	2	20	6	1	2	15		100
Urochloa mosambicensis	55	30	55	72	80	86	58	39	111	73	153	41	45	45	56	65	45	54	52	77	66	115	1473
Total:	111	134	102	128	137	116	110	146	212	233	203	141	121	116	122	145	100	100	134	155	214	200	3180

Appendix 6: The number of tree assessments conducted at each site during the dry and wet season. Assessments conducted at the treatment site are separated according to the eight nutrient treatments; C refers to the control, N refers to nitrogen addition, P refers to phosphorus addition, NP refers to nitrogen and phosphorus addition and ( ) refers to water addition. Nomenclature follows Schmidt *et al.* 2002.

	Dry Season											Wet Season											
	Treatment Site								Control Site 1	Control Site 2	Control Site 3	Treatment Site								Control Site 1	Control Site 2	Control Site 3	Total:
	C	(C)	N	(N)	NP	(NP)	P	(P)				C	(C)	N	(N)	NP	(NP)	P	(P)				
Acacia nilotica	4	5	4	6	1	5	2	2	6	6	1	6	6	6	6	4	5	4	1	8	8	1	97
Acacia tortilis	3	5	3	4	6	3	5	3	6	6	4	6	5	6	6	6	4	6	5	6	4	4	106
Dichrostachys cinerea	4	5	3	5	5	6	6	5	8	9	9	4	6	6	6	6	6	6	6	9	9	9	138
Total:	11	15	10	15	12	14	13	10	20	21	14	16	17	18	18	16	15	16	12	23	21	14	341