

**BACKING THE BURN**

**Carbon Sequestration in South African Mesic Grasslands Through Sustainable Fire  
Management**

**by**

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“The care of the Earth is our most ancient and most worthy, and after all our most pleasing responsibility. To cherish what remains of it and to foster its renewal is our only legitimate hope.”

- Wendell Berry -

## PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Grassland Science, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by the Red Meat Research & Development SA (RMRDSA).

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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## DECLARATION 1: PLAGIARISM

I, Robyn Elizabeth Nicolay, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

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a) their words have been re-written but the general information attributed to them has been referenced;

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(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

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## DECLARATION 2: PUBLICATIONS

Chapters 2 and 3 have been submitted to peer-reviewed journals, and chapters 4 and 5 are intended for submission as papers to appropriate scientific journals. My three supervisors have been included as co-authors because of their significant scientific input. Work presented at conferences has been included below. \* Indicating corresponding author.

### Submitted to journal for review:

#### Chapter 2

1. Nicolay RE\*, Tedder MJ, Mkhize NR, Kirkman KP. Submitted April 2023. Increased fire frequency improves soil organic carbon stocks and sequestration in a South African mesic grassland. Submitted to *Fire Ecology*. FECO-D-23-00042R1

The study analysed the effects of different fire frequencies and seasons on soil organic carbon (SOC) stocks, total nitrogen (TN), and carbon to nitrogen (C:N) ratios at Ukulinga research farm, Pietermaritzburg. I collected and analysed the carbon data and wrote the paper. Ukulinga grassland fire experiment is a long-standing trial, designed by late Professor JD Scott. Co-authors gave edits and statistical assistance on the manuscript.

### Accepted for publication:

#### Chapter 3

2. Nicolay RE\*, Tedder MJ, Mkhize NR, Kirkman KP. Accepted 13-May-2024. Fire suppression interacts with soil acidity to maintain stable recalcitrant pyrogenic carbon fractions in South African mesic grassland soils. Submitted to *African Journal of Range & Forage Science*. TARF-2023-0127.R2

Investigation of the effect of prescribed fires on the accumulation of refractory OC and BC fractions in grassland soils. I collected and analysed the carbon data and wrote the paper. Carbon samples were fractionated with assistance from the Department of Mechanical Engineering, Durban University of Technology (DUT) Durban University of Technology. Co-authors gave edits and statistical assistance on the manuscript.

## Presented research at conference:

### Chapter 2

3. Nicolay RE\*, Tedder MJ, Mkhize NR, Kirkman KP. 2021. Changes in fire frequency influence soil organic carbon sinks in South African mesic grasslands. Paper presentation at Grassland Society of Southern Africa's 56<sup>th</sup> annual congress, 26<sup>th</sup> July to 30<sup>th</sup> July 2021, Zoom online conference. Presented by RE Nicolay.

The study analysed the effects of different fire frequencies and seasons on soil organic carbon (SOC) stocks, total nitrogen (TN), and carbon to nitrogen (C:N) ratios at Ukulinga research farm, Pietermaritzburg. I collected and analysed the carbon data and wrote the paper. Co-authors gave edits and statistical assistance on analysis.

### Chapter 3

4. Nicolay RE\*, Tedder MJ, Mkhize NR, Kirkman KP. 2023. Fire suppression interacts with soil acidity to maintain stable recalcitrant pyrogenic carbon fractions in South African mesic grassland soils. Paper presentation at Grassland Society of Southern Africa's 58<sup>th</sup> annual congress, 25<sup>th</sup> July to 27<sup>th</sup> July 2023, Buffelspoort, South Africa. Presented by RE Nicolay.
5. Nicolay RE\*, Tedder MJ, Mkhize NR, Kirkman KP. 2023. Fire suppression interacts with soil acidity to maintain stable recalcitrant pyrogenic carbon fractions in South African mesic grassland soils. Paper presentation at Ecological Society of America's 57<sup>th</sup> annual meeting, 6<sup>th</sup> August to 11<sup>th</sup> August 2023, Portland Oregon, United States of America. Presented by RE Nicolay.

The study investigated the effect of prescribed fires on acidification of the soil, and its influence on the accumulation and breakdown of refractory OC and BC fractions within South African mesic grassland soils. I collected and analysed the carbon data and wrote the paper. Co-authors gave edits and statistical assistance on analysis.



Signed: Robyn Elizabeth Nicolay

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## ABSTRACT

Emphasising the ancient origins of the grassland biome in South Africa, much research has supported the role of grasslands in biodiversity, ecosystem services, and economic benefits for local populations. Despite this, the grassland biome faces challenges. It is highly transformed, poorly conserved, and urgently in need of preservation and restoration. Fire-adapted mesic grasslands are distinct ecologically from arid-adapted climatic climax grassland communities in that they are adapted to frequent defoliation, necessitating burning to maintain productivity and biodiversity. To fully understand the role of grasslands in the global climate change arena, it is necessary to quantify the impacts of grassland management, including fire and livestock grazing, on carbon exchange (source versus sink).

Research in this thesis looks particularly at management with prescribed fire as a form of defoliation in mesic grasslands. To do this, I quantified soil total carbon stocks, fractionated stable pyrogenic carbon stocks, and soil carbon sequestration rates in various fire regime treatments at the Ukulinga Grassland Fire Experiment (UGFE), Pietermaritzburg. Additionally, I examined the impact of fire frequency on grazed grassland at Wakefield research farm, quantifying carbon stocks and release while exploring potential mechanisms behind observed patterns in this grazed system. Lastly, I monitored Eddy Covariate carbon flux data over a four-year period at research catchment six, Cathedral Peak, KwaZulu-Natal, to understand seasonal and interannual flux within mesic grassland and observe patterns in source versus sink dynamics in these ecosystems. Research has emphasised the necessity of frequent fires to maintain grassy biomes and sustain the role of biotic and abiotic factors in this biome, through biochemical soil alteration in the form of ash deposition, and the alteration of above and below ground biomass. The complexity of managing this grassland is emphasized by the need to balance the impact of herbivory, prescribed fires, and the nature of biomass accumulation in these biomes, all of which influence carbon cycling.

Differences were observed between different prescribed burning regimes. Substantial differences in soil organic carbon (SOC) and total nitrogen (TotN) stocks at different soil depths were observed, with the highest stocks observed in the top 5 and 10 cm of soil across all treatments. Annual winter and spring burns exhibit the highest SOC stocks and wider C:N ratios. Triennial burns display the lowest sequestration rates in the top 0 to 5 cm of soil, with negative rates within the 5 to 10 cm horizon. Over a period of 20 years, SOC sequestration

increased in a 70-year-old experiment with no signs of stabilization within the 0 to 5 cm soil horizon, but SOC loss is noted below 5 cm in areas burnt triennially. Increased fire frequency in grassland also caused a reduction in the stable fraction of black carbon (BC), and contrastingly - increased levels of BC quantified in grassland burnt infrequently or excluded from fire. This pattern may be due to reduced alkaline ash deposition and subsequently greater soil acid saturation, suggested to result in increased pyrogenic carbon particulate size and reduced breakdown of this carbon in the soil.

When considering the inclusion of livestock and grazing into grassland managed with prescribed fire, findings showed no significant differences in SOC and TotN levels between annually burned grasslands and those excluded from fire. Grassland managed with annual burning showed greater soil respiration rates compared to unburned sites, indicating greater soil microbial activity and root turnover. Annual burning and heavy grazing were both associated with reduced aboveground biomass accumulation compared to the adjoining unburned grassland. Additionally, annually burnt grasslands exhibit reduced aboveground biomass lignin and fibre percentage relative to adjacent unburned areas. Findings highlighted that increased fire frequency in grazed grassland influences livestock grazing behaviours through improvement of forage palatability and available biomass, contributing to greater belowground carbon turnover. Considering the mechanisms governing carbon dynamics in fire-dependent grassland, four years of flux data showed that following rainfall events, increased soil water content is linked to a rapid rise in soil respiration, aligning with heightened biological and photosynthetic activities during warmer growing seasons. These processes determine the rate and variability of grassland uptake and release of CO<sub>2</sub>. The findings support evidence that mesic grasslands managed with regular long term prescribed fires consistently act as carbon sinks, absorbing more carbon than they emit over periods exceeding 70 years.

Findings from this research advocate for management practices utilizing a frequent burn regime, suggesting that such practices maintain persistent carbon sinks in South African mesic grasslands. This approach enhances the resilience and capacity of mesic grasslands to act as effective and consistent carbon sinks, even in the face of potential future climate change impacts. The evidence from these studies shows that prescribed fire during late winter / spring in South African mesic grasslands should enhance carbon sequestration and the role of these grasslands as a carbon sink.

## ABBREVIATIONS AND ACRONYMS

**AFR100:** African Forest Landscape Restoration Initiative

**BAU:** Business as usual

**BC:** Black Carbon

**BD:** Bulk Density

**C:** Carbon

**CO<sub>2</sub>:** Carbon Dioxide

**DM:** Dry matter

**DOC:** Dissolved organic carbon

**FLR:** Forest Landscape Restoration

**HM:** Holistic Management

**IPCC:** Intergovernmental Panel on Climate Change

**GHG:** Greenhouse Gases (CO<sub>2</sub> = carbon dioxide; NO= nitrous oxide; CH<sub>4</sub>= methane)

**SDGs:** Sustainable Development Goals

**SOC:** Soil Organic Carbon

**SOC<sub>seq</sub>:** Soil Organic Carbon Sequestration

**SOM:** Soil Organic Matter

**OC:** Organic Carbon

**UGFE:** Ukulinga Grassland Fire Experiment

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# 1. INTRODUCTION

## 1.1 Global Carbon Efforts and the Importance of Grassland and Soil Conservation

Encompassing 52.5 million km<sup>2</sup>, global grasslands store approximately one-third of terrestrial carbon stocks, with ~90% of this carbon stored belowground as root biomass and soil organic carbon (Bai and Cotrufo, 2022). Understanding belowground carbon storage in these biomes is not only vital in supporting their role in soil carbon sequestration but also emphasizes the broader impact on global carbon dynamics and its role in climate change mitigation (Bai *et al.*, 2013; Bai and Cotrufo, 2022). Soils and soil organic carbon (SOC) have been recognised as crucial components of the Sustainable Development Goals (SDGs) to foster a global food system that balances agriculture, biodiversity conservation, and climate action (FAO, 2020). Biodiversity loss has significant effects on ecosystem functions, particularly carbon (C) cycling. Soil communities are characterized by extensive taxonomic and functional diversity, playing a crucial role in C fluxes between the atmosphere and terrestrial ecosystems (De Graaff *et al.*, 2015). Research has indicated that reductions in soil biodiversity notably affect soil C cycling processes. Specifically, a decline in microbial diversity significantly diminishes soil C respiration, whereas a loss in soil faunal diversity adversely impacts plant tissue decomposition (De Graaff *et al.*, 2015). SOC content has been highlighted as a key indicator of soil fertility and grassland productivity, with SOC sequestration considered crucial for climate change mitigation and lowering atmospheric CO<sub>2</sub> concentration (Tessema *et al.*, 2020). Understanding grassland soils' capacity to store carbon is vital in developing climate change mitigation strategies and addressing soil health concerns (Conant, 2010; Tulau and McInnes-Clarke, 2015).

Within the SDGs, soils are identified as vulnerable resources, faced with challenges of land degradation, biodiversity loss, and heightened food production demands (Smith *et al.*, 2020). The established link between SOC, climate adaptation and mitigation emphasize the urgency of maintaining and increasing SOC stocks beyond reducing greenhouse gas emissions (Nakicenovic and Swart, 2000). By maintaining and increasing SOC, grassland biomes benefit from soil health, fertility, water storage, and plant resilience to drought (FAO, 2020).

## **1.2 The South African Grassland Biome**

Covering about 28% of the higher-lying eastern regions in South Africa, the grassland biome encompasses a range of ecosystem subtypes influenced by factors such as precipitation, temperature, and altitude (Rutherford *et al.*, 2006; Tainton, 1999) (Figure 1.1). These subtypes are shaped by the edaphic interaction of fire, grazing, and climatic conditions. The South African grassland biome incorporates mesic grasslands, characterized by moderate moisture levels, arid grasslands adapted to drier conditions, and areas displaying distinctive fire climax characteristics. Fire-adapted mesic grasslands are found specifically in cooler temperatures with moderate to high summer rainfall exceeding 600 mm (Rutherford *et al.*, 2006; Tainton, 1999). In these regions, soil leaching is moderate to high, potentially resulting in infertile conditions (Tainton, 1999). The grassland community in mesic regions is a secondary development, largely influenced by the restraining impact of fire, preventing the natural climax of other vegetation types like savannah and forest. These fire-adapted mesic grasslands are distinct ecologically from arid-adapted climatic climax grassland communities found in the drier inland plateau and west of the Drakensberg escarpment (Tainton, 1999). The term climatic climax grassland reflects arid or cold conditions inhibiting the development of woody communities, even without the influence of fire (Tainton, 1999).

In the South African grassland biome, fire-adapted mesic grassland is adapted to frequent defoliation, necessitating burning to maintain productive grassland (Bond *et al.*, 2003). Determining the most appropriate time for burning becomes crucial in this context. The growth of these grassland biomes primarily occurs between October and March, with rapid spring growth accounting for a substantial portion (70-80%) of the season's total growth by the end of December (Tainton, 1999). Due to relatively favourable moisture conditions, these grasslands have undergone intensive development. Given that this region encompasses major river catchments crucial for South Africa's water provision, maintaining the stability of vegetation in these areas becomes paramount (Wigley *et al.*, 2009; Moodley *et al.*, 2023).

### ***1.2.1 The Role of Fire in a South African Grassland***

The management of grassland biomes has been a contentious topic within grassland ecology and management, marked by ongoing debates, particularly surrounding opposing views of the use of prescribed fires. In a South African context, Eurocentric bias has influenced perceptions

of ideal landscapes, historically favouring tree-dominated environments and discouraging the use of fire in grass-dominated biome (Kraus, 2010). This bias dates to colonial influences (Hall, 1934), and has led to a misrepresentation of grasslands as ecologically insignificant ecosystems. The suppression of fire in fire-dependent grasslands, rooted in Eurocentric ideologies, has far-reaching consequences, including increased fuel buildup and intensified fire risks (Trollope and Trollope, 2010). Early colonial decrees against veld burning, dating back to Van Riebeeck in 1652, contributed to fire suppression, ignoring the anthropological use of fire in these landscapes for over 50,000 years prior (Hall, 1934). An example of this historical bias is seen in afforested catchments within Cathedral Peak uKhahlamba-Drakensberg Park, South Africa, where the natural grassland vegetation of the first three Catchments was replaced by *Pinus patula* between 1951 and 1969 (Nanni, 1970; Stephens *et al.*, 2020). Advocating against the use of prescribed fire in fire prone grasslands, has continued with the 'Holistic Management' approach, emphasising the role of large herbivores in maintaining grassland health through the 'herd effect' or short-duration, high-intensity grazing (Savory and Butterfield, 2016; Gosnell *et al.*, 2020). This philosophy remains controversial and mostly lacks robust empirical support (Gosnell *et al.*, 2020).

Grasslands have historically been managed with fire by subsistence pastoralists and ranchers for millennia (Du Toit and Cumming, 1999). In recent decades, the coexistence of wildlife and livestock that once characterized grassland has been disrupted by changes in grazing species, practices, fire intensities, and fire frequencies, influenced by factors such as subdivision and degradation (Du Toit and Cumming, 1999; Russell *et al.*, 2018). The complexity of managing these grasslands is emphasized by the need to balance herbivorous impact, biomass accumulation, and grassland productivity, all of which influence carbon cycling (Trollope and Trollope, 2010).

Historically, communal grasslands were viewed as mismanaged, but this perception is widely challenged today (Vetter, 2005; Allsopp *et al.*, 2007). This traditional view has led to widespread interventions aimed at reducing livestock numbers to prevent degradation, based on the assumption that overgrazing is inevitable in communal pastoral systems (Allsopp, 2013). Further contributions to the breakdown of traditional resource management structures were now seen to contribute to this degradation. It is now seen that these historical perceptions are based on flawed ecological and economic assumptions, disputing the notion that these communal rangelands are inherently mismanaged (Vetter, 2005). Effective and sustainable management

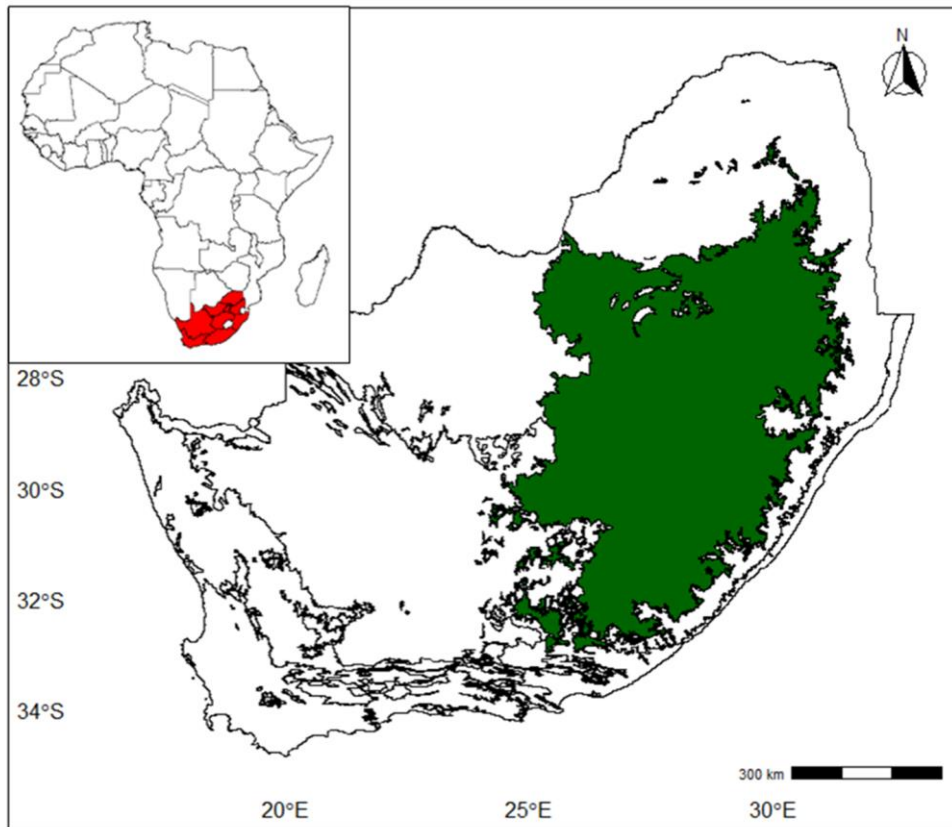
of these rangelands requires more than just establishing collective rules, but involves the adoption of farming practices that align with the objectives and realities of the communities involved (Allsopp *et al.*, 2007). Considering these complexities of managing grassland and the ecological implications across landscapes, decisions must fundamentally be guided by scientific principles, and recognize that there is no universal solution applicable to all situations. Management strategies should remain flexible, adapting to the specific characteristics of the landscape and the prevailing circumstances.

### ***1.2.2 Degradation, Mitigation and the Bonn Challenge.***

In recent years, an extensive analysis of degraded lands globally, driven by desktop studies and remote sensing, has laid the foundation for global initiatives like The Bonn Challenge (Gichuki *et al.*, 2019; Giuliani *et al.*, 2020). The Bonn Challenge is a high-profile attempt to initiate a global response to increasing greenhouse gas levels, in a global effort to restore 150 million hectares of degraded land by 2020, and 350 million hectares by 2030 (Gichuki *et al.*, 2019; Stanturf *et al.*, 2019). Driven by climatic envelope modelling, which assumes that if a tree can grow in a particular area, it should, as well as by the assumption that forests represent a universal endpoint of ecological succession. it has been proposed that massive-scale tree planting will rejuvenate degraded landscapes and sequester significant carbon reserves, thereby combating and potentially reversing rising greenhouse gas levels (Unruh, 2008; Stanturf *et al.*, 2019; Anderegg *et al.*, 2020).

Current afforestation efforts are primarily targeting developing nations in Africa, Asia, and South America, focusing efforts on expanding tree cover in response to perceived degradation or underutilization of vast grasslands within drylands and grassy biome (Niles *et al.*, 2002; Boone *et al.*, 2018; Vetter, 2020). These perceptions, whether fuelled by a lack of awareness of the vulnerability and immense value of our grassland biome, or the potential to capitalize on existing carbon credit allocations, have prompted appeals to enhance tree cover in grassy biomes (Boone *et al.*, 2018; Vetter, 2020). This perception is mostly rooted in historical theories on forests and desertification and continues to be repeated through contemporary science-policy frameworks (Guha, 1983; Davis and Robbins, 2018). From the perspective of grassland management, this global Forest Landscape Restoration (FLR) initiative raises a crucial concern around the target-driven nature of these afforestation efforts, often involving substantial funding (Vetter, 2020).

Notably, 45% of the areas designated for the Bonn Challenge are intended to be monoculture plantations primarily aimed at profitable enterprises, posing a new set of challenges for tropical grasslands and savannas (Bond *et al.*, 2019; Lewis *et al.*, 2019; Mani *et al.*, 2021). This raises not only a threat to pastoral livelihoods but also the risk of ecological damage, with limited potential to effectively mitigate climate change (Vetter, 2020). The African Forest Landscape Restoration Initiative (AFR100), contributing to The Bonn Challenge, aims to restore 100 million hectares of land in Africa by 2030, with South Africa committing to the restoration of 3.6 million hectares (Bond *et al.*, 2019; Mani *et al.*, 2021). Although South Africa's specified priority interventions do not explicitly detail the type of re-vegetation, the underlying theme of both AFR100 and the Bonn Challenge revolves around reforestation and afforestation (Gichuki *et al.*, 2019; Lewis *et al.*, 2019; Stanturf *et al.*, 2019). Afforestation attempts in naturally treeless biomes are contested by Bond *et al.* (2019), challenging the notion that widespread tree planting will yield the intended results. Emphasizing the ancient origins of the grassland biome in Africa, research has supported their vital role in biodiversity, ecosystem services, and economic benefits for local populations in their un-afforested state (Lehmann *et al.*, 2019; Buisson *et al.*, 2022). A critical consideration emerges for the importance of conserving natural grassland on a global level, rather than transforming them into forests (Davis and Robbins, 2018; Buisson *et al.*, 2022). In the face of these challenges, a paradigm shift towards prioritizing the conservation of natural grassland is required.



**Figure 1.1:** The extent of the southern Africa grassland biome extends into Lesotho and Eswatini. Created using R studio version 4.3.1 (R Core Team, 2023) using the *ggspatial* package. Shapefiles for grassland cover obtained from the South African National Biodiversity Institute (2006–2018) (Rutherford *et al.*, 2006).

### 1.3 Carbon Pools and Feedback Mechanisms in Grassland Biome

Carbon cycling processes in grassland biomes encompass both aboveground and belowground components and contribute to the sequestration and release of carbon (Ammann *et al.*, 2007; Abberton, 2010). Vegetation plays a role as a carbon sink through photosynthesis, converting atmospheric carbon dioxide into organic compounds (Fatichi *et al.*, 2014). It's now acknowledged that ecosystem fertility depends on efficient carbon cycling among plants, microbes, and organic matter (Vermeire *et al.*, 2021). Microbial biomass serves as both a carbon sink, contributing toward the pool of labile carbon, and a source, releasing carbon during biomass turnover through decomposition of plant material and microbial respiration (Brookes, 2001; Ingram *et al.*, 2005). The balance between these processes determines the net carbon flux

in the ecosystem. Positive and negative feedback mechanisms influence the net carbon flux in grassland. Positive feedback amplifies its effects, potentially leading to increased carbon release or sequestration (Friedlingstein *et al.*, 2003). Negative feedback, on the other hand, stabilizes the system, counteracting changes and maintaining equilibrium (Friedlingstein *et al.*, 2003).

Soil organic matter (SOM) formation influences the soil microbiome decomposition activity, water and nutrient retention, and plant root distribution and activity (Frouz, 2018). The decomposing litter contributes to the accumulation of OM, serving as an energy and nutrient reservoir in the soil that buffers ecosystems against adverse ecological disturbances such as drought events (Christensen, 2001). These feedback mechanisms are influenced by various factors such as land use, vegetation type, and climatic conditions (Figure 1.2). Considering the key role of soil microbe activity in the carbon cycle, it is essential to explore the impacts of management practices, including prescribed fire and grazing factors that may influence microbial activity and biomass (Vermeire *et al.*, 2021).

One of the critical questions in grassland carbon dynamics revolves around the stability of soil carbon. Total SOC comprises labile and stable forms, determined by its transformation within the pedosphere (Christensen, 2001). While some carbon remains resistant to decomposition, forming stable SOM, other fractions are more susceptible to microbial breakdown, contributing to the continuous cycling of carbon within the soil (Condon *et al.*, 2010).

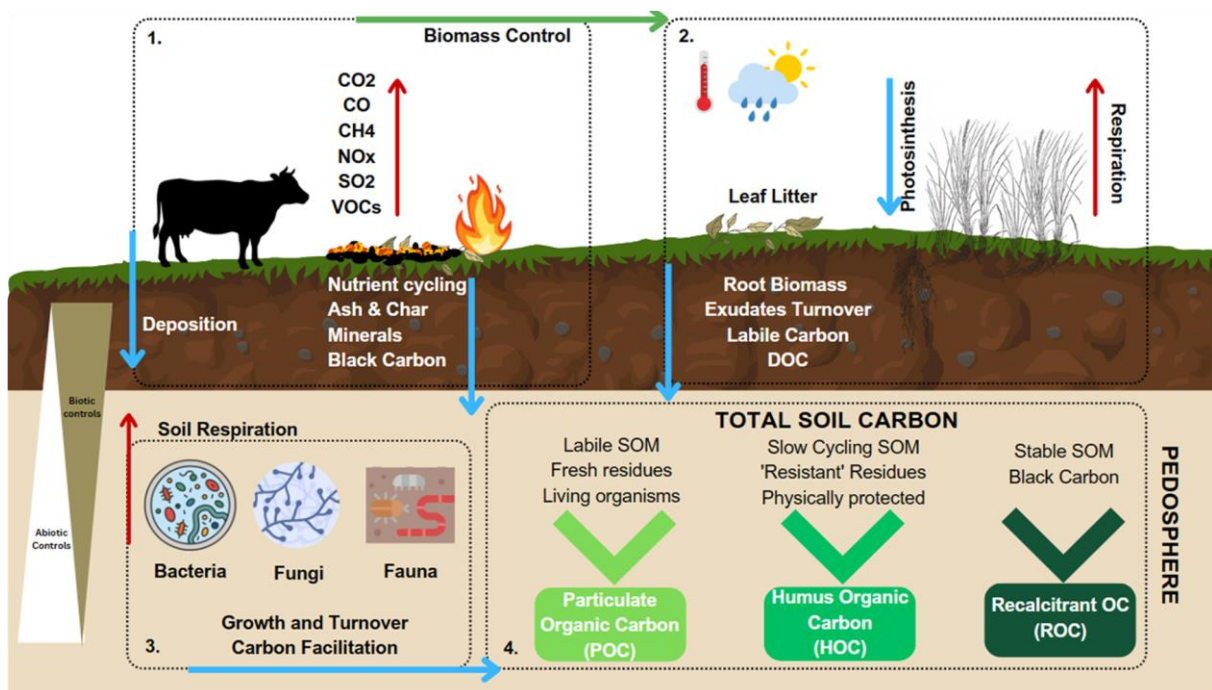
### ***1.3.1 Effective Measuring of Soil Carbon***

Global efforts in the promotion of soil carbon have emphasized the crucial need for standardized, robust, and easily applicable platforms to assess changes in SOC and GHG removals across diverse agricultural systems (Nakicenovic and Swart, 2000; FAO, 2020). Despite methodological advancements, accurately measuring and reporting SOC status and trends remains challenging, highlighting the urgent necessity for effective worldwide measurement, reporting, and verification tools (Conant *et al.*, 2011; FAO, 2020).

The global symposium on soil organic carbon in 2017 emphasized the importance of developing a standardised protocol for monitoring SOC sequestration and GHG emissions in agricultural landscapes, offering practical recommendations for effective implementation (Minasny *et al.*, 2017). Standardized monitoring protocols ensure that measurements and data

collection are reliable, high-quality, and consistent across various research initiatives. This uniformity enables meaningful comparisons between studies, regions, and over time, fostering a more comprehensive and accurate understanding of SOC dynamics (Conant, 2010; Jandl *et al.*, 2014; Bispo *et al.*, 2017).

The primary objective of employing standardized monitoring protocols is to establish a foundation for evidence-based policymaking, ensuring that decisions are grounded in reliable scientific information (Jandl *et al.*, 2014). The development of a standardized protocol allows for consistent monitoring over time, facilitating the assessment of progress in SOC sequestration efforts.



**Figure 1.2:** The carbon cycle in grassland biomes exposed to fire and grazing as forms of defoliation, visually describing the connectivity between influencing factors. 1. Ungulate grazing and fire represent defoliation processes driving carbon turnover. Ungulates introduce carbon through dung, urine, and trampling of vegetation, whilst releasing gases into the atmosphere. Fire contributes to carbon sinks by altering soil chemistry through nutrient cycling, ash, and char deposition, whilst releasing carbon into the atmosphere through the combustion of vegetation. 2. Defoliation supports grassland vegetation turnover (aboveground and belowground biomass). Compensatory growth and decomposition release carbon through respiration, while photosynthesis and root biomass contribute labile and dissolved carbon fractions, influencing the grassland status as a carbon source or sink. Climate conditions further modulate photosynthesis and respiration rates. 3. Soil microbes (bacteria, fungi, fauna) thrive due to the influences of grazing, fire, and grassland biomass turnover, playing a primary role in

carbon facilitation. 4. Three soil carbon fractions—particulate organic carbon (POC), humus organic carbon (HOC), and recalcitrant organic matter (ROC)—represent the cumulative carbon pool influenced by both bottom-up abiotic factors and top-down biotic factors in the carbon cycle. Figure adapted from (Bai and Cotrufo, 2022).

#### **1.4 How do Grassland Biomes and Management Fit into the Carbon Cycle?**

Evidence points towards grassland biome soils having the largest terrestrial reservoir of carbon, with the capacity to store double the amount of stable carbon than the atmosphere in the form of decomposed plant detritus and residues (Lorenz and Lal, 2018; Dobson *et al.*, 2022). The potential of regeneration largely depends on how a grassland biome is managed. Grassland biomes serve as critical components in the global carbon cycle, contributing significantly to carbon sequestration through the growth, accumulation, and turnover of biomass, particularly in the extensive root systems of grasses (Fynn *et al.*, 2003). Because of the turnover of extensive root material below the surface, the primary processes governing the carbon cycle within grassland ecosystems take place in the soil (Gang *et al.*, 2011).

The impact of management practices on carbon flux in grassland has shown in recent studies that the source and sink of carbon in this biome varies according to the growth cycles of grassland vegetation and the mechanisms that control this biomass turnover (Gang *et al.*, 2011; Fatichi *et al.*, 2014). Grassland biome management, particularly through prescribed fires and appropriate grazing practices, is seen as a mechanism capable of enhancing soil carbon sequestration in that whilst causing decreases in aboveground biomass and litter inputs, the turnover of root material in the soil is increased (Fynn *et al.*, 2003). A synthesis of various studies, (Mcsherry and Ritchie, 2013; Wang *et al.*, 2016; Conant *et al.*, 2017), indicates an average increase of  $0.47 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  in soil carbon sequestration with appropriate fire and grazing management. As well as a notable 10% increase in soil carbon concentration following changes in management practices related to appropriate grazing and fire implementation (Conant *et al.*, 2017). It is also important to consider the role of soil properties in determining the amount of SOC that can be stored. Factors such as soil texture, mineral composition, and SOM content can significantly influence the capacity of soils to sequester carbon (Gross and Harrison, 2019). Moreover, temporal variability—such as seasonal fluctuations, climate variability, and short-term disturbances—can obscure true long-term changes in environments that are highly variable (Weltzin *et al.*, 2020).

Disturbances are inherent in grassland ecosystems, playing a primary role in influencing the vegetation dynamics and carbon balance of grassland over the long term (Conant, 2010). Traditional South African grassland management, characterized by biomass removal by fire, grazing or both, changes in vegetation structure and composition, and alterations in soil function, integrates disturbance to foster sustainable and high-quality grazing (O'Connor, 2005). While well-managed disturbances can contribute positively to carbon dynamics, incorrect management practices, such as excessive forage consumption and poorly managed fire regimes, can deplete carbon stocks in both soils and vegetation (Smith *et al.*, 2008; Conant, 2012). Poorly managed fire regimes could constitute controlled fires applied either too frequently or not frequent enough, as well as inappropriate timing, where prescribed fires are applied in inappropriate seasons. Timing should consider the natural fire regime of the ecosystem and the specific needs of the grassland vegetation.

To fully understand the role of grassland in the global climate change arena, it is necessary to quantify the impacts of grassland management including fire and livestock grazing on greenhouse gas emissions and the sequestration and storage of organic carbon (OC) (source versus sink).

### **1.5 Grassland Biome Carbon Potential in a Changing Climate**

Understanding the factors that determine whether grasslands function as carbon sources or sinks involves considering various elements influencing grassland vegetative turnover, including defoliation, seasonal variations, photosynthetic processes, and external factors such as rainfall and temperature (Picone *et al.*, 2003; Findlay *et al.*, 2022). With a changing climate, increasing atmospheric CO<sub>2</sub> and subsequent rising temperatures, the photosynthetic rate in plants is altered (Hall and Scurlock, 1991). Initially, warmer temperatures may boost photosynthesis, leading to increased carbon uptake by grasses, however, prolonged heat stress can negatively affect photosynthetic efficiency (Hall and Scurlock, 1991). Variability in precipitation patterns, including changes in the timing and increase rainfall, can influence water availability and increase soil moisture in grassland ecosystems. Insufficient precipitation may limit plant growth, thereby reducing carbon sequestration (Archibald *et al.*, 2009; Knapp *et al.*, 1998).

Variations in temperature and moisture conditions can influence the activity of soil microbes responsible for decomposing organic matter (Millard and Singh, 2010). Shifts in

microbial activity have direct implications for the breakdown of plant material, affecting the storage of carbon in the soil (Thoresen *et al.*, 2021). The current and anticipated risks associated with changing climates, such as hotter and drier weather leading to more frequent and intense fires highlight the importance of effective landscape strategies, to mitigate the extent and damage caused by highly intense fire occurrences, thereby reducing associated carbon losses (González-Pérez *et al.*, 2004; Orians and Milewski, 2007).

There are contrasting dynamics between tree-dominated ecosystems and grassland ecosystems, based on the longevity and volatility of aboveground vs belowground carbon storage in forests vs grassland biomes (Henry *et al.*, 2011). When appropriately managed, frequently prescribed grassland fires burn with low intensity, and belowground carbon remains stable, as well as contributing to environmental hazard reduction, adding to the resilience of these ecosystems in predicted climate change scenarios (Tulau and McInnes-Clarke, 2015). Considering the factors that transform grassland into carbon sources or sinks, it is important to consider grassland ecological objectives. Fire management decisions should not be solely based on short-term objectives without considering broader ecological sustainability of the grassland, which can lead to imbalances and degradation.

## **1.6 Problem Statement**

While it is clear that mesic grassland biomes play an important role on a local and global level in the ecosystem services they provide, a better understanding of the intricate relationship between fire and grazing, and the potential of this grassland biome to sequester and store carbon is required to strengthen management decision making.

Despite the recognized significance of mesic grassland as contributors to net carbon sinks, the impact of anthropogenic intervention, together with historically Eurocentric perspectives on grazing restrictions and fire suppression, has obscured management approaches. These ingrained perceptions, rooted in historical theories on forests and desertification, persist in contemporary science-policy frameworks, creating a notable gap in comprehending how anthropological interventions, particularly fire and grazing, influence carbon dynamics within southern African grassland.

One key challenge is aligning traditional land management practices with global climate change goals. Anthropologically managed grassland must balance sustaining livestock farming

and wildlife conservation goals whilst supporting its role as a carbon sink. The introduced dimension of prescribed fire in these settings requires a thorough examination of their impact on carbon stocks and emissions.

The issue is compounded by a lack of comprehensive studies on the long-term effects of these practices on mesic grassland soils. In this context, it is important to explore the impacts of long-term prescribed fire on soil acidity, stable carbon fractions, and their implications for pastoral ecosystems. Moreover, the interactions between fire and grazing, influencing soil organic carbon stocks and soil respiration, need closer examination. Understanding these interactions is important for optimizing anthropogenic managed grassland as a carbon sink. By doing so, we hope to contribute toward informed decision-making in the sustainable management of mesic grassland amid the challenges posed by climate change and evolving land-use patterns.

### **1.7 Project Aims**

This research has the primary aim of estimating carbon stocks and emissions (source vs sink) within mesic grassland managed under prescribed fire regimes. The following research aims will guide our investigation:

*i. Quantifying the Potential of Anthropologically Managed Grassland as a Carbon Sink*

We look at understanding the capability of anthropologic managed grassland to maintain carbon stocks and promote sequestration. Through measurements and modelling, we aim to establish current carbon stocks and sequestration rates in mesic grassland within scenarios involving long-term exclusion from fire, varying seasonal burns, and differences in burn frequency.

*ii. Effects of Prescribed Fire on the Turnover and Stability of Soil Carbon in Mesic Grassland*

A study was undertaken to explore the impacts of 70 years of prescribed fire on mesic grassland soils in South Africa. Our focus for this aim is to understand how fire frequency influences soil acidification and the subsequent accumulation and turnover of biologically stable carbon fractions (refractory OC and BC). This investigation involves quantifying recalcitrant carbon fractions and soil acidity status in mesic grassland, examining scenarios of long-term fire exclusion, varying seasonal burns, and differences in burn frequency.

*iii. Synergistic Effects Between Fire and Grazing*

This aim investigates the inclusion of grazing large ruminants as a secondary source of defoliation in mesic grassland, and how this influences grassland potential as a carbon sink or source. We look at understanding the mechanisms driving grassland capacity as a carbon sink and source in these scenarios. This extends to the contributions of fire regime frequency to soil organic carbon stocks, soil respiration, and their influence on livestock grazing habits. We also look at the grazing potential of the grassland under both scenarios and how they contribute to the carbon cycle.

*iv. Developing Recommendations for Perceptions of South African Mesic Grassland as a Carbon Sink or Source with Predicted Changes in Climate*

Here we aim to understand South African mesic grassland managed by prescribed fire, and how they behave as a carbon sink or source. The flux of carbon in these grassland systems is expected to be responsive to environmental variables, thus facilitating more informed and adaptive approaches to address the fostering of carbon cycles in mesic grassland ecosystems.

The findings of this research could potentially contribute to the development of anthropologically managed grassland biome for climate change mitigation and carbon management strategies. The results have the potential to enhance the management practices of these grassland, improving their capacity to preserve carbon stocks, promote sequestration, and simultaneously support the productivity of extensive ruminant production mesic grassland.

It is essential to recognize the role of fire as a management tool in extensive African grassland ecosystems. Findings from this study are expected to guide land management strategies, fostering the restoration of carbon balances in grassland, and ensuring the long-term sustainability of agricultural grazing systems.

The findings from the long-term fire experiments aim to offer recommendations for burn frequencies that can support SOC sinks in inherently nitrogen-deficient mesic grassland. The results examining the implications of grazing have the potential to significantly influence the determination of fire frequency and grazing pressures applied by land managers. These results are vital for making well-informed decisions in managing carbon offsets and promoting sustainable land practices.

## 1.8 Thesis Outline

Chapter One provides an overview of grassland ecosystems and their crucial role in the global carbon cycle. It includes the impact of management practices on carbon sequestration and how climate change influences carbon dynamics. The review examines the available literature on seasonal variations, photosynthesis, and external elements such as rainfall and temperature in determining whether grasslands act as carbon sources or sinks. Here, we highlight the effects of rising temperatures on plant photosynthesis and the role of precipitation patterns in influencing soil water availability and, consequently, plant growth and carbon sequestration. The review focuses on the implications of changing climate scenarios, particularly the increased risk of fires in hotter and drier conditions, and the importance of well-planned landscape interventions to mitigate fire occurrences and carbon losses.

The central theme revolves around how mesic grassland ecosystems contribute to the carbon cycle, particularly under management practices of prescribed fire regimes and grazing. Here I address gaps in existing literature, specifically exploring the interactions and impacts of practices in South African grassland. Identified gaps include understanding the combined effects of controlled fires and grazing, examining extremes between the two, and discerning how predicted climatic changes influence carbon dynamics in South African mesic grassland. Moreover, a significant gap in quantifying and modelling long-term carbon sequestration, particularly isolating stable carbon fractions following long-term application of fire in South African mesic grassland, was identified.

Chapter two addresses aim one, by quantifying the potential of anthropologically managed grassland, to maintain carbon stocks and promote sequestration. The aims were achieved by firstly quantifying the effects of different fire frequencies and seasons on soil organic carbon (SOC) stocks, total nitrogen (TotN), and carbon to nitrogen (C: N) ratios in a mesic grassland ecosystem in a South African mesic grassland managed with long-term prescribed fire. Secondly by modelling the sequestration of carbon in these long-term trials, using carbon data collected from the same trial 20 years prior, to establish change in carbon over time.

Chapter three addresses aim two by quantifying the accumulation of biologically stable soil carbon fractions and possible underlying mechanisms driving South African mesic grassland managed with long-term fire, to act as a stable sink of soil carbon. This was done by

achieving the following objectives: isolating stable fraction refractory organic carbon and black carbon at Ukulinga Research Farm on the Ukulinga Grassland Fire Experiment (UGFE), a long-term grassland fire experiment with over 70 years of continuous prescribed fire and fire exclusion treatments to understand the effect of frequency and seasonal fire variation on the accumulation and maintenance of these stable pyrogenic forms of soil carbon. To further understand the secondary mechanisms that drive these changes, which according to the literature are suggested to be linked with deposition and acidification of soils following fire application. This was achieved by modelling acid saturation and pH within treatments and against quantified black carbon.

Chapter four addresses aim number three, looking at how extremes in the application of fire, fire exclusion and grazing influence soil organic carbon stocks, affect soil respiration rates and influence livestock grazing behaviour concerning biomass availability and palatability. We achieved this from the following objectives: Soil organic carbon and TotN were quantified in sites burnt annually and grazed heavily, representing extreme defoliation in pyric herbivorous management. In contrast, measurements were taken in sites excluded from fire entirely and subject to selective grazing over an extended period. We further measured soil respiration, accounting for possible carbon release in either of the scenarios and investigated secondary mechanisms which may drive these trends by quantifying available biomass, and lignification of available biomass.

Chapter five addresses aim number four to determine the responsiveness of mesic grassland to changing environmental and seasonal factors and its subsequent role as a carbon sink or source. The objective of this chapter was to determine the carbon flux in mesic grassland and how they release and sequester carbon in response to changing moisture availability by modelling variation in carbon flux inter-annually over four years, seasonally and monthly. We modelled seasonal soil moisture content as a possible explanatory mechanism and modelled the response of carbon flux to factors driving photosynthetic activity, namely radiation, temperature, latent heat, and rainfall.

Chapter six presents a synthesis of the findings of the study and how they relate to and augment the current understanding of carbon sinks and sources in southern African mesic grassland managed with fire and grazing.

## 2 FIRE FREQUENCY INFLUENCES SOIL ORGANIC CARBON STOCKS AND SEQUESTRATION IN A SOUTH AFRICAN MESIC GRASSLAND

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### 2.1 Abstract

Grasslands serve as crucial terrestrial carbon sinks, storing substantial amounts of organic carbon (OC) in aboveground vegetation, roots, and soils. However, effective carbon sequestration in grasslands depends on management practices, particularly anthropogenic fires, which yield both positive and negative carbon feedbacks. South African mesic grasslands are fire-adapted ecosystems, that rely on fire as a key ecological force shaping and sustaining them. Fire influences carbon sequestration by removing above-ground biomass, redistributing nutrients, and contributing inputs through thermal mineralization. This study assessed the impact of varying fire frequencies and seasons on soil organic carbon (SOC) stocks, total nitrogen (TotN), and carbon-to-nitrogen (C:N) ratios in a South African mesic grassland. Results indicated substantial differences in the main effects at 5 cm depth increments (up to 30 cm) and burn treatments, but no significant interaction between treatment and depth. SOC and TotN exhibited the greatest stocks in the top 5 and 10 cm respectively of soil across all treatments, with decreasing amounts at deeper soil depths. Annual winter and spring burns exhibited the highest SOC stocks and wider C:N ratios, while reduced stocks were evident in biennial spring and autumn burns. Triennial burns displayed the lowest sequestration rates in the top 5 cm of soil, with negative rates below that. Aside from triennial burns in the 5 – 10 cm soil horizon, SOC sequestration increased over time within all treatments, with no signs of stabilization, aside from triennial burns where SOC loss was noted below 5 cm. These findings reinforce the positive role that regular grassland fires can play in carbon sequestration.

**Key Words:** *Sequestration, Carbon Storage, C: N Ratio, Soil Horizons, Prescribed burns*

## 2.2 Introduction

Grasslands play a substantial role as a terrestrial carbon sink, storing significant amounts of carbon both in aboveground vegetation as well as roots and soils (Bikila *et al.*, 2016; Ward *et al.*, 2016). The potential of these grasslands to store organic carbon (OC) is greatly dependent on how they are managed (Ward *et al.*, 2016), with the application of anthropogenic applied fires inducing either self-reinforcing and self-regulating carbon feedbacks (Materchera *et al.*, 1998; Snyman, 2002). Prescribed fire management is commonly practised in South African grasslands (Van Wilgen *et al.*, 1990; Oluwole *et al.*, 2008), and due to carbon (C) and nitrogen (N) losses from biomass during burns, there is scepticism on the long-term sustainability of fire managed systems (Materchera *et al.*, 1998; Snyman, 2002). With the recent noted rise in atmospheric carbon dioxide (CO<sub>2</sub>), it has become necessary to understand the influence of fire on natural grassland and their role in the sequestration of carbon.

The potential of grasslands to mitigate climate change has been extensively explored, emphasizing the crucial role of soils as long-term organic carbon (OC) reservoirs within terrestrial ecosystems (Pellegrini *et al.*, 2020; Schrumpf *et al.*, 2011; Soussana *et al.*, 2004; Ward *et al.*, 2016). Research indicates that changes in carbon levels within ecosystems are characterized by dynamic equilibria in biochemical processes at various levels, rather than the existence of large, permanent carbon pools (Bikila *et al.*, 2016; Ward *et al.*, 2016). This dynamism is primarily linked to shifts in ecosystem conditions and management practices. Rather than having sizable, stable carbon reservoirs, ecosystems are composed of numerous smaller, interconnected carbon pools in a continuous state of flux.

While numerous studies have investigated the carbon storage and sequestration potential of grassland systems, there remains ongoing debate about the rate at which soil organic carbon (SOC) levels change in response to land use conditions and management practices (Petersen *et al.*, 2013; Paustian *et al.*, 2019). Previous studies have suggested that the potential for carbon storage and sequestration in these systems may reach a steady state equilibrium around 20 years after changes in management practices (Eggleston *et al.*, 2006; Goglio *et al.*, 2015; Poeplau, 2019). However, ongoing discussions persist regarding the accumulation of carbon in grasslands over extended periods, with some evidence showing that carbon continues to accumulate for up to 200 years following initial land use changes (Poeplau *et al.*, 2011; Vellinga *et al.*, 2013).

South African mesic grasslands are largely fire-prone ecosystems with defined wet and dry seasons and owe their existence to fire (Morris *et al.*, 1992; Skarpe, 1992; Mucina *et al.*, 2006). Both natural and anthropogenic managed fires play an important ecological role in shaping grasslands in their present form (Tainton *et al.*, 1978; Fynn *et al.*, 2003), influencing physical, chemical, and biological soil properties (Badía *et al.*, 2014). These are particularly noticeable in the top few centimetres of soil, as a result of organic matter (OM) combustion and structural changes in vegetation (Badía *et al.*, 2014). Fire additionally acts as a driver for fluctuations in carbon sequestration, through the removal of above-ground biomass, redistribution of nutrients on large spatial scales and direct contribution of inputs through thermal mineralisation (Hartshorn *et al.*, 2009; Pausas and Bond, 2020; Vermeire *et al.*, 2021). Additionally, fire directly influences soil microbial communities by changing microbial biomass, shifting bacterial and fungal community composition, and modification of the functional processes carried out by these microbes (Vermeire *et al.*, 2021) These feedbacks, either positive or negative, are primarily reliant on the season and frequency of fire (Bikila *et al.*, 2016).

Previous studies have differed in assumptions, suggesting that large nutrient and carbon pools are retained in ash deposits following the burning of biomass (Materchera *et al.*, 1998; Manson *et al.*, 2007; Jones *et al.*, 2019), however, regular burning has been recorded to reduce carbon in the top layers of soil, through the reduction of organic matter content and microbial biomass (Snyman, 2002; Fynn *et al.*, 2003). Moreover, measuring soil nitrogen is necessary as soil carbon and total nitrogen (TotN) play a reciprocal role in microbial activity and edaphic diversity, vital for nutrient cycling in grassland systems. The carbon-to-nitrogen (C:N) ratio is an indicator of soil health, and how microbial activity contributes to the efficient breakdown of organic matter (Venter, Scott, *et al.*, 2017). To understand the long-term impacts of fire on soil carbon pools, it is necessary to directly quantify carbon pools in these grassland systems to greater depths over extended periods. This understanding will facilitate management strategies aimed at increasing carbon sequestration in grasslands and will allow for modelling the impacts of climate-induced changes to fire regimes.

While it is understood that the distribution of organic C and total N within mesic grassland soil horizons are altered by the occurrence of fire, the impact of fire frequency and season of burn on these biophysical processes and the resulting stability of SOC stocks remain poorly understood. Furthermore, despite considerable research over the years, much uncertainty

exists concerning the effects of fire on SOC. The Ukulinga Grassland Fire Experiment initiated in 1950 at Ukulinga Research Farm, Pietermaritzburg, provided the ideal opportunity to examine OC stocks and turnover following a synthesis in 2000 by Fynn *et al.* (2003), over 20 years previously. This paper will establish current OC and N stocks within varying fire frequency treatments and estimate the change in C sequestration over time using available data from Fynn *et al.* (2003) as a baseline. We hypothesised that i) increased fire frequency reduces the concentration of SOC stored in grassland soils to depths of 30 cm, and ii) increased fire frequency influences the reciprocal relationship between SOC and TotN cycling processes within grassland soils to depths of 30 cm, and iii) carbon stocks stabilise and reach equilibrium after around 20 years of treatment application and will have stabilised by years of prescribed fire applications. The test for stability was measured over the last 20 years to detect changes in stocks and establish stabilisation or lack thereof.

Recent reports by the IPCC (Eyring *et al.*, 2021) noted the uncertainties of shifts in carbon turnover and land use change regarding its importance on land carbon fluxes. The IPCC identified limitations in accurately assessing the implications of changes in carbon (C) storage resulting from different land use and management options (Eyring *et al.*, 2021). These limitations are poorly incorporated in carbon cycle schemes. This paper aims to address this by quantifying the potential of anthropologically managed grasslands, to maintain carbon stocks and promote sequestration.

## 2.3 Methods

### 2.3.1 Site Description and Experimental Layout

The Ukulinga Grassland Fire Experiment (UGFE) is located at the University of KwaZulu-Natal Research Farm, Ukulinga, situated in Pietermaritzburg (-29.667° S, 30.399 E) at an altitude of 843 m. Soils at the site were classified as sandy clay loam (Table 2.1), orthic A over soft plinthic B horizon (Soil Classification Working Group, 1991). Mean annual rainfall is recorded as 790 mm, most of which falls in the summer months. Mean maximum and minimum temperatures range from 26.4 °C in February to 8.8 °C in July, respectively. The vegetation of the area is classified as KwaZulu-Natal Hinterland Thornveld (Mucina and Rutherford, 2006a) which is an open savanna. The native grass species in the area all use the C4 photosynthetic pathway.

Established in 1950, the UGFE was initiated to test the effect of various combinations of burning treatments on mesic grasslands (Tainton *et al.*, 1978). Treatments are allocated to plots measuring  $18.3 \times 13.7$  m, replicated three times within a full factorial split plot, randomised block design. The experimental design involves manipulating two factors: burn and mow treatments, with two levels each (burned vs unburned, mowed vs unmowed) resulting in four utilisation treatments combinations, namely utilisation A (No mowing), Utilisation B (one cut early in the season when grass is at 20 cm), utilisation C (one cut at the end of February), and utilisation D (Two cuts, one at B and again at C). The experimental plots were arranged in a randomized block design with each block consisting of four whole plots, one for each treatment combination. The blocks were assigned randomly to one of three replicates of the design. Nine sub-treatments within Utilisation treatment A (no mow) were included for the purpose of this study (Appendix A). Sub-treatment plots are ungrazed, with treatments consisting of annual burning in winter or spring, and biennial and triennial burning in winter, spring, or autumn. Control treatments are unburnt (Appendix A).

**Table 2.1:** Soil physico-chemical properties of UGFE, Pietermaritzburg South Africa. Near-infrared spectroscopy screening was used for estimating soil particle size and soil texture classification. Soil chemical properties acquired from the UKZN repository on collated UGFE soil data.

Properties	Value
Clay (%)	23.67
Sand (%)	56.13
Silt (%)	26.1
Texture class	Sandy Clay Loam
pH	4.46
P (mg/kg)	2.62
K (mg/kg)	69.60
Ca (mg/kg)	909.21
Mg (mg/kg)	363.84
exch acid (meq/100g)	0.32
cations (meq/100g)	8.03
acid sat. (%)	4.34
Zn (mg/kg)	0.66
Mn (mg/kg)	17.72

### 2.3.2 Quantification of Total Carbon and Nitrogen

Soils were collected in May 2021 before treatment burns, using a hand-held soil corer (core  $\varnothing = 7.4$  cm). Soils were sampled randomly, with four representative sampling cores extracted per subplot treatment, and composited to determine SOC variability using standard error within treatments. Soil core samples were reported to a 30 cm depth in compliance with Intergovernmental Panel on Climate Change (IPCC) recommendations, using methods described by (FAO, 2018). Appropriate depth error was reported in sites with shallow soils. Cores were separated into 5 cm sections, representing six increments, and combined to give a composite sample representative of each subplot. Soils were dried at 65°C for 48 Hours. Dried samples were weighed, milled, homogenized, and sieved to 0.5 mm. Samples below fine earth (<0.5 mm) were analysed for total SOC and TotN using a Leco TruMac CNS analyser. Organic carbon ( $g\ C\ kg\ soil^{-1}$ ) and nitrogen ( $g\ N\ kg\ soil^{-1}$ ) concentration was estimated by converting percentage carbon fraction present in the soil to parts per thousand by multiplying by a factor of ten. The SOC stocks and TotN for the sample depths were summed for each treatment plot to give total C stocks (Mg) per hectare to a depth of 30 cm.

### 2.3.3 Estimation of Soil Organic Carbon Stocks

Soil Organic Carbon (SOC) stock present in a sample was estimated as a representative of each treatment. SOC is expressed as Mg C/ hectare (ha) for each stratum (cm) and calculated using methods described by (FAO, 2020).

Equation 2.1:

$$SOC_i stock (Mg\ C\ ha^{-1}) = OC^i \times BD^i \times (1 - vG^i) \times t^i \times 0.1$$

Where  $SOC_i\ stock$  is soil organic carbon stocks ( $Mg\ C\ ha^{-1}$ ) of the fine soil fraction (< 0.5 mm) within depth increments  $i$ ,  $OC^i$  is the Organic Carbon content ( $g\ C\ kg^{-1}$ ) of the fine soil fraction (< 0.5 mm) in depth increment  $i$ .  $BD^i$  is the bulk density calculated as the mass of the soil per total volume of the soil sample of the depth increment  $i$  ( $g\ soil\ cm^{-3}\ soil$ ).  $vG^i$  is the volumetric coarse fragment content of the depth increment  $i$  ( $g\ coarse\ fragment\ g^i\ soil$ ),  $t$  is the depth (cm) of the sampled site. 0.1 is the conversion factor for converting  $mg\ C\ cm^{-2}$  to  $Mg\ C\ ha^{-1}$ .

### 2.3.4 Estimation of SOC Sequestration

To quantify the change in SOC over time in response to fire frequency, average yearly sequestration rates of the specified period are estimated using the top 10 cm of soil organic carbon (g C / Kg Soil) measurements from 2000 (Fynn *et al.*, 2003) as a baseline. Further SOC and TotN readings were used to model the change in OC over time (20 Years). Estimation of SOC sequestration change per unit area is expressed as Mg C ha<sup>-1</sup> and calculated using equations described by (FAO, 2020).

Equation 2.2:

$$\Delta SOC_{seq} (g C ha^{-1}) = SOC_{is} - SOC_{bau}$$

Where  $\Delta SOC_{seq}$  is the SOC sequestration rates per unit area,  $SOC_{is}$  is the SOC stock at 0-10 cm depth 20 years following initial carbon capture (2022),  $SOC_{bau}$  is the SOC stock at 0-10 cm depth 20 years prior to follow-up measurement (2000).

### 2.3.5 Statistical Analysis

A hierarchical linear mixed model analysis was conducted to examine the effects of the frequency and season of burn within soil depths, and their interaction on SOC stock, total N (TotN), C:N ratio and sequestration rates. This model accounts for the nested structure of the data, with observations nested within replicates, and replicates further nested within soil depth categories, and fitted to account for non-independence within soil depth samples (Gili *et al.*, 2013). Data was analysed using the *lme4* package in R studio (R Core Team, 2023). The fixed effects included nested variables fire frequency and seasonal burn, soil Depth, carbon sequestration rate and their interaction, while random effects accounted for the variation within blocks and soil depth levels. Due to sampling depth from the baseline year (2000) being measured to a depth of 10 cm, carbon stocks to the same depth were observed for comparisons at year 2021.

Additionally, a stepwise regression analysis was performed to assess the significance of nested treatments of frequency and season alone on SOC stock, TotN, C:N ratio and sequestration rates. Diagnostic checks were performed to assess the assumptions of the models which were met. Checks comprised of assessments of linearity, normality of residuals,

homogeneity of variance, independence of residuals, and collinearity among predictors, which were met.

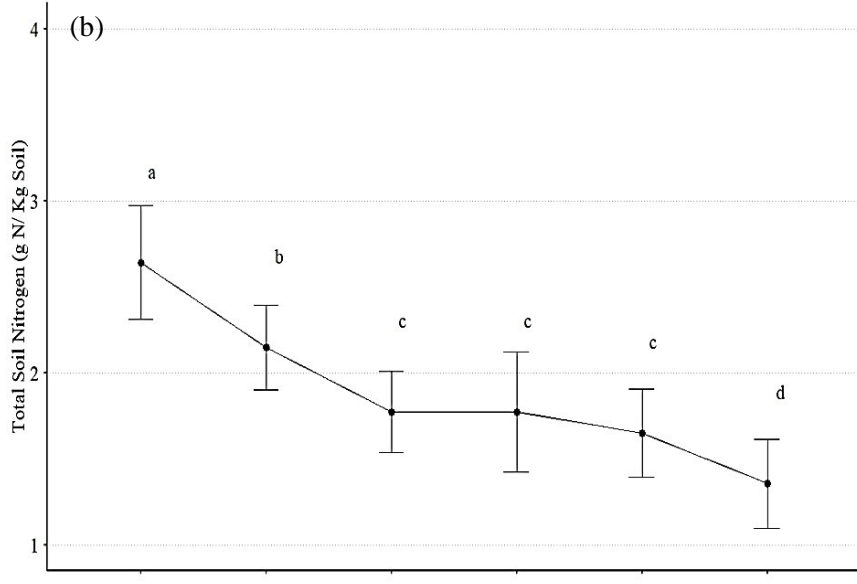
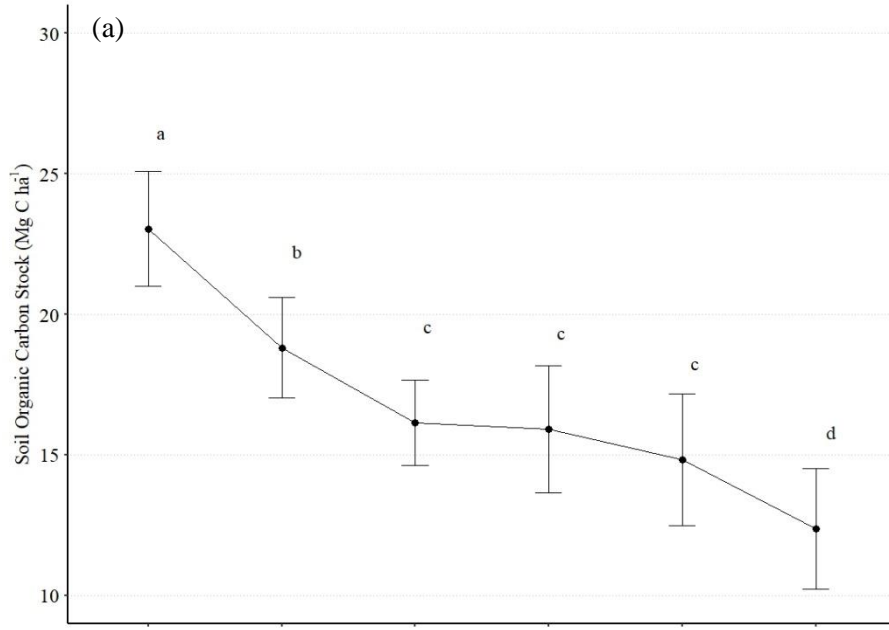
## 2.4 Results

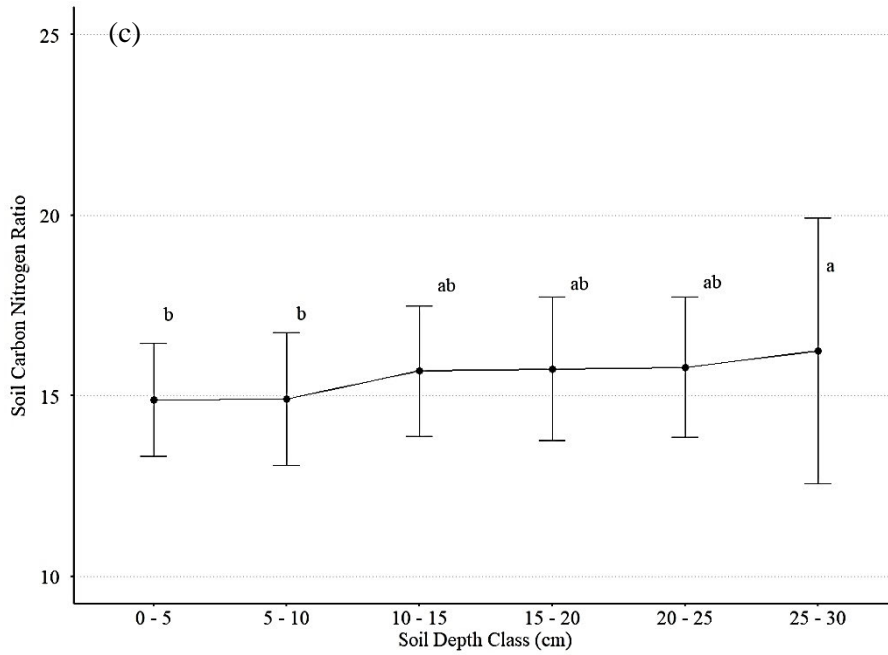
### 2.4.1 Total Carbon and Nitrogen Status Under Varying Fire Regimes

Soil organic carbon (SOC) stocks ( $\beta = -0.396$ ,  $t_{5,419} = -8.006$ ,  $p = 0.02$ ) and TotN ( $\beta = -0.046$ ,  $t_{5,419} = -8.006$ ,  $p < 0.001$ ) significantly reduced with increasing soil depth (Figure 2.1). Biennial spring ( $\beta = -0.639$ ,  $t_{8,425} = -3.468$ ,  $p < 0.001$ ) and winter ( $\beta = -1.482$ ,  $t_{8,425} = -4.553$ ,  $p < 0.001$ ) burns and triennial autumn ( $\beta = -3.174$ ,  $t_{8,425} = -3.427$ ,  $p < 0.001$ ) and winter ( $\beta = -2.131$ ,  $t_{8,425} = -2.373$ ,  $p = 0.01$ ) burns show significantly reduced SOC stock, while annual winter burns show significantly increased SOC stock ( $\beta = 0.995$ ,  $t_{8,425} = 2.145$ ,  $p < 0.001$ ). Biennial autumn burns did not show a significant effect on SOC stock ( $p = 0.072$ ); however, they exhibited a strong negative trend ( $\beta = -3.007$ ). The overall model is significant ( $p < 0.001$ ), indicating that the combination of treatment variables explains the variation in SOC stock (Figure 2.2).

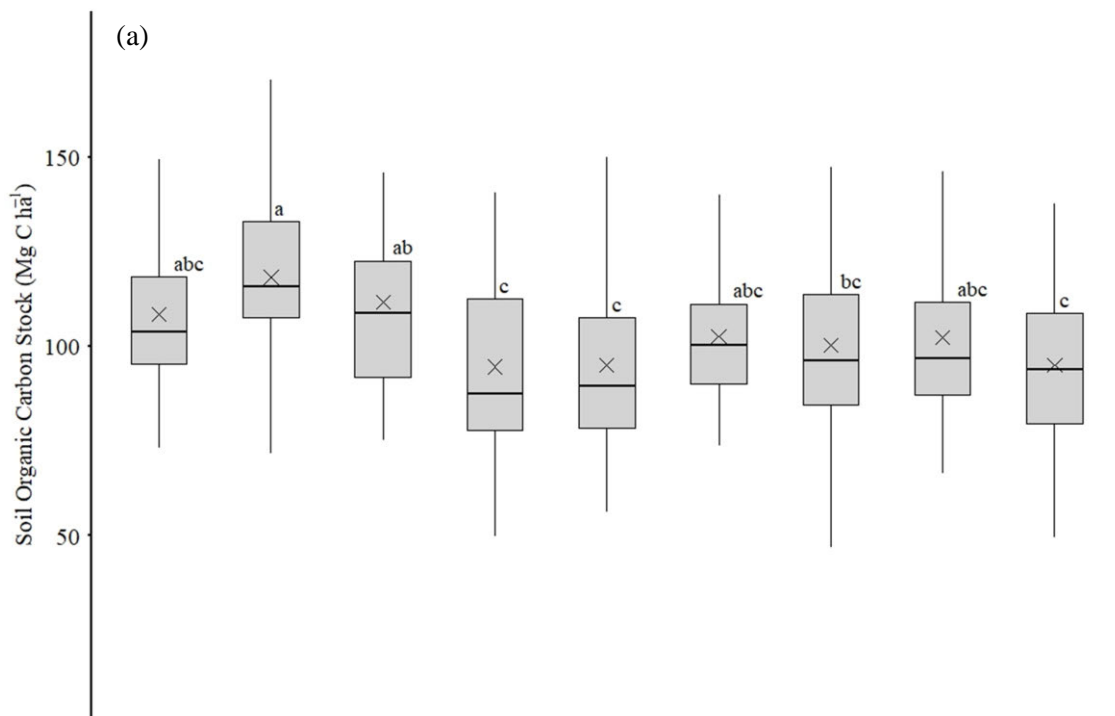
Of all the burn treatments, only the no burn control had a significant effect on soil C:N ratio ( $\beta = -4.310$ ,  $t_{8,425} = -4.178$ ,  $p < 0.001$ , Figure 2.2). Soil depth showed marginal positive effects on C:N ratio ( $\beta = 0.003$ ,  $t_{5,419} = -8.006$ ,  $p = 0.05$ ) suggesting that as soil depth increases, C:N ratio will increase slightly (Figure 2.1).

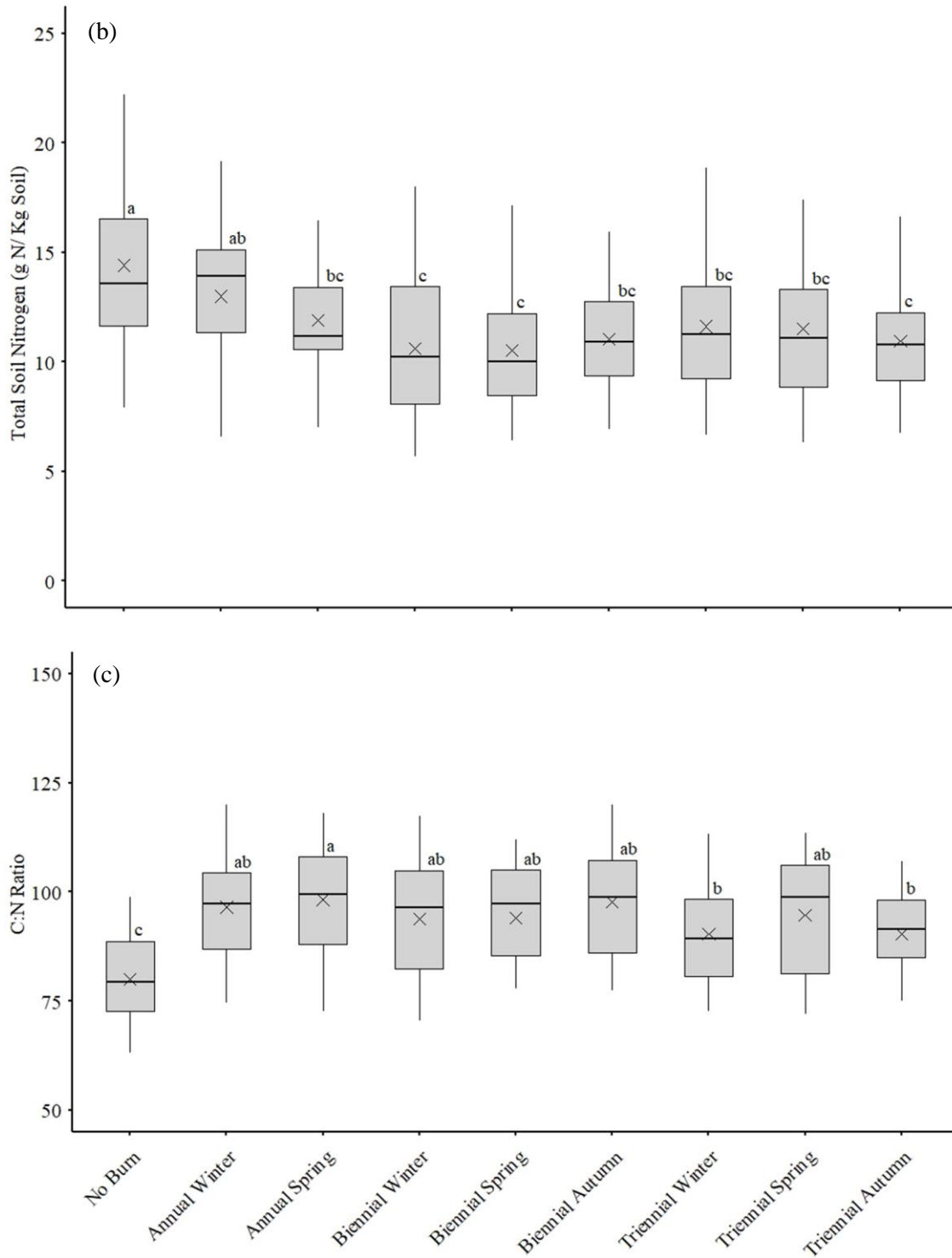
No burn and annual winter burns had a significantly greater TotN compared to other treatments ( $\beta = 0.740$ ,  $t_{8,425} = 5.089$ ,  $p < 0.001$ ,  $\beta = 0.275$ ,  $t_{8,425} = 2.023$ ,  $p = 0.04$  respectively). The interaction between no burn and soil depth had a statistically significant effect on TotN ( $\beta = -0.020$ ,  $t_{40,425} = -2.287$ ,  $p = 0.02$ ), indicating that the impact of burn exclusion on TotN varied depending on the soil depth. The final stepwise regression model had a residual standard error of 0.3134 and an adjusted R-squared of 0.6571, indicating good model fit.





**Figure 2.1:** Mean ( $\pm$ SE) values for (a) total soil organic carbon stocks ( $\text{Mg C ha}^{-1}$ ), (b) total organic nitrogen ( $\text{g N Kg soil}$ ), and (c) soil C:N ratio, at 0 - 5 cm depth increments to a maximum depth of 30 cm. Results show means comparisons (LSD) using untransformed data. Treatments with letters in common are not significantly different.



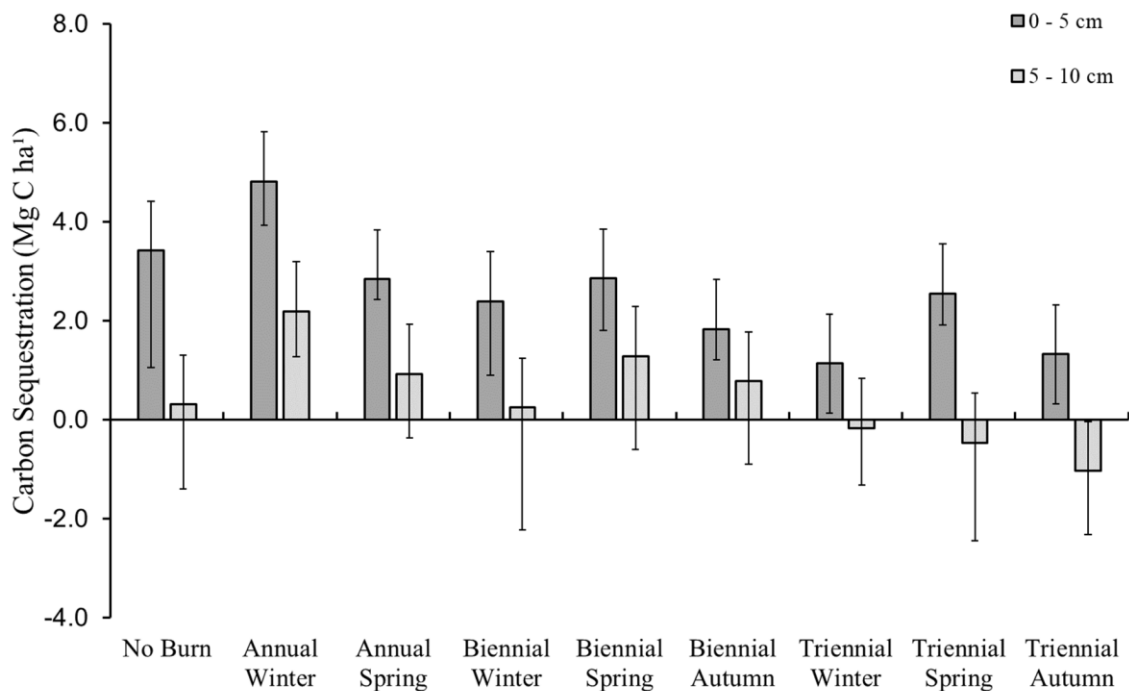


**Figure 2.2:** Mean ( $\pm$ SE) values for (a) total soil organic carbon stocks ( $\text{Mg C ha}^{-1}$ ), (b) total organic nitrogen ( $\text{g N Kg soil}$ ), and (c) soil C:N ratio, at varying burn frequencies and season applications to a total depth of 30 cm. Results show means comparisons (LSD) using untransformed data. Treatments with letters in common are not significantly different. Sites are located at UGFE, Pietermaritzburg South Africa.

### 2.4.2 Carbon Sequestration Over 20 Years

The effect of frequency and season did not significantly contribute to any of the variation in SOC sequestration rates, however stepwise regression showed that Depth alone is a significant predictor of SOC<sub>seq</sub> ( $t_{1,36} = -3.371$ ,  $p = 0.001$ ). Trends observed in sequestration rates within frequency and season indicate triennial burn frequencies were the only treatments exhibiting negative sequestration in the 5 - 10 cm soil depth (Figure 3).

Over a 20-year period, triennial burns in winter ( $1.1 \pm 1.0$  Mg C ha<sup>-1</sup>), spring ( $2.5 \pm 1.7$  Mg C ha<sup>-1</sup>), and autumn ( $1.3 \pm 1.1$  Mg C ha<sup>-1</sup>) exhibited the lowest rates of sequestration in the top 5 cm of soil. Triennial burns have negative sequestration rates in winter ( $-0.16 \pm 2.0$  Mg C ha<sup>-1</sup>), spring ( $-0.46 \pm 3.42$  Mg C ha<sup>-1</sup>), and autumn ( $-1.03 \pm 2.23$  Mg C ha<sup>-1</sup>) in the 5 - 10 cm soil depth (Figure 2.3).



**Figure 2.3:** Changes in carbon sequestration (SOC<sub>seq</sub> Mg ha<sup>-1</sup>) between the period of 2000 and 2021 on the Ukulinga Grassland Fire Experiment, KwaZulu-Natal. Quantification of carbon changes observed within 5 cm depth increments, to a maximum depth of 10 cm. Sites are located at UGFE, Pietermaritzburg South Africa.

## 2.5 Discussion

This research studied the effects of prescribed burn frequency and season on the availability of SOC stocks, self-reinforcing and self-regulating carbon feedbacks and the rate at which SOC sequesters within a long-term fire experiment in a mesic grassland. These were observed in soils to depths of 30 cm. We expected to observe differences in SOC stocks between varying treatments of applied fire, as removal of vegetative litter layer as well as the input of charred material resulting from fire disturbances, change the quantitative and qualitative nature of soils organic component (Certini *et al.*, 2011). Previous research has indicated the seasonal timing and frequency of applied fire contribute to changes in SOC and TotN concentrations (Fynn *et al.*, 2003; Manson *et al.*, 2007; Findlay *et al.*, 2022). We found that SOC stocks varied significantly between fire treatments, as well as showed significant decreasing stocks with increasing depths, to a depth of 30 cm.

Random intercepts were observed at two levels: soil depth within replicates and replicate alone were expected. A significant effect of block was accounted for within the degrees of freedom, signifying an environmental gradient influencing carbon stocks. In this experiment, soil depth and underlying shale was a limiting factor, with block 2 having shallower soils than block 1 and 3.

While some burn treatments exhibit significant changes in TotN, particularly the no burn control and annual winter burns, they do not show significant corresponding shifts in SOC. This implies that nitrogen is likely impacted more by combustion processes or redistribution within the soil in contrast to how carbon responds to burn treatments (Manson *et al.*, 2007).

### 2.5.1 Transformation of Carbon and Nitrogen Fractions

Similar studies at Ukulinga emphasised the importance of the timing of burning in grasslands on the accumulation of SOC and TotN within the top 5 cm of soil, where results indicated no decrease and an accumulation of in OC with annual and biennial spring burning (Fynn *et al.*, 2003). This may be attributed to the temperature of prescribed fires, these fires' burn durations, and their relative rate of spread which could influence recovery of edaphic microbes and their contribution to litter decomposition and carbon turnover (Vermeire *et al.*, 2021). This being dependent on the nature of above ground biomass as well as actively growing foliage present at varying seasons (Bond and van Wilgen, 1996; Ohrtman *et al.*, 2012). Ohrtman *et al.* (2012)

has shown spring fires in grasslands with a combination of both dormant and green foliage, maintained average temperatures and burn durations which account for accumulation of carbon and nitrogen in the soil. Exposure of organic materials to low combustion temperatures are known to contribute to increased production of pyrogenic based OM input (Alexis *et al.*, 2010). This is particularly observed in the top layers of soil, most exposed to the disturbance and where volatilisation of available nitrogen also takes place during burning as seen in unburnt treatments, where N concentration is at its greatest in the absence of fire.

### ***2.5.2 Capacity for Carbon and Nitrogen Turnover and Storage***

As native mesic grasslands are inherently low in nitrogen, N concentrations observed are expectedly low, owing to rapid growth and uptake by C4 grasses as well as volatilisation resulting from applied burns (Ojima *et al.*, 1994; Blair, 1997; Felton *et al.*, 2020). We expected total N to be concentrated in the top 5 cm of soil, decreasing with depth as N mineralises as seen in similar studies by Fynn *et al.* (2003), and where C:N ratios widened within soil horizons. This mineralizable N accumulation would be enhanced due to elevated soil temperatures occurring in prescribed burns (Ojima *et al.*, 1994; Fynn *et al.*, 2003). This might not be the case in unburnt treatments, where we would expect a TotN build up because of reduced volatilisation and N uptake, but contrary lowered mineralisation and availability. The TotN measurement in the soil may too, have been skewed by soil processing and heat application, potentially leading to an underestimation of the available TotN. In unburnt treatments where there is significant litter accumulation, reduced atmospheric inputs and overall, later ‘green-up’ of new grass growth could explain greater concentrations of total N in unburnt treatments (Ojima *et al.*, 1994). While TotN correlated positively with organic carbon (Supplementary Figure 2.1), no notable differences were seen within treatments, but rather treatments burnt in spring showed significantly greater SOC stocks. Because of the greater total N concentration observed in the unburnt control, carbon nitrogen ratios were expected to be reduced in plots burnt infrequently, however the notable increase in C:N ratios with greater depths suggests the soil organic materials are becoming less N-eutrophic (Findlay *et al.*, 2022).

### ***2.5.3 Long-Term C and N Sequestration in Soils***

Soil Organic C sequestration increased significantly between 2000 and 2021 and did not show indications of stabilisation. However, there is significant variation, and the differences may

simply reflect seasonal differences as only two time points were considered in this study. Additionally, there were no significant differences in SOC changes between treatments. It was additionally seen that treatments burnt triennially had C loss in soils below 5 cm. Carbon sequestration in the subsoil, the biodegradability of vegetative matter and degradability of roots attributed to lignin content, are relevant in the formation of chemically recalcitrant forms of C (Lorenz and Lal, 2005). Prolonged periods between burns in the triennial treatments would influence the nature of biomass build up and species composition of the site, resulting in changes in burn temperature and rate of spread. The resulting accumulation of combustible dead biomass and vegetative material turnover may contribute to varying lignin contents in available above and belowground biomass. This may influence the generation of chemically recalcitrant forms of PyOM, resulting in net C loss (Lorenz and Lal, 2005).

Soil texture may also play a role by influencing water retention, nutrient availability, and carbon persistence (English *et al.*, 2005; Hyvarinen *et al.*, 2023). Clay content, in particular, can affect C storage capacity due to its ability to stabilize organic matter and protect it from microbial decomposition. Changes in the amount of summer precipitation may interact with soil texture to affect community and ecosystem processes such as carbon turnover in temperate grasslands and savannas (English *et al.*, 2005).

For instance, in Ukulinga, the soil physicochemical characteristics include a clay content of 23.67%, a sand content of 56.13%, and a silt content of 26.10%. Compared to other grassland sites such as the open grazing lawn habitat of Hluhluwe iMfolozi Park (Hyvarinen *et al.*, 2023) with a clay content of 35%, or the grasslands within the Potshini catchment, KZN, dominated by Moist Highveld Sourveld characterized by a soil clay content of 15% (Dlamini *et al.*, 2014), this clay percentage is relatively moderate. High clay content generally enhances carbon sequestration by providing more binding sites for organic matter, thus reducing its degradability and increasing its persistence in the soil (Wiesmeier *et al.*, 2019).

The resulting rates of decomposition would therefore influence subsoil C inputs and subsequent SOC sequestration. This research established that carbon and nitrogen are more susceptible to turnover and C fraction change in the upper horizons of soils, where they respond more rapidly to prescribed fire management practices. While C sequestration was observed primarily in the upper layers of soil, it has been proposed that SOC may occur in more recalcitrant forms in deeper soil horizons (Lorenz and Lal, 2005; Findlay *et al.*, 2022). These

recalcitrant forms in deeper horizons have the greatest potential for C sequestration and are important in the mitigation of greenhouse effects resulting from the greater rate SOC of turnover (Lorenz and Lal, 2005).

## 2.6 Conclusions

Grassy biomes play a substantial role as a terrestrial C sink, storing up to a third of the world's C in their soils and contributing significantly to the global sequestration of C (Conant, 2010; Vetter, 2020). This includes South African mesic grasslands which are utilised and managed with prescribed seasonal burns in both a wildlife setting as well as by farmers for extensive livestock production. The application of fire in grassy ecosystems, can significantly impact the cycling of SOC and SON, and any changes in management could have significant implications for this biome (Schuman *et al.*, 2002; Findlay *et al.*, 2022).

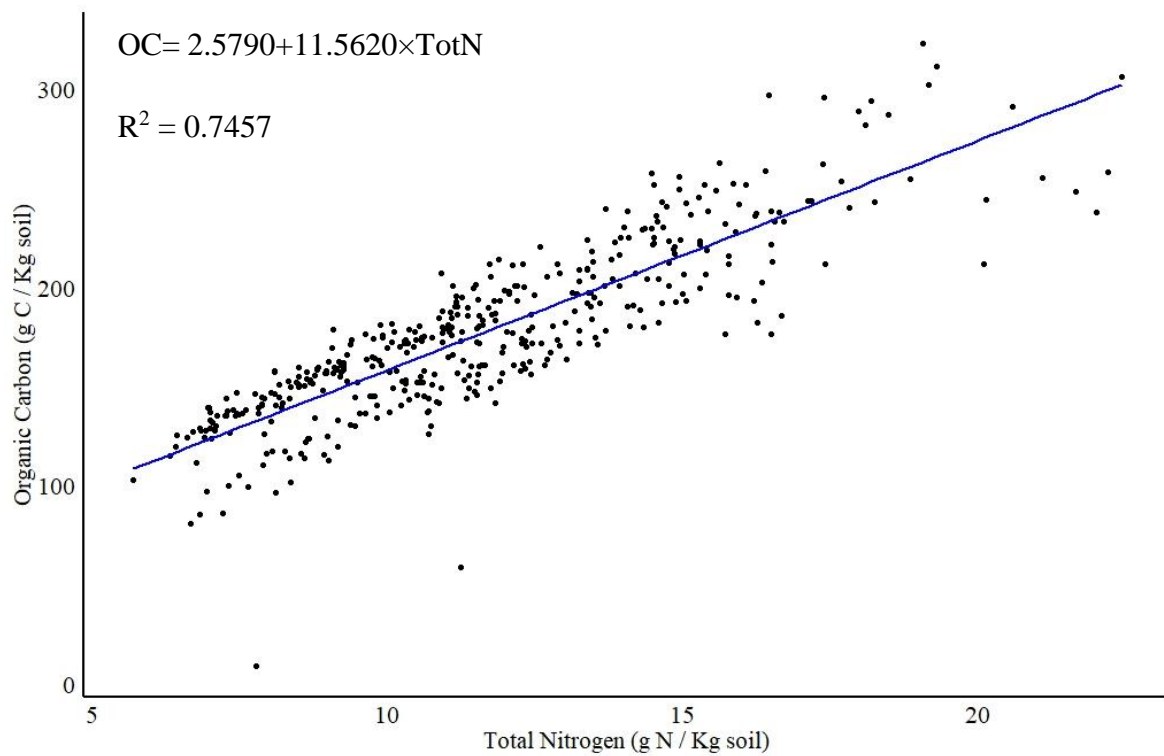
The consequences of prescribed burns on C and N were seen in this research where the greatest differences in C turnover were seen in treatments burnt annually, biannually, or triennially in spring, notably in the first 5 cm of soil. Soil organic carbon (SOC) stocks and TotN levels showed significant declines with increasing depth, indicating susceptibility of topsoil to management practices and disturbances, such as prescribed burning. While some burn treatments exhibit significant changes in TotN, particularly the no burn control and annual winter burns, they do not show significant corresponding shifts in SOC. Greater SOC accumulation in frequently burnt grasslands and increased nitrogen concentrations in unburned areas suggest the presence of species adapted to low TotN conditions, contributing to soil organic matter in C4 dominant mesic grasslands. Changes in C accumulation in the upper horizon indicated that the topsoil is more susceptible to changes, because of management practices. This was expected as these soils are most exposed to disturbances, and where volatilisation of available nitrogen also takes place during burning.

Upper soil horizons would additionally have greater microbial activity. We expected total N to be concentrated in the top cm of soil, decreasing with depth as N mineralises, as reflected in the greater C:N ratios widening within deeper horizons. In agreement with similar studies (Ojima *et al.*, 1994; Fynn *et al.*, 2003), N concentration is at its greatest in the absence of fire, opposed to accumulation of C in grasslands burnt frequently. This disproportionate accumulation suggests the presence of dominant species adapted to low N concentrated soils

contributing to the bulk soil organic matter in C4 dominant mesic grasslands. The altered distribution of organic matter contribution in grasslands burnt in Spring, imply this would be the preferred timing of prescribed fires, when considering management decisions. Further investigation into the implications of stable C isotope composition of C3 and C4 plants under various fire management applications may be valuable in understanding organic geochemical process of organic carbon conversion into persistent pyrogenic forms of carbon, as well as its role in its contribution to the combustion continuum within mesic grassland ecosystems managed with fire.

Soil Organic C sequestration increased significantly between 2000 and 2021 which is an indication that the carbon levels have not stabilised over 70 years since the treatments were implemented. This disproves our hypothesis that suggests SOC stocks stabilise and reach equilibrium over a period of 20 years of treatment application. It was additionally seen that treatments burnt triennially showed a loss of SOC in soils below 5 cm over the 20-year time period. This loss might be attributed to lower biological activity, potentially linked to TotN being in a form that is less accessible to microbes, thereby reducing the overall decomposition and incorporation of organic matter into the soil. These results provide useful insight into potential rates of sequestration in management decisions relating to grassland continuation and productivity, land rehabilitation or conversion to pastoral use. Further research could establish if SOC storage in grasslands burnt infrequently would indeed alter the nature of future burns, either prescribed or natural, through varied burn intensity and rate of spread. This would address whether these alterations are indeed detrimental to the sequestration of C, or in fact contribute to the occurrence of more recalcitrant forms of C in deeper soil horizons. Given the size and volatility of C and N sinks in grassland systems, and the effects of fire on C and N balances, it is imperative to better understand the potential effects of management practices on mitigating detrimental offsets and promote sustainable grassland practices. Therefore, there is a need to further understand potential management strategies suitable for the enhancement of subsoil sequestration whilst considering the sustainability of grasslands ability to retain this carbon in occurrence of accidental fire or lightning strike.

## 2.7 Supplementary Figures



**Supplementary Figure 2.1:** Scatter plot depicting the relationship between total nitrogen (TotN) and organic carbon (OC) in the soil as a function of burning frequency in the Ukulinga Grassland Fire Experiment (UGFE), KwaZulu-Natal. The blue line represents the fitted linear regression model ( $OC = 2.5790 + 11.5620 \times TotN$ ,  $p < 0.001$ ), demonstrating a strong positive association between TotN and OC. The R-squared indicates that approximately 74.57 % of the variability in organic carbon can be explained by the linear relationship with total nitrogen.

### 3 FIRE SUPPRESSION INTERACTS WITH SOIL ACIDITY TO MAINTAIN STABLE RECALCITRANT PYROGENIC CARBON FRACTIONS IN SOUTH AFRICAN MESIC GRASSLAND SOILS

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#### 3.1 Abstract

Grassland ecosystems have evolved with fire, which plays a fundamental role in shaping and maintaining the ecological integrity of grassy biomes. Although carbon sequestration is predominantly observed in the upper layers of grassland soils, there is a suggestion that carbon produced during pyrogenic events may occur in more recalcitrant forms within deeper soil horizons. Notably, refractory organic matter (refractory OM) and black carbon (BC) fractions are recognized globally as highly stable components of soil carbon. Here, we investigated the effect of prescribed fires on the accumulation of refractory OM and BC fractions within South African mesic grassland soils, to a depth of 15 cm. The results of the study indicated that an increased frequency of pyrogenic events resulted in a reduction of BC in the soil and that BC remains relatively stable in the soil, showing consistent levels across soil horizons. Additionally, it was observed that there were no significant differences in refractory OM within the burn treatments, but there was a significant decrease in refractory OM with increasing depth increments below 5 cm. Observed greater levels of BC quantities were observed in burn exclusion and prescribed burns with a greater burn interval, suggested as primarily driven by a decline in soil pH. This increase in BC is suggested to be related primarily to available organic matter as well as acid saturation associated with triennial burns and the absence of fire specifically.

**Keywords:** *Black Carbon, Pyrogenic Carbon, Refractory organic matter, Nitrification, Microbial Biomass*

### 3.2 Introduction

Fire is a major determinant of the ecology and distribution of Africa's savanna and grassland vegetation and is often used as a management tool in these biomes, commonly used for livestock production, and conservation areas (Archibald *et al.*, 2009; Bond and Keeley, 2005; Higgins *et al.*, 2000; Tainton, 1999). The management of grassland through prescribed fires plays a crucial role in the sequestration and turnover of carbon fractions within the soil horizons, particularly considering the frequency and season of burns (Archibald *et al.*, 2009; Bodí *et al.*, 2014; Wang *et al.*, 2017). Studies have shown that increased burn frequency increases soil pH by deposition of alkaline ash, thus decreasing soil acid saturation and consequently improving extractable nutrient availability (Snyman, 2004; Oluwole *et al.*, 2008; Bodí *et al.*, 2014; Agbeshie *et al.*, 2022).

Recent studies have highlighted the potential of recalcitrant pyrogenic carbon ( $C_{py}$ ) in contributing to soil organic carbon (SOC) pools in grasslands (Soon and Cotrufo, 2015; Guo *et al.*, 2022). These forms of C exhibit high resistance to decomposition (Alexis *et al.*, 2010) and can be found in both surface and subsurface soil horizons (Masiello, 2020; Findlay *et al.*, 2022). However, the influence of fire frequency, seasonality, and soil depth on the distribution of  $C_{py}$  fractions, as well as their quantification and contribution to the overall sequence of combustion processes within managed mesic grasslands, remains poorly understood (Wang *et al.*, 2017; Stavi, 2019). Understanding the factors controlling  $C_{py}$  formation and retention in soils is crucial for accurate quantification of C storage in terrestrial ecosystems and the development of effective C sequestration and management strategies.

Black carbon (BC) is thermally altered organic matter produced from the incomplete combustion of vegetation and represents a highly stable fraction of total SOC, playing a vital role in long-term carbon storage (Gao *et al.*, 2017; Thomas *et al.*, 2018; Agbeshie *et al.*, 2022). Interest in quantifying BC in terrestrial environments has grown due to its potential impacts on biogeochemical processes such as improved nutrient cycling, facilitation of microbial activity, and stabilization and turnover of organic materials (Baldock and Smernik, 2002; Sánchez-García *et al.*, 2021). The BC spectrum ranges from partially charred plant material to graphite and soot with no consensus on distinct boundaries within this range (Schmidt *et al.*, 2000; Masiello and Druffel, 2003). Black carbon (BC) exists in a continuum of forms, from partially altered plant residues that withstand combustion to submicron particles formed by the

condensation of gases released during combustion (Baldock and Smernik, 2002; Glaser and Amelung, 2003). Recalcitrant pyrogenic carbon ( $C_{py}$ ) fractions are largely resistant to mineralisation and decomposition and form a highly stable fraction of total soil organic matter (SOM), contributing to slow-cycling carbon pools in the soil which include BC (Glaser and Amelung, 2003). However, despite BC being acknowledged as a persistent component of the total soil carbon pool, studies have highlighted its natural degradation, suggesting soil microbes may have the capacity of solubilizing char material (Masiello and Druffel, 2003; Schmidt *et al.*, 2000; Scott *et al.*, 1986). Notably, recent observations on BC decomposition in charcoals exposed to microbial inoculums revealed that decomposition rates decreased with increasing charring temperatures (Baldock and Smernik, 2002; Czimczik and Masiello, 2007).

Fire patterns (frequency and intensity) in the grassland biome change in response to human population size, land use, and climatic changes in Africa (Bond *et al.*, 2005), and an understanding of the impact of such changes will be needed to predict their consequences and contributions to stable C pools (Scheintaub *et al.*, 2009). The spread and intensity of grassland fires are affected by climatic factors, season and frequency of burn, fuel load and fuel composition (Mapiye *et al.*, 2008). These factors influence the duration of the fire and the production of BC (Lorenz and Lal, 2018). Fires with warmer temperatures tend to produce more BC than those with cooler temperatures, as the elevated temperatures promote the decomposition of organic compounds derived from lignin into simpler forms that can be easily converted into BC (DeLuca *et al.*, 2006; Bird *et al.*, 2015). Additionally, a reduction in burn frequency has been observed to correlate with elevated quantities of black carbon (BC). This is suggested to be attributed to prolonged exposure to heightened temperatures resulting from increased fuel loads. Consequently, the greater fire intensity stemming from these conditions promotes enhanced lignin decomposition, contributing to the greater levels of BC (Knicker *et al.*, 2008).

Fire-induced soil disturbance is influenced primarily by factors such as fire intensity, frequency of burn, fuel load, and soil properties (Knicker *et al.*, 2008; Bodí *et al.*, 2014). Low-intensity fires, accompanied by alkaline mineral ash deposition comprising inorganic carbonates (calcium, magnesium, sodium, potassium, silicon and phosphorus), can lead to alterations in soil chemistry, including increased soil pH and subsequent nutrient availability (Bodí *et al.*, 2014; Agbeshie *et al.*, 2022). While microbial communities are largely resilient to low-intensity fire disturbances (Docherty *et al.*, 2012), soil pH influences carbon dynamics in

grassland ecosystems by determining microbial activity and nutrient availability. Increased acidity in grassland soils may hamper microbial activity, reducing the decomposition and accumulation of organic matter (Fynn *et al.*, 2003; Sinsabaugh, 2010). A neutral soil pH can promote the retention and stabilization of carbon in the soil, while lesser pH values can hinder these processes through the direct influence of pH on the abundance and composition of major soil microbial taxa, fungi and bacteria (Lauber *et al.*, 2009; Rousk *et al.*, 2010).

To address this, we conducted a study to investigate the effects of 70 years of prescribed fire on mesic grassland soils in South Africa. Specifically, we aimed to determine how fire frequency affects soil acidification and subsequent accumulation or breakdown of refractory OM and BC fractions in the soil. We hypothesized that in a fire-prone mesic grassland system, withholding fire would lead to higher fuel loads, resulting in higher charring temperatures when fires do occur. This would stabilize refractory organic matter (OM) and black carbon (BC) in the soil, leading to their accumulation. Conversely, with frequent fires, lower fuel loads would result in lower charring temperatures, enabling some decomposition of BC and ash deposition, which would maintain a neutral pH and promote microbial activity, ultimately reducing the amount of refractory OM and BC retained in the soil. To test these hypotheses, we measured soil acid saturation and pH, and quantified soil refractory OM and BC within the first 15 cm soil horizon. We compared the effects of long-term applied fire regimes using the fire exclusion treatment as a reference point, and discussed the underlying mechanisms that may account for the directional shifts in refractory OC and BC in response to long-term prescribed fire frequency.

### **3.3 Materials and Methods**

#### ***3.3.1 Study Site and Experimental Setup***

The Ukulinga Grassland Fire Experiment (UGFE) is located at the Ukulinga Research Farm, University of KwaZulu-Natal, Pietermaritzburg (-29.667° S, 30.399 E) at an altitude of 843 m. The soil type at the site is classified as a sandy clay loam (Westleigh soil form comprising an orthic A horizon over a soft plinthic B horizon) and orthic A horizon over hard rock (shale) in shallower areas (Mispah soil form) (Soil Classification Working group, 1991). The mean annual rainfall at the site is 790 mm, with precipitation largely occurring during the summer months. The mean maximum and minimum temperatures range from 26.4 °C in February to

8.8 °C in July, respectively. The vegetation in the area is categorised as KwaZulu-Natal Hinterland Thornveld (Mucina and Rutherford, 2006b), an open savanna. The indigenous grass species in the area follow the C4 photosynthetic pathway. The Ukulinga grassland plateau area would have experienced regular burning prior to 1950, likely resulting in the deposition of recalcitrant forms of carbon. While there is a record of the A1 (Unburnt control) plot in Rep 1 burning accidentally in 1957, there are no other records indicating accidental fires affecting any of the other A1 plots at any time.

The UGFE was established in 1950 to examine the effects of different combinations of burning treatments on mesic grassland (Tainton *et al.*, 1978). The experimental design involved the manipulation of two factors: burning and summer utilisation (mowing), with two levels each (burned vs unburned, mowed vs unmowed), resulting in four utilisation treatment combinations, namely Utilisation A (No mowing), Utilisation B (one cut early in the season when grass is at 20 cm, usually December), Utilisation C (one cut at the end of February), and Utilisation D (two cuts, one at B and another at C). Each block consisted of four plots, one for each whole plot treatment, arranged in a randomised block design, and each block has one of each whole plot treatment (Appendix A).

For this study, ten subplot treatments within utilisation treatment A were included. Subplots measuring 18.3 × 13.7 m were used, with treatments allocated to the plots in a full factorial split-plot, randomised block design that was replicated three times. The entire trial is ungrazed, with treatments consisting of annual (An) burning in winter or spring and biennial (Bi) and triennial (Tri) burning in winter, spring, or autumn. Contrasting removal exclusion treatments were included as burn exclusion (no burn) and a treatment looking at winter defoliation without fire (annual mowing first week in August, with mown grass removed using a hay rake) was included. Spring defoliations are applied in the week following the first 12.5 mm rainfall. Winter burns are applied in the first week of August.

### ***3.3.2 Pyrogenic Carbon Quantification Methods***

In May 2021, before treatment burns, soils were collected using a hand-held soil corer with a core diameter of 2.8 cm. Random soil sampling was conducted, with four representative sampling cores extracted per treatment, to assess the variability of recalcitrant C<sub>py</sub> fractions within treatments. Soil core samples were collected to a 15 cm depth according to methods

described by the Food and Agriculture Organization (FAO, 2020). In cases where shallow soils were encountered, appropriate depth error was reported. The soil cores were separated into 5 cm sections, resulting in a representation of three soil depths. The soil samples were then dried at 65 °C for 48 hours, and then weighed, homogenized, and sieved to a particle size of 0.5 mm (fine earth). Soil was tested for acid saturation and pH at the KwaZulu-Natal Department of Agriculture and Rural Development (KZNDARD) Analytical Laboratory. The analysis involved preparing a soil-water suspension and using a pH electrode or indicator to measure the pH value.

Soil C fractions were analysed using a Thermogravimetric analyser (TGA) (Universal V4.5A TA) and recording percentage weight drops. Samples were heated at 20°C per minute, from 30 °C to 650°C under a flow of 20% O<sub>2</sub> in 80% pure nitrogen atmosphere (Dell'Abate *et al.*, 2000; Lopez-Capel *et al.*, 2005; Plante *et al.*, 2011). A pretrial of soil samples was subjected to differential scanning calorimetry (DSC) under both nitrogen (N) and pure air atmospheres. Analysis of the resulting data revealed that the soil samples exhibited more prominent exothermic peaks under N atmosphere compared to pure air. Based on these findings, N was identified as the optimal atmosphere for DSC analysis of the soil samples. Recalcitrant C<sub>py</sub> was recorded as lost between 475-650 °C. Recalcitrant C<sub>py</sub> was separated into refractory OC and BC (475 °C to 550 °C and 550 °C to 650 °C respectively). Fractions of refractory OC ( $g\ C\ kg\ soil^{-1}$ ) and BC ( $g\ C\ kg\ soil^{-1}$ ) concentration were estimated by converting the percentage carbon fraction present in the soil to parts per thousand by multiplying by a factor of ten.

### 3.3.3 Statistical Analysis

Data were analysed using R Studio, statistical software (R Core Team, 2023), using packages *DescTools* (Signorell, 2017), *magrittr* (Bache and Wickham, 2022), *dplyr* (Wickham *et al.*, 2023), *tidyverse* (Wickham *et al.*, 2019), and *dabestr* (Ho *et al.*, 2019). To compare the effects of fire frequency and defoliation among specific treatments and depths, we analysed results by first conducting a two-way ANOVA in which soil depth and treatment were tested using the refractory OC and BC in each plot as the dependent variable. Following this ANOVA, an overall comparison between differences among each treatment with the interaction of soil depth was tested using a *post hoc* Tukey HSD test. Probability values were adjusted in a pairwise t-test using the Bonferroni correction and Holm adjustments, to account for type 1 errors. Where the ANOVA revealed significant differences within main effects, the least significant Fisher's

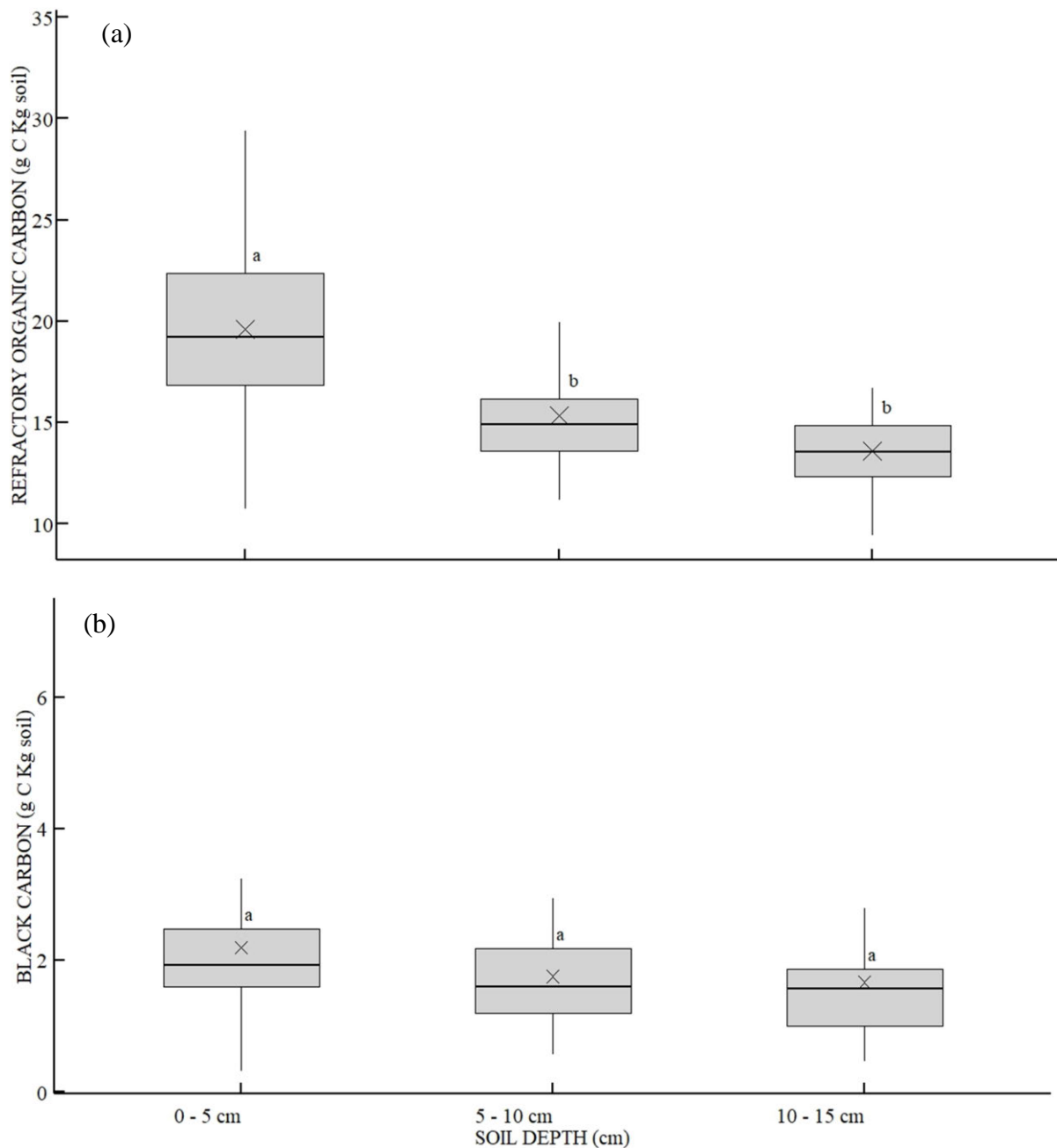
test (LSD) for  $p \leq 0.05$  was used to distinguish means. Refractory OC was log-transformed, and BC was square root transformed, assumptions of normality and homogeneity of variance were satisfied using the Shapiro-Wilk normality test ( $p > 0.05$ ).

To establish a relationship between the pH status of the soil and BC levels at the UGFE, a correlation analysis was conducted using Pearson's product-moment correlation coefficient. In examining the refractory OC and BC data, we initially applied the outlier labelling rule to identify outliers, using a multiplier of 2.2 as suggested by Hoaglin and Iglewicz (2012). Winsorization was then performed on these outliers (Tukey, 1962), where the outliers were assigned the same value as the highest, most reliable value in the data set. statistical significance was further assessed using a one-way ANOVA. Acid saturation was analysed within the prescribed burn and mow treatments at the UGFE using a one-way ANOVA followed by a *post hoc* Tukey HSD test.

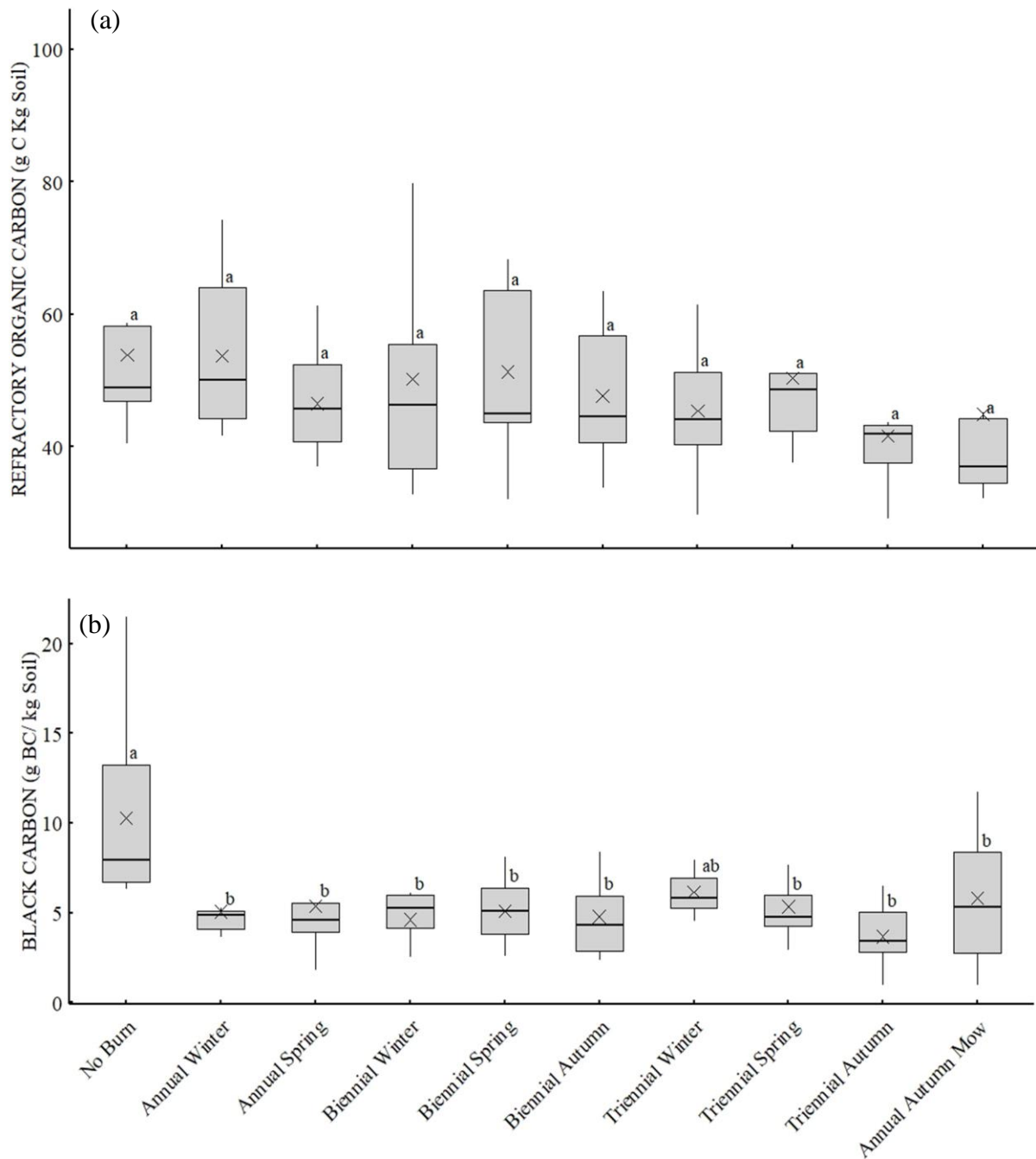
### 3.4 Results

Results of the ANOVA revealed a significant effect of soil depth on refractory OC ( $F_{2, 60} = 20.41$ ,  $P < 0.001$ ), with refractory OC decreasing with increasing soil depth. Refractory OC was highest in the top 0 – 5 cm of soil ( $19.62 \pm 0.929$  g/ kg Soil), followed by a decrease in the second horizon of 5 – 10 cm soil ( $15.34 \pm 0.596$  g/ kg Soil), and a further decrease in the third horizon of 10 – 15 cm ( $13.61 \pm 0.430$  g/ kg Soil). Only the burning and mowing treatments significantly affected BC ( $F_{8, 60} = 3.58$ ,  $P < 0.001$ ), while it remained relatively stable throughout the soil profile ( $1.87 \pm 0.180$  g/ kg soil) (Figure 3.1).

The greatest quantities of BC were seen in grassland that were left unburnt and undefoliated entirely ( $10.27 \pm 2.141$  g/ kg Soil). Grassland plots excluded from fire but mowed annually in the first week of August ( $5.82 \pm 1.871$  g/ kg Soil), as well as those subjected to infrequent triennial burns in winter ( $6.13 \pm 0.474$  g/ kg Soil) (Figure 3.2) also showed relatively greater accumulation of BC in their soils. In contrast, grassland sites that experienced frequent burns, both annually and biennially, exhibited the lowest accumulation of BC (Figure 3.2). The accumulation of refractory OC was not influenced by prescribed fires and exclusion of burns.



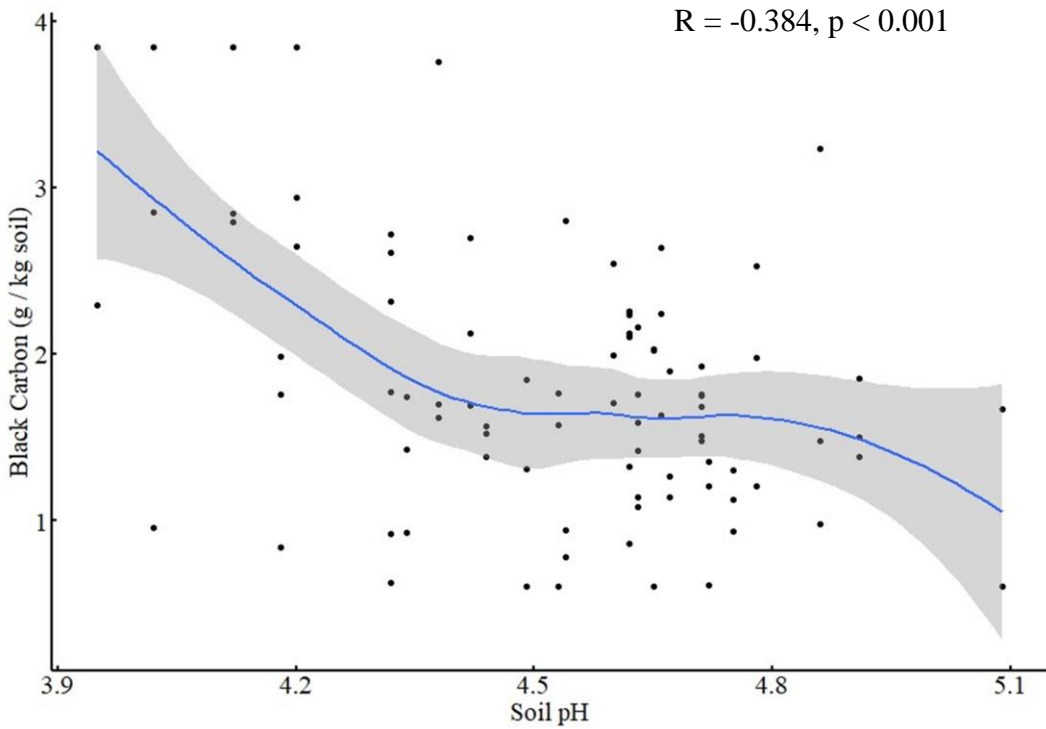
**Figure 3.1:** Boxplots showing the distribution of refractory OC (a) and BC (b) concentrations (g C Kg soil) across three soil depth horizons: 0 - 5 cm, 5 – 10 cm, and 10 – 15 cm. The central line within each box represents the median, with the box denoting the interquartile range (IQR). Outliers are indicated as individual points. Means are depicted by 'X' symbols. Results show mean comparisons (LSD). Treatments with letters in common are not significantly different.



**Figure 3.2:** Boxplots showing the effects of long-term burning and mowing of mesic grassland on refractory OC (a) and BC (b) concentrations (g C Kg soil) in the soil to a sampling depth of 15 cm. The central line within each box represents the median, with the box denoting the interquartile range (IQR). Outliers are indicated as individual points. Mean values for BC (g C Kg soil) are denoted by 'x' symbols along with standard error (SE) bars. Results show mean comparisons (LSD). Treatments with letters in common are not significantly different.

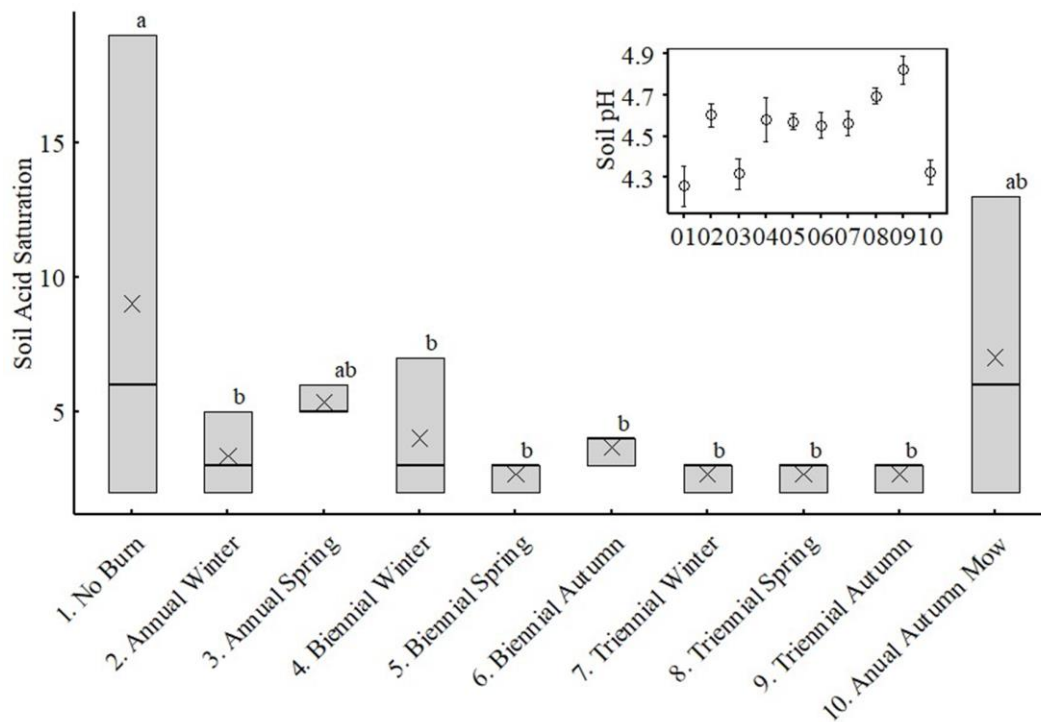
Table 3.1: ANOVA table showing the variation between sample means in refractory OC, BC and acid saturation within prescribed fire treatments at UGFE, and depths to 15 cm.

	Refractory Organic Carbon		Black Organic Carbon		Acid Saturation		df
	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	
Treatment	1.51	0.16	3.583	<b>&lt;0.001</b>	3.506	<b>0.002</b>	9
Depth	20.41	<b>&lt;0.001</b>	3.403	0.05	0	1.00	2
<i>Treatment * depth</i>	1	0.77	1.306	0.23	0	1.00	18
Residuals							60
Total							89



**Figure 3.3:** The observed trends showing a negative relationship between BC and soil pH at UGFE, Pietermaritzburg. The 95% confidence interval provides a range of plausible values for the true correlation in the population.

Correlation analysis revealed a significant, negative correlation between BC and soil pH ( $r = -0.384$ ,  $p < 0.001$ ), indicating a moderate relationship between the variables. As pH increases and the soil becomes less acidic, BC decreases (Figure 3.3). Fire treatments significantly affected soil acid saturation ( $F_{9,80} = 4.67$ ,  $p < 0.001$ ). Soils that have been excluded from burning entirely, and those with a greater interval between burns have shown greater acid saturation and reduced pH than those exposed to fire (Figure 3.4). Soils that showed the greatest acid saturation and acidification were those excluded from burns, or those treatments burnt triennially. These treatments also demonstrated the greatest BC accumulation within the soil with little variation deeper into the soil horizons.



**Figure 3.4:** Acid saturation ratios and soil pH between burn and mowed treatments at UGFE, Pietermaritzburg. Mean ( $\pm$ SE) ratio values show comparisons (LSD) and soil pH means are shown in the secondary graph, with treatment numbers corresponding with those in the primary graph. Treatments with letters in common are not significantly different and apply to both acid saturation and pH.

### 3.5 Discussion

Prescribed fires play a crucial role in determining biomass accumulation in grassland. Regular burning usually results in less lignified aboveground biomass (Resco de Dios, 2020). In grassland, the type and amount of aboveground biomass differs between fire regimes. Factors like the frequency of burning can change woody encroachment and grass lignin content. These, in turn, influence flammability and the intensity of the fire (Hmielowski *et al.*, 2014; Resco de Dios, 2020). Increased intervals between fire events change the nature of the accumulated biomass, with greater lignification and fuel load, including greater quantities of moribund material (Resco de Dios, 2020). This leads to fires that burn longer and more intensely than those that have shorter burn intervals (Patrick and Dalton, 2007; Resco de Dios, 2020).

Fire exclusion and reduced burn frequency associated with more intense burns can lead to reduced total carbon accumulation as the decomposition and turnover of above and belowground biomass are reduced (Pellegrini *et al.*, 2017, 2021, 2022). Lignified biomass is less prone to decomposition, resulting in a slower build-up of carbon stocks in the soil (Lewis *et al.*, 1982; Kimetu *et al.*, 2008). Considering this, we expected ROC fractions to accumulate more in grassland burnt less frequently, as observed in the triennial burns during winter ( $2.04 \pm 0.14$  g BC/kg Soil). However, we did not expect the highest accumulation of stable fraction BC in grassland excluded from fire entirely, as seen in the burn exclusion ( $10.27 \pm 2.141$  g BC/kg Soil) and the annually mown treatments ( $5.82 \pm 1.871$  g BC/kg soil).

We quantified soil acidity levels within the various prescribed fire treatments to understand the influence of fire on soil pH and acid saturation of grassland soils. Prescribed grassland fire can induce changes in soil chemistry through the deposition of mineral and ash materials, contributing to reduced soil acidity (Manson *et al.*, 2007; Bodí *et al.*, 2014). The ash contains alkaline compounds, such as calcium, potassium, and magnesium, which can increase soil pH and reduce acidity (Manson *et al.*, 2007). This alkaline effect can counteract soil acidification processes, particularly in mesic grassland where the vegetation and soil naturally tend to be more acidic (Agbeshie *et al.*, 2022). The influence of ash deposition on soil pH and acidity is context-dependent and can vary based on factors such as the composition of the burned vegetation and the characteristics of the underlying soil (Thomas, 1996; Agbeshie *et al.*, 2022). It could be further suggested that fire frequency in grassland systems and its associated burn intensity and rate of spread could influence nitrogen (N) loading (Manson *et al.*, 2007;

Docherty *et al.*, 2012). Nitrogen is highly volatile and with the exclusion of fire, the deposition of N and resulting nitrification could reduce the soil pH and soil microbial communities (Docherty *et al.*, 2012). This would explain the high acid saturation and low pH (acid soils) seen in plots left unburnt and unmowed since 1950 and those unburnt and mowed annually. Highly acidic grassland soils can significantly reduce the decomposition of organic material, primarily by limiting soil microbial activity and enzymatic processes (Wang *et al.*, 2020; Agbeshie *et al.*, 2022). Prior to 1950, the whole site is likely to have been burnt frequently. Implementation of the treatments in 1950 may have started the soil acidification process, slowed the breakdown and cycling of BC in the unburnt and infrequently burnt treatments. This can result in a slower breakdown of organic material, leading to reduced nutrient release and cycling in the soil.

Soil microbial biomass typically declines following a high-intensity fire (Raison *et al.*, 1986; Agbeshie *et al.*, 2022) but is largely dependent on the severity and frequency of burning. However, these decreases are often followed by rapid microbial recovery (Pellegrini *et al.*, 2017; Agbeshie *et al.*, 2022). Significantly increased microbial biomass on the UGFE have been observed, in plots burnt annually, but only to a depth of 4 - 10 cm, when compared to grassland left unburnt, suggesting that frequent fires increase microbial activity in deeper soil horizons specifically (Fynn *et al.*, 2003).

Considering the influence of ash deposition on soil processes, analysing soil acid saturation and pH levels could help explain the response in black carbon (BC) accumulation in plots where burning is excluded. As a response to low pH and high acid saturation the decomposition rate of organic material may be reduced (Manson *et al.*, 2007), resulting in the accumulation of partially decomposed or more Recalcitrant  $C_{py}$  (Pellegrini *et al.*, 2022). The accumulation of BC in burn exclusion treatments may occur due to the microbial community being less efficient in breaking down complex organic compounds under increased acidic conditions (Bodí *et al.*, 2014).

Particle size of BC which can show variations based on different burn treatments, should also be considered. Specifically, coarse BC particle sizes could be linked to scenarios where burns are excluded, correlating with diminished microbial activity in the soil (Herring, 1985; Schmidt *et al.*, 2000). Alternatively, finer BC particle sizes are typically associated with environments that experience frequent fires and heightened microbial activity (Herring, 1985;

Schmidt *et al.*, 2000). As a result, coarse BC particles may be more readily detectable at elevated concentrations in the soil, reflecting the impact of the burn frequency and microbial interactions on BC distribution. The Ukulinga grassland plateau area would have experienced regular burning prior to 1950, likely resulting in the deposition of recalcitrant forms of carbon. While there is a record of the A1 plot in Rep 1 burning accidentally in 1957, there are no other records indicating accidental fires affecting any of the other A1 plots at any time. The maintenance of this recalcitrant BC in burn exclusion treatments may have occurred due to the microbial community being less efficient in breaking down complex organic compounds under increased acidic conditions (Bodí *et al.*, 2014).

Black carbon (BC) tends to be relatively stable and resistant to decomposition (Lehmann *et al.*, 2008). Therefore, it may remain consistent throughout the soil horizons, as seen in this study where there was no variation in accumulation of carbon within the horizons to a depth of 15 cm. On the other hand, the significant variation in the accumulation of refractory organic carbon within the soil layers can be attributed to several factors. Firstly, mineral and ash deposits resulting from prescribed fire can enhance nutrient availability and pH levels in the top layer of soil (Wang *et al.*, 2020; Agbeshie *et al.*, 2022). These deposits can promote the accumulation of refractory organic carbon by supplying favourable conditions for the formation and stabilization of this carbon fraction. The combined effects of prescribed fire, mineral and ash deposits, and soil acidity likely contribute to the observed maintained quantity of refractory organic carbon in the top 5 cm of soil, followed by a decrease and stabilization in the next 5-10 cm of soil and deeper. The surface layer receives direct inputs of organic matter from vegetation and combustion residues, leading to a greater accumulation of refractory organic carbon (Pellegrini *et al.*, 2017). Moving deeper into the soil, decomposition processes and the influence of mineral and ash deposits become less pronounced, resulting in reduced levels and stabilization of refractory organic carbon. Black carbon (BC) is recognized as a continuum, comprising of charred biomass, which may still be microbially degradable, to more stable soot fractions (Knicker, 2007). Refractory OC fractions however, can differ from BC and result from secondary combustion, formed through the condensation of heterocyclic volatiles, likely via a radical mechanism (Knicker, 2007). These refractory fractions are suggested to be more resistant to degradation by microbial activity compared to organic compounds from non-fire sources (Knicker, 2007). Depression in microbial activity resulting in soil acidity fluctuations between fire treatments would then not influence the rate of decomposition of this particular

fraction, as it would BC. The surface layer of these grasslands directly receives organic matter inputs from these heterocyclic combustion residues, which may have minimal impact on the size of the refractory soil organic matter (SOM) pool through microbial cycling (Pellegrini *et al.*, 2017).

Fire-adapted ecosystems often show a greater allocation of carbon to belowground biomass, such as roots and rhizomes, which contributes to post-fire recovery and resilience (Resco de Dios, 2020). The decrease in refractory OC with increasing depth seen in this study is consistent with the concept of SOC persistence discussed by Schmidt *et al.* (2011). SOC is an important ecosystem property that contributes to soil fertility and C storage. However, SOC persistence can vary depending on factors such as climate, vegetation type, and land use (Schmidt *et al.*, 2011; Angst *et al.*, 2021). Refractory OC can act as a source of nutrients and organic matter that supports the re-establishment of vegetation and enhances soil properties for plant growth following a fire event (Batjes, 1999; Schmidt *et al.*, 2011). Here, the decrease in refractory OC observed with increasing depth suggests that SOC persistence may be reduced at greater soil depths. It can then be inferred that the observed trend of refractory organic carbon accumulation in the top layer of soil, influenced by prescribed fire and associated factors, may contribute to the post-fire recovery and resilience of fire-adapted ecosystems.

### **3.6 Conclusion**

Ash deposition from fires, rich in alkaline compounds, has been suggested to play a role in modulating soil pH and acidity. This was seen on the UGFE, where the absence of fire supported heightened acid saturation and reduced pH. The reduced pH correlated with the accumulation of BC in the soil, with greater acidity resulting in maintained quantities of BC. This is suggested to be a result of reduced microbial activity, resulting in slower decomposition, and reduced nutrient release and cycling in the soil. It was also suggested that environments experiencing frequent fires may have finer BC particle sizes and lower charring temperatures, which can enable decomposition and microbial activity, thereby diluting the detection of overall BC. As a result, coarse BC particles may be more readily detectable at elevated concentrations in the soil, reflecting the impact of the burn frequency and microbial interactions on BC distribution. Black carbon particle size fractionation may be beneficial in further understanding the distribution of stable fraction recalcitrant  $C_{py}$  in grassland soils exposed to fire as there were

constraints in characterizing BC particulate size due to limitations in time and resources during this research.

The accumulation of refractory OC within the soil layers showed significant variation, with greater levels observed in the top 5 cm of soil followed by a decrease and stabilization in the next 5-10 cm. The surface layer receives direct inputs of organic matter from vegetation and combustion residues, promoting the accumulation of refractory organic carbon. The observed decrease in refractory OC with increasing soil depth suggests that soil organic matter persistence may be reduced at greater depths. However, the accumulation of refractory OC in the top layer can support the recovery and resilience of fire-adapted ecosystems by providing nutrients and organic matter for vegetation establishment. This supports the idea that  $C_{py}$  can show variations in response to burn frequency, and while stable forms of BC may occur in grassland burnt infrequently, the trade-off of acidic soil conditions in these scenarios may inhibit overall carbon particulates and turnover of fractions.

## 4 FIRE, GRAZING AND CARBON IN MESIC GRASSLANDS

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### 4.1 Abstract

Fire and herbivory are important drivers of ecosystem processes within South African mesic grasslands. However, the frequency of applied fires and the grazing management, stocking rate, timing and duration of burns on these grasslands are coupled in their ecological responses. In such ecosystems, variations in fire frequency can influence vegetation dynamics, impacting the grazing selection patterns. This study investigates the mesic grassland dynamics between annual winter burning and total burn exclusion in grazed grassland. We look at how fire and grazing influence soil organic carbon stocks, affect soil respiration rates and influence livestock grazing behaviour in relation to biomass availability and palatability. The research site, Wakefield Farm in Fort Nottingham, KwaZulu-Natal, adopts a no-burn approach, utilizing cattle grazing as the primary means of defoliation. The grazed and annually burnt fire breaks at the site offer an opportunity to observe the impacts of both fire and grazing.

Results indicate no significant differences in soil organic carbon (SOC) and total nitrogen (TotN) levels between the fire breaks and unburned grasslands, but substantial differences in soil carbon-to-nitrogen (C:N) ratios were observed based on depth and burning regime. Soil organic carbon (SOC) stocks reduced with increasing soil depth. Soil respiration showed greater totals in the fire breaks compared to unburned sites. Annually burnt fire breaks led to reduced aboveground biomass accumulation following grazing, suggesting heavy grazing of annually burned areas relative to the unburned areas. These annually burnt grasslands also exhibited reduced aboveground biomass lignin and fibre percentage relative to the adjacent unburned areas. Firebreaks showed higher aboveground net primary productivity (ANPP) in the enclosure cages, implying that fire enhances productivity.

**Keywords:** *Annual grassland fire, grazing, Lignification, Carbon flux, Pyric herbivory*

## 4.2 Introduction

The historical occurrence of fire in South African mesic grasslands significantly influences vegetation structure, composition, and overall functioning, leading to adaptations in both plant and animal species to these fire regimes (Archibald *et al.*, 2005; O'Connor, 2005; Venter, Hawkins, *et al.*, 2017). Grassland plant species, in particular, have developed mechanisms such as increased lignification or extensive underground root systems to thrive in fire-prone environments (Orians and Milewski, 2007). Research on the Nutrient-Poverty/Intense-Fire Theory has associated carbon (C)-rich, defensive compounds like lignin with reduced herbivory rates and the accumulation of fuel in fire-prone arid grassy biomes (Orians and Milewski, 2007). While this defence mechanism may contribute to slow nutrient cycling, it could influence soil nutrient availability for plant uptake and elevate the risk of high-intensity fires.

Grazing, as a natural disturbance, aids in reducing dominant plant species cover, fostering the coexistence of a diverse range of plants with different growth strategies (Olf and Ritchie, 1998). Moreover, grazing and prescribed fires play a crucial role in removing excess biomass and moribund material, thereby reducing the risk of catastrophic fires (Belsky, 1992; De Deyn *et al.*, 2008). However, the frequency of applied fires and grazing pressure in these grasslands are linked with shifts in ecological responses. Grasslands protected from fire and subjected to low stocking rate have been shown to exacerbate the impact of fire by increasing the fuel load in grassland systems. Similarly, grazing at a high stocking rate may reduce fuel loads and plant biomass, mitigating the effects of the Nutrient-Poverty/Intense-Fire Theory on defensive compound build-up.

However, the high number of herbivores in heavily stocked systems, relative to available forage, can lead to intensive, non-selective grazing particularly if herbivores are concentrated at high densities, contributing to soil structure degradation and changes in species composition (Chamane *et al.*, 2017). In mesic grasslands, fire enhances forage quality by stimulating nutrient-rich vegetation regrowth and reducing the dominance of coarse, fibrous, moribund plant material (Sargent, 2016). Controlled burns can mimic natural fire regimes, rejuvenating the grassland and preventing the encroachment of woody vegetation (O'Connor, 2005; Sargent, 2016).

Considerable research has recognised fire and grazing as integrated factors, in effective management approaches that combine burning, grazing, and periods of rest (Fuhlendorf *et al.*,

2009; O'Connor *et al.*, 2010; Carbutt and Kirkman, 2022). These integrated approaches promote pyric herbivory as a unified management approach and are now often supported and applied in grassland agroecosystems. The synergistic effects of fire and grazing together can induce substantial shifts in vegetation composition and structure, altering the quality of available forage in grassland and modifying carbon cycling dynamics both in aboveground and belowground biomass (De Deyn *et al.*, 2008).

Herbivore preference for palatable regrowth in frequently burnt grasslands, especially when bordering unburnt grasslands, may influence nutrient cycling processes through excessive livestock deposition, trampling, and compaction (Johnson and Matchett, 2001; Bardgett and Wardle, 2003; Garibaldi *et al.*, 2007). Changes in vegetative composition with frequent fire may interfere with the selective grazing habits and preferences of ungulates, potentially leading to reductions in soil microbial activity linked to grazing-induced depressions in plant carbon inputs to grassland soils (Sankaran and Augustine, 2004). Conversely, complete fire exclusion in a pyro-herbivorous agroecosystem can have opposing effects. Without the influence of fire, certain plant species may become dominant, potentially reducing plant diversity and palatability, thereby changing grazing habits and overall utilization of the grassland habitat by livestock (Johnson and Matchett, 2001; Bardgett and Wardle, 2003). The accumulation of dead plant material over time, in the absence of fire and grazing, poses a greater risk of increased burn intensity and burn temperatures when a fire is eventually reintroduced, either accidentally or intentionally through management interventions (Wilcox *et al.*, 2022).

Ongoing research at Wakefield farm, KwaZulu-Natal, focuses on interaction between fire and grazing management in a mesic grassland agroecosystem. Wakefield Farm currently employs a no-burn approach, with most of the grasslands protected from fire for over 15 years and relying on grazing cattle as the primary source of defoliation. This complete pyric exclusion will be compared to annually burned grasslands on the farm, burned as mandatory fire breaks along paddock borders. These fire breaks are grazed as part of the adjacent unburnt paddocks. This research aims to investigate the changes in mesic grasslands resulting from different fire frequencies, specifically comparing frequently burnt grasslands with fire-protected grasslands subjected to cattle grazing. The study focuses on the following objectives; 1) Assess the impact of fire frequency on SOC stocks and soil respiration, and 2) Evaluate the influence of fire and fire exclusion on livestock grazing habits, biomass availability, and forage palatability considering the confounding factor of higher grazing pressure in burnt areas. We hypothesize

that frequent burning will lead to an increase in SOC stock and soil respiration due to increased microbial activity and nutrient availability from ash deposition. Conversely, fire exclusion will result in reduced SOC accumulation due to reduced decomposition rates. Additionally, we hypothesize that fire frequency will influence livestock grazing habits, with frequently burnt areas providing more palatable forage compared to fire-protected grasslands also subjected to cattle grazing. Consequently, these altered grazing habits will further influence changes in soil carbon and respiration, with concentrated grazing in frequently burnt areas potentially limiting SOC through increased soil disturbance and compaction.

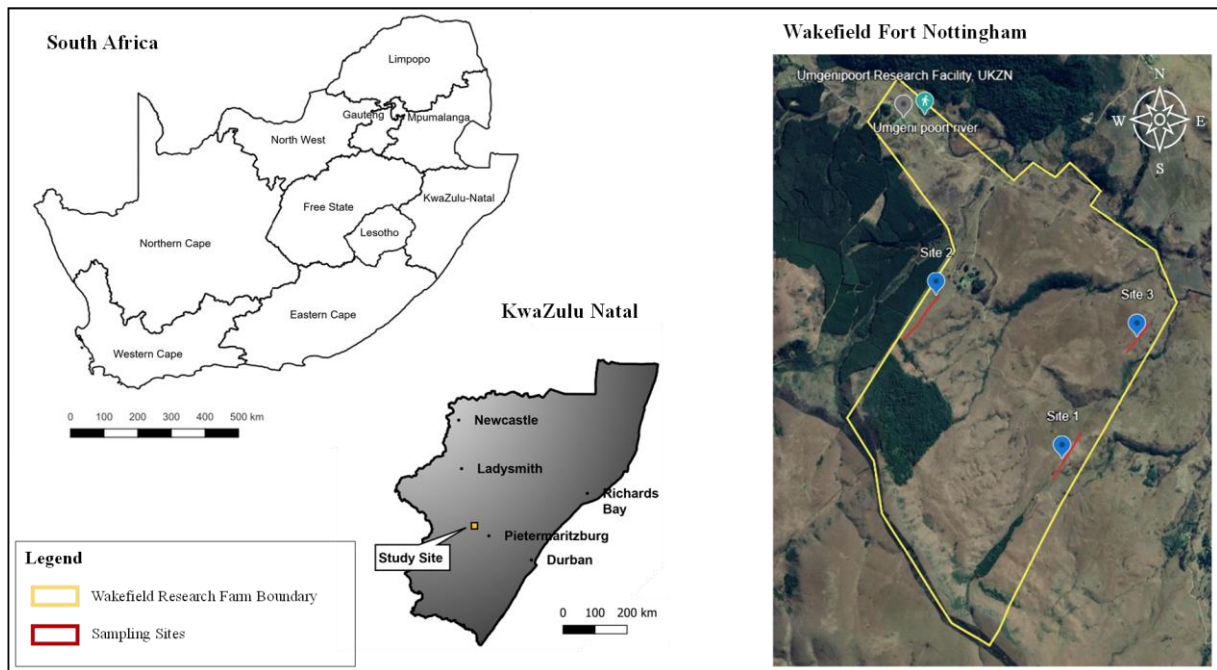
### 4.3 Methods

#### 4.3.1 Site and Transect Selection

The study was conducted at Wakefield farm (29°48' E; 29°89' S), between October 2021 and September 2023. Wakefield Farm is a cattle farm managed by Oppenheimer Generations Research and Conservation and forms part of the University of KwaZulu-Natal Umgeniport Research Facility. The site is located within the Umgeni Municipality, KwaZulu-Natal, South Africa (Figure 4.1), bordering the Umgeni River to the east (approximately 1370 m to 1780 m in altitude). Wakefield is characterised by primarily open mesic grassland dominated by C4 grasses, largely *Alloteropsis semialata*, *Aristida junciformis*, *Tristachya leucothrix*, *Eragrostis curvula* and *Eragrostis plana*. Two vegetation types dominate the Wakefield farm, Mooi River Highland Grassland and Southern Mistbelt forest with mostly shallow and poorly drained soils (Mucina and Rutherford, 2006b). The region experiences a hot and wet climate in summer, with cold and dry winters. Temperatures range from a mean minimum of 5 °C in the winter months to a mean maximum of 28 °C in the summer months. On average, the annual rainfall is approximately 800-1000 mm. The region experiences summer rainfall, with most precipitation occurring between October and March.

The property comprises predominantly grazed grassland over a varied topography, adjacent to the Umgeni River. Grassland management excludes burning except for firebreaks along the farm's boundaries which are burnt annually in June. Indigenous Nguni cattle are grazed as a single herd at a stocking rate of 1.5 ha/ large stock unit (LSU), grazing for short periods at relatively high density, followed by long recovery periods, and supplemented with lucerne and *Eragrostis* bales as well as salt mineral licks in the dry winter season.

Samples were taken along paired transects at three pre-determined sites within the farm (Figure 4.1). Transects were georeferenced and run parallel within the annually burnt firebreaks, and unburned paddocks. Site 1 is located next to Boundary Gate 1 ( $29^{\circ}30'29''\text{S}$ ,  $29^{\circ}54'01''\text{E}$ ), site 2 behind the farmhouse ( $29^{\circ}29'54''\text{S}$ ,  $29^{\circ}54'01''\text{E}$ ), and site 3 is on the plateau along the back boundary ( $29^{\circ}30'11''\text{S}$ ,  $29^{\circ}54'47''\text{E}$ ).



**Figure 4.1:** Location of the Wakefield farm study site, part of the University of KwaZulu-Natal Umgeni poort Research Facility, Fort Nottingham, KwaZulu-Natal South Africa. Locations of the three sample sites are indicated along the farm boundaries, showing the network of firebreaks. Paired biomass and soil samples were collected on both annually burnt and burn exclusion sites in these sites (QGIS and Google Earth).

#### 4.3.2 Soil Carbon Quantification

Soil samples were collected on all three sites at the start of the growing season, October 2021. At each site, 10 paired soil samples (annual burn and burn exclusion) were extracted along a transect using a bucket auger. Each of the 10 soil samples was taken at depth increments of 5 cm to a maximum depth of 15 cm (0 – 5 cm, 5 – 10 cm, 10 – 15 cm). Soil bulk density was calculated for each soil depth using bulk density rings. Soils were then dried at  $65^{\circ}\text{C}$  for 48 Hours, homogenized, and sieved to 0.5 mm. Soil samples were not composited. Fine earth ( $< 0.5$  mm) samples were analysed for total carbon and nitrogen using a Leco TruSpec CN and

Leco TruMac CNS analyser. Organic carbon ( $g\ C\ kg\ soil^{-1}$ ) and nitrogen ( $g\ N\ kg\ soil^{-1}$ ) concentration was estimated by converting the percentage carbon fraction present in the soil to parts per thousand by multiplying by a factor of ten. Soil organic carbon (SOC) stock present in a sample was estimated as representative of each treatment. SOC is expressed as Mg C/hectare (ha) for a nominated stratum (cm) and calculated using methods described by the Food and Agriculture Organization (FAO) (FAO, 2018).

*Equation 4.1:*

$$SOC_i\ stock\ (Mg\ C\ ha^{-1}) = OC^i \times BD^i \times (1 - vG^i) \times t^i \times 0.1$$

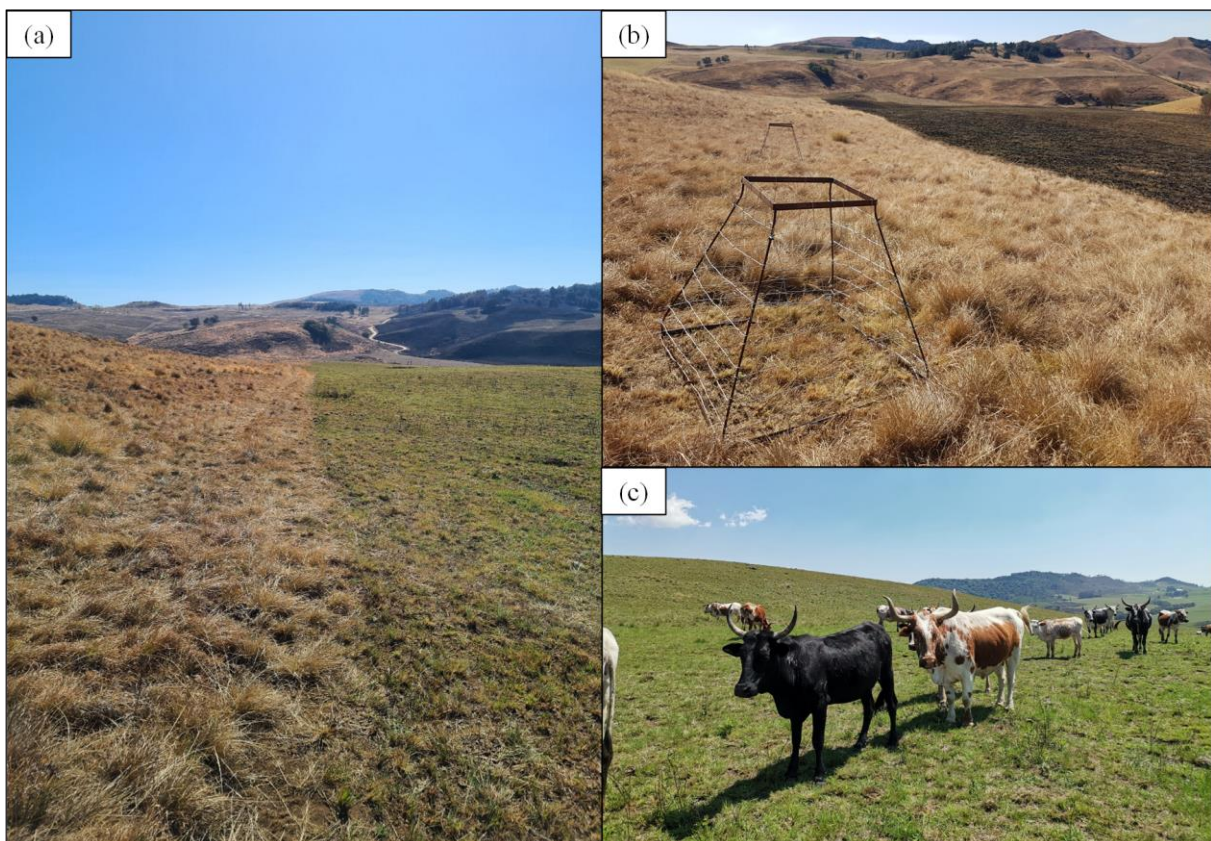
Where  $SOC_i\ stock$  is soil organic carbon stocks ( $Mg\ C\ ha^{-1}$ ) of the fine soil fraction (< 0.5 mm) within depth increments  $i$ ,  $OC^i$  is the organic carbon content ( $g\ C\ kg^{-1}$ ) of the fine soil fraction (< 0.5 mm) in depth increment  $i$ .  $BD^i$  is the bulk density calculated as the mass of the soil per total volume of the soil sample of the depth increment  $i$  ( $g\ soil\ cm^{-3}\ soil$ ).  $vG^i$  is the volumetric coarse fragment content of the depth increment  $i$  ( $g\ coarse\ fragment\ g^i\ soil$ ),  $t$  is the depth (cm) of the sampled site. 0.1 is the conversion factor for converting  $mg\ C\ cm^{-2}$  to  $Mg\ C\ ha^{-1}$ .

### **4.3.3 Aboveground Standing Biomass and Digestibility**

Total aboveground standing biomass (ASB) was measured for each site to establish the quantity of forage available and assess whether grazing was occurring at comparable levels between the burnt and unburnt sites. ASB was calculated using a disk pasture meter (DPM), recording 200 biomass height (cm) points along each transect, on both the annually burnt and burn exclusion grassland sites. Calibration readings were calculated at each site by recording 30 biomass heights on both the annual burn and burn exclusion sites, clipping standing aboveground biomass cover within the DPM ring, oven drying the biomass clippings at 65 °C for 48 hours and a regression was developed to convert readings to DM, estimating ASB in  $kg\ DM\ ha^{-1}$ . ASB was calculated using the DPM and calibration method at the beginning of the growing season (October 2021) and again at the end of the growing period (March 2022), with the difference between the two representing total standing aboveground biomass accumulation following grazing.

Grazing exclusion cages were placed at the sites and were utilized to assess the impact of grazing on biomass accumulation over the growing season, on the burnt and unburnt sites. Grazing exclusion cages were built and six were placed on transects that were burnt annually and six cages placed on those sites were excluded from burning (Figure 4.2). Grazing exclusion cages were built as a 1 m x 1 m base, tapering to 0.5 m x 0.5 m at the top, with a height of 70 cm and enclosed with 10 x 10 cm weld mesh. These were placed directly after annual burning in June or placed on plots mown to crown height in treatments excluded from burning.

To assess the nutritional quality of the available forage between the burnt and unburnt sites, 30 aboveground standing biomass clippings were collected per site at the end of the growing season, March 2022. This included 15 samples from annually burnt grazed areas, and 15 samples from unburnt areas. These samples were analysed for acid detergent lignin (ADL) and acid detergent fibre (ADF) content of the ASB.



**Figure 4.2:** Photos depicting a typical representation of one of the sample sites at Wakefield farm, Fort Nottingham, KwaZulu-Natal. Image a) shows green regrowth along the annually burnt firebreak on the right, and thicker, more moribund grassland stands to the left that has

been excluded from fire entirely. Image b) shows the grazing exclusion cages that were erected along each of the three transects, on both burnt (to the left) and recently burnt grassland to the right. Grazing exclusion cages were used to account for grazing effects at the site and started at point zero, with grazing exclusion cages put up directly following a burn on the annually burnt sites and mowing existing biomass on the unburnt site before placement of the cages. Image c) shows that following prescribed burns, cattle preferentially graze on a small firebreak which is then subjected to abnormally high grazing pressure relative to the larger adjacent unburnt area.

#### **4.3.4 Soil Respiration**

A LICOR 6400 portable flux system was used to monitor the respiration of the paired transect sites in the growing season between the two burn scenarios. Soil CO<sub>2</sub> emissions are measured from three random points per site, with 150 readings per treatment. The measurements were done using a LI-Cor 6400 gas exchange system (LI-Cor, Lincoln, NE, U.S.A) fitted with a soil respiration chamber. This established the variation in the soil CO<sub>2</sub> emissions between grassland treatments excluded from fire and those burnt annually (Abdalla *et al.*, 2021).

#### **4.3.5 Statistical Analysis**

The statistical analysis was performed in R Studio version 4.3.1 (R Core Team, 2023), and focused on soil organic carbon (SOC) stocks, soil total nitrogen (TotN), soil C:N ratio, aboveground standing biomass (ASB) following grazing, grazing exclusion cage biomass, soil respiration, and aboveground biomass digestibility (ADF and ADL).

Two-way ANOVA models were employed to investigate the impact of burning regime and soil depth on the SOC Stock, Soil N and soil C:N ratio. A post hoc analysis using Tukey's method with Bonferroni correction to assess pairwise differences between treatment groups was then used. The normality of residuals was assessed using the Shapiro-Wilk test, a plot of residuals against fitted values was examined for homoscedasticity and a Levene's test was conducted to formally assess the homogeneity of variances.

Aboveground standing biomass accumulation following a period of grazing over the growing season as well as biomass within the grazing exclusion cages was analysed using an independent samples t-test to compare the differences between annually burnt and burn exclusion sites. Assumptions of normality were assessed using the Shapiro-Wilk test and homogeneity of variances was examined using Levene's test.

Soil respiration data for annually burnt sites and burn exclusion sites did not meet the assumption of normality following transformation, so a nonparametric Wilcoxon rank sum test with continuity correction was used.

The nutritional quality and digestibility of aboveground biomass were assessed in both annually burnt and unburnt mesic grassland, using Acid Detergent Lignin (ADL) and Acid Detergent Fibre (ADF) percentages as key indicators. The analysis of ADL involved an independent two-sample t-test, with normality and equality of variances confirmed through the Shapiro-Wilk and Levene's Test, respectively. For ADF data, despite log transformation, normality assumptions were not met. A nonparametric analysis was conducted using the Wilcoxon rank sum test with continuity correction.

To summarise the results and the relationships within the data, principal component analysis (PCA) was performed using the *FactoMineR* and *factoextra* R packages. The analysis focused on the variables, ungrazed biomass, ADF, ADL, TotN, ratio, SOC, carbon flux, and ASB and the resulting principal components were examined for their contribution to the overall variance in the data.

## 4.4 Results

### 4.4.1 Soil Carbon Status

Differences were observed in soil C:N ratios for the main effect of soil depth (0-5 cm = 15:1, 5-10 cm = 15.5:1, 10-15 cm = 16:1) ( $F_{2,171} = 16.45$ ,  $p < 0.01$ , Table 4.1), as well as differences in sites burnt annually = 15:1, and those left unburnt = 16:1 ( $F_{1, 171} = 8.33$ ,  $p < 0.01$ ). There were no significant interactions between the main effects (), as well as differences in sites burnt annually = 15:1, and those left unburnt = 16:1 ( $F_{1, 171} = 8.33$ ,  $p < 0.01$ ). There were no significant interactions between the main effects (Table 4.1).

Soil organic carbon (SOC) stocks and soil N showed no significant effect of a burning regime or the interactions. However, soil depth affected SOC stocks and soil N ( $F_{2,171} = 43.7$ ,  $p < 0.01$  and  $F_{2,171} = 50.31$ ,  $P < 0.001$ , respectively) (Figure 4.3). SOC demonstrated a decline in the upper 5 cm before stabilizing, while soil N content exhibited a decreasing trend with increasing soil depth, reaching its lowest point at the maximum sampling depth of 15 cm. Within the specified increments, SOC ranged between  $22.2 \pm 1.48$  and  $32.1 \pm 2.34$  Mg C ha<sup>-1</sup>

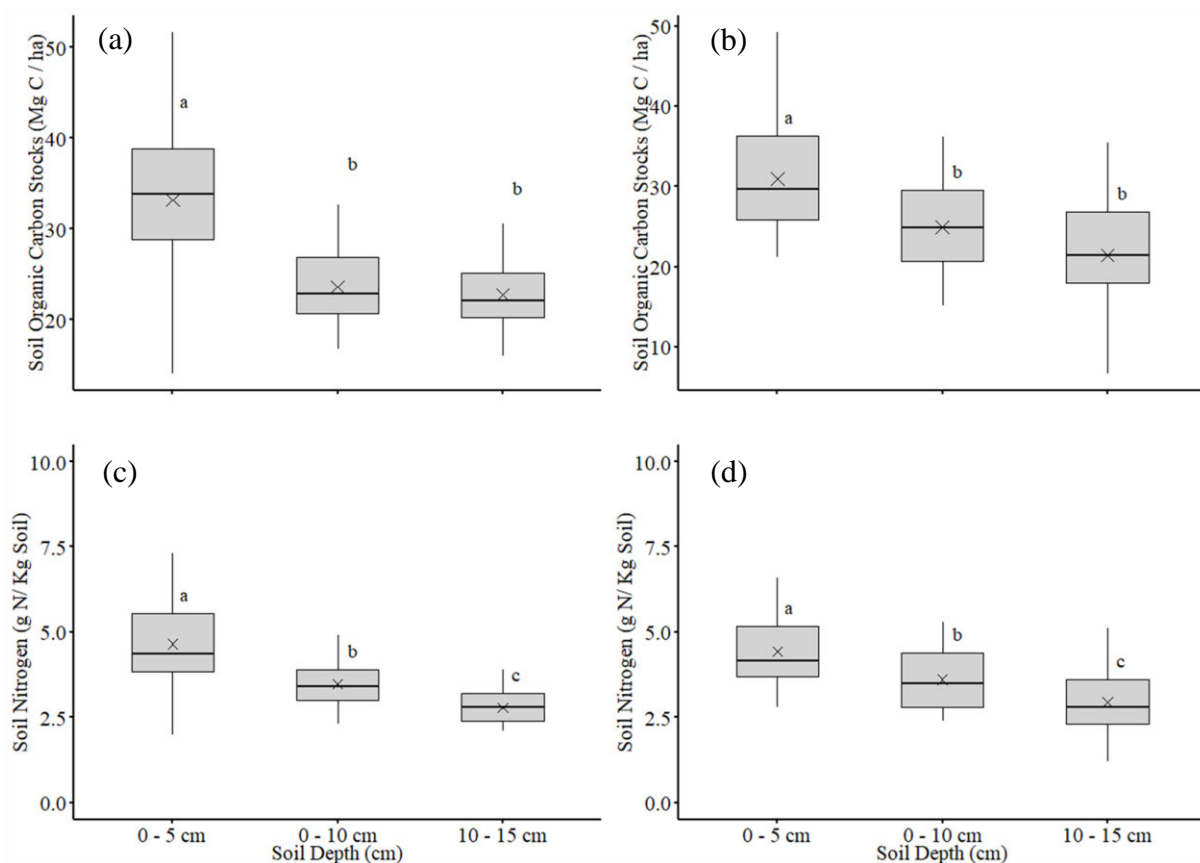
within the soil depth. Similarly, soil N content ranged between  $2.8 \pm 0.21$  and  $4.5 \pm 0.36$  g N/kg within the soil depth (Figure 4.3).

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**Table 4.1:** ANOVA table for soil organic carbon stocks (Mg C ha<sup>-1</sup>), soil nitrogen (g N Kg soil<sup>-1</sup>) and for soil carbon-nitrogen ratios at Wakefield farm, showing significant variation in C:N ratio main effects, but not interactions of these main effects. No variation in SOC stocks was found between the two burn scenarios.

	df	SOC Stock		Soil Nitrogen		C:N ratio	
		F-value	p	F-value	p	F-value	p
Burning (B)	1	0.74	0.39	0.009	0.92	8.3	<b>0.004**</b>
Depth (D)	2	46.01	<b>&lt;0.001***</b>	50.31	<b>&lt;0.001***</b>	16.8	<b>&lt;0.001***</b>
B * D	2	1.42	0.25	0.76	0.47	2.712	0.07
Residuals	171						
Total	176						

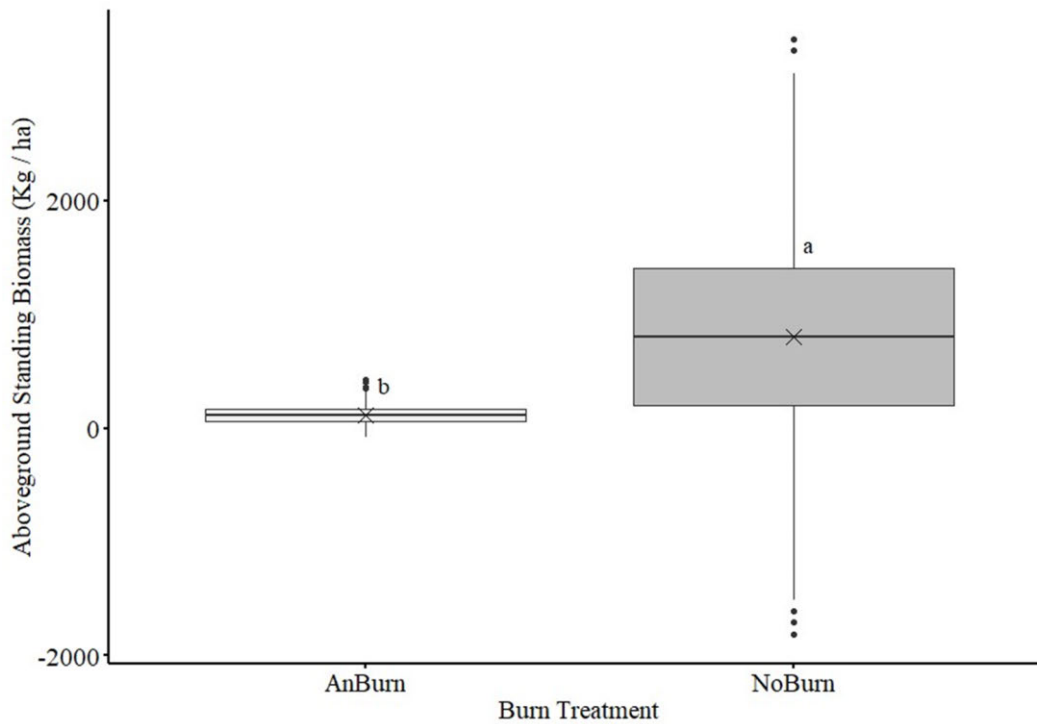


**Figure 4.3:** Soil organic carbon stocks ( $\text{Mg C ha}^{-1}$ ) under varying management of fire protected (a) grassland, and annually burnt (b) grassland, as well as soil nitrogen ( $\text{g N kg}^{-1}$ ) in fire-protected (c) grassland and grasslands burnt annually (d) status at Wakefield farm in Fort Nottingham, KwaZulu-Natal. The figure illustrates significant decreases in soil organic carbon within deeper soil horizons, up to a maximum depth of 15 cm. Letters in common are not significant.

#### 4.4.2 Aboveground Standing Biomass Status Between the Growing Seasons

Grazed annually burnt and unburnt sites exhibited significant variations in aboveground standing biomass (ASB) following a grazing season ( $t_{607.4} = -17.6$ ,  $p < 0.001$ ) (Figure 4.4). Grassland sites subjected to annual burning demonstrated reduced post-grazing biomass, likely due to preferential grazing over the growing season ( $114.1 \pm 3.28 \text{ kg/ha}$ ) compared to sites left unburnt ( $802.9 \pm 38.99 \text{ kg/ha}$ ).

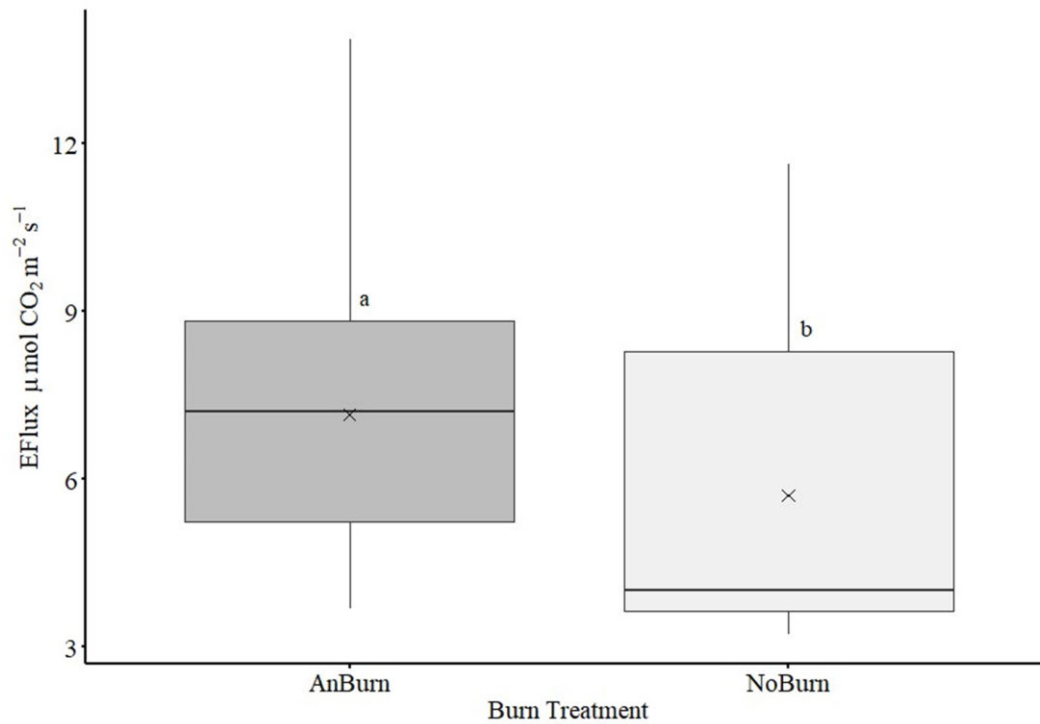
Grazing exclusion cages placed in the sites were utilized to assess the impact of grazing on biomass accumulation. Sites excluded from grazing showed no significant differences ( $t_{10}=0.71$ ,  $p = 0.5$ ) in biomass between burnt annually sites ( $6480 \pm 409.8 \text{ kg/ ha}$ ) and those left unburnt ( $5626 \pm 658.2 \text{ kg/ha}$ ) (Supplementary Figure 4.1).



**Figure 4.4:** Aboveground standing biomass (ASB) accumulation following a season of grazing (Kg/ha) for transects burnt annually (AnBurn) and transects excluded from burns (NoBurn). Sites located at Wakefield farm, Fort Nottingham, KwaZulu-Natal.

#### 4.4.3 Soil Respiration

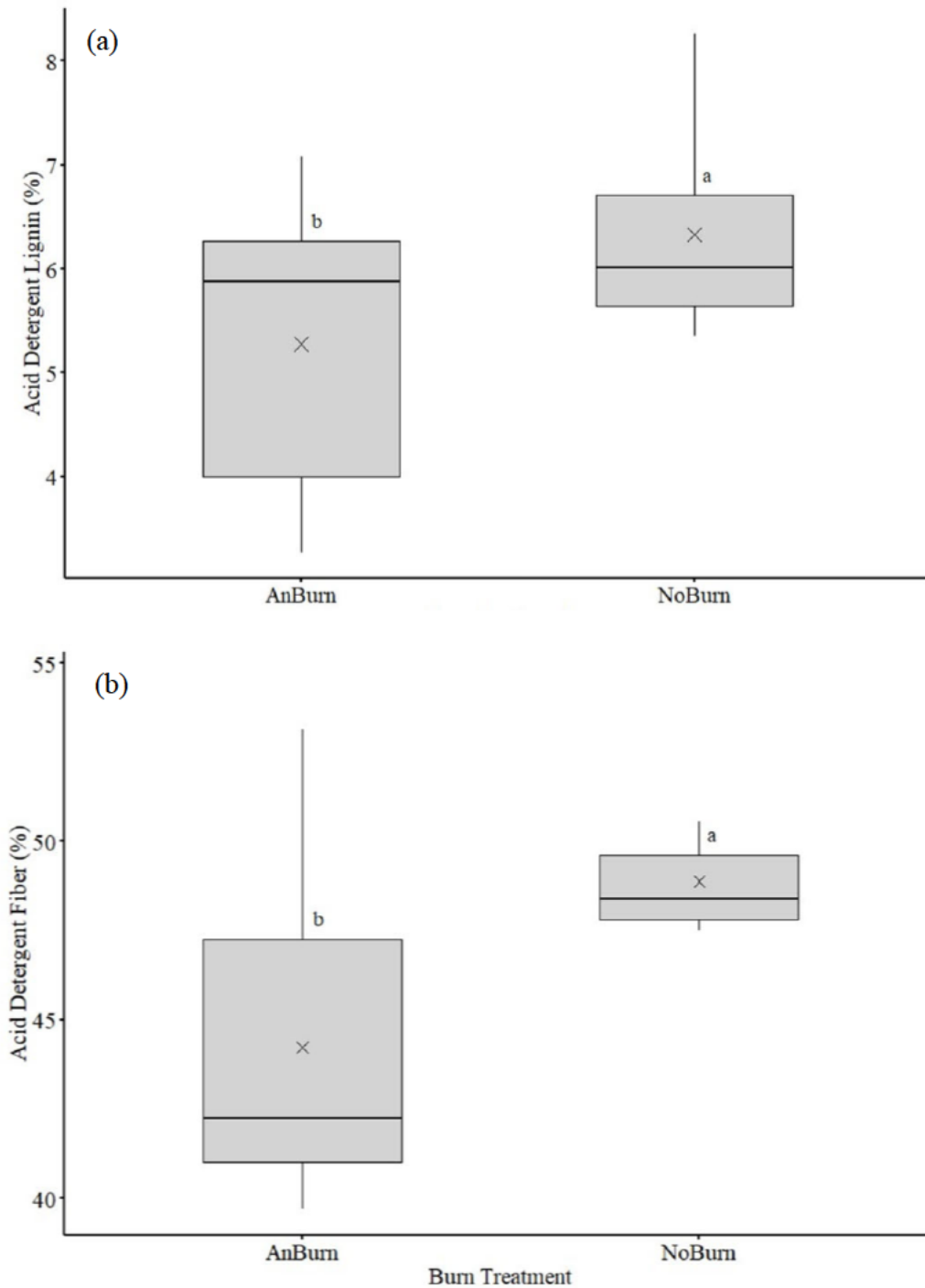
Soil respiration exhibited a significant increase of 13 % in sites subjected to annual burning ( $7.24 \pm 0.24 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), compared to sites left unburnt ( $5.54 \pm 0.27 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (Figure 4.5) ( $W = 25444$ ,  $P < 0.001$ ).



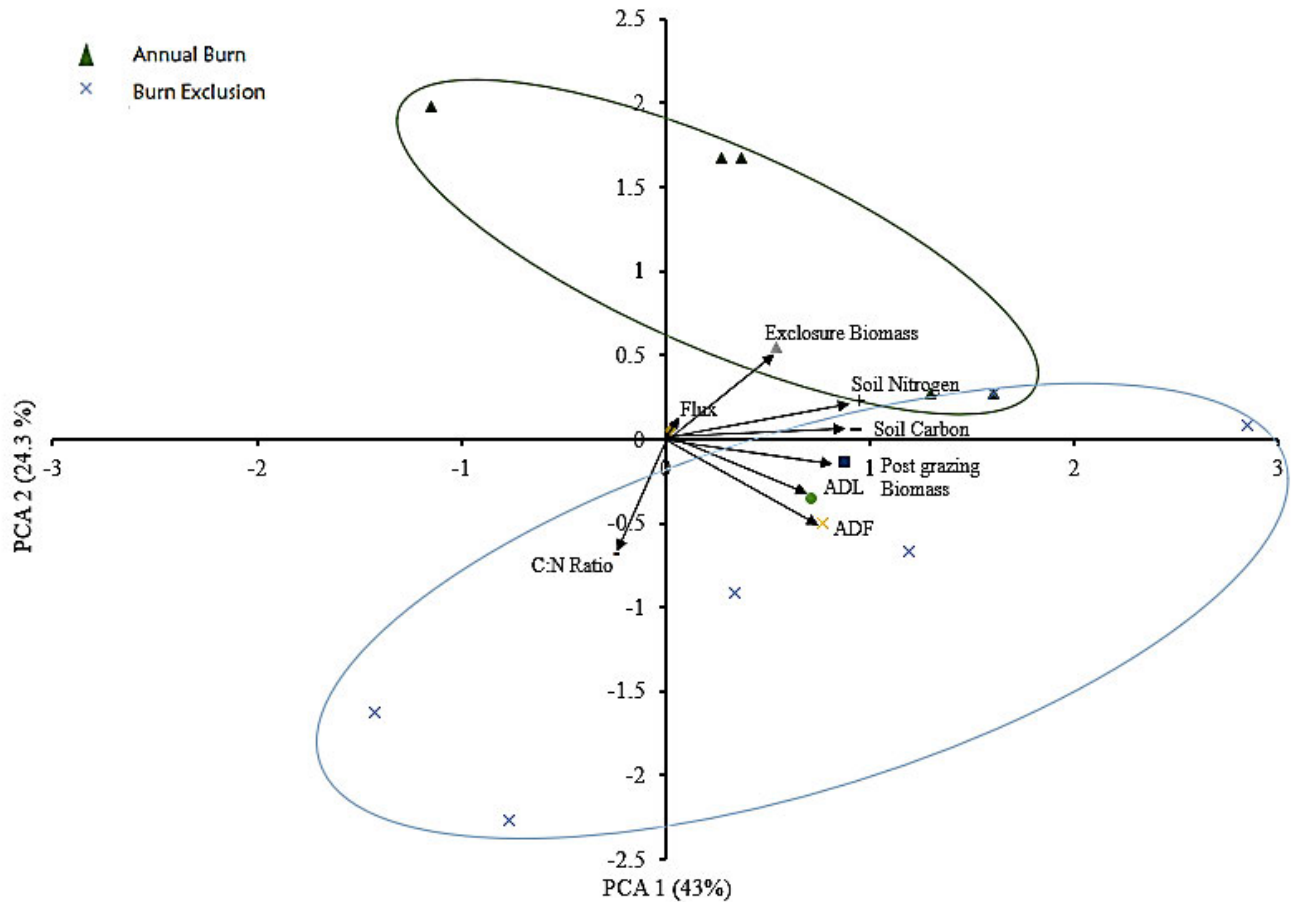
**Figure 4.5:** Differences in soil respiration rates ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) in sites burnt annually (AnBurn) and those left unburnt (NoBurn). Sites located at Wakefield farm, Fort Nottingham, KwaZulu-Natal.

#### 4.4.4 Grassland Digestibility

Grasslands subjected to annual burning exhibited significantly reduced proportions of aboveground biomass ADL ( $5.28 \pm 0.27 \%$ ), compared to grasslands left unburnt ( $6.33 \pm 0.35 \%$ ; Figure 4.6) ( $t_{248.7} = -2.47$ ,  $P = 0.02$ ). Additionally, grasslands burnt annually demonstrated significantly less levels of aboveground biomass ADF ( $44.23 \pm 1.12 \%$ ), in contrast to grasslands left unburnt ( $48.88 \pm 1.03 \%$ ; Figure 4.6) ( $t_{23.8} = -3.6$ ,  $P < 0.001$ ).



**Figure 4.6:** Boxplots illustrating Grassland digestibility assessed through a) Acid Detergent Lignin percentage (ADL%) and b) Acid Detergent Fiber percentage (ADF%) These patterns were observed within different groups; annually burnt and unburnt sites, at Wakefield farm in Fort Nottingham, KwaZulu-Natal.



**Figure 4.7:** Individual and treatment variables factor map (PCA) between grasslands excluded completely from fire, and grasslands burnt annually, at Wakefield farm, Fort Nottingham, KwaZulu-Natal South Africa. The first two axes explained 67.3% of the variation among variables. Variables indicated as Acid detergent Fibre (ADF), acid detergent lignin (ADL), Accumulated standing biomass after a season of grazing by livestock (Accumulated Biomass), soil organic carbon (SOC), soil nitrogen, grazing exclosure biomass is standing biomass excluded from grazing by livestock over the growing season, soil respiration as represented as carbon Flux (Flux).

#### 4.4.5 *Principal Component Analysis Between Variables*

PC1 captures the majority of the variance in the data. Soil N, soil carbon and post-grazing biomass were strongly correlated with axis 1, describing the majority of the variability between sites (Figure 4.7). In PCA 1, positive values for variables such as ADF and post-grazing biomass are associated with grasslands burnt annually, while negative values for C:N ratio are associated more with grasslands excluded from fire. In PCA 2, positive values correspond to higher soil N

content and enclosure biomass, and negative values indicate elevated levels of soil respiration (flux) and accumulated biomass post-grazing.

## 4.5 Discussion

This study examined the dynamics of frequent burning and fire exclusion on a South African mesic grassland. The two treatments comprised of grasslands that were completely excluded from the application of fire for 15 years and annually burnt fire breaks bordering the farm. The annually burnt fire breaks formed part of the unburnt paddocks, which were grazed at a stocking rate of 1.5 ha/LSU. The fire breaks resulted in preferential non-selective grazing on burnt grassland sites and selective grazing on unburnt grassland sites, despite the relatively high stocking densities during grazing events. This research observed the interactions between fire management and grazing, focusing on how contrasts in fire management strategies may influence grazing behaviours, soil carbon dynamics, biomass palatability and accumulation as well as sources of carbon through soil respiration.

### 4.5.1 Soil Organic Carbon Sinks

Frequent fire and fire exclusion, as well as high-intensity and low-intensity grazing, can have significant implications for the potential of grasslands to act as terrestrial carbon sinks (Chamane *et al.*, 2017). This study aimed to detect differences in carbon accumulation between the two fire treatments. While there were no significant differences in SOC stocks and N between grasslands burnt annually and those left unburnt. However there were significant differences observed in soil SOC stocks and soil N based on soil depth (0-5 cm, 5-10 cm, 10-15 cm), with quantities decreasing with increasing depth. This distribution in the soil profile is well documented and expected (Franzluebbbers *et al.*, 2000). The observed narrower soil C:N ratios in annually burnt grasslands (15:1) compared to unburnt grasslands (16:1), which also narrowed with increasing depth, suggesting that fire frequency influences SOC and TotN cycling. This indicates that fire management practices may play a significant role in shaping soil nutrient dynamics and overall soil health in these grazed grassland systems. In this context, the C:N ratio represents the relative availability of carbon to nitrogen in the soil, with a narrower ratio indicating greater nitrogen availability, potentially influenced by factors such as increased organic deposition from livestock and a return of greater N material to the soil (Fynn *et al.*, 2003; Manson *et al.*, 2007).

Grass-dominated biomes are generally characterized by a greater proportion of carbon relative to nitrogen, influencing decomposition processes within these ecosystems (Neary *et al.*, 1999; Aerts *et al.*, 2003). Over time, organic matter undergoes partial decomposition, resulting in a greater proportion of carbon compared to nitrogen, as reflected in widening C:N ratios with increasing soil depth. The wide C:N ratio of litter from native grassland, coupled with decreasing microbial biomass activity with depth (Schuman *et al.*, 1999), leads to greater nitrogen immobilization, indicating less net mineralization near the soil surface (Schuman *et al.*, 1999). The frequency of burning can also impact the composition and activity of plant and microbial communities, influencing carbon and nitrogen inputs and turnover rates in the soil (Raison *et al.*, 1986; Fynn *et al.*, 2003; Manson *et al.*, 2007). Widening C:N ratios with reduced burning frequency, suggest that grassland productivity becomes nitrogen-limited in these grazed treatments.

SOC stocks did not differ between annually burnt and unburnt grassland. However, significant variation was observed based on soil depth. SOC stocks decreased with increasing soil depth. Although frequent burning may lead to carbon losses through combustion, the redistribution of organic matter within the soil horizons may compensate for these losses (Sitaula *et al.*, 2004). The movement of organic residues from the burned surface to deeper soil horizons, through processes like incorporation by fauna or physical translocation, can help maintain overall SOC stocks (Lorenz and Lal, 2005; De Deyn *et al.*, 2008).

The absence of significant differences in SOC stocks does not imply that there are no impacts of frequent burning on soil carbon dynamics. The narrow C:N ratio observed in frequently burnt grasslands suggests alterations in C and N cycling, even if it does not manifest as significant changes in overall SOC stocks. In this context, the main source of C addition in annually burnt sites is likely root turnover, while leaf litter plays more of a role in the unburnt sites (Johnson and Matchett, 2001; Richards *et al.*, 2011).

#### **4.5.2 Soil Respiration**

Soil respiration is a vital component of the carbon cycle, but high soil respiration rates without corresponding carbon input can lead to carbon loss and should be managed carefully to maintain soil health and carbon sequestration potential (Wen *et al.*, 2018). Soil respiration at Wakefield Farm exhibited a significant increase of 13% in sites subjected to annual burning (7.24  $\mu\text{mol}$

CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) compared to sites left unburnt (5.54 μmol CO<sub>2</sub> m<sup>-1</sup> s<sup>-1</sup>). Soil respiration is largely driven by greater microbial activity and elevated soil respiration is often detected in frequently defoliated grasslands (Millard and Singh, 2010). Microorganisms play a crucial role in decomposing organic matter, and grasslands burnt frequently may increase its root turnover which subsequently impacts microbial activity and soil respiration rates (Six *et al.*, 2004; Frouz, 2018). This suggests that microbial activity may be notably heightened in grassland sites subjected to frequent burning, primarily attributed to a greater turnover of roots (Six *et al.*, 2004; Frouz, 2018).

This phenomenon signifies more rapid carbon cycling within this ecosystem and that for carbon release during the respiration process, there must be a corresponding uptake of carbon (De Deyn *et al.*, 2008; Millard and Singh, 2010). However, soil respiration is the primary pathway through which CO<sub>2</sub> is released from the soil to the atmosphere within the carbon cycle and it may have negative consequences for carbon sequestration if carbon inputs do not correspond to or surpass carbon lost through respiration (Davidson, 2009; Smith *et al.*, 2015). Combustion of aboveground biomass may further lead to the deposition of charred organic material, ash, and partially burned plant residues, which can serve as a source of easily decomposable carbon for soil microorganisms (Bai *et al.*, 2013). This increased availability of labile carbon substrates can enhance microbial activity and subsequent respiration rates (Bai *et al.*, 2013; Smith *et al.*, 2015). However, our study's once-off measurements may limit the interpretation due to the influence of temperature and moisture variability over time and space.

#### ***4.5.3 Preferential Grazing and Biomass Variation***

Grassland sites along the firebreak demonstrated reduced aboveground standing biomass (ASB) accumulation following grazing, over the growing season (114 kg/ha) compared to sites left unburnt (802 kg/ha). These extremes in fire management led to differences in herbivory patterns, as grazers exhibited pronounced preferences for burnt grassland sites while selectively grazing unburnt grassland sites (Cummings *et al.*, 2007). There were no significant differences in annually burnt and unburnt sites where grazing exclusion cages were used, representing greater grazing intensity and area selectivity on the burnt sites. In the paddocks used for the study, cattle preferentially graze on the proportionally small firebreaks which are then subjected to greater grazing pressure relative to the larger adjacent unburnt area. This heightened grazing pressure can be attributed to the preference of cattle for the relatively greater quality grasses

regenerating within these burnt zones, compared to grasses that have been excluded from fire (Franzese and Ghermandi, 2012). Grazing exclusion cages placed at the sites were utilized to assess the impact of grazing on biomass accumulation over the growing season. With the exclusion of grazing, there were no significant differences in biomass between grassland treatments burnt annually and those left unburnt. Despite statistical non-significance at the 5% level, firebreaks consistently showed a trend of higher ANPP than the adjacent unburnt grassland, suggesting that fire may play a role in enhancing grass growth.

#### **4.5.4 Grassland Digestibility**

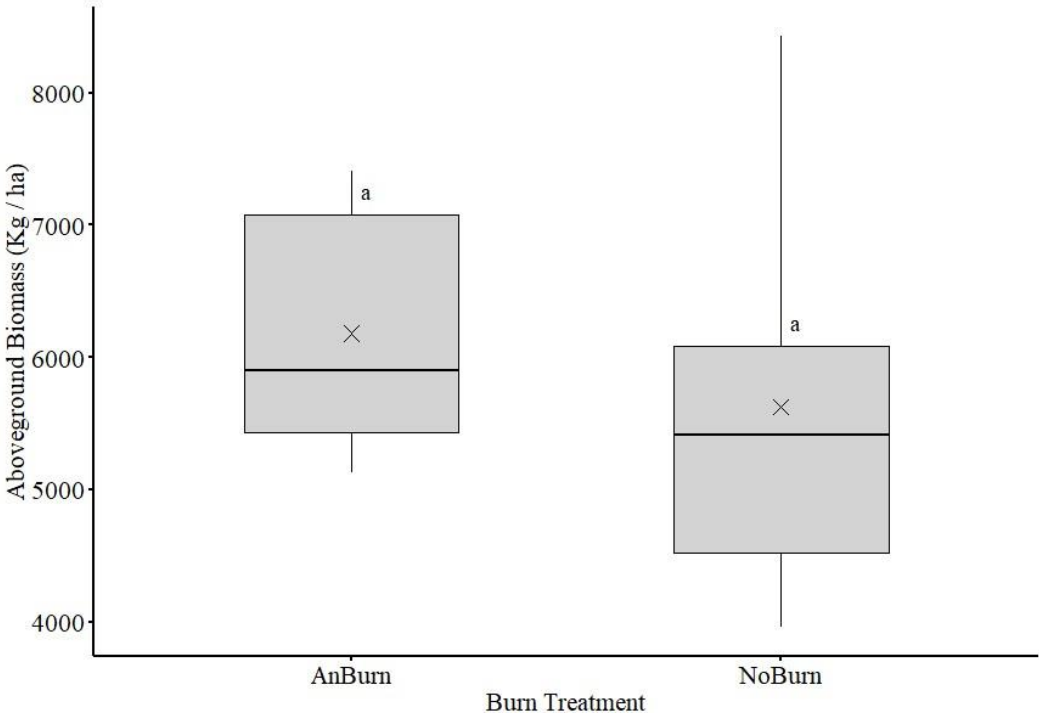
Frequently burnt firebreaks exhibited significantly reduced proportions of lignin in the aboveground biomass (5.28%), compared to grasslands protected from fire (6.33%). Grazing and fire defoliation can stimulate regrowth and increase the water content in the aboveground biomass due to the growth of younger, more succulent plant tissues (Orr *et al.*, 2023). Reduced lignin and fibre percentages in frequently burnt sites result in improved forage quality, increased palatability, and increased intake for livestock (Coleman *et al.*, 2016; Orr *et al.*, 2023). However, given the alternative of grasslands left unburnt, maintaining higher quality, livestock are more likely to select these frequently burnt firebreaks, resulting in heavily non-selective grazing and selectively grazed unburnt paddocks.

#### **4.6 Conclusions**

The interaction between herbivory and fire can have complex effects on soil carbon dynamics and are considered important drivers of ecosystem processes within Southern African Mesic Grasslands (Venter, Hawkins, *et al.*, 2017). The influence of fire dynamics in a grazed system can be driven by changes in herbivore foraging site selection. Here we compared annual fires on smaller firebreaks bordering fire-protected paddocks. We observed an increased soil respiration rate in annually burnt grasslands, suggesting heightened microbial activity in grassland sites experiencing frequent burning. This increase could be primarily attributed to an augmented turnover of roots, signifying a more rapid carbon cycling within this ecosystem, and contributing to increased carbon release during the respiration process. We also observed annual burning reducing ADL and ADF % compared to unburnt areas. This reduction would enhance forage quality, palatability, and livestock intake. Thus, when given the option between the two scenarios, livestock tended to preferentially graze the burnt firebreaks, leading to

heavily nonselective grazing. Considering the observed rapid cycling in annually burnt grassland, and that these firebreaks enhanced overall grassland productivity; frequent fire as a management tool should be recommended in mesic grasslands.

**4.7 Supplementary Figure**



**Supplementary Figure 4.1:** No variation was observed in aboveground biomass excluded from grazing, using the livestock grazing cages in annually burnt and unburnt grazed grasslands. Sites located along three paired transects on Wakefield farm, Fort Nottingham. Grazing exclusion cages were utilized to account for the grazing factor on aboveground biomass within the treatments.

## 5 BIENNIAL BLAZE: FIRE MANAGED MESIC GRASSLANDS SEQUESTER CARBON

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### 5.1 Abstract

Mesic grasslands have the potential to sequester significant amounts of carbon, and the mechanisms around how carbon is gained and lost in these fire-dependant systems remain highly debated. Global studies have consistently identified grasslands as potential carbon sinks, absorbing carbon at rates surpassing CO<sub>2</sub> emissions, however, grassland productivity and turnover of carbon is dependent on a combination of environmental factors. Following rainfall events, an increase in soil water content has been linked to a rapid increase in soil respiration, aligning with heightened biological and photosynthetic activities during warmer growing seasons. These processes determine the rate and variability of grassland uptake and release of CO<sub>2</sub>. Further, projections of future elevated temperatures and extremes in climatic conditions associated with climate change and climatic slow variables, such as Subtropical Indian Ocean Dipole (SIOD) and El Niño Southern Oscillation cycles, may change the potential of grassy ecosystems as carbon sinks or sources. In this study, we analysed four years of CO<sub>2</sub> flux data from research Catchment Six, Cathedral Peak, South Africa, and observed the variation in source vs sink of CO<sub>2</sub> in these mesic montane grasslands over the measured time period. The outcomes solidify the evidence supporting the mesic grassland ecosystem's role as a carbon sink, absorbing more carbon than it emits. Results showed pronounced variability in the uptake and release of carbon over the warmer growing season, but overall net carbon gains when considering the balance of carbon in these systems. This study supports management practices utilising biennial fire, which appears to maintain persistent carbon sinks at this research site, enhancing the resilience and capacity of these ecosystems to act as effective carbon sinks.

**Keywords:** *Carbon Sequestration, Climate Change, Seasonal Fire Frequency, El Niño, Soil Moisture*

## 5.2 Introduction

Fire, an integral component of global grassland ecosystems, is increasingly influenced directly and indirectly by anthropogenic activities. The influence of fire regimes on biodiversity and biogeochemistry remains a subject of heated debate, particularly in the context of recurrent fires as crucial disturbances in the grassland biome and how these disturbances uptake and release carbon (Archibald *et al.*, 2005, 2012; Bond *et al.*, 2005; Slette *et al.*, 2021). Understanding the dynamics of CO<sub>2</sub> flux in mesic grasslands is pivotal for understanding the broader context of carbon management, and climate change mitigation. Global studies have consistently identified mesic grasslands as carbon sinks, absorbing carbon at rates surpassing CO<sub>2</sub> emissions (Archibald *et al.*, 2009; Knapp *et al.*, 2002; Lorenz and Lal, 2005; Milne *et al.*, 2016; Zhang *et al.*, 2011). While mesic grasslands have emerged as significant sources of carbon sequestration, the quantum of net gain or net loss of carbon in these fire-managed systems remain questioned (Scurlock and Hall, 1998; Ciais *et al.*, 2011; Wang *et al.*, 2019).

Previous investigations within temperate grasslands observed that the majority of annual soil CO<sub>2</sub> flux occurs during the growing season (Archibald, Kirton, *et al.*, 2009). In this context, flux refers to the movement or exchange of CO<sub>2</sub> between the soil and the atmosphere. This is closely associated with increased precipitation, soil moisture, and temperatures, specifically air temperature, photosynthetic radiation and latent heat exchange (Bremer *et al.*, 1998; Knapp *et al.*, 1998; Harper *et al.*, 2005; Slette *et al.*, 2021). Moreover, fire as a means of defoliation has been identified as a factor influencing soil CO<sub>2</sub> flux in temperate grasslands, with the influence of fire and seasonal differences in climate amplifying flux rates compared to unburned grassland (Knapp *et al.*, 1998; Johnson and Matchett, 2001; Slette *et al.*, 2021). These fluctuations in carbon can be attributed to the interchange of plant growth cycles, precipitation, and temperature within seasons, affecting photosynthesis and decomposition rates (Archibald *et al.*, 2009). Considering this, previous research has emphasised the significance of directly quantifying multiple ecosystem processes, rather than assuming uniform responses of different fluxes to disturbances (Slette *et al.*, 2021).

Plant productivity is predominantly controlled by temperature, effective moisture (a combination of soil moisture and potential evapotranspiration), and nutrient availability (Bachelet *et al.*, 2004). However, in semi-arid systems, soil water content (SWC) is often limited and is, therefore, a key determinant of grassland ecosystem processes (Archibald *et al.*,

2009). Following rainfall events, an increase in SWC has been linked to a rapid increase in soil respiration, aligning with heightened biological and photosynthetic activities during warmer growing seasons (Briggs and Knapp, 1995; Bond *et al.*, 2003; Slette *et al.*, 2021). We would expect soil moisture to initially be greater at the start of the growing season (spring) in mesic climates (Everson and Everson, 2016). However, we would also anticipate greater variation in soil water content during warmer months, and it should be noted that grasslands have been seen to exhibit moisture retention capabilities, even in dormant low rainfall periods, acting as reservoirs between growing seasons (Archibald *et al.*, 2009a; Scurlock and Hall, 1998).

Previous studies in grassland show that the majority of annual soil CO<sub>2</sub> flux occurs during the growing season. Notably, soil CO<sub>2</sub> flux tends to escalate with increased precipitation, soil moisture, and warmer temperatures during this period (Bremer *et al.*, 1998; Knapp *et al.*, 1998; Harper *et al.*, 2005; Slette *et al.*, 2021). Models predict that anthropogenic climate change will bring elevated temperatures and extremes in climatic conditions (Albritton, 2001; Knapp *et al.*, 2002). Studies have emphasized the impact of these changing conditions on the moisture balance and temperature of these grassland systems and how this may influence the efficiency of the ecosystem's ability to assimilate carbon (Migliavacca *et al.*, 2021). Predictions in climate change, along with the further influence of climatic slow variables such as El Niño Southern Oscillation cycles and the Subtropical Indian Ocean Dipole (SIOD), (Holmgren *et al.*, 2001; Philippon *et al.*, 2012; Jo *et al.*, 2022), affect the variability of climatic factors such as rainfall, and may lower SWC due to higher evapotranspiration (Nakicenovic and Swart, 2000). These factors, along with Predicted temperature increases of 2.5°C by 2050 highlight the need for climate change mitigation and carbon management. Evaluating the potential of grassy ecosystems as carbon sinks or sources under varying conditions is therefore important. With these projected climatic changes, understanding how soil moisture, temperature, and fire shape carbon dynamics in mesic grassland ecosystems is focal for predicting their future as carbon sinks (Everson and Everson, 2016).

To look at these interactions, we examined data from the Cathedral Peak research Catchment Six, a high altitude biennially burnt grassland, over four years. Based on literature, it was predicted that the effects of frequent spring fires on C storage in montane mesic grasslands would present as follows: i) inter-annual variation in CO<sub>2</sub> flux over four years would present as an overall sink of C rather than a source, ii) CO<sub>2</sub> flux between seasons and months would be more prominent during the active growing periods, attributed partly to soil moisture

content, and iii) environmental conditions influencing terrestrial heat exchange, act as primary drivers for predicting carbon source vs sink in these grassy biomes. This paper aims to contribute toward perceptions of South African mesic grasslands, the application of frequent fire and their function as a carbon sink or source. We hypothesize that frequent spring fires will enhance carbon sequestration during active growing periods and that environmental conditions will significantly influence carbon dynamics in these grasslands.

## 5.3 Methods

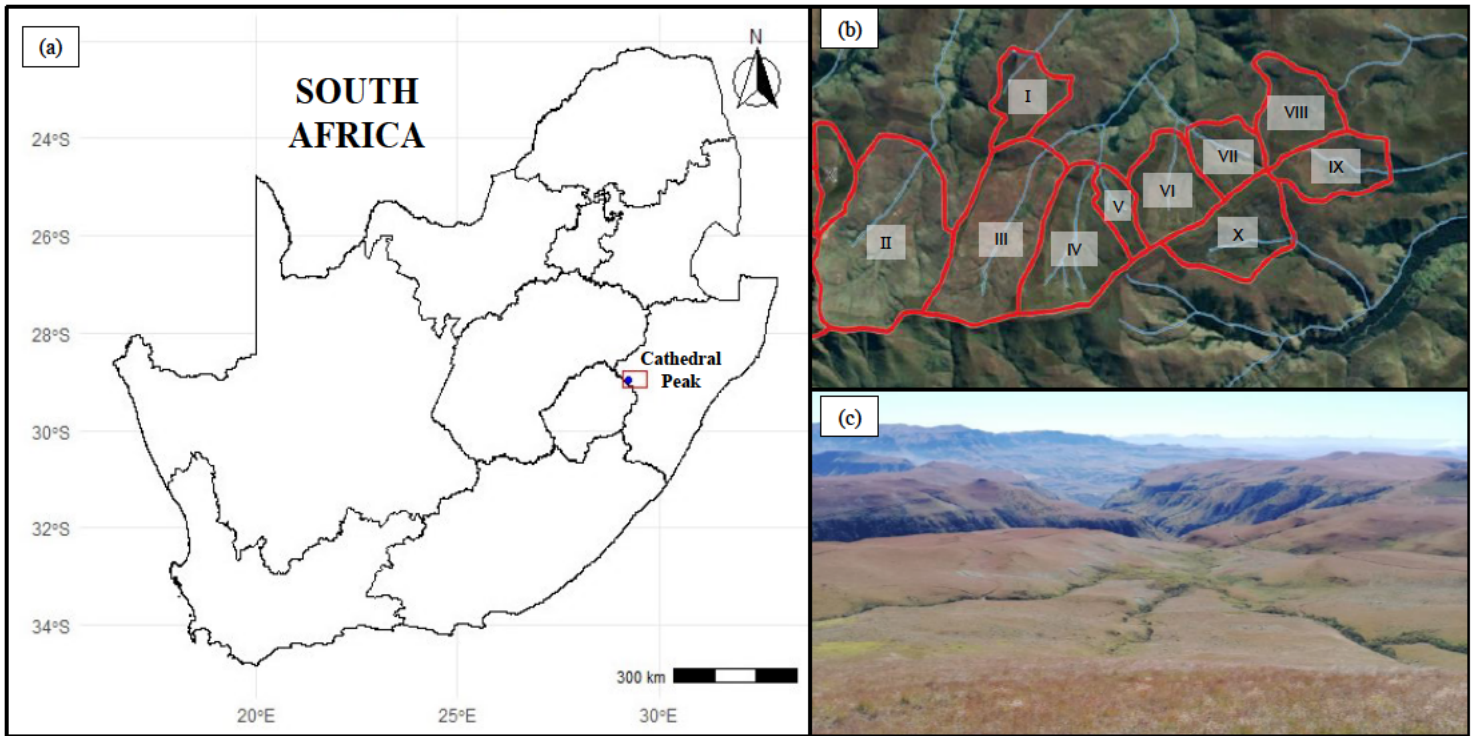
### 5.3.1 Study Site and Data Collection

The South African Environmental Observation Network (SAEON) runs an Eddy Covariance (EC) tower, established in 2016 in Cathedral Peak Catchment Six, forming part of the Long-Term Ecological Research (LTER) network. The Cathedral Peak research catchments are made up of fifteen sites (named I – XV) within the uKhahlamba-Drakensberg Park (29°00'S, 29°15'E). Catchment Six reflects broader characteristics in the study area and serves as a major contributor to streamflow (Toucher *et al.*, 2020). Unlike most catchments at Cathedral Peak, which were historically afforested between 1951 and 1963, Catchment Six has remained a grassland, burned every second spring (Nanni, 1970). Establishing a long-term monitoring site here would ensure continuity and consistency in data collection at this location (Figure 5.1).

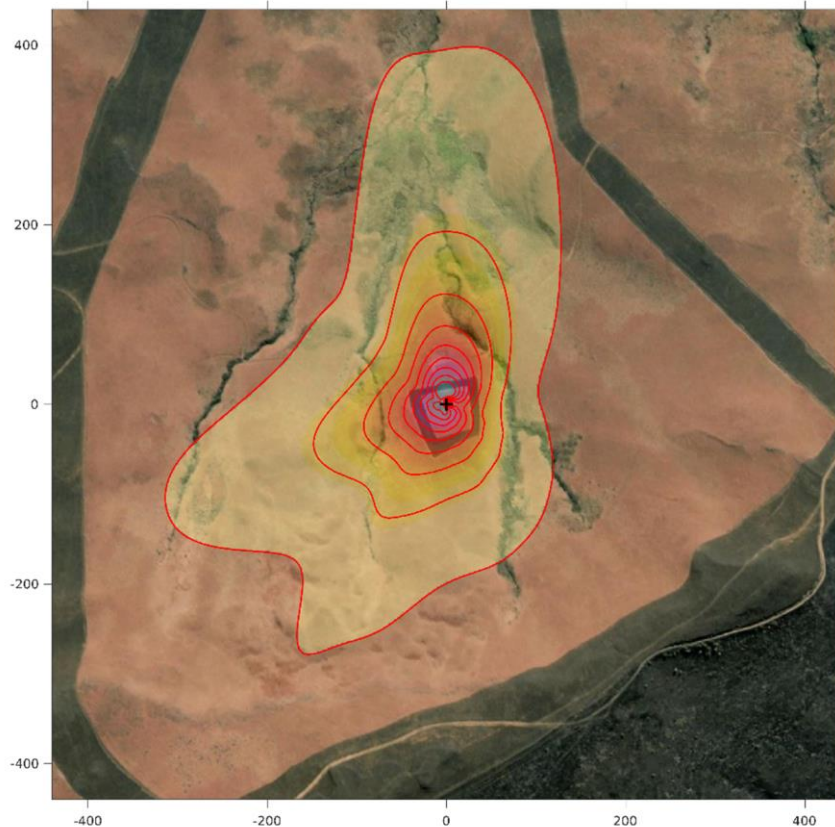
Catchment Six geology is underlain by basaltic lavas over Clarens Sandstone, with acidic, highly leached soils (Toucher *et al.*, 2016; Kibirige *et al.*, 2023). Vegetation is primarily mesic grassland, dominated by perennial C4 grass species (Mucina and Rutherford, 2006b). Precipitation is concentrated in the summer growing season with a mean annual temperature of 13.8 °C (Everson and Everson, 2016). Seasonal maximum and minimum temperatures at Cathedral Peak were  $T_{\text{Max}} = 29.7$  °C,  $T_{\text{Min}} = 18.2$  °C in hottest month of February, and  $T_{\text{Max}} = 22.7$  °C,  $T_{\text{Min}} = -4.9$  °C in the coldest month of July. Mean annual precipitation (MAP) is 1 380 mm which falls predominantly between October and March, with occasional winter snowfall between May and August (Gordijn *et al.*, 2018). Catchment Six includes a total area of 0.667 km<sup>2</sup>, an altitude of 2073 meters above sea level (m.a.s.l.) and a north–northwest aspect (Kibirige *et al.*, 2023).

This experiment reports four years of continuous measurements, including net turbulent CO<sub>2</sub> flux, latent heat, net radiation, air temperature, and precipitation at Catchment Six. The measurements were collected using an eddy covariance (EC) flux tower equipped with a 3-D sonic anemometer (CSAT3A, Campbell) and an open-path gas analyser for H<sub>2</sub>O and CO<sub>2</sub> fluxes (EC150, Campbell), integrated into the EASYFLUX DL CR6OP system for the CR6 and Open-Path Eddy-Covariance System. These sensors were positioned 6 meters above the ground and connected to an electronics panel (EC100, Campbell), which was coupled to a Campbell datalogger (CR3000). The Campbell EasyFlux-DL program was used for EC data collection. Eddy covariance (EC) flux data quality is assessed on a scale from 0 (best quality) to 9 (lowest quality). Quality grades reflect the reliability of the measurements, with greater values indicating reduced reliability. Specifically, data quality grades 7 to 9 are considered low quality readings and are excluded from scientific analysis, due to factors such as instrument malfunctions, extreme outliers, or measurement inconsistencies that fall outside of the expected range (Loescher *et al.*, 2006). These grades are excluded to enhance the robustness and accuracy of flux findings. Excluding poor quality data ensures that the analysis is based on reliable and accurate measurements, reducing the potential for erroneous conclusions (Loescher *et al.*, 2006). In this study, approximately 28% of the data were excluded based on these criteria. The EC flux tower footprint was determined using the Flux Footprint Prediction (FFP) software (Kljun *et al.*, 2015), employing a two-dimensional parameterization to understand the spatial context of the data and ensuring that the measurements reflect the intended study area.

Catchment Six is managed with an annual fire break burn around the perimeter in late winter and biennial burns between August and October before the spring rains, over the remainder of the catchment where the EC Flux Tower is located (Carbutt and Thompson, 2021) (Figure 5.2). The study used 4-year observed soil water content % (SWC) data collected from probes positioned at a depth of 1 meter below the soil surface. Probes were installed in pairs with a ~ 50 m spacing (Kibirige *et al.*, 2023). Total aboveground standing biomass (ASB) was calculated at the end of a growing season in March 2022, along three transects within Catchment Six. ASB was calculated by from 600-disc pasture meter (DPM) readings, calibrated in situ to determine the available standing fuel to be burnt in the upcoming catchment burn scheduled for August 2022. Calibration readings were calculated at each site by recording the DPM reading, clipping 30 ASB within the DPM ring, and oven drying the biomass clippings at 65 °C for 48 hours. A regression was developed to convert readings to DM, estimating ASB in kg DM ha<sup>-1</sup>.



**Figure 5.1:** Location of the study site and experimental plots in South Africa (a), with the ten hydrological research catchment sites at Cathedral Peak, uKhahlamba-Drakensberg Park. The Eddy Covariate Flux Tower is located in Catchment Six (b) and was used in this experiment. Images were created using R, QGIS and Google Earth Pro. Photo of Catchment Six (c) where the eddy covariate flux tower is located (Photo by Robyn Nicolay).



**Figure 5.2:** Two-dimensional parameterisation of Flux Footprint estimates for Cathedral Peak Catchment Six, burnt biennially. The red dot depicts the tower location with a receptor height of 6 m. Colours used to represent the concentration of flux readings and footprint contour lines are shown in steps of 10 % from 10 to 90 %. Image generated using data from the Eddy Covariate tower and FFP software (Kljun *et al.*, 2015).

### 5.3.2 Statistical Analysis

Data from the eddy covariance flux tower was analysed over four years between September 2019 and August 2023, each year (September – August) representing a single replication in each of the two ANOVA analyses. The statistical analysis was conducted using R Studio version 4.3.1 (R Core Team, 2023).

Multivariate Analysis of Variance (MANOVA) models were used to assess the impact of the interaction between continuous response variable soil water content (SWC) and factorial covariant season on the response continuous response variable CO<sub>2</sub> flux. To ensure the validity of the MANOVA models, diagnostic plots were generated to assess normality of residuals and homogeneity of variances. Post-hoc pairwise comparisons using t-tests with Bonferroni correction were conducted to investigate differences in SWC and CO<sub>2</sub> flux, across seasons. The

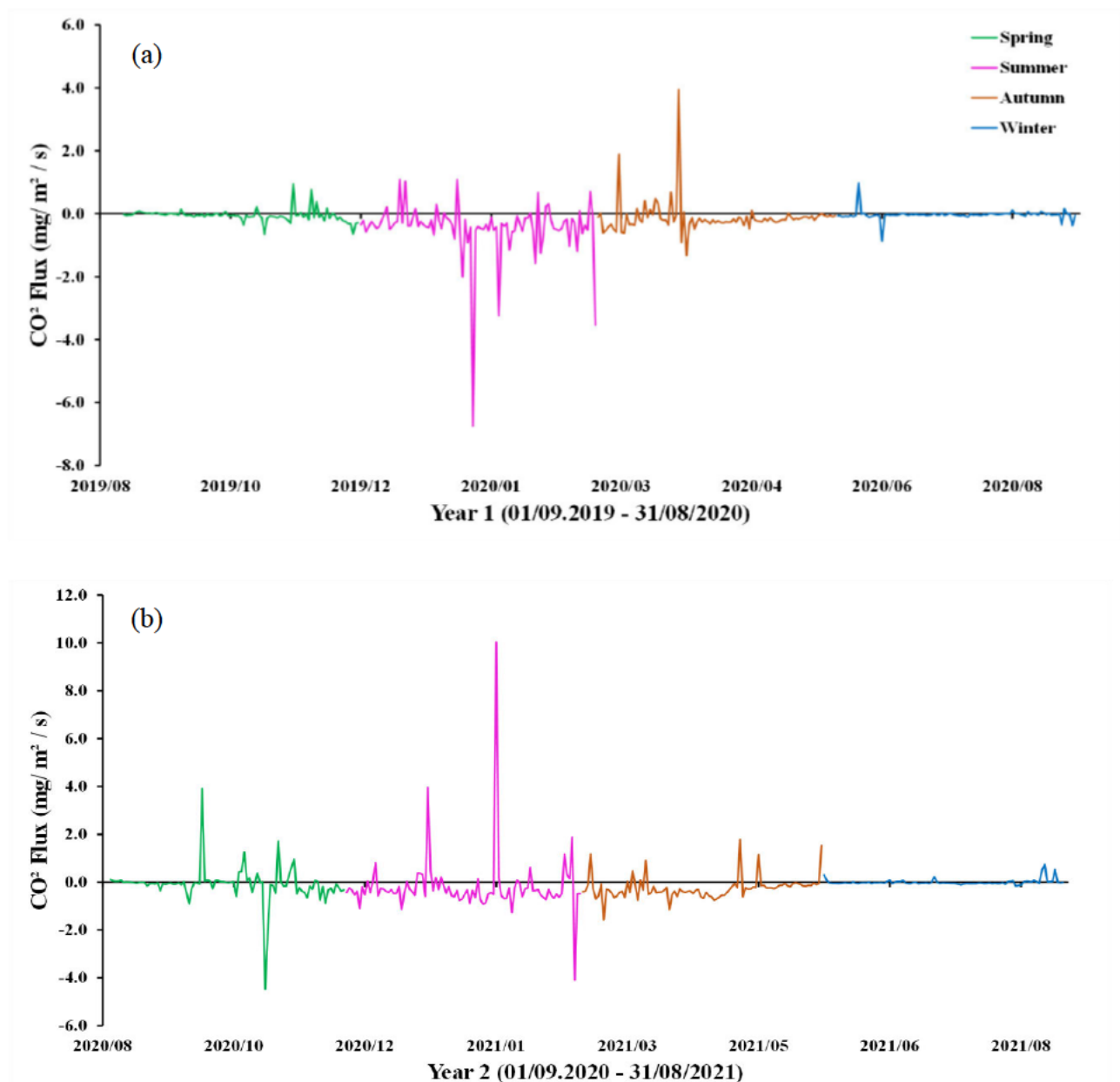
impact of SWC percentage within seasons was evaluated through a one-way ANOVA, subsequently followed by a Tukey HSD test. Assumptions of normality were examined using the Shapiro-Wilk test.

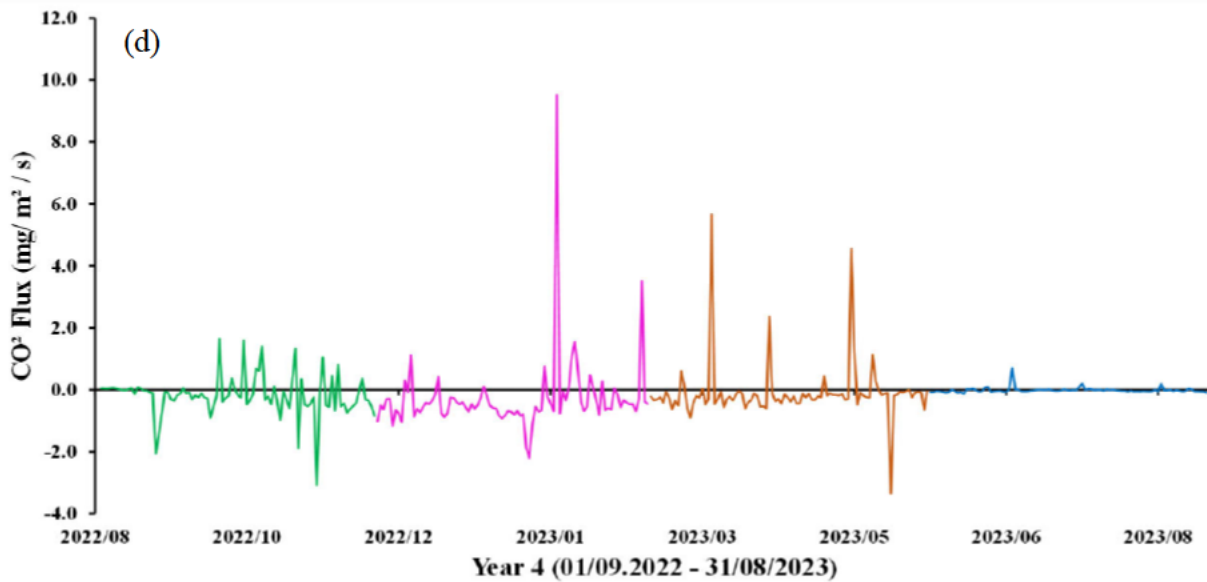
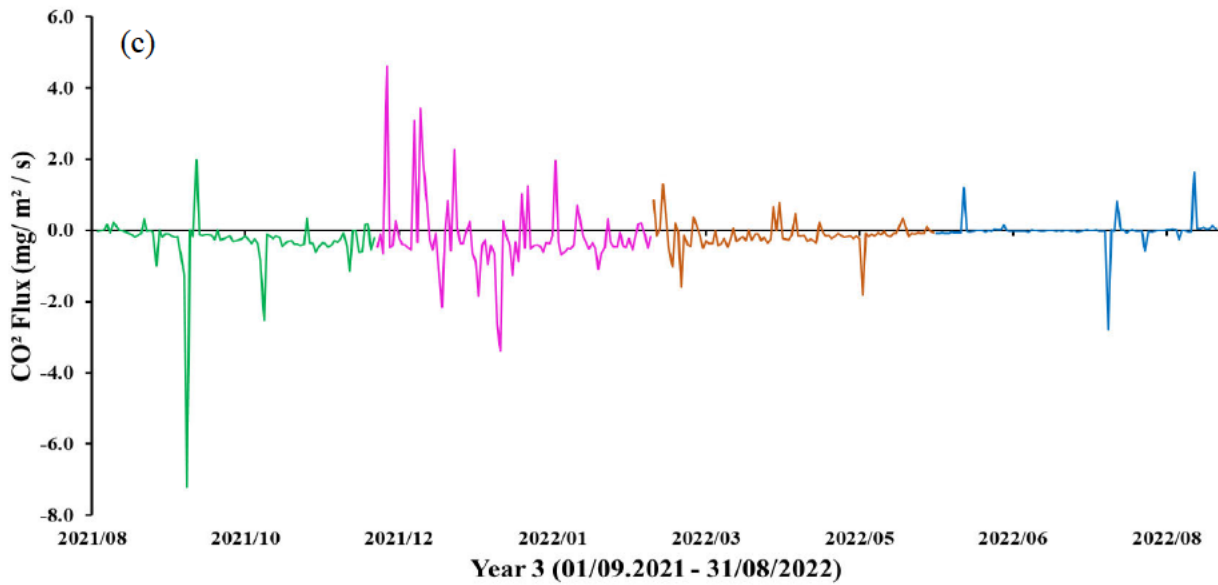
A one-way ANOVA was used to evaluate the relationship between season and CO<sub>2</sub> flux. Post-hoc pairwise comparisons were conducted with Bonferroni, and Holm adjustment methods. The assumptions of the one-way ANOVA were checked using the Shapiro-Wilk normality test, indicating that data are normally distributed ( $p = 0.2$ ), including checks for normality of residuals using Q-Q plots and homogeneity of variances through residual plots, in order to ensure the validity of the analysis. The Kruskal-Wallis rank sum test was used as a non-parametric analysis to determine whether there were statistically significant effects of months on the balance of CO<sub>2</sub> flux.

We fitted a linear mixed model in R using the *lme4* and *nlme* packages and estimated using maximum likelihood (ML) and nonlinear optimization with box constraints (nlminb), to predict CO<sub>2</sub> flux with net radiation, temperature, latent heat and rainfall. Rainfall was found to be nonsignificant within the model, as a main effect or as an interaction, so was removed, improving the fit of the model. The model included date as a random effect. The Levene's test showed that the variance was distributed evenly ( $p > 0.05$ ). Standardized parameters were obtained by fitting the model on a standardized version of the dataset. 95% Confidence Intervals (CIs) and p-values were computed using a Wald t-distribution approximation.

## 5.4 Results

The balance of carbon uptake and release in Catchment Six of Cathedral Peak, was recorded and analysed over four years, with annual CO<sub>2</sub> flux ranging from a balance of  $-898.94 \pm 0.031$  mg CO<sub>2</sub> m<sup>2</sup> s (53% uptake) to  $-1342.86 \pm 0.037$  mg CO<sub>2</sub> m<sup>2</sup> s (136% uptake). The biennial burn in year four, on the 24th of August 2022, burnt an estimated standing biomass of  $2576.51 \pm 17.807$  kg DM/ ha<sup>-1</sup>, but little variation was seen in the carbon flux in August 2022 (Figure 5.3). The Inter-annual variability between the four observed years all showed a greater carbon uptake than carbon release in the grassland system, with the greatest flux in the growing seasons and little variability in the dormant winter months (Figure 5.3).





**Figure 5.3:** Inter-annual variability between the four observed years, a) year 1 (01 September 2019 – 31 August 2020) with an annual balance of  $-1342.86 \pm 0.037$  mg CO<sub>2</sub> m<sup>2</sup> s, or 136% more of a carbon sink compared to what emits that year. b) year 2 (01 September 2020 – 31 August 2021), with an annual balance of  $-898.94 \pm 0.031$  mg CO<sub>2</sub> m<sup>2</sup> s, or 53% more of a carbon sink compared to what respire that year. c) year 3 (01 September 2021 – 31 August 2022), with an annual balance of  $-1133.10 \pm 0.030$  mg CO<sub>2</sub> m<sup>2</sup> s, or 88% more of a carbon sink compared to what emits that year. d) year 4 (01 September 2022 – 31 August 2023), with an annual balance of  $-1087.68 \pm 0.028$  mg CO<sub>2</sub> m<sup>2</sup> s, or 70% more of a carbon sink compared to what emits that year. Variability in daily CO<sub>2</sub> flux within seasons is seen with the greatest variance observed in summer growing months, and the least amount of variability in daily CO<sub>2</sub> flux observed in the winter dormant periods. Negative values indicate a sink of atmospheric

CO<sub>2</sub> and values greater than zero indicate a source of carbon release. Readings from Catchment Six, Cathedral Peak.

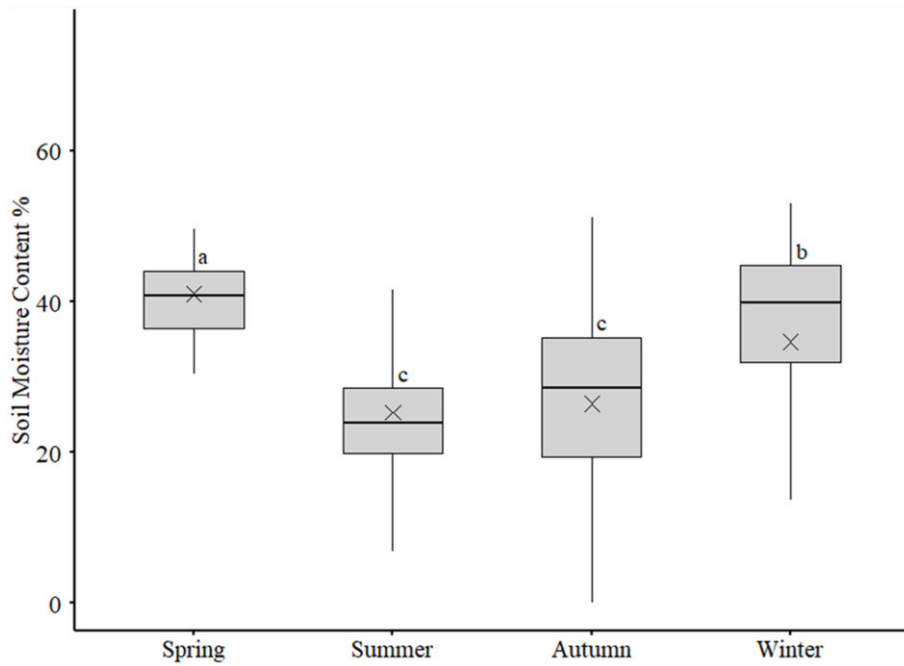
Season and soil moisture content (SWC) had a significant interaction on the balance of CO<sub>2</sub> flux within the grassland ( $F_{3,756} = 3.99, p = 0.007$ ), indicating that soil moisture content variation within wet and dry seasons had a significant impact on the observed differences in CO<sub>2</sub> flux balances (Table 5.1). Post-hoc tests showed that for SWC, significant differences were found between Spring and Autumn ( $p < 0.001$ ), Summer and Autumn ( $p < 0.001$ ), and Winter and Summer ( $p < 0.001$ ). Regarding CO<sub>2</sub> flux, significant differences were observed between Summer and Autumn ( $p = 0.013$ ), Winter and Autumn ( $p < 0.001$ ), and Winter and Summer ( $p < 0.001$ ). The ANOVA suggests that the main effect of season on SWC is statistically significant and large ( $F_{3, 791} = 60.05, p < 0.001; 95\% \text{ CI } [0.15,1.00]$ ) (Table 5.2, Figure 5.4).

**Table 5.1:** MANOVA table showing the variation between sample means in CO<sub>2</sub> flux balances as a response to soil water content and season at Cathedral Peak research Catchment Six.

	df	Mean sq	F	P
Soil Water content % (SWC)	1	3.886	11.82	<b>&lt;0.0001***</b>
Season	3	4.770	14.51	<b>&lt;0.0001***</b>
SWC × Season	3	1.312	3.99	<b>0.007**</b>
Residuals	756	0.34		
Total	763			

**Table 5.2:** ANOVA table showing the variation of soil water content (%) within seasons in grasslands at Cathedral Peak research Catchment Six.

	df	Mean sq	F	P
Seasons	3	8186	60.05	<b>&lt;0.0001***</b>
Residuals	791	136		
Total	794			

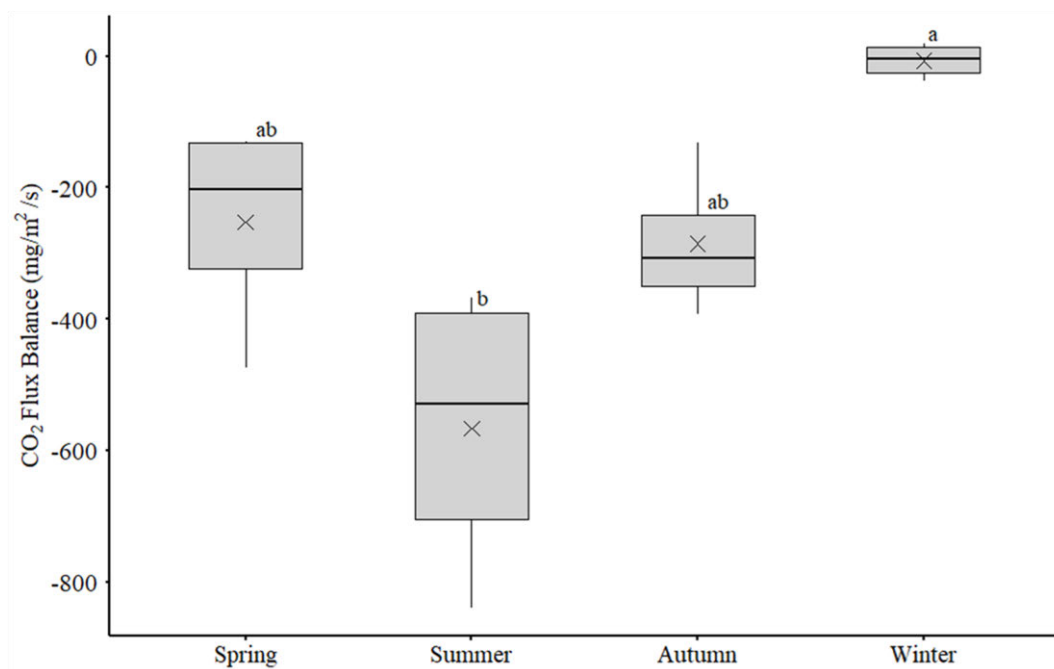


**Figure 5.4:** Variation in soil water content % (SWC) to a depth of 1 m within seasons is seen predominantly in Spring and Summer when MAP is greatest. SWC is seen to remain relatively high in the dry periods, with no differences between Autumn and Summer. The greatest SWC % is seen in the spring months. Data were taken between September 2019 and August 2023 from research Catchment Six, Cathedral Peak.

One-way ANOVA (Table 5.3) showed significant variation of CO<sub>2</sub> flux balances within seasons ( $F_{3, 12} = 9.32$ ,  $p = 0.003$ ). Post-hoc pairwise comparisons further highlighted specific pairwise differences in balance of CO<sub>2</sub> flux among seasons (Figure 5.5). Notably, summer ( $-566.6 \pm 106.1$  mg CO<sub>2</sub> m<sup>2</sup> s) and winter ( $-7.0 \pm 106.1$  mg CO<sub>2</sub> m<sup>2</sup> s) significantly differed in the effect on the absorption of CO<sub>2</sub> flux measured, compared to autumn ( $-285.3 \pm 106.1$  mg CO<sub>2</sub> m<sup>2</sup> s) and spring ( $-252.5 \pm 106.1$  mg CO<sub>2</sub> m<sup>2</sup> s) ( $p < 0.05$ ). This indicates greater variability and absorption of carbon during the peak growing season of Summer, while the least variation and minimal source of carbon absorption was seen in the dormant Winter period (Figure 5.5). The greatest variation in soil moisture content was also seen in spring and summer (Figure 5.4) These results suggest that the seasonal variations and related soil moisture have a notable influence on the observed differences in whether carbon is either absorbed or released.

**Table 5.3:** ANOVA table showing the variation within seasons over four years, on the balance of CO<sub>2</sub> flux in grasslands at Cathedral Peak research Catchment Six.

	df	F	P
Season	3	9.32	<b>0.002**</b>
Residual	12		
Total	15		

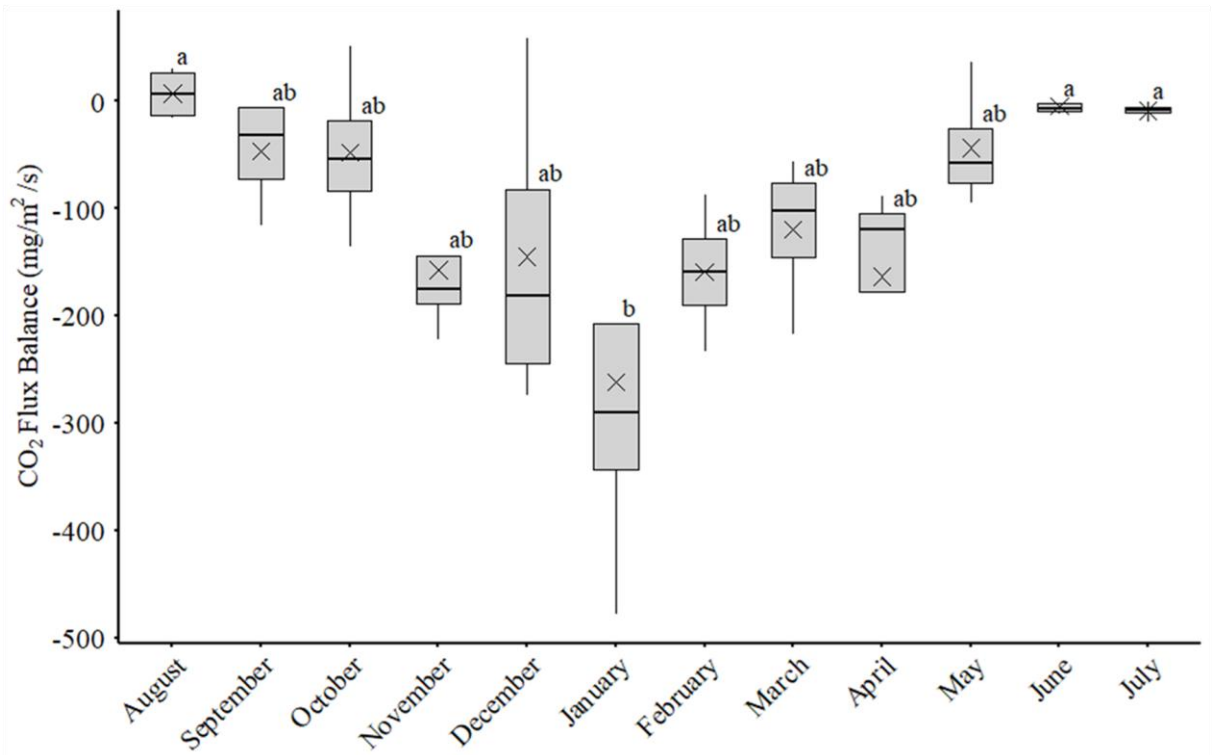


**Figure 5.5:** Seasonal variation in the balance of CO<sub>2</sub> source vs sink, with negative figures denoting a sink of carbon and a positive figure denoting a source of carbon emission. Based on data from Cathedral Peak research Catchment Six, uKhahlamba-Drakensberg Park.

Kruskal-Wallis rank sum test demonstrated a significant effect of the month on the balance of CO<sub>2</sub> flux ( $\chi^2_{11,36} = 22.88$ ,  $p = 0.018$ ) (Table 5.4). Post-hoc pairwise comparisons with Bonferroni adjustment revealed specific pairwise differences among months. January, assumed to be the peak growth period, showed to be significantly different in the balance of CO<sub>2</sub> flux compared to June, July and August (Bonferroni-adjusted  $p < 0.05$ ). These findings indicate that specific months significantly influenced the balance of CO<sub>2</sub> flux values, notably the primary growing period between November and April where the greatest sink of atmospheric carbon is observed as well as the greatest variability in flux readings (Figure 5.6).

**Table 5.4:** Table showing the variation within months over four years, on the balance of CO<sub>2</sub> flux in grasslands at Cathedral Peak research Catchment Six.

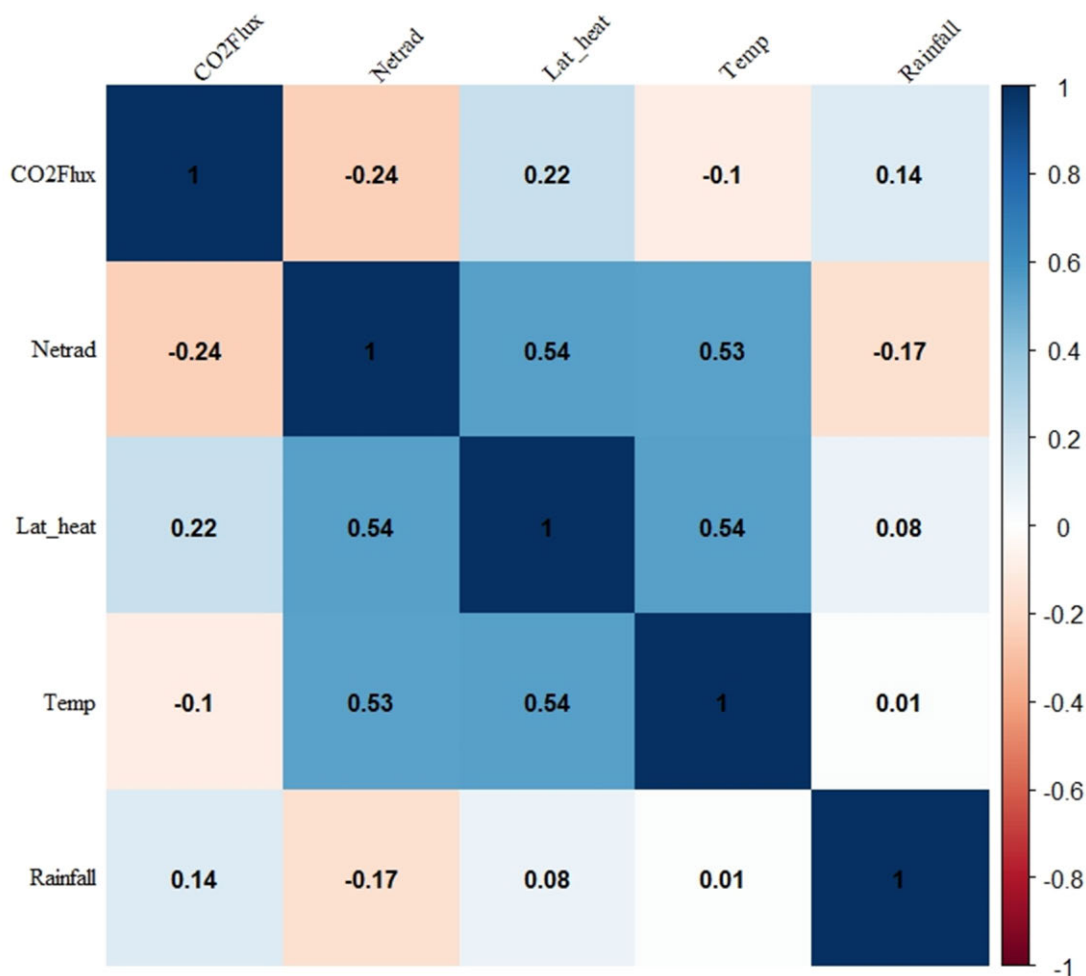
	df	$\chi^2$	P
Month	11	22.88	<b>0.018*</b>
Residual	36		
Total	47		



**Figure 5.6:** Monthly variation in the balance of CO<sub>2</sub> source vs sink, with negative figures denoting a sink of carbon from the atmosphere and a positive figure denoting a source of carbon emission. Based on data from Cathedral Peak, Catchment Six, uKhahlamba-Drakensberg Park.

The multiple regression model indicated that several variables were significantly associated with CO<sub>2</sub> flux (Figure 5.7). Net radiation, latent heat and air temperature remained important predictors of CO<sub>2</sub> flux (Figure 5.8, Table 5.5). The linear mixed-effects model (LMM) featured a fixed-effects structure incorporating net radiation, temperature, latent heat,

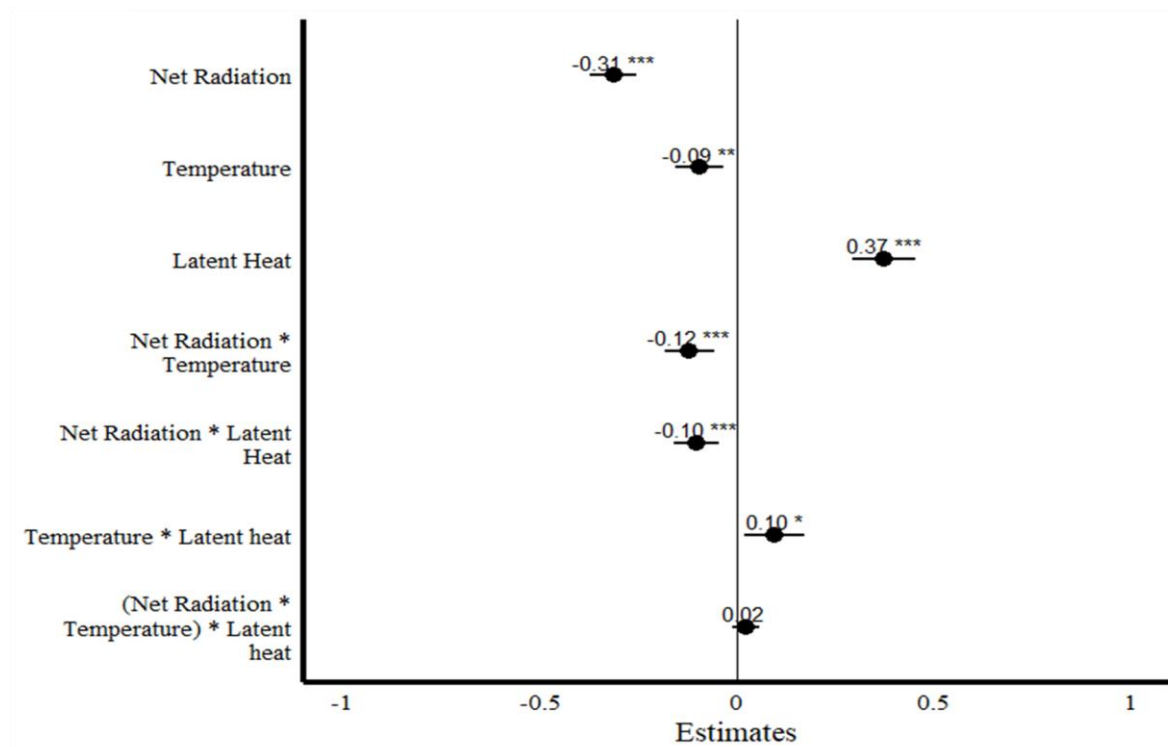
and their interactions. Notably, net radiation exhibited a strong negative relationship with CO<sub>2</sub> flux ( $\beta = -0.3136$ ,  $p < 0.0001$ ), while latent heat had a positive effect ( $\beta = 0.3735$ ,  $p < 0.0001$ ). The interactions between net radiation: temperature, net radiation: latent heat, and temperature: latent heat also showed significant effects (Table 5.5). The model's R-squared values ( $R^2 = 0.28$ ), indicate that approximately 28.86% of the variance in CO<sub>2</sub> flux can be explained by the model. The model's total explanatory power was moderately significant ( $R^2 = 0.29$ ) and related to the fixed effects alone. The model's intercept, corresponding to net radiation = 0, temperature = 0 and latent heat = 0 is at -0.09 (95% CI [-0.14, -0.03],  $t_{938} = -3.24$ ,  $p = 0.001$ ).



**Figure 5.7:** The correlation matrix depicts the relationship between variables measured in a southern African mesic montane grassland ecosystem, Cathedral Peak site, Catchment Six. CO<sub>2</sub>Flux represents the carbon flux, Netrad corresponds to net radiation, Lat\_heat signifies latent heat, Temp denotes temperature, and Rainfall indicates precipitation. Correlation coefficients range from -1 to 1, with shades of blue indicating the strength and direction of correlations, while numbers represent correlation values.

**Table 5.5:** Presentation of the results of a Linear Mixed Model analysis for various environmental factors and their interactions on CO<sub>2</sub> flux in a grassland system, based on data at the Cathedral Peak site, Catchment Six. The table includes estimates ( $\beta$ ), standard errors (SE), 95% confidence intervals (CI) as well as significant values for each variable and interaction term.

	$\beta$	SE	95% CI		$t_{(938)}$	p
			Lower	Upper		
Net Radiation (NR)	-0.31	0.030	-0.37	-0.25	-10.17	< <b>0.0001</b> ***
Temperature (T°)	-0.09	0.031	-0.16	-0.03	-2.98	<b>0.003</b> **
Latent Heat (LH)	0.37	0.041	0.29	0.45	8.98	< <b>0.0001</b> ***
NR × T°	-0.12	0.032	-0.18	-0.06	-3.68	< <b>0.0001</b> ***
NR × LH	-0.10	0.029	-0.16	-0.04	-3.47	<b>0.015</b> *
T° × LH	0.10	0.038	0.02	0.17	2.50	<b>0.021</b> *
NR × T° × LH	0.02	0.017	-0.01	0.06	1.22	0.222



**Figure 5.8:** The interaction effects on CO<sub>2</sub> flux as estimated by the fitted statistical model. Each line represents an interaction term involving varying environmental factors that contribute to the sink or source of carbon in the Cathedral Peak grassland ecosystem in Catchment Six, managed with biennial fire. Negative beta coefficients suggest an increase in carbon release,

and positive beta coefficients suggest an increase in carbon absorption or a decrease in carbon release. The plot illustrates the estimated coefficients with their corresponding p-values, indicating the strength and statistical significance of these interactions. These interactions show how CO<sub>2</sub> flux responds to the combined influences of these environmental factors. The horizontal lines and dots represent effect estimates and their significance, respectively.

## 5.5 Discussion

Global studies have shown mesic grassland ecosystems consistently operate as a carbon sink, absorbing more carbon than they emit (Archibald *et al.*, 2009a; Knapp *et al.*, 2002; Zhang *et al.*, 2011). The results from this study at Cathedral Peak Research Catchment Six over four years, confirmed the importance of mesic grassland ecosystems as a notable sink for carbon. Here, we have seen 53% and 135% more carbon absorbed compared to what was emitted within the four-year study period in grasslands that are managed with biennial spring fire regimes. The greatest absorption of carbon was in the warm, wet summer months, while a reduced flux in these systems was seen in dormant winter periods where catchments were cooler and received less rainfall (Toucher *et al.*, 2020). Greater CO<sub>2</sub> flux during the growing periods is suggested to be attributed to plant growth cycles and precipitation and temperature fluctuations, influencing photosynthesis and decomposition rates (Archibald *et al.*, 2009a).

The beneficial effect of fire on the growth and production of montane grasslands has been established, contributing to the turnover of carbon above and below ground (Everson *et al.*, 1998; Everson and Everson, 2016). The most important result of this study was that there were no significant losses in the carbon of grasslands that had received over 35 years of biennial spring burning, contrary to some perceptions that African grassland burning contributes to net carbon emissions (Ciais *et al.*, 2011). Carbon emissions from fire events would likely result in a spike in CO<sub>2</sub> flux data, however, the recorded net carbon balance over the experimental time period of 4 years suggests that any emissions were effectively offset by carbon sequestration processes within the grassland ecosystem. Catchment Six sequestered 88% more carbon than it emitted during its biennial burn in August 2022, where a standing biomass of  $2576.51 \pm 17.807$  kg DM/ha<sup>-1</sup> was burned. Fire as a primary defoliation method in this catchment is likely to promote plant growth via nutrient cycling, increased photosynthetic rates, and carbon allocation to belowground biomass after burning, explaining the greater flux during this period. Conversely, the dormant winter months showed minimal flux and made only a minor

contribution to the carbon sink as respiration and carbon turnover as a product of photosynthesis during this dormant period would be reduced.

Prior research indicates that soil moisture plays a crucial role in regulating the pace and duration of various ecosystem processes, particularly those related to carbon cycling in arid and semi-arid systems (Archibald *et al.*, 2009a; Migliavacca *et al.*, 2021). When there is an increase in SWC% specifically following spring rainfall, there is an expected rapid rise in soil respiration, taking several days for grasslands to achieve peak photosynthetic activity (Huxman *et al.*, 2004; Archibald, Kirton, *et al.*, 2009). The occurrence of more rainfall events in warmer growing seasons (Spring and Summer) is associated with fluctuations in grasslands releasing and absorbing carbon, indicating heightened biological activity during these periods (Huxman *et al.*, 2004). Despite greater SWC% variations in warmer summer months, SWC% content did not significantly decrease during the dormant seasons. This implies that, even during dormant seasons when grasslands exhibit minimal biological activity, they have the capacity to retain soil moisture. The stored moisture becomes available for utilization when temperatures rise in the subsequent growing seasons (Balocchi *et al.*, 2004). The mechanism through which grasslands retain this moisture during dormancy may involve retention in the soil facilitated by ground cover and low temperatures limiting evaporation. These patterns in seasonal flux, where carbon source, sink and SWC% highlight interactions between vegetation, climate, and carbon cycling, are consistent with previous findings (Bouvet *et al.*, 2018). Our findings also demonstrate that certain months, notably those within the primary growing period from November to April, play a pivotal role in determining the ecosystem's CO<sub>2</sub> flux. The greatest absorption of atmospheric carbon was observed during these months, accompanied by substantial variability in the balance of CO<sub>2</sub> flux readings. This variability is likely attributed to the interchange of factors such as heightened temperature, precipitation, and plant phenology including variations in leaf emergence, flowering, and senescence, during these primary growing months (Archibald, Kirton, *et al.*, 2009). Changes in environmental conditions along with events of drought years on temporal variability could see altered carbon responses by these grasslands as this has been found to heavily impact similar ecoregions (Zhang *et al.*, 2011).

Evidence suggests that elevated extremes in temperatures and atmospheric CO<sub>2</sub> can be anticipated as part of projected anthropogenic climate change (Albritton, 2001; Knapp *et al.*, 2002). Studies also indicate the impact of aridity and temperature on ecosystem efficiency in utilizing assimilated carbon (Migliavacca *et al.*, 2021). In temperate systems, incoming solar

radiation (PAR) and temperature act as primary drivers for predicting photosynthesis and respiration (Archibald, Kirton, *et al.*, 2009). Consequently, greater positive net radiation values, reflecting increased incoming solar radiation exceeding outgoing longwave radiation, are linked to elevated surface temperatures in grassland ecosystems (Baldocchi *et al.*, 2004; King *et al.*, 2015). These conditions can influence photosynthesis which can be constrained under elevated CO<sub>2</sub> and temperature (Crafts-Brandner and Salvucci, 2000). Additionally, debates exist regarding the potential water-saving influences of stomatal closure under elevated CO<sub>2</sub>, as nutrient uptake can be negatively impacted. The observed significant negative relationship between net radiation, temperature, and CO<sub>2</sub> flux, combined with the positive influence of latent heat, highlights interactions between climate variables and the sequestration of carbon (Schwalm *et al.*, 2010). The sensitivity of the ecosystem to these environmental conditions is suggested to determine the grassland's capacity as a carbon sink or source and their interaction with local feedback mechanisms at the biosphere-atmosphere interface (Zhang *et al.*, 2011; Brunsell and Wilson, 2013).

Previous studies have shown that more extreme shifts in ecosystem variability were accompanied by reduced soil CO<sub>2</sub> flux and lesser CO<sub>2</sub> uptake by dominant grass species in similar ecosystems (Knapp *et al.*, 2002). The influence of this on high-altitude amplification is yet to be considered in southern African grassland systems, as larger trends in temperature ranges based on energy balances at the surface of the earth (Ohmura, 2012; Ripple *et al.*, 2023) are likely to respond to projected climatic changes in the future. How exactly these grass-dominated ecosystems will respond is unclear, as studies have shown increases in temperatures favour the expansion of C<sub>4</sub> grasses especially if such climate warming is accompanied by reduced precipitation or soil moisture (Parker-Allie *et al.*, 2009; Hulme *et al.*, 2014).

Considering these projected changes in climate, correct application and timing of grassland fire regimes is imperative for the maintenance of carbon sinks in these grassy ecosystems for the offset of atmospheric carbon (Bracho *et al.*, 2021). However, we do acknowledge the limitation in the lack of varying fire frequencies in this study. Future research could explore the long-term effects of a range of fire management strategies on CO<sub>2</sub> flux and assess the resilience of the observed carbon sink behaviour in response to changing environmental conditions.

## 5.6 Conclusion

The outcomes of our study at Cathedral Peak Research Catchment Six solidify the evidence supporting the mesic grassland ecosystem's potential role as a carbon sink, absorbing more carbon than it emits. Over the four years, we consistently observed substantial carbon flux into grasslands managed with biennial spring fires. Notably, we found a significantly greater carbon sink compared to CO<sub>2</sub> emissions, even in the year of a burn. The pronounced CO<sub>2</sub> flux during warm, wet summer months underscores the impact of temperature, precipitation, and plant phenology on photosynthesis and decomposition rates. Recognizing these seasonal fluctuations is essential when evaluating the overall carbon balance of an ecosystem, emphasizing the dynamic nature of ecosystems and the influence of environmental factors on carbon sequestration potential.

Additionally, this research highlights the importance of soil moisture in regulating ecosystem processes. We observed a significant effect and variation in SWC%, paralleled with significant carbon sinks in corresponding wet and dry seasons. Fire as a crucial element in maintaining the composition and structure of the Drakensberg montane grasslands, did not negate the carbon sequestration potential of these grasslands. Despite increased carbon outputs from fire, the grassland remained a net carbon sink. The findings of this study has contributed to understanding Southern African mesic montane grasslands' potential to reduce atmospheric CO<sub>2</sub> concentrations, having significant regional and global implications in perceiving fire as a management tool in these grassy biomes. The resilience of these grasslands to biennial spring fires highlights their capacity to mitigate carbon emissions, contributing to broader climate change mitigation efforts in Southern Africa.

## 6 CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

### 6.1 Introduction

The grassland biome is recognized as a crucial terrestrial reservoir of carbon, having the potential to store double the amount of stable carbon than the atmosphere itself (Lorenz and Lal, 2018; Dobson *et al.*, 2022). However, the regeneration and preservation of carbon pools in this biome hinges on effective management. Inappropriate or uninformed management, particularly in relation to the nature, frequency, and intensity of disturbances, can hinder the grassland's ability to maintain soil organic carbon (SOC) balances (Dlamini *et al.*, 2014; Buisson *et al.*, 2022). Misconceptions about grassland ecology and the role of fire and grazing in these ecosystems pose threats to ecosystem functionality, services, and the livelihoods of local pastoralists (Davis and Robbins, 2018).

The management of the grassland biome remains a contentious topic in rangeland ecology, marked by ongoing debates on the use of prescribed fires. To comprehend the role of grasslands in the global climate change arena, it becomes imperative to quantify the impacts of fire management on the sequestration of organic carbon.

This research investigated how prescribed fire affects carbon storage, release, and sequestration in mesic grasslands, both in grazed and ungrazed systems. The aim was to understand its role as a source or sink and contribute to fire management strategies in pastoral agroecological systems. The overarching goal was to propose recommendations for sustainable grassland management and actively contribute to mitigating the impacts of climate change in these anthropogenic managed systems.

### 6.2 Summary of Contributions

#### *i. Does Anthropologically Managed Grassland Act as a Carbon Source or Sink?*

Annually burnt grassland stored the most carbon, with only marginal differences between biennial, triennial burns, and burn exclusion plots following ~70 years of fire treatment application (Chapter 2). The differences in carbon storage in annually burnt areas is attributed to heightened root turnover and microbial activity, evidenced by increased soil respiration rates (Chapter 4). In contrast, burn exclusion plots showed a greater buildup of lignified aboveground

biomass, potentially altering the nature of accidental or infrequently prescribed fires, towards increased intensity and duration, which could lead to greater total carbon losses and reduced microbial activity (Chapter 4).

While burn exclusion plots exhibited similar total carbon storage capacities as biennial and triennial prescribed burn treatments, they showed greater soil nitrogen concentration, possible nitrification and increased soil acidity (Chapter 3). These carbon pools may not necessarily be less stable or prone to microbial degradation; instead, burnt grasslands may support microbial activity by regulating optimal soil environments, thus facilitating the turnover of these fractions within the carbon pools. The elevated acidity in burn exclusion areas, possibly attributed to reduced ash deposition, and reduced volatility of soil nitrogen, suggests that although carbon exists in increased amounts in the soil, turnover, decomposition, and nutrient availability may be hindered, with a potential increase in carbon particulate matter. This suggests a trade-off in soil carbon storage and availability in a ‘use it or lose it’ scenario, where the soil might act as a reservoir with limited turnover potential. Overall, frequent burning is beneficial for sustaining grassland health and its potential as a carbon sink. This is further supported by the modelling of long-term carbon flux in montane mesic grassland burnt biennially, affirming the biome's consistent role as a carbon sink—absorbing more carbon than it emits (Chapter 5).

*ii. What are the effects of prescribed fire on the turnover and stability of soil carbon in mesic grassland?*

Annual burning in mesic grassland demonstrated the greatest total carbon storage (Chapter 2), primarily attributed to improved soil health (Chapter 3). However, when examining specific stable pyrogenic carbon fractions, grassland excluded from fire showed the greatest detectable quantities of black carbon (BC) in their soils (Chapter 3). While this suggests potential benefits in soil fertility and water-holding capacity associated with BC, there were constraints in characterizing BC particulate size due to limitations in time and resources during this research.

Ash deposition from fires, rich in alkaline compounds, plays a crucial role in modulating soil pH and acidity. The absence of fire led to heightened acid saturation and reduced pH, correlating with increased BC accumulation. This is proposed to result from reduced microbial activity, slowing decomposition, and nutrient cycling in the soil (Chapter 3). The finer black carbon particles in annually burnt grassland could be less detectable, indicating the influence

of burn frequency and microbial interactions on BC breakdown and distribution (Chapter 3). This observation aligns with evidence of greater microbial activity and carbon turnover in annually burnt grassland, supported by increased soil respiration levels (Chapter 4).

While increased soil respiration suggests heightened microbial activity and greater turnover of belowground biomass, facilitating carbon turnover, the composition of aboveground biomass in annually burnt systems supports rapid carbon turnover by reducing the lignification of vegetative matter (Chapter 4).

Contrastingly, unburnt and triennially burnt grassland exhibited carbon sequestration losses in lesser soil horizons, indicating reduced root and soil carbon turnover. In these scenarios, increased aboveground biomass accumulation may make these fire-prone grasslands susceptible to greater fire intensity and duration, potentially hindering soil microbial activity and limiting carbon turnover (Chapter 3). Further, the significant increase in SOC sequestration of annual and biennial grassland between 2000 and 2021 contradicts theories challenging the notion that SOC stocks stabilize and reach equilibrium over 20 years (Chapter 2).

In summary, while BC is detectable in greater levels under fire exclusion, the exclusion of fire limits the grassland potential to turnover stable fraction pyrogenic carbon fractions. I would then support the application of frequent fire as a sustainable option as a long-term management tool, promoting soil health and carbon turnover in mesic grassland.

*iii. Does the frequency of fire in mesic grassland influence its potential as a carbon sink or source in a grazed system?*

When looking at the fire frequency in mesic grassland that has been grazed, there were no discernible differences in SOC between annually burnt and grasslands protected from fire with the inclusion of grazing. The main source of C addition in annually burnt sites is likely root turnover, while leaf litter plays more of a role in the unburnt sites. Annually burnt grasslands, when compared to grassland protected from fire, exhibit enhanced palatability due to reduced lignin and fibre percentages (Chapter 4). This palatable regrowth encourages increased grazing pressure, leading to notable impacts on nutrient cycling processes through livestock deposition, trampling, and compaction.

The effects of annually burnt grasslands showed greater soil respiration rates, indicating increased microbial activity and carbon turnover (Chapter 4). This trend may be further

facilitated considering these grasslands are grazed year-round, with assumed concentration in the summer growth periods.

For pastoral farmers and rangers, the practice of frequent burning proves beneficial. Frequently burnt firebreaks demonstrated reduced lignin and fibre in aboveground biomass, resulting in improved forage quality and increased palatability for livestock. This, coupled with higher aboveground net primary productivity (ANPP) in enclosure cages, suggests that fire enhances overall grassland productivity (Chapter 4), making it a valuable management tool.

Annually burnt plots exhibited 9.1% more carbon ( $118.40 \text{ Mg C ha}^{-1}$ ) compared to unburnt plots ( $108.40 \text{ Mg C ha}^{-1}$ ), and 24.35% more carbon on average than the biennially burnt plots (Chapter 2) in ungrazed grasslands at UGFE. At Wakefield, where grasslands were excluded from fire ( $26.61 \text{ Mg C ha}^{-1}$ ) or burnt annually ( $25.77 \text{ Mg C ha}^{-1}$ ) in winter (Chapter 4), the presence of grazing cattle showed no significant differences between the two scenarios and a 3.21% increase in carbon in the unburnt sites. These findings are consistent with studies by Beringer *et al.* (2015) and O'Connor *et al.* (2010). This is suggested to be influenced by differences in climate and soil type between the two sites. Patterns could also be attributed to deposition and trampling of organic matter from livestock, as well as greater belowground carbon turnover shown by increased respiration rates in annually burnt and grazed grassland (Chapter 4). With the incorporation of grazing by cattle at 1.5 ha/ LSU which is appropriate for this region, alongside frequent burning, the pattern of carbon differences changed.

***iv. Does the potential of anthropogenic managed grassland as a carbon sink change with variations in climate?***

In the context of South African mesic grasslands, the potential of anthropogenic managed grassland as a carbon sink is significantly influenced by variations in climate (Chapter 5). This holds implications for management decisions regarding burning mesic grasslands, especially considering predicted changes in climatic conditions driven by global climate change trends.

Observations indicate that frequent burning enhances the storage and sequestration of carbon, with notable differences in carbon turnover observed in treatments burnt annually or biennially in spring (Chapter 2). Findings over four years of observed grassland carbon flux, consistently reveal substantial carbon sequestration in grasslands managed with biennial spring fires (Chapter 5).

The role of soil moisture is seen as a key determinant of carbon sinks in these ecosystems. Notably, soil moisture consistently peaks in the spring months, coinciding with greater sequestration rates and variability of carbon flux during the same period (Chapter 5). This has implications for management decisions, especially in determining the correct application and timing of burning. With projected climate change scenarios introducing variable and unpredictable temperatures and rainfall, understanding the relationship between soil moisture and carbon sinks becomes crucial. Data from the study can serve as a valuable tool in predicting how future climate scenarios may impact CO<sub>2</sub> flux under different fire management strategies (Chapter 5).

When subject to annual burning, firebreaks have been found in previous studies to not only enhance grassland productivity, and rapid carbon turnover but also prove sustainable in maintaining plant diversity (O'Connor *et al.*, 2004; Beringer *et al.*, 2015). Considering these findings, frequent fire as a management tool is recommended for grazed mesic grasslands.

The consistent finding is that anthropogenic managed grasslands, consistently act as carbon sinks, storing more carbon than they emit. Frequent spring burns, when soil moisture is greatest are identified as the most appropriate timing for burning grasslands, supporting long-term sequestration, storage, and turnover of carbon. This becomes particularly relevant when considering predicted variations in climate, where extreme changes in rainfall and temperature may impact plant phenology, photosynthesis, and decomposition rates.

### **6.3 Management Recommendations for Anthropogenic Managed Grassland**

In mesic grasslands, the implementation of frequent spring burns for optimal carbon storage, turnover, and sequestration is recommended. Evidence suggests that biennial spring fires are also particularly effective in promoting substantial carbon sequestration, as well as creating conditions conducive to soil health for carbon sequestration. This includes mineral ash deposition, which subsequently reduces existing soil acidity. This reduction in acidity is crucial as it prevents potential decreases in soil microbe activity and belowground biomass turnover. Evaluating burning regimes, with an emphasis on annual or biennial burns, is advised, as observed differences in carbon turnover indicate that these frequencies are more conducive to effective carbon management.

Monitoring soil moisture levels and aboveground biomass as a controller of fire intensity, especially during wet and dry seasons, is essential, as they play a vital role in determining carbon sinks. Soil moisture data can inform decisions on the timing and frequency of prescribed burns to maximize carbon sequestration potential. The monitoring of soil moisture could then be more critical in the context of predicted changes in climate. Adjusting fire management plans based on projected climate scenarios can be beneficial in optimizing carbon flux under changing environmental conditions.

#### **6.4 Study Limitations and Scope for Future Research**

In fire-prone ecosystems, protecting vegetation from fire results in a significant accumulation of biomass and aboveground carbon, posing risks of detrimental release when fire becomes inevitable (Bradstock *et al.*, 2012). Regular prescribed grassland fires, known for their low intensity, contribute to environmental hazard reduction and enhance ecosystem resilience in the face of predicted climate change scenarios (Tulau and McInnes-Clarke, 2015). The dynamics of grassland fires, influenced by climatic factors, season, and burn frequency, impact fuel load, fuel composition, and fire regimes. Less frequent fires tend to accumulate greater aboveground carbon, which is volatile and susceptible to loss during fire events, potentially leading to increased carbon loss and reduced soil moisture. Anticipated changes in climate, marked by extreme weather events, raise concerns about heightened wildfire occurrences, particularly in fire-prone ecosystems, leading to increased fire severities (Francos and Úbeda, 2021). Future research should investigate the alteration of SOC storage in grasslands subjected to infrequent burns, examining the potential effects of varied burn intensity and rate of spread in prescribed or accidental fires. Additionally, it is important to explore the impact of fire treatments on microbial diversity, as well as soil enzyme activities, and how these factors are linked to carbon sequestration processes (Vermeire *et al.*, 2021).

#### **6.5 Final Comments and Summary Conclusions**

This study has highlighted the trade-off between carbon storage and availability in mesic grasslands, emphasizing the limitations of burn exclusion and extended fire return intervals on long-term carbon turnover and sequestration potential. Mesic grasslands managed with fire consistently are found to be carbon sinks, particularly when subjected to frequent spring burns, reinforcing their potential in mitigating climate change impacts.

Not only does the application of frequent fire regimes contribute to long-term carbon sequestration, but it also proves beneficial for pastoral farmers by improving forage quality and overall grassland productivity. The observed increase in soil respiration rates in annually burnt grasslands further supports the positive influence of frequent burning on soil health and carbon turnover.

Evidence from this research advocates for the use of frequent fire regimes to maintain and enhance the role of South African Mesic Grassland as vital carbon sinks with predictions on changing climate. Overall, the intentional application of frequent fire regimes has proved to be a sustainable management practice, enhancing the grasslands' capacity for carbon sequestration, storage, and turnover.

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**APPENDIX A: UKULINGA GRASSLAND FIRE EXPERIMENT (UGFE),  
PIETERMARITZBURG, SOUTH AFRICA**

REP 1										
B	B	B	B	B	B	B	B	B	B	B
11	6	9	8	2	10	1	3	4	7	5
A	A	A	A	A	A	A	A	A	A	A
1	8	11	2	6	3	9	4	7	5	10
C	C	C	C	C	C	C	C	C	C	C
8	2	11	9	6	4	10	3	5	1	7
D	D	D	D	D	D	D	D	D	D	D
1	8	10	2	4	11	7	5	3	6	9

REP 2										
D	D	D	D	D	D	D	D	D	D	D
2	7	6	4	10	5	11	8	1	9	3
A	A	A	A	A	A	A	A	A	A	A
9	8	2	11	6	7	5	4	3	10	1
C	C	C	C	C	C	C	C	C	C	C
5	4	10	2	11	8	6	1	9	3	7
B	B	B	B	B	B	B	B	B	B	B
8	5	11	7	10	6	3	1	2	4	9

REP 3										
B	B	B	B	B	B	B	B	B	B	B
4	5	6	9	2	10	8	11	7	3	1
C	C	C	C	C	C	C	C	C	C	C
1	2	6	3	7	9	4	10	11	5	8
D	D	D	D	D	D	D	D	D	D	D
4	1	5	7	2	3	11	10	6	9	8
A	A	A	A	A	A	A	A	A	A	A
2	9	1	7	3	10	8	5	6	4	11

13.7 m

18.3 m

The Ukulinga Grassland Fire Experiment (UGFE) was established in 1950 to examine the effects of different combinations of burning treatments on mesic grasslands. The experimental design involved the manipulation of two factors: burning and mowing, with two levels each (burned vs unburned, mowed vs un-mowed), resulting in four utilisation treatment combinations, namely Utilisation A (No mowing), Utilisation B (one cut early in the season when grass is at 20 cm, usually December), Utilisation C (one cut at the end of February), and Utilisation D (two cuts, one at B and another at C).

For this thesis, subplot treatments A1 to A10 within Utilisation treatment A (Highlighted) were included. Sub plots measuring 18.3 × 13.7 m were used, with treatments allocated to the plots in a full factorial split-plot, randomised block design that was replicated three times. The entire trial is un-grazed.

