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The Feeding Ecology of Eastern Black Rhinoceroses (Diceros bicornis michaeli) in southern Serengeti national Park, Tanzania.

Philbert Ngoti

Natural Resources Management
Submission date: June 2017
Supervisor: Bente Jessen Graae, IBI

Norwegian University of Science and Technology
Department of Biology

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Supervisors

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DEDICATION

To Park Wardens and Rangers of the rhino projects in Serengeti National Park

To my parents (MICHAEL and MARY), you are the foundation of my education

To my wife, AGNESS and son, PHILIP; you are the basis of my motivation to pursue the post graduate studies

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ABSTRACT

This study has been carried out to improve the knowledge on the feeding ecology and impacts of fire on black rhinoceroses browse abundance in southern Serengeti National Park. It attempts to enlighten on forage preferences of the megaherbivore and investigate the effects of fire on abundance of their preferred vegetation species. Plant species were sampled in 64 rhino foraging plots, 300 random plots, and 198 plots with known occurrence of fire history to estimate abundances and extent of plant species browsed and the preferred species response to fire history. Additionally, a total of 15 fresh black rhinoceroses dungs were collected for DNA metabarcoding analysis to ascertain relative plant species composition of the animals' diet. Across all 64 rhino foraging plots, rhinoceroses preferred 9 plant species: 5 forbs (Crotalaria barkae, Justicia betonica, Indigofera basiflora, Achyranthes aspera, Indogofera volkensii), 3 shrubs (Ziziphus abyssinica, Hibiscus species, Abutilon species), and 1 tree (Acacia sieberiana). A total of 72 taxa were identified in the rhinoceros's dung by DNA analysis, and the most important genera found are Crotalaria, Indigofera, Solanum, Euphorbia and Vachellia. The study concludes that; black rhinoceroses mainly prefer forbs; DNA metabarcoding is an efficient method to study rhino forage preference, and frequent fires may decrease preferred rhino forage. To understand the response of preferred species to fire, it is important to make manipulative studies with fire to comprehend how species grow and reproduce with and without fire.

Keywords: Utilization, fire, black rhinoceros, abundance, occurrence, fire frequency

INTRODUCTION

Serengeti National Park (SENAPA) used to have as many as 400 to 700 free-ranging Eastern Black Rhinoceroses (hereinafter referred to as black rhino) in the 1970s (Frame, 1980; Metzger et al., 2007) making it one of the most important rhino range in Tanzania. Illegal killing of the rhinos significantly reduced the population to less than ten individuals (Arcese et al., 1995; Borner, 1981; Metzger et al., 2007; Sinclair, 1995) throughout the park by the 1980s. Efforts to manage and raise the remnant population began in Moru as a suitable area (Borner, 1981) in southern Serengeti in 1995. To date, fewer than 55 black rhinos survive in the whole of SENAPA and less than 40 individuals can be found in the Moru area. The World Conservation Union (IUCN) regard the subspecies (*Diceros bicornis michaeli*) as "critically endangered" while the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) lists the subspecies in Appendix I (Emslie & Brooks, 1999) prohibiting international trade in specimens of the subspecies.

Studies on rhino feeding ecology provide insights into assessing carrying capacities important for defining stocking rates to ensure maximum reproductive success (Ausland et al., 2001; Emslie, 1999; Goddard, 1968, 1970; Hall-Martin et al., 1982; Mukinya, 1977; Oloo et al., 1994) for practical rhino conservation. According to Luske et al. (2009) thorough understanding of the black rhino diet can be used to determine key plant species as an early warning indicators of food limitation and of threat to preferred species. Further, a knowledge on rhino diet improves our understanding of food selection by black rhino species (Muya & Oguge, 2000) and facilitate research on diet overlap with other herbivores (De Boer & IJdema, 2007) to help in minimizing food competition with the critically endangered species in enclosed areas in the wild. Black rhino feeds on a wide variety of available plant species but prefers herbs and shrubs (Goddard, 1968, 1970; Mukinya, 1977; Oloo et al., 1994), and noticeably legumes (Goddard, 1968, 1970) while either feeding on grasses in low abundances (Goddard, 1968, 1970) or consuming grasses together with shrubs and herbs instead of taking them separately (Mukinya, 1977).

Fire is an integral part of ecosystem management (Botkin, 1990; Morgan et al., 1994) in Africa and is used as one of the management tools to influence vegetation composition and structure (Holdo et al., 2009). Fire may improve the quality of forage for large herbivores (Hassan et al., 2008) by increasing the nutritive quality of resprouting tissues, net primary productivity and richness (Hassan, 2011). On the other hand, fire is known to reduce woody biomass (Holdo et al., 2009) and could potentially reduce rhino forage quantity and quality. Fire also affects composition and number of plant species. Wangari (2016) found higher number and cover of herbaceous species in burnt sites than in unburnt sites. Fire minimizes the height of the fire-sensitive species making rhinos to feed on plants less than 2m high (Mukinya, 1977). In African savanna like Serengeti, fire strongly affects the relative biomass of woody and herbaceous vegetation and cause spatiotemporal variation in tree biomass (Bond, 2005; Scholes & Walker, 2004). This study seeks to understand the effects of fire on the abundance of preferred species of rhino in southern SENAPA. Initially, the study attempts to comprehend the vegetation preference of the rhinos, as the knowledge on their browsed preference by actual assessment of the quantities of browse consumed through direct observation (Matipano, 2003) in the wild is scarce.

OBJECTIVES

The broad goal of this study is to improve an understanding of rhino forage preference in SENAPA and examine how fire can influence the preferred forage species for this iconic and critically endangered species.

Specifically, the study seeks: -

1. To describe forage preferences of black rhinos
2. To investigate the effects of fire on the abundance of those plants that are most preferred by black rhinos

METHODS

Study area

The study was conducted in Moru rhino area in southern SENAPA. The study area is 643.16km^2 and located between 34º30' to 35ºE and 2º30' to 3ºS (*Figure 1*). Elevation ranges from 1,656m at Simba Kopjes in the east to 2,017m at Itonjo hills in the south-west of the study area (Hopcraft, 2008). SENAPA has two rainy seasons determined by intertropical convergence zone: short rains in November – December and long rains from March to May with areas to the south having lower average rainfall (Norton-Griffiths, 1979) and a rainfall gradient of 514 – 688mm (Norton-Griffiths et al., 1975). The dry season occurs from June to October (L. Brown & Cochemé, 1973;

Krebs, 1999; Norton- Griffiths et al., 1975) and is associated with fires on the grassland (Glogiewicz & Baez, 2001). The soils are volcanic, alkaline, have abundant organic matter and are less easily leached (McNaughton et al., 1988; Wit, 1978). Grassland is extensive in the eastern part of the study area and woodlands, dominated largely by species of *Acacia* (Herlocker, 1976), occur in the western regions of the study area (Norton- Griffiths et al., 1975). Widespread burning every year is common in SENAPA (Hassan et al., 2008; Norton-Griffiths, 1979) and the surrounding game reserves from the end of the wet season through the dry season.

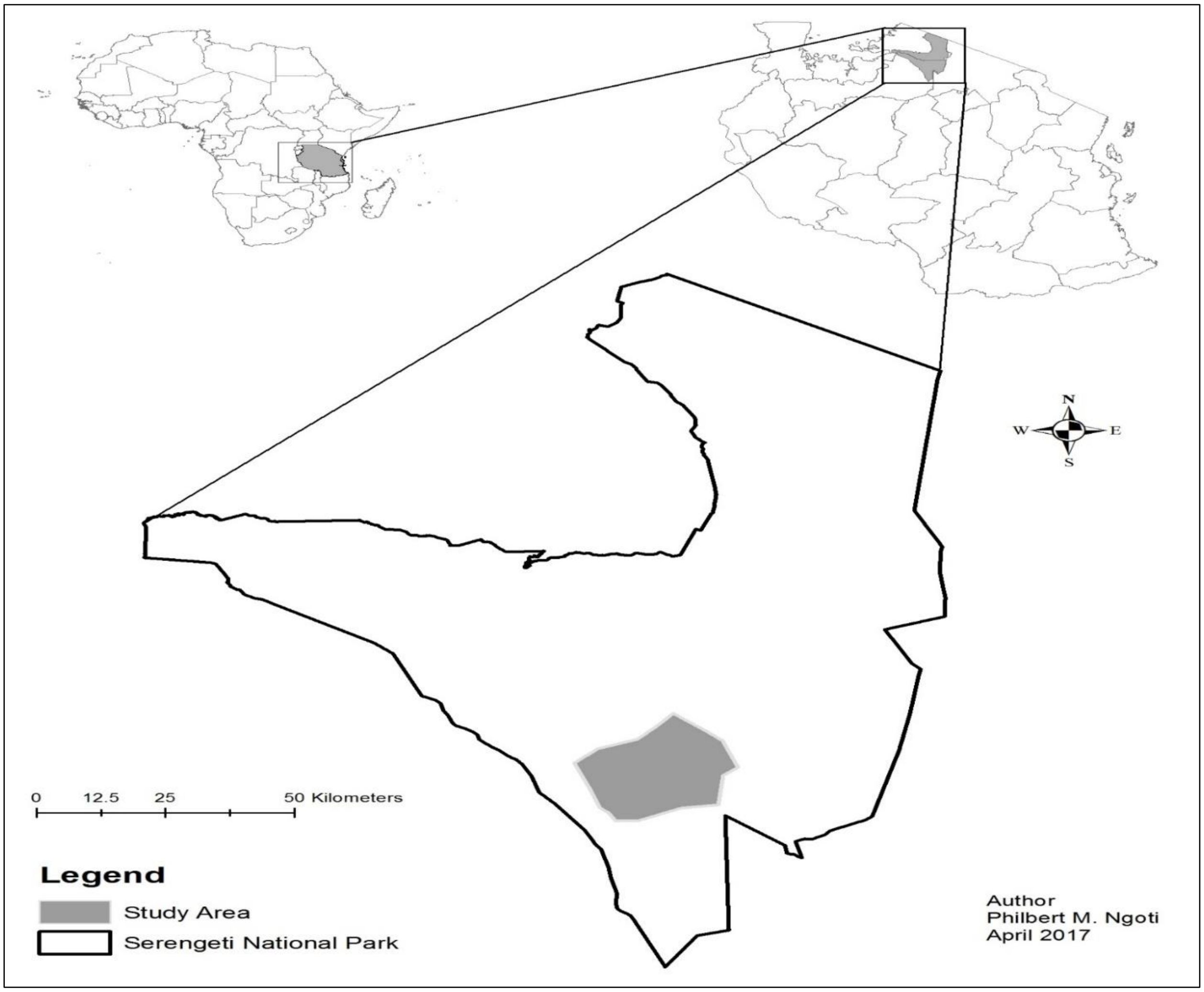

Figure 1: Map of Serengeti National Park showing a study area, and Africa and Tanzania as inset maps

Study animal

Eastern black rhinoceros (*Diceros bicornis michaeli*) is one of the four recognized black rhino subspecies in Africa (Emslie & Brooks, 1999) belonging in the class *Mammalia*, order *Perissodactyla* and family *Rhinocerotidae* (Estes, 1991; Mills & Hes, 1997). The subspecies (*Diceros bicornis michaeli*) are known to be aggressive. Their historical distribution is mainly in East Africa, and in Tanzania they ranged into northern-central areas (Emslie & Brooks, 1999) including SENAPA as one of the ranges for the Eastern African subspecies (Metzger et al., 2007). These rhinos are browsers, have comparatively narrow mouth with a prehensile lip enabling them to feed on woody vegetation (Oloo et al., 1994) and occasionally grazing on grass (Mabinya et al., 2002). Because of their conspicuously mouthparts they are often referred to as hook-lipped rhinos (Emslie & Brooks, 1999). Black rhino feeding is noticeably distinct as it clips off vegetation to leave a scissor-like cut stump (Oloo et al., 1994; Ritchie, 1963). The rhinos are usually solitary and live in a home range with food abundance, water (Goddard, 1968; Mukinya, 1977; Tatman et al., 2000) and food quality (Muya & Oguge, 2000). They are less active during the day and become active in the mornings and evenings when they regularly feed and drink (Mukinya, 1977).

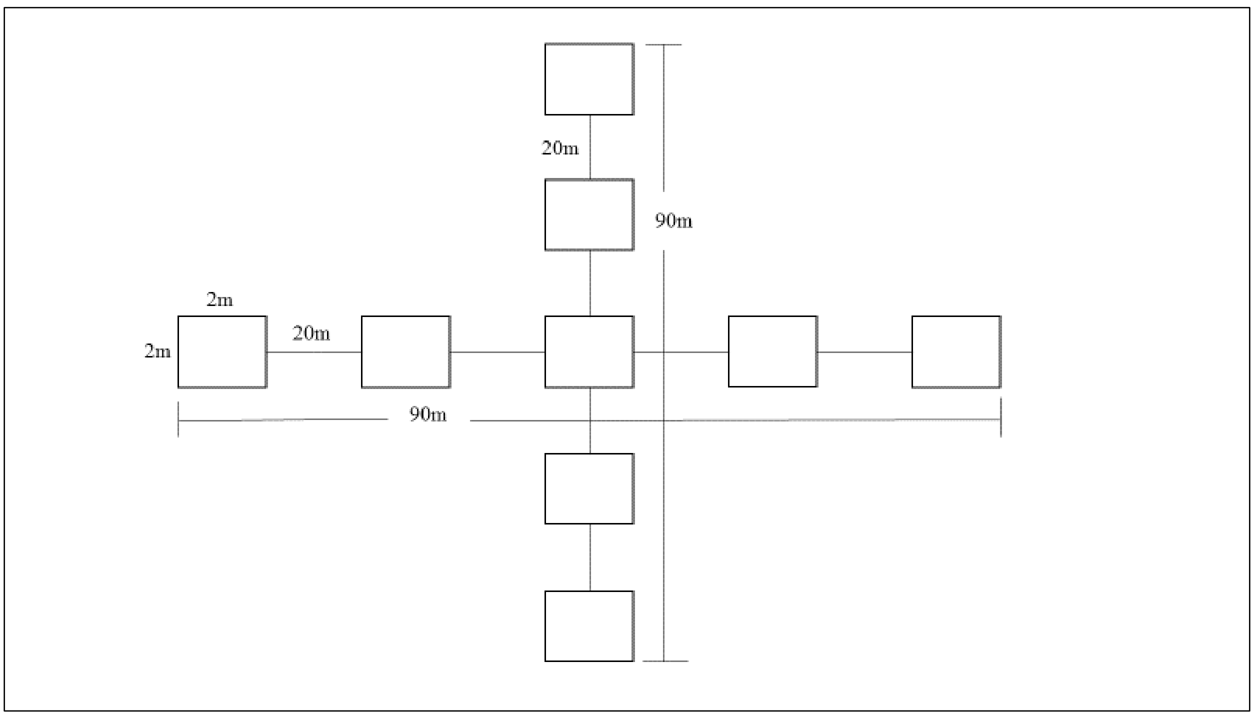

Figure 2: Showing 2 x 2m plots; two 90 m long perpendicular line transects (for fire plots); 90m long line transect (for vegetation plots)

STUDY DESIGN

Data collection and selection of plots and sites

Vegetation was sampled using three different approaches, hereafter referred to as "rhino foraging plots", "vegetation plots" (from random line transects) and "fire plots" (from random perpendicular line transects). All vegetation samples were collected between the dates of 19th June 2016 to 15th August 2016 corresponding to dry season (L. Brown & Cochemé, 1973; Norton-Griffiths, 1979; Norton- Griffiths et al., 1975).

Rhino foraging plots

A total of 64 rhino foraging plots (2x2m) were put in areas where rhinos were physically seen feeding. Feeding rhinos were located by rhino monitoring staff working in the study area. Rhino identities; cover and proportion of plant species browsed were recorded when the animals vacated from the plots. Cover was measured visually by estimating the percent of each plant species present in the plot. Similarly, the proportion in percentage of each species browsed was quantified within each plot sampled and recorded accordingly.

Vegetation and fire line transects

ArcGIS version 10.3 (Desktop, 2014) was used to generate 60 random vegetation plots at least 180m apart in the study area polygon. At each location, five 2 x 2m plots (*Figure 2*) were established 20m apart along a 90m long transect, for a total of 300 random vegetation plots.

In order to ensure that our random plots covered a range of fire histories, an additional 22 sites were sampled that had not burnt for 1, 3, 5 or 15 years across similar habitat, soils (McNaughton et al., 1988) and average rainfall (Norton-Griffiths, 1979). Fire history was established with the Moderate Resolution Imaging Spectroradiomater (MODIS) Land Collection 5, total annual burned area mapping product (Roy et al., 2008) at a spatial resolution of 250m between 2000 and 2016 (Dempewolf et al., 2007). At each of the 22 sites, two 90m line transects (*Figure 2*), set perpendicular to each other, containing nine (2 x 2m) plots distanced at 20m apart, were used to sample a total of 198 fire plots. Four sites not burned for 3 years could not be sampled as they were burnt with late dry season fires set by the park staff. New sites were selected in cases where the coordinates fell on top of mountains. The first transect direction (the second transect set perpendicular to the first) was determined by both Garmin GPSMAP®64 (Desch et al., 2016) and the random number returns from an excel spreadsheet with number 1 being the bottom and 8 the top, rotating clockwise corresponding to directions North and North-West respectively. From these two transects, only percent cover of the species present in the plots were measured and recorded similarly as in the rhino foraging plots.

Plant species from the vegetation and fire plots were identified to the lowest taxonomic level using Wild Flowers of East Africa (Blundell, 1992); Guide to grasses of Southern Africa (Oudtshoorn, 1999); Field guide to common trees & shrubs of East Africa (Dharani, 2002); Field guide to Acacias of East Africa (Dharani, 2006), and Field guide to trees of Southern Africa (Van Wyk & Van Wyk, 1997) guide books and with the help of local vegetation experts. Unidentified plants during field work were collected in envelopes and identified in the Serengeti Wildlife Research Centre herbarium.

Fresh rhino dung

To compliment the knowledge of preferred species, 15 spatially overlapping fresh rhino dung (*Figure 3*) samples (4 dungs from known and 11 from unknown individuals) were collected for DNA metabarcoding and stable-isotope analyses to confirm the vegetation plot data with the barcoding methods. Fresh samples from known individuals were collected from rhinos observed defecating, and unknown individuals' samples were opportunistically collected and identified by appearance. Using gloves all 15 samples were put in tubes and 96% ethanol added to kill bacterial microbes. After two months, the samples were removed from the tubes, dried in air for approximately 10 seconds, and put in new tubes containing silica, tightened and sent to SPYGEN laboratory in France for DNA metabarcoding to genetically determine diet composition and the relative proportions of different plant species browsed by rhinos. At the laboratory, first, total DNA was extracted from about 10 mg of fecal sample using the DNeasy Mini Stool Kit (Qiagen GmbH) following the manufacturer's instructions. The DNA extracts were recovered in a total volume of 200 μL. Mock extractions without samples were systematically performed to monitor possible contaminations. Second, DNA amplifications were carried out using the universal plant primers gh *trn*L gene (Taberlet et al., 2007). For each sample the DNA amplification was repeated twice. After amplification, all samples were purified using the MinElute PCR purification kit

(Qiagen GmbH) and pooled for the pyrosequencing run (Illumina Hiseq). Each sample was recognized by a specific six base long tag for assignation of sequences to samples during bioinformatic segregation of sequences.

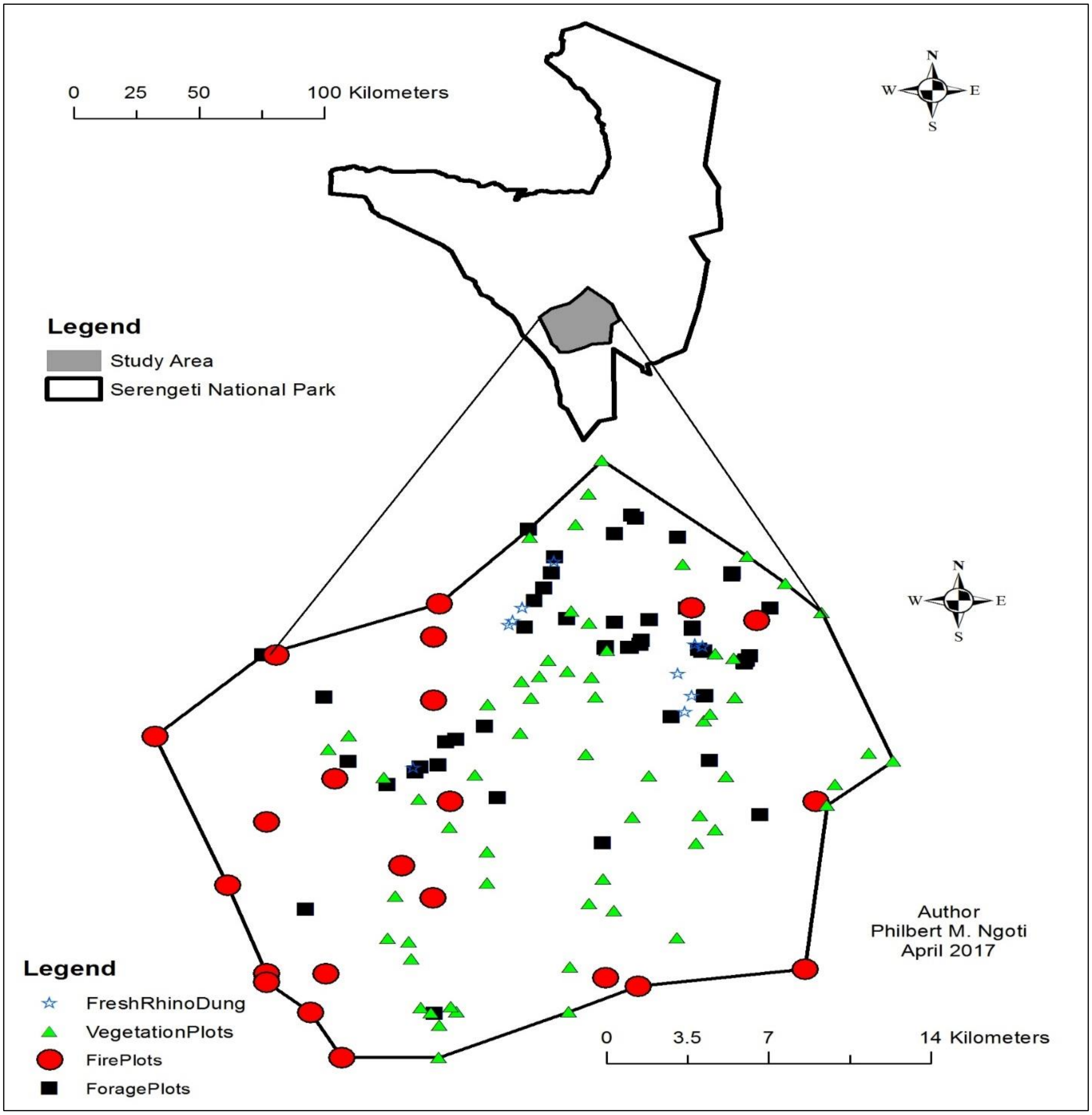

Figure 3: Map showing all sampling locations in the study area

Statistical analysis

All statistical analyses were performed in R version 1.0.136. Plant species utilization by rhinos was obtained by multiplying consumption (% browsed) of each plant species by the respective percent cover. Mean percent browsed (MPB>1%) and mean percent utilization (MPU>1%) were calculated in R for each rhino foraging plot sampled. Hereafter, the term occurrence is used to mean the number of plots across the landscape.

Indicator species analysis was conducted using the 'multipatt' command from the *indicspecies* R package to investigate if particular plant species were highly associated with the foraging locations selected by rhinos. The species by plot matrix for all plots sampled (rhino foraging, vegetation and fire plots) served as input data and plot type (either a rhino forage plot or not) as the cluster variable; the test statistic was selected with the func = "IndVal.g" option and 999 permutations were conducted to generate p-values for each species.

Each plant species that was established as being statistically associated with rhino foraging locations was then analyzed in relation to fire history using plant species distribution and abundance as response variables and fire history as a predictor. To analyze species distribution, the presence or absence of a species as a binomial response was used in two separate logistic regressions with fire frequency and time since last fire as predictors. To analyze species abundance, linear regression was used to assess the linear relationship between percent cover in plots in which the species was found versus fire frequency or time since last fire. False discovery rate by Benjamin Hochberg method was used to adjust the p-values for multiple comparisons.

For the analysis of rhino dung samples, the relative read abundance (RRA) denote the number of sequence of each plant species divided by the final number of sequence in that sample (Kartzinel et al., 2015). Frequency of species in the sample (FSS) will refer to number of times each plant species occurs across the 15 samples.

RESULTS

Plant species preference of black rhinos

In total, 133 plant species were recorded in all plot types. Of these, 56 species were recorded in the rhino foraging plots, and 97 species were recorded in the vegetation plots (*Appendix I*).

Indicator analysis identified 17 plant species that were significantly associated with the rhino foraging plots when compared to the vegetation and fire plots (*Table 1*).

A total of 9 plant species available in the rhino foraging plots were browsed with MPB >1%. These species comprised the forbs: *Crotalaria barkae*, *Justicia betonica*, *Indigofera basiflora*, *Achyranthes aspera*, and *Indigofera volkensii*; the shrubs: *Ziziphus abyssinica*, *Abutilon species*, and *Hibiscus species*; and a tree: *Acacia sieberiana* (*Figure 4, Appendix I*). All these species also had MPU>1% except the forb *Indigofera volkensii* (*Figure 4*).

Table 1: Plant species significantly associated in the rhino foraging plots

Species	Growth form	Test statistic	P value
Indigofera basiflora	Forb	0.772	0.001***
Bothriochloa insculpta	Grass	0.565	0.001***
Hibiscus species	Shrub	0.547	0.001***
Acacia sieberiana	Tree	0.525	0.001***
Dolichos trolobus	Forb	0.506	0.001***
Digitaria scalarum	Grass	0.433	0.001***
Sporobolus africanus	Grass	0.425	0.001***
Achyranthes aspera	Forb	0.411	0.001***
Indigofera volkensii	Forb	0.402	0.001***
Balanite aegyptica	Tree	0.347	0.001***
Acacia drepanoloboum	Tree	0.31	0.001***
Abutilon species	Shrub	0.301	0.001***
Cymbopogon caesius	Grass	0.284	0.038*
Crotalaria barkae	Forb	0.272	0.001***
Orthosiphon parvifolius	Forb	0.253	0.023*
Setaria varticillata	Grass	0.247	0.002**
Ziziphus abyssinica	Shrub	0.174	0.011*

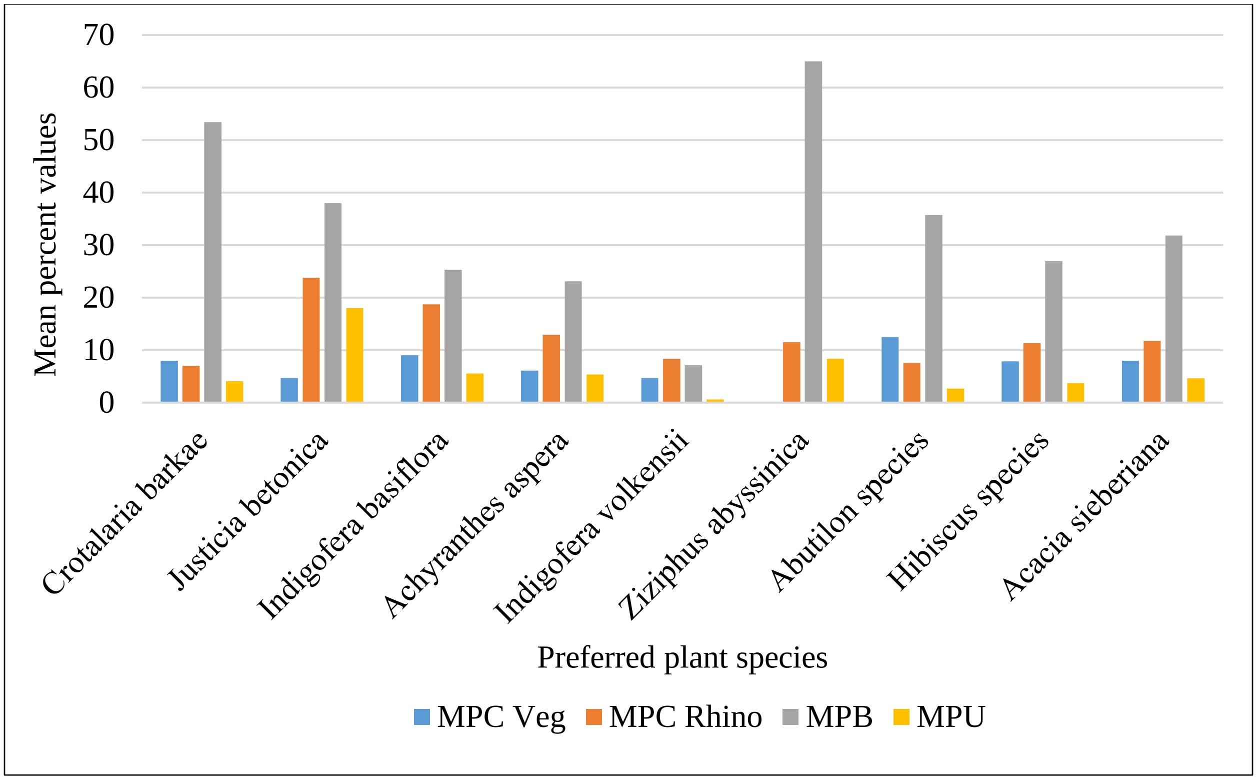

Figure 4: Mean percent values of the preferred plant species (first five forbs, followed by three shrubs, and lastly one tree) in the rhino foraging and vegetation plots; MPC Rhino – Mean percent cover in the rhino foraging plots; MPC Veg – Mean percent cover in the random vegetation plots; MPB – Mean percent browsed; MPU – Mean percent utilized

Plant species from the DNA analysis

A total of 72 taxa from 35 genera were present in the 15 rhino dung samples. Genera *Indigofera*, *Vachellia* and *Crotalaria* were more abundant in the samples. Several other taxa, including the genera *Solanum*, *Euphorbia*, *Achyranthes*, *Phyllanthus*, *Hibiscus*, *Neonotonia*, *Jucticia*, *Plumbago* and the PACMADE clade had plenty occurrences in at least 5 samples (FSS > 5) with relative read abundance > 1% (*Figure 5*, *Appendix II*). Seven genera from the rhino forage analysis overlaps with the DNA analysis results. One species (*Ziziphus abyssinica*) from the forage analysis was not found in the samples, while 28 genera from the rhino dung samples deviated from the rhino foraging analysis results (*Figure 5*, *Appendix II*).

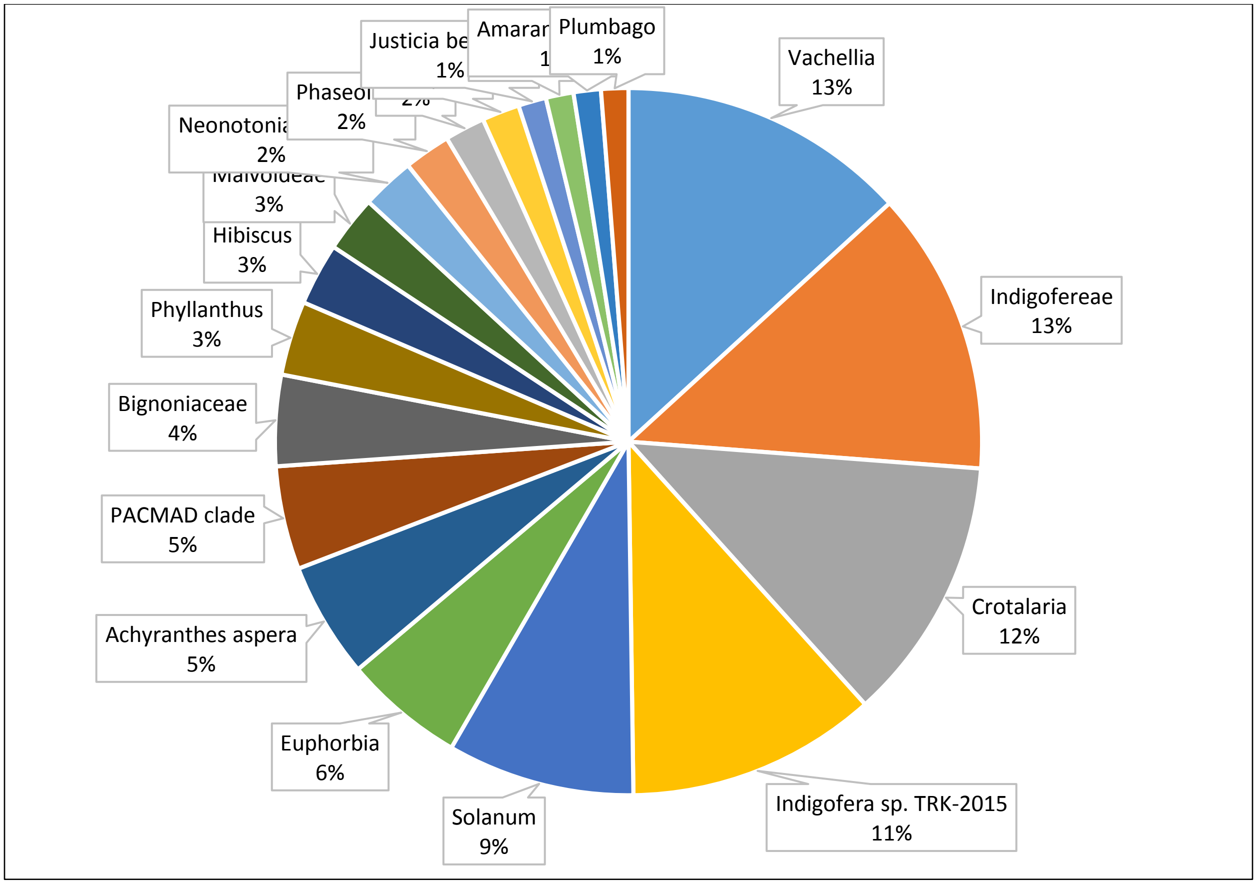

Figure 5: Relative read abundance of the 20 out of 72 taxa found in the fresh rhino dung by DNA metabarcoding method

Effects of fire on the preferred plant species

The presence of the forb *Justicia betonica* is significantly associated (slope = 0.2221 ± 0.065, p = 0.007; *Table 2*) with higher fire frequency, and its abundance increases across a fire frequency gradient (slope = 0.6273 ± 0.2453; p = 0.044). The forb *Indigofera basiflora* occurs significantly less in areas with higher fire frequency (slope = -0.13565 ± 0.0497, p = 0.033) but its abundance is not associated with fire frequency (slope = 0.2439 ± 0.4555, p = 0.667). The occurrence of the shrub *Solanum incanum* is unrelated to fire frequency (slope = -0.01445 ± 0.0449, p = 0.746), but in those locations where it occurs its abundance increases with fire frequency (slope = 0.5066 ± 0.1684, p = 0.028). The presence and abundance of remaining preferred plant species are not affected by fire frequency (*Table 2*). None of the preferred plant species are significantly affected by time since the areas have been lastly burned (data not shown).

Table 2: Statistical table for fire frequency from logistic regression for presence/absence and linear regression for abundance of the preferred plant species across fire frequency gradient

FIRE FREQUENCY			**Species presence versus absence (logistic regression)**				**Species abundance (linear regression)**			
Species	**Functional type**	**N**	**Estimate ± SE**	**Z**	**P**	**Ajusted P**	**Estimate ± SE**	**t**	**P**	**Adjusted P**
Solanum incanum	Forb	146	-0.0145 ± 0.0449	-0.323	0.746	0.821	0.5066 ± 0.1684	3.01	0.0031	0.028
Achyranthes aspera	Forb	37	0.1236 ± 0.0701	1.763	0.078	0.215	0.7505 ± 0.2839	2.64	0.0122	0.055
Justicia betonica	Forb	40	0.2221 ± 0.065	3.418	0.0006	0.007	0.6273 ± 0.2453	2.53	0.0146	0.044
Euphorbia inaequilatera	Forb	10	-0.1207 ± 0.1640	-0.736	0.461	0.724	-0.09376 ± 0.0733	-1.28	0.237	0.356
Indigofera basiflora	Forb	139	-0.1365 ± 0.0497	-2.746	0.006	0.033	0.2439 ± 0.4555	0.54	0.593	0.667
Crotalaria barkae	Forb	2	0.1533 ± 0.2747	0.558	0.577	0.793	NA	NA	NA	NA
Indigofera volkensii	Forb	56	-0.1687 ± 0.0752	-2.245	0.025	0.092	0.2307 ± 0.3382	0.68	0.498	0.640
Hibiscus species	Shrub	51	-0.0991 ± 0.0733	-1.352	0.176	0.323	-0.4533 ± 0.3280	-1.38	0.173	0.389
Abutilon species	Shrub	9	0.1842 ± 0.1284	1.434	0.151	0.332	0.8057 ± 0.5432	1.48	0.1815	0.327
Ziziphus abyssinica	Shrub	1	10.1837 ± 0.5487	-0.335	0.737	0.901	NA	NA	NA	NA
Acacia sieberiana	Tree	4	-0.0065 ± 0.2289	-0.029	0.977	0.977	0.1926 ± 0.7460	0.26	0.82	0.820

DISCUSSION

While seventeen species were characteristic of the rhino foraging plots, 9 of the species were clearly preferred by the rhinos. Rhinos were browsing more forbs than other plant functional groups and there was a good overlap in species and genera preferred by rhinos as per analyses with DNA in dung and by observations of foraging animals in the field. In addition to the field observed preferred species, the DNA analysis showed that species of the genera *Solanum* and *Euphorbia* are also important food for rhinos in the study area. Only three of the preferred plant species from the observational study seems to be sensitive to fire. *Justicia betonica* is positively influenced by fire frequency whereas *Indigofera basiflora* and *Solanum incanum* appear to have lesser occurrences and abundances in plots with higher fire frequency.

Rhino forage species

Black rhinos fed on forbs, shrubs and trees (*Figure 4*). However, they preferred forbs that form 56% of the preferred species in the rhino foraging plots, and 35% of the abundant species in the rhino foraging plots. This implies that rhinos consume species that are common in the rhino areas. This finding is unfamiliar and contrary to other studies in East and Southern Africa (D. H. Brown, 2008; Buk, 2004; Buk & Knight, 2010; Frame, 1980; Ganqa et al., 2005; Goddard, 1968, 1970; Mukinya, 1977; Oloo et al., 1994) which suggest shrubs to be the black rhino's preferred food.

The forb species *Indigofera basiflora*, *Crotalaria barkae* and *Achyranthes aspera* are more abundant in both rhino foraging plots and the genera of these species are also found well represented in the fresh rhino dungs analyzed (*Figure 5, Table 1, Appendix II*). Genera *Indigofera* and *Crotalaria* are more plenty in the rhino dungs forming major part of the diet. However, even though *Crotalaria barkae* is common in the rhino plots, it has low occurrence in plots across the landscape indicating that rhinos are actively searching for them.

Forbs *Indigofera basiflora*, *Justicia betonica* and genus *Euphorbia* are important preferred rhino species (*Figure 4, 5, Appendix I*). From the DNA study, genera *Indigofera* and *Euphorbia* are highly preferred and *Justicia betonica* is highly browsed when encountered in the rhino plots. Even though, the occurrence of *Justicia betonica* is low and that rhinos highly feed on them, then it seems that rhinos are actively searching for this species. It has been shown in Ngorongoro forest habitat, Tanzania that rhinos distinctly prefer *Justicia betonica* in the dry season (Goddard, 1968).

Shrubs *Hibiscus species*, *Ziziphus abyssinica*, *Abutilon species* and genus *Solanum* are also important food for black rhinos (*Figure 4, 5, Appendix I*). These species and the taxa are highly browsed from both observational and DNA studies. Despite its low occurrence, *Ziziphus abyssinica* is highly browsed once came across, indicating that rhinos are vigorously pursuing them on the landscape. *Ziziphus abyssinica* may be an important species for rhinos as it has been found in Augrabies Fall National Park in South Africa where (Buk, 2004) found *Ziziphus* to be among the 10 most important food plants that made up 88.4% of the rhino food. *Hibiscus species*, *Abutilon species* and *Solanum incanum* though plenty and common on the landscape, rhinos are also eating more of them showing their importance as diet.

The tree *Acacia sieberiana* has high occurrence in the rhino plots (*Appendix I*), and genus *Vachellia* (genus for *Acacia species*) occupy higher proportions in the fresh rhino dung (*Figure 5*). This implies that, rhinos consume more *Acacia sieberiana* and other *Acacia species* (*Figure 4, 5*) once encountered on the landscape. *Acacia species* have been found as one of the rhino food in the studies mentioned above.

The DNA and observation methods used in this study yield good outcomes that relatively complement each other. DNA study reveals higher species composition and greater species richness (*Figure 5, Appendix II*). All genera, except *Ziziphus*, from the observational study overlapped with those from the DNA study, whereas 28 genera from the DNA study (*Appendix II*) were not found in the observational study. Absence of these genera in the observational study could be due to that, rhinos were not seen feeding on species from these genera because rhinos feed on them at night (Estes, 1991; Mukinya, 1977). It could also be related to errors in identifying the plant species browsed by rhinos. DNA study reveals accurate relative proportions of plant species eaten. While *Indigofera basiflora* seems to be consumed in high quantities in the observational study, the DNA study discloses higher quantities of genus *Indigofera* in the rhino diet. Relatively, the DNA study is efficient and more robust as it has been found in studies (Kartzinel et al., 2015; Newmaster et al., 2013; Pompanon et al., 2012; Soininen et al., 2009; Willerslev et al., 2014) for herbivores diet assessment.

The impact of fire frequency on rhino forage plants

Presence and abundance of *Justicia betonica* increased by 22% and 63% respectively in the fire plots. Probably, frequent fires promote growth, take away dead tissues, and increase space (Anderson et al., 2007; Archibald & Hempson, 2016; Bond & Keeley, 2005) for *Justicia betonica* to enhance its abundance (Wangari, 2016) across the landscape. Furthermore, fire exhibits contrasting effects to the forb *Indigofera basiflora* and the shrub *Solanum incanum* (*Table 2*). Frequent fires reduce the presence of the forb by 14% and increase the abundance (Holdo et al., 2009; Wangari, 2016) of the shrub by 50%. Because higher fire frequency increases the abundances of *Justicia betonica* and *Solanum incanum*, then frequent fires are important for these two species. The study indicates the negative impact of fire on the important species *Indigofera basiflora*. Because this species makes big portion of the rhino food in both study methods, and that frequent fires reduce its occurrence, then fire harms *Indigofera basiflora* on the landscape. But, to test the importance of fire on this species, there is a need to make experimental studies with different fire treatments. There is however no evidence of the impact of frequent fires to other rhino preferred plant species.

CONCLUSION

This study finalizes that, black rhinos prefer forbs and that, *Indigofera basiflora*; *Crotalaria barkae* and *Achyranthes aspera* are more important food for rhino. Other important browse are *Hibiscus species*; *Acacia sieberiana*; *Indigofera volkensii*; *Justicia betonica*; *Ziziphus abyssinica*; *Abutilon species*, and species of the genera *Solanum* and *Euphorbia*. DNA metabarcoding method is an accurate and effective method for studying forage preference of black rhinos. Frequent fires positively influence *Justicia betonica* and *Solanum incanum* by increasing their cover, and reduce the occurrences of *Indigofera basiflora*. Other preferred species are not affected by fire frequency.

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APPENDICES

Appendix I: A table of abundance and percent occurrence between the rhino foraging plots, random vegetation plots and fire plots: N – number of observations/plots; MPC – mean percent cover, MPB – mean percent browsed.

SPECIES	GROWTH FORM	RHINO PLOTS N=64			VEGETATION PLOTS N=60 (300 plots)		FIRE PLOTS N=198	
		%OCCURRENCE	MPC	MPB	%OCCURRENCE	MPC	%OCCURRENCE	MPC
Abutilon species	Shrub	10.94	7.57	35.71	0.33	15.00	2.53	10.80
Acacia drepanolobium	Tree	12.50	14.38	0.00	2.33	10.57	2.53	7.00
Acacia gerrardii	Tree	0.00	0.00	0.00	0.00	0.00	1.01	8.50
Acacia kirkii	Tree	0.00	0.00	0.00	0.00	0.00	0.51	3.00
Acacia robusta	Tree	3.13	25.00	0.00	2.33	11.14	2.02	13.00
Acacia sieberiana	Tree	28.13	11.78	31.83	1.33	8.00	0.00	0.00
Acacia species	Tree	0.00	0.00	0.00	0.00	0.00	0.51	5.00
Acacia tortilis	Tree	0.00	0.00	0.00	1.33	7.75	3.54	15.29
Achyranthes aspera	Forb	20.31	12.92	23.08	6.33	6.11	8.59	7.82
Aeschynomene cristata	Forb	0.00	0.00	0.00	0.33	5.00	0.00	0.00
Albizia harveyi	Tree	0.00	0.00	0.00	1.00	4.00	3.03	8.33
Aristida adscensionis	Grass	0.00	0.00	0.00	0.00	0.00	0.51	5.00
Aristida congesta	Grass	0.00	0.00	0.00	6.67	5.45	0.00	0.00
Aristida kenyensis	Grass	0.00	0.00	0.00	0.00	0.00	0.51	3.00
Aristida species	Grass	0.00	0.00	0.00	0.67	11.00	0.00	0.00
Aspilia mossambicensis	Forb	1.56	5.00	0.00	2.33	14.00	1.01	17.50

SPECIES	GROWTH FORM	RHINO PLOTS N=64			VEGETATION PLOTS N=60 (300 plots)		FIRE PLOTS N=198	
		% OCCURRENCE	MPC	MPB	% OCCURRENCE	MPC	% OCCURRENCE	MPC
Balanites aegyptica	Tree	12.50	7.13	0.00	1.00	4.33	0.51	4.00
Barleria grandicalyx	Forb	0.00	0.00	0.00	0.00	0.00	2.02	5.75
Blepharis hildebrandtii	Forb	3.13	0.00	0.00	5.00	7.13	0.00	0.00
Blepharis linariifolia	Forb	0.00	5.50	0.00	0.00	0.00	0.00	0.00
Blepharis species	Forb	0.00	0.00	0.00	4.67	5.93	1.01	3.50
Boscia augustifolia	Tree	0.00	0.00	0.00	0.00	0.00	0.51	2.00
Bothriochloa insculpta	Grass	45.31	6.72	0.00	30.33	6.24	3.54	8.00
Bothriocline tomentosum	Shrub	0.00	0.00	0.00	5.67	5.76	0.00	0.00
Brachiaria brizantha	Grass	0.00	0.00	0.00	0.67	3.50	0.00	0.00
Brachiaria deflexa	Grass	0.00	0.00	0.00	0.33	3.00	0.00	0.00
Brachiaria eruciformis	Grass	0.00	0.00	0.00	0.33	3.00	0.00	0.00
Brachiaria serrata	Grass	0.00	0.00	0.00	0.33	5.00	0.00	0.00
Brachiaria species	Grass	0.00	0.00	0.00	0.67	1.50	0.00	0.00
Cassia fallacina	Forb	0.00	0.00	0.00	0.33	3.00	3.03	8.67
Cassia species	Forb	0.00	0.00	0.00	1.33	4.50	1.52	2.00
Chloris gayana	Grass	0.00	0.00	0.00	1.33	5.25	40.40	24.43
Chloris pycnothrix	Grass	18.75	8.75	0.00	46.33	14.83	1.52	11.67
Chloris roxburghiana	Grass	1.56	2.00	0.00	0.00	0.00	14.65	13.38
Chrysochloa orientalis	Grass	0.00	0.00	0.00	8.67	5.19	0.00	0.00
Commelina africana	Forb	1.56	5.00	0.00	0.33	7.00	1.52	9.67
Commelina benghalensis	Forb	6.25	4.00	0.00	4.67	2.21	5.05	4.10

SPECIES	GROWTH FORM	RHINO PLOTS N=64			VEGETATION PLOTS N=60 (300 plots)		FIRE PLOTS N=198	
		% OCCURRENCE	MPC	MPB	% OCCURRENCE	MPC	% OCCURRENCE	MPC
Commelina petersii	Forb	0.00	0.00	0.00	1.67	2.20	0.00	0.00
Commiphora africana	Tree	0.00	0.00	0.00	2.67	11.75	5.05	4.60
Conyza species	Forb	0.00	0.00	0.00	0.00	0.00	2.02	6.25
Crotalaria barkae	Forb	7.81	7.00	53.40	3.13	8.00	0.00	0.00
Crotalaria brevidens	Forb	7.81	0.00	0.00	0.67	8.00	0.00	0.00
Crotalaria rhizoclada	Forb	0.00	0.00	0.00	0.00	0.00	0.51	1.00
Cucumis prophetarum	Forb	0.00	0.00	0.00	0.67	2.50	0.00	0.00
Cymbopogon caesius	Grass	14.06	6.78	0.00	9.33	12.43	0.00	0.00
Cymbopogon prolixus	Grass	0.00	0.00	0.00	0.33	5.00	0.00	0.00
Cynodon dactylon	Grass	62.50	11.40	0.18	55.33	10.07	39.90	17.71
Cynodon nlemfuensis	Grass	0.00	0.00	0.00	0.00	0.00	3.03	18.83
Dactyloctenium aegyptium	Grass	0.00	0.00	0.00	1.33	3.50	1.52	6.33
Dactyloctenium australe	Grass	0.00	0.00	0.00	0.67	2.50	0.00	0.00
Dichrostachys cinerea	Tree	0.00	0.00	0.00	0.00	0.00	1.01	8.00
Digitaria longiflora	Grass	1.56	3.00	0.00	0.00	0.00	0.00	0.00
Digitaria macroblephara	Grass	1.56	10.00	0.00	7.00	7.81	12.12	11.13
Digitaria scalarum	Grass	9.00	10.47	0.13	0.00	11.15	0.51	7.00
Digitaria ternata	Grass	0.00	0.00	0.00	0.67	2.50	0.00	0.00
Dolichos oliveri	Forb	34.38	0.00	0.00	24.00	7.32	0.00	0.00
Dolichos trilobus	Forb	0.00	9.86	0.00	0.00	0.00	3.03	8.33
Dyschoriste radicans	Forb	0.00	0.00	0.00	0.00	0.00	1.01	1.50

SPECIES	GROWTH FORM	RHINO PLOTS N=64			VEGETATION PLOTS N=60 (300 plots)		FIRE PLOTS N=198	
		% OCCURRENCE	MPC	MPB	% OCCURRENCE	MPC	% OCCURRENCE	MPC
Enneapogon cenchroides	Grass	0.00	0.00	0.00	12.33	10.38	0.51	1.00
Eragrostis racemosa	Grass	0.00	0.00	0.00	5.67	6.29	1.52	2.33
Eragrostis species	Grass	0.00	0.00	0.00	1.00	6.00	33.33	0.00
Eragrostis tenuifolia	Grass	25.00	6.06	0.00	28.33	4.21	0.51	7.91
Euphorbia inaequilatera	Forb	0.00	4.00	0.00	0.00	0.00	0.00	0.00
Euphorbia inna	Forb	1.56	0.00	0.00	3.33	1.70	0.00	0.00
Eustachys paspaloides	Grass	10.94	6.29	0.00	23.67	7.89	0.00	0.00
Grewia fallax	Tree	0.00	0.00	0.00	0.00	0.00	0.00	3.00
Gutenbergia cordifolia	Forb	51.56	7.97	0.00	57.33	6.63	46.97	13.53
Gutenbergia petersii	Forb	0.00	0.00	0.00	0.67	7.50	1.52	7.67
Harpachne schimperi	Grass	0.00	0.00	0.00	2.00	3.50	0.51	2.00
Heliotropium steudneri	Forb	0.00	0.00	0.00	0.33	3.00	0.00	0.00
Heteropogon contortus	Grass	0.00	0.00	0.00	2.33	7.71	0.51	2.00
Hibiscus species	Forb	7.81	11.35	26.96	6.33	7.89	6.06	8.42
Hyparrhenia filipendula	Grass	0.00	0.00	0.00	1.00	8.33	0.00	0.00
Hyperthelia dissoluta	Grass	1.56	3.00	0.00	0.33	12.00	0.00	0.00
Indigofera basiflora	Forb	73.44	18.72	25.32	31.00	9.00	22.73	16.36
Indigofera bogdanii	Forb	0.00	0.00	0.00	0.33	5.00	0.00	0.00
Indigofera volkensii	Forb	21.88	8.36	7.14	14.00	4.67	7.07	9.14
Ipomea mombassana	Forb	0.00	0.00	0.00	0.00	0.00	0.51	10.00
Ipomea species	Forb	0.00	0.00	0.00	0.33	5.00	0.00	0.00

SPECIES	GROWTH FORM	RHINO PLOTS N=64			VEGETATION PLOTS N=60 (300 plots)		FIRE PLOTS N=198	
		% OCCURRENCE	MPC	MPB	% OCCURRENCE	MPC	% OCCURRENCE	MPC
Justica anselliana	Forb	0.00	0.00	0.00	0.33	15.00	0.00	0.00
Justicia betonica	Forb	7.81	23.80	38.00	6.00	4.67	10.10	7.80
Justicia matemensis	Forb	25.00	5.94	0.00	55.00	4.77	5.05	5.60
Justicia species	Forb	1.56	2.00	0.00	0.00	0.00	0.00	0.00
Kohautia aspera	Forb	1.56	2.00	0.00	0.00	0.00	0.00	0.00
Leucas deflexa	Forb	6.25	4.25	0.00	17.67	3.42	16.16	5.16
Lippia javanica	Shrub	3.13	7.50	0.00	1.33	4.50	0.51	36.00
Louditia pedicellata	Grass	0.00	0.00	0.00	2.33	8.29	0.00	0.00
Maerua parvifolius	Shrub	1.56	2.00	0.00	0.00	0.00	0.00	0.00
Maerua triphylla	Shrub	0.00	0.00	0.00	0.00	0.00	1.52	18.33
Melhania ovata	Forb	0.00	2.00	0.00	0.00	0.00	0.51	5.00
Melhania parviflora	Forb	1.56	0.00	0.00	3.67	4.27	0.00	0.00
Microchloa caffra	Grass	0.00	0.00	0.00	5.00	6.60	0.00	0.00
Ormocarpum trichocarpum	Shrub	0.00	0.00	0.00	0.33	5.00	1.01	14.00
Orthosiphon parvifolius	Forb	6.25	2.83	0.00	2.67	5.63	0.51	2.00
Orthosiphon rubicundulus	Forb	3.13	0.00	0.00	0.33	15.00	0.00	0.00
Orthosiphon species	Forb	0.00	0.00	0.00	0.00	0.00	1.52	2.67
Panicum atrosanguineum	Grass	0.00	0.00	0.00	0.00	0.00	1.01	1.50
Panicum coloratum	Grass	0.00	0.00	0.00	0.00	0.00	16.16	8.44
Panicum maximum	Grass	0.00	0.00	0.00	0.67	4.00	5.05	8.40
Pennisetum mezianum	Grass	76.56	13.16	0.43	75.00	13.96	73.74	25.05

SPECIES	GROWTH FORM	RHINO PLOTS N=64			VEGETATION PLOTS N=60 (300 plots)		FIRE PLOTS N=198	
		% OCCURRENCE	MPC	MPB	% OCCURRENCE	MPC	% OCCURRENCE	MPC
Pennisetum stramineum	Grass	0.00	0.00	0.00	0.00	0.00	0.51	15.00
Persicaria setosula	Forb	0.00	23.00	0.00	0.00	0.00	0.00	0.00
Plectranthus caninus	Forb	0.00	0.00	0.00	0.00	0.00	0.51	1.00
Plectranthus lanuginosus	Shrub	0.00	0.00	0.00	0.67	2.00	0.00	0.00
Polygonum setulosum	Forb	3.13	0.00	0.00	1.67	9.40	0.00	0.00
Portulaca foliosa	Succulent	0.00	0.00	0.00	0.00	0.00	1.01	6.00
Portulaca kermesina	Forb	0.00	0.00	0.00	0.33	1.00	0.00	0.00
Psilotrichum elliottii	Forb	0.00	0.00	0.00	0.00	0.00	0.51	3.00
Sedge species	Sedge	0.00	0.00	0.00	0.33	3.00	0.00	0.00
Setaria pumila	Grass	1.56	1.00	0.00	3.33	5.90	0.00	0.00
Setaria sphacelata	Grass	1.56	5.00	0.00	6.33	4.74	0.51	6.00
Setaria verticillata	Grass	6.25	8.00	0.00	0.67	3.00	0.00	0.00
Sida cuneifolia	Forb	4.69	3.33	0.00	11.00	4.24	13.64	6.19
Sida ovata	Shrub	0.00	0.00	0.00	0.00	0.00	0.51	3.00
Solanum incanum	Shrub	40.63	5.85	0.00	28.00	4.79	28.28	7.77
Sonchus oleraceaus	Forb	1.56	0.00	0.00	1.33	2.75	0.00	0.00
Sonchus species	Forb	1.56	4.00	0.00	0.00	0.00	0.00	0.00
Sporobolus africanus	Grass	23.44	4.73	0.00	11.67	4.69	0.00	0.00
Sporobolus festivus	Grass	0.00	0.00	0.00	8.00	9.42	0.00	0.00
Sporobolus fimbriatus	Grass	3.13	9.00	0.00	3.33	9.30	1.52	9.00
Sporobolus ioclados	Grass	34.38	3.91	0.00	28.67	5.31	7.07	12.36
Sporobolus panicoides	Grass	0.00	0.00	0.00	0.33	3.00	0.00	0.00

SPECIES	GROWTH FROM	RHINO PLOTS N=64			VEGETATION PLOTS N=60 (300 plots)		FIRE PLOTS N=198	
		% OCCURRENCE	MPC	MPB	% OCCURRENCE	MPC	% OCCURRENCE	MPC
Sporobolus pellucidus	Grass	0.00	0.00	0.00	4.33	13.31	5.56	7.91
Sporobolus pyramidalis	Grass	6.25	4.25	0.00	3.00	3.00	10.61	7.62
Sporobolus species	Grass	1.56	15.00	0.00	0.00	0.00	0.00	0.00
Sporobolus stapfianus	Grass	1.56	5.00	0.00	0.33	5.00	2.02	28.75
Tephrosia pumila	Forb	4.69	4.33	0.00	4.00	6.08	0.00	0.00
Themeda triandra	Grass	73.44	9.85	0.02	64.67	10.30	50.51	15.72
Vernonia glabra	Forb	0.00	0.00	0.00	1.33	12.00	0.00	0.00
Vernonia myriantha	Forb	1.56	0.00	0.00	0.67	3.50	0.00	0.00
Vernonia species	Forb	0.00	20.00	0.00	0.33	1.00	0.00	0.00
Vigna oblongifolium	Forb	9.38	8.00	0.00	2.33	12.43	25.76	6.69
Ziziphus abyssinica	Shrub	3.13	11.50	65.00	0.00	0.00	0.51	5.00

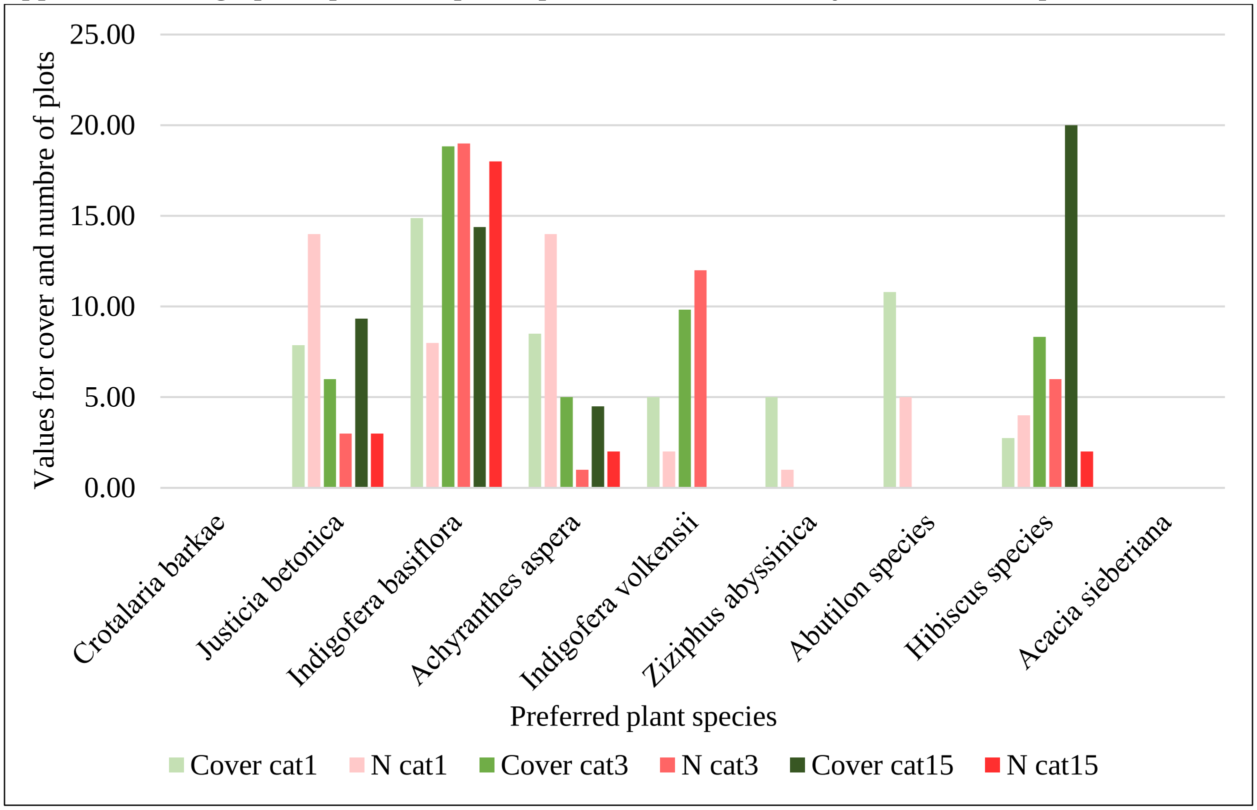
Appendix II: A detailed table of plant species from the DNA metabarcoding analysis of the fresh rhino dung: FSS – frequency of sequence in the sample; RRA – relative read abundance.

Order	Family	Genus	Taxon	FSS	RRA
Fabales	Fabaceae	Vachellia	Vachellia	13	1.695
Fabales	Fabaceae	NA	Indigofereae	12	1.666
Fabales	Fabaceae	Crotalaria	Crotalaria	4	1.553
Fabales	Fabaceae	Indigofera	Indigofera sp. TRK-2015	15	1.468
Solanales	Solanaceae	Solanum	Solanum	14	1.099
Malpighiales	Euphorbiaceae	Euphorbia	Euphorbia	5	0.707
Caryophyllales	Amaranthaceae	Achyranthes	Achyranthes aspera	15	0.678
Poales	Poaceae	NA	PACMAD clade	13	0.607
Lamiales	Bignoniaceae	NA	Bignoniaceae	8	0.533
Malpighiales	Phyllanthaceae	Phyllanthus	Phyllanthus	8	0.436
Malvales	Malvaceae	Hibiscus	Hibiscus	10	0.364

Order	Family	Genus	Taxon	FSS	RRA
Malvales	Malvaceae	NA	Malvoideae	8	0.329
Fabales	Fabaceae	Neonotonia	Neonotonia wightii	11	0.310
Fabales	Fabaceae	NA	Phaseoleae	11	0.274
Boraginales	Cordiaceae	Cordia	Cordia	7	0.236
Lamiales	Lamiaceae	NA	Lamiaceae	7	0.220
Lamiales	Acanthaceae	Justicia	Justicia betonica	6	0.164
Fabales	Fabaceae	NA	Fabaceae	9	0.162
Caryophyllales	Amaranthaceae	NA	Amaranthaceae	8	0.160
Caryophyllales	Plumbaginaceae	Plumbago	Plumbago	5	0.160
Lamiales	Acanthaceae	Dicliptera	Dicliptera magaliesbergensis	6	0.148
Malvales	Malvaceae	Abutilon	Abutilon mauritianum	9	0.141
Malvales	Malvaceae	Pterospermum	Pterospermum heterophyllum	7	0.123
Gentianales	Apocynaceae	NA	Asclepiadoideae	5	0.119
Fabales	Fabaceae	NA	Mimosoideae	5	0.119
Lamiales	Oleaceae	Jasminum	Jasminum	8	0.117
Commelinales	Commelinaceae	Commelina	Commelina erecta	4	0.117
Cucurbitales	Cucurbitaceae	NA	Cucurbitaceae	8	0.103
Celastrales	Celastraceae	NA	Celastraceae	5	0.103
Solanales	Convolvulaceae	Ipomoea	Ipomoea cairica	7	0.100
Malvales	Malvaceae	Grewia	Grewia sp. Mada141	2	0.090
Lamiales	Verbenaceae	NA	Lantaneae	6	0.078
Sapindales	Sapindaceae	NA	Sapindaceae	4	0.069
Fabales	Fabaceae	Glycyrrhiza	Glycyrrhiza	5	0.061
Lamiales	Acanthaceae	Hypoestes	Hypoestes	6	0.061
Poales	Poaceae	Cenchrus	Cenchrus	4	0.060
Caryophyllales	Amaranthaceae	Achyropsis	Achyropsis avicularis	8	0.049
Poales	Poaceae	NA	Paniceae	4	0.044
Malvales	Malvaceae	Grewia	Grewia	2	0.039
Asterales	Asteraceae	NA	Asteraceae	5	0.039
Brassicales	Capparaceae	Cadaba	Cadaba	3	0.031
Lamiales	Acanthaceae	Justicia	Justicia debilis	2	0.031
Boraginales	Ehretiaceae	NA	Ehretiaceae	3	0.031
Lamiales	Acanthaceae	NA	Ruellieae	1	0.029
Fabales	Fabaceae	NA	Papilionoideae	5	0.028
Fabales	Fabaceae	Glycine	Soja	1	0.024
Fabales	Fabaceae	NA	Caesalpinieae	1	0.018
Poales	Poaceae	Themeda	Themeda	2	0.017

Order	Family	Genus	Taxon	FSS	RRA
Sapindales	Urticaceae	NA	Anacardiaceae	2	0.015
Caryophyllales	Polygonaceae	NA	Polygonoideae	2	0.014
Malvales	Malvaceae	Sida	Sida sp. TRK-2015	3	0.013
Fagales	Betulaceae	Alnus	Alnus	1	0.013
Sapindales	Burseraceae	NA	Burseraceae	1	0.013
Fabales	Fabaceae	NA	Desmodieae	3	0.011
Lamiales	Acanthaceae	Dyschoriste	Dyschoriste radicans	1	0.011
Gentianales	Rubiaceae	Gardenia	Gardenia volkensii	2	0.011
Poales	Poaceae	NA	Poeae Chloroplast Group 2 (Poeae type)	1	0.010
Rosales	Urticaceae	Urtica	Urtica	1	0.009
Asterales	Asteraceae	NA	Asteroideae	1	0.008
Caryophyllales	Aizoaceae	Zaleya	Zaleya	1	0.007
Lamiales	Lamiaceae	NA	Ocimeae	1	0.007
Malpighiales	Euphorbiaceae	Acalypha	Acalypha	2	0.007
Asparagales	Xanthorrhoeaceae	Aloe	Aloe	1	0.006
Fabales	Fabaceae	NA	Dalbergieae	2	0.005
Gentianales	Rubiaceae	NA	Rubiaceae	1	0.005
Fabales	Fabaceae	NA	Acacieae	1	0.004
Malvales	Malvaceae	Sida	Sida tenuicarpa	1	0.004
Apiales	Apiaceae	NA	Apioideae	1	0.003
Lamiales	Verbenaceae	Priva	Priva curtisiae	1	0.003
Solanales	Convolvulaceae	NA	Convolvulaceae	1	0.003
Solanales	Convolvulaceae	NA	Ipomoeeae	1	0.003
Poales	Poaceae	NA	Pooideae	1	0.003

Appendix III: A graph of preferred plant species in the fire history on the landscape



Mean percent cover and number of plots of the preferred plant species in 1, 3 and 15 years fire categories; Cover – mean percent cover; N – number of plots; cat – Fire category