Postgraduate Thesis submitted for the degree of Masters of Research in Conservation Biology

The effect of megaherbivore guild intactness on the distribution of plant spinescence

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Abstract

A comparative approach was used to investigate the vertical distribution of the plant defence trait, spinescence, in response to the presence or absence of megaherbivores. The loss of megaherbivores across Europe 10,000 years ago led to a loss of ecological functions and processes. Pleistocene rewilding offers a solution involving the reintroduction of descendants of Pleistocene fauna and flora with the hope of restoring these lost ecological functions. This study compared the vertical distribution (length and density) of thorns on *Acacia* sp. in the Zambezi National Park in Zimbabwe (ZNP) with an intact megaherbivore guild, to hawthorn (*Crataegus monogyna*) and blackthorn (*Prunus spinosa*) trees in a rewilding site in England that has lost all megaherbivore species, to explore the potential of restoring this ecological relationship. The length of thorns above 2.5 metres (ZNP median = 2.3 cm, SD = 1.5; Knepp median = 3.5 cm, SD = 2.1) was longer in both study sites than below 1 metre (ZNP median = 1.3 cm, SD = 1.1; Knepp median = 1.6cm, SD = 1.8). The density was higher below 1 metre in Knepp (median = 9.5, SD = 6.4) than above 2.5 metres (median = 1, SD = 4.1). The density of thorns above 2.5 metres in ZNP (median = 22, SD = 6.9) was significantly higher than the density of thorns above 2.5 metres in Knepp. Evidence suggests European species are still able to produce and distribute thorns in a similar way to *Acacia* sp. in Africa, suggesting they have ecologically adapted to the absence of megaherbivores but have not evolutionarily adapted and would be capable of sustaining a reintroduced megaherbivore population in the future.

1. Introduction

The loss of megaherbivores across Europe during the quaternary megafauna extinction has led to a decline in ecological processes and ecosystem function (Johnson, 2009). By reintroducing ecological equivalents of lost megaherbivores, their function within an ecosystem can be restored. However, herbivores have a large impact on flora species and in particular how plants defend themselves. One of the longest running arms races involves the co-evolution of large herbivores and plants over the past 16 million years (Bradshaw *et al*¸ 2003; Charles – Dominique *et al*, 2016). The interaction between plants and herbivores varies significantly based on certain traits: for example, body mass and browsing height induces strong structural defence responses (Young *et al*, 2003). In particular, the size of megaherbivores, will have an effect on the structure and distribution of spinescence (spines, thorns and prickles) (Cooper and Owen-Smith, 1986; Young *et al*, 2003). With the lack of megaherbivores across Europe over the past 10,000 years, the evolution of these structures is likely to differ from structures found on plants in an ecosystem that has retained megaherbivores. In this study megaherbivores are considered ≥1000kg and lacking effective predators; whereas large herbivores are described as having a body size of 45 – 999kg (Malhi *et al*, 2016).

Plants have many different forms of defence, both chemical and structural, to allow them to thrive in environments where herbivores are common (Hanley *et al*, 2007). Chemical defences consist of secondary metabolites such as tannins and alkaloids and are used to defend against all types of herbivores regardless of size. Structural defences, such as spines, thorns and a thickened cuticle (Hanley *et al*, 2007), are used to defend against larger herbivores. This study will focus on the spinescence trait, widely regarded to be induced by herbivory from megaherbivores and large herbivores, and how this trait has changed in the absence of megaherbivores.

1.1. Co-evolution of plant-herbivore interactions

60 million years ago mammalian megafauna species expanded causing plants to develop new adaptations in response to these new species. During the Miocene, a land bridge between Eurasia and Africa allowed megaherbivores, such as rhinoceros and suids, to enter Africa (Bibi, 2013). There was a second invasion in the mid Miocene where mammals such as horned bovids and antlered giraffoids entered Africa. Novel plant adaptations in the Miocene across Africa include the rapid accumulation and diversification of spiny plant lineages in response to the diversification of bovids (Charles-Dominique *et al*, 2016). This suggests the novel mode of

feeding by bovids selected for the spinescence trait in plants (Bibi, 2013). Megaherbivores found in Europe included the grazing woolly mammoth and woolly rhinoceros, and in the early Pleistocene there would have also been browsing straight-tusked elephant and woolly rhinoceros (Owen-Smith, 1987; Kurtén, 1968) which would have contributed to the development of spinescence in European plant species such as *Prunus spinosa* (blackthorn). However, more research is needed to understand how spinescence evolved in Europe.

Other plant adaptations that have evolved due to the expansion of mammalian herbivores include structural defences such as spinescence, thickened cuticles, wide angled branches and smaller leaves. These have developed from varying methods of feeding by different types of herbivores. For example, browsing by mammals in Africa led to the formation of the spinescence trait including thorns and prickles present on the trunk and branches and spines found on leaves. However, within the same ecosystem, megaherbivores have adapted to these defensive traits which has then impacted how plants present defences. Elephants are much more destructive and are indiscriminate in what they browse when using their trunks (Guy, 1976). Elephants are able to browse across all heights and it has been reported that their browse heights overlap with impala below two metres (Guy, 1976). In contrast, giraffes will selectively browse above 2.5 metres creating high levels of browsing pressure at taller heights. In addition to this, the giraffe has adapted to the long thorns of acacia species by having thickened papillae on their tongue (Emura *et al*, 2013) and thick saliva which protects their mouths. They also have a prehensile tongue (Estes, 1991) which allows them to wrap around branches and pull leaves off. Small-medium sized herbivores such as impala have smaller mouthparts (Owen-Smith, 1982) and will be able to eat around large thorns that are more effective against megaherbivores. Impala and other large herbivores will not be able to browse above 2.5 metres as they are limited to their body size. However, these species will reside in larger groups and can place high amounts of browsing pressure lower down. These methods of browsing are very different when compared to other parts of the world. For example, large bird browsers, such as moas and elephant birds, that were present in New Zealand and Madagascar, caused the independent evolution of wide angled branches, smaller leaves and divaricate growth form as an adaptation to avoid being eaten (Bond & Silander, 2007).

Megaherbivores were the dominant herbivore across ecosystems; their large body size made them capable of disrupting ecosystem structure by altering vegetation at a landscape scale (Owen-Smith, 1992; Malhi *et al*, 2016). Their populations are limited by bottom-up trophic interactions associated with forage availability (Malhi *et al,* 2016). However, over the last

50,000 years there has been a rapid loss in megafauna, with a global loss of 177 large mammal species (more than or equal to 10kg), 19 of which were lost in Europe (Sandom *et al*, 2014a). This event has been termed the quaternary megafauna extinction. There are few places that still retain the megafauna as they were in their previous state, one such example is in Africa, although current megafauna are in functional decline (Malhi *et al*, 2016). The biggest decline in megafauna can be seen across North and South America with a total loss of 43 extinct large mammals (Sandom *et al*, 2014a). The key impacts of megafaunal loss are on the physical structure of vegetation communities (Bakker *et al*, 2016). There has been a significant loss in open vegetation and habitat heterogeneity after the loss of megafauna in Europe (Johnson, 2009). This suggests defensive traits that were adaptive in an environment with megafauna may now be maladaptive after the extinction, in turn suggesting the extinction of megaherbivores has led to the decoupling of megaherbivore and plant interactions over much of the world. This study explores the idea that plant spinescence has decoupled from megaherbivores since the quaternary megafauna extinction in Europe.

1.2. Herbivore induced plant reactions

Large herbivores are able to influence the heterogeneity of ecosystems by creating a cyclic change in vegetation communities (Olff *et al*, 2008). Megaherbivores, proboscideans in particular, can create more open landscapes through destructive behaviours during herbivory (Charles-Dominique & Owen-Smith, 2016). Their large body size means they can destroy woody vegetation and consume large amounts of foliage (Malhi *et al*, 2016). This directly disrupts vegetation structure through trampling and high levels of breakage and is considered ecosystem engineering. Before the quaternary extinction, megafauna were major controls in vegetation composition and structure throughout Europe (Bradshaw *et al*, 2003). There was a significant increase in forest cover in Australia, North America, and Europe caused by the extinction of Pleistocene megafauna (Owen-Smith, 1987; Vera, 2000; Malhi *et al*, 2016). Bakker *et al* (2004) reported that European vegetation responded to modern cattle and horse grazing by establishing a shifting mosaic of grasses, shrubs and trees similar to the conditions thought to have existed during interglacial periods during the Pleistocene. This suggests a reintroduction of megaherbivores across Europe could induce a similar response from the vegetation. Although it would appear cattle grazing establishes these shifting mosaics, the reintroduction of megaherbivores could provide more trophic complexity which promotes top-down trophic interactions (Svenning *et al*, 2016).

Ecosystem engineering by megafauna has both negative and beneficial impacts on the environment. It can negatively impact individual trees, particularly woody species, but can benefit biodiversity at an ecosystem level. For example, Shannon *et al* (2008) reported that African elephants in Kruger National Park uprooted 1,500 adult trees per year per individual elephant. Their destructive behaviours appear to have little benefit on the ecosystem, however, when large herbivores have been removed from African savannahs, Keesing and Young (2014) have reported an increase in smaller species such as rodents and venomous snakes, which has the potential to create inhospitable landscapes for humans and livestock, for example by increasing tick densities. Megaherbivores benefit the ecosystem by allowing for a more diverse community of vegetation with bottom-up and top-down regulation that can suppress the accumulation of woody vegetation and allow more habitat for grasses and forbs (Connor & Page, 2014). They can also enhance nutrient cycling, diminish competition with herbaceous vegetation, and reduce rodent densities (Danell *et al*, 2006; Bakker *et al,* 2016). In Europe, however, the megafauna populations dwindled to extinction 13,000 years ago which led to unbalanced flora and fauna communities (Martin, 2007) due to the co-evolution of plants with larger mammals.

When megafauna persist in high abundances they can impact the woody species composition by promoting browsing-tolerant vegetation (Wigley *et al*, 2014). For example, in African savannahs there has been a change in plant species composition due to browsing. Acacia species have developed spinescence to deter herbivory by megaherbivores and have come to dominate savannahs (Wigley *et al*, 2014). In Europe, many species also use thorns to deter herbivory, including hawthorn (*Crataegus monogyna*) and blackthorn (*Prunus spinosa*). Other species produce structural defences in the form of prickles and spines. These adaptations are structural, and the spinescence trait has been found to be mainly associated with two functional types of mammals; large browsers and medium-sized mixed feeders (Charles-Dominique, 2016). In modern Africa these groups include the Giraffidae family, proboscideans, perrisodactyla, and bovids (Werdelin & Sanders, 2010). In Europe these groups contained proboscideans such as the straight-tusked elephant (*Elephas antiquus*) (Malhi *et al,* 2016), perissodactyla, bovids and cervid species, before the quaternary megafauna extinction these groups were also far more diverse.

Plants are able to distribute their spinescence non-randomly so as to maximise protection from specific herbivores (Burns, 2013). This allows them to protect areas that are under high levels of browsing pressure. A plant's ability to alter its form and function in response to ecological or environmental change is known as 'heteroblasty'. Previous studies have reported that plants have decreased their structural defences vertically which may be as a result of growing out of reach of the tallest herbivores (Young, 1987; Burns, 2013). This suggests that in an ecosystem with no megaherbivores, plants will have reduced structural defences above the browsing height of the next tallest herbivore or will inhibit the production of defences completely above a certain height. Since the quaternary megafauna extinction there has been little to no megafauna in Europe which poses the question as to whether plants with the spinescence trait have reduced spinescence in response to this lack of herbivory. There has been little study into whether plants have evolved their defences alongside the lack of megaherbivores across Europe. If plants are still able to produce thorns above the height of the next tallest herbivores in ecosystems that have not had megafauna since the late quaternary extinction, it may lead to the possibility of Pleistocene rewilding.

1.3. Pleistocene Rewilding

Rewilding has many definitions, the most inclusive being to provide a strategy that restores natural systems and resources with little management (Keestra *et al*, 2018). This includes large-scale reintroductions of freely-roaming large herbivores to restore 'naturalistic grazing' (Hodder & Bullock, 2009). Pleistocene rewilding refers to the reintroduction of descendants of Pleistocene megafauna or their close ecological equivalents (Donlan *et al*, 2006; Jørgensen, 2015) to restore ecological and evolutionary processes. For example, the close ecological equivalent of the straight tusked elephant would be the Asian elephant (*Elephas maximus*) or African elephant (*Loxodonta africana*). The functional importance of extinct megafauna has had a lack of attention (Donlan *et al*, 2006), and their functional roles are thought to be important (Owen-Smith, 1988). This suggests the focus of conservation biology should look towards restoring species interactions and ecological function by restoring species (or substitutes) from the Pleistocene era. Reintroductions of megaherbivores to areas that were historically part of that species range can benefit both species protection as well as restoring natural ecosystem processes (Zekker *et al*, 2017). However, there are noticeable differences between ecosystems in Europe and Africa and it is unrealistic to assume communities today are functionally similar to communities over 10,000 years ago (Rubenstein *et al*, 2006). Therefore, studies need to focus on whether ecosystems are capable of sustaining megafauna if they are reintroduced, specifically looking at whether there has been a reduction in defence mechanisms such as spinescence in the absence of megafauna.

Many rewilding projects refer to the Pleistocene as an ecological baseline (Zeller *et al*, 2017) such as the Pleistocene Park in northern Siberia (Lorimer *et al*, 2015). Other rewilding projects in Europe use domesticated forms of extinct species and wild ungulates. For example, the Knepp Estate rewilding project aims to replicate near-natural grazing by using a variety of free-roaming large herbivores (Greenaway, 2006). This site is situated in the South East of England, and has already implemented a rewilding approach with the hope of seeing an improvement in biodiversity and ecological processes (Knepp.co.uk). By reintroducing large herbivores to the landscape they can create a variation in structural diversity in the vegetation which benefits the biodiversity of plants and animals (Olff *et al*, 2008). Grazing and browsing by herbivores creates disturbance and subsequently this disturbance has the potential to change the plant community. For example, the interaction between plant species at one point of their life cycle are competitively displaced by another species (Olff *et al*, 2008) because of disturbance. The positive association between herbivore grazing and ecological processes suggests that the reintroduction of large and megaherbivores will also benefit the ecosystem by increasing biodiversity and creating cyclic changes in habitat (Olff *et al*, 2008). As Knepp currently has a population of large herbivores it provides an opportunity to examine whether, in the absence of megaherbivores, the plant species would still be able to endure intensive browsing pressure through the vertical distribution of structural defence traits.

The Zambezi National Park, although not conducting a rewilding project, holds a mostly intact megafauna guild with little management. The evaluation of African ecosystems provides essential insight into plant-megaherbivore interactions, and becomes increasingly more important as rewilding projects with reintroductions are implemented across Europe (Zeller *et al*, 2017). The Zambezi National Park can be used as a comparison to the Knepp Estate as the key interactions are still present. The National Park contains two megaherbivore species that can browse above 2.5 metres, elephants (*Loxodonta africana*) and giraffe (*Giraffa giraffe*), and seven small-medium sized herbivores that browse below 2.5 metres (To The Victoria Falls, 2012; Du Toit, 1990). If both sites in England and Africa present spinescence similarity, it is arguable that the spinescence defence trait has not yet completely disappeared from European plant species allowing for the possibility of restoring this ecological interaction through Pleistocene rewilding.

This study looks at the impact of herbivory by megaherbivores on density and length of spinescence, focusing on thorns, defined as the woody, sharp-pointed branches (Hanley *et al*, 2007), between two ecosystems. It specifically looks at whether an ecosystem still distributes this defence trait vertically in the absence of megaherbivores (Knepp Estate, Britain) in the same way as an ecosystem that has retained its megaherbivores (Zambezi National Park, Zimbabwe). This comparative approach improves the understanding of ecological interrelations between two geographical regions and evolutionary differences between European plants and African plants.

1.4. Hypotheses

This study was conducted based on the following hypotheses:

H_{a1} There will be a significant increase in density of spinescence on trees with higher browsing pressure.

H_{a2} There will be a significant increase in length of spinescence above 2.5 metres in the Zambezi National Park.

H_{a3} There will be a significant decrease in length of spinescence above 2.5 metres in the Knepp Estate.

H_{a4} There will be a significant increase in density of thorns above 2.5 metres in an ecosystem that has retained its megafauna compared to an ecosystem that has lost all megafauna.

H_{a5} There will be a significant relationship between herbivore field use and vertical distribution of spinescence, with a decrease in spinescence above the height of the most frequent herbivore.

2. Methods

2.1. Study sites:

2.1.1. Knepp Estate**:**

Data were collected within the southern block of the Knepp Estate. The estate is 1416 hectares of mostly heavy clay soil and lies just south of Horsham in West Sussex (Lat: 50° 58’ 18.298” N, Long: 0° 21’ 46.394” E; Figure 1). Historically the estate was used as a hunting park in the 11th century by King John and became intensively farmed after World War 2. The park is split into 3 blocks (northern, middle, and southern) and has retained its original field system consisting of fields that are an average of four hectares in size and bordered by hedgerows. The middle block began restoration in 2001 when 202 hectares were taken out of arable production. The northern block was taken out of agriculture in 2003 and the Southern Block fields were slowly taken out of production between the years 2001 to 2006 (Appendix 1). This study used 19 randomly selected fields within the southern block of Knepp (Figure 2). The vegetation community is very different to that seen in the northern and middle blocks of the estate which introduced herbivores as soon as the fields were taken out of production. The Southern Block was passively rewilded (no human intervention or management to allow ecological succession (Nogués-Bravo *et al*, 2016)) allowing scrub and woodland habitats to take hold in the absence of herbivores.

There is a diverse mixture of broadleaved trees and conifers throughout the southern block, including: oak (*Quercus robur*), hazel (*Corylus avellana*), grey willow (*Salix cinerea*), field maple (*Acer campestre*), hawthorn (*Crataegus monogyna*), hornbeam (*Carpinus betulus*), holly (*Ilex aquifolium*), wych elm (*Ulmus glabra*), sweet chestnut (*Castanea sativa*), silver birch (*Betula pendula*), blackthorn (*Prunus spinosa*), ash (*Fraxinus excelsior*), alder (*Alnus glutinosa*), beech (*Fagus sylvatica*), elder (*Sambucus nigra*), and sycamore (*Acer pseudoplatanus*). The ground flora varies from field to field, which is thought to depend on amount of grazing in each field (Ryland, 2016). There are a variety of large herbivore species in the southern block of the estate, Exmoor ponies (*Equus ferus caballus*), fallow deer (*Dama dama)*, Longhorn cattle (*Bos primigenius)*, red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and Tamworth pigs (*Sus scrofa domesticus*). The fields selected were taken out of agricultural production in different years (Figure 2). The herbivore species randomly chosen to measure browsing pressure were red deer, fallow deer and Longhorn cattle. Mammalian herbivores will provide varying amounts of browsing pressure because of their herd size and timing of browsing (Ånöstam, 2017) that will cover all levels of browsing pressure. Common tree species with the spinescence functional trait were hawthorn, blackthorn, dog rose (*Rosa canina*), field rose (*Rosa arvensis*), bramble (*Rubus fruticosus*) and holly. The species that were selected to collect spinescence data were the hawthorn and blackthorn. Data collection took place between 25th May 2018 and 15th June 2018. A total of 75 trees were recorded, five trees in nine fields and three trees in ten fields were recorded from the 19 selected fields in Knepp (Figure 2).

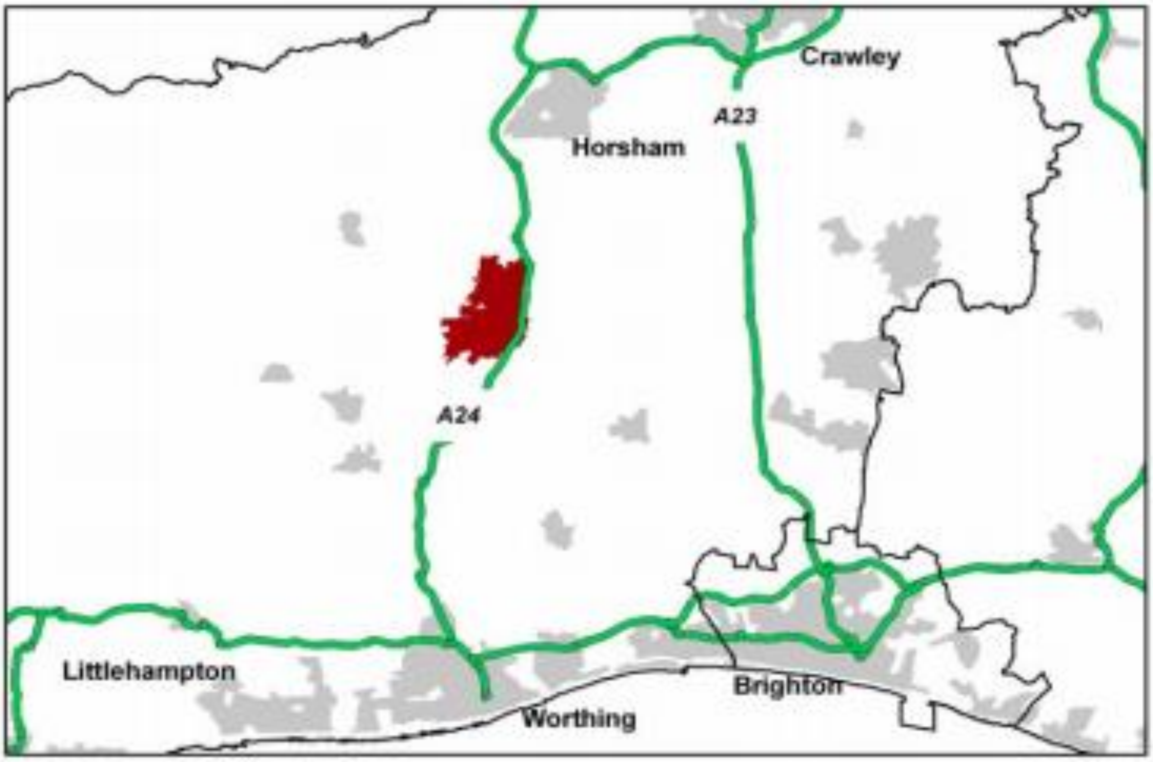

Figure 1: Location of Knepp Estate (red) (Greenaway, 2006)

Figure 2: The southern block of Knepp and the fields that were randomly selected for data collection. They were set aside for rewilding in different years (orange = 2000, green = 2003, blue = 2004, purple = 2005, pink = 2006, yellow = permanent pasture, red = woodland)

2.1.2. Zambezi National Park – Zimbabwe

The Zambezi National Park (Lat: -17° 54’ 40.41” S, Long: 25° 48’28.976” E; Figure 3) is made up of 56,000 hectares, located in the North-Western corner of Zimbabwe, bordering Botswana to the West and Zambia to the North. The climate is warm and semi-arid which leads to a habitat that consists of mainly savannah (bushveld) with a mixture of mopane woodland and shrub woodland (van Wyk, 2013). The vegetation types within the park can be divided into 5 distinct types, acacia (14%), terminalia (0.5%), teak (60%), mixed (22.5%) and grassland (3%) (Mandinyenya, 2018; Figure 4). The megaherbivore species focused on in this study site were elephant and giraffe, and the small – medium sized herbivore studied was impala (*Aepyceros melampus*). These species have different browsing behaviours and will place varying levels of browsing pressure upon plants due to these behaviours. The spinescence trait is most distinctive on acacia species which are common browse plants of many African herbivores and these tree species were chosen for data collection. Data were collected over five days, 6th April 2018 to

10th April 2018 and a total of 46 trees were recorded (29 trees were in acacia, 12 in mixed, 4 in Terminalia and 1 in riverine).

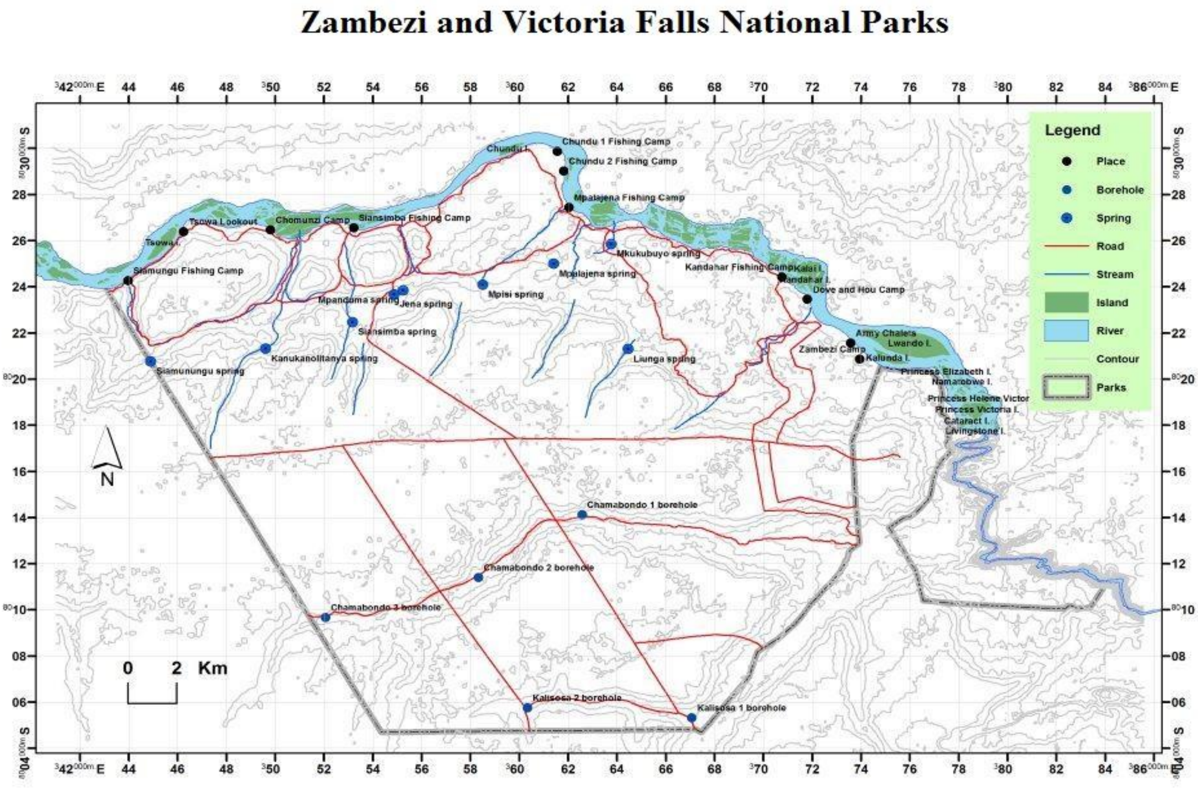

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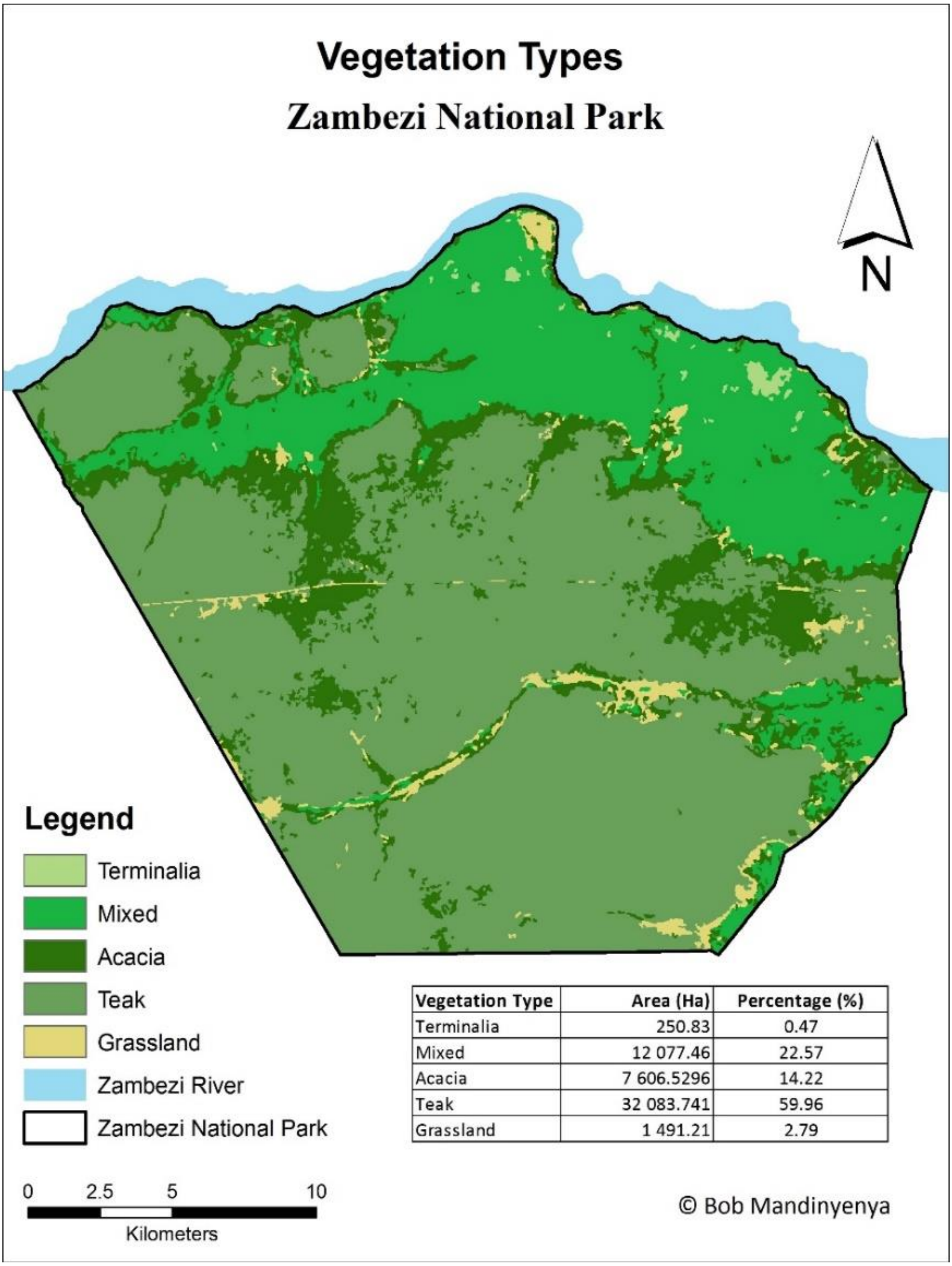
Figure 1: Location of Zambezi National Park in relation to Victoria Falls in Zimbabwe. Zambia borders the park to the North of the Zambezi River and Botswana borders the Western side of the park



Vegetation Type	Area (Ha)	Percentage (%)
Terminalia	250.83	0.47
Mixed	12 077.46	22.57
Acacia	7 606.5296	14.22
Teak	32 083.741	59.96
Grassland	1 491.21	2.79

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Figure 2: Map of vegetation types within the Zambezi National park; Terminalia, Mixed, Acacia, Teak and grassland

2.2. Vegetation Surveys

Vegetation surveys were conducted using random point sampling in both study sites. These surveys were conducted in 19 randomly selected fields in the Southern block of the Knepp Estate and within acacia, mixed and terminalia vegetation types in the Zambezi National Park (ZNP). Five trees were randomly selected to cover all levels of browsing pressure in each field in Knepp, and randomly selected trees (chosen using a random number generator) across ZNP were chosen to cover all areas within the three vegetation types. Browsing pressure was assessed for both study sites based on the categories described in Table 1, relating to tree architecture. Elephant, giraffe and impala refers to ZNP; Longhorn cattle, red deer and fallow deer refers to the Knepp Estate. Thorn length and density were measured below 1 metre and above 2.5 metres. Two branches below 1 metre were selected through a random number generator to measure density and length of spinescence in areas accessible to all browsing herbivores, and two branches above 2.5 metres which are only accessible to megaherbivores. To record thorn density, all thorns within 30cm of the tip of the branch were recorded, thorns that had been removed through damage were not counted. To measure length, all thorns within a 10cm section were measured. The 10cm section was located 5cm from the tip of the branch. Ten fields were randomly selected from the 19 fields in the southern block of the Knepp Estate and within these fields, three trees were randomly chosen to determine at what height the spinescence density changed. Measurements were taken every 30cm between 1 metre and 2.5 metres using the same technique as described above.

Table 1: Scale (1-5) of damage induced by browsing from elephants, giraffe and impala (1 being the least amount of damage and lowest browsing pressure, and 5 being the most amount of damage and highest browsing pressure)(Appendix 2).

Scale	Elephant	Giraffe	Impala
1	No damage	No Sculpting above 2.5m	No damage below 1m
2	1-2 broken branches	1-2 branch ends sculpted above 2.5m	1 – 2 branches browsed below 1m
3	3-7 broken branches, no trunk damage	3-7 branch ends sculpted above 2.5m	3-7 branch ends browsed below 1m
4	≥ 10 broken branches, and some trunk damage	Noticeable browse line due to sculpting. sculpted	Slightly noticeable browse-line below 1m
5	Trunk highly destroyed with few unbroken branches	Highly noticeable browse-line at the top, very sculpted	Highly noticeable browse-line at the bottom of the tree
Scale	**Long Horned Cattle**	**Red Deer**	**Fallow Deer**
1	No damage	No sculpting/damage	No sculpting/damage
2	Noticeable rubbing on trunk	1-2 branch ends browsed	1-2 branch ends browsed
3	Rubbing and 1-2 broken branches	3-7 branch ends browsed, some may be broken	3-7 branch ends browsed, some may be broken
4	2-10 broken branches	Noticeable browse-line	Noticeable browse-line
5	≥ 10 broken branches, with some trunk damage	Highly noticeable browse-line	Highly noticeable browse-line

2.3. Herbivore assemblage

To determine whether large herbivores impact the height at which spinescence density changes, herbivore assemblage was assessed. In the Knepp Estate the herbivore assemblage was determined by placing 25 camera traps in 10 randomly selected fields of the 19 fields already selected for vegetation surveys in the Southern Block to be able to determine distribution of herbivores. The frequency of field use by large herbivores was recorded to determine whether the use of each field is dominated by one herbivore species. The average height of the dominating herbivore (Table 2) was then compared to the height at which mean spinescence density is reduced.

Table 2: Average height (metres) of large herbivore species found in the Knepp Estate (The British Deer Society, 2018; The Cattle Site, 2014)

Height	**Fallow Deer**		**Red Deer**		**Longhorn Cattle**	
	Male	Female	Male	Female	Male	Female
Shoulder	0.94	0.91	1.37	1.22	1.50	1.30
Head	~1.45	~1.40	~1.60	~1.50	1.50	1.40

2.4. Data Analysis

All analysis was conducted in RStudio version 1.1.383 (R Core Team, 2018) using the "Tidyverse" (Wickham, 2017), "nortest" (Gross & Ligges, 2015), "multcomp" (Hothorn *et al*, 2008) and "car" (Fox & Weisberg, 2011) packages. To determine data normality the Lilliefors (Kolmogorov-Smirnov) normality test was run on Knepp and ZNP data separately and together. The results of the normality tests are described in Table 3.

Table 3: Normality Test scores and P values for data collected in the Knepp Estate, Zambezi National Park and all data together (density of thorns per branch and length of thorns)

	Knepp		**ZNP**		**Both**	
	Density	Length	Density	Length	Density	Length
Test Statistic	0.17072	0.15172	0.092658	0.11011	0.11419	0.11849
P Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The following tests were used to address each hypothesis:

H_{a1}: Differences in spinescence density based on the scale of browsing pressure were determined using an ANOVA for ZNP and a Kruskal-Wallis rank-sum test in Knepp.

H_{a2}: A Wilcoxon rank sum test was used to determine whether the length of thorns were longer above 2.5m compared to below 1m in the ZNP ecosystem. A Wilcoxon rank sum test was also used to determine any differences in density below 1 metre compared to above 2.5 metres.

H_{a3}: A Wilcoxon rank sum test was used to determine whether there was a decrease in thorn length above 2.5m compared to below 1m in the Knepp ecosystem. A Wilcoxon rank-sum test was used to determine whether density of thorns changed by height (1 metre and 2.5 metres) in the Knepp Estate.

H_{a4}: To test whether the density of spinescence above 2.5m were different between ZNP and Knepp, a Mann Whitney U test was used.

H_{a5}: To determine whether there was a relationship between height and when spinescence density changed in different fields a multiple linear regression was used.

3. Results

3.1. Length and Density of Thorns in the Zambezi National Park

The length of thorns below one metre were significantly shorter (median = 1.3cm, SD = 1.1) compared to above 2.5 metres (median = 2.3cm, SD = 1.5; W = 196600, $p < 0.001$; Figure 5). There was no significant difference in the density of thorns at different heights (W = 3862.5, p = 0.053). The density of thorns below one metre was not significantly more (median = 24, SD = 9.8) than the density of thorns above 2.5 metres (median = 22, SD = 6.9; Figure 6). There was no significant relationship between the density of spinescence and the level of browsing pressure (F = 0.4555, p = 0.5006; Figure 7).

3.2. Length and Density of Thorns in the Knepp Estate

The length of thorns was significantly shorter below one metre (median = 1.6cm, SD = 1.8) compared to above 2.5 metres (median 3.5cm, SD = 2.1; W = 11590, $p < 0.01$; Figure 5). Density of thorns were significantly higher below one metre (median = 9.5, SD = 6.4) compared to the density above 2.5 metres (median = 1, SD = 4.1; W = 12086, $p < 0.001$; Figure 6). There was a significant relationship between the density of spinescence and the level of browsing pressure ($x^2 = 86.968$, $p < 0.001$; Figure 7). There was a significant negative linear relationship between the density of spinescence and height (f = 10.51, $p < 0.001$; Figure 8).

3.3. Zambezi National Park compared to Knepp Estate

Density of thorns above 2.5 metres in ZNP was significantly different to the density of thorns above 2.5 metres in Knepp (W = 245, $p <0.001$; Figure 6). Density of thorns in the Zambezi National Park (median = 23 thorns/30cm, SD = 8.6) was higher than the density in the Knepp Estate (median = 5 thorns/30cm, SD = 6.3).

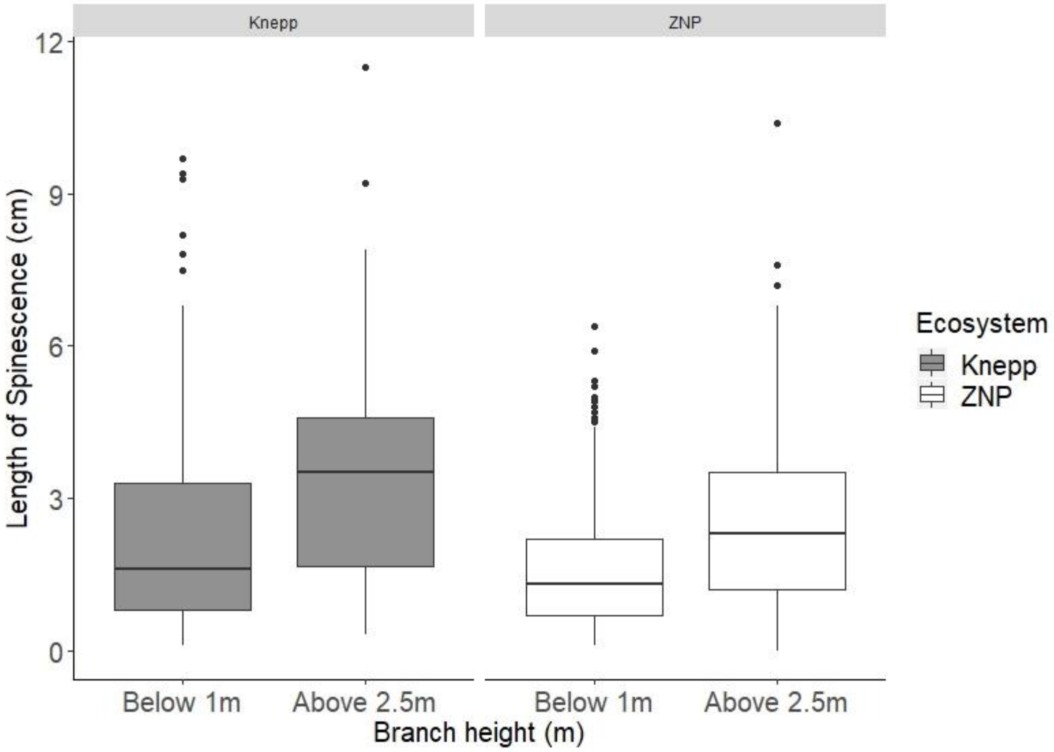

Figure 3: Length of thorns (cm) at two browsing heights (1 metre and 2.5 metres) on Acacia species in the Zambezi National Park and Hawthorn and Blackthorn trees in Knepp. A Wilcoxon rank -sum shows that the thorn length below 1 metre (median = 1.3 cm) is significantly smaller than the thorn length above 2.5m (median = 2.3 cm) in ZNP which has a broader range (W = 196600, P < 0.001). Length of thorns below 1m (median = 1.6cm) was significantly smaller than length of thorns above 2.5m (3.5cm) in Knepp (W = 11590, p < 0.01).

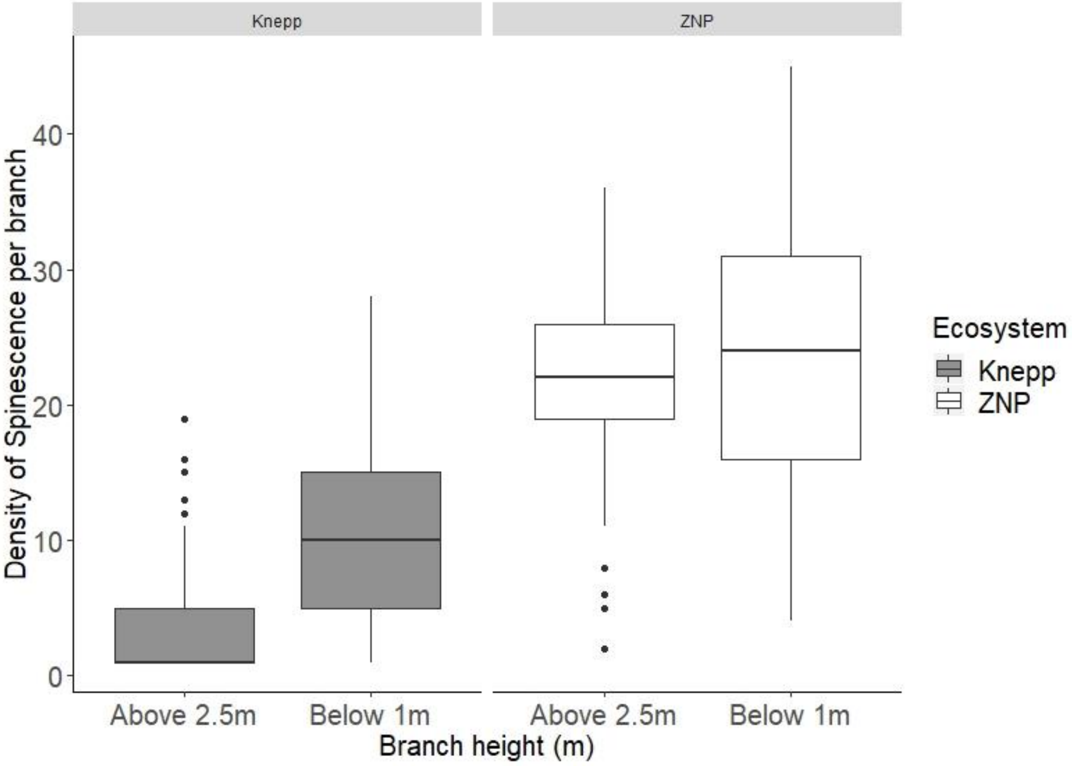

Figure 6: Density of thorns below 1 metre and above 2.5 metres was not significantly different in ZNP (W = 3862.5, p = 0.05274). Density of thorns below 1 metre (median = 24) was not significantly different than 2.5 metres (median = 22). Density of thorns in the Knepp Estate below 1 metre and above 2.5 metres were significantly different (W = 12086, p< 0.001). Density below 1m (median = 9.5) was significantly higher than above 2.5 (median = 1)

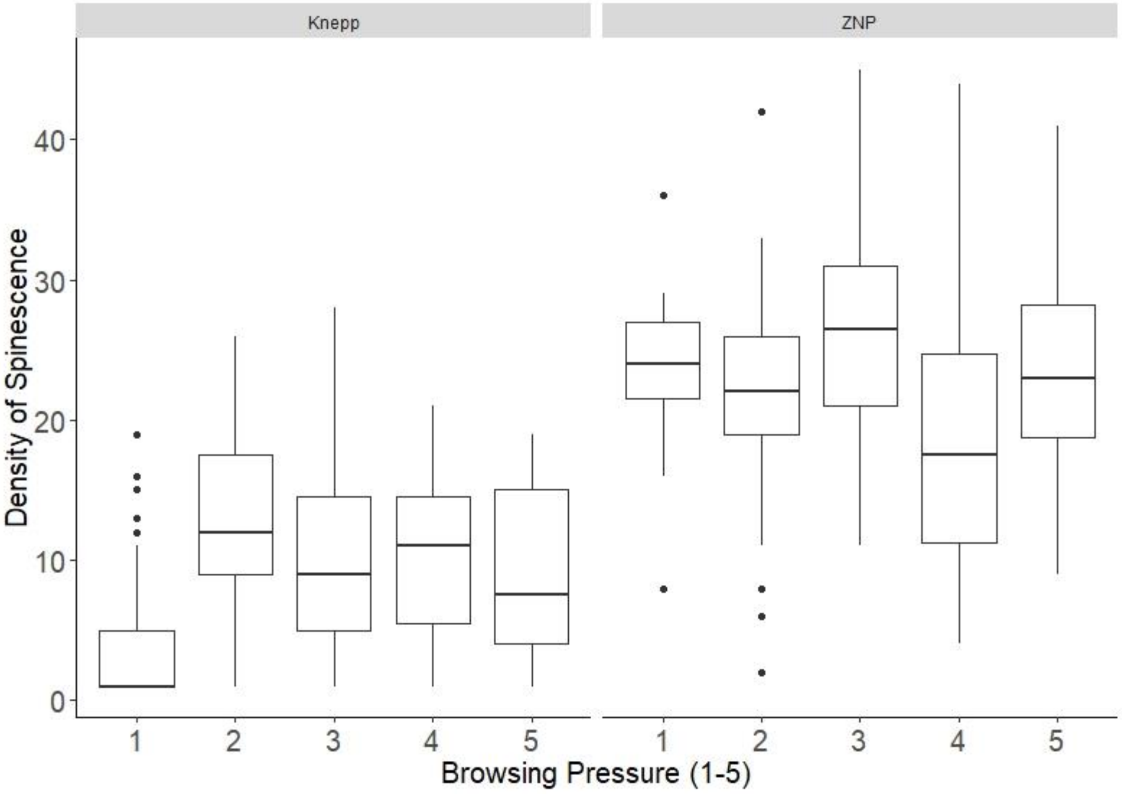

Figure 7: Browsing pressure (1-5) of herbivory on the density of thorns between two ecosystems (ZNP and Knepp). There was no observed significance between the densities of thorns and browsing pressure in ZNP, however, there was a significance in the Knepp Estate.

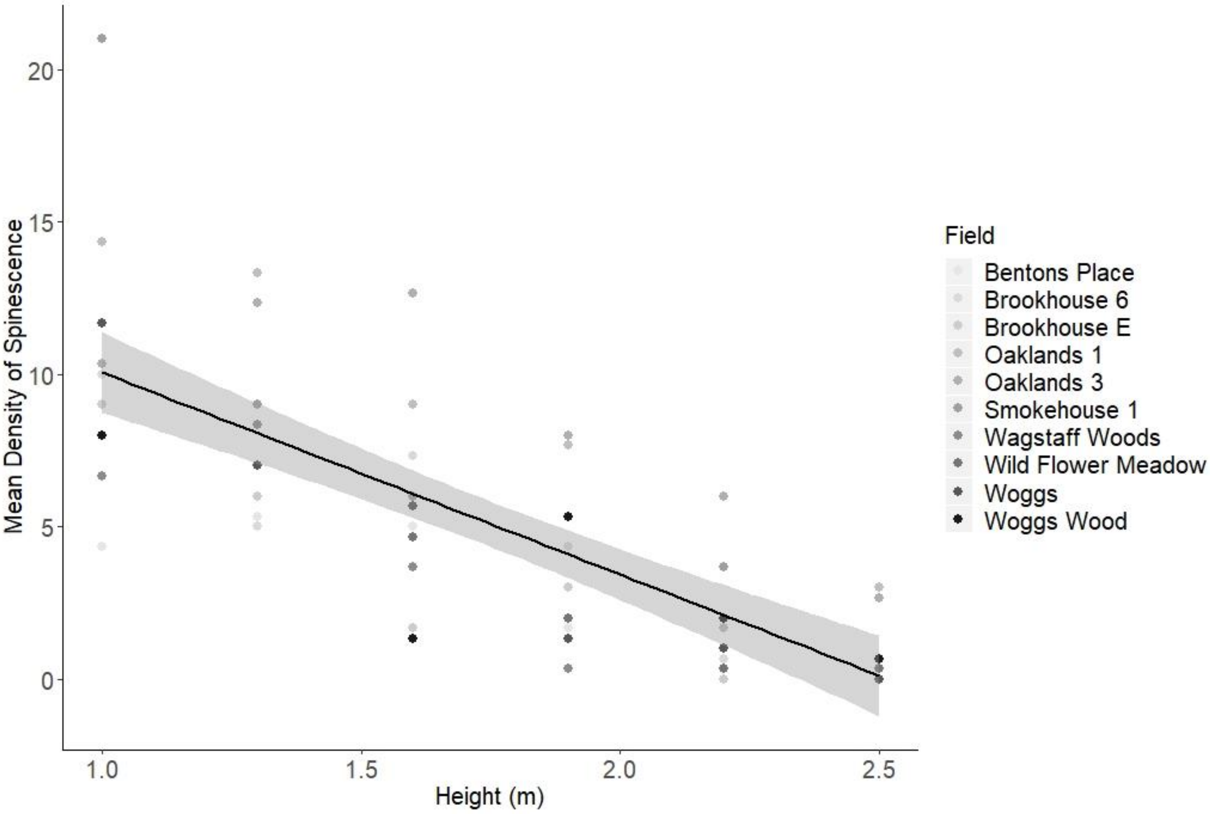

Figure 8: Mean density of spinescence at 30cm vertical intervals along a tree in different fields of the Knepp Estate. The single trend line reflects the linear model with error shading representing the 95% confidence limits.

4. Discussion

This study has found that there are similarities in the way thorns are presented with differing megaherbivore guild intactness. The length of thorns in both sites were longer above 2.5 metres suggesting European species have not evolutionarily adapted to the absence of megaherbivores since their extinction. However, the density of spinescence is higher below 1 metre in Knepp, whereas the densities at both heights in ZNP were not significantly different suggesting European species are ecologically adapting to the absence of megaherbivores. Due to the difference in herbivore guilds and browsing methods in each ecosystem the likely causes of varying thorn densities and thorn lengths will have different explanations in each site.

4.1. Zambezi National Park

4.1.1. Vertical Distribution of Thorn Length and Density

The results suggest that the length of thorns increases over vertical variations in acacia species in the Zambezi National Park, supporting hypothesis two (H_{a2}), however, the density of thorns does not differ between these two heights. This could suggest that longer thorns are better at preventing herbivory from megaherbivores which have evolved adaptations against thorns, and the high densities of thorns are effective at preventing herbivory from all herbivores. Adaptations that megaherbivores have evolved to counter the increased length of thorns include trunks on elephants which allows them to wrap around branches to pull leaves off, and prehensile tongues on giraffe (Estes, 1991). Small thorns are unlikely to cause a problem for these methods of browsing, whereas large thorns will. The smaller thorns below one metre may be more effective at deterring herbivory from smaller herbivores such as the impala. Impala are more selective in the way they browse, their smaller mouthparts allow them to eat around larger thorns and select leaves (Owen-Smith, 1982). The small thorns may impact this behaviour more effectively than longer thorns.

In previous studies the browsing height of elephants has been disputed, suggesting they overlap with impala browsing heights (Makhabu *et al*, 2005), this suggests the similar thorn density below one metre to above 2.5 metres could be as a result of the elephants selectively browsing below one metre by using their trunks (Owen-Smith, 1998). The browse heights of impala and giraffe, however, do not overlap, suggesting giraffe benefit from browsing above 2.5 metres as a result of the scramble competition hypothesis (Woolnough & du Toit, 2001). The scramble competition hypothesis states taller species are displaced vertically due to the effect of selective feeding from smaller species within a browsing guild (Woolnough & du Toit, 2001). In this case impala will reduce the quality of forage due to their feeding mechanisms, therefore making it more beneficial for giraffe to browse at taller heights. The adaptations giraffe have to browse on plants with thorns may have led to the evolution of longer thorns, therefore, if they are browsing above 2.5 metres because of displacement the longer thorns will be at that height. Elephants may have adapted their browsing behaviour to browse below one metre to avoid the longer thorns higher up.

4.1.2. Browsing Pressure

There was no observed significant difference between the thorn densities at different browsing pressures in ZNP so we are proving the null hypothesis with regard to hypothesis one (H_{a1}) in this study site, suggesting increased browsing pressure does not lead to increases in thorn densities. However, the method produced for this study did not take into account damaged thorns, including cases where thorns had been stripped from the branches leaving only "nodes". These are indicators of browsing pressure which should be included in future studies. The methodology for ZNP was not as clear as expected which may have influenced the results, due to the unexpected presence of elephant damage below one metre. By elephants damaging the main trunk, branches above one metre are being brought down to heights where impala are placing high amounts of pressure. In this case it would be unclear whether the high browsing pressure was due to elephants or impala. Clearer scales would be needed that can identify the species which caused the damage. The results here indicate browsing pressure does not influence thorn density as the pressure can vary across all vertical variations.

4.2. Knepp Estate

4.2.1. Vertical Distribution of Thorn Length and Density

The length of thorns in the Knepp Estate on hawthorn and blackthorn trees also increased above 2.5 metres, therefore we can reject hypothesis three (H_{a3}). The density of thorns also differed between the two heights, however, there was an increase in density below one metre. The presence of longer thorns above 2.5 metres suggests European plant species may still be able produce thorns that are of an adequate length to protect from megaherbivores with different methods of browsing. For example, if elephants were reintroduced across Europe, the longer thorns would still be able to deter browsing from this species. This increased length may be an induced response to the tallest herbivores on the estate (red deer) reaching above 2.5 metres, causing the plant to present a defence as if megaherbivores were present. The height selection of certain species will differ amongst large herbivores in Knepp like they do between megaherbivores in Africa. For example, red deer will browse above the height of fallow deer due to their larger body size. Observations from the camera traps show that the large herbivores were not restricted to their head height and would stand on their hind legs to reach leaves (Appendix 3). This could indicate why there are still low densities of longer thorns present above 2.5 metres since the extinction of megaherbivores which could have easily reached that height.

It is unclear why thorns are present above 2.5 metres due to the lack of megaherbivores but it does suggest that there may be a “buffer zone”, where plants distribute spinescence. This “buffer zone” would ensure herbivory does not occur in the area surrounding those that are being browsed. However, there are studies that clearly indicate plants can be branch specific (Young *et al*, 2003) in how they distribute defences, and will not place resources into a defence where it is not needed. This suggests that the spinescence above 2.5 metres is as a result of browsing at that height and not a response to browsing in other areas.

The resource allocation into defences may be lower above 2.5 metres due to the higher densities of thorns below one metre. This could be due to the resource availability theory, which states that species in resource-poor environments invest more into defence (Endura & Coley, 2010). The Knepp Estate is made up of heavy clay soil that made the environment difficult to grow crops. Plant species that have taken over since passive rewilding may have adapted to the resource-poor environment by investing more into structural defences to avoid using the limited resources to replace leaves. The increased spinescence density below 1 metre could also be a result of browsing being focussed around this height because of the height of the herbivores that are present.

The variation in densities across the trees could be an example of a delayed induced response (Alves-Silva & Del Claro, 2016). The delayed induced response is a reaction to a period of high browsing pressure, where a tree will display more defences the season or year after this high browsing pressure. When compared to heights above the largest herbivore there is no delayed induced response because of the lack of spinescence. However, because browsing pressure may have been high during the summer months the previous year the amount of spinescence may have increased the following year.

4.2.2. Browsing Pressure

There was an observed significant difference between the thorn densities and increased browsing pressure in Knepp, therefore we can accept hypothesis one (H_{a1}) in regard to this study site. A browsing pressure score of one did not induce large densities of thorns when compared to a score of two, this is likely due to most trees having a score of one above 2.5 metres where no browsing occurred. There were very few trees that showed a browsing pressure score of one below one metre, as these heights are easily accessed by all herbivores on the estate. This suggests that browsing pressure does influence how a plant allocates defences vertically. It does not suggest, however, that European plant species could adapt to the increased browsing pressure caused by megaherbivores if they were reintroduced as we cannot assume a high level of browsing pressure caused by a megaherbivore is similar to the same level of browsing pressure by a large herbivore. Therefore, European plants are able to respond to the changing browsing impacts of large herbivores but this may be on a much smaller scale than if megaherbivores were present.

4.2.3. Herbivore Field use on Thorn Density

Results indicated that there was a clear negative correlation between the density of spinescence and height in the Knepp study site. As height increased, spinescence decreased, however, there were no significant differences between fields, suggesting that the height of the most frequent herbivore in each field does not affect the height at which spinescence density decreases or stops, and so we can reject hypothesis five (H_{a5}) . For most fields the most frequent herbivore was the fallow deer, whose maximum browse height is 1.6 metres (The Deer Initiative, 2008). Fallow deer herds adopt ‘core areas’ that they favour and any movement of herds will be between these sites. Their habitat selection varies between seasons (Apollonio *et al*, 1998) suggesting they will place browsing pressure in different areas of the park in different seasons due to their ‘core areas’ and habitat selection. If this herbivore had a larger influence on where spinescence density decreased the number of thorns would vary between those fields that had a high frequency of fallow deer and fields that did not. The density of spinescence would also have been similar at all heights up to 1.6 metres in the fields and then reduced in the fields that form part of their core areas in those seasons. However, there is a steady decline up until 2.5 metres where the number of thorns drops to zero in all fields. This suggests all large herbivores present have an impact on where spinescence is distributed and not one particular species, therefore, suggesting plants could ecologically adapt to an introduction of megaherbivores.

4.3. Zambezi National Park compared to the Knepp Estate

There was a significant increase in the density of thorns above 2.5 metres in ZNP (median = 22) compared to Knepp (median = 1) so we can accept hypothesis four (H_{a4}). This suggests that with the lack of megaherbivores in the rewilding site, the plants have adapted to distribute spinescence only in areas under browsing pressure. Previous studies in European holly show that spinescence decreases when leaf size increases as a result of no browsing (Obeso, 1997). This suggests they are placing more energy into leaf production and less into defensive traits. There could be a similar process happening in hawthorn and blackthorn species. As browsing does not occur above 2.5 metres to place pressure on the defensive trait, they are able to place more resources into other areas such as leaf production. Observations above 2.5 metres in Knepp showed much larger leaves to those seen below 1 metre, whereas leaf sizes in ZNP were similar across all heights.

Since the quaternary megafauna extinction there has been an increase in closed and semi-closed forest across Europe (Bradshaw *et al*, 2003). In unmanaged reserves, the likelihood of reforestation is high, suggesting the population of herbivores in Knepp may not inflict enough browsing pressure on plants to prevent reforestation, thus allowing the conversion of open grassland back to closed forest. In this way European environments are suffering from the lack of megaherbivores. The lack of browsing pressure is shown in the density of spinescence, they are much lower in Knepp compared to ZNP even under high amounts of pressure. This suggests plants in Europe have ecologically adapted to the loss of megaherbivores but have not evolutionarily adapted to their absence. They show the ability to produce thorns higher up but generally do not because there is not a form of pressure placed at those heights, but the thorns that are present are of a length that would inhibit megaherbivore browsing.

4.4. Limitations and further research

This study provides a baseline for further studies when looking at how browsing pressure influences spinescence distribution. A more thorough methodology that can clearly identify browsing pressure caused by different herbivore species, and take into account the disparities between browsing from large herbivores and browsing from megaherbivores, is needed. This could be done by creating a larger scale (1 – 10) that is more detailed for each megaherbivore species. Camera traps could also be used to identify browsing behaviours by different herbivores and the intensity of browsing.

There were many other factors that may have influenced the distribution of thorns and so it is not certain whether the spinescence trait and herbivory from megaherbivores has completely decoupled in European plants. Longer studies, over at least 2 years, would be required to understand the impact of the delayed induced response on the distribution of spinescence. The inequality between both sites due to season and herbivore size will impact the delayed induced response and would need to be taken into account in future studies. Other sites must also be studied across Europe which are not limited in their resource availability. If more resources are being placed into increased thorn density there is likely to be a trade off in leaf size, a suitable future study could look into whether leaves are larger at heights with decreased thorn density. Other possible studies could explore the density of thorns in relation to jaw width of herbivores to see if there is an ecological difference between densities of thorns at heights browsed by herbivores with smaller mouthparts to the density of thorns at heights browsed by herbivores with larger mouthparts. This would take into account the evolutionary adaptations herbivores have developed in response to plant defences.

5. Conclusions:

This approach identifies similar characteristics of spinescence distribution between functionally different ecosystems. The similarity in thorn length at different heights between the Zambezi National Park and the Knepp Estate suggests that European plant species still retain the ability to present a defensive trait that is effective against megaherbivores and that they have not evolutionarily adapted to the absence of megaherbivores. The difference in the density of spinescence above 2.5 metres between both sites indicates that European plant species are likely to have ecologically adapted to the extinction of megaherbivores by reducing the thorn density at heights where it is not required. The disparity in browsing pressure between large herbivores and megaherbivores is clearly identified in this study due to the differences in density because of browsing pressure and would need to be studied further to understand whether European plants could withstand increased damage from reintroduced megaherbivores.

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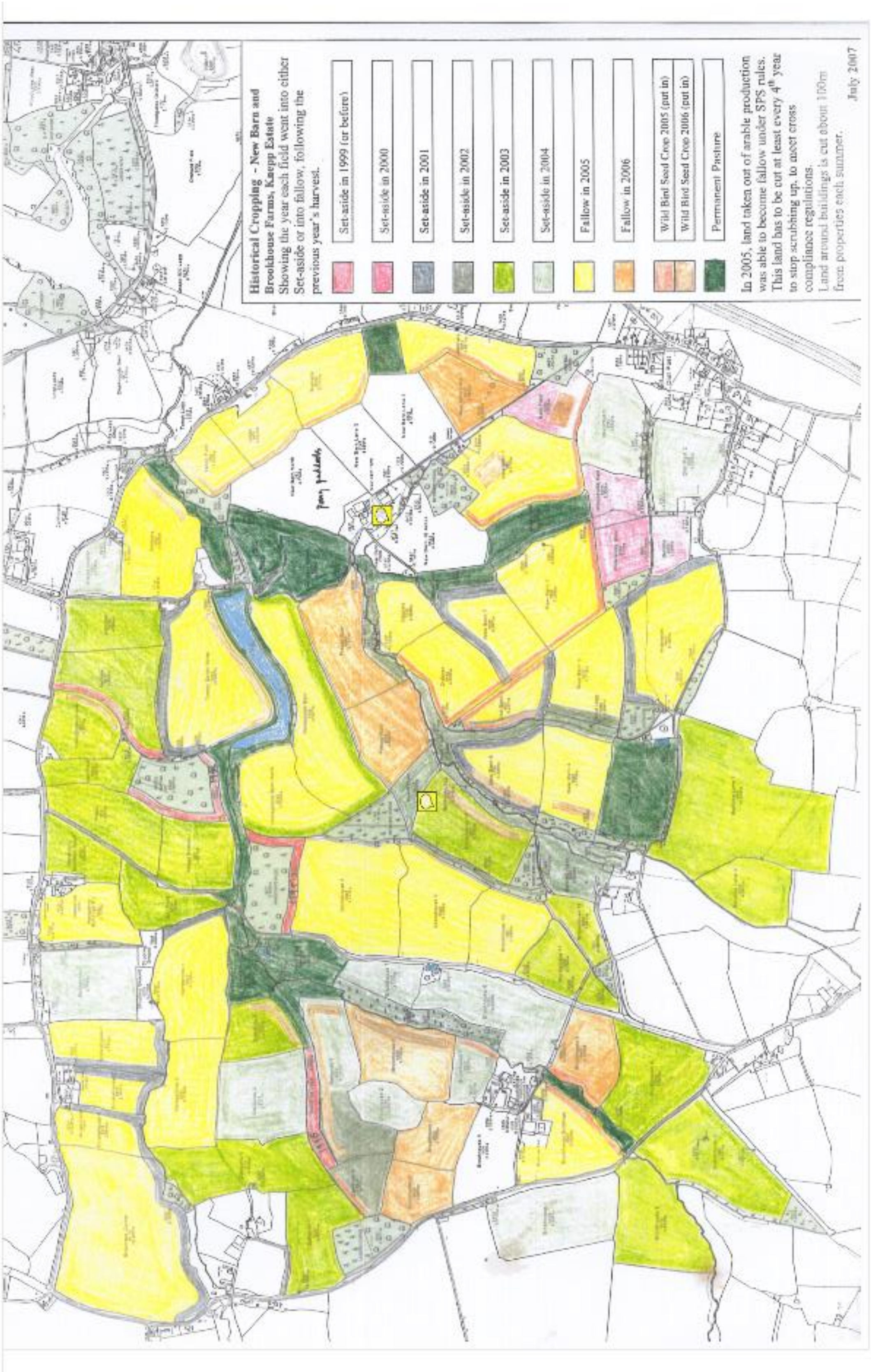
Appendices:

Appendix 1

Year fields were taken out of production in the southern block of Knepp Estate



Appendix 2:

Browsing pressure level 1 across all heights

Browsing pressure 2 above 2.5m due to sculpting from giraffe, and browsing pressure level 5 from impala browsing

Browsing pressure level 5 at both heights due to giraffe and impala browsing

Browsing pressure level 3 due to giraffe above 2.5m, and browsing pressure level 4 for elephant damage below 1m

Browsing pressure level 5 below 1m caused by browsing from any species, and browsing pressure level 1 above 2.5m

Appendix 3: