

**Impacts of fire and herbivory on soil
nutrient cycling and microbial communities
in savanna ecosystems**

Mary Jennifer Cvetkovic-Jones



This thesis is submitted for the degree of Doctor of Philosophy

June 2024

Lancaster Environment Centre, Lancaster University



Artwork by Kaia Benskin, age 7 ½

For Granny

Declaration

This thesis has not been submitted in support of an application for another degree at this or any other university. It is the result of my own work and includes nothing that is the outcome of work done in collaboration except where specifically indicated. Many of the ideas in this thesis were the product of discussion with my supervisors: Prof Carly Stevens (Lancaster University), Prof Kate Parr (Liverpool University) and Dr Jeanette Whitaker (UK Centre of Ecology & Hydrology).

This thesis word length is 31,110 (excluding table legends, figure captions and reference lists) and therefore does not exceed the permitted maximum.

Statement of Authorship

This thesis has been prepared in the alternative format, as a set of four papers presented in Chapters 2-5. These chapters have co-authors in addition to my supervisory team. Please find below details of these publications. Chapters 1 and 6 are introductory and discussion chapters and are not intended for submission.

Chapter 2

Cvetkovic-Jones, M, Strydom, T, Stevens, C.J, Whitaker, J, Ostle, N, Parr, C.L. Soil microbes exhibit resilience to long-term savanna fire regimes. *In revision with Plant and Soil.*

Chapter 3

Cvetkovic-Jones, M, Stevens, C.J, Whitaker, J, Parr, C.L, Strydom, T. Savanna soil microbes are resilient to moderate intensity fire regimes: a year-long time since fire study tracking soil respiration post-burn. *In preparation.*

Chapter 4

Cvetkovic-Jones, M, Stevens, C.J, Whitaker, J, McCleery R, Ostle, N, Parr, C.L. Little effect of mammalian herbivores on savanna soil microbial communities and nutrient cycling. *In preparation.*

Chapter 5

Cvetkovic-Jones, M, Stevens, C.J, Whitaker, J, Archibald S., Situngu S., Strydom T., Staver A.C, Ostle, N, Parr, C.L. The individual and interactive effect of fire and herbivory on savanna soil properties. *In preparation.*



Mary Jennifer Cvetkovic-Jones



Professor Carly J Stevens (primary supervisor)

Lancaster University, UK, June 2024

Abstract

Savanna vegetation dynamics are driven by fire, herbivory, and climate, but their effects on soil properties are poorly understood. Specifically, the potential individual and interactive impacts of these drivers on the soil biogeochemical resilience remains unknown. Savanna ecosystems are increasingly vulnerable to global change and human activity, with predicted future changes in fire, herbivory, and climatic regimes. These drivers have the capacity to significantly alter savanna biodiversity and biogeochemical function, with cascading impacts on human welfare. The consequences of fire-herbivory interactions on soil are difficult to separate due to their synergistic nature, and understanding has been compounded by a lack of fire-herbivory experiments investigating both above and belowground processes. To address this gap, experiments were conducted in the lab and field within Kruger National Park, South Africa, across different rainfall gradients, fire intensities, soil types and herbivore presence. The effects of fire, herbivory and their interactions on soil carbon, nutrient concentrations, and microbial communities were quantified to determine the variability in savanna soil properties and test the biogeochemical resilience of the soils. There was limited evidence that changing fire or herbivory regimes had an effect on soil nutrients or microbial activity, particularly in sandy soils. In contrast, changes in soil nitrate and microbial community composition were observed following a single fire-herbivory event on clay soils. Overall, there was strong evidence that savanna soils are extremely resilient to fire and large mammal herbivory. These findings contribute to unravelling the complex mechanisms underlying fire-herbivory interactions in savanna ecosystems. They also underscore the unique nature of savanna ecosystems compared with temperate ecosystems, emphasizing the necessity for more focussed experiments in arid and tropical savannas to understand how both abiotic and biotic factors influence ecosystem functioning. Such understanding is crucial for predicting how global change will shape the future of savanna ecosystems.

Acknowledgements

I have loved my PhD experience and wish I could do it all again. However, it wasn't the long, hot days in the field, the perpetual struggle with lab equipment, or the regular R battles that made this journey memorable. Instead, it was the wonderful people who I was lucky enough to work with over the past four years.

Firstly, I extend my deepest appreciation to my supervisors, Prof Carly Stevens, Prof Kate Parr, Dr Jeanette Whitaker, and Prof Nick Ostle. Carly, your unwavering support transformed my PhD. Thank you for all the chats, invaluable insights, exceptional proof reading, trip to Florence and for nurturing my growth as a scientist. Kate, thank you for my love of fires and savannas. I'm so very grateful for you pushing me to spend more and more time in Kruger, it now feels like a second home. Jeanette, thank you for all your words of encouragement, problem solving and constant support. Nick, promoting scientific independence and securing the funding for such an interesting PhD project is truly appreciated. Thank you to my wonderful examiners, Dr Kimberly Simpson and Professor Jos Barlow for my viva and insightful comments.

I extend my thanks to my funders, Envision DTP. Special appreciation for Dr Catherine Baxendale, Sohila Raqib and Olivia Lawrenson for all the administrative support. Additional thanks to all of the LEC technical team and specifically the teaching technicians! Thank you to the Scientific Services team in Kruger National Park, especially Dr Tercia Strydom. Tercia, completing my PhD alongside you was a privilege, thank you for all the Kruger knowledge, endless support and looking after me every time I appeared in the bush (particularly when I got cholera!). Thank you to Adolf, Nikisha, Samantha, Sipokhazi, and Patricia for the game guard, lab and admin support every trip. Thank you to the game guards, Isaac, Dudu and Khensani for keeping me safe every trip. The academic savanna community has been so welcoming and extra appreciation is extended to Prof Sally Archibald, Dr Sivu Situngu, Prof Carla Staver, Prof Bob McCleery, Dr Lawrence Kruger, and Dr Navashni Govender for all your collaborative support on my PhD projects.

To my colleagues and friends in the LEC Sustainable soils group, A28 soils lab group, my new and old office mates, and lunch friends, it was a pleasure to do my PhD alongside you all. Special thanks to Hannah for all the lifts to work and making our tiny corner of the office so much fun – enjoy not having to crash your chair into me every day- you have really dragged me through the final 12 months. Thanks to Cristina for being my office friend when we were

all alone as we crawled through the aftermath of COVID. I am also so grateful to the CEH PSI group, particularly Sam and Stella, for their support and coffee breaks.

To all my friends, near and far, thank you for your support. Dr Rosanne Broyd, thank you for all the technical help, Wednesday stats calls, and running round the Lake District with me. To Dr C, J, and of course AJ, thanks for your endless motivation and care packages. We first met when I started my savanna adventures and feel very lucky to still be supported by you all now. Thank you to Megan for getting me running again and encouraging me to write up my thesis and train for an ultramarathon simultaneously. Thanks to Dr Clare Benskin, for your unwavering support, funny stories every morning over coffee and being my biggest cheerleader. Thank you to my goddaughter Kaia for the front-page artwork and for always making me laugh.

To my in-laws, Vesna, Andy, Marnie and Rob, your constant support and grounding presence reminding me I was only becoming a Dr of Mud is appreciated. I am very proud to become the first Dr Cvetkovic-Jones. To my parents, Jennie and Graham, and my family, thank you for your unwavering support in every life path I have taken, both pre- and post- PhD. I'm so glad I came home to Lancaster. Granny, your encouragement echoes through every opportunity I pursued – I hope you enjoy this extra coffee table book.

Lastly, to my wonderful husband, Morgan, your love, humour, and endless support sustained me through all of my PhD challenges. You will never realise how much your morning coffee, three-legged cat antics (the biggest thanks go to Tripod and Bella!), and silly accents got me through my PhD. Enjoy this thesis on our paper wedding anniversary.

Contents

List of Tables.....	xiii
List of Figures.....	xvi
List of Abbreviations and Acronyms	xx
1. General Introduction	1
1.1. Savannas	2
1.2. Fire, Herbivory and Climate	2
1.3. The importance of soils for ecosystem functioning.....	4
1.4. Fire impacts on soil properties.....	5
1.4.1. Single fire event vs fire regime.....	5
1.4.2. Fire effects on physical soil properties	6
1.4.3. Fire effects on chemical soil properties	6
1.4.4. Fire effects on biological soil properties.....	7
1.5. Herbivore impacts on soil properties	8
1.6. Fire x herbivory impact on soil properties.....	10
1.7. Global Change	11
1.8. Kruger National Park	12
1.8.1 Climate.....	12
1.8.2. Fauna.....	13
1.8.3. Fire occurrence.....	15
1.8.4. Geology.....	15
1.9. Thesis Aims and Objectives.....	18
1.10. Thesis Structure	19
2. Soil microbes exhibit resilience to long-term savanna fire regimes	20
2.1. Introduction.....	21
2.2. Methods.....	24
2.2.1. Study Area.....	24
2.2.2. Soil sampling	26
2.2.3. Soil Biotic and Abiotic Analyses	26
2.2.4. Soil Water Holding Capacity	27
2.2.5. Basal and Substrate-Induced Respiration	28
2.2.6. Respiration measurements	29
2.2.7. Statistical Analysis.....	29

2.3. Results.....	29
2.3.1. Soil characteristics along the fire gradient.....	29
2.3.2. Substrate-induced respiration response along the fire gradient.....	31
2.4. Discussion.....	32
2.4.1. Fire regime effects on microbial community composition.....	33
2.4.2. Fire regime effects on soil nutrients.....	34
2.4.3. Fire regime effects on substrate-induced respiration.....	35
2.4.4. Conclusion.....	37
2.5. Acknowledgments.....	38
3. Savanna soil microbes show high resilience to moderate intensity fires: a year-long time-since-fire study tracking soil respiration post-burn.....	40
3.1. Introduction.....	41
3.2. Methods.....	43
3.2.1. Study area.....	43
3.2.2. Prescribed burns.....	43
3.2.3. Phospholipid Fatty Acid Analysis.....	44
3.2.4. Soil respiration.....	45
3.2.5. Gravimetric Soil Moisture.....	46
3.2.6. Statistical Analysis.....	47
3.3. Results.....	47
3.3.1. Microbial community composition.....	47
3.3.2. Soil Respiration following prescribed burns.....	48
3.3.3. Annual Fluctuations in Soil Moisture with TSF.....	50
3.4. Discussion.....	51
3.4.1. Impact of Fire on Soil Microbial Community.....	51
3.4.2. Impact of Fire on Soil Respiration.....	52
3.4.3. Impact of soil moisture on soil respiration.....	56
3.4.4. Conclusion.....	57
3.5. Acknowledgments.....	57
4. Little effect of mammalian herbivores on savanna soil microbial communities and nutrient cycling.....	58
4.1. Introduction.....	59
4.2. Methods.....	62
4.2.1. Study Area.....	62
4.2.2. Experimental Design.....	62

.....	63
4.2.3. Pre-Treatment Sampling	64
4.2.4. Post Treatment Sampling	64
4.2.5. Abiotic and biotic analyses	64
4.2.6. Statistical Analysis	66
4.3. Results.....	66
4.3.1. Pre-Treatment Analyses	66
4.3.2. Post Treatment Analyses	67
4.4. Discussion.....	70
4.4.1. Herbivore density and intensity	71
4.4.2. Fire effects on herbivore impacts.....	71
4.4.3. Herbivore impacts on soil nutrient levels.	72
4.4.4. Herbivore effects on soil microbial community	74
4.4.5. Conclusion	76
4.5. Acknowledgments.....	76
5. The individual and interactive effect of fire and herbivory on savanna soil properties.....	78
5.1. Introduction.....	79
5.2. Methods.....	81
5.2.1. Study Area.....	81
5.2.2. Pre-Treatment Soil Sampling.....	82
5.2.3. Prescribed Burns in 2021	83
5.2.4. Post Treatment Soil Sampling.....	85
5.2.5. Soil Biotic and Abiotic Analyses	85
5.2.6. Prescribed burns in 2022.....	86
5.2.7. Soil Respiration.....	88
5.2.8. Gravimetric Soil Moisture	88
5.2.9. Vegetation Sampling	88
5.2.10. Statistical Analysis	88
5.3. Results.....	89
5.3.1. Pre-Treatment Soil Properties	89
5.3.2. Post Treatment Soil Properties.....	90
5.3.3. Impacts of fire and herbivory treatments on soil respiration	95
5.3.4. Grass Biomass Consumption	98
5.4. Discussion.....	98
5.4.1. Impact of fire x herbivory on soil microbial communities	99

5.4.2. Impact of fire x herbivory on soil nutrients	100
5.4.3. Impact of fire x herbivory on soil respiration	101
5.4.4. Conclusion	103
5.5. Acknowledgments.....	104
6. General Discussion	105
6.1. Key Findings.....	106
6.2. General Conclusions	107
6.2.1. Contribution of soil microbial communities to savanna ecosystems.....	107
6.2.2. Soil Resilience or Resistance within Kruger National Park	109
6.2.3. Impacts of fire and herbivory on other savanna systems.....	110
6.3. Management Implications.....	111
6.4. Recommendations for Future Research	113
6.5. Concluding remarks	115
7. References.....	118
8. Appendix	138

List of Tables

Table 1.1 – Global temperature thresholds (°C) for biological disruption in the soil environment across a range of ecosystems (adapted from Neary et al., 2005).....	8
Table 2.1 - Average fuel load (kg ha ⁻¹), fire intensity (kW m ⁻¹) and fire return interval (years) across the three regimes; higher intensity burns, lower intensity burns and fire suppression. Data was averaged from using data from 2005-2021. Fuel load was indexed as disc pasture meter settling height as per Zambatis et al. (2006). Fire intensity was calculated as per Byram's (1959) fire line intensity equations (Fire intensity = heat yield * ROS * biomass). Fuel load and fire intensity values was averaged using SANParks data from 2005-2021. Fire return interval values were averaged using from all recorded fire instances between 1968-2021.....	25
Table 2.2 - Effects of the different fire regimes on soil abiotic and biotic properties (mean ± SE) analysed by linear model. Soil abiotic properties include C: N ratio, soil moisture, soil pH, ammonium (µg NH ₄ g ⁻¹ soil dwt), nitrate (µg NO ₃ g ⁻¹ soil dwt). Soil biotic properties include microbial biomass C and N (µg g ⁻¹ soil dwt) and PLFA measurements (nmol g ⁻¹ soil dwt).....	30
Table 2.3 – Average (± SE) respiration response (mg C g ⁻¹ soil fwt) to increasing water holding capacity (WHC) for the three different fire regimes: higher intensity, lower intensity, and fire suppression.....	31
Table 3.1 - Fire behaviour measurements across the four prescribed burns replicates; Napi (Nap), Mbiyamti (Mby), Nwaswitshaka (Nwa) and Skukuza (Skz). Measures included fuel load (kg ha ⁻¹), rate of spread (m s ⁻¹), fuel moisture content (%), air temperature (°C), thermocouple fire temperatures aboveground and belowground at 1cm and 5cm (°C), % of plot burnt, fire intensity (kW m ⁻¹) and final burning comments.....	44
Table 3.2 - Effects of October biennial burns and fire suppression regimes on mean (±SE) PLFA biomarker concentrations (nmol g ⁻¹ soil dwt), one-month after fire.....	48
Table 4.1 - Pre-treatment data for mean (±SE) soil properties measured for Full (no herbivores), Mega (megaherbivores excluded) and Open (all	

herbivores present) treatments. Soil abiotic properties include soil pH, total C and N (%), C: N ratio, and total phosphorus (mg kg^{-1}).....	66
Table 4.2 - Pre-treatment microbial community composition represented as mean (\pm SE) PLFA biomarkers (nmol g^{-1} soil dwt) for Full (no herbivores), Mega (megaherbivores excluded) and Open (all herbivores present) treatments.....	67
Table 4.3 - Effects of different mammalian herbivory regimes on soil properties (mean \pm SE) analysed by linear model for Full (no herbivores), Mega (megaherbivores excluded) and Open (all herbivores present). Soil abiotic properties include soil moisture, bulk density, soil pH, C: N ratio, total P (mg kg^{-1}), ammonium ($\mu\text{g NH}_4 \text{ g}^{-1}$ soil dwt), and nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt). Soil biotic properties include microbial biomass C ($\mu\text{g g}^{-1}$ soil dwt) and PLFA measurements (nmol g^{-1} soil dwt).....	70
Table 5.1 - Fire behaviour measures across the four burnt plots in May 2021, including percentage of plot burnt (area burnt assessed over 100 plot locations), average fuel moisture content (%), fire temperature ($^{\circ}\text{C}$) aboveground using three sets of thermocouples with two wires aboveground, fire temperature ($^{\circ}\text{C}$) belowground with a wire at 5cm, grass biomass measurements pre burn, post burn and the difference (kg ha^{-1}). Mean values recorded \pm SEM. Data collected by Archibald et al. (private comms).....	84
Table 5.2 - Survey of pre-treatment area, under four plant species and bare ground, on soil properties (mean \pm SE), analysed by linear model. Soil abiotic properties include C: N ratio, soil pH, soil moisture (%), ammonium ($\mu\text{g NH}_4 \text{ g}^{-1}$ soil dwt), nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt) and total phosphorus (mg kg^{-1}). Soil biotic properties include PLFA measurements (nmol g^{-1} soil dwt).....	90
Table 5.3 - Effects of different fire and herbivory regimes, one-year post-burn and herbivore exclusion, on soil properties ($M \pm$ SE) analysed by linear model. Soil abiotic properties include C: N ratio, soil pH, soil moisture (%), ammonium ($\mu\text{g NH}_4 \text{ g}^{-1}$ soil dwt), nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt) and total phosphorus (mg kg^{-1}). Soil biotic properties include microbial biomass C ($\mu\text{g g}^{-1}$ soil dwt) and PLFA measurements (nmol g^{-1} soil dwt). Significant values are in bold.....	94

Table 5.4 – Grass biomass (kg ha^{-1}) measured one year after the first prescribed burn (2022) and the second prescribed burn (2023). No data was collected on the unburnt plots in 2023 due to logistical constraints.....	98
Table 6.1 – Comparison of the average PLFA biomarker data across the three sites (Lower Sabie sites is the combination of Chapter 4 and 5). Variations in soil type, annual rainfall and month sampled are listed above chapter averages of PLFA biomarker data (nmol g^{-1} soil dwt).....	108
Table 8.1 – Total PLFA biomarkers (nmol g^{-1} soil dwt) across the two fire regimes for the four EBP replicates (mean \pm SE). Mbiyamiti (Mby) had much higher PLFA biomarker concentrations on the fire suppression plots (highlighted in bold), compared to the other replicates.....	138

List of Figures

Figure 1.1 – Global map of savanna grasslands (yellow) located across Africa, South America, and Australia. Image from Pennington et al. (2018).....	2
Figure 1.2 - Proportion of dry matter consumed by either fire (*, black line) or herbivory (+, grey line) along the rainfall gradient. At annual rainfall levels below 500 mm, herbivory is the dominant consumer, whereas between 800 – 1800 mm fire is the dominant consumer. In the grey shaded areas, either consumer could dominate. Figure adapted from Archibald and Hempson (2016).....	3
Figure 1.3 - Average temperature and precipitation within Skukuza, KNP, with temperature on the left axis and precipitation levels on the right axis. Figure produced by Meteoblue (2024).....	13
Figure 1.4 - A selection of photographs collected during my time in Kruger NP as part of this thesis, to demonstrate the wide range of animals found (picture credit: Morgan Cvetkovic-Jones)	14
Figure 1.5 – Season of fire influences fire behaviour within KNP, with a) fuel moisture content higher in the summer and autumn (wet season) and lower in winter and spring (dry season), prior to experimental burns. This results in b) higher fire intensities in winter and spring, in comparison to summer and autumn. Figure taken from Govender et al. (2006).....	15
Figure 1.6 – KNP covers ~20,000 km ² of land, encompassing various vegetational and geological landscapes. KNP is split into granitic and basaltic soils in an East-Western split. The shapes represent sampling sites of the different chapters within this thesis: ‘circle’ = Pretoriuskop EBPs sampled in Chapter 2; ‘star’ = Skukuza EBPs for Chapter 3; and ‘triangle’ = basaltic supersites investigated in Chapter 4 and 5. Maps adapted from SANParks (2024).....	17

Figure 2.1 – Pictures from Pretorioskop area on KNP on the a) fire suppression plot, b) lower intensity, lower frequency burns burnt every three years in December and c) higher intensity burns burnt every three years in August (picture credit: M Cvetkovic-Jones) 25

Figure 2.2 - SIR response to glucose substrate addition (2.0 mg C g⁻¹ soil f. wt.) for the three different fire regimes; higher intensity (orange), lower intensity (yellow), and fire suppression (grey) over a period of 32 days. Data represent the mean ± SE of additional CO₂ flux (SIR-BR). SIR, substrate-induced respiration, BR, basal respiration..... 32

Figure 3.1 - Pictures of the experimental burn plots, immediately post burn, on Napi October biennial burn plot (Picture credit: T Strydom)..... 46

Figure 3.2 - Temporal variation in soil respiration rates (mg CO₂-C m⁻² hr⁻¹) over one year following prescribed burns (orange) and fire suppression (green) regimes. Average measurements (n=5, ± SE) displayed at one-week prior to the burns and at day 9, 16, 22, 36, 86, 129, 192, 262 and 358 post-burn. The blue dashed lines indicate the timing of the rainfall events. The blue lines represent precipitation over the year, from local weather data in Skukuza. Calendar months in the wet season are highlighted in blue, with dry season in red. 48

Figure 3.3 - Soil moisture showed a positive relationship with soil respiration rates for fire (orange) and fire suppression (green), respectively. R² values quantifies the proportion of SE variance that can be explain by soil moisture..... 50

Figure 3.4 - Temporal gravimetric soil moisture dynamics for one year following a burn for the biennial burn treatment (orange) and fire suppression (green) regimes. The blue dashed lines indicate the timing of the rainfall events. The first rains arrived within one week of the prescribed burns. The second blue line indicates a large notable rainfall event in February. Calendar months in the wet season are highlighted in blue, with dry season in red..... 51

Figure 3.5 - Pictures demonstrating grass re-growth post-burn at week one, week two and month two, month five and month seven after fire. The difference between dry and wet season grass is evident between month five which was following a heavy rainfall wet season and month seven which is six weeks into the dry season.

It is also noticeable that the ash is still visible at one week after fire, but not week three. Picture credits: M Cvetkovic-Jones and T Strydom..... 55

Figure 4.1 - Herbivory exclusion treatments for a) Full enclosure (no herbivores can access), b) Mega (megaherbivores excluded, large herbivores can access) and c) Open (open to all herbivores). Picture credit: Mary Cvetkovic-Jones, April 2022..... 65

Figure 4.2 - The response of soil ammonium ($\mu\text{g NH}_4 \text{ g}^{-1}$ soil dwt) concentration after four years of exclusion for three experimental treatments; Full (excluding all herbivores), Mega (excluding megaherbivores, accessible by large herbivores) and Open (open to all herbivores). Whilst showing a descriptive trend, the differences were not significant ($p=0.13$)..... 68

Figure 4.3 - The response of soil nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt) concentration to three experimental treatments; Full (excluding all herbivores), Mega (excluding megaherbivores, accessible by large herbivores) and Open (open to all herbivores), after four years of treatment..... 69

Figure 5.1 - Study timeline spanning two years of sampling. Initial surveys and soil biogeochemical and microbial analyses were conducted prior to prescribed burns in May 2021. Following these early season and lower intensity burns, herbivore enclosures were erected. In April 2022, one-year post-burn and herbivore exclusion, plots were re-sampled for biogeochemical and microbial analyses. In September 2022, late season, higher intensity burns were performed on the plots, with soil respiration rates (indicated by arrows) measured over the subsequent nine months..... 82

Figure 5.2 - Pictures from the site following the first set of early season burns in May 2021; a) low intensity early season burn (picture credit, S Archibald), b) 2 m² enclosure installed immediately after the burns, surrounded by *Themeda triandra*, pictured in April 2022 (picture credit, T Strydom)..... 83

Figure 5.3 - Pictures from the site following the second set of late season burns in September 2022; a) prescribed burns at the end of the dry season in September 2022, b) the burn plots at three weeks post-burn with blue wildebeest grazing and c) difference in grass height and biomass with and without herbivory four months post-burn (Photo credit, T Strydom, January 2023)..... 87

Figure 5.4 - Effects of fire x herbivory, fire, herbivory and no fire-no herbivory treatments on soil nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt) levels after a single early season burn and one year of herbivore exclusion. Letters denote significance at $p \leq 0.01$ level..... 91

Figure 5.5 - Effects of fire x herbivory, fire, herbivory and no fire-no herbivory treatments on soil a) total PLFA, b) total fungal, c) total bacteria, d) fungal: bacteria ratio, e) gram positive bacteria, f) gram negative bacteria biomarkers (nmol g^{-1} soil dwt), one year post burn and herbivore exclusion..... 93

Figure 5.6 - Effects of fire x herbivory, fire, herbivory and no fire-no herbivory treatments on soil microbial biomass ($\mu\text{g g}^{-1}$ soil dwt), one-year post-burn and herbivore exclusion. Whilst this trend is not significant ($p=0.61$), it shows a descriptive trend that matches descriptive trends in total PLFA biomass..... 94

Figure 5.7 – The impact of fire and herbivory treatments on soil respiration ($M \pm \text{SE}$) after one early season burn (2021) and one late season burn (2022), and ~17 months of herbivore exclusion. Soil respiration was measured on day prior to treatment (day -1) and at 7, 21, 35 and 252-days post late season burn 2022..... 96

Figure 5.8 - The impact of fire and herbivory treatments after one early season burn and one late season burn, and ~17 months of herbivore exclusion, on soil moisture sampled at the corresponding time points as soil respiration..... 97

Figure 5.9 - Over the period of nine months, soil moisture explained more of the variance on the fire plots with (38%) and without (58%) herbivory, than the unburnt plots, with (11%) and without (18%) herbivory..... 98

Figure 6.1 – African continent mapping of a) area burned, b) current herbivore densities, c) past herbivore densities (without elephants) and d) defining areas where fire or herbivory dominate. Taken from Archibald and Hempson (2016).... 111

List of Abbreviations and Acronyms

°C	Degree Celsius (unit of temperature)
ANOVA	Analysis of variance (statistical test)
BR	Basal Respiration
BROWSE	Biodiversity Research on Wildlife and Savanna Ecosystems
C	Carbon
CaCl ₂	Calcium Chloride
CaSO ₄	Calcium Sulphate
CO ₂	Carbon dioxide
DPM	Disc Pasture Meter
dwt	Dry weight
EBP	Experimental Burn Plots
F: B	Fungal: Bacterial ratio
fwt	Fresh weight
GAMM	Generalised Additive Mixed Model
GP: GN	Gram Positive bacteria: Gram Negative bacteria
H ₂ SO ₄	Sulphuric Acid
ha	Hectare
IRGA	Infrared Gas Analyser
K ₂ SO ₄	Potassium Sulphate
KCl	Potassium Chloride
KNP	Kruger National Park
MgSO ₄	Magnesium Sulphate
N	Nitrogen
NaCl	Sodium Chloride
NH ₄ ⁺	Ammonium
nmol	Nanomoles (unit of molar mass)
NO ₃ ⁻	Nitrate
P	Phosphorus
p	Probability value
pH	Potential for hydrogen

PLFA	Phospholipid Fatty Acid
ppm	Parts per million
PyC	Pyrogenic Carbon
R ²	Correlation coefficient
ROS	Rate Of Spread
SANParks	South African National Parks
SE	Standard error
SIR	Substrate Induced Respiration
TSF	Time Since Fire
µg	Microgram (unit of mass)
WHC	Water Holding Capacity

1. General Introduction



Picture credit: Jennifer Marlon

1.1. Savannas

Savannas cover 20% of the global landscape (Lehmann and Parr, 2016), and are located primarily across Africa, South America, and Australia (Figure 1.1). These tropical grassy biomes are characterized by C₄ grass species, with C₃ tree and shrub species interspersed. Savannas are strongly influenced by fire and herbivory, both analogous drivers of savanna ecosystem structure, through the direct consumption of aboveground vegetation. Fire and herbivory are an essential part of savanna ecosystem functioning, sustaining large populations of herbivores and frequently burning. Climatic conditions, including differing precipitation and nutrient conditions, also exert strong influences on the grass-tree interactions (Scholes and Archer, 1997). African savannas are among the most extensive savanna systems, and are home to wildlife, livestock, and also human populations. African savannas are ecologically, culturally and economically important (Eriksen and Watson, 2009), sustaining vital ecosystem services and providing economic growth (von Maltitz and Scholes, 2013). With a population increase across the continent predicted to reach over two billion by 2040 (Leong et al., 2018), land use change within savanna landscapes is increasingly occurring in order to support communities and increase gross domestic product. It is therefore essential to understand how key drivers such as fire, herbivory and climate, influence savanna ecosystem functioning, particularly in the face of global change.

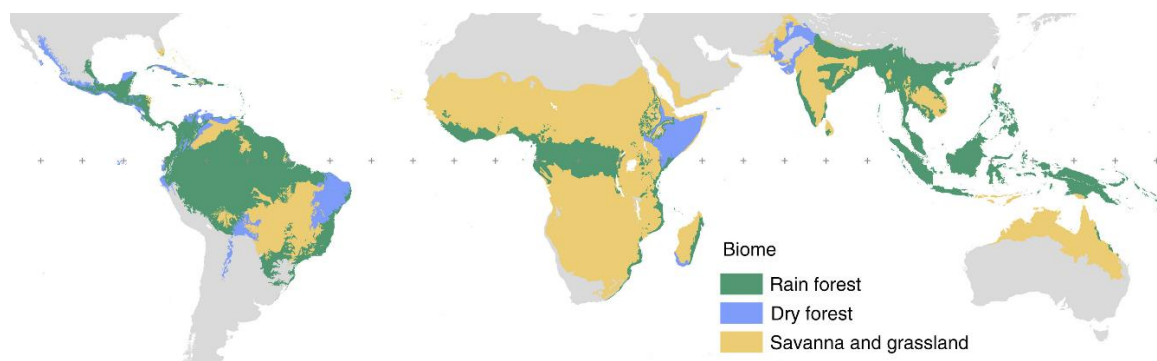


Figure 1.1 – Global map of savanna grasslands (yellow) located across Africa, South America, and Australia. Image from Pennington et al. (2018).

1.2. Fire, Herbivory and Climate

In African savannas, fire regimes and herbivory have co-evolved with both grasses and woody plants showing adaptation and specialisation to either frequently burnt or heavily grazed communities (Archibald and Bond, 2003). Plant species have adapted to fire regimes, with growth and reproductive attributes linked to fire cycles (Ribeiro et al., 2019) and have also

adapted to herbivory by developing defences (Sebata, 2013). However, traditionally, savanna landscape structure was thought to be determined by climate driven conditions, often referred to as a ‘green world’ view. Bond (2005) then suggested that both fire regimes (‘black world’) and herbivore grazing (‘brown world’) could play an equal role in shaping savanna ecosystems, as competing consumers. Both fire and herbivores compete for the herbaceous layer and depending on environmental conditions, either process could dominate. Fire is less likely to occur on heavily grazed land due to removal of plant biomass (fuel load) and decreases in plant flammability (Archibald and Hempson, 2016a; Hempson et al., 2015). In contrast, in less-heavily grazed areas, fire can become dominant, promoting fire-adapted grasses that are less preferable for herbivore consumption. African-wide analysis has shown that in drier, higher nutrient conditions, herbivory seems to dominate, in opposition to fire which dominates in higher rainfall, lower nutrient conditions (Archibald and Hempson, 2016a). In contrast, where annual rainfall levels fall between 500 – 800 mm, neither consumer is dominant (Figure 1.2). This suggests that savanna ecosystems within these annual rainfall parameters can switch between a ‘brown’ or ‘black’ world depending on the mix of herbivory and fire, relative to abiotic factors. There are many dynamic interplays of precipitation, soil type and properties, herbivore species, behaviour and population size, all of which have multiple feedback loops (Archibald and Hempson, 2016a) that may influence which driver is the dominant consumer (detailed review: Smit and Coetsee, 2019).

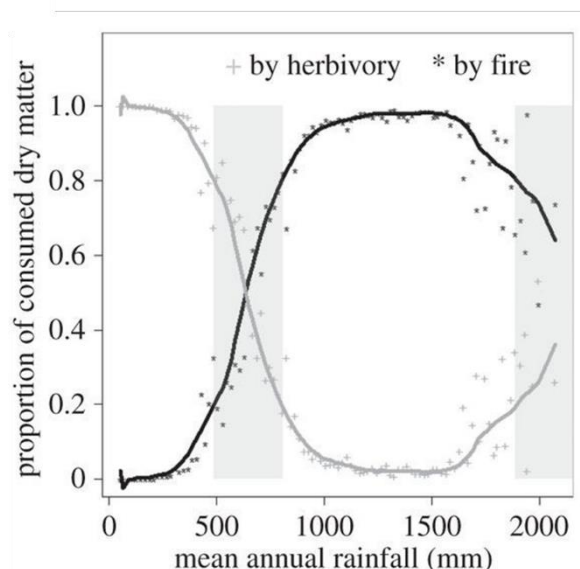


Figure 1.2 - Proportion of dry matter consumed by either fire (*, black line) or herbivory (+, grey line) along the rainfall gradient. At annual rainfall levels below 500 mm, herbivory is the dominant consumer, whereas between 800 – 1800 mm fire is the dominant consumer. In

the grey shaded areas, either consumer could dominate. Figure adapted from Archibald and Hempson (2016).

There is abundant debate about the abiotic and biotic control of savanna biome existence, with views polarized into two camps: the top-down control of fire and herbivory and the bottom-up control of water and soil nutrients. How fire and herbivory shape plant community structure and function above-ground are well understood, but there is a paucity of knowledge regarding the impacts belowground, on soil biogeochemical functioning.

1.3. The importance of soils for ecosystem functioning

Soils are vital for ecosystem functioning, playing a crucial role in plant growth, nutrient cycling, water storage and carbon sequestration (Bardgett, 2005; Blum, 2005; Doelman and Eijssackers, 2004; Smith et al., 2015). Soils are a reservoir of essential elements and nutrients, including carbon (C), nitrogen (N), and phosphorus (P), as well as micronutrients including iron (Fe), manganese (Mn) and zinc (Zn). Soil nutrient cycling, by plants and soil organisms, is essential for plant growth and development (Bardgett and van der Putten, 2014; Cogger and Brown, 2016; Marschner and Rengel, 2007). In the global C cycle, soils act as both CO₂ sources and sinks, with plants taking up CO₂ during photosynthesis and transferring C to the soil through both litter and root exudates (Retallack, 2003; Schlesinger and Andrews, 2000; Stuart Chapin III et al., 2009). Additionally, soils play a significant role in the hydrological cycle, storing and filtering water, preventing floods, and holding water during droughts (Hillel, 2012). Moreover, soils provide a habitat for a wide range of organisms, from soil microbes to invertebrates, facilitating decomposition, nutrient cycling, pest control and soil structure formation (Blume et al., 2016; Thies and Grossman, 2023; Voroney, 2007). Healthy soils sustain diverse soil populations, enhancing ecosystem functioning. Protecting, conserving, and **understanding** soils is essential for maintaining global ecosystem health and biodiversity, as well as sustaining food production, mitigating climate change and protecting vulnerable biomes.

Soils have been found to be as important as fire in determining vegetation structure in African savannas (Staver et al., 2017), but very little is known about savanna soil functioning, particularly in comparison with temperate systems. Savanna soils cover a range of soil textures, organic matter content, structure, nutrient content, are subjected to varying precipitation levels and supporting a diverse range of vegetation species. Typically we observe globally lower soil C and nutrient levels in African savanna soils (Hengl et al., 2017; Rughöft et al., 2016), but there is limited information available on soil nutrient and C content in African savannas and,

therefore, many predictions are likely underestimates (Ernst et al., 2024). With savannas covering nearly 1/5th of the world's landmass, their role in the global C cycle is of high importance. Assumptions of soil processes developed in temperate systems are often applied to arid savanna soils, without accounting for seasonal rainfall, frequent burning, and large populations of herbivores, which play a unique and significant role in driving savanna ecosystem functioning, and potentially soil processes.

1.4. Fire impacts on soil properties

In many ecosystems around the world, fire is an important and natural part of the environment, influencing ecosystem structure and function. Fire-prone ecosystems rely on fires to sustain functioning: for example, many plant species have adapted to be fire-resilient, with germination more successful following fire (Crosti et al., 2006; Dairel and Fidelis, 2020; Pausas and Lamont, 2022). As fire influences aboveground composition, this can influence how animals use the landscape and also impact soil properties. In a recent Living Planet Report by the World Wildlife Fund (WWF), fire regime shifts have been identified as a critical factor to monitor in soil conservation (Almond et al., 2021). Before discussing the impact of fire on soil properties, it is important to define the different types of fire events that can occur.

1.4.1. Single fire event vs fire regime

Single fires are individual events, with a specific intensity, duration, and behaviour. Fire regimes refer to long-term patterns of fire occurrence, which encompass a large variety of fire dynamics, with potentially different fire frequencies, intensities, size, and seasonality, alongside changes in temporal and spatial distribution. Long term patterns of fire regimes can even include ecosystems with no fire occurrence. The literature tends to split the two types of fire, with single fire events often studied in more temperate regions with reactive and immediate suppression fire management. The fire return intervals in these ecosystems may be very long and so it is easier to study single fire events than manipulate or alter the regime. In contrast, fire regimes are usually studied in more tropical systems, where repeated burns are a natural part of ecosystem functioning and is required to manage ecosystem health, resilience, and biodiversity over time. When there is repeated burning in a landscape, prescribed burning is widely applied in conservation areas to achieve specific management aims, for example, creating fire breaks to protect life and property, or in order to break up the fuel load and prevent very large fires.

It is important to understand both the short-term effects of a single fire and the long-term, decadal impacts from fire regimes in order to predict the effects of altered fire regimes on

savanna ecosystems and improve understanding of how best to manage savannas. The short- and long-term effects of single fires and fire regimes on soil C, nutrient storage and microbial dynamics are poorly known. Below, I will briefly discuss the current literature on the impacts of fire on physical, chemical, and biological soil properties. More detail on the mechanisms by which this occurs is given in the introductions of Chapters 2 and 3.

1.4.2. Fire effects on physical soil properties

The effect of savanna fires on soil structure and hydrology in African savannas is largely unknown. It is important to note that most savanna pyrohydrology studies are from regions such as South America and Australia, where fires tend to burn at much higher intensities (~2800-18,000 kW m⁻¹; Robichaud, 2000; Williams et al., 2003), than southern African fires (~1900 kW m⁻¹; Govender et al., 2006; Strydom *et al.*, 2019). Globally, fire has been found to directly influence soil structure, with more intense fires causing decomposition of organic matter from high temperatures, disrupting soil aggregates and soil stability (Mataix-Solera et al., 2011; Šimanský, 2015). Generally, lower intensity fires (for example, < 2000 kW m⁻¹), that are more likely to occur in African savanna systems, do not result in changes in aggregate stability, but the outcome is dependent on soil texture, microbial biomass, and soil organic matter (Mataix-Solera et al., 2011). However, prescribed burns in Southern Africa have been shown to impact soil hydrology and water infiltration, with burns reducing the speed of water infiltration in comparison to unburnt soils (Strydom et al., 2019). This was hypothesised to be as a result of ash filling pore spaces, thus reducing infiltration (Mallik et al., 1984; Strydom et al., 2019). Negative impacts on water infiltration and soil structure, alongside the removal of aboveground vegetation, can result in burnt landscapes being at a higher soil erosion risk (Fonseca et al., 2017; Parente et al., 2022; Stoof et al., 2015).

1.4.3. Fire effects on chemical soil properties

Fire can shape the biogeochemistry of the soil, with frequent burns shown to alter soil C and N storage. Several savanna studies have demonstrated that frequently burned plots deplete soil C and N (Abreu et al., 2017; Bird et al., 2000; Mills and Fey, 2004; Pellegrini et al., 2020, 2014). The decline in soil C and N as a result of frequent burning can be attributed to several simultaneous processes. Firstly, and perhaps most simply, input of organic matter to the soil is reduced as a result of combustion of aboveground material (Knicker, 2007; Mills and Fey, 2004). Secondly, the exposed post-fire environment results in greater soil temperatures and increased soil moisture due to reduced interception of rainfall by vegetation, increasing the rate of drying-wetting cycles (Borken and Matzner, 2009). When soils become dry from increased

sun exposure and then rewetted by precipitation, there is a burst of decomposition, mineralization and release of inorganic N and CO₂ (Birch, 1958). Finally, nutrient levels can be affected through changes in aboveground biomass and composition, thereby altering plant inputs into the soil (Knicker, 2007; Muqaddas et al., 2015; Pellegrini et al., 2014).

The range of soil nutrient outcomes observed post-fire is highly varied due to the number of influential factors including fire frequency, intensity, fuel load, season, precipitation, vegetation type and soil type. For example, when assessing the long term impacts of changes in fire frequency on soil C stocks using two soils of strongly contrasting texture (sand versus clay), results showed C at sandy sites being 35-50% lower than at comparable clay sites (Bird et al., 2000). Furthermore, Pellegrini et al. (2015) observed a decline in soil C and N following repeated burns, whereas similar studies in the same area performed by Coetsee et al. (2010) and Holdo et al. (2012) found neutral effects of fire on soil C and N. The main differences between the experiments were the soil type, precipitation conditions and fire return interval.

1.4.4. Fire effects on biological soil properties

Biologically, the soil provides a habitat for a wide range of organisms, including soil microbes, termites, ants, dung beetles and worms. The primary agents of decomposition are microbes – fungi and bacteria – and soil invertebrates. Fire can impact microbial composition and activity with cascading impacts on decomposition rates (Dooley and Treseder, 2012; Dove et al., 2020; Dove and Hart, 2017; Pressler et al., 2019; Wang et al., 2009, 2012), but biome and fire type play a major role in the degree of impact. A global meta-analysis found that the microbial communities in savanna and grasslands are less vulnerable to fire than forested temperate systems, mainly due to the less intense fire types (Dooley and Treseder, 2012).

Immediately post burn, there may be a fertilizing effect of the nutrient-rich alkaline ash. An increase in pH has been shown to have a positive effect on the biological recovery of soils (Bodí et al., 2014; Knicker, 2007; Pyne, 2019; Rousk et al., 2010; Sánchez-García et al., 2021), which has been suggested to increase labile C concentration, providing increased substrate for microbial growth. Similarly, the increased soil temperature that follows from removal of the aboveground vegetation with burning has also been suggested to increase microbial activity (Hart et al., 2005; Neary et al., 1999; Treseder et al., 2004).

Increased soil temperature during burns, which vary with fire temperature, severity, and rate of spread (ROS), can cause biological disruption in the soil (Table 1.1), with microbial mortality generally occurring above 60°C (Neary et al., 2005). Fungi are usually more sensitive to

temperature increases than bacteria. Microbial, root and seed mortality, alongside organic matter, and nutrient volatilization, can have cascading effects for soil processes and post-fire recovery. However, burns in African savannas often move at a faster ROS than more temperate systems (Govender et al., 2006; Strydom et al., 2019), thus temperature increases do not occur for extended periods of time, reducing the possibility for biological disruption on all soil components.

Table 1.1 – Global temperature thresholds (°C) for biological disruption in the soil environment across a range of ecosystems (adapted from Neary *et al.*, 2005)

Soil component	Threshold Temp (°C)	Reference
Plant roots	48	Hare (1961)
Fungi – wet soils	60	Dunn et al. (1985)
Fungi – dry soils	80	Dunn et al. (1985)
Bacteria – wet soil	80	Dunn and DeBano (1981)
Bacteria – dry soil	90	Dunn and DeBano (1981)
Seeds – wet soil	70	Martin et al. (1975)
Seeds – dry soil	90	Martin et al. (1975)
Organic matter distillation	200	DeBano et al. (1998)
Nutrient volatilization	200	Neary et al. (2005)

Fire can also directly affect soil fauna (Certini et al., 2021; Moyo, 2022). Generally, vertebrates can escape higher temperatures by moving away from the fire or burrowing deeper into the soil, but invertebrates, and microbes, which have very limited to no mobility are often most affected. However, fire often does not have a large impact on soil fauna in savannas (Certini et al., 2021), with ant and termite abundance unaffected by fire in southern African savannas (Hockridge et al., 2023; Parr et al., 2004). This mainly depends on whether taxa can find refuge from the fire or not; ants and termites can't move their colony but remain unaffected as their underground nesting habits buffer them from the fire (Parr and Andersen, 2008).

1.5. Herbivore impacts on soil properties

Another important driver of savanna ecosystems is mammalian herbivory. Herbivory encompasses the consumption of plant material by a wide range of animals, including grazers and browsers, across various ecosystems. Generally, grazing is a specific form of herbivory focussed on consuming grasses and herbaceous plants, whereas browsers consume different parts of woody vegetation. Herbivory can have complex and interconnected effects on soil

properties, influencing soil nutrient dynamics, C storage, microbial communities, and ecosystem functioning. This thesis focusses on savanna meso- and mega- herbivores (>20 kg) and does not cover smaller herbivores, such as grasshoppers and termites, but it is important to acknowledge the ecological impact invertebrate herbivores play in savanna plant communities and soil processes (Bunney et al., 2024; Jouquet et al., 2005; Parr et al., 2004).

The impact of grazing and herbivory on savanna soils is not well understood. Globally, studies have found strong negative, positive or neutral grazing effects on soil biogeochemistry (see review: McSherry and Ritchie, 2013), with more detail in the introduction of Chapter 4. For example, grazing removes aboveground vegetation, thus increasing bare ground, accelerating soil drying and erosion (Li et al., 2008a; Steffens et al., 2008). In contrast, grazing may stimulate fine, shallow roots of grasses, which can compensate for the reduced aboveground C inputs to soils as a result of herbivore consumption (Derner et al., 2019; Wigley et al., 2020). The multifactorial nature of soil type, vegetation species composition, precipitation, herbivore species, mammal size, grazing intensity and study duration make it difficult to separate the interactive influences in order to manage soils on grazed land.

Savannas host a range of herbivores of different sizes, which impose different effects on soil properties related to aboveground consumption, excretion and physical disturbance (Holdo and Mack, 2014). One of the most obvious impacts of large mammal herbivores are the direct impacts of trampling, influencing soil compaction and disturbance (Derner et al., 2019). Increased soil compaction from larger mammals can result in decreased aggregate stability, decreased water infiltration and increased water and wind erosion (Bardgett and Wardle, 2003; Greenwood and McKenzie, 2001). Additionally, herbivores varying in body size produce dung and urine at different quantities, spatial distribution, and nutrient stoichiometry (Cech et al., 2008; le Roux et al., 2020; McNaughton et al., 1997; Sitters et al., 2014b), thereby exerting potentially cascading impacts on the soil.

Herbivore species also play a crucial role in shaping aboveground vegetation. Grazers shape the grasslands and prevent the dominance of woody species through selective grazing of grasses, maintaining conditions that limit the spread of trees and shrubs, whilst browsers feed on more woody vegetation including leaves, shoots, shrubs, bushes, and trees. Thus, the proportion of grazers to browsers can directly influence vegetation dynamics and alterations in plant composition may affect the microbial communities of soils, although these grazing effects on microbes have yet to be elucidated in arid soils (Marcos et al., 2019).

1.6. Fire x herbivory impact on soil properties

In savanna ecosystems, fire can attract grazing herbivores concentrating the herbivores on the post-burn landscape (Donaldson et al., 2018; Singh et al., 2024). This interaction of fire and herbivory has been suggested as a plausible explanation for the mix of grassland and scattered trees/shrubs that is characteristic in these ecosystems (Van Langevelde et al., 2003). Although the effects of fire and herbivory on savanna ecosystems are often addressed independently, as discussed above, research has shown that the two drivers work synergistically, with the impacts of fire difficult to separate from post-fire herbivory effects (Shackleton and Scholes, 2000). Synergy refers to the interaction between multiple drivers or factors that combine to produce an outcome greater than the sum of their individual effects (Côté et al., 2016). Fire x herbivory, or often referred to as pyric herbivory, produced effects that are not simply the sum of the individual effects of fire and herbivory (Smit and Coetsee, 2019), it is more likely a multiplicative synergy, the combined effect of these drivers is greater than the sum of their individual impacts (Côté et al., 2016). Effective management of savanna systems is only possible with better understanding of the interactive role of fire and herbivory in structuring savanna ecosystems.

The attraction of herbivores to recently burnt grass demonstrates that fires can be used to concentrate grazers, rather than dispersing them, with cascading effects for ecosystem processes. Post-fire vegetation is higher in N, a typically limiting nutrient for herbivores, and lower in carbohydrates and fibre, making them more digestible (Goldammer and Ronde, 2004). As a result, freshly burnt land is more attractive to herbivores, resulting in high-intensity grazing during regrowth. Beyond the direct effects of fire, such as ash production altering soil inputs, increased herbivory alters the fuel load of the next fire, affecting both the fire return interval and intensity. Additionally, as herbivores consume aboveground biomass, they change the quality and quantity of soil input through their dung and urine. The increased herbivory may also impact physical soil properties through increased compaction. More details on these interactive mechanisms are provided in Chapter 5.

The interaction of fire and grazing can have cascading effects on the structure, diversity and functioning of savanna landscapes and can be used in savanna landscape management. One management example is using fire x herbivory in the creation of grazing lawns (Donaldson et al., 2018; Singh et al., 2024). A mosaic of lawn (shorter grass) and taller grass patches in savanna ecosystems is critical for biodiversity conservation and switching from one grass state

to another can be facilitated through carefully managed fire and grazing regimes. By concentrating grazers post-burn, these lawns - areas of shorter grass as a result of the increased grazing levels – can form. However, the effects of fire x herbivory on belowground soil properties in savannas is unknown. In Mediterranean systems, pyric herbivory has shown to increase soil fungi and bacteria diversity as well as nutrient availability, with grazing enhancing post-burn soil recovery (Canals et al., 2024; San Emeterio et al., 2023). It can be hypothesised that in African savannas, fire x herbivory might similarly enhance soil microbial diversity and nutrient cycling, potentially leading to improved soil health and resilience. Fire can no longer be considered in isolation from herbivory effects, particularly in African savannas, and controlled studies are needed to separate the impact of fire and herbivory on soil properties.

1.7. Global Change

African savannas are vulnerable to global change, and are projected to experience severe impacts of climate change (Adenle et al., 2017; Ayanlade et al., 2023; Conway and Vincent, 2021). Global change, encompassing climate fluctuations, alterations in land use management, and anthropogenic interventions are altering both fire and herbivory regimes. Over the past decade, fire regimes have changed in intensity and frequency as a result of climate and land-use change, with predictions of further change as temperatures rise (Calvin et al., 2023). Fire regimes also alter as savanna grass communities and their traits change in response to global climate change (Simpson et al., 2022), changing fuel load and flammability parameters. Global change also impacts herbivore behaviour, for example, climate-induced woody encroachment affects zebra visibility and behaviour in Kenyan savannas (Chen et al., 2021; Riginos, 2015). Climate change has also been linked to shifts in herbivore assemblage composition (Smit and Prins, 2015a), placing savanna megaherbivores at risk of extinction (Pringle et al., 2023; Staver et al., 2021). Due to the potential additive or multiplicative synergistic relationship between fire and herbivory, changes in fire regimes will also have implications for herbivory and vice versa. Altered fire and herbivore regimes will have consequences for vegetation composition, C stocks and biodiversity, with feedbacks to climate, biogeochemical cycles and human health (Andela et al., 2017; Bowman et al., 2009; Lelieveld et al., 2015; Lohman et al., 2007; Pellegrini et al., 2018).

Future warming and elevated atmospheric CO₂ levels, patterns of past land use and land management strategies, along with the physical heterogeneity of landscapes, could produce complex patterns of soil nutrient and microbial activity. Improved management of savanna systems is only possible with better understanding of the individual and interactive roles of fire

and herbivory on soil biology in structuring savanna ecosystems. Understanding the abiotic and biotic controls on ecosystem functioning is essential for predicting how global change will influence savanna ecosystems (Bond, 2008).

1.8. Kruger National Park

In order to investigate the individual and interactive impacts of fire, herbivory and climate within a tropical savanna ecosystem, all research was performed within Kruger National Park (KNP), a semi-arid savanna in Southern Africa. At $\sim 20,000 \text{ km}^2$, roughly the same size as Wales, KNP lies in the Northeastern corner of South Africa. Bordering Mozambique to the East and Zimbabwe to the North, the park is a globally important site in terms of its diverse ecology and the application of advanced management techniques. With at least 147 mammal and 1980 plant species (du Toit et al., 2003), it is a globally significant site for studying the heterogeneity of savanna ecosystems. KNP is managed by South African National Parks (SANParks, ZA government) which oversees conservation efforts, visitor management, park infrastructure and scientific studies.

1.8.1 Climate

KNP has wet summers and dry winters, with the dry season typically occurring from May to September (Figure 1.3). During this time, rainfall is minimal, and temperatures are cooler. The wet season occurs from October to April, characterized by higher temperatures and increased rainfall. This seasonality influences both fire occurrence and animal movements, with animals more likely to be concentrated near watering holes in the dry season and inclined to travel further distances in the wet season. With the distinct wet and dry season pattern, there is also a general North-South gradient within KNP of increasing rainfall, at $\sim 350 \text{ mm}$ per annum in the north and a much wetter $\sim 750 \text{ mm}$ per annum in the South.

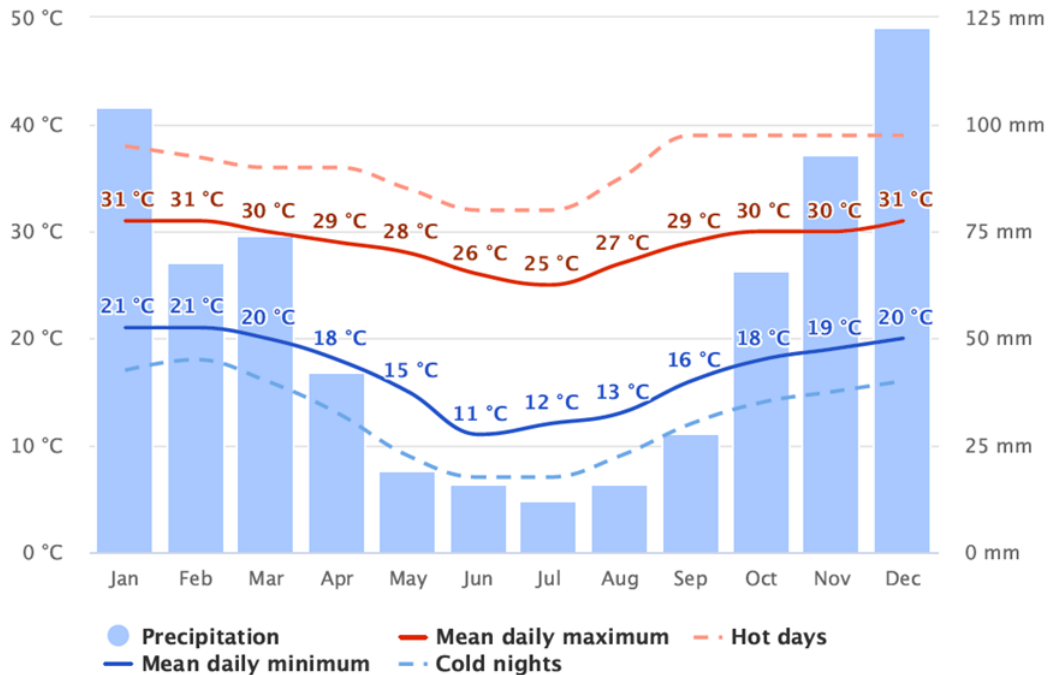


Figure 1.3 - Average temperature and precipitation within Skukuza, KNP, with temperature on the left axis and precipitation levels on the right axis. Figure produced by Meteoblue (2024).

1.8.2. Fauna

KNP is a biodiversity hotspot, with a rich assembly of plant and animal species. Understanding the fauna and flora of the park is essential for conservation efforts and ecosystem management, particularly with fire and herbivores as drivers. KNP's rich mammal diversity encompasses the 'big five' - African elephant (*Loxodonta africana*), African buffalo (*Syncerus caffer*), lion (*Panthera leo*), leopard (*Panthera pardus*) and black (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*), as well as supporting other prominent species such as giraffe (*Giraffa giraffa*), zebra (*Equus quagga*), hippopotamus (*Hippopotamus amphibius*), cheetah (*Acinonyx jubatus*), wild dog (*Lycaon pictus*), impala (*Aepyceros melampus*), kudu (*Tragelaphus strepsiceros*), wildebeest (*Connochaetes taurinus*), hyenas (*Crocuta crocuta*) and jackals (*Lupulella mesomelas*) (Figure 1.4). Beyond mammals, over 500 bird species have been recorded within the park, which also hosts a wide range of reptiles, amphibians, and invertebrates.



Figure 1.4 - A selection of photographs collected during my time in Kruger NP as part of this thesis, to demonstrate the wide range of animals found (picture credit: Morgan Cvetkovic-Jones)

0 1.8.3. Fire occurrence

1 Fires are a natural part of KNP, however due to management goals, tourism and livelihoods
 2 within the park, fire is managed within the park. Most fires within Kruger are prescribed,
 3 though of course wildfires occur as a result of lightning or human behaviour as well. KNP has
 4 an average fire return interval of two to four years, depending on fuel load and landscape
 5 (Govender et al., 2006). KNP employs a comprehensive fire management strategy, which also
 6 encompasses long-term Experimental Burn Plots (EBPs): since 1954, four different areas of
 7 the park with varying vegetation and soil properties have been scientifically burnt at different
 8 frequencies and intensities for nearly seventy years (Biggs et al., 2003). Prescribed burning in
 9 different seasons within KNP can change the fire intensity (Figure 1.5), due to differences in
 10 fuel moisture and load (Govender et al., 2006). In the dry season, vegetation dries out, making
 11 fires more intense, whereas vegetation in the wet season is usually lusher and greener, thus
 12 reducing fire risk.

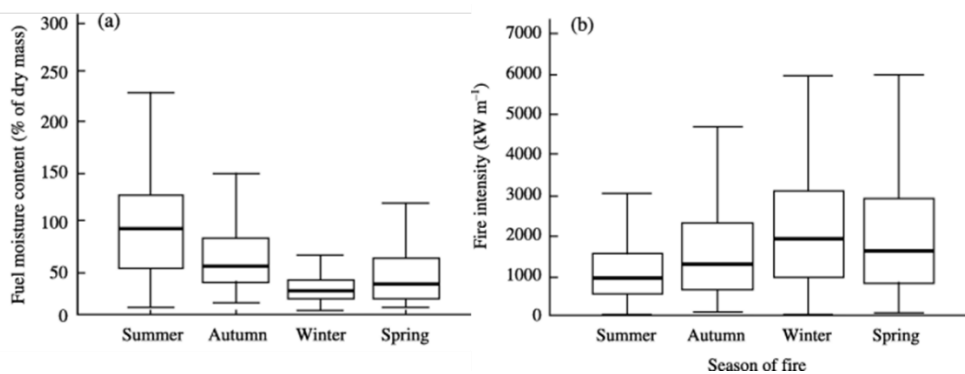


Figure 1.5 – Season of fire influences fire behaviour within KNP, with a) fuel moisture content higher in the summer and autumn (wet season) and lower in winter and spring (dry season), prior to experimental burns. This results in b) higher fire intensities in winter and spring, in comparison to summer and autumn. Figure taken from Govender et al. (2006).

13

14 1.8.4. Geology

15 KNP is predominantly underlain by granitic soils to the West and basaltic soils to the East
 16 (Figure 1.6c). The basaltic soils are a fine grained, clayey, darker coloured volcanic igneous
 17 rock, in contrast to the granitic soils which are coarse grains, sandy, lighter coloured plutonic
 18 igneous rock (SANParks, 2024). These soil types exhibit differences in soil content: basaltic
 19 soils have higher nutrient content, resulting in higher grass production with fewer trees. The
 20 elevated levels of grass productivity can also lead to higher intensity fires due to the larger fuel
 21 load. The granitic soils are much more porous, with better water infiltration and sometimes

22 deeper soils, so tend to have more woody cover and lower grass production. Throughout KNP,
23 there is a variety of vegetation types, with each type supporting a unique assemblage of plant
24 species adapted to that specific area's environmental conditions, soil types and fire regimes.
25 Rivers, including Sabie, Limpopo, Crocodile and Olifants, play a significant role in shaping
26 the topography of Kruger, and also influence fire and herbivory regimes.

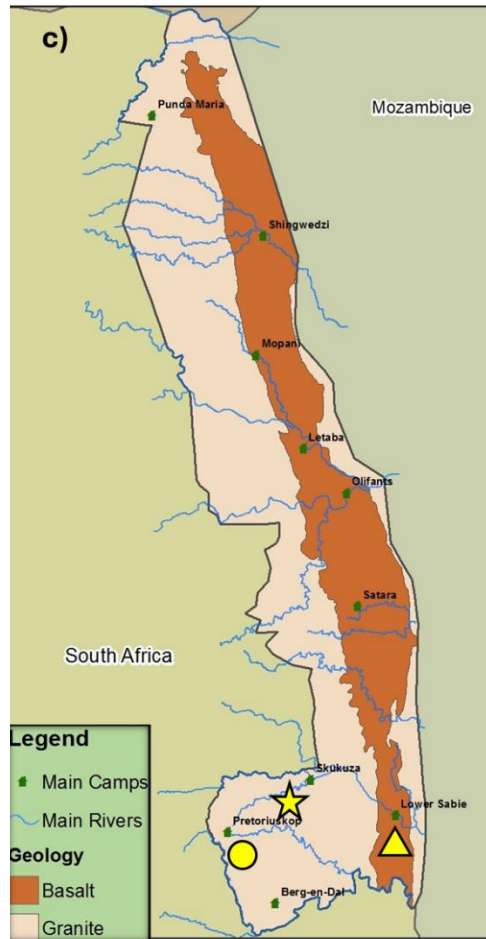





Figure 1.6 – KNP covers ~20,000 km² of land, encompassing various vegetational and geological landscapes. KNP is split into granitic and basaltic soils in an East-Western split. The shapes represent sampling sites of the different chapters within this thesis:  = Pretoriuskop EBPs sampled in Chapter 2;  = Skukuza EBPs for Chapter 3; and  = basaltic supersites investigated in Chapter 4 and 5. Maps adapted from SANParks (2024).



1.9. Thesis Aims and Objectives

The main aim of this thesis is to improve understanding of the effects of fire and herbivory on savanna soil biology, C cycling and resilience to disturbance. Thus, within KNP, samples were collected across different soil textures, vegetation types and rainfall gradients, all factors which influence fire and herbivore behaviour, in order to answer the overarching question:

How does fire, herbivory, and climate affect soil properties in the short and long term in Southern Kruger National Park?

This was investigated by addressing the following sub-questions:

1. *How do fires affect soil chemical properties and nutrient availability, such as total C, N and P, soil pH, nitrate, and ammonium, in the short and long term on granitic and basaltic soils?*
2. *How do fires affect soil microbial properties, such as biomass, community composition and soil respiration, in the short and long term?*
3. *How do herbivores affect soil biological and chemical soil properties? Does herbivore body size have a significant impact on soil nutrients and microbial community?*
4. *Do fire and herbivory interact to produce synergistic effects on soil biological and chemical properties?*

In order to begin to answer these questions, this PhD thesis is divided into four data chapters:

In Chapter 2, the biotic and functional resilience of savanna soils to long-term changes in fire regime intensity were investigated. Using sandy soil from KNP's long-term EBPs, maintained for nearly seven decades, a substrate-induced respiration experiment was performed, alongside the examination of a full suite of biogeochemical and microbial parameters.

In Chapter 3, the impact of a single fire event on soil respiration, at varying Time Since Fire intervals was explored. Soil respiration was measured for a year following a moderate intensity burn on sandy soils. Tracking respiration over different seasons, we also investigated interactive factors of soil microbial community composition and soil moisture.

In Chapter 4, the impact of herbivore body size on basaltic soil properties is reported. Using large scale enclosure experiments on basaltic soils, the effect of differing herbivory exclusion levels on the biogeochemical and microbial properties of the soils was tested.

In Chapter 5, the interactive and individual impacts of an early season burn and large mammal herbivory on basaltic soil abiotic and biotic properties was analysed. Using a combination of controlled burns and herbivore exclosures, pre- and post-treatment samples were collected for soil biogeochemical and microbial community composition data. In the following year, the short-term effects of burns with or without subsequent herbivory in a nine-month soil respiration campaign were tracked.

Chapter 6 provides a synthesis of the key findings, with future recommendations for scientific research and management implications.

1.10. Thesis Structure

This thesis is submitted with the data chapters (Chapters 2-5) presented as manuscripts. Chapter 2 is currently under review in *Plant and Soil*. Chapters 3, 4 and 5 are intended for submission in peer-reviewed journals.

References are included at the end of the thesis, with supplementary information at the end of each chapter.

2. Soil microbes exhibit resilience to long-term savanna fire regimes

In Review in Plant and Soil

Cvetkovic-Jones, M. ^{1,2}, Strydom, T. ³, Stevens, C.J. ¹, Whitaker, J. ², Ostle, N. ¹, Parr, C.L. ^{4,5,6}

¹Lancaster Environment Centre, Lancaster University, Library Avenue, Bailrigg, Lancaster, LA1 4YQ, United Kingdom

²UK Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster, LA1 4AP, United Kingdom

³Scientific Services, South African National Parks, Private Bag X402, Skukuza, 1350, South Africa

⁴School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa

⁵School of Environmental Sciences, University of Liverpool, Liverpool, L69 3GP, United Kingdom

⁶Department of Zoology & Entomology, University of Pretoria, Pretoria, South Africa



Picture credit: Robert Cvetkovic-Jones

2.1. Introduction

Globally, fire plays a significant role in shaping ecosystems. Projections anticipate an increase in fire frequency and intensity (Flannigan et al., 2013; Jolly et al., 2015; Moritz et al., 2012), due to climate change, atmospheric warming, land use alterations and human activities (Marlon et al., 2008; Romps et al., 2014; Syphard et al., 2017). Altered fire regimes as a result of global change could have positive feedbacks on climate change (Jones et al., 2022) and long-term impacts on terrestrial C and nutrient stocks (Pellegrini et al., 2018). Therefore, it is vital that we understand the consequences of fire on ecological functioning and specifically, fires influence on soil properties. Fire alters soil physical, chemical, and biological properties, with fire intensity and frequency factors largely influencing the magnitude of change (Bento-Gonçalves et al., 2012; Verma and Jayakumar, 2012). Directly, fire affects soil through high temperatures, removal of organic matter, pH changes, nutrient volatilization, and input of ash (Knicker, 2007). Indirectly, fire mediates changes in the soil through altered aboveground biomass, including plant diversity and composition, consequently impacting soil abiotic and biotic factors (McLauchlan et al., 2020). Subsequently, these changes can impact soil microbial communities, altering their composition and activity and affecting their functions in nutrient cycling and decomposition processes (Pressler et al., 2019; Whitman et al., 2019; Yang et al., 2020).

There are well understood links between fire and aboveground ecology (McLauchlan et al., 2020). For example, increasing fire frequency and intensity has been shown to negatively correlate with woody biomass and tree cover (Belsky, 1994; Higgins et al., 2007; Lehmann et al., 2014; Staver et al., 2011). Aboveground; fire removes biomass, exposing the soil, thereby reducing plant competition, promoting plant growth and productivity rates, resulting in changes in plant abundance and diversity (He and Lamont, 2018). The more intense and frequent the fire, the more aboveground biomass is combusted, resulting in denuded soil surfaces exposed to direct sunlight, lower soil organic matter concentrations, increased volatilization of nutrients and altered soil thermal regulation (Pellegrini et al., 2015; Reich et al., 2001; Treseder et al., 2004). Consequently, in comparison to lower intensity fires, there may be greater impacts on soil microbial processes and nutrient availability, leading to potentially longer recovery periods (Whitman et al., 2019). Moreover, the aboveground shifts in vegetation composition may also influence future fires. An increase in grass biomass alongside a decrease in woody biomass results in an increase of flammable biomass and may cause more frequent fires in the future (Treseder et al., 2004), creating a positive feedback loop.

Fire can have positive or negative impacts on the soil microbial community, depending on factors such as fire intensity, frequency, and ecosystem type. Higher temperatures in the soil during fires may cause microbial mortality, with temperatures over 50 °C being shown to be fatal for microbes (Neary et al., 1999). During fires above 200 °C, volatilization of nutrients can occur (Knicker, 2007), reducing nutrient availability (Zhao et al., 2015), in turn inhibiting microbial growth and activity. Conversely, fire can also have positive effects on the soil. Following fire, ash deposition provides a fertilising effect (Pyne, 2019) and is a source of readily available nutrients, increasing labile C and inorganic N availability (Mataix-Solera et al., 2009). Post fire increases in substrate availability and N mineralisation may promote microbial growth and stimulate an increase in microbial activity. Moreover, in post-fire soil environments, fire-resistant microbes can survive and aid in nutrient cycling and organic matter decomposition (Pérez-Valera et al., 2018).

Typically, increasing fire frequency and intensity decreases both microbial diversity and biomass and shifts microbial community composition (Dooley and Treseder, 2012; Dove and Hart, 2017; Pressler et al., 2019). For example, fungi can be more susceptible to heat damage than bacteria, and thus may be more affected by fire (Liu et al., 2023). Furthermore, ash deposition has been shown to increase the pH of soils as well as producing labile C, which favours bacterial communities (Rousk et al., 2010). Pyrogenic carbon (PyC), produced by incomplete combustion of organic matter during burns (Bird et al., 2015; Saiz et al., 2018) is an important component of recalcitrant C pools, acting as a C sink (Santín et al., 2016). PyC has been shown to increase soil pH and nutrient availability (Biederman and Harpole, 2013) and have significant impacts on microbial community composition (Khodadad et al., 2011; Whitman et al., 2016). Thereby, soil C pools shift with fire, altering the quality and availability of C in the soil. Fungi are better decomposers of recalcitrant C (Boer et al., 2005), alongside gram-positive bacteria, but gram-negative bacteria have been shown to be more dependent on simple, labile C compounds (Fanin et al., 2019). The responses of microbial communities to fire can persist for a range of time after a fire, for example, from months in grasslands to years in forest ecosystems, depending on range of factors including ecosystem type, soil type, fire intensity, fire frequency and nutrient loss (Dooley and Treseder, 2012; Gui et al., 2023; Pressler et al., 2019).

Fire regimes characterized by varying frequencies and intensities of fire could have long-term effects on ecosystems. However, studying these impacts at an ecosystem level is challenging, as it requires sustained manipulation of fire over long periods to observe changes in microbial communities, nutrient composition, and vegetation.. Many fire studies are often short term, small scale or focus on single fires (Gomes et al., 2018; Parr and Chown, 2003), which may not accurately reflect the ecological effects of cumulative fires over time (Malkinson et al., 2011; Moretti et al., 2002).

While the interaction between fire regimes and aboveground dynamics in savannas has been well researched (Burkepile et al., 2016; Eby et al., 2014; Higgins et al., 2007; Smith et al., 2013; Wilcox et al., 2020), much less is known about how different fire regimes affect biological soil properties, particularly microbial communities, over extended periods. Understanding fire's effects on microbial community composition, soil respiration and nutrient pools is critical for developing effective conservation and fire management strategies, especially in the context of escalating global change. Specifically, research is needed to uncover the mechanisms that regulate microbial community responses and functions following fire.

We used the longest fire manipulation experiment globally, the Experimental Burn Plots (EBPS) in Kruger National Park (KNP), South Africa, which have manipulated fire frequency and intensity to study fire dynamics at an ecosystem level for 68 years. The long-term effects of changing intensity and frequency of fire regimes on soil nutrient concentrations, microbial biomass, and microbial community composition were quantified to address the question 'do higher intensity, more frequent fires have a greater effect on soil abiotic and biotic properties?' It was hypothesised that higher intensity, more frequent burns, would reduce nutrient availability, decrease microbial community size and shift to a more bacterial-dominated community. Fires often favour bacterial communities due to the resulting increase in pH and labile C, and fungi have been found to be more vulnerable to burns due to the increased temperatures (Liu et al., 2023; Rousk et al., 2010). The activity of microbial communities has rarely been investigated in sandy savanna soils, particularly with the influence of fire. Therefore, we also investigated how differences in fire intensity and frequency influenced respiration of active soil microbes in response to a water or C source by conducting both a water holding capacity (WHC) and a C-substrate addition experiment. It was hypothesised that the higher intensity burns would have the greatest microbial functional response to substrate

addition, with microbes more readily accessing short term labile C sources (Reischke et al., 2014; Sánchez-García et al., 2021).

2.2. Methods

2.2.1. Study Area

The study was conducted on the Experimental Burn Plots in KNP, South Africa, an ongoing long-term experiment that was established in 1954 (Biggs et al., 2003). The sites were created to study the impact of different fire regimes, and subsequent herbivory, on contrasting savanna landscapes. The EBPs comprise of 14 plots, laid out in a strip, and repeated across four different locations within the park. Each seven ha (~ 380 m x 180 m) plot represents a different burning regime (season and frequency combination (Trollope et al., 1998)). We selected three plots for the investigation, controlling for frequency as much as possible; August triennial (referred to as higher intensity), December triennial (referred to as lower intensity) and a control plot (referred to as fire suppression), where fire has been suppressed as much as possible since 1954. Burning in different seasons controls the intensity of fire (Govender et al., 2006; Smith et al., 2013). August burns are at the end of the dry season, so usually there is a high fuel load with low moisture content, so fires burn at higher intensities (Table 2.1). Contrastingly, December burns are in the middle of the wet season, so fuel loads are often smaller and wetter, with fires burning at a lower intensity. The most recent experimental burns in these fire regimes prior to sampling were burnt in 2021. The average fire return interval for KNP is around three years (Govender et al., 2006), but it is not possible to completely control fire frequency when burning across seasons, as whilst burns are attempted triennially, burns are not always successful (Table 2.1).

Table 2.1 - Average fuel load (kg ha^{-1}), fire intensity (kW m^{-1}) and fire return interval (years) across the three regimes; higher intensity burns, lower intensity burns and fire suppression. Data was averaged from using data from 2005-2021. Fuel load was indexed as disc pasture meter settling height as per Zambatis et al. (2006). Fire intensity was calculated as per Byram's (1959) fire line intensity equations (Fire intensity = heat yield * ROS * biomass). Fuel load and fire intensity values was averaged using SANParks data from 2005-2021. Fire return interval values were averaged using from all recorded fire instances between 1968-2021.

Fire Measurements	Fire Regime		
	Higher Intensity Burns	Lower Intensity Burns	Fire Suppression
Fuel Load	3824	1670	X
Fire Intensity	2670	1030	X
Fire Return Interval	2.92	4.29	24.19

Out of the four savanna landscape types within KNP, we focussed on the sandy, granitic soils (average of 3.9% clay, 9.5% silt, 86.6% sand), which underlay *Terminalia* woodland, in the Pretoriuskop area (Figure 2.1). With an average rainfall of 705 mm, the dominant tree species are *Dichrostachys cinerea* and *Terminalia sericea*, with the grasses dominated by *Hyperthelia dissoluta* and *Setaria sphacelata* (Gertenbach, 1983; Low and Rebelo, 1996). Within the Pretoriuskop area, the fire treatments were applied to plots in three block replicate locations: Kambeni (33°26'E, 25°15'S), Numbi (31°20'E, 25°13'S) and Shabeni (31°23'E, 25°12'S).



Figure 2.1 – Pictures from Pretoriuskop area on KNP on the a) fire suppression plot, b) lower intensity, lower frequency burns burnt every three years in December and c) higher intensity burns burnt every three years in August (picture credit: Mary Cvetkovic-Jones).

2.2.2. Soil sampling

In April 2022, soil cores were collected, to a depth of eight cm, for analysis of basal respiration and substrate-induced respiration (SIR). In each replicate plot, soils were sampled at four points along a transect approximately every 90 m, with a 10 m road buffer at either end of the plot. At each of the four sampling points, two cores were taken for the SIR assay (totalling 72 cores: 3 fire treatments x 3 locations x 4 transect sampling points x 2 assay cores) using plastic piping (8 x 4 cm internal diameter). The ends of the cores were sealed upon collection with rubber lids and kept upright. Soil cores were maintained intact for the SIR experiment to preserve soil structural properties, more closely representing field conditions. In each plot, one additional core was collected in the same way along the transect for determining maximum soil WHC, field soil moisture content and field % WHC.

In April 2023, soil samples were collected, at the same soil depth of 8 cm, for soil characterisation of abiotic and biotic properties using approximately the same transect path and the same methods, then stored at 4 °C prior to analyses. While soils were not sampled within the same year they were collected within the same season. Using a long-term fire experiment that nearly spans seven decades, the soil properties are unlikely to change significantly in this interval.

2.2.3. Soil Biotic and Abiotic Analyses

Soil pH was measured using a 10.0 ± 0.5 g subsample of fresh soil, mixed with 25 ml of deionised H₂O, using a pH meter (InLab Expert Pro-ISM). Soil moisture was determined by using aliquots 1 ± 0.1 g of fresh soil, dried at 105°C for 48 hours. Moisture content was calculated as a percentage of the dry weight. Soil ammonium ($\mu\text{g NH}_4 \text{ g}^{-1}$ soil dwt) and nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt) was calculated as a measure of inorganic N in the soil which is directly available to the plants. 5 ± 0.5 g subsamples were extracted using 25 ml of 2M KCl and analysed on an AA3 HR Auto Analyser (Seal Analytica, UK). Total soil C and N was measured determined using a Elementar Vario EL CUBE CN analyser. Microbial biomass C and N ($\mu\text{g g}^{-1}$ soil dwt) was measured on 5 ± 0.5 g soil subsamples using the chloroform fumigation-incubation method (Brookes et al., 1985). Microbial biomass C and N was analysed using a 5000A TOC Analyser (Lancaster University, Shimadzu, Japan). Microbial biomass was

calculated as the difference between fumigated and unfumigated soil, using general correction factors of 0.45 and 0.54 for microbial biomass C and N, respectively (Brookes et al., 1985; Joergensen and Mueller, 1996; Wu et al., 1990).

Phospholipid Fatty Acid (PLFAs) analysis was performed to measure both the soil microbial community size and composition (nmol g^{-1} soil dwt.). Total lipid was extracted from ~ 1.5 g of freeze-dried soil subsamples and using a modified Bligh and Dyer (1959) method (Frostegård et al., 1991), lipids were collected on a column. Neutral and glycolipids were eluted from the column with chloroform and acetone and discarded. Polar lipids were eluted with methanol and collected. These lipids were quantified and identified by chromatographic retention time and mass-spectral comparison using 19:0 (methyl nonadecanoate fatty acid) as an internal standard. Gas Chromatography was carried out on Agilent Technologies (UK) 6890N GC Network System, equipped with Agilent 7683 Series Injector. Gram-negative bacteria was identified by cyclopropyl saturated (7cy-17:0, 7,8cy-19:0) and monosaturated fatty acids (16:1 ω 5, 16:1 ω 7, 18:1 ω 7) and gram positive were identified by ester-linked branched-chain fatty acids (15:0i, 15:0a, 16:0i, 7Me-17:0, 17:0i, 17:0a) (Rinnan and Bååth, 2009; Whitaker et al., 2014). Fungi were identified using 18:2 ω 6,9 and 18:1 ω 9 fatty acids, indicative of ectomycorrhizal and saprotrophic fungi (Kaiser et al., 2010). Finally, total PLFA values were identified using all PLFAs measured (14:0, 16:1, 16:0, 17:1 ω 8, br17:0, br18:0, 18:1 ω 5, 18:0, 19:1; plus, the other fatty acids listed above). The ratios of gram-positive to gram-negative (GP: GN) and fungal: bacterial (F: B) were calculated to determine the relative abundances of each group.

2.2.4. Soil Water Holding Capacity

Soil WHC was determined following Ohlinger (1995). The fresh weight of the 36 cores collected for determining soil WHC were recorded before immersion in deionised water for 24 hours to reach maximum saturation. Saturated weight of each core was recorded after leaving the cores to freely drain for ~ 15 hours to remove excess water. The cores were dried in an oven at 105 °C for 48 hours to determine dry weight. Maximum soil WHC ($\text{g water g soil}^{-1}$) was determined by dividing the mass of water in the saturated cores by the dry weight of soil. Field WHC ($\text{g water g soil}^{-1}$) was calculated by dividing field soil moisture content (%) by maximum WHC and multiplying by 100.

2.2.5. Basal and Substrate-Induced Respiration

Soil cores sampled from across the fire regime plots were used to quantify the effects of fire intensity and frequency on basal respiration (BR) and the active microbial respiration of the soils through a WHC manipulation experiment and a C-SIR experiment, under controlled laboratory conditions. The base of each core was covered with filter paper and mesh, then secured with tape to prevent material loss. Cores were mounted on large plastic weigh boats and placed upright in 1 L Mason jars. Cores were installed in a controlled temperature room maintained at 21 °C for the duration of the experiment. This is the average annual air temperature across Pretoriuskop and additionally the average air temperature for April, the month the samples were collected (Zambatis, 2006).

All 72 cores in the experimental incubation were adjusted to 40 (\pm 3) % WHC, the average field WHC (monitored at least every two days). For soil % WHC adjustment, synthetic rain (~ pH 4.97) was prepared using sterilised deionised water with additions of sodium chloride (NaCl: 0.00052 g L⁻¹), calcium chloride dihydrate (CaCl₂.2H₂O: 0.00024 g L⁻¹), calcium sulphate dihydrate (CaSO₄.2H₂O: 0.000228 g L⁻¹), magnesium sulphate heptahydrate (MgSO₄.7H₂O: 0.00042 g L⁻¹) and sulphuric acid (98 % H₂SO₄: 0.00061 g L⁻¹) based on available chemical composition data on rain collected from Skukuza, KNP, South Africa (31°59'E, 24°99'S), in the same region as the study plots (Mphepya et al., 2006). After an equilibration period of three days, respiration rates were measured at 40 (\pm 3) % soil WHC. Then, all treatment cores were raised to 60 (\pm 3) % soil WHC and allowed to stabilise for a further three days. Following measurement of respiration rates at 60 % WHC, all treatment cores were raised to 80 (\pm 3) % soil WHC, with another three-day stabilisation period before respiration rates were measured. Paired treatment cores remained at 40 % WHC throughout. Following this, all treatment cores were air dried back down to 40% soil WHC.

Following basal respiration sampling, (amount of CO₂ released from the soil with no substrate addition), a C substrate was added to one of the paired samples; Alpha-D-glucose (96%), 2 mg g⁻¹ soil fwt. In order to ensure we could elicit a response from the lower BR values, 2 mg g⁻¹ soil fwt glucose was selected as it had the greatest CO₂ increase in a similar study by Whitaker et al., (2014) Glucose was added in a solution (1 ml of synthetic rain) to the top of the soil core using a syringe just below the surface of the soil, to ensure maximum dispersal through the intact core. The equivalent amount of synthetic rain was added to the control paired sample by the same method. Following this, respiration measurements were taken at one, two, seven, 10-

, 14-, 21-, 28- and 32-days post addition on both the glucose addition and control cores. The cores were held at 40 (± 3) % WHC throughout the SIR experiment.

2.2.6. Respiration measurements

CO₂ sampling was conducted using an EGM-4 IRGA (PP Systems, USA). Jars were sealed using custom lids fitted with an inlet and outlet pipe, using silicon grease to ensure an airtight seal. CO₂ concentrations (ppm) were recorded at 4-5 second intervals for 150 seconds. CO₂ concentration data for each measurement was cut to the final 120 second to ensure a period of linear increase over time. Rate of change in ppm was calculated using linear regression and CO₂ respiration rates were calculated as described in Holland *et al.* (1999). Additional flux was calculated by subtracting the CO₂ flux of the paired control core (basal respiration, BR) from the CO₂ flux of the glucose manipulated core (SIR), SIR-BR.

2.2.7. Statistical Analysis

All statistical analyses were conducted in R version (4.1.1) and significance in all tests was considered at the $p \leq 0.05$ level. Differences in soil characteristics (pH, C and N content, C:N ratio, microbial Biomass of C and N, nitrate, and ammonium) and microbial community composition (PLFAs) were determined using a linear model, with fire regime as the predictor variable and block replication as a random effect. Data were first averaged by plot level replications within plots to control for spatial pseudo replication in field sampling (n=9).

The effect of fire regime on WHC respiration rates were determined using a linear model, with block replication as a random effect. The effect of fire regime on SIR rates were determined by generalised additive models with repeated measures performed using *gamm4* (Wood, 2022). Generalised additive mixed models (GAMMs) were used with fire regime as a fixed effect, day as non-linear random effects (spline, *gamm4* function; Wood, 2022), block, nested Core ID (to avoid spatial pseudo replication) and respiration block (to account for effects in the incubation design) as random effects. When model residuals did not satisfy assumption of normality, data were square root transformed.

2.3. Results

2.3.1. Soil characteristics along the fire gradient

Soil abiotic properties in the surface soil did not vary significantly across the different fire regimes (Table 2.2). Total soil C ($p=0.73$) and N ($p=0.36$) and the soil C: N ratio ($p=0.85$) did not differ significantly between the three fire regimes. Soil pH also did not change significantly

between fire regimes ($p=0.72$). Additionally, ammonium ($p=0.87$) and nitrate ($p =1.00$) remained unaffected across the fire regimes.

Table 2.2 - Effects of the different fire regimes on soil abiotic and biotic properties (mean \pm SE) analysed by linear model. Soil abiotic properties include C: N ratio, soil moisture, soil pH, ammonium ($\mu\text{g NH}_4 \text{ g}^{-1}$ soil dwt), nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt). Soil biotic properties include microbial biomass C and N ($\mu\text{g g}^{-1}$ soil dwt) and PLFA measurements (nmol g^{-1} soil dwt).

Soil properties	Fire Regime			F (2,6)	p value
	Higher Intensity	Lower Intensity	Fire Suppression		
Total C (%)	1.43 (± 0.16)	1.70 (± 0.44)	1.73 (± 0.18)	0.33	0.73
Total N (%)	0.32 (± 0.01)	0.33 (± 0.02)	0.35 (± 0.01)	1.22	0.36
Soil C: N	4.42 (± 0.41)	4.9 (± 0.93)	4.78 (± 0.27)	0.17	0.85
Soil Moisture	12.9 (± 0.97)	10.9 (± 2.08)	10.4 (± 0.77)	0.91	0.45
Soil pH	6.74 (± 0.11)	6.61 (± 0.10)	6.73 (± 0.14)	0.35	0.72
Ammonium	11.4 (± 4.34)	9.04 (± 1.68)	11.4 (± 4.11)	0.14	0.87
Nitrate	14.2 (± 9.86)	14.0 (± 4.14)	14.0 (± 7.58)	0.00	1.00
Microbial biomass C	59.8 (± 6.74)	63.2 (± 19.20)	51.40 (± 28.3)	0.09	0.91
Microbial biomass N	54.1 (± 4.09)	45.0 (± 6.81)	47.2 (± 12.9)	0.30	0.75
Total PLFA	11.1 (± 1.17)	17.7 (± 7.55)	30.8 (± 15.1)	1.06	0.40
Fungal PLFA	0.00 (± 0)	0.39 (± 0.24)	0.66 (± 0.27)	2.60	0.15
Bacterial PLFA	5.88 (± 1.04)	9.28 (± 3.89)	21.2 (± 12.9)	1.07	0.40
Gram-positive PLFA	1.83 (± 0.52)	3.49 (± 1.91)	14.2 (± 10.9)	1.10	0.39
Gram-negative PLFA	4.05 (± 0.52)	5.79 (± 1.99)	7.02 (± 1.98)	0.89	0.46
F: B PLFA	0.00 (± 0)	0.04 (± 0.02)	0.02 (± 0.01)	1.88	0.23
GP: GN PLFA	0.44 (± 0.10)	0.50 (± 0.10)	0.94 (± 0.49)	0.89	0.46

Furthermore, there was no evidence that soil biotic properties differed across the three fire regimes (Table 2.2). Microbial biomass C ($p=0.91$) and N ($p=0.75$) did not significantly differ across the fire treatments. The concentration of PLFA biomarkers for total ($p=0.40$), fungal ($p=0.15$) and bacterial ($p=0.40$) PLFAs and gram-positive ($p=0.39$) and gram-negative ($p=0.46$) bacterial PLFAs did not vary significantly between the three fire regimes. The overall concentration of fungal PLFAs across all three fire regimes was very low at an average of $0.35 \text{ nmol g}^{-1} \text{ soil dwt.}$, with no fungal PLFAs detected in the higher intensity burn plots.

The ratio of F:B PLFA did not significantly alter across the differing fire regimes ($p=0.23$), but the low ratio indicate that bacteria are a dominant component of the microbial community at all fire regimes. Similarly, the ratio of gram positive (GP): gram negative (GN) PLFA was not significantly different across the different treatments ($P=0.46$). Overall, these results indicate little changes in both soil abiotic and biotic properties between the three long-term fire regimes.

2.3.2. Substrate-induced respiration response along the fire gradient

Increasing the WHC of the soils from 40 to 60 to 80 % (Table 2.3) did not significantly change soil respiration rates across the three fire regimes ($F_{2,24} = 1.23$, $p=0.31$).

Table 2.3 – Average (\pm SE) respiration response ($\text{mg C g}^{-1} \text{ soil fwt}$) to increasing water holding capacity (WHC) for the three different fire regimes: higher intensity, lower intensity, and fire suppression.

Water Holding Capacity (%)	Fire Regime		
	Higher Intensity	Lower Intensity	Fire Suppression
40	2.78 (± 1.99)	2.34 (1.60)	8.36 (4.54)
60	3.33 (± 1.67)	6.65 (3.27)	3.89 (1.55)
80	4.13 (± 2.45)	1.51 (0.85)	0.57 (0.30)

For all the fire-regime treatments, the mean CO_2 flux prior to substrate addition was $5.5 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ (Figure 2.2). Following the addition of $2 \text{ mg C g}^{-1} \text{ soil f. wt.}$ of glucose, additional soil CO_2 flux increased in all three fire regimes. Usually after the addition of a C substrate, there is a lag phase, growth phase and decline after peak levels (Reischke et al., 2014). Within the lag phase, the microbial respiration remains unchanged from the pre-substrate levels. This may have happened within the first 24 hours and was not captured under our time measurements. The CO_2 respiration peak for all three treatments occurred on Day 10, reaching $480 \pm 40.08 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ for the fire suppression plots. The size of the peak was similar in the lower intensity burns at $446 \pm 78.82 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ however, the higher

intensity burns peaked at $775 \pm 54.8 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$. Following peak levels, CO_2 respiration levels declined to basal rates over the next 22 days. The SIR response over a period of 32 days was significantly larger in the higher intensity burns than the lower intensity burns and fire suppression ($p < 0.001$, Figure 2.2).

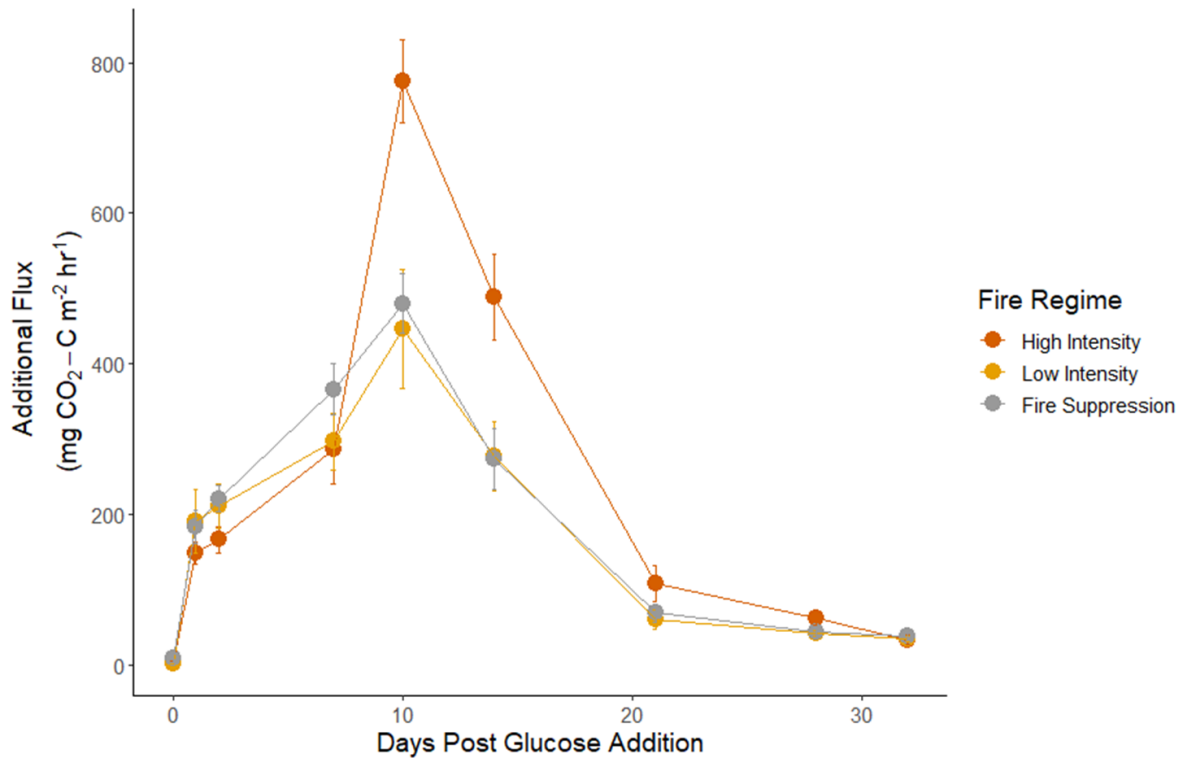


Figure 2.2 - SIR response to glucose substrate addition ($2.0 \text{ mg C g}^{-1} \text{ soil f. wt.}$) for the three different fire regimes; higher intensity (orange), lower intensity (yellow), and fire suppression (grey) over a period of 32 days. Data represent the mean \pm SE of additional CO_2 flux (SIR-BR). SIR, substrate-induced respiration, BR, basal respiration.

2.4. Discussion

The unique 68-year EBPs provide an opportunity to explore the effects of fire regimes on savanna nutrient and microbial dynamics. Our results show that over half a century of repeated burning had little effect on soil nutrients and microbial community composition, relative to fire suppression. These results support previous savanna research within KNP, showing that fire has little impact on granitic soil nutrients (Coetsee et al., 2010; Holdo et al., 2012; Strydom et al., 2019).

2.4.1. Fire regime effects on microbial community composition

We observed that the soils in this study behaved differently to the soils of other ecosystems. Much of the research around fire and microbial communities has been located in temperate systems (Dooley and Treseder, 2012), resulting in limited knowledge on fire effects on microbial communities in savanna ecosystems. First, the overall concentrations of PLFA biomarkers in this study were extremely low. Microbial communities can be influenced by soil texture, with PLFA biomarker levels often lower in sandy soils, compared with more clay rich soils (Bach et al., 2010; Roberts et al., 2011). However, the concentration of total PLFA biomarkers in these soils was up to 65 times lower than other ecosystems (Lombao et al., 2013; Moore-Kucera and Dick, 2008; Wang et al., 2016; Wu et al., 2010). Second, the number of fungal PLFA biomarkers was an order of magnitude lower than similar soil types within South Africa (Kotzé et al., 2017). These low levels suggest that these soils are extremely inert and may not sustain the size of microbial communities seen in more temperate systems (Noronha et al., 2017; Rughöft et al., 2016). The low concentrations also make it difficult to identify potential shifts in microbial community composition between the different fire regimes. Relatively few studies have used PLFA to quantify fire effects on soil microbial communities (Dooley and Treseder, 2012), particularly in savanna ecosystems, with PLFAs never having been characterised within KNP. It should be noted that PLFA biomarkers have their limitations when quantifying microbial community composition as there are several cases where the same biomarkers can indicate two very different functional microbial groups (Frostegård et al., 2011; Olsson and Lekberg, 2022) For example, when there is no known fungi in the soil, 18:1 ω 9 is sometimes detected as a fungal biomarker due to its presence in bacteria also (Schoug et al., 2008).

Generally, increasing fire intensity decreases total bacterial and fungal biomass and diversity (Dove and Hart, 2017). We observed no changes in total PLFA biomarkers, or proportional changes across all the microbial groups, in response to fire intensity or frequency. Similarly, another prescribed burn study observed no change in the total PLFAs with burning treatments, but conversely did see a moderate effect on community composition, with an increase in gram-positive and negative bacteria biomarkers and a decrease in fungal PLFAs in burned areas (Ponder et al., 2009). As expected, bacteria make up the largest proportion of the microbial community in this system. Ash deposition has been shown to increase the pH of soils, particularly with fire temperatures over 450 °C (Knicker, 2007), favouring bacterial communities (Rousk et al., 2010). Thus, we could expect to see an increased pH alongside a

higher proportion of bacterial PLFAs in the hotter, more frequent burns. In this study, both the soil pH and bacterial PLFA remained unaffected across the fire regimes. In addition, the microbial biomass C and N remained unchanged across the differing fire regimes. Whilst significant decreases in soil microbial biomass have been reported after prescribed forest fires (Muqaddas et al., 2015), increasing fire intensity has been observed to have no effect on microbial biomass (Reinhart et al., 2016). A global meta-analysis studying the impact of fire on microbial biomass found that across all biomes, fire reduced microbial biomass by 33%, but there was a lot of inconsistency between the biomes and the fire type, where reduced microbial biomass was observed with wildfires, but remain unaltered in prescribed fires (Dooley and Treseder, 2012). The study also found that savannas and grasslands are less susceptible to fire-related microbial biomass loss than other ecosystem types. The short duration and prescribed nature of the savanna burns, with a lack of heat penetrating the soil during the burns, may contribute to fire not having an impact on microbial biomass or any direct changes to the microbial community.

2.4.2. Fire regime effects on soil nutrients

Our findings show that the investigated fire regimes did not affect soil C or N (and therefore soil C:N ratio), irrespective of the fire intensity or frequency. The lack of change in soil C and N content between the fire treatments is consistent with the lack of change observed in the microbial community. Similarly, soil nitrate and ammonium also did not differ across the treatments. Prescribed burning can break down organic matter in the soil, releasing nutrients into the soil, increasing nutrient availability (Alcañiz et al., 2018), however this may only be observed temporarily. On the other hand, fires can volatilize nutrients from the soil, a particular problem in sandy soils, such as those in this study (Knicker, 2007; Meng et al., 2023). Within KNP, differences in soil C and N have been observed between fire suppression, annual burns and triennial burns, however, these burns occurred in the Satara landscape, under different soil texture conditions (Pellegrini et al., 2015). Conversley, also within KNP, Coetsee et al. (2010) found that fire management had little impact on N dynamics and Zhou et al. (2022) observed that changes in fire frequencies did not affect total soil organic C storage. The nutrient poor nature of the sandy soil in KNP has been previously reported by Rughöft et al. (2016), where comparison with global grassland surveys found C and N contents up to seven times lower in KNP granitic soils. This is further confirmed in other studies characterising KNP's low nutrient levels (Aranibar et al., 2003; Coetsee et al., 2012).

Whilst the nutrient concentrations, microbial biomass, activity, and community composition belowground is intrinsically linked with changes aboveground we show that it does not necessarily determine the belowground response. Studies on the same fire regimes within the Pretoriuskop EBPs observed significant changes in tree numbers, grass community structure and diversity (Higgins et al., 2007; Smith et al., 2013). Fire season has shown to have a significant effect on grass community structure, and fire frequency a significant impact on grass dominance, with grass dominance highest in fire suppression (Smith et al., 2013). Fire frequency and season also significantly changes grass species composition with lower intensity burns (December) having a higher diversity. Similarly, when fire suppression and higher intensity burns (August triennial) were compared, fire suppression had a higher aboveground diversity. In order to survive in semi-arid systems, both the microbes and plant communities have tight nutrient cycles, competing with each other for the same limiting nutrients (Bardgett et al., 2003; Dijkstra et al., 2012), resulting in aboveground vegetation composition shifts (Burkepile et al., 2016; Eby et al., 2014; Higgins et al., 2007; Smith et al., 2013; Wilcox et al., 2020), but a lack of nutrient availability and microbial biomass belowground, which remains unaffected by fire regimes.

2.4.3. Fire regime effects on substrate-induced respiration

The granitic soils across all the fire regimes had low basal respiration rates, with an average of 5.5 mg CO₂-C m² hr⁻¹ (Fioretto et al., 2005; McCulley et al., 2007; Wüthrich et al., 2002). However, these granitic soils within southern KNP have been shown to have lower soil respiration rates than the basaltic soils in central KNP (Makhado and Scholes, 2011) and the low basal respiration levels are not entirely unusual for savanna soils (Andersson et al., 2004; Sánchez-García et al., 2021). The small CO₂ fluxes may reflect both the size and activity of the microbial community, confirmed by our low PLFA and biomass data.

We observed that increasing soil WHC had no effect on soil respiration. The addition of synthetic rain was expected to yield increased respiration rates (Birch, 1958), as soil microbes can be extremely sensitive to changes in soil moisture levels. However, our results suggest that these microbes are either not moisture limited or that these microbial communities are very tolerant to changes in soil moisture, perhaps due to inherent drought tolerance to cope with the annual dry season and episodic drought events (Leizeaga et al., 2021).

The increased SIR response observed in all three fire treatments following the addition of a simple C substrate suggest that the soils may be C limited. Peak fluxes were observed at 10 days post-C substrate addition for all three fire regimes. This is a longer period than typically observed in other SIR studies, where peak responses can be seen within 24-48 hours post substrate addition (Reischke et al., 2014; Whitaker et al., 2014). The delay in reaching peak levels could be due to microbial dormancy. Dormant microbes are found in unfavourable environmental conditions, such as C-limited soils, and whilst they maintain basic metabolism, producing CO₂, they do not grow in number and/or biomass (Joergensen and Wichern, 2018). The ability to transition between active and dormant metabolic states can be an effective survival strategy for soil microbes with the dormant microbes expending just enough energy to stay metabolically alert for immediate use of any substrate that enters the soil (De Nobili et al., 2001). Substrate availability is often limited in duration and so the microbial community must be able to take advantage of resources when available and rapidly switch to active metabolism. Moreover, dormant soil microbes have been observed to not only recycle their own cell-membrane PLFA, but also the PLFA of decaying neighbouring microbes (Dippold and Kuzyakov, 2016), which may reduce the specificity of indicator PLFAs (Joergensen, 2022). As the soils in this study may have a large population of dormant microbes, this may interfere with the precision of allocating PLFA biomarkers to specific microbial groups.

We also observed that the higher intensity, more frequent fires had the greatest SIR response, nearly double that of the response of lower intensity, lower frequency fires and fire suppression at peak respiration rates. Due to observing no differences in the soil nutrient status or the microbial community composition across the fire regimes, one explanation for the different respiration responses may be subtle changes in the soil microbial community structure, not detected by PLFAs, causing a subtle shift in the soil C cycle response. These subtle changes could result in enhanced microbial activity, a shift in carbon use efficiency as a result of utilising higher proportions of labile C sources and changes in C cycling rates; resulting in increased soil respiration observed in the higher intensity burn plots. These subtle changes in the soil microbial communities could contribute to rapid C cycle responses and the resilience of systems to fire variance. These dynamics need to be elucidated with investigation of the microbial community composition at a more detailed level (16S and ITS) and extracellular enzyme activity to further understand the microbial processes influenced by different fire managements. Furthermore, glucose is one of the simplest C compounds that generates one of the greatest additional CO₂ fluxes (Reischke et al., 2014; Whitaker et al., 2014) but ash can

have a similar effect. It is widely assumed that ash deposition increases soil respiration, by providing readily available soil nutrients (Matáix-Solera et al., 2009), including labile C; a potential C substrate to microbes. Thus, in this experiment, the C-substrate could have acted as a proxy for ash deposition post-fire. A similar experiment was performed by Sánchez-García *et al* (2021) on soils also from Pretoriuskop in KNP and showed CO₂ fluxes trebled- post fire, with the addition of ash and rewetting, highlighting the importance of ash in post-fire C cycling and may also provide an explanation for the different responses between the long-term fire regimes. The higher intensity burns will produce higher quantities of ash, burning a higher proportion of the aboveground biomass, in comparison to the lower intensity burns (Strydom, 2023). Higher amounts of ash may result in an increase in the amount of labile C, resulting in a subtle shift in the microbial processing of the increased available soil C, allowing them to increase their metabolism, and thus respiration, to higher rates compared with the lower intensity burns and fire suppression.

Conversely, the C substrate could have also acted as a proxy for PyC produced during the burns. PyC is produced during burns, as part of incomplete combustion of organic matter. Similarly to ash, Whitman et al. (2016) found that PyC addition significantly increased CO₂ fluxes in the first twelve days post addition. The higher intensity, more frequent burns could have higher proportions of PyC due to the higher repetition of burning and the microbes may be able to act more readily to substrate addition. However, lower intensity burns have been observed to produce more PyC due to the higher levels of incomplete combustion (Oliveira et al., 2011). PyC was not quantified in this study, however with sandy soils when clay content is less than 5 %, less than 6 % of the soil organic C is usually PyC (Reisser et al., 2016). Clay content was an average of 3.9%, so large amounts of PyC are unlikely to remain stored in the soil. Better understanding of the role of PyC in savannas is imperative for a clearer mechanistic comprehension of soil C dynamics.

2.4.4. Conclusion

Repeated prescribed fires alter aboveground vegetation and we expected this to be translated belowground, following nearly seven decades of differing fire regimes. However, we show with 68 years of repeated triennial fire regimes or fire suppression, the soil abiotic and biotic properties remain the same, demonstrating high resilience in savanna soils. The resilience of soils to fire has been observed previously within KNP, with N (Aranibar et al., 2003; Coetsee et al., 2012), C (Coetsee et al., 2010; Zhou et al., 2022) and soil hydraulic conductivity (Strydom et al., 2019) being shown to remain unaffected by fire regimes as well as ant species

richness and abundance (Parr et al., 2004), termite mounds (Hockridge et al., 2023) and rodent abundance (MacFadyen et al., 2012). Research focussing on prescribed savanna fires, outside of KNP, have also seen minimal impact on soil chemical and microbial properties (Andersson et al., 2004), particularly in comparison to grassland wildfire (Fultz et al., 2016). Fire suppression has been suggested as a management tool to prevent C and N losses in savanna ecosystems (Archer et al., 2001; Williams et al., 2004), however our data contradicts this. It is important to prevent suppression of prescribed fires as a management tool as not only do the savannas become much more susceptible to wild, uncontrollable fires that will likely have negative impacts on soil C stocks, nutrient availability, and microbial communities (Alcañiz et al., 2018; Homann et al., 2011; Pereira et al., 2021), but fire suppression could have much larger impacts on the savanna biome by altering the aboveground composition. When fire is suppressed, it leads to an increase in the levels of woody encroachment and can result in the dominance of more fire-sensitive forest species resulting in a biome shift from savanna to forest (Khavhagali and Bond, 2008).

2.5. Acknowledgments

We gratefully acknowledge the logistical support provided by South African National Parks Staff in Kruger National Park and the Fire Team for their repeated application of these experimental fires that keep such a long-term project running. We thank Sam Walrond and Theo Ndlovu for assistance with data collection in the field and Dr Clare Benskin, Dr Dafydd Elias, Dr Samuel Robinson, Stella Linnekogel, and Nyxon Rockliffe-Fidler for their laboratory support. We gratefully acknowledge Dr Rosanne Broyd for her help with the statistical analyses and thank Dr Angeliki Kourmouli for her comments on the final manuscript.

3. Savanna soil microbes show high resilience to moderate intensity fires: a year-long time-since-fire study tracking soil respiration post-burn

Cvetkovic-Jones, M. ^{1,2}, Stevens, C.J. ¹, Whitaker, J. ², Parr, C.L. ^{3,4,5}, Strydom, T. ⁶

¹Lancaster Environment Centre, Lancaster University, Library Avenue, Bailrigg, Lancaster, LA1 4YQ, United Kingdom

²UK Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster, LA1 4AP, United Kingdom

³School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa

⁴School of Environmental Sciences, University of Liverpool, Liverpool, L69 3GP, United Kingdom

⁵Department of Zoology & Entomology, University of Pretoria, Pretoria, South Africa

⁶Scientific Services, South African National Parks, Private Bag X402, Skukuza, 1350, South Africa



Picture credit: Jennie Hodgson

3.1. Introduction

Soil respiration, the process through which CO₂ is released to the atmosphere from the soil, is a major part of the global C cycle (Luo and Zhou, 2006; Raich et al., 2002). The CO₂ respired is a combination of microbial and root and soil organism respiration, which respond to abiotic drivers that influence plant growth and microbial activity, including temperature, moisture, nutrient availability, and vegetation productivity. Thus, soil respiration exhibits sensitivity to climatic variations, with elevated temperature and moisture levels stimulating biological activity within the soil and consequently increasing soil respiration rates up to a certain threshold. After the threshold of temperature or moisture is reached, soil respiration rates typically decline due to factors such as enzyme denaturation, oxygen limitation, or microbial stress. The fact soil respiration increases with temperature and moisture has prompted predictions of warming-induced increases in soil CO₂ fluxes, potentially exacerbating climate change (Cox et al., 2000).

Savannas are a widespread, important biome that are characterised by distinct wet and dry seasons, where the seasonal fluctuations in soil moisture have significant impacts on soil respiration (Makhado and Scholes, 2011; Richards et al., 2012). Furthermore, savannas are flammable, with natural fire regimes acting as key drivers shaping both aboveground and belowground processes (Bond and Keeley, 2005; Pellegrini et al., 2018; Sankaran et al., 2008; Scholes and Archer, 1997b). In ecosystems without fire, C assimilated into the soil by photosynthesis is respired back to the atmosphere through both aboveground and belowground respiration processes. In fire-prone ecosystems, fire events disrupt this cycle by consumption of aboveground biomass, thereby preventing C from entering the soil through this pathway. Additionally, fire may affect other soil properties involved in CO₂ flux production, including microbial community composition. The high temperatures during a fire can reduce soil respiration through microbial and root mortality (Neary et al., 2005), and a consequent reduction in microbial biomass (Adkins et al., 2020; Dooley and Treseder, 2012; Liu et al., 2023). Moreover, the burns could cause a shift to a more bacterial dominated community, as bacteria are less vulnerable to higher temperatures compared to fungi (Liu et al., 2023; Rousk et al., 2010). This shift impacts substrate consumption, with bacteria preferentially using labile C, whilst fungi favour recalcitrant C (Boer et al., 2005; Fanin et al., 2019). Soil respiration is approximately proportional to the amount of respiration substrate (soil organic matter, dead roots, exudates, necromass) present. Therefore, shifts in microbial community composition and

substrate type can alter soil C cycling, and consequently impact soil respiration in regularly burnt ecosystems. Nevertheless, fire can have positive impacts on soil respiration, such as the fertilizing effect of ash increasing soil microbial activity (Pyne, 2019; Sánchez-García *et al.*, 2021; Mataix-Solera *et al.*, 2009). Therefore, studies on the effects of fire on soil respiration can be varied with negative (Michelsen *et al.*, 2004; Richards *et al.*, 2012), neutral (Scholes and Andreae, 2000) and positive (Andersson *et al.*, 2004) impacts observed. Despite the importance of soil respiration in the terrestrial C cycle, studies investigating the impact of fire on soil respiration in savannas are limited.

Time-since-fire (TSF) is a fundamental concept when investigating the impact of fires on ecosystem processes, in understanding fire return intervals, plant, and soil diversity as well as post-burn dynamics (Czimczik *et al.*, 2006; Giorgis *et al.*, 2021; Haslem *et al.*, 2012; Morgan and Lunt, 1999; Smucker *et al.*, 2005), all of which may influence soil respiration rates. Thus, the soil respiration response to TSF can serve as an indicator of soil recovery and resilience post-burn. When fire detrimentally affects soil respiration post-burn, the timeline of recovery depends on a multitude of factors, including burn intensity, soil properties and soil type, climate, resilience of the vegetation and microbial community (Gui *et al.*, 2023; Wright *et al.*, 2021; Xu *et al.*, 2022). Consequently, there can be considerable variability among global studies in the recovery times for soil respiration following fire events, but savanna/grassland recovery from fire has been estimated to be the fastest at 0.35 years, whilst in tropical and temperate forests recovery typically take 1.20 and 2.98 years (Gui *et al.*, 2023).

To understand how savanna fires impact soil respiration in the short-term and the resilience of soil function in the longer term, soil respiration was monitored for one-year post-burn. The study compared the response of biennial burns with fire suppressed plots, that have not been burnt for nearly 70 years. The longest fire manipulation experiment globally, the Experimental burn plots in KNP, South Africa, was used, which manipulates fire frequency and intensity to study fire dynamics at an ecosystem level. Following experimental burns in October 2022, soil CO₂ rates were measured weekly, for the first month, seeking immediate insights into any short-term shifts. After one month, the monitoring transitioned to monthly monitoring and then quarterly monitoring. This approach allowed for the assessment of both TSF impact on soil respiration, alongside the natural variations in moisture across different seasons. Additionally, soil microbial composition data was collected to understand whether potential changes in composition were driving changes in soil respiration. The study addressed three aims: firstly

to determine whether prescribed burns result in changes to savanna soil microbial communities. I hypothesise that the burns will significantly alter the composition and diversity of microbial communities, with a decrease in fungal concentrations, due to changes in soil temperature and aboveground composition. Secondly, I aimed to assess whether prescribed burns result in a short-term reduction in soil respiration. I hypothesise that prescribed burns will lead to a short-term reduction in soil respiration rates as a result of reduced microbial activity and carbon availability. The final aim is to determine the rates of recovery of soil respiration and the importance of soil moisture as a driver over the one-year post-burn. It is hypothesised that soil respiration will gradually recover over the year following the burn, with soil moisture being a key driver of this recovery process.

3.2. Methods

3.2.1. Study area

The study was conducted on the Experimental Burn Plots (EBP) in Kruger National Park (KNP), South Africa, an ongoing long-term experiment that was established in 1954 (Biggs et al., 2003). The sites were created to study the impact of different fire regimes, and subsequent herbivory, in contrasting savanna landscapes. The EBPs comprise of repeated seven ha (~ 380 m x 180 m) plots, across four different locations within KNP, with each plot representing a different burning regime (Trollope et al., 1998). We selected two fire regimes for this study, October biennial (burnt at the end of the dry season every two years) and a fire suppression plot, where fire has been suppressed as much as possible since 1954. Out of the four savanna landscape types within KNP, we focussed on the sandy, granitic soils, in the Skukuza area. With an average rainfall of 572 mm, the dominant tree species are *Combretum collinum* and *Combretum zeyheri*. Within the Skukuza area, the fire treatments were applied to plots in four replicate locations: Skukuza (Skz: 25°10'S, 31°47'E), Mbiyamti (Mby: 25°13'S, 31°41'E), Napi (Nap: 25°11'S, 31°40'E) and Nwaswitshaka (Nwa: 25°11'S, 31°38'E).

3.2.2. Prescribed burns

The fires burnt at the end of dry season in late October 2022 and were applied by the KNP Scientific Services Team. In order to measure fire behaviour (Table 3.1), we used five thermocouple loggers placed immediately prior to the fire along a transect down the centre of the plot, with replications ~60 m apart. Out of the four thermocouple wires, two were placed aboveground in opposite directions with one facing East and the other facing West, and two were placed belowground at one and five cm. The third thermocouple unit failed in the field, so only four data points from each burn were included (Table 3.1). Fire behaviour information

was collected during the burns including rate of spread (ROS, m s^{-1}), area burnt (%), air temperature ($^{\circ}\text{C}$), fuel moisture content (%) and fuel load (kg ha^{-1}). Fuel moisture content was calculated by collecting three replicates of grass biomass, before drying at 60°C for 48 hours. Fuel load is subsequently calculated by dividing the mass of the load by the area of the plot. Fire intensity (kW m^{-1}) was calculated as per Byram's (1959) fire line intensity equations (Fire intensity = heat yield * ROS * biomass).

Table 3.1- Fire behaviour measurements across the four prescribed burns replicates; Napi (Nap), Mbiyamti (Mby), Nwaswitshaka (Nwa) and Skukuza (Skz). Measures included fuel load (kg ha^{-1}), rate of spread (m s^{-1}), fuel moisture content (%), air temperature ($^{\circ}\text{C}$), thermocouple fire temperatures aboveground and belowground at 1cm and 5cm ($^{\circ}\text{C}$), % of plot burnt, fire intensity (kW m^{-1}) and final burning comments.

Fire Behaviour Measures	Nap	Mby	Nwa	Skz
Time of Day	09:57	11:34	13:07	14:28
Fuel Load	2085	3087	2424	2281
Rate of Spread	0.31	0.30	0.35	0.25
Fuel Moisture Content	44.7	31.6	49.4	18.6
Air Temperature	29	34	34	36
Fire Temp AG	419	377	355	596
Fire Temp BG (1cm)	33.5	35	41	41
Fire Temp BG (5cm)	31	33.5	39	38
% burnt	65	90	95	100
Fire intensity	1091	1564	1433	963
Comments	Patchy burn, so fire torches were used to reburn unburnt sections.	Clean burn, highly successful	Clean burn, highly successful	Clean burn, highly successful

3.2.3. Phospholipid Fatty Acid Analysis

Phospholipid Fatty Acid (PLFAs) analysis was conducted on three soil samples (8 cm deep) from each plot, at ~ 120 m apart down the centre of the plot. This analysis was performed to measure both the soil microbial community abundance and composition (nmol g^{-1} soil dry weight.). Initially, total lipid was extracted from ~ 1.5 g of freeze-dried soil subsamples and using a modified Bligh and Dyer (1959) method, as described by Frostegård *et al.* (1991). The

extracted lipids were then eluted down to neutral and glycolipids using a column, with chloroform and acetone and discarded. Polar lipids were subsequently eluted with methanol and collected. These lipids were quantified and identified by chromatographic retention time and mass-spectral comparison utilising 19:0 (methyl nonadecanoate fatty acid) as an internal standard. Gas Chromatography was carried out on Agilent Technologies (UK) 6890N GC Network System, equipped with Agilent 7683 Series Injector. Gram-negative bacteria was identified by cyclopropyl saturated (7cy-17:0, 7,8cy-19:0) and monosaturated fatty acids (16:1 ω 5, 16:1 ω 7, 18:1 ω 7) and gram positive were identified by ester-linked branched-chain fatty acids (15:0i, 15:0a, 16:0i, 7Me-17:0, 17:0i, 17:0a) (Rinnan and Bååth, 2009; Whitaker et al., 2014). Fungi were identified using 18:2 ω 6,9 and 18:10 ω 9 fatty acids, indicative of ectomycorrhizal and saprotrophic fungi (Kaiser et al., 2010). Finally, total PLFA values were identified using all PLFAs measured (14:0, 16:1, 16:0, 17:1 ω 8, br17:0, br18:0, 18:1 ω 5, 18:0, 19:1; plus, the other fatty acids listed above). The ratios of gram-positive to gram-negative (GP: GN) and fungal: bacterial (F: B) were calculated to assess the relative abundances of each microbial group.

3.2.4. Soil respiration

CO₂ sampling in the field was conducted regularly for 12 months following the burn using an EGM-4 IRGA with SRC-1 soil respiration chamber (15 cm height x 10 cm diameter) (PP Systems, USA). The chamber was placed on the soil in the field and CO₂ concentration (ppm) was measured for a period of 60 seconds with a 15 second dead band (Figure 5.1c). This was replicated five times along a transect through the centre of the plot, with each transect point approximately ~60 m apart with a minimum 50 m road buffer. Locations were marked with a peg and soil respiration were taken from approximately the same location at each time point. All transect locations were in the open, away from tree canopies and termite mounds. Measurements were taken in the morning with time of day recorded. Air temperature (°C) was also measured alongside soil respiration measurements, using either a Kestrel Wet Bulb Logger (Nielson-Kellerman, USA) or a HOBO 1-800 Logger (Onset Computer Corporation, USA). The rate of change in CO₂ ppm was determined via linear regression and CO₂ respiration rates were calculated as described in Holland *et al.* (1999).

Soil respiration in the field was measured at several time points across the year. Pre-burn soil respiration was measured one week prior to the burns. Following the burns, soil respiration was measured at nine TSF intervals of one, two and three weeks followed by one, two, four, six, eight and twelve months. This ensured we captured short term changes immediately following

the burns, but also that we captured natural variations in soil respiration across wet and dry seasons.

3.2.5. Gravimetric Soil Moisture

Alongside soil respiration, at the same time and transect points, cores (5 cm depth, 4.5 cm dia) were sampled for soil moisture analysis. Soils were dried at 105 °C with weights recorded (g). Gravimetric soil moisture was calculated as dry soil weight subtracted from fresh soil weight and then divided by the dry soil weight (g).



Figure 3.1 - Pictures of the experimental burn plots, immediately post burn, on Napi October biennial burn plot (Picture credit: T Strydom).

3.2.6. Statistical Analysis

All statistical analyses were conducted in R (4.1.1) and significance was reported at $p \leq 0.05$ level. Normality of microbial PLFA concentrations, soil respiration rates and soil moisture were checked using QQ plots, residuals and Shapiro Wilks tests.

The effect of prescribed burns on soil respiration over time was determined by generalised additive models (GAMs) with repeated measures performed using *gamm4* (Wood & Scheipl, 2020). Generalised additive mixed models (GAMMs) were used with fire regime as a fixed effect, TSF as non-linear random effects (spline, *gamm4* function; Wood & Scheipl, 2020), with replicate as a random effect. When model residuals did not satisfy assumption of normality, data were square root transformed. The same GAM analysis was repeated with soil moisture as the fixed effect, but all parameters remained the same. Furthermore, a linear regression analysis was used to model the relationship between soil respiration and soil moisture, for both fire regimes.

The effect of prescribed burns on soil PLFAs was determined using a general linear model, with replicate as a random effect. Data were first averaged by plot level replications within plots to control for spatial pseudo replication in field sampling.

3.3. Results

3.3.1. Microbial community composition

There were no significant differences in the concentrations of PLFAs biomarkers for any of the microbial functional groups between the burnt and fire suppression plots at one-month after burning (Table 3.2). There was no evidence of a difference in total PLFA concentrations ($p=0.36$), with concentrations of $12.2 (\pm 1.32)$ for burnt treatments and $16.2 (\pm 3.84)$ nmol g^{-1} soil dwt for the fire suppression regimes. The concentration of fungal PLFAs was much smaller than bacterial PLFAs, with fungal biomarkers undetected in the Napi replicate plots. The very low concentrations of fungal biomarkers resulted in both treatments F: B ratio below 0.05. The abundance of gram-positive bacteria was approximately double that of the gram-negative community, with no significant differences observed between treatments ($p=0.40$ and 0.38 respectively), resulting in a GP: GN ratio of ~ 0.5 .

Table 3.2 - Effects of October biennial burns and fire suppression regimes on mean (\pm SE) PLFA biomarker concentrations (nmol g^{-1} soil dwt), one-month after fire.

PLFA functional group	October burns	Fire Suppression	p value
Total PLFAs	12.2 (\pm 1.32)	16.2 (\pm 3.84)	0.36
Fungal	0.20 (\pm 0.20)	0.43 (\pm 0.34)	0.58
Bacterial	6.41 (\pm 0.83)	8.57 (\pm 2.11)	0.38
Fungal: Bacterial ratio	0.02 (\pm 0.02)	0.03 (\pm 0.02)	0.76
Gram Positive	4.33 (\pm 0.49)	5.61 (\pm 1.25)	0.40
Gram Negative	2.07 (\pm 0.36)	2.76 (\pm 0.67)	0.38
GP: GN	0.49 (\pm 0.05)	0.49 (\pm 0.01)	0.93

3.3.2. Soil Respiration following prescribed burns

Prior to the prescribed burns in October, marking the end of the dry season, the lowest respiration rates of the entire annual study were recorded, overall averaging 36.8 ± 1.9 (SE) $\text{mg CO}_2\text{-C m}^{-2} \text{hr}^{-1}$, with minimal difference observable in soil respiration between the two treatments (Figure 3.2). While we observed that TSF had a significant effect on soil respiration ($p < 0.001$), fire regime did not affect soil respiration ($p = 0.54$).

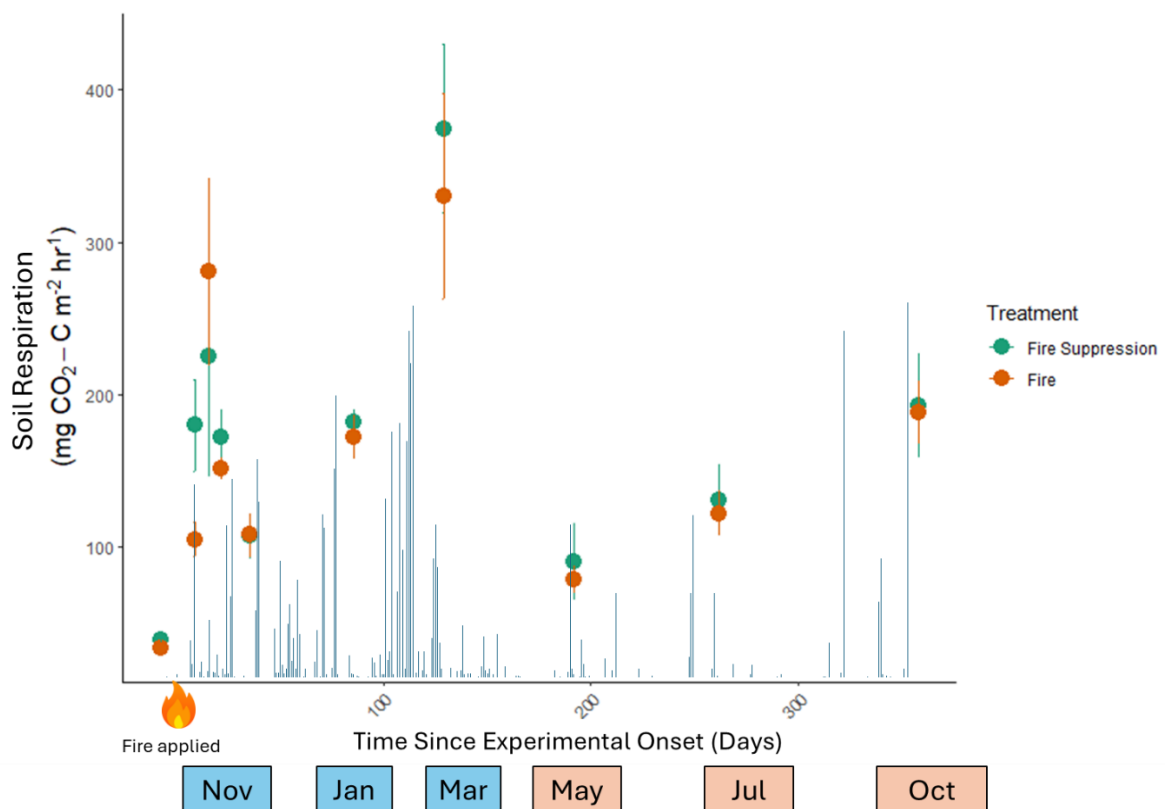


Figure 3.2 - Temporal variation in soil respiration rates ($\text{mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$) over one year following prescribed burns (orange) and fire suppression (green) regimes. Average measurements ($n=5, \pm \text{SE}$) displayed at one-week prior to the burns and at day 9, 16, 22, 36, 86, 129, 192, 262 and 358 post-burn. The blue dashed lines indicate the timing of the rainfall events. The blue lines represent precipitation over the year, from local weather data in Skukuza. Calendar months in the wet season are highlighted in blue, with dry season in red.

Over the year, soil respiration rates in both the burnt and unburnt plots exhibited seasonal variations, with the wet season generally having higher rates and greater variability, while the dry season had lower rates and reduced variability. Although the fire treatment did not significantly impact soil respiration rates, distinct differences were observed at various time points. Immediately following the burns, soil respiration rates at one-week TFS were lower in the fire treatment plots ($115.8 \pm 5.9 \text{ SE mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$) compared with the fire suppressed plots ($180 \pm 31.6 \text{ SE mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$). However, by week two TFS, while total respiration values were higher compared with week one, no differences were observed between the two treatments. Throughout the rest of the year, limited differences between the two treatments persisted. Soil respiration rates were greatest in March, coinciding with the end of the wet season, peaking at $374.7 (\pm 38.8 \text{ SE})$ and $222.7 (\pm 49.8 \text{ SE}) \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ for fire suppressed and burnt plots, respectively. Soil respiration then declined in May as the dry season commenced to an average of $84.6 (\pm 6.3 \text{ SE}) \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$. After a full year of soil respiration monitoring, rates in October 2023 were $\sim 150 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ greater than in 2022.

Soil moisture content was positively correlated with respiration rates in the burned plots (Figure 3.3), where 30% of variation in soil respiration could be explained by soil moisture ($F(1,38) = 16.36, p > 0.01$) This is slightly greater than fire suppression, where 23% of soil respiration variance can be explained by soil moisture ($F(1,38) = 11.32, p > 0.01$).

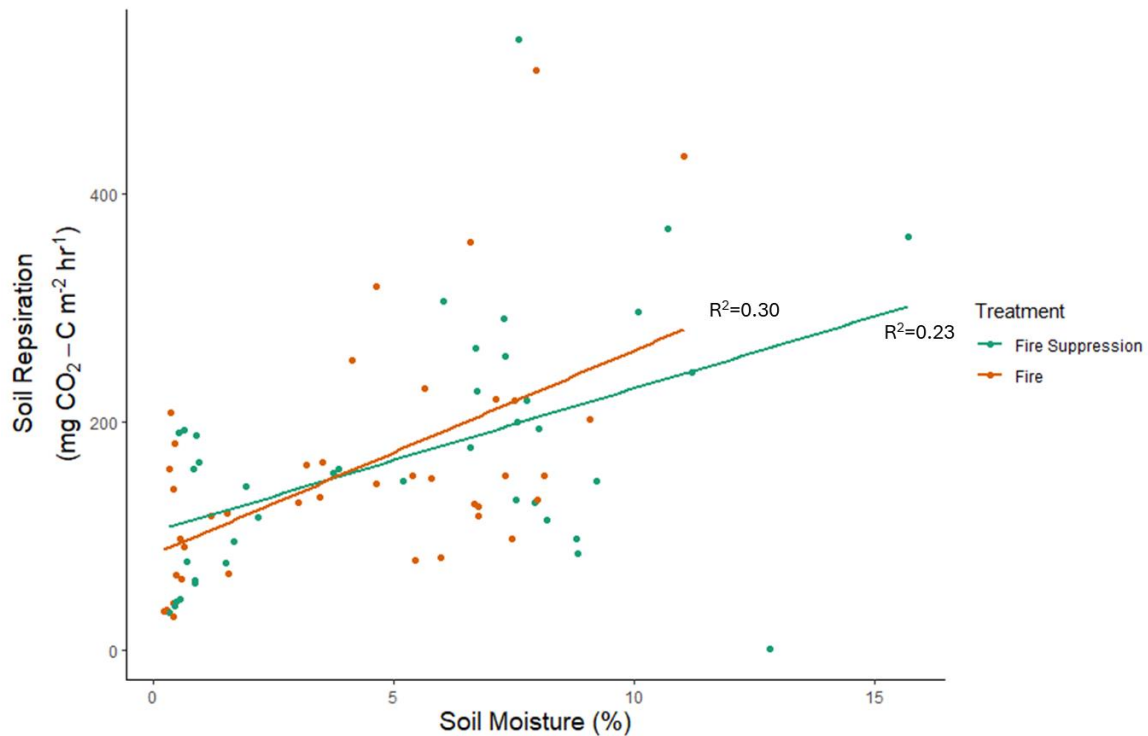


Figure 3.3 - Soil moisture showed a positive relationship with soil respiration rates for fire (orange) and fire suppression (green), respectively. R^2 values quantifies the proportion of SE variance that can be explain by soil moisture.

3.3.3. Annual Fluctuations in Soil Moisture with TSF

Unburned plots tended to have higher soil moisture compared with burned plots (Figure 3.4), but this difference was not significant ($p=0.16$). Soil moisture levels were at their lowest prior to the burns, averaging 0.4%. Following the fires, rainfall occurred within the first nine days as the wet season commenced, resulting in an increase to 7.6 % on the fire suppression plots and 6.6% on the burnt plots. Two weeks after the fire, soil moisture levels rose again to 10.5% and 7.8% respectively, following further rainfall. Subsequently, soil moisture levels gradually decreased, reaching an average of 0.6 % in January, following a mid-wet season ‘drought’. Another peak in soil moisture occurred in March, after a large amount of rainfall in February which caused flooding within areas of KNP, with levels reaching 9.9% for the fire suppression plots and 6.9% for the burnt plots. Throughout the remainder of the annual survey, soil moisture remained between 6 - 10 %, with the exception of May, when a decline to below 1% was observed. Notably, soil moisture levels were also substantially higher in October 2023 compared to 2022 (Figure 3.4).

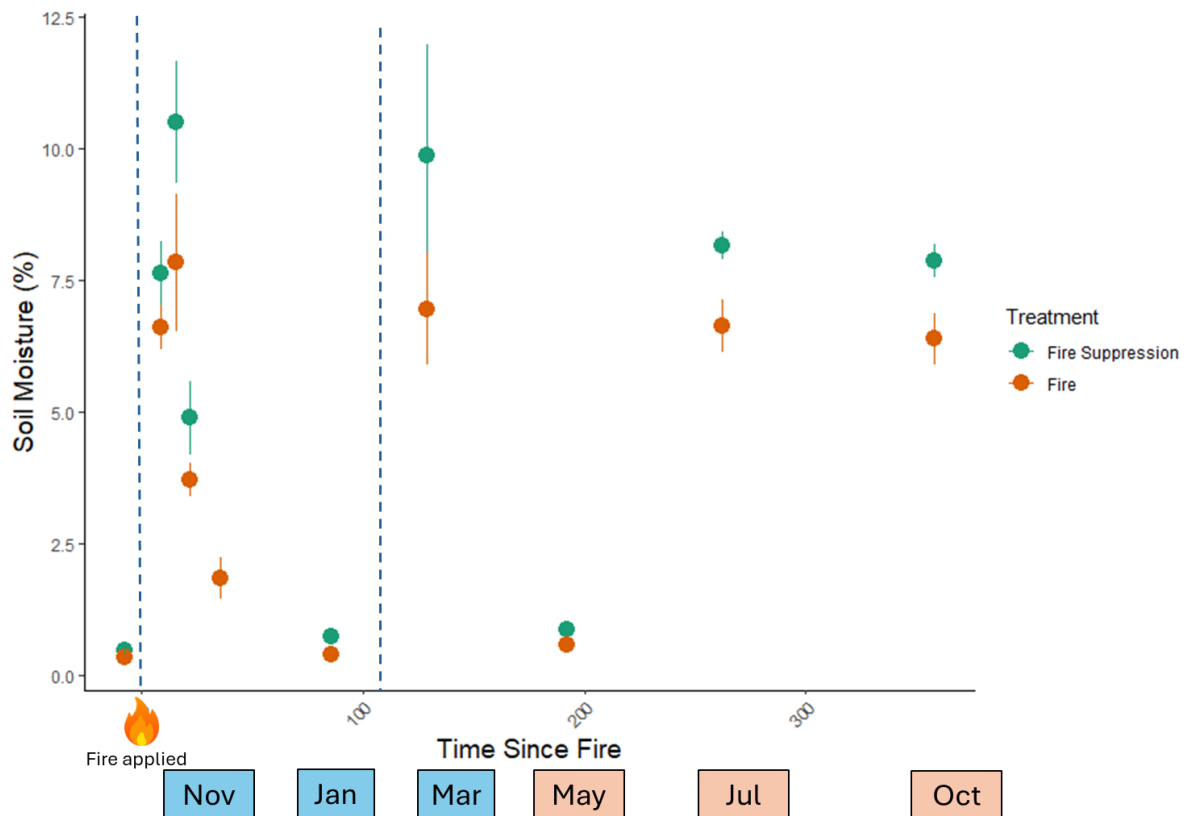


Figure 3.4 - Temporal gravimetric soil moisture dynamics for one year following a burn for the biennial burn treatment (orange) and fire suppression (green) regimes. The blue dashed lines indicate the timing of the rainfall events. The first rains arrived within one week of the prescribed burns. The second blue line indicates a large notable rainfall event in February. Calendar months in the wet season are highlighted in blue, with dry season in red.

3.4. Discussion

3.4.1. Impact of Fire on Soil Microbial Community

We observed no impact of burning on soil microbial abundance and community composition at the microbial functional group level one-month post-burn. Assessing microbial community composition at only one time point provides us with only a snapshot of soil microbial community composition, unlike temporally over the year with the soil respiration data. At the point of collection of soil microbial community composition, we also observed very little differences between the respiration rates of the two regimes. This aligns with the understanding that soil respiration is linked to microbial biomass (Wang et al., 2003), so microbial community composition is most likely not contributing to soil respiration variations.

Our results demonstrate that the microbial communities in sandy soils are resilient or resistant to burning, which we also observed with both high and low intensity fires in Pretoriuskop EBPs within KNP (Chapter 2). The soils have lower concentrations of microbial biomarkers, up to 65 times smaller, compared with more temperate ecosystems (Lombao et al., 2013; Moore-Kucera and Dick, 2008; Wang et al., 2016; Wu et al., 2010). These low concentrations suggest that these soils do not sustain the size of microbial communities of the same size as those in more less arid systems (Noronha et al., 2017; Rughöft et al., 2016). Despite the reduced microbial community size, the ecosystem compensates by shifting carbon cycling through alternative or more efficient pathways. These pathways could involve more resilient microbial species, increased reliance on non-microbial processes (such as root respiration), or enhanced carbon use efficiency by the remaining microbial community in order to sustain soil respiration rates. PLFA biomarkers have been used to quantify fire effects on soil microbial communities in limited studies (review: Dooley and Treseder, 2012), particularly in savannas. Furthermore, PLFAs have never been performed within KNP apart from within this thesis (Chapter 2; Chapter 4; Chapter 5). While observational trends in our study suggests higher PLFA concentrations in fire suppression plots compared to burned plots, this variance may be influenced by the Mbiyamti string, which exhibited significantly higher PLFA concentrations on the fire suppression plots (Appendix Figure 8.1). This difference is possibly attributed to distinct soil type characteristics. The Mbiyamti plots contain large granite rock outcrops and boulders, leading to hydromorphic soils that differ slightly from those in other plots (Venter and Govender, 2012). These soil characteristics can significantly impact soil microbial composition (Bach et al., 2010; Roberts et al., 2011).

3.4.2. Impact of Fire on Soil Respiration

The prescribed burns in this study were applied in the Spring season (1 Sep-30 Nov). Prescribed burns of the EBPs in KNP have been characterised into different fire intensities with season (Govender et al., 2006). Each of the prescribed burn intensities in our study (1091, 1564, 1433 and 963 kW m⁻¹) burnt between the previously reported KNP fire intensities of low (712 kW m⁻¹) and moderate (1570 kW m⁻¹) fire intensities, which is characteristic of spring season burns (Govender et al., 2006). The average annual fluctuations in soil respiration observed following the moderate-intensity fire and suppression regimes ranged from 37 – 375 mg CO₂-C m⁻² hr⁻¹. These rates are within the range of other studies within a similar *Combretum* savanna area of KNP, at 21- 226 mg CO₂-C m⁻² hr⁻¹ (Makhado and Scholes, 2011) and 62 – 375 mg CO₂-C m⁻² hr⁻¹.

$^2 \text{ hr}^{-1}$ (Fan et al., 2015). These are also within the range of other semi-arid and mesic savannas (Butler et al., 2012; Gupta and Singh, 1981; Michelsen et al., 2004).

We observed little effect of the moderate intensity prescribed burns on soil respiration in the year following the burns, in comparison with the fire suppression regime. Any effect of fire on soil respiration had disappeared within two weeks post fire. The neutral impact of fire suggests strong soil resilience to fire. Gui et al. (2023) determined in a global meta-analysis of fire on soil respiration, that moderate severity fires had negligible effects on soil respiration, particularly in grassland/savanna systems. This was also highlighted in a literature study by Scholes and Andreae (2000), where soil CO_2 fluxes in Africa were not significantly affected by burning of dry savanna sites, but not necessarily wet savanna sites (Morris et al., 1982), suggesting that the lower soil moisture content overrides any effect of fire in the dry season. Similarly, soil respiration was neutrally affected by fire in the dry season in Australian savannas (Richards et al., 2012), but there was a significant interaction between fire and soil respiration in the wet season.. Similar minimum and maximum soil respiration rates ($69\text{-}360 \text{ mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$) to our study were also recorded. Thus, the semi-arid, lower rainfall nature and soil moisture of Southern African savannas, in comparison to more tropical savannas, may prevent significant interactions between fire and soil respiration.

Overall, the increase in soil respiration at week one TSF can be attributed to the arrival of the seasonal rains (Figure 3.2 & 3.4). However, immediately following the burns we observed lower soil respiration rates on the fire treatment plots suggesting a subtle direct effect of fire on soil respiration, albeit with a quick recovery rate. Fire consumes aboveground vegetation (Bond and Keeley, 2005), especially in open areas where respiration rates were measured, prompting a potential shift in resource allocation towards mitigating plant damage and survival in the following days post-burn. Root exudates, comprising organic compounds such as enzymes and amino acids, exhibit considerable variability depending on plant health. Since the release of root exudates is closely related to plant photosynthesis (Badri and Vivanco, 2009), levels of root exudation may decrease following a disturbance such as fire. Rapid regrowth of plant species post-burn is characteristic of savanna and grassland ecosystems (Liu et al., 2022; Pereira et al., 2016) and was observed in our study (Figure 3.5). This rapid resprouting may cause plants to divert resources away from root exudation during this period. Given that root exudates are a primary C and energy source for microbes, a decline in their availability due to fire-induced plant stress would lead to a reduction in soil respiration. However, the observed rapid regrowth of grass, suggests a potential reallocation to root exudation within two weeks

post-burn, likely recovering microbial activity and soil respiration to pre-burn rates. If this hypothesised root exudate mechanism is at play, it is important to note that while microbes are the primary beneficiaries of root exudates due to their direct interactions with the plant roots and the rhizosphere, these exudates also indirectly support the entire soil web, including arthropods, by providing essential nutrients and shaping soil conditions (Hiltbold et al., 2013; Scheunemann et al., 2015). Consequently, alterations in root exudation patterns following fire events could impact the respiration rates of other soil organisms, thereby influencing overall soil respiration dynamics.

Other immediate impacts on soil respiration following a fire include microbial and root death. However, our belowground thermocouple data revealed that our soil temperatures did not exceed 41°C at 1 cm and 39°C at 5cm, with such peaks lasting for less than two seconds and immediately returning to pre-burn level. Neary et al., (2005) collated data sets indicating temperature thresholds for biological disruptions by fire, with plant roots showing minimum disruption at 48°C, and fungi at 60-80°C. Thus, direct mortality effects of the fire on soil respiration seem unlikely given the burn conditions.

In contrast, soil respiration has been shown to increase after fire following a pulse of nutrients and microbial substrate from ash deposition (Pyne, 2019; Sánchez-García et al., 2021). Ash infiltration into the soil can be aided by precipitation, removed by grazing herbivores, or dispersed elsewhere by wind, complicating measurement, and quantification of the impact of ash on the soil. As evidenced by Figure 3.5, ash residue was still present on the soil surface nine days post burn. In addition to ash, particularly in moderate intensity fires, charcoal may be generated through incomplete combustion (Ping et al., 2022). While charcoal cannot be used directly by microbes, it can raise the soil pH, which could increase microbial activity and soil respiration (Carter et al., 2018; Wardle et al., 2008). The beneficial effects of ash and charcoal on the soil respiration may offset any potential concurrent reduction in soil respiration from impacts such as root exudates and C pool consumption (Gui et al., 2023), resulting in the neutral response of soil respiration to fire.

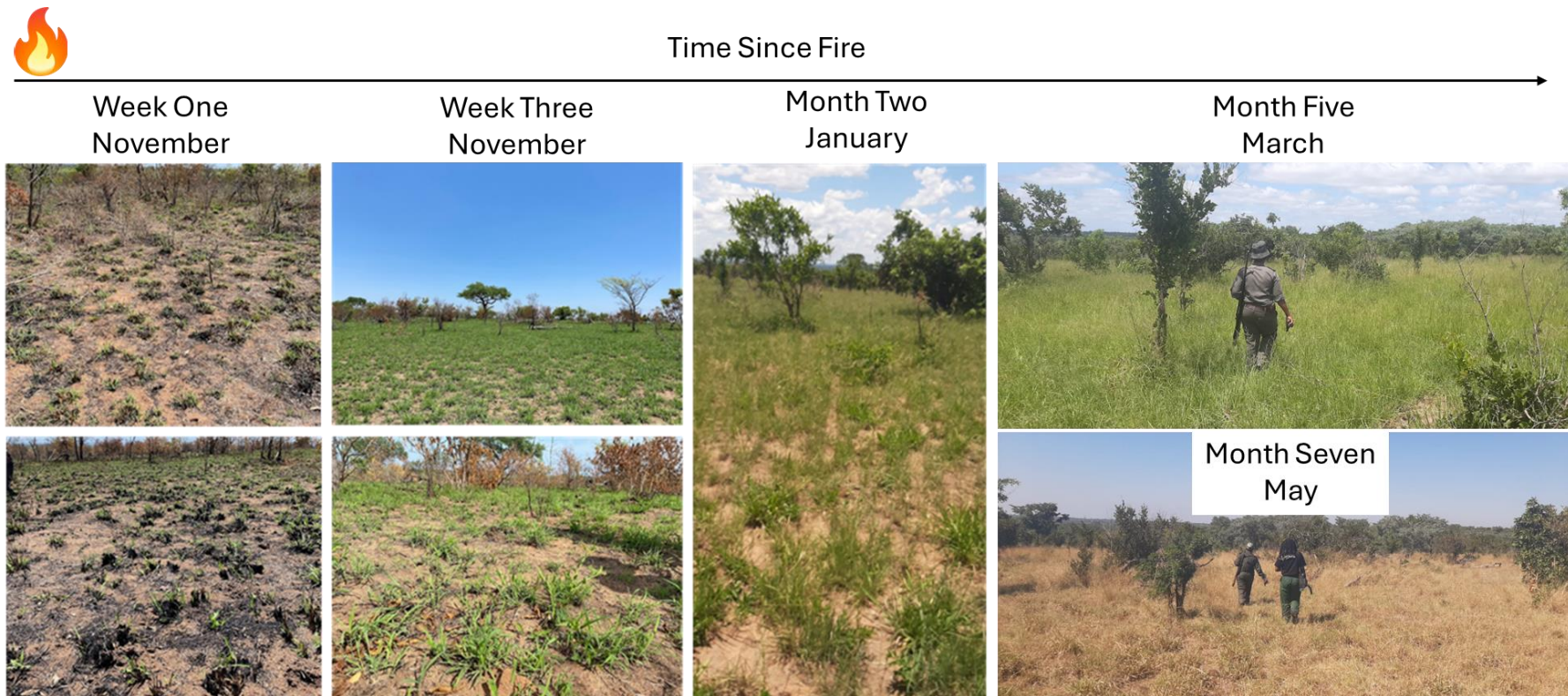


Figure 3.5 - Pictures demonstrating grass re-growth post-burn at week one, week two and month two, month five and month seven after fire. The difference between dry and wet season grass is evident between month five which was following a heavy rainfall wet season and month seven which is six weeks into the dry season. It is also noticeable that the ash is still visible at one week after fire, but not week three. Picture credits: M Cvetkovic-Jones and T Strydom.

3.4.3. Impact of soil moisture on soil respiration

As we monitored soil respiration over the course of a year, we expected to observe changes in respiration rates with environmental conditions, such as precipitation and air temperature. The ‘Birch effect’ occurs where increasing soil moisture levels initially enhance soil respiration rates, but beyond a certain threshold that is dependent on factors such as soil type, ecosystem, vegetation and environmental conditions, further increases in soil moisture can suppress soil respiration (Birch, 1958). In this study, soil respiration increases with soil moisture, but as gravimetric soil moisture levels stayed below 15% in this study, it suggests the Birch threshold was not reached. A positive relationship between soil moisture and respiration have been observed in other studies within Southern KNP, also on sandy soils of a similar moisture (Fan et al., 2015; Makhado and Scholes, 2011; Williams et al., 2009).

We found that 23% ($R^2 = 0.23$) of soil respiration variation could be explained by soil moisture on the fire suppression plots and 30% ($R^2 = 0.3$) of the burn plots. This is similar to other studies in this area of KNP, with an R^2 value of 0.26 in the *Combretum* savanna in a similarly located site (Makhado and Scholes, 2011). However, this is lower than the R^2 value of 0.39 modelled from flux data also collected on KNP’s sandy soils (Fan et al., 2015), however soil moisture was measured to a much deeper level of 61 cm. In our study, soil moisture was measured manually using five cm deep soil cores, before drying. This may not have been a deep enough to get an accurate assessment of soil moisture as whilst October to April is wet season in KNP, it also has higher air temperatures, so water may evaporate quickly from the top five cm of the soil (Figure 1.3, Chapter 1). The soils in this study are also granitic, so the water will drain through very quickly (Strydom et al., 2019). At the two October time points in this study, soil respiration rates are very different, influenced by very different soil moisture (Figure 3.2 and 3.4). This highlights the effect of rainfall trends on soil respiration and how it can alter annually. Our data supports the evidence that precipitation events are an important component of the C cycle in African savannas. The impact of soil moisture on soil respiration is important to understand, as rainfall trends in Africa are changing (Alahacoon et al., 2021), with periods of aridity increasing (Lickley and Solomon, 2018), which will significantly impact C fluxes (Ernst et al., 2024). With soil respiration response to seasonal precipitation events being an important element of the C cycle in African savannas (Fan et al., 2015), it is essential to

continue understanding soil moisture impact on soil respiration, specifically in the face of global change.

3.4.4. Conclusion

Soil respiration is influenced by several factors, including the availability of respiration substrate, which tends to be low in KNP (Rughöft *et al.*, 2016; Coetsee *et al.*, 2010; Aranibar *et al.*, 2003; Chapter 2). Our understanding of soil microbial functioning is based on temperate ecosystems, and this does not necessarily translate to semi-arid savannas. With moderate intensity prescribed fires, soil respiration response is neutral for one-year post-fire, with soil moisture having a larger effect on soil respiration than fire occurrence. Further research investigating the impact of fire within a week following the burns would yield greater understanding, particularly focussing on changes in root exudation following fire. A deeper understanding of savanna C cycling in response to prescribed burning is imperative before making fire management decisions. This study highlights, again, the resilience of the sandy soil microbial communities following burns (Chapter 2) and can be used to help improve fire and conservation management in savanna ecosystems.

3.5. Acknowledgments

We gratefully acknowledge the logistical support provided by South African National Parks Staff in Kruger National Park and the Fire Team for their repeated application of these experimental fires that keep such a long-term project running. We thank Ikageng Olifant, Duduzile Mzimba and Khensani Mkhonto for assistance with data collection in the field.

4. Little effect of mammalian herbivores on savanna soil microbial communities and nutrient cycling

Cvetkovic-Jones, M.^{1,2}, Stevens, C.J.¹, Whitaker, J.², McCleery R.A.³, Ostle, N.¹, Parr, C.L.^{4,5,6}

¹Lancaster Environment Centre, Lancaster University, Library Avenue, Bailrigg, Lancaster, LA1 4YQ, United Kingdom

²UK Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster, LA1 4AP, United Kingdom

³ Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL, USA

⁴School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa

⁵School of Environmental Sciences, University of Liverpool, Liverpool, L69 3GP, United Kingdom

⁶Department of Zoology & Entomology, University of Pretoria, Pretoria, South Africa



Picture Credit: Marnie Cvetkovic-Jones

4.1. Introduction

Herbivores, particularly large herbivores, are among the most prevalent components of terrestrial ecosystems. Their effects on aboveground plant community structure and function has been widely studied, however whilst the responses vary and the mechanisms remain uncertain (Bakker et al., 2016; Kerley and Landman, 2006; Poulsen et al., 2018), there is even less understanding of how herbivores influence belowground properties. Soil microbial communities and nutrient dynamics are crucial in governing essential ecosystem functions, such as primary production and decomposition. These processes are influenced by aboveground organisms and their activities (Bardgett and Wardle, 2003; Wardle et al., 2004). Although general links between aboveground activities and belowground soil processes are well established, identifying the specific factors that drive this connection remains challenging. Large herbivores can affect soil processes by multiple pathways: directly, through trampling, dung, and urine deposition and indirectly, by influencing plant communities, nutrient distribution, microbial communities, soil erosion and water infiltration (Bardgett and Wardle, 2003; Greenwood and McKenzie, 2001; Pringle et al., 2023). When studied across a range of systems, herbivores have been observed to have negative, positive, and neutral effects on soil health (Andriuzzi and Wall, 2017; Forbes et al., 2019; McSherry and Ritchie, 2013; Milchunas and Lauenroth, 1993), depending on soil type, vegetation species composition, precipitation, herbivore species, mammal size, grazing intensity, and study duration, making it difficult to separate the interactive influences. For example, large herbivores can have a positive impact on the transport of C and nutrients, moving assimilated nutrients into the soil through dung. However, high levels of herbivory can negatively impact soil nutrient levels by decreasing vegetation and increasing bare ground, which accelerates soil drying and erosion, culminating in decreased soil C (Li et al., 2008b; Steffens et al., 2008; Wigley et al., 2020). Herbivore grazing may stimulate fine, shallow roots of grasses, which can compensate for the reduced aboveground C inputs to soils as a result of herbivore consumption (Derner et al., 2019). Conversely, herbivore movements and activities (grazing, browsing, walking) can cause direct physical disturbance to the soil. This causes compaction and increases the amount of bare ground, influencing water infiltration, soil moisture and potentially even vegetation dynamics. Herbivores not only have an impact on the physical and chemical properties of the soil but play a significant role in shaping

the soil microbial community. Dung deposition, containing partially digested plant material, can act as a nutrient source for soil microbes (Bardgett et al., 1998), by releasing C, N and P in a form that enhances microbial activity and diversity in soil. Dung can also help maintain fungal communities, with the absence of dung having been observed to reduce fungi proliferation (Bardgett et al., 1997). Alteration of soil structure and aeration because of physical disturbance can also influence microbial community structure, abundance, and diversity (Bardgett and Wardle, 2003). Furthermore, herbivores alter the composition and structure of aboveground vegetation by selective consumption of specific plant species (Olf et al., 2002), influencing plant-microbe associations.

Herbivore body size can play a large role in herbivore impacts on soil processes. Megaherbivore populations have a disproportionate impact on plant species composition, plant biomass and fire suppression (Pringle et al., 2023). For example, larger herbivores produce higher quantities of dung and urine, which is distributed less widely, where smaller herbivores produce smaller dung pellets but more widely distributed (le Roux et al., 2020; Le Roux et al., 2018). Furthermore, large herbivores cause higher soil compaction (van Klink et al., 2015) and the size of herbivore influences the plants selected in their diet (Olf et al., 2002). Herbivore body size effects are particularly relevant in African savannas, where there is a diverse, large mammalian herbivore community that have an important role in shaping ecosystem processes (Malhi et al., 2016; Owen-Smith, 1987; Pringle et al., 2023; Ripple et al., 2015).

There have been several long-term herbivore exclusion studies set up in African savannas (Goheen et al., 2010; Guy et al., 2021; Long et al., 2017; Moncrieff et al., 2011; Pringle et al., 2007; Sankaran and Augustine, 2004; Sitters et al., 2020a; van der Plas et al., 2016; Wigley et al., 2020; Young et al., 2003), however due the multifactorial nature of large herbivore effects, and the difficulty of manipulating large herbivores at an ecosystem scale, impacts can be difficult to quantify (Pringle et al., 2023; Terborgh, 1988). Soil data in herbivory exclusion experiments is often lacking, complicating the process of separating environmental constraints (Staver et al., 2021). Large herbivore mammals (over 20 kg) are particularly vulnerable to global change, with megaherbivores (over 1000 kg) at the greatest risk of extinction (Gill, 2014; Pringle et al., 2023; Ripple et al., 2015). The extinction of megaherbivores in South American savannas has led to significant changes in vegetation structure, carbon dynamics, and overall ecosystem function

(Doughty et al., 2016). This highlights the critical role of megaherbivores in maintaining savanna ecosystems and underscores the potential impacts their loss could have on South African savannas. Understanding how different herbivore groups shape soil properties in savanna ecosystems is essential for conserving biodiversity, sustainable land management, fire management, climate change mitigation and ensuring ecosystem resilience in the face of environmental challenges.

To achieve a better understanding of both the impact of herbivory, and the contribution of different herbivore groups, on soil properties, we utilised the BROWSE (Biodiversity Research on Wildlife and Savanna Ecosystems) project in Kruger National Park (McCleery et al., submitted). BROWSE uses replicated enclosure treatments to separate the impacts of megaherbivore (>1000 kg), large herbivore (>20 kg) and absence of mammal herbivores on ecological processes. In 2017, before the enclosures were established, we assessed a limited range of soil properties across the plot locations to establish the natural variability of the experimental area.

After four years of enclosure treatment, the impact of different herbivory regimes on soil nutrient concentrations, microbial biomass, and microbial community composition was assessed to address two questions. Firstly, ‘does the absence of herbivores decrease soil nutrient availability and result in a shift towards a more bacteria-dominated microbial community?’ It was hypothesised that the exclusion of herbivores would lead to a decrease in the size of the microbial community, specifically the fungal community, and soil nutrient availability as a result of reduced dung and urine inputs. Secondly, megaherbivores have a disproportionate impact on aboveground vegetation compared to other large herbivores due to their increased body size. The study aimed to assess the contributions of megaherbivores to altered soil properties, due to changes in dung and urine deposition, soil compaction and altered plant composition. Megaherbivores have been shown to reverse the negative effects on soil of cattle grazing in Kenyan savannas (Sitters et al., 2020a) Thus, more specifically, we hypothesize that without megaherbivores, and in the presence of only large herbivores, we would observe a reduction in nutrient availability and fungal biomass, in comparison to all herbivores present. It was hypothesised that megaherbivores larger body size would have positive affects on soil nutrients and microbial community size.

4.2. Methods

4.2.1. Study Area

The study area was located at the intersection of the Lower Sabie and Crocodile Bridge regions of Kruger National Park, South Africa (25°15'S, 31°58'E). The soils are nutrient rich basaltic soils with a rainfall average of 500 – 700 mm per annum (Hijmans et al., 2005; Smit et al., 2013b). The dominant grass species of the site include *Themeda triandra* and *Megathurus maximum*. The shrubs were dominated by *Dichrostachys cinerea* and *Gymnosporia senegalensis* (Jones et al., 2022). The tree canopy was mostly comprised of *Senegalia nigrescens*, *Sclerocarya birrea caffra*, and *Vachellia gerrardii*. Before treatment, overstory canopy cover was 3.5 % shrub cover, with the height of woody vegetation between 0.5 and 5 m and limited bare ground (McCleery et al., 2018). The study area was previously rotationally burned as part of fire management strategies, at a mean return interval of 4 to 5 years (Smit et al., 2013b). Within the BROWSE project, prescribed burns occurred on these plots in May 2020.

This area of KNP has an average grazer metabolic biomass of 627 kg km⁻² (Metabolic biomass = total biomass x average metabolic rate x activity factor; McCleery et al., submitted). Megaherbivores present on our site include elephants (*Loxodonta Africana*), southern giraffe (*Giraffa giraffa*) and white rhino (*Ceratotherium simum*) (Abraham et al., 2019; McCleery et al., 2018). Elephants are by far the most frequent megaherbivore at an abundance of 0.9 km² (Chase et al., 2016), with giraffe being detected 75% less than elephants, and white rhino extremely uncommon from the beginning of the BROWSE study (McCleery et al., submitted). Large herbivores found commonly in the site include plains zebra (*Equus quagga burchellii*), cape buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*) and blue wildebeest (*Connochaetes taurinus*).

4.2.2. Experimental Design

Exclosures were erected in January 2018, set out in three replicate blocks. Within each block, there are three treatment plots: a 'Full' exclosure (excluding all mammalian herbivores), a partial 'Mega' exclosure (excluding megaherbivores, i.e. >1000kg) and a control 'Open' plot (no exclusion). All plots are 70 m x 70 m (0.49 ha).

To prevent any mammalian herbivores grazing on the full exclosure, electrified fences at a height of 2.5 m were erected (Figure 4.1). The mega exclosures are also electrified but the fence is erected from a height of 2.5 m, which allows smaller animals, such as impala,

zebra and wildebeest to graze, but prevents larger megaherbivores (elephant, giraffe, rhino) from entering the plot. Fences were checked for damage, every 14 days.



Figure 4.1 - Herbivory exclusion treatments for a) Full enclosure (no herbivores can access), b) Mega (megaherbivores excluded, large herbivores can access) and c) Open (open to all herbivores). Picture credit: Mary Cvetkovic-Jones, April 2022.

4.2.3. Pre-Treatment Sampling

At each of the nine plots, soil samples were collected in December 2017 by the BROWSE field team, prior to the erection of the exclosures. Samples were taken to a depth of 15 cm using a hammer core (15 cm x 5 cm). Three samples were taken in a vertical transect 20 m apart across the centre of the plot, with a 10 m edge effect. Upon collection, the soil samples were transported to a KNP laboratory where they were stored in a freezer at -18°C. In 2021, they were shipped to Lancaster University, UK and immediately analysed upon arrival.

Samples were defrosted and subsampled to be freeze dried or dried for respective analyses. Soil was defrosted for determination of soil pH. Freeze dried samples were used for PLFA analysis and dried samples determined total soil C, N and P.

4.2.4. Post Treatment Sampling

Soil was re-sampled in May 2022, four years and six months after the start of the experiment. Samples were taken to a depth of 15 cm using a soil auger. Ten samples were taken within each plot to attempt to capture the heterogeneous nature of savanna soil. Samples were taken along two parallel transects of five samples each at 15 m apart with a 5 m exclosure edge effect buffer. Upon collection, samples were stored at 4°C before being transported to Lancaster University, UK using ice gel packs. Samples were stored at 4°C prior to biogeochemical analyses. Freeze dried samples were used for PLFA analysis and dried samples for total C, N and P. All other analyses were performed on fresh soil.

Three samples per plot were taken for bulk density in a transect along the centre of the plot. Bulk density was calculated from dry mass and volume of soil excluding stones. Mass and volume of stones were measured by water displacement.

4.2.5. Abiotic and biotic analyses

Soil pH was measured using a 10.0 ± 0.5 g subsample of fresh soil, mixed with 25 ml of deionised H₂O, using a pH meter (InLab Expert Pro-ISM). Soil moisture was determined by using aliquots 1 ± 0.1 g of fresh soil, dried at 105°C for 48 hours. Moisture content was calculated as a percentage of the dry weight. Soil ammonium and nitrate was calculated as a measure of inorganic N in the soil which is directly available to the plants.

5 ± 0.1 g subsamples were extracted using 25 ml of 2M KCl. Samples were filtered through Grade 42 Whatman filters. Plant available N was analysed on an AA3 HR Auto Analyser (Seal Analytica, UK). Total soil C and N was measured by drying subsamples at 60°C and ball milling before measurement of C and N content of 20 mg on Elementar Vario EL CUBE CN analyser. Total soil phosphorus was measured through digestion of air dried, ball milled samples with a sulphuric acid-hydrogen peroxide digestion mixture. 0.2 ± 0.01 g samples were heated at 400 °C for three hours, before being diluted with MilliQ water to an acid content of 5% v/v. The samples were then vortexed and filtered through Grade 42 Whatman filters. Inorganic P was analysed on the auto analyser (Lancaster University, Seal Analytical AA3).

Microbial biomass C was measured on 5 ± 0.5 g soil subsamples using the chloroform fumigation-incubation method (Brookes et al., 1985). Using paired subsamples; one of the samples was fumigated with chloroform for 18 hours before both paired subsamples were extracted via shaking in 25 ml of 0.5M K₂SO₄. Following shaking, the samples were filtered through Grade 42 Whatman filters. Microbial biomass C was analysed using a 5000A TOC Analyser (Lancaster University, Shimadzu, Japan). Microbial biomass C was calculated as difference between fumigated and unfumigated soil, using general correction factors of 0.45 for microbial biomass C (Brookes et al., 1985; Joergensen and Mueller, 1996; Wu et al., 1990).

Phospholipid Fatty Acid (PLFA) analysis was performed using a modified Bligh and Dyer (1959) method (Frostegård et al., 1991) and collected on a column. Total lipid was extracted from ~ 1.5 g of freeze-dried soil subsamples. Neutral and glycolipids were eluted from the column with chloroform and acetone and discarded. Polar lipids were eluted with methanol and collected. These lipids were quantified and identified by chromatographic retention time and mass-spectral comparison using 19:0 (methyl nonadecanoate fatty acid) as an internal standard. Gas Chromatography was carried out on Agilent Technologies (UK) 6890N GC Network System, equipped with Agilent 7683 Series Injector. Gram-negative bacteria were identified by cyclopropyl saturated (7cy-17:0, 7,8cy-19:0) and monosaturated fatty acids (16:1 ω 5, 16:1 ω 7, 18:1 ω 7) and gram positive bacteria were identified by ester-linked branched-chain fatty acids (15:0i, 15:0a, 16:0i, 7Me-17:0, 17:0i, 17:0a) (Rinnan and Bååth, 2009; Whitaker et al., 2014). Fungi were identified using 18:2 ω 6,9 and 18:10 ω 9 fatty acids, indicative of ectomycorrhizal and saprotrophic fungi (Kaiser et al., 2010). Finally, total PLFA values were identified

using all PLFAs measured (14:0, 16:1, 16:0, 17:1 ω 8, br17:0, br18:0, 18:1 ω 5, 18:0, 19:1; plus, the other fatty acids listed above). The ratios of gram-positive to gram-negative (GP: GN) and fungal: bacterial (F: B) were calculated to demonstrate the relative abundances of each group.

4.2.6. Statistical Analysis

All statistical analyses were conducted in R (4.3.1) and significance was reported at the $p \leq 0.05$ level. The effect of herbivory regime, both pre-treatment and post-treatment, on soil abiotic and biotic measures were determined using general linear models with plot level replications controlled as random variables. Data points were first averaged within plots to control for spatial pseudo replication in field sampling. Normality of all the soil properties was checked using QQ plots, residuals and Shapiro-wilk tests. Soil pH and bulk density were log transformed prior to analysis.

4.3. Results

4.3.1. Pre-Treatment Analyses

In the samples collected in 2017 prior to the erection of the exclosures, we found no significant pre-existing differences across the plots (Table 4.1 and 4.2). There were no disparities in soil pH, which averaged 6.71 (± 0.05 SE). The average soil C: N content stood at 9.23 (± 0.36), with total C at 2.86 (± 0.24) % and total N at 0.3 (± 0.02) %. Although total P did not exhibit significant differences, its distribution was heterogenous, averaging 554 (± 43.84) mg kg⁻¹.

Table 4.1 - Pre-treatment data for mean (\pm SE) soil properties measured for Full (no herbivores), Mega (megaherbivores excluded) and Open (all herbivores present) treatments. Soil abiotic properties include soil pH, total C and N (%), C: N ratio, and total phosphorus (mg kg⁻¹).

Soil property	Full	Mega	Open	p value
pH	6.82 (± 0.09)	6.68 (± 0.06)	6.62 (± 0.12)	0.35
Total C (%)	2.86 (± 0.39)	2.8 (± 0.49)	2.96 (± 0.53)	0.96
Total N (%)	0.303 (± 0.03)	0.28 (± 0.04)	0.32 (± 0.04)	0.72
C: N	8.94 (± 0.46)	9.81 (± 0.72)	8.94 (± 0.74)	0.58
Total Phosphorus	518 (± 128)	510 (± 36.3)	636 (± 19.6)	0.48

The microbial community composition also did not vary prior to the herbivore treatments (Table 4.2), with an average total PLFA biomarker concentration of 20.71 (± 2.67) nmol g⁻¹ soil dwt. Bacteria biomarkers averaged 9.84 (± 1.35) nmol g⁻¹ soil dwt, while fungal concentrations average 2.63 (± 0.32) nmol g⁻¹ soil dwt, resulting in a fungal: bacterial ratio of 0.29 (± 0.05). Gram negative bacteria PLFA biomarkers were greater than gram positive at 5.39 (± 1.08) and 3.69 (± 0.47) nmol g⁻¹ soil dwt respectively, resulting in an average GP: GN of 1.59 (± 0.30). Pre-treatment PLFA biomarkers were on average lower than those we observed post-treatment. This is likely due to the nature of how the samples were stored frozen prior to the PLFA analysis.

Due to these differences in the collection methods and handling of the pre-treatment and post-treatment samples, they could not be directly compared for nutrient or microbial community analysis.

Table 4.2 - Pre-treatment microbial community composition represented as mean (\pm SE) PLFA biomarkers (nmol g⁻¹ soil dwt) for Full (no herbivores), Mega (megaherbivores excluded) and Open (all herbivores present) treatments.

PLFA biomarkers	Full	Mega	Open	p value
Total	20.9 (± 5.78)	18.5 (± 0.00)	21.2 (± 3.93)	0.96
Bacterial	10.5 (± 2.39)	9.15 (± 0.00)	9.77 (± 2.53)	0.90
Fungal	2.87 (± 0.50)	1.85 (± 0.00)	2.65 (± 0.58)	0.67
F: B	0.30 (± 0.08)	0.23 (± 0.00)	0.31 (± 0.10)	0.91
Gram Positive	5.34 (± 2.25)	4.47 (± 0.00)	5.74 (± 1.72)	0.95
Gram Negative	4.52 (± 0.26)	3.24 (± 0.00)	3.00 (± 0.88)	0.35
GP: GN	1.16 (± 0.44)	1.38 (± 0.00)	2.09 (± 0.47)	0.42

4.3.2. Post Treatment Analyses

Following four and a half years of exclusion treatment, soil abiotic and biotic properties sampled in 2022, did not vary significantly with different herbivore regimes (Table 4.3). Soil pH was an average of 6.80 across the three treatments, with no significant difference among treatments ($p=0.96$). Soil moisture also did not alter significantly in line with herbivore presence ($p=0.49$). Likewise, bulk density remained did not vary with increasing herbivore presence ($p=0.62$).

Total C ($p=0.99$) and total N ($p=0.59$) did not alter across the treatments, averaging at 2.68% and 0.15% respectively. As there were no changes in the proportions of C and N, C: N ratio remained unchanged across the three exclosure treatments ($p=0.88$). Total phosphorus showed a slight decreasing trend as herbivores were excluded; however the differences were not significant ($p=0.48$), and pre-treatment phosphorus levels were also highly variable.

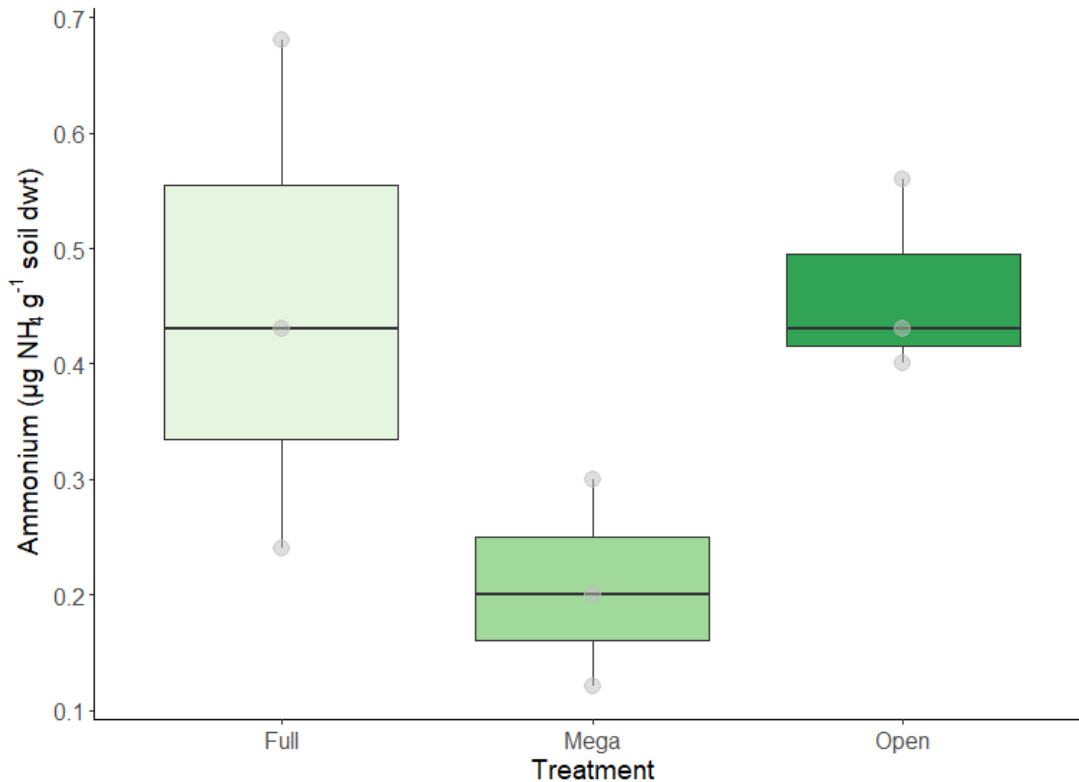


Figure 4.2 - The response of soil ammonium ($\mu\text{g NH}_4 \text{g}^{-1}$ soil dwt) concentration after four years of exclusion for three experimental treatments; Full (excluding all herbivores), Mega (excluding megaherbivores, accessible by large herbivores) and Open (open to all herbivores). Whilst showing a descriptive trend, the differences were not significant ($p=0.13$).

Ammonium and nitrate concentrations did not significantly alter between the three treatments. However, we did observe a descriptive trend with ammonium ($p=0.13$), where full and open remained at a similar level of $\sim 0.45 \mu\text{g NH}_4 \text{g}^{-1}$ soil dwt, but megaherbivore exclusion decreased by over 50% to an average of $0.21 \mu\text{g NH}_4 \text{g}^{-1}$ soil dwt (Figure 4.2). This was mirrored, to a lesser extent, with the soil nitrate concentrations (Figure 3), remaining mostly consistent across the differing herbivory regimes ($p=0.50$).

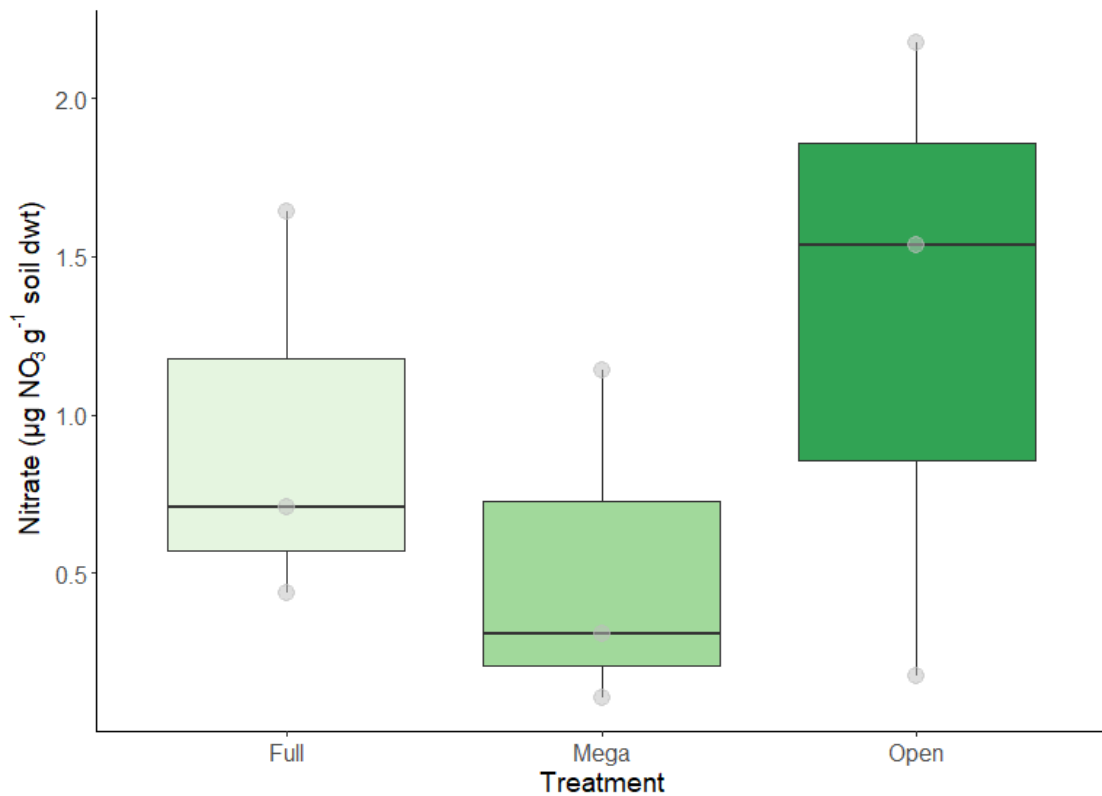


Figure 4.3 - The response of soil nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt) concentration to three experimental treatments; Full (excluding all herbivores), Mega (excluding megaherbivores, accessible by large herbivores) and Open (open to all herbivores), after four years of treatment.

Microbial biomass C did not differ among treatments ($p=0.98$). Similarly, we did not see any significant differences in microbial community composition. Total PLFA biomarkers remained constant in both the size of the microbial community ($p=0.64$) and the composition, with no difference observed between bacterial PLFA biomarkers ($p=0.55$) and fungal biomarkers ($p=0.65$). Bacteria were the dominant microbial group, with a fungal: bacterial PLFA ratio of less than 0.1 across all the treatments ($p=0.76$). Within the bacterial PLFAs, we saw a greater concentration of gram-positive bacteria than gram negative in each of the herbivory regimes, but no differences across the treatments respectively ($p=0.57, 0.65$). Thus, we observed an average GP: GN ratio of 0.73 ($p=0.99$).

Table 4.3 - Effects of different mammalian herbivory regimes on soil properties (mean \pm Standard Error) analysed by linear model for Full (no herbivores), Mega (megaherbivores excluded) and Open (all herbivores present). Soil abiotic properties include soil moisture, bulk density, soil pH, C: N ratio, total P (mg kg^{-1}), ammonium ($\mu\text{g NH}_4 \text{g}^{-1}$ soil dwt), and nitrate ($\mu\text{g NO}_3 \text{g}^{-1}$ soil dwt). Soil biotic properties include microbial biomass C ($\mu\text{g g}^{-1}$ soil dwt) and PLFA measurements (nmol g^{-1} soil dwt).

Soil Property	Full	Mega	Open	p value
Soil Moisture	24.9 (± 1.24)	28.7 (± 3.57)	30.9 (± 4.46)	0.49
Bulk Density	1.15 (± 0.05)	1.18 (± 0.02)	1.21 (± 0.04)	0.62
pH	6.78 (± 0.19)	6.84 (± 0.16)	6.8 (± 0.17)	0.96
Total C (%)	2.68 (± 0.24)	2.65 (± 0.46)	2.72 (± 0.25)	0.99
Total N (%)	0.15 (± 0.01)	0.14 (± 0.01)	0.15 (± 0.00)	0.59
C: N	17.9 (± 0.81)	19.0 (± 1.92)	18.4 (± 1.89)	0.88
Total Phosphorus	278 (± 67.3)	345 (± 76.5)	440 (± 115)	0.48
Microbial Biomass C	82.4 (± 21.9)	87.7 (± 22.2)	88.4 (± 25.1)	0.98
Ammonium	0.45 (± 0.13)	0.21 (± 0.05)	0.46 (± 0.05)	0.13
Nitrate	0.93 (± 0.37)	0.52 (± 0.32)	1.3 (± 0.59)	0.50
Total PLFA	48.9 (± 2.68)	48.8 (± 5.55)	56.2 (± 8.66)	0.64
Bacterial PLFA	23.9 (± 0.59)	26.4 (± 3.00)	29.2 (± 4.7)	0.55
Fungal PLFA	2.12 (± 0.53)	1.49 (± 0.70)	2.39 (± 0.77)	0.65
F: B	0.09 (± 0.02)	0.06 (± 0.03)	0.07 (± 0.02)	0.76
Gram Positive PLFA	10.2 (± 0.76)	10.9 (± 1.01)	12.5 (± 2.21)	0.57
Gram Negative PLFA	13.5 (± 1.25)	15.4 (± 2.17)	16.5 (± 2.93)	0.65
GP: GN	0.75 (± 0.16)	0.73 (± 0.06)	0.72 (± 0.12)	0.99

4.4. Discussion

The BROWSE experiment provides an opportunity to explore the effects of herbivore presence and body size on savanna soil nutrients and microbial dynamics. Our results show that nearly four years of herbivore absence had little effect on soil nutrients and microbial community composition. These findings highlight the complexity of factors influencing mammalian herbivores, and their impacts on soil nutrient and microbial dynamics in savanna ecosystems.

4.4.1. *Herbivore density and intensity*

The minimal response seen belowground may be due to insufficient activity of the large mammalian grazers. The basaltic soils in the BROWSE area of KNP have been observed to be highly productive (Munyati and Ratshibvumo, 2010), suggesting that herbivore densities may need to be much higher here, in comparison to other systems, to see an effect of herbivory on aboveground biomass. The KLEE experiment in Mpala Research Centre in Kenya has a slightly lower grazer metabolic biomass of 502 kg km⁻² (Staver et al., 2021) in comparison to this study at 627 kg km⁻² (McCleery et al., submitted). However, the ungrazed aboveground biomass at KLEE is nearly six times smaller than our study, at 555 kg ha⁻¹ versus 3200 kg ha⁻¹, thus proportionally there is much greater grass biomass per kg of grazer within our study (McCleery et al., submitted). Furthermore, the Hluhluwe-iMfolozi experiment within South Africa has a higher grazer metabolic biomass of 751 kg km⁻² but an ungrazed aboveground biomass of only 195 kg ha⁻¹ (Staver et al., 2021; Staver and Bond, 2014; McCleery et al., submitted). Therefore, the changes observed due to herbivory in other studies may be attributed to higher grazer: grass biomass intensity. Additionally, the productive nature of the BROWSE basaltic soils in our study, might mean there is insufficient mammalian herbivore biomass to cause significant changes in soil abiotic and biotic properties. In comparison to other enclosure studies, which are often erected in areas where grazing effects are intense (Staver et al., 2021), areas with lower herbivore grazing intensities may not see such comparable, marked differences in soil properties.

4.4.2. *Fire effects on herbivore impacts*

Unlike other large herbivore and megaherbivore exclusion experimental studies (Charles et al., 2021, 2017; McCauley et al., 2018; Pringle et al., 2014; Riginos et al., 2018; Sankaran et al., 2013), the BROWSE plots did not see rapid changes in grass biomass, three years into the exclusion experiment (McCleery et al., submitted). However, one year into the experiment in 2019, the mega and full plots did have a significant increase in grass biomass in comparison to the open plots (McCleery et al., submitted). A prescribed burn in 2020 was burnt over a large area, covering all of the exclusion plots, and removed the majority of grass biomass across all three treatments. The following year in 2021, grass biomass had returned to pre-experimental levels, but with no significant differences between the three herbivore exclusion treatments. Many African savanna exclusion studies often do not have experimental fires as a factor due to their confounding effects

(Augustine and McNaughton, 2004; Goheen et al., 2018; Long et al., 2017; Sitters et al., 2017; Staver et al., 2021). Although increased grazing post-fire could potentially exacerbate herbivore effects on soil, the large-area fire in 2020 likely had a homogenizing effect on the landscape (Archibald et al., 2005; Donaldson et al., 2018), making this unlikely. In this landscape, fire may be a larger driver of aboveground vegetation dynamics, with herbivores having a comparatively smaller influence. Archibald and Hempson) found that fire and herbivory are competing consumers of vegetation dynamics in African savannas, with rainfall influencing which driver could dominate. The precipitation levels of our study fall within the 500-800 mm rainfall band where either driver could dominate. There is a major knowledge gap on the interactive role of fire and herbivory in savanna ecosystems, specifically on belowground effects on soil properties, which are discussed more thoroughly in Chapter 5 and 6 of this thesis.

4.4.3. Herbivore impacts on soil nutrient levels.

Mammalian herbivory has been shown to alter C: N: P stocks in savanna soils (Holdo and Mack, 2014; Munjonji et al., 2020; Sitters et al., 2020a; Wigley et al., 2020), with animal body size influencing the ratios of nutrients supplied to plants and soil (le Roux et al., 2020), via the distribution and quantity of animal waste products across the landscape (Cech et al., 2008; McNaughton et al., 1997). Despite this, we found limited changes across any of the three primary soil nutrients as a direct consequence of herbivore management. Firstly, total soil C levels remained similar among the three treatments. Conversely, prior savanna studies have shown substantial increases in soil C of 54% in the absence of herbivores for 17 years, with these gains primarily driven by C4 grasses (Wigley et al., 2020), however, this was observed on sandy soils in Kenya. Soil texture has been observed to be a larger indicator of changes to soil properties than herbivory levels (Andrés et al., 2016; Kotzé et al., 2017), with sandy soils more sensitive to grazing/herbivory intensification. Thus, shifts in C levels may be more obvious in sandy soils, unlike the clay soils in this study. Our total C finding has been observed in a similar length grazing exclusion study where seven years of grazing exclusion in upland grassland restoration project, UK, observed changes in plant communities aboveground but no effect on soil C stocks (Medina-Roldán et al., 2012). The authors suggested that whilst a certain level of grazing may be compatible with soil C storage, a longer period of time may be required to observe changes in C.

Secondly, there were no differences in total N across treatments, but we did observe an interesting, but not significant, trend emerged in plant available N, specifically ammonium. Our study found lower levels of ammonium in the presence of smaller herbivores (megaherbivore exclusion), and higher levels of NH_4 in the complete presence and absence of herbivores. N remains the most limiting and important soil nutrient in sub-Saharan Africa (Saito et al., 2019) and herbivores may increase or decrease soil N through dung and urine deposition. There are differences in the N: P stoichiometric ratios between the dung of large and small herbivores, but also grazers and browsers. Smaller herbivores and browser dung is generally higher in N content compared to megaherbivores and grazers (le Roux et al., 2020; Sitters et al., 2014a). Changes in the body size or grazer/browser composition of an herbivory community will shift the stoichiometric ratio of nutrients deposited into the soil. Therefore, the subtle differences seen in ammonium concentrations may not be because of body size, but due to herbivore feeding method. Grazers, such as zebras and wildebeest, fall into the category of smaller herbivores, while browsers such as giraffe and elephant, are megaherbivores. The lower content of N in the dung of smaller herbivore grazers in comparison to the megaherbivore browsers may result in a subtle reduction in soil ammonium when megaherbivores are excluded. This difference may be minimal as dung input is just a single factor that could impact changes in soil nutrient levels.

Thirdly, total P levels remained similar across the enclosure treatments, supported by Sitters *et al.*, (2020), who also found that total P pools remained unchanged across different animal size herbivory treatments. However, herbivores release P in the form of dung and urine (Sitters et al., 2017, 2014a) and so it is hypothesised that removal of herbivores would lead to a decline in total P. Le Roux et al., (2020) showed that dung from larger herbivores contained lower quantities of phosphorus and higher N:P ratio. Thus, the combination of reduction of dung biomass and herbivore body size may result in a decline in total P as herbivore exclusion increases and could become more apparent over a longer enclosure period. The effects of soil P have been less well-studied, but are just as important as C and N for savanna soil dynamics due to P limitation (Holdo and Mack, 2014).

The impact of herbivores can be influenced by the nutrient status of the soil. Bardgett and Wardle (2003) suggested that differences in soil nutrient status may determine the net direction of herbivore effects on nutrient cycling, such that positive effects occur under

fertile soil and negative effects under infertile soil conditions. This principle can also apply to more extreme environments, for example, in a low arctic meadow ecosystem, the effects of grazing removal were more marked at higher nutrient availabilities after 10 years of grazing removal (Francini et al., 2014). It is possible that in extreme environments such as arid savannas and subarctic forests, slower nutrient turnover rates may weaken the link between the aboveground and belowground processes. Consequently, the soil in these environments might exhibit high resilience or resistance to changes in herbivory, requiring much longer periods to observe differences. The four-year time-period of this study may not be sufficient to detect changes in these lower nutrient, arid soils.

Herbivory exclusion has previously been shown to have an influence on soil moisture and bulk density. Our study observed little difference in both soil moisture and bulk density across the different herbivore treatments. Conversely, also within KNP, a 35 year herbivore exclusion experiment observed that areas outside of exclusions experienced higher soil moisture retentions (Holdo and Mack, 2014), which was driven by bulk density changes. Denser soils with higher bulk densities often have a lower saturated conductivity and thus lower infiltration rates. Bulk density was higher with the presence of herbivores as large mammals increase compaction through trampling. The exclusion time period was over seven times longer than our study and whilst the specific duration for significant soil compaction will depend on environmental conditions and herbivore intensity, we may need a longer exclusion period than four years to observe significant differences.

Finally, we observed no differences in soil pH across the herbivory regimes. This finding is supported by other studies where herbivory did not have a significant effect on pH in savanna ecosystems (Angassa et al., 2012; Britz and Ward, 2007; Kgosikoma, 2013; Okullo and Moe, 2012).

4.4.4. Herbivore effects on soil microbial community

There were no herbivore effects on soil microbial community composition, irrespective of body size exclusion. Soil microbial communities play a fundamental role in ecosystems and studies are only just beginning to investigate how microbes mediate herbivore-environment interactions. It has not yet been established whether herbivore behaviour and microbial response are closely related and how herbivore-induced changes aboveground

are linked to changes in microbial biomass and microbial community composition. Reported effects of herbivory and grazing management on microbial activity and PLFA concentration are often inconsistent. This has been suggested to be due to the combination of the negative effects of grazing versus the positive effects of added nutrients through dung and urine on microbial communities (Oates et al., 2012). There are limited savanna exclosure studies that are looking at belowground properties (Staver et al., 2021) and particularly microbial communities in savanna ecosystems. This was additionally highlighted by Andriuzzi and Wall (2017) who performed a global meta-analysis investigating responses of belowground communities to large aboveground herbivores, where only two out of the 44 grassland studies were African savannas.

Large herbivores can impact microbes via direct pathways (dung and urine) or indirect (habitat modification). We hypothesised a decrease in the total PLFA biomarkers along the herbivore management gradient from the open plots to the mega and then full plots, as dung and urine inputs reduce. However, we observed no differences in total PLFA biomarkers across the three treatments. We also hypothesised that we would see a more bacteria dominated microbial community with the removal of herbivores. The absence of dung has been observed to reduce fungi proliferation (Bardgett et al., 1997). We also observed no shifts in the community composition, with both the fungal: bacteria and GP: GN biomarker ratio remaining unchanged across the treatments. A study investigating the effects of grazing management on microbial community composition in a semi-arid system observed that microbial communities responded more strongly to differences in soil texture than grazing management (Kotzé et al., 2017). PLFA concentrations recorded on clayey soils are usually higher in numbers than sandy soils, but the total PLFA biomarkers observed in this study are up to 10 times lower than sandy savanna ecosystems in South Africa and 26 times smaller than clayey grasslands in SA (Kotzé et al., 2017). To the best of our knowledge, outside of this thesis, PLFA biomarkers have never been used to measure the impact of herbivory within KNP before.

A number of other studies have failed to see differences in PLFA biomarkers with different grazing treatments. With smaller mammal grazing, after seven years, PLFAs were not systematically affected by grazing across the temperate grasslands (Ford et al., 2013). Similarly, Ward et al. (2007) observed that with 50 years of grazing and fire manipulation on peatlands, aboveground changes occurred but alongside minimal changes in soil microbial properties. Any changes belowground were attributed to

burning, not grazing and changes in C dynamics were primarily related to changes in vegetation community structure. Thus, perhaps in similarity to this study, fire was a stronger driver of soil microbial community composition than herbivory. On the other hand, grazing has been shown to influence PLFA biomarkers, with just under forty years of sheep exclusion reducing PLFA fungi biomarker abundance, thus shifting the F: B ratio (Bardgett et al., 1997), linked to changes in root exudates, microbial activity and dung deposition. With a much shorter time frame, Aldezabal et al. (2015) found that soil microbial communities in Atlantic mountain grasslands were very sensitive to livestock grazing after five years. Furthermore, winter herbivore exclusion methods impacted aboveground vegetation which further influenced changes in microbial community (Peschel et al., 2015). Further research is necessary to comprehensively understand how changes in large mammal herbivory could shift savanna microbial community composition.

Correspondingly to the PLFA biomarkers, we saw no changes in microbial biomass across the treatments. Within African savannas, Sankaran and Augustine (2004) observed that with only two years of cattle exclusion, microbial biomass was greater in soil within exclosures. This was attributed to the herbivores diverting plant C away from the soils. However, cattle grazing is often at higher and more intense densities, which could have a stronger and faster influence on the size of the microbial community.

4.4.5. Conclusion

We show that short-term removal of large herbivore and megaherbivores has little effect on soil properties, which may be exacerbated by the presence of a prescribed burn or low herbivore-grass biomass densities. The minimal changes to belowground properties as a result of different herbivore treatments are consistent with the limited aboveground changes observed in other studies on KNP's BROWSE plots. To comprehensively understand the long-term effects of body size on soil properties, specifically within KNP, additional soil data is needed over a longer time period. Furthermore, this study highlights the need for further research to understand the complex interactions between herbivores, fire, and soil dynamics in savanna ecosystems.

4.5. Acknowledgments

We gratefully acknowledge the BROWSE team, with special recognition for Dr Lawrence Kruger for the pre-treatment sampling of the sites in 2018. Additionally, we extend our

thanks to KNP Scientific Services game guards for continued maintenance of the exclosures. I also extend my appreciation to Sam Walrond and Theo Ndlovo for their help in the collection of samples, as well as Anna Hook and Alice Scotland for their assistance in the lab.

5. The individual and interactive effect of fire and herbivory on savanna soil properties

Cvetkovic-Jones, M.^{1,2}, Stevens, C.J.¹, Whitaker, J.², Archibald S.³, Situngu S.³,
Strydom T.⁴, Staver A.C.⁵, Ostle, N.¹, Parr, C.L.^{3,6,7}

¹Lancaster Environment Centre, Lancaster University, Library Avenue, Bailrigg, Lancaster, LA1 4YQ, United Kingdom

²UK Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster, LA1 4AP, United Kingdom

³School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa

⁴Scientific Services, South African National Parks, Private Bag X402, Skukuza, 1350, South Africa

⁵Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA

⁶School of Environmental Sciences, University of Liverpool, Liverpool, L69 3GP, United Kingdom

⁷Department of Zoology & Entomology, University of Pretoria, Pretoria, South Africa



Picture credit: Mary Cvetkovic-Jones

5.1. Introduction

Fire and herbivory are key drivers influencing the spatial and temporal variations in woody and grass biomass that characterise savannas (Bond et al., 2005; Higgins et al., 2007; Staver et al., 2021; Van Langevelde et al., 2003), alongside other abiotic properties such as rainfall and soil properties. Fire and herbivory are known drivers of ecosystem dynamics, influencing the composition, density, and structure of aboveground plant communities (Bardgett and Wardle, 2003; Higgins et al., 2007; McLauchlan et al., 2020; Pringle et al., 2023; Staver et al., 2021), but may also alter the physical, chemical and biological properties belowground (Alcañiz et al., 2018; Gordon et al., 2019; Knicker, 2007; Pringle et al., 2023; Sitters et al., 2020b). Savanna studies have often focussed on the impacts of fire and herbivory independently, however it is increasingly apparent that the combined effects of fire x herbivory are not the sum of the separate individual effects (Bond et al., 2005; Bond and Keeley, 2005; Fuhlendorf and Engle, 2004; Sankaran et al., 2005; Smit and Coetsee, 2019) and should be viewed as a single driver (Fuhlendorf and Engle, 2004; Smit and Coetsee, 2019). Understanding the fire and herbivory synergistic interaction is essential for managing and conserving fire-prone ecosystems, where herbivores also play a significant ecological role.

Fires often occur over large areas, exerting a homogenous impact on the savanna landscape (Archibald et al., 2005). Recently burnt grass increases grazer pressure as herbivores are attracted to the fresh re-growth. However, in the aftermath of such large burns, individual grasses are under reduced grazing pressure as grazing is dispersed, consequently promoting unpalatable, fire-adapted plant communities (Archibald et al., 2005; Archibald and Bond, 2004). However, smaller repeated fires, concentrate grazers at higher densities, promoting short-grass areas, or grazing lawns (Donaldson et al., 2018; Singh et al., 2024). Grazing lawns are crucial components of savanna landscape heterogeneity, serving as vital habitats for biodiversity and playing a key role in sustaining large herbivore populations (Hempson et al., 2015), primarily attributed to the higher quality of grasses found on these lawns (Cromsigt et al., 2017). Grazing lawns are an important part of savanna fire and herbivore management (KNP Fire Protocol, 2019), but there is limited understanding of belowground activities and processes during the development of grazing lawns.

Immediately post-burn, herbivores are attracted to the highly nutritious regrowth, which is high in N and low in carbs and fibre (Goldammer and Ronde, 2004), concentrating herbivores on recently burnt areas (Donaldson et al., 2018). The increased herbivore grazing densities could further alter the quality and quantity of inputs to the soil through trampling, dung, and urine deposition. Dung and urine can provide an input of N and phosphorus (P) to the soil, often limiting nutrients in savannas, promoting a fungal-dominating community (Bardgett et al., 1997; Brachmann, 2023). The increase in herbivores as a result of the burn may also cause trampling of ash into the soil, increasing labile C and increasing soil pH (Mataix-Solera et al., 2009; Pyne, 2019). However, herbivore grazing can reduce soil nutrient levels by removing vegetation and also increasing bare ground. Herbivores also influence plant dynamics, affecting regrowth, altering interactions between plants and soils. Furthermore, the consumption of grass biomass alters the fuel load, time to the next fire, fire spread and fire intensity (Archibald and Hempson, 2016a). Herbivores may also preferentially consume plant species that differ in flammability, indirectly influencing fire behaviour (Anderson et al., 2007). To add further complexity, there are many abiotic factors that affect fire, herbivory and soil properties including herbivore species, population sizes, soil type, rainfall, fire season, fire size and fire intensity, which all have multiple feedback loops (Archibald and Hempson, 2016a; Donaldson et al., 2018; Smit and Coetsee, 2019; Van Langevelde et al., 2003). The feedback loops are often context specific so can result in either positive or negative impacts on the soil nutrients, microbial community, and activity. This repeated interaction of fire x herbivory over repeated burn cycles can further strengthen this interaction.

In African savannas, there have been several long-term, large fire (Biggs et al., 2003; Kimuyu et al., 2017; Mills and Fey, 2004; van Wilgen et al., 2022; Wilgen et al., 2007) and long-term herbivore studies (Goheen et al., 2010; Guy et al., 2021; Long et al., 2017; Moncrieff et al., 2011; Pringle et al., 2007; Sankaran and Augustine, 2004; Sitters et al., 2020a; van der Plas et al., 2016; Wigley et al., 2020; Young et al., 2003). Whilst there are savanna studies investigating both fire x herbivory on aboveground impacts (Donaldson et al., 2018; Ngugi et al., 2022; Singh et al., 2024), comprehensive investigations into the belowground consequences of fire x herbivore interactions, especially following smaller-scale, repeated fires, are limited.

To explore the individual and interactive effects of fire and herbivory on soil nutrients and microbial communities in savanna grasslands, we applied an early season fire in May 2021 followed by a late season fire in September 2022, with and without herbivore exclusion. Before and after the early season fire in May 2021 we took soil samples to examine the interactive effects of fire x herbivory on soil nutrient concentrations, microbial biomass, and microbial community composition to address two questions: Firstly, ‘does the interactive effect of fire x herbivory increase soil nutrient availability and result in a shift to a more fungal-dominated microbial community?’ Fungi is often more sensitive to burning initially, but has been shown to recover in the presence of herbivory (San Emeterio et al., 2023). Following the late season fires in September 2022 we monitored soil respiration for nine months to observe the interactive effects of fire x herbivory on the resilience of soil microbial functioning, asking ‘is the resilience of soil microbial functioning to repeated fire, influenced by the presence or absence of herbivory?’ We expected to observe an initial decrease in soil respiration with fire x herbivory immediately post-fire as a direct impact of burning, followed by increased respiration rates as time since fire increases, as microbial activity recovers in the presence of herbivory.

5.2. Methods

5.2.1. Study Area

The study was located at between Lower Sabie and Crocodile Bridge (31°54' E, 25°13' S) within Kruger National Park (KNP), South Africa. The site is located within the southern basalt region of KNP, with an annual precipitation averages 610 mm, accompanied by a mean annual temperature of 22 °C (Hijmans et al., 2005; Smit et al., 2013b). The site exhibits a mean fire return interval of 4.05 years (Smit et al., 2013b) and sustains an average herbivore biomass of 44 kg ha⁻¹ (Smit et al., 2013a). The dominant grass species are *Themeda triandra* and *Megathyrsus maximum*. Megaherbivores present at our site include elephants (*Loxodonta Africana*), southern giraffe (*Giraffa giraffa*) and white rhino (*Ceratotherium simum*) (Abraham et al., 2019; Chase et al., 2016; McCleery et al., 2018). Other large herbivores found commonly in the site include plains zebra (*Equus quagga burchellii*), cape buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*) and blue wildebeest (*Connochaetes taurinus*).

This study investigated the response of soil C, nutrient concentrations, microbial community composition and soil respiration to differing combinations of fire and

herbivory. The study involved two separate burning events, starting with early season burns in 2021, followed by herbivore exclusions, and culminating with a late-season burn in 2022. Figure 5.1 provides an overview of the study timeline, which is then elaborated upon below in the Methods.

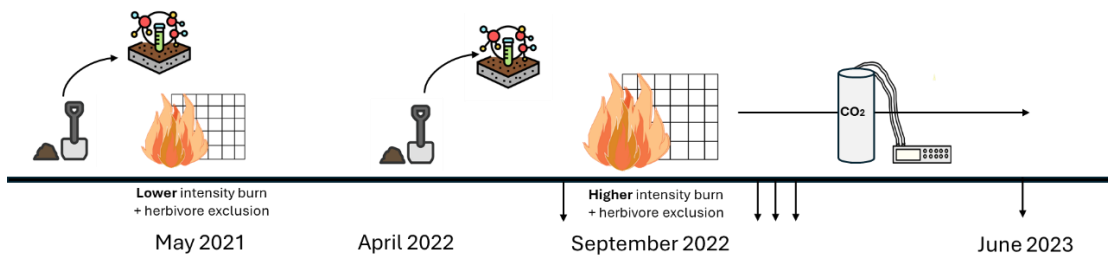


Figure 5.1 - Study timeline spanning two years of sampling. Initial surveys and soil biogeochemical and microbial analyses were conducted prior to prescribed burns in May 2021. Following these early season and lower intensity burns, herbivore exclusions were erected. In April 2022, one-year post-burn and herbivore exclusion, plots were re-sampled for biogeochemical and microbial analyses. In September 2022, late season, higher intensity burns were performed on the plots, with soil respiration rates (indicated by arrows) measured over the subsequent nine months.

5.2.2. Pre-Treatment Soil Sampling

In May 2021, initial soil samples were gathered across the study site to assess baseline levels and variation in soil biogeochemical and microbial properties. These pre-treatment samples were sampled from the upper layer (0-10 cm depth) directly beneath four grass species: *Themeda triandra*, *Panicum coloratum*, *Urochloa mosambicensis*, *Bothriochloa insculpta*, and also beneath bare ground (total of 80 samples), across the study site. We chose to sample directly under these four different species because we anticipated that changes in the soil rhizosphere of the species would be most evident after only one burn and one year of herbivory. Subsequently, subsamples were shipped immediately to Lancaster University for analysis.

No significant differences in any soil biogeochemical or microbial properties between the grass species (Table 5.2) or across the site were found. Furthermore, when species regenerated post fire, the experimental site became dominated by *Themeda triandra*, thereby rendering the species-based approach irrelevant. Dominance by *Themeda triandra* following a burn is not unusual, as it relies on periodic burning and is known to rapidly resprout post burn, with both fire and herbivory necessary for its persistence in an ecosystem (Bond et al., 2003; Dunning et al., 2017; Morgan and Lunt, 1999).



Figure 5.2 - Pictures from the site following the first set of early season burns in May 2021; a) low intensity early season burn (picture credit, S Archibald), b) 2 m² enclosure installed immediately after the burns, surrounded by *Themeda triandra*, pictured in April 2022 (picture credit, T Strydom).

5.2.3. Prescribed Burns in 2021

Following pre-treatment sampling in May 2021, eight experimental plots (100 m x 100 m) were established. In May 2021, four plots were burnt with early dry season fires, whilst

four remained unburnt (Figure 5.2a). May is at the beginning of the dry season, so fires are often of a lower intensity than later in the dry season, as grass is often greener and wetter (Govender et al., 2006). Fires were burnt at a low intensity (Table 5.1) and in the days immediately following the fires, a reinforced steel cage enclosure (2 m x 2 m x 1.2 m) was randomly placed in each of the eight plots (Figure 5.2b). This resulted in four separate treatments: Fire + Herbivory, Fire, Herbivory, No Fire-No Herbivory.

During the burns, fire behaviour measurements were collected across the four burnt plots (Table 5.1). Percentage of the plot burnt was calculated by identifying whether the area of 100 plot locations was burnt or not burnt. Fuel moisture content (%) was calculated using three replicates of grass biomass dried at 60°C for 48 hours. Fire temperature was assessed using thermocouples replicated five times across the plot. Two of the thermocouple wires were aboveground facing in opposite directions (East and West) and the remaining two wires were placed at 1 cm and 5 cm belowground. Grass biomass measurements were collected pre and post burn using a disc pasture meter, calibrated to KNP (Zambatis et al., 2006).

Table 5.1 - Fire behaviour measures across the four burnt plots in May 2021, including percentage of plot burnt (area burnt assessed over 100 plot locations), average fuel moisture content (%), fire temperature (°C) aboveground using three sets of thermocouples with two wires aboveground, fire temperature (°C) belowground with a wire at 5cm, grass biomass measurements pre burn, post burn and the difference (kg ha⁻¹). Mean values recorded ±Standard Error. Data collected by Archibald et al, (personal comms, 2023).

Fire Behaviour Measures	Experimental Fire 1	Experimental Fire 2	Experimental Fire 3	Experimental Fire 4
% of plot burnt	96	74	97	78
Fuel Moisture Content (%)	54.10	50.92	58.04	49.97
Fire temp 1 cm aboveground	258.75 (±34.98)	292.83 (±85.06)	326.17 (±76.12)	361.33 (±54.22)
Fire temp 5 cm belowground	19.75(±0.96)	20.67(±0.67)	21.00(±0.58)	19.00(±0.00)
Grass biomass pre-burn	4240	4771.8	4973.1	3778.5
Grass biomass post-burn	2192.4	2814.5	2410.5	2667.0

Grass biomass burn reduction	- 2047.6	- 1957.3	- 2563.3	- 1111.5
------------------------------	----------	----------	----------	----------

5.2.4. Post Treatment Soil Sampling

In May 2022, one year after the first set of burns, soil samples were collected from the plots. Soils were sampled to a depth of 10 cm under *Themeda triandra*. Five samples were collected in an X shape within the enclosure on each of the eight plots. A further five samples were also collected in an X shape outside of the enclosure, 5 m away from the enclosure (for a total of 80 samples). Upon collection, samples were transported to a KNP laboratory, where they were stored at 4 °C before being transported to Lancaster University, UK using ice gel packs. Once at the university samples were stored at 4 °C and analysed within 14 days of collection.

5.2.5. Soil Biotic and Abiotic Analyses

All pre-treatment samples collected in 2021 and post-treatment soil samples from 2022 underwent the same soil biotic and abiotic analyses, except for microbial biomass C, which was not performed on pre-treatment samples due to soil transportation and laboratory instrumentation issues. Soil pH was measured using a 10.0 ± 0.5 g subsample of fresh soil, mixed with 25 ml of deionised water, using a pH meter (InLab Expert Pro-ISM). Soil moisture was determined by using aliquots 1 ± 0.1 g of fresh soil, dried at 105°C for 48 hours. Moisture content was calculated as a percentage of the dry weight. Soil ammonium and nitrate was calculated as a measure of inorganic N in the soil which is directly available to the plants. 5 ± 0.1 g subsamples were extracted using 25 ml of 2 mol/l KCl. samples were filtered through Grade 42 Whatman filters. Inorganic N was analysed on an AA3 HR Auto Analyser (Seal Analytica, UK). Total soil phosphorus was measured through digestion of air dried, ball milled samples with a sulphuric acid-hydrogen peroxide digestion mixture. 0.2 ± 0.01 g samples were heated at 400 °C for three hours, before being diluted with MilliQ water to an acid content of 5% v/v. The samples were then vortexed and filtered through Grade 42 Whatman filters. Inorganic P was analysed on the auto analyser (Lancaster University, Seal Analytical AA3). Total soil C and N was measured by drying subsamples at 80°C and ball milling before measurement of C and N content of 20 mg on Elementar Vario EL CUBE CN analyser. Microbial biomass C was measured on 5 ± 0.5 g soil subsamples using the chloroform fumigation-incubation method (Brookes et al., 1985). Using paired subsamples one of the samples was fumigated with chloroform for 18 hours before both of the paired subsamples

were extracted via shaking in 25ml of 0.5M K₂SO₄. Following shaking, the samples were filtered through Grade 42 Whatman filters. Microbial biomass C was analysed using a 5000A TOC Analyser (Lancaster University, Shimadzu, Japan). Microbial C was calculated as difference between fumigated and unfumigated soil, using the general correction factor of 0.45 for microbial biomass C (Brookes et al., 1985; Joergensen and Mueller, 1996; Wu et al., 1990).

Phospholipid Fatty Acid (PLFAs) analysis was performed to measure both the soil microbial community size and composition (nmol g⁻¹ soil dwt.). Total lipid was extracted from ~ 1.5 g of freeze-dried soil subsamples and using a modified Bligh and Dyer (1959) method (Frostegård et al., 1991), lipids were collected on a column. Neutral and glycolipids were eluted from the column with chloroform and acetone and discarded. Polar lipids were eluted with methanol and collected. These lipids were quantified and identified by chromatographic retention time and mass-spectral comparison using 19:0 (methyl nonadecanoate fatty acid) as an internal standard. Gas Chromatography was carried out on Agilent Technologies (UK) 6890N GC Network System, equipped with Agilent 7683 Series Injector. Gram-negative bacteria was identified by cyclopropyl saturated (7cy-17:0, 7,8cy-19:0) and monosaturated fatty acids (16:1 ω 5, 16:1 ω 7, 18:1 ω 7) and gram positive were identified by ester-linked branched-chain fatty acids (15:0i, 15:0a, 16:0i, 7Me-17:0, 17:0i, 17:0a) (Rinnan and Bååth, 2009; Whitaker et al., 2014). Fungi were identified using 18:2 ω 6,9 and 18:10 ω 9 fatty acids, indicative of ectomycorrhizal and saprotrophic fungi (Kaiser et al., 2010). Finally, total PLFA values were identified using all PLFAs measured (14:0, 16:1, 16:0, 17:1 ω 8, br17:0, br18:0, 18:1 ω 5, 18:0, 19:1; plus, the other fatty acids listed above). The ratios of gram-positive to gram-negative (GP:GN) and fungal: bacterial (F: B) were calculated to determine the relative abundances of each group.

5.2.6. Prescribed burns in 2022

In September 2022, the same four plots, now containing the exclosures, were burnt again (Figure 5.3). The four unburnt plots did not have prescribed fires applied. September is at the end of the dry season, so fires are often of a hotter intensity as the grass has dried out (Govender et al., 2006). Usually, with higher intensity fires we observe a greater amount of biomass consumed and lower patchiness. However, fire intensity data were not collected from these fires due to logistical issues. Satellite measures of fire radiative

power (FRP) or burn scars on trees were not used as a proxy for fire intensity due to concerns over the accuracy due to spatial resolution and number of trees on the burn plots.



Figure 5.3 - Pictures from the site following the second set of late season burns in September 2022; a) prescribed burns at the end of the dry season in September 2022, b) the burn plots at three weeks post-burn with blue wildebeest grazing and c)

difference in grass height and biomass with and without herbivory four months post-burn (Picture credit, T Strydom, January 2023).

5.2.7. Soil Respiration

Following the September 2022 burns, soil respiration measurements were conducted on all burnt and unburnt plots, replicated five times inside and outside the enclosure, in the same X shape design, referred to *Section 5.2.4*. Respiration rates were assessed at multiple time points: one day prior to the burns, one week, three weeks, five weeks and nine months post-burn. CO₂ sampling in the field was conducted using an EGM-4 IRGA with an SRC-1 soil respiration chamber (15 cm height x 10 cm diameter) (PP Systems, USA). The chamber was placed on the soil, between the grass tussocks, and CO₂ concentration (ppm) was recorded for a period of 60 seconds with a 15 second dead band. The rate of change in ppm was determined via linear regression and CO₂ respiration rates were calculated as described in Holland et al. (1999).

5.2.8. Gravimetric Soil Moisture

Simultaneously, gravimetric field soil moisture was collected to a depth of 8 cm during the respiration sampling at the same five time points. Samples were dried at 105°C for 48 hours in the KNP laboratory and moisture content was calculated as a percentage of the dry weight.

5.2.9. Vegetation Sampling

Disc Pasture Meter measurements (DPM) were used to assess grass biomass on all plots. Measurements were conducted both before and directly after the burns in May 2021, followed by additional assessments prior to the burns in September 2022 and after the burns in September 2023. Unfortunately, in September 2023, unburnt plots grass biomass was not collected due to logistical restrictions. Grass biomass (kg ha⁻¹) was indexed as DPM settling height (Zambatis et al., 2006). Due to size limitations within the enclosure, five replicates were obtained from inside the enclosures, while 100 replicates were performed outside the enclosure.

5.2.10. Statistical Analysis

All statistical analyses were conducted in R (4.1.1) with significance reported at $p \leq 0.05$ level. Normality was checked using QQ plots, residuals, and Shapiro Wilks tests. The effect of fire and/or herbivory pre- and post- treatment on soil biogeochemical parameters

(pH, soil moisture, C and N content and ratio, microbial biomass C, nitrate and ammonium) and microbial community composition (PLFAs) and grass biomass was determined using a general linear model, incorporating plot replication as a random effect. To address spatial pseudo replication in field sampling, data were initially averaged by soil core replications within plots.

The effect of fire x herbivory on soil respiration and soil moisture were analysed using separate generalised additive models (GAMs) with repeated measures performed using *gam4* (Wood & Scheipl, 2020). Generalised additive mixed models (GAMMs) were employed with fire and herbivory regime as a fixed effect, time since fire as a non-linear random effect (spline, *gam4* function; Wood & Scheipl, 2020), plot replication and nested plot respiration replicates (to mitigate spatial pseudo replication) as random effects. To improve normality, soil respiration data were square root transformed prior to analyses.

The relationship between soil respiration and soil moisture for all treatments was assessed using a linear regression analysis.

5.3. Results

5.3.1. Pre-Treatment Soil Properties

There were no significant differences across any of the pre-treatment soil analyses, under the four grass species and bare ground (Table 5.2). Due to the monoculture regrowth of *Themeda triandra*, the species-based approach was discontinued.

Soil abiotic properties did not vary across the species prior to treatment application. There was no evidence for a difference in soil pH (6.94 ± 0.03 SE) with different grass species ($p=0.87$). Soil moisture did not vary across the treatments, sitting between 18-20 % ($p=0.88$). Total C and N also did not vary pre-treatments ($p=0.73$ and 0.88 respectively). As there were no differences in total C and N, we saw limited variation in C: N ratio, at an average of 10.38 ($p=0.69$). Furthermore, we saw no significant variation in total P ($p=0.64$), with an average of 537 mg kg^{-1} . Although pre-treatment plant available nitrate exhibited heterogeneity, the differences were not significant ($p=0.38$), while ammonium levels remained consistent across species ($p=0.91$).

When analysing pre-treatment microbial community composition, we found our pre-treatment PLFA concentrations were nearly half the concentration of our post-treatment

microbial levels, although both were sampled at the same time of year, suggesting a potential influence of various factors such as soil transport, storage duration, or seasonal variations. We did not observe any significant differences between total PLFA biomarkers ($p=0.82$), fungal biomarkers ($p=0.48$) and bacterial biomarkers ($p=0.98$) under different plant species. Similarly, the distribution of gram-positive bacteria biomarkers ($p=0.95$), gram-negative bacteria ($p=0.90$) and the gram-positive: gram-negative (GP: GN) bacteria ($p=0.44$) did not vary, indicating a uniform microbial community composition prior to the fire and herbivory treatments.

Table 5.2 - Survey of pre-treatment area, under four plant species and bare ground, on soil properties (mean \pm SE), analysed by linear model. Soil abiotic properties include C: N ratio, soil pH, soil moisture (%), ammonium ($\mu\text{g NH}_4 \text{ g}^{-1}$ soil dwt), nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt) and total phosphorus (mg kg^{-1}). Soil biotic properties include PLFA measurements (nmol g^{-1} soil dwt).

Soil Properties	Bare Ground	<i>Bothriochloa insculpta</i>	<i>Panicum coloratum</i>	<i>Themeda triandra</i>	<i>Urochloa mosambicensis</i>	p value
Soil Moisture	19.1 (± 1.89)	20.4 (± 1.24)	19.0 (± 1.43)	20.2 (± 1.56)	18.7 (± 0.97)	0.88
pH	6.98 (± 0.08)	6.95 (± 0.05)	6.97 (± 0.11)	7.01 (± 0.10)	6.88 (± 0.07)	0.87
Total C (%)	3.28 (± 0.17)	3.49 (± 0.17)	3.17 (± 0.20)	3.28 (± 0.17)	3.28 (± 0.13)	0.73
Total N (%)	0.32 (± 0.01)	0.32 (± 0.01)	0.32 (± 0.01)	0.31 (± 0.01)	0.32 (± 0.01)	0.88
C: N	10.3 (± 0.37)	10.8 (± 0.33)	10.0 (± 0.44)	10.4 (± 0.45)	10.4 (± 0.31)	0.69
Total Phosphorus	544 (± 37)	521 (± 21.5)	527 (± 25.5)	578 (± 43)	516 (± 23.3)	0.64
Ammonium	2.59 (± 1.30)	1.68 (± 0.87)	4.09 (± 2.82)	3.10 (± 1.14)	3.00 (± 1.96)	0.91
Nitrate	4.9 (± 2.84)	1.27 (± 0.60)	3.77 (± 0.86)	1.40 (± 0.40)	2.88 (± 1.28)	0.38
Total PLFA	32.2 (± 4.86)	40.6 (± 8.40)	36.4 (± 4.59)	35.3 (± 5.82)	42.8 (± 9.62)	0.82
Bacterial PLFA	17.7 (± 3.05)	19.2 (± 4.31)	19.5 (± 2.92)	18.1 (± 2.83)	20.2 (± 4.22)	0.98
Fungal PLFA	4.60 (± 1.16)	7.83 (± 1.76)	5.42 (± 1.00)	5.77 (± 1.20)	7.55 (± 2.16)	0.48
F: B	0.28 (± 0.05)	0.43 (± 0.05)	0.30 (± 0.04)	0.31 (± 0.03)	0.36 (± 0.04)	0.31
GP PLFA	11.1 (± 1.90)	12.2 (± 3.08)	11.2 (± 2.26)	9.96 (± 2.07)	12.4 (± 2.77)	0.95
GN PLFA	5.64 (± 1.52)	5.79 (± 1.89)	7.23 (± 1.17)	7.20 (± 1.35)	6.61 (± 1.39)	0.90
GP: GN	2.72 (± 0.62)	1.60 (± 0.47)	1.87 (± 0.44)	1.58 (± 0.42)	1.99 (± 0.36)	0.44

5.3.2. Post Treatment Soil Properties

Following the early season burns in 2021 and one year of herbivore exclusion, no differences were observed in soil pH across the four fire and herbivore treatments

($p=0.60$) (Table 5.3). Soil moisture levels also did not vary among treatments, and were slightly higher than pre-treatment levels, at 24-27 % ($p=0.42$).

No significant differences were observed in the levels of C, N or P across the four treatments. Total C and total N did not differ across the four treatments ($p= 0.34$ and 0.44 respectively). Thus, the C: N ratio exhibited no significant variations across the fire-herbivory regimes ($p=0.41$). However, it is noted that fire x herbivory had much less variation than the other three treatments (Table 5.3). Total P ($p=0.41$) also showed no significant differences across the four treatments.

There were also no significant differences in ammonium levels across the fire-herbivory treatments ($p=0.56$). However, plant available nitrate was significantly higher in the fire x herbivory treatment than the other three treatments ($p=0.01$). The fire x herbivory interaction substantially increased nitrate concentration, reaching approximately three times higher concentrations, with limited differences across the other three treatments (Figure 5.4).

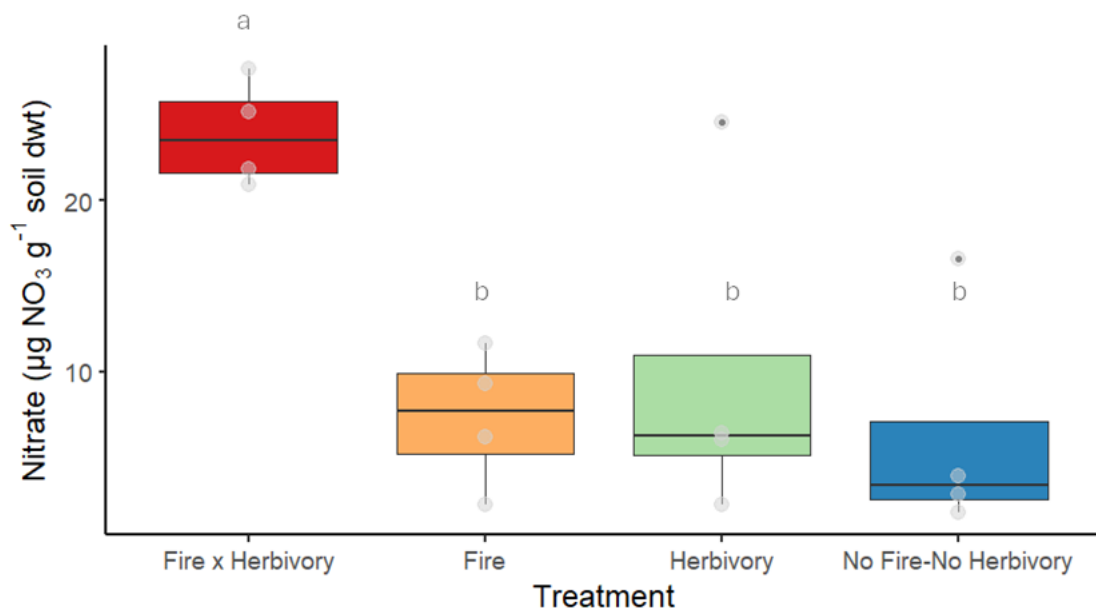


Figure 5.4 - Effects of fire x herbivory, fire, herbivory and no fire-no herbivory treatments on soil nitrate ($\mu\text{g NO}_3 \text{ g}^{-1}$ soil dwt) levels after a single early season burn and one year of herbivore exclusion. Letters denote significance at $p \leq 0.01$ level.

We observed no significant differences in the microbial community composition, but notably did observe descriptive trends in the PLFA biomarker concentrations. Total

PLFAs exhibited a general downwards trend ($p=0.25$), starting with the highest concentrations when both drivers, fire x herbivory, were present and gradually decreasing until no drivers present (Figure 5.5a). Similar trends were observed with fungal biomarkers ($p=0.11$) (Figure 5.5b). Bacterial PLFAs showed very limited differences across the four treatments ($p=0.77$) (Figure 5.5c). Notably, there was very little variation in the bacterial community for the fire x herbivory treatment compared with the other three treatments. The subtle shift in the fungal community composition led to limited change in the fungal: bacterial ratio between the treatments ($p=0.31$) (Figure 5.5d).

This subtle downward trend persisted with gram-positive bacteria ($p=0.15$) (Figure 5.5e). With gram-negative populations, there is no evidence for any difference among the treatments ($p=0.91$, Figure 5.5f). Thus, there were no significant impacts to the GP: GN ratio, resulting in a ratio close to 1 ($p=0.38$).

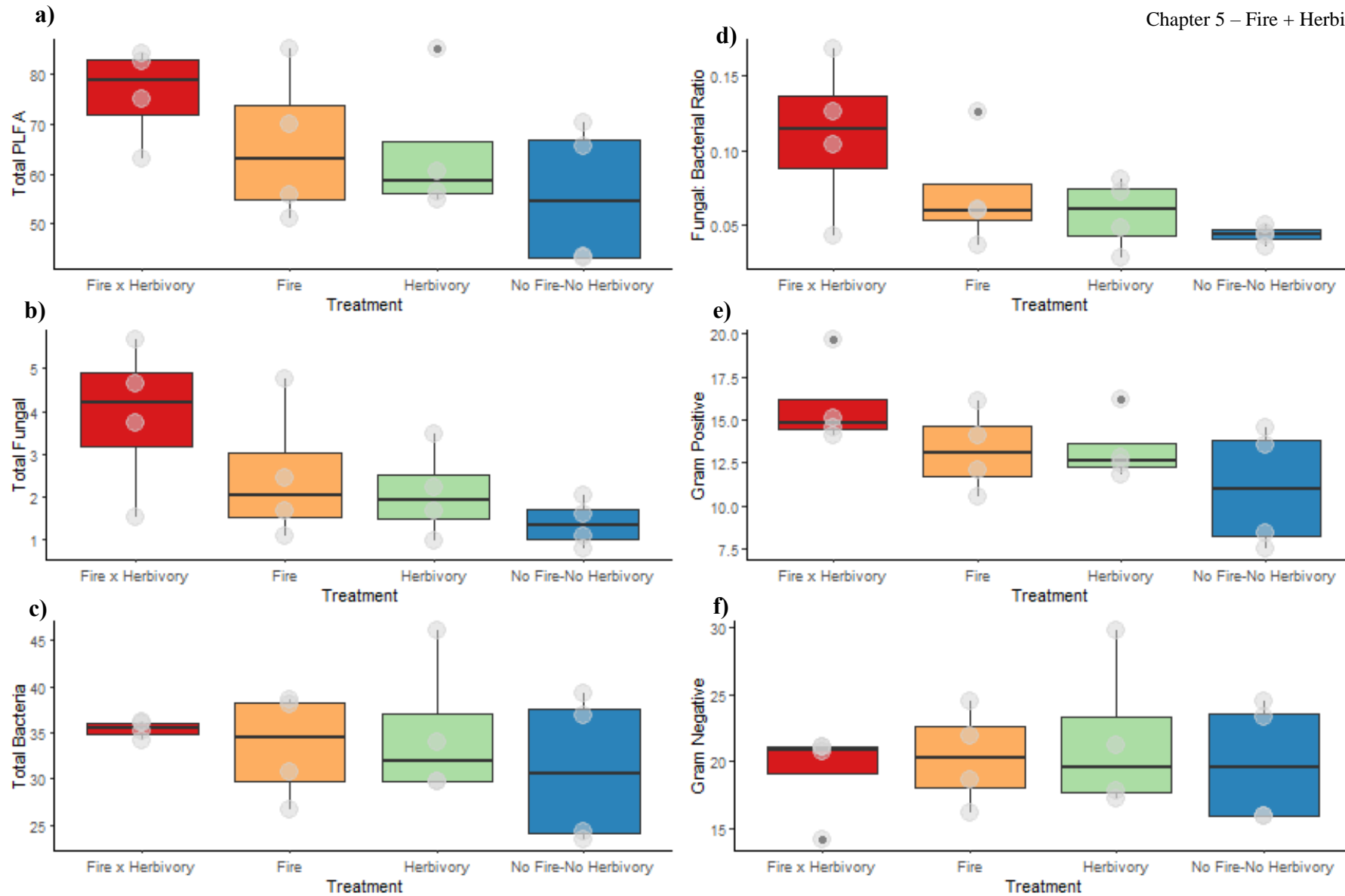


Figure 5.5 - Effects of fire x herbivory, fire, herbivory and no fire-no herbivory treatments on soil a) total PLFA, b) total fungal, c) total bacteria, d) fungal: bacteria ratio, e) gram positive bacteria, f) gram negative bacteria biomarkers (nmol g^{-1} soil dwt), one year post burn and herbivore exclusion.

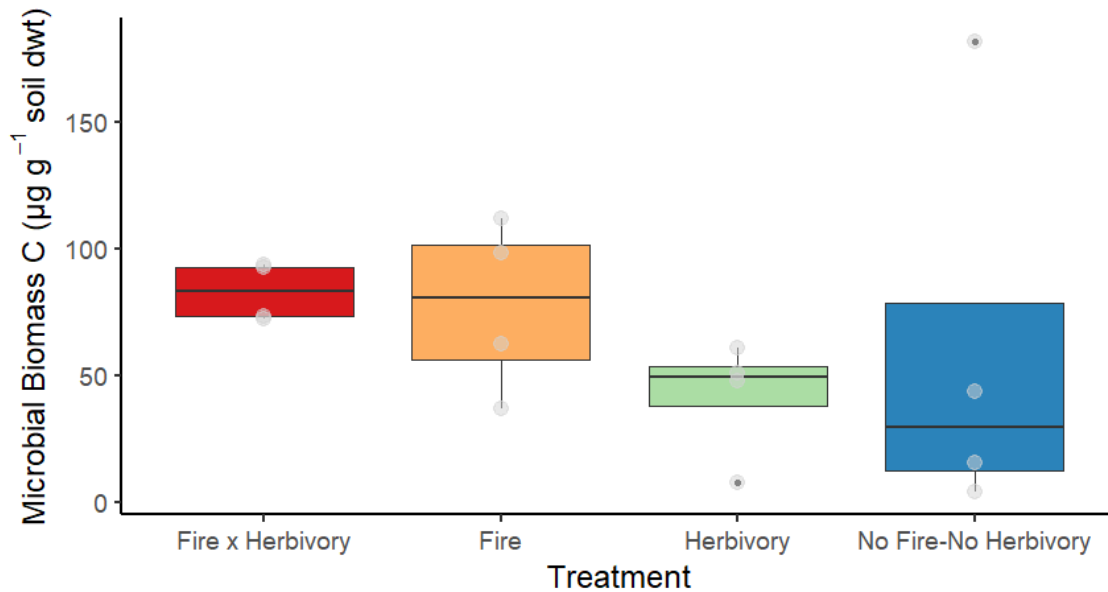


Figure 5.6 - Effects of fire x herbivory, fire, herbivory and no fire-no herbivory treatments on soil microbial biomass ($\mu\text{g g}^{-1}$ soil dwt), one-year post-burn and herbivore exclusion. Whilst this trend is not significant ($p=0.61$), it shows a descriptive trend that matches descriptive trends in total PLFA biomass.

Minimal differences were observed in microbial biomass C among the fire-herbivore treatments ($p=0.61$). However, a similar descriptive trend to total PLFAs was noted (Figure 5.6), supporting the microbial community dynamic shifts, in response to fire and herbivory.

Table 5.3 - Effects of different fire and herbivory regimes, one-year post-burn and herbivore exclusion, on soil properties ($M \pm SE$) analysed by linear model. Soil abiotic properties include C: N ratio, soil pH, soil moisture (%), ammonium ($\mu\text{g NH}_4 \text{g}^{-1}$ soil dwt), nitrate ($\mu\text{g NO}_3 \text{g}^{-1}$ soil dwt) and total phosphorus (mg kg^{-1}). Soil biotic properties include microbial biomass C ($\mu\text{g g}^{-1}$ soil dwt) and PLFA measurements (nmol g^{-1} soil dwt). Significant values are in bold.

Soil Properties	Fire x Herbivory	Fire	Herbivory	No Fire - No Herbivory	p value
Soil Moisture	23.9 (± 1.48)	24.0 (± 1.18)	26.9 (± 1.76)	24.6 (± 0.93)	0.42
pH	6.86 (± 0.06)	6.80 (± 0.19)	6.89 (± 0.11)	7.02 (± 0.04)	0.60
Total C (%)	2.95 (± 0.08)	2.83 (± 0.16)	3.34 (± 0.232)	3.01 (± 0.27)	0.34

Total N (%)	0.15 (± 0.01)	0.15 (± 0.01)	0.17 (± 0.01)	0.15 (± 0.01)	0.44
C: N	19.9 (± 0.18)	19.2 (± 0.69)	20.1 (± 0.82)	20.7 (± 0.53)	0.41
Total Phosphorus	476 (± 111)	407 (± 53.5)	331 (± 33.8)	344 (± 20.3)	0.41
Microbial Biomass C	82.9 (± 5.85)	77.4 (± 17.1)	42.0 (± 11.7)	61.3 (± 41.0)	0.61
Ammonium	1.34 (± 1.04)	0.61 (± 0.23)	0.83 (± 0.35)	1.78 (± 0.54)	0.56
Nitrate	23.9 (± 1.57)	7.32 (± 2.04)	9.8 (± 5.01)	6.25 (± 3.46)	0.01
Total PLFA	76.0 (± 4.75)	65.4 (± 7.58)	64.1 (± 6.97)	55.6 (± 7.14)	0.25
Bacterial PLFA	35.4 (± 0.46)	33.5 (± 2.9)	34.9 (± 3.84)	31.0 (± 4.13)	0.77
Fungal PLFA	3.89 (± 0.88)	2.49 (± 0.81)	2.08 (± 0.53)	1.38 (± 0.27)	0.11
F: B	0.11 (± 0.03)	0.07 (± 0.02)	0.06 (± 0.01)	0.04 (± 0.00)	0.09
GP PLFA	15.8 (± 1.28)	13.2 (± 1.21)	13.3 (± 0.98)	11.0 (± 1.79)	0.15
GN PLFA	19.3 (± 1.71)	20.3 (± 1.84)	21.5 (± 2.88)	19.9 (± 2.32)	0.91
GP: GN	2.29 (± 1.59)	0.67 (± 0.04)	0.65 (± 0.05)	0.54 (± 0.03)	0.38

5.3.3. Impacts of fire and herbivory treatments on soil respiration

Following the late-season burns in 2022, both treatment ($p=0.012$, $F=3.66$) and time since fire ($p<0.01$, $F=3.38$) had a significant effect on soil respiration (Figure 5.7). Prior to the burns, herbivory alone had the highest soil respiration, a potential legacy effect of the 2021 burns. Following the burns, there was a decline in respiration on all treatments and a convergence of treatments at week one where the soil respiration rates were notably similar. However, at week three and week five post-burn, a divergence is apparent between the fire and no-fire treatments, with fire treatments having reduced respiration rates in comparison. This divergence was further amplified after the rainy season in month nine, where distinct differences were observed among the treatments. The no-fire treatments, both with and without herbivory, maintained similar respiration rates to those observed at week five post-burn at $115 (\pm 4.44)$ and $115 (\pm 5.43)$ $\text{mg CO}_2\text{-C m}^2 \text{ hr}^{-1}$ respectively. Conversely, the fire plots displayed higher soil respiration rates, with the fire-only treatment reaching the highest peak at $218 (\pm 15.6)$ $\text{mg CO}_2\text{-C m}^2 \text{ hr}^{-1}$, followed by fire x herbivory at $165 (\pm 5.64)$ $\text{mg CO}_2\text{-C m}^2 \text{ hr}^{-1}$.

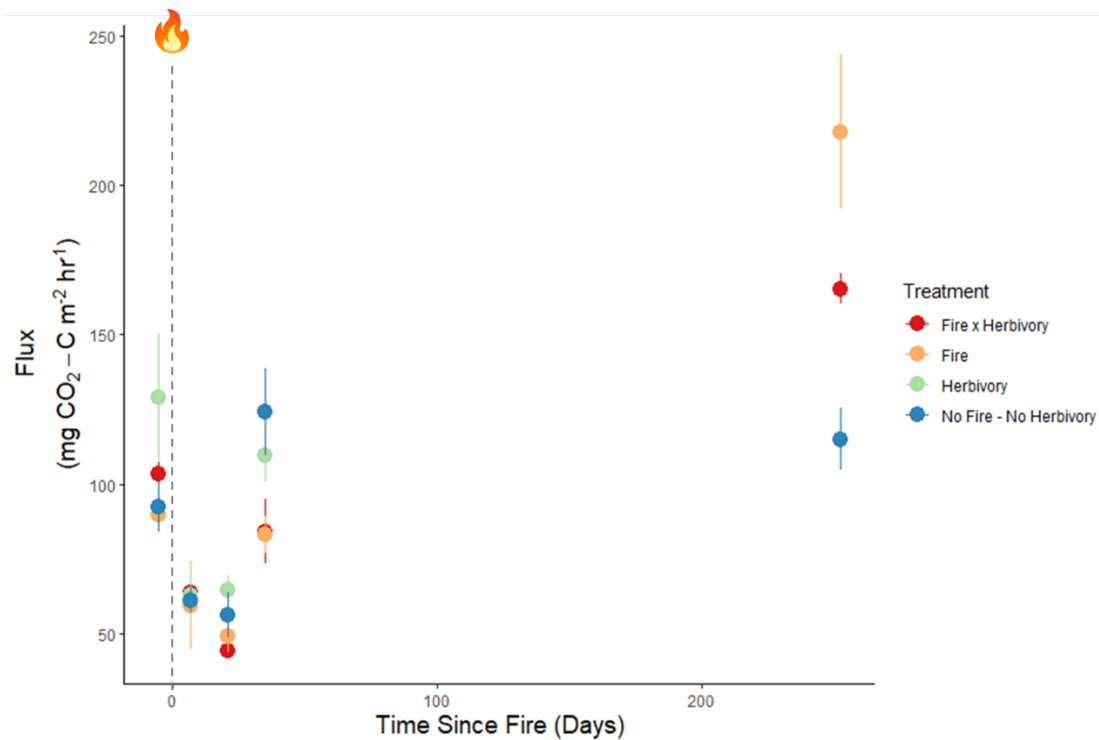


Figure 5.7 – The impact of fire and herbivory treatments on soil respiration ($M \pm SE$) after one early season burn (2021) and one late season burn (2022), and ~17 months of herbivore exclusion. Soil respiration was measured on day prior to treatment (day -1) and at 7, 21, 35 and 252-days post late season burn 2022.

The decline in soil respiration observed in the following weeks post-burn generally corresponded to changes in soil moisture. Both treatment ($p < 0.01$) and time since fire ($p < 0.01$) had a significant effect on soil moisture (Figure 5.8). Soil moisture declined from pre-fire rates in the first three weeks post-burn. Rainfall was recorded at week four, resulting in a slight increase in soil moisture on the unburnt plots by week five post-burn. By month nine post-burn, which followed the rainy season in June 2023, average soil moisture level reached peak levels among all time points. At this time point, the fire x herbivory treatment had much lower soil moisture levels than the other three treatments.

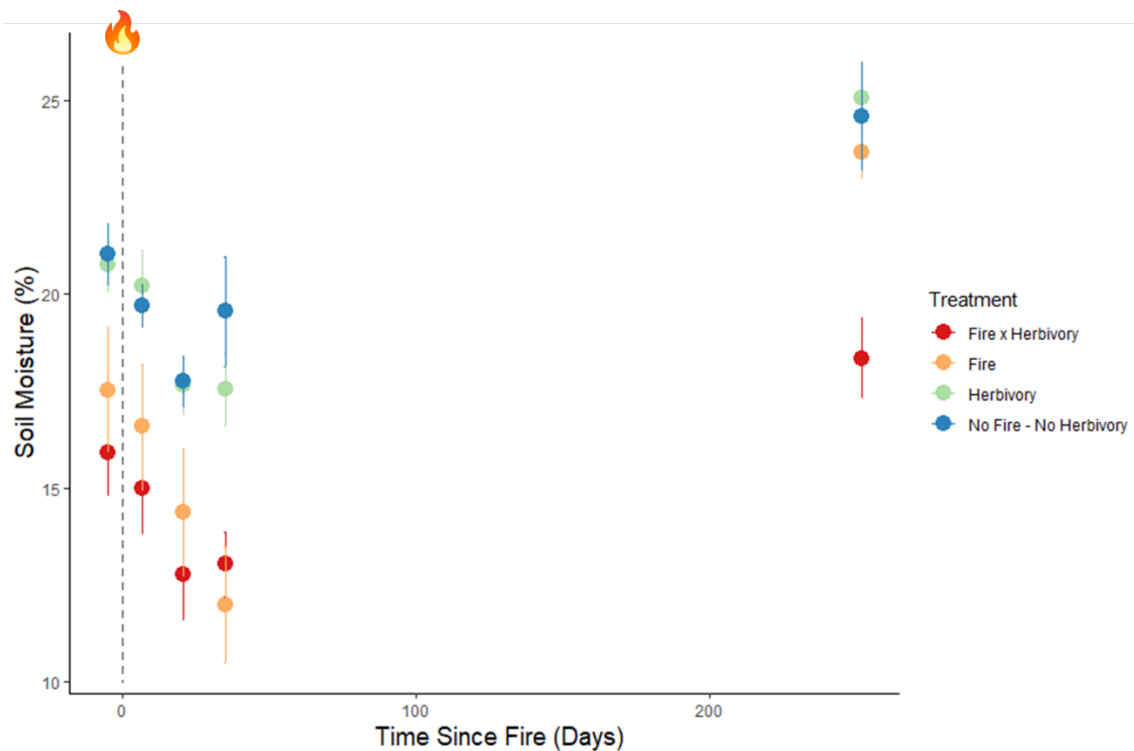


Figure 5.8 - The impact of fire and herbivory treatments after one early season burn and one late season burn, and ~17 months of herbivore exclusion, on soil moisture sampled at the corresponding time points as soil respiration.

We observed a consistent positive linear relationship between soil respiration rates across all four treatments (Figure 5.9). Soil moisture explained a significant portion of the variability in soil respiration flux, with approximately 38% and 58% of the variability accounted for in fire treatments, with and without herbivory respectively. However, in the unburnt plots, the relationship was weaker. Specifically, in plots without fire and herbivory, soil moisture explained 18% of the variability in soil respiration flux while in plots with herbivory, it explained 11%.

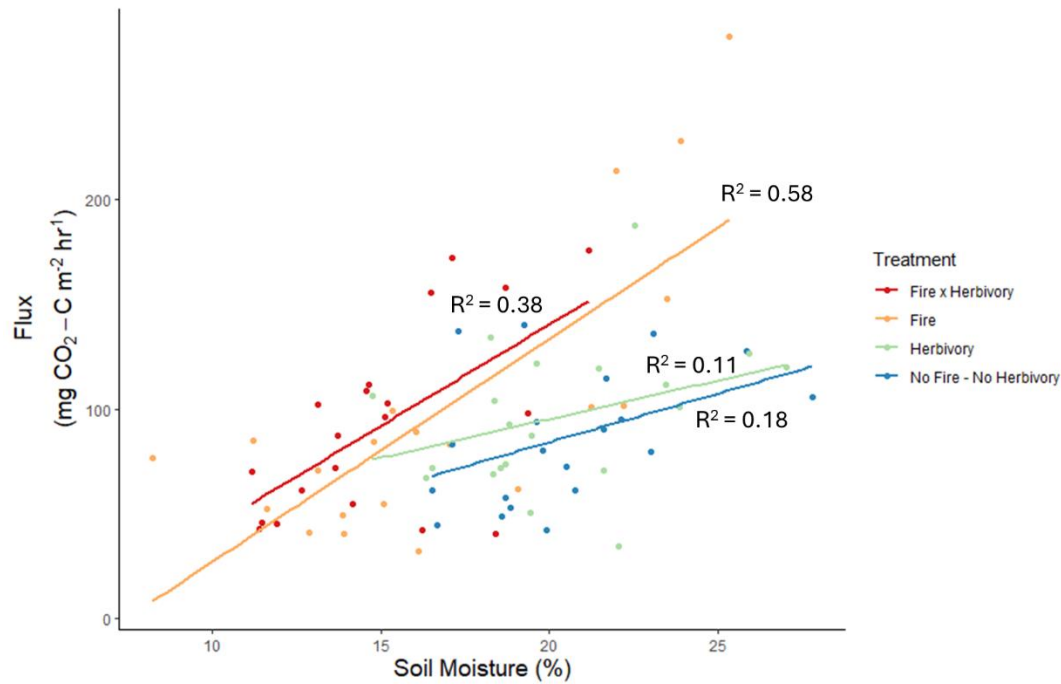


Figure 5.9 - Over the period of nine months, soil moisture explained more of the variance on the fire plots with (38%) and without (58%) herbivory, than the unburnt plots, with (11%) and without (18%) herbivory.

5.3.4. Grass Biomass Consumption

Grass biomass was higher on the no herbivory treatments, identifying herbivores are grazing around the plots (Table 5.4). In 2022, herbivore consumption was not significantly different on the burnt and unburnt plots ($p=0.35$), suggesting grazing lawns have not yet formed.

Table 5.4 – Grass biomass (kg ha^{-1}) measured one year after the first prescribed burn (2022) and the second prescribed burn (2023). No data was collected on the unburnt plots in 2023 due to logistical constraints.

	Post 2021 burn: 2022		Post 2022 burn: 2023	
	Prescribed Burn 1	Unburnt	Prescribed burn 2	Unburnt
Herbivory	4238 (± 96)	4177 (± 243)	3555 (± 157)	X
No Herbivory	5551 (± 250)	5185 (± 190)	6442 (± 354)	X
Total Herbivore Consumption	1313 (± 203)	1008 (± 221)	2888 (± 436)	X

5.4. Discussion

Very little is understood about the impact of fire x herbivory on soil properties in savanna ecosystems. This study explores the interactive interplay between small-scale, patchy fires and

the subsequent increased herbivory on microbial community composition, soil nutrients and soil respiration. Our findings reveal that fire x herbivory has subtle effects on microbial community composition, more specifically fungal concentrations, and greatly increases soil nitrate following one early season, lower intensity, burn and one year of subsequent herbivory. Furthermore, repeated small-scale fires have mid-term impacts on soil respiration that change as time since fire increases. Our study emphasises the potential effectiveness of smaller, patchy burns and the subsequent potential increased herbivory densities for inducing shifts in microbial community composition and activity, increasing soil heterogeneity.

Previous studies have highlighted how such repeated fire x herbivory cycles can reshape aboveground vegetation, with grazing lawns forming within five years of repeated annual burning (Donaldson et al., 2018; Singh et al., 2024). Grazing lawns feature uniformly short grasses and forbs, which remains low and dense due to continuous grazing pressure. In the time frame of this study, local expertise and grass biomass levels do not support the formation of grazing lawns. However, the lower grass biomass on the fire + herbivory plots, over the other three treatments, does support the concentration of herbivores on the burnt plots following a small burn.

5.4.1. Impact of fire x herbivory on soil microbial communities

Our study demonstrates minimal interactive effects of fire x herbivory on soil microbial composition, particularly in direct comparison to the total absence of fire and herbivory. We observed that total PLFA biomarkers were highest when both drivers were present. Individually, fire and herbivory showed similar concentration, with the lowest concentrations of total PLFA biomarkers observed when no drivers were present. Prior to the burn, we did not see any differences between any of the microbial functional groups across the survey area. Increased size of microbial community has been observed and reported in savannas when both fire and grazing is present (Ruess and McNaughton, 1987; Singh et al., 1991). In these observations, microbial increases were attributed to increased root turnover in burned and grazed grasslands, where turnover provide an additional resource of dead roots for microbes.

Compensatory growth by plants is often stimulated after herbivory, which can alter aboveground and belowground C allocation patterns, affecting root growth and exudation (Bardgett et al., 1998; Frank et al., 2000; Stritar et al., 2010) and thus microbial communities. Whilst aboveground composition data was not collected at this site, a shift post-fire to dominance by *Themeda triandra* was observed. Further to this, other savanna studies have also

shown that fire and grazing drive distinct aboveground changes (Bond et al., 2005; Higgins et al., 2007; Staver et al., 2021; Van Langevelde et al., 2003). Such shifts in aboveground vegetation as a result of changing drivers may alter root exudates (Bardgett et al., 1998), which serve as conduits for communication between plants and associated microbes (Preece and Peñuelas, 2020). Compounds lost from the plant roots by exudation also are a high-quality nutrient source for growth of soil microbes. Thus, increased root exudation, stimulated by increased herbivory following fire, may sustain higher microbial concentrations and favour shifts in microbial community composition on the fire + herbivory treatment.

The potential mechanism outlined above is supported by a longer term fire + herbivore experiment also within KNP, where fungal diversity and abundance increased in response to fire + herbivory, and were driven by changes in aboveground vegetation (Vermeire et al., 2021). Bacterial community diversity and abundance were found to decrease in the presence of fire + herbivory, driven by changes in soil nutrients. The absence of changes in soil nutrients in our study could explain why no differences were observed in the bacterial community. It is important to highlight that the shifts in microbial community composition in our study are minor and significantly limited, and alongside data collected by Vermeire et al., (2021), the modest, trending changes observed in soil microbes below ground compared to aboveground changes within KNP, suggest overall microbial resilience, or resistance, to fire and herbivory as drivers.

5.4.2. *Impact of fire+ herbivory on soil nutrients*

Shifts in soil nutrients as a result of fire + herbivory has been observed in savannas (Pellegrini et al., 2014, 2018). However, the only soil nutrient to show a significant difference among treatments was soil nitrate. Nitrate was over three times higher for fire + herbivory compared with the other treatments. An increase in nitrate with fire + herbivory has been observed on pasture sites in Australia, where following 40 years of sheep grazing and an unexpected wildfire, there was a big increase in nitrate in the month following the fire, which was not observed on plots that had not been grazed (i.e. fire only) (Prendergast-Miller et al., 2017). Additionally, Singh *et al.* (1991) found that burning and grazing increased inorganic N in Indian savannas, further supporting our findings. In our study, it was likely that the small fires acted to concentrate grazers, which increased inputs and has cascading impacts on soil N. Grazing animals typically use between 10-35 % of the N they consume, and the remainder is returned to the soil through dung and urine (Bussink, 1994; Sitters and Olde Venterink, 2021a). The majority of N is excreted via urine, which is soluble in the soil and thus easily assimilated by

microbes and plants, in the form of nitrate and ammonium (Sitters et al., 2017). Increased nitrate levels thereby may be as a result of the concentration of grazers. Additionally, the increased grazing reduces plant biomass, decreasing plant uptake of N from the soil, increasing N availability in the soil (Anderson et al., 2006; Bardgett and Wardle, 2003). Similarly, plant species composition may change with the increased grazing, which may have higher N content, affecting soil N cycling and nitrate levels. Observing any effects on soil properties following just one early season burn and one year of herbivory was surprising and highlights the importance of understanding the impacts of fire + herbivory on savanna ecosystems.

There were no significant changes in any other soil nutrients among the treatments. Our prescribed burns were of a low intensity, where aboveground temperatures did not rise above a maximum of 619 °C and were on average 305 °C. Furthermore, at 5cm depth in the soil, temperatures did not rise above 21 °C. Thus, it was unlikely that the low intensity burns in this study had any direct effects on the soil. Furthermore, the study area is on the southern basaltic plains of KNP, which is known for its highly productive systems (Munyati and Ratshibvumo, 2010), but low herbivore densities (McCleery et al., submitted). Herbivore densities may need to be much larger to have a visible impact on other soil nutrient levels (see data in Chapter 4). A study within KNP on two long-term ecological research sites, in Nkuhlu and Letaba, also did not see any significant differences between herbivory, fire and fire + herbivory for soil total C and N, or soil pH and moisture (Thoresen et al., 2021). Further to this, they measured for a host of micronutrients and also observed no significant differences between the treatments. Thus, one early season fire and the subsequent herbivory may not be a long enough period to see large shifts in soil nutrients.

5.4.3. Impact of fire + herbivory on soil respiration

When examining the impact of fire + herbivory on soil respiration following a late season burn, it's crucial to consider the cumulative and compounding effects of the previous early season burn and ~17 months of prior herbivore exclusion. The impact of a single fire event will likely be different from those after repeated burns (Malkison et al., 2011; Moretti et al., 2002); the respiration rates were only measured following the second round of burning and grazing exclusion. The respiration rates in this study sat between ~ 50 and 200 mg CO₂-C m² hr⁻¹, which globally are low soil respiration rates, but not in comparison to other savannas (Raich and Tufekciogul, 2000; Wang and Fang, 2009). Other studies within the Skukuza region of KNP have observed similar rates, with Fan *et al.* (2015) observing slightly higher rates at 62-375 mg CO₂-C m² hr⁻¹ in the Skukuza region and slightly lower rates of 25-250 mg CO₂-C m² hr⁻¹ have

also been recorded by Makhado and Scholes, 2011 and also in Chapter 3 of this thesis. Similar rates have also been recorded in Indian savannas, between 10-200 mg CO₂-C m² hr⁻¹ (Gupta and Singh, 1981). When comparing soil respiration rates across other biomes, CO₂ flux data within sub Saharan Africa, and savannas, are often not included (Bahn et al., 2010; Hursh et al., 2017).

Following the late season burns, our initial observations suggest that there are no immediate effects within the first week post-burn on soil respiration and microbial function. At three weeks and particularly five weeks post-burn, the fire treatments exhibited lower soil respiration rates compared with the unburnt treatments. The addition or removal of herbivory in the short term (one to five weeks post-burn) has limited impact on soil respiration. While fires have the potential to rapidly increase CO₂ fluxes through release of stored C (Beringer et al., 2015; Hao et al., 1996; Singh, 2022), such effects are likely to happen when the fire is still smouldering and unlikely to persist seven days post-burn.

Major drivers of soil respiration include temperature, soil moisture and gross primary productivity (Bahn et al., 2010; Hursh et al., 2017; Xu et al., 2004). Soil moisture plays a significant role in modulating soil respiration by influencing the activity and metabolism of soil microbes; increases in soil moisture may contribute to increased decomposition rate of the recalcitrant C pools, thus increasing soil respiration (Chen et al., 2018; Chen and Frank, 2020; Mueller et al., 2017; Wilson et al., 2018). We observed that soil moisture levels of the fire plots were significantly lower than the unburned plots, regardless of herbivory presence. Fires can promote lower soil moisture by reduced infiltration via various mechanisms including soil water repellency (Doerr et al., 2009), ash deposition clogging soil pores (Mallik et al., 1984), removal of aboveground vegetation increasing surface run off (Sidle et al., 2007), and vegetation denudation exposing the soil to direct sunlight, leading to heightened evaporation rates and further reducing soil moisture levels. Soil texture can also play a role: fire impacts on soil moisture are more prominent on clay soils, like in our study, than sandy soils (Erickson and White, 2008).

However, by nine months post-burn (June 2023), in the middle of the dry season, treatment effects of soil moisture are not mirrored by respiration rates. At this time point, soil moisture content for fire + herbivory was significantly lower than the other treatments. The persisting lower soil moisture nine months post-burn for fire + herbivory is plausibly due to the additional consumption of aboveground vegetation by the herbivores, keeping evaporation rates and run

off heightened. But the fire + herbivory treatment has a greater soil respiration rate at nine months post-burn, than herbivory and no fire-no herbivory, suggesting additional factors are at play. Dung and urine inputs of the concentrated fire + herbivory treatment may have a positive effect on soil respiration, overriding the effect of reduced soil moisture. Increased degradation of the recalcitrant soil C pool through dung and urine inputs (Blagodatsky et al., 2010; Piñeiro et al., 2010), or through trampling and breaking up organic material (Kauffman et al., 2004) could increase soil respiration rates by increasing microbial substrate availability. However, respiration rates of fire + herbivory do not reach the peak rates of fire alone, as respiration rates can also decline with herbivore-driven vegetation removal, as a reduction in plant biomass may not only reduce root respiration and thus soil respiration (Kotroczó et al., 2023), but could also root exudation and substrate availability for microbes, potentially lowering soil respiration rates.

The fire treatment alone had the greatest soil respiration rates at nine months post-burn. The production of ash has been shown to have an initial fertilising effect on soil respiration, causing an increase in microbial activity and soil respiration through labile C availability (Pyne, 2019; Matáix-Solera et al., 2009). Sánchez-García et al. (2021) observed that when adding ash to post-fire soil, there was three times increase in CO₂ flux. The pulse of CO₂ was observed within 48 hours, however, this was under controlled lab conditions, where it is firstly difficult to accurately predict the quantities of ash that may enter the soil in the field, and secondly, occurred after wetting in soil, which would only occur following precipitation in the field. However, increased specific respiration in an African savanna has been measured on fire plots and plots with ash addition, 210 days after the treatment, indicating a possible higher C availability for microbes (Andersson et al., 2004). Further research is required to understand the contribution for ash to soil C pools and microbial activity following a burn, specifically at different time since fire intervals. Pyrosugar analyses of ash could be an excellent method of tracking sugars in ash, from the burnt vegetation, into the soil and providing valuable information on ash's potential role in soil C cycling, nutrient cycling, and microbial activity.

5.4.4. Conclusion

In conclusion, our study demonstrates that fire + herbivory impact on soil properties is not the sum of the individual effects. While direct impacts of fire on soil properties appear limited, the subsequent increased density of herbivore grazing exerts a significant impact on soil nitrate and respiration rates, with more subtle impacts on microbial community composition. Specifically, smaller, patchy burns coupled with intensified herbivore activity, could result in

compensatory growth, altering C allocation patterns, subsequently impacting microbial activity. Nonetheless, changes in soil microbial communities and nutrients are modest, suggesting that longer-term studies may be necessary to observe significant shifts, integral for predicting long-term consequences, especially amidst global change.

5.5. Acknowledgments

We gratefully acknowledge Dr Navashni Govender, Nothando Ngoebni, Shedron Mukhumo, Nokuhkanya Mpanza and Dr Laurence Kruger for helping with the set-up of the experimental designs and enclosure building logistics. We thank Prof Carla Staver for the funding of the experimental burns. Additionally, we thank the Davies lab at Harvard for their generous support during fieldwork. Special thanks are also due to Working on Fire for their expertise guidance in executing our experimental fires, particularly without pre-burnt fire breaks. I also extend my appreciation to Sam Walrond and Theo Ndlovo for their help in the collection of samples, as well as Anna Hook and Alice Scotland for their assistance in the lab.

6. General Discussion



Picture credit: Mary Cvetkovic-Jones

At the beginning of this thesis, the overarching question was posed:

How does fire and herbivory affect soil properties in Southern Kruger National Park?

In this final chapter, the key findings will be briefly discussed, followed by general conclusions drawn from all four data chapters, before discussing management implications and concluding with recommendations for future research.

6.1. Key Findings

1. *How do fires affect soil chemical properties and nutrient availability, such as total C, N and P, soil pH, nitrate, and ammonium, in the short and long term on granitic and basaltic soils?*

Soil chemical properties and nutrient availability remained unchanged despite long-term fire regimes on granitic sandy soils (Chapter 2). Similarly, following a single fire event, minimal changes in soil nutrients were observed in the basaltic clay soils (Chapter 5). Therefore, neither the type of fire event nor the soil type exerted a significant impact on soil chemical properties and nutrient availability.

2. *How do fires affect soil microbial properties, such as biomass, community composition and soil respiration, in the short and long term?*

The findings revealed that both fire regime and single fire events had limited effects on microbial community composition, regardless of soil type. However, a notable fire effect was observed on microbial function, specifically C-substrate induced respiration rates were greatest in soils with higher intensity fire regimes, indicating that higher intensity burns may cause subtle alterations in soil abiotic properties and subsequently influence the soil C cycle response (Chapter 2). Additionally, fire presence decreased soil respiration rates following burns on the granitic sandy soils, recovering within one week (Chapter 3). In contrast, on the basaltic clay soils, soil respiration was lower on the burn plots than unburnt plots for one month, before having higher levels at month nine, highlighting the importance of considering time since fire (Chapter 5).

3. *How do herbivores affect soil biological and chemical soil properties? Does herbivore body size have a significant impact on soil nutrients and microbial community?*

No evidence was found of any soil biogeochemical impacts from either megaherbivore or meso-herbivores presence (Chapter 4). Neither soil nutrients nor soil microbial community

composition were affected by four years of full herbivore and megaherbivore exclusion. However, the interactive effect of herbivory x fire following burns on soil nitrate and soil respiration was observed.

4. Do fire and herbivory interact to produce synergistic effects on soil biological and chemical properties?

The study found positive impacts of the interaction of fire + herbivory on soil biogeochemistry (Chapter 5). A three-fold increase in soil nitrate levels were observed following a single fire event and one year of herbivory. Additionally, descriptive trends indicated subtle increases in microbial functional groups, with fire + herbivory having greater concentrations of PLFA biomarkers compared to individual fire or herbivory events, or no drivers at all. Nine months post a single fire event, the interaction of fire + herbivory resulted in greater soil respiration fluxes than unburnt plots, however, the subsequent herbivory had a dampening effect on soil respiration. This thesis suggest that the effects of fire and herbivory are multiplicative synergy, not additive.

6.2. General Conclusions

KNP's diverse fire and herbivory regimes provided an opportunity to explore the individual and interactive effects of fire and herbivory on savanna soil nutrient and microbial dynamics. The findings collated in this thesis fill vital knowledge gaps and provide a comprehensive picture of fire and herbivore impacts to soil biota. Below, I discuss the general conclusions of the thesis.

6.2.1. Contribution of soil microbial communities to savanna ecosystems

Much of the current understanding on soil biogeochemical and microbial processes has been developed in temperate systems but may not necessarily accurately represent more arid systems. As site-specific climatic and topographic factors play a large role in shaping microbial communities, it is not easy to predict the impact of fire and herbivory on soil microbial properties. For example, soil type (Austin et al., 2004; Kotzé et al., 2017) and moisture (Evans et al., 2022; Manzoni et al., 2012) play significant roles in shaping microbial communities. In chapters 4 and 5, the highest soil microbial PLFA concentrations were found in the basaltic clay soils, which were approx. three times greater than the granitic sandy soils (Table 6.1).

Rainfall also impacted PLFA biomarker concentrations, where Pretoriuskop levels were higher than Skukuza, as whilst both are granitic, Skukuza is drier.

Table 6.1 – Comparison of the average PLFA biomarker data across the three sites (Lower Sabie sites is the combination of Chapter 4 and 5). Variations in soil type, annual rainfall and month sampled are listed above chapter averages of PLFA biomarker data (nmol g⁻¹ soil dwt).

	Pretoriuskop (Chp2)	Skukuza (Chp3)	Lower Sabie (Chp4 &5)
Soil Type	Granitic Sandy	Granitic Sandy	Basaltic Clay
Annual Rainfall (mm)	705	572	610
Month Sampled	April	November	April
Total PLFA	19.7	14.2	59.3
Bacterial PLFA	12.2	7.49	30.6
Fungal PLFA	0.4	0.3	2.3

However perhaps of greatest note, were the low levels of soil microbial biomass, community size and activity, specifically, the extremely low, sometimes non-existent, fungal communities. This finding was both surprising and difficult to quantify with very limited published data on soil microbial communities from within KNP and other African savannas for comparison. However, insights gained from discussions with Ana Novoa (personal comms, 2024) and collaboration with Alice Walker (2021), lend support to the observation that fungal communities in the soil of southern KNP and adjacent private reserves are nearly non-existent.

Fungi usually play a vital role in soil health; as primary decomposers, with their network of hyphae creating aggregates and maintaining soil structure. Additionally, mycorrhizal fungi enhance plant productivity through symbiotic relationships, while also contributing to disease suppression, bioremediation, and C sequestration (Finlay and Thorn, 2019; Frac et al., 2018; Ritz and Young, 2004). The microbial community as a whole play fundamental roles in plant health, nutrient cycling, and the global C cycle. Thus, with such small microbial communities and potentially high microbial dormancy (Chapter 2), it prompts reflections on the significance of the soil microbial community in the functioning of African savanna ecosystems.

The findings from this thesis suggest that the soil microbial communities in KNP may not contribute to soil and ecosystem functioning to the extent observed in other ecosystem types

(Aislabie and Deslippe, 2013; Fierer et al., 2021; Miransari, 2013). In semi-arid systems like these, both microbial and plant communities contend for the same limited nutrients, leading to tight nutrient cycles. Microbes may have to enter a period of dormancy when there is limited substrate for survival, resulting in a sustained small community size and reduced contribution to decomposition and nutrient cycling, particularly in granitic sandy soils. However, further research is imperative to deepen our understanding of soil microbial communities in savanna ecosystems.

If the findings from this thesis indicate there are limiting microbial processes occurring in savanna soils, it suggests there must be alternative mechanisms facilitating the decomposition of plant litter and the recycling of C and nutrients to make them available for plant uptake. It would be interesting to investigate the role of invertebrates, alongside microbes, further particularly since Bunney et al. (2024) recently highlighted the importance of termites for decomposition within savannas and advocated for the inclusion of termite decomposition in global C models, alongside microbes as primary agents of wood, grass, and dung turnover.

6.2.2. Soil Resilience or Resistance within Kruger National Park

Ecosystem resistance refers to the ability to persist or withstand a disturbance, whereas resilience is defined as the ability to recover once a disturbance ends (Lake, 2013). Determining whether the soil in KNP exhibits resistance or resilience to fire and herbivory necessitates a temporal examination of the data to determine if the soil remained unaffected following fire and/or herbivory or underwent a recovery period. In Chapter 2, 4 and 5, when investigating microbial community composition and nutrient levels, single sampling campaigns were conducted. Consequently, where in most cases there were no discernible differences across the treatments, the distinction between resistance and resilience remains inconclusive. However, in Chapter 3, soil respiration was monitored over the year post-burn, there was an initial decline in respiration rates post-burn, indicating the microbial community displayed resilience to the fire and rapidly recovered. Similarly, in Chapter 5, when assessing soil respiration with fire + herbivory over a nine-month period, a similar initial decline in rates following the burn was observed, which subsequently recovered in the months following, further suggesting resilience.

The high resilience of soils to fire and herbivory has been seen within KNP with soil nutrients (Aranibar et al., 2003; Coetsee et al., 2012, 2010; Vermeire et al., 2021; Zhou et al., 2022), soil hydrology (Strydom et al., 2019), invertebrate, and rodent abundance (Hockridge et al., 2023; MacFadyen et al., 2012; Parr et al., 2004). Throughout this thesis, the lack of changes to soil

biogeochemical properties under differing fire and herbivore regimes, especially when contrasted to notable aboveground changes reported in the savanna literature (Burkepile et al., 2016; Eby et al., 2014; Higgins et al., 2007; Smith et al., 2013; Wilcox et al., 2020), suggest that the soils in KNP are resilient to fire and herbivory. While this resilience is positive for mitigating short-term changes in fire and herbivory regime induced by global change, it raises concerns regarding long-term prospects (Vermeire et al., 2021). There is a possibility that soil microbes may struggle to adapt to long-term enduring shifts, such as higher temperatures, changing precipitation patterns, drought, and overall increased climate variability (Alahacoon et al., 2021; Lickley and Solomon, 2018; Stevens et al., 2022), posing significant challenges to their resilience.

6.2.3. Impacts of fire and herbivory on other savanna systems

This thesis focussed only on sampling within KNP in South Africa. With savannas comprising 20% of the world, the factors influencing them vary greatly across different regions, including fire, herbivory, and climate, even within Africa itself (Figure 6.1). Hence, it is necessary to ask the same questions within other savanna systems, both within and outside of Africa.

Our sampling was confined to a relatively narrow rainfall range (500-700mm), where either fire or herbivory could potentially exert significant influence. Consequently, it's imperative to include drier savannas, with rainfall under 500mm, where herbivory typically dominates (e.g. mixed/acacia savannas), as well as wetter savannas receiving over 800 mm rainfall, where fire tends to play a prominent role (e.g. broadleaf African savannas). Given the crucial role of rainfall in soil processes and our findings (Table 6.1), I predict that higher rainfall savannas are more likely to support larger and more active microbial communities. Consequently, these wetter savannas may exhibit different belowground outcomes, as fire and herbivory may interact with soil processes through different mechanisms.

However, savannas with different wet-dry seasons to Southern Africa, such as those in Kenya and the Cerrado of Brazil, or savannas that receive significantly more rainfall, like those in the Congo Basin, or those in Northern Australian savannas, characterised by a monsoonal climate in their distinct wet season, will have different impacts of fire and herbivory on soil processes. Many factors influence soil processes and health, each context-dependent, but micro-scale drivers of soil processes can have significant macro-scale impacts. Therefore, studying these interactions under different climatic regimes is essential for predicting and mitigating the broader environmental effects of climate change. Additionally, further data collection within

savannas allows for their inclusion in global models and meta-analyses. Currently, savannas are often overlooked due to minimal data, leading to inadequate generalisations across this diverse biome.

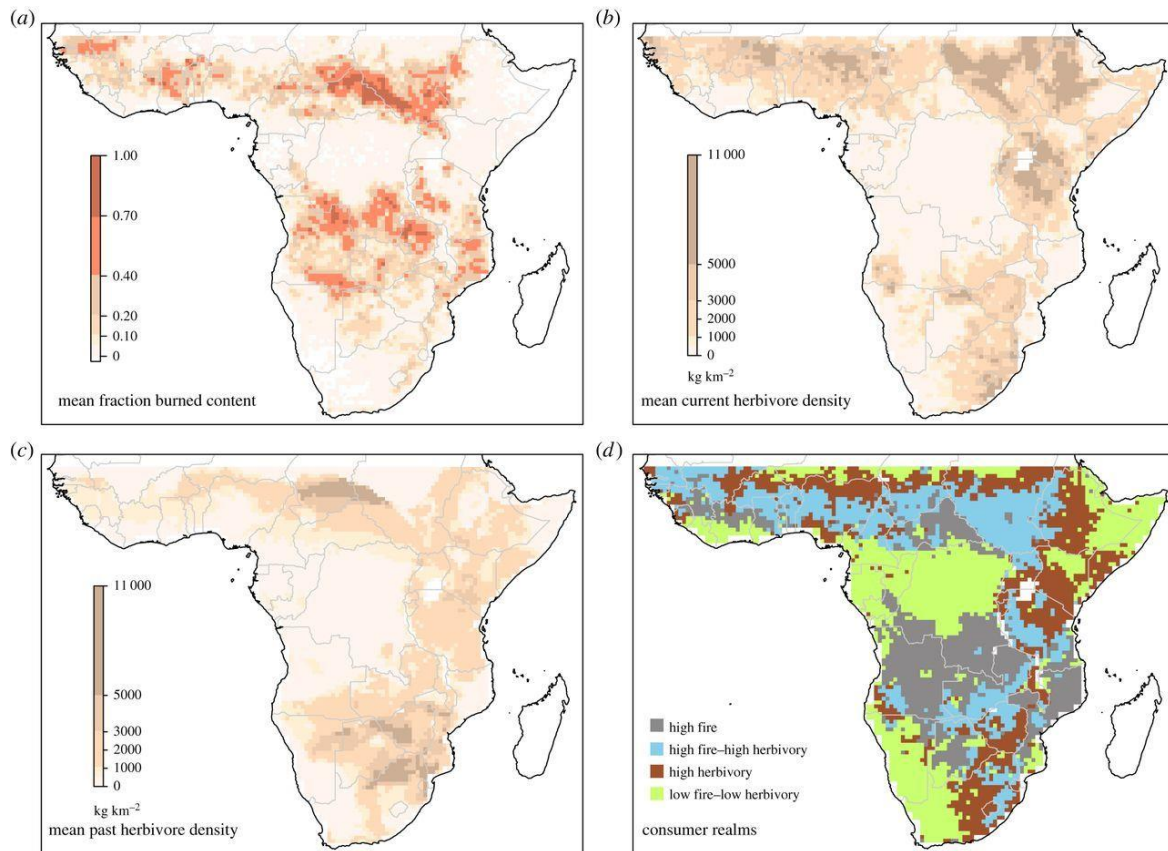


Figure 6.1 – African continent mapping of a) area burned, b) current herbivore densities, c) past herbivore densities (without elephants) and d) defining areas where fire or herbivory dominate. Taken from Archibald and Hempson (2016).

6.3. Management Implications

Fire is used for a range of purposes in conservation areas like KNP, where managing fire intensity and frequency can achieve management goals. Grazing lawns are part of SANParks management objectives to increase landscape heterogeneity and grazer numbers on the southern basalt plains (KNP Fire Protocol 2019). The findings in Chapter 5, highlight the importance of considering the impact of herbivore grazing post-burn, and the potential for using fire regimes to manipulate grazers. Specifically, the fire + herbivory treatment resulted in a threefold increase in soil nitrate alongside small shifts in soil microbial community

composition. By conducting smaller burns (1 ha), we were able to increase soil heterogeneity, a result not observed in larger experimental burn plots (7ha), as discussed in Chapter 2 and 3. Thereby, a mosaic of grazing lawns can enhance soil biogeochemical heterogeneity, supporting KNP management objectives.

Additionally, our findings suggest that the low stocking densities on the southern basalts do not significantly impact soil properties (Chapter 4). Using small fires to promote grazing lawns in this area of KNP could help increase grazer numbers, an important goal for conservation and tourism. The basalt plains provide the ideal location for this approach. In order to get a better estimate of stocking densities on the basalt plains, camera traps could be used to assess grazer activities. Future work could leverage this technology to monitor seasonal variations, concentrations on burnt plots, grazing intensity and assess the impact of grazing on vegetation and savanna ecosystem dynamics.

Globally, there have been suggestions that increasing C sequestration in savannas would require fire suppression (Sawyer et al., 2018), promoted in fire management policies (Batista et al., 2018; Bridge et al., 2005). Fire exclusion to increase C stocks has been associated with species loss (Abreu et al., 2017) and woody encroachment (Smit and Prins, 2015b). Furthermore, fire suppression in savannas could result in a shift in biome to forest in mesic areas (Khavhagali and Bond, 2008). Fire bans are often unsuccessful as they result in more accidental, uncontrolled burns in the years following, making fires more severe and ultimately accentuating climate change effects (Kreider et al., 2024; Machado et al., 2024). The balance of fire activity in savannas and grasslands should be a trade-off between conservation and climate mitigation (Andela et al., 2017). Our findings demonstrate that within Southern KNP, on both granitic and basalt soil types, there is minimal effect of fire intensity and frequency on soil chemical and biological properties, with fire suppression not showing an increase in soil C, or microbial biomass, supporting prescribed burning across a range of seasons.

This study only investigated Southern KNP, where the southern granitic soils cover 12% of the park and the southern basalt plains an even smaller proportion. The rest of the park has different soil types, landscapes, vegetations and climate, which could have varying outcomes of changing fire and herbivory regimes on soil properties, which should be considered when determining management plans.

6.4. Recommendations for Future Research

The findings from this thesis have revealed areas for future research that could further improve our understanding of fire and herbivory effects on savanna ecosystem functioning.

One factor affecting soils exposed to fire is the regular input of ash. While the theory and current understanding surrounding ash inputs have been discussed throughout this thesis (Chapter 2, 3 and 5), there is limited literature to support these ideas, raising further research questions. These include, ‘what is the specific impact of ash on microbial activity?’, ‘how quickly do microbes utilise the ash?’, and ‘how much ash enters the soil, versus being dispersed by wind or removed by grazing herbivores?’ An interesting approach to begin exploring these questions is through pyrosugars found in ash. Levoglucosan, for example, is a pyrosugar produced by the pyrolysis of cellulose and hemicellulose during biomass burning, making it a robust marker for characterisation and quantification of burning (Bhattarai et al., 2019; Ruan et al., 2020; Tan et al., 2023). Levoglucosan has been shown to be metabolized by various fungi and bacteria using levoglucosan kinases. Future studies should isolate levoglucosan from savanna soils, for an indication of how much ash enters the soils and is utilised by microbes, to explore the link between ash, fire-driven pyrosugar production and microbial activity. In addition, during fieldwork for this thesis, it was noted that grazing herbivores are attracted to the ash, licking it up alongside consumption of the fresh regrowth post-burn. The gut microbes of herbivores may be capable of directly metabolizing pyrosugars such as levoglucosan and may benefit from the C in the pyrosugars. Understanding ash’s role post-burn, particularly in synergy with herbivore grazing, is essential for enhancing our mechanistic understanding of soil processes subsequent to fire x grazing in savannas.

With less than five years of megaherbivore exclusion, we observed no impact of different herbivory levels on soil properties (Chapter 4), however the BROWSE study is set to continue as a long-term exclusion study, which may eventually result in differences in soil properties. Understanding the individual contributions of different herbivore effects, such as compaction and excreta, is vital to the overall picture. Studies where dung and urine are applied to experimental plots have been conducted in agricultural research globally (Chadwick et al., 2018; Jiang et al., 2012; Lombardi et al., 2022). While the role of dung and urine in the savanna ecosystem has been investigated in relation to nutrient feedbacks (Augustine et al., 2003), tree-grass competition (Sitters and Olde Venterink, 2021b) and spatial distribution (Veldhuis et al., 2018), studies that add dung and urine to savanna exclusion plots are extremely limited. This approach would help untangle the direct and indirect mechanisms of mammalian herbivory.

Furthermore, the BROWSE plots should continue with minimal fire interventions to further separate the impacts of fire and herbivory.

This thesis focussed on belowground activities only, as savanna studies have primarily focussed on fire and herbivory effects on plants and aboveground vegetation. However, more studies investigating savanna plant-soil interactions are required to fully understand the effect of drivers on savanna ecosystem functioning. For example, further research exploring the contribution of root exudation in savanna systems would help unravel savanna plant-soil interactions. Root exudates were hypothesised to cause short-term shifts in soil respiration and soil microbial communities following fire and herbivory across both soil types (Chapter 3 and 5). However, root exudates have not been well-studied in savannas, particularly in response to frequent burning (see review: Zhou, 2023). Thereby it is recommended that future studies investigate root exudation in the week post burn and their role in the interaction of fire + herbivory.

Furthermore, this thesis investigated soil in open areas, deliberately excluding sampling under trees or canopies in order to reduce confounding factors, when there is so little prior knowledge on the driver's effect on soil. However, a study also based in KNP showed that there is much higher nutrient and C storage below trees (Zhou et al., 2022), suggesting we may see different response to fire and herbivory underneath trees compared to open areas.

Gaining a deeper understanding of the tree-grass-soil is vital for savanna conservation. The phenomenon of woody encroachment, characterised by the increase in density of woody plants and trees at the expense of the grass layer, poses a substantial threat to savannas, shifting the vitally important savanna ecosystems towards forest biomes (Archer et al., 2017; Devine et al., 2017; Smit and Prins, 2015b; Stevens et al., 2017). To counteract this shift, fire and herbivory is used as a tool to prevent woody encroachment (Archer et al., 2017; Scholtz et al., 2022; Wilcox et al., 2018). Thus, understanding the interplay of trees alongside these drivers, within the context of plant-soil interactions is paramount for savanna conservation. Future studies should encompass both open areas and tree canopies to comprehensively explore these dynamics and inform effective conservation strategies.

In future studies, using interaction terms in Generalized Additive Models (GAMs) can help explore complex, nonlinear relationships between environmental drivers, such as the combined effects of fire frequency and soil moisture on soil respiration (Chapter 3). Interaction terms in GAMs allow for flexible modelling of how these variables influence the result, particularly

when the variables are not linear. This approach would provide more detailed insights in savanna ecosystem processes, accounting for temporal variability.

6.5. Concluding remarks

Savannas are vital biomes that are at risk and vulnerable to anthropogenic actions; and key drivers including fire, herbivory and climatic regimes are shifting with global change. Understanding the intricate interactions between fire and herbivory on all abiotic and biotic factors is essential. The findings that have emerged from this thesis contribute to the understanding of the individual and interactive impacts of fire, herbivory on savanna soil nutrient and microbial dynamics. The studies demonstrate the resilience and resistance of savanna soils to these drivers and challenge the adequacy of our understanding of microbial processes developed in temperate soils, which may not accurately represent the dynamics in arid environments with complex drivers. Additionally, this highlights the need for more soil studies based in African savannas and advocates for the inclusion of African savannas in global reviews and meta-analyses. This thesis has begun to unravel the potential multiplicative synergistic mechanisms of fire and herbivory on soil properties, but closing these knowledge gaps, through the quantification of savanna soil heterogeneity and designing future studies that extricate the mechanisms and drivers that underlie them, is imperative. Enhanced understanding of savanna systems is pivotal for conserving and protecting these remarkable ecosystems.

7. References

- Abraham, J.O., Hempson, G.P., Staver, A.C., 2019. Drought-response strategies of savanna herbivores. *Ecol. Evol.* 9, 7047–7056. <https://doi.org/10.1002/ece3.5270>
- Abreu, R.C.R., Hoffmann, W.A., Vasconcelos, H.L., Pilon, N.A., Rossatto, D.R., Durigan, G., 2017. The biodiversity cost of carbon sequestration in tropical savanna. *Sci. Adv.* 3, e1701284. <https://doi.org/10.1126/sciadv.1701284>
- Adenle, A.A., Ford, J.D., Morton, J., Twomlow, S., Alverson, K., Cattaneo, A., Cervigni, R., Kurukulasuriya, P., Huq, S., Helfgott, A., Ebinger, J.O., 2017. Managing Climate Change Risks in Africa - A Global Perspective. *Ecol. Econ.* 141, 190–201. <https://doi.org/10.1016/j.ecolecon.2017.06.004>
- Adkins, J., Docherty, K.M., Gutknecht, J.L.M., Miesel, J.R., 2020. How do soil microbial communities respond to fire in the intermediate term? Investigating direct and indirect effects associated with fire occurrence and burn severity. *Sci. Total Environ.* 745, 140957. <https://doi.org/10.1016/j.scitotenv.2020.140957>
- Aislabie, J., Deslippe, J.R., 2013. Soil microbes and their contribution to soil services. *Ecosyst. Serv. New Zealand - Cond. Trends Manaaki Whenua Press, Lincoln, New Zealand.*
- Alahacoon, N., Edirisinghe, M., Simwanda, M., Perera, E.N.C., Nyirenda, V.R., Ranagalage, M., 2021. Rainfall variability and trends over the African continent using TAMSAT data (1983–2020): Towards climate change resilience and adaptation. *Remote Sens.* 14, 96. <https://doi.org/10.3390/rs14010096>
- Alcañiz, M., Outeiro, L., Francos, M., Úbeda, X., 2018. Effects of prescribed fires on soil properties: A review. *Sci. Total Environ.* 613–614, 944–957. <https://doi.org/10.1016/j.scitotenv.2017.09.144>
- Aldezabal, A., Moragues, L., Odriozola, I., Mijangos, I., 2015. Impact of grazing abandonment on plant and soil microbial communities in an Atlantic mountain grassland. *Appl. Soil Ecol.* 96, 251–260. <https://doi.org/10.1016/j.apsoil.2015.08.013>
- Almond, R.E.A., Grooten, M., Petersen, T., 2021. Living planet report 2020-bending the curve of biodiversity loss. *Nat. Resour. Environ.* 35, 62–62.
- Andela, N., Morton, D.C., Giglio, L., Chen, Y., Werf, G.R. van der, Kasibhatla, P.S., DeFries, R.S., Collatz, G.J., Hantson, S., Kloster, S., Bachelet, D., Forrest, M., Lasslop, G., Li, F., Mangeon, S., Melton, J.R., Yue, C., Randerson, J.T., 2017. A human-driven decline in global burned area. *Science* 356, 1356–1362. <https://doi.org/10.1126/science.aal4108>
- Anderson, R.H., Fuhlendorf, S.D., Engle, D.M., 2006. Soil Nitrogen Availability in Tallgrass Prairie Under the Fire–Grazing Interaction. *Rangel. Ecol. Manag.* 59, 625–631. <https://doi.org/10.2111/05-088R2.1>
- Anderson, T.M., Ritchie, M.E., Mayemba, E., Eby, S., Grace, J.B., McNaughton, S.J., 2007. Forage Nutritive Quality in the Serengeti Ecosystem: The Roles of Fire and Herbivory. *Am. Nat.* 170, 343–357. <https://doi.org/10.1086/520120>
- Andersson, M., Michelsen, A., Jensen, M., Kjølner, A., 2004. Tropical savannah woodland: effects of experimental fire on soil microorganisms and soil emissions of carbon dioxide. *Soil Biol. Biochem.* 36, 849–858. <https://doi.org/10.1016/j.soilbio.2004.01.015>
- Andrés, P., Moore, J.C., Simpson, R.T., Selby, G., Cotrufo, F., Deneff, K., Haddix, M.L., Shaw, E.A., de Tomasel, C.M., Molowny-Horas, R., Wall, D.H., 2016. Soil food web stability in response to grazing in a semi-arid prairie: The importance of soil textural heterogeneity. *Soil Biol. Biochem.* 97, 131–143. <https://doi.org/10.1016/j.soilbio.2016.02.014>
- Andriuzzi, W.S., Wall, D.H., 2017. Responses of belowground communities to large aboveground herbivores: Meta-analysis reveals biome-dependent patterns and critical research gaps. *Glob. CHANGE Biol.* 23, 3857–3868. <https://doi.org/10.1111/gcb.13675>
- Angassa, A., Sheleme, B., Oba, G., Treydte, A.C., Linstädter, A., Sauerborn, J., 2012. Savanna land use and its effect on soil characteristics in southern Ethiopia. *J. Arid Environ.* 81, 67–76. <https://doi.org/10.1016/j.jaridenv.2012.01.006>
- Aranibar, J.N., Macko, S.A., Anderson, I.C., Potgieter, A.L.F., Sowry, R., Shugart, H.H., 2003. Nutrient cycling responses to fire frequency in the Kruger National Park (South Africa) as indicated by Stable Isotope analysis. *Isotopes Environ. Health Stud.* 39, 141–158. <https://doi.org/10.1080/1025601031000096736>
- Archer, S., Boutton, T.W., Hibbard, K.A., 2001. 1.9 - Trees in Grasslands: Biogeochemical Consequences of Woody Plant Expansion, in: Schulze, E.-D., Heimann, M., Harrison, S., Holland, E., Lloyd, J., Prentice, I.C., Schimel, D. (Eds.), *Global Biogeochemical Cycles in the Climate System*. Academic Press, San Diego, pp. 115–137. <https://doi.org/10.1016/B978-012631260-7/50011-X>
- Archer, S.R., Andersen, E.M., Predick, K.I., Schwinning, S., Steidl, R.J., Woods, S.R., 2017. Woody plant encroachment: causes and consequences. *Rangel. Syst. Process. Manag. Chall.* 25–84.

- Archibald, S., 2023. private comms.
- Archibald, S., Bond, W., 2003. Growing tall vs growing wide: Tree architecture and allometry of *Acacia karroo* in forest, savanna, and arid environments. *Oikos* 102, 3–14. <https://doi.org/10.1034/j.1600-0706.2003.12181.x>
- Archibald, S., Bond, W.J., 2004. Grazer movements: spatial and temporal responses to burning in a tall-grass African savanna. *Int. J. Wildland Fire* 13, 377–385. <https://doi.org/10.1071/WF03070>
- Archibald, S., Bond, W.J., Stock, W.D., Fairbanks, D.H.K., 2005. Shaping the Landscape: Fire–Grazer Interactions in an African Savanna. *Ecol. Appl.* 15, 96–109. <https://doi.org/10.1890/03-5210>
- Archibald, S., Hempson, G.P., 2016a. Competing consumers: contrasting the patterns and impacts of fire and mammalian herbivory in Africa. *Philos. Trans. R. Soc. B Biol. Sci.* 371, 20150309. <https://doi.org/10.1098/rstb.2015.0309>
- Archibald, S., Hempson, G.P., 2016b. Competing consumers: contrasting the patterns and impacts of fire and mammalian herbivory in Africa. *Philos. Trans. R. Soc. B Biol. Sci.* 371, 20150309. <https://doi.org/10.1098/rstb.2015.0309>
- Augustine, D.J., McNaughton, S.J., 2004. Regulation of shrub dynamics by native browsing ungulates on East African rangeland. *J. Appl. Ecol.* 41, 45–58. <https://doi.org/10.1111/j.1365-2664.2004.00864.x>
- Augustine, D.J., McNaughton, S.J., Frank, D.A., 2003. Feedbacks Between Soil Nutrients and Large Herbivores in a Managed Savanna Ecosystem. *Ecol. Appl.* 13, 1325–1337. <https://doi.org/10.1890/02-5283>
- Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., Ravetta, D.A., Schaeffer, S.M., 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* 141, 221–235. <https://doi.org/10.1007/s00442-004-1519-1>
- Ayanlade, A., Smucker, T.A., Nyasimi, M., Sterly, H., Weldemariam, L.F., Simpson, N.P., 2023. Complex climate change risk and emerging directions for vulnerability research in Africa. *Clim. Risk Manag.* 40, 100497. <https://doi.org/10.1016/j.crm.2023.100497>
- Bach, E.M., Baer, S.G., Meyer, C.K., Six, J., 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. *Soil Biol. Biochem.* 42, 2182–2191. <https://doi.org/10.1016/j.soilbio.2010.08.014>
- Badri, D.V., Vivanco, J.M., 2009. Regulation and function of root exudates. *Plant Cell Environ.* 32, 666–681. <https://doi.org/10.1111/j.1365-3040.2009.01926.x>
- Bahn, M., Reichstein, M., Davidson, E.A., Grünzweig, J., Jung, M., Carbone, M.S., Epron, D., Misson, L., Nouvellon, Y., Rouspard, O., Savage, K., Trumbore, S.E., Gimeno, C., Curiel Yuste, J., Tang, J., Vargas, R., Janssens, I.A., 2010. Soil respiration at mean annual temperature predicts annual total across vegetation types and biomes. *Biogeosciences* 7, 2147–2157. <https://doi.org/10.5194/bg-7-2147-2010>
- Bakker, E.S., Gill, J.L., Johnson, C.N., Vera, F.W.M., Sandom, C.J., Asner, G.P., Svenning, J.-C., 2016. Combining paleo-data and modern exclosure experiments to assess the impact of megafauna extinctions on woody vegetation. *Proc. Natl. Acad. Sci.* 113, 847–855. <https://doi.org/10.1073/pnas.1502545112>
- Bardgett, R., 2005. *The Biology of Soil: A Community and Ecosystem Approach*. OUP Oxford.
- Bardgett, R. D., Keiller, S., Cook, R., Gilburn, A.S., 1998. Dynamic interactions between soil animals and microorganisms in upland grassland soils amended with sheep dung: a microcosm experiment. *Soil Biol. Biochem.* 30, 531–539. [https://doi.org/10.1016/S0038-0717\(97\)00146-6](https://doi.org/10.1016/S0038-0717(97)00146-6)
- Bardgett, R.D., Leemans, D.K., Cook, R., Hobbs, P.J., 1997. Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biol. Biochem.* 29, 1285–1294. [https://doi.org/10.1016/S0038-0717\(97\)00019-9](https://doi.org/10.1016/S0038-0717(97)00019-9)
- Bardgett, R.D., Streeter, T.C., Bol, R., 2003. Soil Microbes Compete Effectively with Plants for Organic-Nitrogen Inputs to Temperate Grasslands. *Ecology* 84, 1277–1287. [https://doi.org/10.1890/0012-9658\(2003\)084\[1277:SMCEWP\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[1277:SMCEWP]2.0.CO;2)
- Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511. <https://doi.org/10.1038/nature13855>
- Bardgett, R.D., Wardle, D.A., 2003. Herbivore-Mediated Linkages Between Aboveground and Belowground Communities. *Ecology* 84, 2258–2268. <https://doi.org/10.1890/02-0274>
- Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. *Soil Biol. Biochem.* 30, 1867–1878. [https://doi.org/10.1016/S0038-0717\(98\)00069-8](https://doi.org/10.1016/S0038-0717(98)00069-8)
- Batista, E.K.L., Russell-Smith, J., França, H., Figueira, J.E.C., 2018. An evaluation of contemporary savanna fire regimes in the Canastra National Park, Brazil: Outcomes of fire suppression policies. *J. Environ. Manage.* 205, 40–49. <https://doi.org/10.1016/j.jenvman.2017.09.053>
- Belsky, A.J., 1994. Influences of Trees on Savanna Productivity: Tests of Shade, Nutrients, and Tree-Grass Competition. *Ecology* 75, 922–932. <https://doi.org/10.2307/1939416>

- Bento-Gonçalves, A., Vieira, A., Úbeda, X., Martín, D., 2012. Fire and soils: Key concepts and recent advances. *Geoderma*, Fire effects on soil properties 191, 3–13. <https://doi.org/10.1016/j.geoderma.2012.01.004>
- Beringer, J., Hutley, L.B., Abramson, D., Arndt, S.K., Briggs, P., Bristow, M., Canadell, J.G., Cernusak, L.A., Eamus, D., Edwards, A.C., Evans, B.J., Fest, B., Goergen, K., Grover, S.P., Hacker, J., Haverd, V., Kanniah, K., Livesley, S.J., Lynch, A., Maier, S., Moore, C., Raupach, M., Russell-Smith, J., Scheiter, S., Tapper, N.J., Uotila, P., 2015. Fire in Australian savannas: from leaf to landscape. *Glob. Change Biol.* 21, 62–81. <https://doi.org/10.1111/gcb.12686>
- Bhattacharai, H., Saikawa, E., Wan, X., Zhu, H., Ram, K., Gao, S., Kang, S., Zhang, Q., Zhang, Y., Wu, G., Wang, X., Kawamura, K., Fu, P., Cong, Z., 2019. Levoglucosan as a tracer of biomass burning: Recent progress and perspectives. *Atmospheric Res.* 220, 20–33. <https://doi.org/10.1016/j.atmosres.2019.01.004>
- Biederman, L.A., Harpole, W.S., 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *GCB Bioenergy* 5, 202–214. <https://doi.org/10.1111/gcbb.12037>
- Biggs, R., Biggs, H.C., Dunne, T.T., Govender, N., Potgieter, A.L.F., 2003. Experimental burn plot trial in the Kruger National Park: history, experimental design and suggestions for data analysis. *Koedoe* 46, 1–15. <https://doi.org/10.4102/koedoe.v46i1.35>
- Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. *Plant Soil* 10, 9–31. <https://doi.org/10.1007/BF01343734>
- Bird, M.I., Veenendaal, E.M., Moyo, C., Lloyd, J., Frost, P., 2000. Effect of fire and soil texture on soil carbon in a sub-humid savanna (Matopos, Zimbabwe). *Geoderma* 94, 71–90. [https://doi.org/10.1016/S0016-7061\(99\)00084-1](https://doi.org/10.1016/S0016-7061(99)00084-1)
- Bird, M.I., Wynn, J.G., Saiz, G., Wurster, C.M., McBeath, A., 2015. The Pyrogenic Carbon Cycle. *Annu. Rev. Earth Planet. Sci.* 43, 273–298. <https://doi.org/10.1146/annurev-earth-060614-105038>
- Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., Kuzyakov, Y., 2010. Model of apparent and real priming effects: Linking microbial activity with soil organic matter decomposition. *Soil Biol. Biochem.* 42, 1275–1283. <https://doi.org/10.1016/j.soilbio.2010.04.005>
- Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911–917. <https://doi.org/10.1139/o59-099>
- Blum, W.E.H., 2005. Functions of Soil for Society and the Environment. *Rev. Environ. Sci. Biotechnol.* 4, 75–79. <https://doi.org/10.1007/s11157-005-2236-x>
- Blume, H.-P., Brümmer, G.W., Fleige, H., Horn, R., Kandeler, E., Kögel-Knabner, I., Kretzschmar, R., Stahr, K., Wilke, B.-M., 2016. Soil Organisms and Their Habitat, in: *Soil Organisms and Their Habitat*. Springer, pp. 87–122. https://doi.org/10.1007/978-3-642-30942-7_4
- Bodí, M.B., Martín, D.A., Balfour, V.N., Santín, C., Doerr, S.H., Pereira, P., Cerdà, A., Mataix-Solera, J., 2014. Wildland fire ash: Production, composition and eco-hydro-geomorphic effects. *Earth-Sci. Rev.* 130, 103–127. <https://doi.org/10.1016/j.earscirev.2013.12.007>
- Boer, W. de, Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiol. Rev.* 29, 795–811. <https://doi.org/10.1016/j.femsre.2004.11.005>
- Bond, W., 2005. Large parts of the world are brown or black: A different view on the ‘Green World’ hypothesis. *J. Veg. Sci.* 16, 261–266.
- Bond, W.J., 2008. What Limits Trees in C4 Grasslands and Savannas? *Annu. Rev. Ecol. Evol. Syst.* 39, 641–659. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173411>
- Bond, W.J., 2005. Large parts of the world are brown or black: A different view on the ‘Green World’ hypothesis. *J. Veg. Sci.* 16, 261–266. <https://doi.org/10.1111/j.1654-1103.2005.tb02364.x>
- Bond, W.J., Keeley, J.E., 2005. Fire as a global ‘herbivore’: the ecology and evolution of flammable ecosystems. *Trends Ecol. Evol.* 20, 387–394. <https://doi.org/10.1016/j.tree.2005.04.025>
- Bond, W.J., Midgley, G.F., Woodward, F.I., 2003. What controls South African vegetation — climate or fire? *South Afr. J. Bot., Special Issue — Acocks’ Veld Types* 69, 79–91. [https://doi.org/10.1016/S0254-6299\(15\)30362-8](https://doi.org/10.1016/S0254-6299(15)30362-8)
- Bond, W.J., Woodward, F.I., Midgley, G.F., 2005. The global distribution of ecosystems in a world without fire. *New Phytol.* 165, 525–538. <https://doi.org/10.1111/j.1469-8137.2004.01252.x>
- Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Glob. Change Biol.* 15, 808–824. <https://doi.org/10.1111/j.1365-2486.2008.01681.x>
- Bowman, D.M.J.S., Balch, J.K., Artaxo, P., Bond, W.J., Carlson, J.M., Cochrane, M.A., D’Antonio, C.M., DeFries, R.S., Doyle, J.C., Harrison, S.P., Johnston, F.H., Keeley, J.E., Krawchuk, M.A., Kull, C.A., Marston, J.B., Moritz, M.A., Prentice, I.C., Roos, C.I., Scott, A.C., Swetnam, T.W., Werf, G.R. van der, Pyne, S.J., 2009. Fire in the Earth System. *Science* 324, 481–484. <https://doi.org/10.1126/science.1163886>

- Brachmann, C., 2023. Trophic interactions in the tundra: Impacts of large mammal herbivory on carbon processes and fungal communities. Gothenburg University Library.
- Bridge, S.R.J., Miyanishi, K., Johnson, E.A., 2005. A critical evaluation of fire suppression effects in the boreal forest of Ontario. *For. Sci.* 51, 41–50. <https://doi.org/10.1093/forestscience/51.1.41>
- Britz, M.-L., Ward, D., 2007. The effects of soil conditions and grazing strategy on plant species composition in a semi-arid savanna. *Afr. J. Range Forage Sci.* 24, 51–61. <https://doi.org/10.2989/AJRFS.2007.24.2.1.155>
- Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* 17, 837–842. [https://doi.org/10.1016/0038-0717\(85\)90144-0](https://doi.org/10.1016/0038-0717(85)90144-0)
- Bunney, K., Robertson, M., Eggleton, P., Twine, W., Parr, C., 2024. Quantifying the role of termite decomposition in a mesic savanna. *Biotropica* e13333. <https://doi.org/10.1111/btp.13333>
- Burkepile, D.E., Thompson, D.I., Fynn, R.W.S., Koerner, S.E., Eby, S., Govender, N., Hagenah, N., Lemoine, N.P., Matchett, K.J., Wilcox, K.R., Collins, S.L., Kirkman, K.P., Knapp, A.K., Smith, M.D., 2016. Fire frequency drives habitat selection by a diverse herbivore guild impacting top–down control of plant communities in an African savanna. *Oikos* 125, 1636–1646. <https://doi.org/10.1111/oik.02987>
- Bussink, D.W., 1994. Relationships between ammonia volatilization and nitrogen fertilizer application rate, intake and excretion of herbage nitrogen by cattle on grazed swards. *Fertil. Res.* 38, 111–121. <https://doi.org/10.1007/BF00748771>
- Butler, A., Meir, P., Saiz, G., Maracahipes, L., Marimon, B.S., Grace, J., 2012. Annual variation in soil respiration and its component parts in two structurally contrasting woody savannas in Central Brazil. *Plant Soil* 352, 129–142. <https://doi.org/10.1007/s11104-011-0984-7>
- Byram, G., 1959. Combustion of forest fuels. *For. Fire Control Use* 61–89.
- Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P.W., Trisos, C., Romero, J., Aldunce, P., Barrett, K., Blanco, G., Cheung, W.W.L., Connors, S., Denton, F., Diongue-Niang, A., Dodman, D., Garschagen, M., Geden, O., Hayward, B., Jones, C., Jotzo, F., Krug, T., Lasco, R., Lee, Y.-Y., Masson-Delmotte, V., Meinshausen, M., Mintenbeck, K., Mokssit, A., Otto, F.E.L., Pathak, M., Pirani, A., Poloczanska, E., Pörtner, H.-O., Revi, A., Roberts, D.C., Roy, J., Ruane, A.C., Skea, J., Shukla, P.R., Slade, R., Slangen, A., Sokona, Y., Sörensson, A.A., Tignor, M., Van Vuuren, D., Wei, Y.-M., Winkler, H., Zhai, P., Zommers, Z., Hourcade, J.-C., Johnson, F.X., Pachauri, S., Simpson, N.P., Singh, C., Thomas, A., Totin, E., Arias, P., Bustamante, M., Elgizouli, I., Flato, G., Howden, M., Méndez-Vallejo, C., Pereira, J.J., Pichs-Madruga, R., Rose, S.K., Saheb, Y., Sánchez Rodríguez, R., Ürgé-Vorsatz, D., Xiao, C., Yassaa, N., Alegría, A., Armour, K., Bednar-Friedl, B., Blok, K., Cissé, G., Dentener, F., Eriksen, S., Fischer, E., Garner, G., Guivarch, C., Haasnoot, M., Hansen, G., Hauser, M., Hawkins, E., Hermans, T., Kopp, R., Leprince-Ringuet, N., Lewis, J., Ley, D., Ludden, C., Niamir, L., Nicholls, Z., Some, S., Szopa, S., Trewin, B., Van Der Wijst, K.-I., Winter, G., Witting, M., Birt, A., Ha, M., Romero, J., Kim, J., Haites, E.F., Jung, Y., Stavins, R., Birt, A., Ha, M., Orendain, D.J.A., Ignon, L., Park, S., Park, Y., Reisinger, A., Cammaramo, D., Fischlin, A., Fuglestvedt, J.S., Hansen, G., Ludden, C., Masson-Delmotte, V., Matthews, J.B.R., Mintenbeck, K., Pirani, A., Poloczanska, E., Leprince-Ringuet, N., Péan, C., 2023. IPCC, 2023: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland. Intergovernmental Panel on Climate Change (IPCC). <https://doi.org/10.59327/IPCC/AR6-9789291691647>
- Canals, R.M., Múgica, L., Duran, M., San Emeterio, L., 2024. Restorative Pyric Herbivory Practices in Shrub-Encroached Grasslands Enhance Nutrient Resource Availability and Spatial Heterogeneity. <https://doi.org/10.2139/ssrn.4767854>
- Carter, Z.W., Sullivan, B.W., Qualls, R.G., Blank, R.R., Schmidt, C.A., Verburg, P.S., 2018. Charcoal increases microbial activity in eastern Sierra Nevada forest soils. *Forests* 9, 93. <https://doi.org/10.3390/f9020093>
- Cech, P.G., Kuster, T., Edwards, P.J., Olde Venterink, H., 2008. Effects of Herbivory, Fire and N₂-fixation on Nutrient Limitation in a Humid African Savanna. *Ecosystems* 11, 991–1004. <https://doi.org/10.1007/s10021-008-9175-7>
- Certini, G., Moya, D., Lucas-Borja, M.E., Mastrodonardo, G., 2021. The impact of fire on soil-dwelling biota: A review. *For. Ecol. Manag.* 488, 118989. <https://doi.org/10.1016/j.foreco.2021.118989>
- Chadwick, D.R., Cardenas, L.M., Dhanoa, M.S., Donovan, N., Misselbrook, T., Williams, J.R., Thorman, R.E., McGeough, K.L., Watson, C.J., Bell, M., Anthony, S.G., Rees, R.M., 2018. The contribution of cattle urine and dung to nitrous oxide emissions: Quantification of country specific emission factors and implications for national inventories. *Sci. Total Environ.* 635, 607–617. <https://doi.org/10.1016/j.scitotenv.2018.04.152>

- Charles, G.K., Porensky, L.M., Riginos, C., Veblen, K.E., Young, T.P., 2017. Herbivore effects on productivity vary by guild: cattle increase mean productivity while wildlife reduce variability. *Ecol. Appl.* 27, 143–155. <https://doi.org/10.1002/eap.1422>
- Charles, G.K., Riginos, C., Veblen, K.E., Kimuyu, D.M., Young, T.P., 2021. Termite mound cover and abundance respond to herbivore-mediated biotic changes in a Kenyan savanna. *Ecol. Evol.* 11, 7226–7238. <https://doi.org/10.1002/ece3.7445>
- Chase, M.J., Schlossberg, S., Griffin, C.R., Bouché, P.J.C., Djene, S.W., Elkan, P.W., Ferreira, S., Grossman, F., Kohi, E.M., Landen, K., Omondi, P., Peltier, A., Selier, S.A.J., Sutcliffe, R., 2016. Continent-wide survey reveals massive decline in African savannah elephants. *PeerJ* 4, e2354. <https://doi.org/10.7717/peerj.2354>
- Chen, A., Reperant, L., Fischhoff, I.R., Rubenstein, D.I., 2021. Increased vigilance of plains zebras (*Equus quagga*) in response to more bush coverage in a Kenyan savanna. *Clim. Change Ecol.* 1, 100001. <https://doi.org/10.1016/j.ecochg.2021.100001>
- Chen, J., Frank, D.A., 2020. Herbivores stimulate respiration from labile and recalcitrant soil carbon pools in grasslands of Yellowstone National Park. *Land Degrad. Dev.* 31, 2620–2634. <https://doi.org/10.1002/ldr.3656>
- Chen, J., Luo, Y., García-Palacios, P., Cao, J., Dacal, M., Zhou, X., Li, J., Xia, J., Niu, S., Yang, H., Shelton, S., Guo, W., van Groenigen, K.J., 2018. Differential responses of carbon-degrading enzyme activities to warming: Implications for soil respiration. *Glob. Change Biol.* 24, 4816–4826. <https://doi.org/10.1111/gcb.14394>
- Coetsee, C., Bond, W., February, E., 2010. Frequent fire affects soil nitrogen and carbon in an African savanna by changing woody cover. *Oecologia* 162, 1027–34. <https://doi.org/10.1007/s00442-009-1490-y>
- Coetsee, C., Jacobs, S., Govender, N., 2012. An Overview of Nitrogen Cycling in a Semiarid Savanna: Some Implications for Management and Conservation in a Large African Park. *Environ. Manage.* 49, 387–402. <https://doi.org/10.1007/s00267-011-9779-0>
- Cogger, C., Brown, S., 2016. Soil Formation and Nutrient Cycling, in: *Sowing Seeds in the City: Ecosystem and Municipal Services*. Springer Netherlands, Dordrecht, pp. 25–52. https://doi.org/10.1007/978-94-017-7453-6_2
- Conway, D., Vincent, K., 2021. *Climate Risk in Africa: Adaptation and Resilience*. Springer Nature. <https://doi.org/10.1007/978-3-030-61160-6>
- Côté, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their importance in conservation. *Proc. R. Soc. B Biol. Sci.* 283, 20152592. <https://doi.org/10.1098/rspb.2015.2592>
- Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A., Totterdell, I.J., 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* 408, 184–187.
- Cromsigt, J.P.G.M., Archibald, S., Owen-Smith, N., 2017. *Conserving Africa's Mega-Diversity in the Anthropocene: The Hluhluwe-iMfolozi Park Story*. Cambridge University Press.
- Crosti, R., Ladd, P.G., Dixon, K.W., Piotta, B., 2006. Post-fire germination: The effect of smoke on seeds of selected species from the central Mediterranean basin. *For. Ecol. Manag.* 221, 306–312. <https://doi.org/10.1016/j.foreco.2005.10.005>
- Czimczik, C.I., Trumbore, S.E., Carbone, M.S., Winston, G.C., 2006. Changing sources of soil respiration with time since fire in a boreal forest. *Glob. Change Biol.* 12, 957–971. <https://doi.org/10.1111/j.1365-2486.2006.01107.x>
- da Silva, D.M., Batalha, M.A., 2008. Soil–vegetation relationships in cerrados under different fire frequencies. *Plant Soil* 311, 87–96. <https://doi.org/10.1007/s11104-008-9660-y>
- Dairel, M., Fidelis, A., 2020. How does fire affect germination of grasses in the Cerrado? *Seed Sci. Res.* 30, 275–283. <https://doi.org/10.1017/S0960258520000094>
- De Nobili, M., Contin, M., Mondini, C., Brookes, P.C., 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biol. Biochem.* 33, 1163–1170. [https://doi.org/10.1016/S0038-0717\(01\)00020-7](https://doi.org/10.1016/S0038-0717(01)00020-7)
- DeBano, L.F., Neary, D.G., Ffolliott, P.F., 1998. *Fire Effects on Ecosystems*. John Wiley & Sons.
- Derner, J.D., Augustine, D.J., Frank, D.A., 2019. Does Grazing Matter for Soil Organic Carbon Sequestration in the Western North American Great Plains? *Ecosystems* 22, 1088–1094. <https://doi.org/10.1007/s10021-018-0324-3>
- Devine, A.P., McDonald, R.A., Quaife, T., Maclean, I.M.D., 2017. Determinants of woody encroachment and cover in African savannas. *Oecologia* 183, 939–951. <https://doi.org/10.1007/s00442-017-3807-6>
- Dijkstra, F.A., Augustine, D.J., Brewer, P., von Fischer, J.C., 2012. Nitrogen cycling and water pulses in semiarid grasslands: are microbial and plant processes temporally asynchronous? *Oecologia* 170, 799–808. <https://doi.org/10.1007/s00442-012-2336-6>

- Dippold, M.A., Kuzyakov, Y., 2016. Direct incorporation of fatty acids into microbial phospholipids in soils: Position-specific labeling tells the story. *Geochim. Cosmochim. Acta* 174, 211–221. <https://doi.org/10.1016/j.gca.2015.10.032>
- Doelman, P., Eijsackers, H.J.P., 2004. *Vital Soil: Function, Value and Properties*. Elsevier.
- Doerr, S.H., Shakesby, R.A., MacDonald, L.H., 2009. Soil water repellency: a key factor in post-fire erosion, in: *Fire Effects on Soils and Restoration Strategies*. CRC Press, pp. 213–240.
- Donaldson, J.E., Archibald, S., Govender, N., Pollard, D., Luhdo, Z., Parr, C.L., 2018. Ecological engineering through fire-herbivory feedbacks drives the formation of savanna grazing lawns. *J. Appl. Ecol.* 55, 225–235. <https://doi.org/10.1111/1365-2664.12956>
- Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. *Biogeochemistry* 109, 49–61. <https://doi.org/10.1007/s10533-011-9633-8>
- Doughty, C.E., Faurby, S., Svenning, J.-C., 2016. The impact of the megafauna extinctions on savanna woody cover in South America. *Ecography* 39, 213–222. <https://doi.org/10.1111/ecog.01593>
- Dove, N.C., Hart, S.C., 2017. Fire Reduces Fungal Species Richness and In Situ Mycorrhizal Colonization: A Meta-Analysis. *Fire Ecol.* 13, 37–65. <https://doi.org/10.4996/fireecology.130237746>
- Dove, N.C., Safford, H.D., Bohlman, G.N., Estes, B.L., Hart, S.C., 2020. High-severity wildfire leads to multi-decadal impacts on soil biogeochemistry in mixed-conifer forests. *Ecol. Appl.* 30, e02072. <https://doi.org/10.1002/eap.2072>
- du Toit, J.T., Rogers, K.H., Biggs, H.C., 2003. The Kruger experience: ecology and management of savanna heterogeneity. *Kruger Exp. Ecol. Manag. Savanna Heterog.*
- Dunn, P.H., Barro, S.C., Poth, M., 1985. Soil moisture affects survival of microorganisms in heated chaparral soil. *Soil Biol. Biochem.* 17, 143–148. [https://doi.org/10.1016/0038-0717\(85\)90105-1](https://doi.org/10.1016/0038-0717(85)90105-1)
- Dunn, P.H., DeBano, L.F., 1981. Fire's effect on biological and chemical properties of chaparral soils.
- Dunning, L.T., Liabot, A.-L., Olofsson, J.K., Smith, E.K., Vorontsova, M.S., Besnard, G., Simpson, K.J., Lundgren, M.R., Addicott, E., Gallagher, R.V., Chu, Y., Pennington, R.T., Christin, P.-A., Lehmann, C.E.R., 2017. The recent and rapid spread of *Themeda triandra*. *Bot. Lett.* 164, 327–337. <https://doi.org/10.1080/23818107.2017.1391120>
- E. Evans, S., D. Allison, S., V. Hawkes, C., 2022. Microbes, memory and moisture: Predicting microbial moisture responses and their impact on carbon cycling. *Funct. Ecol.* 36, 1430–1441. <https://doi.org/10.1111/1365-2435.14034>
- Eby, S., Burkepille, D.E., Fynn, R.W.S., Burns, C.E., Govender, N., Hagenah, N., Koerner, S.E., Matchett, K.J., Thompson, D.I., Wilcox, K.R., Collins, S.L., Kirkman, K.P., Knapp, A.K., Smith, M.D., 2014. Loss of a large grazer impacts savanna grassland plant communities similarly in North America and South Africa. *Oecologia* 175, 293–303. <https://doi.org/10.1007/s00442-014-2895-9>
- Erickson, H.E., White, R., 2008. *Soils Under Fire: Soils Research and the Joint Fire Science Program*. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station.
- Eriksen, S.E.H., Watson, H.K., 2009. The dynamic context of southern African savannas: investigating emerging threats and opportunities to sustainability. *Environ. Sci. Policy, Thematic set: The sustainability of southern African savannas: threats and opportunities* 12, 5–22. <https://doi.org/10.1016/j.envsci.2008.10.009>
- Ernst, Y., Archibald, S., Balzter, H., Chevallier, F., Ciaï, P., Fischer, C.G., Gaubert, B., Higginbottom, T., Higgins, S., Lawal, S., Lacroix, F., Lauerwald, R., Lourenco, M., Martens, C., Mengistu, A.G., Merbold, L., Mitchard, E., Moyo, M., Nguyen, H., O'Sullivan, M., Rodríguez-Veiga, P., Rosan, T., Rosentreter, J., Ryan, C., Scheiter, S., Sitch, S., Stevens, N., Tagesson, T., Tian, H., Wang, M., Woon, J.S., Zheng, B., Zhou, Y., Scholes, R.J., 2024. The African Regional Greenhouse Gases Budget (2010–2019). *Glob. Biogeochem. Cycles* 38, e2023GB008016. <https://doi.org/10.1029/2023GB008016>
- Fan, Z., Neff, J.C., Hanan, N.P., 2015. Modeling pulsed soil respiration in an African savanna ecosystem. *Agric. For. Meteorol.* 200, 282–292. <https://doi.org/10.1016/j.agrformet.2014.10.009>
- Fanin, N., Kardol, P., Farrell, M., Nilsson, M.-C., Gundale, M.J., Wardle, D.A., 2019. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. *Soil Biol. Biochem.* 128, 111–114. <https://doi.org/10.1016/j.soilbio.2018.10.010>
- Fierer, N., Wood, S.A., Bueno de Mesquita, C.P., 2021. How microbes can, and cannot, be used to assess soil health. *Soil Biol. Biochem.* 153, 108111. <https://doi.org/10.1016/j.soilbio.2020.108111>
- Finlay, R.D., Thorn, R.G., 2019. *The Fungi in Soil*, in: *Modern Soil Microbiology*, Third Edition. CRC Press.
- Fioretto, A., Papa, S., Pellegrino, A., 2005. Effects of fire on soil respiration, ATP content and enzyme activities in Mediterranean maquis. *Appl. Veg. Sci.* 8, 13–20. <https://doi.org/10.1111/j.1654-109X.2005.tb00624.x>
- Flannigan, M., Cantin, A.S., de Groot, W.J., Wotton, M., Newbery, A., Gowman, L.M., 2013. Global wildland fire season severity in the 21st century. *For. Ecol. Manag., The Mega-fire reality* 294, 54–61. <https://doi.org/10.1016/j.foreco.2012.10.022>

- Fonseca, F., de Figueiredo, T., Nogueira, C., Queirós, A., 2017. Effect of prescribed fire on soil properties and soil erosion in a Mediterranean mountain area. *Geoderma* 307, 172–180. <https://doi.org/10.1016/j.geoderma.2017.06.018>
- Forbes, E.S., Cushman, J.H., Burkepile, D.E., Young, T.P., Klope, M., Young, H.S., 2019. Synthesizing the effects of large, wild herbivore exclusion on ecosystem function. *Funct. Ecol.* 33, 1597–1610. <https://doi.org/10.1111/1365-2435.13376>
- Ford, H., Rousk, J., Garbutt, A., Jones, L., Jones, D.L., 2013. Grazing effects on microbial community composition, growth and nutrient cycling in salt marsh and sand dune grasslands. *Biol. Fertil. Soils* 49, 89–98. <https://doi.org/10.1007/s00374-012-0721-2>
- Fraç, M., Hannula, S.E., Belka, M., Jędryczka, M., 2018. Fungal Biodiversity and Their Role in Soil Health. *Front. Microbiol.* 9. <https://doi.org/10.3389/fmicb.2018.00707>
- Francini, G., Liiri, M., Männistö, M., Stark, S., Kytöviita, M.-M., 2014. Response to reindeer grazing removal depends on soil characteristics in low Arctic meadows. *Appl. Soil Ecol.* 76, 14–25. <https://doi.org/10.1016/j.apsoil.2013.12.003>
- Frank, D.A., Groffman, P.M., Evans, R.D., Tracy, B.F., 2000. Ungulate stimulation of nitrogen cycling and retention in Yellowstone Park grasslands. *Oecologia* 123, 116–121. <https://doi.org/10.1007/s004420050996>
- Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. *Soil Biol. Biochem.* 43, 1621–1625.
- Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. *J. Microbiol. Methods* 14, 151–163. [https://doi.org/10.1016/0167-7012\(91\)90018-L](https://doi.org/10.1016/0167-7012(91)90018-L)
- Fuhlendorf, S.D., Engle, D.M., 2004. Application of the fire–grazing interaction to restore a shifting mosaic on tallgrass prairie. *J. Appl. Ecol.* 41, 604–614. <https://doi.org/10.1111/j.0021-8901.2004.00937.x>
- Fultz, L.M., Moore-Kucera, J., Dathe, J., Davinic, M., Perry, G., Wester, D., Schwilk, D.W., Rideout-Hanzak, S., 2016. Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: Two case studies in the semi-arid Southwest. *Appl. Soil Ecol.* 99, 118–128. <https://doi.org/10.1016/j.apsoil.2015.10.023>
- Gertenbach, W.P.D., 1983. Landscapes of the Kruger National Park. *Koedoe* 26, 9–121. <https://doi.org/10.4102/koedoe.v26i1.591>
- Gill, J.L., 2014. Ecological impacts of the Late Quaternary megaherbivore extinctions. *New Phytol.* 201, 1163–1169. <https://doi.org/10.1111/nph.12576>
- Giorgis, M.A., Zeballos, S.R., Carbone, L., Zimmermann, H., von Wehrden, H., Aguilar, R., Ferreras, A.E., Tecco, P.A., Kowaljow, E., Barri, F., Gurvich, D.E., Villagra, P., Jaureguiberry, P., 2021. A review of fire effects across South American ecosystems: the role of climate and time since fire. *Fire Ecol.* 17, 11. <https://doi.org/10.1186/s42408-021-00100-9>
- Goheen, J.R., Augustine, D.J., Veblen, K.E., Kimuyu, D.M., Palmer, T.M., Porensky, L.M., Pringle, R.M., Ratnam, J., Riginos, C., Sankaran, M., Ford, A.T., Hassan, A.A., Jakopak, R., Kartzinel, T.R., Kurukura, S., Louthan, A.M., Odadi, W.O., Otieno, T.O., Wambua, A.M., Young, H.S., Young, T.P., 2018. Conservation lessons from large-mammal manipulations in East African savannas: the KLEE, UHURU, and GLADE experiments. *Ann. N. Y. Acad. Sci.* 1429, 31–49. <https://doi.org/10.1111/nyas.13848>
- Goheen, J.R., Palmer, T.M., Keesing, F., Riginos, C., Young, T.P., 2010. Large herbivores facilitate savanna tree establishment via diverse and indirect pathways. *J. Anim. Ecol.* 79, 372–382. <https://doi.org/10.1111/j.1365-2656.2009.01644.x>
- Goldammer, J.G., Ronde, C.D., 2004. *Wildland Fire Management Handbook for Sub-Saharan Africa*. African Minds.
- Gomes, L., Miranda, H.S., Bustamante, M.M. da C., 2018. How can we advance the knowledge on the behavior and effects of fire in the Cerrado biome? *For. Ecol. Manag.* 417, 281–290. <https://doi.org/10.1016/j.foreco.2018.02.032>
- Gordon, I., Prins, H., Mallon, J., Puk, L., Miranda, E., Starling-Manne, C., van der Wal, R., Moore, B., Foley, W., Lush, L., Maestri, R., Matsuda, I., Clauss, M., 2019. The Ecology of Browsing and Grazing in Other Vertebrate Taxa. pp. 339–404. https://doi.org/10.1007/978-3-030-25865-8_15
- Govender, N., Trollope, W.S.W., Van Wilgen, B.W., 2006. The effect of fire season, fire frequency, rainfall and management on fire intensity in savanna vegetation in South Africa. *J. Appl. Ecol.* 43, 748–758. <https://doi.org/10.1111/j.1365-2664.2006.01184.x>
- Greenwood, K.L., McKenzie, B.M., 2001. Grazing effects on soil physical properties and the consequences for pastures: a review. *Aust. J. Exp. Agric.* 41, 1231–1250. <https://doi.org/10.1071/ea00102>

- Gui, H., Wang, J., Hu, M., Zhou, Z., Wan, S., 2023. Impacts of fire on soil respiration and its components: A global meta-analysis. *Agric. For. Meteorol.* 336, 109496. <https://doi.org/10.1016/j.agrformet.2023.109496>
- Gupta, S.R., Singh, J.S., 1981. Soil respiration in a tropical grassland. *Soil Biol. Biochem.* 13, 261–268. [https://doi.org/10.1016/0038-0717\(81\)90060-2](https://doi.org/10.1016/0038-0717(81)90060-2)
- Guy, T.J., Hutchinson, M.C., Baldock, K.C.R., Kayser, E., Baiser, B., Staniczenko, P.P.A., Goheen, J.R., Pringle, R.M., Palmer, T.M., 2021. Large herbivores transform plant-pollinator networks in an African savanna. *Curr. Biol.* 31, 2964–2971.e5. <https://doi.org/10.1016/j.cub.2021.04.051>
- Hao, W.M., Ward, D.E., Olbu, G., Baker, S.P., 1996. Emissions of CO₂, CO, and hydrocarbons from fires in diverse African savanna ecosystems. *J. Geophys. Res. Atmospheres* 101, 23577–23584. <https://doi.org/10.1029/95JD02198>
- Hare, R.C., 1961. Heat effects on living plants. USDA For. Serv. South. For. Exp. Stn. Occas. Pap. 183.
- Hart, S.C., DeLuca, T.H., Newman, G.S., MacKenzie, M.D., Boyle, S.I., 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *For. Ecol. Manag., Forest Soils Research: Theory, Reality and its Role in Technology* 220, 166–184. <https://doi.org/10.1016/j.foreco.2005.08.012>
- Haslem, A., Avitabile, S.C., Taylor, R.S., Kelly, L.T., Watson, S.J., Nimmo, D.G., Kenny, S.A., Callister, K.E., Spence-Bailey, L.M., Bennett, A.F., Clarke, M.F., 2012. Time-since-fire and inter-fire interval influence hollow availability for fauna in a fire-prone system. *Biol. Conserv.* 152, 212–221. <https://doi.org/10.1016/j.biocon.2012.04.007>
- He, T., Lamont, B.B., 2018. Baptism by fire: the pivotal role of ancient conflagrations in evolution of the Earth's flora. *Natl. Sci. Rev.* 5, 237–254. <https://doi.org/10.1093/nsr/nwx041>
- Hempson, G.P., Archibald, S., Bond, W.J., Ellis, R.P., Grant, C.C., Kruger, F.J., Kruger, L.M., Moxley, C., Owen-Smith, N., Peel, M.J.S., Smit, I.P.J., Vickers, K.J., 2015. Ecology of grazing lawns in Africa. *Biol. Rev.* 90, 979–994. <https://doi.org/10.1111/brv.12145>
- Hengl, T., Leenaars, J.G.B., Shepherd, K.D., Walsh, M.G., Heuvelink, G.B.M., Mamo, T., Tilahun, H., Berkhout, E., Cooper, M., Fegeus, E., Wheeler, I., Kwabena, N.A., 2017. Soil nutrient maps of Sub-Saharan Africa: assessment of soil nutrient content at 250 m spatial resolution using machine learning. *Nutr. Cycl. Agroecosystems* 109, 77–102. <https://doi.org/10.1007/s10705-017-9870-x>
- Higgins, S.I., Bond, W.J., February, E.C., Bronn, A., Euston-Brown, D.I.W., Enslin, B., Govender, N., Rademan, L., O'Regan, S., Potgieter, A.L.F., Scheiter, S., Sowry, R., Trollope, L., Trollope, W.S.W., 2007. Effects of Four Decades of Fire Manipulation on Woody Vegetation Structure in Savanna. *Ecology* 88, 1119–1125. <https://doi.org/10.1890/06-1664>
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25, 1965–1978. <https://doi.org/10.1002/joc.1276>
- Hillel, D., 2012. *Soil and Water: Physical Principles and Processes*. Elsevier.
- Hiltbold, I., Bernklau, E., Bjostad, L.B., Alvarez, N., Miller-Struttman, N.E., Lundgren, J.G., Hibbard, B.E., 2013. Chapter Three - Nature, Evolution and Characterisation of Rhizospheric Chemical Exudates Affecting Root Herbivores, in: *Advances in Insect Physiology, Behaviour and Physiology of Root Herbivores*. Academic Press, pp. 97–157. <https://doi.org/10.1016/B978-0-12-417165-7.00003-9>
- Hockridge, E.G., Singh, J., Boucher, P.B., Davies, A.B., 2023. Resistance of termite mounds to variation in long-term fire regimes across semi-arid African savannas. *J. Anim. Ecol.* 92, 1294–1305. <https://doi.org/10.1111/1365-2656.13830>
- Holdo, R.M., Mack, M.C., 2014. Functional attributes of savanna soils: contrasting effects of tree canopies and herbivores on bulk density, nutrients and moisture dynamics. *J. Ecol.* 102, 1171–1182. <https://doi.org/10.1111/1365-2745.12290>
- Holdo, R.M., Mack, M.C., Arnold, S.G., 2012. Tree canopies explain fire effects on soil nitrogen, phosphorus and carbon in a savanna ecosystem. *J. Veg. Sci.* 23, 352–360. <https://doi.org/10.1111/j.1654-1103.2011.01357.x>
- Holland, E.A., Robertson, G.P., Greenberg, J., Groffman, P.M., Boone, R.D., Gosz, J.R., 1999. Soil CO₂, N₂ and CH₄ exchange., in: *Standard Soil Methods for Long-Term Ecological Research*. Oxford University Press, pp. 185–201.
- Homann, P.S., Bormann, B.T., Darbyshire, R.L., Morrisette, B.A., 2011. Forest Soil Carbon and Nitrogen Losses Associated with Wildfire and Prescribed Fire. *Soil Sci. Soc. Am. J.* 75, 1926–1934. <https://doi.org/10.2136/sssaj2010-0429>
- Hursh, A., Ballantyne, A., Cooper, L., Maneta, M., Kimball, J., Watts, J., 2017. The sensitivity of soil respiration to soil temperature, moisture, and carbon supply at the global scale. *Glob. Change Biol.* 23, 2090–2103. <https://doi.org/10.1111/gcb.13489>

- Jiang, Y., Tang, S., Wang, C., Zhou, P., Tenuta, M., Han, G., Huang, D., 2012. Contribution of Urine and Dung Patches from Grazing Sheep to Methane and Carbon Dioxide Fluxes in an Inner Mongolian Desert Grassland. *Asian-Australas. J. Anim. Sci.* 25, 207–212. <https://doi.org/10.5713/ajas.2011.11261>
- Joergensen, R.G., 2022. Phospholipid fatty acids in soil—drawbacks and future prospects. *Biol. Fertil. Soils* 58, 1–6. <https://doi.org/10.1007/s00374-021-01613-w>
- Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EN} value. *Soil Biol. Biochem.* 28, 33–37. [https://doi.org/10.1016/0038-0717\(95\)00101-8](https://doi.org/10.1016/0038-0717(95)00101-8)
- Joergensen, R.G., Wichern, F., 2018. Alive and kicking: Why dormant soil microorganisms matter. *Soil Biol. Biochem.* 116, 419–430. <https://doi.org/10.1016/j.soilbio.2017.10.022>
- Jolly, W.M., Cochrane, M.A., Freeborn, P.H., Holden, Z.A., Brown, T.J., Williamson, G.J., Bowman, D.M.J.S., 2015. Climate-induced variations in global wildfire danger from 1979 to 2013. *Nat. Commun.* 6, 7537. <https://doi.org/10.1038/ncomms8537>
- Jones, M.M., Fletcher Jr, R., Kruger, L., Monadjem, A., Simelane, P., McCleery, R., 2022. Drought limits large trees in African savannas with or without elephants. *Austral Ecol.* 47, 1668–1684. <https://doi.org/10.1111/aec.13244>
- Jones, M.W., Abatzoglou, J.T., Veraverbeke, S., Andela, N., Lasslop, G., Forkel, M., Smith, A.J.P., Burton, C., Betts, R.A., van der Werf, G.R., Sitch, S., Canadell, J.G., Santín, C., Kolden, C., Doerr, S.H., Le Quéré, C., 2022. Global and Regional Trends and Drivers of Fire Under Climate Change. *Rev. Geophys.* 60, e2020RG000726. <https://doi.org/10.1029/2020RG000726>
- Jouquet, P., Barré, P., Lepage, M., Velde, B., 2005. Impact of subterranean fungus-growing termites (Isoptera, Macrotermitinae) on chosen soil properties in a West African savanna. *Biol. Fertil. Soils* 41, 365–370. <https://doi.org/10.1007/s00374-005-0839-6>
- Kaiser, C., Frank, A., Wild, B., Koranda, M., Richter, A., 2010. Negligible contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2 ω 6,9 and 18:1 ω 9. *Soil Biol. Biochem.* 42, 1650–1652. <https://doi.org/10.1016/j.soilbio.2010.05.019>
- Kauffman, J.B., Thorpe, A.S., Brookshire, E.N.J., 2004. Livestock Exclusion and Belowground Ecosystem Responses in Riparian Meadows of Eastern Oregon. *Ecol. Appl.* 14, 1671–1679. <https://doi.org/10.1890/03-5083>
- Kerley, G.I., Landman, M., 2006. The impacts of elephants on biodiversity in the Eastern Cape Subtropical Thickets: elephant conservation. *South Afr. J. Sci.* 102, 395–402.
- Kgosikoma, E., 2013. Grazing management systems and their effects on savanna ecosystem dynamics: A review. *J. Ecol. Nat. Environ.* 5, 88–94. <https://doi.org/10.5897/JENE2013.0364>
- Khavhagali, V., Bond, W., 2008. Increase of woody plants in savannah ecosystems. *Grassroots Newsl. Grassl. Soc. South. Afr.* 8, 21–24.
- Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biol. Biochem.* 43, 385–392. <https://doi.org/10.1016/j.soilbio.2010.11.005>
- Kimuyu, D.M., Sensenig, R.L., Chira, R.M., Githaiga, J.M., Young, T.P., 2017. Spatial scales influence long-term response of herbivores to prescribed burning in a savanna ecosystem. *Int. J. Wildland Fire* 26, 287–295. <https://doi.org/10.1071/WF16152>
- Knicker, H., 2007. How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. *Biogeochemistry* 85, 91–118. <https://doi.org/10.1007/s10533-007-9104-4>
- Kotroczó, Z., Makádi, M., Kocsis, T., Béni, Á., Várбірó, G., Fekete, I., 2023. Long-Term Changes in Organic Matter Content and Soil Moisture Determine the Degree of Root and Soil Respiration. *Plants* 12, 251. <https://doi.org/10.3390/plants12020251>
- Kotzé, E., Sandhage-Hofmann, A., Amelung, W., Oomen, R.J., du Preez, C.C., 2017. Soil microbial communities in different rangeland management systems of a sandy savanna and clayey grassland ecosystem, South Africa. *Nutr. Cycl. Agroecosystems* 107, 227–245. <https://doi.org/10.1007/s10705-017-9832-3>
- Kreider, M.R., Higuera, P.E., Parks, S.A., Rice, W.L., White, N., Larson, A.J., 2024. Fire suppression makes wildfires more severe and accentuates impacts of climate change and fuel accumulation. *Nat. Commun.* 15, 2412. <https://doi.org/10.1038/s41467-024-46702-0>
- Lake, P.S., 2013. Resistance, Resilience and Restoration. *Ecol. Manag. Restor.* 14, 20–24. <https://doi.org/10.1111/emr.12016>
- Lawes, M.J., Richards, A., Dathe, J., Midgley, J.J., 2011. Bark thickness determines fire resistance of selected tree species from fire-prone tropical savanna in north Australia. *Plant Ecol.* 212, 2057–2069. <https://doi.org/10.1007/s11258-011-9954-7>

- Le Roux, E., Kerley, G.I.H., Cromsigt, J.P.G.M., 2018. Megaherbivores Modify Trophic Cascades Triggered by Fear of Predation in an African Savanna Ecosystem. *Curr. Biol.* 28, 2493–2499.e3. <https://doi.org/10.1016/j.cub.2018.05.088>
- le Roux, E., van Veenhuisen, L.S., Kerley, G.I.H., Cromsigt, J.P.G.M., 2020. Animal body size distribution influences the ratios of nutrients supplied to plants. *Proc. Natl. Acad. Sci.* 117, 22256–22263. <https://doi.org/10.1073/pnas.2003269117>
- Lehmann, C.E.R., Anderson, T.M., Sankaran, M., Higgins, S.I., Archibald, S., Hoffmann, W.A., Hanan, N.P., Williams, R.J., Fensham, R.J., Felfili, J., Hutley, L.B., Ratnam, J., San Jose, J., Montes, R., Franklin, D., Russell-Smith, J., Ryan, C.M., Durigan, G., Hiernaux, P., Haidar, R., Bowman, D.M.J.S., Bond, W.J., 2014. Savanna Vegetation-Fire-Climate Relationships Differ Among Continents. *Science* 343, 548–552. <https://doi.org/10.1126/science.1247355>
- Lehmann, C.E.R., Parr, C.L., 2016. Tropical grassy biomes: linking ecology, human use and conservation. *Philos. Trans. R. Soc. B Biol. Sci.* 371, 20160329. <https://doi.org/10.1098/rstb.2016.0329>
- Leizeaga, A., Hicks, L.C., Manoharan, L., Hawkes, C.V., Rousk, J., 2021. Drought legacy affects microbial community trait distributions related to moisture along a savannah grassland precipitation gradient. *J. Ecol.* 109, 3195–3210. <https://doi.org/10.1111/1365-2745.13550>
- Lelieveld, J., Evans, J.S., Fnais, M., Giannadaki, D., Pozzer, A., 2015. The contribution of outdoor air pollution sources to premature mortality on a global scale. *Nature* 525, 367–371. <https://doi.org/10.1038/nature15371>
- Leong, D.P., Teo, K.K., Rangarajan, S., Lopez-Jaramillo, P., Avezum Jr, A., Orlandini, A., 2018. World Population Prospects 2019. Department of Economic and Social Affairs Population Dynamics. New York (NY): United Nations; 2019 (<https://population.un.org/wpp/Download/>, accessed 20 September 2020). The decade of healthy ageing. Geneva: World Health Organization. World 73, 362k2469.
- Li, C., Hao, X., Zhao, M., Han, G., Willms, W.D., 2008a. Influence of historic sheep grazing on vegetation and soil properties of a Desert Steppe in Inner Mongolia. *Agric. Ecosyst. Environ.* 128, 109–116. <https://doi.org/10.1016/j.agee.2008.05.008>
- Li, C., Hao, X., Zhao, M., Han, G., Willms, W.D., 2008b. Influence of historic sheep grazing on vegetation and soil properties of a Desert Steppe in Inner Mongolia. *Agric. Ecosyst. Environ.* 128, 109–116. <https://doi.org/10.1016/j.agee.2008.05.008>
- Lickley, M., Solomon, S., 2018. Drivers, timing and some impacts of global aridity change. *Environ. Res. Lett.* 13, 104010. <https://doi.org/10.1088/1748-9326/aae013>
- Liu, W., Zhang, Z., Li, J., Wen, Y., Liu, F., Zhang, W., Liu, H., Ren, C., Han, X., 2023. Effects of fire on the soil microbial metabolic quotient: A global meta-analysis. *CATENA* 224, 106957. <https://doi.org/10.1016/j.catena.2023.106957>
- Liu, Z., Liu, K., Shi, X., Ryan Lock, T., Kallenbach, R.L., Yuan, Z., 2022. Changes in grassland phenology and growth rate, rather than diversity, drive biomass production after fire. *Agric. For. Meteorol.* 322, 109028. <https://doi.org/10.1016/j.agrformet.2022.109028>
- Lohman, D., Bickford, D., Sodhi, N., 2007. Environment. The burning issue. *Science* 316, 376. <https://doi.org/10.1126/science.1140278>
- Lombao, A., Martín Jiménez, Á., Barreiro, A., Fontúrbel Lliteras, M.T., Carballas, T., Vega Hidalgo, J.A., Jiménez, E., Fernández Filgueira, C., Díaz-Raviña, M., 2013. Microbial biomass estimated by phospholipid fatty acids (PLFA pattern) in a soil with different post-fire treatments (seeding, mulching) one year after the experimental fire. *FuegoRED* 4, 9–12. <https://doi.org/10.13039/501100010801>
- Lombardi, B., Alvarado, P.I., Ricci, P., Buraschi, L.M., Viduzzi, G., Palladino, R.A., Gonda, H.L., Juliarena, M.P., 2022. Combination of cattle urine and dung patches synergically increased nitrous oxide emissions from a temperate grassland under wet conditions. *Agric. Ecosyst. Environ.* 340, 108147. <https://doi.org/10.1016/j.agee.2022.108147>
- Long, R.A., Wambua, A., Goheen, J.R., Palmer, T.M., Pringle, R.M., 2017. Climatic variation modulates the indirect effects of large herbivores on small-mammal habitat use. *J. Anim. Ecol.* 86, 739–748. <https://doi.org/10.1111/1365-2656.12669>
- Low, A.B., Rebelo, A.G., 1996. Vegetation of South Africa, Lesotho and Swaziland. Pretoria (South Africa) Dept. of Environmental Affairs and Tourism.
- Luo, Y., Zhou, X., 2006. CHAPTER 2 - Importance and Roles of Soil Respiration, in: *Soil Respiration and the Environment*. Academic Press, Burlington, pp. 17–32. <https://doi.org/10.1016/B978-012088782-8/50002-4>
- MacFadyen, D.N., Avenant, N.L., van der Merwe, M., Bredenkamp, G.J., 2012. The influence of fire on rodent abundance at the N’washitshumbe enclosure site, Kruger National Park, South Africa. *Afr. Zool.* 47, 138–146. <https://doi.org/10.3377/004.047.0116>

- Machado, M.S., Berenguer, E., Brando, P.M., Alencar, A., Oliveras Menor, I., Barlow, J., Malhi, Y., 2024. Emergency policies are not enough to resolve Amazonia's fire crises. *Commun. Earth Environ.* 5, 1–5. <https://doi.org/10.1038/s43247-024-01344-4>
- Makhado, R.A., Scholes, R.J., 2011. Determinants of soil respiration in a semi-arid savanna ecosystem, Kruger National Park, South Africa. *Koedoe* 53, 8 pages. <https://doi.org/10.4102/koedoe.v53i1.1041>
- Malhi, Y., Doughty, C.E., Galetti, M., Smith, F.A., Svenning, J.-C., Terborgh, J.W., 2016. Megafauna and ecosystem function from the Pleistocene to the Anthropocene. *Proc. Natl. Acad. Sci.* 113, 838–846. <https://doi.org/10.1073/pnas.1502540113>
- Malkinson, D., Wittenberg, L., Beeri, O., Barzilai, R., 2011. Effects of Repeated Fires on the Structure, Composition, and Dynamics of Mediterranean Maquis: Short- and Long-Term Perspectives. *Ecosystems* 14, 478–488. <https://doi.org/10.1007/s10021-011-9424-z>
- Mallik, A.U., Gimingham, C.H., Rahman, A.A., 1984. Ecological Effects of Heather Burning: I. Water Infiltration, Moisture Retention and Porosity of Surface Soil. *J. Ecol.* 72, 767–776. <https://doi.org/10.2307/2259530>
- Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* 196, 79–91. <https://doi.org/10.1111/j.1469-8137.2012.04225.x>
- Marcos, M.S., Bertiller, M.B., Olivera, N.L., 2019. Microbial community composition and network analyses in arid soils of the Patagonian Monte under grazing disturbance reveal an important response of the community to soil particle size. *Appl. Soil Ecol.* 138, 223–232. <https://doi.org/10.1016/j.apsoil.2019.03.001>
- Marlon, J.R., Bartlein, P.J., Carcaillet, C., Gavin, D.G., Harrison, S.P., Higuera, P.E., Joos, F., Power, M.J., Prentice, I.C., 2008. Climate and human influences on global biomass burning over the past two millennia. *Nat. Geosci.* 1, 697–702. <https://doi.org/10.1038/ngeo313>
- Marschner, P., Rengel, Z., 2007. *Nutrient Cycling in Terrestrial Ecosystems*. Springer Science & Business Media.
- Martin, R.E., Miller, R.L., Cushwa, C.T., 1975. Germination Response of Legume Seeds Subjected to Moist and Dry Heat. *Ecology* 56, 1441–1445. <https://doi.org/10.2307/1934712>
- Matáix-Solera, J., C. Guerrero, F. Garcia-Orenes, G.M. Barcenas, M.P. Torres, 2009. *Forest fire effects on soil microbiology*. CRC Press.
- Matáix-Solera, J., Cerdà, A., Arcenegui, V., Jordán, A., Zavala, L.M., 2011. Fire effects on soil aggregation: A review. *Earth-Sci. Rev.* 109, 44–60. <https://doi.org/10.1016/j.earscirev.2011.08.002>
- McCauley, D.J., Graham, S.I., Dawson, T.E., Power, M.E., Ogada, M., Nyingi, W.D., Githaiga, J.M., Nyunja, J., Hughey, L.F., Brashares, J.S., 2018. Diverse effects of the common hippopotamus on plant communities and soil chemistry. *OECOLOGIA* 188, 821–835. <https://doi.org/10.1007/s00442-018-4243-y>
- McCleery, R., Monadjem, A., Baiser, B., Fletcher, R., Vickers, K., Kruger, L., 2018. Animal diversity declines with broad-scale homogenization of canopy cover in African savannas. *Biol. Conserv.* 226, 54–62. <https://doi.org/10.1016/j.biocon.2018.07.020>
- McCleery, R.A., submitted. Large herbivorous mammals direct and indirect effects on animal communities are context dependent. *Ecology*.
- McCulley, R.L., Boutton, T.W., Archer, S.R., 2007. Soil Respiration in a Subtropical Savanna Parkland: Response to Water Additions. *Soil Sci. Soc. Am. J.* 71, 820–828. <https://doi.org/10.2136/sssaj2006.0303>
- McLauchlan, K.K., Higuera, P.E., Miesel, J., Rogers, B.M., Schweitzer, J., Shuman, J.K., Tepley, A.J., Varner, J.M., Veblen, T.T., Adalsteinsson, S.A., Balch, J.K., Baker, P., Batllori, E., Bigio, E., Brando, P., Cattau, M., Chipman, M.L., Coen, J., Crandall, R., Daniels, L., Enright, N., Gross, W.S., Harvey, B.J., Hatten, J.A., Hermann, S., Hewitt, R.E., Kobziar, L.N., Landesmann, J.B., Loranty, M.M., Maezumi, S.Y., Mearns, L., Moritz, M., Myers, J.A., Pausas, J.G., Pellegrini, A.F.A., Platt, W.J., Roozeboom, J., Safford, H., Santos, F., Scheller, R.M., Sherriff, R.L., Smith, K.G., Smith, M.D., Watts, A.C., 2020. Fire as a fundamental ecological process: Research advances and frontiers. *J. Ecol.* 108, 2047–2069. <https://doi.org/10.1111/1365-2745.13403>
- McNaughton, S.J., Banyikwa, F.F., McNaughton, M.M., 1997. Promotion of the Cycling of Diet-Enhancing Nutrients by African Grazers. *Science* 278, 1798–1800. <https://doi.org/10.1126/science.278.5344.1798>
- McSherry, M.E., Ritchie, M.E., 2013. Effects of grazing on grassland soil carbon: a global review. *Glob. Change Biol.* 19, 1347–1357. <https://doi.org/10.1111/gcb.12144>
- Medina-Roldán, E., Paz-Ferreiro, J., Bardgett, R.D., 2012. Grazing exclusion affects soil and plant communities, but has no impact on soil carbon storage in an upland grassland. *Agric. Ecosyst. Environ.* 149, 118–123. <https://doi.org/10.1016/j.agee.2011.12.012>

- Meng, C., Liu, Y., Su, W., Zhang, J., Luo, Y., Zhang, Q., Di, H., Tang, C., Xu, J., Li, Y., 2023. Nutrient availability and microbial traits constrained by soil texture modulate the impact of forest fire on gross nitrogen mineralization. *For. Ecol. Manag.* 541, 121067. <https://doi.org/10.1016/j.foreco.2023.121067>
- Meteoblue, 2024. Simulated historical climate & weather data for Skukuza [WWW Document]. meteoblue. URL https://www.meteoblue.com/en/weather/historyclimate/climatemodelled/skukuza_south-africa_954955 (accessed 5.9.24).
- Michelsen, A., Andersson, M., Jensen, M., Kjøller, A., Gashew, M., 2004. Carbon stocks, soil respiration and microbial biomass in fire-prone tropical grassland, woodland and forest ecosystems. *Soil Biol. Biochem.* 36, 1707–1717. <https://doi.org/10.1016/j.soilbio.2004.04.028>
- Milchunas, D.G., Lauenroth, W.K., 1993. Quantitative Effects of Grazing on Vegetation and Soils Over a Global Range of Environments. *Ecol. Monogr.* 63, 327–366. <https://doi.org/10.2307/2937150>
- Mills, A.J., Fey, M.V., 2004. Frequent fires intensify soil crusting: physicochemical feedback in the pedoderm of long-term burn experiments in South Africa. *Geoderma* 121, 45–64. <https://doi.org/10.1016/j.geoderma.2003.10.004>
- Miransari, M., 2013. Soil microbes and the availability of soil nutrients. *Acta Physiol. Plant.* 35, 3075–3084. <https://doi.org/10.1007/s11738-013-1338-2>
- Moncrieff, G.R., Chamaille-Jammes, S., Higgins, S.I., O’Hara, R.B., Bond, W.J., 2011. Tree allometries reflect a lifetime of herbivory in an African savanna. *Ecology* 92, 2310–2315. <https://doi.org/10.1890/11-0230.1>
- Moore-Kucera, J., Dick, R.P., 2008. PLFA Profiling of Microbial Community Structure and Seasonal Shifts in Soils of a Douglas-fir Chronosequence. *Microb. Ecol.* 55, 500–511. <https://doi.org/10.1007/s00248-007-9295-1>
- Moretti, M., Conedera, M., Duelli, P., Edwards, P.J., 2002. The effects of wildfire on ground-active spiders in deciduous forests on the Swiss southern slope of the Alps. *J. Appl. Ecol.* 39, 321–336. <https://doi.org/10.1046/j.1365-2664.2002.00701.x>
- Morgan, J.W., Lunt, I.D., 1999. Effects of time-since-fire on the tussock dynamics of a dominant grass (*Themeda triandra*) in a temperate Australian grassland. *Biol. Conserv.* 88, 379–386. [https://doi.org/10.1016/S0006-3207\(98\)00112-8](https://doi.org/10.1016/S0006-3207(98)00112-8)
- Moritz, M.A., Parisien, M.-A., Batllori, E., Krawchuk, M.A., Van Dorn, J., Ganz, D.J., Hayhoe, K., 2012. Climate change and disruptions to global fire activity. *Ecosphere* 3, 1–22. <https://doi.org/10.1890/ES11-00345.1>
- Morris, J.W., Bezuidenhout, J.J., Furniss, P.R., 1982. Litter Decomposition, in: Huntley, B.J., Walker, B.H. (Eds.), *Ecology of Tropical Savannas*. Springer, Berlin, Heidelberg, pp. 535–553. https://doi.org/10.1007/978-3-642-68786-0_25
- Moyo, S., 2022. Community Responses to Fire: A Global Meta-Analysis Unravels the Contrasting Responses of Fauna to Fire. *Earth* 3, 1087–1111. <https://doi.org/10.3390/earth3040063>
- Mpheyya, J.N., Galy-Lacaux, C., Lacaux, J.P., Held, G., Pienaar, J.J., 2006. Precipitation Chemistry and Wet Deposition in Kruger National Park, South Africa. *J. Atmospheric Chem.* 53, 169–183. <https://doi.org/10.1007/s10874-005-9005-7>
- Mueller, P., Granse, D., Nolte, S., Do, H.T., Weingartner, M., Hoth, S., Jensen, K., 2017. Top-down control of carbon sequestration: grazing affects microbial structure and function in salt marsh soils. *Ecol. Appl.* 27, 1435–1450. <https://doi.org/10.1002/eap.1534>
- Munjonji, L., Ayisi, K.K., Mudongo, E.I., Mafeo, T.P., Behn, K., Mokoka, M.V., Linstädter, A., 2020. Disentangling Drought and Grazing Effects on Soil Carbon Stocks and CO₂ Fluxes in a Semi-Arid African Savanna. *Front. Environ. Sci.* 8. <https://doi.org/10.3389/fenvs.2020.590665>
- Munyati, C., Ratshibvumo, T., 2010. Differentiating geological fertility derived vegetation zones in Kruger National Park, South Africa, using Landsat and MODIS imagery. *J. Nat. Conserv.* 18, 169–179. <https://doi.org/10.1016/j.jnc.2009.08.001>
- Muqaddas, B., Zhou, X., Lewis, T., Wild, C., Chen, C., 2015. Long-term frequent prescribed fire decreases surface soil carbon and nitrogen pools in a wet sclerophyll forest of Southeast Queensland, Australia. *Sci. Total Environ.* 536, 39–47. <https://doi.org/10.1016/j.scitotenv.2015.07.023>
- Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999. Fire effects on belowground sustainability: a review and synthesis. *For. Ecol. Manag.* 122, 51–71. [https://doi.org/10.1016/S0378-1127\(99\)00032-8](https://doi.org/10.1016/S0378-1127(99)00032-8)
- Neary, D.G., Ryan, K.C., DeBano, L.F., 2005. Wildland fire in ecosystems: effects of fire on soils and water. *Gen Tech Rep RMRS-GTR-42-Vol4* Ogden UT US Dep. Agric. For. Serv. Rocky Mt. Res. Stn. 250 P 042. <https://doi.org/10.2737/RMRS-GTR-42-V4>
- Ngugi, M.W., Kimuyu, D.M., Sensenig, R.L., Odadi, W.O., Kiboi, S.K., Omari, J.K., Young, T.P., 2022. Fire and Herbivory Interactively Suppress the Survival and Growth of Trees in an African Semiarid Savanna. *Fire* 5, 169. <https://doi.org/10.3390/fire5050169>

- Noronha, M.F., Lacerda Júnior, G.V., Gilbert, J.A., de Oliveira, V.M., 2017. Taxonomic and functional patterns across soil microbial communities of global biomes. *Sci. Total Environ.* 609, 1064–1074. <https://doi.org/10.1016/j.scitotenv.2017.07.159>
- Novoa, A., 2024. Personal communication.
- Oates, L.G., Balsler, T.C., Jackson, R.D., 2012. Subhumid pasture soil microbial communities affected by presence of grazing, but not grazing management. *Appl. Soil Ecol.* 59, 20–28. <https://doi.org/10.1016/j.apsoil.2012.03.020>
- Ohlinger, R., 1995. Maximum water-holding capacity. *Methods Soil Biol.* 385–386.
- Okullo, P., Moe, S.R., 2012. Termite activity, not grazing, is the main determinant of spatial variation in savanna herbaceous vegetation. *J. Ecol.* 100, 232–241. <https://doi.org/10.1111/j.1365-2745.2011.01889.x>
- Olf, H., Ritchie, M.E., Prins, H.H.T., 2002. Global environmental controls of diversity in large herbivores. *Nature* 415, 901–904. <https://doi.org/10.1038/415901a>
- Oliveira, E., Kobziar, L., Putz, F.E., 2011. Fire ignition patterns affect production of charcoal in southern forests. *Int. J. Wildland Fire* 20, 474–477. <https://doi.org/10.1071/WF10061>
- Olsson, P.A., Lekberg, Y., 2022. A critical review of the use of lipid signature molecules for the quantification of arbuscular mycorrhiza fungi. *Soil Biol. Biochem.* 166, 108574. <https://doi.org/10.1016/j.soilbio.2022.108574>
- Owen-Smith, N., 1987. Pleistocene extinctions: the pivotal role of megaherbivores. *Paleobiology* 13, 351–362. <https://doi.org/10.1017/S0094837300008927>
- Parente, J., Girona-García, A., Lopes, A.R., Keizer, J.J., Vieira, D.C.S., 2022. Prediction, validation, and uncertainties of a nation-wide post-fire soil erosion risk assessment in Portugal. *Sci. Rep.* 12, 2945. <https://doi.org/10.1038/s41598-022-07066-x>
- Parr, C.L., Andersen, A.N., 2008. Fire resilience of ant assemblages in long-unburnt savanna of northern Australia. *Austral Ecol.* 33, 830–838. <https://doi.org/10.1111/j.1442-9993.2008.01848.x>
- Parr, C.L., Chown, S.L., 2003. Burning issues for conservation: A critique of faunal fire research in Southern Africa. *Austral Ecol.* 28, 384–395. <https://doi.org/10.1046/j.1442-9993.2003.01296.x>
- Parr, C.L., Robertson, H.G., Biggs, H.C., Chown, S.L., 2004. Response of African Savanna Ants to Long-Term Fire Regimes. *J. Appl. Ecol.* 41, 630–642. <https://doi.org/10.1111/j.0021-8901.2004.00920.x>
- Pausas, J.G., Lamont, B.B., 2022. Fire-released seed dormancy - a global synthesis. *Biol. Rev.* 97, 1612–1639. <https://doi.org/10.1111/brv.12855>
- Pellegrini, A., Hedin, L., Staver, A., Govender, N., 2014. Fire alters ecosystem carbon and nutrients but not plant nutrient stoichiometry or composition in tropical savanna. *Ecology*. <https://doi.org/10.1890/14-1158.1>
- Pellegrini, A., Hobbie, S., Reich, P., Jumpponen, A., Brookshire, E.N., Caprio, A., Coetsee, C., Jackson, R., 2020. Repeated fire shifts carbon and nitrogen cycling by changing plant inputs and soil decomposition across ecosystems. *Ecol. Monogr.* 90. <https://doi.org/10.1002/ecm.1409>
- Pellegrini, A.F.A., Ahlström, A., Hobbie, S.E., Reich, P.B., Nieradzick, L.P., Staver, A.C., Scharenbroch, B.C., Jumpponen, A., Anderegg, W.R.L., Randerson, J.T., Jackson, R.B., 2018. Fire frequency drives decadal changes in soil carbon and nitrogen and ecosystem productivity. *Nature* 553, 194–198. <https://doi.org/10.1038/nature24668>
- Pellegrini, A.F.A., Hedin, L.O., Staver, A.C., Govender, N., 2015. Fire alters ecosystem carbon and nutrients but not plant nutrient stoichiometry or composition in tropical savanna. *Ecology* 96, 1275–1285. <https://doi.org/10.1890/14-1158.1>
- Pennington, R.T., Lehmann, C.E.R., Rowland, L.M., 2018. Tropical savannas and dry forests. *Curr. Biol.* 28, R541–R545. <https://doi.org/10.1016/j.cub.2018.03.014>
- Pereira, P., Bogunovic, I., Zhao, W., Barcelo, D., 2021. Short-term effect of wildfires and prescribed fires on ecosystem services. *Curr. Opin. Environ. Sci. Health* 22, 100266. <https://doi.org/10.1016/j.coesh.2021.100266>
- Pereira, P., Cerdà, A., Lopez, A.J., Zavala, L.M., Mataix-Solera, J., Arcenegui, V., Misiune, I., Keesstra, S., Novara, A., 2016. Short-Term Vegetation Recovery after a Grassland Fire in Lithuania: The Effects of Fire Severity, Slope Position and Aspect. *Land Degrad. Dev.* 27, 1523–1534. <https://doi.org/10.1002/ldr.2498>
- Pérez-Valera, E., Verdú, M., Navarro-Cano, J.A., Goberna, M., 2018. Resilience to fire of phylogenetic diversity across biological domains. *Mol. Ecol.* 27, 2896–2908. <https://doi.org/10.1111/mec.14729>
- Peschel, A.R., Zak, D.R., Cline, L.C., Freedman, Z., 2015. Elk, sagebrush, and saprotrophs: indirect top-down control on microbial community composition and function. *Ecology* 96, 2383–2393. <https://doi.org/10.1890/15-0164.1>
- Piñeiro, G., Paruelo, J.M., Oesterheld, M., Jobbágy, E.G., 2010. Pathways of Grazing Effects on Soil Organic Carbon and Nitrogen. *Rangel. Ecol. Manag.* 63, 109–119. <https://doi.org/10.2111/08-255.1>

- Ping, X., Chang, Y., Liu, M., Hu, Y., Huang, W., Shi, S., Jia, Y., Li, D., 2022. Carbon Emission and Redistribution among Forest Carbon Pools, and Change in Soil Nutrient Content after Different Severities of Forest Fires in Northeast China. *Forests* 13, 110. <https://doi.org/10.3390/f13010110>
- Ponder, F., Tadros, M., Loewenstein, E.F., 2009. Microbial properties and litter and soil nutrients after two prescribed fires in developing savannas in an upland Missouri Ozark Forest. *For. Ecol. Manag.* 257, 755–763. <https://doi.org/10.1016/j.foreco.2008.10.009>
- Poulsen, J.R., Rosin, C., Meier, A., Mills, E., Nuñez, C.L., Koerner, S.E., Blanchard, E., Callejas, J., Moore, S., Sowers, M., 2018. Ecological consequences of forest elephant declines for Afrotropical forests. *Conserv. Biol.* 32, 559–567. <https://doi.org/10.1111/cobi.13035>
- Preece, C., Peñuelas, J., 2020. A Return to the Wild: Root Exudates and Food Security. *Trends Plant Sci.* 25, 14–21. <https://doi.org/10.1016/j.tplants.2019.09.010>
- Prendergast-Miller, M.T., de Menezes, A.B., Macdonald, L.M., Toscas, P., Bissett, A., Baker, G., Farrell, M., Richardson, A.E., Wark, T., Thrall, P.H., 2017. Wildfire impact: Natural experiment reveals differential short-term changes in soil microbial communities. *Soil Biol. Biochem.* 109, 1–13. <https://doi.org/10.1016/j.soilbio.2017.01.027>
- Pressler, Y., Moore, J.C., Cotrufo, M.F., 2019. Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. *Oikos* 128, 309–327. <https://doi.org/10.1111/oik.05738>
- Pringle, R.M., Abraham, J.O., Anderson, T.M., Coverdale, T.C., Davies, A.B., Dutton, C.L., Gaylard, A., Goheen, J.R., Holdo, R.M., Hutchinson, M.C., Kimuyu, D.M., Long, R.A., Subalusky, A.L., Veldhuis, M.P., 2023. Impacts of large herbivores on terrestrial ecosystems. *Curr. Biol.* 33, R584–R610. <https://doi.org/10.1016/j.cub.2023.04.024>
- Pringle, R.M., Goheen, J.R., Palmer, T.M., Charles, G.K., DeFranco, E., Hohbein, R., Ford, A.T., Tarnita, C.E., 2014. Low functional redundancy among mammalian browsers in regulating an encroaching shrub (*Solanum campylacanthum*) in African savannah. *Proc. R. Soc. B-Biol. Sci.* 281, 20140390. <https://doi.org/10.1098/rspb.2014.0390>
- Pringle, R.M., Young, T.P., Rubenstein, D.I., McCauley, D.J., 2007. Herbivore-initiated interaction cascades and their modulation by productivity in an African savanna. *Proc. Natl. Acad. Sci.* 104, 193–197. <https://doi.org/10.1073/pnas.0609840104>
- Pyne, S.J., 2019. Fire: a brief history. Univ. Wash. Press.
- Raich, J.W., Potter, C.S., Bhagawati, D., 2002. Interannual variability in global soil respiration, 1980–94. *Glob. Change Biol.* 8, 800–812. <https://doi.org/10.1046/j.1365-2486.2002.00511.x>
- Raich, J.W., Tufekciogul, A., 2000. Vegetation and soil respiration: Correlations and controls. *Biogeochemistry* 48, 71–90. <https://doi.org/10.1023/A:1006112000616>
- Reich, P.B., Peterson, D.W., Wedin, D.A., Wrage, K., 2001. Fire and Vegetation Effects on Productivity and Nitrogen Cycling Across a Forest–Grassland Continuum. *Ecology* 82, 1703–1719. [https://doi.org/10.1890/0012-9658\(2001\)082\[1703:FAVEOP\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[1703:FAVEOP]2.0.CO;2)
- Reinhart, K.O., Dangi, S.R., Vermeire, L.T., 2016. The effect of fire intensity, nutrients, soil microbes, and spatial distance on grassland productivity. *Plant Soil* 409, 203–216. <https://doi.org/10.1007/s11104-016-2957-3>
- Reischke, S., Rousk, J., Bååth, E., 2014. The effects of glucose loading rates on bacterial and fungal growth in soil. *Soil Biol. Biochem.* 70, 88–95. <https://doi.org/10.1016/j.soilbio.2013.12.011>
- Reisser, M., Purves, R.S., Schmidt, M.W.I., Abiven, S., 2016. Pyrogenic Carbon in Soils: A Literature-Based Inventory and a Global Estimation of Its Content in Soil Organic Carbon and Stocks. *Front. Earth Sci.* 4. <https://doi.org/10.3389/feart.2016.00080>
- Retallack, G.J., 2003. Soils and Global Change in the Carbon Cycle over Geological Time. *Treatise Geochem.* 5, 605. <https://doi.org/10.1016/B0-08-043751-6/05087-8>
- Ribeiro, N., Ruecker, G., Govender, N., Macandza, V., Pais, A., Machava, D., Chauque, A., Lisboa, S.N., Bandeira, R., 2019. The influence of fire frequency on the structure and botanical composition of savanna ecosystems. *Ecol. Evol.* 9, 8253–8264. <https://doi.org/10.1002/ece3.5400>
- Richards, A.E., Dathe, J., Cook, G.D., 2012. Fire interacts with season to influence soil respiration in tropical savannas. *Soil Biol. Biochem.* 53, 90–98. <https://doi.org/10.1016/j.soilbio.2012.05.009>
- Riginos, C., 2015. Climate and the landscape of fear in an African savanna. *J. Anim. Ecol.* 84, 124–133. <https://doi.org/10.1111/1365-2656.12262>
- Riginos, C., Porensky, L.M., Veblen, K.E., Young, T.P., 2018. Herbivory and drought generate short-term stochasticity and long-term stability in a savanna understory community. *Ecol. Appl.* 28, 323–335. <https://doi.org/10.1002/eap.1649>
- Rinnan, R., Bååth, E., 2009. Differential Utilization of Carbon Substrates by Bacteria and Fungi in Tundra Soil. *Appl. Environ. Microbiol.* 75, 3611–3620. <https://doi.org/10.1128/AEM.02865-08>

- Ripple, W.J., Newsome, T.M., Wolf, C., Dirzo, R., Everatt, K.T., Galetti, M., Hayward, M.W., Kerley, G.I.H., Levi, T., Lindsey, P.A., Macdonald, D.W., Malhi, Y., Painter, L.E., Sandom, C.J., Terborgh, J., Van Valkenburgh, B., 2015. Collapse of the world's largest herbivores. *Sci. Adv.* 1, e1400103. <https://doi.org/10.1126/sciadv.1400103>
- Ritz, K., Young, I.M., 2004. Interactions between soil structure and fungi. *Mycologist* 18, 52–59. <https://doi.org/10.1017/S0269915X04002010>
- Roberts, B.A., Fritschi, F.B., Horwath, W.R., Scow, K.M., Rains, W.D., Travis, R.L., 2011. Comparisons of Soil Microbial Communities Influenced by Soil Texture, Nitrogen Fertility, and Rotations. *Soil Sci.* 176, 487. <https://doi.org/10.1097/SS.0b013e31822769d3>
- Robichaud, P.R., 2000. Fire effects on infiltration rates after prescribed fire in Northern Rocky Mountain forests, USA. *J. Hydrol.* 231–232, 220–229. [https://doi.org/10.1016/S0022-1694\(00\)00196-7](https://doi.org/10.1016/S0022-1694(00)00196-7)
- Romps, D.M., Seeley, J.T., Vollaro, D., Molinari, J., 2014. Projected increase in lightning strikes in the United States due to global warming. *Science* 346, 851–854. <https://doi.org/10.1126/science.1259100>
- Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* 4, 1340–1351. <https://doi.org/10.1038/ismej.2010.58>
- Ruan, Y., Mohtadi, M., Dupont, L.M., Hebbeln, D., van der Kaars, S., Hopmans, E.C., Schouten, S., Hyer, E.J., Schefuß, E., 2020. Interaction of Fire, Vegetation, and Climate in Tropical Ecosystems: A Multiproxy Study Over the Past 22,000 Years. *Glob. Biogeochem. Cycles* 34, e2020GB006677. <https://doi.org/10.1029/2020GB006677>
- Ruess, R.W., McNaughton, S.J., 1987. Grazing and the Dynamics of Nutrient and Energy Regulated Microbial Processes in the Serengeti grasslands. *Oikos* 49, 101–110. <https://doi.org/10.2307/3565559>
- Rughöft, S., Herrmann, M., Lazar, C.S., Cesarz, S., Levick, S.R., Trumbore, S.E., Küsel, K., 2016. Community Composition and Abundance of Bacterial, Archaeal and Nitrifying Populations in Savanna Soils on Contrasting Bedrock Material in Kruger National Park, South Africa. *Front. Microbiol.* 7, 1638. <https://doi.org/10.3389/fmicb.2016.01954>
- Saito, K., Vandamme, E., Johnson, J.-M., Tanaka, A., Senthilkumar, K., Dieng, I., Akakpo, C., Gbaguidi, F., Segda, Z., Bassoro, I., Lamare, D., Gbakatchetche, H., Abera, B.B., Jaiteh, F., Bam, R.K., Dogbe, W., Sékou, K., Rabeson, R., Kamissoko, N., Mossi, I.M., Tarfa, B.D., Bakare, S.O., Kalisa, A., Baggie, I., Kajiru, G.J., Ablede, K., Ayeva, T., Nanfumba, D., Wopereis, M.C.S., 2019. Yield-limiting macronutrients for rice in sub-Saharan Africa. *Geoderma* 338, 546–554. <https://doi.org/10.1016/j.geoderma.2018.11.036>
- Saiz, G., Goodrick, I., Wurster, C., Nelson, P.N., Wynn, J., Bird, M., 2018. Preferential Production and Transport of Grass-Derived Pyrogenic Carbon in NE-Australian Savanna Ecosystems. *Front. Earth Sci.* 5. <https://doi.org/10.3389/feart.2017.00115>
- San Emeterio, L., Baquero, E., Antón, R., Jordana, R., Múgica, L., Sáez, J.L., Virto, I., Canals, R.M., 2023. Pyric herbivory increases soil microbial diversity but has a site-dependent effect on soil mesofauna in the mid-term. *Agric. Ecosyst. Environ.* 356, 108632. <https://doi.org/10.1016/j.agee.2023.108632>
- Sánchez-García, C., Santín, C., Doerr, S.H., Strydom, T., Urbanek, E., 2021. Wildland fire ash enhances short-term CO₂ flux from soil in a Southern African savannah. *Soil Biol. Biochem.* 160, 108334. <https://doi.org/10.1016/j.soilbio.2021.108334>
- Sankaran, M., Augustine, D.J., 2004. Large Herbivores Suppress Decomposer Abundance in a Semiarid Grazing Ecosystem. *Ecology* 85, 1052–1061. <https://doi.org/10.1890/03-0354>
- Sankaran, M., Augustine, D.J., Ratnam, J., 2013. Native ungulates of diverse body sizes collectively regulate long-term woody plant demography and structure of a semi-arid savanna. *J. Ecol.* 101, 1389–1399. <https://doi.org/10.1111/1365-2745.12147>
- Sankaran, M., Hanan, N.P., Scholes, R.J., Ratnam, J., Augustine, D.J., Cade, B.S., Gignoux, J., Higgins, S.I., Le Roux, X., Ludwig, F., Ardo, J., Banyikwa, F., Bronn, A., Bucini, G., Caylor, K.K., Coughenour, M.B., Diouf, A., Ekaya, W., Feral, C.J., February, E.C., Frost, P.G.H., Hiernaux, P., Hrabar, H., Metzger, K.L., Prins, H.H.T., Ringrose, S., Sea, W., Tews, J., Worden, J., Zambatis, N., 2005. Determinants of woody cover in African savannas. *Nature* 438, 846–849. <https://doi.org/10.1038/nature04070>
- Sankaran, M., Ratnam, J., Hanan, N., 2008. Woody cover in African savannas: The role of resources, fire and herbivory. *Glob. Ecol. Biogeogr.* 17, 236–245. <https://doi.org/10.1111/j.1466-8238.2007.00360.x>
- SANParks, 2024. Savanna Research Unit – Scientific Services [WWW Document]. SANParks. URL <https://www.sanparks.org/conservation/scientific-services/data-information-resources/maps/savanna-research-unit> (accessed 5.2.24).
- Santín, C., Doerr, S.H., Kane, E.S., Masiello, C.A., Ohlson, M., de la Rosa, J.M., Preston, C.M., Dittmar, T., 2016. Towards a global assessment of pyrogenic carbon from vegetation fires. *Glob. Change Biol.* 22, 76–91. <https://doi.org/10.1111/gcb.12985>

- Sawyer, R., Bradstock, R., Bedward, M., Morrison, R.J., 2018. Fire intensity drives post-fire temporal pattern of soil carbon accumulation in Australian fire-prone forests. *Sci. Total Environ.* 610–611, 1113–1124. <https://doi.org/10.1016/j.scitotenv.2017.08.165>
- Scheunemann, N., Digel, C., Scheu, S., Butenschoen, O., 2015. Roots rather than shoot residues drive soil arthropod communities of arable fields. *Oecologia* 179, 1135–1145. <https://doi.org/10.1007/s00442-015-3415-2>
- Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. *Biogeochemistry* 48, 7–20. <https://doi.org/10.1023/A:1006247623877>
- Scholes, M., Andreae, M.O., 2000. Biogenic and Pyrogenic Emissions from Africa and Their Impact on the Global Atmosphere. *Ambio* 29, 23–29.
- Scholes, R.J., Archer, S.R., 1997a. Tree-Grass Interactions in Savannas. *Annu. Rev. Ecol. Syst.* 28, 517–544. <https://doi.org/10.1146/annurev.ecolsys.28.1.517>
- Scholes, R.J., Archer, S.R., 1997b. Tree-Grass Interactions in Savannas. *Annu. Rev. Ecol. Syst.* 28, 517–544. <https://doi.org/10.1146/annurev.ecolsys.28.1.517>
- Scholtz, R., Donovan, V.M., Strydom, T., Wonkka, C., Kreuter, U.P., Rogers, W.E., Taylor, C., Smit, I.P., Govender, N., Trollope, W., Fogarty, D.T., Twidwell, D., 2022. High-intensity fire experiments to manage shrub encroachment: lessons learned in South Africa and the United States. *Afr. J. Range Forage Sci.* 39, 148–159. <https://doi.org/10.2989/10220119.2021.2008004>
- Schoug, Å., Fischer, J., Heipieper, H.J., Schnürer, J., Håkansson, S., 2008. Impact of fermentation pH and temperature on freeze-drying survival and membrane lipid composition of *Lactobacillus coryniformis* Si3. *J. Ind. Microbiol. Biotechnol.* 35, 175–181. <https://doi.org/10.1007/s10295-007-0281-x>
- Sebata, A., 2013. Woody Plant-Herbivore Interactions in Semi-Arid Savanna Ecosystems. *Herbivory*. <https://doi.org/10.5772/48400>
- Shackleton, C.M., Scholes, R.J., 2000. Impact of fire frequency on woody community structure and soil nutrients in the Kruger National Park. *Koedoe* 43, 75–81. <https://doi.org/10.4102/koedoe.v43i1.210>
- Sidle, R.C., Hirano, T., Gomi, T., Terajima, T., 2007. Hortonian overland flow from Japanese forest plantations—an aberration, the real thing, or something in between? *Hydrol. Process.* 21, 3237–3247. <https://doi.org/10.1002/hyp.6876>
- Šimanský, V., 2015. Changes in Soil Structure and Soil Organic Matter Due to Different Severities of Fire. *Ekológia Bratisl.* 34, 226–234. <https://doi.org/10.1515/eko-2015-0022>
- Simpson, K.J., Archibald, S., Osborne, C.P., 2022. Savanna fire regimes depend on grass trait diversity. *Trends Ecol. Evol.* 37, 749–758. <https://doi.org/10.1016/j.tree.2022.04.010>
- Singh, J., Donaldson, J.E., Archibald, S., Parr, C.L., Voysey, M.D., Davies, A.B., 2024. Small-scale fires interact with herbivore feedbacks to create persistent grazing lawn environments. *J. Appl. Ecol.* 1365-2664.14645. <https://doi.org/10.1111/1365-2664.14645>
- Singh, R.S., Srivastava, S.C., Raghubanshi, A.S., Singh, J.S., Singh, S.P., 1991. Microbial C, N and P in Dry Tropical Savanna: Effects of Burning and Grazing. *J. Appl. Ecol.* 28, 869–878. <https://doi.org/10.2307/2404213>
- Singh, S., 2022. “Forest fire emissions: A contribution to global climate change.” *Front. For. Glob. Change* 5. <https://doi.org/10.3389/ffgc.2022.925480>
- Sitters, J., Bakker, E.S., Veldhuis, M.P., Veen, G.F., Olde Venterink, H., Vanni, M.J., 2017. The Stoichiometry of Nutrient Release by Terrestrial Herbivores and Its Ecosystem Consequences. *Front. Earth Sci.* 5. <https://doi.org/10.3389/feart.2017.00032>
- Sitters, J., Kimuyu, D.M., Young, T.P., Claeys, P., Olde Venterink, H., 2020a. Negative effects of cattle on soil carbon and nutrient pools reversed by megaherbivores. *Nat. Sustain.* 3, 360–366. <https://doi.org/10.1038/s41893-020-0490-0>
- Sitters, J., Maechler, M.-J., Edwards, P.J., Suter, W., Olde Venterink, H., 2014a. Interactions between C : N : P stoichiometry and soil macrofauna control dung decomposition of savanna herbivores. *Funct. Ecol.* 28, 776–786. <https://doi.org/10.1111/1365-2435.12213>
- Sitters, J., Maechler, M.-J., Edwards, P.J., Suter, W., Venterink, H.O., 2014b. Interactions between C : N : P stoichiometry and soil macrofauna control dung decomposition of savanna herbivores. *Funct. Ecol.* 28, 776–786. <https://doi.org/10.1111/1365-2435.12213>
- Sitters, J., Olde Venterink, H., 2021a. Stoichiometric impact of herbivore dung versus urine on soils and plants. *Plant Soil* 462, 59–65. <https://doi.org/10.1007/s11104-021-04960-7>
- Sitters, J., Olde Venterink, H., 2021b. Herbivore dung stoichiometry drives competition between savanna trees and grasses. *J. Ecol.* 109, 2095–2106. <https://doi.org/10.1111/1365-2745.13623>
- Sitters, J., Wubs, E.R.J., Bakker, E.S., Crowther, T.W., Adler, P.B., Bagchi, S., Bakker, J.D., Biederman, L., Borer, E.T., Cleland, E.E., Eisenhauer, N., Firn, J., Gherardi, L., Hagenah, N., Hautier, Y., Hobbie, S.E., Knops, J.M.H., MacDougall, A.S., McCulley, R.L., Moore, J.L., Mortensen, B., Peri, P.L., Prober, S.M., Riggs, C., Risch, A.C., Schütz, M., Seabloom, E.W., Siebert, J., Stevens, C.J., Veen,

- G.F., 2020b. Nutrient availability controls the impact of mammalian herbivores on soil carbon and nitrogen pools in grasslands. *Glob. Change Biol.* 26, 2060–2071. <https://doi.org/10.1111/gcb.15023>
- Smit, I.P.J., Coetsee, C., 2019. Interactions Between Fire and Herbivory: Current Understanding and Management Implications, in: *The Ecology of Browsing and Grazing II*, Ecological Studies. Springer International Publishing, Cham, pp. 301–319. https://doi.org/10.1007/978-3-030-25865-8_13
- Smit, I.P.J., Petersen, R., Riddell, E.S., Cullum, C., 2013a. Kruger National Park research supersites : establishing long-term research sites for cross-disciplinary, multiscaled learning : original research. *Koedoe Afr. Prot. Area Conserv. Sci.* 55, 1–7. <https://doi.org/10.4102/koedoe.v55i1.1107>
- Smit, I.P.J., Prins, H.H.T., 2015a. Predicting the Effects of Woody Encroachment on Mammal Communities, Grazing Biomass and Fire Frequency in African Savannas. *PLOS ONE* 10, e0137857. <https://doi.org/10.1371/journal.pone.0137857>
- Smit, I.P.J., Prins, H.H.T., 2015b. Predicting the Effects of Woody Encroachment on Mammal Communities, Grazing Biomass and Fire Frequency in African Savannas. *PLOS ONE* 10, e0137857. <https://doi.org/10.1371/journal.pone.0137857>
- Smit, I.P.J., Smit, C.F., Govender, N., Linde, M. van der, MacFadyen, S., 2013b. Rainfall, geology and landscape position generate large-scale spatiotemporal fire pattern heterogeneity in an African savanna. *Ecography* 36, 447–459. <https://doi.org/10.1111/j.1600-0587.2012.07555.x>
- Smith, M.D., van Wilgen, B.W., Burns, C.E., Govender, N., Potgieter, A.L.F., Andelman, S., Biggs, H.C., Botha, J., Trollope, W.S.W., 2013. Long-term effects of fire frequency and season on herbaceous vegetation in savannas of the Kruger National Park, South Africa. *J. Plant Ecol.* 6, 71–83. <https://doi.org/10.1093/jpe/rts014>
- Smith, P., Cotrufo, M.F., Rumpel, C., Paustian, K., Kuikman, P.J., Elliott, J.A., McDowell, R., Griffiths, R.I., Asakawa, S., Bustamante, M., House, J.I., Sobocká, J., Harper, R., Pan, G., West, P.C., Gerber, J.S., Clark, J.M., Adhya, T., Scholes, R.J., Scholes, M.C., 2015. Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. *SOIL* 1, 665–685. <https://doi.org/10.5194/soil-1-665-2015>
- Smucker, K.M., Hutto, R.L., Steele, B.M., 2005. Changes in Bird Abundance After Wildfire: Importance of Fire Severity and Time Since Fire. *Ecol. Appl.* 15, 1535–1549. <https://doi.org/10.1890/04-1353>
- Staver, A.C., Abraham, J.O., Hempson, G.P., Karp, A.T., Faith, J.T., 2021. The past, present, and future of herbivore impacts on savanna vegetation. *J. Ecol.* 109, 2804–2822. <https://doi.org/10.1111/1365-2745.13685>
- Staver, A.C., Archibald, S., Levin, S.A., 2011. The Global Extent and Determinants of Savanna and Forest as Alternative Biome States. *Science* 334, 230–232. <https://doi.org/10.1126/science.1210465>
- Staver, A.C., Bond, W.J., 2014. Is there a ‘browse trap’? Dynamics of herbivore impacts on trees and grasses in an African savanna. *J. Ecol.* 102, 595–602. <https://doi.org/10.1111/1365-2745.12230>
- Staver, A.C., Botha, J., Hedin, L., 2017. Soils and fire jointly determine vegetation structure in an African savanna. *New Phytol.* 216, 1151–1160.
- Steffens, M., Kölbl, A., Totsche, K.U., Kögel-Knabner, I., 2008. Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia. *Geoderma* 143, 63–72. <https://doi.org/10.1016/j.geoderma.2007.09.004>
- Stevens, N., Bond, W., Feurdean, A., Lehmann, C.E.R., 2022. Grassy Ecosystems in the Anthropocene. *Annu. Rev. Environ. Resour.* 47, 261–289. <https://doi.org/10.1146/annurev-environ-112420-015211>
- Stevens, N., Lehmann, C.E.R., Murphy, B.P., Durigan, G., 2017. Savanna woody encroachment is widespread across three continents. *Glob. Change Biol.* 23, 235–244. <https://doi.org/10.1111/gcb.13409>
- Stoof, C.R., Ferreira, A.J.D., Mol, W., Van den Berg, J., De Kort, A., Drooger, S., Slingerland, E.C., Mansholt, A.U., Ferreira, C.S.S., Ritsema, C.J., 2015. Soil surface changes increase runoff and erosion risk after a low–moderate severity fire. *Geoderma* 239–240, 58–67. <https://doi.org/10.1016/j.geoderma.2014.09.020>
- Stritar, M.L., Schweitzer, J.A., Hart, S.C., Bailey, J.K., 2010. Introduced ungulate herbivore alters soil processes after fire. *Biol. Invasions* 12, 313–324. <https://doi.org/10.1007/s10530-009-9624-z>
- Strydom, T., Riddell, E.S., Rowe, T., Govender, N., Lorentz, S.A., le Roux, P.A.L., Wigley-Coetsee, C., 2019. The effect of experimental fires on soil hydrology and nutrients in an African savanna. *Geoderma* 345, 114–122. <https://doi.org/10.1016/j.geoderma.2019.03.027>
- Stuart Chapin III, F., McFarland, J., David McGuire, A., Euskirchen, E.S., Ruess, R.W., Kielland, K., 2009. The changing global carbon cycle: linking plant–soil carbon dynamics to global consequences. *J. Ecol.* 97, 840–850. <https://doi.org/10.1111/j.1365-2745.2009.01529.x>
- Syphard, A.D., Keeley, J.E., Pfaff, A.H., Ferschweiler, K., 2017. Human presence diminishes the importance of climate in driving fire activity across the United States. *Proc. Natl. Acad. Sci.* 114, 13750–13755. <https://doi.org/10.1073/pnas.1713885114>

- Tan, Z., Yuan, Y., Gu, M., Han, Y., Mao, L., Tan, T., Wu, C., Han, T., 2023. Levoglucosan and its isomers in terrestrial sediment as a molecular markers provide direct evidence for the low-temperature fire during the mid-Holocene in the northern Shandong Peninsula of China. *Quat. Int.* 661, 22–33. <https://doi.org/10.1016/j.quaint.2023.05.010>
- Terborgh, J., 1988. The Big Things that Run The World-A Sequel to E. O. Wilson. *Conserv. Biol.* 2, 402–403.
- Thies, J.E., Grossman, J.M., 2023. The Soil Habitat and Soil Ecology, in: *Biological Approaches to Regenerative Soil Systems*. CRC Press.
- Thoresen, J., Vermeire, M.-L., Venter, Z., Wolfaard, G., Krumins, J.A., Cramer, M., Hawkins, H.-J., 2021. Fire and herbivory shape soil arthropod communities through habitat heterogeneity and nutrient cycling in savannas. *Glob. Ecol. Conserv.* 25, e01413. <https://doi.org/10.1016/j.gecco.2020.e01413>
- Treseder, K.K., Mack, M.C., Cross, A., 2004. Relationships Among Fires, Fungi, and Soil Dynamics in Alaskan Boreal Forests. *Ecol. Appl.* 14, 1826–1838. <https://doi.org/10.1890/03-5133>
- Trollope, W.S.W., Potgieter, A.L.F., Biggs, H.C., Trollope, L.A., 1998. Experimental Burning Plots (EBP) trial in the major vegetation types of the Kruger National Park. Scientific Report No. 09/ 1998. South African National Parks, Skukuza, RSA.
- van der Plas, F., Howison, R.A., Mpanza, N., Cromsigt, J.P.G.M., Oloff, H., 2016. Different-sized grazers have distinctive effects on plant functional composition of an African savannah. *J. Ecol.* 104, 864–875. <https://doi.org/10.1111/1365-2745.12549>
- van Klink, R., Schrama, M., Nolte, S., Bakker, J.P., WallisDeVries, M.F., Berg, M.P., 2015. Defoliation and Soil Compaction Jointly Drive Large-Herbivore Grazing Effects on Plants and Soil Arthropods on Clay Soil. *Ecosystems* 18, 671–685. <https://doi.org/10.1007/s10021-015-9855-z>
- Van Langevelde, F., Van De Vijver, C.A.D.M., Kumar, L., Van De Koppel, J., De Ridder, N., Van Andel, J., Skidmore, A.K., Hearne, J.W., Stroosnijder, L., Bond, W.J., Prins, H.H.T., Rietkerk, M., 2003. Effects of Fire and Herbivory on the Stability of Savanna Ecosystems. *Ecology* 84, 337–350. [https://doi.org/10.1890/0012-9658\(2003\)084\[0337:EOFAHO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[0337:EOFAHO]2.0.CO;2)
- van Wilgen, B.W., Strydom, T., Simms, C., Smit, I.P.J., 2022. Research, monitoring, and reflection as a guide to the management of complex ecosystems: The case of fire in the Kruger National Park, South Africa. *Conserv. Sci. Pract.* 4, e12658. <https://doi.org/10.1111/csp2.12658>
- Veldhuis, M.P., Gommers, M.I., Oloff, H., Berg, M.P., 2018. Spatial redistribution of nutrients by large herbivores and dung beetles in a savanna ecosystem. *J. Ecol.* 106, 422–433. <https://doi.org/10.1111/1365-2745.12874>
- Venter, F.J., Govender, N., 2012. A geomorphic and soil description of the long-term fire experiment in the Kruger National Park, South Africa. *Koedoe Afr. Prot. Area Conserv. Sci.* 54, 1–10.
- Verma, S., Jayakumar, S., 2012. Impact of forest fire on physical, chemical and biological properties of soil: A review.
- Vermeire, M.-L., Thoresen, J., Lennard, K., Vikram, S., Kirkman, K., Swemmer, A.M., Te Beest, M., Siebert, F., Gordijn, P., Venter, Z., Brunel, C., Wolfaard, G., Krumins, J.A., Cramer, M.D., Hawkins, H.-J., 2021. Fire and herbivory drive fungal and bacterial communities through distinct above- and belowground mechanisms. *Sci. Total Environ.* 785, 147189. <https://doi.org/10.1016/j.scitotenv.2021.147189>
- von Maltitz, G.P., Scholes, R.J., 2013. Vulnerability of southern African biodiversity to climate change, in: *Climate Change and Vulnerability and Adaptation*. Routledge, pp. 49–64.
- Voroney, R.P., 2007. 2 - THE SOIL HABITAT, in: Paul, E.A. (Ed.), *Soil Microbiology, Ecology and Biochemistry* (Third Edition). Academic Press, San Diego, pp. 25–49. <https://doi.org/10.1016/B978-0-08-047514-1.50006-8>
- Walker, A., 2021. personal communication.
- Wang, C., Wang, G., Wang, Y., Rafique, R., Ma, L., Hu, L., Luo, Y., 2016. Fire Alters Vegetation and Soil Microbial Community in Alpine Meadow. *Land Degrad. Dev.* 27, 1379–1390. <https://doi.org/10.1002/ldr.2367>
- Wang, L., Okin, G.S., Caylor, K.K., Macko, S.A., 2009. Spatial heterogeneity and sources of soil carbon in southern African savannas. *Geoderma* 149, 402–408. <https://doi.org/10.1016/j.geoderma.2008.12.014>
- Wang, Q., Zhong, M., Wang, S., 2012. A meta-analysis on the response of microbial biomass, dissolved organic matter, respiration, and N mineralization in mineral soil to fire in forest ecosystems. *For. Ecol. Manag.* 271, 91–97. <https://doi.org/10.1016/j.foreco.2012.02.006>
- Wang, W., Fang, J., 2009. Soil respiration and human effects on global grasslands. *Glob. Planet. Change, Changes in land use and water use and their consequences on climate, including biogeochemical cycles* 67, 20–28. <https://doi.org/10.1016/j.gloplacha.2008.12.011>
- Wang, W.J., Dalal, R.C., Moody, P.W., Smith, C.J., 2003. Relationships of soil respiration to microbial biomass, substrate availability and clay content. *Soil Biol. Biochem.* 35, 273–284. [https://doi.org/10.1016/S0038-0717\(02\)00274-2](https://doi.org/10.1016/S0038-0717(02)00274-2)

- Ward, S.E., Bardgett, R.D., McNamara, N.P., Adamson, J.K., Ostle, N.J., 2007. Long-Term Consequences of Grazing and Burning on Northern Peatland Carbon Dynamics. *Ecosystems* 10, 1069–1083. <https://doi.org/10.1007/s10021-007-9080-5>
- Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological Linkages Between Aboveground and Belowground Biota. *Science* 304, 1629–1633. <https://doi.org/10.1126/science.1094875>
- Wardle, D.A., Jonsson, M., Mayor, J.R., Metcalfe, D.B., 2016. Above-ground and below-ground responses to long-term nutrient addition across a retrogressive chronosequence. *J. Ecol.* 104, 545–560. <https://doi.org/10.1111/1365-2745.12520>
- Wardle, D.A., Nilsson, M.-C., Zackrisson, O., 2008. Fire-Derived Charcoal Causes Loss of Forest Humus. *Science* 320, 629–629. <https://doi.org/10.1126/science.1154960>
- Whitaker, J., Ostle, N., Nottingham, A.T., Cahuana, A., Salinas, N., Bardgett, R.D., Meir, P., McNamara, N.P., 2014. Microbial community composition explains soil respiration responses to changing carbon inputs along an Andes-to-Amazon elevation gradient. *J. Ecol.* 102, 1058–1071. <https://doi.org/10.1111/1365-2745.12247>
- Whitman, T., Pepe-Ranney, C., Enders, A., Koechli, C., Campbell, A., Buckley, D.H., Lehmann, J., 2016. Dynamics of microbial community composition and soil organic carbon mineralization in soil following addition of pyrogenic and fresh organic matter. *ISME J.* 10, 2918–2930. <https://doi.org/10.1038/ismej.2016.68>
- Whitman, T., Whitman, E., Wooley, J., Flannigan, M.D., Thompson, D.K., Parisien, M.-A., 2019. Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. *Soil Biol. Biochem.* 138, 107571. <https://doi.org/10.1016/j.soilbio.2019.107571>
- Wigley, B.J., Augustine, D.J., Coetsee, C., Ratnam, J., Sankaran, M., 2020. Grasses continue to trump trees at soil carbon sequestration following herbivore exclusion in a semiarid African savanna. *Ecology* 101, e03008. <https://doi.org/10.1002/ecy.3008>
- Wilcox, B.P., Birt, A., Fuhlendorf, S.D., Archer, S.R., 2018. Emerging frameworks for understanding and mitigating woody plant encroachment in grassy biomes. *Curr. Opin. Environ. Sustain., Environmental change issues* 2018 32, 46–52. <https://doi.org/10.1016/j.cosust.2018.04.005>
- Wilcox, K.R., Koerner, S.E., Hoover, D.L., Borkenhagen, A.K., Burkepille, D.E., Collins, S.L., Hoffman, A.M., Kirkman, K.P., Knapp, A.K., Strydom, T., Thompson, D.I., Smith, M.D., 2020. Rapid recovery of ecosystem function following extreme drought in a South African savanna grassland. *Ecology* 101, e02983. <https://doi.org/10.1002/ecy.2983>
- Wilgen, B.W. van, Govender, N., Biggs, H.C., 2007. The contribution of fire research to fire management: a critical review of a long-term experiment in the Kruger National Park, South Africa. *Int. J. Wildland Fire* 16, 519–530. <https://doi.org/10.1071/WF06115>
- Williams, C.A., Hanan, N., Scholes, R.J., Kutsch, W., 2009. Complexity in water and carbon dioxide fluxes following rain pulses in an African savanna. *Oecologia* 161, 469–480. <https://doi.org/10.1007/s00442-009-1405-y>
- Williams, R.J., Gill, A.M., Moore, P.H.R., 2003. Fire Behavior, in: Andersen, A.N., Cook, G.D., Williams, R.J. (Eds.), *Fire in Tropical Savannas: The Kapalga Experiment*. Springer, New York, NY, pp. 33–46. https://doi.org/10.1007/0-387-21515-8_3
- Williams, R.J., Hutley, L.B., Cook, G.D., Russell-Smith, J., Edwards, A., Chen, X., 2004. Viewpoint: Assessing the carbon sequestration potential of mesic savannas in the Northern Territory, Australia: approaches, uncertainties and potential impacts of fire. *Funct. Plant Biol.* 31, 415–422. <https://doi.org/10.1071/fp03215>
- Wilson, C.H., Strickland, M.S., Hutchings, J.A., Bianchi, T.S., Flory, S.L., 2018. Grazing enhances belowground carbon allocation, microbial biomass, and soil carbon in a subtropical grassland. *Glob. Change Biol.* 24, 2997–3009. <https://doi.org/10.1111/gcb.14070>
- Wood, S., Scheipl, F., 2022. gamm4: Generalized Additive Mixed Models using “mgcv” and “lme4.” R.
- Wright, J., DeLaMater, D., Simha, A., Ury, E., Ficken, C., 2021. Changes in Prescribed Fire Frequency Alter Ecosystem Carbon Dynamics. *Ecosystems* 24, 640–651. <https://doi.org/10.1007/s10021-020-00540-5>
- Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. *Soil Biol. Amp Biochem.* 22, 1167–1169. [https://doi.org/10.1016/0038-0717\(90\)90046-3](https://doi.org/10.1016/0038-0717(90)90046-3)
- Wu, Y., Yu, X., Wang, H., Ding, N., Xu, J., 2010. Does history matter? Temperature effects on soil microbial biomass and community structure based on the phospholipid fatty acid (PLFA) analysis. *J. Soils Sediments* 10, 223–230. <https://doi.org/10.1007/s11368-009-0118-5>
- Wüthrich, C., Schaub, D., Weber, M., Marxer, P., Conedera, M., 2002. Soil respiration and soil microbial biomass after fire in a sweet chestnut forest in southern Switzerland. *CATENA* 48, 201–215. [https://doi.org/10.1016/S0341-8162\(01\)00191-6](https://doi.org/10.1016/S0341-8162(01)00191-6)

- Xu, L., Baldocchi, D.D., Tang, J., 2004. How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature. *Glob. Biogeochem. Cycles* 18. <https://doi.org/10.1029/2004GB002281>
- Xu, S., Eisenhauer, N., Pellegrini, A.F.A., Wang, J., Certini, G., Guerra, C.A., Lai, D.Y.F., 2022. Fire frequency and type regulate the response of soil carbon cycling and storage to fire across soil depths and ecosystems: A meta-analysis. *Sci. Total Environ.* 825, 153921. <https://doi.org/10.1016/j.scitotenv.2022.153921>
- Yang, S., Zheng, Q., Yang, Y., Yuan, M., Ma, X., Chiariello, N.R., Docherty, K.M., Field, C.B., Gutknecht, J.L.M., Hungate, B.A., Niboyet, A., Roux, X.L., Zhou, J., 2020. Fire affects the taxonomic and functional composition of soil microbial communities, with cascading effects on grassland ecosystem functioning. *Glob. Change Biol.* 26, 431–442. <https://doi.org/10.1111/gcb.14852>
- Young, T.P., Stanton, M.L., Christian, C.E., 2003. Effects of natural and simulated herbivory on spine lengths of *Acacia drepanolobium* in Kenya. *Oikos* 101, 171–179. <https://doi.org/10.1034/j.1600-0706.2003.12067.x>
- Zambatis, N., Zacharias, P., Morris, C., Derry, J., 2006. Re-evaluation of the disc pasture meter calibration for the Kruger National Park, South Africa. *Afr. J. Range Forage Sci.* 23, 85–97. <https://doi.org/10.2989/10220110609485891>
- Zhao, Y., Wang, Y.Z., Xu, Z.H., Fu, L., 2015. Impacts of prescribed burning on soil greenhouse gas fluxes in a suburban native forest of south-eastern Queensland, Australia. *Biogeosciences* 12, 6279–6290. <https://doi.org/10.5194/bg-12-6279-2015>
- Zhou, Y., 2023. Root traits in response to frequent fires: Implications for belowground carbon dynamics in fire-prone savannas. *Front. Plant Sci.* 14. <https://doi.org/10.3389/fpls.2023.1106531>
- Zhou, Y., Singh, J., Butnor, J.R., Coetsee, C., Boucher, P.B., Case, M.F., Hockridge, E.G., Davies, A.B., Staver, A.C., 2022. Limited increases in savanna carbon stocks over decades of fire suppression. *Nature* 603, 445–449. <https://doi.org/10.1038/s41586-022-04438-1>

8. Appendix

Table 8.1 – Total PLFA biomarkers (nmol g⁻¹ soil dwt) across the two fire regimes for the four EBP replicates (mean ± SE). Mbiyamiti (Mby) had much higher PLFA biomarker concentrations on the fire suppression plots (highlighted in bold), compared to the other replicates.

EBP Replicate	Fire Regime	
	Fire Suppression	Oct B2 Burns
Mby	27.7 (±4.71)	10.90 (±1.58)
Nap	12.6 (±2.05)	12.60 (±1.72)
Nwa	13.2 (±1.80)	15.80 (±2.60)
Skz	11.4 (±1.73)	9.66 (±0.85)