ASSESSING SOIL SEED BANK DIVERSITY IN BUSH ENCROACHED SAVANNA RANGELAND, LIMPOPO PROVINCE, SOUTH AFRICA

by

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Declaration

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Pasture Science) has not been previously submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

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Dedication

I dedicate this dissertation to my daughter Rabopape Motlatso Tshegofatso Faith and my parents Rabopape Selabaledi David and Rabopape Sewela Virginia.

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Abstract

Savanna rangelands are ecosystems which are characterized by the co-existence of scattered trees and shrubs with a continuous grass layer. However, the grass and tree balance has been highly altered as a result of disturbances caused by bush encroachment. Encroaching woody species have been shown to decrease species richness and abundance of the seed bank and ground-layer diversity. So far little is known on the effect of bush encroachment and soil depth on the soil seed bank diversity in savanna rangelands. The objectives of this mini-dissertation were to (1) determine the influence of soil depth on soil seed bank diversity in bush encroached savanna rangelands, and (2) determine the relationships between soil seed bank herbaceous vegetation and physicochemical properties in encroached rangeland.

In order to address these objectives, a savanna rangeland was demarcated into two encroachment gradients spanning from open to encroached rangeland. Within each encroachment gradient, six plots of 10 m x 10 m were randomly selected, whereby soil sampling and herbaceous vegetation were carried out and determined. In each replicate plot per encroachment level, five soil samples were randomly collected at 0-10 and 10-20 cm depths. The number of seedlings of different species emerging from the soil samples was used as a measure of the number of viable seeds in the soil and the composition of the seed bank using the seedling emergence method.

The total seed densities showed significant differences ($P<0.05$) in the 0-10 cm depth layer in the open rangeland and encroached rangeland. Bush encroachment significantly ($P<0.05$) decreased the seed density of perennial grasses, specifically in 0-10 cm depth layer. Further, species diversity increased with bush encroachment in the 10-20 cm depth layer. Menhinick's richness index showed no significant difference in the open and encroached rangeland, while species evenness decreased in the 0-10 cm depth layer and increased at 10-20 cm depth.The study also revealed negative correlations between organic carbon, calcium, clay, silt and forbs while mean weight diameter (MWD), a measure of soil aggregate stability was positively correlated with forbs. The canonical correspondence analysis (CCA) showed that pH, phosphorus, potassium and calcium were positively correlated to *Eragrostis curvula* and magnesium was negatively correlated to *Panicum maximum.* In open rangeland, CCA revealed that clay content was negatively correlated with species evenness while magnesium was negatively correlated to the Shannon Weiner index. Further, silt content was positively correlated with species richness and evenness. In the encroached rangeland, the CCA showed a negative correlation between magnesium and the Shannon Weiner index. The Sørensen's index between soil seed banks and aboveground vegetation was low with index values of 0.22 and 0.24 in open and encroached rangeland, respectively.

Keywords: Bush encroachment, soil depth, soil seed bank, seed density, species richness, diversity, evenness, functional groups, soil properties, savanna and rangelands.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Background

Savanna rangelands are ecosystems characterized by the co-existence of scattered trees and shrubs with a continuous grass layer (Sankaran *et al*., 2004). However, it has been reported that the grass and tree balance has been highly altered as a result of disturbance (Savadogo *et al.,* 2008) in the form of bush encroachment (Ward, 2005). In recent times, it has been reported that for the past 60 years, savannas throughout the world have been altered by an increase in woody plants (Devine *et al*., 2017).

In South Africa, it has been reported that this widespread of woody plant species has been occurring since 1940 (Stevens *et al*., 2017), and its existence has been increasing at a tremendous rate since 1993 (Ward *et al*., 2014). This increase of woody plants species into previously open grasslands leads to bush encroachment. Bush encroachment is defined here as the suppression of palatable herbaceous plants by undesirable woody plants often unpalatable to livestock (Ward, 2005). Intensive woody plant encroachment into formerly open savanna rangelands decreases the existence of herbaceous plants and forms recurring patches which alter the structure and composition of naturally occurring plant communities (Wangen and Webster, 2006). Consequently, this alters ecosystem functioning. The undesirable increase of woody plants also results in the suppression of palatable and productive grasses (Ward, 2005), thereby reducing forage production which is the main feed for livestock. The reduction in biomass productivity decreases the profitability of these South African rangelands, thereby threatening livestock production. This poses a challenge to pastoral farmers as it affects the sustainability of communal rangelands (Tokozwayo, 2016). Furthermore, woody plants increase aboveground net primary productivity (ANPP) far above existing levels (Knapp *et al*., 2008), which is often correlated with a decline in plant species diversity in many herbaceous communities.

A decline in plant species diversity adversely affects soil seed bank diversity in woody plant encroached rangelands. Soil seed banks are the sum of viable seeds within the soil and in the litter layer on the soil surface (Thompson and Grime, 1979). Importantly, soil seed banks are the main representation of the regenerative potential of vegetation in areas that have experienced severe disturbance and display a view of the past environment and vegetation that was established previously (Bakker *et al*., 1996; Thompson, 2000).

1.2. Problem statement

Bush encroachment into previously open savanna rangelands leads to a decrease in seed production of understory grasses through microsites under canopies (Bakker *et al*., 2014). The undesirable increase of woody plants also results in the suppression of palatable and productive grasses (Ward, 2005). In South Africa, suppression of herbaceous vegetation continuously threatens livestock production especially in communal rangelands (Tokozwayo, 2016) as it leads to a decline in grazing capacity (Mogashoa *et al*., 2020). Livestock production in communal rangelands remains a fundamental foundation for rural livelihood, as livestock rely on these rangelands for their feed (Tokozwayo, 2016). Furthermore, woody plants increase aboveground net primary productivity (ANPP) far above existing levels (Knapp *et al*., 2008), which is often correlated with a decline in plant species diversity in many herbaceous communities. Moreover, bush encroachment limits seed dispersal, recruitment and establishment of understory grass (Gabay *et al*., 2012). A decline in plant species diversity adversely affects soil seed bank diversity in bush encroached rangelands.

1.3. Rationale

Rangelands form a part of the major source of forage for grazing herbivores and provide great economic, social, cultural and biological values (Belayneh and Tessema, 2017). Land cover transformation caused by the encroachment of woody species into previously open grasslands changes availability of resources by suppressing the development of herbaceous species within an area (Guido *et al*., 2017). The most notable changes entail the availability of light energy which is required for seed production and contributes to seed input in the soil seed bank (Scott *et al*., 2010; Bakker *et al*., 2014). Such disturbances induce changes in the composition of vegetation, which can result in the decrease of species diversity and richness (Yayneshet *et al*., 2009). Encroaching woody plants have also been shown to decrease species richness and abundance of the seed bank and ground-layer diversity (Price and Morgan, 2008). It is therefore crucial that in order to deepen our understanding of plant diversity, studies should not only focus on current above-ground vegetation cover, but also on species composition of underlying soil seed banks, which partly reflect the regional history of former vegetation types (Simpson *et al*., 1989). Soil seed banks are the aggregations of viable seeds in the soil potentially capable of replacing adult plants (Baker, 1989; Thompson and Grime, 1979). Soil seed bank are the main representation of the regenerative potential of vegetation in areas that have experienced severe disturbance and reflect a view of the past environment and vegetation that was established previously (Bakker *et al*., 1996; Thompson, 2000). Soil seed banks can alter grassland composition (Rice 1989), restore species-rich pastures (McDonald *et al*., 1996) and maintain floristic diversity in ecosystems (Willems, 1983). It serves as the potential pool of propagules for regeneration of grasses after any form of disturbance (Laura and Brenda, 2000; Snyman 1998). Improved understanding of seed bank diversity in savanna grasslands could also assist in designing conservation and restoration programs (Zaghloul, 2008) since soil seed banks can contribute substantially to the composition of future plant communities (Meissner and Facelli, 1999). It also plays a key role in sustaining native plant populations following disturbance (Thompson and Grime, 1979).

1.4. Purpose of the study

1.4.1. Research questions

1) What are the effects of bush encroachment on species density and species richness in savanna rangelands?
2) How do soil physicochemical properties from different soil depths influence soil seed bank diversity?
3) What is the relationship between the emerging herbaceous vegetation and physicochemical properties from bush-encroached rangelands?

1.4.2. Aim

The study seeks to investigate how bush encroachment influences soil seed bank diversity in savanna rangelands.

1.4.3. Objectives

The objectives of the study were to:

i) determine the influence of soil depth on soil seed bank diversity in bush encroached savanna rangelands.

ii) determine the relationship between soil seed bank herbaceous vegetation and physicochemical properties in encroached rangeland.

1.5. Dissertation structure

The dissertation is organized into five chapters with Chapter 1 outlining the background of the study, objectives and research questions. Chapter 2 reviews the existing literature on work that has been done on the topic. Chapter 3 addresses the influence of bush encroachment and soil depth on soil seed banks as well as implications for restoration. Chapter 4 addresses bush encroachment and soil depth effects on soil seed bank diversity in savanna rangelands. Chapter 5 presents a summary of the findings of the study and recommendations.

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CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Rangelands comprise of indigenous vegetation dominated by grasses, forbs or shrubs (Allen *et al*., 2011). In South Africa, rangelands cover more than 80% of the land surface (Fajji *et al.,* 2017). South African rangelands play a crucial role by contributing to the development of the economy through agriculture, particularly livestock production, which contributes substantially to food security (Meissner *et al*., 2013). Livestock provides a wide range of nutritious, protein-rich foodstuff, such as eggs, meat, milk and honey, which can be processed into a variety of products (Lesoli *et al*., 2013). In communal rangelands, livestock production is the main cornerstone for rural livelihoods and livestock are reliant on these rangelands for the forage.

South African savanna rangelands have been reported to be threatened by bush encroachment (Stevens *et al*., 2017; Moleele and Perkins, 1998; Devine *et al*., 2017). Bush encroachment is the increase in density, cover and biomass of woody plants across savanna rangelands (Ratajczak *et al*., 2012; Maestre *et al*., 2016; Guido *et al*., 2017). The increase in woody plant density reduces the grazing capacity of animals, thereby threating the livelihood of commercial and communal game farmers (Lesoli *et al*., 2013). The problem of bush encroachment is considered to be particularly acute in communal rangelands of South Africa where human and livestock population densities are very high and consequently heavy grazing, which is often considered to lead to bush encroachment is common (Kraaij and ward, 2006). Furthermore, bush encroachment increases aboveground net primary productivity (ANPP) far above existing levels (Knapp *et al*., 2008), and this is often associated with a decline in plant species diversity. A decline in plant species diversity adversely affects the soil seed bank diversity of bush encroached rangelands (Savadogo *et al*, 2017). The

2.2 Factors driving bush encroachment

Researchers have persistently attempted to explore the driving factors triggering encroachment of woody plants in the semi-arid environments (Archer *et al*., 1995; Briske *et al*., 2003; Ward, 2005). There are number of triggers and drivers of vegetation change in the savanna rangelands (Figure 2.1). Bush encroachment in savannas is mainly associated with overstocking, atmospheric carbon dioxide, precipitation availability and fire suppression (Devine *et al*., 2017).

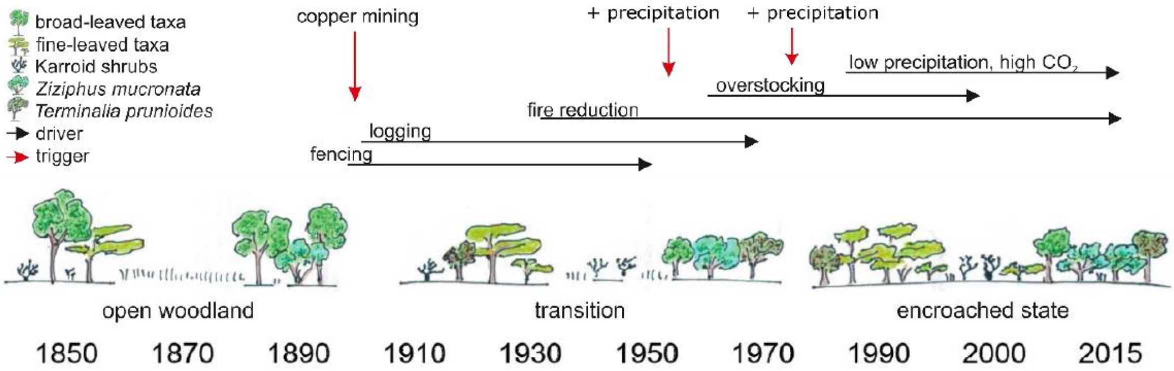

Figure 2.1. Network of triggers and drivers of savanna vegetation change (Tabares *et al*., 2019).

2.2.1 Overstocking

In semi-arid savannas, plant composition is dependent upon the types of herbivore species and their grazing frequency and intensity (Allred *et al*., 2012). Most studies have identified overgrazing as a driving factor of bush encroachment (Archer *et al*., 2017; Sankaran *et al*., 2008; van Auken, 2009). According to van Auken (2009), this factor favours the establishment and growth of woody species. Smit (2004) reported that increased stocking rates promotes the reduction of growth and reproductive rate of herbaceous species thus influencing competition among different plant species. According to Moleele and Perkins (1998) degradation of rangelands, including encroachment of bushes, is linked with high density of cattle around the water points such as boreholes (wells) and kraals in semi-arid African savannas. Moreover, Abule *et al*. (2007) noted that besides heavy grazing, expansion of cultivation and inhibition of animals' mobility are also important factors contributing to bush encroachment. It should be stressed that overgrazing is the dominant factor leading to bush encroachment since it removes grass species and favours growth of woody plants (Skarpe, 1990). Grazing was also described to contribute to bush encroachment through seed dispersal of encroaching woody plant species (Scholes and Archer, 1997). However, there are also studies that dispute against the over-grazing proposition. For example, Oba *et al*. (2000) found that heavy grazing was not related significantly to the cover of bush in semi-arid rangelands of Borana and that the adopted mitigation protocols such as decreasing livestock concentrations in periods of low rainfall were unsuccessful in decreasing bush encroachment (Ward, 2005).

2.2.3 Elevated levels of atmospheric carbon dioxide

Amplified atmospheric CO_2 levels have been deemed as the reason for encroachment of C_3 woody plants into grasslands and savannas throughout arid and semi-arid rangelands (Idso, 1992; Polley *et al*., 1992). Atmospheric CO_2 concentration facilitates the growth and functioning of plants, which is known to increase woody plant cover. An increase in CO_2 increases photosynthetic rates of woody species by 30-50% compared to grasses (Eamus and Palmer, 2007). According to Eldridge *et al*. (2011), an increase in global CO_2 concentration may have promoted C_3 species of woody plants at the cost of C_4 grasses.

2.2.2 Precipitation availability

Precipitation has a major influence on species richness and productivity in savanna grasslands (Chen *et al*., 2015). Precipitation is a defining characteristic of bush encroachment in semi-arid savannas. Due to the highly irregular precipitation and the vanishing of palatable grasses during the dry seasons, trees and shrubs become an important part in semi-arid environments (Gemedo *et al*., 2006). Under such environments, bush encroachment is always related with annual and inter-annual moisture variabilities (Briske *et al*., 2003; Angassa and Oba, 2007). At local scales, a continual wet period (i.e., a high amount of mean annual precipitation in multiple and consecutive years) can effectively promote a considerable increase in the cover of woody vegetation (Kerstin *et al*., 2005; Sankaran *et al*., 2005). For example, the encroaching of *A. mellifera* needs a minimum of three years of successive higher-than-average precipitation to recruit successfully. The importance of precipitation in controlling woody plant growth is well demonstrated by Sankaran *et al*. (2005) who reported that the percentage of woody plants is linearly correlated with the mean annual rainfall in African savannas. Specifically, an increase in soil moisture allows the seedlings of woody plant species to stay alive and to establish into bush coppices.

2.2.4 Fire reduction

Fire plays a key role in managing savanna rangelands (Bond and Archibald, 2003). Fire reduces the height and cover of woody plants resulting in tree mortalities which help to prevent woody plant encroachment (Hoffmann and Solbrig, 2003). Without fire, woody plants increase in density, height and cover and this causes bush encroachment in savanna rangelands. According to Trollope (1980) and Bond *et al*. (2003), long term absence of fire allows for increases in woody plant seedlings and saplings to develop to fire resistant stages. Therefore, bush encroachment triggered by fire reduction leads to reductions in fine fuel and less intense fires in future burnings, and this further promotes woody encroachment (Devine *et al*., 2017).

2.3 Effect of bush encroachment on herbaceous vegetation and soil properties

Over the past 100 years till present, there has been a directional shift towards increased abundance of woody plant species worldwide (Sala and Maestre, 2014), a phenomenon referred to as bush encroachment (Stevens *et al*., 2017). The effect is brought about by a change in the savanna state from open woodlands to dense savanna. An increase in the woody plant density beyond a critical density results in the suppression of herbaceous plants, mainly due to severe competition for available soil water, thus lowering the yield of the herbaceous layer and also the grazing capacity consequently affecting livestock management (O'Connor, 1991; Smit and Rethman, 1998). Herbaceous species compete with woody plants for resources (e.g. water) in the topsoil, and reduction of herbaceous growth by grazing also reduces the competitive vigour of herbaceous species, potentially enhancing woody plant growth (Hoffman and Ashwell, 2001; Riginos *et al*., 2009). This increases density of woody species resulting in the reduction of biomass production that in turn decreases food security as livestock enterprises are affected (Keno and Suryabhagavan, 2014). Bush encroachment does not only affect the ecosystem structure, but also alters ecosystem processes such as nutrient cycling as well as soil chemical and physical properties, thereby negatively affecting the primary productivity of many arid and semi-arid savanna rangelands (Belayneh and Tessema, 2017). A review of the existing literature indicates that woody encroachment may have variable effects on soil organic matter. For example, comparative studies (i.e., between encroached and non-encroached sites) conducted in African (Gill and Burke 1999; Hudak *et al*., 2003) and North American rangelands (Springsteen *et al*., 2010) have shown that woody encroachment may increase the organic matter content of the soil. While other studies have reported either a decline (Wessman *et al*., 2004) or no net change (Smith and Johnson 2003) in soil organic matter in response to woody encroachment. Soil carbon (C) and nitrogen (N) increased 100– 500% during 130 years of woodland development in areas that were once grassland (Liao e*t al*., 2006). In contrast to the studies reported above, Mesele *et al*. (2006) found that bush encroached areas have less organic matter content than the other fields in a Borana rangeland. Dalle *et al*. (2006) found that P, pH, Mg, Ca, CEC and silt were also positively correlated with woody plants in the semi-arid Borana lowlands, southern Oromia, Ethiopia.

2.4. The importance of soil seed banks in savanna rangelands

Soil seed bank is a reflection of past vegetation and a contributor of future community structure and dynamics (Johannsmeier, 2009). Soil seed banks are important determinants of the initial floristic composition following disturbance (Savadogo *et al*., 2017). Soil seed bank can be classified into two types. The first consists of transient seeds which persist in the soil for less than a year. The second entails persistent seeds, which persist for five years or more (Gioria and Pysek, 2016). Soil seed banks play key role in maintaining genetic and ecological diversity of rangelands.

The soil seed bank also influences the function of the temporal and spatial patterns of the disturbance regime, which in turn affects both ecosystem resistance and resilience (Snyman, 2004). Developing strategies which may help conserve, restore and manage disturbed rangelands is essential for the provision of goods and services through livestock production. However, these costs can be avoided if enough seeds of desirable species are found in the soil seed bank (Savadogo *et al*., 2017). Knowledge of soil seed bank dynamics is important for understanding the development and dynamics of rangelands and this can be used as a management tool for conservation.

2.5. Impact of bush encroachment on soil seed bank and soil seed bank diversity

In recent times, it has been reported that rangelands are threatened by degradation associated with soil erosion, invasion of alien plant and bush encroachment (Lesoli *et al*., 2013). Bush encroachment is considered to be one of the most extensive form of land degradation in South Africa (Joubert *et al*., 2009), negatively impacting both wild and domestic herbivores. The undesirable increase of woody plants results in the suppression of palatable and productive grasses (Ward, 2005). The suppression of herbaceous vegetation threatens livestock production and the sustainability of rangelands (Oba *et al*., 2000). The suppressive effect of encroaching woody species reduces forage production, which is valuable to livestock. This leads to a decline in profitability as woody plants decrease the forage quality of rangelands (Ward, 2005).

Species diversity can be assessed using the richness (number of species in each area) and evenness (relative abundance or biomass distributed among species) (Wilsey and Stirling, 2007). Increasing woody plant species may adversely affect soil seed banks (Savadogo *et al*., 2017). For instance, a study conducted by Price and Morgan (2008) which assessed the impact of an indigenous shrub *Leptospermum scoparium* (Myrtaceae) on herb-rich *Eucalyptus camaldulensis* woodlands in southern Australia, reported that the encroaching shrubs reduced species richness and abundance of the seed bank and decreased ground-layer diversity. It has also been reported that woody plant species dominate above ground vegetation of encroached areas, while the soil seed bank is dominated by grasses and herbaceous vegetation (Chapano *et al*., 2013). Snyman (2013) mentioned that savanna rangelands have large persistent seed banks, often with species that show no correlation with the aboveground vegetation.

2.6. Effect of bush encroachment and soil depth on soil seed bank density

Seed germination and emergence are influenced by the position of seeds in the soil profile (Sheldon, 1997). The position of seeds in the soil seed bank profile can affect seedling emergence by influencing germination via environmental factors such as light, moisture, oxygen and temperature (Traba *et al*., 2004). Generally, there is a decrease in density and richness of the soil seed bank as well as a change in composition along different soil depth layers. This was confirmed by a study conducted by Kebede *et al*. (2012), assessing soil seed bank and seedlings bank composition and diversity at the Wondo Genet Moist Afromontane Forest. The authors reported a decline in species richness for seedlings that germinated from the soil seed bank at varying soil depth intervals. A total number of 54 species germinated from the uppermost soil layer (0-3 cm), 32 from the middle layer (3-6 cm) and 24 species from the deeper layer (6-9 cm). Soil seed bank density also declined progressively with soil depth. The density of germinated seedlings was 375 seedlings/m^2 in the upper layer, 180 m^{-2} in the middle layer and 104 seedlings/m^2 in the deeper layer. This pattern is assumed to reflect the input of seeds at the surface of the soil. Stark *et al.* (2003) also reported a lower grass seed bank density under shrub encroached grasslands.

However, (Eshete *et al.,* 2020) reported a high grass seed density in Afar Grazing lands of Ethiopia encroached by *Prosopis juliflora*. The high seed density was due to some woody plant covers characterized by stands that enabled them to protect seeds from being blown away by wind or washed out by runoff. Larvorel *et al.* (1993) postulated that most of the viable seeds are usually denser in the first few centimeters of the surface via contribution by the aboveground vegetation. Another possibility for the differences in seed bank densities across the soil depth may be due to the ability of seeds to penetrate the soil profile (Thompson *et al.,* 1993). Muvengwi and Ndagurwa (2015) documented that 98% of soil samples collected below 15 cm of the soil contains no seeds, and this may be as a result of seed predation and dormancy. The deeply buried seeds remain dormant for a long period because light as a germination cue does not penetrate too deep in the soil (Benvenuti *et al*, 2001).

2.7. Impact of physico-chemical properties on soil seed bank

Since soil characteristics affect the community composition and diversity, it is important to study the soil physico-chemical properties (Heydari *et al*., 2013). Soil characteristics are also important because they have an impact on soil seed bank composition and seed germination capacity. Soil physical and chemical properties have the ability to alter the growth of certain plant species. As such, these attributes may explain the presence and relative abundance of plant species (Lousadal *et al*., 2013). Heydari *et al*. (2013) found a low number of species in the soil seed banks of disturbed sites compared to undisturbed sites. This was linked to the compaction of soil caused by anthropogenic disturbances associated with livestock trampling. This is because disturbed sites are characterised by low amounts of litter and organic matter. Another soil properties that influences soil seedbank diversity is soil texture. Work done by Lousada *et al*. (2013), found a positive correlation between density of the soil seed banks of weeds under clayey textured soils and a negative correlation in sandy textured soil. A study conducted by Tessema *et al*. (2012) revealed that the differences in soil texture leads to variation in species composition, number of species and success of seed germination.

2.8. Relationship between soil seed bank and aboveground vegetation

Investigating the relationship between soil seed bank and aboveground vegetation is important because it helps in understanding the previous management of a rangeland in question, to understand the history of the existing aboveground vegetation and to determine conservation and management potential of the rangeland (Lopez-Marino *et al*., 2000). It has been reported that grasses are less abundant or completely absent in the soil seed bank and are most abundant in the aboveground vegetation due to their reliance on vegetative recovery (Bakoglu *et al*., 2009) and production of short persistence seeds (Shaukat *et al*., 2004). If there is high correspondence between the soil seed bank and above-ground vegetation, then soil seed bank will be effective in conserving and managing disturbed plant communities (Gomaa, 2012). This is because soil seed banks provide new seedlings for re-establishment of plant communities after any kind of disturbance (Naghipour *et al*., 2015). A correlation between the soil seed bank composition and aboveground vegetation is attributed to lack of seed dispersal away from the parent plant and long seed persistence (Henderson *et al*., 1988). Heydari *et al*. (2013) observed low similarities between soil seed banks and aboveground vegetation in disturbed and undisturbed areas. In contrast, studies conducted by Chapano *et al. (*2013) and Diaz Villa *et al*. (2003) found an increase, while Peco *et al*. (1998) reported no change. Improved knowledge of seed bank dynamics, and their correlation with standing vegetation and disturbances is important for improved understanding of plant community dynamics and development of management practices that are appropriate for managing disturbed vegetation (Savadogo *et al*., 2017).

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CHAPTER 3

INFLUENCE OF BUSH ENCROACHMENT AND SOIL DEPTH ON SOIL SEED BANK: IMPLICATIONS FOR RESTORATION

Abstract

Bush encroachment has been described as the gradual conversion of grasslands to woody or shrub dominated savannas. In recent years, many grasslands and savannas around the world have experienced an increase in woody plant cover at the expense of herbaceous cover and production. Bush encroachment affects the soil seed bank by limiting seed dispersal, recruitment and establishment of understory grass, thereby decreasing species density. The objective of the study was to assess the effect of bush encroachment and soil depth on seed density, grass composition of the soil seed bank and to explore the relationship between species and soil properties. Sampling was done at two encroachment levels established in open and bush encroached rangelands. Soil samples were collected at two depth intervals (0-10 and 10-20 cm) within the encroachment levels. The soil seed bank was determined using the seedling emergence method. A total of six grass species emerged from the soil seed bank. Seed densities declined significantly ($P<0.05$) at 0-10 cm depth layer in the open and encroached rangelands. Bush encroachment significantly ($P<0.05$) decreased the densities of perennial and weak perennial grasses at the 0-10 cm depth layer. The canonical correspondence analysis (CCA) revealed that pH, phosphorus, potassium and calcium were positively correlated to *Eragrostis curvula* while magnesium was negatively correlated to *Panicum maximum.* The analysis also revealed a negative correlation between organic carbon, calcium, clay, silt and forbs while mean weight diameter was positively correlated to forbs.

Keywords: Bush encroachment, soil depth, soil seed bank, seed density, soil properties, savanna and rangelands.

3.1. Introduction

Bush encroachment is the gradual conversion of grasslands to woody and shrub dominated savannas (Hudak *et al*., 2003). In recent years, many grasslands and savannas around the world have experienced an increase in woody plant cover at the expense of herbaceous vegetation (Van Auken, 2000). This is caused by the higher competition between woody plants and herbaceous species for light, soil moisture and nutrients (Mckinney and Goodell, 2010). The increase in encroaching woody plants results in the suppression of palatable grasses and herbs which are often found to be unpalatable to domestic livestock (Ward, 2005). The dominant tree cover substantially suppresses the growth of high-value herbaceous forage species in the understory, reduces indigenous plant biodiversity and alters rangeland ecosystem functions (Rundel *et al*., 2014; Scholes and Archer, 1997). By supressing the growth of palatable grasses, bush encroachment further reduces the carrying capacity for livestock (Ward, 2005). This leads to a decrease in productivity and profitability of rangelands (Mugasi *et al*., 2000). Moreover, woody plants increase aboveground net primary productivity (ANPP) far above existing levels (Knapp *et al*., 2008), which is often correlated with a decline in plant species diversity in many herbaceous communities. A decline in plant species diversity adversely affects the soil seed bank of woody plant encroached rangelands (Görzen *et al*., 2019).

Soil seed bank is all viable seeds present on or in the soil, and the associated surface litter is considered crucial for the persistence of many flora species following ecosystem disturbance (Fenner and Thompson 2005). Most importantly, the soil seed bank not only indicates the past vegetation composition but also potential future communities and acts as a reservoir for conservation and restoration of plant species diversity (Bakker *et al*., 1996). Bush encroachment affects the soil seed bank by limiting seed dispersal, recruitment and establishment of understory grass (Pugnaire and Lázaro 2000; Gabay *et al*., 2012), therefore decreasing species density.

Soil depth is another important factor that influences the soil seed bank. Seed burial depth has the potential to affect seedling emergence by influencing germination (Bewley and Black, 1994; Baskin and Baskin, 2001). The impact of bush encroachment on soil seed bank at varying soil depth layers is poorly documented in the South African rangelands, therefore, it is crucial to gain an understanding of the relationships between bush encroachment and soil seed bank dynamics at varying soil depth layers since this relationship is vital in restoring seeds and establishing seedlings of grass species in encroached grasslands. The objective of this study was to assess the effect of bush encroachment and soil depth on seed density, species composition and to explore the relationship between the species and inherent soil properties.

3.2. Materials and Methods

3.2.1. Description of study site

The study was conducted at Bela Bela's Towoomba Pasture Research Station (28°21 'E, 24°25'S) in Waterberg District of Limpopo Province, South Africa (Figure 3.1). The climate of the study area consists of a mean annual rainfall and temperature of 629 mm and 29˚C (Mills *et al*., 2017), respectively. Soil in the study area is mostly red loam of the Bainsvlei form (Orthic A/red apedal B/soft plinthic B) (Soil Classification Working Group, 1991) or Plinthic (IUSS Working Group WRB, 2014). The vegetation of the study area is classified as Springbokvlakte Thornveld (Mucina and Rurtherford, 2006). The tree layer consists of *Dichrostachys cinerea,* and *Acacia* species. The grass layer is dominated by *Eragrostis* species (*E. barbinodis and E. rigidior*), *Panicum maximum, Themeda triandra* and *Heteropogon contortus* (Table 3.1).

Table 3.1. Site characteristics of open and bush-encroached grassland

Intensity	Tree density Trees. ha^{-1}	Location	Elevation (m.a.s.l)	Slope (%)	Soil form	Species
Open	800	S23º50,66' E29º41,97'	1236-	0-2	Bansvlei	Trees: *Vachellia karoo, Vachellia gerrardi, cineria, Dovylis caffra, Grewia flava, Gymnosporia* Grasses: *Panicum maximum, Aristida congesta, Heteropogon contortus, Eragrostis rigidior Hyperthelia dissolute, Cymbopogon pospichilli Schmidtia pappophroides*
Encroached	1700	S23º50,673' E29º41,948'	1234-	0-2	Bansvlei	Trees: *Vachellia karoo, Vachellia gerrardi, Dichrostachys cineria, Flueggea virosa.* Gasses: *Panicum maximum, Aristida congesta, Schmidtia pappophroides, Cymbopogon pospichilli.*

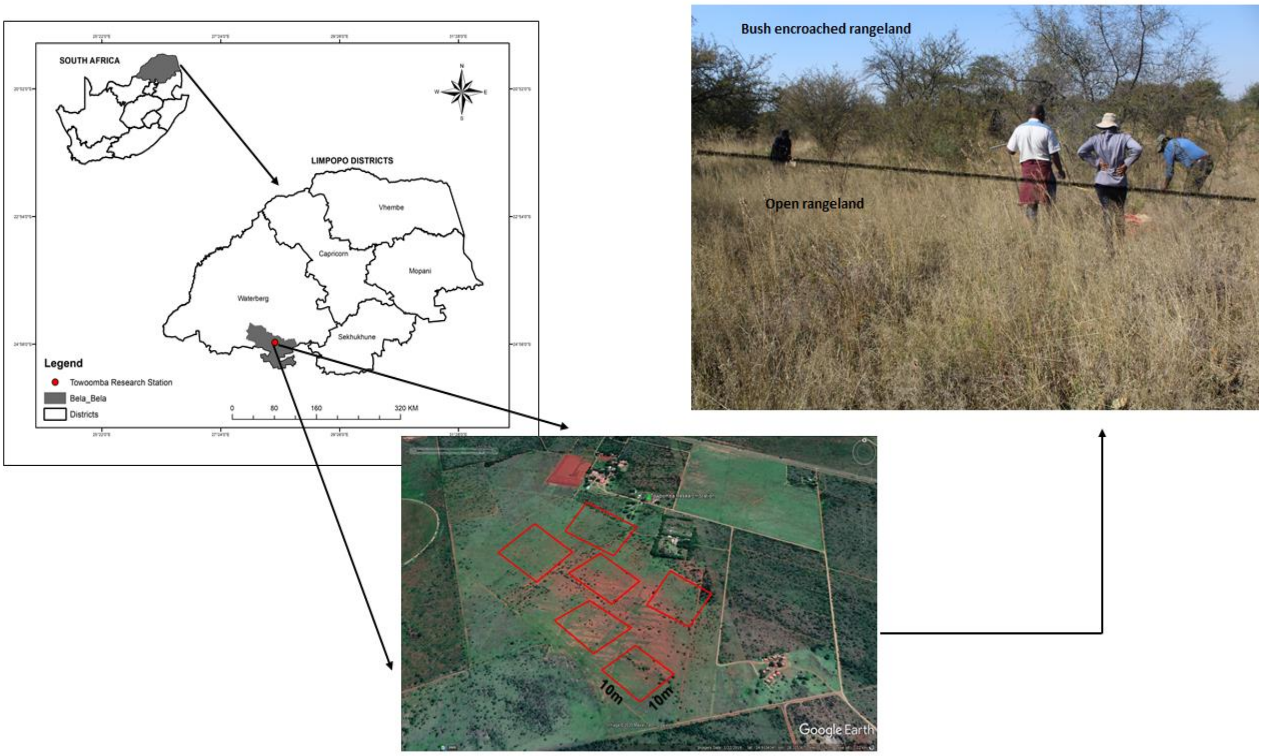


Figure .3.1 Map of the study area, Towoomba Pasture Research Station, Waterberg District, Limpopo Province, South Africa

3.2.2. Site selection and sampled plots

Field soil and vegetation surveys were carried out across the rangeland. Both surveys were conducted along the encroachment intensity ranging from open to bush encroached savanna rangeland. In each bush encroachment (BE) level, six 10 m x 10 m plots were randomly selected and marked on the same plinthic soil type (bainsvlei), with similar elevation and landform.

3.2.3. Vegetation survey and soil seed bank sampling

All woody species occurring within each 10 m x 10 m plot per encroachment level were counted and identified following van Wyk and van Wyk (1997). The diameter (longest and shortest) of individual trees were measured and recorded using a 2 m rod. Plant height and lowest browsable height (LBH) were also measured and recorded using the same 2 m rod. In each replicate plot, per encroachment level, five soil samples were randomly collected at 0-10 and 10-20 cm depths, which entails the position of transient and persistent seed banks, giving a total of 120 soil samples (2 BE levels x 6 plots x 5 soil samples x 2 depths). In addition, undisturbed core samples for bulk density determination were collected following the same sampling strategy. The collected soil samples were bulked per soil depth and per encroachment level, giving a total of 24 samples (BE levels x 2 depths x 6 plots). Bulked soil samples were extruded into polythene sample bags, sealed and taken to laboratory where they were air dried and passed through a 2-mm aperture metal sieve to remove visible plant material for seed bank germination and soil chemical and physical analysis.

3.2.4. Soil analysis

In the laboratory, soil aggregate stability was determined using the wet-sieving method (Six *et al*., 1998). Bulk density was determined from the intact soil cores extracted from the field following Blake and Hartge (1986). Soil pH was determined in a 1:2.5 solution ratio in 1 M KCl suspension using a glass electrode. Exchangeable cations Ca and Mg were first extracted in 1M KCl, while P, K, were extracted in an Ambic 2 extractant containing 0.25M NH_4HCO_3. The detection of the extracted cations was done by inductively coupled plasma optical emission spectrometry (ICP-OES) using an Optima 7300DV spectrometer (Perkin Elmer, Inc., 2 Shelton, CT). Organic carbon content was determined by the Walkley-Black method (Walkley and Black, 1934) (Table 3.2).

3.2.5. Soil seed bank experiment

Table 3.2 shows the basic characteristics of soil samples used for the soil seed bank experiment. The number of seedlings of different species emerging from the soil samples was used as a measure of the number of viable seeds in the soil and the composition of the seed bank (Roberts, 1981). The seedling emergence method was used to estimate the number of seeds in the soil. The seedling emergence method was used because it is more appropriate than actual identification of seeds (Gross, 1990; Espeland *et al*., 2010). It determines the relative abundance of viable seeds that can germinate and excludes the non-viable seeds (Poiani and Johnson, 1988). The germination experiment was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo (23^{o} 53' 10''S, 29^{o} 44' 15''E). The minimum and maximum ambient temperatures are 21 ^{o}C and 28 ^{o}C, respectively. In the greenhouse**,** a completely randomized design (CRD) was used whereby three plastic pots (size= 20 cm) were used per composite soil sample, per soil depth and encroachment level, totaling 72 plastic pots (2 BE level x 2 depths x 6 plots x 3 replications) (Figure 3.2). The soil was spread over sand and hygromix to a depth of 30 mm. Pots were examined every 3 days for the first 2 months and thereafter weekly until the end of the experiment (Tessema *et al*., 2012). Seedlings started to emerge after 1 week, and were allowed to grow until they were identifiable. Thereafter, they were identified, counted, recorded and discarded. Each pot was hand-watered regularly until field capacity. The experiment was carried out for a period of 6 months (September 2019-February 2020). This is because the number of emerging seedlings, particularly grasses and forbs declined considerably after this period.

Figure 3.2. Experimental layout of the seedling emergence experiment in the glasshouse

Table 3.2. Characteristics of soil used for potting in the greenhouse

Soil properties	0-10 cm		10-20 cm	
	Open	Encroached	Open	Encroached
pH (KCl)	5.23 ± 0.05	4.45 ± 0.07	5.18± 0.05	4.50± 0.05
OC g/kg	6.17 ± 0.37	5.33 ± 0.39	5.92± 0.52	5.97± 0.31
Clay%	6.83 ± 0.46	11.50 ± 0.96	10.33± 1.41	13.67± 2.22
Silt%	4.83 ± 0.67	7.17 ± 0.58	4.33± 0.95	9.67± 2.28
Sand%	88.33 ± 0.73	81.33± 1.08	85.33± 1.22	76.67± 2.11
P mg/L	3.00 ± 0.12	1.17 ± 0.11	2.67± 0.49	1.00± 0.00
K mg/L	258.50 ± 23.86	193.83 ± 16.01	251.17± 40.64	163.00± 14.97
Ca mg/L	508.25 ± 11.32	489.67 ± 35.15	473.00± 10.79	486.58± 69.72
Mg mg/L	209.42 ± 4.28	162.42 ± 6. 66	214.33± 5.17	177.67± 6.30
Textural class	sand	loamy sand	sand	loamy sand

3.2.6. Statistical analysis

The number of seedlings was expressed as seed density per m^2. Species composition was calculated using EXCEL (Microsoft, 2013). Student t-test was applied to compare the density of the soil seed bank and life form species density (i.e. weak perennials and perennials) among the encroachment levels along soil depth gradient at the significance level of $P \leq 0.05$. Statistical analyses were performed using SAS (version 9.4, 2019). Canonical correspondence analysis (CCA) was performed using species composition data and soil variables (pH, organic carbon, phosphorus, Potassium, calcium, magnesium, clay content, silt content and mean weight diameter). Before analysis, species composition matrix was transformed using square root sqrt(x) transformation to reduce the influence of the most abundant species. Canonical correspondence analysis (CCA) was conducted using the vegan package (Oksanen et al., 2019) in RStudio of R Version 3.5.1 (R Core Team, 2018). A permutation test (999 cycles, function ANOVA.cca in Vegan package) was performed to test which variables explained a significant part of the variation in seed bank composition.

3.3. Results

3.3.1. Seed bank density and functional groups in open and encroached rangelands

The total seed bank density in the open and encroached savanna rangelands at 0-10 cm and 10-20 cm depth layer is shown in Figure 3.3. In the 0-10 cm layer, bush encroachment significantly ($P<0.05$) decreased seed bank density. In the 10-20 cm layer, there were no significant differences ($P>0.05$) between open and the bush encroached grassland. Soil seed bank density of perennial and weak perennial grasses at 0-10 cm and 10-20 cm soil depth layers in the open and encroached rangeland are presented in Figure 3.4. Bush encroachment significantly ($P<0.05$) decreased the seed density of weak perennial species in the 0-10 cm depth layer, while there was no impact ($P>0.05$) in the 10-20 cm layer (Figure 3.4a). Bush encroachment also decreased ($P<0.05$) the presence of perennial grasses in the 0-10 cm depth layer, while in the 10-20 cm depth layer there was no apparent change ($P>0.05$) when comparing open and bush encroached grassland (Figure 3.4b).

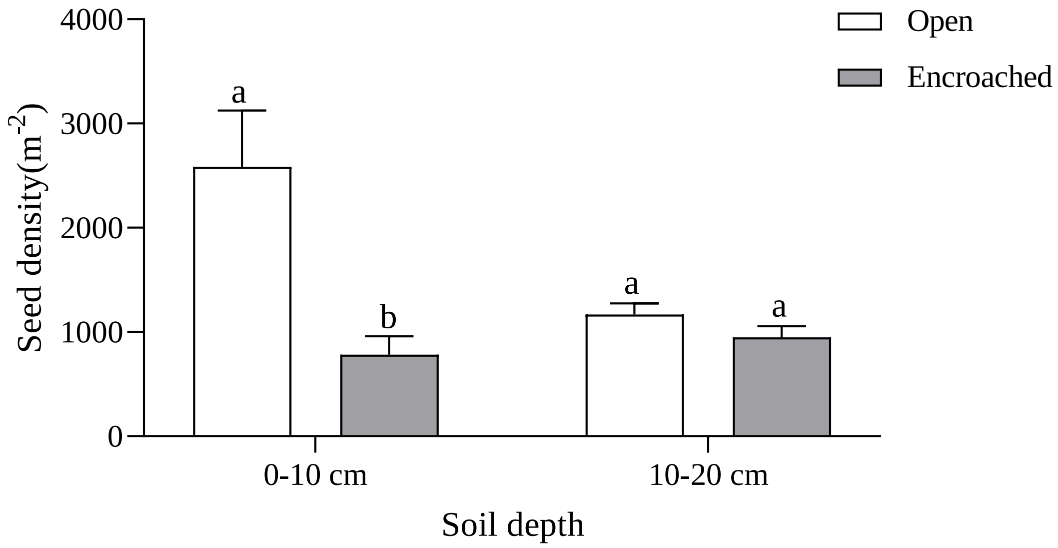
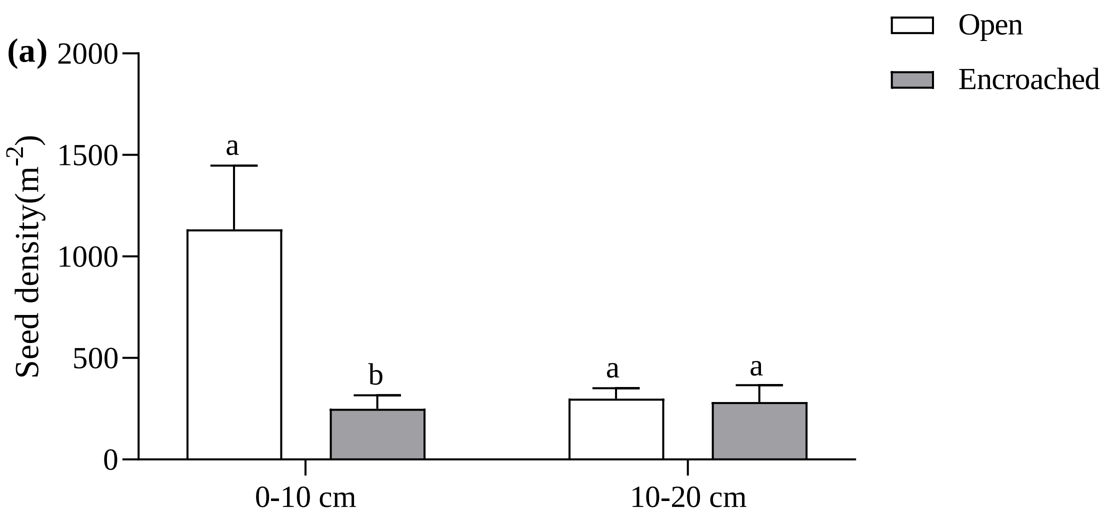


Figure 3.3. Effect of bush encroachment on seed density at various soil depths. Means with different superscripts indicate significant differences between treatments $P \leq 0.05$



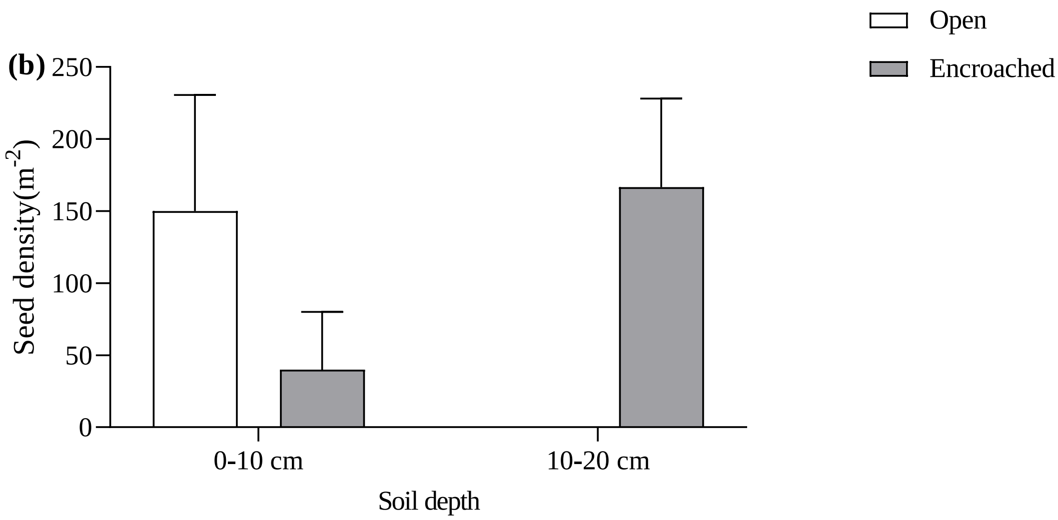


Figure 3.4. Effect of bush encroachment on seed density of **(a)** Weak perennial grasses and **(b)** Perennials grasses at various soil depths. Means with different superscripts indicate significant differences between treatments P ≤ 0.05

3.3.2. Soil seed bank composition in open and encroached rangeland

A total of 4 grass species emerged from the soil seed bank in the open rangeland while in the encroached rangeland 6 species were observed over the period of the study (Table 3.3). The proportion of increaser II species was larger than decreaser species in both rangelands. In the 0-10 cm depth layer of the encroached rangeland, the grass layer was dominated by *Eragrostis curvula* (74.3%), forbs (22.9%), *Panicum maximum* (1.4%) and *Pogonarthria squarossa (*1.4%), while the open grassland soil was dominated by forbs (50.3%), *Eragrostis curvula* (42.6%), *Panicum maximum* (4.5%), *Eragrostis rigidior* (1.3%) and *Pogonarthria squarossa* (1.3%). The 10-20 cm soil layer of the bush encroached rangeland was dominated by forbs (57.1%), *Eragrostis curvula* (22.2%), *Digitaria eriantha* (12.7%), *Eragrostis rigidior* (4.8%) and *Urochloa mosambicensis* (3.2%), while the dominant grasses in the open grassland soil were forbs (74.3%), *Eragrostis curvula* (18.5%) and *Eragrostis rigidior* (7.1%).

Table 3.3. Ecological status and relative abundance of grass species and forbs from the seed bank of open and bush encroached rangeland at 0-10 cm and 10-20 cm soil depths

Species	Ecological status	0-10 cm Relative abundance (%)		10-20 cm Relative abundance (%)	
		Open	Encroached	Open	Encroached
Eragrostis	Inc II	42.6	74.3	18.6	22.2
Panicum	Dec	4.5	1.4	0.0	0.0
Pogonarthria	Inc II	1.3	1.4	0.0	0.0
Eragrostis	Inc II	1.3	0.0	7.1	4.8
Urochloa	Inc II	0.0	0.0	0.0	3.2
Digitaria	Dec	0.0	0.0	0.0	12.7
Forbs		50.3	22.9	74.3	57.1

Note: Inc II-Increaser II species, Dec-decreaser species, (-) indicates absence of species (Oudtshoorn, 2014)

3.3.3. Relationships between soil seed bank composition and soil variables

Figure 3.5 Shows a Canonical correspondence analysis (CCA) of soil seed bank composition in relation to soil variables (pH, organic carbon, phosphorus, calcium, magnesium, clay, silt and mean weight diameter). The first two axes of the Canonical correspondence analysis (CCA) biplot explained 56% of the total variation in open rangeland (Figure 3.4a). The Canonical correspondence analysis (CCA) revealed that organic carbon, calcium, clay and, silt were negatively correlated with forbs while mean weight diameter was positively correlated with forbs, even though, the Canonical correspondence analysis (CCA) model was not significant (P=0.662. F=0.819). In encroached rangeland, the first two axes of the CCA biplot explained 39% of the total variation (Figure 3.4b). The Canonical correspondence analysis (CCA) showed that pH, phosphorus, potassium and calcium were positively correlated to *Eragrostis curvula* while magnesium was negatively correlated to *Panicum maximum*, even though CCA model was not significant (P=0.875, F=0.6473)

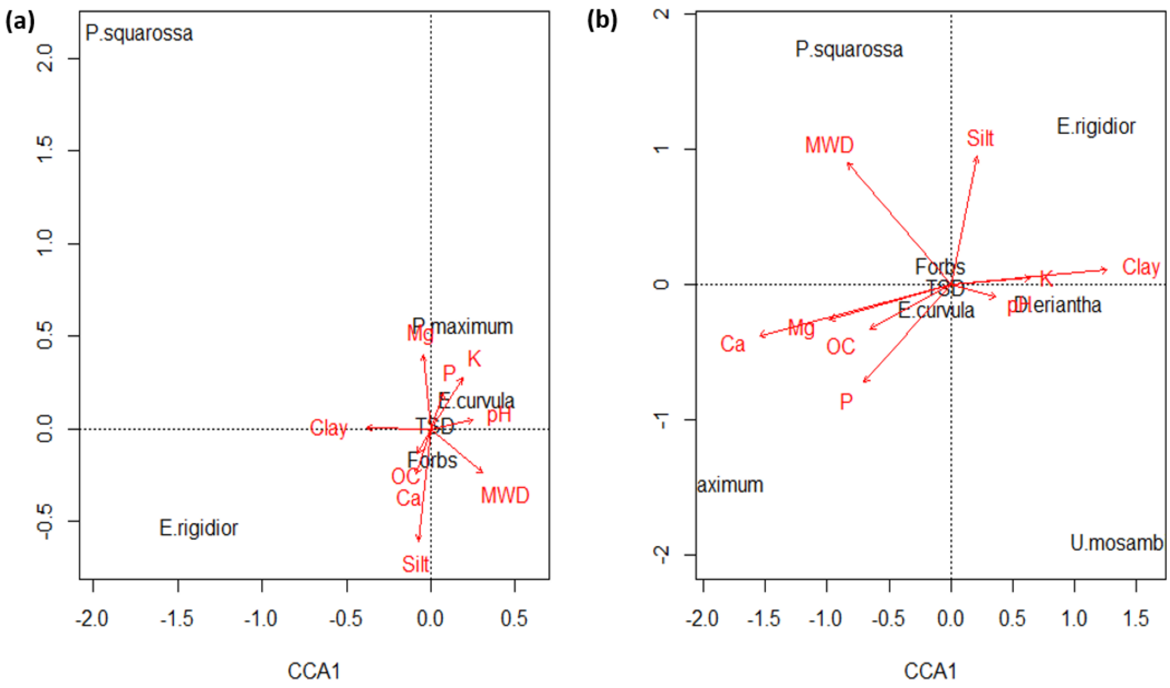


Figure.3.5. Canonical correspondence analysis (CCA) between soil seed bank herbaceous vegetation and environmental factors in (a) open rangeland and (b) encroach rangeland. *Key to species*: *E.curvula=Eragrostis curvula, D.eriantha=Digitaria eriantha, Eragrostis rigidior, U. mosambicensis=Urochloa mosambicensis E. rigidior= Eragrostis rigidior, P. maximum=Panicum maximum, P.squarossa= Pogonarthria squarossa. Key to soil properties: K=Potassium, P=Phosphorus, Mg=Magnesium, OC=organic carbon, Ca=Calcium, pH, Clay, Silt, MWD=Mean weight diameter. TSD=Total seed density*

3.4 Discussion

3.4.1. Effect of bush encroachment on soil seed bank density and functional groups across various soil depths in the open and encroached rangeland

Woody plants often negatively affect the density of resident seed banks (Holmes, 2002; Wearne and Morgan 2006; Giantomasi *et al*., 2008; French *et al.,* 2011; Gaertner *et al*., 2011; González-Muñoz *et al*., 2011; Marchante *et al*., 2011). This study confirmed that indeed bush encroachment in savanna rangelands considerably affects soil seed bank density. In this study it was found that woody plant encroachment reduces seed density in the encroached rangeland in the uppermost soil layer (0-10 cm). This could be attributed to the shade imposed by woody plants which limits seed production of grass species (Van Calster *et al*., 2008; Tessema *et al*., 2017). The low seed bank size of grasses in encroached sites is in line with the findings of Bakker *et al*. (1996) who confirmed that bush encroachment reduced soil seed bank size only in the upper soil depth layer in dry alva grasslands. Mndela *et al*. (2019) studied a shrub encroached semi-arid savanna rangeland in Springbok thornveld of Mpumalanga Province of South Africa and found a depletion of seed density in the topsoil layer (0-10 cm). Bossuyt *et al*. (2006) also reported a relatively low seed density in the encroached calcareous grasslands soils in Southern Belgium. The low seed production and poor establishment of plants between woody canopies as a result of low light are reported to be the main causes of low seed bank densities (Bakker *et al*., 2014).The present study also revealed that seed densities of weak and strong perennials increase with an increase in bush encroachment in the uppermost soil layer. This was consistent with results of Tedder *et al*. (2012) who found seeds of perennial grasses to be dominant in the soil seed bank sampled at the upper 10 cm depth layer, in the Okavango Delta, Botswana. This is, however, in contrast to other studies. For example, a study conducted by Mndela *et al*. (2020) at Maseding and Kgomokgomo in Bojana District Municipality, North-West Province found annual species to be dominating the seed bank. While, Figueroa *et al*. (2004) found annual grasses to be dominating the seed bank in the Mediterranean shrubland of Chile where the authors were investigating the composition, size and dynamics of the seed bank.

3.4.2. Effect of bush encroachment on dominating species in the soil seed bank

In the present study *Eragrostis spp* (i.e. *Eragrostis curvula* and *Eragrostis rigidior)* dominated the bush encroached rangeland at uppermost soil layer. Similarly, Kassahun *et al*. (2009) found *Eragrostis spp* (*Eragrostis cilianensis* and *Eragrostis nubica*) to be dominating the seed bank in the rangelands of the Somali region, eastern Ethiopia. The dominance of *Eragrostis curvula* can be explained by the ability of the species to produce large quantity of seeds with full survival ability (du Toit and Alard, 1995; Snyman, 2013). Moreover, the dominance of the species could also be because *Eragrostis* species have small seeds that become more buried faster into the soil to escape seed predation (Thompson *et al*., 1998; Snyman, 2013). According to Parsons and Cuthbertson (2001), *Eragrostis curvula* produces large amounts of seeds that readily spread into disturbed areas. The results of this study also showed the dominance of increaser II species and forbs in the bush encroached rangeland in uppermost soil layer (0-10 cm). The findings concur with those found by Mndela *et al*. (2020) who reported higher levels of increaser II species and forbs in bush encroached rangeland in North-West Province of South Africa. Forbs dominate the seed bank of encroached ecosystems as due to their shade-intolerant properties (Shiferaw *et al*., 2018b). Erfanzadeh *et al*. (2020) found forbs to be most abundant plants in the soil seed bank composition at 0-10 cm upper layer in semi-arid regions encroached by different shrub species. This suggests that the savanna rangeland previously experienced anthropogenic disturbance, in particular overgrazing. Grazing reduces the suppressive effect and seed production of grass component, thereby allowing forbs to reproduce and contribute to the abundance of the soil seed bank (Solomon *et al*., 2006). According to van der Walt (2009), most of the increaser II species and forbs that emerge in the soil seed bank are weeds of croplands, indicating that these rangelands are abandoned cultivated lands that were encroached by woody plants following abandonment. D’Souza and Barnes (2008) also reported the abundance of forbs in the seed bank in a study observing the impact of woody plant on soil seed bank in Central Texas Savanna, USA.

3.4.3. Relationships between species composition and soil physical and chemical properties

Soil is a storage for soil nutrients and moisture which support plant growth (Kidane and Pieterse, 2006). Soil physical and chemical properties regulate the availability of water and nutrients which influence the nature of plant communities and their soil seed banks (Greig-Smith, 1983; Schlesinger *et al*., 1990). Additionally, changes in the soil chemistry may directly boost the change of the plant species composition and result in delay of soil seed bank (Bossuyt and Hermy, 2004; D'Odorico *et al*., 2012). For example, previous work has shown that soil pH directly affects the growth and survival of species in the aboveground plant community due to effects on the availability of essential mineral nutrients, and on soil microbial communities (Stephenson and Rechcigl, 1991). Grime (1973) showed that species richness decreases with both increasing acidity and alkalinity.

The present study found a negative correlation between clay content and forbs in the open rangeland. The negative relation between forbs and clay content in the study area may be as a result of soil compaction caused by livestock over grazing or over stocking (Solomon *et al*., 2007) which dries out the clay content. Dry clay content results in a compacted soil surface that prevents water penetration into the soil which has a negative impact on soil seed bank availability. Soil compaction also reduces water infiltration and aeration. A compacted soil surface promotes poor plant respiration and soil seed germination unless the soil crust is broken (Abdel-Megid *et al*., 1987) Van der wehuizen *et al*., 1999). Furthermore, in our study soil organic carbon was negatively correlated with forbs. Litter accumulation constrain soil seed bank composition by affecting litter layer quality and establishment of late seral plants (Schmidt *et al*., 2009). Closed-canopy rangelands with multiple layers of vegetation are less favourable to germination and survival of herbaceous species. Hence, herbaceous plants make a limited contribution to the ground vegetation and seed bank. In the encroached rangeland, magnesium was found to be negatively correlated to *Panicum maximum*, indicating that this grass species increases in soil with magnesium content. According to Skoglund (1992), recruitment from the seed bank is restricted to periods with favourable conditions of soil parameters controlling seed germination. Consequently, a combination of environmental conditions may determine seed accumulation in the soil. Hence, soil parameters not only influence the aboveground vegetation species composition, but also the composition of the soil seed banks and the seed germination.

3.5. Conclusion

This study quantified the effect of bush encroachment on seed bank density at two depth layers, 0-10 cm and 10-20 cm and explored the interaction between species composition and inherent soil properties in the open and encroached rangeland. Bush encroachment reduced seed density in the 0-10 cm depth layer. Bush encroachment also influences the species composition of the soil seed bank. The study revealed a negative association between organic carbon, calcium, clay, silt and forbs while mean weight diameter was positively correlated with forb. Soil pH, phosphorus, potassium and calcium were positively correlated to *Eragrostis curvula* while magnesium was negatively correlated to *Panicum maximum*. The findings of this study demonstrate the impact of bush encroachment on the diversity of savanna rangelands. More research should be conducted to get a clear understanding on seed bank dynamics, aboveground vegetation and their interaction with soil properties. Such studies will provide more understanding on the seed bank dynamics and will help in further planning of rangeland management strategies.

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CHAPTER 4

BUSH ENCROACHMENT AND SOIL DEPTH EFFECTS ON THE SOIL SEED BANK DIVERSITY IN A SAVANNA RANGELAND

Abstract

Savanna grasslands are characterized by the co-dominance of trees and grasses. The tree grass balance found in savannas is highly disturbed because of bush encroachment. The impacts of bush encroachment on seedbank diversity at different soil depth intervals remain poorly understood. This study assessed the effect of bush encroachment and soil depth on seed bank diversity in a savanna rangeland. The study was conducted at Bela Bela's Towoomba Pasture Research Station in Waterberg District of Limpopo Province, South Africa. Within each encroachment gradient six plots of 10 m x 10 m were randomly selected, whereby soil sampling and herbaceous vegetation were carried out and determined. In each replicate plot per encroachment level, five soil samples were randomly collected at 0-10 and 10-20 cm depths. The number of seedlings of different species emerging from the soil samples was used as a measure of the number of viable seeds in the soil and the composition of the seed bank. The results obtained in this study revealed that the Shannon-Weiner index increased in the 10-20 cm depth layer. The Menhinick's richness index showed no significant difference when comparing open and encroached rangeland, while species evenness decreased in the 0-10 cm depth layer, but increased in the 10-20 cm. In open rangeland, the Canonical correspondence analysis (CCA) showed that clay content was negatively correlated with species evenness and magnesium was negatively correlated to Shannon Weiner index. Silt content was positively correlated with species richness and evenness. In the encroached rangeland, the Canonical correspondence analysis (CCA) showed a negative correlation between magnesium and the Shannon Weiner index. The Sørensen's index between soil seed bank and aboveground vegetation was low with index values of 0.22 and 0.24 in open and encroached rangeland, respectively. Taken together, the results reveal that soil seedbank diversity is controlled by edaphic factors, specifically soil depth and associated soil texture and nutrients.

Keywords: Bush encroachment, soil depth, soil seed bank, species richness, diversity, evenness, soil properties, savanna, and rangelands.

4.1. Introduction

Savanna grasslands are characterized by the co-dominance of trees and grasses (Scholes and Walker, 1993, Sankaran *et al*., 2005). According to the competition-based models by (Walter 1971; Walker *et al*., 1981), the coexistence of grasses and trees in savannas results because of the spatial and temporal niche differences between trees and grasses that serve to concentrate intra-relative to inter-life form competition (Chesson And Huntly 1997; Chesson 2000; Amarasekare, 2003). Trees and grasses coexist in savannas because of their differential ability to acquire and partition limiting resources. For the most part, the models have focused on plant-available moisture, rather than plant-available nutrients as the main resource limiting plant growth in savannas (Walter 1971; Walker *et al*., 1981; Fernandez-Illescas and Rodriguez-Iturbe, 2003; van Langevelde *et al*., 2003). Higgins *et al*. (2000) argues that the critical problem for savanna trees is demographic and not competitive in nature. Here, trees and grasses persist in savannas because of climatic variability and disturbances such as fire and grazing which limit successful tree seedling germination, establishment and transition to mature size classes (Menaut *et al*., 1990; Hochberg *et al*., 1994; Jeltsch *et al*., 2000; Higgins *et al*., 2000; van Wijk and Rodriguez-Iturbe, 2002).

The tree grass balance in savannas is highly disturbed because of bush encroachment (Ward, 2005; Angass and Oba, 2010). Encroachment of woody plants alters the composition of vegetation resulting in decreasing species diversity and richness (Yayneshet *et al*., 2009). Soil seed bank diversity has also been reported to decrease with soil depth (Mndela *et al*., 2019). It is assumed that this pattern reflects regular seed input at the surface and a gradual decline in viability as seeds age and move vertically down the soil profile. This is because older seeds have more time to become deeply buried and depth distribution is often a reasonably good indicator of seed longevity (Thompson *et al*., 1997; Bekker *et al*., 1998). Seed banks can substantially contribute to the composition of future plant communities (Meissner and Facelli, 1999). It is therefore important to understand the diversity level of the soil seed bank because it is essential for designing conservation and restoration programs (Zaghoul, 2008) In fact, the success of restoring disturbed ecosystems is largely dependent upon the composition and diversity of the soil seed bank (Luzuriaga *et al*., 2005). It is also crucial to understand the relationship between soil seed banks and aboveground vegetation (Caballero *et al*., 2008; Ma *et al*., 2013) as the seed bank size is largely dependent on the seed input from the local vegetation, as seeds persist over a long period and dispersal from nearby areas (Olano *et al*., 2012; Siebert and Drebber, 2019). The objective of this study was to assess the effect of bush encroachment and soil depth on the seed bank diversity in a savanna rangeland and to evaluate the relationship between the soil seed bank and aboveground vegetation.

4.2. Materials and Methods

4.2.1. Study site

The study was conducted at Bela Bela's Towoomba Pasture Research Station (28°21 'E, 24°25'S) in Waterberg District of Limpopo Province, South Africa. The climate of the study area consists of a mean annual rainfall and temperature of 629 mm and 29˚C (Mills *et al*., 2017) respectively. For further details related to site description, refer to Chapter 3, section 3.3.1.

4.2.2. Site selection and experimental layout

A soil and vegetation survey were carried out in a savanna rangeland. Both surveys were conducted along an encroachment gradient encompassing open and bush encroached savanna rangelands. In each bush encroachment level (BE), six 10 m x 10 m plots were randomly selected and marked on the same plinthic soil (Bainsvlei) with similar elevation and landform.

4.2.3. Vegetation survey and soil seed bank sampling

All woody species found within each 10 m x 10 m plot per encroachment level were counted and identified following van Wyk and van Wyk (1997). The longest and shorted diameter of individual tree species were measured and recorded using a 2 m rod. Plant height and lowest browsable height (LBH) were also measured and recorded using the same 2 m rod. In each replicate plot per encroachment level, five soil samples were randomly collected at 0-10 cm and 10-20 cm depths. These soil depth intervals have been shown to have both transient and persistent seed banks (Masocha and Dube, 2018). The overall number of samples collected was 120 (2 BE levels x 6 plots x 5 soil samples x 2 depths). Soils were then bulked per soil depth and per encroachment level, giving a total of 24 samples (BE levels x 2 depths x 6 plots). Bulked soil samples were extruded into polythene sample bags, sealed and taken to laboratory where they were air dried and passed through a 2-mm aperture metal sieve to remove visible plant material for seed bank germination and soil chemical and physical analysis.

4.2.3.1 Herbaceous vegetation

Within each 10 m x 10 m plot, six 0.5 m × 0.5 m quadrats were used to collect herbaceous vegetation. All herbaceous species within each quadrant were identified and recorded. Grasses in each quadrant were identified and recorded following van Oudtshoorn (2014). To determine the aboveground biomass, identified grass species were harvested above 3 cm from the soil surface using grass cutting shears. Similar grass species were arranged and placed in the same paper bag. The collected grass samples were oven dried at 60°C for 48 hours. Aboveground forb species composition was not recorded as forbs are generally a minor component of the sward in more open areas and tree dominated sites.

4.2.4 Soil analysis

Soil texture was determined using the hydrometer method (Bouyoucos, 1962). Soil aggregate stability was determined using the wet-sieving method (Six *et al*., 1998). Intact soil cores were extracted from the field to determine bulk density following Blake and Hartge (1986). Soil pH was determined in a 1:2.5 solution ratio in 1 M KCl suspension using a glass electrode. Exchangeable cations Ca and Mg were first extracted in 1M KCl, while P, K, were extracted in an Ambic 2 extractant containing 0.25M NH_4HCO_3. The detection of the extracted cations was done by inductively coupled plasma optical emission spectrometry (ICP-OES) using an Optima 7300DV spectrometer (Perkin Elmer, Inc., 2 Shelton, CT). Organic carbon content was determined by the Walkley-Black method (Walkley and Black, 1934).

4.2.5. Soil seed bank experiment

The number of seedlings of different species emerging from the soil samples was used as a measure of the number of viable seeds in the soil and the composition of the seed bank (Roberts, 1981). The seedling emergence method was used to estimate the number of seeds in the soil. The seedling emergence method was used because it is more appropriate than actual identification of seeds (Gross, 1990; Espeland *et al*., 2010). It determines the relative abundance of viable seeds that can germinate and excludes the non-viable seeds (Poiani and Johnson, 1988). The germination experiment was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo (23^{o} 53’ 10’’S, 29^{o} 44’ 15’’E). In the greenhouse**,** a completely randomized design (CRD) was used to layout the pot experiment. Three plastic pots (size= 20 cm) were used per composite soil sample per soil depth and encroachment level, totaling 72 plastic pots (2 BE level x 2 depths x 6 plots x 3 replications). The soil was spread over sand and hygromix to a depth of 30 mm. Pots were examined every 3 days for the first 2 months and thereafter weekly until the end of the experiment following Tessema *et al*. (2012). The minimum and maximum ambient temperatures in the greenhouse are 21 ^{o}C and 28 ^{o}C respectively. Seedlings started to emerge after 1 week and were allowed to grow until they were identifiable. Thereafter, the emerged plant species were identified, counted recorded then discarded. Each pot was hand-watered regularly until field capacity. The pot experiment was monitored for a period of 6 months starting from September 2019 to February 2020.

4.2.6. Calculation of ecological indices

The diversity of the soil seed bank was estimated by calculating the Shannon-Wiener diversity index (Krebs, 1989), using the following formula:

$$H' = -\sum [(n_i/N) \times \ln (n_i/N)]$$

Where *H’* is the Shannon-Wiener index (the measure of species diversity), n_i is the number of seedlings per treatment, N is the total number of seedlings and ln is the natural log.

The Simpson's diversity index (Simpson, 1949) was also calculated using the following formula:

$D = \sum_i \{n_i \times (n_i - 1)\} / (N \times (N - 1)$ and was expressed in the output as both 1-D and 1/D

Species richness in each encroachment level was estimated using the Menhinick's index (Magurran, 2004), which is calculated as follows:

$$D = \frac{S}{\sqrt{N}}$$

Where S is the number of different species represented within each encroachment level, and N is the total number of species in each encroachment level.

Species evenness in each bush encroached level was estimated using the Pielou's evenness index (Magurran, 2004), which is calculated as follows:

$$\mathrm{J} = \frac{\mathrm{H}}{\ln \mathrm{S}}$$

Where H is the shannon-Wiener diversity index and S is the total number of species in each encroachment level.

Similarity between seed bank samples and above ground vegetation was determined using Sørensen's index:

S = 2c/ (a+b+2c)

Where, a is the number of species present only in the seed banks, b is the number of species present only in above ground vegetation and c, the number of species present in vegetation and seed bank samples (Sorensen, 1948). Forbs were rarely presented in the above ground vegetation and therefore, similarity was calculated only for grass species.

4.2.7. Data analysis

The calculation of Menhinick's index, Shannon-Wiener diversity index, Pileou's evenness index, Simpson index, and Sørensen's index were done using Microsof Excel (Microsoft, 2013). The diversity data was tested for normality using Shapiro-Wilk and Levene's tests before the analysis. The Shannon-Wiener diversity index and Pileou's evenness index data were transformed using $\log_{10}$ (X+1). Student t-test was applied to compare the Menhinick's index, Shannon-Wiener diversity index, Pileou's evenness index, and Simpson index among the encroachment levels along soil depth intervals at the significance level of $P \leq 0.05$. Statistical analyses were performed using SAS version 9.4.

To examine a possible relationship between the soil seed bank diversity (Menhinick's index, Shannon-Wiener diversity index, Pileou's evenness index, Simpson index and soil variables (pH, organic carbon, phosphorus, potassium, calcium, magnesium, clay content, silt content and mean weight diameter, a measure of soil aggregate stability), a Canonical correspondence analysis (CCA) was performed. Canonical correspondence analysis (CCA) was performed using the vegan package (Oksanen et al., 2019) in RStudio of R Version 3.5.1 (R Core Team, 2018). A permutation test (999 cycles, function ANOVA.cca in Vegan package) was performed to test for the variables that explained a significant part of the variation in seed bank diversity.

To explore the relationship between the soil seed bank composition and species composition of the vegetation, a multivariate ordination was conducted using Non-metric multi-dimensional scaling (NMDS). The NMDS was used to explore the pattern of seed bank composition among the encroachment levels. The NMDS was performed based on Bray-Curtis distance matrices. The statistical significance of the NMDS results were tested using an ADONIS test (999 permutations) using the vegan package (Oksanen *et al*., 2019) in RStudio of R Version 3.5.1 (R Core Team, 2018).

4.3. Results

4.3.1. Influence of encroachment and soil depth on seed bank diversity

Shannon-Wiener diversity did not differ significantly ($P \leq 0.05$) between the open and bush encroachment rangeland for both soil depth intervals (0-10 cm and 0-20 cm) (Figure 4.1a). As shown in Figure 4.1 b, bush encroachment did not affect Simpson's diversity index at topmost soil layer (0-10 cm). Bush encroachment significantly reduced Simpson's diversity in the deeper soil layer (10-20 cm). Species richness was not statistically different among the bush encroachment levels at various soil depths (0-10 and 0-20 cm) (Figure 4.2 a). Figure 4.2 b shows no significant ($P \leq 0.05$) effect of bush encroachment on species evenness in the uppermost soil layer (0-10 cm). In 10-20 cm depth layer, species evenness significantly ($P \leq 0.05$) increased with increase in bush encroachment.

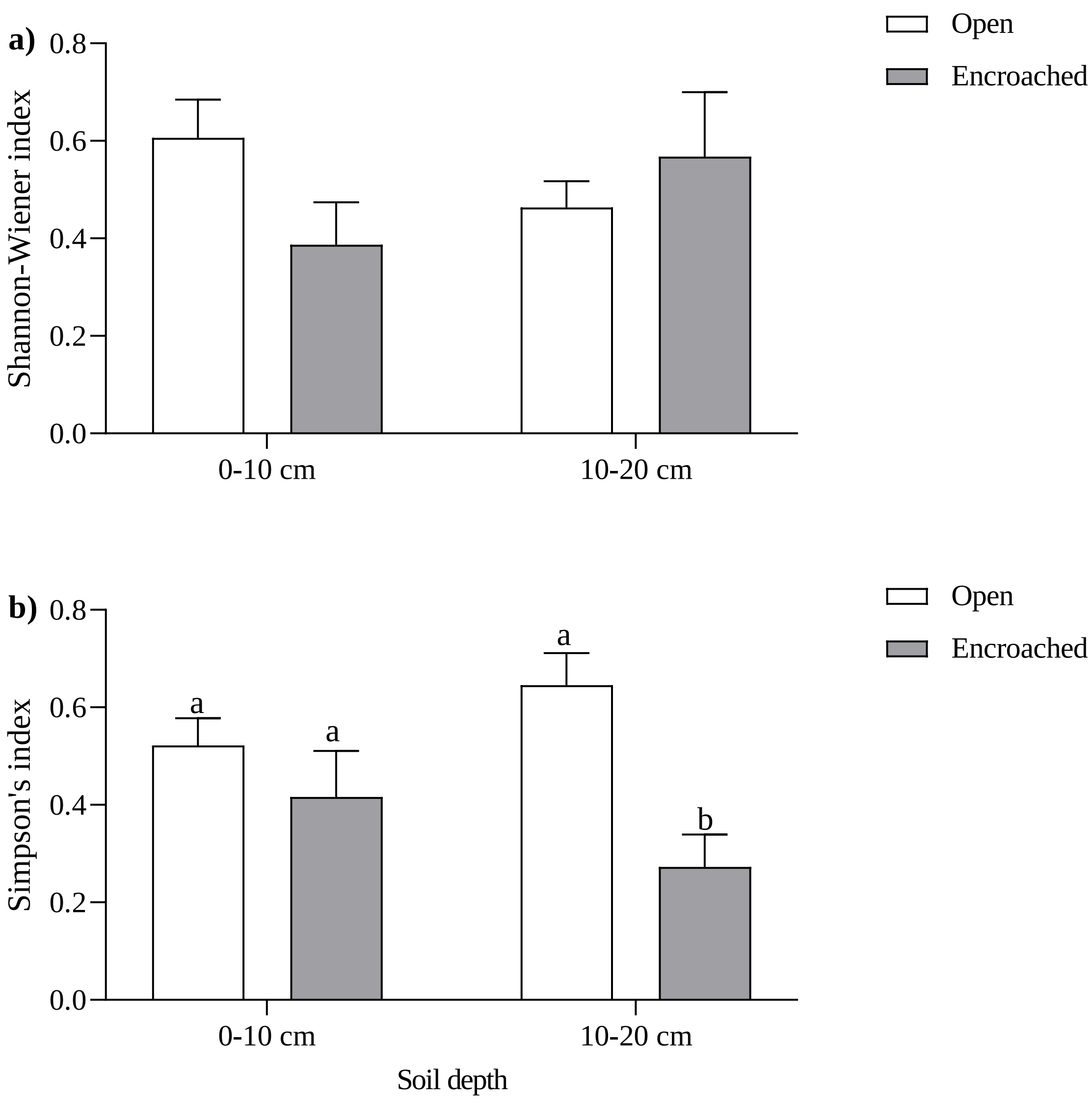

Figure 4.1. The diversity of the soil seed bank at different soil depth in open and encroached rangeland. Means with different superscripts indicate significant differences between treatments P ≤ 0.05

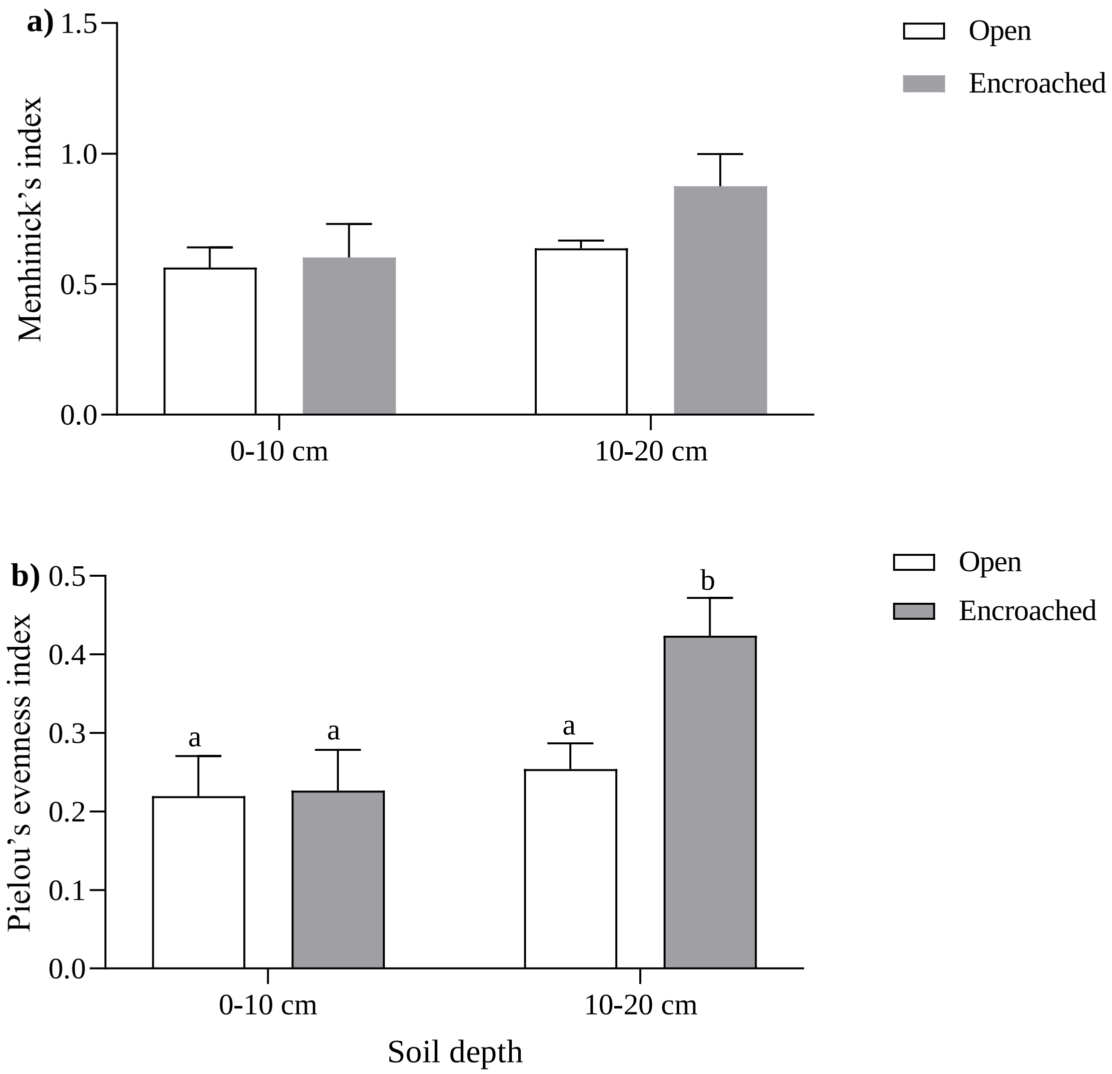

Figure. 4.2. Effect of encroachment level and soil depth on species richness and evenness. Means with different superscripts indicate significant differences between treatments P ≤ 0.05

4.3.2. Relationship between seed bank diversity and environmental factors

To visualize the relationship between soil seed bank diversity and environment parameters of the soil properties, a Canonical correspondence analysis (CCA) was performed for open and encroached rangeland (Figure 4.3). In open rangeland, the first two axes of the CCA biplot explained 96% of the total variation (Figure 4.3a). The Canonical correspondence analysis (CCA) showed that clay was negatively correlated with species evenness and magnesium was negatively correlated to Shannon Weiner index (Figure 4.3a). The silt content was positively correlated with species richness and evenness. The Canonical correspondence analysis (CCA) model was significant (P=0.047, F=7.524). The first two axes of the CCA biplot explained 80% of the total variation in the bush encroached rangeland (Figure 4.3b). The Canonical correspondence analysis (CCA) revealed a negative correlation between magnesium and the Shannon Weiner index, even though, the Canonical correspondence analysis (CCA) model was not significant (P= 0.473, F=1.0779)

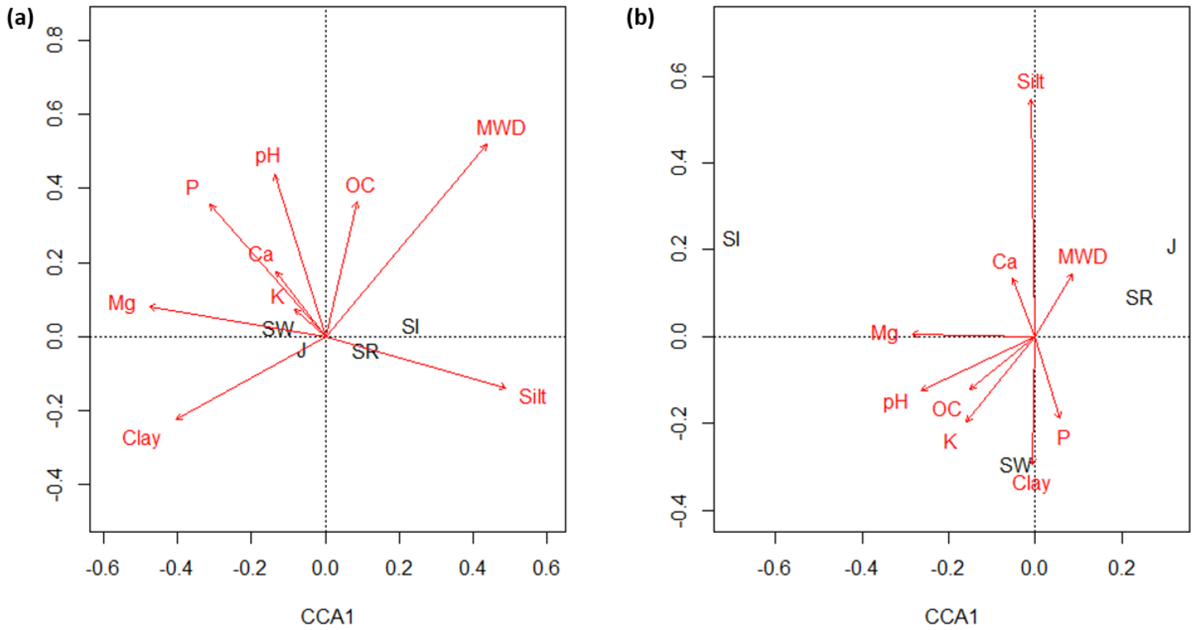

Figure 4.3. Canonical Correspondence Analysis (CCA) between soil seed bank diversity and environmental factors in (a) Open rangeland and (b) Encroached rangeland. *Key to soil properties: K=Potassium, P=Phosphorus, Mg=Magnesium, OC=organic carbon, Ca=Calcium, pH, Clay, Silt, MWD=Mean weight diameter. Key to Seed bank diversity: SR*= Menhinick's *richness* index*, SW*= Shannon Weiner index.

4.3.3. Relationship between the seed banks and aboveground vegetation

Sørensen's indices were calculated between the soil seed banks and above-ground vegetation in each encroachment level using species composition data on species presence and absence. The Sørensen's index between soil seed banks and aboveground vegetation was low with the index values of 0.22 and 0.24 in open and encroached rangeland, respectively (Figure 4.4). In the NMDS analysis of soil seed bank and aboveground vegetation, the ordination revealed a separation in species composition in open rangeland whereas encroached rangeland did not show any separation (Figure 4.5). The NMDS ordination analysis of soil seed bank species composition did not show a separation along the encroachment gradient (Figure 4.6).

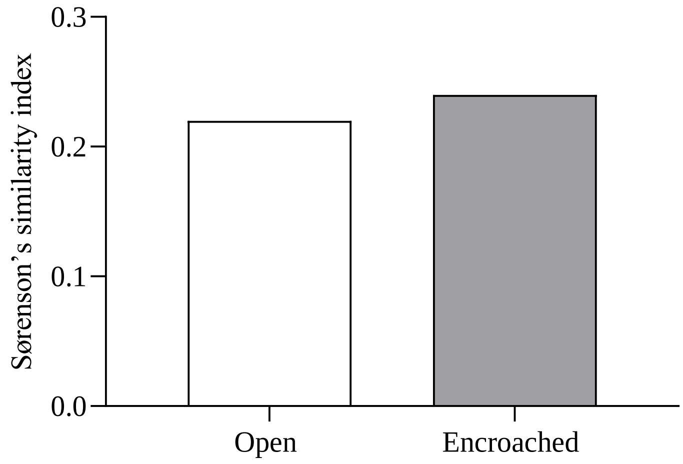

Figure 4.4. Similarity index between soil seed bank and aboveground vegetation in open and encroached rangelands

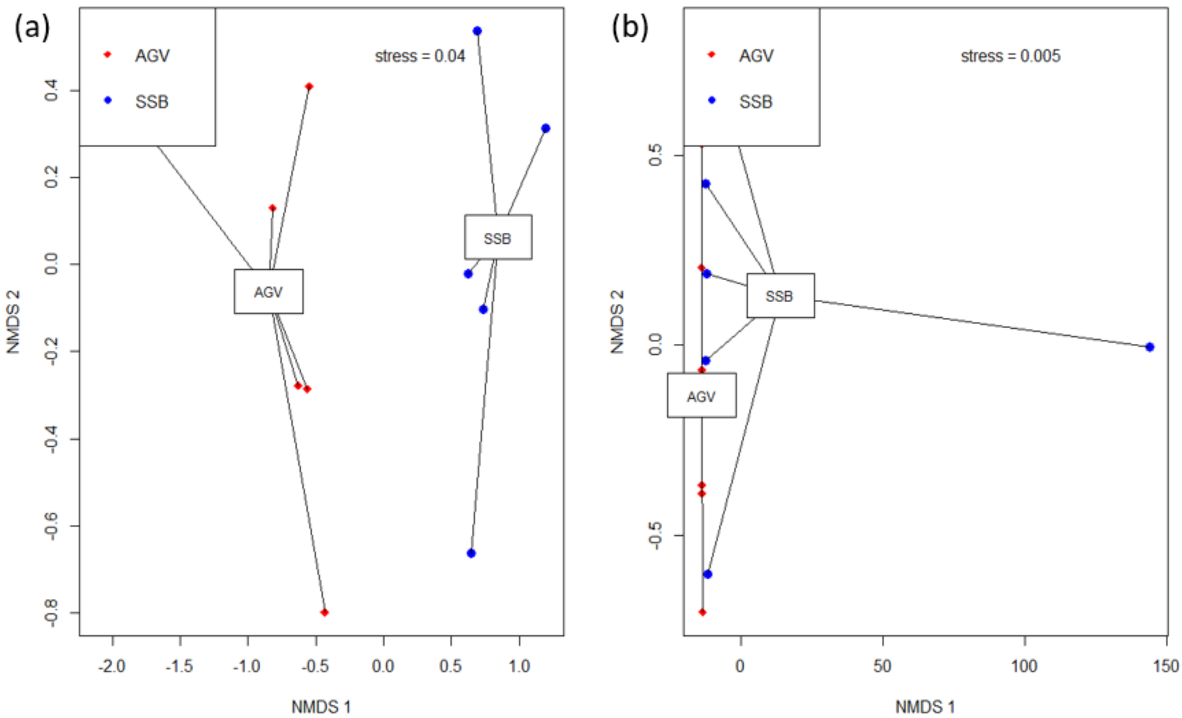

Figure 4.5. Nonmetric multidimensional scaling (NMDS) ordination of soil seed banks and above-ground vegetation in (a) open rangeland and (b) encroached rangeland. Ordination is based on species composition data; there were 6 plots in each

encroachment level. Text shows centroids for encroachment level. The lines connect plot points to their centroids. SSB= Soil Seed Bank, AGV= Aboveground Vegetation.

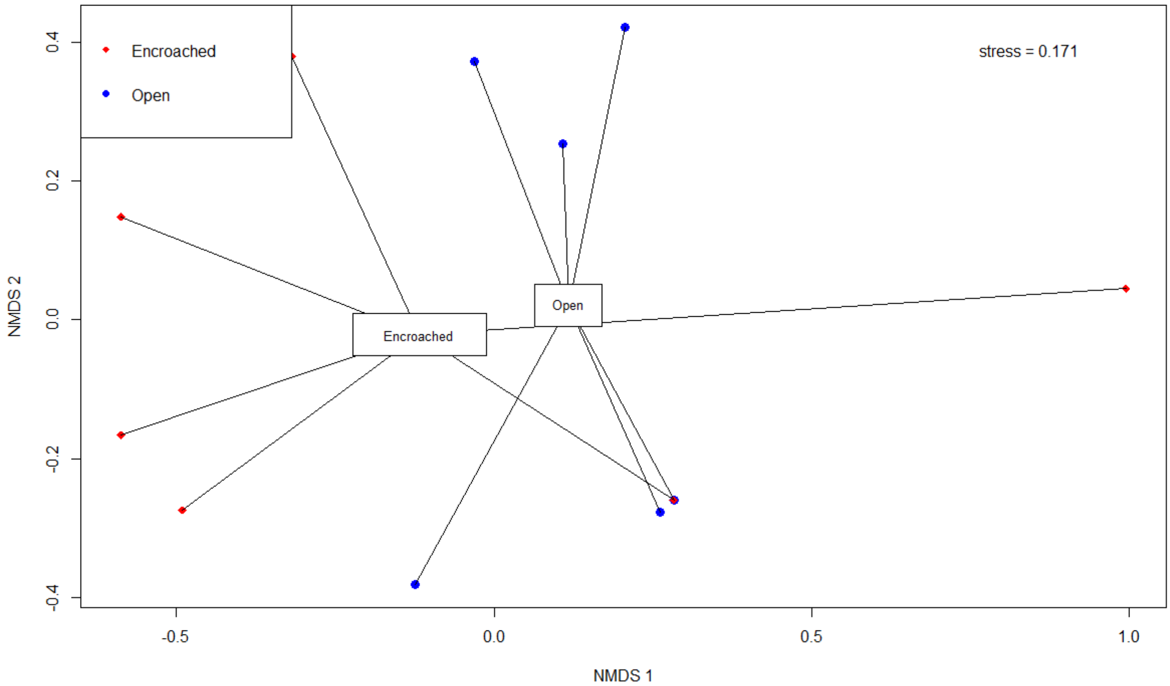

Figure 4.6. Non-metric multidimensional scaling (NMDS) for soil seed bank composition of open and encroached rangeland. Ordination is based on species composition data; there were 6 plots in each encroachment level. Text shows centroids for encroachment level. The lines connect plot points to their centroids.

4.4. Discussion

4.4.1. Effect of bush encroachment and soil depth on species diversity

The Shannon wiener diversity index revealed that bush encroachment decreased seed bank diversity in the 0-10 cm soil depth layer. The results of this study concur with Shiferaw *et al*. (2019), who reported a decrease in seed bank diversity in the uppermost layer of *Prosopis juliflora* encroached rangeland in Afar region, Northeast Ethiopia. In the bush encroached rangeland, the decrease in species diversity may be due to the shade effects of the canopy of woody plants, which reduce seed productivity. Bush encroachment increased seed bank diversity in the 10-20 cm depth layer. The increase in species diversity may be linked to the persistence of seeds. Grassland ecosystems can retain viable seed banks for decades under woody plant cover (Donelan and Thompson 1980; Bakker *et al*., 1996; Davies and Waite 1998; Kalamees and Zobel 1998). However, it is important to understand that persistence of the soil seed bank may vary widely depending on the germination requirements and plant-related seed characteristics. For example, some species may germinate soon after seed set while dormancy release for other species requires extended exposure to germination (Baskin and Baskin, 2014). Furthermore, seed bank dominated by perennial grasses produce longer-lived seeds which stay viable in the soil for longer periods (Kellerman, 2004), hence the increase in diversity in deeper soil depths.

4.4.2. Effect of bush encroachment and soil depth on species evenness and richness

In this study, bush encroachment did not have an impact on the species richness in the 0-10 cm and 10-20 cm depth layers. Similar observations were found by Mndela *et al*. (2019) who also reported no significant differences in the species richness under encroached rangeland of Makapanstad in the North West Province. However, the results are in contrast with other studies which reported an increase (D'souza and Barnes, 2008; Tessema *et al*., 2017) and decrease (Görzen *et al*., 2019) in species richness of the soil seed bank in encroached ecosystems. The non-impact was despite substantial post encroachment changes in the aboveground vegetation as well as the seed bank composition. This indicates that seed rain and seed persistence of some of the native species is likely to leave the soil seed bank unaltered, at least until major changes in the vegetation occurs (Gioria *et al*., 2014). Bush encroachment did not have an impact on species evenness in the 0-10 cm depth layer in the present study.

Similar observations were also made by Tererai (2012), who reported no significant difference in species evenness in the top layer of encroached area at the Berg River, north-east of Cape Town in the Western Cape of South Africa. Bush encroachment, however, resulted in an increase in the 10-20 cm depth layer. The reason for the increase could be due to the less disturbances down the soil profile layers (Olano *et al*., 2012).

4.4.3. Relationship between seed bank diversity and soil properties

The relationship between soil physical, chemical properties and plant vegetation have an impact on the geographic distribution and diversity of plants (Abbasi-kesbi *et al*., 2017). Soil texture directly influences the composition of soil seed banks through its effects on the horizontal and vertical movements of seeds (Chambers and MacMahon, 1994). In this study, we have found that clay content of the open rangeland soil was negatively correlated with species evenness. The decrease in species evenness with an increase in clay contented can be explained by that clayey soils lack adequate pore spaces, and this negatively affects aeration and promotes greater variation of species patterns in the soil seed bank (Focht, 2001). Moreover, magnesium was found to be negatively correlated with the Shannon Weiner diversity index in both the open and encroached rangeland, suggesting that Shannon Weiner diversity index is lower in soil rich in magnesium content.

4.4.4. Similarity between the soil seed bank and aboveground vegetation

In this study, the Sørensen’s index between soil seed banks and aboveground vegetation was low with index values of 0.22 and 0.24 in open and encroached rangeland, respectively. The NMDS analysis of the soil seed bank and aboveground vegetation indicated that in the open rangeland there was a separation in species composition while in the encroached there was no separation observed. This was a clear indication that the species composition among the seed bank communities was small relative to the aboveground vegetation communities in the savanna rangeland. The low similarity index between soil seed banks and aboveground vegetation in encroached rangelands has also been reported by other studies. For instance, Tessema *et al*. (2017), also reported a low similarity index between the soil seed bank and aboveground in the semi-arid savanna of the Babile Elephant sanctuary, in Ethiopia.

In this study the number of species germinated in the soil seed bank was lower than the number of species recorded in the aboveground vegetation. In the open rangeland, grass species such as *Schmidtia pappophroides, Heteropogon contortus, Cymbopogon pospischilli and Aristida congesta* were well represented in the aboveground but absent in the soil seed bank. This was also apparent in the encroached rangeland where grass species such as *Schmidtia pappophroides, Eragrostis superba, Heteropogon contortus, Cymbopogon pospischilli, Hyperthelia dissolute, themeda triandra, Enneapogon cenchroides were* only observed in the aboveground vegetation. In both the open and encroached rangeland, the correspondence between the soil seed bank and aboveground vegetation was almost non-existent. Similar observations were found by other studies elsewhere (Tedder *et al*., 2012; Tessema *et al*., 2017; Lang and Halpen, 2007). The low similarity could be due to the frequent occurrence of perennial grasses in the aboveground vegetation, which may have been as a result of short dormancy period of perennial grasses as compared to annual species (Tessema *et al*., 2012). Poor correspondence between the seed bank and aboveground vegetation may be due to seed predation (Baskin and Baskin, 1998; Crowley and Garnet, 1999; Marone *et al*., 2000), reliance on vegetative reproduction (Baker, 1989) and lack of dormancy mechanisms (Esmailzadeh *et al*., 2011). In addition, differences in hard seeded coat, germination ability, and mortality of seeds could contribute to the low similarity between the species composition of the soil seed banks and understory vegetation in semi-arid African savannas (Godefroid *et al*., 2006). Peco *et al*. (1998) and Egan and Ungar (2000) reported that grasslands dominated by perennial grasses have few similarities between soil seed bank and vegetation which was also apparent in the present study.

4.5. Conclusion

This study showed that bush encroachment and soil depth affects the diversity and evenness of the soil seed bank. Bush encroachment, however, did not have an impact on species richness. The similarity in species composition between the soil seed bank and the aboveground vegetation was low in both open and encroached rangelands. This study also showed that this change was depended on the soil depth from which the seeds were sampled in the profile. The study also revealed that the germination of species is directly linked to soil physical and chemical properties. More research is still required especially in the savanna rangelands of South Africa to improve our understanding of soil seedbank dynamics in these ecosystems that are prone to bush encroachment.

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CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Bush encroachment has long been reported to alter the grass and tree balance in savanna grasslands. This leads to a disturbance of the ecosystem functioning of savannas. The undesirable increase of woody plants results in the suppression of palatable and productive grasses, thereby reducing forage production, which is the main feed for livestock. The reduction in biomass productivity decreases the profitability of rangelands. Encroaching woody species have also been shown to decrease species richness and abundance of the seed bank and ground-layer diversity. The main objective of this study was to investigate how bush encroachment influences soil seed bank diversity in savanna rangelands. The results obtained in this study revealed that bush encroachment affects the seed density and diversity (richness and evenness) of the soil seed bank. This study showed that this change was depended on the soil depth from which the seeds were sampled in the profile. The study also revealed that the germination of species is directly linked to soil physical and chemical properties. The study further outlines the importance of considering several traits of the soil seed bank, such as species composition, seed density and persistence, in order to understand seed bank dynamics of plant communities disturbed by bush encroachment.

Bush plant encroachment in the savanna rangeland is accompanied by several changes in the soil seed bank. Given the importance of soil seed banks in the restoration of vegetation following disturbance, the findings of this study have several potential implications for restoration of the grassland into which woody plants are encroaching the seed bank dominated by forbs. Therefore, seeds of forbs may be unable to rapidly drive the transition from bush encroached to perennial grass cover that represents good fodder value in the savanna rangeland. However, more studies need to be carried out in other local habitats of savanna rangelands in South Africa to improve our understanding of the role of soil seed bank in the restoration of encroached areas as well as constraints associated with natural regeneration. In addition, seed bank sampling on a single period may miss transient species, therefore seasonal seed bank dynamics should be followed in future studies. It is also required that an in-depth study be conducted in the future to establish more relationships between the seed bank and inherent soil properties. This will assist in designing conservation and restoration programs since soil seed banks can contribute substantially to the composition of future plant communities, and can also play a critical role in sustaining native plant populations following disturbance.

APPENDICES

Appendix 1: T test for seed density in the open and encroached rangeland at 0-10 cm and 10-20 cm soil depths

Variable: Seed density

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	783.3	426.2	174.0	0	1200.0
Open	6	2583.3	1325.8	541.2	1000.0	4100.0
Diff (1-2)		-1800.0	984.7	568.5		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		783.3	336.0	1230.6	426.2	266.1	1045.4
Open		2583.3	1192.0	3974.6	1325.8	827.6	3251.6
Diff (1-2)	Pooled	-1800.0	-3066.8	-533.2	984.7	688.0	1728.1
Diff (1-2)	Satterthwaite	-1800.0	-3189.9	-410.1			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	-3.17	0.0101
Satterthwaite	Unequal	6.0226	-3.17	0.0193

Variable: Seed density

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	950.0	258.8	105.7	600.0	1300.0
Open	6	1166.7	265.8	108.5	800.0	1600.0
Diff (1-2)		-216.7	262.4	151.5		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		950.0	678.4	1221.6	258.8	161.6	634.8
Open		1166.7	887.7	1445.6	265.8	165.9	652.0
Diff (1-2)	Pooled	-216.7	-554.2	120.8	262.4	183.3	460.4
Diff (1-2)	Satterthwaite	-216.7	-554.2	120.9			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	-1.43	0.1831
Satterthwaite	Unequal	9.9929	-1.43	0.1831

Appendix 2: T test for perennial grass seed density in the open and encroached rangeland at 0-10 cm and 10-20 cm soil depths

Variable: Perennials

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	33.3333	81.6497	33.3333	0	200.0
Open	6	150.0	197.5	80.6226	0	500.0
Diff (1-2)		-116.7	151.1	87.2417		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		33.3333	-52.3527	119.0	81.6497	50.9664	200.3
Open		150.0	-57.2469	357.2	197.5	123.3	484.4
Diff (1-2)	Pooled	-116.7	-311.1	77.7199	151.1	105.6	265.2
Diff (1-2)	Satterthwaite	-116.7	-325.1	91.7755			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	-1.34	0.2108
Satterthwaite	Unequal	6.6609	-1.34	0.2250

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	5.85	0.0750

Variable: Perennials

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	166.7	150.6	61.4636	0	400.0
Open	6	0	0	0	0	0

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Diff (1-2)		166.7	106.5	61.4636		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		166.7	8.6694	324.7	150.6	93.9773	369.3
Open		0	0	0	0	.	.
Diff (1-2)	Pooled	166.7	29.7172	303.6	106.5	74.3841	186.8
Diff (1-2)	Satterthwaite	166.7	8.6694	324.7			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	2.71	0.0219
Satterthwaite	Unequal	5	2.71	0.0422

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	Infty	<.0001

Appendix 3: T test for Weak perennial grass seed density in the open and encroached rangeland at 0-10 cm and 10-20 cm soil depths

Variable: Weak perennials

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	250.0	164.3	67.0820	0	500.0
Open	6	1133.3	768.5	313.8	200.0	2100.0
Diff (1-2)		-883.3	555.7	320.8		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		250.0	77.5601	422.4	164.3	102.6	403.0
Open		1133.3	326.8	1939.9	768.5	479.7	1885.0
Diff (1-2)	Pooled	-883.3	-1598.2	-168.4	555.7	388.3	975.3
Diff (1-2)	Satterthwaite	-883.3	-1687.8	-78.8779			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	-2.75	0.0204
Satterthwaite	Unequal	5.4562	-2.75	0.0366

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	21.88	0.0041

Variable: Weak perennials

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	283.3	204.1	83.3333	0	600.0
Open	6	300.0	126.5	51.6398	200.0	500.0
Diff (1-2)		-16.6667	169.8	98.0363		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		283.3	69.1182	497.5	204.1	127.4	500.6
Open		300.0	167.3	432.7	126.5	78.9568	310.2
Diff (1-2)	Pooled	-16.6667	-235.1	201.8	169.8	118.6	298.0
Diff (1-2)	Satterthwaite	-16.6667	-241.1	207.8			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	-0.17	0.8684
Satterthwaite	Unequal	8.3465	-0.17	0.8691

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	2.60	0.3170

Appendix 4: T test for Shannon diversity index in the open and encroached rangeland at 0-10 cm and 10-20 cm soil depths

Variable: Shannon diversity index

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	0.3873	0.2126	0.0868	0	0.6537
Open	6	0.6065	0.1921	0.0784	0.3986	0.8879
Diff (1-2)		-0.2192	0.2026	0.1170		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		0.3873	0.1641	0.6104	0.2126	0.1327	0.5215
Open		0.6065	0.4049	0.8081	0.1921	0.1199	0.4712
Diff (1-2)	Pooled	-0.2192	-0.4799	0.0414	0.2026	0.1416	0.3556
Diff (1-2)	Satterthwaite	-0.2192	-0.4802	0.0418			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	-1.87	0.0904
Satterthwaite	Unequal	9.8991	-1.87	0.0907

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	1.22	0.8295

Variable: Shannon diversity index

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	0.5679	0.3233	0.1320	0	0.8697
Open	6	0.4632	0.1316	0.0537	0.3242	0.7100
Diff (1-2)		0.1047	0.2468	0.1425		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		0.5679	0.2286	0.9072	0.3233	0.2018	0.7930
Open		0.4632	0.3251	0.6014	0.1316	0.0822	0.3228
Diff (1-2)	Pooled	0.1047	-0.2129	0.4222	0.2468	0.1725	0.4332
Diff (1-2)	Satterthwaite	0.1047	-0.2364	0.4457			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	0.73	0.4796
Satterthwaite	Unequal	6.6133	0.73	0.4879

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	6.03	0.0706

Appendix 5: T test for Simpson's diversity Index in the open and encroached rangeland at 0-10 cm and 10-20 cm soil depths

Variable: Simpson's diversity Index

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	0.4165	0.2311	0.0944	0	0.6111
Open	6	0.5221	0.1367	0.0558	0.3895	0.7179
Diff (1-2)		-0.1056	0.1899	0.1096		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		0.4165	0.1740	0.6591	0.2311	0.1443	0.5669
Open		0.5221	0.3787	0.6656	0.1367	0.0853	0.3353
Diff (1-2)	Pooled	-0.1056	-0.3499	0.1387	0.1899	0.1327	0.3332
Diff (1-2)	Satterthwaite	-0.1056	-0.3578	0.1466			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	-0.96	0.3581
Satterthwaite	Unequal	8.1172	-0.96	0.3632

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	2.86	0.2738

Variable: Simpson's diversity Index

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	0.2725	0.1629	0.0665	0	0.4667
Open	6	0.6453	0.1613	0.0658	0.4909	0.9286
Diff (1-2)		-0.3729	0.1621	0.0936		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		0.2725	0.1016	0.4434	0.1629	0.1017	0.3994
Open		0.6453	0.4761	0.8146	0.1613	0.1007	0.3956
Diff (1-2)	Pooled	-0.3729	-0.5814	-0.1644	0.1621	0.1132	0.2844
Diff (1-2)	Satterthwaite	-0.3729	-0.5814	-0.1644			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	-3.98	0.0026
Satterthwaite	Unequal	9.9991	-3.98	0.0026

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	1.02	0.9836

Appendix 6: T test for Species richness in the open and encroached rangeland at 0-10 cm and 10-20 cm soil depths

Variable: Species richness

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	0.6019	0.3142	0.1283	0	0.9045
Open	6	0.5642	0.1874	0.0765	0.3482	0.8944
Diff (1-2)		0.0378	0.2587	0.1493		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		0.6019	0.2722	0.9316	0.3142	0.1961	0.7705
Open		0.5642	0.3675	0.7608	0.1874	0.1170	0.4597

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Diff (1-2)	Pooled	0.0378	-0.2950	0.3705	0.2587	0.1807	0.4539
Diff (1-2)	Satterthwaite	0.0378	-0.3054	0.3810			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	0.25	0.8054
Satterthwaite	Unequal	8.1591	0.25	0.8066

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	2.81	0.2814

Variable: Species richness

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	0.8748	0.3028	0.1236	0.3333	1.2060
Open	6	0.6368	0.0747	0.0305	0.5547	0.7500
Diff (1-2)		0.2380	0.2205	0.1273		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		0.8748	0.5570	1.1925	0.3028	0.1890	0.7426
Open		0.6368	0.5584	0.7152	0.0747	0.0466	0.1833
Diff (1-2)	Pooled	0.2380	-0.0457	0.5216	0.2205	0.1541	0.3870
Diff (1-2)	Satterthwaite	0.2380	-0.0789	0.5549			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	1.87	0.0912
Satterthwaite	Unequal	5.6069	1.87	0.1143

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	16.42	0.0081

Appendix 7: T test for Species evenness in the open and encroached rangeland at 0-10 cm and 10-20 cm soil depths

Variable: Species evenness

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	6	0.2265	0.1268	0.0518	0	0.3912
Open	6	0.2194	0.1251	0.0511	0	0.3454
Diff (1-2)		0.00711	0.1259	0.0727		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		0.2265	0.0935	0.3595	0.1268	0.0791	0.3109
Open		0.2194	0.0881	0.3507	0.1251	0.0781	0.3068
Diff (1-2)	Pooled	0.00711	-0.1549	0.1691	0.1259	0.0880	0.2210
Diff (1-2)	Satterthwaite	0.00711	-0.1549	0.1691			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	10	0.10	0.9240
Satterthwaite	Unequal	9.9983	0.10	0.9240

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	5	1.03	0.9776

Variable: Species evenness

Encr	N	Mean	Std Dev	Std Err	Minimum	Maximum
Encroach	5	0.4239	0.1071	0.0479	0.2647	0.5233
Open	6	0.2539	0.0800	0.0326	0.1561	0.3960
Diff (1-2)		0.1700	0.0930	0.0563		

Encr	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Encroach		0.4239	0.2909	0.5570	0.1071	0.0642	0.3079
Open		0.2539	0.1700	0.3378	0.0800	0.0499	0.1961
Diff (1-2)	Pooled	0.1700	0.0426	0.2974	0.0930	0.0640	0.1698
Diff (1-2)	Satterthwaite	0.1700	0.0341	0.3059			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	9	3.02	0.0145
Satterthwaite	Unequal	7.3138	2.93	0.0209

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	4	5	1.80	0.5339

Appendix 8: Permutation test for Canonical Correspondence Analysis (CCA) in (1) Open and (2) Encroached rangeland

(1) Permutation test for cca under reduced model
Permutation: free
Number of permutations: 999

Model: cca(formula = abundance.matrix2 ~ pH + OC + P + K + Ca + Mg + Clay + Silt + MWD, data = Fixed1)

	Df	ChiSquare	F	Pr(>F)
Model	9	0.37271	0.8197	0.662
Residual	2	0.10105		

Call:
cca(formula = abundance.matrix2 ~ pH + OC + P + K + Ca + Mg + Clay + Silt + MWD, data = Fixed1)

Partitioning of scaled Chi-square:

	Inertia	Proportion
Total	0.4738	1.0000
Constrained	0.3727	0.7867
Unconstrained	0.1010	0.2133

(2) Permutation test for cca under reduced model
Permutation: free
Number of permutations: 999

Model: cca(formula = abundance.gxash ~ pH + OC + P + K + Ca + Mg + Clay + Silt + MWD, data = Fixed3)

	Df	ChiSquare	F	Pr(>F)
Model	9	0.81743	0.6473	0.875
Residual	2	0.28061		

Call:
cca(formula = abundance.gxash ~ pH + OC + P + K + Ca + Mg + Clay +   Silt + M
WD, data = Fixed3)

Partitioning of scaled Chi-square:

	Inertia	Proportion
Total	1.0980	1.0000
Constrained	0.8174	0.7444
Unconstrained	0.2806	0.2556

Eigenvalues, and their contribution to the scaled Chi-square

Appendix 9: Bootstrap Test for NMDS in (1) open rangeland and (2) encroach rangeland

(1) Call:
adonis(formula = data_1 ~ VTP, data = data_2, permutations = 999,   method = "br
ay")

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
VTP	1	1.8123	1.81226	11.38	0.53228	0.001	***
Residuals	10	1.5925	0.15925		0.46772		
Total	11	3.4047			1.00000		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(2) Call:
adonis(formula = data_3 ~ VTP, data = data_4, permutations = 999,   method = "bray")

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

	Df	SumsOf Sqs	Mean Sqs	F.Model	R2	Pr(>F)	
VTP	1	1.9235	1.92346	12.53	0.55614	0.001	***
Residuals	10	1.5351	0.15351		0.44386		
Total	11	3.4586			1.00000		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 10: Permutation test for Canonical Correspondence Analysis (CCA) in (1) open rangeland and (2) encroach rangeland

(1) Model: cca(formula = abundance.matric5 ~ pH + OC + P + K + Ca + Mg + Clay + Silt + MWD, data = Fixed5)

```
         Df ChiSquare     F Pr(>F)
Model     9 0.0206225 7.524  0.047 *
Residual  2 0.0006091
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Permutation test for cca under reduced model
Marginal effects of terms
Permutation: free
Number of permutations: 999

Model: cca(formula = abundance.matric5 ~ pH + OC + P + K + Ca + Mg + Clay + Silt + MWD, data = Fixed5)

```
         Df  ChiSquare      F Pr(>F)
pH        1 0.00020261 0.6653  0.511
OC        1 0.00025210 0.8278  0.459
P         1 0.00003672 0.1206  0.837
K         1 0.00009724 0.3193  0.672
Ca        1 0.00024603 0.8079  0.483
Mg        1 0.00027303 0.8965  0.432
Clay      1 0.00008121 0.2667  0.696
Silt      1 0.00001741 0.0572  0.916
MWD       1 0.00039613 1.3007  0.349
Residual  2 0.00060909
```

Call:
cca(formula = abundance.matric5 ~ pH + OC + P + K + Ca + Mg +   Clay + Silt + MWD, data = Fixed5)

Partitioning of scaled Chi-square:

```
                Inertia Proportion
Total         0.0212316    1.00000
Constrained   0.0206225    0.97131
Unconstrained 0.0006091    0.02869
```

Eigenvalues, and their contribution to the scaled Chi-square

(2) Model: cca(formula = abundance.matrix7 ~ pH + OC + P + K + Ca + Mg + Clay + Silt + MWD, data = Fixed6)

```
         Df ChiSquare      F Pr(>F)
Model     9  0.177515 1.0779  0.473
Residual  2  0.036597
```

Permutation test for cca under reduced model
Marginal effects of terms
Permutation: free
Number of permutations: 999

Model: cca(formula = abundance.matrix7 ~ pH + OC + P + K + Ca + Mg + Clay + Silt + MWD, data = Fixed6)

	Df	ChiSquare	F	Pr(>F)	
pH	1	0.018534	1.0129	0.431	
OC	1	0.068226	3.7285	0.057	.
P	1	0.033859	1.8504	0.234	
K	1	0.036444	1.9916	0.195	
Ca	1	0.085576	4.6767	0.046	*
Mg	1	0.097172	5.3104	0.055	.
Clay	1	0.005870	0.3208	0.778	
Silt	1	0.040357	2.2055	0.184	
MWD	1	0.020177	1.1027	0.359	
Residual	2	0.036597			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Call:
cca(formula = abundance.matrix7 ~ pH + OC + P + K + Ca + Mg + Clay + Silt + MWD, data = Fixed6)

Partitioning of scaled Chi-square:

	Inertia	Proportion
Total	0.2141	1.0000
Constrained	0.1775	0.8291
Unconstrained	0.0366	0.1709

Eigenvalues, and their contribution to the scaled Chi-square