

Effects of herbivore-induced disturbances on above- and belowground community heterogeneity within a semi-arid sodic African savanna

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Abstract

Large mammalian herbivores (LMH) function as significant drivers of change within consumer-controlled grassy biomes, essentially maintaining Africa's open ecosystems through numerous top-down and bottom-up feedbacks. These feedbacks fundamentally drive and maintain invaluable ecological processes and cycles, subsequently regulating system structure and functioning. Yet, heterogeneous savanna ecosystems are faced with anthropogenic-induced changes to native LMH communities with which these systems evolved. The most common change to herbivore communities includes the switch towards single-species pastoralism at the expense of a diverse suite of wild African herbivores. Such dramatic changes in native herbivore communities might affect several underlying structural and functional constituents of these herbivore-adapted systems through top-down trophic cascades. Numerous studies have reported on the compartmentalised effects of native LMH on vegetation structure and edaphic properties. Given the link between these structural constituents and associated soil microbes (bacteria and fungi), herbivory is potentially a major driver of belowground microbial community structure and activity, and subsequently, soil function. However, little is known about the long-term impact of herbivory, and particularly the loss thereof, on bacterial and fungal community composition and potential implications for microbially-mediated decomposition.

Considering the close association between herbivory and preferentially foraged sodic vegetation, sodic patches are suggested to exhibit changes related to altered herbivory intensity over short time scales. The Nkuhlu research exclosures within the Kruger National Park (KNP), South Africa, provided an ideal setting to explore spatial and temporal heterogeneity patterns within intensively utilised herbivore-driven sodic patches, and how these patterns are affected by changes in native herbivore communities. This study was specifically designed to elucidate the effects of herbivory, or the loss thereof from a herbivore-adapted system, on belowground microbial community structure and associated soil-based decomposition of detrital plant material, and how these potential belowground changes are related to observed changes in vegetation structure and edaphic properties.

Sampling was conducted within five sites, each comprising five experimental plots, located across three herbivore treatments of varying intensity within the Nkuhlu exclosures. Successful retrieval of tea bags after the application of the extended, site-specific version of the Tea Bag Index (TBI) highlighted the value of this approach to quantify decomposition in a disturbance-driven savanna ecosystem. Results revealed that decomposition rate (k) was

highest in the presence of all LMH, irrespective of incubation time (3-, 6-, 9- and 12-months) with significant variation occurring after a six- and nine-month period. Conversely, stabilisation factor (S) was highest under complete herbivore exclusion across all four incubation periods with significantly higher values attained after the three- and six-month periods. Global comparison of TBI-based decomposition data revealed substantial differences between varying sites, illustrating the importance of an improved understanding of the complex functioning of semi-arid African savannas and how natural disturbances, such as herbivory play a valuable role in maintaining ecological processes, including carbon cycling. Next-Generation Sequencing (NGS) of soil-based genomic material for combined dry and wet seasons revealed a positive association between bacterial α -diversity and herbivore-induced disturbances, with the highest values obtained in the presence of all LMH. Conversely, fungal richness was highest in the sites from which herbivores were excluded for ~18 years, whereas diversity was highest under intermediate levels of herbivore disturbances. With seasonality as an added variable, substantial variation in both bacterial and fungal α -diversity indices were revealed, including significantly higher fungal richness and diversity under complete herbivore exclusion for the dry season. Moreover, heat tree analysis revealed significant changes in relative abundance of both bacterial and fungal taxa across the three herbivore treatments. Local scale vegetation sampling consisted of herbaceous biomass and species composition in an attempt to link observed changes in microbial community composition and soil function with changes in vegetation structure. Linking aboveground and belowground results revealed that herbaceous- and bacterial α -diversity measures together with decomposition rate (k) were positively associated with herbivore-induced disturbances. Conversely, herbaceous biomass, fungal α -diversity measures and stabilisation factor (S) had a positive association with herbivore exclusion.

In the face of increasing land-use change and subsequent disturbance to carbon cycling, this study emphasises the importance of effectively managing heterogeneous savanna herbivore communities as a major determinant of system heterogeneity (above- and belowground) and associated nutrient cycling. This study could potentially be used as a framework to advance holistic ecological research and to develop bottom-up management strategies, at least for sodic patches within the KNP.

Key words: *land-use change; herbivore loss; nutrient-rich; soil function; decomposition; Tea Bag Index (TBI); belowground microbial communities; microbial activity; nutrient cycling*

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Spr. 2:6-7

“Dit is die Here wat wysheid gee. Sy woorde gee kennis en insig. Hy laat hulle wat reg leef, sukses behaal.”

Table of content

Abstract	i
Acknowledgements	iii
List of abbreviations	ix
List of symbols	xi
List of figures	xii
List of tables	xvii
Chapter 1: Introduction	1
1.1 Preface	1
1.2 Herbivory and savanna heterogeneity	2
1.3 Rationale	3
1.4 Aims, objectives and hypotheses	5
1.5 Format of study	6
Chapter 2: Literature review	10
2.1 Semi-arid savannas	10
2.2 Herbivory as a driver of savanna dynamics	11
2.2.1 Effects of herbivory on aboveground structure	13
2.2.2 Effects of herbivory on belowground structure	16
2.2.2.1 Effects of herbivory on soil physicochemical properties	16
2.2.3 Effect of herbivory on soil-based microbial community structure	19
2.2.4 Effects of herbivory on soil function	25
2.2.5 Quantification of decomposition	27
2.3 Conclusion	30
Chapter 3: Study Area	31
3.1 Locality and description	31

3.2 Climate	33
3.2.1 Rainfall	33
3.2.2 Temperature	34
3.3 Geology	35
3.4 Topography	36
3.5 Soil	38
3.6 Fauna and Flora	40
3.6.1 Vegetation composition	41
3.6.2 Herbivore communities	42
Chapter 4: Experimental- and sampling design	43
4.1 Introduction	43
4.2 Experimental design	43
4.2.1 Nkuhlu experimental site	43
4.2.1.1 Fire treatments	44
4.2.1.2 Experimental plots	45
4.3 Field surveys and sampling	46
4.4 Data preparation	48
4.5 Data analyses	48
Results and discussion	
Chapter 5: Extension and validation of the Tea Bag Index as a viable approach to quantify decomposition in a semi-arid African savanna	51
5.1 Preamble	51
5.2 Introduction	51
5.3 Materials and Methods	52
5.3.1 Statistical analyses	53
5.4 Results	54
5.5 Discussion	55

5.6 Conclusion	57
Chapter 6: Decomposition rates of standardised organic material across herbivore treatments	59
6.1 Preamble	59
6.2 Introduction	59
6.3 Materials and Methods	60
6.3.1 Statistical analyses	60
6.4 Results	60
6.5 Discussion	62
6.6 Conclusion	64
Chapter 7: Potential decomposition rate and stabilisation factor of standardised plant material across varying herbivore intensities	66
7.1 Preamble	66
7.2 Introduction	66
7.3 Materials and Methods	68
7.3.1 Statistical analyses	68
7.4 Results	69
7.4.1 Decomposition rate (k) and stabilisation factor (S) of organic material	69
7.4.2 Soil properties	71
7.5 Discussion	73
7.5.1 Influence of herbivory on decomposition	73
7.5.2 Influence of time on decomposition	75
7.6 Conclusion	76
Chapter 8: Soil microbial community structure across various herbivore intensities	78
8.1 Introduction	78
8.2 Materials & Methods	79

8.2.1 Soil sampling	79
8.2.2 DNA extraction	79
8.2.3 High-throughput sequencing of bacterial and fungal ribosomal markers and data processing	79
8.2.4 Bioinformatic and Diversity Analyses	80
8.2.5 Deposition of Sequences	81
8.2.6 Statistical analyses	81
8.3 Results	82
8.3.1 Bacterial Diversity and Community Differentiation	82
8.4 Discussion	91
8.5 Conclusion	93
 Chapter 9: Linking above- and belowground systems: Linking changes in vegetation structure to belowground processes	 95
9.1 Preamble	95
9.2 Introduction	95
9.3 Materials and Methods	96
9.3.1 Statistical analyses	100
9.4 Results	100
9.4.1 Herbaceous biomass	100
9.4.2 Floristic and functional trait patterns	102
9.4.3 Soil properties	105
9.4.4 Changes in decomposition rate and microbial community structure	105
9.5 Discussion	105
9.5.1 Herbivore-induced changes to aboveground structure	105
9.5.2 Linking changes in above- and belowground structure and function	107
9.6 Conclusion	112
 Chapter 10: Conclusion	 113
10.1 Main findings	113

10.2 The way forward	116
10.2.1 Recommendations for the sodic zone of the Nkuhlu exclosures, KNP	116
References	118

Appendices:

Appendix A: Supplementary figure supporting Chapter 4: Experimental layout of the Nkuhlu exclosures with fire and no fire treatments

Appendix B: Supplementary table supporting Chapter 4: T-test results for fire vs no fire treatments and accompanying *p*-values

Appendix C: Supplementary tables supporting Chapter 8: Heat tree analysis and accompanying Wilcoxon *p*-values

C1: Heat tree With all LMH vs Elephant exclosure -Bacteria

C2: Heat tree_LMH exclosure vs With all LMH_table -Bacteria

C3: Heat tree_LMH exclosure vs With all LMH_table -Bacteria

C4: Heat tree_With all LMH vs Elephant exclosure -Fungi

C5: Heat tree LMH exclosure vs Elephant exclosure -Fungi

C6: Heat tree LMH exclosure vs with all LMH -Fungi

Appendix D: Published Article (Chapter 5)

Appendix E: Published Proceedings Article (Chapter 6)

List of abbreviations

Abbreviation	Meaning
ABS	alternate biome state
ANOVA	One-Way Analysis of Variance
ASL	above sea level
ASV	amplicon sequence variants
CEC	cation exchange capacity
DADA2	Divisive Amplicon Denoising Algorithm v. 2
DPM	disk pasture meter
IDH	intermediate disturbance hypothesis
ITS	internal transcribed spacer
IUP	intensively utilised patches
KNP	Kruger National Park
LMH	large mammalian herbivores
LDMC	leaf dry matter content
NCBI	National Centre for Biotechnological Information
NGS	Next-Generation Sequencing
NPP	net primary productivity
PAST	Paleontological Statistics Software
QIIME2	Quantitative Insight into Microbial Ecology version 2
SLA	specific leaf area
SANParks	South African National Parks

SOM	Soil organic material
SRA	Sequence Read Archives
TBI	Tea Bag Index

List of symbols

Symbol	Meaning
C	Carbon
Ca ⁺²	calcium
C:N ratio	carbon:nitrogen ratio
N	nitrogen
Na ⁺	sodium
NH ₄ ⁺	ammonium
NO ₃ ⁻	nitrate
P	phosphorus
PO ₄ ⁻³	phosphate
S ⁻²	sulphur

List of figures

Figure no.	Figure description	Page no.
Chapter 2		
Figure 1	Diagrammatic representation of the influence of herbivory on terrestrial nutrient cycling (Adapted from Bardgett <i>et al.</i> , 1998).	19
Figure 2	Effects of herbivore-induced disturbances (i.e. defoliation) and root exudation on decomposition pathways and associated microbial community structure (Adapted from Bardgett <i>et al.</i> , 1998).	22
Figure 3	Above- and belowground systems are fundamentally linked through decomposition. This link between belowground and aboveground compartments produces a positive feedback response (dotted line) to the plant community under herbivore pressure (A) and a negative feedback in their absence (B) (Adapted from Wardle <i>et al.</i> , 2004).	27
Chapter 3		
Figure 4	Location of rest camps and river systems within the KNP relative to the study area (i.e. Nkuhlu experimental site) as well as a key map of southern Africa indicating the positioning of the KNP.	31
Figure 5	Layout of the Nkuhlu experimental site and its location relative to the Sabie River, tourist roads and the Nkuhlu picnic site in the Kruger National Park. Key map indicating the location of the Nkuhlu exclosures in relation to the town of Skukuza.	32
Figure 6	Actual monthly rainfall (mm) from February 2017 to February 2020 as recorded at the Skukuza Meteorological Station (Data courtesy of the South African Weather Service).	34
Figure 7	Actual mean monthly minimum, maximum and average temperatures (°C) from February 2017 to February 2020 as recorded at the Skukuza Meteorological Station (Data courtesy of the South African Weather Service).	35

Figure 8	Geological map of the KNP indicating the location of the Nkuhlu experimental site in relation to the different geological formations.	36
Figure 9	Schematic representation of the granitic catena present within the Nkuhlu exclosures indicating the varying terrain morphology (Adapted from Alard, 2009).	37
Figure 10	Aerial view of the Nkuhlu experimental site indicating the distribution of the sodic zone within the exclosures.	38
Figure 11	Location of the Nkuhlu experimental site relative to the different vegetation units within the KNP.	40
Figure 12	Vegetation map of the Nkuhlu exclosures indicating the location of the Sabie River, seasonal streams, smaller tributaries and the various plant communities relative to the three exclosure units (Adapted from Siebert & Eckhardt, 2008).	41

Chapter 4

Figure 13	Aerial view of the Nkuhlu exclosures indicating the three herbivore treatments and location of sampled plots (1-5) within the sodic zone.	44
Figure 14	Experimental layout of the Nkuhlu long-term research exclosures indicating the five sampled sites and associated plots located across the three herbivore treatments within the sodic zone.	45
Figure 15	Positioning of (a) temporary experimental plots within the sodic zone of each herbivore treatment of the Nkuhlu exclosures, and (b) layout of plots for the purpose of decomposition-based sampling. A detailed explanation of the experimental plot layout is provided in Chapter 5. (Adapted from Erasmus <i>et al.</i> , 2021).	47

Chapter 5

Figure 16	Experimental plot layout consisting of a paired tea bag design across four incubation periods.	53
Figure 17	Mean number of successfully recovered tea bags over four incubation periods. Significant differences ($p < 0.05$) between tea bags are indicated with different lowercase letters.	54

Figure 18	Mean number of tea bags infested by fungi (a), and damaged by termites (b) across three herbivore treatments. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters	55
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Chapter 6

Figure 19	Mean decomposition rates (k) and stabilisation factor (S) of a subtropical, semi-arid southern African savanna in the Kruger National Park. This system is divided into three herbivore treatments (coloured circles). Whiskers indicate standard error of the mean based on the results obtained from 250 tea bags (125 green & 125 rooibos) over a three-month incubation period. Black dots represent global Tea Bag Index references adapted from Keuskamp <i>et al.</i> (2013) and Becker and Kuzyakov (2018).	61
Figure 20	Mean decomposition rate $-k$ (a) stabilisation factor $-S$ (b) of standardised litter after a three-month incubation period across three herbivore treatments at the Nkuhlu exclosures study site, Kruger National Park. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters.	62

Chapter 7

Figure 21:	Boxplot indicating the median (central line in box), interquartile range (box) and extreme (whiskers) decomposition rate $-k$ (a, c, e, g) and stabilisation factor $-S$ (b, d, f, h) values of standardised litter after a 3-, 6-, 9- and 12-month incubation period across three herbivore treatments within the Nkuhlu exclosures, Kruger National Park. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b).	70
Figure 22	Mean differences between herbivore treatments (i.e. presence or absence) for selected soil properties (a-f). Significant differences ($p < 0.05$) between treatments are indicated with different lowercase letters (a and b).	72

Chapter 8

- Figure 23:** Mean bacterial (a) and fungal (b) richness and Shannon-Wiener diversity index values across three herbivore treatments within the Nkuhlu exclosures, Kruger National Park. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b). **83**
- Figure 24:** Bacterial (a) and fungal (b) phylum composition in terms of relative abundance over two seasons (October 2019 dry season and January 2020 wet season) and across three herbivore treatments (With all LMH, Elephant exclosure, LMH exclosure) within a semi-arid sodic savanna ecosystem. **86**
- Figure 25:** Heat tree analysis depicting variations in bacterial (a-c) and fungal (d-f) community composition (from phylum to genus) for combined dry and wet seasons between herbivore treatments (With all LMH, Elephant exclosure, LMH exclosure). Nodes indicate the hierarchical structure of taxa with significantly altered taxa displayed by name at the corresponding node. Red branches are indicative of an increase in a specific treatment compared to another treatment, whilst green (for bacteria) and blue (for fungi) branches indicate a decrease. **89**
- Figure 26:** Unsupervised random forest classification which utilises the three different herbivore treatments (With all LMH, Elephant exclosure, LMH exclosure) as classes and abundance values as classifiers along a decision tree. Y-axis represents the 14 most important classifying variables, whilst the z-axis represents the mean decrease accuracy (MDA) where MDA is the measure of loss of accuracy if the y-axis variable is removed. A mini heatmap (red, yellow and blue squares in order of highest to lowest values) indicates abundance within treatments. **90**

Chapter 9

- Figure 27** DPM-based biomass measurements (kg/ha) for the dry -October 2019 (a) and wet -January 2020 (b) season across three herbivore treatments within the Nkuhlu exclosures, Kruger National Park. **101**

Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b).

- Figure 28** Dry weight biomass measurements (kg/ha) for grasses (a) and forbs (b) across three herbivore treatments within the Nkuhlu exclosures, Kruger National Park. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b). **101**
- Figure 29** Comparison of grass and forb dry weight biomass measurements (kg/ha) across three herbivore treatments, (a) With all LMH, (b) Elephant exclosure and (c) LMH exclosure. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b). **102**

List of tables

Table no	Table Description	Page no
Chapter 7		
Table 1	One-way ANOVA results and mean values for soil attributes and leaf litter from the soil A-horizon across herbivore treatments. Significant variance across treatments is indicated in bold and with an asterisk (*). Data were obtained from Van Coller et al. (in prep) and are presented here with courtesy of the authors.	71
Chapter 8		
Table 2	Bacterial and fungal α -diversity (observed richness and Shannon-Wiener diversity index) over two seasons (October 2019 dry season and January 2020 wet season) and across three herbivore treatments (With all LMH, Elephant exclosure, LMH exclosure). Values are means and $\pm$ standard deviation per treatment. Significant differences between herbivore treatments are represented by lowercase letters and bold characters (i.e. a and b) as measured by One-way Analysis of Variance together with <i>post hoc</i> Tukey HSD tests.	84
Chapter 9		
Table 3	Plant functional traits associated with decomposability of herbaceous detrital material included in this study and motivation for trait selection.	98
Table 4	Individual frequencies depicting the dominant species for the five life form classes across three herbivore treatments. Only the top five dominant species are represented in this table.	104
Table 5	Frequency values (%) of functional plant traits related to decomposition of detrital plant material across three herbivore treatments.	105

Chapter 1

Introduction

1.1 Preface

Savannas encompass approximately 20% of the earth's land surface, constituting one of the largest biomes in the world (Scholes & Walker, 1993; Mucina & Rutherford, 2006). The Savanna Biome is unique amongst other terrestrial biomes, due to the co-dominance of two contrasting plant growth forms, trees and grasses (Mucina & Rutherford, 2006; Pausas & Bond, 2020). Thought to be consumer-controlled, savannas have evolved to become disturbance-dependent, sharing a strong relation to multiple agents of disturbance (Scholes & Archer, 1997; Adler *et al.*, 2001; Smith *et al.*, 2012; Pausas & Bond, 2020). Savannas are mostly associated with heterogeneous landscapes, defined as having strongly seasonal plant communities rich in shade-intolerant species and characterised by a relatively continuous herbaceous layer (forb-grass mixture), and a discontinuous woody component of varying density (Scholes & Archer, 1997; Adler *et al.*, 2001; Venter *et al.*, 2003; Mucina & Rutherford, 2006; Smith *et al.*, 2012; Pausas & Bond, 2020). The strong seasonality of savanna rainfall is clearly reflected in vegetation patterns, and the unique wet and dry cycles have a substantial influence on plant cover, fire regimes and dynamics within animal populations (Venter *et al.*, 2003; Mucina and Rutherford, 2006).

Dynamics of savanna structure and distribution are determined and maintained by multiple site-specific global and/or local regulators (Adler *et al.*, 2001; Bond *et al.*, 2003; Smith *et al.*, 2012; Pausas & Bond, 2020; Thoresen *et al.*, 2021). These include climatic and elevation variability, edaphic properties as well as aboveground plant consumers in the form of fire and vertebrate herbivory, interacting across scales driving system heterogeneity (Bergström & Skarpe, 1999; Adler *et al.*, 2001; Pickett *et al.*, 2003; Jacobs & Naiman, 2008; Sankaran *et al.*, 2008; Smith *et al.*, 2012; Pausas & Bond, 2020; Vermeire *et al.*, 2021). Landscape and resource heterogeneity are key determinants of above- and belowground biodiversity and structure, subsequently driving various essential ecological processes and system functioning (Bardgett *et al.*, 1998; Adler *et al.*, 2001; Pickett *et al.*, 2003; Smith *et al.*, 2012; Eldridge *et al.*, 2019; Pausas & Bond, 2020; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021).

1.2 Herbivory and savanna heterogeneity

African savannas are inhabited by some of the richest large mammalian fauna, including some of the last remaining megaherbivores together with the largest congregation of ungulate species found anywhere in the world (Mucina & Rutherford, 2006). As a global consumer of plant-based biomass, vertebrate herbivory is considered to be a primary agent of disturbance and an important ecosystem modifier (Aguiar & Sala 1999; Adler *et al.*, 2001; Venter *et al.*, 2003; Jacobs & Naiman, 2008; Sankaran *et al.*, 2008; Pausas & Bond, 2020). Herbivores are capable of driving change in landscape and resource heterogeneity across spatial and temporal scales, through several direct and indirect pathways (Bardgett *et al.*, 1998; Jacobs & Naiman, 2008; Smith *et al.*, 2012; Holdo & Mack, 2014; Lui *et al.*, 2016; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021). System heterogeneity is an important regulating factor in ecosystem structure and functioning. (Caldwell & Pearcy 1994; Pickett & Cadenasso, 1995; Aguiar & Sala, 1999; Adler *et al.*, 2001; Du Toit, 2003; Eldridge *et al.*, 2019). This is especially true for semi-arid ecosystems where the patchy nature of system structure has an essential role in the maintenance of productivity through concentrating limiting resources (Pickett & Cadenasso, 1995; Aguiar & Sala 1999; Adler *et al.*, 2001). Herbivory is therefore considered to be an essential mechanism in maintaining Africa's open ecosystems through generating various stabilising feedbacks which drive and maintain invaluable ecological processes and cycles (Bergström & Skarpe, 1999; Du Toit, 2003; Grant & Scholes, 2006; Jacobs & Naiman, 2008; Pausas & Bond, 2020).

The process of decomposition is fundamental to the effective functioning of terrestrial ecosystems as it forms the link between above- and belowground nutrient cycling, essentially driving system structure and functioning through regulating nutrient availability (Swift *et al.*, 1979; Bardgett *et al.*, 1998; Chapin *et al.*, 2002; Wang & D'Odorico, 2008; Cotrufo *et al.*, 2015; Eldridge *et al.*, 2017; Petraglia *et al.*, 2019). Decomposition is directly influenced by detrital quantity and quality, edaphic properties as well as the abundance and composition of decomposer communities (Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Certini, 2005; Hatterschwiler *et al.*, 2005; Brennan *et al.*, 2009; Schrama *et al.*, 2013; Eldridge *et al.*, 2019). However, these determinants do not occur in isolation, as such they are capable of influencing or being influenced by various prevailing environmental conditions and/or actors. Subsequently, decomposition-related processes can also be affected indirectly by various site-specific drivers such as herbivory through top-down trophic cascades (Adler *et al.*, 2001; Jacobs & Naiman, 2008; Smith *et al.*, 2012; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021).

Numerous studies have reported on the effects of LMH on system structure with regards to plant productivity, -community and chemical composition as well as edaphic properties (Bardgett *et al.*, 1998; Adler *et al.*, 2001; Grant & Scholes, 2006; Jacobs & Naiman, 2008; Scogings *et al.*, 2012; Van Coller *et al.*, 2013; Holdo & Mack, 2014; Lui *et al.*, 2016). Considering the link between vegetation structure, soil conditions and soil microbes, herbivore-induced disturbances has the potential to influence several regulatory constituents of microbial community structure (Bardgett *et al.*, 1998; Wardle *et al.*, 2002; Nilsson *et al.*, 2008; Maestre *et al.*, 2015; García- Palacios *et al.*, 2016; Eldridge & Delgado-Baquerizo, 2018; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). Moreover, considering potential changes to microbial community structure, it is suggested that LMH can function as major determinants of biogeochemical cycles such as decomposition, essentially influencing soil function (Bardgett *et al.*, 1998; Bardgett & Wardle, 2003; Eldridge *et al.*, 2019; Erasmus *et al.*, 2022).

1.3 Rationale

Large parts of protected African savannas are faced with numerous challenges, including heavy utilisation pressure from single-species domestic livestock, crop cultivation and rural expansion (Du Toit, 2003; Koerner *et al.*, 2014; Ratnam *et al.*, 2019). These disturbances may result in the loss of native heterogeneous savanna herbivore communities (albeit the diverse suite of LMH, or elephants only), which contribute towards the structural and functional degradation of these systems (Jacobs & Naiman, 2008; Koerner *et al.*, 2014; Wigley *et al.*, 2020). Mismanagement of native and/or domesticated herbivore grazing regimes can cause substantial changes to both soil and vegetation structure, and therefore landscape and resource heterogeneity across multiple contexts and spatial scales (Zhao *et al.*, 2011; Koerner *et al.*, 2014; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021).

The effects of herbivore-induced disturbances on soil- and vegetation heterogeneity are relatively well known (Bardgett *et al.*, 1998; Aguiar & Sala 1999; Adler *et al.*, 2001; Grant and Scholes, 2006; Jacobs & Naiman, 2008; Holdo & Mack, 2012; Van Coller *et al.*, 2013). However, the impact and extent to which variation in these system determinants affect microbial community structure, and associated soil function remain limited (Bardgett *et al.*, 1998; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). The link between environmental heterogeneity and soil microbial heterogeneity remains an important knowledge gap in our current understanding of microbial responses to variations in environmental drivers (Bardgett *et al.*, 1998; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). Despite extensive use of enclosure experiments to investigate herbivore impacts, there is little consensus on their direct and indirect effects on soil function, particularly on microbial-mediated decomposition and

inherently, above- and belowground carbon sequestration (Bardgett *et al.*, 1998; Semmartin *et al.*, 2008; Eldridge *et al.*, 2019; Tuomi *et al.*, 2019; Teo *et al.*, 2020; Erasmus *et al.*, 2021; Erasmus *et al.*, 2022). It has been suggested that the paucity of information available on decomposition can potentially be ascribed to the range of complex, cross-scale interactions that collectively regulate African savanna structure and function (Becker & Kuzyakov, 2017; Teo *et al.*, 2020). Assessments of ecosystem functioning, such as the Tea Bag Index (TBI), are becoming increasingly valuable especially for application in monitoring programmes in areas exposed to increasing intensities of land-use and/or climate change. The TBI provides an easily applicable, standardised approach to quantify belowground decomposition of plant-based material through the use of readily available tea bags (Keuskamp *et al.*, 2013).

Sodic patches situated within the semi-arid savanna of the KNP serve as a good example of a highly dynamic, herbivore-adapted system based on edaphic variability and a strong herbivory feedback (Khomu & Rogers, 2005; Grant & Scholes, 2006; Jacobs & Naiman, 2008; Scogings *et al.*, 2012; Van Coller *et al.*, 2013). These patches are prominent intensively utilised patches (IUP) in granitic landscapes that are structurally open areas, associated with the production of high-quality forage (Khomu & Rogers, 2005; Grant & Scholes, 2006; Scogings *et al.*, 2012). Sodic vegetation is considered to be preferentially foraged by LMH, resulting in congregated herbivore activity promoting a positive nutrient feedback loop (Naiman *et al.*, 2003; Grant & Scholes, 2006). Strong interactive effects between herbivory and sodic vegetation dynamics have suggested that these patches are capable of exhibiting change related to a release from grazing pressure over short periods of time (Khomu & Rogers, 2005; Jacobs & Naiman, 2008). The Nkuhlu experimental site located within the KNP, South Africa, provided an opportunity to examine the potential influence of complete (all LMH) or partial (elephants only) herbivore loss on above- and belowground heterogeneity and associated essential ecological processes (decomposition) within dynamic sodic patches. Determining the effects of herbivory on microbial diversity and abundance is an essential management tool that can aid in understanding and predicting potential effects of land-use change with regards to herbivory, or the loss thereof on microbially mediated soil function and inherent carbon cycling. This study was specifically designed to further contribute to current knowledge pertaining to the effects of herbivore loss on ecologically important herbivore-preferred nutrient hot-spots in African savanna ecosystems.

1.4 Aims, objectives and hypotheses

This study aims to assess the effects of herbivory (or the loss thereof) on potential soil-based decomposition of standardised detrital plant material and soil microbial community structure, and how these belowground processes are related to observed changes in vegetation structure and edaphic properties in a herbivore-driven, nutrient-rich semi-arid sodic African savanna ecosystem.

In this study, herbivore loss was divided into two intensity classes, referred to as treatments and defined accordingly: (1) complete herbivore loss referring to the loss of all LMH and (2) partial herbivore loss referring to the loss of elephants and giraffes only.

Objectives, accompanied by specific hypotheses are outlined below. Note that the objectives and hypotheses will be highlighted and strengthened in the introductory sections of each of the Results chapters of the dissertation.

Objectives of the study were to:

1. Examine the potential of the TBI as a viable approach to quantify decomposition in a disturbance-driven semi-arid African savanna across different herbivore treatments and over a period of one year, and to adapt the protocol for application in African ecosystems where applicable (*Chapter 5*).
2. Through the use of the proposed adapted version of the TBI (*Chapter 5*), assess three-month soil-based decomposition results across herbivore treatments, and compare these results to globally distributed TBI-derived decomposition data from a variety of terrestrial ecosystems (*Chapter 6*). Here, it was hypothesised that TBI-based decomposition rates (k) will be highest on a (i) local scale, i.e. in the presence of LMH, whilst on a (ii) global scale, northern hemisphere systems will have higher decomposition rates (k) when compared to this southern hemisphere-based semi-arid savanna as a result of varying regulatory factors, such as climatic, landscape and resource heterogeneity that differ substantially across spatial scales (*Chapter 6*).
3. Determine the effect of varying herbivore treatments on potential decomposition rate (k) and stabilisation factor (S) of detrital plant material through application of the extended TBI approach across four incubation periods (three-, six-, nine- and twelve-months). Disturbance-prone, seasonal ecosystems are subject to constant change, necessitating the use of varying incubation periods as a means to represent the full complement of subsystem dynamics. Given the environmental conditions under which

the TBI was developed (i.e. in boreal and temperate ecosystems), it is hypothesised that decomposition rate (k) will be slower (i.e. significant changes will only be detected after longer incubation periods) than is suggested in the original protocol (three-months) (*Chapter 7*).

4. Considering changes in decomposition (k) and stabilisation (S) in response to herbivory treatments, Chapter 8 aimed to assess the effects of herbivory on soil microbial community structure through the amplification of bacterial 16S rRNA and the fungal ITS region (Next-Generation Sequencing -NGS) from soil-based Environmental DNA. Given the link between soil microbes (bacteria and fungi), vegetation structure and edaphic properties, it is hypothesised that long-term changes in herbivore communities will have significant effects on soil microbial community composition. Microbial communities' variable metabolic capacity and tolerance towards disturbance led to the hypothesis that bacterial α -diversity will be highest in the presence of LMH, whilst herbivore exclusion will favour fungal α -diversity (*Chapter 8*).
5. To ultimately bring together the observed effects of varying herbivore treatments on top-down and bottom-up controls, including site-specific plant species, biomass and functional trait changes, and soil physicochemical properties (*Chapter 9*). As a result of the range of complex top-down trophic cascades and bottom-up feedback responses within semi-arid savanna ecosystems, it is hypothesised that herbivore-induced changes to aboveground vegetation structure (productivity and composition) can be linked with belowground changes to microbial community structure (bacteria and fungi) and associated soil function (decomposition and stabilisation) (*Chapter 9*).

1.5 Format of study

This dissertation conforms to the guidelines set for a standard dissertation at the North-West University¹ in South Africa. This dissertation encompasses ten chapters, of which one (Chapter 5) was prepared, accepted and has already been published in a scientific journal, while the second (Chapter 6) has been prepared and accepted for publication as a proceedings article for the Joint XXIV International Grassland and XI Rangeland Congress 2021. Both manuscripts have been attached as appendices (B and C, respectively) to this dissertation.

¹ See section 2.1 of the manual for Post Graduate Studies (www.nwu.ac.za/library/documents/manualpostgrad.pdf)

Since each of the study objectives addresses a specific research question that requires materials and methods that are not applicable to other objectives, each specific objective represents a different chapter under the Results and Discussion section of the dissertation. Each of these Results chapters will therefore include a detailed methods section pertaining to a specific research question. Literature cited throughout the ten chapters were combined into a single list of references at the end of this dissertation.

Chapter 2: Literature review

This chapter provides a comprehensive outline of existing literature pertaining to the effect of herbivore-induced disturbances on above- and belowground community heterogeneity and how it relates to soil function. It further provides detailed background information relating to key aspects of this study, including semi-arid savannas and herbivory as major ecosystem drivers. This chapter further emphasises the necessity for long-term soil-based monitoring in the KNP as current literature is limited with regards to the effect of disturbance agents, especially herbivores, or the loss thereof on essential ecological processes (e.g. decomposition and stabilisation of detrital plant material) relating to soil function and subsequent carbon (C) cycling and associated climate change.

Chapter 3: Study area

A general description of the study area pertaining to relevant components including the location and description of the study area, climate, geology, topography, soil, fauna and flora is provided in this chapter.

Chapter 4: Experimental- and sampling design

A comprehensive outline of the experimental design as well as a general description of the methods that were used to obtain and analyse floristic-, decomposition- and soil microbial genomic data is provided in this chapter. Each of the *Results* chapters will include details on the materials and methods that are relevant to that particular objective of the study.

Results and Discussion

Chapter 5: Extension and validation of the Tea Bag Index as a viable approach to quantify decomposition in a semi-arid, African savanna

Application of the internationally standardised TBI approach as described by Keuskamp *et al.* (2013) is tested and evaluated as a viable means to quantify decomposition in an African context. This chapter further entails the development of an extended, site-specific version of the original TBI approach to facilitate the application of TBI-based decomposition studies in a semi-arid African savanna. This unit has been accepted and published in the *South African Journal of Science* (Erasmus *et al.*, 2021; Appendix D).

Chapter 6: Decomposition rates of detrital plant material across herbivore treatments in a semi-arid savanna

Application of the extended, site-specific TBI approach (Erasmus *et al.*, 2021) as a means to examine the effect of varying herbivore intensities in a semi-arid sodic savanna on potential decomposition rate (k) and stabilisation factor (S) of detrital plant material after a three-month incubation period. Moreover, this chapter attempts to compare obtained three-month TBI results to a global dataset of TBI-derived decomposition data from different ecosystems. This unit has been converted into a manuscript for an international congress (i.e. Joint XXIV International Grassland and XI Rangeland Congress 2021) and accepted for publication as a proceedings article (Erasmus *et al.*, 2022; Appendix E).

Chapter 7: A detailed account of potential decomposition rate and stabilisation factor at several incubation lengths across herbivore treatments

This chapter addresses the temporal nature of decomposition dynamics in a semi-arid, African savanna through the application of the extended TBI approach over the course of one year with four three-month intervals (3-, 6-, 9- and 12-months). In this chapter both decomposition rate (k) and stabilisation factor (S) obtained over four incubation periods and across different herbivore treatments are compared to examine the effect of herbivory on decomposition dynamics. This chapter elaborates on changes in decomposition across a temporal scale.

Chapter 8: Soil microbial community structure across various herbivore intensities

This chapter provides a description of the soil microbial community composition of the sodic soils relevant in this investigation. Results report on changes within community structure in terms of α -diversity measures, phylum composition and relative abundance of bacterial and fungal taxa across varying herbivore treatments derived from soil-based genomic analyses. The possibility of seasonal-driven variation in bacterial and fungal community composition is also discussed.

Chapter 9: Linking above- and belowground systems: Linking changes in vegetation structure to belowground processes

As herbivores actively alter herbaceous biomass and species composition, this chapter serves to provide a link between herbivore-induced changes to aboveground vegetation structure, edaphic properties and belowground microbial community composition and related soil function (i.e. decomposition and stabilisation).

Chapter 10: Conclusion

The main findings from chapters 6, 7, 8 and 9 are presented as a combined aboveground-belowground approach and interpreted in terms of the effects of LMH (or the loss thereof) on the structure and function of a semi-arid African savanna ecosystem. The importance of LMH in ecologically sensitive areas, such as sodic patches are emphasised especially due to the lack of knowledge pertaining to the dynamics of these systems. This study also indicates potential knowledge gaps and recommendations on how to address these.

Chapter 2

Literature review

2.1 Semi-arid savannas

Savannas occupy roughly 20% of the world's terrestrial surface and represent the most widespread ecosystem within tropical- and subtropical regions (Scholes & Walker, 1993; Mucina & Rutherford, 2006). Covering approximately 40% of the African continent and >30% of South Africa, the Savanna Biome is also the largest biome in the country (Mucina & Rutherford, 2006). Southern African savannas constitute a prime example of an interactive social-ecological system with numerous individuals and communities depending on the associated ecosystem goods and services for their livelihood (Sandhage-Hofmann, 2016; Ratnam *et al.*, 2019; Scheiter *et al.*, 2019). This includes wildlife and livestock forage, wood products, mineral fluxes and several socio-cultural-based services (Sandhage-Hofmann, 2016; Scheiter *et al.*, 2019). A continuous provision of these valuable services requires sustainable use and effective top-down and bottom-up management strategies to maintain essential ecological function (Vermeire *et al.*, 2021). This study is primarily focused on semi-arid, sub-tropical southern African savanna ecosystems and will for the sake of conciseness be referred to as *savannas* throughout the study.

Savannas are described as water-limited systems that are highly dependent on rainfall seasonality (Scholes & Archer, 1997). Semi-arid savannas predominantly occur at low elevation and are generally characterised by two distinctive seasons, namely a hot, sporadically wet growth season and a warm, dry, non-growing season (Venter *et al.*, 2003). The influence and subsequent importance of rainfall variability as a system determinant is accentuated in low-elevation, semi-arid ecosystems (Venter *et al.*, 2003; Sankaran *et al.*, 2008; Becker & Kuzyakov, 2018). The effects of seasonal rainfall are clearly reflected in the pulse-nature of vegetation patterns and associated animal activity (Venter *et al.*, 2003; Mucina & Rutherford, 2006). The unique wet and dry cycles related to the seasonal nature of savannas have a marked effect on several structural-based factors including vegetation cover, fire regimes and animal population dynamics (Venter *et al.*, 2003).

The distribution of savannas is fundamentally determined by cross-scale interactions between regional climate and edaphic properties (Scholes & Archer, 1997; Augustine &

McNaughton, 2006; Mucina & Rutherford, 2006; Semmartin *et al.*, 2008). These interactions essentially define and affect vegetation structure and subsequently, the role of above- and

belowground drivers such as herbivory and soil microbes (Bardgett *et al.*, 1998; Bergström & Skarpe, 1999; Bond & Keeley, 2005; Semmartin *et al.*, 2008; Becker & Kuzyakov, 2018; Eldridge *et al.*, 2019). Water availability is the primary limiting growth factor in semi-arid regions and is mainly regulated by the physical properties of soil (Scholes & Archer, 1997; Bergström & Skarpe, 1999; Venter *et al.*, 2003). Prevailing soil conditions, and by association the factors influencing soil properties (e.g. herbivory), have the capability to mediate rainfall efficiency and determine above- and belowground structure (Bergström & Skarpe, 1999; Venter *et al.*, 2003; Khomo & Rogers, 2005).

Vegetation structure of savannas consists of a dynamic coexistence between two contrasting life forms in which herbaceous and woody plants share dominance in structure and function (Scholes & Archer, 1997; Venter *et al.*, 2003; Smith *et al.*, 2012). In these systems, herbaceous species form a relatively continuous matrix with a discontinuous woody component of varying density. Here, the grass component functions as the main source of forage for the diverse community of native LMH and domestic livestock (Scholes & Archer, 1997; Venter *et al.*, 2003; Sankaran *et al.*, 2008). There exists a dynamic relationship between the herbaceous and woody component which is driven and maintained by various site-specific determinants including climatic and elevation variability, soil conditions, nutrient availability, fire and herbivory (Scholes & Archer, 1997; Venter *et al.*, 2003; Sankaran *et al.*, 2008; Sankaran & Anderson, 2009; Smith *et al.*, 2012).

Cross-scale interactions between rainfall variability and other site-specific factors i.e. edaphic properties and aboveground herbivory are responsible for driving change in above- and belowground community heterogeneity, ultimately influencing ecosystem functioning (Bond & Keeley, 2005; Augustine & McNaughton, 2006; Semmartin *et al.*, 2008; Lui *et al.*, 2016; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021).

2.2 Herbivory as a driver of savanna dynamics

African savannas are inhabited by the Earth's richest large mammalian fauna, including the largest congregation of ungulate species found anywhere in the world (Venter *et al.*, 2003; Mucina & Rutherford, 2006; Jacobs & Naiman, 2008). Herbivores therefore constitute a large part of savanna trophic structure and is intimately linked to various ecological processes and system functioning (Bergström & Skarpe, 1999; Grant & Scholes, 2006; Augustine & McNaughton, 2006; Van Coller *et al.*, 2013; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021).

Herbivores and their associated effects are essential for the sustained conservation of natural systems as they are capable of altering both above- and belowground structural heterogeneity

through various top-down mechanisms (Adler *et al.*, 2001; Augustine & McNaughton, 2006; Grant & Scholes, 2006; Jacobs & Naiman, 2008; Lui *et al.*, 2016; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021). Generally regarded as ecosystem modifiers, herbivores have the potential to alter the physical state of a system via numerous direct and indirect processes including foraging, trampling and nutrient enrichment (Adler *et al.*, 2001; Venter *et al.*, 2003; Augustine & McNaughton, 2006; Sankaran *et al.*, 2008; Semmartin *et al.*, 2008; Waldram *et al.*, 2008). Herbivores are therefore considered to be a key component of dynamics within savannas as they are capable of actively influencing and altering landscape heterogeneity and resource availability (Adler *et al.*, 2001; Grant & Scholes, 2006; Augustine & McNaughton, 2006; Jacobs & Naiman, 2008; Waldram *et al.*, 2008; Alard, 2009).

Southern African savannas are under increasing threat from mostly anthropogenic-derived disturbances including game and livestock farming as well as cultivation practices and rural development (Venter *et al.*, 2003; Mucina & Rutherford, 2006; Scheiter *et al.*, 2019; Ratnam *et al.*, 2019). Such activities disrupt or completely destroy essential ecological processes by altering system structure through numerous mechanisms including overgrazing, erosion and the loss of biodiversity (Eldridge *et al.*, 2019; Ratnam *et al.*, 2019). The global environment is changing, posing savanna ecologists with growing challenges, such as the loss of heterogeneous savanna herbivore communities to single-species domestic livestock (Koerner *et al.*, 2014; Ratnam *et al.*, 2019). Understanding the effects of large mammalian herbivory and/or its loss on savanna ecosystems is necessary to effectively manage these natural systems. Plant community and species diversity responses in response to grazing by large herbivores is strongly influenced by management practices (Hickman *et al.*, 2004). However, soil microbial community responses towards top-down disturbances and subsequent impact on soil function are less known (Bardgett *et al.*, 1998; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). An imbalance in native or domestic herbivore community structure can result in decreased resource connectivity and corresponding loss of ecological functioning and essential ecosystem services through changes to above- and belowground structure (Augustine & McNaughton, 2006; Jacobs & Naiman, 2008; Koerner *et al.*, 2014; Eldridge *et al.*, 2019). This is often the result of improper land use practices and/or poor management strategies (Jacobs & Naiman, 2008; Semmartin *et al.*, 2008; Burns *et al.*, 2009; Koerner *et al.*, 2014; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021).

Due to the high level of connectivity within savanna trophic structure, the influence and overall impact of disturbances such as herbivore loss may be exacerbated (Venter *et al.*, 2003; Grant and Scholes, 2006; Alard, 2009; Ratnam *et al.*, 2019; Teo *et al.*, 2020). However, naturally occurring savanna disturbance regimes have resulted in increased system (above- and

belowground) resilience, subsequently reducing the impact of disturbances on system structure and functioning (Alard, 2009; Vermeire *et al.*, 2021).

There remains uncertainty about the scales at which ecological processes are affected by large herbivores (Asner *et al.*, 2009). Understanding how large herbivores change terrestrial ecosystems through alteration to both above- and belowground structure and associated function is key to well-balanced management strategies (Rogers, 1997; Aguiar & Sala, 1999; Adler *et al.*, 2001; Shannon *et al.*, 2008; Asner *et al.*, 2009). Savanna conservation relies on the maintenance of interactive ecological mechanisms pertaining to both top-down drivers (e.g. herbivory) and bottom-up controls (e.g. soil nutrient cycling) of system structure and function. (Rogers, 1997; Aguiar & Sala, 1999; Adler *et al.*, 2001; Rogers, 2003; Eldridge & Delgado-Baquerizo, 2018). This poses a challenging dilemma to stakeholders to find a balance between the requirements of large herbivore populations and the sustainable management of essential ecosystem processes and functioning (Adler *et al.*, 2001; Shannon *et al.*, 2008; Asner *et al.*, 2009). Sustainable management of savanna ecosystems requires a proper understanding of combined above- and belowground mechanistic feedbacks between native LMH, vegetation, soil and the decomposition subsystem, which are key determinants of system functioning (Bardgett & Wardle, 2003).

2.2.1 Effects of herbivory on aboveground structure

Native LMH serve as primary drivers of change within savanna ecosystem structure through actively altering aboveground standing biomass, herbaceous and woody plant diversity as well as plant chemical composition (Olf & Ritchie, 1998; Grant & Scholes, 2006; Jacobs & Naiman, 2008; Riginos & Grace, 2008; Scogings *et al.*, 2012; Van Coller *et al.*, 2013). Productive ecosystems tend to attract high levels of LMH activity (Jacobs & Naiman, 2008), subsequently allowing for greater herbivore-induced modification (Bardgett & Wardle, 2003; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021).

Herbivory acts as an agent of disturbance through several associated physical mechanisms including biomass consumption and hoof action (Olf & Ritchie, 1998; Adler *et al.*, 2001; Jacobs & Naiman, 2008; Riginos & Grace, 2008; Van Coller *et al.*, 2013). Herbivores are capable of reducing standing biomass and canopy cover of selected herbaceous and woody plant species by browsing and by creating grazing patches, wallowing points and game paths (Adler *et al.*, 2001; Jacobs & Naiman, 2008; Riginos & Grace, 2008; Savadogo *et al.*, 2008; Van Coller *et al.*, 2013). Through several direct and indirect mechanisms pertaining to foraging and trampling, herbivory has the potential to reduce local extinction rate by actively regulating

the effects of competitive exclusion related to high biomass (Olf & Ritchie, 1998; Adler *et al.*, 2001; Savadogo *et al.*, 2008). By removing competitively dominant species, herbivory allows for more species that are functionally different to coexist (Olf & Ritchie, 1998; Adler *et al.*, 2001; Savadogo *et al.*, 2008). However, herbivores can also enhance extinction processes by means of preferentially consuming high-quality vegetation, increasing the abundance of a few grazing tolerant species (Savadogo *et al.*, 2008).

A reduction in biomass has a major impact on the quantity and distribution of detrital plant material that is available to detritivores, and ultimately enters the decomposition subsystem (Bardgett *et al.*, 1998; Lui *et al.*, 2016; Eldridge *et al.*, 2019; Teo *et al.*, 2020). A reduction in woody canopy cover allows more light to penetrate through to the herbaceous understory, facilitating the survival of more species (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Riginos & Grace, 2008; Van Coller *et al.*, 2013). Reduced canopy cover also relates to increased soil temperatures as more light reaches the surface (Holdo & Mack, 2014; Becker and Kuzyakov, 2018; Petraglia *et al.*, 2019).

Herbivory increases overall spatial heterogeneity, indirectly promoting plant diversity (Olf & Ritchie, 1998; Adler *et al.*, 2001; Jacobs & Naiman, 2008; Van Coller *et al.*, 2013). LMH are able to further alter floristic composition of herbaceous species via the facilitation of several ecological processes. Examples include seed dispersal, soil nutrient enrichment through urination and defecation as well as hoof action which promotes the formation of various soil microhabitats capable of supporting less common plant species (Riginos & Grace, 2008). Plant species richness is therefore regulated by the interactions between various system disturbances and productivity (Pollock *et al.*, 1998; Jacobs & Naiman, 2008; Van Coller *et al.*, 2013). However, the effects and extent of herbivore-induced disturbances on system structure in terms of productivity and diversity is dependent on several site-specific factors ranging from the variability in precipitation and edaphic properties to the size and composition of native LMH communities (Augustine & McNaughton, 2006; Jacobs & Naiman, 2008; Riginos & Grace, 2008).

The relationship between LMH, productivity and diversity is central to the effective conservation and management of biodiversity and the subsequent maintenance of ecosystem functioning (Michalet *et al.*, 2006; Jacobs & Naiman, 2008; Van Coller & Siebert, 2015). The intermediate disturbance hypothesis (IDH), often observed to be hump-shaped, predicts that relatively high herbaceous species richness will occur at intermediate levels of disturbance or competition and subsequently biomass (Pollock *et al.*, 1998; Michslet *et al.*, 2006; Jacobs & Naiman, 2008). In accordance with the IDH, when biomass levels are high and disturbances in the form of grazing pressure is low, only a few dominant herbaceous and/or woody species

will replace the majority of other species due to competition for resources (Jacobs & Naiman, 2008; Van Coller & Siebert, 2015).

A relaxation of herbivore pressure has been noted to cause significant reductions in herbaceous species richness (Jacobs & Naiman, 2008; Van Coller *et al.*, 2013; Van Coller & Siebert, 2015). This can primarily be ascribed to the increase in grass biomass due to reduced herbivory, suggesting that herbivores are essential to maintain low levels of biomass and corresponding species diversity (Grant and Scholes, 2006; Jacobs & Naiman, 2008; Van Coller *et al.*, 2013; Van Coller & Siebert, 2015). This is readily seen in herbivore-adapted systems where system structure is mainly driven and maintained by herbivore-induced disturbances and associated feedbacks (Grant and Scholes, 2006; Jacobs & Naiman, 2008; Van Coller *et al.*, 2013). Moreover, it is generally acknowledged that removal of herbivores from an herbivore-adapted system results in an increase in aboveground biomass, both in the herbaceous and woody vegetation layers (Grant and Scholes, 2006; Jacobs & Naiman, 2008; Van Coller *et al.*, 2013; Van Coller & Siebert, 2015). Increased woody biomass, i.e. bush encroachment, may potentially have severe implications for system functioning and resilience (Van Coller *et al.*, 2013; Van Coller & Siebert, 2015). Bush encroachment can essentially affect soil function (decomposition) by influencing the quality of detrital material (more recalcitrant materials such as secondary metabolites, lignin etc present), increased shading and subsequently lower soil surface temperatures (Scogings *et al.*, 2012). This facilitates increased litter stabilisation, resulting in a slower nutrient turn-over and the subsequent formation of soil organic material (SOM) (Keuskamp *et al.*, 2013; Erasmus *et al.*, 2022).

LMH have the ability to actively select for high-quality forage through sustained herbivory pressure and selective feeding (Grant and Scholes, 2006; Jacobs & Naiman, 2008; Semmartin *et al.*, 2008; Alard, 2009; Scogings *et al.*, 2012). Sodic patches in the KNP is a good example of a herbivore-adapted system, based on sustained herbivore pressure and a strong herbivory feedback (Grant and Scholes, 2006; Jacobs & Naiman, 2008; Alard, 2009; Van Coller *et al.*, 2013). In these systems congregated herbivore activity results in high utilisation of sodic vegetation which maintain foliage at an immature stage (McNaughton, 1994). Immature foliage has low carbon(C):nitrogen(N) ratios, rendering vegetation more palatable and promoting continual grazing (McNaughton, 1994; Alard, 2009). It has been found that plant regrowth post herbivore-induced defoliation often contains increased nutrient concentrations, i.e. N and phosphorus (P), within aboveground biomass (Semmartin *et al.*, 2008; Scogings *et al.*, 2012).

Defoliation by herbivores stimulate various chemical-based changes within heavily grazed vegetation (Semmartin *et al.*, 2008; Scogings *et al.*, 2012). This includes decreased carbon-

based secondary metabolites (e.g. recalcitrant compounds such as tannins and polyphenols), decreased carbon:nitrogen (C:N) ratios and increased nutrient content within vegetation tissue (Semmartin *et al.*, 2008; Scogings *et al.*, 2012). This subsequently renders highly utilised savanna vegetation more palatable, nutrient-rich and labile (Grant and Scholes, 2006; Jacobs & Naiman, 2008; Alard, 2009; Scogings *et al.*, 2012). On the contrary, it has been suggested that sustained long-term herbivory pressure could over time result in the selection of grazing-resistant genotypes containing various grazing-resistant traits (Painter *et al.*, 1993; Semmartin *et al.*, 2008). This includes greater nutrient allocation to roots (Bardgett *et al.*, 1998) and higher lignin:N ratios in aboveground plant tissue (Painter *et al.*, 1993; Semmartin *et al.*, 2008).

2.2.2 Effects of herbivory on belowground structure

In heterogeneous savanna landscapes (Scholes & Archer, 1997), structural variation originating from herbivore-induced disturbances and the subsequent discontinuous nature of vegetation cover, results in spatial variation of soil properties (Holdo & Mack, 2014). This includes variation in a range of physical and chemical soil properties, resulting either directly or indirectly from LMH activity related to consumption, excretion and physical disturbance (Holdo & Mack, 2014; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021). These variations have the ability to exert control over biogeophysical processes and cycles (e.g. decomposition and nutrient cycling) through influencing and altering soil-based microbial community structure and associated constituents (Sankaran *et al.*, 2008; Holdo & Mack, 2008; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). The magnitude of herbivore-induced effects on soil properties is thought to be exacerbated in productive ecosystems (e.g. nutrient hot-spots, such as sodic sites in the granitic Lowveld) which attract congregated LMH activity, subsequently allowing for greater modification of soil properties (Bardgett & Wardle, 2003; Vermeire *et al.*, 2021).

2.2.2.1 Effects of herbivory on soil physicochemical properties

Disturbances caused by native LMH on one trophic level e.g. alteration to aboveground vegetation structure, can initiate a trophic cascade that has the ability to indirectly influence numerous physicochemical soil attributes through various top-down mechanisms (Bardgett *et al.*, 1998; Gongalsky & Persson, 2013; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021). Soil spatial heterogeneity through variation in chemical and physical properties, has been found to be strongly dependent on the activity of foraging herbivore assemblages, which in turn are driven by vegetation diversity and structure (Lui *et al.*, 2016). Lui *et al.* (2016) suggested that herbivore assemblages, with regards to species diversity and abundance, are

important in maintaining spatial heterogeneity in terms of vegetation- and soil structure together with associated ecological processes.

Herbivory has the capability to alter soil resource (nutrient) heterogeneity through several mechanisms related to changes in plant diversity and the spatial patterns of vegetation composition, subsequently influencing the spatiotemporal distribution of detrital plant material (Augustine & Frank, 2001). Changes in vegetation structure, such as decreasing standing biomass will affect the accumulation of surface detrital plant material (Dormaar & Carefoot, 1996; Willms & Chanasys, 2006). This material has the ability to provide shade to the soil but also to hold moisture, subsequently keeping the soil moist for longer (Dormaar & Carefoot, 1996; Willms & Chanasys, 2006). Bare soil is more prone to erosion as a result of decreased plant rooting and decreased microbial biofilm which bind soil particles. Biotic and abiotic disturbances, such as wind, rain and animal activity, may therefore lead to greater risks of topsoil erosion, predominantly the fertile A-horizon (Venter *et al.*, 2003; Khomo & Rogers, 2005). Moreover, bare soil allows for higher rates of leaching which results in lower levels of accessible nutrients within superficial soil profiles (Isichei & Muoghalu, 1992; Bardgett & Wardle, 2003).

Defoliation has been noted to induce rapid changes in belowground nutrient allocation with regards to C and N, which affects the chemical composition of root tissue and subsequent quality of belowground detrital material and root exudates (Bardgett *et al.*, 1998; Hamilton & Frank, 2001; Wang *et al.*, 2018). This strongly influences the amount and availability of soil extractable C in the rhizosphere resulting in profound modification of soil physicochemical properties (Bardgett *et al.*, 1998; Hamilton & Frank, 2001; Bardgett & Wardle, 2003; Bardgett & Wardle, 2010; Crocker, 2015; Vandegehuchte *et al.*, 2015). Moreover, this has a direct influence on rhizosphere-based microbial community structure and activity (Bardgett & Wardle, 2003; Bardgett & Wardle, 2010; Crocker, 2015).

Native LMH directly influence soil physicochemical properties through two fundamental pathways, i.e. trampling and nutrient enrichment via defecation and urination (Bardgett *et al.*, 1998; Bardgett *et al.*, 2001; Bardgett & Wardle, 2003; Sankaran & Augustine, 2004; Hamza & Anderson, 2005; Semmartin *et al.*, 2008; Schrama *et al.*, 2013). Trampling reduces the porosity of soil through compaction, restricting vegetation rooting as well as the movement of water and gasses through the soil (Qadir & Schubert, 2002; Hamza & Anderson, 2005; Schrama *et al.*, 2013). This negatively affects the diffusion capacity of water, gasses (e.g. oxygen, carbon dioxide etc.) and nutrients (Qadir & Schubert, 2002; Hamza & Anderson, 2005). Compaction greatly affects hydrological properties, decreasing water infiltration and internal drainage (Qadir & Schubert, 2002; Hamza & Anderson, 2005; Holdo & Mack, 2014).

Under wet conditions, slow internal drainage can lead to soil anoxia resulting in increased denitrification and subsequently reduced accessible soil N (Qadir & Schubert, 2002; Hamza & Anderson, 2005). Yet, it has been suggested that due to higher levels of compaction, water potentially remains within the top 10 cm of soil for longer periods post precipitation event, essentially sustaining vegetation with superficial rooting (Holdo & Mack, 2014). Conversely, Holdo and Mack (2014) found that the layer of topsoil from subcanopy areas, generally associated with higher levels of SOM and less compaction, would drain much faster than in open areas. This observed pattern is the potential outcome of variations in soil bulk density (result of compaction) where more compacted soil has a lower saturated conductivity and subsequently slower infiltration rate and internal drainage (Holdo & Mack, 2014). Holdo and Mack (2014) further found that patterns of variation in bulk density clearly match soil moisture patterns on a granite parental material. It is suggested that native LMH have the potential to shift ecosystem limitations from moisture towards nutrients through decreasing the rate at which moisture drains from the upper soil layer after precipitation events (Holdo & Mack, 2014).

It is generally acknowledged that herbivores have the ability to enhance plant-available N through forage consumption, digestive breakdown and the subsequent excretion of easily accessible and degradable faeces and urine (Bardgett *et al.*, 1998; Sankaran & Augustine, 2004; Eldridge *et al.*, 2019). This fast cycle is typically referred to as the herbivore-decomposition pathway (Figure 1). Ungrazed plants passively return nutrients to the soil through the slow process of natural litter fall and subsequent detrital decomposition (Bardgett *et al.*, 1998; Knops *et al.*, 2002). Labile compounds originating from the passive return of plant litter are easily broken down and become readily accessible while most of the material forms part of the SOM compartment resulting in a slow turnover (Figure 1) (Bardgett *et al.*, 1998; Johnson & Matchett, 2001; Knops *et al.*, 2002; Bakker *et al.*, 2004). Herbivores are able to shortcut this slow process through mechanical and chemical degradation linked to hoof action, consumption and digestion in a relatively fast cycle (Figure 1) (Bardgett *et al.*, 1998; Bakker *et al.*, 2004). Excretions of LMH can directly influence patch-specific nutrient pools through returning readily accessible nutrients in the form of urine (i.e. N in inorganic form) and/or dung (i.e. high-quality detritus with low C:N ratios) enhancing N cycling (Bardgett *et al.*, 1998; Johnson & Matchett, 2001; Bakker *et al.*, 2004; Semmartin *et al.*, 2008; Holdo & Mack, 2014). In the short-term, excreta are expected to substantially increase nutrient patchiness at small spatial scales (Afzal & Adams, 1992; Lui *et al.*, 2016). This mechanism of nutrient enrichment has a substantial effect in areas with high herbivore population densities (Johnson & Matchett, 2001; Sankaran & Augustine, 2004; Jacobs & Naiman, 2008; Semmartin *et al.*, 2008; Holdo & Mack, 2014). However, the scale of N redistribution varies for different sized herbivores and

subsequent community composition (Bakker *et al.*, 2004). Large herbivores deposit faeces in large quantities in a few patches, while small herbivores produce smaller amounts of faeces in the form of small pellets that are widely distributed over an area (Bakker *et al.*, 2004). Small pellets are better suited for fast leaching and decomposition as they have a larger surface area, the incumbent nutrients are therefore more readily available to plants than the larger, patch-specific faecal deposits (Bakker *et al.*, 2004).

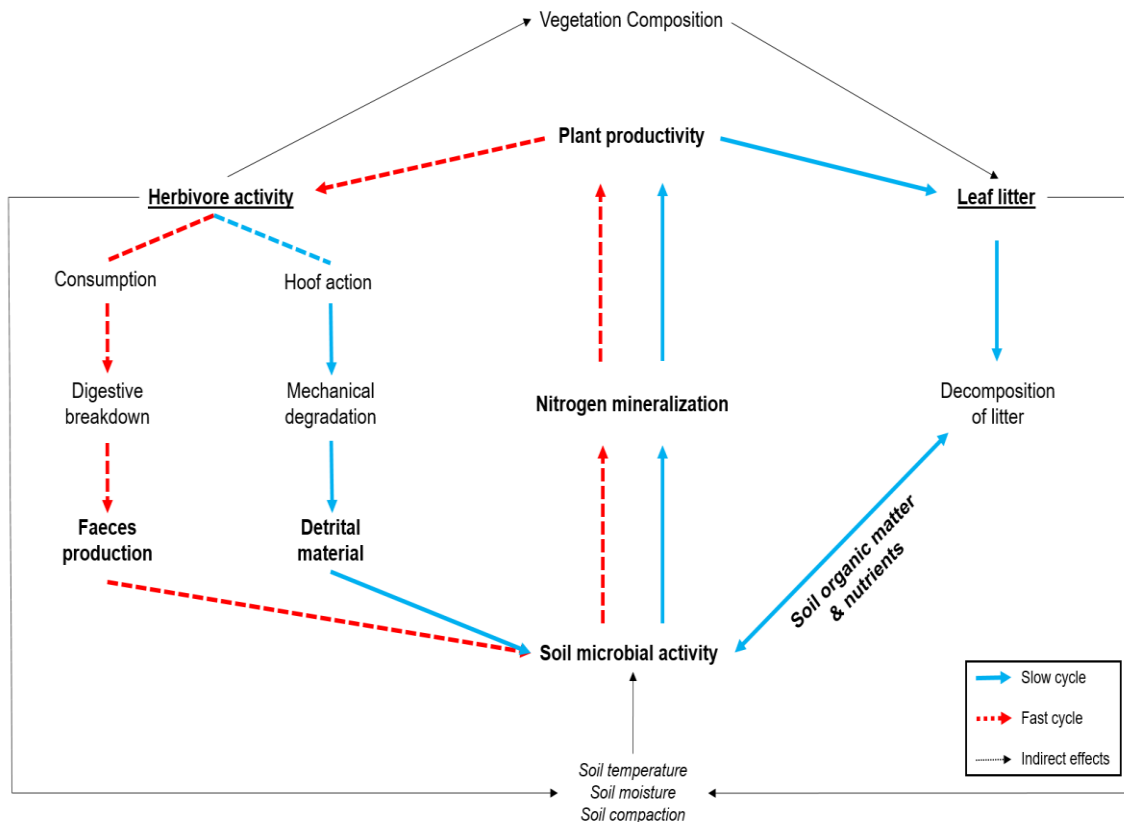


Figure 1: Diagrammatic representation of the influence of herbivory on terrestrial nutrient cycling (Adapted from Bardgett *et al.*, 1998).

2.2.3 Effect of herbivory on soil microbial community structure

Terrestrial ecosystems consist of distinct above- and belowground compartments, generally considered to be separate with limited interactions and are subsequently studied independently (Bardgett *et al.*, 1998). However, trophic cascades originating in one of the compartments have the potential to directly and/or indirectly affect the other through various top-down and bottom-up controls (Bardgett *et al.*, 1998; Bardgett & Wardle, 2003; Lui *et al.*, 2016; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021). It has been

suggested that indirect biotic and abiotic interactions between compartments may be more important in regulating community-level attributes than direct effects (Bardgett *et al.*, 1998).

Studies considering inter-compartmental interactions between aboveground herbivory and soil biota typically focus on direct effects based on detrital inputs to the soil associated with plant litter, faeces, urine and carrion (Bardgett *et al.*, 1993; Ruess & Seagle, 1994; Bardgett *et al.*, 1998). However, indirect effects such as plant responses to aboveground herbivory have been suggested to indirectly influence microbial communities and associated processes i.e. decomposition and nutrient cycling (Bardgett *et al.*, 1998; Eldridge *et al.*, 2019; Erasmus *et al.*, 2022). This effect predominantly arises from changes in detrital litter quality, including chemical composition as well as physiological changes related to altered patterns of C allocation and root exudation (Bardgett *et al.*, 1998; Bardgett & Wardle, 2003; Hart *et al.*, 2005; Crocker, 2015; Vandegehuchte *et al.*, 2015; Eldridge *et al.*, 2019; Tuomi *et al.*, 2019; Vermeire *et al.*, 2021). Herbivore-induced alterations to aboveground vegetation structure can either increase or decrease soil microbial biomass and diversity (Hart *et al.*, 2005; Pressler *et al.*, 2019). However, this depends on the extent of the disturbance and how it impacts dominant plants, ecosystem productivity and the association between plants and soil microorganisms (Vermeire *et al.*, 2021). Due to the symbiotic relationship between mycorrhiza and plant roots, the overall impact of herbivore-induced disturbances on vegetation and soil structure will be much greater for these communities (Hart *et al.*, 2005; Crocker, 2015). Aboveground herbivory has the potential to indirectly influence soil microbial community structure through alterations to several regulatory constituents, including vegetation structure (i.e. biomass production) and composition (i.e. community and chemical e.g. secondary metabolites and C:N ratios), input of non-detrital substrates (i.e. root exudates) and belowground root biomass and chemical composition (Bardgett *et al.*, 1998; Bardgett & Wardle, 2003; Liu *et al.*, 2016; Eldridge *et al.*, 2019; Pressler *et al.*, 2019; Tuomi *et al.*, 2019). These alterations substantially impact the physicochemical characteristics of soil, and can subsequently also influence the microorganisms which depend thereon (Pressler *et al.*, 2019).

Detrital plant material serves as the primary source of nutrients for soil microorganisms (Bardgett *et al.*, 1998; Pressler *et al.*, 2019). Through microbially mediated metabolic activity, this material is broken down and released into the soil as available nutrients (Figure 3) (Keuskamp *et al.*, 2013; Eldridge *et al.*, 2019; Petraglia *et al.*, 2019; Tuomi *et al.*, 2019). Wardle and Lavelle (1997) proposed three aspects of detrital plant material that collectively regulate soil microbial biomass and activity: 1) the ratio of carbon to other nutrients (C:N ratios are especially important), 2) quantity of inhibitory compounds (e.g. phenolics) and 3) quantity of recalcitrant compounds (e.g. cellulose, hemicellulose and lignin) in detrital litter. It is therefore expected that any herbivore-induced effects on detrital litter quality could potentially regulate

microbial community structure and associated processes and cycles i.e. decomposition and nutrient cycling (Bardgett *et al.*, 1998; Liu *et al.*, 2016; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021).

Aboveground herbivory can affect root carbon flow in two ways: 1) short-term alterations in the pattern of root exudation and 2) long-term changes in root biomass (Bardgett *et al.*, 1998). Available soil C is a major determinant of microbial biomass and activity (Bardgett *et al.*, 1998; Eldridge *et al.*, 2019). Variation within aforementioned C pools as a result of altered C allocation through indirect, herbivore-induced trophic cascades can potentially influence rhizosphere-based microbial community structure and associated processes (Bardgett *et al.*, 1998; Vandegehuchte *et al.*, 2015; Vermeire *et al.*, 2021). Photosynthetically derived C-based compounds stored belowground in root carbohydrate pools can be released in the rhizosphere as high-quality exudates (Bardgett *et al.*, 1998; Vandegehuchte *et al.*, 2015). These exudates serve as a key nutrient source for rhizosphere-based microbes (Bardgett *et al.*, 1998; Vandegehuchte *et al.*, 2015). Changes to the pattern and composition of exudates can have a substantial impact on microbial community structure and activity related to soil function (Bardgett *et al.*, 1998). Moreover, the quantity and quality of root exudates are affected by several factors including plant species, physiological state, age and prevailing environmental conditions (Holland *et al.*, 1996; Bardgett *et al.*, 1998).

Shand *et al.* (1994) reported that regular defoliation of grass swards had a negative effect on the concentration of both inorganic and organic P in the soil. This can generally be ascribed to plant responses to aboveground herbivory in which more C was allocated belowground resulting in increased root exudates and C availability, stimulating microbial activity and the subsequent immobilisation of P (Bardgett *et al.*, 1998). According to Holland *et al.* (1996), aboveground herbivore-induced disturbances i.e. grazing, promotes the short-term allocation of C assimilates in root tissue. This response action could protect C reserves against further loss i.e. herbivory (Bardgett *et al.*, 1998). The mechanism is thought to allow for the rapid mobilisation of stored C reserves for the purpose of aboveground regrowth (Holland *et al.*, 1996). Further studies showed that heavy grazing in grassland ecosystems resulted in soil microbial community composition dominated by bacteria, whereas lower levels of grazing favoured fungal populations (Figure 3) (Bardgett *et al.*, 1996). Vermeire *et al.* (2021) similarly reported that herbivore-based disturbances more negatively impacted fungal communities compared to bacterial communities. Subsequently, it has been suggested that heavy grazing intensity and associated heightened herbivore-induced disturbances with regards to defoliation, favours fast cycles dominated by bacteria and labile substrates (Figure 2) (Bardgett *et al.*, 1996). Conversely, light grazing and associated low disturbances, support

slower cycles which are dominated by fungi and recalcitrant compounds (Bardgett *et al.*, 1998).

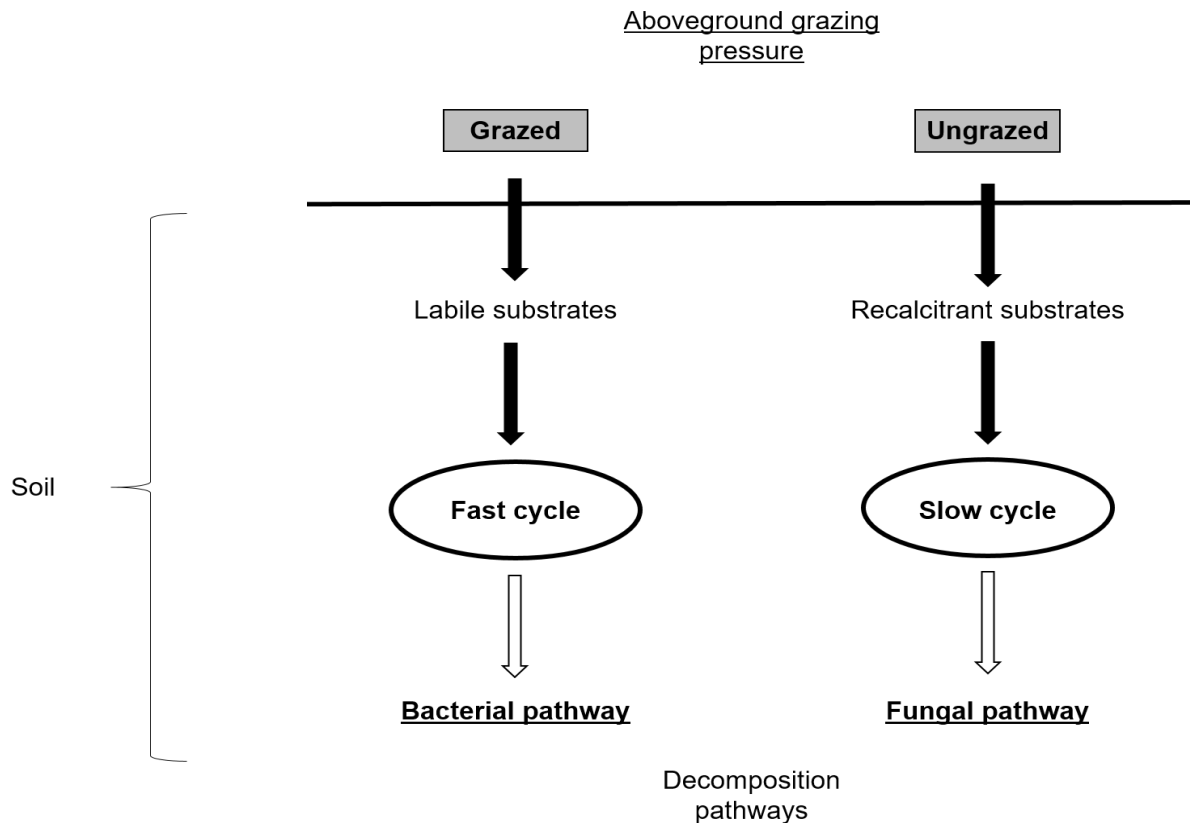


Figure 2: Effects of herbivore-induced disturbances (i.e. defoliation) and root exudation on decomposition pathways and associated microbial community structure (Adapted from Bardgett *et al.*, 1998).

Soil microorganisms rely on vegetation roots as an essential source of C (Bardgett *et al.*, 1998). However, several studies have reported on the ability of aboveground herbivory to decrease root biomass (McNaughton *et al.*, 1983; Ruess, 1988; Georgiadis *et al.*, 1989; Holland & Detling, 1990; Seagle *et al.*, 1992; Chaieb *et al.*, 1996). Wardle (1992) noted that short-term C input generally results in a rapid increase in microbial biomass. However, over longer periods Zak *et al.* (1994) found that increased net primary productivity (NPP) has an enhancing effect on microbial biomass. Conversely, Wardle *et al.* (1995) found that increased NPP can result in reductions of microbial biomass. These contradictory findings are thought to be driven by the collective effects of vegetation and soil heterogeneity on microbial communities (Van Veen *et al.*, 1989; Bardgett *et al.*, 1998; Liu *et al.*, 2016; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). Vegetation can promote microbial biomass through contributing towards nutrient availability (i.e. detrital plant litter and root exudates) and preferred environmental conditions (i.e. large rhizosphere, soil moisture etc.). However, vegetation can

also impede microbial biomass by actively competing with soil microbes for available nutrients (Van Veen *et al.*, 1989; Liu *et al.*, 2016). According to Holland and Detling (1990), long-term, sustained heavy grazing can potentially decrease belowground C allocation, resulting in reduced decomposable root C and higher levels of N mineralisation.

LMH grazing is a major determinant of soil spatial heterogeneity (Augustine & Frank, 2001; Lin *et al.* 2010; Eldridge *et al.*, 2019). However, the extent of herbivore-induced effects can vary substantially with herbivore community composition, population density, grazing intensity and spatial scales (Augustine & Frank, 2001; Lin *et al.* 2010). In undisturbed savanna ecosystems, multiple LMH species can coexist (Jacobs & Naiman, 2008). Through several direct and indirect mechanisms (e.g. selective feeding), these rich herbivore assemblages can dramatically regulate soil heterogeneity (Eldridge *et al.*, 2019). In the long-term, changes to the pattern of litter distribution, quantity and quality, root exudates and adsorption can result in substantial modification of soil properties, subsequently influencing microbial community structure (Crocker, 2015). Herbivores can directly influence soil properties and microbial communities through soil nutrient enrichment (i.e. deposits of faeces and urine), concentrating nutrients into nutrient-enriched patches (Bardgett *et al.*, 1998; Orwin *et al.*, 2009; Liu *et al.*, 2016; Eldridge *et al.*, 2019). Moreover, the effect of herbivory on vegetation structure can have a substantial impact on soil microbial community structure through altering certain abiotic soil factors which control microbial density and activity (Bardgett *et al.*, 1998; Liu *et al.*, 2016; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021).

Soil microbial communities consist of varying proportions of fungi and bacteria which have substantially different requirements with regards to habitat and resources within soil (Chenu *et al.*, 2002; Gadd, 2007). Fungi are classified as being obligate aerobes and heterotrophs, predominantly involved in the breakdown of detrital material i.e. decomposition (Antunes & Koyama, 2017). Fungi are mainly found within superficial soil horizons where they are typically associated with the roots of plants (Antunes & Koyama, 2017). Heterotrophic fungi rely on plants for nutrients, with different types of fungi relying on different aspects of plants. Saprotrophic fungi rely on detrital material, symbiotrophic fungi on plant metabolites and pathotrophs on the plant itself. Changes within plant host populations will subsequently have a dramatic impact on fungal biomass and activity (Antunes & Koyama, 2017). This is especially true for mycorrhizal fungi as they form close associations with vegetation (Antunes & Koyama, 2017).

Bacteria have a more versatile metabolism which allows them to survive under various environmental conditions ranging from oxic to anoxic (Vermeire *et al.*, 2021). They are generally heterotrophic, predominantly relying on labile substrates but can also be autotrophic

(Paul, 2014). On a global scale, edaphic properties have been identified as a major determinant of bacterial community composition (Kaiser *et al.*, 2016; Bahram *et al.*, 2018). On the other hand, fungi tend to follow biogeographic patterns, similar to that of plants and are generally influenced more by climatic factors (Bahram *et al.*, 2018). Vermeire *et al.* (2021) gave evidence that herbivore-induced disturbances can alter bacterial community structure through changes in edaphic properties, while fungal communities are affected by alterations to vegetation structure. It was found that aboveground disturbances had a greater impact on fungal communities than on bacterial communities. This can predominantly be ascribed to the lower tolerance of fungi towards certain environmental conditions (e.g. higher soil temperatures and reduced A-horizon due to erosion), often induced by herbivore activity (Baldrian *et al.*, 2012; Eldridge *et al.*, 2019; Pressler *et al.*, 2019; Vermeire *et al.*, 2021).

Microbes have developed numerous strategies to survive varying environmental conditions (Andrews & Harris, 1986). Such adaptations include that of r- and K-strategists that pertain to reproduction, growth and nutrient acquisition (Andrews & Harris, 1986). Species that are r-selected, capitalise on temporary nutrient sources through high growth rates and their ability to rapidly reproduce and colonise unexploited resources (Andrews & Harris, 1986). However, r-strategists are less specialised and are unable to compete for resources at high population densities (Andrews & Harris, 1986). On the contrary, K-strategists have developed to be efficient competitors at high population densities and in nutrient-poor environments (Andrews & Harris, 1986). The r-strategists are often abundant in early successional stages when labile resources are readily available, while K-strategists tend to thrive in the latter successional stages when recalcitrant material dominates (Andrews & Harris, 1986).

Herbivory affects soil microbial communities through several direct and indirect mechanisms. These effects can have a pulse nature -the short-term result of non-detrital inputs such as root exudates, dung and urine. Other effects may be long-lasting resulting from changes in the pattern, quantity and quality of detrital plant material, alterations in belowground biomass and changes in vegetation structure (Figure 3) (Bardgett *et al.*, 1998; Liu *et al.*, 2016; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). Through these mechanisms aboveground herbivory can potentially have either a negative or positive effect on soil microbial community structure. It is suggested that productive ecosystems which are capable of supporting congregated LMH activity, promote a positive nutrient feedback loop whereby labile nutrients are rapidly returned to the soil, subsequently resulting in increased soil microbial activity (Figure 3) (Vermeire *et al.*, 2021). However, due to the large spatial variation in terrestrial ecosystems, it is difficult to predict the exact manner in which herbivory will affect soil microbial communities and associated ecological processes. Effects within dynamic African savannas are especially difficult to predict as a result of the complex interactions between numerous top-down and

bottom-up controls that collectively regulate these systems (Erasmus *et al.*, 2021; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021).

2.2.4 Effects of herbivory on soil function

Decomposition is fundamental to the effective functioning of terrestrial ecosystems as it forms the link between above- and belowground nutrient cycling (Figure 3) (Eldridge *et al.*, 2019; Petraglia *et al.*, 2019). It is especially important in nutrient-limited systems where it is responsible for concentrating valuable nutrients, contributing towards a patchy structure. Moreover, decomposition is a key constituent of soil respiration and subsequent carbon flux and hence, a major role player in both local and global climate regulation (Wang & D'Odorico, 2008; Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Eldridge *et al.*, 2019).

Decomposition can essentially be described as the accretion of metabolically derived biochemical reactions, resulting in the degradation of complex organic compounds into constituent organic and inorganic components (Wang & D'Odorico, 2008; Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Eldridge *et al.*, 2019; Petraglia *et al.*, 2019). Soil-based decomposition of plant material encompasses numerous regulatory factors and a complex network of actors functioning at different scales across various pathways (Wang & D'Odorico, 2008; Eldridge *et al.*, 2019). Fundamentally, decomposition is mediated by soil microorganisms and subsequently subjected to similar regulatory constituents. This primarily includes climatic and edaphic variations, together with numerous site-specific factors such as vegetation structure which influences detrital quantity, quality and distribution as well as soil properties, and agents of disturbance (e.g. LMH) (Schrama *et al.*, 2013; Eldridge *et al.*, 2019; Ochoa-Hueso *et al.*, 2019). It is important to note that these factors do not occur in isolation, therefore they are capable of influencing or being influenced by various prevailing environmental factors. As such, decomposition and inherent soil respiration (i.e. carbon flux potential) is a function of both climatic and structural factors and subsequently the disturbance thereof (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Ochoa-Hueso *et al.*, 2019; Petraglia *et al.*, 2019). Decomposition-based processes are often assisted by detritivorous activity (e.g. termites), predominantly associated with subtropical African savannas (Leitner *et al.*, 2018; Sundsdal *et al.*, 2020; Teo *et al.*, 2020; Erasmus *et al.*, 2021).

The process of decomposition is responsible for three essential ecosystem functions, namely mineralisation, formation of SOM and soil-CO₂ efflux (Swift *et al.*, 1979; Badalucco & Kuikman, 2000; Schlesinger & Andrews, 2000; Jassal *et al.*, 2007; Wang & D'Odorico, 2008). The level

to which these functions are carried out can to a large extent regulate the structural and functional characteristics of an ecosystem (Swift *et al.*, 1979; Paudel *et al.*, 2015).

Mineralisation refers to the conversion of inaccessible organic-bound nutrients originating from the above- and belowground detrital pool to an accessible inorganic form (Wang & D'Odorico, 2008). This contributes towards the recycling of finite nutrients within the local as well as global system (Coûteaux *et al.*, 1995; Badalucco & Kuikman, 2000; Wang & D'Odorico, 2008). Partial decomposition and subsequent transformation of detrital material through microbial metabolic activity is responsible for the formation of SOM (Cotrufo *et al.*, 2015). The long-term C balance of an ecosystem is dependent on the proportion of detrital input that is incorporated and stabilised into SOM rather than being mineralised and consequently circulated (Wang & D'Odorico, 2008; Cotrufo *et al.*, 2015). SOM is an essential and dynamic C reservoir with regards to the global C cycle, storing more C than both vegetation and the atmosphere combined (Davidson & Janssens, 2006; Wang & D'Odorico, 2008).

During the process of decomposition, metabolic-derived CO₂ is released into the atmosphere as soil-based decomposer organisms respire together with vegetation roots and micro-fauna, also referred to as soil respiration (Schlesinger & Andrews, 2000; Jassal *et al.*, 2007; Wang & D'Odorico, 2008). Furthermore, soil-CO₂ efflux contributes to the global C cycle and atmospheric CO₂ concentration which profoundly affect climatic conditions (Raich & Schlesinger, 1992; Davidson & Janssens, 2006; Jassal *et al.*, 2007; Wang & D'Odorico, 2008). Decomposition is central in regulating ecosystem structure and function as it is the principal pathway and a major determinant of nutrient cycling and the transfer of above-ground C (Swift *et al.*, 1979; Moretto *et al.*, 2001; Chapin *et al.*, 2002; Wang & D'Odorico, 2008; Paudel *et al.*, 2015; Didion *et al.*, 2016; Ochoa-Hueso *et al.*, 2019).

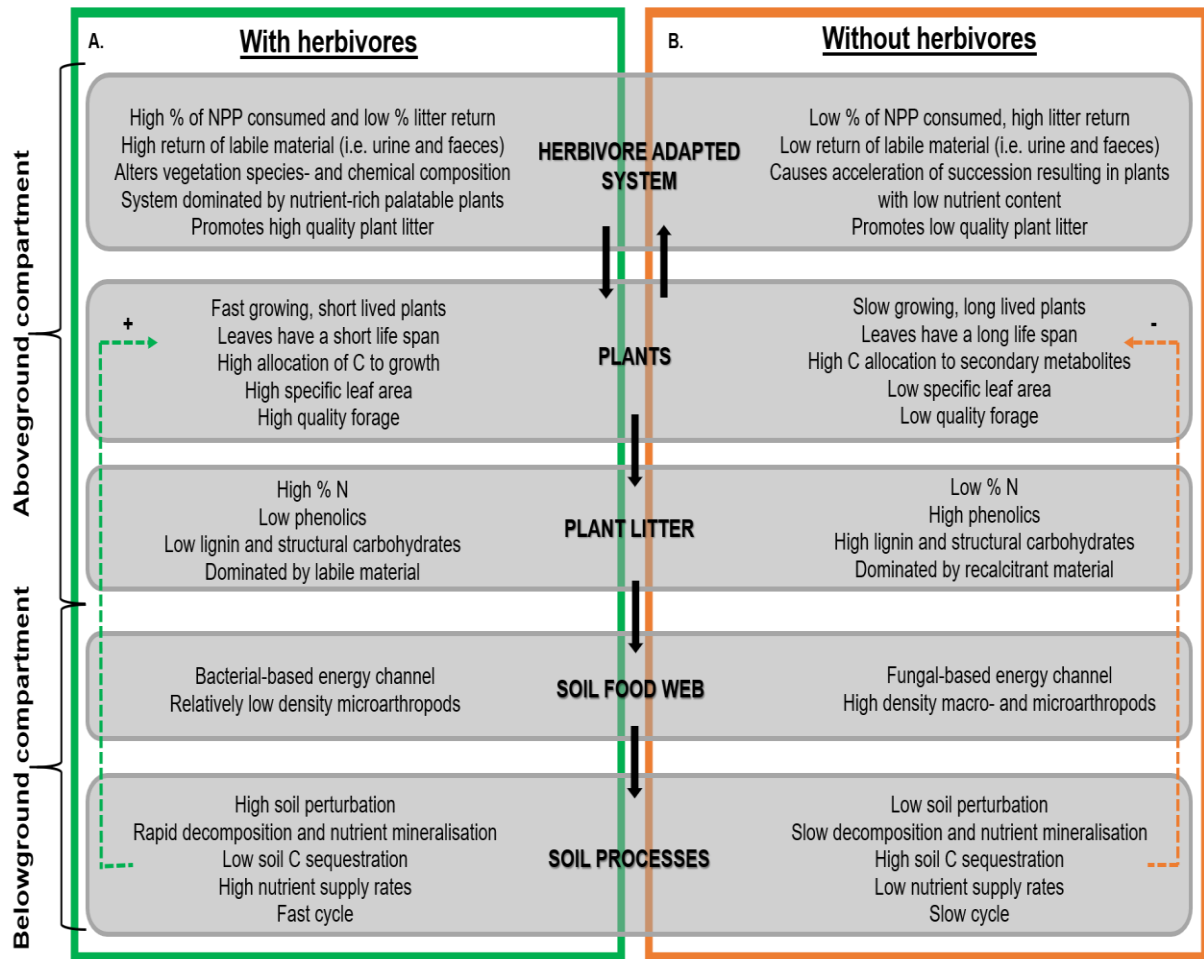


Figure 3: Above- and belowground systems are fundamentally linked through decomposition. This link between belowground and aboveground compartments produces a positive feedback response (dotted line) to the plant community under herbivore pressure (A) and a negative feedback in their absence (B) (Adapted from Wardle *et al.*, 2004).

2.2.5 Quantification of decomposition

While governed by various environmental factors, decomposition is primarily regulated by the chemical composition of site-specific leaf litter (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Elumeeva *et al.*, 2018). Detrital material rich in labile compounds tend to favour decomposition, whilst materials with high C:N ratios and high lignin content impedes microbially mediated breakdown (Keuskamp *et al.*, 2013).

Cross-site comparison of natural litter decomposition and intrinsic drivers thereof is subsequently compromised by variability in detrital chemistry (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Elumeeva *et al.*, 2018; Leitner *et al.*, 2018; MacDonald *et al.*, 2018; Petraglia *et al.*, 2019; Teo *et al.*, 2020; Erasmus *et al.*, 2021).

Attempts to examine the role of environmental drivers on decomposition of detrital plant material have previously depended on time and labour-intensive methods such as the use of cotton strips or natural leaf litter mixtures which fail to provide high resolution data required for global-scale modelling and comparison (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Becker & Kuzyakov, 2017; MacDonald *et al.*, 2018; Teo *et al.*, 2020). Developed as a citizen-science drive in the Northern hemisphere, Keuskamp *et al.* (2013) provided an easily applicable method to identify and quantify the effect of environmental drivers on decomposition using a single sampling time, namely the TBI approach. This approach is an internationally standardised, user-friendly method to collect comparable, globally distributed decomposition data. Standardised test kits include commercially available tetrahedron-shaped non-woven polypropylene tea bags (i.e. Lipton® rooibos and green tea) -initially woven nylon mesh tea bags were used but are no longer produced (Keuskamp *et al.*, 2013). Tea bags were specifically selected based upon differences in chemical composition (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Elumeeva *et al.*, 2018; MacDonald *et al.*, 2018). Green tea represents high quality organic matter (i.e. low C:N ratios) while rooibos tea represents low quality organic matter (i.e. high C:N ratios) (Keuskamp *et al.*, 2013). Tea bags therefore act as proxies for labile and recalcitrant compounds in natural occurring organic matter (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Elumeeva *et al.*, 2018; MacDonald *et al.*, 2018). Using standardised organic litter (i.e. tea leaves) enables the TBI to identify and examine environmental drivers of decomposition without the ambiguous effects of site- and species-specific litter –with reference to litter bag studies (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Becker & Kuzyakov, 2017; Elumeeva *et al.*, 2017). The TBI therefore serves as a reference which facilitates data comparison across spatial scales (Didion *et al.*, 2016; Becker & Kuzyakov, 2017). The TBI consists of two parameters describing (1) decomposition rate (k –rate at which detrital plant material is lost) and (2) stabilisation factor (S –sequestration of detrital plant material) (Keuskamp *et al.*, 2013). Both k and S are determined by using built-in formulas (Equation 1 and 2) provided by Keuskamp *et al.* (2013) in the TBI 2.0 NW data sheet for non-woven tea bags (refer to <http://www.teatime4science.org/>).

k is calculated from mass loss of tea material (W) after incubation time (t), assuming double-exponential decomposition due to faster decomposition of the hydrolysable fraction (a) and relative increase of the recalcitrant fraction ($1-a$) over time (Equation 1).

$$W(t) = ae^{-kt} + (1-a) \quad [\text{Eqn 1}]$$

During decomposition, components of labile compounds stabilise and become more recalcitrant. The stabilisation of detrital plant material depends on environmental factors and results in a deviation of the actual decomposed fraction (i.e. limit value $-a$) from the

hydrolysable fraction (i.e. chemically labile –H). S can therefore be interpreted as the inhibiting effect of environmental factors on decomposition of the labile fraction with a_g referring to the decomposed fraction and H_g the hydrolysable fraction of green tea (Equation 2).

$$S = 1 - a_g/H_g \quad [\text{Eqn 2}]$$

The use of tea bags as standardised test kits presents numerous advantages, none more valuable than cross-site comparison of decomposition and the associated implications for carbon flux management (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Pouyat *et al.*, 2017; Petraglia *et al.*, 2019; Erasmus *et al.*, 2021; Blume-werry *et al.*, 2021). However, recent studies have suggested that the application of the original TBI can lead to erroneous conclusions regarding drivers of decomposition between sites (Pouyat *et al.*, 2017; Seelen *et al.*, 2019). The reasoning for this is due to the labile nature of tea litter with regards to the high amount of water-soluble compounds (Pouyat *et al.*, 2017; Seelen *et al.*, 2019; Blume-werry *et al.*, 2021). As such, tea litter i.e. tea bags are thought to leach faster and subsequently lose much more weight compared to natural, site-specific plant material (Pouyat *et al.*, 2017; Seelen *et al.*, 2019). The affinity of tea bags towards leaching is thought to be exacerbated in soils with a high moisture content (Blume-werry *et al.*, 2021). Variation in decomposition rates between systems that are predominantly driven by precipitation and soil moisture will subsequently only be observable in plant material which is susceptible to high leaching (Blume-werry *et al.*, 2021).

To account for this potential flaw, Pouyat *et al.* (2017) and Seelen *et al.* (2019) suggested that tea bags should be soaked and thus leached prior to incubation. However, according to Blume-werry *et al.* (2021) there has been no studies which tested whether standardised tea litter (green and rooibos tea) leach and subsequently lose more weight than other natural plant litter. Moreover, it has not been tested whether these potential differences in leaching susceptibility could cloud the results of decomposition studies under varying moisture regimes. Including leaching as a step to the TBI approach would substantially decrease comparability between studies.

The study by Blume-werry *et al.* (2021) also found that pre-leached tea bags lost less mass after incubation in comparison to standard (non-leached) tea bags as water-soluble compounds were already leached/lost. Moreover, differences between pre-leached and standard tea bags were similar in moist and dry soils. It was suggested that the pre-treatment step of leaching is not necessary for accurate comparison between sites. It is, however, important to note that the majority of tea bag mass loss generally observed over short periods (e.g. generally accepted three-month period for global comparison) can be ascribed to natural

leaching processes rather than microbial decomposition (Pouyat *et al.*, 2017; Elumeeva *et al.* 2018; MacDonald *et al.* 2018; Petraglia *et al.* 2019; Blume-werry *et al.*, 2021; Erasmus *et al.*, 2021). Yet, other plant material also experiences leaching of naturally occurring water-soluble compounds as part of normal decomposition processes (Blume-werry *et al.*, 2021). Tea bags are subsequently able to act as suitable proxies for natural litter (Keuskamp *et al.*, 2013). The affinity of tea bags towards leaching therefore does not pose the major problem it was initially thought to (Blume-werry *et al.*, 2021). Furthermore, due to temporal changes affecting decomposition i.e. variation in rate and extent due to changes in detrital substrate composition it is suggested that decomposition studies, in particular the TBI, can be adapted to include longer incubation periods to elucidate decomposition dynamics as noted by Erasmus *et al.* (2021).

2.3 Conclusions

African savannas are under severe pressures arising from changing land-use practices and unsustainable management strategies resulting in dramatic shifts within aboveground agents of disturbance such as LMH communities. Herbivory has the ability to directly and indirectly influence soil function (i.e. decomposition) through various reinforcing feedback responses and trophic cascades. Alteration to the rate and extent of decomposition may potentially have a substantial impact on ecosystem structure and subsequent functioning. It is therefore essential to develop management strategies that focus on both top-down drivers and bottom-up controls of savanna ecosystems as a means to effectively manage these complex socio-ecological systems. This is especially true for dynamic and ecologically sensitive systems such as sodic patches which are in general misunderstood due to their denuded appearance.

This review indicates the various mechanisms by which herbivore-induced disturbances can affect above- and belowground heterogeneity and associated soil function. It further suggests the importance of long-term monitoring programs focussing on aboveground disturbances and the associated direct and indirect trophic cascades that impact soil function.

Chapter 3

Study Area

3.1 Locality and description

The study was conducted in the Kruger National Park, South Africa, which is located within the Savanna Biome of South Africa. A semi-arid savanna ecosystem at the Nkuhlu long-term experimental monitoring site ($24^{\circ}58'S$, $31^{\circ}46'E$) was selected to address the research questions related to this study. The Nkuhlu experimental site is situated on the northern bank of the perennial Sabie River, approximately 18 km downstream from Skukuza in the southern section of the KNP, South Africa (Siebert & Eckhardt, 2008) (Figure 4).

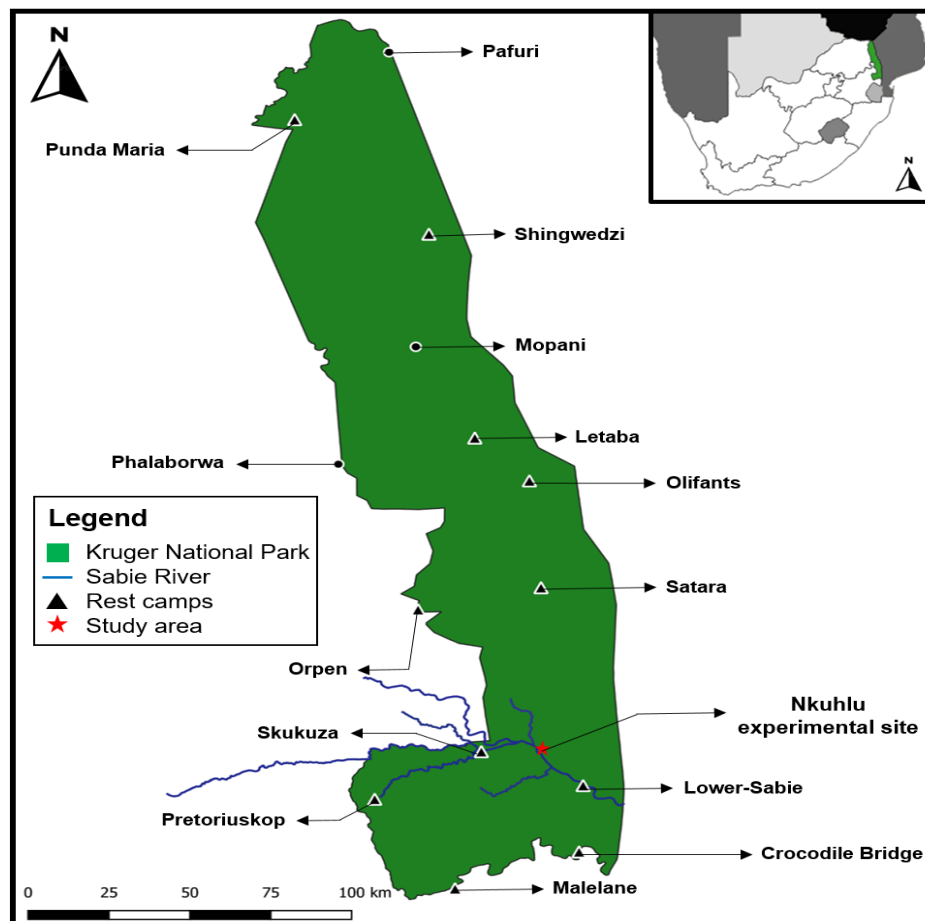


Figure 4: Location of rest camps and river systems within the KNP relative to the study area (i.e. Nkuhlu experimental site) as well as a key map of southern Africa indicating the positioning of the KNP.

The Nkuhlu experimental site (Figure 5), also referred to as the 'Nkuhlu exclosures' was constructed in 2001 through a collaborative effort by South African National Parks (SANParks) and a team of international scientists (Siebert & Eckhardt, 2008). This project formed part of a large-scale, long-term exclusion experiment based on the removal of certain ecosystem modifiers (e.g. herbivory and fire) in an attempt to determine their effect on spatial and temporal heterogeneity patterns of herbaceous and woody vegetation in a semi-arid, southern African savanna (O'Keefe & Alard, 2002).

The Nkuhlu exclosures encompass an area of 139 ha and is divided into three smaller units, each subjected to a different treatment of fire and herbivory (O'Keefe & Alard, 2002). Detailed description of the experimental design is provided in Chapter 4, Experimental- and sampling design.



Figure 5: Layout of the Nkuhlu experimental site and its location relative to the Sabie River, tourist roads and the Nkuhlu picnic site in the Kruger National Park.

3.2 Climate

This semi-arid subtropical African savanna falls within the Lowveld bushveld zone (south and central KNP) and is primarily characterised by two distinct seasons, i.e. a hot growing season with sporadic precipitation from October to April, and a mild to warm, dry non-growing season (Mucina & Rutherford, 2006; Williams *et al.*, 2009).

Long-term climatic records indicate that this region is prone to cycles of excessive wet- and dry periods (Venter *et al.*, 2003; Jacobs & Naiman, 2008). Occasional tropical cyclones, originating in the Mozambique Channel have the potential to deliver up to half of the annual mean rainfall within a short period of time (Jacobs & Naiman, 2008). Such events can last for several days, which may cause extensive flooding. The last century-scale flood occurred in 2000, altering much of the active river channel (Jacobs & Naiman, 2008). Climatic variation after the flood resulted in a major dry period (2002) in which only 51% of the annual mean rainfall fell (Jacobs & Naiman, 2008). Climatic projections indicate that the climate of the KNP will become significantly warmer (2–6 °C) together with altered rainfall patterns during the twenty-first century, mainly due to anthropogenic-induced disturbances (Venter *et al.*, 2003; Mucina & Rutherford, 2006). The recent drought of 2015-2017 represents a prime example of projected climatic variations (Smith & Fitchett, 2020).

3.2.1 Rainfall

Rainfall within the study area (Figure 6) varies from 500–700 mm per year with a maximum evapotranspiration of 6 mm per day measured during the hot, growing season in October (Grant & Scholes, 2006; Williams *et al.*, 2009). Annual mean rainfall is 561 mm as measured at the Skukuza Meteorological Station, approximately 30 km west of the Nkuhlu exclosures (Siebert & Eckhardt, 2008). The majority of annual rainfall falls in the wet season as thunderstorms of short duration, i.e. lasting from a few minutes to a few hours (Venter *et al.*, 2003). Short bursts of high intensity rainfall often result in soil erosion, especially on sparsely vegetated, unprotected soil (Venter *et al.*, 2003; Khomo & Rogers, 2005; Grant & Scholes, 2006).

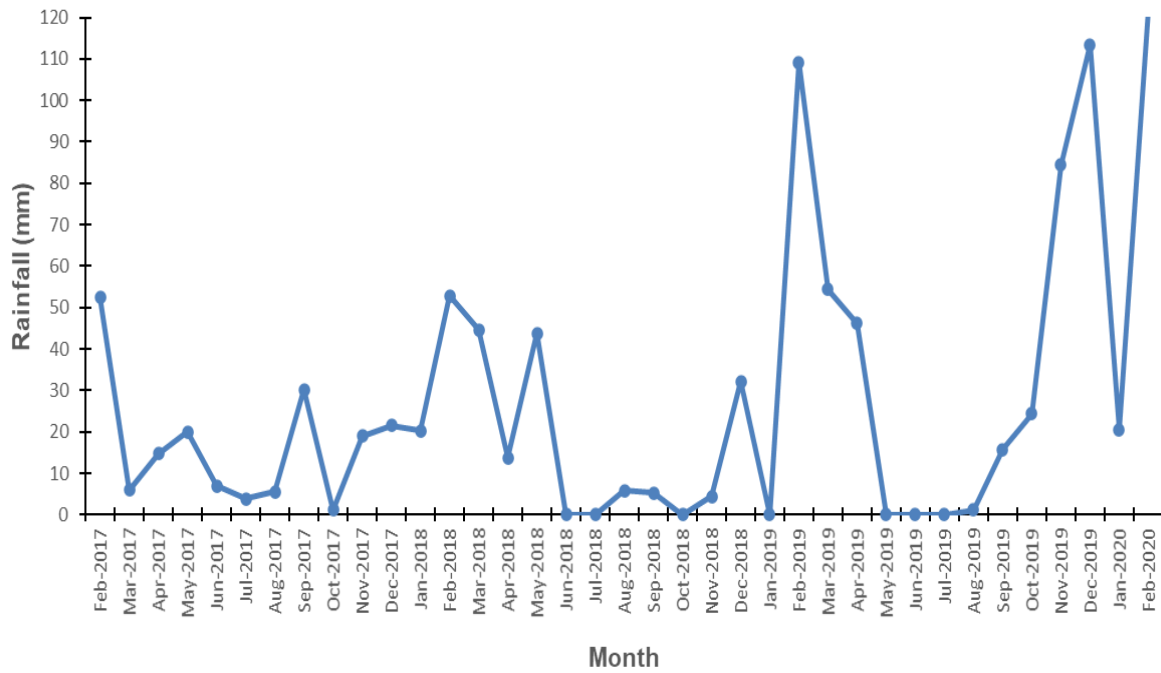


Figure 6: Actual monthly rainfall (mm) from February 2017 to February 2020 as recorded at the Skukuza Meteorological Station (Data courtesy of the South African Weather Service).

3.2.2 Temperature

Temperature patterns for the study area vary from hot summers (October–April) to mild, generally frost-free winters (May–September) (Venter *et al.*, 2003). The average daily temperature in Skukuza is 21.9 °C with daily averages ranging from 15.7 °C in June to 26.6 °C in January (Scogings *et al.*, 2012). Seasonal temperatures range from an average minimum of 5.6 °C in winter to an average maximum of 32.6 °C in summer (Venter *et al.*, 2003). High average temperatures and potential evapotranspiration can predominantly be ascribed to high solar radiation recorded in the KNP, typical of low elevation systems (Venter *et al.*, 2003; Becker & Kuzyakov, 2018).



Figure 7: Actual mean monthly minimum, maximum and average temperatures (°C) from February 2017 to February 2020 as recorded at the Skukuza Meteorological Station (Data courtesy of the South African Weather Service).

3.3 Geology

The geology of the study area consists of foliated metamorphic rock known as granitic gneiss (Figure 6), typically characterised by compositional banding, comprising of bands of feldspar and/or quartz grains within an interlocking texture (Venter, 1990; Venter *et al.*, 2003; Grant & Scholes, 2006). Observed layers or bands within gneissic granite are the result of different mineral depositions with varying compositions (Venter, 1990; Venter *et al.*, 2003). Compositional banding is not always detectable within gneissic granite as mineral depositions often appear as random clumps within the substrate (Venter, 1990; Venter *et al.*, 2003).

Weathering of gneissic granite is generally associated with the formation of greyish- to reddish-brown soil with varying physicochemical properties (Venter, 1990; Venter *et al.*, 2003; Mucina & Rutherford, 2006). Archaean granite and gneiss in the study area resulted in the formation of sandy soil in the uplands and clayey soil with a disproportionately high sodium content in the lowlands (the 'sodic zone'), with specific reference to the footslopes of the catena (Venter *et al.*, 2003; Mucina & Rutherford, 2006; Siebert & Eckhardt, 2008).

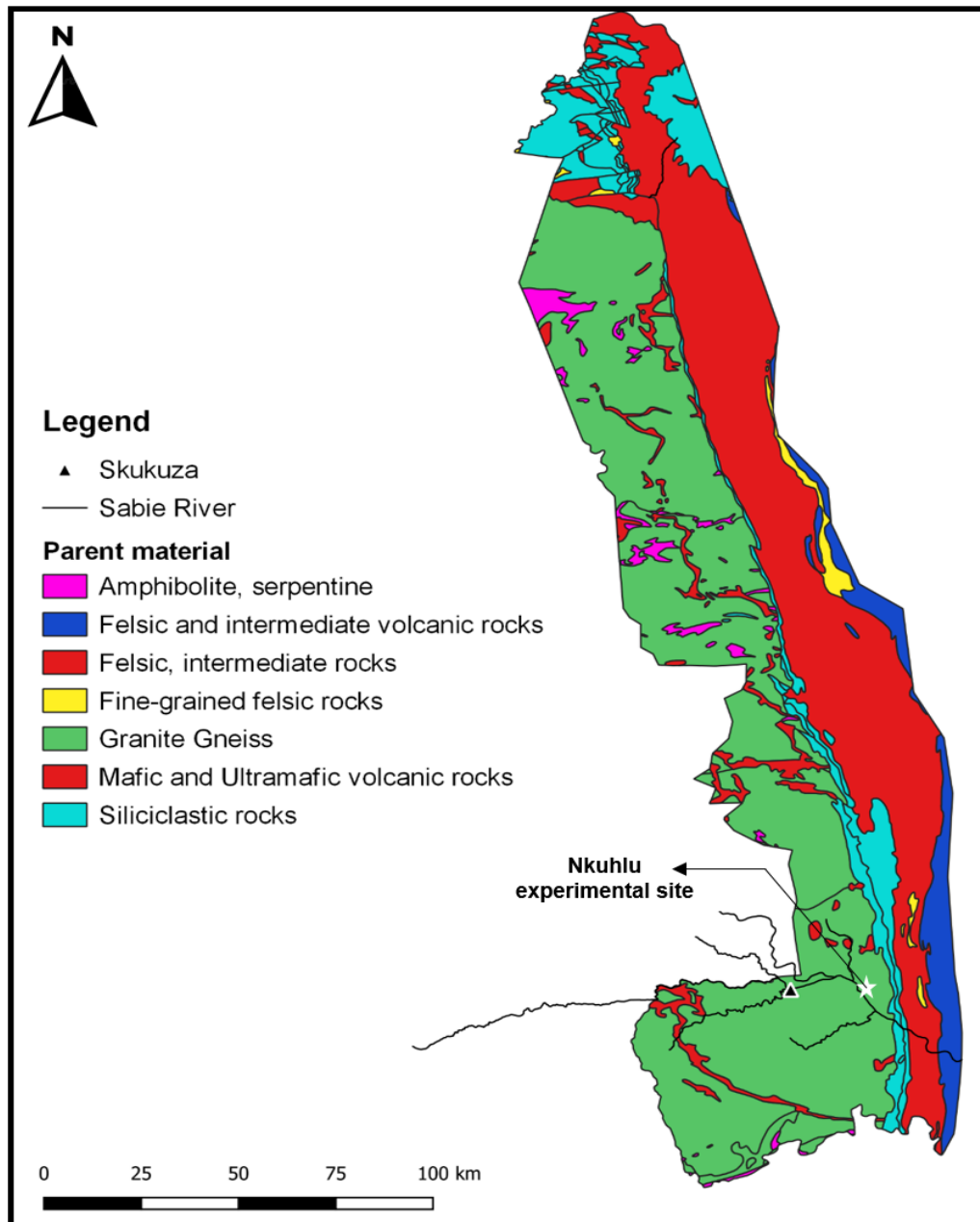


Figure 8: Geological map of the KNP indicating the location of the Nkuhlu experimental site in relation to the different geological formations.

3.4 Topography

The Nkuhlu exclosures are situated within a heterogeneous, slightly undulating granitic catena at an altitude ranging between 200 and 230 m above sea level (Venter *et al.*, 2003; Alard, 2009). Typical of a granite-derived landscape, these exclosures include a complete sequence of terrain morphology ranging from crests to footslopes (Venter *et al.*, 2003; Alard, 2009) (Figure 9).

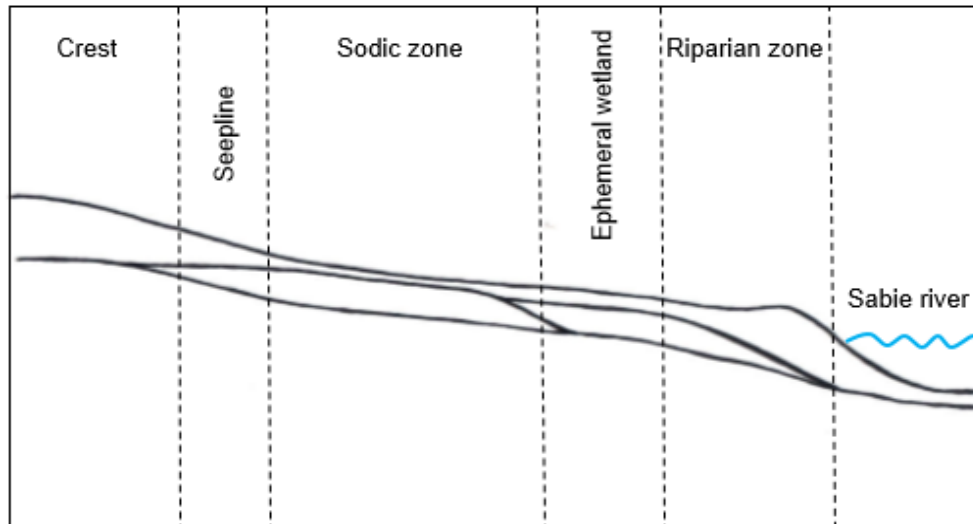


Figure 9: Schematic representation of the granitic catena present within the Nkuhlu exclosures indicating the varying terrain morphology (Adapted from Alard, 2009).

Soils vary from shallow, coarse sandy soil on the crests to deep, sandy loam on the footslopes and along the Sabie River (Siebert & Eckhardt, 2008), having a general composition of approximately 65% sand, 30% clay, and 5% silt (Williams *et al.*, 2009). Soils overlay weathering granite which out-crops in certain places (Williams *et al.*, 2009). The study site, located within the exclosures is situated beneath the seep-line, on the footslopes of the catena (Figure 9) and is characterised by sodium-rich, deep, duplex soil referred to as the sodic zone (Figure 10) (Venter *et al.*, 2003; Khomo & Rogers, 2005; Grant & Scholes, 2006; Siebert & Eckhardt, 2008).

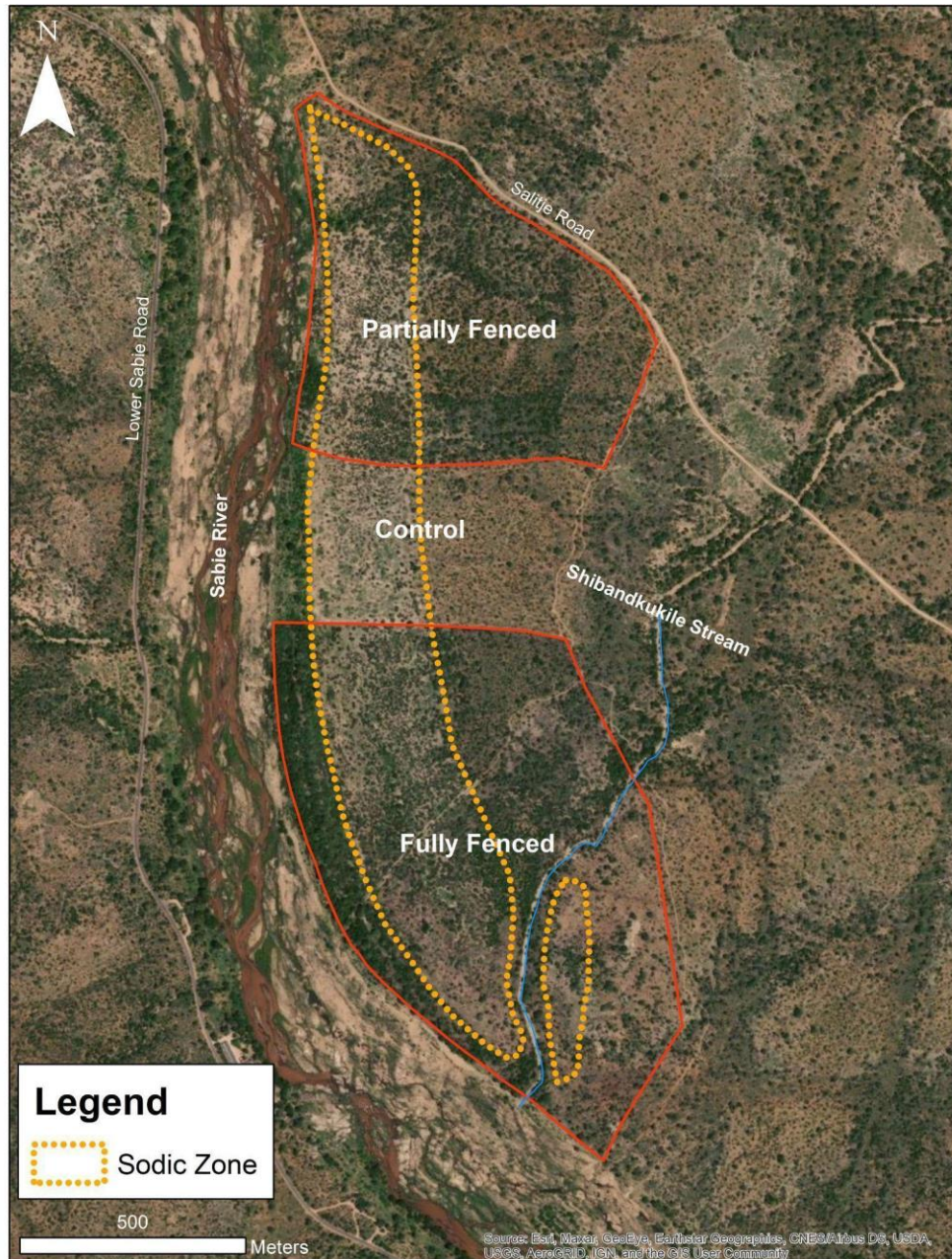


Figure 10: Aerial view of the Nkuhlu experimental site indicating the distribution of the sodic zone within the exclosures.

3.5 Soil

Sodium-rich, deep, duplex soil on the footslopes is typical of granite-derived, semi-arid landscapes (Venter *et al.*, 2003; Grant & Scholes, 2006; Siebert & Eckhardt, 2008). These soils tend to be sparsely vegetated with herbaceous cover interspersed by extensive bare patches with significant crusting (Venter *et al.*, 2003; Khomo & Rogers, 2005; Alard, 2009).

Sodic soil contains dispersed clay particles together with a high concentration of available nutrients including nitrogen (N), phosphorus (P) and sodium (Na) (Qadir & Schubert, 2002;

Khomo & Rogers, 2005; Grant & Scholes, 2006; Alard, 2009). The duplex structure of sodic soil comprises of two distinct layers, namely (i) a coarsely textured sandy loam A-horizon (< 15 cm) which is capable of supporting a variety of low trophic level organisms (soil microbes, detritivores and plants), overlying a (ii) compacted B-horizon of almost impervious calcium-rich heavy clay which often contributes towards vegetation mortality and limiting rooting of woody species (Qadir & Schubert, 2002; Khomo & Rogers, 2005; Grant & Scholes, 2006).

Sodic soil is characterised by a disproportionately high concentration of sodium (i.e. sodium adsorption ratio above 13) in the cation exchange complex (CEC) originating from sodium releasing parent material such as granite (Qadir & Schubert, 2002; Khomo & Rogers, 2005; Grant & Scholes, 2006). Sodic soil is further characterised by a pH greater than 8.5 and electrical conductivity (EC) of < 4.0 dS/m. The accumulation of sodium can potentially displace various essential elemental cations (e.g. magnesium, calcium (Ca) and potassium, resulting in stronger competition between soil microbes and plants for available nutrients (Qadir & Schubert, 2002; Khomo & Rogers, 2005; Kaspari *et al.*, 2014).

Hyper-accumulation of exchangeable sodium often results in poor physical soil conditions that are typically associated with sodic soil (Qadir & Schubert, 2002; Khomo & Rogers, 2005; Alard, 2009). In soils with a high sodicity, clay particles tend to expand when water is applied, causing dispersion of these particles which extensively weakens soil aggregation, resulting in structural failure and closing-off of soil pores (Qadir & Schubert, 2002; Venter *et al.*, 2003; Khomo & Rogers, 2005). This property of sodic soil restricts the movement of water and gasses through the soil (hydraulic conductivity), having an adverse effect on soil-based diffusion of water and nutrients as well as the osmotic capability of microbial organisms (Qadir & Schubert, 2002; Khomo & Rogers, 2005; Eldridge *et al.*, 2019). The closing of soil pores can affect soil moisture of sodic soil in two fundamental ways: 1) a slow infiltration rate leading to soil moisture content dropping below that of vegetation and microbial requirements (Qadir & Schubert, 2002; Khomo & Rogers, 2005) and 2) slow internal drainage that results in soil becoming susceptible to waterlogging and in turn creating an anoxic environment (Qadir & Schubert, 2002; Khomo & Rogers, 2005).

Weakened soil aggregates are prone to erosion, since such erosion events are triggered and enhanced by water- and wind-flow, anthropogenic or animal activity as well as a shortage of soil-binding root-systems and low-density microbial biofilm (Qadir & Schubert, 2002; Venter *et al.*, 2003; Khomo & Rogers, 2005; Alard, 2009). Surface erosion often leads to the loss of the original A-horizon which contains the highest concentration of SOM and is better suited for organismal activity (Qadir & Schubert, 2002; Venter *et al.*, 2003; Khomo & Rogers, 2005).

3.6 Fauna and Flora

The study site is located within the Lowveld Bioregion of the Savanna Biome and forms part of the Granite Lowveld vegetation unit (SVI 3) (Figure 11) (Mucina and Rutherford, 2006). The Granite Lowveld is characterised by tall shrubland with few trees and a herbaceous layer predominantly composed of C4 graminoid species suited to tolerate the hot growing season (Mucina & Rutherford, 2006; Siebert & Eckhardt, 2008). Dominant graminoids are characterised as palatable, nutritious bulk growers (Venter *et al.*, 2003; Mucina & Rutherford, 2006; Alard, 2009).

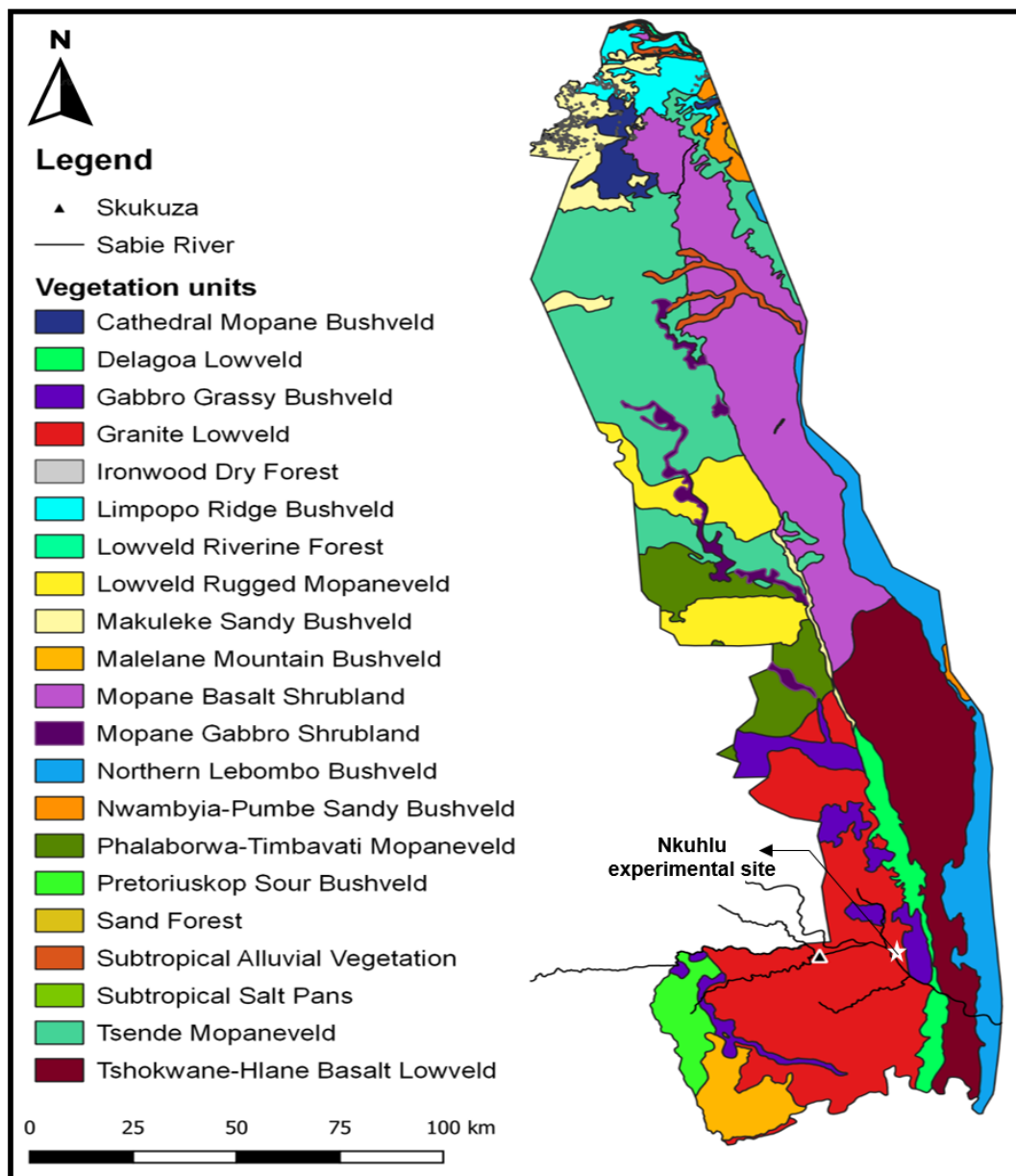


Figure 11: Location of the Nkuhlu experimental site relative to the different vegetation units within the KNP.

Plant physiology in this ecosystem resembles a pulse pattern that corresponds with the occurrence of rainfall and the unique wet and dry cycles of the area (Venter *et al.*, 2003; Grant & Scholes, 2006; Alard, 2009). These pulses of metabolic activity tend to last a few days up to several weeks following the occurrence of rain, after which activity slowly decreases (Venter *et al.*, 2003; Grant & Scholes, 2006; Alard, 2009).

3.6.1 Vegetation composition

Siebert and Eckhardt (2008) described the vegetation of the Nkuhlu exclosures and identified five main plant communities, of which this study was undertaken in the *Sporobolus nitens-Euclea divinorum* Dry Sodic Savanna which consist of two sub-communities (Figure 12, 1.1 and 1.2).

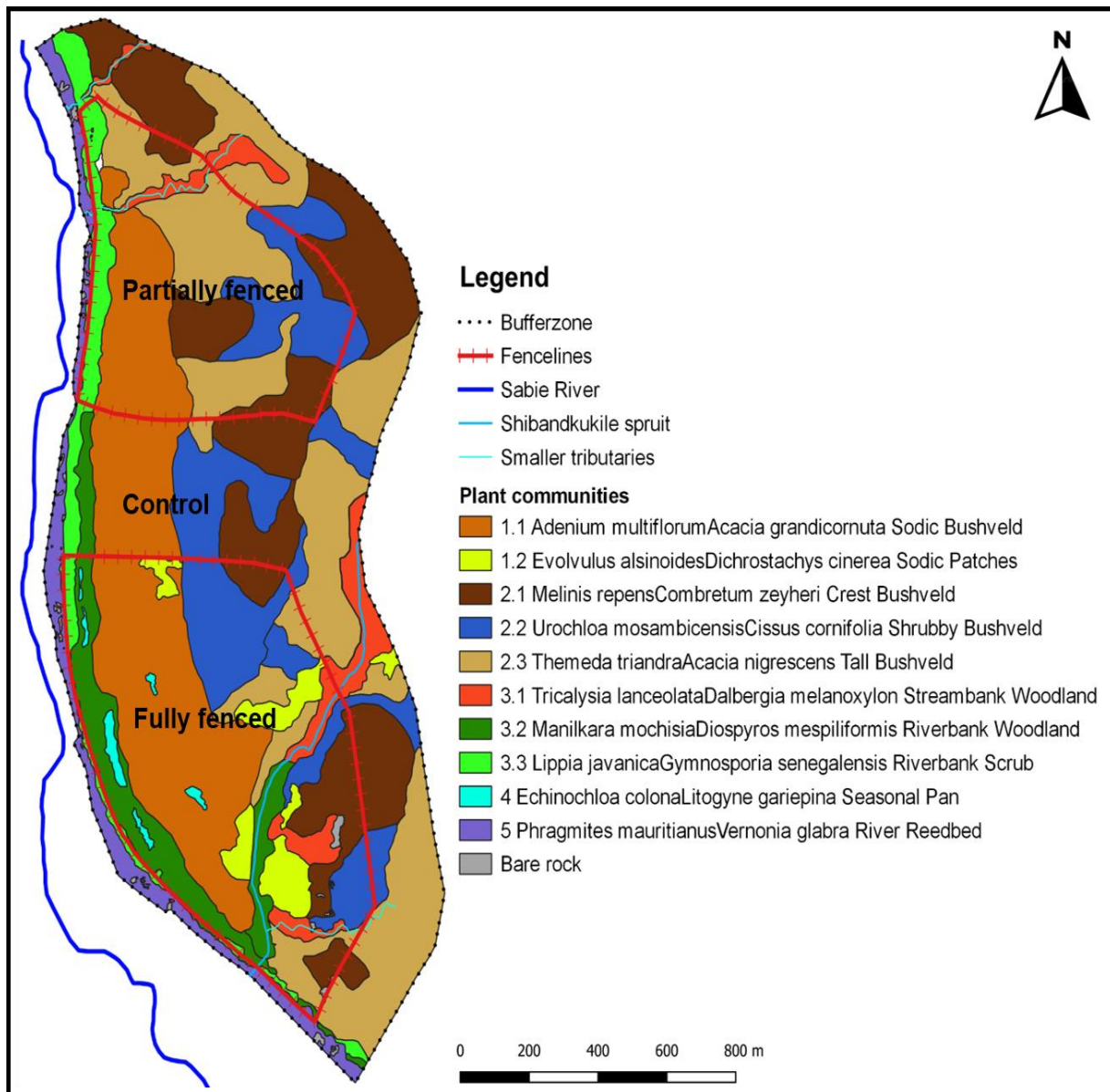


Figure 12: Vegetation map of the Nkuhlu exclosures indicating the location of the Sabie River,

seasonal streams, smaller tributaries and the various plant communities relative to the three enclosure units (Adapted from Siebert & Eckhardt, 2008).

The sodic plant community covers approximately 23% of the Nkuhlu enclosures (Siebert & Eckhardt, 2008). Situated on a relatively fertile substrate the vegetation is generally associated with palatable, nutrient-rich forage that supports congregated herbivore activity (Venter *et al.*, 2003; Grant & Scholes, 2006; Siebert & Eckhardt, 2008; Alard, 2009; Scogings *et al.*, 2014). Sodic patches on the footslopes tend to be dominated by fine-leaved, nitrogen-fixing species such as *Vachellia grandicornuta* that are known for their mycorrhizal associations and subsequent high nitrogen content (Venter *et al.*, 2003; Siebert & Eckhardt, 2008; Scogings *et al.*, 2014). The herbaceous layer of the sodic zone is dominated by forb species such as *Abutilon austro-africanum*, *Portulaca kermesina* and *Ocimum americanum* while dominant graminoids include, *Chloris virgata*, *Enteropogon monostachyus* and *Sporobolus nitens* (Siebert & Eckhardt, 2008). The sodic plant community is further characterised by a discontinuous woody component which include abundant species such as *Vachellia grandicornuta*, *Euclea divinorum*, *Spirostachys africana* and *Pappea capensis* (Siebert & Eckhardt, 2008).

Sodic patches facilitate unique vegetation and subsequent herbivore activity that differ from upland crests (Grant & Scholes, 2008; Siebert & Eckhardt, 2008). Sodic vegetation plays a major role in LMH survival as it facilitates nutrient requirements and subsequent body maintenance during the dry season (Grant & Scholes, 2006). The vegetation of the sodic zone exhibits distinct structural patterns across herbivore treatments alluding to the direct influence of herbivory or the loss thereof of on system structure (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Siebert & Eckhardt, 2008; Alard, 2009; Van Coller *et al.*, 2013).

3.6.2 Herbivore communities

Sodic patches, also referred to as nutrient hot-spots facilitate high-quality forage (i.e. low C:N ratios) and therefore attract congregated LMH communities, particularly grazers (Cape buffalo [*Syncerus caffer*], hippopotamus [*Hippopotamus amphibious*], blue wildebeest [*Connochaetes taurinus*] and plains zebra [*Equus quagga*]) and mixed feeders (impala [*Aepyceros melampus*], African elephant [*Loxodonta africana*], grey duiker [*Sylvicapra grimmia*] and steenbok [*Raphicerus campestris*]). These herbivore preferred sites are often over-utilised, which contributes towards the characteristically denuded appearance of sodic patches (Venter *et al.*, 2003; Khomo & Rogers, 2005; Grant & Scholes, 2006; Alard, 2009; Scogings *et al.*, 2012; Van Coller *et al.*, 2013; Kaspari *et al.*, 2014).

Chapter 4

Experimental- and sampling design

4.1 Introduction

This chapter provides a detailed outline of the experimental design and general background information on the methods that were used to obtain and analyse data during this study. Methodology relevant to specific research questions is outlined and addressed in detail within each respective Results & Discussion chapter to avoid repetition.

4.2 Experimental design

4.2.1 Nkuhlu experimental site

The Nkuhlu experimental site (referred to as the Nkuhlu exclosures) encompasses an area of 139 ha of semi-arid savanna which is divided into three herbivory treatments (Siebert & Eckhardt, 2008). Treatments include (1) a partially fenced area (hereafter the Elephant exclosure) of 44 ha designed to exclude elephants, but also excluding giraffes due to their body size, (2) an unfenced area (hereafter the Control site) of 25 ha representing an area of natural, undisturbed semi-arid savanna which allows for unrestricted movement of all LMH and (3) a fully fenced area (hereafter the LMH exclosure) of 70 ha designed to exclude all herbivores larger than a hare (Figure 13 & 14) (O'Keefe & Alard, 2002). This experimental setting provided the opportunity to test the effects of complete and/or partial release of herbivore pressure from a herbivore-adapted system (Siebert & Eckhardt, 2008).

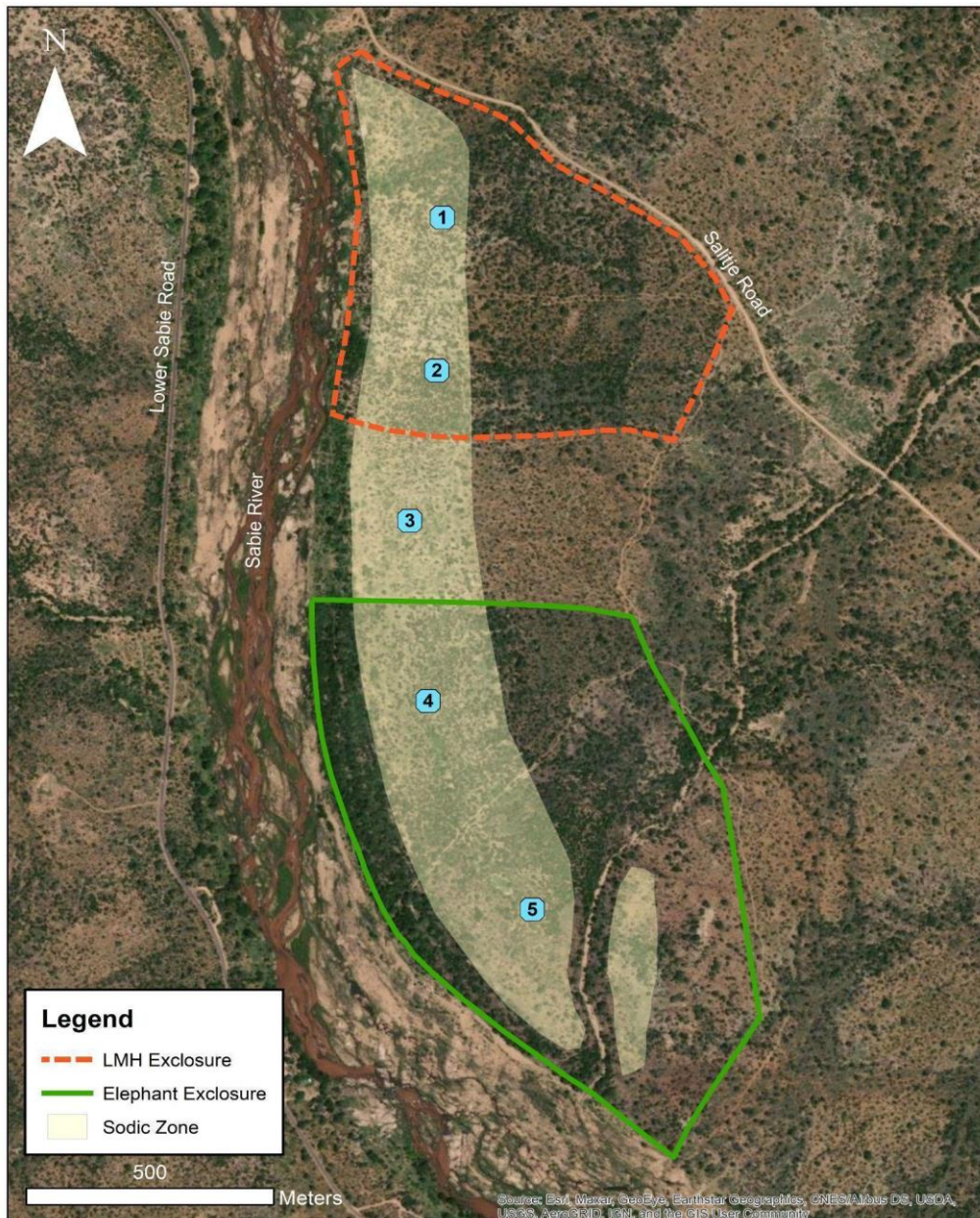


Figure 13: Aerial view of the Nkuhlu exclosures indicating the three herbivore treatments and location of sampled sites (1-5) within the sodic zone.

LMH exclosure, all LMH excluded; Elephant exclosure, elephants (and giraffes) excluded; Sodic zone, sodium-rich, deep, duplex soil associated with the footslopes of undulating granitic landscapes.

4.2.1.1 Fire treatments

Each of the three treatments are further divided into a fire and no-fire area, resulting in a total of six herbivory-fire combinations (Figure 14) (O'Keefe & Alard, 2002). Located within the larger KNP burn blocks (i.e. fire management units), occurrence of fire in these treatments are dependent on fire management strategies set out by the KNP (Van Coller *et al.*, 2018). Prescribed burn events occurred approximately once every five years between June and

October, with four burn events taking place between the establishment of the exclosures and sampling i.e. 2002, 2007, 2012, 2017 (Vermeire *et al.*, 2021). Although fire is a major driver of savanna ecosystem dynamics (Van Coller *et al.*, 2013; Pausas & Bond, 2020; Vermeire *et al.*, 2021), it was not considered for the purpose of this study. The primary reason for the exclusion of fire as a variable pertains to the design constraints of the Nkuhlu exclosures. The main focal point of the study, the sodic zone, does not extend into the sixth herbivore treatment i.e. the Control site that is exposed to fire. As such, fire was not included as it would result in an unbalanced experimental design. A detailed graphical representation of the Nkuhlu exclosure layout and the associated constraints of the six herbivore-fire treatments are provided as supplementary material in Appendix A, Figure A1. Furthermore, evidence has been found that the fuel load within intensively utilised sodic patches, characterised by palatable forage, are kept relatively low as a direct result of higher grazing pressure (Van Wilgen *et al.*, 2003; Govender *et al.*, 2006). As such, it is often the case that fire, within the sodic zone, does not represent a continuous burn as a result of high structural patchiness of the system. Moreover, initial statistical analyses revealed no significant variation between fire and no fire treatments within the Elephant- and LMH exclosure, further justifying the pooling of these sites. Statistical results are provided as supplementary material in Table B1 of Appendix B.

4.2.1.2 Experimental plots

Surveys were conducted within five sites (Figure 13), each comprising five plots (2.75 m x 1.5 m; Figure 15) located across the three herbivore treatments (Elephant exclosure, Control site with all LMH present, LMH exclosure; Figure 14), resulting in a total of 25 plots.

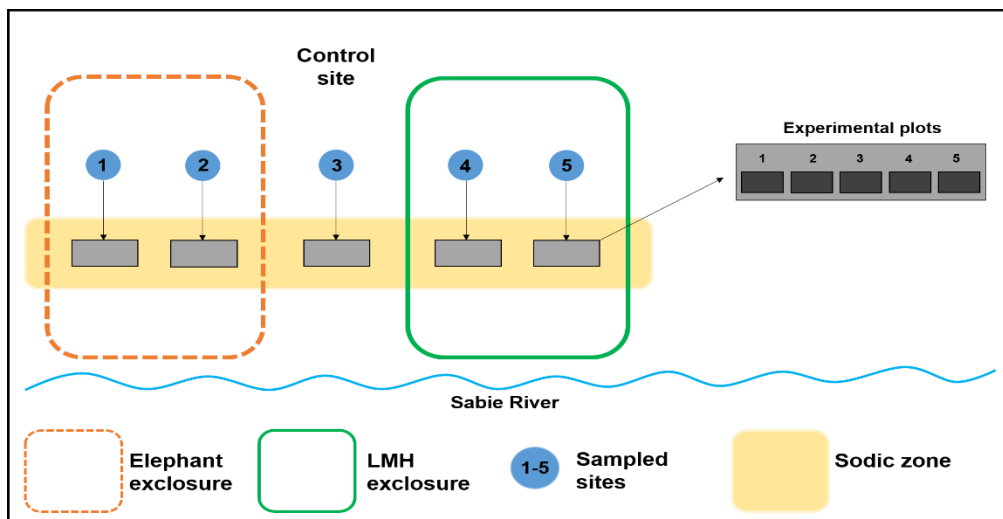


Figure 14: Experimental layout of the Nkuhlu long-term research exclosures indicating the five sampled sites and associated plots located across the three herbivore treatments within the sodic zone.

4.3 Field surveys and sampling

This study was initiated in January 2019 and concluded in January 2020 with sampling taking place along four intervals of three months, each representing a different season. At the time of data collection, herbivores have been excluded from the LMH enclosure for approximately 18-19 years. Sampling encompassed the collection of aboveground productivity (i.e. herbaceous biomass), plant species composition and functional traits data, decomposition data as well as soil samples for the purpose of soil microbial genomic analyses. Soil physicochemical properties of the studied site have also been sampled for the purpose of another research project in which data were pooled into the larger herbivore treatments (i.e. herbivore presence or absence) and analysed accordingly (Van Coller *et al.*, *in prep*). Results obtained from soil analyses will be used to facilitate the interpretation of observed patterns of change in potential soil-based decomposition and stabilisation as well as belowground microbial community composition. Observed patterns of changes in vegetation structure and edaphic properties are contextualised in terms of soil function as key regulatory constituents of belowground processes and structure.

Chapter 5, 6 & 7

Decomposition-based sampling conformed to the extended, site-specific TBI approach as described by Erasmus *et al.* (2021) -Appendix D. Initiated in January 2019 with the burial of 1 000 tea bags across three herbivore treatments (Figure 14) within 25 experimental plots (Figure 15), this study ran for a period of one year with sampling taking place along four intervals of three months. The development and validation of this extended approach is comprehensively described in Chapter 5. Application of the extended TBI approach from a global comparative perspective (Erasmus *et al.*, 2022; Appendix E) is described in Chapter 6, while the application thereof along a temporal gradient is described in Chapter 7.

Chapter 8

Soil samples were collected during the dry (October 2019) and wet (January 2020) season of this particular savanna ecosystem. Soil samples (25 per season, i.e. one composite sample per plot; Figure 15) were taken at a depth of up to 8 cm and stored at -80 °C to maintain DNA integrity. Extracted DNA was analysed through the application of NGS techniques. A detailed account on the methodology used to identify microbial taxa across the three herbivore treatments is presented in Chapter 8.

Chapter 9

Primary productivity was estimated for each of the three herbivore treatments (Figure 14) by using a Lowveld Savanna-calibrated disk pasture metre (DPM). DPM readings were recorded for both the dry (October) and wet (January) season and converted to kg/ha. Additional productivity data in the form of aboveground herbaceous biomass clippings were collected in January 2020 for a more accurate approximation of dry weight of aboveground phytomass during the wet season. Clippings were dried and separated into forbs and grasses where after they were weighed and converted to kg/ha. Plant species composition data (sampled in January 2020) were collected from five 50 m transects that were positioned in close proximity to the decomposition and soil microbial sites. Local scale floristic data were collected to better understand the observed patterns in decomposition, stabilisation and microbial community composition. The floristic analyses are therefore not aimed at describing vegetation patterns across the herbivore treatments. A detailed account of the herbaceous floristic patterns in the sodic zone has already addressed floristic responses to herbivory in the studied site (Van Coller *et al.*, 2013).

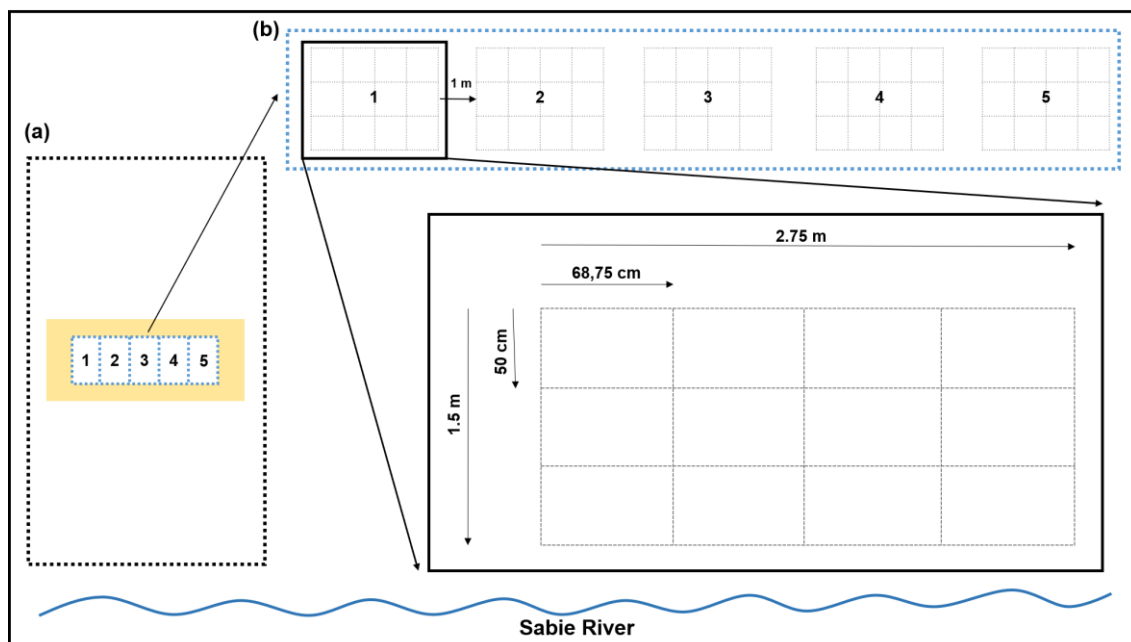


Figure 15: Positioning of (a) temporary experimental plots within the sodic zone of each herbivore treatment of the Nkuhlu exclosures, and (b) layout of plots for the purpose of decomposition-based sampling. A detailed explanation of the experimental plot layout is provided in Chapter 5. (Adapted from Erasmus *et al.*, 2021).

4.4 Data preparation

Decomposition data (TBI data) were recorded in the TBI 2.0 NW data sheet for non-woven tea bags provided by Keuskamp *et al.* (2013). Floristic data were recorded and consolidated in a Microsoft Excel (2013) database. Vegetation-based data consisted of quantitative floristics (i.e. species counts) whereas biomass data consisted of aboveground primary productivity estimates per hectare. Data were extracted from the separate databases and formatted to be imported into various statistical software –Microsoft Excel (2013), Paleontological Statistics Software (PAST) (Hammer *et al.*, 2001), STATISTICA version 13.3 (TIBCO Software Inc., 2017) and R version 4.1.0 (R Core Team, 2021). Existing soil physicochemical data was already consolidated in Microsoft Excel and analysed. Plant species that could not be identified in the field were collected for accurate identifications from specimens housed at the AP Goossens herbarium of the North-West University, Potchefstroom Campus.

4.5 Data analyses

General background on the statistical methods that were used in this study is presented below. Statistical methods are described in more detail in each relevant chapter. In general, statistical interpretations and assumptions were based on conventional p -values, however, it is important to note that recent studies including that of Cumming (2008), Nuzzo (2014) and Halsey *et al.* (2015) just to name a few, have reported on the frailties of the p -value in significance testing. High p -values that subsequently do not reject the null hypothesis cannot be interpreted as the null hypothesis being true, resulting in an unspecified answer, or “open verdict” (Cohen, 1994). Moreover, with a large sample size, the null hypothesis will inevitably be rejected (Tukey, 1991). Further compounding the fragile nature of the p -value, when significant values are attained at just below 0.05, these values provide extremely weak evidence against the null hypothesis, potentially alluding to a false positive result (Colquhoun, 2017).

Note that Chapter 5 (Published article) was completed during the hard lockdown of 2020, statistical software such as Microsoft Excel and PAST was the only software accessible at that point. The results for Chapter 6 was completed in 2019 as part of my Honnours project, whilst Chapters 7, 8 and 9 were completed at a later stage with newer software.

Chapter 5

Independent Student's *t* tests in Microsoft Excel (2013) were used to determine differences in recovery success of tea bags (green and rooibos) within each incubation period. One-way analysis of variance (ANOVA) was applied in PAST (Hammer *et al.*, 2001) and Microsoft Excel (2013) to test for significant variation in the number of tea bags infested by fungi and damaged by termites across herbivore treatments and over different incubation periods.

Chapter 6

Kruskal-Wallis ANOVA in combination with multiple comparisons of mean ranks –as *post hoc* tests were applied in STATISTICA version 13.3 (TIBCO Software Inc., 2017) to the three-month TBI dataset to determine whether (1) decomposition rate (*k*) and (2) stabilisation factor (*S*) varied significantly across different herbivory treatments within the sodic zone of the Nkuhlu exclosures.

Chapter 7

To assess the effects of herbivory on soil function (decomposition rate -*k* and stabilisation factor -*S*), all statistical analyses were performed using R version 4.1.0 (R Core Team, 2021). Kruskal-Wallis One-way Analysis of Variance (ANOVA) was applied to the 2019-2020 TBI dataset to test for significant variation in decomposition rate (*k*) and stabilisation factor (*S*) of standardised plant material. Where statistically significant variance among treatments were attained, a *post hoc* Dunn test was applied to test for significant differences between treatments.

Chapter 8

Statistical analysis of microbiome data was performed using marker-gene data profiling in MicrobiomeAnalyst (Xia Lab, McGill University, Quebec, Canada). PERMANOVA was used to calculate β -diversity whilst a heat tree analysis together with Wilcoxon signed rank tests were used to examine differences in bacterial and fungal taxa between different treatments. In addition, a random forests classification was used to determine important differentiating factors (genera) between the different herbivore treatments. Alpha diversity indices including the number of observed ASVs and Shannon-Wiener index (*H'*) for bacterial and fungal communities were computed in QIIME2 software (QIIME2 core_2019.10). One-way Analysis of Variance (ANOVA) was applied in R version 4.1.0 (R Core Team, 2021) to test for significant variation among treatments. Tukey's Honestly Significant Difference (HSD) test was applied as *post hoc* analysis.

Chapter 9

To test for significant variation in biomass of the herbaceous layer amongst three different treatments of varying herbivore intensity, data were analysed using a Kruskal-Wallis One-way Analysis of Variance (ANOVA), whilst a Dunn test was applied as *post hoc* analyses to test for significant differences between treatments in R version 4.1.0 (R Core Team, 2021).

Chapter 5

Extension and validation of the Tea Bag Index as a viable approach to quantify decomposition in a semi-arid African savanna

5.1 Preamble

The scientific content of this Chapter was submitted as a manuscript to the South African Journal of Science. The complete published article is provided in the Supplementary Material (Appendix D- Erasmus *et al.*, 2021 - DOI: <https://doi.org/10.17159/sajs.2021/6846>). This Chapter is therefore presented as an extended version of the published paper, but aligned with the format of a dissertation. Overlap in content, certain statements, or important background information was, however, unavoidable.

5.2 Introduction

Decomposition is central to the effective functioning of terrestrial ecosystems as it forms the link between above- and belowground nutrient cycling (Paudel *et al.*, 2015; Didion *et al.*, 2016; Eldridge *et al.*, 2017; Elumeeva *et al.*, 2018; Petraglia *et al.*, 2019; Teo *et al.*, 2020). Research regarding the links between soil-based decomposition of detrital plant material and subsequent soil respiration (carbon flux) is covered extensively for temperate (MacDonald *et al.*, 2018; Tremblay *et al.*, 2018) and boreal (Elumeeva *et al.*, 2018; Petraglia *et al.*, 2019) ecosystems, whilst studies in tropical and subtropical systems, especially in Africa, remain limited (Becker & Kuzyakov, 2017; Teo *et al.*, 2020). The paucity of information available on these mechanistic relationships in African savannas can potentially be ascribed to the wide range of complex interactions between top-down drivers (e.g. climate variability, fire, LMH) and bottom-up controls (e.g. edaphic properties, soil microbial- and detritivore community structure), that collectively regulate savanna structure and function (Ratnam *et al.*, 2019; Sundsdal *et al.*, 2020; Teo *et al.*, 2020; Thoresen *et al.*, 2021).

Assessments of ecosystem functioning are becoming increasingly valuable, especially its application in monitoring programmes in areas exposed to increasing intensities of land-use- and/or climate change. Necessary resources required for such studies are often unavailable in many African countries. However, citizen science projects, such as the TBI has become a useful tool to facilitate ecological research due to relatively easy application, time- and cost-effectiveness as well as public engagement (e.g. Teatime 4 Science, <http://www.teatime4science.org/>) (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Becker &

Kuzyakov, 2017; MacDonald *et al.*, 2018). A detailed description of the TBI approach, development and application thereof is provided in chapter 2 of this dissertation.

Little is known about site-specific disturbance effects (i.e. herbivory) on decomposition and carbon flux. TBI-based decomposition studies can provide valuable information on the extent of these disturbances and their potential effect on essential ecosystem processes and functioning (Paudel *et al.*, 2015; Didion *et al.*, 2016; Becker & Kuzyakov, 2017; Eldridge *et al.*, 2017; MacDonald *et al.*, 2018). The TBI is able to identify and examine environmental drivers of decomposition without ambiguous effects of site-specific litter, serving as a reference which facilitates data comparison across spatial scales (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Becker & Kuzyakov, 2017; Elumeeva *et al.*, 2018; MacDonald *et al.*, 2018).

However, the physical and biological template upon which African systems function varies from most ecosystems in which the TBI has previously been applied (Didion *et al.*, 2016; MacDonald *et al.*, 2018; Tremblay *et al.*, 2018; Elumeeva *et al.*, 2018; Petraglia *et al.*, 2019). This study will reflect on the application of the TBI approach in an African context to facilitate decomposition monitoring. Details on (i) an experimental design aimed at elucidating temporal dynamics, (ii) tea bag losses due to LMH, fungal infestation and termites, (iii) recommended equipment to enhance effective sampling, and (iv) the potential value in African systems are provided.

5.3 Materials and Methods

The TBI was developed and applied in systems where decomposition is primarily dominated by microbial activity (Keuskamp *et al.*, 2013; Sundsdal, 2019; Teo *et al.*, 2020). As such, it does not account for the presence and possible damage caused by savanna-based agents of disturbance, namely native LMH and termites (Leitner *et al.*, 2018; Sundsdal, 2019; Teo *et al.*, 2020). To evaluate the number of samples (tea bags) that were rendered statistically useless due to either physical termite damage and/or fungal infestation, tedious visual inspection was done for each sample before weighing commenced. Characteristic signs of termite damage included small holes in the nylon mesh, often accompanied by soil particles within the tea bags. Moreover, fungal infestation typically comprised a fine black powdery substance on both the out- and inside of the tea bags.

Moreover, decomposition is a time-bound process consisting of different phases (Didion *et al.*, 2016; Elumeeva *et al.*, 2018), primarily regulated by the chemical composition of detrital substrate with respect to labile and recalcitrant ratios (Keuskamp *et al.*, 2013; Becker & Kuzyakov, 2017). Since savannas are highly dynamic systems, regulating factors have the

potential to change over relatively small time intervals, which may influence the rate and extent of decomposition. As such, temporal comparisons are essential to not only observe progression in decomposition phases, but the influence of site-specific factors thereon (Berg *et al.*, 2015; Didion *et al.*, 2016; Ratnam *et al.*, 2019; Sundsdal, 2019; Teo *et al.*, 2020).

To account for potential sample (tea bag) losses as well as the temporal nature of decomposition we applied a paired tea bag design, which included 25 experimental plots across three herbivore treatments, sampled over four seasons (Figure 14, 15 & 16). Each plot consisted of 40 tea bags (20 green- and 20 rooibos; Figure 16) which added up to a 1 000 tea bags in total. Plots comprised of four rows representing different incubation periods (i.e. three-, six-, nine- and twelve-months) to measure temporal dynamics in k (decomposition) and S (stabilisation) (Figure 16), but also to determine the resilience of the TBI in an unexplored system over the course of a year. In an attempt to account for high spatial heterogeneity, typical of semi-arid savanna ecosystems, experimental plot placement accounted for areas under canopy cover (full- and semi-cover) and without canopy cover. Detailed step-wise procedures for the application of this extended TBI approach is described in Appendix D, Erasmus *et al.* (2021).

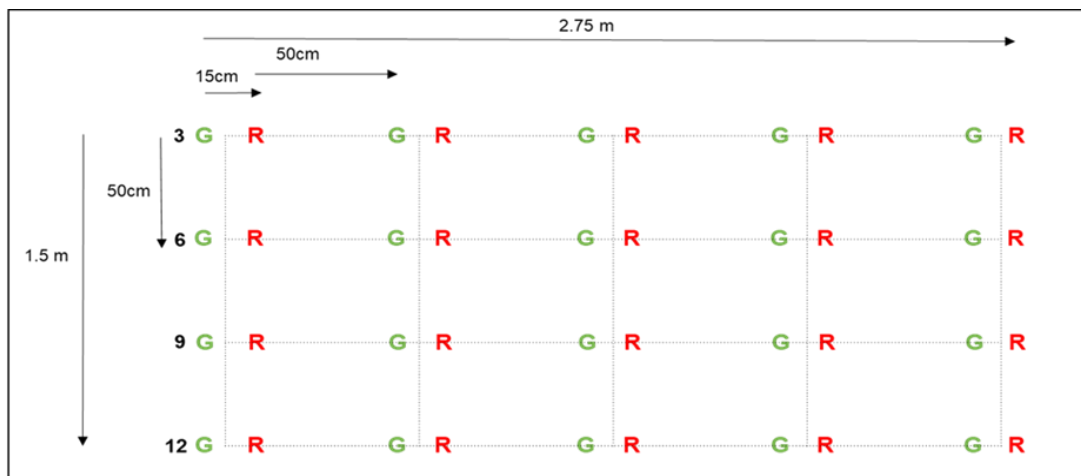


Figure 16: Experimental plot layout consisting of a paired tea bag design across four incubation periods.

G, green tea bag; R, rooibos tea bag

5.3.1 Statistical analyses

Differences in recovery success of green and rooibos tea bags within the four seasonal incubation periods were tested for significance using independent Student's t tests in Microsoft Excel (2013). To test for significant variation in the number of tea bags infested by fungi and damaged by termites across treatments and over the different incubation periods, a one-way

analysis of variance (ANOVA) was applied in Paleontological Statistics Software (PAST) and Microsoft Excel (2013). Significance levels for analyses were set at the standard significance level ($p < 0.05$) enabling confident conclusions made from statistical results.

5.4 Results

Overall recovery success, irrespective of herbivore treatment and incubation period was 78.5%. Results did not reveal significant differences in recovery between green and rooibos tea among most treatments (Figure 17), although significantly less rooibos tea bags could be retrieved after the 6-month incubation period (Figure 17).

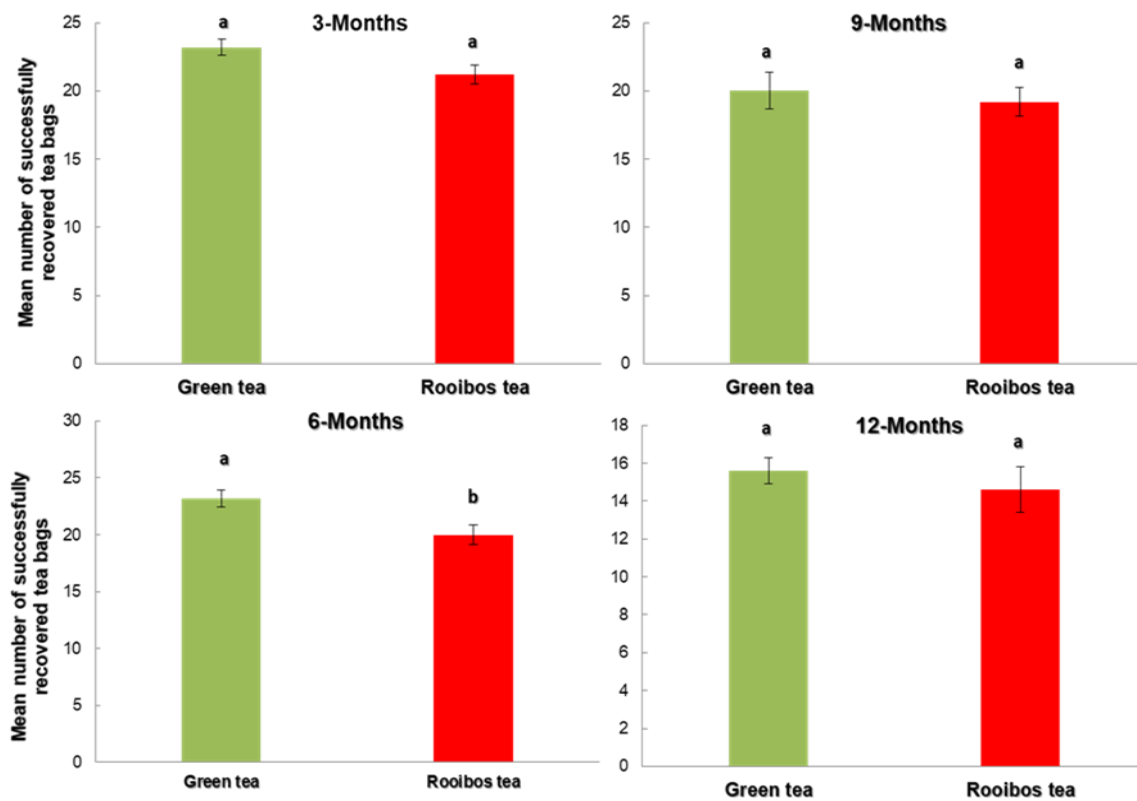


Figure 17: Mean number of successfully recovered tea bags over four incubation periods. Significant differences ($p < 0.05$) between tea bags are indicated with different lowercase letters.

A finely grained black fungus was present on the surface of 42% of recovered green tea bags, while no rooibos tea bags were infested. Fungal infestation showed no significant difference between incubation periods ($p = 0.897$). Fungal infestation and damage by termites were significantly lower in the control site ($p < 0.001$, Figure 18a and $p = 0.003$, Figure 18b). Rooibos tea bags were significantly more damaged by termites than green tea bags ($p = 0.033$).

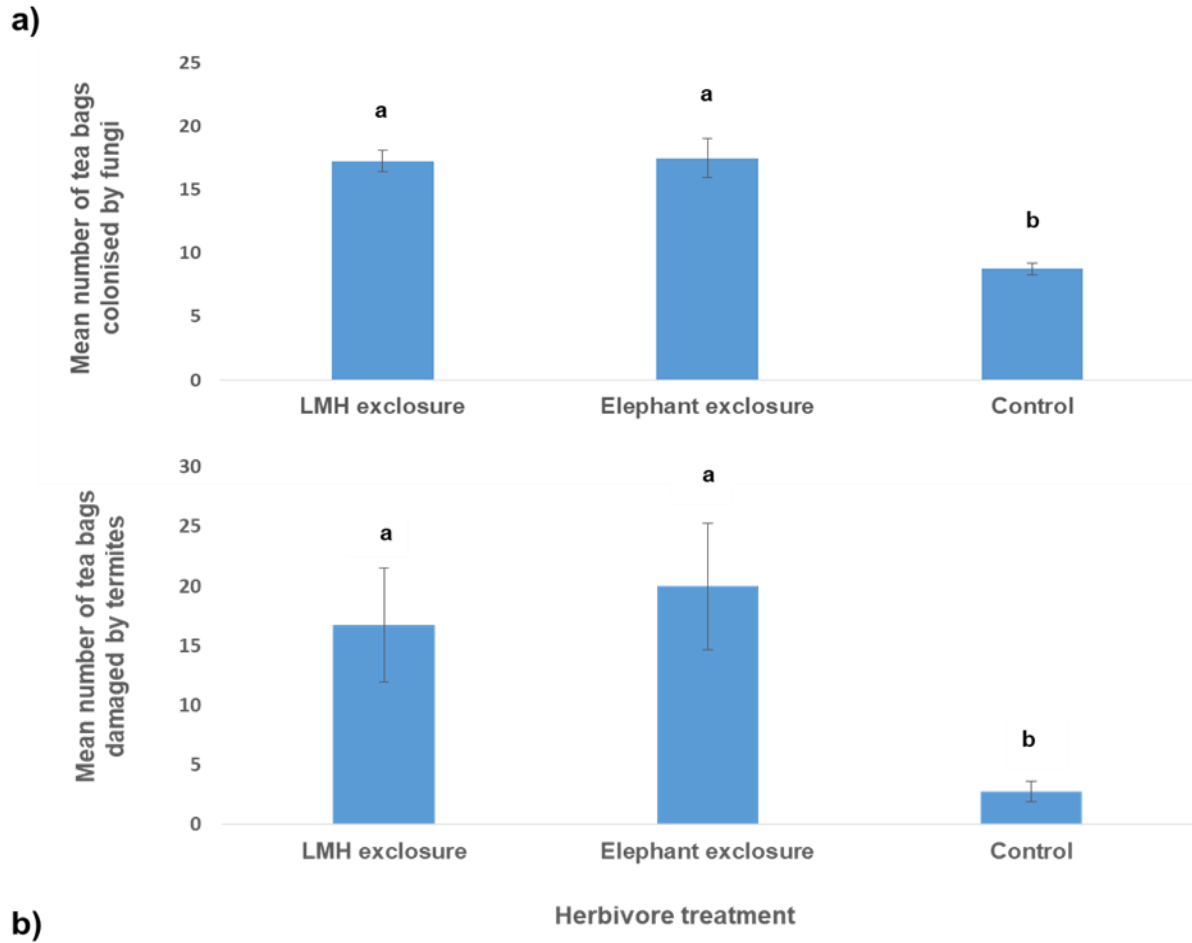


Figure 18: Mean number of tea bags infested by fungi (a), and damaged by termites (b) across three herbivore treatments. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters.

LMH exclusion, all LMH excluded; Elephant exclusion, elephants (and giraffes) excluded; Control, all herbivores present.

5.5 Discussion

Results obtained indicate that retrieval of tea bags (both green and rooibos) is more challenging in the presence of herbivores. Lower retrieval success could be explained by the difficulty in finding markers in sites where above-ground markers are not recommended due to high mammal activity, especially in protected areas. Moreover, detritivore activity in the form of termites has a significant impact on the longevity of tea bags. Teo *et al.* (2020) suggested the use of physical and/or chemical barriers to account for termite-based damage. However, such applications may impede the practicality and standardisation of the TBI approach. Moreover, the application of these barriers can potentially reduce the effectiveness of the TBI approach in measuring decomposition by altering essential regulatory constituents (i.e. microbial- and detritivore communities, soil chemical composition) (Sundsdaal, 2019).

Nutrient-rich systems such as sodic patches in the KNP are dominated by high quality forage capable of supporting high herbivore densities e.g. Control site with all LMH (Grant & Scholes, 2006; Jacobs & Naiman, 2008), with more than 50% of NPP returned to the belowground compartment in the form of easily accessible labile nutrients (i.e. urine and faecal material; Figure 3) (Wardle *et al.*, 2004). Contrariwise, herbivore exclusion results in lower amounts of NPP returned to the soil with the majority of these materials being of a recalcitrant nature (Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Scogings *et al.*, 2014). Fertile soils with a higher labile component have the potential to support bacterial dominated energy channels together with microfauna (e.g. nematodes and protozoa), however, soils characterised by recalcitrant materials tend to be dominated by fungi and arthropods (Wardle *et al.*, 2004). This corresponds with obtained results in which both termite and fungi activity were significantly higher in the absence of herbivores when compared to the Control site with all LMH (Figure 18a & b).

Arthropods, including meso- and macrofauna are an essential component of trophic structure (Coleman *et al.*, 2017). Soil-based arthropods play an important role in soil functioning by aiding microbial-based decomposition through fragmentation of detrital material, creating biopores and distributing detrital material within soil profiles (Coleman *et al.*, 2017). The retrieval of significantly less rooibos tea bags after a six-month incubation period can be ascribed to the influence of seasonality on macrodetritivore activity e.g. termites (Sundsdal *et al.*, 2020). The six-month retrieval period coincided with the dry, non-growing season in the study area (Mucina & Rutherford, 2006). The subsequent seasonal-derived lack in detrital plant material may have resulted in termites predominantly targeting readily available recalcitrant material found in rooibos tea bags. Termites have been observed to continue foraging during the dry seasons in various tropical ecosystems (Veldhuis *et al.*, 2017; Ashton *et al.*, 2019), this is however limited to certain savanna-based species (Leitner *et al.*, 2018). Sundsdal *et al.* (2020) found that macrodetritivore communities have the potential to sustain decomposition of recalcitrant detrital plant litter despite cycles of seasonal water scarcity and shortages in labile litter.

In general, aboveground disturbances such as that associated with herbivore activity has a negative effect on arthropod communities (Bardgett *et al.*, 2002). However, recent studies (Kral *et al.* 2017; Wang *et al.* 2018), including a local example by Thoresen *et al.* (2021), have revealed that due to the multifaceted nature of herbivore disturbances it can result in either positive, negative, or neutral effect. The amplitude of disturbances is thought to be greater with densely congregated herbivore communities (Vandegehuchte *et al.*, 2017). Moreover, a

study done by Wang *et al.* (2018) suggests that certain herbivore assemblages are capable of promoting more disturbances and subsequently having a greater impact on arthropod community structure and activity.

Lower termite-based damage and fungal infestation in the Control site in comparison to the other treatments suggests that the presence of all LMH have a significant negative effect on termite and fungal activity. This can potentially be ascribed to the direct and indirect effects of LMH on several determining factors regulating these communities (Pressler *et al.*, 2019; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021). For termites this includes a reduction in aboveground biomass (i.e. food source), soil compaction, higher soil temperatures, associated lower soil moisture as well as physical damage to termite mounds as the associated vegetation is preferentially foraged by LMH (Grant & Scholes, 2006; Leitner *et al.*, 2018; Pressler *et al.*, 2019; Thoresen *et al.*, 2021).

Fungal communities follow similar biogeographical patterns as plants and are predominantly affected by climatic factors (Vermeire *et al.*, 2021). More specifically, fungal communities are mainly restricted to superficial soil horizons and are closely related to vegetation structure e.g. production and host composition (Pressler *et al.*, 2019; Vermeire *et al.*, 2021; Thoresen *et al.*, 2021). LMH activity in the Control site has resulted in compaction and/or erosion of the preferred porous A-horizon, decreased plant production, altered host composition, higher surface temperatures and lower soil moisture (Jacobs & Naiman, 2008; Siebert & Eckhardt, 2008; Van Coller *et al.*, 2013; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021).

The seemingly high number of replicates used in this small-scale investigation, successfully accounted for tea bag losses ascribed to savanna-based disturbances (i.e. animal, insect and fungal activity). Sufficient recovery (78%) with little differences between green- and rooibos bags will lead to confident conclusions made from TBI output results.

5.6 Conclusion

African savannas function as interactive social-ecological systems with numerous communities depending on associated ecosystem services for their livelihoods. However, these systems are faced with numerous challenges pertaining to the loss of biodiversity and subsequent ecosystem functioning, predominantly arising from unsustainable land-use practices. Ecosystem assessments such as the TBI provide a relatively easy approach to obtain valuable information regarding system disturbances and their potential effect on ecosystem functioning. Yet, the heterogeneous and dynamic nature of African savannas

complicates the application of general research methods, often requiring system-specific approaches. This study highlights the range of obstacles and disturbances that may potentially impact on the successful application of the TBI approach in an African savanna. Results indicate that the presence of certain agents of disturbance i.e. LMH and termites substantially affect the retrieval of tea bags. However, accounting for potential sample losses due to site-specific conditions, this extended version of the TBI approach is more conducive to both the available resources and prevailing environmental conditions in Africa. As most studies aimed at elucidating decomposition are predominantly focused in temperate and boreal ecosystems, more should be done to elaborate on decomposition dynamics and the role of various influencing factors e.g. herbivory on soil function in heterogeneous African savannas.

Chapter 6

Decomposition rates of standardised organic material across herbivore treatments

6.1 Preamble

The scientific content of this Chapter was submitted as a Proceedings Paper to the Joint XXIV International Grassland and XI Rangeland Congress 2021. The content has been revised by the scientific committee, and accepted for publication. The complete published article is provided in the Supplementary Material (Appendix E- Erasmus *et al.*, 2022). This Chapter is therefore presented as an extended version of the accepted paper, but aligned with the format of a dissertation. Overlap in content, certain statements, or important background information was, however, unavoidable.

6.2 Introduction

Large parts of protected African savannas are faced with herbivore loss, albeit the diverse suite of LMH, or elephants only. Given the link between herbivore-induced disturbances and system heterogeneity, such losses might affect several underlying ecological processes and system functioning (Grant & Scholes, 2006; Tuomi *et al.*, 2019; Wigley *et al.*, 2020). This includes soil-based decomposition and stabilisation of detrital plant material together with above- and belowground nutrient cycling (Becker and Kuzyakov, 2018; Tuomi *et al.*, 2019; Wigley *et al.*, 2020).

Despite extensive use of enclosure experiments to investigate herbivore impacts, there is little consensus on their direct and indirect effects on soil function, particularly on decomposition and inherently, above- and belowground carbon sequestration (Eldridge *et al.*, 2019; Tuomi *et al.*, 2019; Wigley *et al.*, 2020). This Chapter aimed to assess three-month soil-based decomposition results across herbivore treatments and compare these results to globally distributed TBI-derived decomposition data from ecosystems across the globe. In this Chapter, the following hypotheses were tested: (i) decomposition rate (k) would be highest in the presence of LMH together with lowest stabilisation factor (S) under similar conditions, whilst (ii) globally distributed decomposition rates (k) will be higher in northern hemisphere-based systems when compared to the studied system.

To test this hypothesis, the extended site-specific TBI approach (Erasmus *et al.*, 2021) was applied across three herbivore treatments within the Nkuhlu enclosures study site for a three-month period to determine the effect of herbivory on k and S of standardised litter. Obtained

TBI results were plotted against a global dataset of TBI-derived decomposition data to serve as a comparison between globally distributed ecosystems.

6.3 Materials and Methods

Methodology conformed to the extended, site-specific version of the TBI approach as described in Teatime in Kruger (Erasmus *et al.*, 2021). As the generally accepted duration, only the three-month incubation period data was considered for this study to facilitate global comparison. Results were compared with TBI values obtained from a diverse range of ecosystems globally (Figure 19).

6.3.1 Statistical analyses

Data did not meet assumptions of normality according to the Kolmogorov–Smirnov and Shapiro–Wilk normality tests. As such, a Kruskal-Wallis ANOVA was applied to the data in Statistica, version 13.3 (TIBCO Software Inc., 2017) to test for significant variation in k and S of standardised litter amongst three herbivore treatments. Where statistically significant variance among treatments were attained, multiple comparisons of mean ranks were applied as *post hoc* tests. Significance levels for all analyses were set at the standard significance level ($p < 0.05$) enabling confident conclusions made from statistical results.

6.4 Results

All measured k - and S -values were within the global TBI reference value range derived from Keuskamp *et al.* (2013) and Becker and Kuzyakov (2018). Both k and S occupied the lower ranges of the global references reflecting the impact of climate as a global regulator of soil function. Of the available TBI data sets at the time of this analysis, only one system, i.e. System 15 (Figure 19) from Tanzania, partially relates to the KNP study area in terms of system structure (Grant & Scholes, 2006; Becker & Kuzyakov, 2018). However, S -values obtained from the KNP study site differed substantially from the Tanzanian savanna values, whereas k -values were comparable (Figure 19). This supports the general view that litter stabilisation (S) is more dependent on system-specific environmental and climatic factors, reflecting the influence of site-specific drivers (Keuskamp *et al.*, 2013; Becker & Kuzyakov, 2018).

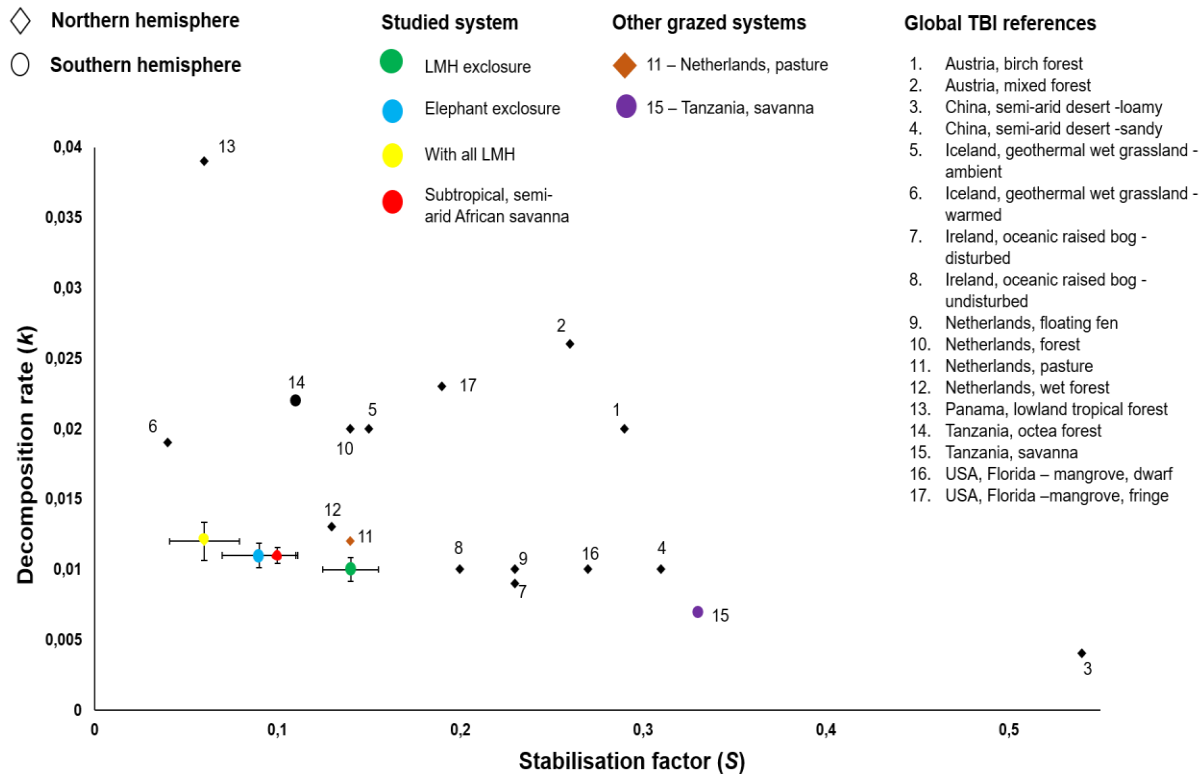


Figure 19: Mean decomposition rates (k) and stabilisation factor (S) of a subtropical, semi-arid southern African savanna in the Kruger National Park. This system is divided into three herbivore treatments (coloured circles). Whiskers indicate standard error of the mean based on the results obtained from 250 tea bags (125 green & 125 rooibos) over a three-month incubation period. Black dots represent global TBI references adapted from Keuskamp *et al.* (2013) and Becker and Kuzyakov (2018).

LMH enclosure, all LMH excluded; Elephant enclosure, elephants (and giraffes) excluded; Control, all herbivores present.

At the KNP study site, mean decomposition rate (k) decreased with increasing stabilisation factor (S) which corresponded with a release in herbivory pressure (Figure 20a). After a 3-month incubation period, k of standardised litter did not vary significantly across herbivore treatments ($p > 0.05$; Figure 20a), whereas S varied significantly across treatments ($p < 0.05$; Figure 15b). S was significantly higher in the LMH enclosure when compared to the control (multiple comparisons: $p < 0.05$; Figure 15b), which corresponds with the lowest k -value in this treatment (Figure 20a).

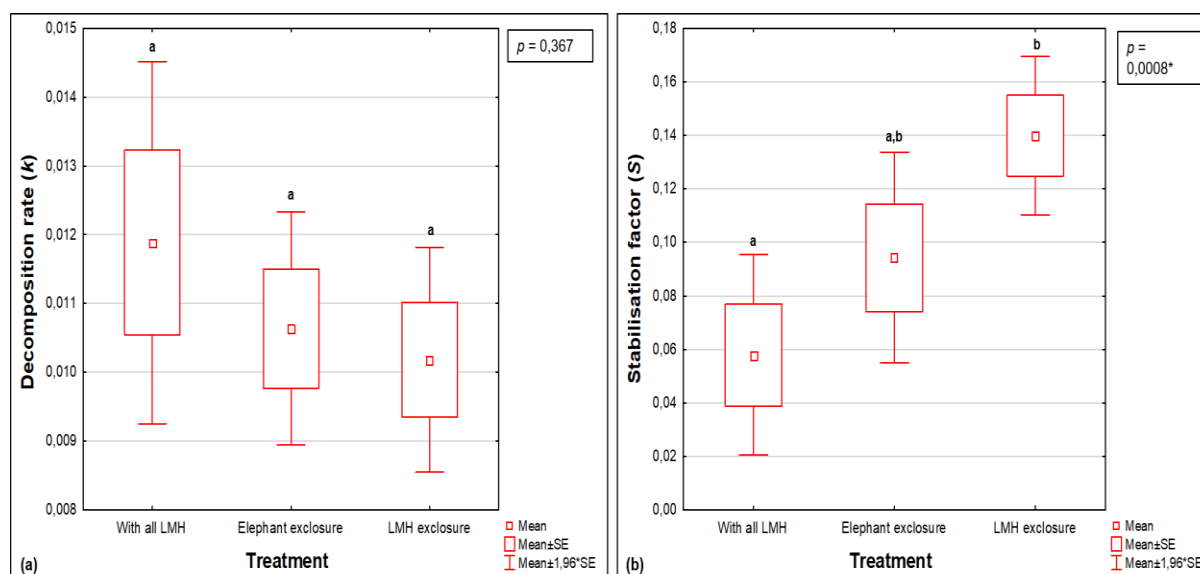


Figure 20: Mean decomposition rate - k (a) stabilisation factor - S (b) of standardised litter after a three-month incubation period across three herbivore treatments at the Nkuhlu exclosures study site, Kruger National Park. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters.

LMH enclosure, all LMH excluded; Elephant enclosure, elephants (and giraffes) excluded; Control, all herbivores present; $\pm 1.96^*$, standard deviations of the mean; SE, standard error; p , $p < 0.05$ indicates a significant difference between treatments.

6.5 Discussion

Results obtained from the study area varied substantially from other systems (Figure 19), which highlights the unique assemblage of global (i.e. climate) and local (i.e. edaphic properties and herbivory) environmental drivers present within the studied system (Grant & Scholes; Jacobs & Naiman, 2008; Vermeire *et al.*, 2021). Differences between k - and S -values from the studied system and its closest relative (i.e. system 15 - Tanzanian savanna, 951 m ASL) may be ascribed to combined effects of higher elevation and climatic variation on soil processes in the Tanzanian savanna (Becker & Kuzyakov, 2018). Systems situated at low elevation, especially in semi-arid environments (such as the study area) are subjected to climatic limitations, such as higher surface temperatures and lower soil moisture availability, which directly influence constituents of the decomposition subsystem (Grant & Scholes, 2006; Becker & Kuzyakov, 2018; Vermeire *et al.*, 2021). The presence of native LMH further adds to variations in soil function through numerous above- and belowground mechanisms which directly and/or indirectly influence constituents of the decomposition subsystem (Grant & Scholes, 2006; Jacobs & Naiman, 2008; García- Palacios *et al.*, 2016; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). Moreover, semi-arid savanna vegetation has developed numerous plant traits to survive under harsh African conditions. Savanna-based plant functional traits fall

within a more conservative spectrum, which is typically associated with small, thin leaves (e.g. low specific leaf area -SLA) that are low in nutrient and moisture content and have high leaf dry matter content (LDMC) values (Cornelissen & Thompson, 1997; Cornelissen *et al.*, 2004; Chillo *et al.*, 2011; Scoffoni *et al.*, 2011; Van Coller *et al.*, 2018). It has been postulated that certain plant functional traits have an 'afterlife' effect in which these traits are capable of influencing and/or determining the decomposability of associated detrital leaf material (Cornelissen & Thompson, 1997; Wardle *et al.*, 1998; Cornelissen *et al.*, 2004; Kazakou *et al.*, 2009). Subsequently, leaves within this conservative spectrum tend to favour a lower decomposition rate. Contrariwise, systems with lower temperatures and higher moisture availability typically consist of vegetation that have plant traits more associated with nutrient acquisition. These plants tend to have larger leaves (higher specific leaf area) with higher moisture and nutrient content (Cornelissen *et al.*, 2004; Liu *et al.*, 2018), subsequently favouring higher decomposition. Functional plant traits in combination with various site-specific drivers therefore provide a plausible explanation for substantial variation in global decomposition rates and stabilisation factor.

Higher decomposition rates (k) in the presence of all LMH (Control) can be ascribed to the ability of LMH to alter above- (i.e. vegetation composition and production) and belowground structure (i.e. edaphic properties, e.g. pH, nutrients, soil temperature and moisture) (Grant and Scholes, 2006; Jacobs & Naiman, 2008; Semmartin *et al.*, 2008; Siebert & Eckhardt, 2008; Van Coller *et al.*, 2013; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021). The impact of herbivory on any one trophic level can initiate a range of trophic cascade which has the potential to promote decomposition through various top-down and bottom-up effects (Gongalsky & Persson, 2013; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021).

High intensity utilisation of savanna vegetation has several effects on plant chemical composition. This includes, 1) decreased carbon-based secondary metabolites (recalcitrant compounds such as tannins and polyphenols), 2) decreased C:N ratios and 3) increased nutrient content within vegetation tissue, rendering highly utilised vegetation more palatable, nutrient-rich and labile (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Siebert & Eckhardt, 2008; Van Coller *et al.*, 2013; Scogings *et al.*, 2014). LMH influence patch-specific soil nitrogen (N) availability through excretions (i.e. urination and defecation) which stimulate microbial metabolic activity and subsequent decomposition of detrital plant material (Grant & Scholes, 2006; Semmartin *et al.*, 2008; Vermeire *et al.*, 2021). System perturbation, typical of high LMH activity, is associated with decreased stabilisation and a subsequent potential decrease in SOM, which result in increased microbial demand for organic carbon, essentially promoting a more rapid detrital turn-over (decomposition) (Bardgett *et al.*, 1998; Semmartin *et al.*, 2008;

Becker & Kuzyakov, 2018). Higher levels of SOM due to high stabilisation (significantly higher S in the LMH enclosure) results in fertile soil with a higher productivity potential (biomass production). Several studies have indicated that absence of herbivores lead to increased standing biomass (Grant & Scholes, 2006; Van Coller *et al.*, 2013). Low soil temperatures associated with the shading effect of high biomass can promote stabilisation as it results in reduced microbial metabolic activity (Keuskamp *et al.*, 2013; Becker and Kuzyakov, 2018; Petraglia *et al.*, 2019). This effect is evident from the results as S of standardised litter increased with a decrease in herbivore pressure. Moreover, the study area is situated within a moisture-limited system (561 mm per year) further impeding microbial activity and subsequent decomposition (Grant & Scholes, 2006; Keuskamp *et al.*, 2013; Becker and Kuzyakov, 2018; Petraglia *et al.*, 2019).

The sodic vegetation zone at the Nkuhlu study site is representative of a productive ecosystem that is dominated by palatable, nutrient-rich vegetation supporting large, congregated herbivore communities (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Siebert & Eckhardt, 2008; Van Coller *et al.*, 2013). It is proposed that in productive ecosystems, the beneficial effects of native LMH may outweigh the negative effects (Vandeghechuchte *et al.*, 2017). In these systems, labile nutrients are rapidly returned to the soil, resulting in increased levels of microbial activity which stimulate decomposition (mineralisation) and subsequent nutrient cycling (Bardgett & Wardle, 2003).

6.6 Conclusion

Decomposition is central to the effective functioning of terrestrial ecosystems as it forms the link between above- and belowground nutrient cycling. Despite the importance of decomposition on a local and global scale, little is known about site-specific disturbance effects such as herbivory or the loss thereof on this essential ecological process and related nutrient cycling. TBI-based decomposition studies provide a relatively easy approach to obtain valuable information regarding system disturbances and their potential effect on ecosystem functioning. This study highlights the complex nature of top-down and bottom-up controls that regulate decomposition and stabilisation of detrital plant material in a semi-arid African savanna. Obtained results indicate the importance of native LMH in maintaining essential ecosystem services in the form of decomposition. Global comparison alluded to the distinct effect of global and local change drivers on soil function. The study site varied substantially from global TBI-references, reflecting the unique ensemble of environmental factors collectively regulating semi-arid savanna functioning.

From a global perspective, the presence of native LMH appears to be detrimental to the effective storing of carbon when compared to other systems. However, herbivore-induced disturbances drive a rapid nutrient turnover, promoting soil respiration, simultaneously facilitating carbon sequestration through driving new plant growth. Removal of native LMH from a herbivore-adapted system resulted in decreased decomposition, and in turn, higher stabilisation of detrital material which may potentially result in a larger proportion of sequestered carbon. Yet, this poses a serious threat to system resilience as herbivore exclusion is also related to reduced diversity. A sudden disturbance to this carbon sink could potentially result in a rapid, unmitigated release of carbon.

More studies should consider disturbance-driven African savannas, as most decomposition and associated carbon flux studies focus on temperate and boreal ecosystems of the northern hemisphere. Studies aimed at elucidating decomposition dynamics and the role of various influencing factors such as herbivory is essential to better understand and effectively manage soil function together with associated carbon cycling.

Chapter 7

A detailed account of potential decomposition rate and stabilisation factor at several incubation lengths across herbivore treatments

7.1 Preamble

Note that detailed soil analyses were not assessed as a separate objective in this study. Van Coller *et al.* (*in prep*) sampled and analysed soil data from the same study sites. Preliminary results were made available by the authors to better understand observed patterns of change in decomposition and microbial community structure.

7.2 Introduction

The process of litter breakdown comprises biotic and abiotic factors that function simultaneously across spatial and temporal scales, fundamentally linking above- and belowground nutrient pools (Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Keuskamp *et al.*, 2013; Eldridge *et al.*, 2019). Decomposition is essential to the effective functioning of terrestrial ecosystems as it is the primary determinant of nutrient cycling (Schlesinger, 1996; Bardgett *et al.*, 1998; Wang *et al.*, 2018; Eldridge *et al.*, 2019). In nutrient-limited systems this process is fundamentally important as it accumulates plant available nutrients, promoting further aboveground production and subsequently maintaining system functioning (Bardgett *et al.*, 1998; Wang & D'Odorico, 2008; Wang *et al.*, 2018; Eldridge *et al.*, 2019). Decomposition is governed by a range of environmental conditions which are subject to constant change, further contributing towards the dynamic nature of the process (Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Eldridge *et al.*, 2019).

Dynamic African savannas are primarily driven by LMH acting as ecosystem modifiers and drivers of change within above- and belowground structure through various trophic cascades (Grant & Scholes, 2006; Van Coller *et al.*, 2013; Holdo & Mack, 2014; Pausas & Bond, 2020; Vermeire *et al.*, 2021). The presence of native LMH adds to variation within soil function through numerous top-down and bottom-up mechanisms which directly and/or indirectly influence constituents of the decomposition subsystem (Bardgett *et al.*, 1998; García-Palacios *et al.*, 2016; Eldridge *et al.*, 2019;

Thoresen *et al.*, 2021; Vermeire *et al.*, 2021). LMH constitute major regulators of biogeochemical cycles (i.e. decomposition) as they link producer and decomposer subsystems through several mechanisms related to consumption, trampling and defecation (Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Wang *et al.*, 2018; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021; Erasmus *et al.*, 2022).

The effect of native LMH on system heterogeneity is readily observable in herbivore-adapted systems such as the sodic patches of the Nkuhlu exclosures in the KNP (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Siebert & Eckhardt, 2008; Van Coller *et al.*, 2013; Van Coller & Siebert, 2015). In these systems, herbivores are capable of actively altering above- and belowground structure through various direct and indirect pathways (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Scogings *et al.*, 2012; Van Coller *et al.*, 2013). Cascading top-down effects associated with herbivore-induced disturbances drive change within key environmental determinants of the decomposition subsystem including vegetation structure and edaphic properties (Bardgett *et al.*, 1998; Zhao *et al.*, 2011; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). It is suggested that due to the dynamic nature of sodic patches (Jacobs & Naiman, 2008), changes in soil function, in this case decomposition as a result of altered top-down drivers, will occur over a relatively short period of time (Erasmus *et al.*, 2022). Decomposition comprises varying stages of breakdown and associated rates with relation to the chemical composition of detrital substrate, alluding to temporal dynamics (Keuskamp *et al.*, 2013; Didion *et al.*, 2016; Blume-werry *et al.*, 2021). As such, once-off measurements of decomposition should be avoided. Moreover, the influence of driving factors such as herbivory on constituents of the decomposition subsystem (e.g. vegetation structure and edaphic properties) vary over time in accordance with population dynamics across spatial and temporal scales. Despite numerous exclosure experiments which aimed to investigate herbivore-induced impacts, little is known about their effects on soil function. This chapter is aimed at determining the effect of varying herbivore treatments on potential decomposition rate (k) and stabilisation factor (S) across four incubation periods (three-, six-, nine- and twelve-months). Given the role of herbivory in promoting system heterogeneity and influencing resource connectivity across scales, it is hypothesised that decomposition rate (k) will be highest under herbivore pressure, whilst stabilisation factor (S) will be highest under herbivore exclusion throughout the four incubation periods. However, given the temporal dynamics of detrital material (changes in chemical composition) and that of semi-arid savanna ecosystems, it is further hypothesised that significant variation across treatments for decomposition rate (k) will occur at longer incubation periods (six- and nine-months) than that suggested for global comparison (three-months). The specific objective for this study was to determine the effect of varying herbivore

treatments and intensities on decomposition rate (k) and stabilisation factor (S) of detrital plant material through the application of the extended, site-specific TBI approach across four incubation periods within the sodic zone of the Nkuhlu exclosures.

7.3 Materials and Methods

Methodology conformed to the extended, site-specific version of the TBI approach as described in Erasmus *et al.* (2021). As a means to elaborate on the temporal dynamics of decomposition in relation to herbivore-induced aboveground disturbances, the suggested four incubation periods (i.e. three-, six-, nine- and twelve-months) have been included in this study.

Physical and chemical properties of soil were not analysed for the purpose of this study. However, a study running parallel to this study, included soil data collection, laboratory analyses and interpretations of the data. Since the data have not yet been accepted for publication, the authors made the data available for use in this chapter, to explain the observed patterns of potential decomposition across herbivore treatments according to soil properties. Soil physicochemical properties in the study area will be discussed with reference to Van Coller *et al.* (*in prep*).

7.3.1 Statistical analyses

To assess the effects of herbivory on edaphic properties and soil function (decomposition rate - k and stabilisation factor - S), all statistical analyses were performed using R version 4.1.0 (R Core Team, 2021). Significance levels for all analyses were set at the standard significance level ($p < 0.05$) enabling confident conclusions made from statistical results. To test the assumptions of normality and homogeneity of variance, the 2019-2020 TBI-dataset was subjected to visual inspection (Kernel density plots and Q-Q plots), Shapiro–Wilk and Bartlett tests. The 2019-2020 TBI-dataset showed equal variance across samples ($p > 0.05$) but did not meet the assumption of normality ($p < 0.05$). As such, a Kruskal-Wallis One-way Analysis of Variance (ANOVA) was applied to the dataset to test for significant variation in decomposition rate (k) and stabilisation factor (S) of standardised plant material across the three herbivore treatments (i.e. Elephant exclosure, Control, LMH exclosure -Figure 14). Where statistically significant variance among treatments were attained, a *post hoc* Dunn test was applied to test for significant differences between treatments.

7.4 Results

7.4.1 Decomposition rate (k) and stabilisation factor (S) of organic material

Across four incubation periods (i.e. three-, six-, nine- and twelve-months) decomposition rate (k) decreased with increasing stabilisation factor (S) (Figure 21a-h). Decomposition rate (k) was consistently highest in the Control site with all LMH present whereas stabilisation factor (S) was consistently highest in the LMH exclosure. Throughout four incubation periods, the Elephant exclosure maintained the middle ground between the Control site and the LMH exclosure for both decomposition rate (k) and stabilisation factor (S).

Both decomposition rate (k) and stabilisation factor (S) varied significantly across the three herbivore treatments after a six-month incubation period (Kruskal-Wallis test: $p < 0.05$; Figure 21c and d). A three-month incubation period was too short to attain significant variation in decomposition rate (k) (Kruskal-Wallis test: $p > 0.05$; Figure 21a), yet, significant variation was revealed for stabilisation factor (S) (Kruskal-Wallis test: $p > 0.05$; Figure 21b). Moreover, a nine-month incubation period was potentially too long to obtain significant variation in stabilisation factor (S) (Kruskal-Wallis test: $p > 0.05$; Figure 21f), but revealed significant variation in decomposition rate (k) (Kruskal-Wallis test: $p < 0.05$; Figure 21e). After a twelve-month incubation period there was no significant variance among treatments for either decomposition rate (k) or stabilisation factor (S) (Kruskal-Wallis test: $p > 0.05$; Figure 21g and h). Where significant variation was attained, *post hoc* analyses revealed significant differences in decomposition rate (k) between the Control site with all LMH present and the LMH exclosure, with significantly higher values in the Control site (Dunn test: $p < 0.05$; Figure 21c and e). Significant differences in stabilisation factor (S) were also obtained for these two treatments (With all LMH and LMH exclosure), but with significantly higher values in the LMH exclosure (Dunn test: $p < 0.05$; Figure 21b and d).

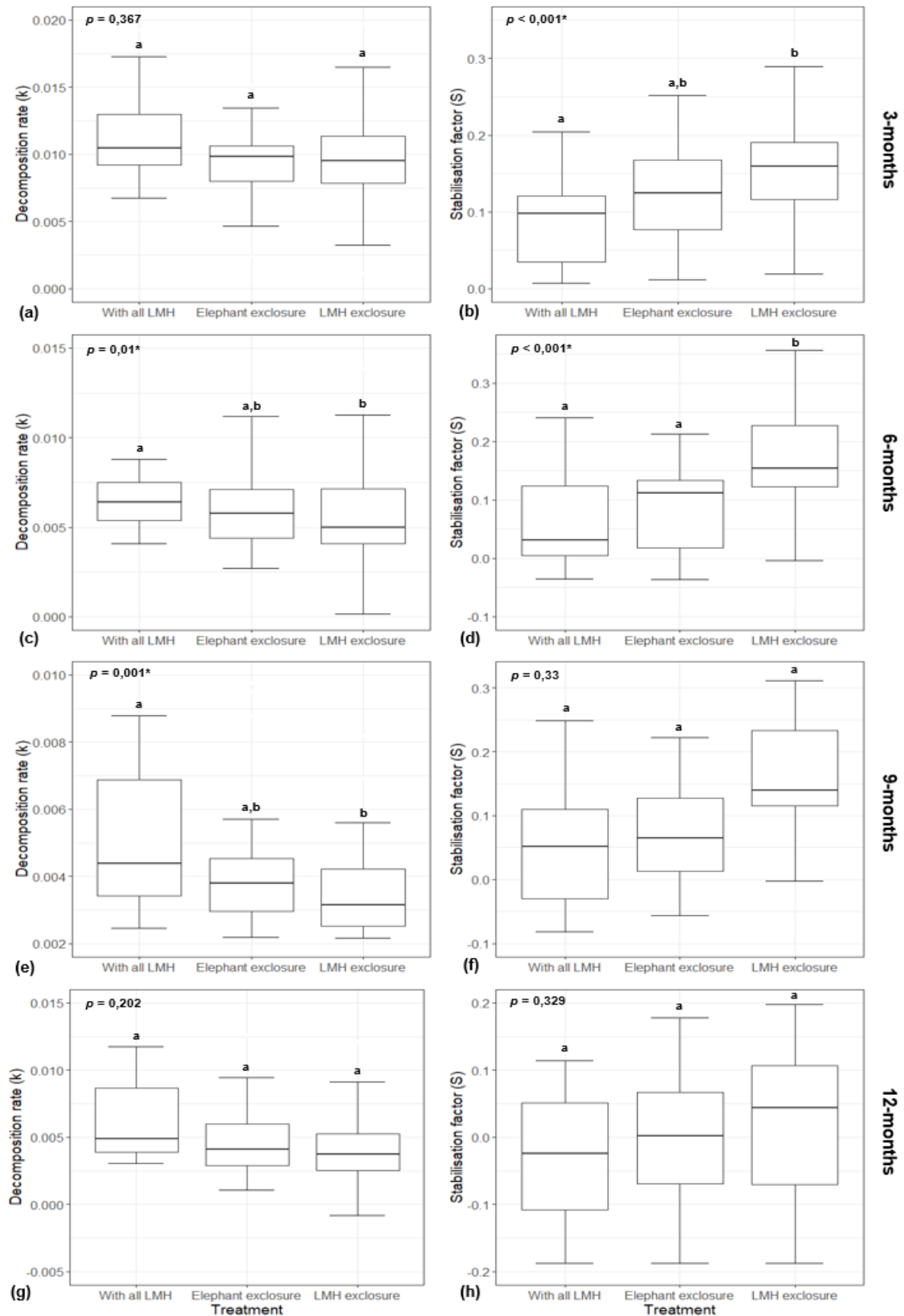


Figure 21: Boxplots indicating the median (central line in box), interquartile range (box) and extreme (whiskers) decomposition rate - k (a, c, e, g) and stabilisation factor - S (b, d, f, h)

values of standardised litter after a 3-, 6-, 9- and 12-month incubation period across three herbivore treatments within the Nkuhlu enclosures, Kruger National Park. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b).

With all LMH (Control), all herbivores present; Elephant enclosure, elephants (and giraffes) excluded; LMH enclosure, all LMH excluded; $p, p < 0.05$ indicates a significant difference between groups as assessed using the Kruskal-Wallis test.

7.4.2 Soil properties

Existing soil analyses of the study area were undertaken for two herbivore treatments (i.e. With herbivores and No herbivores) (Van Coller *et al.*, *in prep*). Data of specific relevance were extracted from the original results, and comprised of several edaphic properties, including pH, CEC, calcium (Ca), phosphate (PO_4), nitrate (NO_3), Soil bulk density and Leaf litter (Table 1).

Results revealed a significant positive association between several soil chemical properties (NO_3 , Ca, PO_4 and CEC and herbivore presence (Figure 22a-d), whilst herbivore exclusion was shown to promote improved soil physical condition in terms of significantly lower soil bulk density (Figure 22e) and significantly higher levels of leaf litter (Figure 22f).

Table 1: One-way ANOVA results and mean values for soil attributes and leaf litter from the soil A-horizon across herbivore treatments. Significant variance across treatments is indicated in bold and with an asterisk (*). Data were obtained from Van Coller *et al.* (*in prep*) and are presented here with courtesy of the authors.

Variable	df	SS	MS	F	p
NO_3^-	22	6.40	0.29	5.02	0.035*
Ca	22	1.14	0.05	4.71	0.040*
CEC	22	0.12	0.01	4.57	0.044*
PO_4^{3-}	22	0.34	0.02	4.77	0.040*
Soil bulk density	22	0.01	0.001	5.27	0.032*
Leaf litter	22	3.63	0.17	25.63	< 0.001*

df, degrees of freedom; SS, sum-of-squares; MS, mean squares; F, F ratio; p , p -value

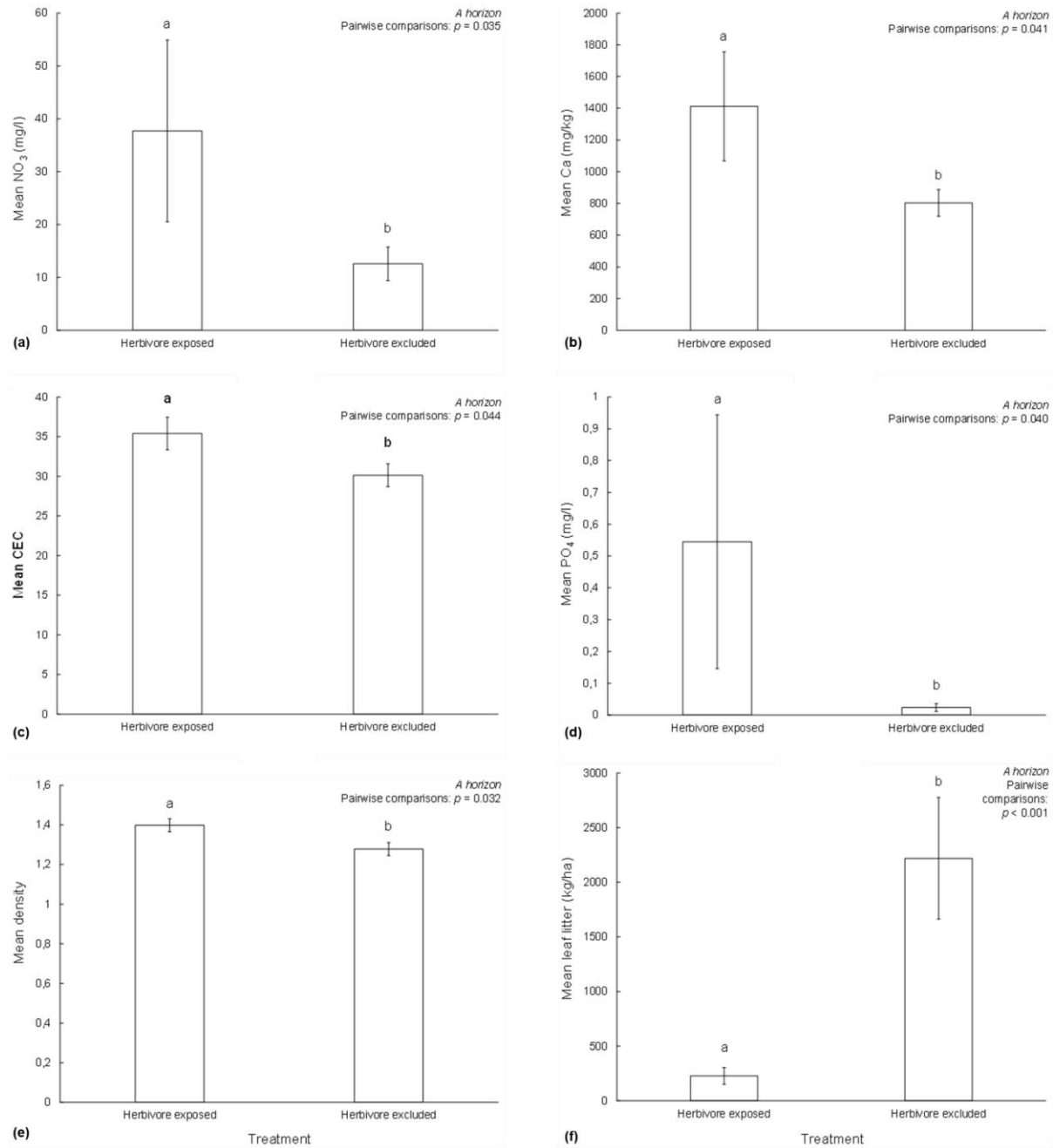


Figure 22: Mean differences between herbivore treatments (i.e. presence or absence) for selected soil properties (a-f). Significant differences ($p < 0.05$) between treatments are indicated with different lowercase letters (a and b).

All herbivores, all herbivores present; No herbivores, all LMH excluded

7.5 Discussion

7.5.1 Influence of herbivory on decomposition

Higher decomposition rates (k) in the presence of LMH, and the highest stabilisation factor (S) in their absence suggests the potential ability of LMH to influence decomposition through various direct and indirect feedback responses. Positive feedbacks between herbivores and the herbaceous layer are evident in nutrient-rich sodic patches given a substantial decrease in herbaceous species richness as aboveground biomass increases in the absence of LMH (Van Coller *et al.*, 2013; Van Coller & Siebert, 2015). These positive feedbacks can be ascribed to the combined effects of herbivory and sodic patches on both vegetation (Van Coller *et al.*, 2013; Van Coller & Siebert, 2015) and decomposition subsystem constituents. Congregated herbivore activity (e.g. Control site) within sodic patches is generally acknowledged to result in a reduction in standing biomass (Jacobs & Naiman, 2008; Van Coller *et al.*, 2013; Van Coller & Siebert, 2015). Reduced plant productivity is known to negatively affect the amount and distribution of detrital substrate that enters the decomposition subsystem (Bardgett *et al.*, 1998; Lui *et al.*, 2016; Eldridge *et al.*, 2019). Mean leaf litter was shown to be significantly lower in the presence of LMH (Van Coller *et al.*, *in prep*), essentially reducing the amount of detrital substrate, creating a C demand within soil microbial communities which promote a faster nutrient turnover (Bardgett *et al.*, 1998; Eldridge *et al.*, 2019). This is evident from the results as decomposition rate (k) was highest in the presence of all LMH. Moreover, reduced herbaceous and woody cover through defoliation and/or trampling (Grant & Scholes, 2006; Van Coller *et al.*, 2013) leads to more bare soil which facilitates higher levels of light penetration. This results in increased photodegradation and higher soil surface temperatures which has the potential to promote litter breakdown and microbial metabolic activity (Keuskamp *et al.*, 2013; Holdo & Mack, 2014; Becker & Kuzyakov, 2018; Eldridge *et al.*, 2019). Furthermore, reduced vegetation and by extension, detrital cover results in a much higher leaching potential as water is allowed to flow unrestricted through the soil profile, subsequently promoting decomposition via its abiotic pathway (Blume-werry *et al.*, 2021).

Herbivory is an important driver of plant species- and chemical composition as herbivores actively select for high quality grazing-tolerant forage (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Siebert & Scogings, 2015). It has been shown that plant regrowth post herbivore-induced defoliation often stimulates increased nutrient concentrations within aboveground biomass (Semmartin *et al.*, 2008; Scogings *et al.*, 2012; Siebert & Scogings, 2015). Moreover, Scogings *et al.* (2012) concluded that high intensity utilisation of savanna-based vegetation resulted in decreased carbon-based secondary metabolites (CBSM's) (e.g.

tannins and polyphenols) and increased N:C concentrations within vegetation tissue. Highly utilised vegetation is subsequently rendered to be more palatable and labile (Scogings *et al.*, 2012; Siebert & Scogings, 2015). Further studies done by Grant and Scholes (2006) as well as Siebert and Scogings (2015) indicate that in semi-arid sodic savanna systems congregated herbivorous activity has a positive feedback response whereby trampling of vegetation, defoliation and excretion contribute to the nutritive state of vegetation. This has a direct influence on the quality of detrital material that enters the decomposition subsystem, subsequently promoting the input of high-quality detritus into the detrital pool (Bardgett *et al.*, 1998; Semmartin *et al.*, 2008; Schrama *et al.*, 2013; Eldridge *et al.*, 2019).

The Nkuhlu study site represents a productive ecosystem dominated by palatable, nutrient-rich vegetation capable of supporting large, congregated herbivore communities (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Van Coller *et al.*, 2013). It is thought that in productive ecosystems, positive effects of native LMH can potentially outweigh their negative effects (Vandegehuchte *et al.*, 2017). Positive nutrient feedbacks resulting from sustained herbivore activity promotes the rapid return of labile nutrients to the soil, facilitating increased levels of microbial activity and subsequent breakdown of detrital material (Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Eldridge *et al.*, 2019). Higher decomposition rates (k) in the presence of all LMH therefore can be ascribed to LMH actively selecting for palatable vegetation high in nutrients which in turn promotes the production of high-quality plant-litter, inherently promoting decomposition (Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Wang *et al.*, 2018; Eldridge *et al.*, 2019; Erasmus *et al.*, 2022).

Defoliation associated with sustained herbivore activity has the potential to induce rapid changes in nutrient allocation to roots with regards to C and N (Bardgett *et al.*, 1998; Wang *et al.*, 2018). This changes the chemical composition of root tissue, and by extension belowground detrital material and also root exudation (Bardgett *et al.*, 1998). Increased root exudation promotes the availability of soil extractable C in the rhizosphere (Bardgett *et al.*, 1998; Vandegehuchte *et al.*, 2015), which may in turn influence decomposition rates by changing belowground detrital chemical composition and subsequently stimulating microbial metabolic activity (Bardgett *et al.*, 1998; Hamilton & Frank, 2001; Bardgett & Wardle, 2003; Bardgett & Wardle, 2010; Crocker, 2015; Vandegehuchte *et al.*, 2015).

Excretions of LMH can directly influence patch-specific nutrient pools through returning readily accessible nutrients in the form of urine (i.e. N in inorganic form) and/or dung (i.e. high-quality detritus with low C:N ratios) to the soil (Bardgett *et al.*, 1998; Bakker *et al.*, 2004; Semmartin *et al.*, 2008; Holdo & Mack, 2014; Eldridge *et al.*, 2019). LMH excretions are capable of stimulating microbial activity and the subsequent breakdown of plant material, resulting in the

production of ammonium (NH_4^+) through mineralisation (Schmidt, 1982; Sankaran & Augustine, 2004; Semmartin *et al.*, 2008; Eldridge *et al.*, 2019). LMH therefore have the capability to increase soil N availability which stimulates further decomposition of organic material (Schmidt, 1982; Sankaran & Augustine, 2004; Semmartin *et al.*, 2008; Eldridge *et al.*, 2019). This is supported by existing soil data from the study area which revealed significantly higher levels of NO_3^- in the presence of all LMH (Van Coller *et al.*, *in prep.*), potentially providing an explanation for significantly higher levels of decomposition through enhanced microbial activity.

Stabilisation of organic matter (stabilisation factor -S) increased along with a decrease in herbivory pressure. Decreased herbivory correlates with higher levels of secondary metabolites which are mainly recalcitrant compounds with a high C:N ratio (Scogings *et al.*, 2012; Scogings *et al.*, 2014). Decomposition is primarily regulated by the chemical composition of detrital substrate (Keuskamp *et al.*, 2013; Eldridge *et al.*, 2019). As such, recalcitrant material associated with secondary metabolites and low-quality forage (low N:C ratios) limits decomposition through inhibiting biological breakdown, promoting the formation of SOM (Keuskamp *et al.*, 2013; Eldridge *et al.*, 2019). Increased SOM results in fertile soil with a higher productivity potential (biomass production) as was observed in the LMH enclosure (Van Coller *et al.*, 2013; Van Coller & Siebert, 2015). Moreover, stabilisation of organic material results in a larger fraction of the initial fixated C to be stored in organic form (i.e. SOM) resulting in a slower carbon cycle (Wang & D'Odorico, 2008; Cotrufo *et al.*, 2015).

7.5.2 Influence of time on decomposition

Decomposition is a time-bound process, essentially regulated by the chemical composition of detrital substrate (Keuskamp *et al.*, 2013; Eldridge *et al.*, 2019; Blume-Werry *et al.*, 2021). The chemical composition of detrital material residing in the decomposition subsystem is subjected to constant change as numerous biotic and abiotic factors influence it. These factors include seasonal-bound climatic determinants such as temperature and precipitation together with detritivore and microbial activity (Becker & Kuzyakov, 2018; Teo *et al.*, 2020; Thoresen *et al.*, 2021; Vermeire *et al.*, 2021). Variation within decomposition rate (k) and stabilisation factor (S) across incubation periods can potentially be ascribed to changes within the chemical composition of standardised detrital material.

Initial stage decomposition in the control site can predominantly be ascribed to leaching associated with precipitation events and/or soil moisture due to the high amount of water-soluble compounds found within standardised tea litter (Blume-Werry *et al.*, 2021). As the

three-month incubation period coincided with the wet season of the study area (Figure 3), high decomposition rates (k) across all three treatments may be ascribed to leaching.

During the process of decomposition, labile compounds originating from detrital material are preferentially targeted and quickly broken down either through biotic and/or abiotic processes (Keuskamp *et al.*, 2013; Eldridge *et al.*, 2019). As time progresses and labile compounds are removed, detrital material stabilises and becomes more recalcitrant, restricting decomposition as it breaks down much slower, if at all (Keuskamp *et al.*, 2013). After the three-month incubation period most of the labile material had been lost, largely due to leaching and/or biotic factors. The material that persisted, was most likely more recalcitrant, and any further decomposition could predominantly be ascribed to biological processes and activity that is representative of prevailing system conditions. After a six- and nine-month incubation period decomposition rate (k) was significantly higher in the Control site with all LMH present when compared to the LMH exclosure. Contrariwise, after six months stabilisation factor (S) was significantly higher for the LMH exclosure in comparison to the Control site with all LMH present. Both the six- and nine-month incubation periods fell within the dry season of the study area. Despite reduced forage, sodic vegetation has the ability to sustain congregated LMH communities throughout the dry season (Jacobs & Naiman, 2008; Van Coller *et al.*, 2013). Sodic patches are therefore preferentially occupied during dry periods (Van Coller *et al.*, 2013). This sustained congregated activity can potentially further promote the positive effects of LMH on decomposition in these areas with regards to nutrient enrichment which stimulate microbial activity. This correlates with significantly higher decomposition rates (k) in the Control site compared to the LMH exclosure during the dry season (Figure 17 & 18).

After a twelve-month incubation period there was no significant difference for either decomposition rate (k) or stabilisation factor (S). This can potentially be ascribed to the chemical composition of the standardised tea litter as most to all of the labile compounds have been broken down, leaving only recalcitrant materials.

7.6 Conclusion

TBI-based decomposition studies provide a relatively easy approach to obtain valuable information regarding decomposition dynamics and the influence of system disturbances. This study highlights the set of complex cross-scale interactions that collectively regulate decomposition and stabilisation (nutrient cycling) in a semi-arid African savanna. Results indicated the importance of native LMH in maintaining ecosystem functioning (in this case, decomposition) through regulating system structure. Variance in decomposition rate (k) across

herbivore- and temporal treatments can potentially be attributed to the range of direct and indirect effects of native LMH on landscape and resource heterogeneity together with the temporal influence on chemical composition of detrital substrate. Studies aimed at elucidating decomposition dynamics and the various regulating factors such as herbivory are essential to better understand and effectively manage soil function in complex African savannas.

Furthermore, the results discussed in this chapter suggest that once-off measurements of decomposition do not represent the full complement of subsystem dynamics in disturbance-prone and seasonal ecosystems, such as African savannas. Globally, a three-month incubation period is generally considered to be sufficient to capture accurate TBI-based decomposition results. However, obtained results indicate that a three-month incubation period is too short in seasonal, African ecosystems, since significant variation in decomposition rate (k) was obtained only after a six-month incubation period. A nine-month incubation period is still feasible, although twelve-months are too long as the weight loss of rooibos tea litter approaches its limit value, potentially leading to inaccurate estimations of k . Since the TBI was tested in European ecosystems, which are remarkably different compared to the drylands of Africa, it is expected that optimal incubation periods may differ among these ecosystems. Subsequently, it is suggested that three TBI-based incubation periods i.e. three-, six- and nine-months should be implemented to accurately capture decomposition dynamics in complex African savannas.

Chapter 8

Soil microbial community structure across various herbivore intensities

8.1 Introduction

African savannas are highly dynamic, consumer-controlled systems with a strong relation to rainfall seasonality and multiple agents of disturbance (Scholes & Archer, 1997; Pausas & Bond, 2020). Within these systems, LMH activity together with seasonal-based wet and dry cycles have a substantial influence on vegetation structure and edaphic properties (Scholes & Archer, 1997; Adler *et al.*, 2001; Jacobs & Naiman, 2008; Van Coller *et al.*, 2013; Holdo & Mack, 2014; Pausas & Bond, 2020). Changes to these structural components has a cross-scale, top-down cascading effect, resulting in alterations to constituent determinants of belowground structure, such as microbial community composition (Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Zhao *et al.*, 2017; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021).

Anthropogenic-driven land-use change pertaining to the loss of the diverse suite of native LMH, especially in African savannas, poses a serious environmental concern (Koerner *et al.*, 2014) with numerous well documented aboveground disturbances (Jacobs & Naiman, 2008; Scogings *et al.*, 2012; Van Coller *et al.*, 2013; Holdo & Mack., 2014). Yet, little is known about the long-term effects of system-specific drivers of change, such as LMH, or the loss thereof, on soil microbial community heterogeneity and whether this is similar to traditional models of disturbance demonstrated in plant communities (Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Zhao *et al.*, 2017; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021).

A recent study conducted by Vermeire *et al.* (2021) within the crest and riparian zone of the Nkuhlu exclosures reported that LMH are capable of inducing significant changes within microbial community composition (bacterial and fungal) through several direct and indirect mechanisms pertaining to alterations in vegetation structure and edaphic properties. Given the evolutionary development of sodic patches with congregated herbivore-induced disturbances, it is expected that these patches, in terms of soil microbial structure, may be more resistant to change.

This Chapter aims to examine the effect of varying herbivore treatments and intensities on soil microbial community structure through the amplification of bacterial 16S rRNA and the fungal ITS region. Given the link between soil microbes, vegetation structure and edaphic properties

(Bardgett *et al.*, 1998; Wardle *et al.*, 2004; Zhao *et al.*, 2011; Yang *et al.*, 2013; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021), it is hypothesised that soil microbial community composition will differ across various herbivore treatments. Microbial communities' variable metabolic capacity and tolerance towards disturbance led to the hypothesis that bacterial α -diversity will be highest in the presence of LMH, whilst herbivore exclusion will favour fungal α -diversity.

8.2 Materials & Methods

8.2.1 Soil sampling

Soil sampling was conducted in the dry (October 2019) and wet (January 2020) season of the southern KNP. Five samples were collected from a 1-8 cm depth with a small spade within each of the five sampled treatments (Figure 14), per season. The spade was thoroughly cleaned with 96% ethanol after each sample to reduce the potential for cross-contamination. Samples were placed in resealable plastic bags and preserved at -80 °C until DNA extraction.

8.2.2 DNA extraction

Genomic analyses were applied to fifty soil samples (25 wet season samples and 25 dry season samples). Microbial DNA was extracted from 0.25 g of defrosted soil using the Macherey-Nagel NucleoSpin® soil kit (Macherey-Nagel, Duren, Germany), following the manufacturer's instructions.

8.2.3 High-throughput sequencing of bacterial and fungal ribosomal markers and data processing

Following DNA isolation, the V3–V4 region of the bacterial 16S rRNA was amplified using the universal bacterial primers 341F (forward) and 805R (reverse) (Klindworth *et al.*, 2013), while for fungi, the ITS region (ITS2) was amplified using the forward primer ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and the reverse primer ITS4 (5'-TCCTC CGCTTATTGATATGC-3') (White *et al.*, 1990). Each forward and reverse primer contained Illumina forward and reverse overhang adapters, respectively (Illumina Inc., Foster City, CA, USA). Library preparation steps were performed as described previously (Ezekiel *et al.*, 2019).

Briefly, the library preparation protocol entailed a first “amplicon PCR” step involving 12.5 ng DNA, 0.2 μ M of each forward and reverse primers, 12.5 μ L of KAPA HiFi HotStart ready Mix [0.5 U DNA polymerase, 0.3 mM dNTPs, 2.5 mM MgCl₂] (Kapa Biosystems, Wilmington, MA, USA) and nuclease-free water (Thermo Fisher Scientific, Waltham, MA, USA) in a final

reaction volume of 25 μ L. Furthermore, PCR amplicons were purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). The libraries were amplified with a limited-cycle PCR program (10 cycles) to add the index 1 (i7) and index 2 (i5) adapters, containing sequences required for cluster generation of the Illumina flow cell. The quality and sizes of the resulting DNA fragments were evaluated on a 2% (w/v) agarose gel. The libraries were quantified with a fluorometric method (Qubit, Life Technologies) and normalized to 4 nM using a standard dilution method. The libraries were pooled, denatured with 0.1 N NaOH, and diluted to the final loading concentration of 4 pMol. An identically treated PhiX Control v3 adapter-ligated library at a low-concentration spike-in of 10% was added as an in-lane positive control for alignment calculations and quantification efficiency. Paired-end sequencing was done on an Illumina MiSeq system (Illumina, San Diego, CA, USA) using a MiSeq Reagent Kit V3 600 cycles.

8.2.4 Bioinformatic and Diversity Analyses

Bacterial and fungal demultiplexed paired-end reads were quality-checked using FastQC software version 0.11.5 (Babraham Institute, United Kingdom). Subsequently, Trimmomatic software (version 0.38) (Bolger *et al.*, 2014) was used to quality-trim paired-reads, including clipping off any Illumina barcodes and eliminating reads with an average quality score (Phred Q score) lower than 20. For the bacterial reads short and low-quality sequences that contained less than 300 bp and ambiguous bases were removed. Whilst for fungi only ambiguous bases were removed. Clean paired reads were merged, depleted of chimeric sequences, and clustered into amplicon sequence variants (ASV) (operational definition for a species) by using the DADA2 (Divisive Amplicon Denoising Algorithm v. 2) denoiser (Callahan *et al.*, 2016). Pruning of ASVs with a low number of sequences (<5) was conducted on a per-sample basis, as an ASV that is common in one sample may occur as a low-abundance contaminant in others due to cross-contamination. The most abundant sequence of each ASV was selected as representative. The denoised reads were assigned taxonomy by the Silva 16S rRNA taxonomy (Release 132) (Quast *et al.*, 2013) using a trained classifier of the V3-V4 region. Furthermore, fungal ITS2 representative sequences were assigned against UNITE v7 sequence database (Kõljalg *et al.*, 2013) using the Bayesian classifier as implemented in MOTHUR (Schloss *et al.*, 2009).

Post quality filtering and the removal of chimera sequences, amplicon sequencing generated a total of 49,628 bacterial 16S rRNA genes (average of $2,626 \pm 387$ per sample) and 296,535 fungal ITS2 (average of $10,887 \pm 268$ per sample) high-quality sequences. Bacterial and fungal α -diversity were estimated by calculating observed richness based on observed OTUs

ASVs and Shannon-Wiener diversity index values per sample, computed in QIIME2 core_2019.10. Moreover, for further analyses, rare microbial taxa were removed, only retaining ASVs with a total relative abundance of > 0.001 of the total reads.

8.2.5 Deposition of Sequences

Sequence data generated for this study are available in the Sequence Read Archives (SRA) of the National Centre for Biotechnological Information (NCBI) under the accession number, PRJNA782813.

8.2.6 Statistical analyses

Statistical analysis of microbiome data was performed using marker-gene data profiling in MicrobiomeAnalyst (Xia Lab, McGill University, Quebec, Canada) (Dhariwal *et al.*, 2017). Recently published methods on data processing, normalisation, and profiling by Chong *et al.* (2020) were utilised for this study. A low count filter was used to filter all features with <4 counts in at least 20% of values. Furthermore, features with $<10\%$ variance, based on the inter-quartile rank, between experimental conditions (With all LMH, Elephant enclosure, and LMH enclosure) as well as seasons (Dry and Wet) were filtered using a low variance filter. Both bacterial and fungal samples were rarefied to even sequencing depth using the minimum library size of each. Finally, for data scaling, total sum scaling was applied.

A Bray-Curtis Index distance method was used to calculate β -diversity in PERMANOVA. Since results based on sequencing of both the V3 and V4 regions and ITS3 and ITS4 regions, respectively, are not reliable for species level, no further analyses were pursued for this taxonomic level. Moreover, altered bacterial and fungal taxa with <10 absolute counts were considered as not biologically relevant and excluded. In addition, a Wilcoxon signed rank test was used to assess differences between the different treatments (i.e. With all LMH vs Elephant enclosure, LMH enclosure vs Elephant enclosure, LMH enclosure vs With all LMH), and displayed visually using heat tree analysis (results from Wilcoxon signed rank tests are provided as supplementary material in Appendix C, Table C1-C6). Furthermore, MicrobiomeAnalyst was utilised to perform the classification of a random forest to elucidate the most important differentiating factors (genera) between the different herbivore treatments.

Observed ASV richness as well as Shannon-Wiener diversity index values were used to calculate α -diversity. All downstream statistical analyses were performed using R software (R Core Team, 2021 -version 4.1.0). Significance levels for all analyses were set at the standard significance level ($p < 0.05$) enabling confident conclusions made from statistical results. To

check the assumptions of normality and homogeneity of variance, bacterial and fungal α -diversity data were subjected to both visual inspection (Kernel density plots and Q-Q plots), Shapiro–Wilk and Bartlett tests. One-way Analysis of Variance (ANOVA) was applied to test for significant variation among the treatments. Where significant variation was obtained a *post hoc* Tukey’s Honestly Significant Difference (HSD) test was applied to further analyse the data.

8.3 Results

8.3.1 Bacterial Diversity and Community Differentiation

Following quality control (i.e. read trimming, low-quality filtering, denoising, and chimeric sequences removal), a total of 38,860 (2073 ± 809 per sample) bacterial and 264,187 (10052 ± 151 per sample) fungal high-quality reads were clustered into ASVs, respectively.

Both the observed richness and Shannon-Wiener diversity index (α -diversity) values for bacteria (Figure 23a and c) and fungi (Figure 23b and d) showed no significant variation (ANOVA: $p = 0.177$, $p = 0.226$ and $p = 0.117$, $p = 0.051$, respectively) across the three herbivore treatments for combined dry and wet seasons. Bacterial α -diversity was highest under herbivore pressure (Treatment with all LMH and Elephant enclosure; Figure 23a and c). On the contrary, fungal richness was highest under herbivore exclusion (LMH enclosure; Figure 23b), whilst diversity was shown to be marginally higher under intermediate levels of disturbance (Elephant enclosure) and lowest in the presence of all LMH (Figure 23d).

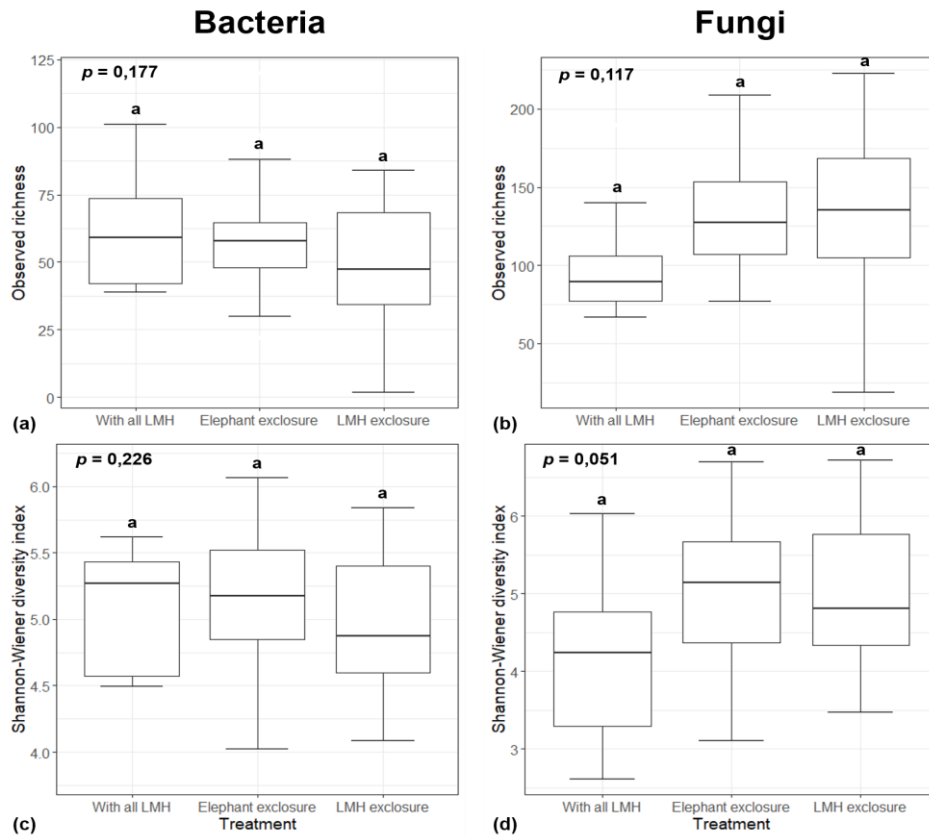


Figure 23: Mean bacterial and fungal richness and Shannon-Wiener diversity index values for combined dry and wet seasons across three herbivore treatments within the Nkuhlu exclosures, Kruger National Park. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b).

Central line in box, median; box, interquartile range; extreme values, whiskers; With all LMH (Control), all herbivores present; Elephant exclosure, elephants (and giraffes) excluded; LMH exclosure, all LMH excluded; p , $p < 0.05$ indicates a significant variance between groups as assessed using One-way ANOVA.

Although not always significant, herbivory modified bacterial and fungal richness and diversity in all treatments and seasons. Significant changes in α -diversity were observed in the dry season for fungal communities across herbivore treatments (Table 2). Herbivore pressure was shown to have a significant negative effect on dry season fungal community richness and diversity (significant decrease of the index with increased herbivore-induced disturbances; ANOVA: $p = 0.0329$ and $p = 0.0363$, respectively). Alpha-diversity measures for the dry season bacterial and wet season fungal communities were shown to be highest under intermediate levels of disturbance (Elephant exclosure). Conversely, wet season bacterial α -diversity was highest in the presence of LMH.

Table 2: Bacterial and fungal α -diversity (observed richness and Shannon-Wiener diversity index) over two seasons (October 2019 dry season and January 2020 wet season) and across three herbivore treatments (With all LMH, Elephant exclosure, LMH exclosure). Values are means and $\pm$ standard deviation per treatment. Significant differences between herbivore treatments are represented by lowercase letters and bold characters (i.e. a and b) as measured by One-way Analysis of Variance together with *post hoc* Tukey HSD tests.

Semi-arid sodic savanna	Season	Treatment	Bacteria		Fungi	
			Richness	Shannon	Richness	Shannon
	Dry	With all LMH	46 $\pm$ 15	4.7 $\pm$ 0.4	100 $\pm$ 51^a	3.8 $\pm$ 1.4^a
		Elephant exclosure	57 $\pm$ 18	5.1 $\pm$ 0.4	147 $\pm$ 35^{ab}	5.1 $\pm$ 1.2^{ab}
		LMH exclosure	44 $\pm$ 12	4.7 $\pm$ 0.4	158 $\pm$ 34^b	5.5 $\pm$ 0.9^b
	Wet	With all LMH	70 $\pm$ 22	5.3 $\pm$ 0.4	102 $\pm$ 24	4.5 $\pm$ 0.5
		Elephant exclosure	65 $\pm$ 27	5.3 $\pm$ 0.6	113 $\pm$ 20	4.9 $\pm$ 0.6
		LMH exclosure	53 $\pm$ 30	4.5 $\pm$ 1.7	109 $\pm$ 52	4.5 $\pm$ 0.6

With all

LMH (Control), all herbivores present; Elephant exclosure, elephants (and giraffes) excluded; LMH exclosure, all LMH excluded; Dry season, October 2019; Wet season, January 2020.

In order of highest abundance, Firmicutes (relative abundance between 6 and 75% depending on the treatment), Proteobacteria (between 5.5 and 43.6%), Actinobacteria (between 4.5 and 32.6%) and Acidobacteria (between 2.9 and 25%) were the predominant bacterial phyla across all three treatments, whilst Bacteroidetes was present at very low abundance (Figure 24a). All phyla had only one representative class (i.e. *Bacilli*, *Alphaproteobacteria*, *Acidobacteriia* and *Bacteroidia*, respectively), except *Actinobacteria* that divided into two classes, *Actinobacteria* and *Rubrobacteria*. Moreover, *Bacilli*, *Acidobacteriia*, *Bacteroidia* and *Rubrobacteria* had one representative order, whereas both *Alphaproteobacteria* and *Actinobacteria* classes were divided into four representative orders (Figure 25a-c).

The dominant fungal phyla were Ascomycota (between 30 and 91%) and Basidiomycota (between 1 and 63%) (Figure 24b). Within the fungal population, five different phyla were observed together with a substantial unidentified grouping. Ascomycota was the predominant phylum and divided into three high proportion classes (i.e. Dothideomycetes, Sordariomycetes and Eurotiomycetes), each of which further gave rise to several high proportion orders (Figure 25d-f).

Both bacterial and fungal phylum compositions showed substantial variation over seasons (dry and wet) and treatments (With all LMH, Elephant exclosure, LMH exclosure). Firmicutes showed a positive correlation with herbivory over both seasons as higher relative abundance values were revealed with an increase in herbivore pressure. In contrast, Proteobacteria appeared to be negatively associated with herbivory, increasing with a release in herbivore pressure over two seasons. Actinobacteria followed a similar trend to that of Proteobacteria, whilst Acidobacteria maintained a higher relative abundance under intermediate levels of disturbance (Elephant exclosure) for the dry and wet season. Relative abundance of Bacteroidetes was shown to be lowest in the dry season and highest in the wet season, moreover, Bacteroidetes was found to be absent from the Control site (With all LMH) in the dry season (Figure 24a).

In contrast to bacterial phylum composition, fungal communities across herbivore treatments and seasons were essentially dominated by two phyla (Ascomycota and Basidiomycota), with several other phylums constituting a much lower relative abundance (Figure 24b). Ascomycota appeared to have a lower relative abundance under intermediate levels of disturbance (Elephant exclosure) for both dry and wet seasons, whilst maintaining a relatively similar abundance under complete herbivore exclusion and the presence of all LMH. Glomeromycota (associated with the mycorrhizal taxa) was shown to be absent under high herbivore-induced disturbances for both seasons, only occurring at very low relative abundances under complete herbivore exclusion for the dry season and both complete and partial herbivore exclusion in the wet season.

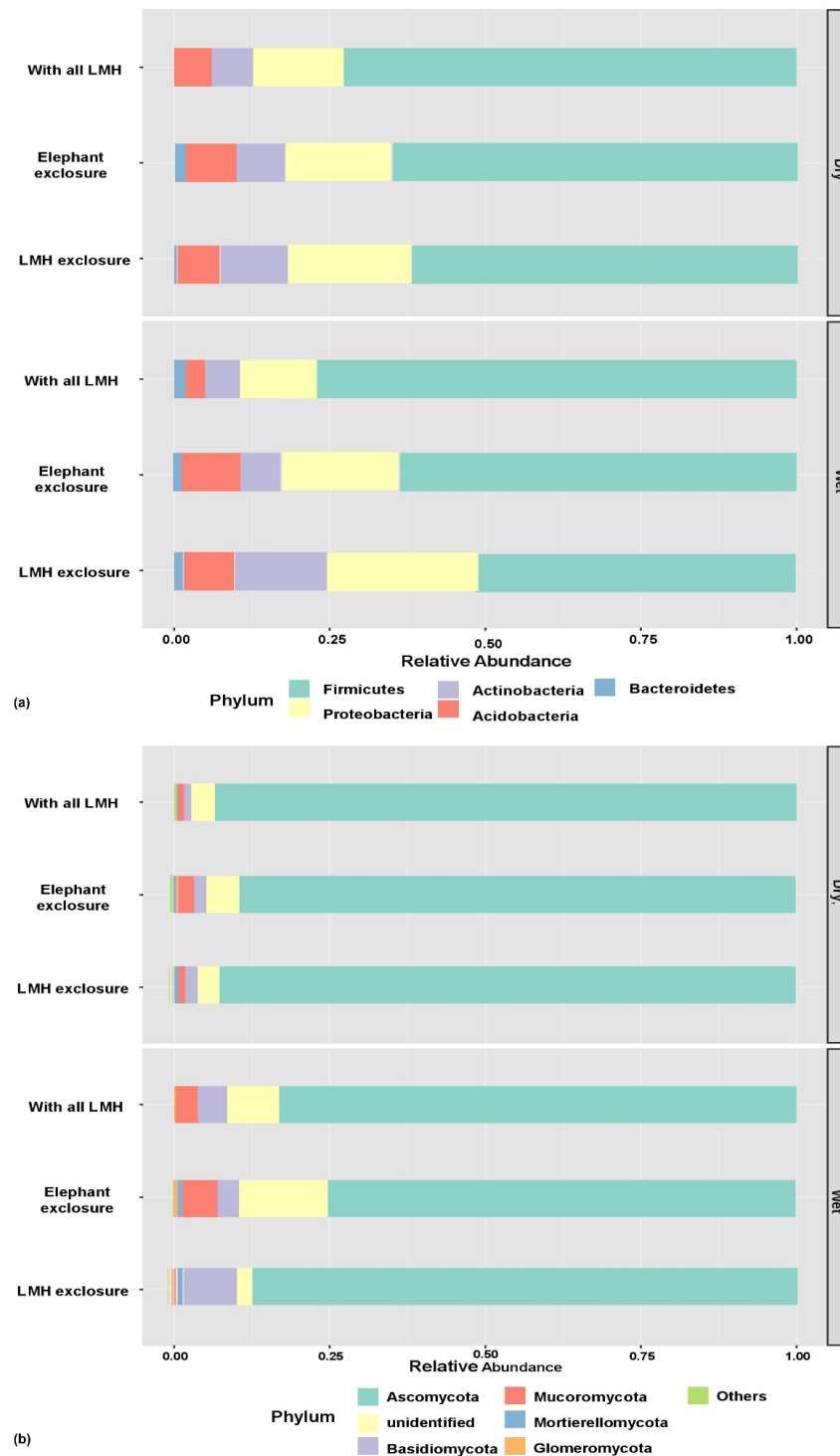


Figure 24: Bacterial (a) and fungal (b) phylum composition in terms of relative abundance over two seasons (October 2019 dry season and January 2020 wet season) and across three herbivore treatments (With all LMH, Elephant enclosure, LMH enclosure) within a semi-arid sodic savanna ecosystem.

With all LMH (Control), all herbivores present; Elephant enclosure, elephants (and giraffes) excluded; LMH enclosure, all LMH excluded.

A Wilcoxon-signed rank test, through the application of heat tree analysis in MicrobiomeAnalyst was utilised to ascertain significant variation in abundance for bacterial (Figure 25a-c) and fungal (Figure 25d-f) populations across herbivore treatments. Heat tree diagrams (Figure 25a-f) indicate variations in terms of taxa abundance and direction of change within specific treatments. Due to the immense amount of altered fungal taxa, not all significant alterations are presented under this section. However, a full compilation of altered bacterial and fungal taxa across the three herbivore treatments are provided as supplementary material (Table S1-S6) which shows the results for all Wilcoxon-signed rank tests and indicate significant variation as different coloured text.

Bacterial composition for the With all LMH vs Elephant enclosure treatment combination (Figure 25a) revealed significant variation in Firmicutes ($p = 0.049$) and Acidobacteria ($p = 0.0439$) on phylum level. Furthermore, significantly altered microbial composition was observed within the classes *Bacilli* ($p = 0.049$) and *Acidobacteriia* ($p = 0.0439$), orders *Bacillales* ($p = 0.049$) and *Solibacterales* ($p = 0.0439$), families *Bacillaceae* ($p = 0.0392$) and *Solibacteraceae* ($p = 0.0439$) as well as the genera *Bacillus* ($p = 0.0392$) and *Bryobacter* ($p = 0.0113$). Moreover, further variation was found within the orders *Frankiales* ($p = 0.0006$) and *Kineosporiales* ($p = 0.016$), families *Acidothermaceae* ($p = 0.0006$), *Kineosporiaceae* ($p = 0.016$) and the genera *Acidothermus* ($p = 0.0006$) and *Kineosporia* ($p = 0.016$). For fungal composition under the same treatment combination, significant alteration in abundance was observed for Ascomycota ($p = 0.0439$) and Mucoromycota ($p < 0.0231$) at phylum level (Figure 25d). Altered fungal composition was further observed within several classes, including *Eurotiomycetes* ($p = 0.0145$), *Dothideomycetes* ($p = 0.0244$), *Mucoromycetes* ($p = 0.0231$) and *Ustilaginomycetes* ($p = 0.013$).

Significant variation in bacterial phylum composition was only observed for Actinobacteria ($p = 0.0215$) for the LMH enclosure vs Elephant enclosure treatment combination (Figure 25b). Microbial composition revealed variation for the class *Actinobacteria* ($p = 0.036$), orders *Frankiales* ($p = 0.0034$), *Micrococcales* ($p < 0.0001$) and *Kineosporiales* ($p = 0.0007$), families *Acidothermaceae* ($p = 0.0034$), *Intrasporangiaceae* ($p < 0.0001$), *Kineosporiaceae* ($p = 0.0007$) and genera *Acidothermus* ($p = 0.0034$), *Lapillicoccus* ($p < 0.0001$) and *Kineosporia* ($p = 0.0007$). Furthermore, the order *Rhizobiales* ($p = 0.0312$), family *Xanthobacteraceae* ($p = 0.0312$) and the genera *Bradyrhizobium* ($p = 0.0312$) as well as *Bryobacter* ($p = 0.017$) showed further variation. Significant variation in fungal phylum abundance were shown for Ascomycota ($p = 0.0038$), Mucoromycota ($p < 0.0001$) and Mortierellomycota ($p = 0.0316$). Further variation in class composition was observed for *Mucoromycetes* ($p < 0.0001$), *Pezizomycetes* ($p = 0.00047$), *Mortierellomycetes* ($p = 0.0316$) and *Ustilaginomycetes* ($p < 0.0001$).

For the LMH enclosure vs With all LMH treatment combination (Figure 25c), significant variation within bacterial composition was revealed for Firmicutes ($p = 0.0051$), Proteobacteria ($p = 0.0165$) and Actinobacteria ($p = 0.0256$) on phylum level. Moreover, alteration in bacterial composition was observed for the classes Bacilli ($p = 0.0051$) and Alphaproteobacteria ($p = 0.0165$), order Bacillales ($p = 0.0051$), Elsterales ($p = 0.0067$), Sphingomonadales ($p = 0.0051$), families Bacillaceae ($p = 0.0051$) as well as Sphingomonadaceae ($p = 0.0412$) and the genera *Bacillus* ($p = 0.0051$) and *Sphingomonas* ($p = 0.0412$). Further compositional variation was revealed for the order Micrococcales ($p = 0.0007$), family Intrasporangiaceae ($p = 0.0007$) and genus *Lapillicoccus* ($p = 0.0007$). Fungal population composition only showed variation in phylum composition for Mortierellomycota ($p = 0.0139$) (Figure 25f). Moreover, further variation was shown on class level for Eurotiomycetes ($p = 0.0244$), Dothideomycetes ($p = 0.0145$), Mortierellomycetes ($p = 0.0139$) and Arthoniomycetes ($p = 0.0406$).

Lastly, analysis of the fungal ITS region yielded several unassigned sequences. This could potentially be ascribed to a lack of ITS sequence updates and/or curation within the UNITE database (López-García *et al.*, 2021).

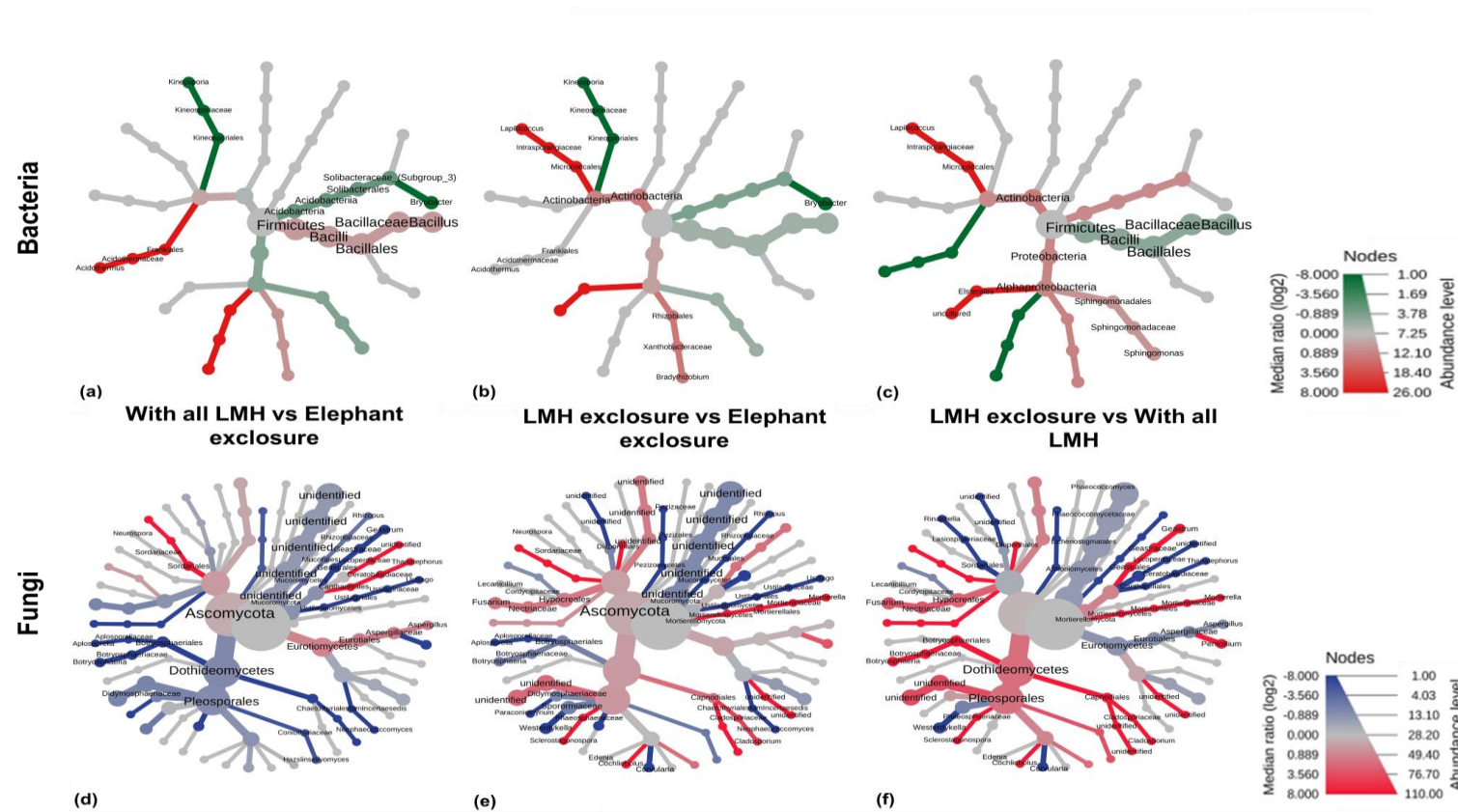


Figure 25: Heat tree analysis depicting variations in bacterial (a-c) and fungal (d-f) community composition (from phylum to genus) for combined dry and wet seasons between herbivore treatments (With all LMH, Elephant exclusion, LMH exclusion). Nodes indicate the hierarchical structure of taxa with significantly altered taxa displayed by name at the corresponding node. Red branches are indicative of an increase in a specific treatment compared to another treatment, whilst green (for bacteria) and blue (for fungi) branches indicate a decrease.

With all LMH (Control), all herbivores present; Elephant exclusion, elephants (and giraffes) excluded; LMH exclusion, all LMH excluded.

Random forests classification for bacteria (OOB error = 0.208, classification error for With all LMH = 0.30, Elephant enclosure = 0.10 and LMH enclosure = 0.278; Figure 26a) and for fungi (OOB error = 0.42, classification error for With all LMH = 0.7, Elephant enclosure = 0.4 and LMH enclosure = 0.3; Figure 26b) revealed *Lapillicoccus*, *Acidothermus*, *Bryobacter*, *Kineosporia* and *Bacillus* as the 5 most important differentiating factors which separate bacterial composition, whilst *Curvularia*, *Botryosphaeria*, *Rhizopus*, *Ustilago*, and *Westerdykella* are the 5 most important differentiating factors which separate fungal community composition of the three different herbivore treatments from one another.

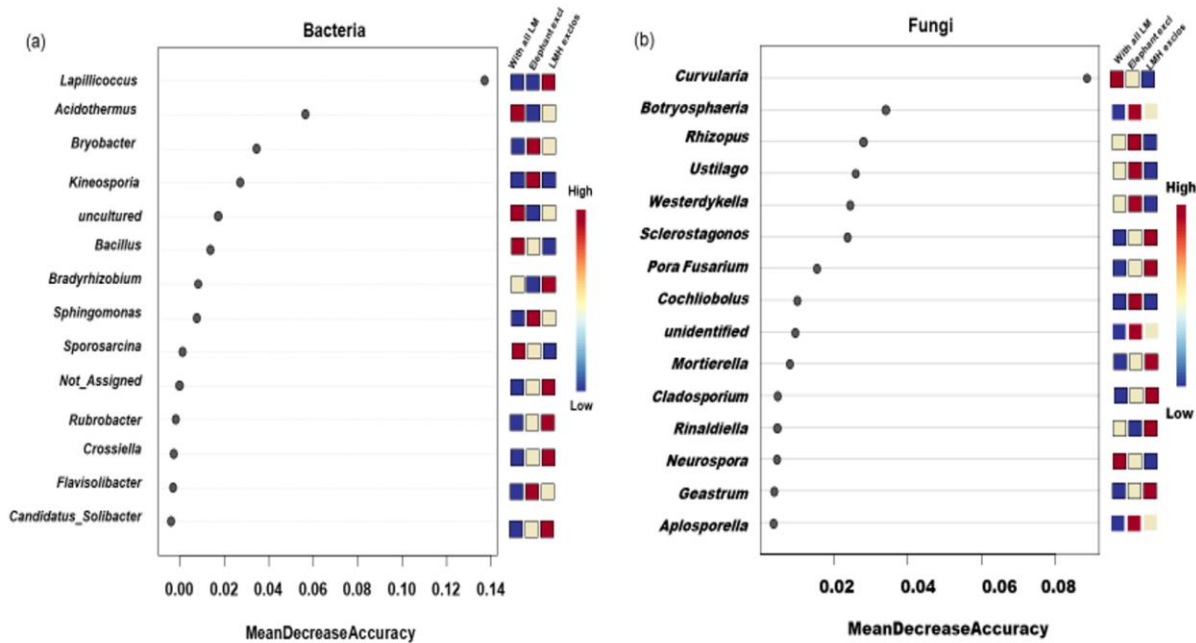


Figure 26: Unsupervised random forest classification of bacterial (a) and fungal (b) communities which utilises the three different herbivore treatments (With all LMH, Elephant enclosure, LMH enclosure) as classes and abundance values as classifiers along a decision tree. The Y-axis represents the 14 (bacteria) and 15 (fungi) most important classifying variables (genera), whilst the z-axis represents the mean decrease accuracy (MDA) where MDA is the measure of loss of accuracy if the y-axis variable is removed. A mini heatmap (red, yellow and blue squares in order of highest to lowest values) indicates abundance within treatments.

With all LMH (Control), all herbivores present; Elephant enclosure, elephants (and giraffes) excluded; LMH enclosure, all LMH excluded.

8.4 Discussion

The results obtained for α -diversity, indicated that LMH activity affects soil microbial composition in terms of richness and diversity. The apparent positive association between herbivore pressure and bacterial richness and diversity (Figure 23a and c) can predominantly be ascribed to the direct and indirect effects of native LMH on system heterogeneity (Bardgett *et al.*, 1998; Wardle *et al.*, 2002; Eldridge *et al.*, 2019; Vermeire *et al.*, 2021). LMH have been suggested to increase landscape and resource heterogeneity through various top-down feedback mechanisms (Bardgett *et al.*, 1998; Jacobs & Naiman, 2008; Pausas & Bond, 2020), essentially promoting niche differentiation and the facilitation of diverse bacterial communities (Bardgett *et al.*, 1998). In contrast, herbivore pressure was shown to have a negative effect on fungal richness (Figure 23b). Fungi have been noted to be more sensitive towards environmental disturbances, such as herbivory, than bacteria (Baldrian *et al.*, 2012; Vermeire *et al.*, 2021). In this study, higher levels of standing biomass associated with herbivore exclusion (Jacobs & Naiman, 2008; Van Coller *et al.*, 2013) (see Chapter 9) together with improved soil conditions (e.g. lower soil compaction and erosion of superficial soil layers) and a larger rhizospheric-area promoted fungal growth, and subsequent richness (Bardgett *et al.*, 1998; Vermeire *et al.*, 2021). Furthermore, diversity measures were shown to be marginally higher in the Elephant enclosure (Figure 23d), suggesting that fungal diversity potentially follows a similar trend than that described by the intermediate disturbance hypothesis (IDH). This can possibly be ascribed to higher resource heterogeneity which provides a habitat for a greater number of different species through niche partitioning. However, due to the amount of LMH-induced disturbances still present within the Elephant enclosure, richness may be suppressed. As expected, fungal diversity was lowest in the presence of all LMH, further supporting the notion that fungi are sensitive to disturbances associated with herbivory. In contrast to these findings, Vermeire *et al.* (2021) reported significant variation in bacterial and fungal richness for both the crest and riparian areas which are located above and below the sodic zone. Moreover, Vermeire *et al.* (2021) indicated significant variation in bacterial diversity in the crest sites. This further reflects the unique nature of the sodic zone and its potential stability with regards to microbial community response to disturbances.

With seasonality (i.e. dry and wet season) as an added variable, there appears to be a greater variation across herbivore treatments (Table 2). For the dry season, α -diversity of fungal communities revealed a significant positive effect in response to herbivore exclusion on richness and diversity. This can potentially be ascribed to system structure in terms of vegetation and edaphic properties across different herbivore treatments which either mitigate or exacerbate seasonal-based moisture limitations. Although not significant, it is interesting to note that dry season bacterial and wet season fungal α -diversity were higher under

intermediate levels of disturbance (Table 2). As plant species richness and diversity have often been described in relation to the IDH with regards to herbivore-induced disturbances, these results allude to a similar response by sodic-based microbial communities. Higher levels of moisture during the wet season can potentially result in a few taxa to dominate, essentially promoting competitive exclusion. Phylum composition (Figure 24b) obtained in this study, indicated two dominant phyla (Ascomycota and Basidiomycota) in the LMH enclosure which limit other phyla and potentially suppress species richness (Table 2). Conversely, the Elephant enclosure consisted of several phyla with higher relative abundance (Figure 24b). In addition, the phylum Mucoromycota was not present within the LMH enclosure, but was revealed to have a substantial relative abundance in comparison with the other phyla in the Elephant enclosure, strengthening the idea that competitive exclusion may be responsible for higher levels of fungal richness in the Elephant enclosure when compared to the LMH enclosure. Changes in vegetation structure may potentially provide a plausible explanation for this apparent shift in bacterial α -diversity from the Control site with all LMH present to the Elephant enclosure. Despite the positive association of bacterial communities and herbivore-induced disturbances, dry season conditions might exacerbate herbivore-related disturbances which has a negative effect on bacterial richness and diversity. More herbaceous and woody cover associated with the Elephant enclosure, when compared to the site with all LMH present (Van Coller *et al.*, 2013), may potentially facilitate relatively higher soil moisture through limiting evapotranspiration. This potential negative effect is supported by obtained bacterial phylum composition (Figure 24a), which indicates that the phylum Bacteroidetes was not present in the Control site with all LMH present, however, Bacteroidetes was present in the Elephant enclosure.

Similar to the findings of Vermeire *et al.* (2021), bacterial and fungal community composition showed a high degree of resistance to herbivore-induced changes, especially when compared to aboveground changes in vegetation structure. Vermeire *et al.* (2021) suggested that this apparent resistance to change in consumer-controlled, and subsequently, disturbance-driven open ecosystems is potentially related to the coevolution of microbial communities alongside herbivore-induced disturbances. Yet, results obtained in this study show greater alteration across treatments under seasonal variation. These herbivore-adapted microbial communities, although resistant, and to some extent dependent on herbivore-induced disturbances, may potentially be co-driven by seasonal variation which regulates community dynamics.

Even though bacterial and fungal α -diversity, in general, was not significantly altered during the present study, herbivores and seasonality have a substantial effect on soil microbial community composition in terms of relative abundance (Figure 24a and b). Previous studies have shown the negative effect of herbivores on certain fungal taxa such as mycorrhiza

(phylum Glomeromycota) through disturbances to vegetation and soil structure (Neary *et al.*, 1999; Baldrian *et al.*, 2012). This finding was supported by the results obtained here, which show the absence of the phylum Glomeromycota in the presence of high LMH activity. Furthermore, heat tree analysis revealed several significantly altered bacterial and fungal taxa (see Appendix C (C1-C6) for significant *p*-values) across the three different herbivore treatments, indicating the ability of native LMH to alter microbial community composition through numerous direct and indirect mechanisms. Moreover, results revealed that *Lapillicoccus* (bacteria) and *Curvularia* (fungi) were consistently the most decreased taxa across the three herbivore treatments.

8.5 Conclusion

Soil microbial communities form the basis of terrestrial trophic structure and are the primary catalysts of nutrient cycling. Given the rise in anthropogenic-induced disturbances and associated land-use change, altered carbon cycling and associated climate change have become a serious environmental concern. A Better understanding of microbial communities and factors driving them such as herbivory has become increasingly valuable as a bottom-up management strategy.

This study highlights the complex dynamic nature of soil microbial communities in sodic soils and their variable responses towards aboveground disturbance such as herbivores and seasonal climatic variations. Herbivore removal was shown to favour fungal richness and diversity, whilst it resulted in lower bacterial richness and diversity. Although α -diversity measures varied across treatments and seasons, the most consistent effect of herbivore-induced disturbances were related to significant changes in bacterial and fungal relative abundance. This can potentially be ascribed to the range of top-down feedbacks pertaining to herbivore-induced disturbances, or the loss thereof. The value of herbivores should therefore not solely be coupled to maintaining aboveground structure, but also in driving microbial community composition and associated soil functioning.

Results obtained from the sodic zone, together with the findings of Vermeire *et al.* (2021) in the adjacent riparian and crest sites reflect the site-specific nature of microbial responses to system disturbances such as herbivory. Given the relationship between soil microbes, vegetation and edaphic properties, the impact of disturbances on these constituent factors needs to be considered in order to better understand these responses. Chapter 9 of this dissertation attempts to link changes in these factors to observed alterations in microbial community composition.

Furthermore, as most studies on microbial composition and response to environmental variables are focused on northern hemisphere systems, more studies should consider increasingly threatened southern hemisphere, herbivore-driven grassy ecosystems. Moreover, in accordance with a previous study by Vermeire *et al.* (2021), observed community resistance towards herbivore-induced disturbances suggests that longer timescales should be considered to elucidate potential effects on community structure.

Chapter 9

Linking above- and belowground systems: Linking changes in vegetation structure to belowground processes

9.1 Preamble

This chapter aims to collate results from belowground patterns (decomposition, stabilisation, and microbial community structure) and to relate the findings to observed patterns in soil properties and local-scale vegetation structure (productivity, plant species composition and functional traits). Floristic analyses were not extensive, although local plant dominance and plant functional trait patterns are discussed in the context of the observed belowground patterns that were discussed in chapters 6, 7 and 8 of this dissertation.

9.2 Introduction

Ecosystems have traditionally been divided into separate, isolated compartments (i.e. above- and belowground) and studied accordingly at the expense of a holistic understanding (Wardle *et al.*, 2004). Herbivores have been implicated in various aboveground structural changes (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Van Coller *et al.*, 2013), yet there is little consensus on how these changes relate to belowground microbial heterogeneity and associated soil function (Bardgett & Wardle, 2003; Wardle *et al.*, 2004; Eldridge *et al.*, 2019; Erasmus *et al.*, 2021; Vermeire *et al.*, 2021; Erasmus *et al.*, 2022). Earlier results from this dissertation (see Chapter 6 and 7) revealed that native LMH, or the loss thereof, have the ability to alter the decomposition and stabilisation of detrital plant material through years of top-down and bottom-up feedbacks. Moreover, in accordance with previous findings by Vermeire *et al.* (2021), Chapter 8 of this dissertation provided evidence that herbivores have a notable influence on soil microbial community structure (bacteria and fungi). These belowground changes can potentially be ascribed to various top-down mechanisms pertaining to altered detrital quantity and quality and edaphic properties, these effects are thought to be exacerbated in highly productive systems, such as sodic patches (Bardgett *et al.*, 1998; Bardgett & Wardle, 2003; Thoresen *et al.*, 2021).

This Chapter aimed to examine the effect of varying herbivore treatments on top-down and bottom-up controls which collectively regulate above- and belowground heterogeneity. As a result of the range of complex top-down trophic cascades and bottom-up feedback responses

within semi-arid savanna ecosystems, it is hypothesised that herbivore-induced changes to aboveground vegetation structure (productivity and composition) can be linked with belowground changes to microbial community structure (bacteria and fungi) and associated soil function (decomposition and stabilisation). To test this hypothesis, aboveground structure (productivity, plant species composition and functional traits) was compared with belowground structure and processes (TBI-based decomposition data -Chapter 6 & 7 and microbial community structure -Chapter 8) across different herbivore treatments with varying intensity. Existing soil physicochemical data of the study area was used to clarify and support patterns of change in above- and belowground communities and processes.

9.3 Materials and Methods

Vegetation sampling was conducted within each of the five herbivory-fire treatments (Figure 9), close to the sites where tea bags were planted and where soil was sampled for microbial analyses. Detailed plant community ecology assessments are beyond the scope of this study, although local plant productivity, floristic and functional patterns are described here to provide a better understanding of the possible connection between vegetation and soil microbial decomposition rates and microbial community structure. Sampling was done within the most representative area near the experimental plots, and not within the plots, as both the TBI procedure and soil sampling caused disturbance to the soil and vegetation. System productivity (biomass) was estimated for the dry (October 2019) and wet (January 2020) season of the study area by taking 20 random readings with a Lowveld Savanna-calibrated DPM. DPM readings were converted to kg/ha in accordance with the latest conversions of DPM-readings to biomass for the Lowveld Savanna as described by Zambatis *et al.* (2006).

To separate the contribution of grasses from forbs to the net dry weight of aboveground phytomass, herbaceous biomass clippings were collected at the peak of the growth season (i.e. in January 2020). Clipped plant material was collected from three randomly placed quadrants (0.5 x 0.5 m) in each of the five sampled sites. Only individuals that were rooted within the quadrant were clipped to ground-level (using secateurs and shears) and placed into brown paper bags (in-field) to absorb moisture and limit insect degradation. Clippings were dried for 72 hours, after which the material was separated into forbs and grasses, weighed and converted to kg/ha.

Plant species (sampled in January 2020) were recorded along a 50 m transect in each of the five herbivory-fire treatments. At each 1 m interval the nearest species was identified and recorded. Species were assigned to specific lifeform classes based upon the structure of the

species in the field (i.e. grass, forb, dwarf shrub, shrub and tree). Species that could not be identified on site were collected and later compared to specimens housed at the AP Goossens herbarium (PUC) of the North-West University, Potchefstroom Campus for accurate identifications.

Functional leaf traits of the dominant plant species were recorded to capture the potential link between plant dominance and detrital leaf material, which in turn will affect decomposition (Cornelissen & Thompson, 1997; Wardle *et al.*, 1998; Cornelissen *et al.*, 2004; Kazakou *et al.*, 2009). Motivation for specific trait selection is provided in Table 3. These selected traits were assigned to plant species using field guides (Van Wyk & Malan, 1998; Van Oudtshoorn, 2015), floras (Germishuizen & Meyer, 2003) and several reputable websites (Biodiversity Explorer, 2018; SANBI, 2018). Plant trait frequencies were calculated as a percentage of the number of individually counted herbaceous species per treatment as a means to account for the potential influence of certain traits on decomposition.

Table 3: Plant functional traits associated with decomposability of herbaceous detrital material included in this study and motivation for trait selection.

Trait group	Trait	Category and numerical score	Motivation for inclusion	References
Life form	Forb or grass	1 = Forb 2 = Grass	Grasses tend to produce more leaf material than forbs, subsequently having a more notable effect on decomposition. Leaves of grasses are nutritionally superior to stems -short grasses therefore have a higher nutrient content than mid and tall grasses. Grasses typically have lower crude protein, phosphorus, and lignin concentrations and higher total fibre and cellulose concentrations than forbs. It is therefore suggested that the digestibility and subsequent breakdown of grasses is generally less than forbs -negatively influencing decomposition. Contrariwise, forbs have higher levels of crude protein, phosphorus and digestibility and lower levels of fibre when in a stage of active growth. Because of their low fibre levels, forb leaves tend to break down quickly -positively influencing decomposition. Moreover, forbs are capable of surviving during periods of drought - increasing in dominance, subsequently maintaining decomposition during dry periods.	Holechek (1984); Germishuizen & Meyer (2003); Rutherford <i>et al</i> (2012); Van Collier <i>et al.</i> (2021)
Lifespan	Annual and perennial	1 = Annual 2 = Perennial	Annual grasses and forbs dominate following a disturbance event, as they have numerous traits which allows them to function as pioneer species in early successional stages. Moreover, annuals tend to fall within the acquisitive end of the leaf economics spectrum which relates to higher leaf N content and a fast leaf turnover. Annuals therefore seem to have a positive association with decomposability. In contrast, perennials fall within the conservative end of the spectrum, relating to higher protection of resources through increased C:N ratios and higher lignin content together with a slow leaf turnover.	Cornelissen <i>et al.</i> (2003)
Photosynthetic pathway	C4/C3	1 = C3 2 = C4	C4 plants (grasses and forbs) are better adapted to warm growing conditions whereas C3 plants favour colder conditions. At comparable growth stages C3 plants are higher in crude protein, phosphorus, digestibility and lower in fibre than warm-season (C4) plants -C3 plants are subsequently better suited for decomposition than C4 plants. It has also been posited that a greater amount of fibre within C4 plants corresponds with greater leaf toughness -rendering them less digestible and less decomposable when compared to C3 plants. Moreover, due to the improved water use efficiency in C4 plants compared to C3 plants, leaves of C4 plants tend to have a lower moisture content associated with higher LDMC values and corresponding lower decomposability.	Ross <i>et al.</i> (2002); Barbehenn <i>et al.</i> (2004)

Physical leaf attributes	Specific leaf area (SLA - mm²)	1 = <10 2 = 10-20 3 = 21-30 4 = 31-40 5 = >40	Leaf thickness plays an important role in leaf and plant functioning and relates to a species' strategy of resource acquisition and use. Thick leaves are indicative of resource conservation and leaf longevity whereas thinner leaves are produced with rapid resource acquisition in mind. High SLA favours decomposition as moisture and nutrients are more readily available in these leaves. Contrariwise, low SLA favours lower decomposition as there is less moisture available and the resources are strongly bound for retention and leaf longevity. SLA represents a trade-off between high levels of defence on one side and high photosynthetic potential on the other. SLA further encompasses other plant traits including leaf physical toughness, palatability and digestibility - subsequently serving as a good reference for decomposition potential.	Cornelissen & Thompson (1997); Wardle <i>et al.</i> (2002); Cornelissen <i>et al.</i> (2004); Santiago (2007); Liu <i>et al.</i> (2018)
Chemical composition / Digestibility	Palatability	1 = Low 2 = Moderate 3 = High	Reflects the nutrient content and digestive potential of plant tissue with regards to lignin, cellulose and CBM. High palatability can therefore be seen as an indication of low amounts of difficult to digest materials (recalcitrant compounds) which also negatively impact soil-based decomposition. High palatability is associated with low C:nutrient ratios which promote decomposition as higher nutrient ratios enhance microbial-based breakdown through facilitating the growth of microbial communities. As such, vegetation palatability and decomposition show a positive correlation.	Holechek (1984); Van Wyk & Malan (1998); Ross <i>et al.</i> (2002); Fioretto <i>et al.</i> (2005); Ha <i>et al.</i> (2008); Kurokawa <i>et al.</i> (2010); Siebert & Scogings (2015); Van Coller <i>et al.</i> (2015); Van Oudtshoorn (2015)
Legume	Nitrogen fixator	1 = Yes 2 = No	Legumes are capable of fixing N₂ through plant-microbe symbioses with Rhizobium bacteria that occur within root nodules. Although most of the fixated N is used in the production of seeds, as the legume dies the remaining N within root nodules are released in the soil. Once the host plant dies, the bacteria are released back into the soil where they either stay put or infect another legume. It has also been noted that legumes maintain a higher foliar N content than non-fixing plants. High foliar N content promotes higher levels of soil-based decomposition of detrital material.	Cornelissen <i>et al.</i> (2003); Ha <i>et al.</i> (2008)

9.3.1 Statistical analyses

To test for significant variation in biomass of the herbaceous layer amongst three different treatments of varying herbivore intensity, data were analysed using R version 4.1.0 (R Core Team, 2021). Significance levels for all analyses were set at the standard significance level ($p < 0.05$) enabling confident conclusions made from statistical results. To test the assumptions of normality and homogeneity of variance, data were subjected to visual inspection (Kernel density plots and Q-Q plots), Shapiro–Wilk and Bartlett tests. As biomass (DPM-based and herbaceous clippings) data did not meet the assumption of normality ($p < 0.05$), a Kruskal-Wallis One-way Analysis of Variance (ANOVA) was applied to the data to test for significant variation across herbivore treatments. Where statistically significant variance among treatments were attained, a *post hoc* Dunn test was applied to test for significant differences between treatments. Differences between grass and forb dry weight biomass within each herbivore treatment was assessed using a paired t-test.

9.4 Results

9.4.1 Herbaceous biomass

Analysis of variances showed a significant negative effect of herbivory on DPM-based biomass measurements among treatments after nine- (October 2019 dry season) and twelve-months (January 2020 wet season) (Kruskal-Wallis test: $p < 0.0001$; Figure 27a and b). Pair-wise comparisons of biomass means revealed significantly higher biomass in the LMH enclosure when compared to treatments exposed to herbivory for both seasons (Dunn test: $p < 0.05$; Figure 27a and b). Similarly, herbivory significantly affected dry weight of grass phytomass (Kruskal-Wallis test: $p < 0.03$; Figure 28a) across treatments, whilst the effect on dry weight of forb phytomass was non-significant (Kruskal-Wallis test: $p > 0.8$; Figure 28b). *Post hoc* analyses revealed that grass dry weight was significantly higher in the LMH enclosure when compared to both the Elephant enclosure and Control site (Dunn test: $p < 0.05$; Figure 28a). Although not significant, forb dry weight followed a similar response (Figure 28b). Dry weight of grass biomass was significantly higher than forb dry weight, but only in the presence of all LMH (Paired t-test: $p < 0.01$; Figure 29a).

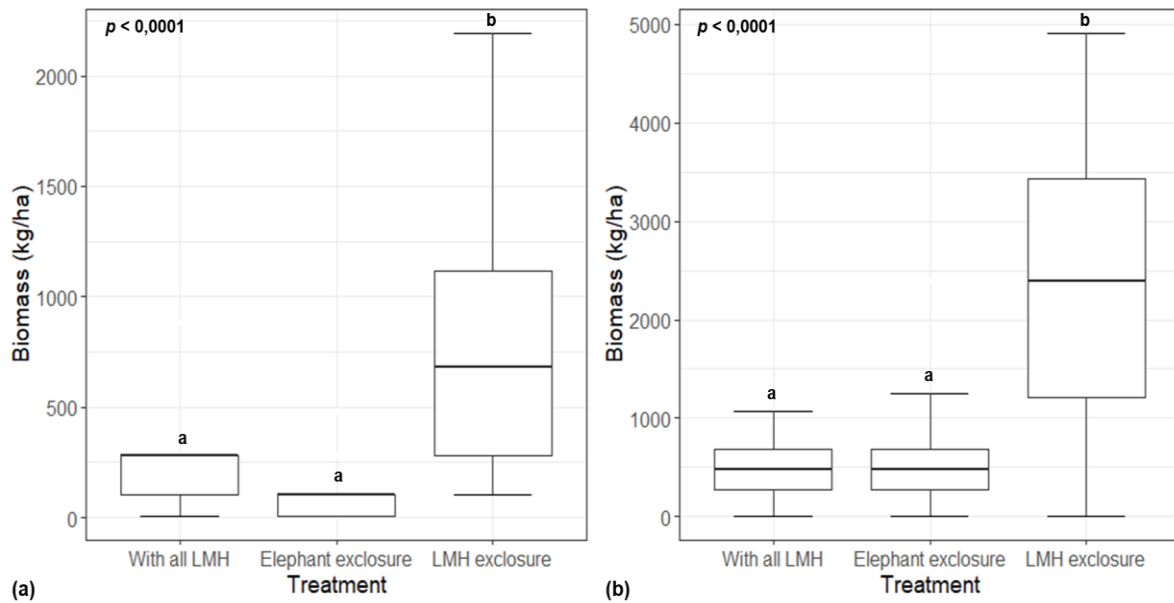


Figure 27: DPM-based biomass measurements (kg/ha) for the dry -October 2019 (a) and wet -January 2020 (b) season across three herbivore treatments within the Nkuhlu enclosures, Kruger National Park. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b).

Central line in box, median; box, interquartile range; extreme values, whiskers; With all LMH (Control), all herbivores present; Elephant enclosure, elephants (and giraffes) excluded; LMH enclosure, all LMH excluded; p , $p < 0.05$ indicates a significant variance between groups as assessed using the Kruskal-Wallis test

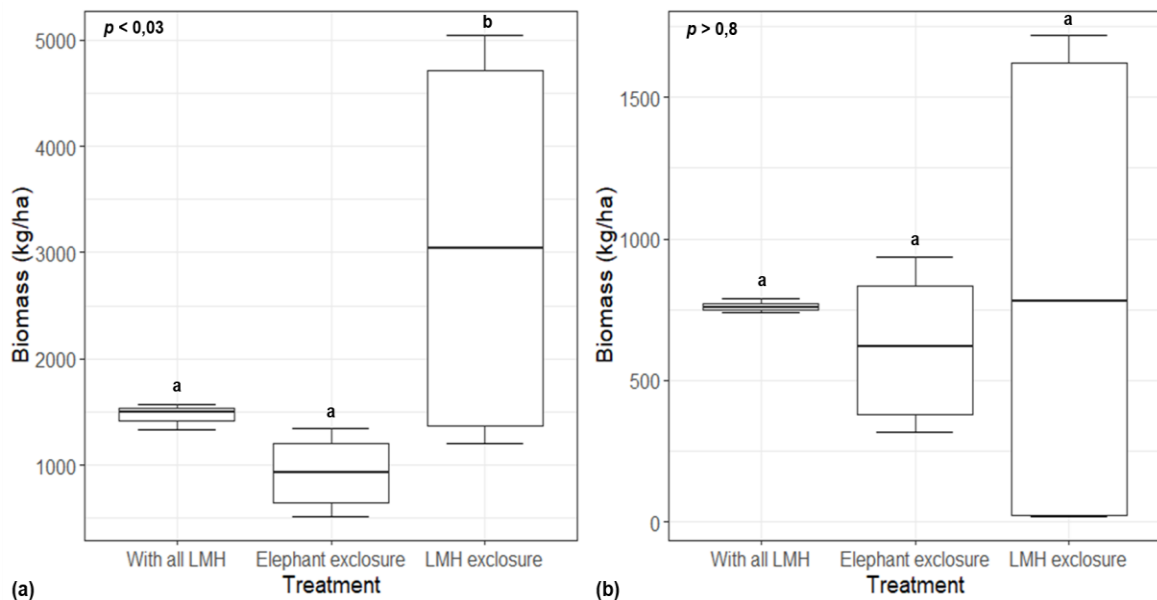


Figure 28: Dry weight biomass measurements (kg/ha) for grasses (a) and forbs (b) across three herbivore treatments within the Nkuhlu enclosures, Kruger National Park. Significant

differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b).

Central line in box, median; box, interquartile range; extreme values, whiskers; With all LMH (Control), all herbivores present; Elephant enclosure, elephants (and giraffes) excluded; LMH enclosure, all LMH excluded; p , $p < 0.05$ indicates a significant variance between groups as assessed using the Kruskal-Wallis test.

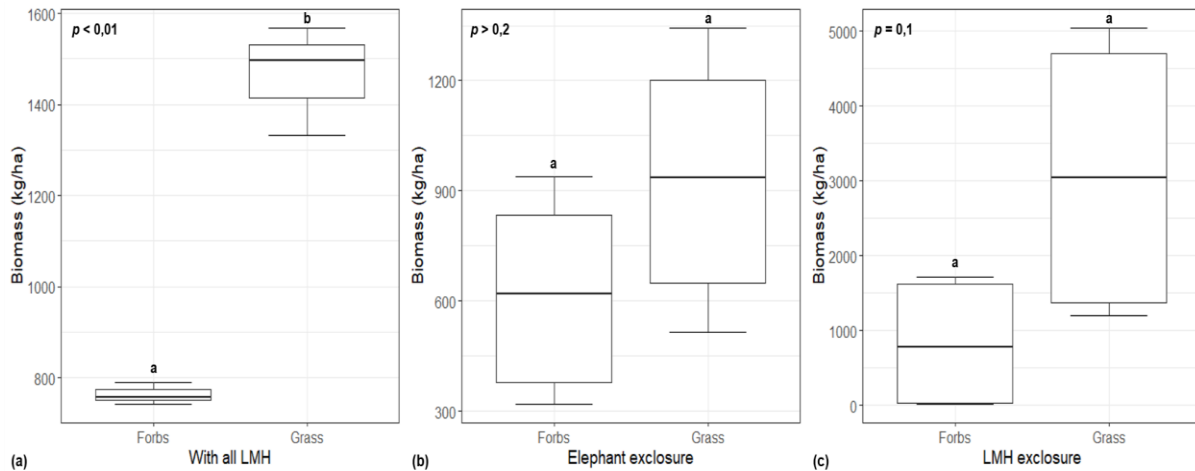


Figure 29: Comparison of grass and forb dry weight biomass measurements (kg/ha) across three herbivore treatments, (a) With all LMH, (b) Elephant enclosure and (c) LMH enclosure. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters (a and b).

Central line in box, median; box, interquartile range; extreme values, whiskers; With all LMH (Control), all herbivores present; Elephant enclosure, elephants (and giraffes) excluded; LMH enclosure, all LMH excluded; p , $p < 0.05$ indicates a significant variance between groups as assessed using a paired t-test.

9.4.2 Floristic and functional trait patterns

Attained frequency values showed differences in dominant species composition across the three different herbivore treatments for all five lifeform classes (Table 4). A total of 53 different herbaceous species were recorded in five transects in the sodic zone of the Nkuhlu enclosures. Trait frequencies revealed that grass and forb species were relatively evenly distributed in terms of abundance per herbivore treatment (Table 4), although the potential inputs of grass leaves to detrital leaf material are higher, based on higher grass dry weight measurements compared to forbs. Furthermore, under grazing pressure where grasses do not become moribund, it can be deduced that grass potentially constitute the majority of detrital material that enters the decomposition subsystem. The Control site with all LMH present showed an equal composition of annual and perennial species (Table 5). Conversely, perennials were more abundant in the Elephant- and LMH enclosure, with highest abundance

in the latter. C4 species dominated across all three herbivore treatments, whilst C3 species was highest under herbivore exclusion. Plants with low SLA values (i.e. 10-20mm²) were more abundant in the presence of herbivores, opposed to the LMH exclosure where higher SLA values (i.e. 31-40 mm²) were recorded. Abundance of species in the high palatability class increased with a release in herbivore pressure. Species abundance within the moderate palatability class increased with LMH activity. Lastly, legumes were only found in the Elephant exclosure and LMH exclosure, but at very low compositional percentages.

It is important to note that for the purpose of this Chapter, the above-mentioned results serve as a means to clarify and support observed changes in soil-based decomposition and microbial community structure at a site-specific scale. As such, these results do not serve to provide detailed explanation of floristic and functional trait patterns within the studied system.

Table 4: Individual frequencies depicting the dominant species for the five life form classes across three herbivore treatments. Only the top five dominant species are presented in this table.

With all LMH									
Tree	Frequency	Shrub	Frequency	Dwarf shrub	Frequency	Forb	Frequency	Grass	Frequency
Species name	%	Species name	%	Species name	%	Species name	%	Species name	%
<i>Rhigozum zambesiaceum</i>	4	<i>Rhigozum zambesiaceum</i>	16	<i>Ocimum americanum</i>	48	<i>Evolvulus alsinoides</i>	36	<i>Eragrostis lehmanniana</i>	30
<i>Euclea divinorum</i>	2	<i>Acacia grandicornuta</i>	6	<i>Abutilon austro-africanum</i>	12	<i>Kyphocarpa angustifolia</i>	12	<i>Urochloa mosambicensis</i>	18
				<i>Hibiscus micranthus</i>	10	<i>Blepharis integrifolia</i>	8	<i>Tragus berteronianus</i>	16
				<i>Indigofera tinctoria</i>	8	<i>Ocimum americanum</i>	8	<i>Sporobolus nitens</i>	14
				<i>Waltheria indica</i>	4	<i>Portulaca hereroensis</i>	6	<i>Aristida congesta subsp. barbicollis</i>	8
Elephant enclosure									
<i>Acacia grandicornuta</i>	11	<i>Acacia grandicornuta</i>	16	<i>Abutilon austro-africanum</i>	32	<i>Blepharis integrifolia</i>	15	<i>Sporobolus nitens</i>	42
<i>Papaea capensis</i>	5	<i>Rhigozum zambesiaceum</i>	6	<i>Indigofera tinctoria</i>	10	<i>Kyphocarpa angustifolia</i>	13	<i>Tragus berteronianus</i>	25
<i>Euclea divinorum</i>	3	<i>Dichrostachys cinerea</i>	5	<i>Hibiscus micranthus</i>	9	<i>Evolvulus alsinoides</i>	10	<i>Urochloa mosambicensis</i>	11
<i>Hippocratea longipetiolata</i>	1	<i>Gymnosporia buxifolia</i>	4	<i>Seddera capensis</i>	7	<i>Phyllanthus parvulus</i>	9	<i>Panicum maximum</i>	7
<i>Zanthoxylum capense</i>	1	<i>Spirostachys africana</i>	3	<i>Ruellia cordata</i>	5	<i>Corbichonia decumbens</i>	7	<i>Brachiaria eruciformis</i>	4
LMH enclosure									
<i>Acacia grandicornuta</i>	9	<i>Dichrostachys cinerea</i>	18	<i>Barleria elegans</i>	17	<i>Commelina benghalensis</i>	21	<i>Panicum maximum</i>	55
<i>Euclea divinorum</i>	7	<i>Rhigozum zambesiaceum</i>	8	<i>Hibiscus micranthus</i>	9	<i>Evolvulus alsinoides</i>	12	<i>Urochloa mosambicensis</i>	36
<i>Spirostachys africana</i>	4	<i>Acacia grandicornuta</i>	7	<i>Ruellia cordata</i>	9	<i>Blepharis integrifolia</i>	5	<i>Sporobolus nitens</i>	2
<i>Papaea capensis</i>	4	<i>Euclea divinorum</i>	5	<i>Abutilon austro-africanum</i>	8	<i>Talinum arnotii</i>	5	<i>Perotis patens</i>	2
<i>Cadaba natalensis</i>	1	<i>Grewia retinervis</i>	2	<i>Seddera capensis</i>	7	<i>Justicia flava</i>	4	<i>Chloris virgata</i>	1

With all LMH (Control), all herbivores present; Elephant enclosure, elephants (and giraffes) excluded; LMH enclosure, all LMH exclude

Table 5: Frequency values (%) of functional plant traits related to decomposition of detrital plant material across three herbivore treatments.

	Functional traits					
Treatment	Life form	Lifespan	Photosynthetic pathway	Specific leaf area (SLA mm ²)	Palatability	Legume
With all LMH	Forb = 50% Grass = 50%	Annual = 50% Perennial = 50%	C3 = 37% C4 = 63%	<10 = 0% 10-20 = 53% 21-30 = 27% 31-40 = 4%	Low = 17% Moderate = 48% High = 34%	= 0%
Elephant enclosure	Forb = 49% Grass = 51%	Annual = 41% Perennial = 59%	C3 = 36% C4 = 64%	<10 = 0% 10-20 = 62% 21-30 = 15% 31-40 = 13%	Low = 21% Moderate = 30% High = 49%	= 1%
LMH enclosure	Forb = 45% Grass = 55%	Annual = 34% Perennial = 66%	C3 = 39% C4 = 61%	<10 = 0% 10-20 = 34% 21-30 = 18% 31-40 = 44%	Low = 4% Moderate = 20% High = 76%	= 1%

With all LMH (Control), all herbivores present; Elephant enclosure, elephants (and giraffes) excluded; LMH enclosure, all LMH excluded.

9.4.3 Soil properties

Existing soil data from the study area, provided in Chapter 7 of this dissertation (Figure 22 and Table 1), was used to clarify and support observed patterns of change in above- and belowground structure.

9.4.4 Changes in decomposition rate and microbial community structure

Results from Chapters 6, 7 and 8 have relevance here. Although results from previous chapters will not be repeated, it will be discussed in the context of the results obtained for plant- and soil data analyses to link observed patterns in above- and belowground processes.

9.5 Discussion

9.5.1 Herbivore-induced changes to aboveground structure

Similar to results reported by Van Coller *et al.* (2013), a release in herbivore pressure had a significant positive effect on herbaceous biomass measured during two consecutive seasons i.e. October 2019 dry season and January 2020 wet season (Figure 27a and b). Van Coller *et al.* (2013) further indicated that high levels of standing biomass has a negative effect on plant

species richness and diversity. These results reflect the importance of LMH in regulating species composition through maintaining aboveground standing biomass at low levels. In comparison to grass biomass, significantly lower forb biomass in the presence of all LMH may potentially allude to the negative effect of herbivory on C3 species (predominantly forbs) which are subjected to high levels of competition from C4 grasses that are better suited to herbivore-induced disturbances pertaining to higher levels of solar radiation and lower soil moisture (Ross *et al.*, 2002; Barbehenn *et al.*, 2004). Another potential explanation might be that forbs are preferentially targeted due to their nutritious state (Siebert & Scogings, 2015), especially in an area, such as the Control site, with overall significantly lower herbaceous biomass (Figure 27a and b).

Results from this Chapter revealed that C4 herbaceous species had a higher compositional percentage in the presence of herbivores (i.e. With all LMH and Elephant enclosure; Table 5). Contrariwise, shade-tolerant plants, such as forbs, are able to survive under dense canopy cover, enabling them to better compete with grasses in tree islands or dense tree stands, typically associated with herbivore removal and woody encroachment e.g. Elephant- and LMH enclosure (Scholes & Archer, 1997; Oba *et al.*, 2000; Ludwig *et al.*, 2004; Kahi *et al.*, 2009). Results reflect this observation as dry weight of forb and grass biomass showed no significant difference under herbivore exclusion (i.e. Elephant- and LMH enclosure) when compared to the Control site with all LMH present (Figure 29a, b and c).

Furthermore, species dominance for the five life form classes differed across sites where soil microbial and decomposition data were sampled (Table 4). Although floristic surveys were not extensive, results still reflected the various direct and/or indirect effects of LMH, particularly on vegetation structure. These differences can be related to direct herbivore-induced disturbances associated with foraging and trampling, but can also be associated with indirect effects pertaining to plant-soil feedbacks. Results from chapter 6 and 7 of this dissertation reflected on the ability of LMH to influence bottom-up controls such as decomposition and associated nutrient cycling (Erasmus *et al.*, 2022).

As would be expected, C4 species dominated the system, corresponding with global predictions of C3 and C4 distribution (Ross *et al.*, 2002; Barbehenn *et al.*, 2004). Treatments in which herbivores were present showed the highest percentage composition for low SLA values (i.e. 10-20mm²), potentially attributed to the drier conditions associated with higher solar radiation as a direct result of herbivore-induced removal of biomass and reduced canopy cover. This is in contrast with other herbivores effects, as low SLA values are in general related to lower decomposition, In line with global predictions, highest percentages were found for the lower SLA values (e.g. 10-20 and 21-30 mm²), corresponding with the drier climatic conditions

associated with semi-arid savanna ecosystems in the southern hemisphere (Wardle *et al.*, 2002; Cornelissen *et al.*, 2004; Chillo *et al.*, 2011; Scoffoni *et al.*, 2011). It was found that annuals, which fall within the acquisitive spectrum of the leaf economic scale (Wright *et al.*, 2004), had a higher compositional percentage in the presence of herbivores. These plants are suggested to have a higher leaf turnover (Wright *et al.*, 2004), which implies that high-quality, labile detrital material is readily returned to the decomposition subsystem. As a result of limited detrital input (significantly lower biomass and leaf litter in the presence of herbivores; Figures 26, 27 and 29f) this material is quickly colonised and utilised due to a high C demand, essentially promoting higher decomposition rates (Figure 16a, c, e, g and i).

9.5.2 Linking changes in above- and belowground structure and function

In accordance with previous studies done by Eldridge *et al.* (2019) and Vermeire *et al.* (2021), results presented in Chapter 8 of this dissertation revealed that LMH have a substantial influence on soil microbial community composition (bacteria and fungi). Although obtained results did not reveal consistent significant effects of herbivory across treatments (Figure 21a-d) it did, however, indicate the importance of cross-scale herbivore-induced disturbances in driving microbial community composition in terms of the abundances of different taxa (Figure 23a-b and Figure 24a-f). The shift between bacterial and fungal α -diversity measures across herbivore treatments can be ascribed to several herbivore-related top-down effects, or the loss thereof, pertaining to changes in both vegetation structure and edaphic properties (Bardgett *et al.*, 1998; Bardgett & Wardle, 2003; Eldridge *et al.*, 2019; Zhao *et al.*, 2019; Vermeire *et al.*, 2021).

Enhanced herbaceous species diversity and quality associated with herbivore presence within sodic patches (Grant & Scholes, 2006; Jacobs & Naiman, 2008; Van Coller *et al.*, 2013), has numerous top-down cascading effects pertaining to constituents of the belowground subsystem. Plant species diversity is associated with varying functional traits, ultimately promoting the formation of high-quality, chemically and physically diverse detrital material, essentially regulating both microbial community structure and associated decomposition (Bardgett *et al.*, 1998; Wardle *et al.*, 2002; Bardgett & Wardle, 2004; Nilsson *et al.*, 2008). Diverse detrital material has the potential to enhance microbial diversity through increased resource heterogeneity which provides a wide variety of nutrient opportunities and subsequent niche partitioning (Bardgett *et al.*, 1998; Wardle *et al.*, 2002; Bardgett & Wardle, 2004). A diverse suite of microbes can promote decomposition as varying microbial functional groups allow for the rapid breakdown of detrital material and associated release of soil available nutrients (Bardgett *et al.*, 1998; Wardle *et al.*, 2002; Bardgett & Wardle, 2004; Goldfarb *et al.*,

2011; Keuskamp *et al.*, 2013; Eldridge *et al.*, 2019). Furthermore, sustained biomass removal induces defensive mechanisms within plants whereby nutrients are increasingly allocated to belowground tissues (Bardgett *et al.*, 1998). High quality root litter together with enhanced root exudation further promotes microbial activity (Bardgett *et al.*, 1998). In accordance with the observations of Bardgett and Wardle (2003), it is suggested that in productive ecosystems dominated by palatable, nutrient-rich vegetation that is capable of supporting congregated herbivore activity (such as the sodic zone), labile nutrients are rapidly returned to the soil through biotic and abiotic pathways. Bacteria preferentially target labile compounds as a nutrient source and are better suited to rapid colonisation of these readily available resources (Goldfarb *et al.*, 2011).

Moreover, LMH activity promotes the formation of microsites (e.g. microclimates and nutrient hot-spots), further enhancing system heterogeneity. Biomass removal results in areas with substantially lower vegetation cover, essentially allowing for more solar radiation to reach the soil surface. In turn, this can aid in photodegradation of leaf litter (promoting decomposition), whilst also raising soil surface temperature which stimulate microbial activity (King *et al.*, 2012). Bacteria, with their highly variable metabolism, are capable of surviving under numerous herbivore-induced environmental conditions (Bardgett *et al.*, 1998; Vermeire *et al.*, 2021). Herbivores can through several mechanisms, indirectly create various soil microsites through alteration to physical and/or chemical soil properties (Bardgett *et al.*, 1998; Eldridge *et al.*, 2019; Thoresen *et al.*, 2021). Studies done by Goldfarb *et al.* (2011) and Vermeire *et al.* (2021) revealed that soil chemical properties are important determinants of bacterial community composition. This is in line with global trends that edaphic properties control bacterial composition (Bahram *et al.*, 2018; Kaiser *et al.*, 2016). Sustained herbivore pressure can alter soil physical conditions through trampling, resulting in soil compaction and erosion of the vulnerable A-horizon. This observation is supported by existing soil data which revealed significantly higher soil bulk density under herbivore presence (Figure 22e). Moreover, bulk density indirectly influences CEC in soils, especially those with a high clay fraction (Xu *et al.*, 2012). Significantly higher CEC (Pairwise comparisons: $p = 0.044$; Figure 22c) in relation to herbivore presence may potentially denote higher levels of organic material (humus) in the soil as a result of higher decomposition rates (k), as observed in Chapter 7 of this dissertation. Furthermore, a high CEC is indicative of higher levels of soil nutrients (Sparks, 2003), alluding to higher bacterial and plant diversity as a result of the heterogeneous soil nutrient pool. Moreover, herbivores can also influence soil chemical conditions through point-source nutrient input. Ammonium (NH_4^+) is a major component of urine and has the potential to stimulate microbial-driven decomposition of organic material which, in turn, also produces NH_4^+ as a by-product. Congregated herbivore activity and associated excretion of urine can therefore be

associated with higher decomposition rates (k) observed in areas exposed to herbivory (Figure 21a, c, e and g). Nitrifying bacteria are present in distinct taxonomic groups which are typically found in high abundance in areas with extensive decomposition and associated availability of NH_4^+ . NH_4^+ is readily taken up by these nitrifying bacteria and converted into NO_3^- through nitrification. Significantly higher levels of NO_3^- (Pairwise comparisons: $p = 0.035$; Figure 22a) observed in the presence of herbivores, potentially alluding to higher bacterial activity as NO_3^- is readily used as an energy source. Existing soil data revealed significantly higher levels of Ca^{+2} in the presence of LMH, potentially ascribed to higher rates of decomposition which, through the breakdown of organic material, mineralises Ca^{+2} and releases it back into the soil. Ca^{+2} is an integral component of bacterial cell wall structure, and is subsequently required for bacterial growth and associated activity. Significantly high levels of Ca^{+2} (Pairwise comparisons: $p = 0.041$; Figure 22b) can therefore contribute to bacterial growth and can potentially be associated with higher bacterial richness and diversity in the presence of herbivores. Similarly, significantly higher PO_4^{+3} (Pairwise comparisons: $p = 0.04$; Figure 22d) in the presence of herbivores corresponds with higher decomposition rates (k) and associated mineralisation of organic phosphorus. Treatments exposed to LMH activity have a greater variation in soil conditions pertaining to physicochemical properties, resource and habitat availability (i.e. greater heterogeneity), ultimately facilitating niche differentiation which corresponds with higher bacterial richness and diversity (Figure 27a and c).

Herbivore exclusion and associated lower herbaceous species diversity (as noted by Van Coller *et al.*, 2013) together with the prevalence of a few dominant species can result in the production of low-quality, homogenous detrital material (Naeem *et al.*, 2000; Bardgett & Wardle, 2003). This potentially has a profound impact on the producer-decomposer codependency which governs essential ecological processes (Naeem *et al.*, 2000; Nilsson *et al.*, 2008). A release in herbivore pressure has often been associated with woody encroachment, further reducing litter quality as detrital material is largely composed of cellulose and lignin-based substrates (Siebert & Eckhardt, 2008; Scogings *et al.*, 2012). Moreover, it was observed that herbivore exclusion resulted in lower bacterial richness and diversity (Figure 27a and c) together with associated decomposition (Figure 16a, c, e and g).

On a global scale, fungal community composition is predominantly regulated by climate (Keitt *et al.* 2016; Oliverio *et al.* 2017). Consequently, fungi have been noted to follow similar biogeographical patterns than that of vegetation (Bui *et al.*, 2020). Given the link between fungi, host plants and the vulnerable top-soil layer, it is expected that disturbances to any one of these constituent factors may ultimately alter fungal community structure (Bardgett *et al.*, 1998; Bui *et al.*, 2020; Vermeire *et al.*, 2021). In contrast to bacterial α -diversity measures, fungal richness and diversity were shown to have a positive association with herbivore

exclusion and corresponding biomass (Figure 21b and d, Figure 27a and b), similar to that of stabilisation of organic material (Figure 16b, d, f and h). In accordance with the findings of Vermeire *et al.* (2021), these results can mainly be attributed to herbivore-induced disturbances to aboveground vegetation structure and physical soil properties. More importantly, a relaxation in herbivore pressure corresponds with significantly lower bulk density (Pairwise comparisons: $p = 0.032$; Figure 22e) and facilitates the reestablishment of topsoil (Walker *et al.*, 1981; Abdalla *et al.*, 2018), allowing for improved fungal growth (Baldrian *et al.*, 2012). Aboveground biomass can also aid fungal communities in providing a relative amount of shading which lowers soil surface temperatures, favouring fungi with their lower thermal tolerance (Baldrian *et al.*, 2012). Moreover, significantly higher measured leaf litter (Pairwise comparisons: $p < 0.001$; Figure 22f) under herbivore exclusion further promotes fungal growth by providing additional shading and retaining more soil moisture. From a belowground perspective, higher levels of biomass (plant growth) together with less soil compaction (Figure 22e) allows for greater vegetation rooting and corresponding fungal associations, subsequently supporting higher fungal richness and diversity (Bui *et al.*, 2020).

Low quality vegetation associated with herbivore removal has been suggested to produce detrital material with higher recalcitrant ratios, essentially promoting fungal growth as they are better suited to the breakdown and utilisation of these materials (Bardgett & Wardle, 2003; Treseder *et al.*, 2016). However, the breakdown of highly complex recalcitrant compounds requires the sustained uptake of C and N to construct catalytic enzymes (Schimel & Bennett, 2004). Existing soil data support this as NO_3^- (commonly used as a source of N by fungi) (Ngwene *et al.*, 2013), was revealed to be significantly lower under herbivore exclusion (Figure 5f). Due to the complex nature of recalcitrant substrates, the rate and extent of decomposition can be impeded (Keuskamp *et al.*, 2013), resulting in a slower nutrient turnover (decomposition) as observed under herbivore exclusion (see Chapter 6 and 7).

A relaxation in herbivore pressure promotes aboveground production (Jacobs & Naiman, 2008; Figure 1a and b) and the subsequent storage of nutrients within these tissues (Bardgett *et al.*, 1998). Without herbivore-induced disturbances promoting a rapid detrital turnover, these nutrients are to an extent locked-up within the aboveground compartment. This observation is supported by existing soil data which revealed significantly higher levels of leaf litter under herbivore exclusion (Figure 22f), denoting lower biotic- (microbial-driven) and abiotic-decomposition (photodegradation). Moreover, functional trait analyses (Table 5) revealed a higher compositional percentage of perennial plants under herbivore exclusion. On the global leaf economic scale, perennials are typically associated with the conservative end of the spectrum, subsequently producing tough leaves with low N content that are retained for longer (Wright *et al.*, 2004). This limits the quantity and quality of detrital material that enters the

decomposition subsystem. A well-known example is that of grass which tends to become moribund if under-grazed, resulting in less detrital material and associated nutrients entering the decomposition subsystem, ultimately resulting in slower nutrient cycling (Bardgett *et al.*, 1998; Wardle *et al.*, 2002; Bardgett & Wardle, 2004).

Soil microbes have been described as being C limited, whilst plants are nutrient limited (Berg, 2000; Harrison *et al.*, 2007). LMH are capable of reducing the amount of detrital plant material that enters the decomposition subsystem through its significant negative effect on herbaceous biomass and associated leaf litter (Figure 27a and b, Figure 22f). This results in higher microbial demand for organic C, promoting a rapid detrital turnover i.e. decomposition (Semmartin *et al.*, 2008; Erasmus *et al.*, 2022; Figure 16a, c, e and g). It has often been suggested that microbes immobilise certain inorganic nutrients, placing them in direct competition with plants (Zhu *et al.*, 2017). Studies done by Jingguo and Bakken (1997) and Hu *et al.* (2001) indicate that plants may suppress microbial nutrient uptake and associated activity through root N immobilisation. Zhu *et al.* (2017) suggested that plants can potentially outcompete microbes as a result of high-density fine root systems within the topsoil layer - often associated with higher levels of herbaceous biomass (e.g. LMH exclosure) and corresponding superficial rooting. Enhanced mycorrhizal associations can aid plants in acquiring nutrients, allowing for greater productivity and biomass (Grant & Grant, 1995). Results obtained in Chapter 8 of this dissertation revealed that the fungal phylum associated with the mycorrhizal taxa (Glomeromycota) was mainly present under herbivore exclusion. This potentially provides an explanation for the lower decomposition rates (k) under herbivore exclusion as significantly higher herbaceous biomass together with re-established topsoil could reduce bacterial activity via plant and/or plant-mycorrhizal competition.

Trait frequencies did not have a substantial direct association with decomposition, although certain traits did favour decomposition. Observed trait frequencies tended to follow global predictions of trait distribution (Cornelissen *et al.*, 2004; Chillo *et al.*, 2011), echoing the relationship between vegetation structure and global climatic patterns (Bond, 2008; Pausas & Bond, 2020). Yet, C4 dominated grassy ecosystems do not unequivocally conform to global patterns (Bond *et al.*, 2008). It has been suggested that the distribution, structure and functioning of these systems can be ascribed to the collective influences of direct and indirect top-down (e.g. herbivory) and bottom-up (e.g. resources) herbivore-induced feedbacks (Bond, 2008; Pausas & Bond, 2020). In accordance with results presented here, soil function in the form of decomposition is similarly regulated by a suite of herbivore-mediated disturbances to the above- and belowground compartments.

9.6 Conclusion

Given the current rate of population growth and subsequent demand for usable land, large parts of natural savannas are under increasing threat, especially prominent native LMH. Within these systems, herbivores are capable of maintaining aboveground structure through various top-down mechanisms. However, limited research has been done on aboveground-belowground feedbacks and the potential effects on both subsystems. It has often been postulated that changes to one of these subsystems can exert a positive, negative, or even neutral influence on the other through various feedback responses, however, this has been suggested to be highly dependent on system context in terms of spatial and temporal scale.

Results presented in this Chapter revealed that herbivory, or the loss thereof, had substantial cross-scale effects on above- and belowground structure together with essential soil function in terms of decomposition and stabilisation. Sustained herbivore pressure was shown to maintain standing biomass at low levels which promote plant species richness and diversity, ultimately facilitating aboveground heterogeneity. Moreover, these top-down feedbacks promote bacterial α -diversity and associated decomposition. These findings are in accordance with previous studies which suggested that congregated herbivore activity promotes the rapid return of labile nutrients to the soil which has a positive effect on bacterial communities and associated soil processes. In contrast, herbivore exclusion was shown to promote herbaceous biomass and associated competitive exclusion. The subsequent prevalence of a few dominant plant species together with woody encroachment results in the production of low-quality homogenous detrital material which favours fungal α -diversity and associated slow nutrient cycling.

Emerging patterns within vegetation structure, microbial communities and soil function observed across the various herbivore treatments potentially alludes to future scenarios related to herbivore removal. These patterns suggest that future herbivore loss can result in decreased plant species diversity together with altered microbial community structure pertaining to a shift from bacterial- (fast nutrient cycle) to fungal-driven decomposition (slow nutrient cycle).

Studies aimed at elucidating aboveground-belowground interactions are critical in improving our knowledge on intercompartmental feedbacks, and more importantly, the effects of anthropogenic-induced disturbances on ecosystem functioning. Knowledge of herbivore-induced cross-scale effects can serve to predict potential effects of land-use change and the development of efficient top-down and bottom-up management strategies.

Chapter 10

Conclusion

Consumer-controlled grassy biomes of southern Africa rely heavily on the presence of coevolved heterogeneous LMH communities as a mechanism which, through numerous stabilising feedbacks, essentially maintain savanna ecosystems. Considering the variety of cross-scale aboveground-belowground interactions, herbivore-induced disturbances can alter several constituents of the belowground subsystem. Yet, very little is known about the impact of herbivory, or the loss thereof from a herbivore-adapted system, on microbial community composition and associated decomposition. The Nkuhlu exclosure in the KNP provided an opportunity to collectively examine the lesser-known effects of herbivory on belowground structure and function and how these potential changes relate to well-known aboveground changes in the dynamic sodic zone.

In its completeness, this study attempted to provide a holistic understanding of intercompartmental feedbacks, and their potential effects on above- and belowground structure and functioning. Moreover, this study provides valuable insight on current knowledge gaps pertaining to soil microbial response towards disturbances and potential implications for soil respiration and associated carbon flux.

10.1 Main findings

The internationally standardised TBI approach provided an easily applicable method to obtain valuable information on decomposition of organic material. Given the initial development and predominant application in temperate and boreal ecosystems, the TBI in its original form did not provide the required specificity and resilience needed to effectively function in a disturbance-driven semi-arid savanna, especially over longer temporal scales. However, various application based amendments pertaining to the burial, retrieval and the amount of samples, yielded a site-specific approach which was shown to account for the various potential disturbances typically associated with disturbance-prone ecosystems. Percentage tea bag retrieval over the period of one year suggests that the extended, site-specific version of the TBI is a viable approach to quantify decomposition over longer periods of time in a disturbance-driven semi-arid African savanna.

The paucity of information available on decomposition is often attributed to the range of complex determinants which collectively regulate the process. The TBI approach is capable of disentangling the effects of herbivory from various other system-specific drivers of decomposition through removing the ambiguity associated with detrital chemistry. After a three-month incubation period, results indicated that LMH influence essential ecological processes such as decomposition rate (k) and stabilisation factor (S) of detrital plant material on a local scale. Although not significant, decomposition rate (k) was found to be highest in the presence of native LMH, whilst stabilisation factor (S) was significantly higher under herbivore exclusion. Moreover, decomposition and stabilisation values from this semi-arid savanna ecosystem occupied a lower range when compared to globally distributed ecosystems. As the majority of studies have been conducted in the northern hemisphere, lower values obtained from the study site can predominantly be ascribed to the combined effect of global drivers such as climate and edaphic properties. It is further suggested that functional plant trait distribution pertaining to C3 and C4 species may contribute towards the observed variation between northern and southern hemisphere systems.

Further analyses revealed the dynamic nature of decomposition over the period of one year, potentially attributed to the range of direct and indirect effects of LMH on system heterogeneity (on landscape and resource level) together with temporal changes in chemical composition of detrital substrate. As a means to facilitate global comparison, the TBI approach predominantly suggests a three-month incubation period. However, it was shown that three months are too short to showcase the full complement of decomposition dynamics in semi-arid savanna ecosystems. Significant variation in decomposition rate (k) occurred after a six- and nine-month incubation period and was consistently highest in the presence of all LMH, whilst stabilisation factor (S) was positively associated with herbivore exclusion, revealing significant variation after three- and six-months. Furthermore, a twelve-month period revealed no significant variation, and was deemed to be too long as it has the potential to result in inaccurate estimates of decomposition rate (k) and stabilisation factor (S). As the TBI approach was developed and mainly tested in systems which bear a substantially different above- (vegetation composition) and belowground structure (microbial community composition) compared to disturbance-prone African savannas, it is suggested that the optimal incubation period to examine decomposition dynamics for semi-arid savanna ecosystems is six- and nine-months.

It was revealed that native LMH can alter bacterial and fungal α -diversity, essentially regulating soil microbial community composition in dynamic sodic patches. Bacterial richness and diversity revealed a positive association with herbivore-induced disturbances, with the highest

values obtained in the presence of all LMH. In contrast, fungal richness was highest under complete herbivore exclusion, whilst diversity was shown to be highest under intermediate levels of disturbance. Moreover, seasonality was revealed to have a major influence on α -diversity measures with fungal richness and diversity showing a significant variation across herbivore treatments in the dry season. Despite the overall lack in significant variation across herbivore treatments and over different seasons, the most consistent effect of herbivory appeared to be related to significant changes in the relative abundance of both bacterial and fungal taxa. Given the stark contrast between aboveground and belowground responses towards herbivory or the loss thereof, sodic microbial communities exhibit a high degree of resistance to herbivore-induced changes, potentially related to the coevolution of microbial communities alongside LMH. In accordance with global predictions, these herbivore-adapted microbial communities appear to be driven by seasonal variation. Yet, herbivore-related activity has a notable effect of aboveground structure, which can potentially either mitigate or exacerbate seasonal effects through facilitating the development of microsites.

Herbivory was shown to have numerous cross-scale top-down effects on sodic ecosystem structure and associated function. The presence of native LMH was revealed to have a negative effect on herbaceous biomass which corresponded with higher levels of bacterial α -diversity and associated decomposition rate (k) of organic material. In contrast, a relaxation in herbivore pressure was found to facilitate higher levels of herbaceous biomass together with fungal α -diversity and stabilisation factor (S) of organic material. Moreover, soil chemical properties were found to support and clarify observed patterns in bacterial community composition, whilst fungal community patterns were better associated with changes in vegetation structure and physical soil properties.

In conclusion, herbivores are deemed to be major drivers of above- and belowground heterogeneity and associated soil function within semi-arid sodic ecosystems of the KNP. Native LMH appear to promote a highly heterogeneous environment capable of supporting high biodiversity, essentially maintained by an underlying fast nutrient cycle driven by bacterial-based decomposition. The removal of herbivores leads to a more homogenous aboveground structure, and through several top-down feedbacks facilitate a shift in microbial composition, favouring fungi and associated slower nutrient turnover. Long-term monitoring is required to elucidate patterns of change in microbial community composition which could potentially aid in predicting disturbance- and seasonal-driven variation in carbon flux.

This study essentially contributes towards a broader understanding of semi-arid sodic savanna response to disturbances and the potential holistic implications for ecosystem structure and function.

10.2 The way forward

It is evident that the effects of native LMH, or potential implications of the loss thereof, stretch much further than generally accepted. Throughout literature the effects of herbivory are compartmentalised, either focusing on aboveground or belowground effects. Numerous studies have provided detailed descriptions of herbivore-induced disturbances on aboveground communities. It is, however, clear that LMH have a substantial impact on soil microbial community composition and associated soil function (decomposition and stabilisation). The diverse suite of native LMH should therefore be managed as a cross-scale determinant of system functioning.

To better understand and effectively manage sodic systems, further studies should be employed to elaborate on the exact mechanisms through which herbivore activity influences belowground microbes and soil function. Such information can aid in the development of above- and belowground conservation strategies specifically aimed at conserving belowground heterogeneity as a means to manage nutrient cycling.

10.2.1 Recommendations for the sodic zone of the Nkuhlu exclosures, KNP

- Highest decomposition rates (k) and bacterial α -diversity measures were associated with highest environmental disturbance (i.e. Control site with all LMH present); herbivore presence therefore promotes a rapid, bacterial-driven nutrient cycle.
- Herbaceous biomass levels increase in the absence of herbivory which, in turn, corresponds with highest fungal α -diversity and stabilisation (S).
- A release in herbivore pressure subsequently results in a belowground shift from a rapid, bacterial-driven cycle to a slower, fungal-driven cycle; indicating that herbivores are necessary to drive and maintain essential soil functioning in terms of decomposition.
- Plant functional traits did not reveal a substantial direct association with decomposition in the highly dynamic sodic zone; trait analyses alone should therefore not be used as an indicator of soil function on a local scale, at least for sodic patches, as these areas are governed by various top-down and bottom-up controls that collectively regulate system functioning.

- Although sodic patches are often regarded as overgrazed and denuded, native herbivores, and associated disturbances, are essential for the maintenance of aboveground structure, and through numerous trophic cascades, microbial community composition and soil function; herbivory should therefore be considered as a major driver of these systems in management practices.

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Appendix A: Experimental layout of the Nkuhlu exclosures with fire and no fire treatments

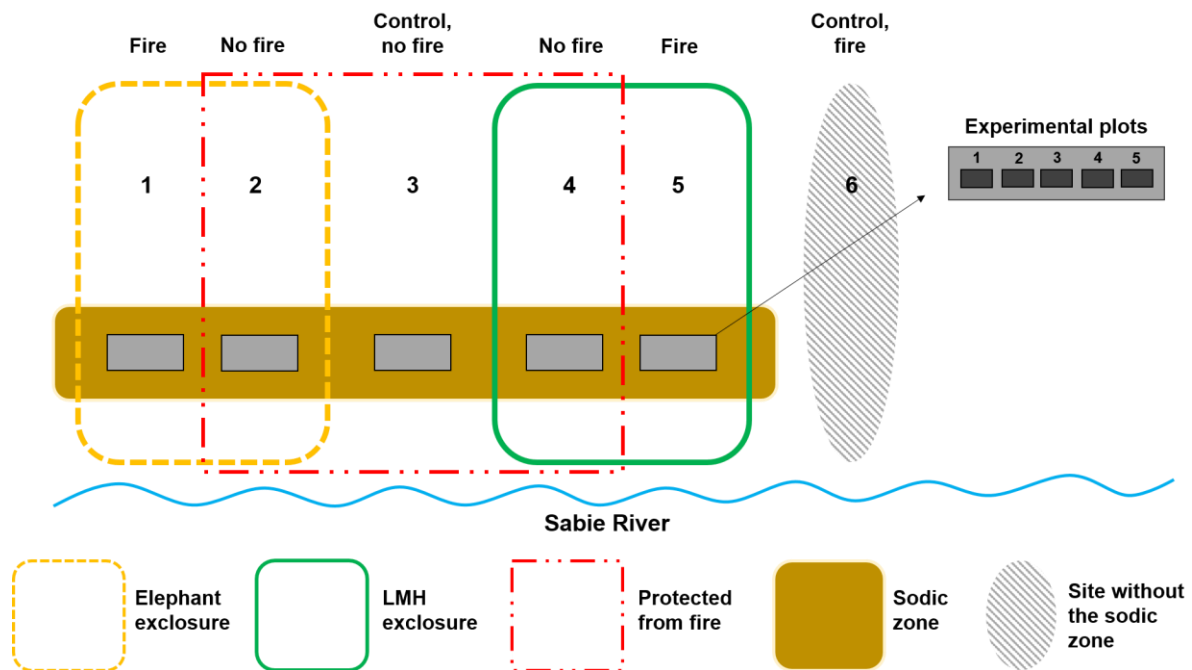


Figure A1: Graphical representation of the six herbivore-fire treatments within the Nkuhlu exclosures. Treatment 6 (Control, fire) was not included in this study as the sodic zone is not present in this area.

Herbivore treatment	Fire treatment	Incubation period	Variable	df	<i>F</i>	<i>p</i>
Elephant enclosure	Fire vs no fire	3-months	<i>k</i>	36	2,14	0,099
			<i>S</i>	36	1,63	0,319
Elephant enclosure	Fire vs no fire	6-months	<i>k</i>	34	1,79	0,198
			<i>S</i>	19	7,46	0,056
Elephant enclosure	Fire vs no fire	9-months	<i>k</i>	22	4,02	0,253
			<i>S</i>	30	1,27	0,217
Elephant enclosure	Fire vs no fire	12-months	<i>k</i>	20	2,21	0,921
			<i>S</i>	20	1,64	0,262
LMH enclosure	Fire vs no fire	3-months	<i>k</i>	38	1,15	0,776
			<i>S</i>	27	4,67	0,001*
LMH enclosure	Fire vs no fire	6-months	<i>k</i>	20	5,41	0,920
			<i>S</i>	30	1,67	0,556
LMH enclosure	Fire vs no fire	9-months	<i>k</i>	26	3,62	0,153
			<i>S</i>	34	1,16	0,636
LMH enclosure	Fire vs no fire	12-months	<i>k</i>	24	1,48	0,819
			<i>S</i>	24	1,26	0,078

Appendix C: Heat tree analysis and accompanying Wilcoxon *p*-values

C1: Heat tree With all LMH vs Elephant enclosure -Bacteria

id,tax_rank,tax_name,treatment_1,treatment_2,log2_median_ratio,median_diff,mean_diff,wilcox_p_value	
ab,r,Root,With all LMH,Elephant enclosure,0,0,0,	
ac,p,Firmicutes,With all LMH,Elephant enclosure,0.33793164,0.156924767,0.194232897,	0.049031029
ad,p,Proteobacteria,With all LMH,Elephant enclosure,-0.71016303,-0.080986549,-0.100350367,	0.074460605
ae,p,Actinobacteria,With all LMH,Elephant enclosure,-0.044371098,-0.001834981,-0.01802129,	0.894881895
af,p,Bacteroidetes,With all LMH,Elephant enclosure,0,0,-0.014193239,	0.315468984
ag,p,Acidobacteria,With all LMH,Elephant enclosure,-1.683453794,-0.062320636,-0.061668001,	0.043975954
ah,c,Bacilli,With all LMH,Elephant enclosure,0.33793164,0.156924767,0.194232897,	0.049031029
ai,c,Alphaproteobacteria,With all LMH,Elephant enclosure,-0.71016303,-0.080986549,-0.100350367,	0.074460605
aj,c,Actinobacteria,With all LMH,Elephant enclosure,0.171296211,0.006577427,-0.000681678,	0.808071006
ak,c,Bacteroidia,With all LMH,Elephant enclosure,0,0,-0.014193239,	0.315468984
al,c,Acidobacteriia,With all LMH,Elephant enclosure,-1.683453794,-0.062320636,-0.061668001,	0.043975954
am,c,Rubrobacteria,With all LMH,Elephant enclosure,0,0,-0.017339613,	0.265995085
an,o,Bacillales,With all LMH,Elephant enclosure,0.33793164,0.156924767,0.194232897,	0.049031029
ao,o,Sphingomonadales,With all LMH,Elephant enclosure,-0.73267884,-0.051980101,-0.085458889,	0.081910911
ap,o,Frankiales,With all LMH,Elephant enclosure,Inf,0.043059908,0.044658908,	0.000645214
aq,o,Rhizobiales,With all LMH,Elephant enclosure,0.494492208,0.009766267,0.001142742,	0.767598422
ar,o,Micropepsales,With all LMH,Elephant enclosure,Inf,0.028235668,0.012060825,	0.223324962
as,o,Pseudonocardiales,With all LMH,Elephant enclosure,0,0,-0.001593588,	0.95493234
at,o,Chitinophagales,With all LMH,Elephant enclosure,0,0,-0.014193239,	0.315468984
au,o,Micrococcales,With all LMH,Elephant enclosure,0,0,-0.00182551,	0.333923855
av,o,Solibacterales,With all LMH,Elephant enclosure,-1.683453794,-0.062320636,-0.061668001,	0.043975954
aw,o,Rubrobacterales,With all LMH,Elephant enclosure,0,0,-0.017339613,	0.265995085
ax,o,Elsterales,With all LMH,Elephant enclosure,0,0,-0.028095044,	0.087414963
ay,o,Kineosporiales,With all LMH,Elephant enclosure,#NAME?, -0.035506256,-0.041921488,	0.016078351
az,f,Bacillaceae,With all LMH,Elephant enclosure,0.33793164,0.156924767,0.194807177,	0.039246577
ba,f,Sphingomonadaceae,With all LMH,Elephant enclosure,-0.73267884,-0.051980101,-0.085458889,	0.081910911
bb,f,Acidothermaceae,With all LMH,Elephant enclosure,Inf,0.043059908,0.044658908,	0.000645214
bc,f,Xanthobacteraceae,With all LMH,Elephant enclosure,0.494492208,0.009766267,0.001142742,	0.767598422
bd,f,Micropepsaceae,With all LMH,Elephant enclosure,Inf,0.028235668,0.012060825,	0.223324962
be,f,Pseudonocardiaceae,With all LMH,Elephant enclosure,0,0,-0.001593588,	0.95493234
bf,f,Chitinophagaceae,With all LMH,Elephant enclosure,0,0,-0.014193239,	0.315468984
bg,f,Intrasporangiaceae,With all LMH,Elephant enclosure,0,0,-0.00182551,	0.333923855
bh,f,Solibacteraceae (Subgroup_3),With all LMH,Elephant enclosure,-1.683453794,-0.062320636,-0.061668001,	0.043975954
bi,f,Rubrobacteriaceae,With all LMH,Elephant enclosure,0,0,-0.017339613,	0.265995085
bj,f,uncultured,With all LMH,Elephant enclosure,0,0,-0.028095044,	0.087414963
bk,f,Planococcaceae,With all LMH,Elephant enclosure,0,0,-0.00057428,	1

Appendix C

bl,f,Kineosporiaceae,With all LMH,Elephant exclosure,#NAME?,-0.035506256,-0.041921488,0.016078351
bm,g,Bacillus,With all LMH,Elephant exclosure,0.33793164,0.156924767,0.194807177,0.039246577
bn,g,Sphingomonas,With all LMH,Elephant exclosure,-0.73267884,-0.051980101,-0.085458889,0.081910911
bo,g,Acidothermus,With all LMH,Elephant exclosure,Inf,0.043059908,0.044658908,0.000645214
bp,g,Bradyrhizobium,With all LMH,Elephant exclosure,0.494492208,0.009766267,0.001142742,0.767598422
bq,g,uncultured,With all LMH,Elephant exclosure,Inf,0.028235668,0.012060825,0.223324962
br,g,Crossiella,With all LMH,Elephant exclosure,0,0,-0.001593588,0.95493234
bs,g,Flavisolibacter,With all LMH,Elephant exclosure,0,0,-0.014193239,0.315468984
bt,g,Lapillicoccus,With all LMH,Elephant exclosure,0,0,-0.00182551,0.333923855
bu,g,Candidatus Solibacter,With all LMH,Elephant exclosure,0,0,-0.014479037,0.53263312
bv,g,Rubrobacter,With all LMH,Elephant exclosure,0,0,-0.017339613,0.265995085
bw,g,Bryobacter,With all LMH,Elephant exclosure,#NAME?,-0.054407955,-0.047188964,0.011322473
bx,g,Sporosarcina,With all LMH,Elephant exclosure,0,0,-0.00057428,1
by,g,Kineosporia,With all LMH,Elephant exclosure,#NAME?,-0.035506256,-0.041921488,0.016078351

C2: Heat tree LMH exclosure vs Elephant exclosure -Bacteria

id,tax_rank,tax_name,treatment_1,treatment_2,log2_median_ratio,median_diff,mean_diff,wilcox_p_value
ab,r,Root,LMH exclosure,Elephant exclosure,0,0,0,
ac,p,Firmicutes,LMH exclosure,Elephant exclosure,-0.17100955,-0.066457125,-0.007896913,0.633352256
ad,p,Proteobacteria,LMH exclosure,Elephant exclosure,0.214413627,0.033380559,0.003131342,0.942434056
ae,p,Actinobacteria,LMH exclosure,Elephant exclosure,1.157327819,0.074546556,0.0529521,0.021591186
af,p,Bacteroidetes,LMH exclosure,Elephant exclosure,0,0,-0.009301061,0.257587402
ag,p,Acidobacteria,LMH exclosure,Elephant exclosure,-0.939647922,-0.043314534,-0.038885468,0.140174781
ah,c,Bacilli,LMH exclosure,Elephant exclosure,-0.17100955,-0.066457125,-0.007896913,0.633352256
ai,c,Alphaproteobacteria,LMH exclosure,Elephant exclosure,0.214413627,0.033380559,0.003131342,0.942434056
aj,c,Actinobacteria,LMH exclosure,Elephant exclosure,0.883861613,0.044102181,0.03966005,0.036015224
ak,c,Bacteroidia,LMH exclosure,Elephant exclosure,0,0,-0.009301061,0.257587402
al,c,Acidobacteriia,LMH exclosure,Elephant exclosure,-0.939647922,-0.043314534,-0.038885468,0.140174781
am,c,Rubrobacteria,LMH exclosure,Elephant exclosure,0,0,0.01329205,0.71667767
an,o,Bacillales,LMH exclosure,Elephant exclosure,-0.17100955,-0.066457125,-0.007896913,0.633352256
ao,o,Sphingomonadales,LMH exclosure,Elephant exclosure,-0.259687248,-0.02150282,-0.045769253,0.388185512
ap,o,Frankiales,LMH exclosure,Elephant exclosure,0,0,0.037090826,0.003482173
aq,o,Rhizobiales,LMH exclosure,Elephant exclosure,1.467009865,0.042151101,0.024812699,0.031277728
ar,o,Micropepsales,LMH exclosure,Elephant exclosure,0,0,0.001010193,0.852542179
as,o,Pseudonocardiales,LMH exclosure,Elephant exclosure,0,0,0.008680156,0.271729671
at,o,Chitinophagales,LMH exclosure,Elephant exclosure,0,0,-0.009301061,0.257587402
au,o,Micrococcales,LMH exclosure,Elephant exclosure,Inf,0.033616925,0.038804383,3.71E-05
av,o,Solibacterales,LMH exclosure,Elephant exclosure,-0.939647922,-0.043314534,-0.038885468,0.140174781
aw,o,Rubrobacterales,LMH exclosure,Elephant exclosure,0,0,0.01329205,0.71667767
ax,o,Elsterales,LMH exclosure,Elephant exclosure,Inf,0.052932636,0.023077704,0.146199698
ay,o,Kineosporiales,LMH exclosure,Elephant exclosure,#NAME?,-0.035506256,-0.044915316,0.000752202
az,f,Bacillaceae,LMH exclosure,Elephant exclosure,-0.194046644,-0.074822633,-0.007302808,0.612869891
ba,f,Sphingomonadaceae,LMH exclosure,Elephant exclosure,-0.259687248,-0.02150282,-0.045769253,0.388185512
bb,f,Acidothermaceae,LMH exclosure,Elephant exclosure,0,0,0.037090826,0.003482173

Appendix C

bc,f,Xanthobacteraceae,LMH exclosure,Elephant exclosure,1.467009865,0.042151101,0.024812699,0.031277728
bd,f,Micropepsaceae,LMH exclosure,Elephant exclosure,0,0,0.001010193,0.852542179
be,f,Pseudonocardiaceae,LMH exclosure,Elephant exclosure,0,0,0.008680156,0.271729671
bf,f,Chitinophagaceae,LMH exclosure,Elephant exclosure,0,0,-0.009301061,0.257587402
bg,f,Intrasporangiaceae,LMH exclosure,Elephant exclosure,Inf,0.033616925,0.038804383,3.71E-05
bh,f,Solibacteraceae_(Subgroup_3),LMH exclosure,Elephant exclosure,-0.939647922,-0.043314534,-0.038885468,0.140174781
bi,f,Rubrobacteriaceae,LMH exclosure,Elephant exclosure,0,0,0.01329205,0.71667767
bj,f,uncultured,LMH exclosure,Elephant exclosure,Inf,0.052932636,0.023077704,0.146199698
bk,f,Planococcaceae,LMH exclosure,Elephant exclosure,0,0,-0.000594105,1
bl,f,Kineosporiaceae,LMH exclosure,Elephant exclosure,#NAME?,-0.035506256,-0.044915316,0.000752202
bm,g,Bacillus,LMH exclosure,Elephant exclosure,-0.194046644,-0.074822633,-0.007302808,0.612869891
bn,g,Sphingomonas,LMH exclosure,Elephant exclosure,-0.259687248,-0.02150282,-0.045769253,0.388185512
bo,g,Acidothermus,LMH exclosure,Elephant exclosure,0,0,0.037090826,0.003482173
bp,g,Bradyrhizobium,LMH exclosure,Elephant exclosure,1.467009865,0.042151101,0.024812699,0.031277728
bq,g,uncultured,LMH exclosure,Elephant exclosure,0,0,0.001010193,0.852542179
br,g,Crossiella,LMH exclosure,Elephant exclosure,0,0,0.008680156,0.271729671
bs,g,Flavisolibacter,LMH exclosure,Elephant exclosure,0,0,-0.009301061,0.257587402
bt,g,Lapillicoccus,LMH exclosure,Elephant exclosure,Inf,0.033616925,0.038804383,3.71E-05
bu,g,Candidatus Solibacter,LMH exclosure,Elephant exclosure,0,0,-0.000457026,1
bv,g,Rubrobacter,LMH exclosure,Elephant exclosure,0,0,0.01329205,0.71667767
bw,g,Bryobacter,LMH exclosure,Elephant exclosure,#NAME?,-0.054407955,-0.038428442,0.017851339
bx,g,Sporosarcina,LMH exclosure,Elephant exclosure,0,0,-0.000594105,1
by,g,Kineosporia,LMH exclosure,Elephant exclosure,#NAME?,-0.035506256,-0.044915316,0.000752202

C3: Heat tree_LMH exclosure vs With all LMH -Bacteria

id,tax_rank,tax_name,treatment_1,treatment_2,log2_median_ratio,median_diff,mean_diff,wilcox_p_value
ab,r,Root,LMH exclosure,With all LMH,0,0,0,
ac,p,Firmicutes,LMH exclosure,With all LMH,-0.508941191,-0.223381892,-0.20212981,0.005176822
ad,p,Proteobacteria,LMH exclosure,With all LMH,0.924576656,0.114367108,0.103481708,0.016500866
ae,p,Actinobacteria,LMH exclosure,With all LMH,1.201698917,0.076381538,0.07097339,0.025699307
af,p,Bacteroidetes,LMH exclosure,With all LMH,0,0,0.004892179,0.971367276
ag,p,Acidobacteria,LMH exclosure,With all LMH,0.743805872,0.019006101,0.022782533,0.710684071
ah,c,Bacilli,LMH exclosure,With all LMH,-0.508941191,-0.223381892,-0.20212981,0.005176822
ai,c,Alphaproteobacteria,LMH exclosure,With all LMH,0.924576656,0.114367108,0.103481708,0.016500866
aj,c,Actinobacteria,LMH exclosure,With all LMH,0.712565402,0.037524754,0.040341727,0.097177467
ak,c,Bacteroidia,LMH exclosure,With all LMH,0,0,0.004892179,0.971367276
al,c,Acidobacteriia,LMH exclosure,With all LMH,0.743805872,0.019006101,0.022782533,0.710684071
am,c,Rubrobacteria,LMH exclosure,With all LMH,0,0,0.030631663,0.292750772
an,o,Bacillales,LMH exclosure,With all LMH,-0.508941191,-0.223381892,-0.20212981,0.005176822
ao,o,Sphingomonadales,LMH exclosure,With all LMH,0.472991593,0.030477281,0.039689636,0.041299247

Appendix C

ap,o,Frankiales,LMH exclosure,With all LMH,#NAME?,-0.043059908,-0.007568082,0.48907721
aq,o,Rhizobiales,LMH exclosure,With all LMH,0.972517657,0.032384834,0.023669957,0.09422973
ar,o,Micropepsales,LMH exclosure,With all LMH,#NAME?,-0.028235668,-0.011050632,0.325510107
as,o,Pseudonocardiales,LMH exclosure,With all LMH,0,0,0.010273744,0.401472783
at,o,Chitinophagales,LMH exclosure,With all LMH,0,0,0.004892179,0.971367276
au,o,Micrococcales,LMH exclosure,With all LMH,Inf,0.033616925,0.040629893,0.000777246
av,o,Solibacterales,LMH exclosure,With all LMH,0.743805872,0.019006101,0.022782533,0.710684071
aw,o,Rubrobacterales,LMH exclosure,With all LMH,0,0,0.030631663,0.292750772
ax,o,Elsterales,LMH exclosure,With all LMH,Inf,0.052932636,0.051172747,0.006719459
ay,o,Kineosporiales,LMH exclosure,With all LMH,0,0,-0.002993828,0.66765098
az,f,Bacillaceae,LMH exclosure,With all LMH,-0.531978284,-0.2317474,-0.202109985,0.005176822
ba,f,Sphingomonadaceae,LMH exclosure,With all LMH,0.472991593,0.030477281,0.039689636,0.041299247
bb,f,Acidothermaceae,LMH exclosure,With all LMH,#NAME?,-0.043059908,-0.007568082,0.48907721
bc,f,Xanthobacteraceae,LMH exclosure,With all LMH,0.972517657,0.032384834,0.023669957,0.09422973
bd,f,Micropepsaceae,LMH exclosure,With all LMH,#NAME?,-0.028235668,-0.011050632,0.325510107
be,f,Pseudonocardiaceae,LMH exclosure,With all LMH,0,0,0.010273744,0.401472783
bf,f,Chitinophagaceae,LMH exclosure,With all LMH,0,0,0.004892179,0.971367276
bg,f,Intrasporangiaceae,LMH exclosure,With all LMH,Inf,0.033616925,0.040629893,0.000777246
bh,f,Solibacteraceae (Subgroup 3),LMH exclosure,With all LMH,0.743805872,0.019006101,0.022782533,0.710684071
bi,f,Rubrobacteriaceae,LMH exclosure,With all LMH,0,0,0.030631663,0.292750772
bj,f,uncultured,LMH exclosure,With all LMH,Inf,0.052932636,0.051172747,0.006719459
bk,f,Planococcaceae,LMH exclosure,With all LMH,0,0,-1.98E-05,0.90429246
bl,f,Kineosporiaceae,LMH exclosure,With all LMH,0,0,-0.002993828,0.66765098
bm,g,Bacillus,LMH exclosure,With all LMH,-0.531978284,-0.2317474,-0.202109985,0.005176822
bn,g,Sphingomonas,LMH exclosure,With all LMH,0.472991593,0.030477281,0.039689636,0.041299247
bo,g,Acidothermus,LMH exclosure,With all LMH,#NAME?,-0.043059908,-0.007568082,0.48907721
bp,g,Bradyrhizobium,LMH exclosure,With all LMH,0.972517657,0.032384834,0.023669957,0.09422973
bq,g,uncultured,LMH exclosure,With all LMH,#NAME?,-0.028235668,-0.011050632,0.325510107
br,g,Crossiella,LMH exclosure,With all LMH,0,0,0.010273744,0.401472783
bs,g,Flavisolibacter,LMH exclosure,With all LMH,0,0,0.004892179,0.971367276
bt,g,Lapillicoccus,LMH exclosure,With all LMH,Inf,0.033616925,0.040629893,0.000777246
bu,g,Candidatus Solibacter,LMH exclosure,With all LMH,0,0,0.014022011,0.56790242
bv,g,Rubrobacter,LMH exclosure,With all LMH,0,0,0.030631663,0.292750772
bw,g,Bryobacter,LMH exclosure,With all LMH,0,0,0.008760522,0.891812728
bx,g,Sporosarcina,LMH exclosure,With all LMH,0,0,-1.98E-05,0.90429246
by,g,Kineosporia,LMH exclosure,With all LMH,0,0,-0.002993828,0.66765098

C4: Heat tree_With all LMH vs Elephant exclosure -Fungi

id,tax_rank,tax_name,treatment_1,treatment_2,log2_median_ratio,median_diff,mean_diff,wilcox_p_value
ab,r,Root,With all LMH,Elephant exclosure,0,0,0,
ac,p,Ascomycota,With all LMH,Elephant exclosure,0.0921810267738643,0.0570619862770618,0.0837898500538382,0.0439193656584961
ad,p,unidentified,With all LMH,Elephant exclosure,-0.695341696164741,-0.0243064421115027,-0.057043024969041,0.00961583810159522
ae,p,Chytridiomycota,With all LMH,Elephant exclosure,0,0,-0.000716447281924501,0.865370330861995
af,p,Mucoromycota,With all LMH,Elephant exclosure,-2.67634077784654,-0.0243939169002455,-0.0291795477801017,0.0231617956149636
ag,p,Basidiomycota,With all LMH,Elephant exclosure,-0.950345121667796,-0.0102196212922425,0.0086170726356705,0.558847515968955
ah,p,Mortierellomycota,With all LMH,Elephant exclosure,0,0,-0.00259584631961857,0.614007162175148
ai,p,Glomeromycota,With all LMH,Elephant exclosure,0,0,-0.00287205633882297,0.0969044446618861
aj,c,Eurotiomycetes,With all LMH,Elephant exclosure,0.998751582701225,0.330204330356061,0.223807628050454,0.0145814538618137
ak,c,Dothideomycetes,With all LMH,Elephant exclosure,-1.3564278776811,-0.176028080314241,-0.145117353195621,0.0244241515605833
al,c,Sordariomycetes,With all LMH,Elephant exclosure,0.373644582399913,0.0279975872867412,0.0264015170524955,0.230725263408922
am,c,unidentified,With all LMH,Elephant exclosure,-0.695341696164741,-0.0243064421115027,-0.057043024969041,0.00961583810159522
an,c,Rhizophlyctidomycetes,With all LMH,Elephant exclosure,0,0,-0.000716447281924501,0.865370330861995
ao,c,Mucoromycetes,With all LMH,Elephant exclosure,-2.67634077784654,-0.0243939169002455,-0.0291795477801017,0.0231617956149636
ap,c,Agaricomycetes,With all LMH,Elephant exclosure,-0.939432455474806,-0.00910165828455685,0.0133161262630228,0.912411300102377
aq,c,Pezizomycetes,With all LMH,Elephant exclosure,-4.96261891872825,-0.00729727046608737,-0.0177580125514633,0.0972151039392309
ar,c,Mortierellomycetes,With all LMH,Elephant exclosure,0,0,-0.00259584631961857,0.614007162175148
as,c,Ustilaginomycetes,With all LMH,Elephant exclosure,-Inf,-0.00351167874749403,-0.00469905362735225,0.0130394025637584
at,c,Orbiliomycetes,With all LMH,Elephant exclosure,0,0,-0.00044429708546945,0.974888034173419
au,c,Arthoniomycetes,With all LMH,Elephant exclosure,0,0,-0.00190508662737367,0.0969044446618861
av,o,Eurotiales,With all LMH,Elephant exclosure,1.11740242774809,0.34627071378903,0.22300346754519,0.0145814538618137
aw,o,Capnodiales,With all LMH,Elephant exclosure,-Inf,-0.00716720785661244,-0.00879542568934011,0.113363142726972
ax,o,Microascales,With all LMH,Elephant exclosure,-Inf,-0.000693802035152636,0.00305106399922838,0.422334693983414
ay,o,Pleosporales,With all LMH,Elephant exclosure,-1.1959203119688,-0.138538035059593,-0.11480000283318,0.0439193656584961
az,o,Dothideales,With all LMH,Elephant exclosure,0,0,-0.00369256021885939,0.358875442514589
ba,o,Hypocreales,With all LMH,Elephant exclosure,-0.891346965545477,-0.00915373355968335,-0.010547980140241,0.209852064211511
bb,o,Botryosphaeriales,With all LMH,Elephant exclosure,-Inf,-0.0143343895618779,-0.0178293644542415,5.44390912111041e-05
bc,o,unidentified,With all LMH,Elephant exclosure,-0.695341696164741,-0.0243064421115027,-0.057043024969041,0.00961583810159522
bd,o,Chaetothyriales,With all LMH,Elephant exclosure,-0.207980863402943,-0.00303135542037145,0.000804160505264776,0.71316383100491
be,o,Trichosphaeriales,With all LMH,Elephant exclosure,0,0,-0.000515146561288983,0.765374421105976
bf,o,Rhizophlyctidales,With all LMH,Elephant exclosure,0,0,-0.000716447281924501,0.865370330861995
bg,o,Sordariales,With all LMH,Elephant exclosure,Inf,0.0137771864545337,0.0158888171952992,0.00594681961794146
bh,o,Xylariales,With all LMH,Elephant exclosure,-0.89397183172752,-0.00124043029463996,-0.00307189949611351,0.461857826516206
bi,o,Diaporthales,With all LMH,Elephant exclosure,0,0,-0.000217722085040506,0.333923855116587
bj,o,Glomerellales,With all LMH,Elephant exclosure,0,0,0.000550503080013074,0.145177573483619
bk,o,Mucorales,With all LMH,Elephant exclosure,-2.67634077784654,-0.0243939169002455,-0.0291795477801017,0.0231617956149636
bl,o,Geastrales,With all LMH,Elephant exclosure,-Inf,-0.00219133205015895,-0.0126537264508597,0.0203261536892711
bm,o,Pezizales,With all LMH,Elephant exclosure,-4.96261891872825,-0.00729727046608737,-0.0177580125514633,0.0972151039392309
bn,o,Mortierellales,With all LMH,Elephant exclosure,0,0,-0.00259584631961857,0.614007162175148
bo,o,Agaricales,With all LMH,Elephant exclosure,0.00321977258850726,1.66564758618936e-05,0.023507470470283,0.551678689368704
bp,o,Ustilaginales,With all LMH,Elephant exclosure,-Inf,-0.00351167874749403,-0.00469905362735225,0.0130394025637584

Appendix C

bq,o,unidentified,With all LMH,Elephant exclosure,0.157400127828801,0.000262890017994285,-0.000501772286426657,0.484405073921104
br,o,Cantharellales,With all LMH,Elephant exclosure,Inf,0.00146790475767717,0.00246238224359946,0.0157535324416255
bs,o,Orbiliales,With all LMH,Elephant exclosure,0,0,-0.00044429708546945,0.974888034173419
bt,o,Lichenostigmatales,With all LMH,Elephant exclosure,0,0,-0.00190508662737367,0.0969044446618861
bu,f,Aspergillaceae,With all LMH,Elephant exclosure,1.13925108870389,0.349258192314205,0.223059422120637,0.0145814538618137
bv,f,Cladosporiaceae,With all LMH,Elephant exclosure,-Inf,-0.000630676084762866,-0.00469576946656314,0.171938502538061
bw,f,Microascaceae,With all LMH,Elephant exclosure,-Inf,-0.000693802035152636,0.00305106399922838,0.422334693983414
bx,f,Coniothyriaceae,With all LMH,Elephant exclosure,-5.68219344123606,-0.00878034162672633,-0.0163204073334462,0.0401120119924237
by,f,Pleosporaceae,With all LMH,Elephant exclosure,-0.923798271992239,-0.00678281024589937,-0.00121918441472299,0.300122890373011
bz,f,Didymellaceae,With all LMH,Elephant exclosure,-Inf,-0.00326664829048204,-0.0132920584417102,0.12647125293021
ca,f,Aureobasidiaceae,With all LMH,Elephant exclosure,0,0,-0.00369256021885939,0.358875442514589
cb,f,Phaeosphaeriaceae,With all LMH,Elephant exclosure,0,0,-0.00334470416518332,0.445499747220962
cc,f,Sporormiaceae,With all LMH,Elephant exclosure,-0.0476734873672283,-0.000539353657803471,-0.0179884206209349,0.86029699381144
cd,f,Didymosphaeriaceae,With all LMH,Elephant exclosure,-1.76005675220685,-0.0275938900212882,-0.0352701102780047,0.00191590730460134
ce,f,Nectriaceae,With all LMH,Elephant exclosure,-1.59168454413934,-0.0112335209707971,-0.00896011793240296,0.20375998049345
cf,f,Botryosphaeriaceae,With all LMH,Elephant exclosure,-Inf,-0.0139482767795493,-0.0163242602632258,0.000375700647916873
cg,f,unidentified,With all LMH,Elephant exclosure,-0.695341696164741,-0.0243064421115027,-0.057043024969041,0.00961583810159522
ch,f,Herpotrichiellaceae,With all LMH,Elephant exclosure,0,0,-0.00119160514228229,0.709741753995744
ci,f,Trichosphaeriaceae,With all LMH,Elephant exclosure,0,0,-0.000515146561288983,0.765374421105976
cj,f,Rhizophlyctidaceae,With all LMH,Elephant exclosure,0,0,-0.000716447281924501,0.865370330861995
ck,f,Sordariaceae,With all LMH,Elephant exclosure,0,0,0.00443432761768493,0.0194069136269711
cl,f,unidentified,With all LMH,Elephant exclosure,-1.64760261935072,-0.0121362155003511,-0.0115767571599036,0.127573301665183
cm,f,Trichocomaceae,With all LMH,Elephant exclosure,0,0,-5.59545754477316e-05,0.858542156510437
cn,f,Chaetothyriaceae,With all LMH,Elephant exclosure,-0.210536385220942,-0.00265351944753809,0.00421368704538836,0.91396093495044
co,f,Sporocadaceae,With all LMH,Elephant exclosure,0,0,-0.000734451196711573,0.223831834064576
cp,f,Cordycipitaceae,With all LMH,Elephant exclosure,0,0,-0.000877409537669058,0.333923855116587
cq,f,unidentified,With all LMH,Elephant exclosure,0,0,-0.000127197039777983,0.524518280213076
cr,f,Glomerellaceae,With all LMH,Elephant exclosure,0,0,0.000550503080013074,0.145177573483619
cs,f,Rhizopodaceae,With all LMH,Elephant exclosure,-2.67634077784654,-0.0243939169002455,-0.0291795477801017,0.0231617956149636
ct,f,Geastraceae,With all LMH,Elephant exclosure,-Inf,-0.00219133205015895,-0.0126537264508597,0.0203261536892711
cu,f,Pezizaceae,With all LMH,Elephant exclosure,-4.96261891872825,-0.00729727046608737,-0.0177580125514633,0.0972151039392309
cv,f,Hypocreaceae,With all LMH,Elephant exclosure,0.280130267792411,0.000452938472352738,-0.000710452670169023,0.982004463130138
cw,f,Mortierellaceae,With all LMH,Elephant exclosure,0,0,-0.00259584631961857,0.614007162175148
cx,f,Lasiosphaeriaceae,With all LMH,Elephant exclosure,Inf,0.00526901062391749,0.0114544895776142,0.0502387291772232
cy,f,Lycoperdaceae,With all LMH,Elephant exclosure,Inf,0.00530676220331393,0.026653295163877,0.0116326521259382
da,f,unidentified,With all LMH,Elephant exclosure,-0.302169964952389,-0.000336738066393125,-0.00233744829940193,0.606216233342809
db,f,unidentified,With all LMH,Elephant exclosure,0,0,-0.00409965622277697,0.0979254820037898
dc,f,unidentified,With all LMH,Elephant exclosure,0.157400127828801,0.000262890017994285,-0.000501772286426657,0.484405073921104
dd,f,Ceratobasidiaceae,With all LMH,Elephant exclosure,Inf,0.00146790475767717,0.00246238224359946,0.0157535324416255
de,f,Orbiliaceae,With all LMH,Elephant exclosure,0,0,-0.00044429708546945,0.974888034173419
df,f,Phaeococcomycetaceae,With all LMH,Elephant exclosure,0,0,-0.00190508662737367,0.0969044446618861
dg,f,ChaetothyrialesfamIncertaedis,With all LMH,Elephant exclosure,-Inf,-0.00140848778604593,-0.00209072435806331,0.00551508837621502
dh,f,Aplosporellaceae,With all LMH,Elephant exclosure,-Inf,-0.000387296669248645,-0.00150510419101567,0.0234470805423998

Appendix C

di,g,Aspergillus,With all LMH,Elephant exclosure,1.15542622069539,0.352495935144917,0.226367594631543,0.0175039499690511
dj,g,Cladosporium,With all LMH,Elephant exclosure,-Inf,-0.000630676084762866,-0.00469576946656314,0.171938502538061
dk,g,Microascus,With all LMH,Elephant exclosure,-Inf,-0.000693802035152636,0.00305106399922838,0.422334693983414
dl,g,Hazslinszkyomyces,With all LMH,Elephant exclosure,-5.68219344123606,-0.00878034162672633,-0.0163204073334462,0.0401120119924237
dm,g,Exserohilum,With all LMH,Elephant exclosure,-0.0508178556663834,-0.000101356874842857,0.000661978409697697,0.964247178090966
dn,g,Aureobasidium,With all LMH,Elephant exclosure,0,0,-0.00369256021885939,0.358875442514589
do,g,Sclerostagonospora,With all LMH,Elephant exclosure,0,0,-0.00334470416518332,0.445499747220962
dp,g,Didymella,With all LMH,Elephant exclosure,0,0,0.00379228118857229,0.413107205228418
dq,g,Penicillium,With all LMH,Elephant exclosure,-Inf,-0.000754375377187689,-0.00330817251090514,0.117655006566824
dr,g,Westerdykella,With all LMH,Elephant exclosure,-2.24436350791311,-0.00804207866012924,-0.00705425938129487,0.216213424944972
ds,g,Hendersonia,With all LMH,Elephant exclosure,0,0,-0.00451982962191988,0.0778238743711169
dt,g,Fusarium,With all LMH,Elephant exclosure,-1.59168454413934,-0.0112335209707971,-0.00896011793240296,0.20375998049345
du,g,Botryosphaeria,With all LMH,Elephant exclosure,-Inf,-0.0139482767795493,-0.0163242602632258,0.000375700647916873
dv,g,Curvularia,With all LMH,Elephant exclosure,-0.423682459466848,-0.00183704245200925,0.00344497070818882,0.627087263425293
dw,g,unidentified,With all LMH,Elephant exclosure,-0.695341696164741,-0.0243064421115027,-0.057043024969041,0.00961583810159522
dx,g,Cochliobolus,With all LMH,Elephant exclosure,0,0,0.000162771844559422,0.966307075684792
dy,g,Paraconiothyrium,With all LMH,Elephant exclosure,-Inf,-0.00270990609199473,-0.00855771983571447,0.251752593715159
dz,g,Cladophialophora,With all LMH,Elephant exclosure,0,0,-0.00119160514228229,0.709741753995744
ea,g,Nigrospora,With all LMH,Elephant exclosure,0,0,-0.000515146561288983,0.765374421105976
eb,g,Rhizophlyctis,With all LMH,Elephant exclosure,0,0,-0.000716447281924501,0.865370330861995
ec,g,Neurospora,With all LMH,Elephant exclosure,0,0,0.00443432761768493,0.0194069136269711
ed,g,unidentified,With all LMH,Elephant exclosure,-1.64760261935072,-0.0121362155003511,-0.0115767571599036,0.127573301665183
ee,g,Talaromyces,With all LMH,Elephant exclosure,0,0,-5.59545754477316e-05,0.858542156510437
ef,g,unidentified,With all LMH,Elephant exclosure,-0.210536385220942,-0.00265351944753809,0.00421368704538836,0.91396093495044
eg,g,Edenia,With all LMH,Elephant exclosure,0,0,-0.00096907575524905,0.655132458160035
eh,g,Neopestalotiopsis,With all LMH,Elephant exclosure,0,0,-0.000734451196711573,0.223831834064576
ei,g,Lecanicillium,With all LMH,Elephant exclosure,0,0,-0.000877409537669058,0.333923855116587
ej,g,unidentified,With all LMH,Elephant exclosure,0,0,-0.000127197039777983,0.524518280213076
ek,g,Colletotrichum,With all LMH,Elephant exclosure,0,0,0.000550503080013074,0.145177573483619
el,g,Rhizopus,With all LMH,Elephant exclosure,-2.67634077784654,-0.0243939169002455,-0.0291795477801017,0.0231617956149636
em,g,Geastrum,With all LMH,Elephant exclosure,-Inf,-0.00219133205015895,-0.0126537264508597,0.0203261536892711
en,g,Trichoderma,With all LMH,Elephant exclosure,0.280130267792411,0.000452938472352738,-0.000710452670169023,0.982004463130138
eo,g,Mortierella,With all LMH,Elephant exclosure,0,0,-0.00259584631961857,0.614007162175148
ep,g,Rinaldiella,With all LMH,Elephant exclosure,Inf,0.00526901062391749,0.0114544895776142,0.0502387291772232
eq,g,unidentified,With all LMH,Elephant exclosure,Inf,0.00530676220331393,0.026653295163877,0.0116326521259382
er,g,Ustilago,With all LMH,Elephant exclosure,-Inf,-0.00351167874749403,-0.00469905362735225,0.0130394025637584
es,g,unidentified,With all LMH,Elephant exclosure,-0.302169964952389,-0.000336738066393125,-0.00233744829940193,0.606216233342809
et,g,unidentified,With all LMH,Elephant exclosure,0,0,-0.00409965622277697,0.0979254820037898
eu,g,unidentified,With all LMH,Elephant exclosure,0.157400127828801,0.000262890017994285,-0.000501772286426657,0.484405073921104
ev,g,Thanatephorus,With all LMH,Elephant exclosure,Inf,0.00146790475767717,0.00246238224359946,0.0157535324416255
ew,g,Arthrobotrys,With all LMH,Elephant exclosure,0,0,-0.00044429708546945,0.974888034173419
ex,g,Phaeococcomyces,With all LMH,Elephant exclosure,0,0,-0.00190508662737367,0.0969044446618861
ey,g,Neophaeococcomyces,With all LMH,Elephant exclosure,-Inf,-0.00140848778604593,-0.00209072435806331,0.00551508837621502
ez,g,Aplosporella,With all LMH,Elephant exclosure,-Inf,-0.000387296669248645,-0.00150510419101567,0.0234470805423998

C5: Heat tree LMH exclosure vs Elephant exclosure -Fungi

id,tax_rank,tax_name,treatment_1,treatment_2,log2_median_ratio,median_diff,mean_diff,wilcox_p_value	
ab,r,Root,LMH exclosure,Elephant exclosure,0,0,0,	
ac,p,Ascomycota,LMH exclosure,Elephant exclosure,0.117650058698741,0.0734813999740214,0.0835188245279877,	0.00388500574214169
ad,p,unidentified,LMH exclosure,Elephant exclosure,-1.32548943368471,-0.0381967159590173,-0.0750275955387823,	0.000179238232815827
ae,p,Chytridiomycota,LMH exclosure,Elephant exclosure,0,0,4.63850819977369e-05,1	
af,p,Mucoromycota,LMH exclosure,Elephant exclosure,-Inf,-0.0289177385433281,-0.0388909190292213,5.55867488384398e-05	
ag,p,Basidiomycota,LMH exclosure,Elephant exclosure,-0.0667375258668246,-0.000957490681458038,0.0280437626106367,0.903071245762519	
ah,p,Mortierellomycota,LMH exclosure,Elephant exclosure,Inf,0.0032832829225707,	0.00392415999978858,
ai,p,Glomeromycota,LMH exclosure,Elephant exclosure,0,0,-0.00161461765240707,0.831000571863856	
aj,c,Eurotiomycetes,LMH exclosure,Elephant exclosure,0.169340695690334,0.0411963854060664,0.0101877898131047,0.904208037873463	
ak,c,Dothideomycetes,LMH exclosure,Elephant exclosure,0.350163591221922,0.0793431306061095,0.0333933812581066,0.639513882406952	
al,c,Sordariomycetes,LMH exclosure,Elephant exclosure,0.232482014673037,0.0165600210256659,0.0569888737723286,0.114254480535856	
am,c,unidentified,LMH exclosure,Elephant exclosure,-1.32548943368471,-0.0381967159590173,-0.0750275955387823,	0.000179238232815827
an,c,Rhizophlyctidomycetes,LMH exclosure,Elephant exclosure,0,0,4.63850819977369e-05,1	
ao,c,Mucoromycetes,LMH exclosure,Elephant exclosure,-Inf,-0.0289177385433281,-0.0388909190292213,5.55867488384398e-05	
ap,c,Agaricomycetes,LMH exclosure,Elephant exclosure,0.0302808534767649,0.00040340525852765,0.0334881580467641,0.674510010326322	
aq,c,Pezizomycetes,LMH exclosure,Elephant exclosure,-Inf,-0.00753904996318601,-0.0184984936132446,	0.000474482043334686
ar,c,Mortierellomycetes,LMH exclosure,Elephant exclosure,Inf,0.0032832829225707,0.00392415999978858,	0.0316498528713269
as,c,Ustilaginomycetes,LMH exclosure,Elephant exclosure,-Inf,-0.00351167874749403,-0.00544439543612741,5.90175758626443e-05	
at,c,Orbiliomycetes,LMH exclosure,Elephant exclosure,0,0,0.00130748507628765,0.439229127415624	
au,c,Arthoniomycetes,LMH exclosure,Elephant exclosure,0,0,0.0017211696639503,0.333228178107879	
av,o,Eurotiales,LMH exclosure,Elephant exclosure,0.0117664163908319,0.00242457257023049,-0.0014434219356983,0.956835119181445	
aw,o,Capnodiales,LMH exclosure,Elephant exclosure,1.84163159980864,0.0185211505638938,0.0392521588375045,	0.0253861423295159
ax,o,Microascales,LMH exclosure,Elephant exclosure,0.587500458486633,0.000348733411052535,0.0113676749649196,0.496836091093632	
ay,o,Pleosporales,LMH exclosure,Elephant exclosure,0.424827104957149,0.0841840248872906,0.0109985713382219,0.758350956319714	
az,o,Dothideales,LMH exclosure,Elephant exclosure,0,0,-0.0063187961955816,0.523652518387882	
ba,o,Hypocreales,LMH exclosure,Elephant exclosure,1.36030337776382,0.0311299925688659,0.043406627441587,	0.00321093063270362
bb,o,Botryosphaeriales,LMH exclosure,Elephant exclosure,-1.78603983255282,-0.0101778909485556,-0.0105385527220383,	0.010868677900534
bc,o,unidentified,LMH exclosure,Elephant exclosure,-1.32548943368471,-0.0381967159590173,-0.0750275955387823,	0.000179238232815827
bd,o,Chaetothyriales,LMH exclosure,Elephant exclosure,-0.0482304292643333,-0.000742375555680332,0.011631211748803,0.694836637354135	
be,o,Trichosphaeriales,LMH exclosure,Elephant exclosure,Inf,0.00054525627044711,0.00189060215739543,0.138487695913946	
bf,o,Rhizophlyctidales,LMH exclosure,Elephant exclosure,0,0,4.63850819977369e-05,1	
bg,o,Sordariales,LMH exclosure,Elephant exclosure,0,0,-0.00104205886304331,0.760021285441685	
bh,o,Xylariales,LMH exclosure,Elephant exclosure,-1.587087467549,-0.00179176633532711,-0.00322760931429595,0.158547486217386	
bi,o,Diaporthales,LMH exclosure,Elephant exclosure,Inf,0.00101761099067184,0.00123910072003784,	0.00294065881931737
bj,o,Glomerellales,LMH exclosure,Elephant exclosure,0,0,0.000586394115166839,0.0921623155276486	
bk,o,Mucorales,LMH exclosure,Elephant exclosure,-Inf,-0.0289177385433281,-0.0388909190292213,5.55867488384398e-05	
bl,o,Geastrales,LMH exclosure,Elephant exclosure,1.7275971389425,0.00506582881544789,0.0330981149613316,0.455391640683846	
bm,o,Pezizales,LMH exclosure,Elephant exclosure,-Inf,-0.00753904996318601,-0.0184984936132446,	0.000474482043334686
bn,o,Mortierellales,LMH exclosure,Elephant exclosure,Inf,0.0032832829225707,0.00392415999978858,	0.0316498528713269
bo,o,Agaricales,LMH exclosure,Elephant exclosure,-Inf,-0.00745500168313416,0.00079514826518258,0.168945853807542	
bp,o,Ustilaginales,LMH exclosure,Elephant exclosure,-Inf,-0.00351167874749403,-0.00544439543612741,5.90175758626443e-05	

Appendix C

bq,o,unidentified,LMH exclosure,Elephant exclosure,1.25359086047909,0.00315704177548244,0.00357177694708301,0.00785977295753782
br,o,Cantharellales,LMH exclosure,Elephant exclosure,0,0,-0.000405105179750025,0.621114691703981
bs,o,Orbiliales,LMH exclosure,Elephant exclosure,0,0,0.00130748507628765,0.439229127415624
bt,o,Lichenostigmatales,LMH exclosure,Elephant exclosure,0,0,0.0017211696639503,0.333228178107879
bu,f,Aspergillaceae,LMH exclosure,Elephant exclosure,0.0396437252683295,0.00809062252397702,0.00257274712833938,1
bv,f,Cladosporiaceae,LMH exclosure,Elephant exclosure,4.75296173263153,0.0163748633433108,0.0372168870650028,0.0398984118370172
bw,f,Microascaceae,LMH exclosure,Elephant exclosure,0.587500458486633,0.000348733411052535,0.0113676749649196,0.496836091093632
bx,f,Coniothyriaceae,LMH exclosure,Elephant exclosure,-2.41567501407951,-0.00727646798954943,-0.0126789210982691,0.0730613878410446
by,f,Pleosporaceae,LMH exclosure,Elephant exclosure,0.00273015940276328,2.71695622684771e-05,0.00288595138949234,0.892237888587045
bz,f,Didymellaceae,LMH exclosure,Elephant exclosure,-Inf,-0.00326664829048204,-0.0165492299654281,0.0885137404647333
ca,f,Aureobasidiaceae,LMH exclosure,Elephant exclosure,0,0,-0.0063187961955816,0.523652518387882
cb,f,Phaeosphaeriaceae,LMH exclosure,Elephant exclosure,Inf,0.00757146094104687,0.0106222772425101,0.00154513272639077
cc,f,Sporormiaceae,LMH exclosure,Elephant exclosure,-1.8812598862011,-0.0120889469801155,-0.0554676284469718,0.000562714580154396
cd,f,Didymosphaeriaceae,LMH exclosure,Elephant exclosure,-1.40330844626483,-0.0243510243521962,-0.029477588978339,0.00047203196596242
ce,f,Nectriaceae,LMH exclosure,Elephant exclosure,1.39852569592749,0.0275085216126853,0.0349792761581852,0.00858439033518297
cf,f,Botryosphaeriaceae,LMH exclosure,Elephant exclosure,-1.95110815862883,-0.0103410082239046,-0.00971674483105802,0.0215215567532658
cg,f,unidentified,LMH exclosure,Elephant exclosure,-1.32548943368471,-0.0381967159590173,-0.0750275955387823,0.000179238232815827
ch,f,Herpotrichiellaceae,LMH exclosure,Elephant exclosure,0,0,0.00367490925175919,0.0825518260905278
ci,f,Trichosphaeriaceae,LMH exclosure,Elephant exclosure,Inf,0.00054525627044711,0.00189060215739543,0.138487695913946
cj,f,Rhizophlyctidaceae,LMH exclosure,Elephant exclosure,0,0,4.63850819977369e-05,1
ck,f,Sordariaceae,LMH exclosure,Elephant exclosure,0,0,0.00201180939692975,0.0432080574396468
cl,f,unidentified,LMH exclosure,Elephant exclosure,2.02740523407265,0.0548442596713069,0.0701100205707694,0.000258541055937033
cm,f,Trichocomaceae,LMH exclosure,Elephant exclosure,0,0,-0.00401616906403768,0.235611795831409
cn,f,Chaetothyriaceae,LMH exclosure,Elephant exclosure,-0.663501208415242,-0.00720434061079527,0.00709848724264925,0.946058708458115
co,f,Sporocadaceae,LMH exclosure,Elephant exclosure,0,0,0.0017000990316261,0.327125929848973
cp,f,Cordycipitaceae,LMH exclosure,Elephant exclosure,0,0,0.00794191132836964,0.037449457602782
cq,f,unidentified,LMH exclosure,Elephant exclosure,Inf,0.000778452436556126,0.00276897709205307,0.00147312469709395
cr,f,Glomerellaceae,LMH exclosure,Elephant exclosure,0,0,0.000586394115166839,0.0921623155276486
cs,f,Rhizopodaceae,LMH exclosure,Elephant exclosure,-Inf,-0.0289177385433281,-0.0388909190292213,5.55867488384398e-05
ct,f,Geastraceae,LMH exclosure,Elephant exclosure,1.7275971389425,0.00506582881544789,0.0330981149613316,0.455391640683846
cu,f,Pezizaceae,LMH exclosure,Elephant exclosure,-Inf,-0.00753904996318601,-0.0184984936132446,0.000474482043334686
cv,f,Hypocreaceae,LMH exclosure,Elephant exclosure,-1.17155943261281,-0.00117524543916693,0.000485439955032178,0.724942278222575
cw,f,Mortierellaceae,LMH exclosure,Elephant exclosure,Inf,0.0032832829225707,0.00392415999978858,0.0316498528713269
cx,f,Lasiosphaeriaceae,LMH exclosure,Elephant exclosure,0,0,-0.00305386825997306,0.144741456906687
cy,f,Lycoperdaceae,LMH exclosure,Elephant exclosure,0,0,-0.000792418011339493,0.323603835455764
cz,f,Ustilaginaceae,LMH exclosure,Elephant exclosure,-Inf,-0.00351167874749403,-0.00544439543612741,5.90175758626443e-05
da,f,unidentified,LMH exclosure,Elephant exclosure,-Inf,-0.00178198084689218,-0.00492770834592205,0.00123478423960282
db,f,unidentified,LMH exclosure,Elephant exclosure,Inf,0.00457485121541299,0.00203527177250174,0.563076620487709
dc,f,unidentified,LMH exclosure,Elephant exclosure,1.25359086047909,0.00315704177548244,0.00357177694708301,0.00785977295753782
dd,f,Ceratobasidiaceae,LMH exclosure,Elephant exclosure,0,0,-0.000405105179750025,0.621114691703981
de,f,Orbiliaceae,LMH exclosure,Elephant exclosure,0,0,0.00130748507628765,0.439229127415624
df,f,Phaeococcomycetaceae,LMH exclosure,Elephant exclosure,0,0,0.0017211696639503,0.333228178107879
dg,f,ChaetothyrialesfamIncertaesedis,LMH exclosure,Elephant exclosure,-Inf,-0.00140848778604593,-0.00191116183765848,0.00107574516774481
dh,f,Aplosporellaceae,LMH exclosure,Elephant exclosure,-Inf,-0.000387296669248645,-0.00082180789098025,0.0332676075721512
di,g,Aspergillus,LMH exclosure,Elephant exclosure,0.0172443841099644,0.00345305466966056,-0.00977480911569612,0.924502083072675

Appendix C

dj,g,Cladosporium,LMH exclosure,Elephant exclosure,4.75296173263153,0.0163748633433108,0.0372168870650028,0.0398984118370172
dk,g,Microascus,LMH exclosure,Elephant exclosure,0.587500458486633,0.000348733411052535,0.0113676749649196,0.496836091093632
dl,g,Hazslinszkyomyces,LMH exclosure,Elephant exclosure,-2.41567501407951,-0.00727646798954943,-0.0126789210982691,0.0730613878410446
dm,g,Exserohilum,LMH exclosure,Elephant exclosure,1.7804247164368,0.00713157494084901,0.0134335847165813,0.0778974399638147
dn,g,Aureobasidium,LMH exclosure,Elephant exclosure,0,0,-0.0063187961955816,0.523652518387882
do,g,Sclerostagonospora,LMH exclosure,Elephant exclosure,Inf,0.00757146094104687,0.0106222772425101,0.00154513272639077
dp,g,Didymella,LMH exclosure,Elephant exclosure,0,0,0.0036814881310144,0.291756114145099
dq,g,Penicillium,LMH exclosure,Elephant exclosure,3.22248557952487,0.00628691613452367,0.0123475562440355,0.0694310865872967
dr,g,Westerdykella,LMH exclosure,Elephant exclosure,-Inf,-0.0101933563287135,-0.012523684455429,3.89393124503612e-05
ds,g,Hendersonia,LMH exclosure,Elephant exclosure,0,0,-0.000865949844284656,0.464607856651141
dt,g,Fusarium,LMH exclosure,Elephant exclosure,1.39852569592749,0.0275085216126853,0.0349792761581852,0.00858439033518297
du,g,Botryosphaeria,LMH exclosure,Elephant exclosure,-1.95110815862883,-0.0103410082239046,-0.00971674483105802,0.0215215567532658
dv,g,Curvularia,LMH exclosure,Elephant exclosure,-Inf,-0.0072187879301098,-0.01666067396734,3.50548865903038e-07
dw,g,unidentified,LMH exclosure,Elephant exclosure,-1.32548943368471,-0.0381967159590173,-0.0750275955387823,0.000179238232815827
dx,g,Cochliobolus,LMH exclosure,Elephant exclosure,Inf,0.0012973137973138,0.00552907065833794,0.000807557773939584
dy,g,Paraconiothyrium,LMH exclosure,Elephant exclosure,-Inf,-0.00270990609199473,-0.0119049508749635,0.0101058942876001
dz,g,Cladophialophora,LMH exclosure,Elephant exclosure,0,0,0.00367490925175919,0.0825518260905278
ea,g,Nigrospora,LMH exclosure,Elephant exclosure,Inf,0.00054525627044711,0.00189060215739543,0.138487695913946
eb,g,Rhizophlyctis,LMH exclosure,Elephant exclosure,0,0,4.63850819977369e-05,1
ec,g,Neurospora,LMH exclosure,Elephant exclosure,0,0,0.00201180939692975,0.0432080574396468
ed,g,unidentified,LMH exclosure,Elephant exclosure,2.02740523407265,0.0548442596713069,0.0701100205707694,0.000258541055937033
ee,g,Talaromyces,LMH exclosure,Elephant exclosure,0,0,-0.00401616906403768,0.235611795831409
ef,g,unidentified,LMH exclosure,Elephant exclosure,-0.663501208415242,-0.00720434061079527,0.00709848724264925,0.946058708458115
eg,g,Edenia,LMH exclosure,Elephant exclosure,0,0,0.00144991982619774,0.0385713803469143
eh,g,Neoestalotopsis,LMH exclosure,Elephant exclosure,0,0,0.0017000990316261,0.327125929848973
ei,g,Lecanicillium,LMH exclosure,Elephant exclosure,0,0,0.00794191132836964,0.037449457602782
ej,g,unidentified,LMH exclosure,Elephant exclosure,Inf,0.000778452436556126,0.00276897709205307,0.00147312469709395
ek,g,Colletotrichum,LMH exclosure,Elephant exclosure,0,0,0.000586394115166839,0.0921623155276486
el,g,Rhizopus,LMH exclosure,Elephant exclosure,-Inf,-0.0289177385433281,-0.0388909190292213,5.55867488384398e-05
em,g,Geastrum,LMH exclosure,Elephant exclosure,1.7275971389425,0.00506582881544789,0.0330981149613316,0.455391640683846
en,g,Trichoderma,LMH exclosure,Elephant exclosure,-1.17155943261281,-0.00117524543916693,0.000485439955032178,0.724942278222575
eo,g,Mortierella,LMH exclosure,Elephant exclosure,Inf,0.0032832829225707,0.00392415999978858,0.0316498528713269
ep,g,Rinaldiella,LMH exclosure,Elephant exclosure,0,0,-0.00305386825997306,0.144741456906687
eq,g,unidentified,LMH exclosure,Elephant exclosure,0,0,-0.000792418011339493,0.323603835455764
er,g,Ustilago,LMH exclosure,Elephant exclosure,-Inf,-0.00351167874749403,-0.00544439543612741,5.90175758626443e-05
es,g,unidentified,LMH exclosure,Elephant exclosure,-Inf,-0.00178198084689218,-0.00492770834592205,0.00123478423960282
et,g,unidentified,LMH exclosure,Elephant exclosure,Inf,0.00457485121541299,0.00203527177250174,0.563076620487709
eu,g,unidentified,LMH exclosure,Elephant exclosure,1.25359086047909,0.00315704177548244,0.00357177694708301,0.00785977295753782
ev,g,Thanatephorus,LMH exclosure,Elephant exclosure,0,0,-0.000405105179750025,0.621114691703981
ew,g,Arthrobotrys,LMH exclosure,Elephant exclosure,0,0,0.00130748507628765,0.439229127415624
ex,g,Phaeococcomyces,LMH exclosure,Elephant exclosure,0,0,0.0017211696639503,0.333228178107879
ey,g,Neophaeococcomyces,LMH exclosure,Elephant exclosure,-Inf,-0.00140848778604593,-0.00191116183765848,0.00107574516774481
ez,g,Aplosporella,LMH exclosure,Elephant exclosure,-Inf,-0.000387296669248645,-0.00082180789098025,0.0332676075721512

C6: Heat tree LMH exclosure vs with all LMH -Fungi

id,tax_rank,tax_name,treatment_1,treatment_2,log2_median_ratio,median_diff,mean_diff,wilcox_p_value	
ab,r,Root,LMH exclosure,With all LMH,0,0,0,	
ac,p,Ascomycota,LMH exclosure,With all LMH,0.0254690319248768,0.0164194136969595,-0.000271025525850566,0.745709196683709	
ad,p,unidentified,LMH exclosure,With all LMH,-0.630147737519973,-0.0138902738475146,-0.0179845705697413,0.226289139369733	
ae,p,Chytridiomycota,LMH exclosure,With all LMH,0,0,0.000762832363922238,0.910008351007011	
af,p,Mucoromycota,LMH exclosure,With all LMH,-Inf,-0.00452382164308256,-0.00971137124911958,0.269167480553511	
ag,p,Basidiomycota,LMH exclosure,With all LMH,0.883607595800971,0.00926213061078444,0.0194266899749662,0.581949451587583	
ah,p,Mortierellomycota,LMH exclosure,With all LMH,Inf,0.0032832829225707,0.00652000631940715,0.0139190506716441	
ai,p,Glomeromycota,LMH exclosure,With all LMH,0,0,0.0012574386864159,0.0969044446618861	
aj,c,Eurotiomycetes,LMH exclosure,With all LMH,-0.829410887010891,-0.289007944949994,-0.21361983823735,0.0244241515605833	
ak,c,Dothideomycetes,LMH exclosure,With all LMH,1.70659146890302,0.255371210920351,0.178510734453727,0.0145814538618137	
al,c,Sordariomycetes,LMH exclosure,With all LMH,-0.141162567726876,-0.0114375662610753,0.030587356719833,0.52996971377781	
am,c,unidentified,LMH exclosure,With all LMH,-0.630147737519973,-0.0138902738475146,-0.0179845705697413,0.226289139369733	
an,c,Rhizophyctidomycetes,LMH exclosure,With all LMH,0,0,0.000762832363922238,0.910008351007011	
ao,c,Mucoromycetes,LMH exclosure,With all LMH,-Inf,-0.00452382164308256,-0.00971137124911958,0.269167480553511	
ap,c,Agaricomycetes,LMH exclosure,With all LMH,0.969713308951571,0.0095050635430845,0.0201720317837414,0.4948161032941	
aq,c,Pezizomycetes,LMH exclosure,With all LMH,-Inf,-0.000241779497098646,-0.000740481061781357,0.097937852072167	
ar,c,Mortierellomycetes,LMH exclosure,With all LMH,Inf,0.0032832829225707,0.00652000631940715,0.0139190506716441	
as,c,Ustilaginomycetes,LMH exclosure,With all LMH,0,0,-0.00074534180877516,0.391960846685801	
at,c,Orbiliomycetes,LMH exclosure,With all LMH,0,0,0.0017517821617571,0.400329940999126	
au,c,Arthoniomycetes,LMH exclosure,With all LMH,0,0,0.00362625629132397,0.0406467444933824	
av,o,Eurotiales,LMH exclosure,With all LMH,-1.10563601135726,-0.343846141218799,-0.224446889480888,0.0234081490192092	
aw,o,Capnodiales,LMH exclosure,With all LMH,Inf,0.0256883584205063,0.0480475845268446,0.00402399760006688	
ax,o,Microascales,LMH exclosure,With all LMH,Inf,0.00104253544620517,0.0083166109656912,0.295781759646388	
ay,o,Pleosporales,LMH exclosure,With all LMH,1.62074741692595,0.222722059946883,0.125798574171401,0.0311152449083484	
az,o,Dothideales,LMH exclosure,With all LMH,0,0,-0.00262623597672221,0.644647114207061	
ba,o,Hypocreales,LMH exclosure,With all LMH,2.25165034330929,0.0402837261285493,0.053954607581828,0.00395239502296215	
bb,o,Botryosphaeriales,LMH exclosure,With all LMH,Inf,0.00415649861332232,0.00729081173220322,0.00227486361699888	
bc,o,unidentified,LMH exclosure,With all LMH,-0.630147737519973,-0.0138902738475146,-0.0179845705697413,0.226289139369733	
bd,o,Chaetothyriales,LMH exclosure,With all LMH,0.15975043413861,0.00228897986469111,0.0108270512435383,0.808721683142508	
be,o,Trichosphaeriales,LMH exclosure,With all LMH,Inf,0.00054525627044711,0.00240574871868442,0.151414374294613	
bf,o,Rhizophyctidales,LMH exclosure,With all LMH,0,0,0.000762832363922238,0.910008351007011	
bg,o,Sordariales,LMH exclosure,With all LMH,-Inf,-0.0137771864545337,-0.0169308760583425,0.00632301146994417	
bh,o,Xylariales,LMH exclosure,With all LMH,-0.693115635821479,-0.000551336040687148,-0.00015570981818244,0.765478328508705	
bi,o,Diaporthales,LMH exclosure,With all LMH,Inf,0.00101761099067184,0.00145682280507834,0.00551508837621502	
bj,o,Glomerellales,LMH exclosure,With all LMH,0,0,3.58910351537652e-05,0.978355992500533	
bk,o,Mucorales,LMH exclosure,With all LMH,-Inf,-0.00452382164308256,-0.00971137124911958,0.269167480553511	
bl,o,Geastrales,LMH exclosure,With all LMH,Inf,0.00725716086560684,0.0457518414121913,0.0122142216222821	
bm,o,Pezizales,LMH exclosure,With all LMH,-Inf,-0.000241779497098646,-0.000740481061781357,0.097937852072167	
bn,o,Mortierellales,LMH exclosure,With all LMH,Inf,0.0032832829225707,0.00652000631940715,0.0139190506716441	
bo,o,Agaricales,LMH exclosure,With all LMH,-Inf,-0.00747165815899605,-0.0227123222051004,0.0925343748960349	
bp,o,Ustilaginales,LMH exclosure,With all LMH,0,0,-0.00074534180877516,0.391960846685801	

Appendix C

bq,o,unidentified,LMH exclosure,With all LMH,1.09619073265029,0.00289415175748815,0.00407354923350966,0.280025424913389
br,o,Cantharellales,LMH exclosure,With all LMH,-Inf,-0.00146790475767717,-0.00286748742334948,0.00360884243447006
bs,o,Orbiliales,LMH exclosure,With all LMH,0,0,0.0017517821617571,0.400329940999126
bt,o,Lichenostigmatales,LMH exclosure,With all LMH,0,0,0.00362625629132397,0.0406467444933824
bu,f,Aspergillaceae,LMH exclosure,With all LMH,-1.09960736343556,-0.341167569790228,-0.220486674992298,0.0293555040691928
bv,f,Cladosporiaceae,LMH exclosure,With all LMH,Inf,0.0170055394280737,0.0419126565315659,0.0168538588630378
bw,f,Microascaceae,LMH exclosure,With all LMH,Inf,0.00104253544620517,0.0083166109656912,0.295781759646388
bx,f,Coniothyriaceae,LMH exclosure,With all LMH,3.26651842715654,0.0015038736371769,0.00364148623517711,0.710456130054052
by,f,Pleosporaceae,LMH exclosure,With all LMH,0.926528431395002,0.00680997980816785,0.00410513580421532,0.52260250751012
bz,f,Didymellaceae,LMH exclosure,With all LMH,0,0,-0.00325717152371793,0.892097706239642
ca,f,Aureobasidiaceae,LMH exclosure,With all LMH,0,0,-0.00262623597672221,0.644647114207061
cb,f,Phaeosphaeriaceae,LMH exclosure,With all LMH,Inf,0.00757146094104687,0.0139669814076934,0.00383796100059717
cc,f,Sporormiaceae,LMH exclosure,With all LMH,-1.83358639883387,-0.011549593322312,-0.0374792078260369,0.0561750206261762
cd,f,Didymosphaeriaceae,LMH exclosure,With all LMH,0.356748305942021,0.00324286566909202,0.00579252129966567,0.234374692214657
ce,f,Nectriaceae,LMH exclosure,With all LMH,2.99021024006684,0.0387420425834824,0.0439393940905881,0.00989716378584263
cf,f,Botryosphaeriaceae,LMH exclosure,With all LMH,Inf,0.00360726855564466,0.00660751543216781,0.00160184672239923
cg,f,unidentified,LMH exclosure,With all LMH,-0.630147737519973,-0.0138902738475146,-0.0179845705697413,0.226289139369733
ch,f,Herpotrichiellaceae,LMH exclosure,With all LMH,0,0,0.00486651439404148,0.0874149631414701
ci,f,Trichosphaeriaceae,LMH exclosure,With all LMH,Inf,0.00054525627044711,0.00240574871868442,0.151414374294613
cj,f,Rhizophlyctidaceae,LMH exclosure,With all LMH,0,0,0.000762832363922238,0.910008351007011
ck,f,Sordariaceae,LMH exclosure,With all LMH,0,0,-0.00242251822075518,0.618429862447267
cl,f,unidentified,LMH exclosure,With all LMH,3.67500785342337,0.066980475171658,0.08168677730673,0.000168616021912798
cm,f,Trichocomaceae,LMH exclosure,With all LMH,0,0,-0.00396021448858995,0.246644931672465
cn,f,Chaetothyriaceae,LMH exclosure,With all LMH,-0.452964823194299,-0.00455082116325719,0.0028848001972609,0.774663296083261
co,f,Sporocadaceae,LMH exclosure,With all LMH,0,0,0.00243455022833767,0.0590999339144129
cp,f,Cordycipitaceae,LMH exclosure,With all LMH,0,0,0.0088193208660387,0.0255949344383818
cq,f,unidentified,LMH exclosure,With all LMH,Inf,0.000778452436556126,0.00289617413183105,0.00945241629374603
cr,f,Glomerellaceae,LMH exclosure,With all LMH,0,0,3.58910351537652e-05,0.978355992500533
cs,f,Rhizopodaceae,LMH exclosure,With all LMH,-Inf,-0.00452382164308256,-0.00971137124911958,0.269167480553511
ct,f,Geastraceae,LMH exclosure,With all LMH,Inf,0.00725716086560684,0.0457518414121913,0.0122142216222821
cu,f,Pezizaceae,LMH exclosure,With all LMH,-Inf,-0.000241779497098646,-0.000740481061781357,0.097937852072167
cv,f,Hypocreaceae,LMH exclosure,With all LMH,-1.45168970040522,-0.00162818391151967,0.0011958926252012,0.762930023474175
cw,f,Mortierellaceae,LMH exclosure,With all LMH,Inf,0.0032832829225707,0.00652000631940715,0.0139190506716441
cx,f,Lasiosphaeriaceae,LMH exclosure,With all LMH,-Inf,-0.00526901062391749,-0.0145083578375873,0.0019793090989348
cy,f,Lycoperdaceae,LMH exclosure,With all LMH,-Inf,-0.00530676220331393,-0.0274457131752165,0.00183835324796513
cz,f,Ustilaginaceae,LMH exclosure,With all LMH,0,0,-0.00074534180877516,0.391960846685801
da,f,unidentified,LMH exclosure,With all LMH,-Inf,-0.00144524278049906,-0.00259026004652011,0.0116326521259382
db,f,unidentified,LMH exclosure,With all LMH,Inf,0.00457485121541299,0.0061349279952787,0.0343994000963092
dc,f,unidentified,LMH exclosure,With all LMH,1.09619073265029,0.00289415175748815,0.00407354923350966,0.280025424913389
dd,f,Ceratobasidiaceae,LMH exclosure,With all LMH,-Inf,-0.00146790475767717,-0.00286748742334948,0.00360884243447006
de,f,Orbiliaceae,LMH exclosure,With all LMH,0,0,0.0017517821617571,0.400329940999126
df,f,Phaeococcomycetaceae,LMH exclosure,With all LMH,0,0,0.00362625629132397,0.0406467444933824
dg,f,ChaetothyrialesfamIncertaesedis,LMH exclosure,With all LMH,0,0,0.000179562520404832,0.524518280213076
dh,f,Aplosporellaceae,LMH exclosure,With all LMH,0,0,0.000683296300035416,0.655132458160035

Appendix C

di,g,Aspergillus,LMH enclosure,With all LMH,-1.13818183658543,-0.349042880475256,-0.236142403747239,0.0206212722102556
dj,g,Cladosporium,LMH enclosure,With all LMH,Inf,0.0170055394280737,0.0419126565315659,0.0168538588630378
dk,g,Microascus,LMH enclosure,With all LMH,Inf,0.00104253544620517,0.0083166109656912,0.295781759646388
dl,g,Hazslinszkyomyces,LMH enclosure,With all LMH,3.26651842715654,0.0015038736371769,0.00364148623517711,0.710456130054052
dm,g,Exserohilum,LMH enclosure,With all LMH,1.83124257210318,0.00723293181569186,0.0127716063068836,0.126676118024914
dn,g,Aureobasidium,LMH enclosure,With all LMH,0,0,-0.00262623597672221,0.644647114207061
do,g,Sclerostagonospora,LMH enclosure,With all LMH,Inf,0.00757146094104687,0.0139669814076934,0.00383796100059717
dp,g,Didymella,LMH enclosure,With all LMH,0,0,-0.000110793057557888,0.84937928123595
dq,g,Penicillium,LMH enclosure,With all LMH,Inf,0.00704129151171136,0.0156557287549407,0.0154542267765664
dr,g,Westerdykella,LMH enclosure,With all LMH,-Inf,-0.00215127766858424,-0.00546942507413409,0.0130047557196077
ds,g,Hendersonia,LMH enclosure,With all LMH,0,0,0.00365387977763522,0.28450008586798
dt,g,Fusarium,LMH enclosure,With all LMH,2.99021024006684,0.0387420425834824,0.0439393940905881,0.00989716378584263
du,g,Botryosphaeria,LMH enclosure,With all LMH,Inf,0.00360726855564466,0.00660751543216781,0.00160184672239923
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eb,g,Rhizophlyctis,LMH enclosure,With all LMH,0,0,0.000762832363922238,0.910008351007011
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ey,g,Neophaeococcomyces,LMH enclosure,With all LMH,0,0,0.000179562520404832,0.524518280213076
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Appendix D: Published Article (Chapter 5)



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Teatime in Kruger: Tailoring the application of the Tea Bag Index approach to an African savanna

Attempts to obtain standardised decomposition data to determine potential drivers of carbon release have evolved from the use of cotton strips and standardised leaf litter mixtures to the most recent Tea Bag Index (TBI). The TBI is an internationally standardised method to collect comparable, globally distributed data on decomposition rate and litter stabilisation, using commercially available tea bags as standardised test kits. As this index was developed as a citizen science project in the northern hemisphere, we aimed to highlight the potential value – and pitfalls – of its application in a subtropical African savanna. We furthermore aimed to expand on existing protocol details and propose amendments to achieve an enhanced understanding of decomposition dynamics across temporal and spatial scales in African ecosystems. Proposed adaptations include extended incubation periods for long-term monitoring studies, the burial of more tea bags to account for potential losses, and the use of additional equipment to enhance effective sampling. These adaptations provide a system-specific protocol which can facilitate studies aimed to understand the interactions between top-down drivers (e.g. herbivory, fire, climate variability) and bottom-up controls (e.g. decomposition) in carbon flux dynamics of savanna ecosystems. Application of the proposed extended protocol in a semi-arid savanna provided results which reinforce the potential value of the TBI in an African context.

Significance:

- The TBI is a relatively easy and cost-effective approach to gather globally distributed data on potential decomposition rate and inherent carbon flux, yet it was developed and primarily tested in boreal and temperate ecosystems.
- The use of more paired tea bag replicates and additional equipment is a viable means to mitigate tea bag losses to several savanna-based agents of disturbance, while enabling confident conclusions made from statistical results and improved estimates of the TBI. High recovery success across disturbance treatments and incubation periods suggest that the TBI can be applied successfully to spatial and temporal decomposition studies.

Introduction

Research on linkages between decomposition and carbon flux is covered extensively for temperate^{1,2} and boreal^{3,4} ecosystems, whilst studies in tropical and subtropical ecosystems, especially in Africa, remain limited^{5,6}. The paucity of information available on these mechanistic relationships in African savannas may be ascribed to complex interactions between top-down (e.g. climate variability, fire, large mammalian herbivores) and bottom-up controls (e.g. soil physical-chemical properties, soil-based microbes and detritivores) that collectively regulate savanna structure and function.^{6,7} While governed by various environmental factors, decomposition is primarily regulated by the chemical composition of site-specific leaf litter. Cross-site comparison of natural litter decomposition and intrinsic drivers thereof is subsequently compromised by variability in detrital chemistry.^{1,3,4,6,8-10}

Attempts to standardise approaches to examine the role of environmental drivers on decomposition have evolved from the use of cotton strips or natural leaf litter mixtures^{1,6,9} to the most recent Tea Bag Index (TBI) approach introduced by Keuskamp et al.⁸ This approach is an internationally standardised, user-friendly method to collect comparable, globally distributed decomposition data. The TBI consists of two parameters describing decomposition rate (k) and stabilisation factor (S).^{5,8} Standardised test kits include commercially available Lipton® tea bags (i.e. rooibos and green tea).⁸ Tea bags were selected based upon differences in chemical composition, with green tea representing high-quality organic matter with low C:N ratios, and rooibos tea a low-quality organic matter with high C:N ratios.^{3,8,9} Tea bags therefore act as proxies for labile and recalcitrant compounds in naturally occurring organic matter.^{1,8,9}

Assessments of ecosystem functioning, such as the TBI, are becoming increasingly valuable, especially for application in monitoring programmes in areas exposed to increasing intensities of land-use and/or climate change. However, the physical and biological template upon which African systems function varies from most ecosystems in which the TBI has been applied.¹⁻⁴ Here, we reflect on applying the TBI in an African context to facilitate decomposition monitoring. Details on (1) an experimental design aimed at elucidating temporal dynamics, (2) tea bag losses due to large mammalian herbivores, fungal infestation and termites, (3) recommended equipment to enhance effective sampling and (4) the potential value in African systems are provided.

Study area

The study was conducted in a semi-arid subtropical savanna at the Nkuhlu exclosures long-term monitoring site (24°58'S, 31°46'E) located in the Kruger National Park, South Africa. This area is characterised by a hot growing season with sporadic precipitation from October to April and a mild to warm, dry non-growing season.¹¹ Average annual rainfall is 561 mm, with temperatures varying from an average minimum of 5.6 °C in winter

to an average maximum of 32.6 °C in summer.¹¹ Situated on the foot slopes of undulating granitic landscapes, the study site is characterised by sodium-rich, deep duplex soil, referred to as the sodic zone. The plant community of the sodic zone is described as a *Sporobolus nitens-Euclea divinorum* Dry Sodic Savanna and is associated with nutrient-rich vegetation.¹¹ Sodic patches therefore produce palatable, high-quality forage capable of supporting large herbivores, including grazers and mixed-feeders.¹¹

Materials and methods

Experimental design

The Nkuhlu exclosures form part of a large-scale, long-term exclusion experiment of ecosystem drivers (i.e. herbivory and fire) to determine their effect on spatial and temporal heterogeneity patterns of vegetation in a semi-arid African savanna.¹¹ These exclosures consist of three herbivore treatments including (1) a partially fenced area (elephant exclosure), (2) an unfenced area (control) and (3) a fully fenced area (large mammalian herbivore exclosure).¹¹ As the TBI was developed and applied in systems in which decomposition is dominated by microbial agents^{6,12}, it does not account for the presence and possible damage caused by savanna-based disturbance agents, such as large mammalian herbivores and termites^{6,10,12}. We applied a paired tea bag design which entailed the burial of 20 green and 20 rooibos tea bags in each of the 25 fixed plots across three herbivore treatments (Figure 1) which added up to 1000 experimental tea bags in total.

Decomposition is a time-bound process consisting of different phases^{3,9}, primarily regulated by the chemical composition of detrital substrate with respect to labile and recalcitrant ratios^{5,8}. Due to the dynamic nature of savannas, regulating factors change over time, influencing the rate and extent of decomposition. Temporal comparisons are therefore essential to observe not only progression in decomposition phases, but the influence of site-specific factors thereon.^{1,6,7,9,12,13} Our suggested extended application includes replicates which represent different incubation periods (i.e. 3-, 6-, 9- and 12-months) to capture dynamics in k and S but also to determine the resilience of the TBI in an unexplored system over the course of a year (Figure 1). All tea bags were buried in January 2019 (summer) and retrieved in intervals of three months, each representing a different season (i.e. April 2019, July 2019, October 2019 and January 2020).

Statistical analyses

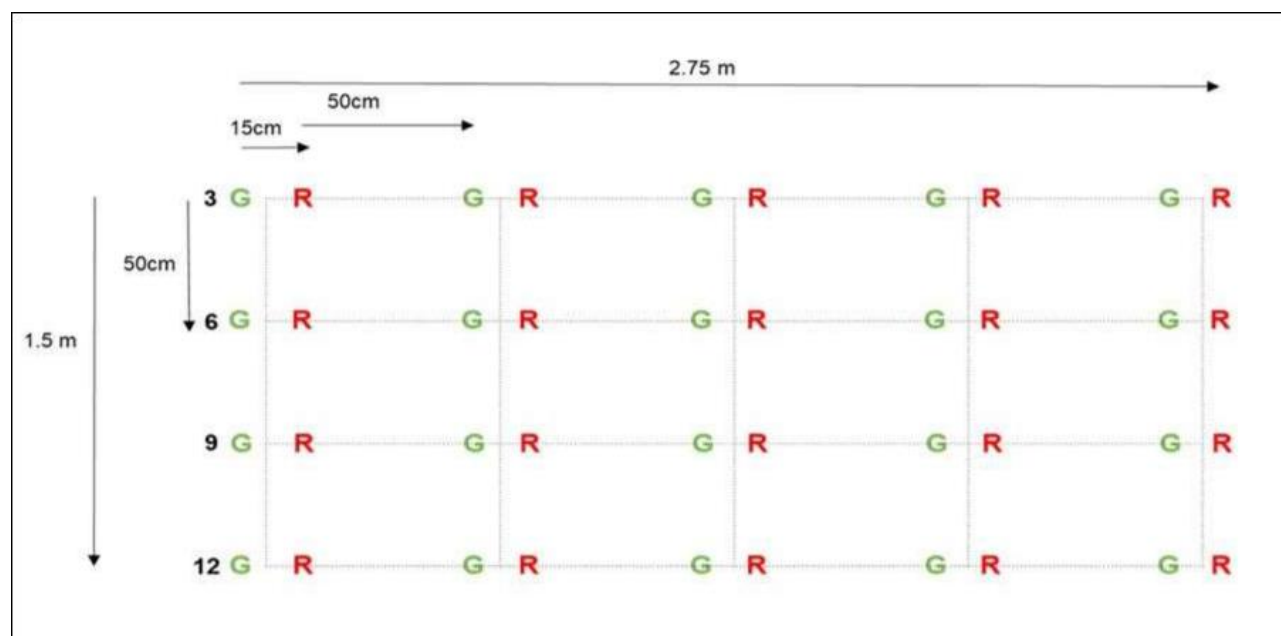
Differences in recovery success of green and rooibos tea bags within each incubation period were tested for significance using independent Student's t tests in Microsoft Excel (2013). To test for significant variation in the number of tea bags infested by fungi and damaged by termites across treatments and over the different incubation periods, a one-way analysis of variance (ANOVA) was applied in Paleontological Statistics Software (PAST) and Microsoft Excel (2013).

Results and discussion

The overall recovery success, irrespective of herbivore treatment and incubation, was 78.5%. Results did not reveal significant differences in recovery between green and rooibos tea among most treatments (Figure 2), although significantly fewer rooibos tea bags could be retrieved after the 6-month incubation period (Figure 2).

A finely grained black fungus was present on 42% of recovered green tea bags, while no rooibos tea bags were infested. Fungal infestation showed no significant difference between incubation periods ($p=0.897$). Fungal infestation and damage by termites were significantly lower in the control site ($p<0.001$, Figure 3a and $p=0.003$, Figure 3b). Rooibos tea bags were significantly more damaged by termites than were green tea bags ($p=0.033$).

From our results, it is evident that the presence of large mammalian herbivores complicates the retrieval of tea bags. This could be ascribed to difficulty in finding markers, because the use of above-ground markers is not recommended in areas with high mammal activity. Moreover, detritivore activity in the form of termites has a significant impact on the longevity of tea bags. To account for termite-based damage, Teo et al.⁶ suggested the use of physical and/or chemical barriers (e.g. metal mesh and termiticide), although such applications may impede the practicality and standardisation of the TBI and furthermore reduce its effectiveness in measuring decomposition by altering regulatory constituents¹². The seemingly high number of replicates used in our small-scale investigation successfully buffered the tea bag losses ascribed to animal, insect and fungal activity. Sufficient recovery (78%) with little differences between green and rooibos tea bags will lead to confident conclusions made from estimates of the TBI.



G, green tea bag; R, rooibos tea bag

Figure 1: Experimental plot layout consisting of a paired tea bag design across four incubation periods.

**Table 1:** Application of the original Tea Bag Index (Keuskamp et al.) and extended version with potential pitfalls and recommendations for African studies

Preparations		
Keuskamp et al.	Potential pitfalls for African study	Recommendations
Order tea bags (Lipton® green and rooibos) – Note that woven nylon mesh tea bag production halted in 2017 and was replaced with non-woven polypropylene tea bags. Measure initial weight of tea bag and subtract weight of empty tea bag. Mark tea bag labels using permanent marker.	Lipton tea bags need to be shipped from the Netherlands, so logistic delays should be expected. Marked labels may deteriorate when buried. Dry organic material can absorb moisture, altering the initial recorded weight.	Order non-woven polypropylene tea bags (Green -EAN 87 22700 05552 5 / EAN 8714100770542 & Rooibos -EAN 87 22700 18843 8) at least 3 months prior to the onset of the experiment. Cover marked labels with transparent adhesive tape to preserve the markings. Store weighed tea bags at room temperature in airtight containers. Mark cupcake holders and brown paper bags (i.e. used for weighing and storage of retrieved bags) in accordance with individual tea bags.
Equipment		
Keuskamp et al.	Potential pitfalls for African study	Recommendations
Permanent marker Small spade Above-ground markers (e.g. stick or plastic marker)	Site-specific conditions (i.e. animal activity, compacted soil and high herbaceous biomass) require specialised equipment to minimise tea bag losses.	Transparent adhesive tape Airtight plastic containers GPS Metal detector Rope quadrant (conforming to experimental plot layout) Flat-head shovel Custom-made auger with 8-cm marker (height = 11 cm; diameter = 4.5 cm) Hammer Metal washers (galvanised steel; 1.5 cm diameter) – 1 per tea bag Resealable plastic bags (3.5 x 2.5 cm) – 1 per metal washer Brown paper bags (18 x 8.5 cm) – 1 per tea bag Cupcake holders (standard size – 5.5 cm bottom diameter) – 1 per tea bag Sieve (45 cm diameter; 3.5 mm mesh) Portable carrier (field equipment and tea bags) Pharmaceutical tablet holders (10 mL)
Experimental design		
Keuskamp et al.	Potential pitfalls for African study	Recommendations
Green and rooibos tea bags buried pairwise (15 cm apart) at 8 cm depth. Between 5 and 32 pairs per site (1 m apart). Recommended incubation period of 90 days.	Potential animal and termite activity might lead to tea bag losses, reducing the effectivity of few replicates. Decomposition processes and savanna systems are dynamic, necessitating extended study periods.	1000 non-woven polypropylene tea bags (500 green and 500 rooibos) buried pairwise (15 cm apart) in five plots (1.5 x 2.75 m) per site. Five sites/ treatments were sampled in this study. Plots followed a grid design (four rows, 50 cm apart), representing different incubation periods in 3-month intervals. Rows contained five pairs of tea bags (five columns) buried 50 cm apart at 8 cm depth.



Burial

Keuskamp et al. ⁸	Potential pitfalls for African study	Recommendations
		Use a rope quadrant to produce exact replicates of plots.
	Obstructions (e.g. vegetation, termite mounds or gullies) might impede exact replicates.	A custom-made mini auger can be used to create holes to bury and retrieve tea bags in hard soil, also limiting disturbance to surrounding soil.
Bury tea bags pairwise (15 cm apart) at a depth of 8 cm.	Soil conditions (e.g. compaction) could make burial of tea bags difficult.	Metal washers in resealable plastic bags and label should be placed directly above tea bags 2 cm below the surface to facilitate location with a metal detector.
Ensure labels are visible above the soil and mark the area.	The study area is subjected to animal activity, therefore the potential effects this might have on the burial and recovery of tea bags should be considered.	Completely fill holes after burial to ensure tea bags are not exposed and thus susceptible to removal by animals or abiotic factors such as water run-off or wind.
	Locating plots for retrieval might be difficult.	Take GPS coordinates at each plot.

[Keuskamp et al.⁸ provide very little detail on the removal, analysis and storage of a high number of replicates.]

Retrieval

Extended procedure for African study	Potential pitfalls	Recommendations
Locate plots with GPS coordinates and tea bags with a metal detector. Rope quadrant can be used to determine exact position of tea bags.	GPS accuracy (5 m radius) impedes exact point location.	Take a picture of the plot with the rope quadrant present. This might help identify significant structures and ease the location of metal washers.
Remove topsoil with a flat-head shovel to expose the label and/or metal washer.	Disturbance to remaining rows (i.e. incubation periods).	Work from the top of the plot (i.e. first row) down so as to not step on the other incubation rows.
Place mini auger directly above the label and screw into the soil up until the 8-cm mark by striking it with a hammer.	High numbers of retrieved tea bags may result in confusing numbers and samples.	Brown paper bags absorb moisture and ensure that the tea bag remains dry and does not attract further fungal and bacterial colonisation.
Remove tea bag from mini auger and place in corresponding cupcake holder and brown paper bag.	Ambient temperature and humidity may influence results.	

Sample analysis

Extended procedure for African study	Potential pitfalls	Recommendations
Place individual tea bags on a sieve to remove excess soil particles and roots.		Gently remove excess particles and debris from tea bags.
Place cupcake holders and corresponding tea bags in an oven and dry at 70 °C for 48 h.	The durability of tea bags decreases with incubation and they can easily tear.	Due to insect activity, mostly termites, some bags might have holes (resulting in the loss of material) and others might have foreign material inside which will affect the final weight. These tea bags should not be included in the TBI data sheet as it will affect results. As far as possible, one person should weigh all tea bags to ensure consistency not only in weighing but also in identifying these 'altered' tea bags.
Weigh empty cupcake holder (to three decimal places) and remember to tare the scale.	Foreign material inside tea bag.	
Cut open the tea bag and pour organic material into the cupcake holder and weigh (to three decimal places).	Ensure that data are recorded on the correct data sheet as there are two different formats (i.e. one for woven nylon tea bags and one for non-woven tea bags).	Visit www.teatime4science.org to download the correct data sheet.
Record weight on the TBI 2.0 NW data sheet for non-woven tea bags.		

Storage

After weighing the organic matter, the sides of the cupcake holder containing the organic matter should be folded and placed in a correspondingly labelled pharmaceutical tablet holder and stored at room temperature for potential use in the future.

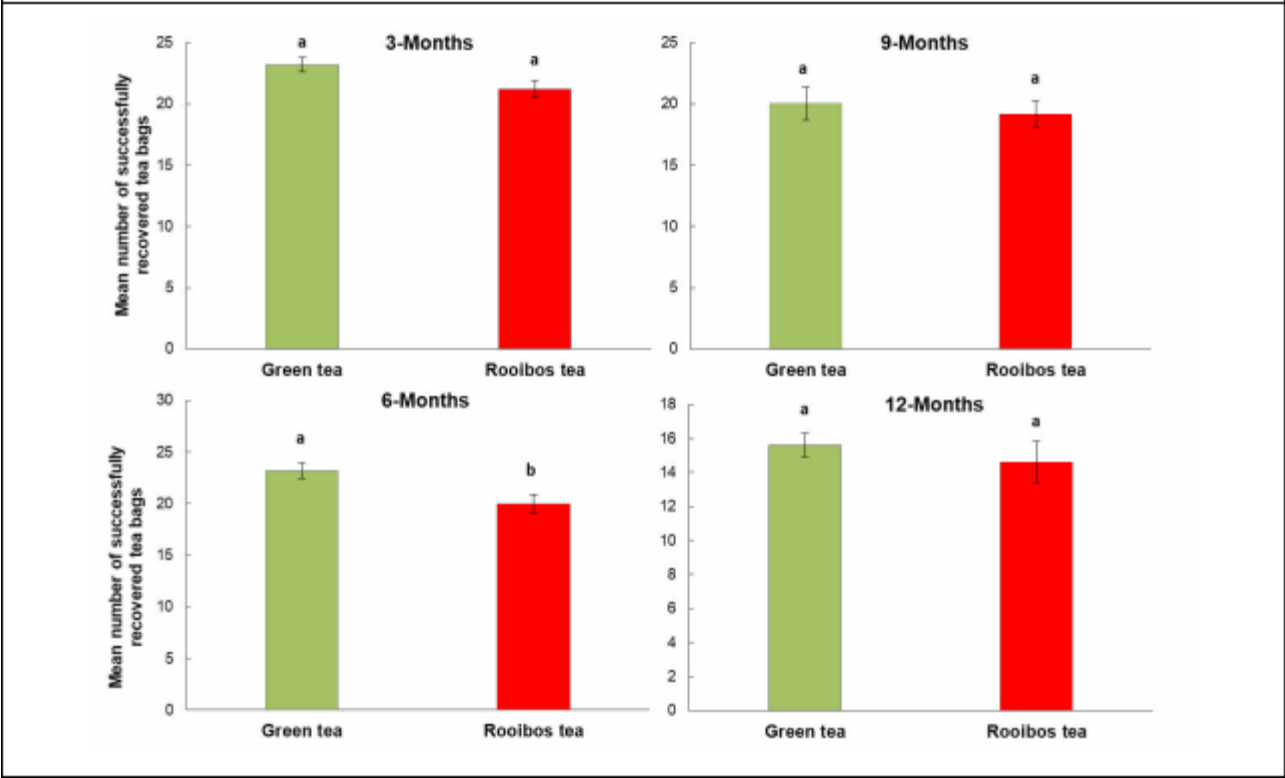
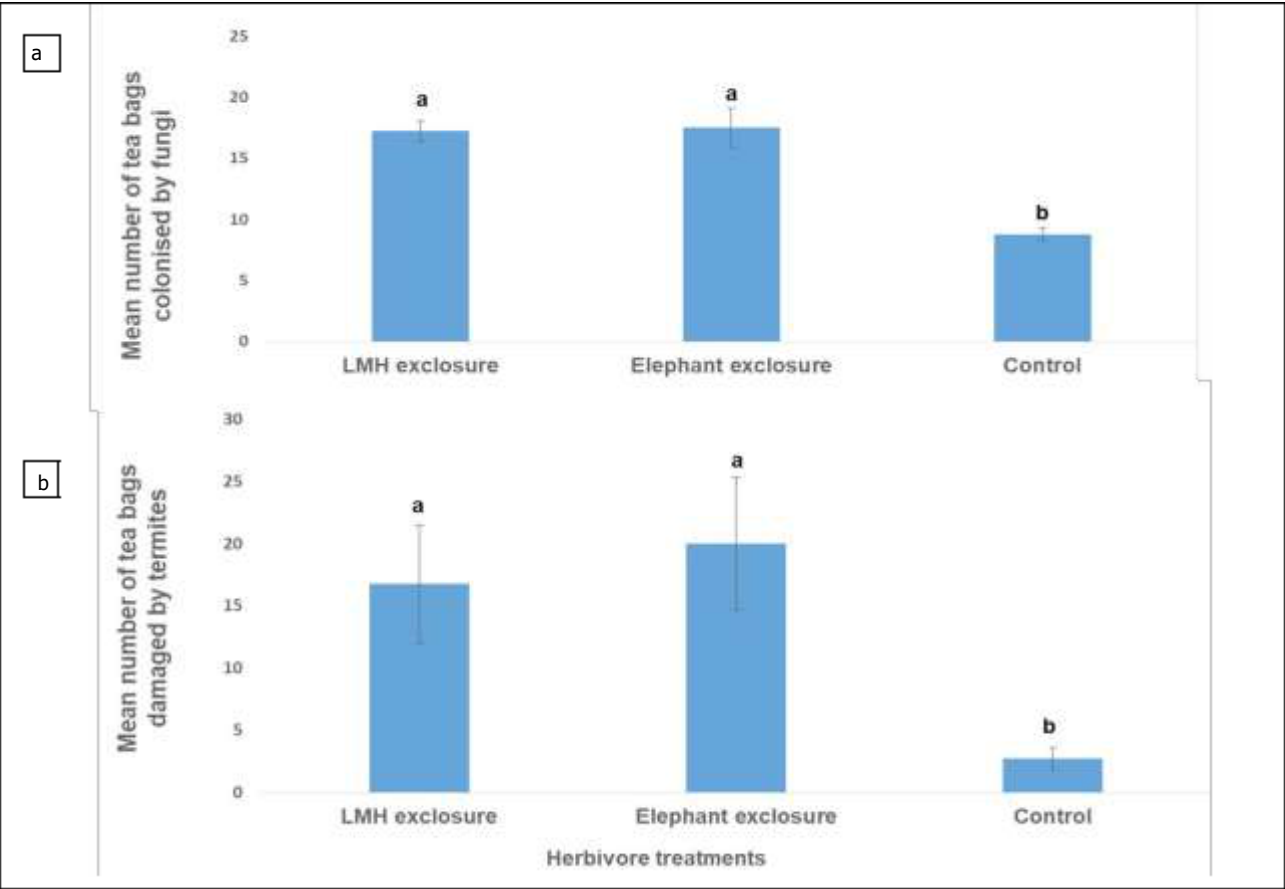


Figure 2: Mean number of successfully recovered tea bags over four incubation periods. Significant differences ($p < 0.05$) between tea bags are indicated with different lowercase letters.



LMH exclusion, all large mammalian herbivores excluded; Elephant exclusion, elephants (and giraffes) excluded; Control, all herbivores present
 Figure 3: Mean number of tea bags (a) infected by fungi and (b) damaged by termites across herbivore treatments. Significant differences ($p < 0.05$) between herbivore treatments are indicated by different lowercase letters.



Value of the TBI in an African context

Decomposition is central to the effective functioning of terrestrial ecosystems as it forms the link between above - and belowground nutrient cycling.^{3,4,6,9,14,15} Yet, little is known about site-specific disturbance effects (i.e. herbivory and fire) on decomposition and carbon flux. TBI -based decomposition studies can provide valuable information on the extent of these disturbances and their potential effect on essential ecosystem processes.^{1,5,9,14,15} The TBI approach has been developed to remove the subjectivity involved in using site- and species-specific litter, only providing information pertaining to the potential decomposition capacity (i.e. deduced from standardised litter) of a specific site or system based on process-driven soil functions.^{1,8,9} However, despite the major differences in chemical composition of standardised (e.g. tea) and local litter, both respond in similar ways to environmental drivers.^{1,9} The TBI is therefore able to identify and examine environmental drivers of decomposition without ambiguous effects of site-specific litter, serving as a reference which facilitates data comparison across spatial scales.^{1,3,5,8,9}

Despite growing concerns about climate change and increased atmospheric CO₂ levels, research on decomposition and soil carbon flux remains limited for Africa.^{5,10} Necessary resources required for such studies are often unavailable in many African countries. However, citizen science projects, such as the TBI, have become a useful tool to facilitate ecological research due to relatively easy application and public engagement (e.g. Teatime 4 Science, <http://www.teatime4science.org/>).^{1,5,8,9}

With the aim to promote efficient decomposition studies, we therefore suggest the application of this extended TBI approach for at least a 9-month duration together with application-based amendments as set out in Table 1. The TBI remains a relatively easy and cost-effective approach which greatly reduces the amount of data to be collected in normal litterbag decomposition studies. As such, this method is more conducive to both the available resources and prevailing environmental conditions in Africa.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

L.L.E.: Methodology, data collection, sample analysis, data analysis, validation, data curation, writing – the initial draft, writing – revisions, project leadership. H.v.C.: Methodology, data collection, sample analysis, data analysis, validation, data curation, writing – the initial draft, writing – revisions, project leadership, student supervision, project management. F.S.: Conceptualisation, writing – the initial draft, writing – revisions, project leadership.

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Appendix E: Published Article (Chapter 6)



Decomposition Rates of Organic Material across Herbivore Treatments in a Nutrient-Rich Semi-Arid Sodic Savanna

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Decomposition rates of organic material across herbivore treatments in a nutrient-rich semi-arid sodic savanna

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Abstract

Decomposition is a major determinant of terrestrial nutrient cycling and therefore an important regulator of ecosystem structure and function. It has been widely documented that large mammalian herbivores (LMH) act as a significant driver of changes to aboveground structure and modifications to edaphic factors. Little is known about the role of herbivory, and particularly the loss thereof, in mediating essential ecological processes in a herbivore-adapted system. The Nkuhlu exclosures, a large-scale, long-term exclusion experiment in the Kruger National Park, South Africa, provided an opportunity to explore the effects of herbivory and/or its long-term exclusion on decomposition and stabilisation of detrital plant material. An extended, site-specific version of the Tea Bag Index approach was used to quantify decomposition rate (k) and stabilisation factor (S) of standardised litter substrate. Two hundred and fifty tea bags (125 green and 125 rooibos tea bags) applied in a paired tea bag design were exposed to three herbivore treatments along the sodic zone of the Nkuhlu exclosures and removed after three months of incubation. Decomposition rates (k) were highest in the presence of LMH and lowest in their absence. Conversely, stabilisation factor (S) was significantly higher in treatments from which herbivores have been excluded for ~18 years. Our study provides evidence that LMH can influence essential ecological processes such as decomposition and stabilisation of detrital plant material. Moreover, results confirmed that ecosystems that evolved with herbivores, are sensitive to herbivore loss as it reduces decomposition rates of plant detritus and hence, decelerates ecosystem nutrient cycling.

Introduction

Savannas are dynamic systems with a high degree of complexity, mainly due to its spatial heterogeneity –the product of system-specific drivers (Grant and Scholes, 2006; Van Coller *et al.*, 2013; Van Coller & Siebert, 2015; Wigley *et al.*, 2020). Savannas are faced with numerous challenges, including heavy utilisation pressure from single-species domestic livestock at the expense of heterogeneous savanna herbivore communities, which contribute towards the structural and functional degradation of these systems (Grant and Scholes, 2006; Van Coller *et al.*, 2013; Tuomi *et al.*, 2019; Wigley *et al.*, 2020). Herbivory is an agent of disturbance and functions as one of the primary drivers of system dynamics in African savannas (Grant and Scholes, 2006; Van Coller *et al.*, 2013; Tuomi *et al.*, 2019). Numerous studies have reported on the ability of large mammalian herbivores (LMH) to alter vegetation structure and edaphic properties of a system (Grant and Scholes, 2006; Van Coller *et al.*, 2013; Van Coller & Siebert, 2015; Wigley *et al.*, 2020). Changes to systems structure has the potential to directly influence and alter soil function in terms of decomposition and stabilisation of detrital plant material (Keuskamp *et al.*, 2013; Becker and Kuzyakov, 2018; Tuomi *et al.*, 2019; Wigley *et al.*, 2020)

In southern African savannas, edaphic factors are stronger determinants of intensively utilised patches (IUP) than animal biomass alone (Grant and Scholes, 2006; Van Coller *et al.*, 2013). Sodic patches are prominent IUP's in granitic landscapes that are structurally open areas, which improve predator vigilance. Soil in these patches have a high sodium (Na) concentration, which produce sodium-enriched forage (Grant and Scholes, 2006; Van Coller & Siebert, 2015). LMH are also responsible for the formation and the maintenance of wallowing points in these systems during the wet season (Grant & Scholes, 2006; Van Coller *et al.*, 2013). Sodic patches are ecologically important as they provide a positive nutrient feedback loop promoted by congregated herbivore activity (Grant and Scholes, 2006; Van Coller *et al.*, 2013). Herbivores are therefore responsible for maintaining invaluable ecological processes and corresponding integrity of sodic systems.

Large parts of protected African savannas are faced with herbivore loss, albeit the diverse suite of LMH, or elephants only (Van Coller *et al.*, 2013; Van Coller & Siebert, 2015; Wigley *et al.*, 2020). Such losses might affect several underlying processes including soil-based decomposition and stabilisation of detrital plant material (Becker and Kuzyakov, 2018; Tuomi *et al.*, 2019; Wigley *et al.*, 2020). Decomposition entails the biochemical-driven degradation of complex organic compounds through mainly, microbial-mediated processes (Keuskamp *et al.*, 2013; Petraglia *et al.*, 2019). Decomposition studies are covered extensively for

temperate and boreal ecosystems, while studies in subtropical ecosystems, especially in Africa, remain limited (Becker and Kuzyakov, 2018; Erasmus *et al.*, 2021).

Despite extensive use of enclosure experiments to investigate herbivore impacts, there is little consensus on their direct and indirect effects on soil function, particularly on decomposition and inherently, above- and belowground carbon sequestration (Tuomi *et al.*, 2019; Wigley *et al.*, 2020). The Nkuhlu enclosures located within the Kruger National Park (KNP) provided an opportunity to examine the potential influence of complete (all LMH) or partial (only elephants) herbivore loss on essential ecological processes. This study therefore aimed to assess the intrinsic role of herbivores in soil-based decomposition and stabilisation in a herbivore-adapted semi-arid, sodic African savanna. We hypothesized that the decomposition rate and stabilisation factor of organic material would differ across different herbivore treatments as herbivores actively alter key regulatory constituents of the decomposition subsystem. To test this hypothesis, we applied the Tea Bag Index (TBI) approach across three herbivore treatments with varying intensity to determine the effect of herbivory on potential soil-based decomposition rate (k) and stabilisation factor (S) of detrital plant material through the quantification of standardised litter.

Methods and Study Site

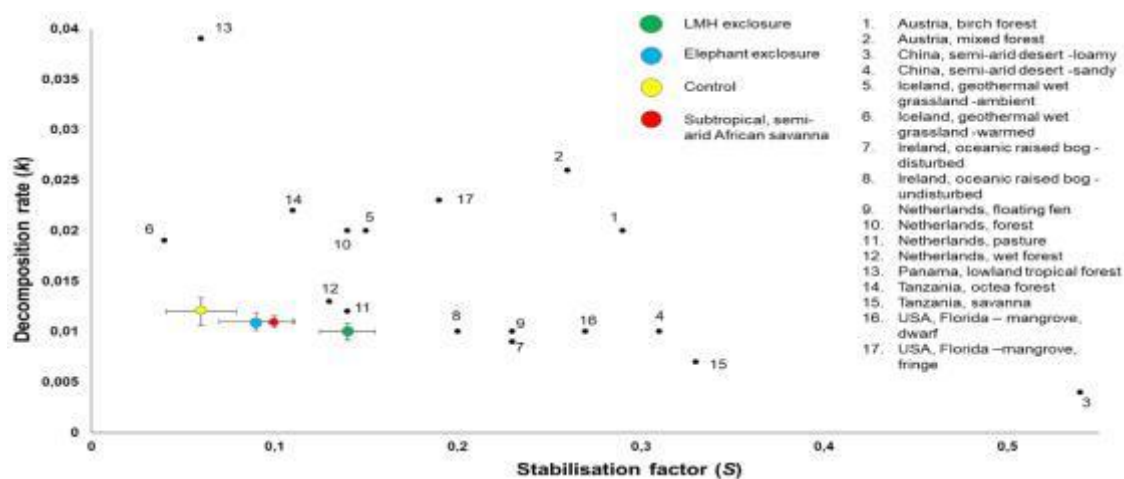
The study was conducted in the semi-arid Dry Sodic Savanna of the Nkuhlu experimental site (24°58'S, 31°46'E) located on the northern bank of the perennial Sabie River approximately 18 km downstream of Skukuza in the KNP, South Africa (Siebert and Eckhardt, 2008). Situated beneath the seepline on the foot slopes of undulating granitic landscapes ranging between 200 and 230 m above sea level (a.s.l.), the study site is characterised by sodium-rich, deep duplex soil, referred to as the sodic zone (Siebert and Eckhardt, 2008). The Nkuhlu enclosures were constructed in 2001 as part of a large-scale, long-term exclusion experiment based on the removal of certain ecosystem drivers (e.g. herbivory and fire) (Siebert and Eckhardt, 2008). Herbivores have therefore been excluded for 18 years at the time of data sampling for this particular study. The experimental site is divided into three herbivory treatments comprising of (1) a partially fenced area (Elephant enclosure) designed to exclude elephants based on body size (also excluding giraffes), (2) an unfenced area (Control) which allows for unrestricted movement of all LMH and (3) a fully fenced area (LMH enclosure) excluding all herbivores larger than a hare (Siebert and Eckhardt, 2008).

The TBI approach introduced by Keuskamp *et al.* (2013) is an internationally standardised, user-friendly method to collect comparable, globally distributed decomposition data. Test kits comprise of commercially available tea bags (i.e. Lipton® rooibos and green tea). Standardised litter (i.e. tea leaves) enables the TBI to identify and examine environmental drivers of decomposition without the ambiguous effects of site-specific litter, serving as a reference which facilitates data comparison across spatial scales (Keuskamp *et al.*, 2013; Becker & Kuzyakov, 2018). The TBI comprises of two parameters describing (1) decomposition rate (k –rate at which detrital plant material is lost) and (2) stabilisation factor (S –sequestration of detrital plant material). Methodology conformed to the extended, site-specific version of the TBI approach as described in Teatime in Kruger (Erasmus *et al.*, 2021). Tea bags (125 green and 125 rooibos tea bags) were buried in a paired design among the three herbivore treatments in a sodic savanna ecosystem. After a three-month incubation period, tea bags were retrieved, followed by the standard protocol to calculate k - and S -values. Results were compared with TBI values obtained from a diverse range of ecosystems globally (Figure 1).

Since the data did not meet assumptions of normality according to Kolmogorov–Smirnov and Shapiro–Wilk normality tests, a Kruskal–Wallis ANOVA was applied to the data in Statistica, version 13.3 (TIBCO Software Inc., 2017) to test for significant variation in k and S of standardised litter amongst three herbivore treatments. Where statistically significant variance among treatments were attained, multiple comparisons of mean ranks were applied as post-hoc tests. Significance levels for all analyses were set at the standard significance level (i.e. $p < 0.05$) enabling confident conclusions made from statistical results.

Results

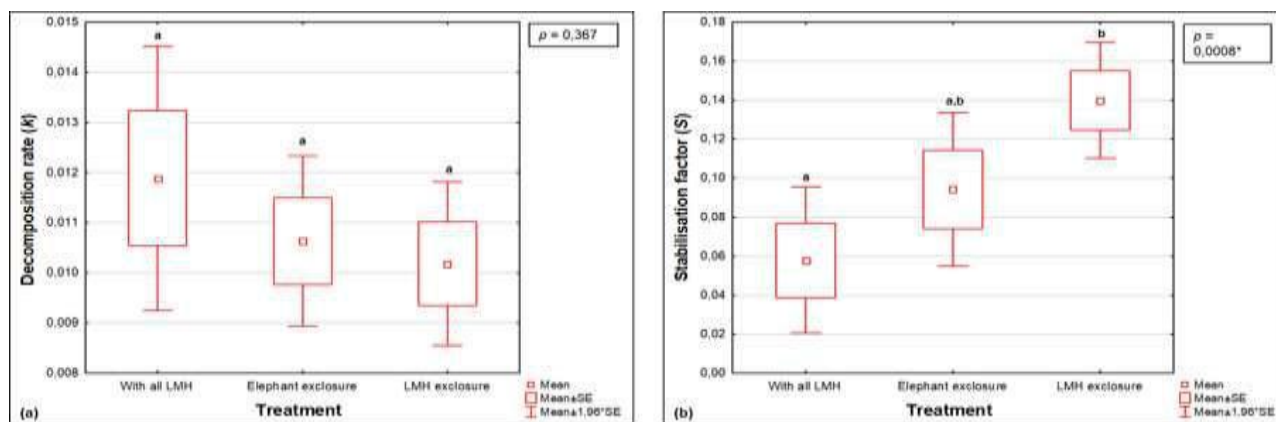
All measured k - and S -values occupied a similar range as that of global TBI references derived from Keuskamp *et al.* (2013) and Becker and Kuzyakov (2018). System 15 (Tanzania, savanna –Figure 1) is the only system that partially relates to the study area in terms of climate and system structure (Siebert & Eckhardt, 2008; Becker & Kuzyakov, 2018). Obtained S -values from the study area differs substantially from that of system 15, whereas k -values are more related (Figure 1). This supports the notion that S is dependent on environmental and climatic factors, reflecting site-specific drivers (Keuskamp *et al.*, 2013; Becker & Kuzyakov, 2018).



LMH exclusion, all LMH excluded; Elephant exclusion, elephants (and giraffes) excluded; Control, all herbivores present.

Figure 1: Mean decomposition rates (k) and stabilisation factor (S) within a subtropical, semi-arid southern African savanna at the Nkuhlu exclosures, Kruger National Park. This system is divided into 3 herbivore treatments (coloured circles). Whiskers indicate standard error of the mean based on the results obtained from 250 tea bags (125 green & 125 rooibos) over a 3-month incubation period. Black dots represent global Tea Bag Index references adapted from Keuskamp *et al.* (2013) and Becker and Kuzyakov (2018).

Mean decomposition rate (k) decreased with increasing stabilisation factor (S) which corresponded with a release in herbivory pressure (Figure 2). After a 3-month incubation period, k of standardised litter did not vary significantly across herbivore treatments ($p > 0.05$; Figure 2a), whereas S varied significantly across treatments ($p < 0.05$; Figure 2b). S was significantly higher in the LMH exclusion when compared to the control (multiple comparisons: $p < 0.05$; Figure 2b), which corresponds with the lowest k -value in this treatment (Figure 2a).



LMH exclusion, all LMH excluded; Elephant exclusion, elephants (and giraffes) excluded; Control, all herbivores present; $\pm 1.96^*$, standard deviations of the mean; SE, standard error; $p, p < 0.05$ indicates a significant differences between groups.

Figure 2: Mean decomposition rate - k (a) and stabilisation factor - S (b) of standardised litter after a 3-month incubation period across three herbivore treatments within the Nkuhlu exclosures, Kruger National Park. Significant differences ($p < 0.05$) between herbivore treatments are indicated with different lowercase letters.

Discussion

Obtained results varied substantially from other systems (Figure 1), echoing the unique ensemble of environmental drivers present within the studied system. Differences between k - and S -values from the studied system and its closest relative (i.e. system 15 - Tanzanian savanna, 951 m a.s.l.) may be ascribed to combined effects of elevation and climatic variation on soil processes (Becker & Kuzyakov, 2018). Systems situated at low elevation, especially in semi-arid environments (such as the study area) are highly subjected to climatic limitations (i.e. temperature and moisture) which directly influence decomposition (Grant & Scholes, 2006; Becker & Kuzyakov, 2018). Higher decomposition rates (k) in the presence of all LMH (control) can be ascribed to the ability of LMH to alter vegetation structure and edaphic properties, specifically to enhance decomposition factors (Grant and Scholes, 2006; Semmartin *et al.*, 2008; Schrama *et al.*, 2013; Van Coller *et al.*, 2013). High intensity utilisation of savanna vegetation results in decreased carbon-based secondary

metabolites (recalcitrant compounds such as tannins and polyphenols), decreased C:N ratios and increased nutrient content within vegetation tissue, rendering highly utilized vegetation more palatable, nutrient-rich and labile (Grant and Scholes, 2006; Siebert & Eckhardt, 2008; Van Coller & Siebert, 2015). LMH influence patch-specific soil nitrogen (N) availability through excretions (i.e. urination and defecation) which stimulate microbial metabolic activity and subsequent decomposition of detrital plant material (Grant and Scholes, 2006; Semmartin *et al.*, 2008). System perturbation, which may be associated with high LMH activity, correlate with decreased stabilisation and a subsequent decrease in soil organic matter (SOM), which result in increased microbial demand for organic carbon and subsequently, a more rapid detrital turn-over (decomposition) (Semmartin *et al.*, 2008; Becker and Kuzyakov, 2018). Increased SOM due to high stabilisation (significantly higher S in the LMH enclosure) results in fertile soil with a higher productivity potential (biomass production). Several studies have indicated that absence of herbivores lead to increased standing biomass (Grant and Scholes, 2006; Van Coller *et al.*, 2013). Low soil temperatures associated with the shading effect of high biomass can promote stabilisation as it results in reduced microbial metabolic activity (Keuskamp *et al.*, 2013; Becker and Kuzyakov, 2018; Petraglia *et al.*, 2019). This effect is evident from the results as S of standardised litter increased with a decrease in herbivore pressure. Moreover, the study area is situated within a moisture-limited system (561 mm per year) further impeding microbial activity and subsequent decomposition (Grant & Scholes, 2006; Keuskamp *et al.*, 2013; Becker and Kuzyakov, 2018; Petraglia *et al.*, 2019).

Decomposition functions as a bottom-up system control and is a major determinant of system dynamics. Yet, little is known about site-specific disturbance effects such as herbivory or the loss thereof on essential ecological processes. Decomposition and associated carbon flux studies are covered extensively in the northern hemisphere, while studies in African systems remain limited. TBI-based decomposition studies provide a relatively easy approach to obtain valuable information regarding system disturbances and their potential effect on ecosystem functioning. Studies aimed at elucidating decomposition dynamics and the role of various influencing factors such as herbivory is essential to better understand and effectively manage soil function together with associated carbon cycling.

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