

INVESTIGATING BLACK RHINOCEROS (*DICEROS BICORNIS*) ECOLOGY TOWARDS IMPROVED CONSERVATION AND MANAGEMENT PRACTICES

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Investigating black rhinoceros (*Diceros bicornis*) ecology towards improved conservation and management practices

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“Do not speak of a rhinoceros if there is no tree nearby.”

-African proverb

Abstract

Savanna systems are facing growing pressures exerted by climate change, ecosystem degradation and the ongoing decline in biodiversity. Particularly, the decline of endangered charismatic megafauna such as large herbivores, has several consequences for such ecosystems. These include impacts such as weakened community stability, modification of food-web dynamics and alteration of nutrient cycles. Often considered as keystone species, large herbivores, exercise considerable influence on whole communities through habitat modification, resource partitioning, and competition. Despite their significant ecological importance and their threatened status, large herbivores have been relatively under-researched due to the inherent challenges associated with studying them, and are now mostly restricted to protected areas. Black rhinoceros (*Diceros bicornis* L.) are among the most endangered large herbivore species on Earth and have received limited scientific attention. The strategy for the recovery of black rhino involves restocking historical ranges with surplus animals from protected areas, as populations reach carrying capacity. This implies promoting population growth in current ranges, and selecting suitable habitats for translocations. Challenges lie not only in identifying factors linked to habitat suitability and maximised growth rate but also in integrating changing conditions, such as shifts in vegetation composition due to climate change and the influence of sympatric herbivores. Population growth is intricately connected not only to environmental abiotic and biotic conditions but also to management actions. Hence research on the species is critical in furthering our ecological knowledge and to ascertain whether management actions are having their desired effect in terms of reaching conservation targets.

This thesis aims at increasing unambiguous knowledge of black rhino ecology, practical to its management and that of its environment, through a contemporary approach with the integration of monitoring techniques and modern methodologies, and three important scopes: space use, foraging and interactions. This work is based on several black rhino populations situated in reserves across KwaZulu-Natal, South Africa, in particular that of Ithala Game Reserve.

In **Chapter I**, I investigate black rhino forage and habitat selection across multiple spatial scales. Knowing that forage selection is an important component linked to herbivore spatial

distribution, I describe forage selection by the means of direct-observation transects on feeding paths and subsequent habitat selection based on monitoring data and ecosystem productivity (NDVI). I compare the nutritional and chemical composition of preferred and avoided plant species through metabolomics (LC-MS) and elemental analysis (CHN). I show that black rhinos' spatial distribution was negatively associated with ecosystem productivity, but positively associated with specific vegetation types that contain highly preferred, chemically distinct, plant species. Black rhinos thus occupy their habitat across space and time through selective foraging on preferred plants.

In **Chapter II**, because understanding interspecific herbivore dynamics within protected areas is crucial for their effective management, using DNA metabarcoding, I investigate resource partitioning between black rhino and three abundant sympatric herbivores, elephant, kudu and impala. While broad categories of foraging strategies can be used to explain coexistence, fine-grained information on seasonal foraging is needed to precisely assess resource partitioning. I describe seasonal diet composition and overlap; and compare foraging strategies between the four herbivores. I quantify the potential encroachment on black rhino dietary niche in Ithala Game Reserve. I found that diet composition and overlap shifted seasonally, where resource scarcity during the dry season generated a more even composition and reduced overlap of diets. Mesoherbivores encroached more on black rhinos than elephants did. In an environment more suited to browsers, the mixed feeders, elephant and impala, maintained nearly solely browsing through the year. This chapter shows that long-standing broad categories of foraging strategies and body size are limited in their use in protected areas and that seasonal strategies are central to managing increasingly threatened populations.

In **Chapter III**, I investigate potential variables driving black rhino forage selection. Foraging behaviour is governed by decisions at various scales and shaped by the perception of morphological and physiological properties of plants. Pre-ingestive cues allow differentiating and choosing between food items. I thus compare the traits and volatile organic compounds (VOCs, through GC-MS) of preferred and avoided plant species determined by feeding-path transects, and examine their relative importance in determining preference or avoidance. This chapter suggests that both morphological and olfactory cues are important for black rhino forage selection. Discriminant volatiles such as Caryophyllene and Hexenol acetate were found to be important across seasons but volatiles alone were not as robust in explaining choice of forage, particularly in the growing season. This chapter provides the first steps to disentangling

factors driving black rhino choice and potential applications to conservation management. Considering the ability to utilise plant odour and morphological cues will aid models pertaining to both the foraging behaviour of black rhino and the ecosystem consequences resulting from their foraging activities.

In **Chapter IV**, I measure the impact of dehorning on black rhino home ranges and the efficacy of dehorning as a poaching deterrent. Because poaching for horns is the biggest threat to black rhino, by proactively dehorning entire rhinoceros populations, conservationists aim to deter poaching and prevent species loss. However, such conservation interventions may have hidden and underestimated effects on animals' behaviour and ecology. Here, I use long-term monitoring data to estimate home ranges before and after dehorning, and trends in dehorning and mortalities. I estimate the effect of such a tool on social interactions based on home-range overlap. While preventative dehorning at these reserves coincided with a nationwide decrease in black rhino mortality from poaching and did not infer increased natural mortality, dehorned black rhinos decreased their home range area and were less likely to engage in social encounters. Dehorning black rhinos as an anti-poaching measure alters their behavioural ecology, although the potential population-level effects of these changes remain to be determined.

In conclusion, this thesis suggests that the black rhino is an intricate species that demonstrates behavioural plasticity to changing ecosystem conditions and management interventions. The ongoing evaluation and adaption of management strategies and consistent monitoring are crucial for ensuring effective conservation efforts. Behavioural ecology, such as space and resource utilisation, can serve as early indicators of concealed consequences and facilitate adaptive management for large herbivores. This thesis furthers our understanding of a critically endangered large herbivore and highlights the need for continued research.

Keywords

Diceros bicornis, foraging ecology, habitat use, DNA metabarcoding, metabolomics, dehorning, home ranges, niche partitioning.

Résumé

Les écosystèmes de savane font face à des pressions croissantes exercées par le changement climatique, la dégradation des écosystèmes et le déclin continu de la biodiversité. En particulier, le déclin des mégafaunes charismatiques en danger telles que les grands herbivores a plusieurs conséquences pour de tels écosystèmes. Celles-ci incluent des impacts tels qu'une stabilité communautaire affaiblie, la modification de la dynamique des chaînes alimentaires et l'altération des cycles des nutriments. Souvent considérés comme des espèces clés, les grands herbivores exercent une influence considérable sur l'ensemble des communautés par le biais de la modification de l'habitat, du partage des ressources et de la compétition. Malgré leur importance écologique et leur statut menacé, les grands herbivores ont été relativement peu étudiés en raison des défis inhérents liés à leur étude, et se trouvent aujourd'hui uniquement dans des zones protégées. Le rhinocéros noir (*Diceros bicornis* L.) est l'une des espèces de grands herbivores les plus en dangers et a reçu une attention scientifique limitée. La stratégie de rétablissement du rhinocéros noir implique de réintroduire des individus des zones protégées en surplus dans leurs aires historiques, à mesure que les populations atteignent leur capacité de charge. Cela implique de favoriser la croissance des populations dans les aires actuelles et de sélectionner des habitats appropriés pour les translocations. Les défis résident non seulement dans l'identification des facteurs liés à la convenance de l'habitat et au taux de croissance maximisé, mais aussi dans l'intégration des conditions changeantes, telles que les changements dans la composition de la végétation dus au changement climatique et l'influence des herbivores sympatriques. La croissance des populations est étroitement liée non seulement aux conditions abiotiques et biotiques de l'environnement, mais aussi aux actions de gestion. Par conséquent, la recherche sur l'espèce est essentielle pour approfondir nos connaissances écologiques et déterminer si les actions de gestion ont l'effet souhaité en termes d'atteinte des objectifs de conservation.

Cette thèse vise à accroître les connaissances univoques de l'écologie du rhinocéros noir, utiles à sa gestion et à celle de son environnement, grâce à une approche contemporaine intégrant des techniques de surveillance et des méthodologies modernes, et trois domaines importants : l'utilisation de l'espace, l'alimentation et les interactions. Ce travail est basé sur plusieurs populations de rhinocéros noirs situées dans des réserves à travers le KwaZulu-Natal, en Afrique du Sud, en particulier celle de Ithala Game Reserve.

Dans le **Chapitre I**, j'étudie l'alimentation et la sélection de l'habitat du rhinocéros noir à travers plusieurs échelles spatiales. En sachant que la sélection de la nourriture est un élément important lié à la distribution spatiale des herbivores, je décris la sélection de la nourriture au moyen de transects d'observation directe sur les sentiers d'alimentation, puis la sélection de l'habitat en fonction des données de monitoring et de la productivité des écosystèmes (NDVI). Je compare la composition nutritionnelle et chimique des espèces végétales préférées et évitées par le biais de la métabolomique (LC-MS) et de l'analyse élémentaire (CHN). Je montre que la distribution spatiale des rhinocéros noirs était négativement associée à la productivité des écosystèmes, mais positivement associée à des types de végétation spécifiques contenant des espèces végétales hautement préférées et chimiquement distinctes. Les rhinocéros noirs occupent donc leur habitat à travers l'espace et le temps en se nourrissant sélectivement de plantes préférées.

Dans le **Chapitre II**, parce que la compréhension des dynamiques interspécifiques des herbivores au sein des zones protégées est cruciale pour leur gestion efficace, le DNA-metabarcoding a été employé pour étudier le partage des ressources entre le rhinocéros noir et trois herbivores sympatriques abondants, l'éléphant, le koudou et l'impala. Bien que des catégories larges de stratégies d'alimentation puissent être utilisées pour expliquer la coexistence, des informations détaillées sur l'alimentation saisonnière sont nécessaires pour évaluer précisément le partage des ressources. Je décris la composition de l'alimentation saisonnière et le chevauchement, et je compare les stratégies d'alimentation entre les quatre herbivores. Je quantifie l'empiétement potentiel sur la niche alimentaire du rhinocéros noir dans Ithala Game Reserve. Je démontre que la composition de l'alimentation et le chevauchement variaient au fil des saisons, la rareté des ressources pendant la saison sèche générant une composition plus uniforme et une réduction du chevauchement des régimes alimentaires. Les mésoherbivores empiétaient davantage sur les rhinocéros noirs que ne le faisaient les éléphants. Dans un environnement plus propice aux brouteurs, les animaux à alimentation mixte, l'éléphant et l'impala, se sont maintenus principalement au broutage tout au long de l'année. Ce chapitre montre que les catégories larges de stratégies d'alimentation et de taille corporelle sont limitées dans leur utilisation dans les zones protégées et que les stratégies saisonnières sont essentielles pour gérer des populations de plus en plus menacées.

Dans le **Chapitre III**, j'étudie les variables potentielles qui influencent la sélection de la nourriture chez le rhinocéros noir. Le comportement alimentaire est régi par des décisions à différentes échelles et façonné par la perception des propriétés morphologiques et physiologiques des plantes. Les signaux pré-ingestifs permettent de différencier et de choisir entre les éléments nutritifs. Ainsi, je compare les caractéristiques et les composés organiques volatils (VOCs, par GC-MS) des espèces végétales préférées et évitées déterminées par des transects de suivi d'alimentation, et j'examine leur importance relative dans la détermination de

la préférence ou de l'évitement. Ce chapitre suggère que les signaux à la fois morphologiques et olfactifs sont importants pour la sélection de la nourriture par le rhinocéros noir. Des VOCs discriminants tels que le caryophyllène et l'acétate d'hexénol se sont révélés importants tout au long des saisons, mais les VOCs seuls ne sont pas aussi robustes pour expliquer le choix de la nourriture, notamment pendant la saison de croissance. Ce chapitre marque les premiers pas dans le déchiffrement des facteurs qui influent sur le choix du rhinocéros noir et dans les applications potentielles à la gestion de la conservation. Tenir compte de la capacité à utiliser l'odeur des plantes et des indices morphologiques bénéficieront les modèles liés au comportement alimentaire du rhinocéros noir ainsi qu'aux conséquences pour l'écosystème découlant de ses activités alimentaires.

Dans le **Chapitre IV**, je mesure l'impact du décornage sur les aires de répartition du rhinocéros noir et l'efficacité du décornage en tant que dissuasion contre le braconnage. Etant donné que le braconnage pour les cornes est la plus grande menace pour le rhinocéros noir, en décornant de manière proactive l'ensemble des populations de rhinocéros, les praticiens visent à dissuader le braconnage et à prévenir la perte d'espèces. Cependant, de telles interventions de conservation peuvent avoir des effets cachés et sous-estimés sur le comportement et l'écologie des animaux. Ici, j'utilise des données de surveillance à long terme pour estimer les aires de répartition avant et après le décornage, ainsi que les tendances en matière de décornage et de mortalité. J'estime l'effet d'un tel outil sur les interactions sociales en fonction du chevauchement des aires de répartition. Bien que le décornage préventif dans ces réserves ait coïncidé avec une diminution nationale de la mortalité du rhinocéros noir due au braconnage et n'ait pas entraîné une augmentation de la mortalité naturelle, les rhinocéros noirs décornés ont réduit leur aire de répartition et étaient moins enclins à participer à des interactions sociales. Le décornage des rhinocéros noirs en tant que mesure anti-braconnage modifie leur écologie comportementale, bien que les effets potentiels au niveau de la population de ces changements restent à déterminer.

En conclusion, cette thèse suggère que le rhinocéros noir est une espèce complexe qui fait preuve de plasticité comportementale en réponse aux conditions changeantes de l'écosystème et aux interventions de gestion. L'évaluation continue et l'adaptation des stratégies de gestion ainsi qu'une surveillance cohérente sont cruciales pour assurer des efforts de conservation efficaces. L'écologie comportementale, telle que l'utilisation de l'espace et des ressources, peut servir d'indicateurs précoces de conséquences cachées et faciliter la gestion adaptative des grands herbivores. Cette thèse approfondit notre compréhension d'un grand herbivore en danger critique d'extinction et souligne la nécessité de poursuivre la recherche.

Mots-clés

Diceros bicornis, écologie alimentaire, utilisation spatiale, DNA metabarcoding, métabolomique, décornage, territoires, partition de niche.

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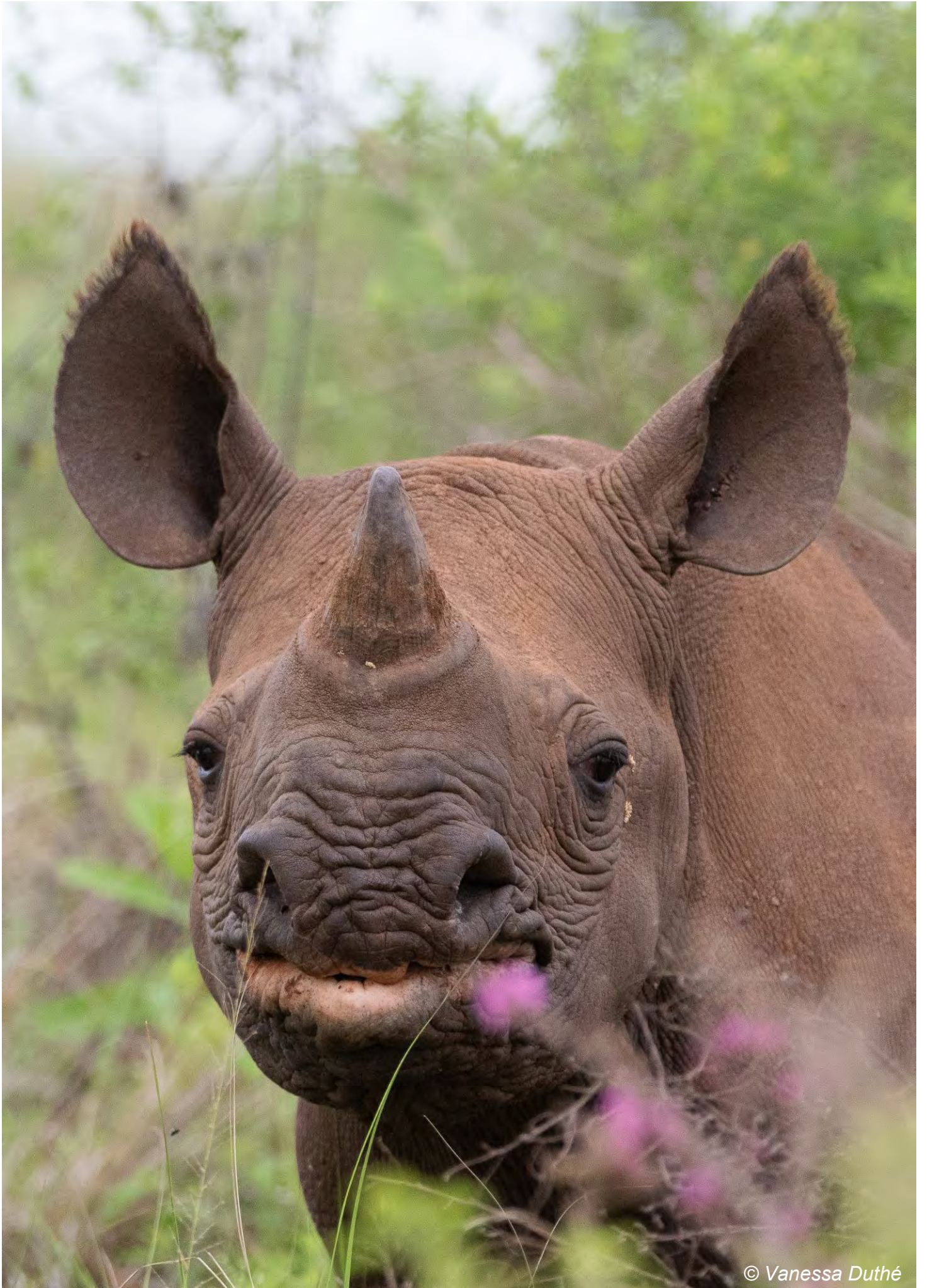
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General Introduction

General Introduction

The ongoing decline in biodiversity and particularly of endangered charismatic megafauna, such as primates, carnivores, and large herbivores (Biggs *et al.*, 2013), has several consequences for ecosystems (Dirzo *et al.*, 2014; Pringle *et al.*, 2023). These include impacts such as weakened community stability, modification of food-web dynamics (Owen-Smith, 1992; Landman, Schoeman & Kerley, 2013; Perino *et al.*, 2019) and alteration of nutrient cycles (Ripple *et al.*, 2015). It is no secret that large herbivores play an important role in regulating terrestrial ecosystems (Pringle *et al.*, 2023). Often considered as keystone species (Pringle *et al.*, 2023), large herbivores, can have vast impacts on whole communities through habitat modification, resource partitioning, and competition (Landman *et al.*, 2013; Ripple *et al.*, 2015). It is estimated that 50% of wild terrestrial mammal biomass is represented by Ungulates which mostly consist of large herbivores with hooves, even-toed (Artiodactyla) and odd-toed (Perissodactyla) (Greenspoon *et al.*, 2023). Despite their significant ecological importance and biomass, large herbivores have been relatively under-researched due to the inherent challenges associated with studying them (Staver & Hempson, 2020; Pringle *et al.*, 2023) and are now mostly restricted to protected areas (Staver & Hempson, 2020). What is more and intensifying the need for immediate action, large herbivores are disproportionately susceptible to extinction, where 60% of mammalian herbivores are threatened, and in particular all 12 species of megaherbivores (Pringle *et al.*, 2023).

The black rhinoceros is no exception. Black rhinoceros (*Diceros bicornis* L.), here after referred to as black rhino, are among the most endangered megaherbivore species on the planet. Listed as critically endangered (IUCN, 2020), the worldwide population since 1960 has declined by an estimated 98% (Emslie & Adcock, 2016). Extensive hunting of the species as well as loss of habitat by clearance of land for settlement, led to a rapid decline and near extinction (Emslie & Adcock, 2016). While the primary threat to the species remains the high demand for rhino horn and associated poaching (Biggs *et al.*, 2013), it is not the sole peril they face. The insufficient availability of suitable habitats is a crucial factor contributing to their endangered status and a threat to survival (Emslie & Adcock, 2016). In this day and age, wild black rhino populations are limited within the bounds of protected areas, which are often fenced and thus limit dispersal (Landman *et al.*, 2013). While conventional restoration projects typically seek

to limit human intervention, a certain degree of management is essential to restore ecosystem processes that have been lost due to human activities (Perino *et al.*, 2019). In this regard, the number of black rhinos within protected areas needs to be managed to remain just under carrying capacity in an effort to maintain and promote population growth (Emslie & Brooks, 1999). This strategy requires selecting animals from among populations which are thriving and translocating them to restock historical ranges that have potential to sustain new populations (Tatman, Stevens-Wood & Smith, 2001; Linklater *et al.*, 2012).

Apart from security concerns, the main challenge for successful translocation plans resides in finding habitats that have all components for maximizing the species growth rate (Odendaal-Holmes, Marshal & Parrini, 2014; Balfour *et al.*, 2019). In characterizing suitable habitats and subsequently, estimating a habitats' potential carrying capacity, many factors are essential to incorporate, including climatic conditions and seasonal variation, substrate fertility, forage selectivity and productivity, and topography (Adcock, 2001). The challenge lies not only in describing these factors but also in integrating changing conditions, such as shifts in vegetation composition due to climate change, the influence of sympatric herbivores, and the spread of invasive species (Owen-Smith, 2002). Previous works have demonstrated that carrying capacity models should be used with caution when determining optimal population numbers for an area, as individual habitat selection of black rhinos were not correlated with predicted carrying capacity values (Morgan, Mackey & Slotow, 2009). For instance, it was suggested that Hluhluwe-IMfolozi Park, also one of the largest populations of black rhino, could be using an ecological carrying capacity of black rhino that is incorrect and as a result of removals, the population could be performing poorly (Nhleko, Parker & Druce, 2017). Population growth is intricately connected not only to environmental abiotic and biotic conditions but also to management actions. Because large herbivores are keystone species and have a high public profile, they are frequently employed as flagship species in conservation management planning (Gordon, Hester & Festa-Bianchet, 2004). Hence research on the species is a priority to ascertain whether management actions are having their desired effect in terms of reaching conservation targets (Ezemvelo KZN Wildlife, 2009). Furthermore, conservation management often consists of an interdisciplinarity approach, where ecological knowledge is sometimes either imperfect or overlooked to the benefit of social, political and economic factors (Mills *et al.*, 2006). It is thus important to not only further ecological knowledge but also to assess management actions.

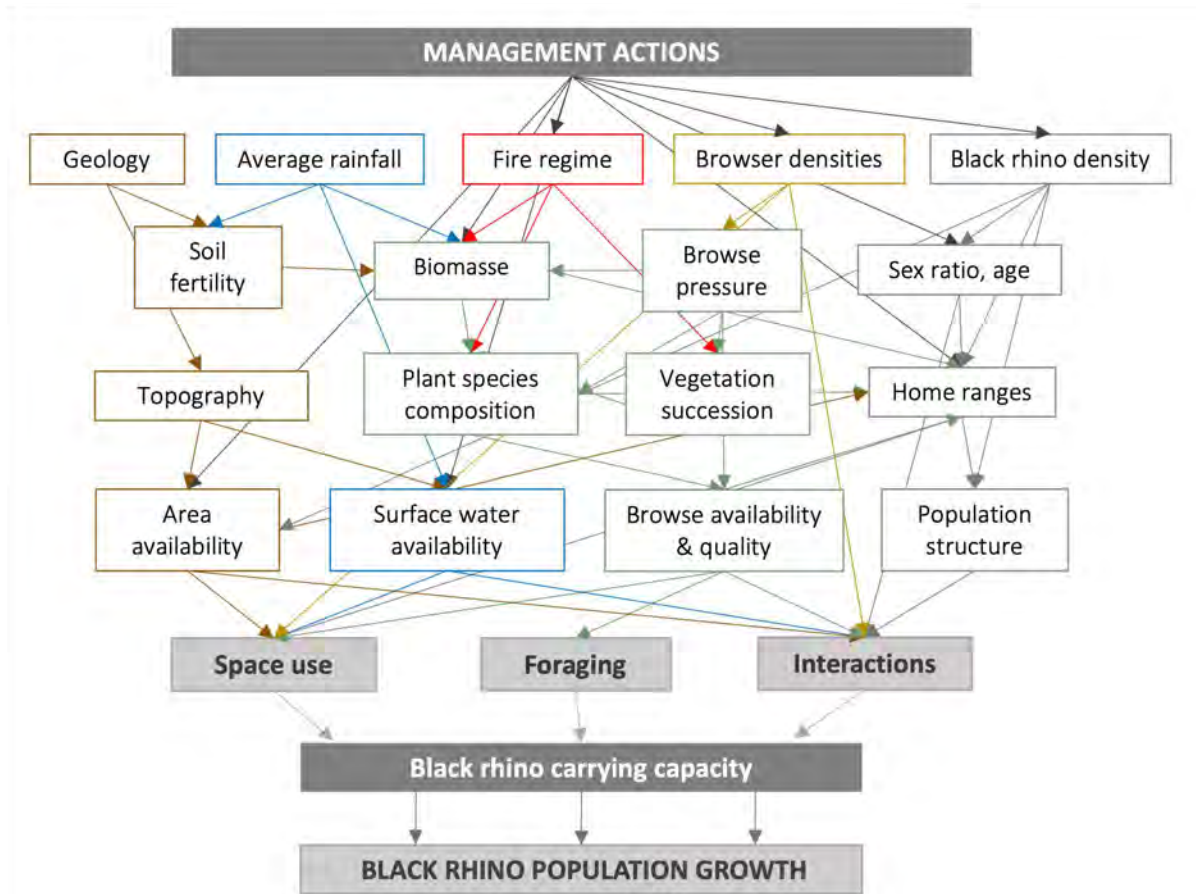


Figure i. Important parameters linked to black rhino population growth. Population growth is intertwined with both abiotic and biotic environmental conditions and management actions.

Movement ecology is fundamental to any ecological process and holds the premises to disentangling complex mechanisms (Nathan, 2008). Habitat selection offers insights into resource distributions and utilisation, interspecific interactions and differences among individuals (Morris, 2003), and thereby informing on an area's capacity to support an animal population with optimal population growth. Optimal foraging strategies influence the way herbivores use their landscape (Grant & Scholes, 2006). Black rhinos, like other large herbivores, forage across several temporal and spatial scales (Morris, 2003; Shrader *et al.*, 2012). Within diverse landscapes, they primarily choose habitat patches based on available resources seeking to maximize their nutrient and energy intake (Belovsky, 1984; Senft *et al.*, 1987; Grant & Scholes, 2006; Owen-Smith, Fryxell & Merrill, 2010); vegetation type and structure are thus particularly important (Lush, Mulama & Jones, 2015). Nonetheless, soils and geomorphic characteristics have a fundamental effect on the structure and quality of plants, hence also influencing habitat selection of large herbivores (Ward, Muller & Shrader, 2017).

Other parameters such as social factors and home ranges (Mace & Harvey, 1983; Mitchell & Powell, 2012), seasons (Mace & Harvey, 1983; Shrader *et al.*, 2012; Seidel *et al.*, 2019), human disturbance and distance to surface water (Odendaal-Holmes *et al.*, 2014) can equally be determinant. However, information precisely characterising black rhino habitat use is still generally scarce (Seidel *et al.*, 2019) and divergent views still prevail about the relative importance of the factors underlying selective foraging of black rhinos, and ultimately how they affect habitat choice (Muya & Oguge, 2000).

Optimal foraging is governed by intake, digestion and food search (Muya & Oguge, 2000). Forage selection is thus influenced by plant physical and chemical characteristics (Freeland & Janzen, 1974), where palatability is determined by the ratio of crude protein, fibre levels that affect digestion rates and secondary metabolites such as tannins or terpenes that can cause toxicosis (Freeland & Janzen, 1974; Cooper, Owen-Smith & Bryant, 1988; Moore & Jung, 2001). However, there is no true consensus among previous work for black rhino (Muya & Oguge, 2000), where the species has been reported to forage according to fibre, protein, leaf:stem ratio, photosynthesizing tissues, total phenols or secondary chemistry (Muya & Oguge, 2000; Ganqa, Scogings & Raats, 2005; van Lieverloo *et al.*, 2009; Buk & Knight, 2010; Scogings, Demmer & Hattas, 2021). Large herbivores rely on pre-ingestive cues for forage selection that can be either visual, gustatory or olfactory (Finnerty *et al.*, 2017; Stutz *et al.*, 2017; Bester, Schmitt & Shrader, 2023). Yet, there is little information regarding the mechanisms black rhinos exercise in relation to food search and selection. In addition, herbivores must adapt their resource use and feeding strategies in relation to temporal and spatial changes in vegetation quality and quantity in seasonal environments (Owen-Smith, 2002). Because the availability of preferred species fluctuates seasonally, in particular owing to deciduous species that lose their leaves in the limiting dry season (Owen-Smith, 2002), large herbivores must adjust their choice pertaining to variable environmental conditions and interactions with coexisting herbivorous species (Owen-Smith, 2002; Cromsigt, Archibald & Owen-Smith, 2017).

The coexistence of ecologically similar large herbivores is principally mediated by resource partitioning (Hutchinson, 1959; Schoener, 1974). The differential use of resources by herbivores can be accomplished by adopting differential diets, consuming different parts of plants or browsing across vertical stratification (Makhabu, Skarpe & Hytteborn, 2006; du Toit & Olff, 2014). In light of seasonal variation of preferred resources, herbivores have two main

strategies: diet shifts and migration (Aikens *et al.*, 2020; Kartzinel & Pringle, 2020; Staver & Hempson, 2020). Because fences restrict migration, diet alteration is expected, particularly for species that present diet flexibility, such as mixed feeders (Codron *et al.*, 2007; Kartzinel & Pringle, 2020; Staver & Hempson, 2020). It is thus important to characterise the overlap in consumed vegetation resources of coexisting large herbivores (Owen-Smith, 2002) and the subsequent potential effects on the habitat. For instance, due to their substantial consumption of forage, elephants can radically alter vegetation structure and composition (Laws, 1970), and it was reported that elephant damage was a predictor for black rhino presence (Lush *et al.*, 2015). Managing sympatric herbivore densities and vegetation communities based on evidence-based information is fundamental to maintaining productive ecosystems and mammal populations (Soto-Shoender *et al.*, 2018).

Management typically seeks to promote endangered species growth and healthy functioning ecosystems. Because large herbivores depend on suitable vegetation composed of palatable species, but also exert top-down control on plant demography (Pringle *et al.*, 2023), habitat management is essential (Soto-Shoender *et al.*, 2018), especially for systems that don't allow migration. Interventions such as burning or bush clearing are common tools to promote the growth of palatable species and environments suited to hosted species assemblages. Density management of herbivore species through carrying capacity calculations in this regard, are one of the pillars of protected area management. Both require extensive knowledge on habitat use, resources and species dynamics. Other interventions more specific to rhino such as selected individual removals or swops and dehornings are common practices, that although appear straightforward, may have further implications for the species. For instance, removals may affect the social structure of populations (Nhleko *et al.*, 2017) and because horns have been reported to be linked to dominance and territorial establishment (Berger & Cunningham, 1998), dehorning may have further reaching consequences than simply deterring poaching.

Overall, it is clear that black rhinos too, suffer from the general neglect of studying “big things” (Pringle *et al.*, 2023) and continued studies are critical. Behaviour ecology fundamentally addresses the adaptive responses of individual organisms to environmental heterogeneity (Owen-Smith, 2002) and rapid human-induced change (Owen-Smith & Cain, 2007; Berger-Tal *et al.*, 2011). In addition, the integration of monitoring techniques with modern methodologies can be effective in providing actionable guidance for the management and conservation of large herbivores (Pringle *et al.*, 2023). On this basis, this thesis aims at increasing unambiguous

knowledge of black rhino ecology, practical to its management and that of its environment, through a contemporary approach and three important scopes: space use, foraging and interactions.

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Aims

Within the framework of this thesis, I aim to investigate the ecological parameters involved in black rhino niche selection and foraging and the impact of a common management intervention. More specifically, I use a combination of field and lab-based methods and relate findings to practical management aspects through four chapters.

In **Chapter I** – *Out of scale out of place: black rhino forage preference across the hierarchal organisation of the savanna ecosystem*, I aim to investigate black rhino forage and habitat selection across multiple scales. I describe forage selection by the means of direct-observation transects on feeding paths and subsequent habitat selection based on monitoring data and ecosystem productivity (NDVI). I compare the nutritional and chemical composition of preferred and avoided plant species through metabolomics (LC-MS) and elemental analysis (CHN).

In **Chapter II** – *Seasonal dietary strategies shape savanna herbivore niche partitioning and management*, using DNA metabarcoding, I investigate resource partitioning between black rhino and three abundant sympatric herbivores, elephant, kudu and impala. I describe seasonal diet composition and overlap; and compare foraging strategies between the four herbivores. I aim to quantify the potential encroachment on black rhino dietary niche in Ithala Game Reserve.

In **Chapter III** – *Prickly preference: the role of plant morphological traits and volatiles as pre-ingestive cues for black rhino forage selection*, I investigate potential variables driving black rhino forage selection. I compare the traits and volatile organic compounds (VOCs, through GC-MS) of preferred and avoided plant species determined by feeding-path transects, and examine their relative importance in determining preference or avoidance.

In **Chapter IV** – *Reductions in home-range size and social interactions among dehorned black rhinoceroses*, I determine the consequences of dehorning for black rhino space use and social interactions and investigate the efficacy of dehorning as a poaching deterrent. I use long-term monitoring data to estimate home ranges before and after dehorning, and trends in dehorning and mortalities. I estimate the effect of such a tool on social interactions based on home-range overlap.

The majority of this thesis' work is based on one black rhino population, that of Ithala Game Reserve, in northern KwaZulu-Natal, South Africa. Chapter IV, however, groups 10 different black rhino populations in KwaZulu-Natal, South Africa.

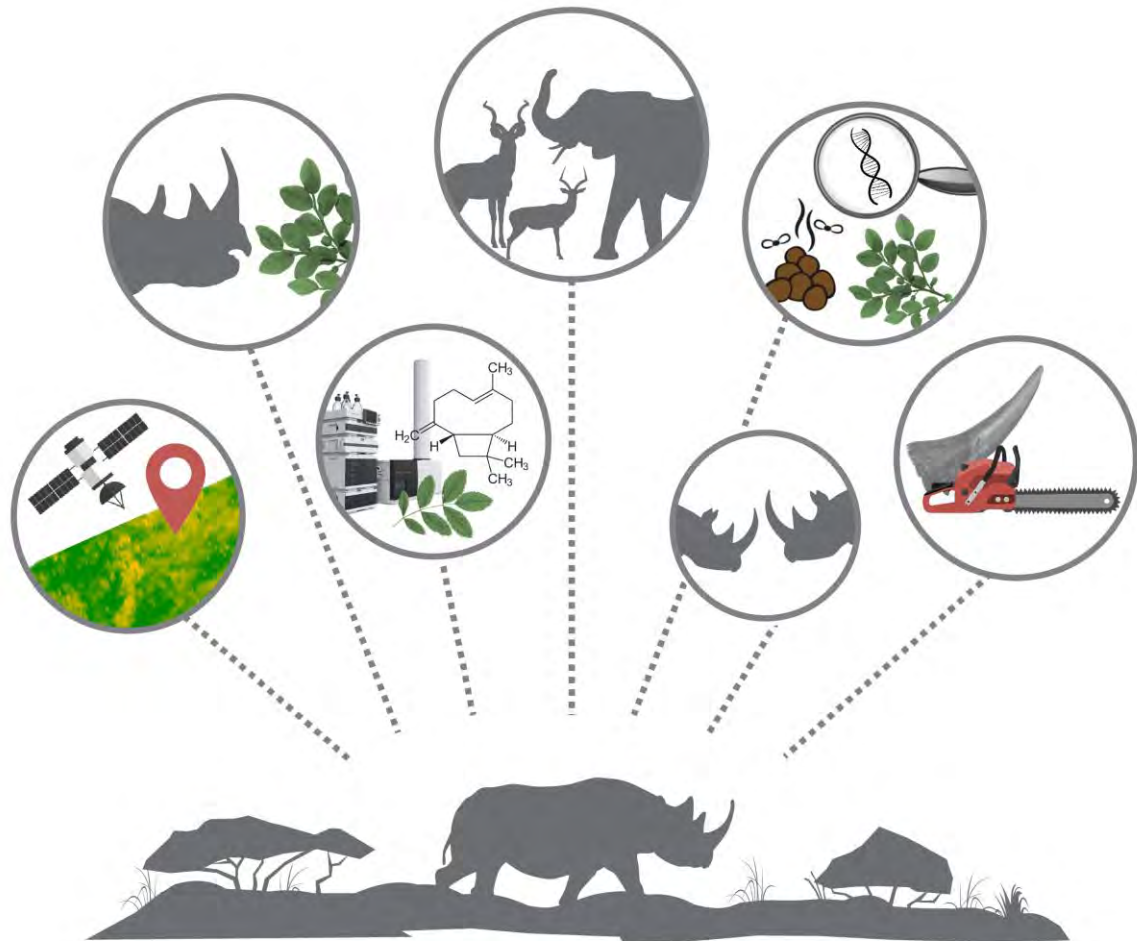


Figure ii. This thesis' work is composed of a combination of field and lab-based methods across different scales and in relation to practical management questions.



Chapter I

Chapter I

Out of scale out of place: black rhino forage preference across the hierarchical organisation of the savanna ecosystem

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Author Contributions

VD, RvdW and SR initiated the project. VD collected the data. GG performed the chemical analyses. EM and VD analysed the data. VD and SR wrote the first version of the manuscript and all authors contributed to the final writing.

Abstract

The successful conservation plans of megaherbivores necessitate precisely characterizing their ecological needs in order to optimize reproduction rates and reintroduction plans. The black rhino (*Diceros bicornis* L.) is among the most endangered species of megaherbivores in Africa and its conservation relies on nature reserves that are bound and habitat-restricted. Therefore, identifying the optimal amount of space this species needs and the factors driving its habitat use are crucial for establishing reserve priority plans. Knowing that forage selection is an important component linked to herbivore spatial distribution, we combined five years of sightings data with observations of rhinos' vegetation type and forage preferences to address their forage selection across multiple spatial scales. We found that black rhinos' spatial distribution was negatively associated with ecosystem productivity, but positively associated with specific vegetation types that contain highly preferred, chemically distinct, plant species. Black rhinos thus occupy their habitat across space and time through selective foraging on preferred plants.

Keywords

Diceros bicornis, endangered species, foraging selectivity, habitat productivity, megaherbivore, metabolomics, plant quality, translocation.

Introduction

The continuously increasing loss of endangered charismatic megafauna diversity (e.g., primates, carnivores, and megaherbivores) (Biggs *et al.*, 2013) has several consequences for respective ecosystems (Dirzo *et al.*, 2014), including weakened community stability, modification of food-web dynamics (Owen-Smith, 1992; Landman, Schoeman & Kerley, 2013), and alteration of nutrient cycles (Ripple *et al.*, 2015). Often considered as keystone species, megaherbivores in particular, can have huge impacts on the whole community through habitat modification, resource partitioning, and competition (Landman *et al.*, 2013; Ripple *et al.*, 2015).

Black rhinoceros (*Diceros bicornis* L.), hereafter referred to as rhinos, are among the most endangered megaherbivore species in the world. Listed as critically endangered (IUCN, 2011), mainly because of poaching, the worldwide population since 1960 has declined by an estimated 98% (Emslie & Adcock, 2016). Poaching for rhino horn is the species' biggest threat, but not the only one. The lack of habitat availability also threatens their survival (Emslie & Adcock, 2016). At present, wild black rhino populations are limited within bounds of protected areas, which are often enclosed and thus restrict migration (Landman *et al.*, 2013). In this context, the number of individuals within protected areas needs to be managed to remain just under carrying capacity in order to maintain and increase the global black rhino population. This strategy comprises selecting animals from populations which have a positive growth rate and translocating them to alternative areas that have potential to sustain new populations (Linklater *et al.*, 2012).

Apart from security concerns, the main challenge for successful translocation plans, resides in finding habitats that have all necessary components for maximizing the species' growth rate (Odendaal-Holmes, Marshal & Parrini, 2014; Balfour *et al.*, 2019). In characterizing suitable habitats and subsequently estimating a habitat's potential carrying capacity, many factors need to be considered, including climatic conditions and seasonal variation, plant productivity, forage selectivity, and topography (Adcock, 2001). However, information precisely identifying black rhino habitat use is still generally not available. Black rhinos, like most large mammalian herbivores, forage across several temporal and spatial scales. In heterogeneous landscapes, they select habitat patches mainly to consume specific plants to maximize nutrient and energy intake (Owen-Smith, Fryxell & Merrill, 2010), however, other factors such as home ranges (Mitchell

& Powell, 2012), human disturbance and distance to surface water (Odendaal-Holmes *et al.*, 2014) play a role. Nonetheless, divergent views still prevail about the relative importance of factors underlying selective foraging of black rhinos and ultimately how they affect habitat choice (Muya & Oguge, 2000).

We adopted a multi-scalar approach to investigate black rhino forage selection and distribution across multiple scales (Figure 1). At the largest scale, we analyzed black rhino populations' spatial densities in relation to ecosystem productivity (Normalized Difference Vegetation INDEX (NDVI)). The species may be selecting highly productive areas in order to maximize biomass intake in minimal time (Bergman *et al.*, 2001). Next, we examined black rhinos' distribution in relation to plant community selection. Specific plant communities may be selected over others for maximizing preferred food intake (Anderson *et al.*, 2018). Then, we analyzed plant species selectivity by establishing seasonal diets through observational transects. Finally, at a molecular level we analyzed plant species' chemical profiles through metabolomics, because black rhinos, like other megaherbivores, may select specific plant species based on their primary and secondary chemical composition (Muya & Oguge, 2000; Ndondo *et al.*, 2004; Anderson *et al.*, 2018). This dataset presents comprehensive information on black rhinos' distribution in relation to foraging across the hierarchical organization of the savanna ecosystem.

Materials and Methods

Study site and vegetation communities

The study was conducted in Ithala Game Reserve (IGR), situated in Northern KwaZulu-Natal (27°30'S, 31°25'E) in South Africa with an area of 296 km². Long-term annual rainfall averages 748 mm and the majority of this falls during the wet season (October-April), particularly, during the summer months (November-April) (Supporting Information – Chapter I **Figure S1**). The reserve lies within the Savanna /Grassland biome and hosts 26 different types of vegetation communities (Van Rooyen & Van Rooyen, 2008) (Supporting Information – Chapter I **Figure S3**), mainly consisting of grasslands, thickets, rocky/open/dense bushveld, woodlands, forests, riparian vegetation, cliffs, scarps and open disturbed patches. Each individual rhino has been marked with a unique set of ear notches for identification and monitoring purposes, which allows the spatial position to be recorded every time an individual is sighted. We used black

rhino monitoring data from 2012 to 2017 for this study. This sensitive data may not be published for the protection of the rhinos, however, the entire population on the reserve was monitored. This resulted in a total of 2371 observation data points, with a median value of 47 observations per rhino for the five years of sightings. Black rhino location data was collected from dusk to dawn by all field rangers stationed across all quadrats of the reserve, thus all parts of the reserve were provided with equal monitoring effort. In particular, since IGR management's goal is to observe each rhino at least once a month, the monitoring effort across all individual rhinos and season should be uniform.

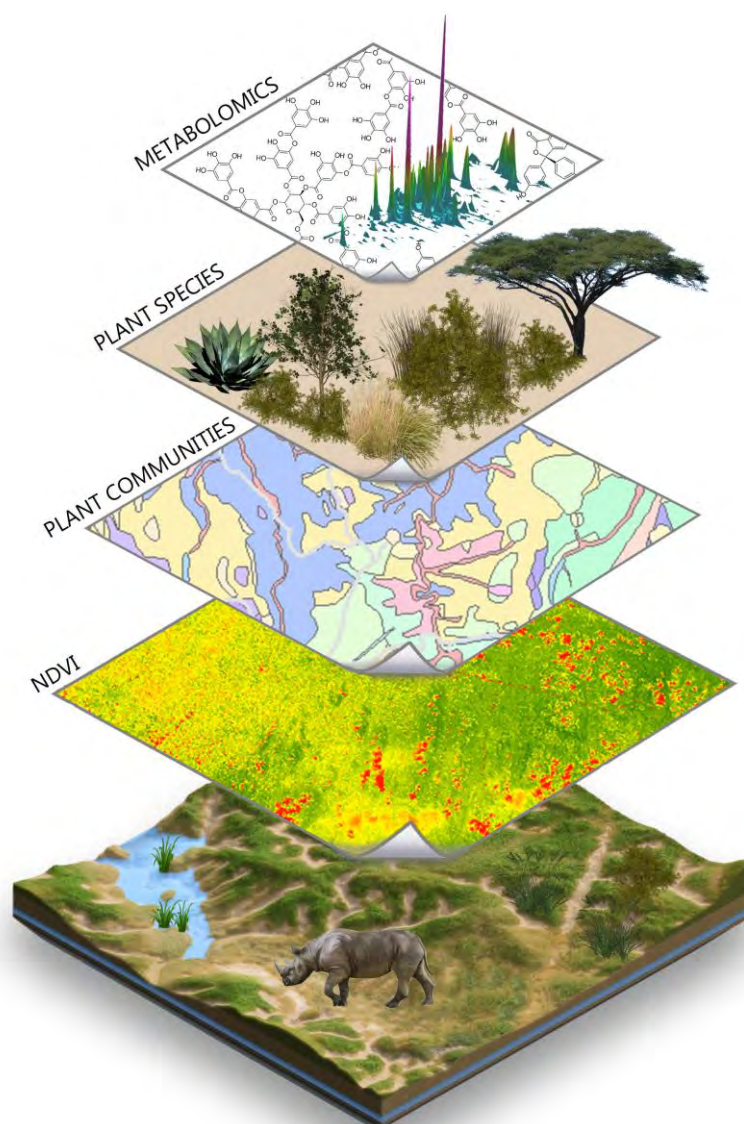


Figure 1. Multi-scalar approach used in this study to explore factors driving black rhino habitat choice and forage selectivity. Each layer represents a scale of analysis applied to the study of the Savanna ecosystem.

Normalized Difference Vegetation INDEX (NDVI) calculation

As a general indicator of ecosystem productivity, or vegetation greenness, we used the Normalized Difference Vegetation INDEX (NDVI) calculated from Landsat TM, assuming that higher NDVI values indicate more productive areas (Berry, Mackey & Brown, 2007). The NDVI was calculated using red (RED) and near-infrared (NIR) spectral bands of Landsat-7 with 30-meter resolution: $NDVI = (NIR - RED)/(NIR + RED)$. We employed Landsat data collected across the 5 years of rhino monitoring (2012-2017) to estimate a monthly average NDVI. Landsat data layers including more than 20 percent of cloud cover were excluded. We next used this dataset to characterize two distinct seasons; dry (May to October) and wet (November to April) (Supporting Information – Chapter I **Figure S2**).

Black rhino diet selectivity

To establish plant species selectivity, plants browsed and those avoided by black rhino individuals were surveyed on feeding paths (Shrader *et al.*, 2012). Feeding black rhinos were located by tracking them or through opportunistic sightings (Supporting Information – Chapter I **Table S1**). To limit autocorrelation, feeding paths from the same individuals were sampled at a minimum of 24 hours apart. Transects were approximately 50 m long and 2 m wide. Tracks determined the start and the direction of transects, predominately backtracking the animal's feeding path. A waypoint was recorded for each transect with the use of a handheld Garmin e-trex 20 GPS. All woody plants, shrubs and trees with a maximum canopy height of 2 m were recorded on the feeding path. Grasses were excluded as black rhinos are predominantly browsers (IUCN, 2011). Forbs were also excluded as we could not ascertain whether they were browsed by the rhinos when missing from the vegetation plot (Kotze & Zacharias, 1993). On the other hand, freshly browsed woody species are recognizable by the lighter colour of the exposed wood, the wetness of remaining branches, and the characteristic way in which black rhinos browse. Black rhinos bite off large twigs in a pruning-shear manner attributable to the morphology of their hook shaped lips, leaving branches pruned at a clear 45 degree angle (Kotze & Zacharias, 1993; Shrader *et al.*, 2012). Along each transect, all woody plant individuals were then scored as browsed or non-browsed for estimating plant species selectivity. Plant species selectivity was quantified by the application of Ivlev's electivity index $E = (r_i - p_i)/(r_i + p_i)$ (Strauss, 1979), where E is the measure of electivity per species, r_i is the sum of browsed individuals of the same species and p_i the relative abundance of the same species. The relative

abundance of each species (p_i) was calculated by dividing the number of times it was encountered by the sum of encounters of all species. Selectivity is given and ranked for each species as the index has a possible range of -1 to +1, with negative values indicating avoidance of the plant species, zero indicating random selection from the environment and positive values indicating active selection (Strauss, 1979).

Metabolomic analyses

To address chemically-based mechanisms of black rhino foraging decisions, we performed untargeted metabolomic analyses of the three most frequently browsed species (*Lantana camara*, *Dichrostachys cinerea* and *Acacia karroo*, see results section), and three of the most frequently avoided species (*Peltophorum africanum*, *Euclea crispa* and *Lippia javanica*). At least 10 leaves from five different individuals were collected for each species. All samples were dried and stored in brown paper bags before performing untargeted metabolomics analyses using UHPLC-QToF-MS as described in the supplementary methods' section. On the same samples we also measured total carbon and nitrogen using a standard elemental analyser (Flash 2000, Thermo Scientific, Waltham, Massachusetts, United States) to calculate the carbon to nitrogen ratio (C/N). First, we predicted that the metabolomics profile would differ between browsed and avoided species. Second, because black rhinos are deficient in nitrogen (as are most animals), we predicted that the browsed plants should contain higher levels of nitrogen content (thus a lower C/N ratio) than avoided plants.

Statistical analyses

First, for each georeferenced rhino sighting, we estimated a specific point density based on the number of individuals observed in a 500 meter radius (Wand, 1994) using the package `pointdensityP` (Evangelista & Beskow, 2018) in R-3.5.3 (R Development Core Team, 2018). Second, we calculated an index of selectivity for each plant community as the average of the species selectivity weighted by their relative abundance. The relative abundance of plant species in each plant community was estimated using transect data (number of stems divided by the cumulative length of transects per plant community). By doing so, we were able to extrapolate precise dietary information obtained during tracking at the local scale to the same plant communities' types. To estimate the influence of NDVI, selectivity and season on rhino density we fitted a Generalized Linear Mixed-Effects Models (`glmer` function in the package

lme4 (Bates *et al.*, 2015)) with *poisson* distribution as: density = NDVI*season + community selectivity*season + random/rhino identity. We visualized the output of the model using prediction of parameters interaction plots and river plots, which are particularly useful to represent interactions across large datasets (Hao *et al.*, 2009), and illustrates the inner works of the model previously discussed.

The metabolome of the six species sampled was analysed using a bidirectional orthogonal partial least square discriminant analysis (OPLS-DA) with Pareto scaling (Thévenot *et al.*, 2015) on the two foraging classes (browsed and avoided). OPLS has a similar predictive capacity compared to PLS by improving discrimination of the predictive components (Pinto, Trygg & Gottfries, 2012). This model results in a two-dimension projection of plant chemical similarity, which allows visualizing rhinos' feeding choice in relation to plant metabolites identity. We used mixed-effect models to test whether rhinos' feeding choice (fixed factor: browsed versus non-browsed) was related to C/N content in plants. Plant species were included in the model as random factor (*lmer* function in the package *lmer4* in R-3.5.3 (Bates *et al.*, 2015)).

Results

Rhino density across NDVI and community selectivity

The spatial distribution analysis showed that rhino density is strongly variable and structured across the landscape (**Figure 2, Figure 3a**). In other words, across the reserve we observed locations that are more frequently occupied (“Aggregated” section in **Figure 3a**) relative to the others (“Dispersed” section of **Figure 3a**). The output of the mixed-effect model showed that black rhino density was negatively correlated with ecosystem productivity (NDVI) and positively correlated with plant community selectivity (**Figure 2, Table 1**). This was also illustrated by the river plot's (**Figure 3**) flow that suggests that areas with higher density of black rhinos are correlated to low NDVI (**Figure 3b**), and plant communities with high global selectivity (**Figure 3c**). The opposite is true when observing the flow starting from the “Dispersed” rhino abundances.

We also found that community selectivity is driven by the balance between highly preferentially browsed (colder colors boxes), non-specific (warmer color boxes) and avoided (red boxes) plant

species (**Figure 3d**). Finally, we observed an effect of season on black rhino density, where in the wet season black rhinos were present in higher numbers in low productivity areas and in plant communities with high selectivity values (**Figure 2, Table 1**) than in the dry season. The wet season corresponds to a higher global productivity (Supporting Information – Chapter I **Figure S2**).

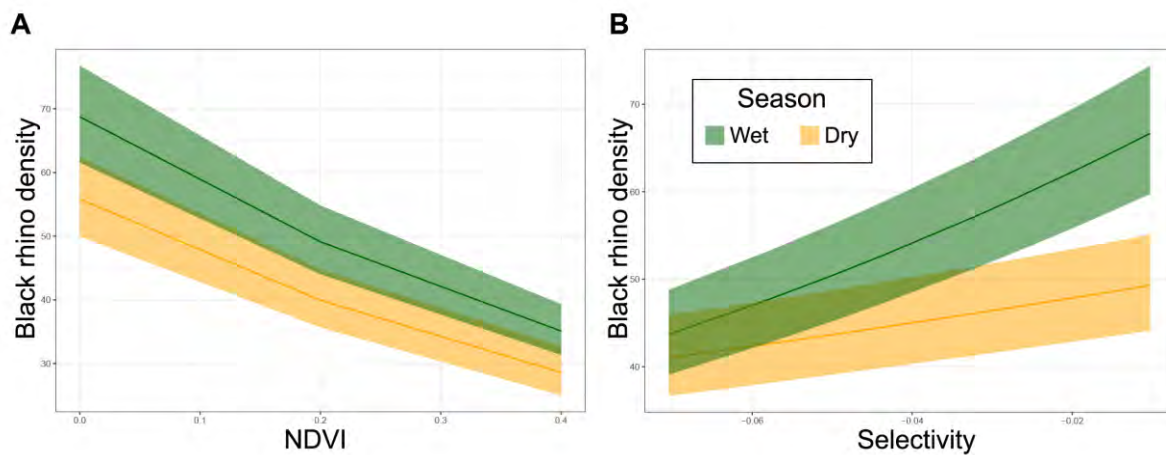


Figure 2. Glmer output displaying; A) the relationship between black rhino density and NVDI; and B) the relationship between black rhino density and average vegetation type selectivity across seasons (yellow = Dry, and green = Wet seasons).

<i>Predictors</i>	RD		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>
(Intercept)	4.126298	55.27 – 69.43	<0.001
Wet Season	0.343625	1.36 – 1.47	<0.001
NDVI	-1.672294	0.15 – 0.24	<0.001
Selectivity	3.057253	10.91 – 41.48	<0.001
Wet Season: NDVI	-0.008477	0.76 – 1.30	0.951
Wet Season: Selectivity	3.97030	25.05 – 112.1	<0.001
Random Effects			
σ^2	0.02		
τ_{00} RhinoID	0.22		
ICC _{RhinosID}	0.92		
Observations	1549		
Marginal R ² / Conditional R ²	0.119 / 0.930		

Table 1. Summary table of the mixed effect generalised linear model for assessing the effect of season (wet and dry), productivity (NDVI), and plant community selectivity on black rhinos' point density. Random effects are individual rhinos (RhinoID).

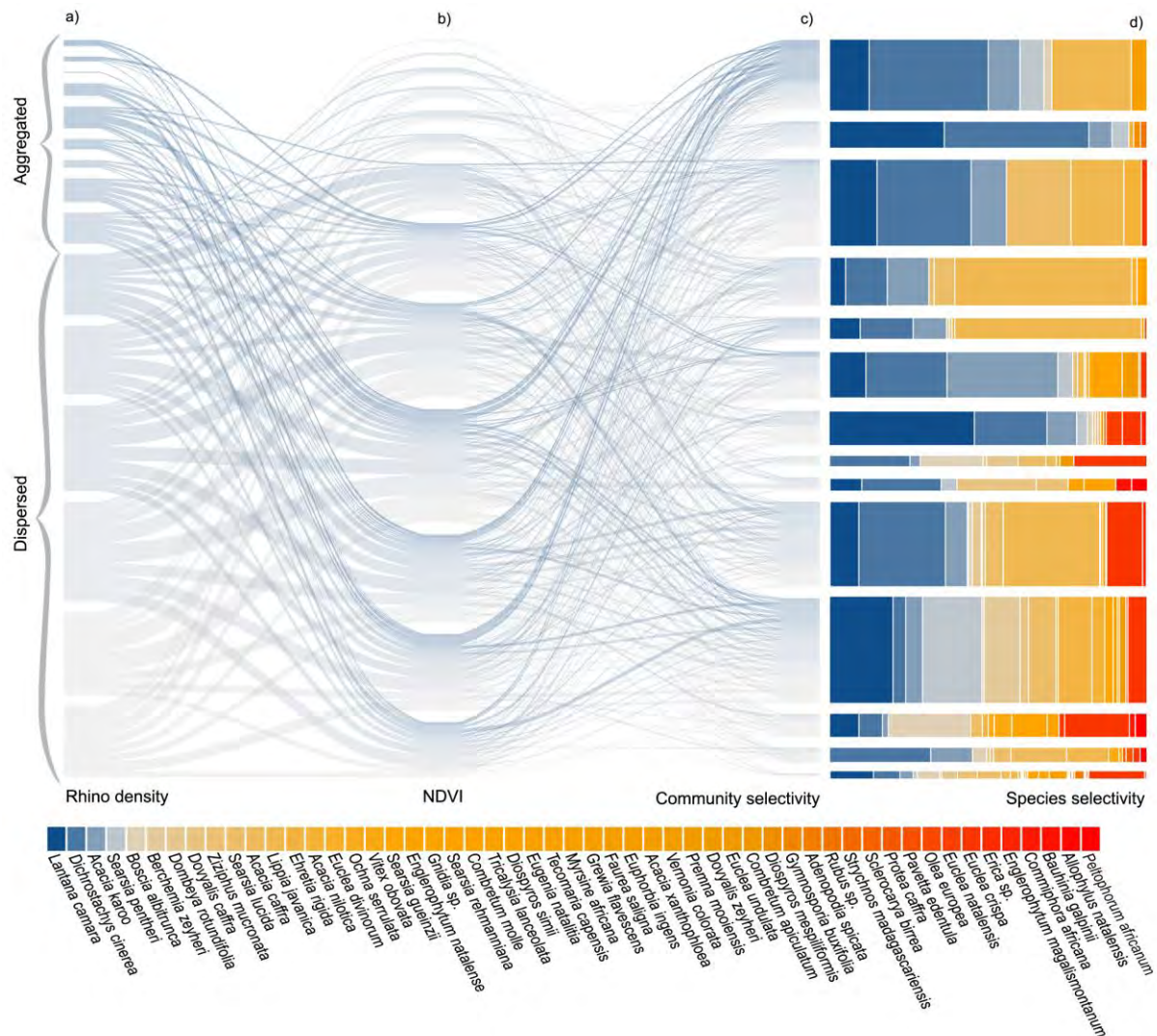


Figure 3. River plot display of the multi-layered model analysis. a) Rhino density is ordered from top to bottom by decreasing density. The width of each line is proportional to the number of rhinos in a given class. b) NDVI is ordered from top to bottom by decreasing values of productivity. c) Community selectivity is ordered from top to bottom according to decreasing vegetation type selectivity. The column d) shows how each vegetation type is constructed according to species selectivity and abundance. Each color represents the average species' selectivity for each species in a given community; blue = more attractive, and red = more repulsive. In the legend, "Aggregated" refers to locations that are more frequently occupied by black rhinos relative to the other locations, which are designed as "Dispersed".

Species-specific forage selectivity

Over the course of two distinct seasons, black rhinos' diet proportions varied significantly (Supporting Information – Chapter I **Figure S4**; Chi-square= 158.95, df = 70, $p < 0.001$), but overall, Ivlev's values for selectivity indicated that black rhinos preferentially browsed on

certain plant species (Supporting Information – Chapter I **Figure S5**). These included *Acacia karroo* (Ivlev value = -0.03), *Dichrostachys cinerea* (Ivlev value = -0.004), and the invasive plant species *Lantana camara* (Ivlev value = 0.03). All other species displayed much smaller Ivlev's values, ranging from complete avoidance (= -1) or non-specific for host plant choice (between -0.5 and -0.95).

The metabolomics results of tested samples (browsed and non-browsed) were plotted by chemical profile dissimilarity with an OPLS-DA. The latter highlighted a chemical discrimination between browsed and non-browsed species showing two clear clusters (**Figure 4A**), indicating that black rhinos select plants to forage based on specific metabolic profiles. On the other hand, while browsed plants have a 70% lower C/N compared to avoided plants (**Figure 4B**), we found no significant difference between browsed and avoided species across the six species tested (mixed-effect model for testing browsing preference effect; $F_{1,24} = 1.41$, $p = 0.37$).

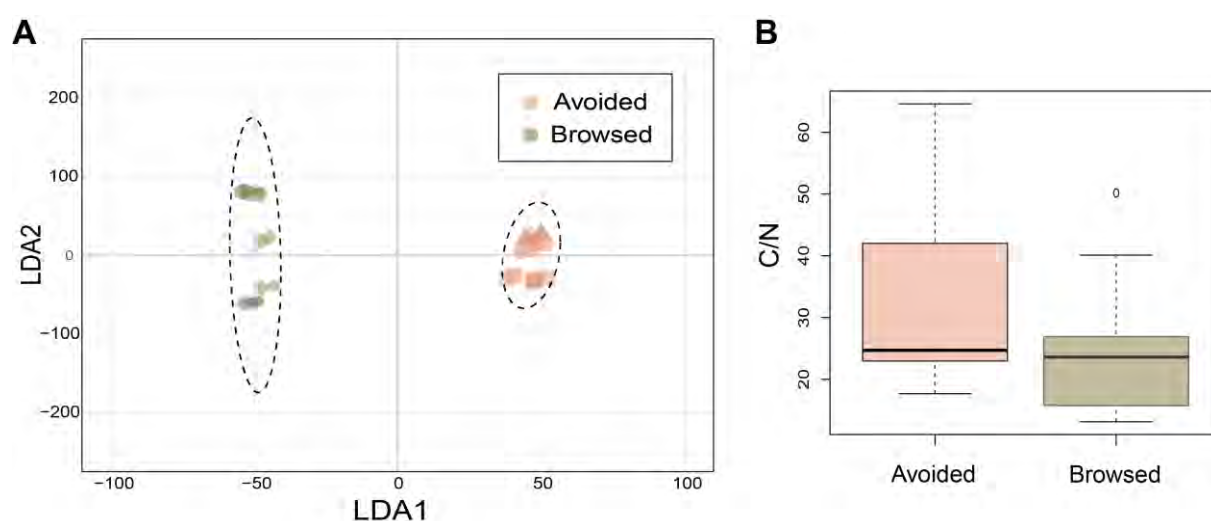


Figure 4. Chemical differences between browsed and avoided plant species. (A) OPLS-DA score plot performed by considering all analysed plant samples (browsed: *Lantana camara*, *Acacia karroo*, *Dichrostachys cinerea*; avoided: *Lippia javanica*, *Euclea crispa* and *Peltophorum africanum*) divided into two classes: browsed (yellow symbols) and avoided (grey symbols). $R2X1 = 11.1\%$, $R2X2 = 11.2\%$. Ellipses show goodness of fit = 99.5%. (B) Boxplots representing average carbon to nitrogen ratio (C/N) for the three most preferred (Browsed, khaki boxes), and the three most avoided (Avoided, salmon boxes) plant species by black rhinos during their foraging paths.

Discussion

Rhino spatial distribution

Through spatial analyses and the river plot, we were able to show that low densities of black rhinos were observed in vast areas that correspond to low selectivity plant communities. These findings concord with the fact that black rhinos are solitary sedentary animals and live alone within home ranges (Burt, 1943; Mitchell & Powell, 2012). Accordingly, to meet the nutritional requirements in low selectivity plant communities, they should maintain large foraging areas (Reid *et al.*, 2007; le Roex *et al.*, 2019). Additionally, we found that high-densities of black rhinos were associated with smaller areas of land and also with plant communities with high selectivity values. This may be because in these areas, nutritional requirements are met faster and therefore they can tolerate sharing their resources with other black rhinos. This effect is particularly marked in the wet season, indicating that during the dry season the decrease of plant biomass could be a limiting factor for rhinos to group more densely.

Effect of productivity and selectivity on black rhino distribution

We found that black rhino density was negatively correlated with NDVI. This goes along with the observations that black rhinos favor open woodland and shrubland for optimal browsing as opposed to closed canopy woodlands (Kotze & Zacharias, 1993; Gadiye & Koskei, 2016). Open woodlands have lower NDVI values due to a lower presence of leafy plants and is therefore consistent to our findings of black rhinos' higher distribution in low NDVI areas. The species has also been reported to utilize dense bush for bedding sites (Anderson *et al.*, 2018), which justifies moderate utilization of other areas. That said, if grassland NDVI values were to be excluded from NDVI plot averages, it is questionable whether black rhinos' choice of plant biomass would still qualify as low. Confirmation would require more intense tracking regimes across the year, and more accurate measurements of plant productivity at each site.

Black rhinos not only choose plant communities with low productivity but also high selectivity values. This finding is likely explained by the fact that they are selective feeders (Muya & Oguge, 2000; Ganqa, Scogings & Raats, 2005), and the general fact that spatial variation in the availability of different food plants drives herbivore distribution (Kartzinel *et al.*, 2015). Furthermore, a diverse and selective diet may be sustained by two constraints that black rhinos face. Firstly, being bulk feeders tolerant of low quality foods (Owen-Smith, 1992), a diverse

diet is necessary for required nutrient intake (Muya & Ouge, 2000). Secondly, because Perissodactyls (odd-toed ungulates which include all rhinos) are unable to benefit from bacterial degradation of toxins (Freeland & Janzen, 1974; Muya & Ouge, 2000), a more diverse diet reduces the effect of ingesting high doses of toxic phytotoxins (Rhoades & Cates, 1976). We also found that the importance of plant community selectivity decreased during the dry season, suggesting that black rhinos must be less selective to cover nutritional requirements during this harsher season. All together this suggests that the ratio between preferred and avoided species determines the strength of community selectivity.

Metabolomics and choice of forage

Previous studies (Ndondo *et al.*, 2004; Van Lieverloo *et al.*, 2009) indicated that black rhinos do not select forage to maximize nutrient intake and/or digestibility. Nutrient intake, measured by C/N ratio, has classically been related to feeding choice in invertebrate herbivores (White, 1984), and to a lesser extent for megaherbivores (Hopcraft *et al.*, 2012). Therefore, secondary metabolites are proposed as possible mechanisms of selection for black rhinos (Anderson *et al.*, 2018). Correspondingly, our preliminary results suggest that black rhinos select their diet through chemistry rather than nutrient uptake. Secondary metabolites, such as phenol-based compounds, seem to play a bigger role than C/N ratio. Black rhino, in largely abandoning good vision capacities, have evolved highly sophisticated olfactory senses. Therefore, host plant choice should be largely mediated by odorous cues, but this hypothesis needs further investigation as well as to determine which compounds drive selection or avoidance.

Study limitations and further steps

A problem encountered by this study, is the limited information on relative abundance of species in vegetation communities available for analysis. To deal with this, abundances on transects were projected across hosting vegetation types. But, microhabitats within vegetation types can be highly variable (Kartzinel *et al.*, 2015) and transect locations were selected according to the presence of black rhinos. This might have led to a misrepresentation of microhabitat selection within a vegetation community. To counter this limitation, a meticulous and exhaustive vegetation survey covering the entire reserve is necessary. Some studies (Hall-Martin, Erasmus & Botha, 1982; Oloo, Brett & Young, 1994) imply that forbs could include an important fraction of black rhino diet. However, because of the unrecognizable way forbs are

browsed due to their herbaceous nature (Kotze & Zacharias, 1993), they were excluded from surveyed vegetation transects. This may have impacted our results on diet proportions during the wet season, as there was a substantial presence of forbs on some of the transects. To resolve this issue and have supplementary accuracy in dietary information, dung samples could be analyzed in conjunction with vegetation surveys. Partially digested plant species fragments can be identified through microhistological examination (Van Lieverloo *et al.*, 2009) or DNA analysis (Pompanon *et al.*, 2012). A recent study (Anderson *et al.*, 2018) showed that DNA analysis and forage plots results correlated well.

As a further matter, black rhino location data is based on sightings collected by rangers on patrol and its use is limited by two constraints. Firstly, because rangers are unable to identify individuals at night, data was usually collected during the day. Secondly, rhinos are less visible when the surrounding vegetation is thick (Walpole, 2002) and in spite of providing equal effort to all areas of the reserve, this could lead to occasionally overlooking rhinos. Black rhinos are diel (Joubert, 1971) and therefore collecting data during both day and night would not only enable finer scale modelling, but also show that feeding preferences, as their behavior (le Roex *et al.*, 2019) might substantially differ across day and night. The use of GPS telemetry would greatly improve the temporality and accuracy of the dataset.

Conservation implications

One of the key results of this study is that black rhino forage selection is driven by specific plant community selectivity and not productivity; and secondary compounds seem to play an important role in plant species selectivity. These findings must be incorporated into black rhino population management strategies and warrant further investigation. By considering precise black rhino habitat use across a hierarchical organization, carrying capacity calculations could alter. The identification of specific plants that black rhinos use preferentially, should assist in the development of more accurate reserve management programs that take into account plant community selectivity and productivity measures.

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Chapter II

Chapter II

Seasonal dietary strategies shape herbivore niche partitioning and management

Submitted to: *Conservation Science and Practice*

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VD, SR and RvdW initiated the project. VD, PS, MF collected and prepared samples. GL performed bioinformatic analyses and VD, PS and MF analyzed the processed data. VD, PS and SR wrote the first version of the manuscript and all authors contributed to the final writing.

Abstract

Protected areas are the last havens for African savanna herbivores. Mostly delimited by fences, these savanna ecosystems can constrain habitat use and species interactions. Hence, understanding interspecific herbivore dynamics within protected areas is crucial for their effective management and persistence into the future. Resource partitioning is central to species assemblage as it can alleviate competition and enable ecologically similar species to coexist. While broad categories of foraging strategies can be used to explain coexistence, fine-grained information on seasonal foraging is needed to precisely assess resource partitioning. We used DNA metabarcoding to quantify seasonal diet breadth, composition, and dietary overlap for the black rhinoceros, the greater kudu, the African savanna elephant, and the impala. Diet composition and overlap shifted seasonally, where resource scarcity during the dry season generated a more even composition and reduced overlap of diets. Mesoherbivores encroached more on the critically endangered black rhinoceros than elephants did. In an environment more suited to browsers, the mixed feeders, elephant and impala, maintained nearly solely browsing through the year. This study shows that long-standing broad categories of foraging strategies and body size are limited in their use in protected areas and that seasonal strategies are central to managing increasingly threatened populations.

Keywords

Diceros bicornis, *Loxodonta africana*, *Tragelaphus strepsiceros*, *Aepyceros melampus*, resource partitioning, seasonal dietary strategies, overlap, DNA metabarcoding.

Introduction

The Protected areas are, for the most part, the last havens for medium to large size African savanna herbivores (Ripple *et al.*, 2015). Yet, savanna ecosystems are dynamic environments where species must adapt to rapidly changing ecological conditions (Skarpe, 1991). Climate change, ecosystem degradation and restricted migration are just a few factors affecting these habitats of importance (Cardillo *et al.*, 2005; Shrader, Pimm & van Aarde, 2010; Cushman *et al.*, 2016; Pekar *et al.*, 2019), which are now mostly delimited by fences (Pekar *et al.*, 2019). Considering the complexity of these factors and growing pressures that savanna ecosystems face, a deeper understanding of herbivore dynamics has never been more crucial for their effective management and persistence into the future (Staver & Hempson, 2020; Downey *et al.*, 2021).

Resource partitioning is the differential use of resources, such as food, by species in the same community (Schoener, 1974; Voeten & Prins, 1999), and is central to species assemblage as it can alleviate competition and enable ecologically similar species to coexist (Hutchinson, 1959; Schoener, 1974). Herbivore dietary niche structure is tied to morphology, body size, and digestive strategy (Olf, Ritchie & Prins, 2002). For instance, larger non-ruminant species can survive on lower quality forage, that is generally more abundant, in comparison to smaller ruminant species (Jarman 1974; Bell 1971). For large mammalian herbivores, dietary strategy can be classified into three broad types of herbivores: grazers (eating predominantly grasses, e.g. white rhinoceros, *Ceratotherium simum*), browsers (eating principally trees and shrubs, e.g. black rhinoceros, *Diceros bicornis*) and mixed feeders (eating grasses and trees, e.g. African elephant, *Loxodonta Africana*) (Landman, Schoeman & Kerley, 2013; Staver & Hempson, 2020). Within each category, diet breadth can vary from broad generalists to strict specialist, as variable environments such as savannas, with exogenous variation in resource growth and with resources located in distinct habitats, promote generalist-specialist coexistence (Abrahams, 2006). Other mechanisms favoring coexistence of large herbivores via the sharing of common resources include browsing stratification, which consists of vertical space partitioning where taller herbivores can access higher vegetation, and microhabitat selection (Makhabu, Skarpe & Hytteborn, 2006; du Toit & Olf, 2014).

Yet, food resources vary spatially and temporally through quantity and quality (Illius & O'Connor, 2000). Large mammalian herbivores have two main strategies in the face of seasonal

variation in vegetation abundance and quality: migration, where seasonally fluctuating resources are followed across the landscape (Aikens *et al.*, 2020), and dietary shifts (Kartzinel & Pringle, 2020; Staver & Hempson, 2020), which are characterized by the alteration in diet composition according to local changes in resource quality and quantity (Codron *et al.*, 2007; Kartzinel & Pringle, 2020). For instance, mixed feeders, can switch from a majority of grazing in the growing season to browsing in the dry season (Codron *et al.*, 2007; Abraham, Hempson & Staver, 2019). Herbivores that present seasonal diet flexibility often dominate savanna ecosystems with large population sizes (Staver & Hempson, 2020). However, to what extent dietary overlap is maintained seasonally still remains to be fully explored (Staver & Hempson, 2020).

Megaherbivores (body size >1000kg) generally constitute most of the animal biomass in savanna ecosystems. Using the largest share of available resources, they shape mesoherbivore communities, as well as ecosystem dynamics (Owen-Smith, 1992; Duffy *et al.*, 2007; Landman *et al.*, 2013). For instance, African elephants often roam at high numbers in protected areas (Wilkinson, Midgley & Cunningham, 2022), sometimes even largely surpassing the intended carrying capacities, with limited removals as the realities of modern-day elephant management in closed systems are challenging (Wilkinson *et al.*, 2022). Due to their substantial consumption of forage, elephants can radically alter vegetation structure and composition. By their impact, elephants can thus be viewed as threats to the conservation and management of other coexisting species (Owen *et al.*, 2006; Guldmond & Van Aarde, 2008; Asner & Levick, 2012). Indeed, other megaherbivore species such as the critically endangered black rhinoceros (IUCN, 2020), which lack the ability to switch to grazing, may be particularly affected in the dry season when resources are scarce, and elephants become predominant browsers. Hence, studies focusing on fine-grained dietary niche analyses can give, in addition to valuable information on resource partitioning (Pompanon *et al.*, 2012), valuable information for the management and regulation of species in protected areas (Pansu *et al.*, 2022).

Despite extensive knowledge of the breadth of possible foraging strategies that support coexistence, diet strategy regarding seasonal overlap is of the least understood mechanisms (Staver & Hempson, 2020). With this work, we asked whether seasonal diet overlap varied according to the availability of resources, in light of facilitating coexistence. In fenced habitats, where migration is not an option, we hypothesized that dietary overlap should be high in periods of abundance and low when resources are scarce, independent from the dietary strategy (Gordon

& Illius, 1989; Mysterud, 2000; Makhabu *et al.*, 2006). However, in periods of scarcity, mixed feeders are expected to have higher dietary overlap with strict browsers due to a higher reduction in grass availability compared to trees and shrubs. We addressed our questions using fecal DNA metabarcoding, and by comparing the seasonal diets and overlap of four predominant browsers (two mixed feeders and two strict browsers) in a fenced environment in South Africa. By providing novel metrics of the seasonal dietary niche of abundant coexisting large mammalian herbivores, this study helps addressing potential management dilemmas regarding the regulation of species in fenced protected areas. In particular, with regards to the highly managed, strict-browsing and critically endangered black rhinoceros.

Material and Methods

Study site and species

The study was conducted in Ithala Game Reserve (IGR), situated in the Northern KwaZulu-Natal region (27°30'S, 31°25'E) of South Africa and spanning an area of 296 km². Long-term annual rainfall averages at 748 mm and the majority of it falls during the wet season (November-April). The reserve lies within the Savanna and Grassland biome and hosts 26 different types of vegetation communities (Van Rooyen & Van Rooyen, 2008), mainly consisting of grasslands, thickets, rocky/open/dense bushveld, woodlands, forests, riparian vegetation, cliffs, scarps and open disturbed patches. The study focuses on four predominant browsing species that are either abundant in IGR or of considerable priority to management (such as the black rhino). As so, the study species were composed of two strict browsers: the black rhinoceros (*Diceros bicornis* L.) and the greater kudu (*Tragelaphus strepsiceros*); and two mixed-feeders: the African savanna elephant (*Loxodonta africana* B.) and the impala (*Aepyceros melampus* L.). An aerial count was performed from a helicopter in September 2021 utilizing North-South transects to determine large mammal numbers for effective wildlife management of IGR. During the time of study, there were 44 black rhinoceros (here after referred to as black rhino), 244 elephants, 415 kudus and 839 impalas (Van Der Westhuizen, 2021).

Sample collection and DNA extraction

A total of 190 fecal samples were collected during the wet and dry seasons of 2021, respectively January to March and July to September. For each species, we gathered a minimum of 15 samples per season in order to represent all main vegetation types in the reserve, except for the black rhino for which 50 samples per season were collected, as this was the focal species of the study. Samples were either collected after observing defecation or when opportunistically found and identified by appearance (Murray 2011). As DNA material rapidly deteriorates, only samples from obviously fresh fecal piles were collected. For each stool, we subsampled different parts of a single dung pile (elephants and black rhinos) or picked up 3 to 4 pellets (impalas and kudus). Upon collection, samples were stored in a cooler box in the field, then 10 g of each sample was immersed in 25 mL of 99% ethanol and mixed by inversion to stop any biological process. After 24 to 36 hours, the ethanol was meticulously poured out and the remaining solid material was left to dry for 48 hours. Then, 5 g of dried fecal sample was stored in 15 g of silica gel beads to preserve DNA material until extraction. QIAamp® PowerFecal® Pro DNA Kit was used to extract DNA from the fecal samples. We followed the QIAGEN protocol except that we added 100 mg of stool material instead of 250 mg. This kit outline indicates that samples are added to a bead beating tube where chemical and mechanical methods are used to homogenize them. When cells are lysed, the Inhibitor Removal Technology® is used to remove PCR inhibitors. Finally, a silica membrane in a spin-column format captures genomic DNA which is washed and eluted, and ready for PCR. All DNA extracts were quantified by a NanoDrop™ 2000/2000c Spectrophotometer and were diluted to have a concentration of 5 ng/μL. Extracted DNA samples were stored at -20 °C until further analyses.

DNA metabarcoding

A DNA metabarcoding approach was used in order to identify plant species present in the diet of the herbivores. We selected the *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) gene as it has been widely accepted as a standard plant DNA barcode by the international body of the Plant Working Group and disposes of a large online database (CBOL Plant Working Group *et al.*, 2009) . In addition, the size of the analyzed barcode should be as short as possible since fecal DNA is highly degraded as it has gone through a whole digestive system (Taberlet *et al.*, 2018). Hence, we used the *rbcLZ1* and *rbcL19b* primers to amplify a 106 bp fragment of the *rbcL* gene (Mallott, Garber & Malhi, 2018). Fecal DNA was PCR

amplified with *rbcL* tagged primers, libraries were prepared with the Illumina TruSeq kit and DNA was sequenced on an Illumina MiSeq V2 platform with paired end 2x150 bp at Fasteris (Life Science Genesupport SA, Switzerland).

Bioinformatic analyses

The bioinformatic analysis enabled the assignment of taxonomically DNA sequences to plant orders, families, genera, tribes, and/or species. The full bioinformatic analyses were automatically processed using DeltaMP v. 0.5 (Lentendu, 2021) on a HPC (“Centre de Calcul de la Faculté des Sciences”, UniNe,) and as described in the supporting information methods’ section. Briefly, rbarcodes and primers were removed from raw sequences with cutadapt (Martin, 2011); reads were quality trimmed with VSEARCH (Rognes *et al.*, 2016); filtered reads were grouped into ASV (amplicon sequence variant) using the error correction model of DADA2 (Callahan *et al.*, 2016); a taxonomy was assigned to each ASV using VSEARCH and NCBI *rbcL* reference sequence of species previously found in the IGR; tag-jump (i.e. the assignment of a sequence to a wrong sample) was controlled and corrected using a combination of positive and negative controls and linear modeling (Schnell, Bohmann & Gilbert, 2015; Taberlet *et al.*, 2018).

Statistical analyses

All data processing and statistical analyses were performed using R Studio® (version 1.4.1106). The number of reads per Amplicon Sequence Variant (ASV) were used to quantify diet composition. The average dietary niche width for each herbivore was defined with ASV richness (number of unique ASVs per sample), evenness (an estimation of the relative abundance of ASVs in the samples (Smith & Wilson, 1996)) and Shannon diversity (diversity of ASVs that integrates relative abundances with ASV counts (Shannon & Weaver, 1948)), using the *vegan* package (Oksanen, 2020). To test for the effect of herbivore species and season on the richness, diversity, and evenness of ASVs, two-ways analyses of variance (ANOVA) were performed. Next, to describe dietary niche (dis)similarities, the Bray-Curtis dissimilarity index (BC) and Pianka’s niche overlap index (PO) (Pianka, 1974) were calculated between each pair of herbivore species. Bray-Curtis dissimilarity index was calculated using the *vegan* package (Oksanen *et al.* 2020) and Pianka’s niche overlap index was calculated with the *spaa* package (Zhang, 2016). To visualize dietary overlap between the four herbivores, nonmetric

multidimensional scaling (NMDS) ordinations based on the Bray-Curtis dissimilarity index were produced. A stress level below 0.2 was considered acceptable (Clarke, 1993). To test for differences among herbivores and by season on ASV composition, a permutational multivariate analysis of variance (perMANOVA) with 999 permutations, using the *adonis2* function in *vegan* was performed. Additionally, perMANOVAs were performed to test for diet overlap between herbivores of the same feeding strategy (browsers, mixed-feeders) for the two seasons, separately. To assess significance of the dietary niche overlap between herbivore species, a null model analysis was conducted with 10'000 iterations and the randomization algorithm RA3 with the *EcoSimR* package (Gotelli et al. 2017). The null model simulates niche overlap if species used resources independently of one another and compares it with the observed resources utilization among all unique pairs of species. The null model analysis was also performed for pairs of species having the same feeding strategy (browsers, mixed-feeders), separately for the two seasons.

Results

Amplification of the *rbcL* DNA barcode was successful for 179 out of the initial 190 fecal samples. Of these, we discarded one sample due to a percentage plant similarity of < 95% and two others, due to poor yield of plant DNA. From the remaining 176 samples, the Illumina sequencing produced a total of 12,390,824 reads, which were reduced to 12,000,037 after filtering processes. The initial 571 ASVs were reduced to 300, as they were not assigned to any plant orders. Of the 300 identified ASVs, 96,3% were identified to the family, 70,7% to the genus and 30,7% to the species level. Finally, we were able to assign ASVs to 52 plant species, 87 genera, 57 families, and 32 orders. The most abundant plant families in terms of read counts were the *Fabaceae* (59,06%), followed by *Ebenaceae* (10,63%) and *Anacardiaceae* (5,3%). Based on the taxonomic classification, we next organized ASVs in two functional categories: grasses or non-grasses. In the dry season, black rhinos consumed 100% of non-grass species, elephant 98.6% of non-grass species and 1.4% of grass species, impala 99.5% of non-grass species and 0.5% of grass species, and kudu 100% of non-grass species. In the wet season black rhinos consumed 99.3% of non-grass species and 0.7% of grass species, elephant 94.2% of non-grass species and 5.8% of grass species, impala 97.7% of non-grass species and 2.3% of grass species and kudu 100% of non-grass species (**Figure 1**).

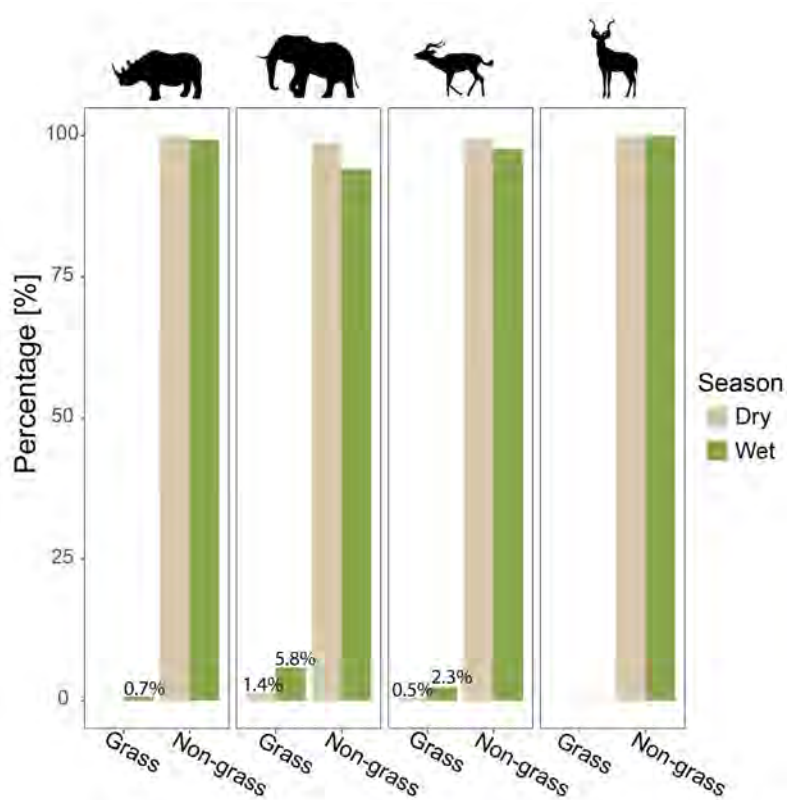


Figure 1. Types of forage (grass or non-grass) consumed by the four herbivores. In order: black rhino, elephant, impala, kudu, across the dry (tan colored bars) and the wet season (green colored bars). Bars represent percentage values based on the number of ASVs found in each sample.

We found that the number of plant species consumed (i.e., plant species richness) was significantly different between the four herbivores (herbivore effect; $F_{3,167} = 19.7$, $p < 0.001$) and the two seasons (season effect; $F_{1,167} = 59.6$, $p < 0.001$; **Figure 2A**), with an interaction effect (interaction effect; $F_{3,167} = 5.6$, $p < 0.001$). Overall, in the wet season, animals ate 20% more species than in the dry season. For evenness we also found a significant effect of species (herbivore effect; $F_{3,167} = 3.5$, $p = 0.016$; **Figure 2B**). Evenness was significantly overall higher during the dry season than in the wet season (season effect; $F_{1,167} = 15.2$, $p < 0.001$), independently of the species identity (interaction effect; $F_{3,167} = 0.2$, $p = 0.882$). The Shannon diversity index was also significantly different between the four herbivores (herbivore effect; $F_{3,167} = 12.9$, $p < 0.001$; **Figure 2C**) but did not differ significantly per season (season effect; $F_{1,167} = 2.4$, $p = 0.120$) and the interaction effect was not significant (interaction effect; $F_{3,167} = 2$, $p = 0.115$).

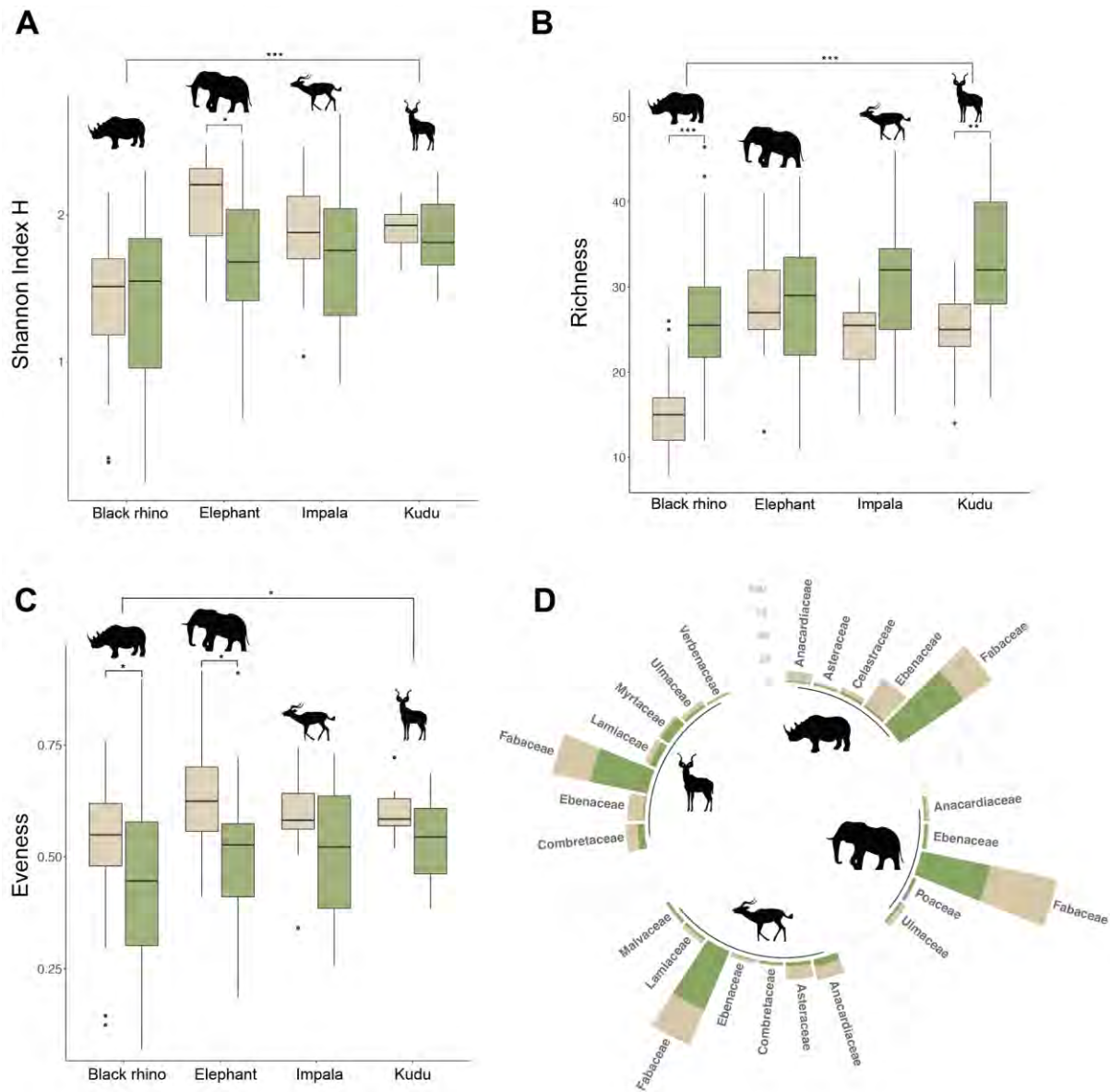


Figure 2. Dietary diversity for the four herbivores (in order: black rhino, elephant, impala, kudu). Shown are boxplot for species richness (A), evenness (B) and diversity (C) for each herbivore over seasons (dry season tan colour, wet season green colour). Asterisks denote significant differences among species or season (TukeyHSD, $p < 0.05$). Panel (D) represents a circular bar chart showing the proportion of plant families consumed (representing $\geq 1\%$ of total diet composition) across the two seasons (dry season tan colour, wet season green colour) for each herbivore species. Similar circular bar charts showing consumed ASVs and tribes (representing $\geq 1\%$ of total diet composition) are shown in Supporting Information - Chapter II (**Figure S1, S2, S3**).

Similar to the diversity results, the four herbivores' diet compositions, based on ASV composition, were significantly different from one species to another (**Figure 3**; herbivore effect; $F_{3,167} = 8.7$, $R^2 = 0.11$, $p = 0.001$), shifted from the wet to the dry season (**Figure 3**; season effect; $F_{1,167} = 26.6$, $R^2 = 0.11$, $p = 0.001$) and where the interaction effect was also significant (interaction effect; $F_{3,167} = 4.1$, $R^2 = 0.05$, $p = 0.001$).

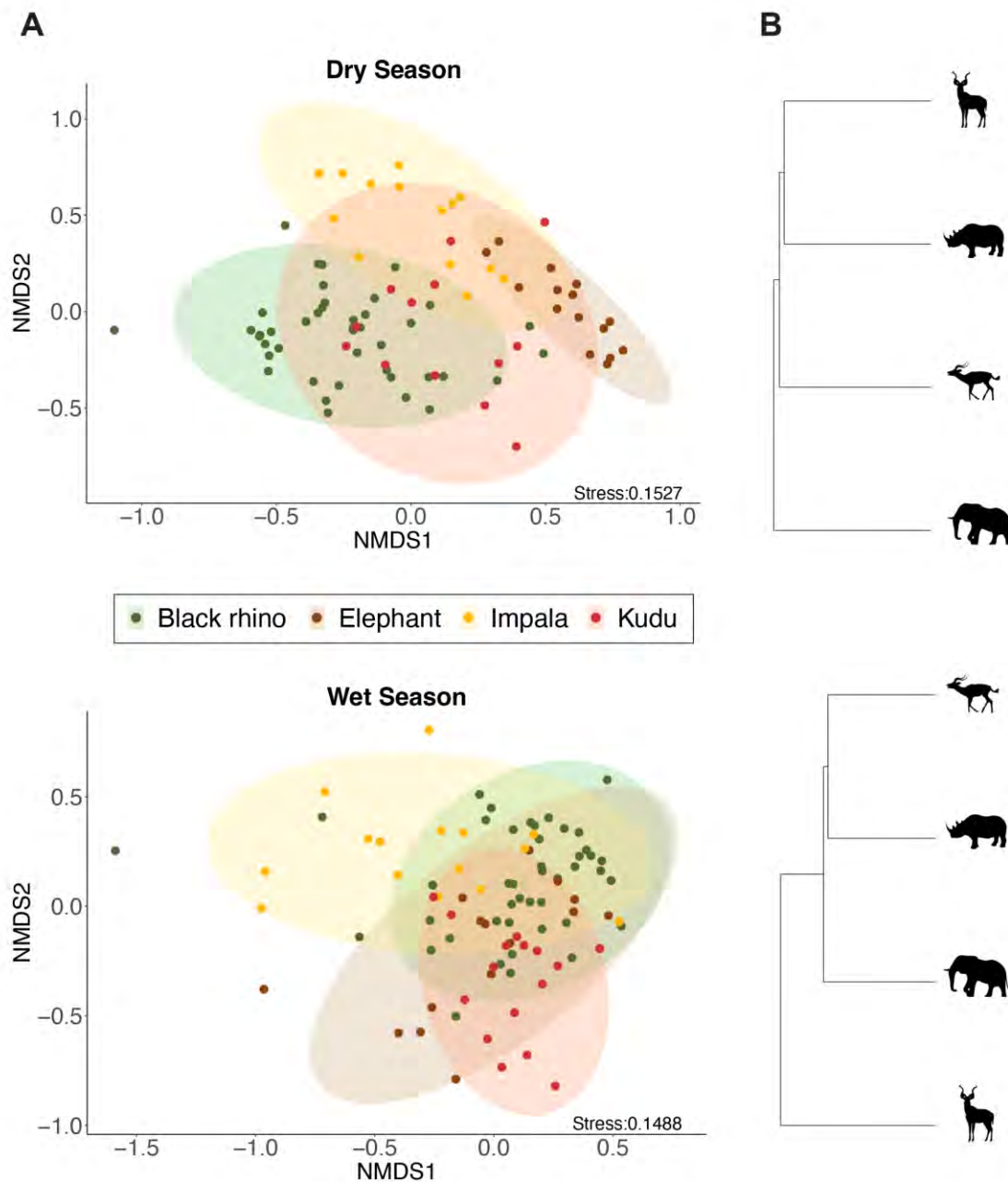


Figure 3. Diet overlaps between the four herbivores (black rhino, elephant, impala, kudu). (A) NMDS plot organizing dung samples with ASV composition based on Bray-Curtis dissimilarity for the dry season (top) and wet season (bottom). Ellipses represent 95% confidence intervals around each

species' data distribution. (B) Dendrograms based on Bray Curtis dissimilarity showing distance between herbivores diet, in the dry (top panel) and in the wet (bottom panel) season.

The niche overlap calculated with Pianka niche overlap indices among all unique pairs of species was of 65% during the dry season and of 84% during the wet season. In addition, they were both significantly lower than the simulated one ($p < 0.001$; Supporting Information – Chapter II **Table S2**), indicating non-random structuration of overlap (**Figure 3A**). In the dry season, overlap with black rhino was the highest with kudu (BC: 0.41, PO: 0.81), followed by impala (BC: 0.43, PO: 0.61) and elephant (BC: 0.54, PO: 0.48) (**Figure 3AB**; Supporting Information – Chapter II **Table S3**). In the wet season overall values shifted, in which, overlap with black rhino was the highest with impala (BC: 0.28, PO: 0.96), followed by elephant (BC: 0.29, PO: 0.94) and kudu (BC: 0.47, PO: 0.73) (**Figure 3A, B**; Supporting Information – Chapter II **Table S4**). Although overlap increased between strict browsers (black rhino and kudu) in the dry season (from wet 72% to dry 81%), diet composition among strict browsers still differed (herbivore effect; $F_{1,112} = 9.87$, $R^2 = 0.06$, $p = 0.001$) during both seasons (season effect; $F_{1,112} = 29.36$, $R^2 = 0.19$, $p = 0.001$) with a significant interaction effect (interaction effect; $F_{1,112} = 2.4$, $R^2 = 0.01$, $p = 0.025$). To the contrary, for mixed-feeders (elephant and impala) overlap decreased (from wet 89% to dry 63%) and remained significantly different (herbivore effect; $F_{1,55} = 8.03$, $R^2 = 0.11$, $p = 0.001$) seasonally (season effect; $F_{1,55} = 5.51$, $R^2 = 0.08$, $p = 0.001$) with a significant interaction effect (interaction effect; $F_{1,55} = 2.2$, $R^2 = 0.03$, $p = 0.025$). For both strategies the niche overlap was significantly lower than the simulated one ($p < 0.001$; Supporting Information – Chapter II **Table S5**, **Table S6**).

Overall, the five most utilized plant species by all four herbivores were the following: *Dichrostachys cinerea* (ASV001), *Acacia sp.* (ASV002), *Diospyros sp.* (ASV003), *Dalbergia sp.* (ASV004) and *Searsia sp.* (ASV005) (**Figure 4**, Supporting Information – Chapter II **Figure S1**). Their proportions varied over seasons, in particular *D. cinerea* in the wet season represented 52.36% of black rhino diet, 37.95% for elephant, 40.72% for impala and 18.54% for kudu; while in the dry season the proportion dropped to 18.21% for black rhino, 13.96% for elephant, 21.60% for impala and 12.89% for kudu (**Figure 4**).

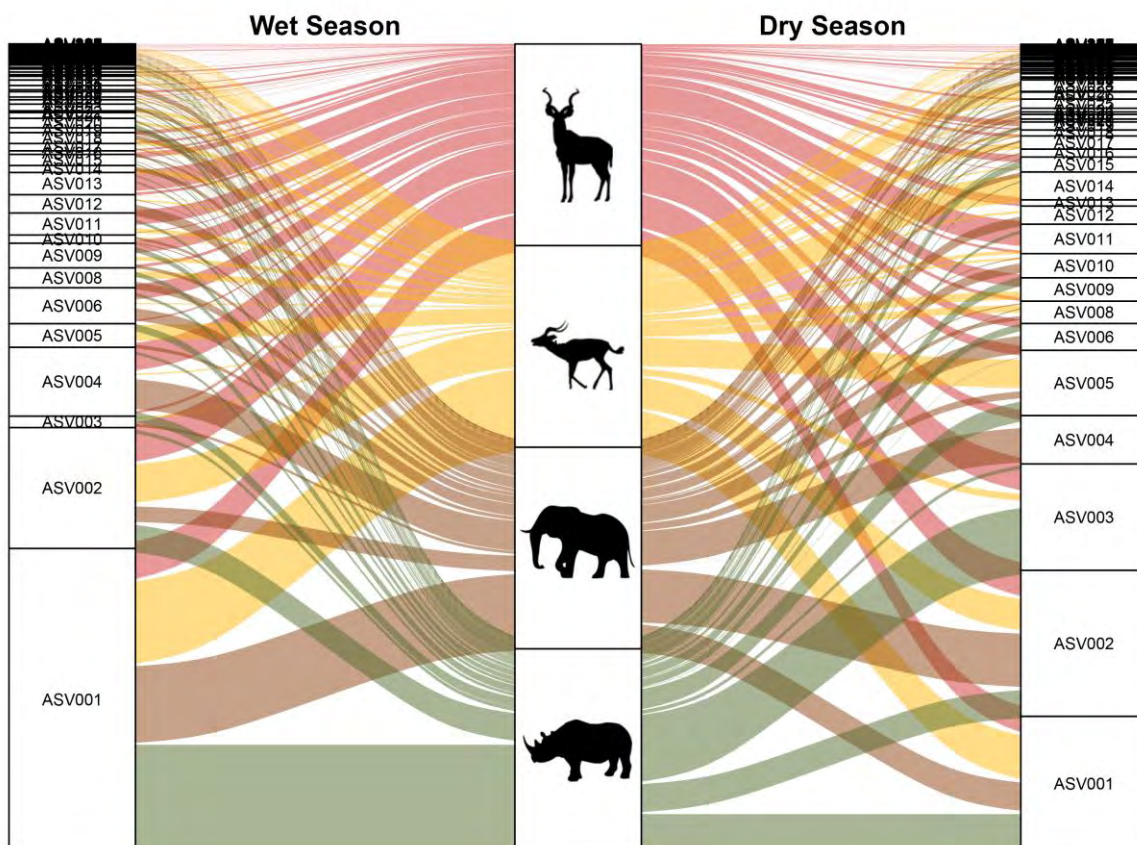


Figure 4. Two-way Riverplot depicting diet composition (percentage) and overlap of the most consumed ASVs by the four herbivores (from top to bottom: kudu, impala, elephant, black rhino). Lines connecting the center to the left shows the wet season's forage composition and overlap, while lines from center to right represent the dry season's forage composition and overlap. The four most consumed ASVs were *Dichrostachys cinerea* (ASV001), *Acacia sp.* (ASV002), *Diospyros sp.* (ASV003), *Dalbergia sp.* (ASV004) and *Searsia sp.* (ASV005).

Discussion

Mechanism of coexistence and competition are important to understand, particularly in fenced habitats, for optimal biological management of such areas. We here confirmed our hypothesis that sympatric herbivores eat variable plant species in differing proportions resulting in diet partitioning, fundamental to coexistence (Pansu *et al.*, 2022). Moreover, we show variable seasonal dietary strategies, likely depending on varying resources in time and space (Staver & Hempton, 2020). Furthermore, we observed that some species, such as mixed feeders, can adapt to local environmental conditions, and switch from grazing to browsing seasonally or annually.

Among the four herbivores, black rhino had the narrowest dietary niche almost solely composed of non-grass species. This finding is consistent with the fact that they are known to be extremely selective browsers (Muya & Oguge, 2000; Ganqa, Scogings & Raats, 2005; Duthé *et al.*, 2020). On the other hand, despite being considered a specialist browser (Kingdon & Hoffmann, 2013), the kudu had the widest dietary niche. Nonetheless, its diet was exclusively constituted of non-grass species implying that this species has a diverse diet in terms of trees, shrubs, and forbs. Moreover, we observed that elephants and impalas had broad dietary niches regarding species richness and diversity, which supports their classification as generalists (Wronski, 2003; Owen-Smith & Chafota, 2012). However, the proportion of consumed grass species for the mixed-feeders was very low in both seasons (Wet: elephants 5.8% and impala 2.3%; Dry: elephants 1.4% and impala 0.5%). In contrast, grazing has been found in some environments to average around 35% for elephant and 50% for impala (Codron *et al.*, 2006), and increasing in the wet season when grass species are more abundant and palatable (Mbatha & Ward, 2010). While grazing slightly increased in the wet season, browsing was clearly preferred in IGR throughout both seasons. Although IGR is composed of a mosaic of different habitats, the reserves holds largely more suitable habitats for browsing species than for grazing species, and mostly offers grass species of poor nutritious quality and very few grazing lawns (Gordijn, Rice & Ward, 2012). To this point, mixed-feeders such as elephant and impala which have the ability to locally adapt to their environment (Codron *et al.*, 2006; Staver & Hempson, 2020), shifted their diet accordingly.

Seasonal variation in resource abundance and quality coerce herbivores to adjust their diets according to environmental conditions (Owen-Smith, 1994; Staver & Hempson, 2020). Our results show a clear shift in diet for all four herbivores. Notably, richness, that is the number of different species consumed, decreased during the dry season where evenness increased. This can be explained, for one part, by the extensive presence of nutritious forbs in the wet season that are particularly represented by the diverse *Fabaceae* family (Singh *et al.*, 2017). Families such as *Ebenaceae* and *Anacardiaceae* were nearly solely consumed in the dry season because of the ability of these species to retain leaves and greenness throughout the year. However, these species are generally less palatable (Scogings *et al.*, 2015) particularly compared to *Fabaceae* species of the *Mimoseae* tribe, forcing the herbivores to broaden their diets and eat more to meet nutritional requirements (Owen-Smith, 1994). Hence, limited resources of low quality could mean that the studied herbivores eat less species but more evenly, as selectivity could not be afforded as time spent foraging had to be increased (Owen-Smith, 1994;

Macandza, Owen-Smith & Cross, 2004). Elephants, however, did not broaden their diet significantly in the dry season. This may be explained by their increased mobility and larger home ranges (Dutoit, 1990), and thus, their ability to mitigate resource depletion more successfully than smaller herbivores, by concentrating their foraging in whatever part of their larger range provides the best forage (O’Kane *et al.*, 2011).

The niche overlap theory predicts that overlap between coexisting species should increase when resources are limiting (Pianka, 1974). Yet, this was not observed in this context. To the contrary, niche overlap between the four herbivores was higher in periods of abundance (wet, 84%) and lower in periods of scarcity (dry, 65%); and was significantly lower than the null model simulation indicating structured feeding strategies according to concurring species. Stronger dietary segregation in the dry season has also been predicted and reported elsewhere (Gordon & Illius, 1989; Mysterud, 2000; Makhabu *et al.*, 2006; Kartzinel *et al.*, 2015), likely facilitating coexistence when resources are limited. However, this was not the case for the strict browsing species, black rhino and kudu, where overlap in the dry season increased between the pair. We could explain this observation by their more restrictive feeding strategy and less abundance in preferred browse. Nonetheless, diet composition remained significantly dissimilar, still indicating minimal encroachment on each other’s niches during the restrictive season. Conversely, high niche overlap suggests low competition and an abundance of forage permitting species sharing non-limiting resources (de Iongh *et al.*, 2011). Not only does plentiful resource allow for coexistence, but other feeding strategies, such as consuming different parts of plant species and vertical stratification, can also contribute to coexistence (Dutoit, 1990; O’Kane *et al.*, 2011; Potter *et al.*, 2022). For instance, a study in Hluhluwe-iMfolozi Park, South Africa, showed that elephants focused on leafy branches, kudus on end branches and buds, and impalas plucked nutritious shoots and leaves (O’Kane *et al.*, 2011). Species diversity in large herbivores can be explained by niche segregation where similar body size should be linked to similar digestive strategy (Redjadj *et al.*, 2014; Kartzinel *et al.*, 2015). However, it has been shown that body type and rumen type were poor predictors of diet composition and quality (Redjadj *et al.*, 2014). Within this framework, similarity of diet was highest between black rhino and the small-bodied impala in the wet season and with kudu in the dry season. From the megaherbivore perspective, elephant and black rhino diets were the most dissimilar when resources were limited in the dry season, and elephants narrowed their niche in terms of species browsed, as similarly observed by O’Kane *et al.* (2011). Overall, less

resources led to less niche overlap, regardless of body sizes, indicating feeding strategies structured according to concurring species.

In the last decades, we have witnessed a worldwide decline of specialist species, where generalist species have effectively replaced the latter (Clavel, Julliard & Devictor, 2011). Mixed feeders are the most abundant herbivores across savanna ecosystems, owing to their adaptive foraging ability (Staver & Hempson, 2020). Because of their high numbers, mixed feeders can have important effects on the vegetation and limit trees and shrubs (Staver & Hempson, 2020). Accordingly, elephants, for instance, have been identified as one of the major drivers of vegetation structure and change in savanna ecosystems (Owen-Smith *et al.*, 2019). By toppling, pollarding, uprooting, or debarking woody plants, the megaherbivore can be particularly destructive and potentially impact other browsers (Calenge *et al.*, 2002; Kohi *et al.*, 2011; Owen-Smith *et al.*, 2019). However, despite their size, smaller bodied mixed feeders may equivalently impact savanna vegetation structure (O’Kane *et al.*, 2012; Staver & Bond, 2014; Staver & Hempson, 2020). In particular, impalas were found to have a central role in woodland dynamics by heavily impacting seedlings, where impact intensity increased with impala density (O’Kane *et al.*, 2012). Where elephants generally select trees to damage based on species and height (Calenge *et al.*, 2002), in IGR their diet was most dissimilar to black rhino in the resource-limited dry season. Although a particular focus on elephants is traditionally given by management, our results highlight the important role of abundant smaller-bodied herbivores.

In light of these findings, we recommend particular attention be paid to abundant meso-herbivores when considering vegetation impact and regulating herbivore densities. Particularly for highly-managed and critically endangered species, such as black rhino, it is vital to consider seasonal niche dynamics. We thus advocate for the use of DNA-metabarcoding as a tool to provide sound and real time evidence, relative to present abiotic and biotic conditions, to correctly estimate actual diet breadth and characterize seasonal overlap within the herbivore guild. The latter will permit responsible adaptive management based on seasonal dietary strategies, which are central to regulating herbivore abundances (Staver & Hempson, 2020), and ultimately assist conserving increasingly threatened herbivore populations and complex ecosystems, such as savannas.

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Chapter III

Chapter III

Prickly preference: the role of plant morphological traits and volatiles as pre-ingestive cues for black rhino (*Diceros bicornis*) forage selection

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VD, SR and RvdW initiated the project. VD, OP, LP, MA collected and prepared samples. GR performed GC-MS analyses and VD, ED, OP, LP, and MA analyzed the processed data. VD wrote the first version of the manuscript.

Abstract

Large mammalian herbivores play crucial ecological roles through foraging in complex habitats. Foraging behaviour is governed by decisions at various scales and shaped by the perception of morphological and physiological properties of plants. Pre-ingestive cues allow differentiating and choosing between food items. Only few studies have focused on mammalian herbivores and the mechanism they employ to select forage. In particular, the critically endangered black rhinoceros (*Diceros bicornis*), a selective forager, has seldomly been considered. We investigated the morphological and chemical differences among highly preferred and avoided plant species by black rhino, by comparing seasonally various traits and volatile organic compounds measured by GC-MS. This study suggests that both morphological and olfactory cues are important for black rhino forage selection. Discriminant volatiles such as Caryophyllene and Hexenol acetate were found to be important across seasons but volatiles alone were not as robust in explaining choice of forage, particularly in the growing season. This study provides the first steps to disentangling factors driving black rhino choice and potential applications to conservation management. Considering the ability to utilise plant odour and morphological cues will enhance models pertaining to both the foraging behaviour of mammalian herbivores and the ecosystem consequences resulting from their foraging activities.

Keywords

Diceros bicornis, plant traits, volatile organic compounds, foraging cues, foraging ecology.

Introduction

Savanna ecosystems are characterized by the extensive presence of large mammalian herbivore species, which play crucial ecological roles (Owen-Smith, 1992; Olff, Ritchie & Prins, 2002; Cromsigt *et al.*, 2009; Ripple *et al.*, 2015). Immediate effects, such as trampling and consumption, drive vegetation succession and heterogeneity in these systems (Owen-Smith, 1992; Landman, Schoeman & Kerley, 2013; Ripple *et al.*, 2015). Correspondingly, plant resources constrain the distribution of herbivores of different sizes (Olff *et al.*, 2002). Thus, it is vital to consider large mammal foraging ecology for the management of habitats of conservation importance and critically endangered species.

Optimal foraging strategies influence the way herbivores use their landscape (Grant & Scholes, 2006) and factors that determine feeding patch selection involve principally forage quantity and quality (Belovsky, 1984; Senft *et al.*, 1987; Grant & Scholes, 2006). A prevailing characteristic observed in the distribution of forage is the inverse correlation between quality and quantity, where the most nutritious items tend to be less abundant (Van Beest *et al.*, 2010). Large herbivores are faced with the trade-off between spending time ingesting low-quality quantity and energy searching for less-common quality (Bergman *et al.*, 2001; Van Beest *et al.*, 2010). To optimise the search for quality and quantity, herbivores have developed various strategies to detect and select palatable species (Bell, 2012). Palatability represents an intricate process that combines pre-ingestive cues (Finnerty *et al.*, 2017), with the post-ingestive impacts of nutrients and toxins (Provenza, Kimball & Villalba, 2000).

Post-ingestive impacts consist of physiological effects perceived following the consumption of different forage (Provenza *et al.*, 2003). Nutritional, structural and chemical composition can affect digestibility and intake rate; and are frequently correlated with forage preference (Freeland & Janzen, 1974; Cooper & Owen-Smith, 1986; Shrader *et al.*, 2012; Schmitt *et al.*, 2018; Bester, Schmitt & Shrader, 2023). For instance, crude protein is important for nutrition (Ulappa *et al.*, 2014), lignin and fibre affect digestibility (Moore & Jung, 2001) and spines and thorns influence bite size and intake rate (Cooper & Owen-Smith, 1986; Wilson & Kerley, 2003). Secondary metabolites such as tannins, terpenes and alkaloids can cause toxicosis with symptoms such as bitter taste and nausea (Freeland & Janzen, 1974; Foley & Moore, 2005). Consequently, plant antiherbivore defences play an important role in determining forage

selection (Endara *et al.*, 2017) and while some of these characteristics serve as deterrents, herbivores have adapted to use them as cues.

Pre-ingestive cues consist of visual, gustatory and olfactory characteristics that permit differentiating between food items (Stutz *et al.*, 2016; Finnerty *et al.*, 2017; Bester *et al.*, 2023). As free-ranging herbivores move across complex sensorial landscapes, they are subject to varying morphological and physiological properties of plant species (Illius & Gordon, 1990; Finnerty *et al.*, 2017). Visual cues and haptic indicators, such as contrasts in colour, texture and plant architecture can assist detecting and selecting suitable foods (Duncan *et al.*, 2007; Castagneyrol *et al.*, 2013; Verdeny-Vilalta, Aluja & Casas, 2015; Finnerty *et al.*, 2017). For instance, greenness of leaves is often correlated with nutrition content and toughness with indigestibility (Duncan *et al.*, 2007). These signals, however, can easily be obstructed by other elements in the landscape (Stutz *et al.*, 2015). Because many plant toxins taste bitter, taste receptors are determinant of diet choice as they enable recognizing bitter compounds and toxic amino acids (Shi *et al.*, 2003; Foley & Moore, 2005); but requires frequent sampling. Olfaction is an important sense for many foraging herbivores (Bedoya-Pérez *et al.*, 2014; Finnerty *et al.*, 2017). Plants release elaborate scent patterns composed of volatile organic compounds (VOCs), for which some are also known as green leaf volatiles (Scala *et al.*, 2013) and detectable by foragers. Volatile compounds such as terpenes can be used to assess leaf quality (Bedoya-Pérez *et al.*, 2014) and distant or visually cryptic preferred forage (Stutz *et al.*, 2016; Schmitt *et al.*, 2018; McArthur *et al.*, 2019). Yet, finding palatable plants amongst an array of vegetation and background odours may be challenging (Stutz *et al.*, 2016), especially where odour can be affected by wind and temperature (Ninkovic, Markovic & Rensing, 2021). Due to the numerous scales at play, disentangling factors involved in detecting and selecting herbivorous forage remains an arduous task.

While many of the above aspects have extensively been studied for herbivorous insects (Bedoya-Pérez *et al.*, 2014; Finnerty *et al.*, 2017; Gélín *et al.*, 2023), studies focused on mammalian herbivores are relatively sparse. One of the least studied large mammals in this regard is the critically endangered black rhinoceros (*Diceros bicornis*). Notably, to improve conservation status, the species is highly managed through translocations to new habitats and meta-population management (Linklater *et al.*, 2006; Linklater & Swaisgood, 2008). Further understanding of black rhino foraging ecology is particularly important for the optimal management of the species and its accommodating habitats.

Here, we investigated the morphological and chemical differences among highly preferred and avoided plant species by black rhino by surveying feeding paths in Ithala Game Reserve, South Africa. We took into consideration seasonal dietary differences and measured plant morphological traits and VOCs through gas chromatography – mass spectrometry (GC-MS). We determined important variables potentially used as cues for the selection of foraged species. We hypothesized that preference would vary according to volatile organic compound profiles and morphological traits. With this study, we take the first steps in the disentanglement of factors driving black rhino forage selection and dietary preferences, and the broader meaning to conservation management and operations.

Material and Methods

Study site

The study was conducted in Ithala Game Reserve (IGR), situated in Northern KwaZulu-Natal (27°30'S, 31°25'E) in South Africa and of 296 km² of size. Long-term annual rainfall averages at 748 mm and the majority of it falls during the wet season (November-April). The reserve lies within the Savanna and Grassland biome and hosts 26 different types of vegetation communities (Van Rooyen & Van Rooyen, 2008), mainly consisting of grasslands, thickets, rocky/open/dense bushveld, woodlands, forests, riparian vegetation, cliffs, scarps and open disturbed patches. The reserve hosts an undisclosed (due to security reasons) number of black rhinoceros (*Diceros bicornis*).

Black rhino forage preference

To establish black rhino forage preference, browsed and avoided plant species were surveyed on feeding paths (Shrader *et al.*, 2012; Duthé *et al.*, 2020). Feeding black rhinos were located and identified by their ear notch combinations. To limit autocorrelation, feeding paths from the same individuals were sampled at a minimum of 24 hours apart. Transects were approximately 50 m long and 2 m wide. Tracks determined the start and the direction of transects, predominately backtracking the animal's feeding path. A waypoint was recorded for each transect with the use of a handheld Garmin e-trex 20 GPS. Transects were performed on each quadrat of the reserve. All woody plants, forbs, shrubs and trees with a maximum canopy height

of 2 m were recorded on the feeding path. Grasses were excluded as black rhinos are predominantly browsers (IUCN, 2011). Freshly browsed woody species are recognizable by the lighter color of the exposed wood, the wetness of remaining branches, and the characteristic way in which black rhinos browse. Black rhinos bite off large twigs in a pruning-shear manner attributable to the morphology of their hook shaped lips, leaving branches pruned at a clear 45-degree angle (Kotze & Zacharias, 1993; Shrader *et al.*, 2012; Duthé *et al.*, 2020). Along each transect, all species were then scored as browsed or non-browsed for estimating plant species selectivity; which was calculated based on Ivlev's electivity index $E=(ri-pi)/(ri+pi)$ (Strauss, 1979), where E is the measure of electivity per species, ri is the sum of browsed individuals of the same species and pi the relative abundance of the same species. The relative abundance of each species (pi) was calculated by dividing the number of times it was encountered by the sum of encounters of all species. Selectivity is given and ranked for each species as the index has a possible range of -1 to +1, with negative values indicating avoidance of the plant species, zero indicating random selection from the environment and positive values indicating active selection (Strauss, 1979). Because black rhino diet varies seasonally (Duthé *et al.*, 2020), preference was established for the dry season and the wet season according to the transects performed in each (N dry=48; N wet= 67). Based on this, abundant (commonly found in areas of black rhino presence) avoided and preferred plant species for each season were selected for functional trait and VOCs analysis (Supporting Information – Chapter III **Figure S1**).

Functional traits

Based on methodology developed by Pérez-Harguindeguy *et al.* (Pérez-Harguindeguy *et al.*, 2016), the following plant functional traits were measured: plant height, branching architecture, leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), toughness and spinescence. Furthermore, we measured chlorophyll, carbon and nitrogen content, as well as the carbon to nitrogen ratio (C/N). At least 5 individuals per species were selected in the field (across all available habitats) for measure in each season (dry: July-September; wet: January-March). When possible, young but fully expanded leaves were measured for leaf traits as they are more photosynthetically active. Leaves affected by pathogens or traces of herbivory were excluded when possible. *Plant height*, measured in centimeters, represents the shortest distance between the ground level and the highest main photosynthetic tissues (excluding inflorescences). A retracting metal tape or a meterstick were used for measure and some bigger trees required height estimation (Pérez-Harguindeguy *et al.*, 2016). *Branching architecture*

refers to how intensively a plant is ramified. This value is measured as the number of ramifications per branch, which is demarcated by a leafless base and presenting secondary branches with leaves. *Leaf area (LA)* is defined as the one-sided surface of a leaf. This value is measured in square millimeters (Pérez-Harguindeguy *et al.*, 2016). The area of five leaves per individual was measured with a portable scanner (AOZBZ® A4 portable scanner 900dpi) and was calculated using a binary threshold on ImageJ (Schneider, Rasband & Eliceiri, 2012). *Specific Leaf Area (SLA)* is the one-sided area of a fresh leaf, divided by its dry mass. This value is measured in square millimeters per milligram (Pérez-Harguindeguy *et al.*, 2016). The mass of each scanned leaf was measured using a precision scale (Kern & Sohn PCB350-3® and Sartorius Secura® 224-1s). *Leaf dry-matter content (LDMC)* is the dry mass of a leaf, divided by its fresh mass. This value was measured in milligrams per gram (Pérez-Harguindeguy *et al.* 2016). *Toughness* or force to punch is the resistance of the leaf tissues to rupture on a reduced contact area (Pérez-Harguindeguy *et al.*, 2016). This value is measured in milligrams per millimeter. To perform this measurement, the leaf is trapped in a mechanism composed of a plexiglass plate and a wooden board bearing holes. Pressure is then applied with a spring dynamometer with a metal tip until the leaf tissue broke. This measurement was always performed on hydrated leaves and veins were avoided. *Leaf chlorophyll* was measured on site, using an optical leaf analyzer (ForceA Dualex®). An average of 3 measures on different spots of the same leaf was performed. *Total leaf carbon (C), hydrogen (H), and nitrogen (N)*, and the subsequently generated *carbon-to-nitrogen ratio (C/N)* was measured on dry leaf material. Samples were milled with a vibro-grinder and steel balls (Retsch® MM400). The powder obtained was then weighed with a precision scale and placed in dedicated tin capsules. Analyses were performed with a standard elemental analyzer (Thermo scientific™ Flash 2000 CHNS/O Analyzer).

Volatile organic compounds (VOCs)

For each plant species, five replicates were sampled in different locations of IGR in both the wet season (January-March) and dry season (July-September). Furthermore, three control replicates without a plant were also collected. In the field, VOCs compounds were collected using small ORBOTM activated charcoal filter connected via a PVC hose to a low flow pocket pump (SKC Pocket Pump). An oven roasting bag (250 mm x 440 mm), made of nalophan, was used to isolate a branch of the plant with some leaves and secured with plastic lock ties. This collection method is also known by with the name of dynamic headspace (Raguso & Pellmyr,

1998). The pump was set on a flow of 200 mL/min. Volatiles were directly pumped for about an hour and a half without any time for accumulation. After collection, the filters were stored in individual zip lock bags and stored during at 5°C, in the fridge, until the day of the extraction. The VOCs collected in the filters were then extracted in the lab for analysis via GC-MS. VOCs were extracted by breaking one side of the filter and eliminating the first part of the charcoal and the synthetic foam. In the white cotton filter, 2 µL of tetralin was injected at a concentration of 9.7 ng/µL as an internal standard. The filter was then rinsed twice with 200 µL of ultra-pure dichloromethane. Finally, 250 µL of the solution obtained from the washing was transferred to a GC vial with an insert. The GC-MS analysis of the VOCs extracts was performed by an Agilent 8890 gas chromatograph coupled with an Agilent 5977B Mass Selective Detector (MSB). A 1.5 µl aliquot of the supernatant was injected in pulsed splitless mode in the Front SS Inlet set to 250 °C with a pressure of 5.9529 psi. Based on electricity impact, the Agilent 5977B Mass Selective Detector (MSD) was set to 280°C. The collected samples mechanically injected in an Agilent HP-5MS UI (Ultra Inert) column (5%-phenyl-methylpolysiloxane), 30 m x 250 µm x 0.25 µm coating thickness, fused silica capillary column. Helium was used as a carrier gas at a constant flow rate of 0.9 mL/min. In the oven, the temperature program was a linear gradient from 40 °C to 270 °C at a rate of 7 °C/min. The raw data were imported to MzMine 2.53 for peak picking. Feature detection was performed using noise levels for MS of 1000. The parameters for ADAP chromatogram builder were: minimum group size in number of scans 5, group intensity threshold 1000, minimum height intensity 500, m/z tolerance 0.015 Da or 15 ppm. The chromatogram deconvolution algorithm used was wavelets (ADAP) and parameters were: threshold 7, minimum feature height 1000, coefficient/area threshold 30, peak duration range 0.02-1.00 min, RT wavelet range 0.00-0.02, m/z center calculation median. The isotope peak grouper parameters were: m/z tolerance 0.015 Da or 15 ppm, retention time tolerance 0.07, maximum number of charges 2, most intense isotope. The join aligner parameters were: m/z tolerance 0.015 Da or 15 ppm, retention time tolerance 0.1, weight for m/z 50, weight for RT 50. No gap-filling was applied. Preliminary identification of compounds in each sample was conducted and coupled with retention time data based on the library NIST 2011 and NIST 17 MS Database with MS Search Program v.2.3 ("NIST Standard Reference Database 1A," 2014).

Statistical analyses

All statistical analyses were performed with R version 4.1.2. For each season, plant traits were plotted for comparison via Principal Component Analysis (PCA) using the package *factoextra*

(Kassambara & Mundt, 2017) and via nonmetric multidimensional scaling (NMDS) ordinations based on Bray-Curtis dissimilarity using the *vegan* package (Oksanen *et al.*, 2020). A permutational multivariate analysis of variance (perMANOVA) with 999 permutations, using the *adonis2* function in *vegan* was performed to test whether traits with species effect could predict preference category (avoided/preferred). Next, a machine learning approach was used to discriminate important traits determining browse category using a Random Forest (RF) with the package *randomForest* (Breiman, 2001) by test and train. Boxplots for the most important variables were then computed and one-way ANOVAs performed with the *aov* function to compare and test differences among avoided and preferred species. When necessary, variables were either log- or square root-transformed to meet homoscedasticity assumptions. For variables that could not meet homoscedasticity assumptions after transformation, a Kruskal-Wallis rank sum test using the *kruskal.test* function was performed. The same process and methodology were applied to VOCs datasets for each season. Next, we tested whether variation among traits and variation among VOCs were correlated in each season with a Mantel test with the function *mantel.rtest* of the *ade4* package (Dray & Dufour, 2007). Additionally, a permutational multivariate analysis of variance (perMANOVA) with 999 permutations, using the *adonis2* function in *vegan* was performed to test whether traits combined with VOCs could predict preference category (avoided/preferred). Finally, considering VOCs and traits together a Random Forest with the *randomForest* package was performed to determine the most discriminant variables for each season. Based on the Random Forest, decision trees were plotted to illustrate factors and mechanisms influencing black rhino choice of forage with the *getTree* function.

Results

Traits

Plant traits in the wet season displayed a clear shift (**Figure 1A**) and were significantly different among preference categories avoided and preferred (preference category effect; $F_{1,43} = 12.2$, $p < 0.001$), and species (species effect; $F_{13,43} = 11.4$, $p < 0.001$). The test classification accuracy of the random forest model was 72.5%. The 3 most important predictors of preference category were SLA, LDMC and spinescence (**Figure 1B**). SLA was significantly higher in preferred than avoided species (log-transformed; $F_{1,56} = 4.1$, $p < 0.001$; **Figure 1Ca**), as was spinescence

(Kruskal-Wallis; $X^2=16.097$; $df= 3$; $p\text{-value}= 0.001$; **Figure 1Cc**) and LDMC was higher in avoided than preferred species ($F_{1,56} = 9.7$, $p < 0.001$; **Figure 1Cb**).

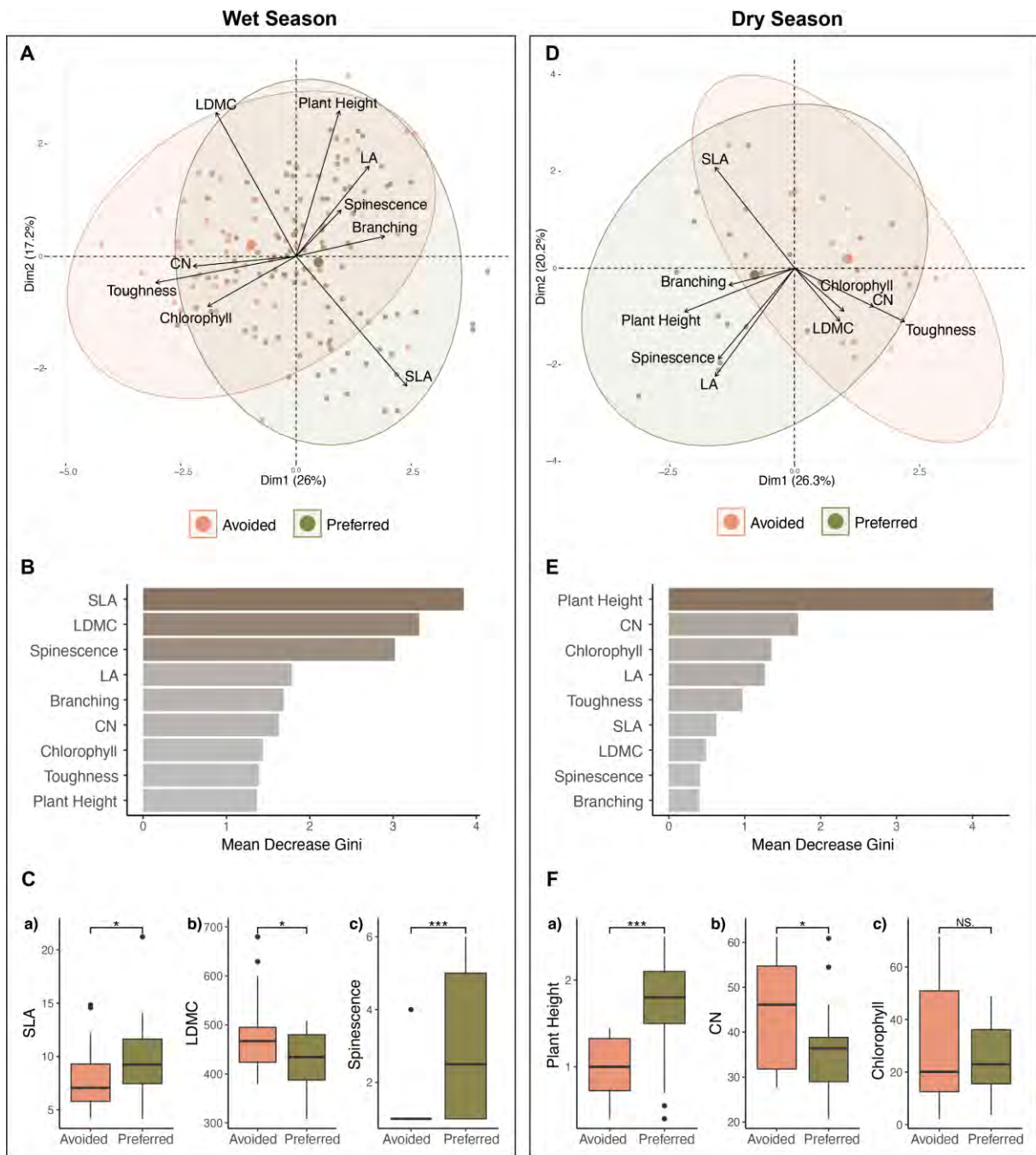


Figure 1. Analysis of traits of plant species avoided and preferred by black rhino. (A) Principal Component Analysis (PCA) biplot organizing avoided (salmon-colour) and preferred plants (khaki-colour) by measured traits for the wet season (A) and dry season (D); ellipses represent 95% confidence intervals around each group’s data distribution. Importance plots derived from Random Forest model for the wet season (B) and dry season (E); traits are ordered by importance based on mean decrease Gini, with the most important at the top of the figure. Boxplots of three most important traits comparing for wet

season (C) and dry season (F) of avoided and preferred species; significance asterisks show significance based on one-way ANOVAs.

Plant traits in the dry season displayed a clear shift (**Figure 1D**) and were significantly different among preference categories avoided and preferred (preference category effect; $F_{1,26} = 8$, $p < 0.001$), and species (species effect; $F_{7,26} = 7.1$, $p < 0.001$). The test classification accuracy of the random forest model was 80%. The 3 most important predictors of preference category were plant height, C/N and chlorophyll (**Figure 1E**). Plant height was significantly higher in preferred species than avoided ones ($F_{1,33} = 17$, $p < 0.001$; **Figure 1Fa**), C/N was significantly higher in avoided species (log-transformed; $F_{1,33} = 6.2$, $p = 0.018$; **Figure 1Fb**), and chlorophyll was non-significantly higher in preferred species than avoided ones (square root-transformed; $F_{1,33} = 0.1$, $p = 0.781$; **Figure 1Fc**).

VOCs

Plant VOCs in the wet season overlapped considerably (**Figure 2A**) and were not significantly different between avoided and preferred species (preference category effect; $F_{1,43} = 0.7$, $p = 0.72$), although species-specific differences were observed (species effect; $F_{13,43} = 1.9$, $p < 0.001$). The test classification accuracy of the random forest model was 37.5%. The 3 most important predictors of preference category were Caryophyllene, Hexenol acetate (X3-Hexen-1-ol-acetate) and D-Limonene (**Figure 2B**). Caryophyllene was not significantly different in preferred and avoided species (log-transformed; $F_{1,32} = 3.7$, $p = 0.063$; **Figure 2Ca**), as was Hexenol acetate (log-transformed; $F_{1,56} = 0.1$, $p = 0.717$; **Figure 2Cb**), and D-Limonene (square root-transformed; $F_{1,56} = 0.02$, $p = 0.902$; **Figure 2Cb**).

VOCs in the dry season displayed a shift (**Figure 2D**) and were significantly different among preference categories avoided and preferred (preference category effect; $F_{1,26} = 3.3$, $p < 0.001$), but was between species (species effect; $F_{7,26} = 1.9$, $p < 0.001$). The test classification accuracy of the random forest model was 66%. The 3 most important predictors of preference category were Caryophyllene, Hexenol acetate (X3-Hexen-1-ol-acetate) and alpha-Pinene (**Figure 2E**). Caryophyllene was significantly higher in preferred species than avoided ones (log-transformed; $F_{1,23} = 21.1$, $p < 0.001$; **Figure 2Fa**), Hexenol acetate was not significantly

different in preferred and avoided species (log-transformed; $F_{1,24} = 4.1$, $p = 0.053$; **Figure 2Fb**),) as was alpha-Pinene (log-transformed; $F_{1,14} = 3.4$, $p = 0.088$; **Figure 2Fc**).

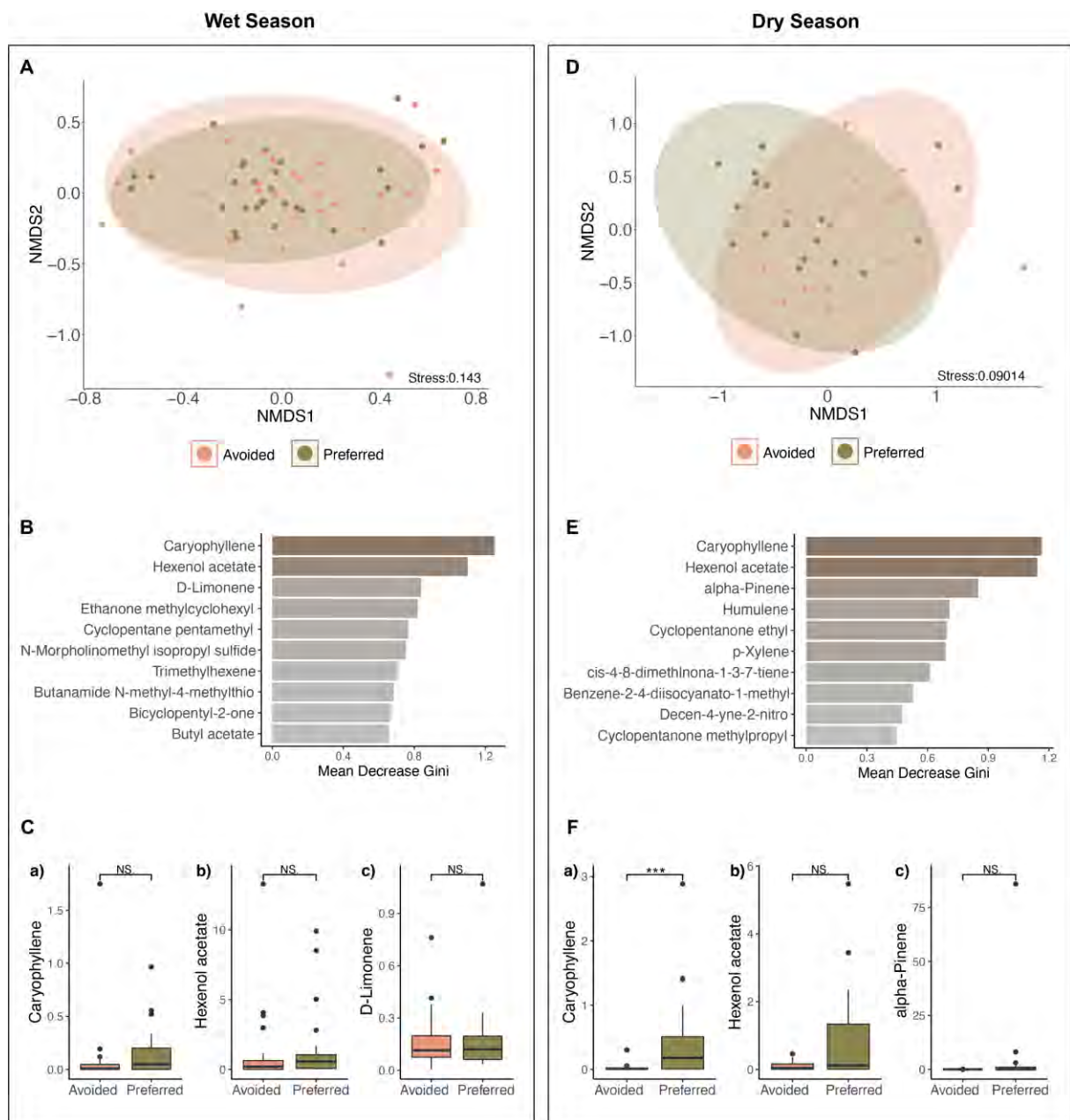


Figure 2. Analysis of VOCs of avoided and preferred plant species. (A) NMDS organizing avoided (salmon-colour) and preferred plants (khaki-colour) by measured VOCs for the wet season (A) and dry season (D); ellipses represent 95% confidence intervals around each group's data distribution. Importance plots derived from Random Forest model for the wet season (B) and dry season (E); VOCs are ordered by importance based on mean decrease Gini, with the most important at the top of the figure. Boxplots of three most important VOCs comparing for wet season (C) and dry season (F) of avoided and preferred species; significance asterisks show significance based on one-way ANOVAs.

Traits and VOCs

In the wet season, the Mantel test indicated a correlation between traits and VOCs (Monte-Carlo test; Obs = 0.22; $p = 0.007$). Variables were significantly different among preference categories avoided and preferred (preference category effect; $F_{1,43} = 12$, $p < 0.001$), and between species (species effect; $F_{13,43} = 11.3$, $p < 0.001$). The test classification accuracy of the random forest model was 77%. The 5 most important predictors of preference category were SLA, LDMC, Caryophyllene, C/N and Hexenol acetate (X3-Hexen-1-ol-acetate) (**Figure 3A**). SLA, Caryophyllene and Hexenol acetate appear to be determinant in the early branches of the illustrated decision tree consequently generated by the Random Forest model (**Figure 3B**).

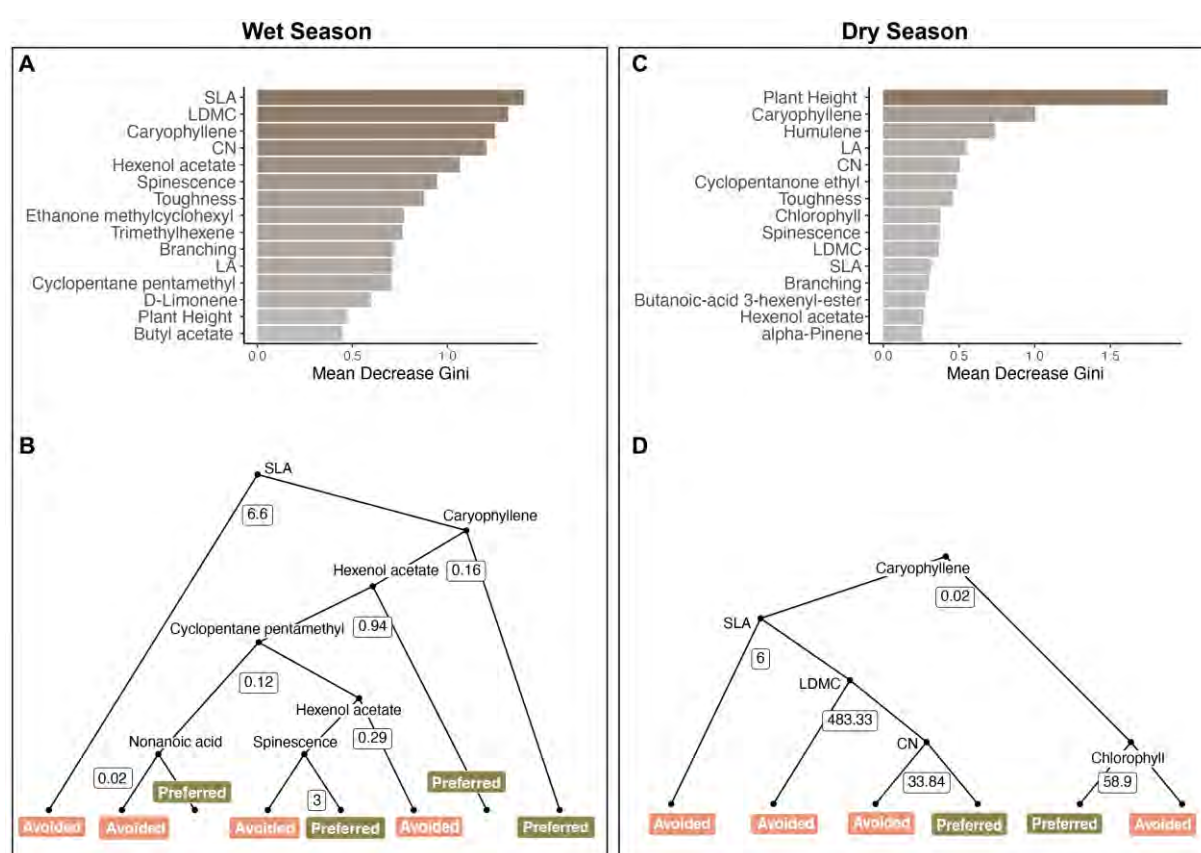


Figure 3. Analysis of the combination of traits and VOCs of avoided and preferred species. Importance plots derived from Random Forest model for the wet season (A) and dry season (C); variables are ordered by importance based on mean decrease Gini, with the most important at the top of the figure. Decision trees generated from Random Forest model for the wet season (B) and dry season (D); leading to preference categories (avoided in salmon, preferred in khaki), where boxed numbers show unscaled equal or above values for branch direction.

In the dry season, the Mantel test indicated no correlation between traits and VOCs (Monte-Carlo test; Obs = -0.06; $p = 0.824$). Variables were significantly different among preference categories avoided and preferred (preference category effect; $F_{1,26} = 7.9$, $p < 0.001$), and between species (species effect; $F_{7,26} = 7.1$, $p < 0.001$). The test classification accuracy of the random forest model was 91.3%. The 5 most important predictors of preference category were plant height, Caryophyllene, Humulene, LA and C/N (**Figure 3C**). Caryophyllene, SLA and Chlorophyll appear to be determinant in the early branches of the illustrated decision tree consequently generated by the Random Forest model (**Figure 3D**).

Discussion

Black rhinos are selective herbivores for which forage preference varies through seasons due to varying availability of preferred resources (Duthé *et al.*, 2020; Chapter II). Our study suggests that black rhinos use a combination of morphological traits and volatile organic compounds as foraging cues. Discriminant traits vary seasonally in rank of importance but remain efficient in predicting preference in both seasons. Discriminant volatiles such as Caryophyllene and Hexenol acetate were found to be important across seasons but VOCs alone were not as robust in explaining choice of forage, in particular in the wet season. The combination of traits and VOCs in both seasons can explain preference more effectively than separated, where overall important components in both groups seem to play key roles in foraging cues and decisions.

Prickly preference

Plant traits have been found to be largely associated with resistance to herbivory and influencing selective foraging (Mattson, 1980; White, 1984; Carmona, Lajeunesse & Johnson, 2011). Traits such as C/N, SLA and LDMC are commonly related to palatability (Schädler *et al.*, 2003; Hall *et al.*, 2020). Increased SLA, as observed here in the preferred species, can be linked to an increase in nutrients and reduced toughness, making these species more attractive to chewing herbivores (Descombes *et al.*, 2020). Relatedly, preferred plants also exhibited a lower C/N ratio, indicating higher Nitrogen content, a limiting nutrient to many herbivores (Mattson, 1980); and lower LDMC, which is associated to reduced toughness (Blumenthal *et al.*, 2020).

Although preferred species were characterised by palatable traits, many were spinescent bearing large thorns or spines. Spinescence reduces leaf and stem removal by vertebrate herbivores by lessening access to edible parts such as leaves and shoots (Cooper & Owen-Smith, 1986; Tomlinson *et al.*, 2016). Spinescence first appeared as a mechanism of defence in savannas when large mammalian herbivores became abundant (Charles-Dominique *et al.*, 2016). While spinescence reduces bite size for many browsing mammals including black rhino (Wilson & Kerley, 2003), it does not deter them (Scogings, Demmer & Hattas, 2021). Black rhino seem to be particularly well equipped to mitigate this defence, where bite size is based purely on mouth morphology (Demment & Greenwood, 1988) and the trade-off of spending more time browsing for higher nutritional gain is worth-while (Wilson & Kerley, 2003; Tomlinson *et al.*, 2016; Wigley, Fritz & Coetsee, 2018). In relation to seasonal conditions, plant height and chlorophyll were particularly discriminant in the dry season. This suggests that black rhino select taller woody species that are more tolerant to drought and can maintain green leaves; as species with low wood density and high specific leaf area typically suffer greater mortality during droughts (Anderegg *et al.*, 2016; Greenwood *et al.*, 2017; Sankaran, 2019).

Follow your nose

Large herbivores make foraging decisions across several scales to select food and maximise nutrient intake (Senft *et al.*, 1987; Owen-Smith, Fryxell & Merrill, 2010). Finding and selecting preferred forage amongst an array of species can be facilitated with the use of plant odours (Bedoya-Pérez *et al.*, 2014). For instance, elephants have been found to use plant odours as cues to select preferred plants (Schmitt *et al.*, 2018; McArthur *et al.*, 2019). Our findings suggest that this could also be the case for black rhino as plant species were distinguishable by odour in both seasons, and that in the dry season black rhino preference categories were discernible based on emitted VOCs. That the wet season did not produce a significant difference between preference categories, may be due to increased noise with a larger array of species in the growing season; a larger breadth of black rhino diet (Supporting Information - Chapter III **Figure S1, Chapter II**) with an increased inclusion of forbs (Anderson *et al.*, 2018; Duthé *et al.*, 2020) not considered in this study, or higher plant quality across species necessitating lower selectivity (Bester *et al.*, 2023). Caryophyllene is an odoriferous sesquiterpene that activates both olfactory and non-olfactory receptors (Koyama *et al.*, 2019). The terpene has also been found to be a major discriminant component among plants familiar to elephants (McArthur *et al.*, 2019) and an important VOC in plant-insect interactions through its role of attraction for

predators of herbivorous insects (Rasmann *et al.*, 2005; Zhang *et al.*, 2020). Another discriminant terpene, Hexenol acetate, also commonly described as a “green leaf volatile” (Yue *et al.*, 2001; Jahangir *et al.*, 2009), has been shown to be attractive to various insects (Lwande *et al.*, 1989; Yue *et al.*, 2001) and is often dominant in nutritious plant species (Arey *et al.*, 1991). This aromatic monoterpene is extensively used in the perfume industry to produce a “fruity-green” odour and taste (García-Garvía *et al.*, 2023). Yet, only Caryophyllene was emitted at significantly higher relative concentrations by preferred plants, suggesting a role in signaling and attraction for black rhino. D-Limonene and alpha-Pinene, which are also strongly odorous monoterpenes, have previously been flagged as potential feeding cues VOCs (Skopec, Adams & Muir, 2019). However due to no significant difference in concentration between preferred and avoided species, it remains unclear whether the latter act as deterrents or attractants or truly have a role in selection. Furthermore, Bester (2023) recently demonstrated that elephants avoided most monoterpenes at high concentrations except for alpha-pinene (Bester *et al.*, 2023), as opposed to goats that preferred *Pistachia lentiscus* shrubs dominated by alpha-Pinene rather than Limonene (Navon *et al.*, 2020).

The combination of traits and VOCs best predicts preference

Because soil moisture and precipitation levels affect VOC emission and physiology (Liu *et al.*, 2017; Simin *et al.*, 2022) and that plant traits are closely linked to VOCs (Dudareva & Pichersky, 2008), it is not surprising that in the growing season (wet season) our results show a correlation between the two. Combining plant traits and volatiles markedly improved the model in both seasons, predicting preference more effectively. The perception of plant odours may be tied to preference as a result of associating food flavours and a conditioned response to past post-ingestive consequences (Duncan *et al.*, 2007; Carmona *et al.*, 2011; Villalba *et al.*, 2015). As such, phenols have been presented as the main post-ingestion drivers of large mammal diet (Nobler *et al.*, 2019; Scogings *et al.*, 2021). Yet, post-ingestive effects work in concert with a range of other factors that assist herbivores in forage selection (Villalba & Provenza, 2000; Duncan *et al.*, 2007). For instance, swamp wallabies (*Wallabia bicolor*) use a combination of visual and olfactory cues to locate preferred forage (Stutz *et al.*, 2016; Finnerty *et al.*, 2017). White-throated woodrats (*Neotoma albigua*), that specialise on cactus, use spinescence as a “rule of thumb” indicator of nutritional quality (Kohl, Miller & Dearing, 2015). Overall, traits seem to better explain preference and have been found to be better predictors of herbivore susceptibility than secondary metabolites (Carmona *et al.*, 2011), possibly owing to

a higher level of conservatism among species than VOCs. However, from a mechanistic approach, plant odour can be detected from a greater distance than visual cues (Bell, 2012; Stutz *et al.*, 2016). In particular, species with poor sight and a keen sense of smell, such as black rhino (Linklater, Mayer & Swaisgood, 2013), may use plant odours to reduce search time and energy expenditure while foraging within and between distant patches (Bell, 2012; Orlando *et al.*, 2020). Black rhinos likely use a combination of volatiles and traits to detect and choose across multiple spatial scales. In particular, volatiles of importance, such as Caryophyllene and Hexenol acetate, often appear determinant in the early branches of the decision trees generated by the Random Forest model. Black rhinos may select specific patches based on volatile cues and successively base their choice on morphological characteristics. That in the wet season volatiles alone could not predict choice, possibly due to noise, may be related to the abundance of green forage and consequently no necessity to intensively search for and between patches. In this scenario, traits are perhaps particularly important for selection, where black rhinos may apply “rule of thumb” associated from previous post-ingestive consequences. Whether nutritious spinescent species are selected due to their spines, their odour or as a learnt choice because of their increased nutrient levels, is yet to be determined. Further experimental studies, particularly choice tests where black rhinos are presented with different forage and treatments, are necessary to ascertain the precise mechanism related to forage selection and disentangle the utilization of traits and volatiles as deterrents or attractants.

Conservation implications

Black rhino conservation efforts have relied significantly on the use of translocations for meta-population management (Linklater *et al.*, 2006; Linklater & Swaisgood, 2008) and expanding the species’ range. Accurate knowledge of black rhino foraging ecology is crucial not only for making informed decisions regarding current and potential new habitats but also for facilitating operations. Notably, further understanding of the mechanism of forage selection is important for three reasons. Firstly, in the context of estimating new habitat suitability and carrying capacity calculations, particularly when diet preferences are unknown for a given area, preliminary assumptions can be derived from easily measurable plant traits and known odour profiles of similar abundant species.

Second, because black rhino are usually accommodated in enclosures (bomas) until transport for release into new habitats (Linklater *et al.*, 2006), dietary preferences and selection are

particularly important for successful adjustment. The animals are fed freshly cut browse, lucerne hay and occasionally supplemented with pelleted domestic ungulate food. While most accommodate to feeding in temporary captivity, empirical evidence shows that some individuals have difficulty eating, sometimes related to habitat of origin. Forage intake could be assisted by manipulating available feed odour and appearance by dousing with discriminant volatiles. Additionally, iron storage disease (hemosiderosis) is frequently documented in long-term captive black rhino (Paglia & Tsu, 2012), particularly when suitable fresh forage is not available. Diets should be established based on multiple criteria (traits, nutrients, secondary chemistry), more inclusive of the characteristics of wild foraged species. Improved dietary knowledge will pave the way for best practices regarding feeding in the bomas and in captivity.

Lastly, for the management of protected areas where vegetation is central, taking into consideration the ability to utilise plant odour and morphological cues will enhance models pertaining to both the foraging behaviour of mammalian herbivores and the ecosystem consequences resulting from their foraging activities.

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Chapter IV

Chapter IV

Reductions in home-range size and social interactions among dehorned black rhinoceroses (*Diceros bicornis*)

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Author Contributions

VD, KO, SR, and ED conceived the ideas. KO, RV, SN, BC, SB, MV, MP, PN, CH, CK spearheaded the dehorning campaigns and provided the data and details of each reserve. VD gathered all the data. EM and VD analysed the dataset. VD, EM, TW and SR led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Abstract

Poaching for horns and tusks is driving declines of megaherbivores worldwide, including the critically endangered African black rhinoceros (*Diceros bicornis*). By proactively dehorning entire rhinoceros populations, conservationists aim to deter poaching and prevent species loss. However, such conservation interventions may have hidden and underestimated effects on animals' behaviour and ecology. Here, we combine >15 years of black rhino monitoring data across 10 South African game reserves, comprising >24,000 sightings of 368 individuals, to determine the consequences of dehorning for black rhino space use and social interactions. While preventative dehorning at these reserves coincided with a nationwide decrease in black rhino mortality from poaching and did not infer increased natural mortality, de-horned black rhinos decreased their home range area by, on average, 11.7 km² (45.5%) and were 37% less likely to engage in social encounters. We conclude that dehorning black rhinos as an anti-poaching measure alters their behavioural ecology, although the potential population-level effects of these changes remain to be determined.

Keywords

Dehorning, conservation, rhinoceros, poaching, home range.

Introduction

The trade of mammal species that possess anatomical features of high value to humans, such as tusks and horns, fuels a multibillion-dollar illicit wildlife industry (Wasser *et al.*, 2018; Scheffers *et al.*, 2019) that poses an immediate threat to the persistence of megafauna globally (Cardillo *et al.*, 2005; Biggs *et al.*, 2013; Ripple *et al.*, 2015; Eikelboom *et al.*, 2020). Rhino horns are sought for cultural and medicinal beliefs (Cheung *et al.*, 2021) throughout south-east Asia, to the extent that they are estimated to be worth \$65,000 kg⁻¹ – more than diamonds or gold (Biggs *et al.*, 2013). Such high demand for rhino horns has encouraged intensive poaching, leading to a >98% decline in numbers of the critically endangered black rhinoceros (*Diceros bicornis* L.) since the 1960s (**Figure 1A**) (Biggs *et al.*, 2013; Eikelboom *et al.*, 2020). Indeed, only ~5,000 black rhino individuals now remain on Earth (IUCN, 2020). In an attempt to curb the poaching crisis and safeguard animals, conservation managers have responded by introducing militarized law enforcement of protected areas (Biggs *et al.*, 2013; Challender & MacMillan, 2014; Muntifering *et al.*, 2017). However, continuing pressure of organized crime over the past two decades has caused many reserves to turn to a short-term solution of dehorning rhinos, which aims to deter poaching by removing the reward (Lee & Roberts, 2016; Rubino & Pienaar, 2018). While the dehorning approach is heralded as a promising tool for the protection of rhino, and has been adopted widely throughout southern Africa, little is known about its implications for black rhino ecology or behaviour (Lindsey & Taylor, 2011).

Black rhinos are solitary animals and live within delimited home ranges (Mitchell & Powell, 2012) that vary over space and time due to changes in resource availability and population demography (Plotz *et al.*, 2016). Black rhinos are thought to be both polygynous and polyandrous (Goddard, 1967; Owen-Smith, 1988; Garnier, Bruford & Goossens, 2001), where dominant males mate with multiple females and females mate with multiple males. Home ranges in both sexes are determined by social interactions (Lent & Fike, 2003), which involve territorial and agonistic behaviours that collectively affect population growth (Adcock, 1994) and are directly related to the presence and characteristics of horns (Nasoori, 2020). For instance, males with larger horns dominate 65% of encounters in male-male interactions (Berger & Cunningham, 1998). Furthermore, male reproductive success is positively correlated with home-range size, and male territories generally do not overlap because intense competition often leads to fatal fights (Cain *et al.*, 2014). Removing horns as a poaching deterrent thus has

clear potential to affect intraspecific competition for mates and space (Linklater & Hutcheson, 2010), reshape population spatial structure and impact black rhino ecology and demography.

Here, we quantify the impact of dehorning on black rhino behaviour by examining data on 24,760 rhino sightings from 368 individuals over 15 years of continuous monitoring (2005 to 2020) in 10 reserves in north-eastern South Africa (Supporting Information – Chapter IV, **Figure S1**). We first summarise trends in both horn removal and black rhino mortality through time, evaluating whether the available data support the premise that dehorning reduces poaching-related fatalities. We then employ spatiotemporal monitoring over the entire study period to determine how dehorning affects black rhino home-range sizes. Finally, we use interaction networks to examine the impact of dehorning on black rhino social interactions.

Material and Methods

Global population

Global black rhinoceros population data from IUCN were used to illustrate the decline of black rhinoceros over time and construct Figure 1A. The data were retrieved on the publicly available IUCN red list of threatened species website:

(<https://www.iucnredlist.org/species/6557/152728945#population>)

Study populations

We gathered monitoring data from 10 reserves in north-eastern South Africa that hosted a total of 368 black rhinos to assess the impact of dehorning on black rhino ecology over space and time. The focal game reserves were the following: Manyoni Private Game Reserve, Ithala Game Reserve, Phinda Private Game Reserve, Pongola Game Reserve, uMkhuze Game Reserve, Weenen Nature Reserve, Thanda Private Game Reserve, Ndumo Game Reserve, Tembe Elephant Park and Somkhanda Community Game Reserve. These reserve range in size from 42 km² to 340 km² (Supporting Information – Chapter IV, **Figure S1**) and each was home to between 4 and 42 black rhinos. The exact number per reserve is not specified here due to security reasons.

Dehorning

Dehorning, which started for most reserves in 2017, involved immobilising individuals prior to horn removal, usually by means of a qualified veterinarian sedating animals from a helicopter by shooting a dart into the animals' rumps. Drugs used for the sedation were a 50/50 ratio of etorphine (e.g. 2 mg for an adult bull) and thiafentanil (e.g. 2 mg for an adult bull) combined with azaperone (40 mg) and hyaluronidase (2,500 iu) with dosage varying according to body size. Once immobilised, rhinos were placed in sternal recumbency and equipped with earplugs and a blindfold to limit sensory input. Chainsaws were then used to cut posterior and anterior horns to leave a stub length of at least 10 cm. Horn stubs were then smoothed with an angle grinder and treated prophylactically for infection and dryness. Sedation was reversed with intravenous naltrexone (10-20x the opioid dose). Respiration was monitored during the entire procedure (<20 minutes). On average, dehorning occurs after every 18 months of horn regrowth.

Monitoring data

In all study populations, each individual black rhino is marked with a unique set of ear notches for identification and monitoring. Monitoring occurs daily across all reserves and, when an individual is sighted and successfully identified, the GPS coordinates of its location are recorded. Demographic data such as birth, death, introduction, and removal dates for each animal are also documented, as are key life events, such as dehorning date, the size of the removed horn, and cause of death. For this study, we used monitoring data encompassing the period 2005 to 2020, which included over 24,760 observations of 368 black rhinos. We assembled multiyear (2005 to 2020) location data into a matrix containing the following information: longitude, latitude, date, rhino ID, state (horned/dehorned), birth date, sex, horn size, and reserve for each observation. Animals that were not dehorned were also included in the analysis as controls. Reserve boundary shapefiles were projected in QGIS and intersected with location data to exclude incorrect GPS coordinates.

Dehorning and poaching rates

We calculated the number of de-horned and horned animals using all individuals present in the reserves over the same period as for mortality calculations (see below) and calculated yearly proportions.

We used mortality data (date and cause) to calculate the number of deaths that occurred naturally and due to poaching collectively in all study populations yearly from when the study reserves started intensively monitoring and investigating cause of death, that is from 2013 to 2020. From the beginning of this period, all rhinos were accounted for monthly, and their fate is known with certainty. In some cases (all of which were horned rhinos), the cause of death could not be determined because the carcass was discovered too late. The latter were categorised as “unknown” and the stacked bar chart in **Figure 1C** is centred on this category so as not to visually affect the other categories. We performed a Chi-square test with the *chisq.test* function in R-3.5.3 to assess the association between horn state (horn, de-horn) and cause of mortality for the entire study period.

We calculated the yearly poaching offtake rate, that is the percentage of rhinos taken out of the populations due to poaching as the number of deaths due to poaching divided by the number of the total population in all reserves accumulated every year. To investigate and compare poaching trends in South Africa and focal populations, we analysed the rhino poaching data (sourced from the Department of Forestry, Fisheries and the Environment (DFFE) of South Africa) along with rhino population numbers (sourced from various Conference of the Parties (CoP) reports for species specific matter for rhinoceroses) for South Africa. We calculated the poaching offtake rates – that is the number of animals illegally harvested divided by the total population – and compared the study sites and South Africa over time. We compared the poaching offtake rate for black rhino only in the study sites and across the whole of South Africa. There was no complete nationwide data set for the yearly number of poached black rhinos in South Africa (such data only exists for white rhino and black rhino combined); however, based on sporadic data for some years where the number of black and white rhinoceros poached were detailed separately, we estimated that in general black rhinos accounted for 10% of all poached rhinos. We conducted a linear regression with the *lm* function in R-3.5.3 to test the correlation between the yearly dehorning frequency (proportion in percentage of de-horned) and the poaching offtake rate in the study sites.

We calculated natural offtake rate of black rhinos in the study sites over the same time period by calculating the yearly number of natural mortalities in study sites divided by the yearly total population of black rhinos in the study sites and compared it to the poaching offtake rate and the proportion of dehorned black rhinos. Changes in offtake rate across years and study sites were tested with ANCOVA, with year as a continuous covariate, sites as a categorical factor,

and a year*site interaction term with the *lm* and *anova* functions in R-3.5.3. Offtake rates were square root-transformed to meet homoscedasticity assumptions.

Changes to home range sizes

For each de-horned animal, we estimated home ranges and calculated their sizes before *versus* after the date they were dehorned. We focused on animals with more than eight months of data (covering dry, wet, and transitional seasons to account for seasonal variation) and 10 sightings in both periods to ensure sufficient home-range resolution (N = 68 individuals) (Supporting Information – Chapter IV, **Figure S5**). To account for temporal home-range shifts that occur naturally during an individual's lifetime (Tatman *et al.*, 2000; Plotz *et al.*, 2016) and avoid overestimating home-range size, we reduced the maximum time window to two years for each period (Supporting Information – Chapter IV, **Figure S3**). We then balanced the number of observations between periods for the same animal by random sampling and calculated home ranges for each period using both 95% kernel density estimators (KDE), which is a non-parametric method that estimates the probability density function of a distribution based on observed data points (Worton, 1989), and with the 95% minimum convex polygon method (MCP), which calculates the minimum convex polygon around a set of points representing the minimum area that contains all the data points and assuming that the animal's home range is convex and symmetrical (Hayne, 1949) with the package *adehabitatHR* (Calenge, 2006) in R-3.5.3 (R Development Core Team, 2018). Home-range change for each animal was quantified as the difference in area covered by each individual rhino between de-horned and horned periods and expressed using effect size (Cohen's D with Hedge's correction) for males and females separately with the package *effsize* (Torchiano, 2020). To ensure that the observed home-range change was not related to natural life-cycle variation or home-range shift, we additionally estimated a control home-range change for rhinos that were never dehorned (N = 120 individuals) (Supporting Information – Chapter IV, **Figure S6**) in the same fashion as for de-horned animals (see Supporting Information – Chapter IV, **Figure S3**).

To estimate the reliability of the chosen sample size (number of individuals) and minimum number of sightings to calculate home ranges, we conducted a sensitivity analysis by progressively filtering individuals based on the minimum number of sightings (>10, >15, >20, >25, >30) and calculated home ranges as described above. The results show the same trend for all groups with intensity increasing with the number of sightings (Supporting Information –

Chapter IV, **Figure S7**). Based on this, we cautiously elected a minimum number of sightings equal to 10 to conserve a large sample size of individuals, more representative of the large number of reserves in the study. As results and conclusions based on the above criteria for MCP and KDE calculations were equivalent (**Figure 2A**; Supporting Information – Chapter IV, **Figure S4**), we only show the visual presentation of home ranges based on MCP calculations (Supporting Information – Chapter IV, **Figure S5**, **Figure S6**). This, to maintain reader clarity and because MCPs are generally considered more robust for the calculation of home ranges based on a limited number of sightings than KDEs (Plotz *et al.*, 2016).

Changes to social interaction networks

To estimate the impact of dehorning on social structure, we constructed within-population interaction networks and compared them prior to and after dehorning events. To build comparable representative networks, we selected populations that possessed more than 15 animals that were present over the same timeframe in horned (that is, less than 5% rhinos de-horned) and de-horned (that is, more than 50% rhinos de-horned) periods. Based on these criteria, we retained three reserves that together contained 74 rhinos for which we had sufficient temporal overlap in the dataset. We then calculated 95% MCPs (and more restrictive core-range 50% MCPs to show robustness of results, see Supporting Information – Chapter IV **Figure S8**) for animals that had a minimum of ten observations in both periods, estimated the total strength of social interactions based on area of overlap between individual rhino home ranges (Schwabe *et al.*, 2015), and computed effect sizes (Cohen's D with Hedge's correction) between horned and de-horned periods at each reserve separately for male-male, female-female, and male-female interactions. Finally, we compiled area overlap data into a pairwise matrix and built an interaction network based on circular layout using the *igraph* package (Csardi & Nepusz, 2006), which we used to derive changes to the number of edges (that is, the number of rhinos engaging in social interactions) and network connectivity/density (that is, density of social interactions) between horned and de-horned networks.

Results

Considering all sites together, the proportion of de-horned rhinos increased rapidly from 0% in 2013 to 63% in 2020 (**Figure 1B**). Concomitantly, the number of natural and poaching-related

mortalities decreased over the same time period (**Figure 1C**). Of the 30 recorded poached rhinos, 9 were killed after the start of the intensified dehorning campaign (from 2017, where the proportion of de-horned rhinos exceeded 30%) and of those, only two animals were de-horned prior to death (**Figure 1C**). The poaching offtake rate, that is, the percentage of black rhinos removed from the study populations due to poaching, declined during this period from 3.97% in 2014 to 0% in 2020, (**Figure 1D**, ANCOVA year effect; $F_{1,12} = 40.97$, $p < 0.001$). Poaching at the study sites (where dehorning was particularly commissioned) declined in concert with a nationwide trend (**Figure 1D**, year by study sites interaction; $F_{1,12} = 2.03$, $p = 0.180$), but was consistently lower than the national average throughout the study period (**Figure 1D**, study site effect; $F_{1,12} = 30.09$, $p < 0.001$). Notably, the frequency of natural mortality also decreased from 2017 to 2020 (**Figure 1C**).

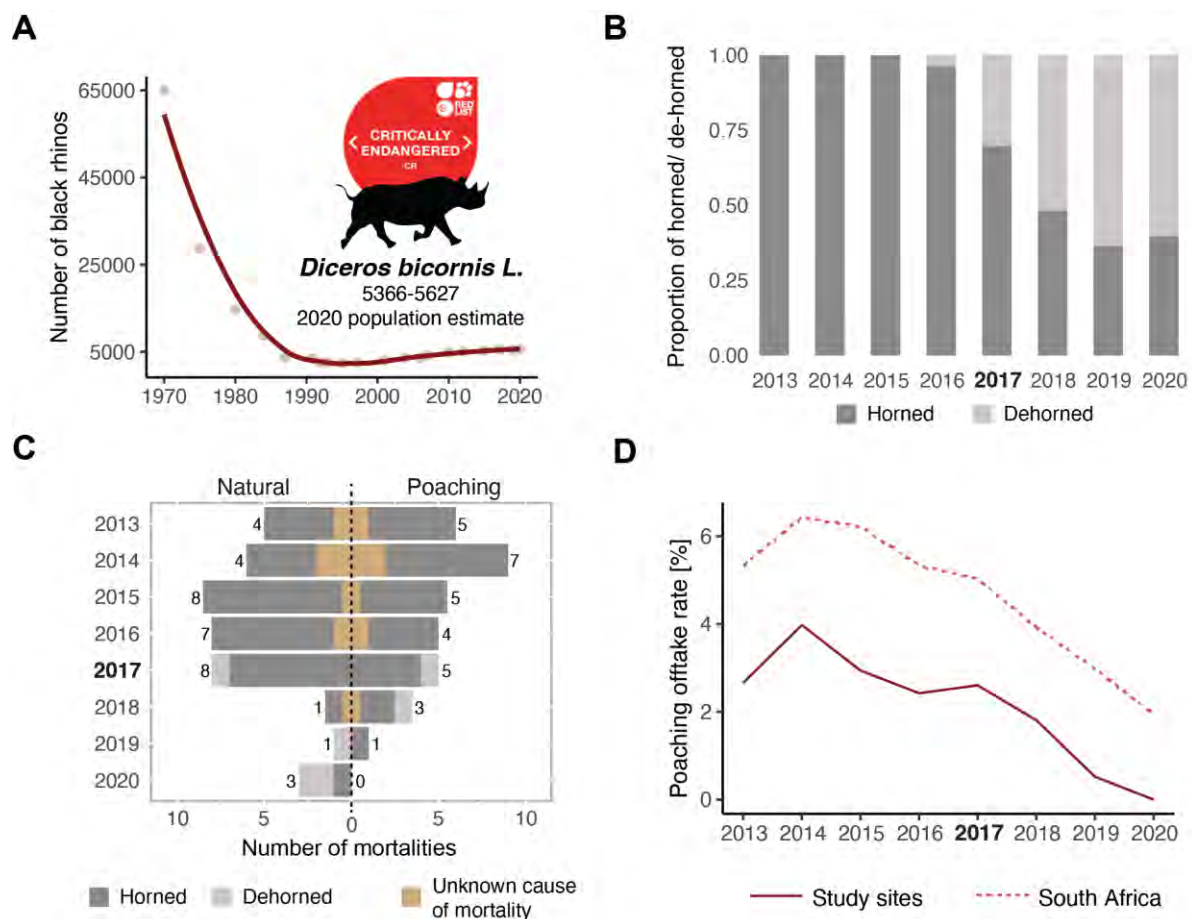


Figure 1. Global population decline of the critically endangered black rhinoceros and recent trends of dehorning and poaching in 10 South African study sites. (A) Plot showing the global decline in the number of black rhinoceros individuals and the 2020 estimate of population size (IUCN, 2020), as highlighted by the red spline. (B) Bar graph displaying the proportion of horned (dark gray) and de-horned (light grey) black rhinos in study sites over time, with the bulk of dehorning campaigns

starting in 2017 (highlighted in bold). (C) Stacked bar chart showing the number of mortalities by year in study sites of horned (dark grey) and de-horned (light grey) by cause (left: natural mortalities; right: poaching-related mortalities). Mortalities of unknown cause (all horned) are displayed in gold and centered. (D) Plot displaying the poaching offtake rate of black rhinos in the study sites compared to that of black rhinos in South Africa over the same period. The poaching offtake rate (solid line for study sites; dotted line for South Africa at large), representing the percentage of black rhinos removed from the population by poaching, was calculated by the yearly number of poached black rhinos divided by the yearly total population of black rhinos.

Our observations collectively suggest that the frequency of de-horned animals is negatively correlated with mortality due to poaching (Supporting Information – Chapter IV **Figure S2A**; linear regression; $R^2 = 0.72$, $F_{1,6} = 19.27$, $p = 0.004$). Yet, it is important to note that these results do not establish a causal relationship between dehorning and poaching. The observed reduction in poaching events almost perfectly paralleled the broader decrease throughout South Africa and may be linked to other variables, such as increased security in game reserves, lower economic incentives for poachers (Holden *et al.*, 2019), and, exceptionally in 2020, COVID lockdown regulations. Relatedly, a census of a black rhino population in Zimbabwe similarly found that rhino individual numbers rose in concert with an intensive dehorning campaign (Kock & Atkinson, 1993; Lindsey & Taylor, 2011; Chanyandura *et al.*, 2021), but dehorning was carried out in synergy with several other interventions, such as a shoot to kill policy (Kagande & Musarurwa, 2014; Chanyandura *et al.*, 2021). While there is therefore no conclusive evidence that dehorning contributed to the reduction in poaching, we also show that there is no evidence of dehorning leading to increased natural mortality (Chi-square test of association between cause of death and horned and de-horned mortalities; $X^2 = 1.422$; $df = 2$; p -value = 0.488). To the contrary, the natural mortality offtake rate sharply declined alongside the poaching offtake rate from 2017 when dehorning became widespread (**Figure 1C** and Supporting Information – Chapter IV, **Figure S2B**).

We examined whether dehorning affects black rhino home range size by comparing the home ranges of de-horned rhinos before *versus* after dehorning ($N = 68$; Methods, Supporting Information – Chapter IV **Figure S5**) to those of a control (not de-horned) group ($N = 120$; Methods, Supporting Information – Chapter IV **Figure S6**). Home ranges of male and female rhinos shrank significantly after dehorning (that is, had a negative effect size; **Figure 2**), with de-horned females displaying an average decrease of 15.42 km^2 (-53.08 %), and de-horned

males an average decrease of 9.13 km² (-38.03 %) compared to horned animals (**Figure 2A**; Supporting Information – Chapter IV **Table S1**). This effect was resolutely independent from the number of sightings used to construct home ranges in both periods and these results remained robust with varying sample sizes of individuals and sightings (Supporting Information – Chapter IV **Table S2**, **Figure S7**). By contrast, horned (control) rhinos actually expanded their home ranges significantly over the study period by 7.38 km² (+57.02%) in average (females: 7.77 km² (+67.55%); males: 7.07 km² (+50.21%) (**Figure 2A,C**; Supporting Information – Chapter IV **Table S1**), accentuating the difference between the two groups. Expansion of horned rhino territories was not caused by animals occupying newly vacant dehorned rhino territory, since 95% of the time the horned control group was separated from the dehorned group in space or time (Methods). Indeed, territory expansions are expected over a rhino’s lifetime (Tatman, Stevens-Wood & Smith, 2000). Our results thus collectively show that de-horned rhinos reduce their home range size, revealing horns as an important determinant of black rhino home-range area.

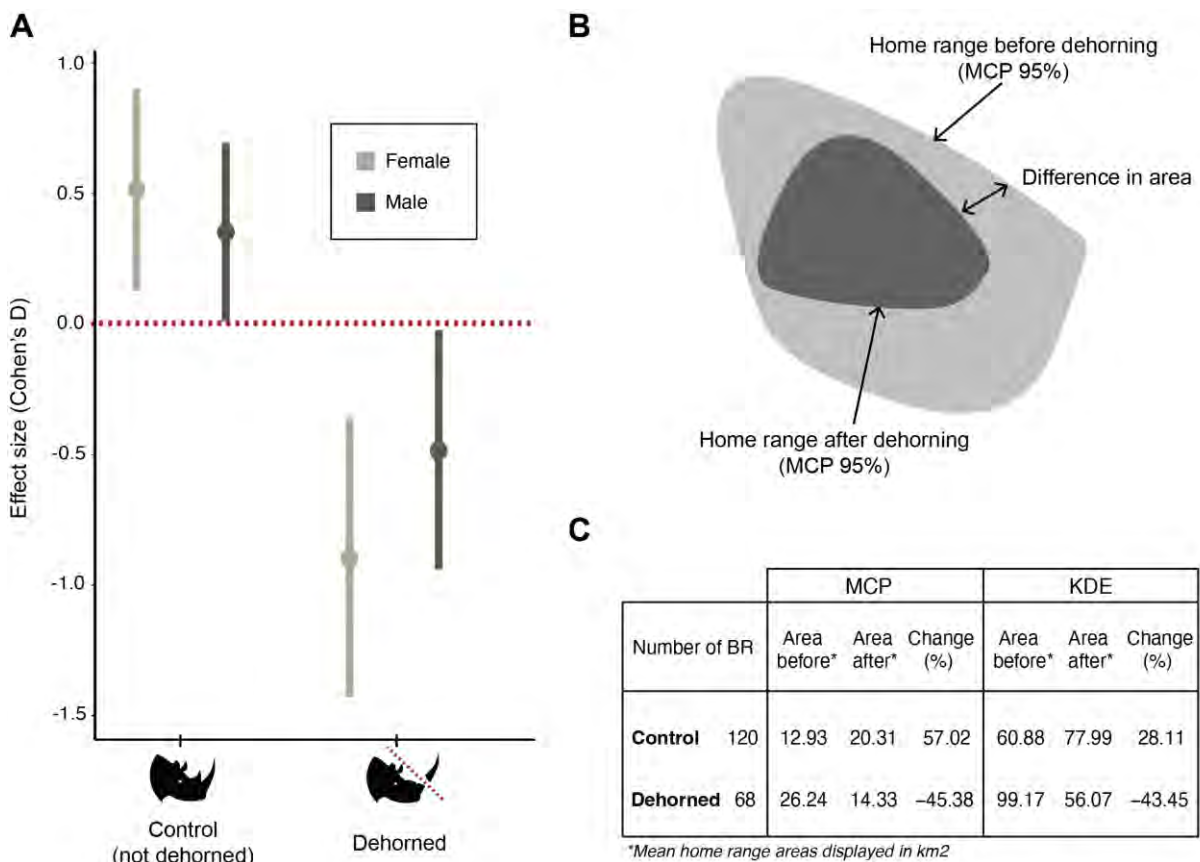


Figure 2. Changes in black rhino home range size after dehorning. (A) Dot plot showing averages (+/- 95 % confidence intervals) change in home range area in horned control animals (left) versus de-

horned animals (right) for both females (tan; control N=53, de-horned N=30) and males (green; control N=67, de-horned N=38) black rhinoceros, derived from minimum convex polygons (MCP 95%) calculations of effect sizes using Cohen's with D Hedge's correction (Methods, see Supporting Information Chapter IV Figure S4 for analogous results using kernel density estimates (KDE)). (B) An example home-range size change for a randomly selected black rhino (MPGRBM-06-05), showing the home range before (light grey) and after (dark grey) dehorning. (C) Summary table showing the mean areas (km²) of control and de-horned black rhino home ranges calculated with 95% minimum convex polygons (MCP) and 95% kernel density estimates (KDE) and the number of black rhino in each group, across all reserves. The change (%) shows the percentage of increase or decrease in area between periods (see Supporting Information Chapter IV Table S1 and Table S2 for tables of mean area by sex and reserve).

Black rhinos are largely solitary and sedentary but possess home ranges that overlap intersexually (Ritchie, 1963; Tatman *et al.*, 2000; Lent & Fike, 2003; Linklater & Hutcheson, 2010), making home-range connectivity critical for maintaining social interactions (Tatman *et al.*, 2000; Linklater, Mayer & Swaisgood, 2013; Schwabe *et al.*, 2015). Particularly for a non-gregarious species, communication through scent is important and thus overlap in territories essential. For instance, adult black rhinos use dung heaps (middens) to identify other individuals, inform competitive interactions, delimit home ranges, and seek potential mates (Tatman *et al.*, 2000; Schwabe *et al.*, 2015). It follows that the observed reductions in black rhino home-range size following dehorning could have a substantial effect on black rhino social interactions. We used the three populations with sufficient consecutive data (Ithala, Manyoni, Phinda) to examine the impact of dehorning on the strength and structure of black rhino social interactions, specifically by performing within-population comparisons of interaction networks before and after dehorning (N = 74 individuals, Methods). Dehorning sharply decreased social-interaction strength in two populations (Cohen's D: Manyoni = -0.62; Phinda = -0.89) and induced smaller decreases in the third population (Cohen's D: Ithala = -0.19) (**Figure 3A**). The impact of dehorning on black rhino social structure was also evident from the interaction network structure (**Figure 3B**), which revealed that dehorning decreased both the total number of social interactions (that is, network density) and the number of rhinos engaging in social interactions (that is, edges of the network diagram) strongly in Manyoni and Phinda and less so in Ithala.

These changes to social interactions varied by sex, with those involving males (particularly male-male interactions) being most affected by dehorning with a significant reduction at all three sites (**Figure 3**). That the Ithala population, the most recently de-horned population (Ithala in 2018, Phinda in 2017, Manyoni in 2016), underwent the smallest changes in social interactions may indicate that there is a time-lag in the black rhino response to dehorning – although this is speculative and requires further validation. These results remained robust with a more restrictive analysis based on core ranges (corresponding to the most central and frequently used areas of the home ranges; Supporting Information – Chapter IV, **Figure S8**). More generally, these findings show that dehorning black rhinos leads to not only a decrease in their home-range size, but also a decline in the number and strength of their social interactions, especially for male rhinos.

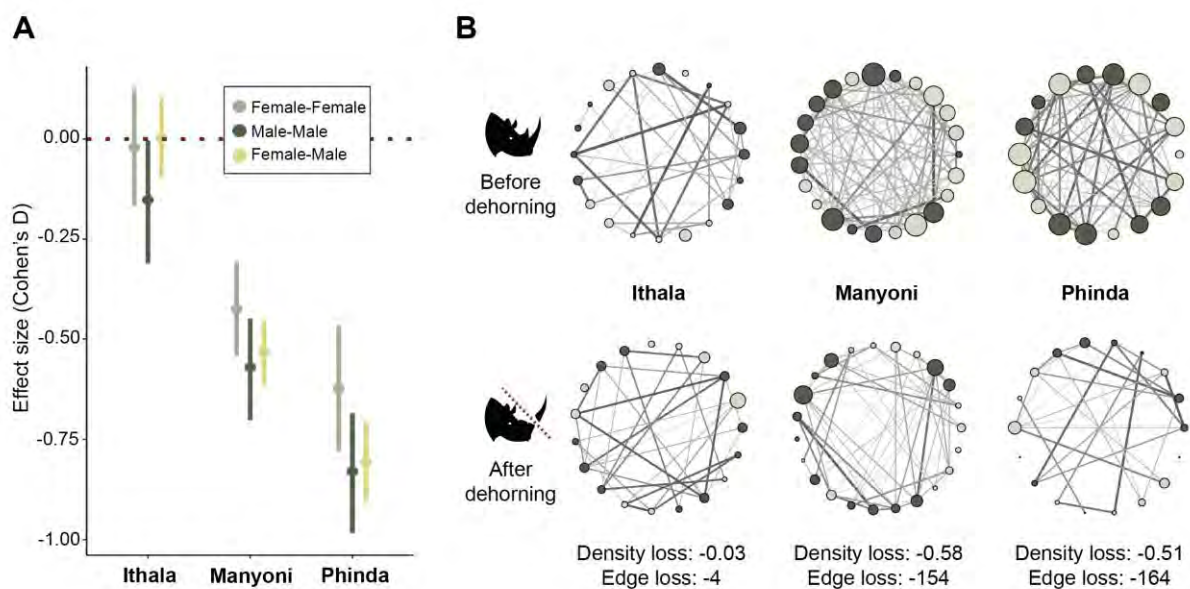


Figure 3. Changes to black rhino social structure after dehorning. (A) Dot plot showing averages (\pm 95 % confidence intervals) change in social interaction strength (effect size; Cohen's D) for female-female (tan), male-male (green) and male-female (yellow) interactions in three reserves (Ithala N=20, Manyoni N=23; Phinda N=18) after dehorning, calculated based on overlap of 95% MCPs. (B) Interaction networks showing social connectivity in the same three populations before (top) and after (bottom) dehorning. Each node (edge) is an individual and lines (edges) represent an interaction between two individuals, with a gradient of line thickness illustrating the strength of interaction (the amount of overlap). The size of the nodes is proportional to the number of connections each individual has. Edge and density loss correspond to the number of disappearing nodes and lines, respectively, after dehorning.

Discussion

Dehorning is an anti-poaching measure that has become popular as a rapid way to safeguard remaining black rhino populations but with hitherto unknown consequences for black rhino ecology (Lindsey & Taylor, 2011). Here, we show that dehorning black rhinos is correlated with a reduction in mortality from poaching and does not induce an increase in natural mortalities. However, causation between dehorning and poaching reduction is yet to be determined and remains a pivotal question for future research. Disentangling complex socio-economic factors is necessary to fully ascertain whether and why dehorning contributes to deterring poaching. The fact that deaths due to poaching were consistently lower through time in the study sites than nationally, could be linked to multiple characteristics such as smaller reserve size, better monitored populations, or less criminal activity in the region at the time.

Black rhinos use horns for a variety of purposes, from pulling down trees and branches (Nasoori, 2020), to scratching the ground for mineral nutrition (Ritchie, 1963), to self-defence (Nasoori, 2020) and enhancing male social status (Berger & Cunningham, 1998). Yet, while it follows that dehorning black rhinos should impact black rhino behaviour, evidence for such impacts to date comes from a small number of studies on a small number of individuals and is not unanimous (Berger *et al.*, 1993; Kock & Atkinson, 1993; Berger & Cunningham, 1998; Kagande & Musarurwa, 2014; Penny *et al.*, 2021). Here, we show that dehorning has clear effects on black rhino behaviour at both the individual and population levels. Such effects must be driven by even more granular cognitive and social behavioural changes, although we are unable to evaluate these in the present study. For instance, if rhinos stripped of their main armament signal vulnerability to others (Berger & Cunningham, 1998), then they may choose to retreat into smaller territories to avoid confrontations with competitors (Linklater & Hutcheson, 2010; Grether *et al.*, 2017; Patton, Campbell & Genade, 2018; Chimes *et al.*, 2022) or other megaherbivore species such as elephants (Landman, Schoeman & Kerley, 2013; Grether *et al.*, 2017). Regardless of the mechanism, by reducing both the size of home ranges and the frequency of social interactions, dehorning rhinos impacts processes important to population dynamics, raising questions about the long-term consequences for the species.

Behavioural plasticity plays an important role in the adaptation, or mal-adaption, of a species to rapidly changed environmental conditions, such as due to anthropogenic disturbance (Schlaepfer, Runge & Sherman, 2002; Sih, 2013). That de-horned black rhinos display

considerably reduced home range sizes and engage in fewer social interactions might have cryptic but powerful population-level consequences for at least three reasons. First, black rhino home ranges are governed by both social interactions and resource availability (Plotz *et al.*, 2016), so dehorning could force animals into ecological traps (Schlaepfer *et al.*, 2002) such as smaller habitats containing less or lower quality browse – with unknown consequences for individual survival and reproduction. Alternatively, smaller home ranges could also mean increased carrying capacity, with reserves able to sustain larger populations, and decreased fighting within the species (Patton *et al.*, 2018; Chimes *et al.*, 2022) possibly even leading to a reduction in natural mortality. Second, changes in the number and strength of black rhino social interactions have the capacity to alter patterns of dominance and reproductive success among individuals – with potentially large implications for demographic processes. Third, by reducing the size of home ranges and limiting explorative behaviours and dispersal (Reid *et al.*, 2007), dehorning may impair black rhino recruitment across landscapes, which is already slow (Reid *et al.*, 2007; Linklater & Shrader, 2017).

These considerations have direct implications for conservation management, which is typically informed by meta-population structure and habitat carrying capacity estimations (Foose, 1993; Hrabar & Toit, 2005; Ferreira *et al.*, 2017). Dehorning could alter carrying capacity calculations directly, while also making the process of selecting candidates for translocation (that is, individuals that show signs of imminent dispersal) more challenging. Despite this, with the more recent (since 2021) escalation in poaching (Nhleko *et al.*, 2021), the lack of evidence that dehorning increases natural mortality, and the lengthy agenda necessary to implement long-term measures (Selier & Di Minin, n.d.), dehorning remains an option readily available to wildlife practitioners to buy time for this critically endangered species.

In conclusion, we suggest that while dehorning may yet prove to be an essential anti-poaching measure, it must be implemented with extreme caution and be accompanied by rigorous monitoring to understand its long-term impacts on black rhino populations and evaluate its true success as a poaching deterrent. Future ecological and behavioural research regarding the ramifications of such a tool must be undertaken imperatively to assess their net effect on black rhino population persistence. In the broader picture, evaluating the effect of conservation interventions and human-induced rapid environmental change through animal behavioural indicators, such as home-range use (Owen-Smith & Cain, 2007; Berger-Tal *et al.*, 2011), is

important as it can serve as an early impact indicator for cryptic consequences (Berger-Tal *et al.*, 2011; Sih, 2013; Greggor *et al.*, 2016) and enables adaptive management.

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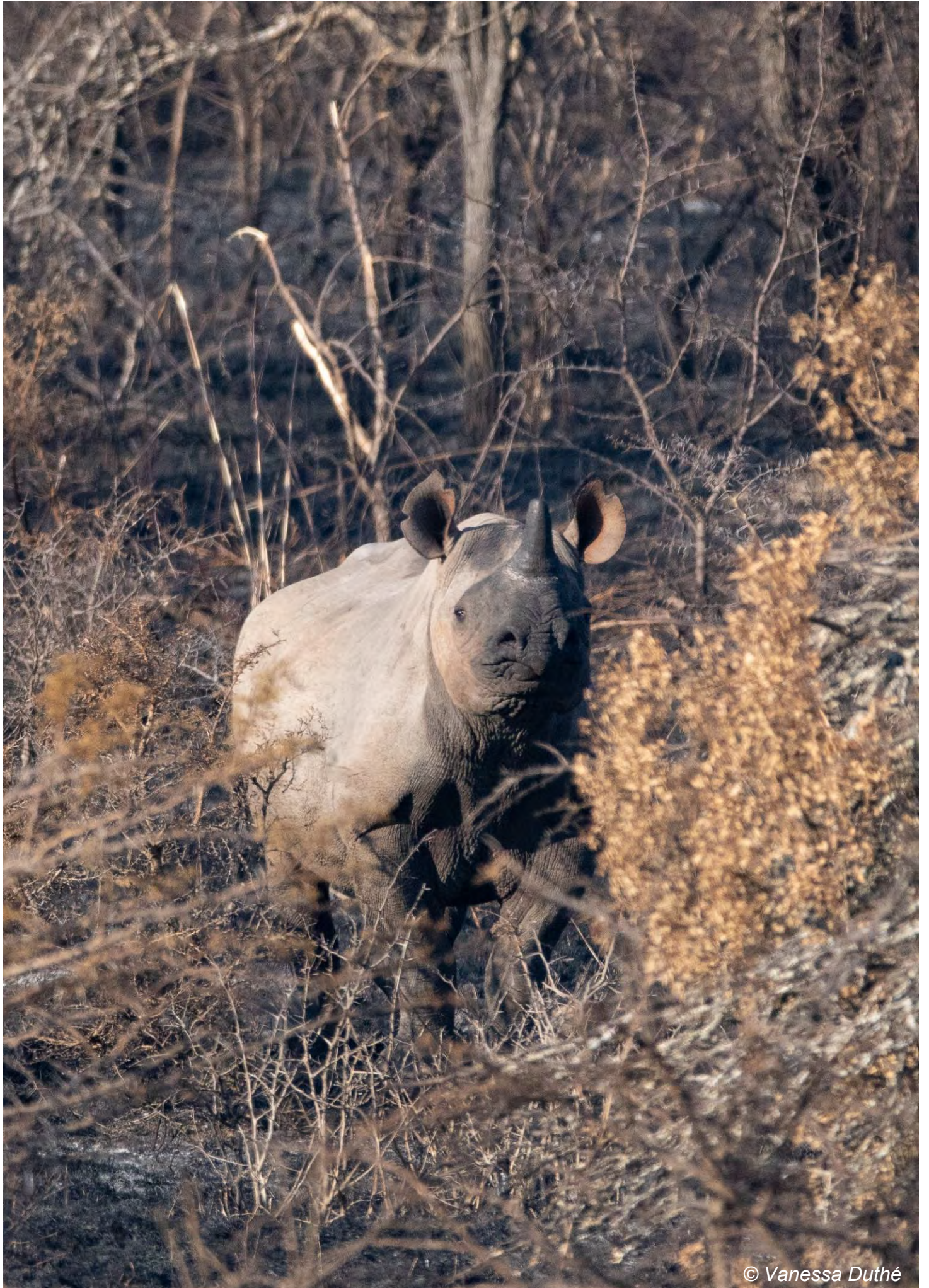
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General Discussion

General Discussion

Approach

Savanna systems are facing growing pressures exerted by climate change and ecosystem degradation (Staver & Hempson, 2020). The continuous loss of charismatic megafauna is not without consequence in such ecosystems (Dirzo *et al.*, 2014; Pringle *et al.*, 2023), where many large herbivores are considered as keystone species (Landman, Schoeman & Kerley, 2013). A deeper understanding of governing ecological processes and interactions is vital for the conservation and management of endangered species and their habitats (Staver & Hempson, 2020). Particularly for species such as the black rhino, where population recovery depends on management actions such as translocations and other operations in line with carrying capacity calculations (Linklater *et al.*, 2006; Linklater & Swaisgood, 2008). This cryptic species is notoriously difficult to study, and in-depth knowledge about its ecology lacks. In addition, many pre-constructed ideas based on inconclusive or insufficient evidence may obstruct science-based decisions regarding its management.

In this dissertation, my approach consisted in investigating three varied but important general topics; forage, space use, and interactions in the aim of increasing unambiguous knowledge of the species' ecology and practical to its management and that of its environment. This thesis shows some of the facets of an incredibly intricate species, characterised by selective foraging, structured space use, mediated interactions and the necessity for responsive biological management in today's world.

The selective forager

While the concept of selective foraging for black rhino is widely accepted (Kotze & Zacharias, 1993; Dierenfeld, du Toit & Braselton, 1995; Muya & Oguge, 2000; Ganqa, Scogings & Raats, 2005; van Lieverloo *et al.*, 2009; Buk & Knight, 2010; Anderson *et al.*, 2018), the mechanisms and factors offering reasoning for choice have been limited or mixed. Studies have reported, from various habitats, black rhino choice to be correlated with total phenols, fibre, protein, leaf:stem ratio, photosynthesizing tissues, or secondary chemistry (Muya & Oguge, 2000; Ganqa *et al.*, 2005; van Lieverloo *et al.*, 2009; Buk & Knight, 2010; Duthé *et al.*, 2020). Beyond

these specificities, results from **Chapters I, II and III** show the extent of this selectivity and the factors driving it. In particular three elements seem to be determinant in shaping diet: plant characteristics, season and coexisting herbivore species. Firstly, black rhino forage according to morphological and physiological characteristics of plant species, such as structure, nutrients and secondary chemistry; and likely detect and select preferred food items by visual and olfactory cues (**Chapters I and III**). Secondly, results from all three chapters show a clear seasonal shift in diet and foraging strategies. Seasonality is an important driving force as it shapes not only resource availability but also plant morphology and physiology (Nelson & Moser, 1994); and consequently, diet, preference and forage search. Thirdly, results from **Chapter II** show structured feeding strategies according to concurring species, particularly in resource scarcity, where dietary overlap between species reduced to support coexistence (Gordon & Illius, 1989; Mysterud, 2000; Makhabu, Skarpe & Hytteborn, 2006; Kartzinel *et al.*, 2015). Although megaherbivores are generally reported to feed on bulk forage of low quality (Owen-Smith, 1992), we show that black rhinos are incredibly selective foragers drawing on explicit parameters and in relation to smaller bodied-herbivores.

The structured navigator

Habitat use for large mammals is generally structured by topography, resources and intra and inter-specific interactions (Cromsigt *et al.*, 2009; Kleist *et al.*, 2021; Freeman *et al.*, 2022; Ang'ila *et al.*, 2023). Elevation and slope are important predictors for animal movements, particularly for species with a substantial body mass as energy expenditure is more important (Ang'ila *et al.*, 2023; Berti *et al.*, 2023). Yet, water availability and resources primarily drive the distribution of large herbivores (Redfern *et al.*, 2003; Smit, Grant & Devereux, 2007). In **Chapter I**, I show that black rhino density is closely related to resources through the selection of habitats of high selectivity and the ratio of avoided and preferred plant species in these areas. However, this Chapter I also reveals a more intricate side to black rhino habitat choice, with the selection of open areas related to low NDVI. While this finding seems surprising for a browsing species known to “sit in thick bush”, it essentially shows the importance of habitat heterogeneity. In the wet season, black rhinos are frequently observed feeding on nutritious forbs in open areas in the reserve. Open areas of this kind are mostly characterised by mesic grasslands scattered with bush and shrubs and that offer an abundance of new shoots and growth (Briggs *et al.*, 2005) of preferred species. Similarly, it was reported that in forest habitats, large herbivores preferably chose habitats with a mosaic of openings and continuous cover (Massé

& Côté, 2012). From a different perspective, tree density has been reported to affect large herbivore habitat use, where predator visibility is diminished in thick clumps of trees and subsequently avoided by most herbivores (Riginos & Grace, 2008; Burkepile *et al.*, 2013). Although the latter may not be relevant to black rhino because of poor eyesight (Linklater, Mayer & Swaisgood, 2013) and the lack of predators (Sinclair, Mduma & Brashares, 2003), the species is affected by disturbance and noise (Odendaal-Holmes, Marshal & Parrini, 2014; Kleist *et al.*, 2021). Notably, it was reported that black rhino retreated to closed savanna and thicket in proximity of human settlements (Odendaal-Holmes *et al.*, 2014). **Chapter IV** indirectly demonstrates the impact of a human induced disturbance on black rhino space use and ultimately their spatial response to fellow rhino. In essence, despite the large body size and low predatory risk, black rhino space use is remarkably complex, where habitat heterogeneity and interactions with neighbours seem key.

The interconnected neighbour

Black rhinos are solitary animals that live within delimited home ranges (Mitchell & Powell, 2012). Home ranges are determined by social interactions (Lent & Fike, 2003) which are governed by competition for mates and space (Linklater & Hutcheson, 2010). Results from **Chapters I** and **IV** support the latter and highlight two aspects mediating black rhino interactions: resources and social hierarchy. **Chapter I** shows that black rhinos, although reclusive, tolerate “grouping” in preferred vegetation types in the growing season when resources are plentiful. **Chapter IV**, on the other hand, presents the basis for a particularly complex social structure where self-perception and of others, is central. It is suggested that strong social bonds form between females and calves, females periodically spend time with males and young animals form loose associations (Tatman, Stevens-Wood & Smith, 2001). In reality, black rhino social fabric appears more intricate than categorised bonds. That black rhinos expanded their range over time indicates a particular social structure based on hierarchy stemming from age, sex and dominance. Because the species is non-gregarious, it is suggested that, similar to white rhino (Marneweck, Jürgens & Shrader, 2018), middens are used as information centres to perceive olfactory messages left by others, informing of sex, age, territorial status and oestrous state. Frequently visited middens in areas of overlap, are thus possibly essential for communication and hierarchy. From a different lens, the pronounced effect of dehorning on home ranges suggests self-perception and the significance of horns in social interactions. Black rhino not only have a more elaborate social structure and perception

than previously thought, but have strong behavioural plasticity in response to environmental change, and which is relatively easily measurable.

Conservation and management implications

The strategy for the recovery of black rhino involves restocking historical ranges with surplus animals from protected areas, as populations reach carrying capacity (Tatman *et al.*, 2001; Linklater *et al.*, 2012). This implies promoting population growth in current ranges, and selecting suitable habitats for translocations (Linklater *et al.*, 2012). In this regard, three management components are essential to optimise through enriched practical ecological knowledge.

Firstly, carrying capacity calculations are central to the strategy and are founded on resource and space use. All four chapters in this dissertation improve our knowledge of the ecological requirements for habitat suitability. While many findings may appear unique to Ithala Game Reserve, some aspects can be generalised. For instance, an estimation of habitat suitability could stem from present plant characteristics rather than solely the familiarity of particular species commonly browsed by black rhino. In addition, heterogenous habitats composed of a mosaic of open and closed areas and considering abundant mesoherbivores in a holistic ecosystemic approach, are pivotal for estimating habitat adequacy.

Secondly, habitat management is essential in maintaining productive ecosystems and mammal populations (Soto-Shoender *et al.*, 2018). Because large herbivores exert top-down control on plant demography (Pringle *et al.*, 2023) and savanna ecosystems are subject to climate change and other pressures (Staver & Hempson, 2020), managing sympatric herbivore densities and vegetation communities is fundamental. Careful consideration must be given to over-abundant herbivores, regardless of body size, as they may significantly degrade preferred habitat and unsustainably encroach on black rhino and other species with narrow dietary niches. As bush encroachment broadens, maintaining heterogenous habitats is crucial for many mammal species (Soto-Shoender *et al.*, 2018). Bush encroachment will likely also affect black rhino by reducing the diversity of habitats and species to feed on. From this perspective, managing herbivore densities holistically and controlled vegetation burning to limit bush encroachment is thus essential to maintain habitat heterogeneity (Smit, 2004).

Thirdly, the success of management operations relies substantially on the diligent implementation of translocations and boma care (Linklater *et al.*, 2006). **Chapters I, II and III** give further insight into diet preferences and requirements; and a possible indication to using volatile organic compounds to induce feeding on presented forage in the bomas. **Chapter IV** highlights the importance of choosing assiduously individuals for translocation, in consideration of a complex social fabric; and the effect on black rhino behavioural ecology of a now common procedure, often carried out in conjunction of most operations.

To conclude, black rhinos are resilient and respond rapidly to changing ecosystem conditions, whether natural or human-induced. For this reason, management interventions must be continuously assessed and persistent monitoring is critical for effective performance. The implications of this thesis extend beyond one species. Behavioural ecology, such as space and resource utilisation, can serve as early indicators of concealed consequences and facilitate adaptive management for large herbivores.

Caveats and outlook

Owing to the difficulty of studying black rhino, and the general limited interest in funding studies focused on large herbivores (Pringle *et al.*, 2023), this work was not without challenge or caveat.

Although large, black rhino are incredibly elusive particularly in thick bush, and thus sightings data entail biases linked to constraints faced by observers. Albeit black rhinos being diel (Joubert, 1971), sightings are generally recorded during the day because of the difficulty of identifying individuals in the dark and increased risk at night. The use of GPS telemetry would resolve the issue of lacking night data and improve temporal finesse to better describe interactions, territorial establishments and enable fine scale modelling. Conventional methods of attaching GPS devices to rhinos have included collars (Alibhai & Jewell, 2001), ankle collars, ear tags and horn implants (Plotz *et al.*, 2016). However, tracking devices are notoriously difficult to attach to rhinos (Hofmeyr, 1998). Collars in particular have proven ineffective and often cause injury due to the particular morphology of rhino with their unusual neck shape: triangular and continuous with the top of the skull (Hofmeyr, 1998; Alibhai & Jewell, 2001). Ear tags have been reported to tear off quite easily (Hofmeyr, 1998), whilst ankle collars can also lead to abrasions causing serious injuries and sometimes even mortalities. Horn

implants have had the greatest success, yet are limited to animals with horns large enough to accommodate transmitters (Hofmeyr, 1998; Plotz *et al.*, 2016) and which are non-dehorned, ruling out the use of horn implants. Recent trials in collaboration with the HEIG-VD (School of Engineering and Management Vaud) indicate that horn pods, under development since the start of this thesis' work, are a prospective solution to tracking rhinos effectively. This method consists in gluing a resistant pod containing a tracking device to the posterior horn stump. Further development and trials are currently under way in the aim of ascertaining functionality and practicality for future research and monitoring.

While diet transects allow precise description of a specific site, extrapolation based on vegetation type and NDVI has its restrictions. Notably, having carried out over 150 direct-observation transects, I found that black rhino exhibited a distinct preference for peculiar sites to forage. Repeatedly, little microhabitats offering a diversity of species particularly in the form of new growth, resembling somewhat a “buffet” but where only certain items were browsed, were picked for foraging. Subtlety of this kind may become imperceptible with extrapolations at a rougher scale, and finer scale measures of diversity should be applied at foraging sites. Light Detection and Ranging (LiDAR) from Unoccupied Aerial Vehicle (UAV) surveys provide a sound measure of detailed vegetation structure at wide scales (Coverdale & Davies, 2023), and although costly, should ideally be implemented in further studies. Additionally, other features such as topography and soil properties should be considered. Preliminary results of our recent soil description campaign show that climatic variables and soil properties are co-structured and could further explain black rhino habitat use (Steiner, 2023).

An undeniable influence on this thesis' findings is the fact that most of the work was focused on one study site. Although Ithala's black rhino population is particularly well-known, well-monitored and productive, the reserve is composed of an extremely complex set of habitats, which can differ significantly from other range habitats through environmental conditions and species assemblage. That impala and elephant were reported to be nearly solely browsing indicates a site specificity, and interactions with other herbivores may vary elsewhere. The comparison between multiple sites would allow a more accurate representation of conditions in range environments.

Despite sampling traits and secondary chemistry for a reasonably representative snapshot of important and abundant plant species for black rhino diet in Ithala, the number of species

included is comparatively restricted in relation to the overall species richness in the ecosystem. Due to the challenge of finding some of the species in the dry season, the season's set is especially limited. The inclusion of more species and replicates would lead to more robust and transferrable results, addressing inter and intra-specific variability. In this context, preliminary evidence suggests that black rhino select individuals according to differences in secondary chemistry within a species (Allisiardi, 2023). Nevertheless, initial trends on important plant characteristics emerge yet powerful, making it a solid foundation to begin with. However, to truly disentangle factors and confirm the true nature of deterrence or attractance of volatiles and other secondary compounds, performing a choice test, where black rhino held in bomas are presented with fresh browse composed of different species (avoided and preferred) and treatments (doused with volatiles of importance, visual manipulation, chemical profiling), is obligatory.

“Ecological sciences are confronted by complexity, contingency, problems of scale, emergent properties, and an urgency to translate basic research into applications” (Pringle *et al.*, 2023). This thesis and specific line of work is no exception and continuous effort and investment is needed to address these challenges. New technologies and innovative ideas such as “the measurement of plant traits and soil chemistry, hyperspectral imaging and LiDAR, subsurface imaging, GPS telemetry, DNA metabarcoding, metabolomics and transcriptomics, flux towers, and machine-learning algorithms” (Pringle *et al.*, 2023) will contribute to gathering difficult information and bridging the gap between scales. As such, this thesis furthers our understanding of a critically endangered large herbivore, by building upon this framework.

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Summary of Findings

The black rhino is an intricate species that demonstrates behavioural plasticity to changing ecosystem conditions and management interventions. The ongoing evaluation and adaption of management strategies and consistent monitoring are crucial for ensuring effective conservation efforts. Furthering scientific comprehension of the species will assist efforts, building onto this work's specific findings:

- Black rhino habitat selectivity is driven by plant community selectivity and low productivity. Black rhino selected vegetation types with high community selectivity, composed of an important ratio of preferred species, and open areas with low NDVI.
- The importance of plant community selectivity decreased in the dry season.
- Plant secondary compounds are more important than nutritional quality (C:N) for forage selection, but nutritional quality (higher C:N ratio) was higher in preferred plants.
- Diet compositions for black rhino but also elephant, kudu and impal shifted seasonally, with broader diets in the wet season. Diet richness was higher and evenness lower in the wet season compared to the dry season.
- Resource partitioning is structured according to concurring herbivore species. Dietary overlap between black rhino, elephant, kudu and impala decreased with fewer resources in the dry season.
- Dietary overlap with black rhino was the highest with kudu in the dry season and impala in the wet season.
- Mixed-feeders' diet composition, impala and elephant, was nearly solely composed of browse through both seasons in Ithala Game Reserve.
- Important seasonal differences were observed among traits and VOCs of avoided and preferred plant species.
- The combination of morphological and olfactory cues best explained preference of plant species.
- Caryophyllene and Hexenol acetate are suggested to be important VOCs for forage choice, but don't drive selection alone.
- Spinescence appears as an important morphological trait but it remains undetermined whether black rhino use it as a "rule of thumb" cue to forage more nutritious species.

Summary of Findings

- Black rhinos increase the area of their home ranges in time.
- Dehorning does not lead to increased natural mortality among dehorned black rhino.
- The increase in the number of dehorned rhinos was correlated with the decrease in mortalities due to poaching, but there was no evidence to indicate causation.
- Dehorned black rhinos decreased the area of their home ranges, where female showed the biggest reduction.
- Dehorning limited social interactions among dehorned black rhino through reduced overlap between home ranges, where male-male interactions were the most affected.



Appendices

Supporting Information - Chapter I

Supporting Information for Chapter I.

Supplementary Methods

Untargeted metabolomics extraction of browsed and avoided plant species

Extraction for browsed/avoided samples was conducted as following: foliage material was ground with a Retsch MM400 tissue lyser at 30 Hz for 5 minutes, 1ml of methanol (HPLC grade) was added to 20mg of dry mass for each sample. Glass beads (2mm) were then added to the solution and agitated for 3 minutes at 30 Hz with the Retsch MM400. After that, samples were centrifuged for 10 minutes at maximum speed (14'000 rpm) and 150 µl of the supernatant was transferred to HPLC vials containing inserts.

Metabolomic analysis was carried out by UHPLC-QToF-MS using an Acquity UPLC coupled to a Synapt G2 QTOF mass spectrometer (Waters). An Acquity UPLC BEH C18 column (50x2.1mm, 1.7 µm; Waters) was employed at a flow rate of 600 µl/min and maintained at a temperature of 40°C. The following gradient with 0.05% formic acid in water as mobile phase A and 0.05% formic acid in acetonitrile as mobile phase B was applied: 2-35% B in 3 min, 35-100% B in 3 min, 100% B for 1.5 min, reequilibration at 2% B for 1.5 min. The injection volume was 2 µl. The QTOF was operated both in electrospray positive and negative modes using the so-called MSE acquisition mode. Mass spectrometric parameters were as follows: mass range 85-1200 Da, scan time 0.15 s, source temperature 120°C, capillary voltage +2.8 or -2.5 kV, cone voltage +25 or -25V, desolvation gas flow and temperature 800 L/h and 400°C, respectively, cone gas flow 20 L/h, collision energy 4 eV (low energy acquisition function) and 10-30 eV (high energy acquisition function). A 500 ng/ml solution of the synthetic peptide leucine-enkephaline in water:acetonitrile:formic acid (50:50:0.1) was infused constantly into the mass spectrometer as internal reference to ensure accurate mass measurements (sub-2ppm). Data was recorded by Masslynx v.4.1. Marker detection was performed using Markerlynx XS (Waters) with the following parameters: initial and final retention time 0.5 and 6.0 min, mass range 85-1200 Da, mass window 0.02 Da, retention time window 0.06 min, intensity threshold 500 counts, automatic peak width and peak-to-peak baseline noise calculation, deisotoping

applied. Data was mean-centered and Pareto scaled before applying multivariate analysis. Markers of interest were tentatively identified based on their molecular formula determination and fragments obtained by collision induced dissociation, and comparison with available databases.

Supplementary Tables

Table S1. Transects for black rhino diet survey. Shown are the 40 transects that were used to follow individual rhinos across two seasons (Wet and Dry), and across 14 vegetation types.

Transect	Season	Rhino	Vegetation type
1	Dry	July	<i>Combretum apiculatum</i> - <i>Euclea schimperi</i> rocky bushveld
2	Dry	Qamukhile	<i>Hyparrhenia hirta</i> - <i>Dichrostachys cinerea</i> old field grassland
3	Dry	Meleni and Masaka	<i>Hyparrhenia hirta</i> - <i>Dichrostachys cinerea</i> old field grassland
4	Dry	Unknown	<i>Trachypogon spicatus</i> - <i>Tristachys leucothix</i> rocky wooded grassland
5	Dry	Phumelele	<i>Hyparrhenia hirta</i> - <i>Dichrostachys cinerea</i> old field grassland
6	Dry	Nyakanyaka, Qalokwakhe	<i>Hyparrhenia hirta</i> - <i>Dichrostachys cinerea</i> old field grassland
7	Dry	Nyakanyaka, Qalokwakhe	<i>Trachypogon spicatus</i> - <i>Themeda triandra</i> - <i>Euclea crispa</i> rocky wooded grassland
8	Dry	Unknown	<i>Hyparrhenia hirta</i> - <i>Sporobolus africanus</i> old field grassland
9	Dry	Sphitiphithi	<i>Hyparrhenia hirta</i> - <i>Dichrostachys cinerea</i> old field grassland
10	Dry	Sphitiphithi	<i>Hyparrhenia hirta</i> - <i>Dichrostachys cinerea</i> old field grassland
11	Dry	Mahlasela	<i>Trachypogon spicatus</i> - <i>Tristachys leucothix</i> rocky wooded grassland
12	Dry	Phidela and Shonophi	<i>Trachypogon spicatus</i> - <i>Tristachys leucothix</i> rocky wooded grassland
13	Dry	Meleni and Masaka	<i>Combretum apiculatum</i> - <i>Euclea schimperi</i> rocky bushveld
14	Dry	Nkonka	<i>Bretonadia salicina</i> - <i>Ficus sycomorus</i> riparian vegetation
15	Dry	Masola	<i>Combretum apiculatum</i> - <i>Euclea schimperi</i> rocky bushveld
16	Dry	Shonaphi	<i>Combretum apiculatum</i> - <i>Bauhinia galpinii</i> open to dense bushveld

17	Dry	Phidela and Shonophi	<i>Hyparrhenia hirta</i> - <i>Sporobolus africanus</i> old field grassland
18	Dry	Vukani, Meleni and Masaka	<i>Ficus sur</i> - <i>Trimeria grandifolia</i> forests
19	Dry	Hashaza	<i>Trachypogon spicatus</i> - <i>Themeda triandra</i> - <i>Euclea crispa</i> rocky wooded grassland
20	Dry	Qhawe and Qolo	<i>Hyparrhenia hirta</i> - <i>Sporobolus africanus</i> old field grassland
21	Dry	Hoshimfe and Golide	<i>Hyparrhenia hirta</i> - <i>Sporobolus africanus</i> old field grassland
22	Dry	Unknown	<i>Olea europea subsp. africana</i> - <i>Euclea schimperi</i> dense bushveld
23	Dry	Magwaza	<i>Ficus glomosa</i> - <i>Euphorbia cooperi</i> wooded rocky outcrops
24	Dry	Hashaza	<i>Olea europea subsp. africana</i> - <i>Euclea schimperi</i> dense bushveld
25	Dry	Mahlathi	<i>Faurea saligna</i> - <i>Searsia harveyi</i> - <i>Cymbopogon excavatus</i> open woodland
26	Dry	Qolo	<i>Combretum apiculatum</i> - <i>Bauhinia galpinii</i> open to dense bushveld
27	Wet	July	<i>Combretum apiculatum</i> - <i>Euclea schimperi</i> rocky bushveld
28	Wet	Sphitiphithi	<i>Trachypogon spicatus</i> - <i>Tristachys leucothix</i> rocky wooded grassland
29	Wet	Unknown	<i>Bretonadia salicina</i> - <i>Ficus sycomorus</i> riparian vegetation
30	Wet	Meleni	<i>Combretum apiculatum</i> - <i>Euclea schimperi</i> rocky bushveld
31	Wet	Unknown	<i>Olea europea subsp. africana</i> - <i>Euclea schimperi</i> dense bushveld
32	Wet	Skhenka	<i>Acacia nilotica</i> - <i>Acacia ataxacantha</i> dense bushveld
33	Wet	Meleni	<i>Hyparrhenia hirta</i> - <i>Dichrostachys cinerea</i> old field grassland
34	Wet	Meleni and Spithipithi	<i>Faurea saligna</i> - <i>Searsia harveyi</i> - <i>Cymbopogon excavatus</i> open woodland
35	Wet	Zidumo and Selesele	<i>Combretum apiculatum</i> - <i>Diospyros lycoides subsp. nitens</i> rocky bushveld
36	Wet	Nenkani, Nyakanyaka	<i>Combretum apiculatum</i> - <i>Bauhinia galpinii</i> open to dense bushveld
37	Wet	Hoshimfe and Golide	<i>Combretum apiculatum</i> - <i>Bauhinia galpinii</i> open to dense bushveld
38	Wet	Masola	<i>Combretum apiculatum</i> - <i>Euclea schimperi</i> rocky bushveld
39	Wet	Meleni	<i>Pterocarpus angolensis</i> - <i>Tetraselago natalensis</i> rocky wooded grassland and open bushveld
40	Wet	Masaka	<i>Combretum apiculatum</i> - <i>Euclea schimperi</i> rocky bushveld

Supplementary Figures

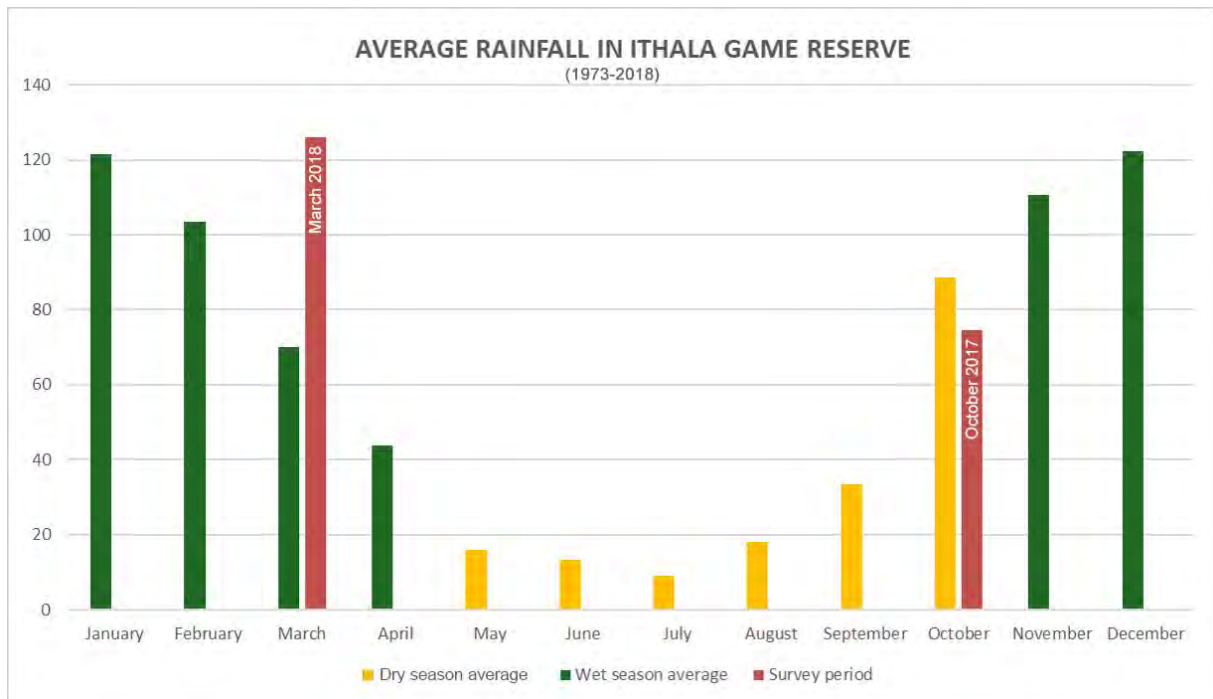


Figure S1. Average monthly rainfall in Ithala Game Reserve (IGR). Highlighted are the dry (yellow bars) and wet (green bars) seasons, as well as the survey periods (red bars)

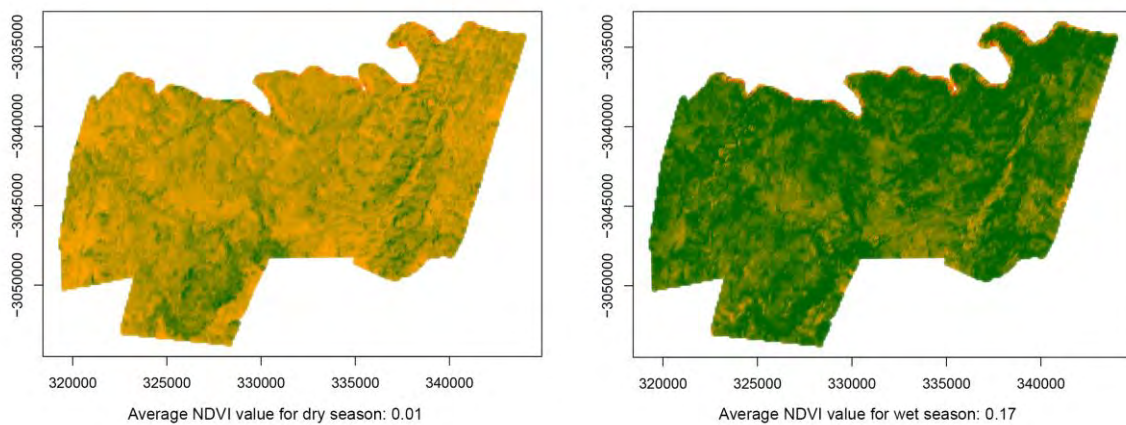


Figure S2. NDVI maps of dry (left panel) and wet (right panel) seasons at Ithala Game Reserve (IGR). Greener pixels indicate higher photosynthetic activity.

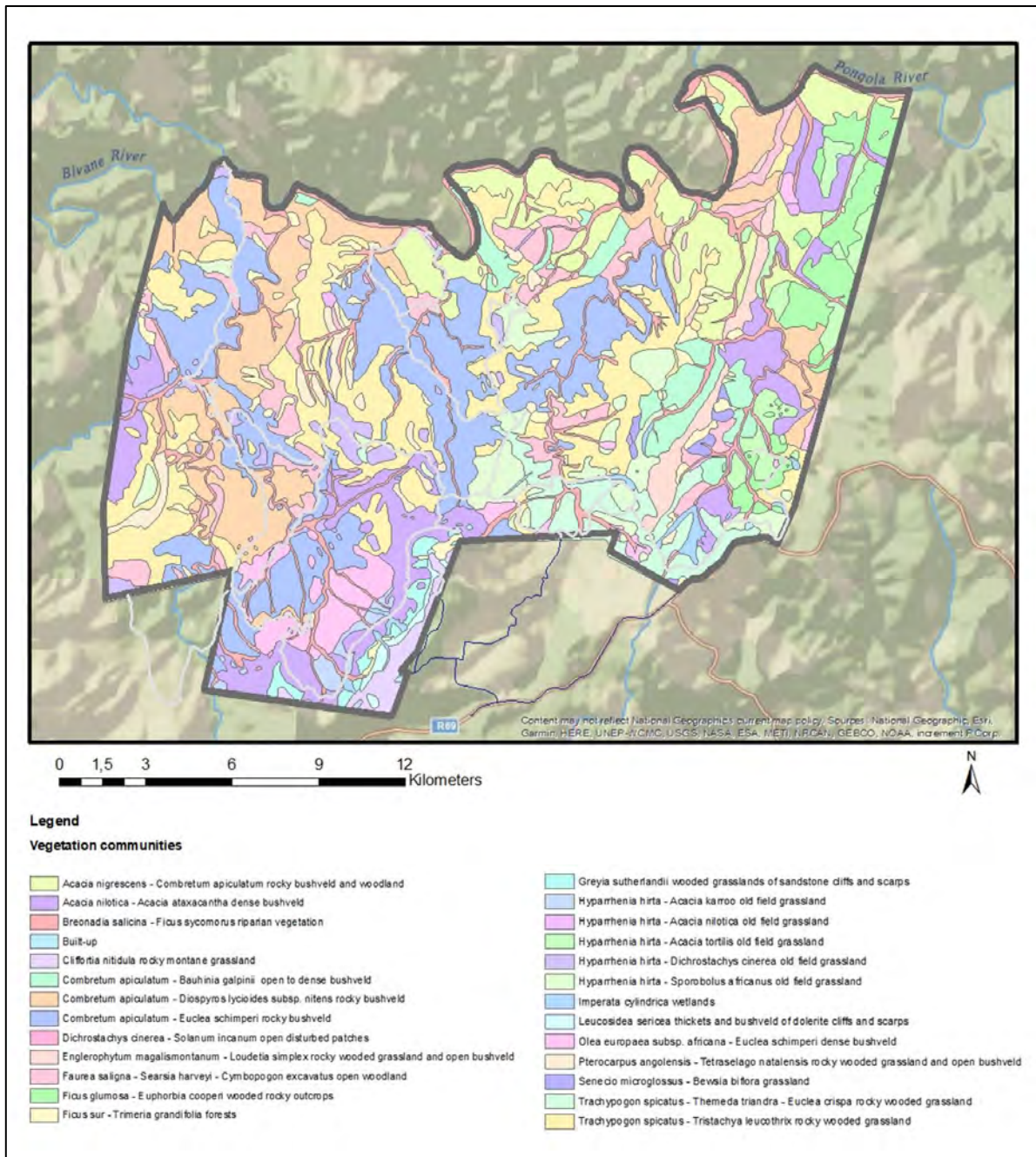


Figure S3. Map of Ithala Game Reserve (IGR) displaying vegetation communities as described by Noel van Rooyen and Gretel van Rooyen in their vegetation monitoring report of Ithala Game Reserve (2010).

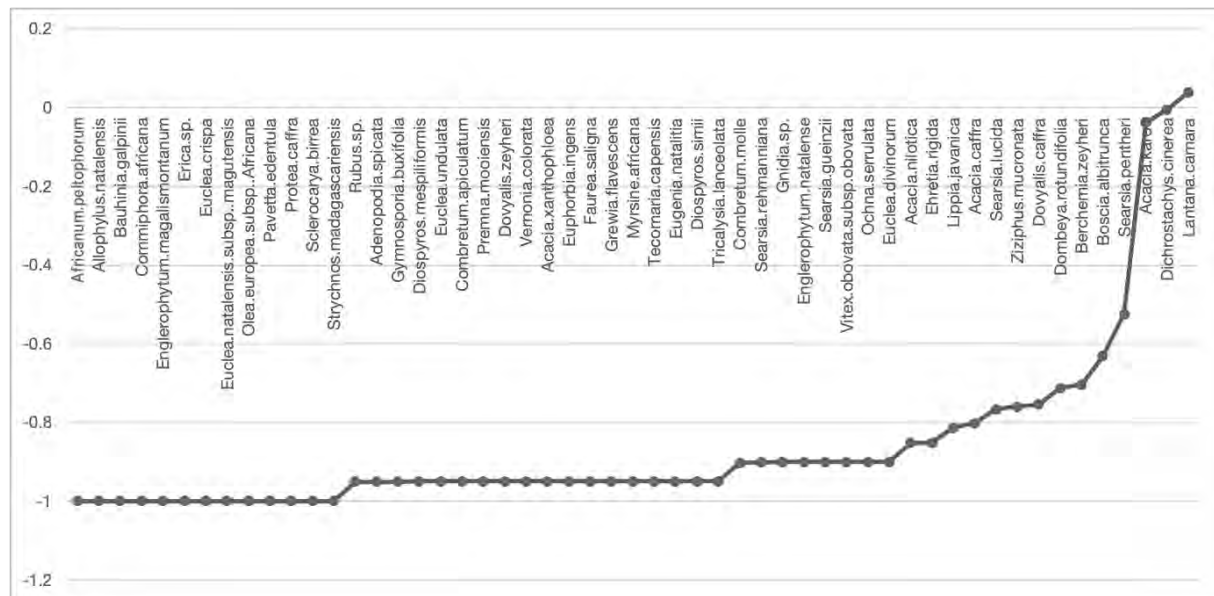


Figure S4. Plant species preference. Shown are the Ivlev's electivity indices for all species encountered along the 40 transects surveyed over two seasons. Positive values indicate high preference, while negative values above -1 indicate low preference, and value = -1 indicate avoidance.

Supporting Information - Chapter II

Supporting Information for Chapter II.

Supplementary Methods

DNA metabarcoding bioinformatical analyses

The full bioinformatic analyses were automatically processed using DeltaMP v. 0.5 (Lentendu, 2021)(Lentendu, 2021) on a HPC (“Centre de Calcul de la Faculté des Sciences”, University of Neuchâtel, Switzerland). Raw Illumina reads were demultiplexed using cutadapt v. 2.10 (Martin, 2011) allowing until 2 mismatches on the barcode sequences. The barcode sequence was stripped off the 5'-end of both R1 and R2 libraries. Every combination of the 16 forward and 25 reverse barcodes was searched for. From the 400 possible barcode pairs, 200 pairs from the 200 samples were found, representing 79.01 % of the 18299786 raw read count, and 200 unexpected pairs were also found (further named sequencing negative controls), accounting for 3.97 % of the raw read count. Sequences from both samples and sequencing negative controls were further analyzed the same way. Primer sequences were then removed from both ends in both libraries using the linked adapter strategy of cutadapt. For that, the full primer at 5'-end was requested to be anchored at sequence 5'-end (after stripping the barcode) and at least 8 nucleotides of the second primer should be found toward the 3'-end of the sequence, allowing until 20 % mismatches over the matching length. This step produced four sets of sequences per sample, that is sequences in both orientation (i.e. originally starting with the forward or the reverse primer) and in both R1 and R2 libraries. After stripping the primer sequences, only the amplified biological sequences were further analyzed. In order to avoid low quality end of reads, only the 80 first nucleotide of each sequence were kept and sequences with a maximum expected error above 0.5 were removed using VSEARCH v. 2.13.6 (Rognes *et al.*, 2016). Amplicon sequence variants (ASV) were then created using the *DADA2 R* package v. 1.14.1 (Callahan *et al.*, 2016). ASV refer to a single DNA sequence and is used as the standard unit of marker-gene analysis and represents significantly biological reality (Callahan, McMurdie & Holmes, 2017). For that, one error model was built for each R1 and R2 library separately and then applied to call ASVs for each orientation in each library, turning the pool option on. Paired

reads in R1 and R2 libraries were assembled with the *dada2 mergePairs* function, with a minimum overlap length of 20 nucleotides and a maximum of 2 mismatches in the overlap region. ASV originally with the reverse primer in the R1 library were reverse complemented using the *seqinr* package v. 3.6-1 (Charif & Lobry, 2007). The presence of chimera was double-checked first with the *dada2* function *removeBimeraDenovo* and with the UCHIME *de-novo* algorithm as implemented in VSEARCH (Edgar *et al.*, 2011).

To assign the taxonomy of each ASV, a reference database of *rbcL* gene was manually created. First, all *rbcL* coding sequences (CDS) were retrieved from the NCBI protein database in form of nucleotide sequence using the NCBI E-utilities and the query "*rbcL*[Gene Name] AND 50:10000[Sequence Length]" (*Entrez Programming Utilities Help*, 2010). Second, only reference sequences assigned to a plant species already observed in Ithala Game Reserve in South Africa were selected from the sequence database. In a few cases, homonyms, as listed on GBIF (gbif.com) were used to allow for proper species name matches between the reserve species list and the NCBI taxonomy. When species name could not be found, genus name was used instead, thus also adding in the database a few sequences of sister species not originally found in the sampled region. The sequences assigned to *A. thaliana* were also added to this reduced database in order to identified sequences from the positive control. Third, the *rbcL* sequences were reduced to the amplified region using the linked adapter strategy of *cutadapt*. Only sequences containing the full sequences of both primers (maximum two mismatches) were kept and only the virtual amplicon (i.e. the region between both primers) was extracted to create the final reference database. Only one exemplar of identical sequence per species was kept. Finally, taxonomy was assigned to each ASV using the VSEARCH option `-usearch_global` without allowing any end-gap. When multiple best matches were found in the database, a consensus taxonomy was resolved at a threshold of 60%.

The final ASV matrix contains 12443133 reads distributed over 388 samples and 901 ASVs: 12232045 reads in 189 biological samples and 897 ASV; 108196 reads in 2 positive controls and 40 ASV; 64083 reads in 9 negative PCR controls and 53 ASVs; 38809 reads in 188 sequencing negative controls and 101 ASVs.

The following tag jump correction methods were written and conceptualized by Dr. Guillaume Lentendu. Tag-jump, that is, the assignment of a sequence to a wrong sample is a known issue in library preparation for metabarcoding and is attested here by the presence of all unexpected

tag combinations at the demultiplexing step (Schnell, Bohmann & Gilbert, 2015). In order to assign back sequences likely originating from tag-jump to their correct sample, all sequencing negative controls were analyzed. Sequences found in these sequencing negative controls can only originate from tag-jump and are more likely to occur in the most abundant ASVs found across all samples.

After removing remaining artifacts (i.e. ASVs with total read count in sequencing negative control of at least half the total read counts in biological samples and positive control; 59 ASVs, 19009 reads), a multiple linear regression was built between the maximum count of an ASV in any sequencing negative control and the interaction between the total count of this ASV over all biological samples and positive control and the maximum count of this ASV in any biological sample or positive control (ANOVA adjusted $R^2 = 0.968$; $p < 0.001$). The 99.9 % upper confidence interval of this multiple linear regression was used as a threshold to determine if the read count of an ASV in a sample was uniquely due to tag-jump or have a proper biological origin. When the read count in one sample was below the threshold of a particular ASV, the read count for this sample was set to 0. All reads from an ASV removed this way were then redistributed proportionally to the samples with reads. After the tag-jump correction, only five sequencing negative controls (out of 188) still have a total of 13 reads in 3 ASVs. In the positive controls, 109144 out of 109150 reads are assigned to a single ASV identified as *A. thaliana*, the 6 other reads to two ASV also identified as *A. thaliana*, while the 37 other ASV were efficiently removed by the tag-jump correction approach. The final corrected matrix contained 12250119 reads distributed in 179 biological samples and 571 ASVs. Nine biological samples had no PCR amplification and were thus retrieved only due to tag-jump.

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Supplementary Tables

Table S1 Overview of the sample size (N), mean Shannon’s diversity index, mean richness, and mean evenness represented by ASVs count with standard error (SE) for each herbivore’s species.

Species	N	Shannon Index	(±SE)	Richness	(±SE)	Evenness	(±SE)
Black Rhino	80	1.40	0.05	19.66	0.85	0.49	0.02
Elephant	30	1.85	0.09	27.9	1.36	0.57	0.03
Kudu	29	1.88	0.04	29.38	1.44	0.56	0.01
Impala	29	1.78	0.09	27.76	1.53	0.54	0.03

Table S2 Table of dietary Pianka’s niche overlap between all four herbivores in general, during the dry and wet seasons and EcoSim simulation.

Season	Lower-tail p = (Obs < Exp)	Upper-tail p = (Obs > Exp)	Standard Effect Size (SES)	Pianka's Index
All	< 0.001*	> 0.999	34.603	0.82
Dry	< 0.001*	> 0.999	27.601	0.65
Wet	< 0.001*	> 0.999	35.554	0.84

	Black Rhino	Elephant	Impala	Kudu
Black Rhino	0	0.48	0.61	0.81
Elephant	0.54	0	0.63	0.69
Impala	0.43	0.57	0	0.67
Kudu	0.41	0.44	0.49	0

Table S3 Table showing the Bray-Curtis dissimilarity (bottom-left; 0: similar; 1: dissimilar) and Pianka's niche overlap index (top-right; 0: no overlap; 1: full overlap) based on the mean ASVs frequency per herbivore during the dry season

	Black Rhino	Elephant	Impala	Kudu
Black Rhino	0	0.94	0.96	0.73
Elephant	0.29	0	0.89	0.78
Impala	0.28	0.4	0	0.75
Kudu	0.47	0.4	0.47	0

Table S4 Table showing the Bray-Curtis dissimilarity (bottom-left; 0: similar; 1: dissimilar) and Pianka's niche overlap index (top-right; 0: no overlap; 1: full overlap) based on the mean ASVs frequency per herbivore during the wet season.

Table S5 Table of dietary Pianka's niche overlap between the strict browsers, black rhino and kudu, during the dry and wet seasons and EcoSim simulation.

Season	Lower-tail p = (Obs < Exp)	Upper-tail p = (Obs > Exp)	Standard Effect Size (SES)	Pianka's Index
Dry	< 0.001*	> 0.999	12.982	0.81
Wet	< 0.001*	> 0.999	12.667	0.72

Table S6 Table of dietary Pianka's niche overlap between the mixed feeder, elephant and impala, during the dry and wet seasons and EcoSim simulation.

Season	Lower-tail $p = (\text{Obs} < \text{Exp})$	Upper-tail $p = (\text{Obs} > \text{Exp})$	Standard Effect Size (SES)	Pianka's Index
Dry	< 0.001*	> 0.999	11.061	0.63
Wet	< 0.001*	> 0.999	17.551	0.89

Supplementary Figures

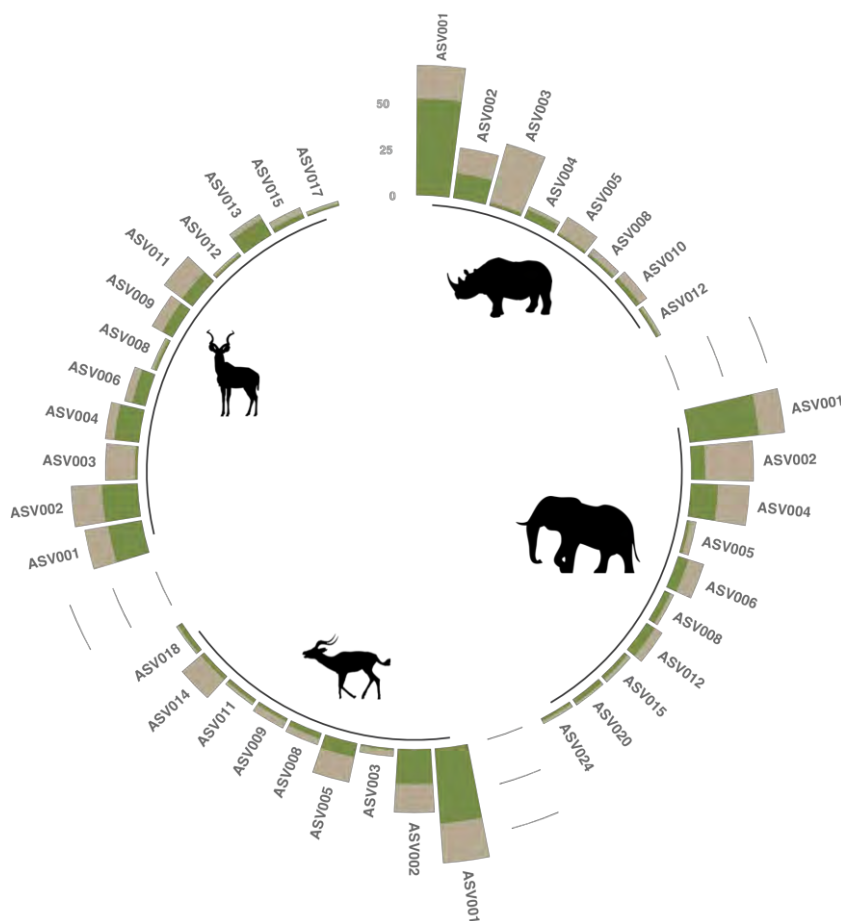


Figure S1. Circular barchart showing ASVs consumed (representing $\geq 1\%$ of total diet composition) and their percentages over seasons (dry season tan colour, wet season green colour) for each herbivore species (black rhino top right, elephant bottom right, impala bottom left and kudu top left).

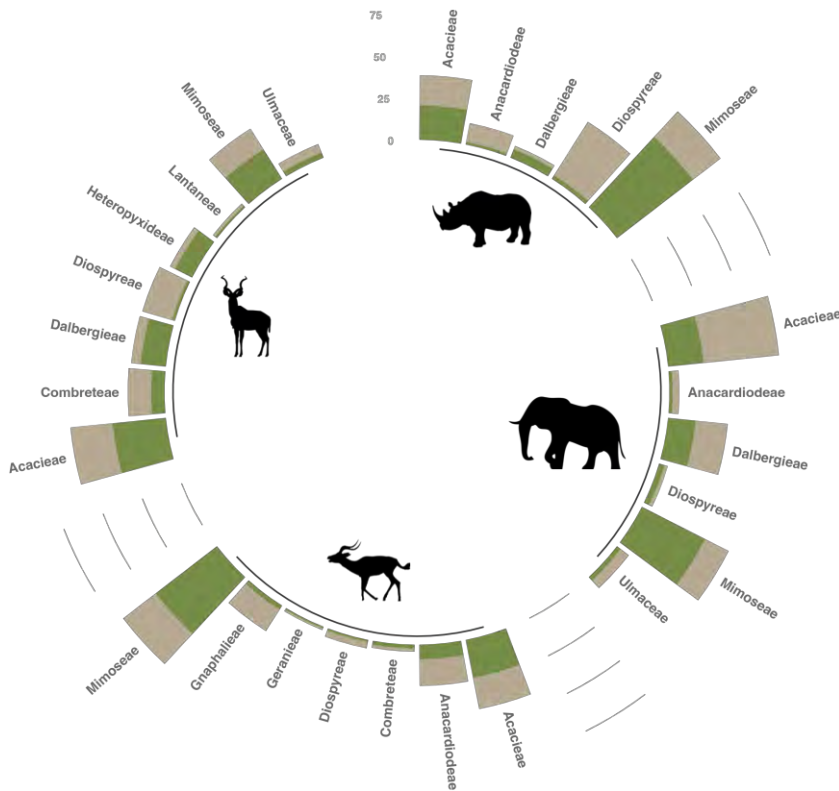


Figure S2. Circular barchart showing plant tribes consumed (representing $\geq 1\%$ of total diet composition) and their percentages over seasons (dry season tan colour, wet season green colour) for each herbivore species (black rhino top right, elephant bottom right, impala bottom left and kudu top left).

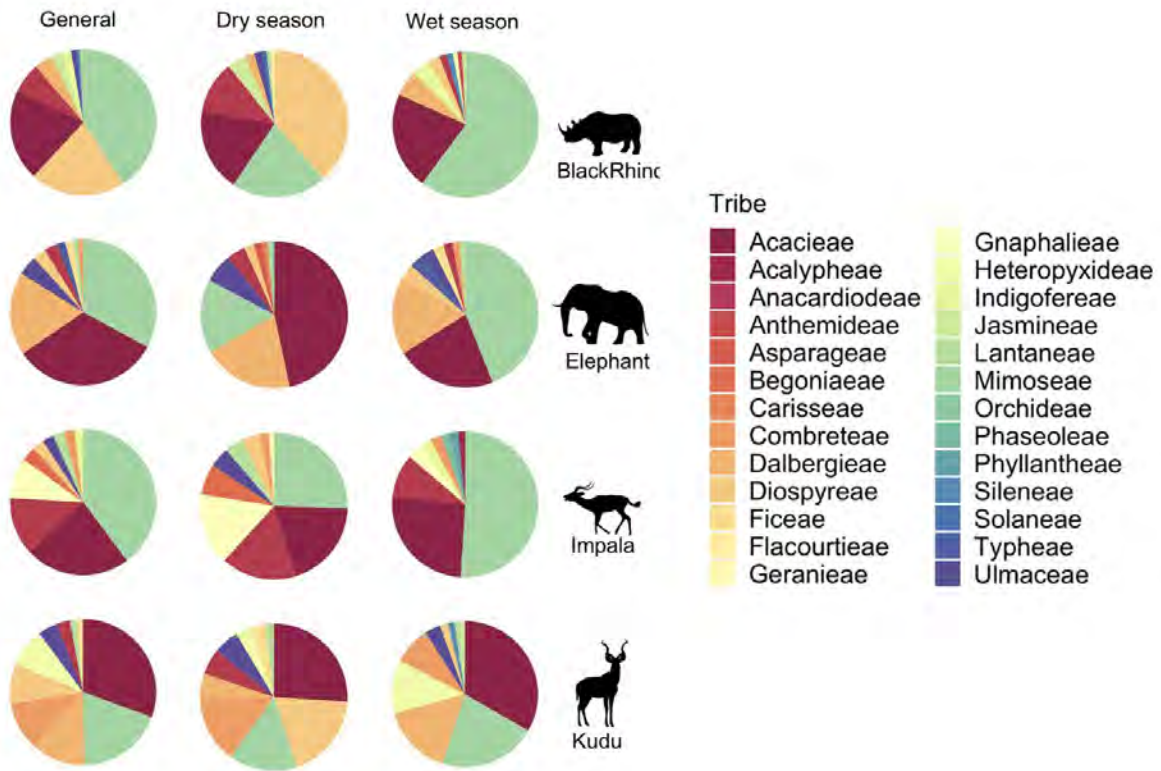


Figure S3. Pie charts showing all tribes consumed overall and across seasons for each herbivore

Supporting Information - Chapter III

Supporting Information for Chapter III.

Supplementary Figures

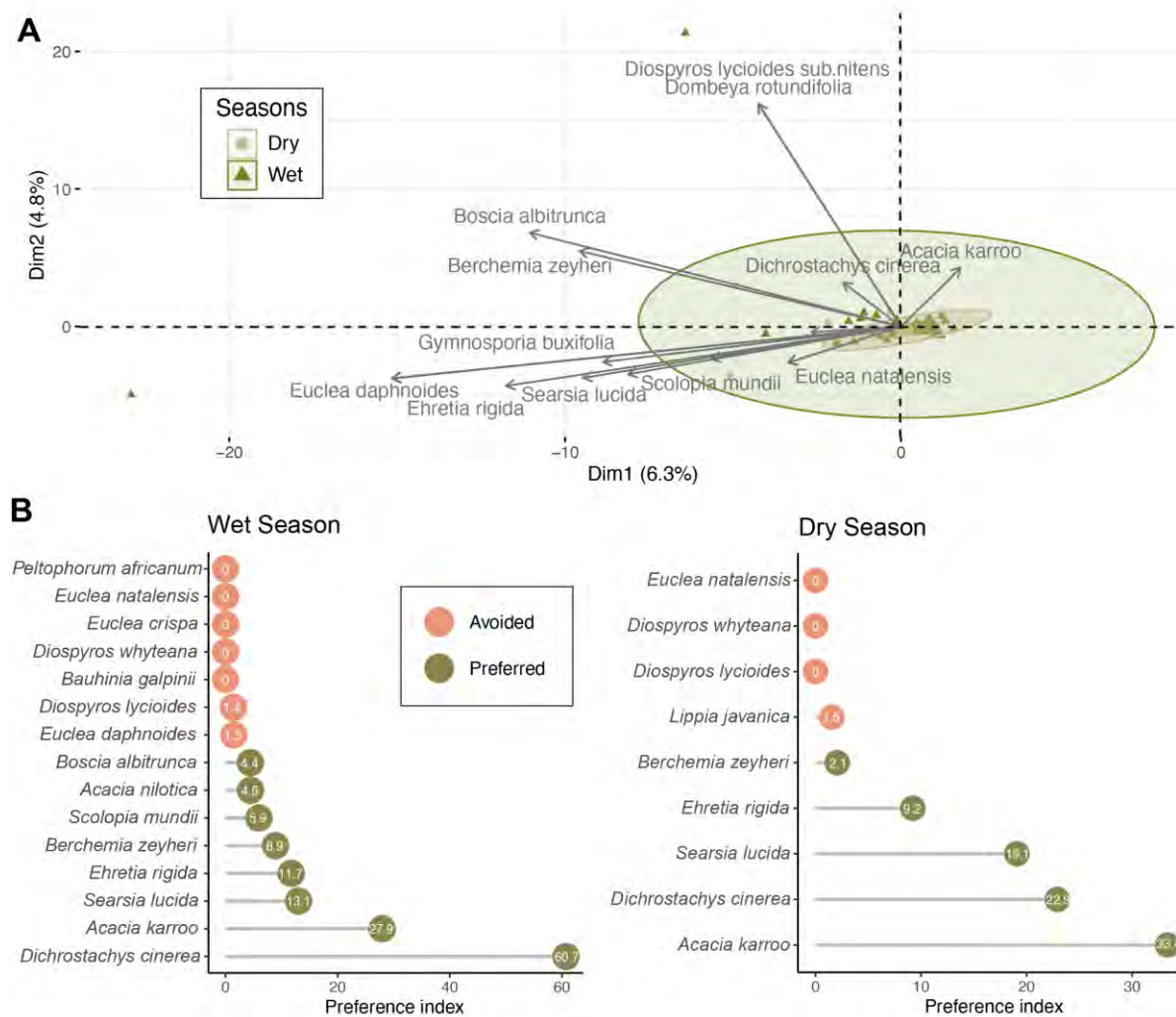


Figure S1. Seasonal black rhino preference and sampled plant species (A) PCA biplot organizing transects by preference (calculated with Ivlev's electivity index) by seasons (wet in green and dry in tan). Vectors (in grey) indicate species 12 plant species (variables) with the highest cos2. (B) Sampled plant species in the wet season and dry season; avoided species and group colored in salmon and preferred in khaki.

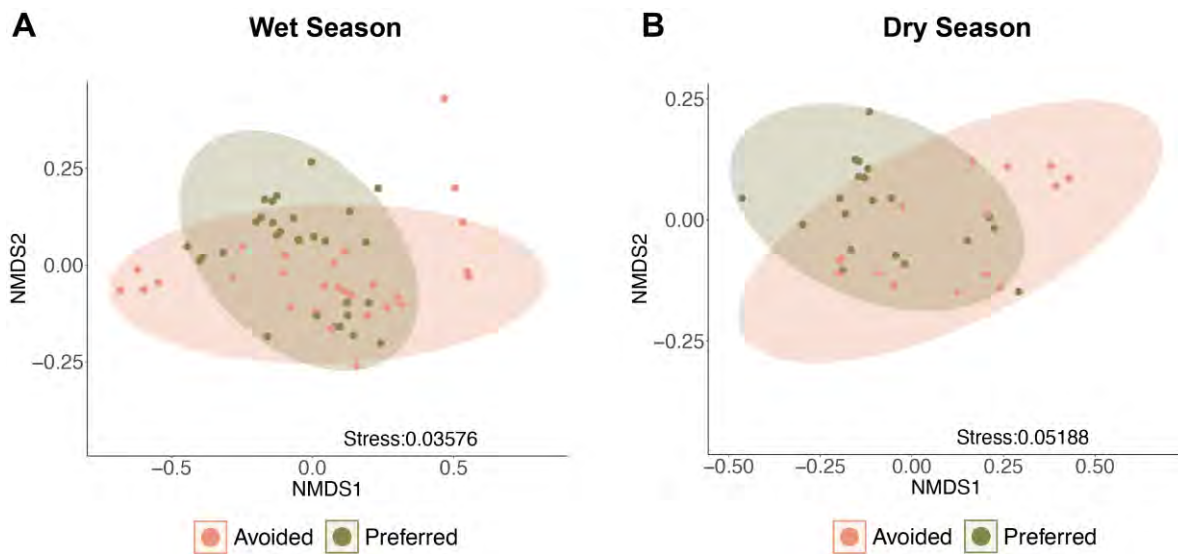


Figure S2. Traits of black rhino avoided and preferred plant species. (A) NMDS plot organizing plant species (avoided colored in salmon and preferred colored in khaki) by traits based on Bray-Curtis dissimilarity for the wet season (A) and dry season (B). Ellipses represent 95% confidence intervals around each group's data distribution.

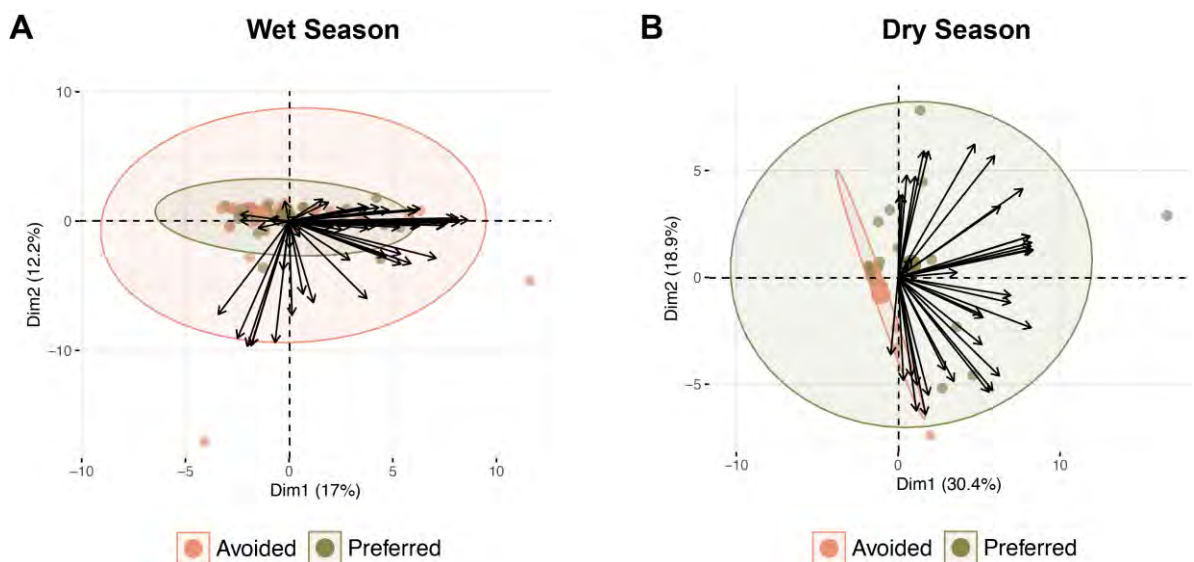


Figure S3. VOCs of black rhino avoided and preferred plant species. (A) Principal Component Analysis (PCA) biplot organizing avoided and preferred plant by volatiles for the wet season (A) and dry season (B). Avoided group and species coloured in salmon and preferred coloured in khaki. Ellipses represent 95% confidence intervals around each group's data distribution.

Supporting Information - Chapter IV

Supporting Information for Chapter IV.

Supplementary Tables

Table S1. Summary tables showing the number of black rhinos retained for analysis, after stringent filtering (described in Methods), in each group and by sex along with their mean home-range areas (km²) and change (%). a) Summary table showing the mean areas (km²) of control and dehorned black rhino home ranges calculated with 95% minimum convex polygons (MCP) and 95% kernel density estimates (KDE) and the number of black rhinos in each group, across all reserves. The change (%) shows the percentage of increase or decrease in area between periods. b) Summary table showing the mean areas (km²) of control black rhino home ranges calculated with 95% minimum convex polygons (MCP) and 95% kernel density estimates (KDE) and the number of black rhinos by sex. The change (%) shows the percentage of increase or decrease in area between periods. c) Summary table showing the mean areas (km²) of dehorned black rhino home ranges calculated with 95% minimum convex polygons (MCP) and 95% kernel density estimates (KDE) and the number of black rhinos by sex. The change (%) shows the percentage of increase or decrease in area between periods

a) Mean areas (km²) of home ranges of control and de-horned black rhinos

		MCP			KDE		
	Number of BR	Area before	Area after	Change (%)	Area before	Area after	Change (%)
Control	120	12.93	20.31	57.02	60.88	77.99	28.11
Dehorned	68	26.24	14.33	-45.38	99.17	56.07	-43.45

b) Mean areas (km²) of home ranges of control black rhinos by sex

		MCP			KDE		
	Number of BR	Area before	Area after	Change (%)	Area before	Area after	Change (%)
Female	53	11.50	19.27	67.55	62.98	73.36	16.49
Male	67	14.07	21.14	50.21	59.22	81.65	37.88

c) Mean areas (km²) of home ranges of de-horned black rhinos by sex

	Number of BR	MCP			KDE		
		Area before	Area after	Change (%)	Area before	Area after	Change (%)
Female	30	29.06	13.64	-53.08	101.54	52.76	-48.04
Male	38	24.01	14.88	-38.03	97.29	58.69	-39.67

Table S2. Summary tables showing the number of black rhinos retained for analysis, after stringent filtering (described in Methods), in each group and by reserve along with their mean home-range areas (km²) and change (%). a) Summary table showing the mean areas (km²) of control black rhino home ranges calculated with 95% minimum convex polygons (MCP) and 95% kernel density estimates (KDE) and the number of black rhinos by reserve. The change (%) shows the percentage of increase or decrease in area between periods. b) Summary table showing the mean areas (km²) of dehorned black rhino home ranges calculated with 95% minimum convex polygons (MCP) and 95% kernel density estimates (KDE) and the number of black rhinos by reserve. The change (%) shows the percentage of increase or decrease in area between periods

a) Mean areas (km²) of home ranges of control black rhinos by reserve

Reserve	Number of BR	MCP			KDE		
		Area before	Area after	Change (%)	Area before	Area after	Change (%)
Ithala	29	9.30	13.08	40.65	35.58	45.33	27.40
Manyoni	20	25.36	22.30	-12.06	110.11	90.46	-17.84
Ndumo	3	5.06	10.85	114.47	69.78	70.60	1.18
Phinda	38	11.64	33.54	188.03	63.65	123.60	94.19
Pongola	3	3.21	11.24	249.76	23.12	52.69	127.90
Tembe	2	36.16	26.32	-27.20	174.17	128.81	-26.04
Thanda	9	7.98	6.26	-21.58	42.40	38.10	-10.14
uMkhuze	5	12.05	10.17	-15.58	67.68	37.62	-44.41
Weenen	11	9.41	10.11	7.40	27.76	34.46	24.14

a) Mean areas (km²) of home ranges of dehorned black rhinos by reserve

Reserve	Number of BR	MCP			KDE		
		Area before	Area after	Change (%)	Area before	Area after	Change (%)
Ithala	14	14.37	12.49	-13.11	51.53	57.19	10.98
Manyoni	26	25.50	16.17	-36.59	91.62	54.58	-40.43
Phinda	18	45.05	15.49	-65.61	169.17	65.01	-61.57
Pongola	2	14.46	8.86	-38.70	101.45	38.18	-62.36
Tembe	1	12.86	10.21	-20.60	97.09	63.44	-34.66
Thanda	2	6.41	10.64	66.05	53.34	52.45	-1.66
Weenen	5	10.94	10.24	-6.40	37.57	35.71	-4.96

Supplementary Figures

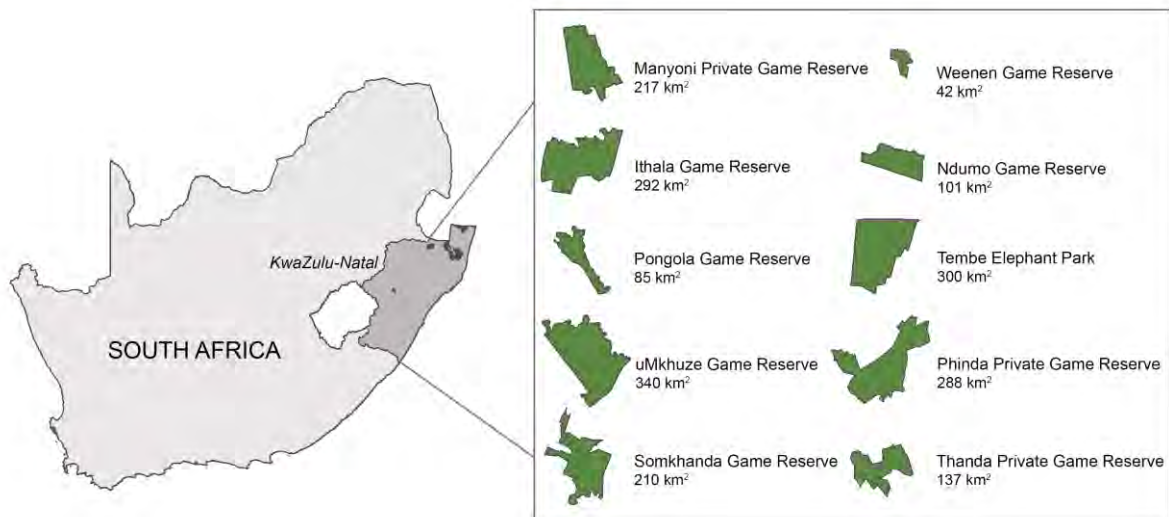


Figure S1. Study sites. Locations and sizes (km²) of the 10 game reserves in KwaZulu-Natal, South Africa, used in this study.

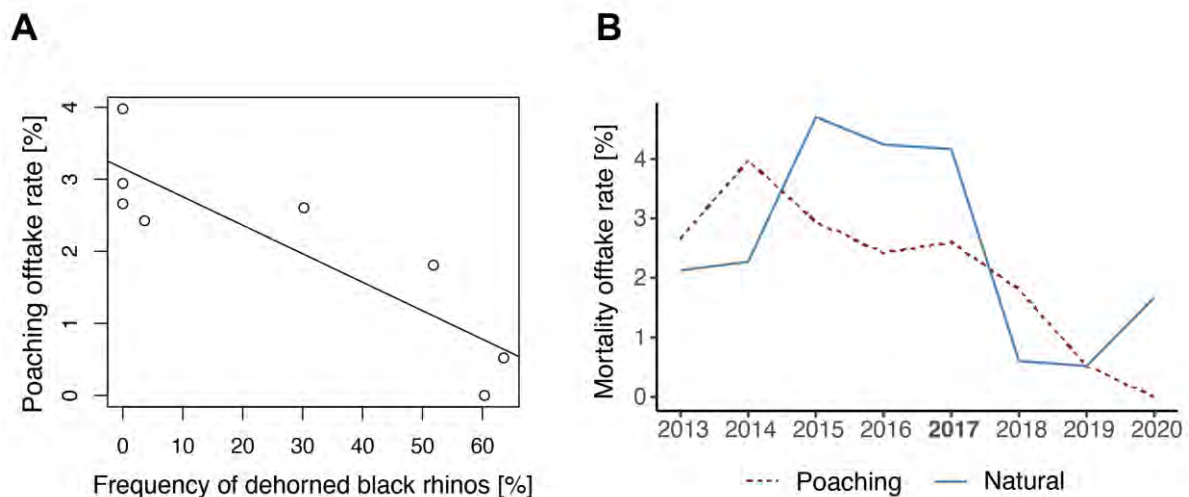


Figure S2. Trends in dehorning and poaching (A) Scatterplot with trend line fit showing the negative correlation between the poaching offtake rate and the frequency of dehorned rhinos (percentage) in the study sites (linear regression; $R^2 = 0.72$, $F_{1,6} = 19.27$, $p = 0.004$). (B) The poaching offtake rate compared to the natural offtake rate of black rhinos in the study sites over the same time period. The poaching offtake rate was calculated by the yearly number of poached black rhinos in study sites divided by the yearly total population of black rhinos in the study sites. The natural offtake rate was calculated the same way based on natural mortalities. The start of intensive dehorning in 2017 is highlighted in a bold font.

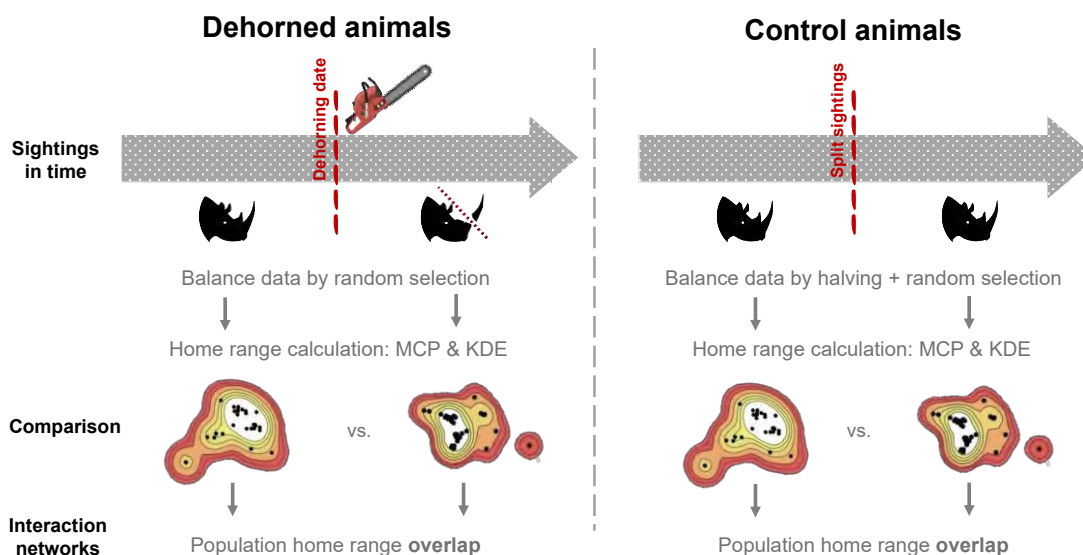


Figure S3. Study design. Workflow used to calculate home-range sizes and social-interaction networks for each rhino during each time period, which included both dehorned animals (before and after dehorning) and horned (control) animals.

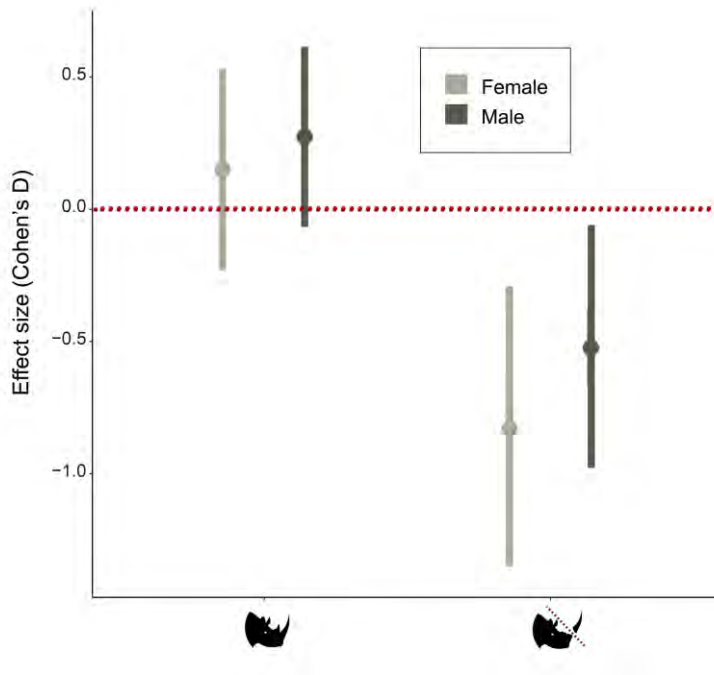


Figure S4 Changes to black rhino home range size before versus after dehorning. Change in home range area in horned control animals versus dehorned animals (females: tan; males: green), derived from 95% kernel density estimates (KDE) calculations of effect sizes using Cohen's D Hedge correction with error bars representing 95% confidence intervals (Methods). Analogous results for home ranges estimated using minimum convex polygons are shown in Figure 2

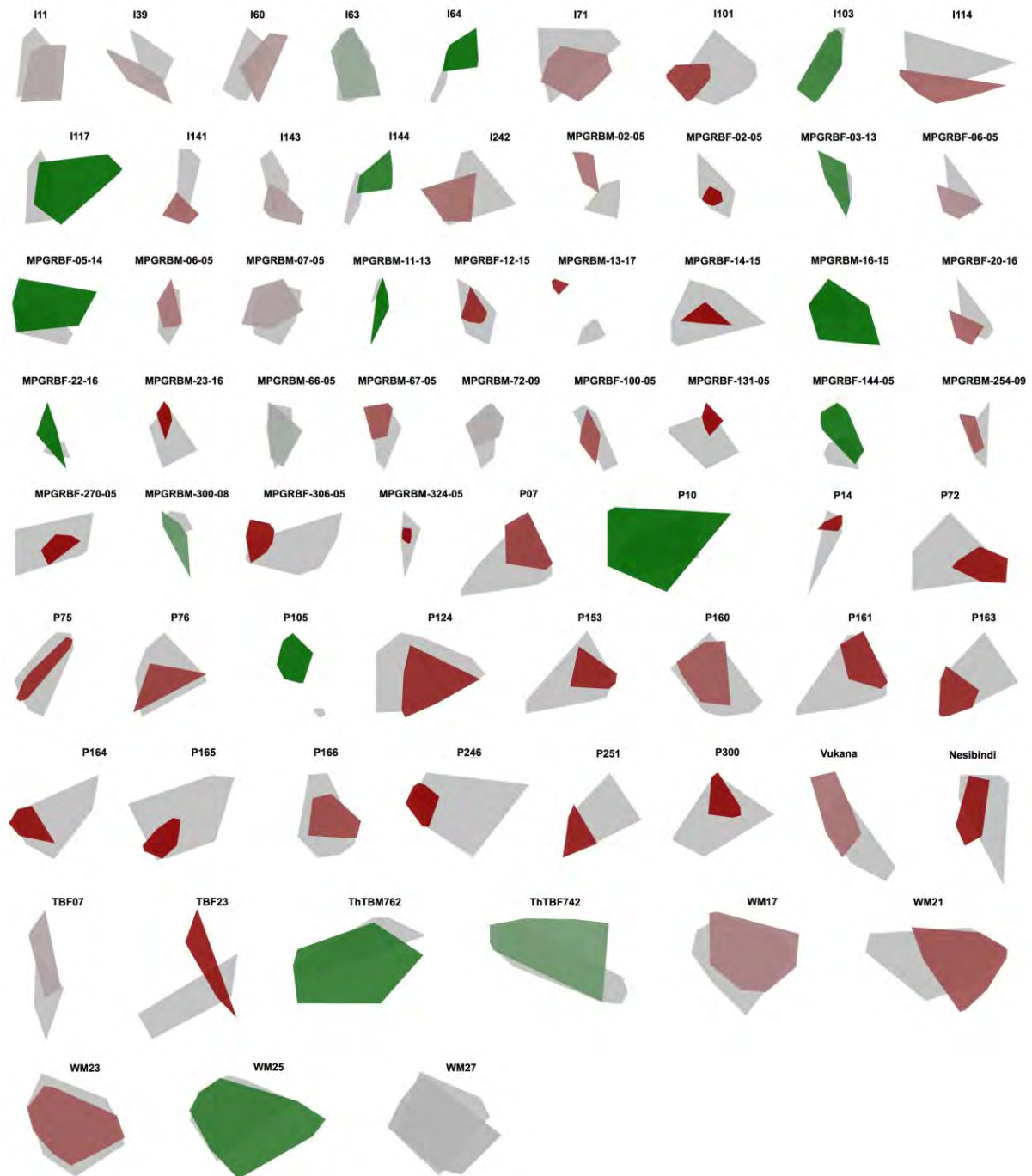


Figure S5. Home-range changes for all dehorned rhinos in this study. Home ranges were calculated with 95% minimum convex polygons (MCP) of sightings of dehorned rhinos. For each rhino (N=68), grey polygons represent horned-period home ranges and shades of green or red represent increased or decreased dehorned-period home ranges, respectively. The gradients of green and red illustrate the amount of increase and decrease in area (the darker, the higher).

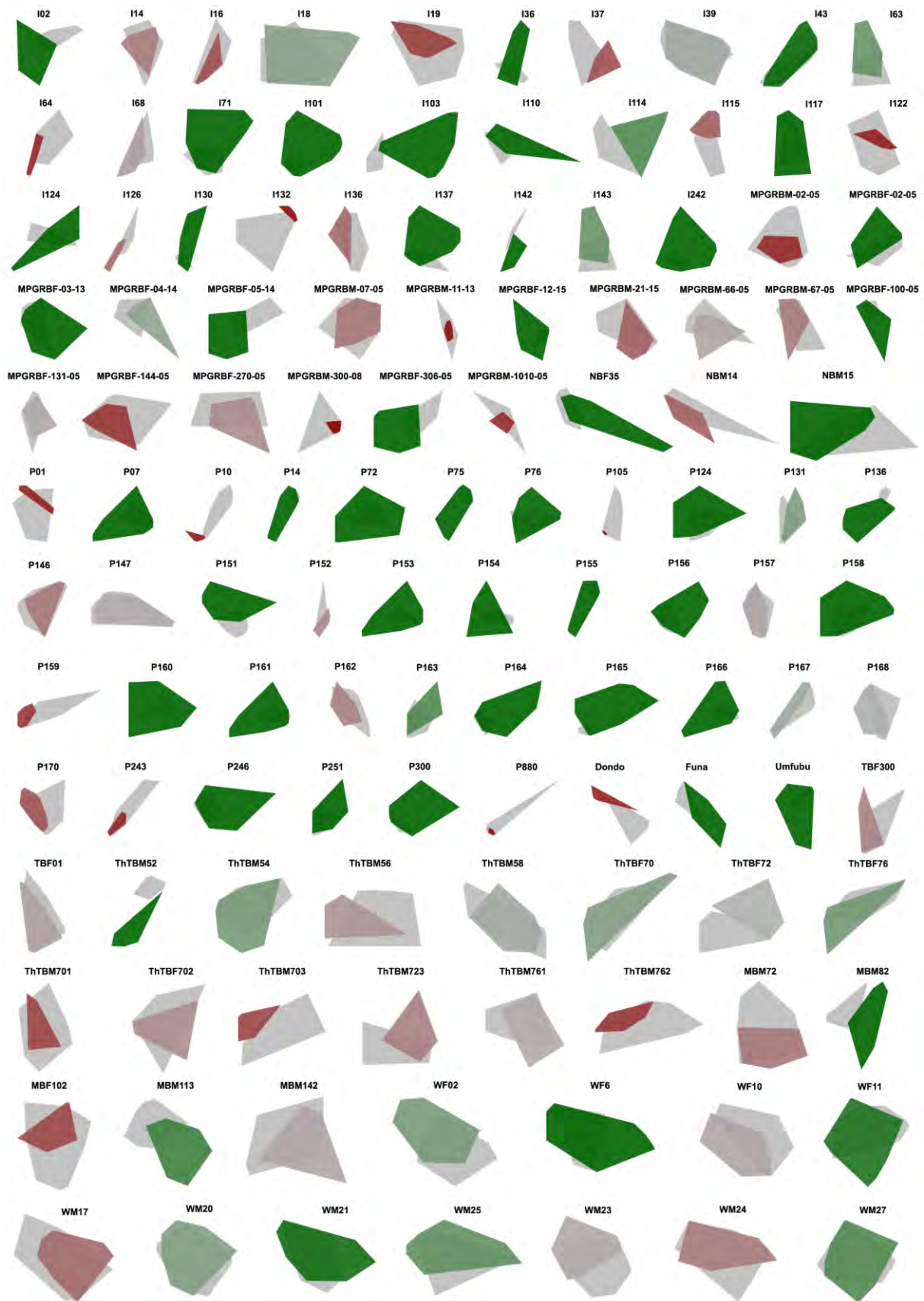


Figure S6. Home-range changes for horned (control) rhinos in this study. Home ranges were calculated using 95% minimum convex polygons (MCP) of control (never dehorned) rhinos. For each

rhino (N=120), grey polygons represent first-period home ranges (corresponding to horned-period home ranges of dehorned individuals) whereas green and red represent increased or decreased second-period (corresponding to post-dehorning for dehorned individuals) home ranges, respectively. The gradients of green and red illustrate the amount of increase and decrease in area (the darker, the higher).

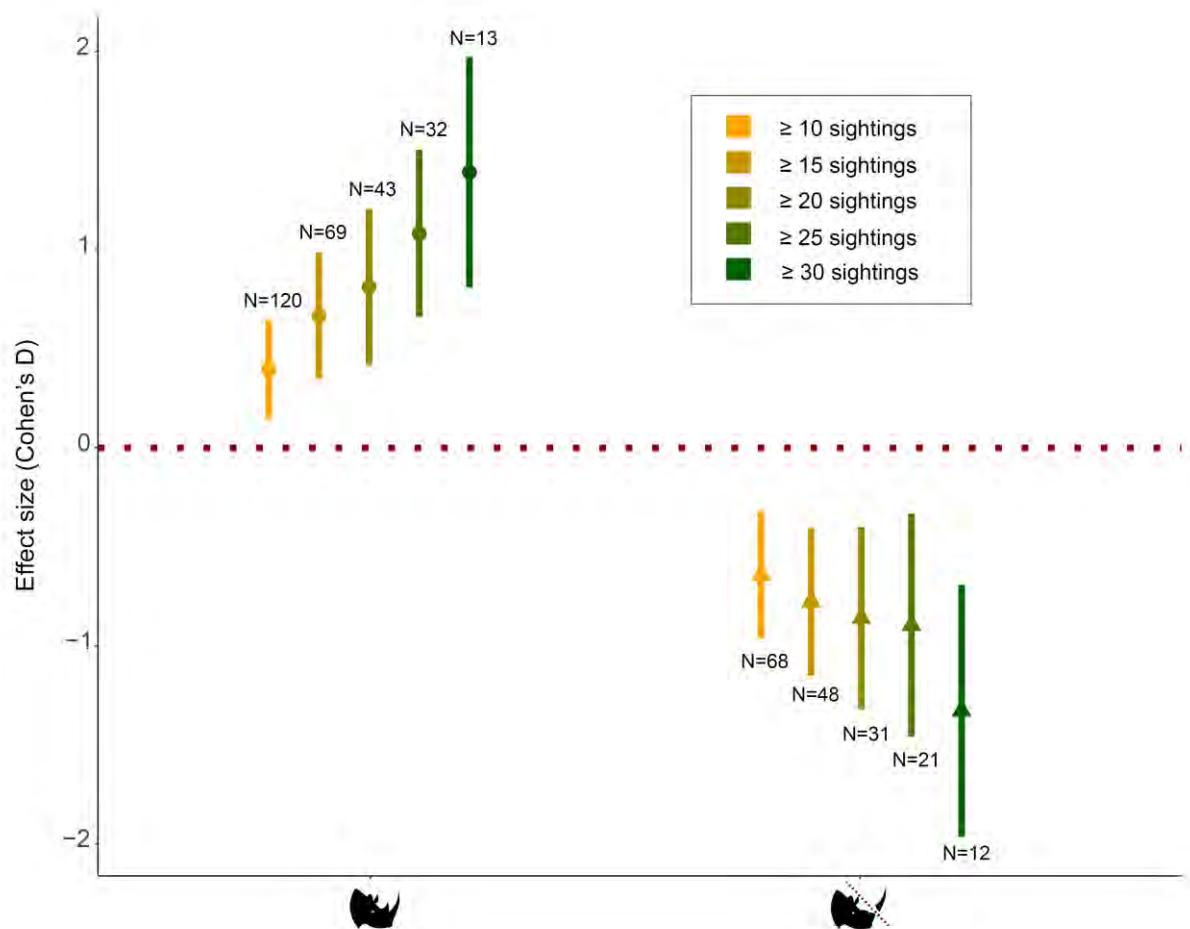


Figure S7 Sensitivity analysis in the number of sightings used to construct home ranges. The dot plot shows averages (+/- 95 % confidence intervals) changes in home range areas in horned control animals versus dehorned animals, derived from 95% minimum convex polygons (MCP 95%) calculations of effect sizes using Cohen's D with Hedge correction with error bars representing 95% confidence intervals (Methods). Each class (from light yellow to dark green gradient) comprises black rhinos with at least 10, 15, 20, 25 and 30 sightings used to construct home ranges in both periods. The number of rhinos in each class is annotated as equal to N above the effect size for control classes and under for dehorned classes. This analysis shows that our results are qualitatively robust to the number of sightings used to estimate home ranges; accordingly, we present results based on the most inclusive threshold (10 sightings) to maximize the number of individuals included.

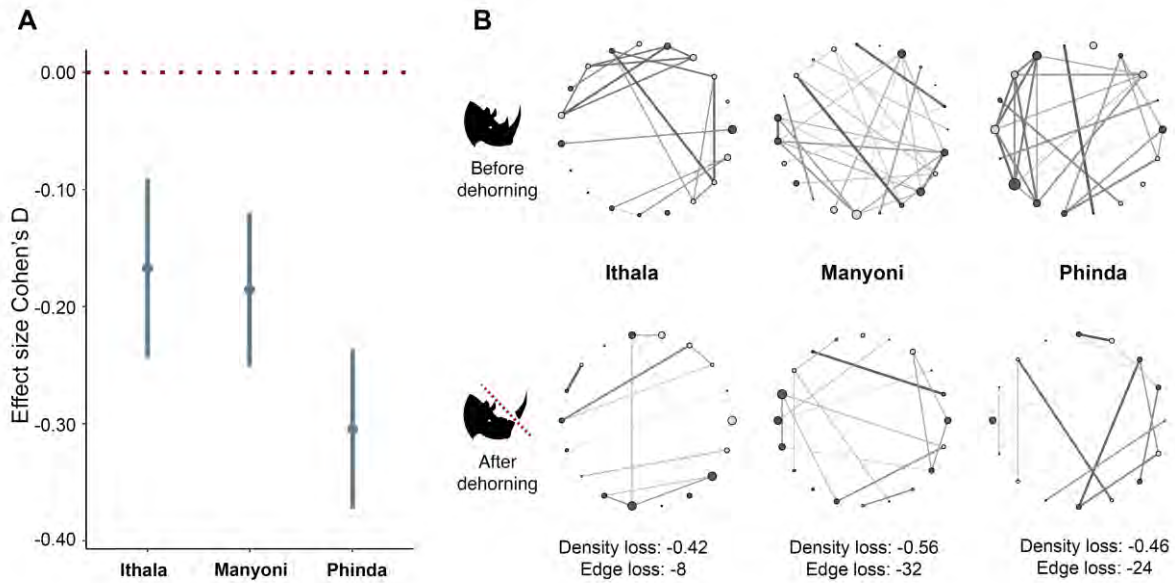


Figure S8. Changes to black rhino social structure after dehorning (supplemental analysis to Figure 3) (a) Dot plot showing averages (\pm 95 % confidence intervals) change in social interaction strength (effect size; Cohen's D) for interactions (all animals) in three reserves (Ithala N=20, Manyoni N=23; Phinda N=18) after dehorning, calculated based on overlap of core ranges using 50% MCPs (versus less restrictive 95% MCP home ranges). (b) Interaction networks showing social connectivity in the same three populations before (top) and after (bottom) dehorning. Each node is an individual and lines (edges) represent an interaction between two individuals, with a gradient of line thickness illustrating the strength of interaction (the amount of overlap). The size of the nodes represents the number of connections each individual has (the larger, the more). Edge and density loss represent the number of disappearing nodes and lines, respectively, after dehorning.



Acknowledgments

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