Dynamics of the soil seed bank over the short-term after bush clearing in a semi-arid shrubland in Springbokvlakte thornveld of South Africa

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Highlights

• Bush clearance increased soil seed bank (SSB) of annual herbaceous species.

• Higher SSB of grasses after bush clearance showed a potential for passive restoration.

• However, dominance by few species reduced seed bank diversity after bush clearance.

• Low SSB densities of woody plants suggested that re-encroachment by reproductive means is

  unlikely.

• Seed bank species distribution was determined largely by soil N after bush clearance.

Abstract

Soil seed bank is a reservoir for passive restoration of rangelands following bush clearance. This study was conducted to assess the effects of bush clearance on the seed bank size, composition, diversity and relationship between soil seed bank (SSB) and above ground vegetation (AGV) in Springbokvlakte thornveld. The study was conducted at Radi communal rangeland, Makapanstad in North-West Province, South Africa with an average rainfall of 459 mm annum^{-1}. The rangeland was encroached at an average density of 6 908 woody plants ha^{-1} by *Vachellia tenuispina, Vachellia tortilis*, *Ziziphus mucronata* and *Asparagus laricinus*, with *V. tenuispina* accounting for 92% of the woody cover. All woody plants within 50×25 m plots were mechanically cut using manual saws and loppers followed by chemical stump treatment using picloram. The cleared and their adjacent uncleared microsites were marked in the middle of a large shrubland and were protected from grazing. Soil samples were collected at three depths (0-10, >10-20 and >20-30 cm) in April (pre-treatment), August, October, December 2016 and, April and August 2017 in bush cleared and uncleared microsites. SSB was determined using the seedling emergence method. Vegetation surveys were conducted in February 2016 (pre-treatment) and 2017 (post-treatment). Thirty-seven species from 16 families emerged from the SSB. Perennial species were the most abundant life-form until December 2016, after which annuals increased to 70 and 71% in April and August 2017, respectively in cleared microsites. *Eragrostis lehmaniana, Jamesbrittenia aurantiaca* and *Aristida bipartita* were the most abundant species until December 2016, thereafter; *Brachiaria eruciformis* dominated by 55 to 61% from April to August 2017 in cleared microsites. Principal component analysis (PCA) revealed that most seed bank species correlated strongly with total soil nitrogen, C:N ratio and nitrates. Total seed densities remained similar from August to December 2016, thereafter; they were 2 to 3-fold higher in cleared compared to uncleared microsites. Seed densities declined significantly ($p < 0.05$) with burial depth in April and August 2017 in cleared microsites. Species

diversity declined with time in cleared relative to uncleared microsites, mainly due to *B. eruciformis* homogenising the SSB in April and August 2017, respectively. In contrast, Sørensen's similarity between SSB and AGV increased with time, being highest in cleared than uncleared microsites from December 2016 to August 2017. Large seed bank densities in cleared microsites indicated high potential for vegetation recovery following bush clearance. However, follow-up management to drive vegetation changes to perennial species dominated state is necessary.

Key words: bush clearance, restoration, seed bank density, diversity, Sørensen's similarity.

1. INTRODUCTION

Increase in canopy cover and density of woody plants above optimal levels reduces herbaceous plant diversity and increases soil erosion (Heras et al., 2016). This is caused by higher competitive effect of woody plants over herbaceous species for light, soil moisture and nutrients (Mckinney and Goodell, 2010). Bush encroachment also reduces seed production of understory grasses through altering the microclimate under canopies (Bakker et al., 2014). Most notable is the reduction in light energy required for seed production thereby reducing seed input into the soil seed bank (Scott et al., 2010; Bakker et al., 2014). Furthermore, bush encroachment limits seed dispersal, recruitment and establishment of the understory grasses (Gabay et al., 2012). However, the burial and storage of seeds in the soil serves as a mechanism by which herbaceous species recover following bush clearance (Lang and Halpern, 2007).

The major challenge facing bush control programmes is the recruitment of woody plants from the soil seed bank following bush clearance (Witkowski and Garner, 2005; Metlen and Fieldler, 2006; Price and Morgan, 2008). Some woody plants have high fecundity and their seeds germinate vigorously to resemble the pre-clearance state (Richardson and Kluge, 2008). Thus, it is crucial to monitor the temporal changes of the size and composition of the soil seed bank after

bush clearance in order to understand the herbaceous plant recovery dynamics and re-encroachment potential of woody plants. Understanding time-series dynamics of the soil seed bank assists in predicting the successional changes of vegetation for decision making towards rangeland improvement (Marchante et al., 2011). Moreover, the time-series assessment helps to capture the effects of climatic stochasticity on the soil seed bank size and composition (Eager et al., 2013).

The seed bank size is largely dependent on the seed input from the local extant vegetation, as seeds persist for a long period and dispersal from nearby areas (Olano et al., 2012). Hence, understanding the relationship between soil seed bank and above ground vegetation components is crucial (Caballero et al., 2008; Ma et al., 2013). The composition of the above ground vegetation changes in time and space causing shifts in the soil seed bank (Ma et al., 2013). The early stages of secondary succession following woody plant clearance are characterised by vigorous colonisation by pioneer herbaceous species. As the above ground succession progresses, the seed bank of pioneers declines gradually and is replaced by seed bank of late successional species (Bossuyt and Hermy, 2004). The decline in seed densities is driven mainly by seed senescence, decay and a general decline of the annual species on the above ground vegetation (Bossuyt and Hermy, 2004). Despite spatial shifts in vegetation, soil seed bank size may vary vertically with soil depth (Holmes, 2002). Such variation is partly determined by the severity of soil disturbance during bush clearance, soil structure, vertical dispersal agents and seed characteristics (Eager et al., 2013; Gelviz-Gelvez et al., 2016). The objectives of the study were to determine: (1) the effects of bush clearance on the soil seed bank size, diversity and composition, (2) the vertical distribution of the seeds in the soil, (3) the relationship between species composition of the above ground vegetation and soil seed bank following bush clearance.

2. MATERIAL AND METHODS

2.1. Description of the study area

The study was conducted at Makapanstad (25^0 $14^\prime$ $36^{\prime\prime}$ S and 28^0 $7^\prime$ $19^{\prime\prime}$ E) in Bojanala District Municipality of North-West Province in South Africa. Radi communal rangeland was selected at Makapanstad. The area is administered under Tribal Authority and the rangeland is used exclusively for grazing. The altitude ranges from 900 to 1 200 m above sea level. The average annual rainfall is 459 mm annum^{-1} (Moerane, 2013). The maximum monthly average temperatures range from 27 - 34^0 C in summer and 20 - 23^0 C in winter and the respective minimum temperatures range from 15 - 16^0 C in summer and 3 - 6^0 C in winter (DIGES, 2012). The rangeland is found on the Central Mixed Bushveld bioregion in Springbokvlakte Thornveld (Mucina et al., 2006). The rangeland was encroached at an average density of 6 908 plants ha^{-1} by *Vachellia tenuispina* (I. Verd.) Kyal & Boatwr, *Vachellia tortilis* (Willd.) Hayne, *Ziziphus mucronata* (Willd.) and *Asparagus laricinus* (Burch.), with *V. tenuispina* accounted for 92% of the woody cover. The local land users attested that the encroachment by *V. tenuispina* occurred more than 40 years ago. The dominant grasses are *Ischaemum afrum* (GF. Gmel.) Dandy, *Aristida bipartita* (Nees.) Trin & Rupr and *Brachiaria eruciformis* (J. E Sm.) Griseb (Mucina et al., 2006). The soil types of the study area are black vertic clays (turf soils), with very loose structure, high calcium carbonate content and poor drainage with high cracking and shrinking potential (DIGES, 2012). The topography is gentle to slightly undulating (Mucina et al., 2006).

2.2. Experimental layout and vegetation survey

In February 2016, three 50 × 50 m bush encroached blocks were randomly selected at Radi. A random selection of the blocks was conducted using 2014 aerial photographs. Sites with similar woody vegetation structure, soil type and topography were selected on the aerial photographs and the coordinates were recorded to peg the blocks. In each block, eight 25 m^2 plots were

marked and woody plants were identified. Three 1.3×1.3 m quadrants were nested diagonally in the corners and centre of each plot to give 72 quadrants. All herbaceous plants within the quadrants were identified to species level. At the end of April 2016, all woody plants were mechanically cleared and removed in half (50×25 m) of each block to make three replicates of bush cleared and uncleared microsites. Woody plants were cut at 6 cm above soil surface using manual saws and loppers followed by chemical stump treatment using picloram (4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid). Cleared woody plants were used to fence the experimental plots to prohibit grazing. In each microsite replicate, four 25 m^2 plots were marked and plants were identified in the quadrants nested within the plots in the similar way as before bush clearance. Because the dominant woody species (*V. tenuispina*) is multi-stemmed and rhizomatous, resprouting occurred during the rainy season and follow-up chemical sprays of picloram were applied at the end of each month from November to March 2017.

2.3. Soil sampling and preparation for seed bank analysis

At the beginning of April 2016 before bush clearance, soil samples of 282.6 cm^3 were randomly collected at 0-10, >10-20 and >20-30 cm burial depths along two 50 m line transects per block, giving 144 samples (3 blocks $\times$ 2 transects $\times$ 8 random points $\times$ 3 depths). After bush clearance, two fixed 50 m line-transects were established 15 m apart in each microsite replicate. Thereafter, soil samples were collected randomly along transects in August, October and December 2016 and, in April and August 2017. The soil samples were collected at similar burial depths as before bush clearance, giving 144 samples (2 microsite types $\times$ 3 replicates $\times$ 2 transects $\times$ 4 random points $\times$ 3 depths). Soil samples were air dried, sieved to remove debris and stored at 5 ^{0}C.

2.4. Soil seed bank experiment

Seedling emergence method was chosen over direct seed identification methods because of its capacity to discriminate non-viable seeds against viable ones (Tessema et al., 2016). Despite

overestimating the seed bank size, direct seed identification methods are time consuming and unable to detect small-sized seeds (Farnsworth et al., 2012). The experiment was conducted in the glasshouse at the University of Pretoria with constant temperatures of 25°C during the day and 10°C at night. The soil samples were bulked for each burial depth per replicate per microsite type (cleared and uncleared microsites). Three samples weighing 1 300 g were pooled from each bulk and placed in three representative trays of 30×20 cm to give 54 trays. The soil layer in the trays was 3 cm.

Germination trays were labelled and distributed randomly on benches and watered to field capacity two times a day (in the morning and afternoon). Germinated seedlings were counted, identified and removed daily. The seedlings were identified to species level and functional groups (forbs, grasses, sedges, succulents and woody plants). The seedlings that were difficult to identify were marked and allowed to grow in the germination trays and identified a week after emergence. On day 4 following cessation of germination, soils were allowed to dry for 2 days, after which the trays were flipped over and watered continuously, twice a day to allow seeds at the bottom to germinate. The seedling emergence was monitored for 3 to 4 months for each germination cycle to give hard-coated seeds enough time to germinate. The plant identification guidelines by van Oudtshoorn (1999) for grasses, Smith et al. (2017) for succulents and van der Walt (2009) for forbs, sedges and legumes were employed. The seedling density was used as proxy for seed density and it was calculated as the number of seedlings per tray divided by the area of a tray and extrapolated to 1 m^2. The species abundances were calculated as the proportion of each species divided by the total number of all species in each treatment multiplied by 100%.

2.5. Soil chemical analysis and physical measurements

Soil samples were collected at 20 cm depth in the opposite corners and the centre of each 25 m^2 plot in each block. Soil samples from the eight 25 m^2 plots were composited by block, sieved and crushed to a fine form. In each composite sample, three representative samples were re-sampled for analysis of soil pH, calcium (Ca), magnesium (Mg), sodium (Na), total carbon (TC) and total nitrogen (TN), potassium (K), nitrite (NO_2), nitrate (NO_3), chloride (Cl), phosphate (PO_4), sulphate (SO_4), and soil fractions (clay, sand and silt). The saturated water paste extract (SWPE) method was used for the analysis of NO_3, Cl, PO_4 SO_4 and NO_2. Soil pH (H_2O) was determined using a pH meter in a 1:5 soil:water suspension. The ammonium acetate method was used for the analysis of Ca, Na, Mg and K (Reeuwijk, 2002). The total carbon (TC) and total nitrogen (TN) were analysed using LECO CR-412 Carbon Analyzer (McDonald et al., 2006) and Kjeldahl method (AOAC, 1990), respectively. The hydrometer method was used to determine clay, silt and sand fractions (Beretta et al., 2014).

The infiltration rate in each block was determined using double ring infiltrometer, whereas soil compaction was measured using dynamic cone penetrometer. For infiltration, a 50 m line transect was established at 25 m inside each block. Therefore, a double ring infiltrometer was hammered 10 cm deep into the soil at four points every 10 m along the line transects. Ten litres of water was poured in and out-side the inner ring and the infiltrated water was measured in 5 minutes intervals until 45 minutes per infiltrometer. To measure soil compaction, two penetrations were taken on opposite corners of each plot. Thereafter, penetrations were averaged per block.

3. Statistical analysis

The Sørensen's similarity index was computed to determine the similarity between the SSB and AGV composition (Chao et al., 2006).

The index was calculated as follows:

$$\text{Sørensen's index} = \frac{2C}{A + B + 2C}$$ …………Equation 1

Here, C is the number of species shared between SSB and AGV in each microsite replicate, A and B is the total number of species per microsite replicate in AGV and SSB, respectively. It is worth noting that from April to December 2016, we used the pre-treatment total number of species for AGV because we assumed that the vegetation within that period had not yet changed. The vegetation survey conducted in February 2017 also confirmed that the vegetation composition did not change until February. The Shannon Wiener's diversity index was used to calculate species diversity (Maguran, 1988). The diversity index was calculated as follows:

$$H' = -\sum_{i=1}^{S} P_i \, ln \, P_i \text{ … … … … … … … … … … .}$$ **Equation 2**

H' = Shannon Wiener's diversity index, S = the total number of species in each microsite replicate, P_i = relative abundance of the ith species. ln = natural log.

Before statistical analysis, univariate analysis was applied to test for normality and homoscedasticity using Shapiro-Wilk and Levene's tests. The data on soil seed bank (SSB) density and similarity index was transformed using $log_{10} (x+1)$ and arcsine (x+0.5), respectively. Thereafter, the data for densities, species richness, diversity and similarity between SSB and AGV was subjected to repeated measures analysis of variance using general linear models (GLMs) of SAS version 9.4 (SAS Institute, 2009). The microsites with two levels (cleared and uncleared) and burial depths (0-10, >10-20 and >20-30 cm) were used as fixed categorical variables while the time of sampling after bush clearance was used as a continuous variable. Block was included as a random factor and pre-treatment densities as covariates in the model. For mean separation, we conducted paired comparisons using t-test (p ≤ 0.05). However, rather than reporting log_{10} and arcsine transformed data, means were back-transformed to the original

scale. Ordination was conducted between species and soil properties using principal component analysis (PCA) with 999 randomisations. Sørensen's distance measure was used to conduct the two-way presence-absence hierarchical clustering, with microsites (cleared and uncleared) being the determinants of the presence or absence of the species. For PCA and clustering, we used the species composition emerged in the SSB of August 2017 because it was the time at which the species richness was higher compared to other sampling periods. The ordination analysis was executed in PC-ORD for Windows 98 version 6.0, MjM software (McCune and Mefford, 2011).

4. RESULTS AND DISCUSSION

4.1 Species composition and richness of the soil seed bank

The plant species abundance (%) and richness of the soil seed bank over time in bush cleared and uncleared microsites at Radi are presented in **Table 1**. A total of 37 species from 16 families, represented by 36 genera emerged in the soil seed bank (SSB) over the period of the study. Forbs were the dominant plant functional group with 17 species followed by grasses with 11 species. In cleared microsites, perennial species were more abundant ($\geq$60%) than annuals from April to December 2016, thereafter, they declined and the abundance of annual species increased substantially to 70 and 71% in April and August 2017, respectively. In uncleared microsites, annual species (52%) were marginally more abundant than perennials (45%) in August 2016. However, from October 2016 to April 2017, the abundance of annuals declined to lower levels than that of perennials. In August 2017, the abundance of annuals was again marginally higher than that of perennials in uncleared microsites. The species richness increased (from 10 to 23 species) linearly ($r^2 = 0.87$, $p < 0.001$) with time in cleared microsites. In contrast, species richness in uncleared microsites averaged to 16 species and there were no significant changes ($p = 0.351$) over time. The increase in abundance of annual species in cleared microsites in April and August 2017 suggested that vegetation recovery following bush clearance is likely

Table 1 Abundance (%) and richness of the soil seed bank species in bush cleared and uncleared microsites at Radi from April 2016 to August 2017.

Species	Family	Plant class	Life form†	Cleared microsites 2016 Apr	Aug	Oct	Dec	2017 Apr	Aug	Uncleared microsites 2016 Aug	Oct	Dec	2017 April	Aug
Vachellia tenuispina (I. Verd.) Kyal & Boatwr.	Fabaceae	Shrub	D	-	1.0	2.0	3.4	-	0.1	3.5	4.0	1.5	-	3.2
Aloe davyana (Schönl.) Glen & Hardy.	Asphodelaceae	Succulent	E	-	-	2.0	1.1	-	-	-	3.2	1.5	-	-
Aristida bipartita (Nees.) Trin. & Rupr.	Poaceae	Grass	P	12.2	8.7	16.0	12.4	4.6	0.7	2.8	3.2	14.9	-	0.7
Aristida conjesta subsp. barbicolis (Roen & Schult)	Poaceae	Grass	WP	-	-	-	-	4.2	5.1	-	-	-	21.2	27.7
Amaranthus hybridus (L.) Timeroy.	Amaranthaceae	Forb	A	-	-	2.0	2.2	0.6	0.2	2.1	-	-	-	-
Balbostylis hispidula (Vahl.) R.W. Haines.	Cyperaceae	Sedge	A	-	-	-	-	-	-	-	6.3	-	-	-
Brachiaria eruciformis (J. E Sm.) Griseb.	Poaceae	Grass	A	-	21.8	8.0	7.9	55.2	60.8	29.2	4.2	3.4	20.3	35.1
Blepharis integrifolia (Schinz.) Oberm.	Acanthaceae	Forb	P	-	-	-	-	-	0.1	-	-	-	-	-
Bothriocloa insulpta (Hochst.) ex A. Rich.	Poaceae	Grass	P	-	1.5	2.0	1.1	0.8	2.9	2.1	-	-	-	1.4
Bidens pilosa (L.) Timeroy.	Asteraceae	Forb	A	-	-	-	-	0.6	-	-	-	-	0.6	-
Commelina benghalensis L.	Commelinaceae	Forb	A	-	-	-	-	-	1.3	-	-	3.0	0.7	-
Chloris virgata (Sw.)	Poaceae	Grass	A	-	-	-	-	-	0.2	-	-	-	-	-
Chochorous asplenifolius (Burch.)	Malvaceae	Forb	P	0.8	8.3	10.0	7.9	15.3	2.2	3.5	4.2	9.0	17.1	2.8
Corbichonia decumbens (Forssk.) Exell.	Molluginaceae	Forb	A	-	-	6.0	2.2	-	-	1.4	4.2	6.0	-	-
Crotalaria heidmannii (Schinz.)	Fabaceae	Legume	A	0.8	-	-	-	-	0.2	-	-	-	-	1.1
Crabbea angustifolia Nees.	Acanthaceae	Forb	P	-	-	4.0	-	-	-	-	2.1	-	-	-
Cynodon dactylon (L.) Pers.	Poaceae	Grass	P	3.3	0.5	-	-	-	-	-	-	-	-	-
Cyperus rotundus L.	Cyperaceae	Sedge	P	-	-	2.0	1.1	-	-	9.7	-	-	-	-
Digitaria eriantha (Stent.) Steud.	Poaceae	Grass	P	-	5.3		-	0.2	14.4	1.4	2.2	-	3.1	2.8
Eragrostis lehmaniana Nees.	Poaceae	Grass	P	24.4	28.6	6.0	24.7	4.2	2.2	16.7	30.5	11.9	9.6	8.4
Indigofera spp	Fabaceae	Legume	P	-	0.5	-	-	-	-	-	-	-	-	-
Justicia flava (Vahl.)	Acanthaceae	Forb	P	-	-	-	-	0.6	0.7	-	-	-	0.7	1.8
Jamesbrittenia aurantiaca (Burch.) Hilliard.	Scrophulariaceae	Forb	P	20.3	3.9	16.0	12.4	1.6	0.1	2.8	22.1	20.9	2.4	-
Kalanchoe brachyloba (Welw.) ex Britten.	Crassulaceae	Succulent	P	-	-	-	1.1	-	-	-	-	-	-	-
Kyphorcarpa angustifolia (Moq.) Lopr.	Amaranthaceae	Forb	A	0.8	1.5	-	-	0.2	0.3	6.9	-	-	-	-
Kohautia virgata (Willd.)	Rubiaceae	Forb	A	-	9.7	16.0	7.9	1.5	-	6.9	-	9.0	1.0	-
Melinis repens (Willd.) Zizka.	Poaceae	Grass	WP	-	-	-	-	-	-	-	-	-	0.7	-
Phylanthus maderaspatensis L.	Phylanthaceae	Forb	P	-	3.9	2.0	9.0	2.7	0.4	6.3	6.3	16.4	5.5	1.8
Rhynconsia minima (L.) DC.	Fabaceae	Legume	P	-	-	-	-	0.2	-	-	-	-	-	-
Spermacoce sinensis (Klotzsch.) Hierm.	Rubiaceae	Forb	A	0.8	1.5	-	2.2	1.5	0.2	0.7	4.2	2.5	5.1	1.1
Schkuhria pinnata (L.) Kuntze ex Thell.	Asteraceae	Forb	A	1.6	-	-	-	0.6	6.7	1.4	1.1	-	0.7	3.9
Sonchus asper (L.) Hill.	Asteraceae	Forb	A	35.0	-	-	-	-	-	-	-	-	-	-
Tragus beteroniunus (Schult.)	Poaceae	Grass	A	-	0.5	2.0	3.4	4.4	0.4	2.1	1.1	-	4.8	6.7
Datura fastuosa (L.) Bernth.	Solanaceae	Forb	A	-	-	-	-	-	0.4	-	-	-	-	-
Hibiscus trionum L.	Malvaceae	Forb	A	-	-	-	-	0.9	0.2	-	-	-	4.1	-
Portulaca quadrifida L.	Portulacaceae	Succulent	A	-	0.5	-	-	-	0.2	0.7	1.1	-	-	0.4
Urochloa mosambicensis (Hack.) Dandy.	Poaceae	Grass	A	-	0.5	-	-	-	-	-	-	-	0.3	1.4
Total abundance of herbaceous perennials (%)				61	61.2	60	69.7	30.2	28.8	45.3	70.6	73.1	60.3	47.1
Total abundance of herbaceous annuals (%)				39	36	34	25.8	69.8	71.2	51.4	22.2	23.9	39.7	49.7
Species richness (number of species)				10	17	15	16	19	23	18	16	12	17	16

†Life form: D = Deciduous, E = Evergreen, WP = Weak perennial, A = Annual and P = Perennial. Apr = April, Aug = August, Oct = October and Dec = December

to be geared by annual species. Our results concurred with Bisteau and Mahy (2005) who found more annual species in the SSB of cleared Pinus forests compared to uncleared sites. A dominance of annual species in the soil seed bank is driven mainly by high fecundity, long seed persistence and short life cycle (Ma et al., 2013). According to Korb et al. (2005) and Gelviz-Gelvez et al. (2016), recruitment and establishment of early successional species requires high light intensity, hence they colonise cleared areas. The time (year and 4 months) for which assessment of the SSB was conducted in this study might not be long enough to allow SSB changes towards perennial species dominated community.

Vachellia tenuispina was the only woody species germinated in the SSB with relatively low abundance of 0.1 to 4%, suggesting that this species produces transient seeds or it reproduces partly by vegetative clones. Previous studies suggest that reproduction of *V. tenuispina* is supported more by rhizomes (Van Wyk and Van Wyk, 2013). Therefore, our results suggested that follow-up management of *V. tenuispina* should be largely directed to resprout control than seedling recruitment. The species that emerged frequently with high abundance over time were *Eragrostis lehmaniana* (Nees.), *Aristida bipartita* (Nees.) Trin. & Rupr, *Jamesbrittenia aurantiaca* (Burch.) Hilliard and *Brachiaria eruciformis* (Sm.) Griseb. However, the soil seed bank of *B. eruciformis* was heavily depleted during rainy months (October and December 2016) but its abundance increased above all other species in April and August 2017 in cleared compared to uncleared microsites. These results suggested that *B. eruciformis* seeds germinated in the rangeland before sampling in October and December 2016. In semi-arid African savannas, a rainfall of 15 to 25 mm may break the dormancy of most grass species (Tessema et al., 2016). Seeds of *B. eruciformis* are highly responsive to early spring rains (McGillion and Storrie, 2006) and high light intensity, typically in clay soils (Ismail, 1985).
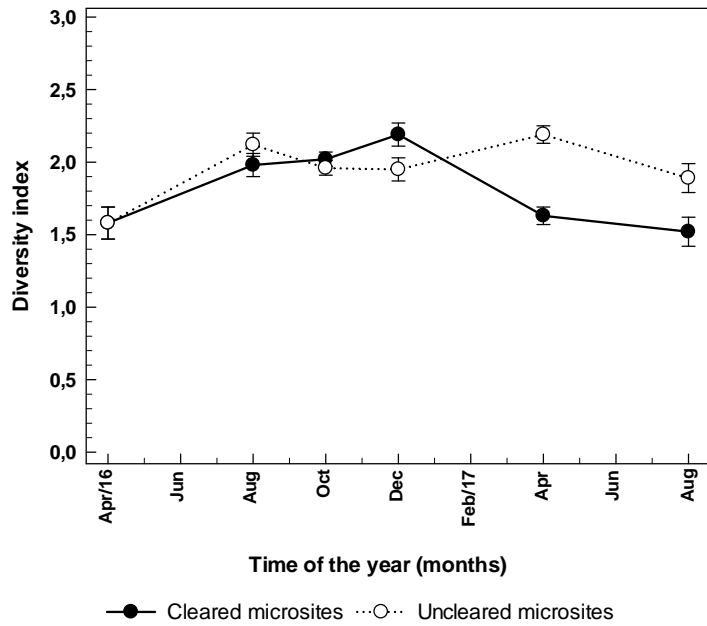
Figure 1 Plant diversity in bush cleared and uncleared microsites over time from April 2016 to August 2017 at Radi.

Key: Apr = April, Jun = June, Aug = August, Oct = October, Dec = December, Feb = February.

4.2. Seed bank diversity over time

The diversity index between cleared and uncleared microsites over time at Radi is shown in **Figure 1**. The microsites and time since bush clearance interacted significantly ($p = 0.007$) for plant diversity. The diversity showed differential response patterns between cleared and uncleared microsites over time. In cleared microsites, diversity increased positively but comparable ($p < 0.05$) to uncleared microsites, from August to December 2016. In April 2017, diversity declined sharply ($p = 0.023$) in cleared, far lower than uncleared microsites. Diversity declined further until August 2017 in cleared microsites. A decline in diversity was mainly caused by high abundance of a few and underrepresentation of most species in the soil seed bank in cleared microsites. This suggested that the highly abundant species might have competed with the other species on the above ground vegetation in cleared microsites thereby suppressing their recruitment and seed production. During senescence, the dominant species (*B. eruciformis*) tends

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to form a mat-like cover that reduces seedling emergence of the plants coexisting in the same habitat (McGillion and Storrie, 2006). Despite reducing seedling emergence, *B. eruciformis* is regarded as a highly competitive grass species that reduces sorghum production by 33 to 60% in Queensland (McGillion and Storrie, 2006).
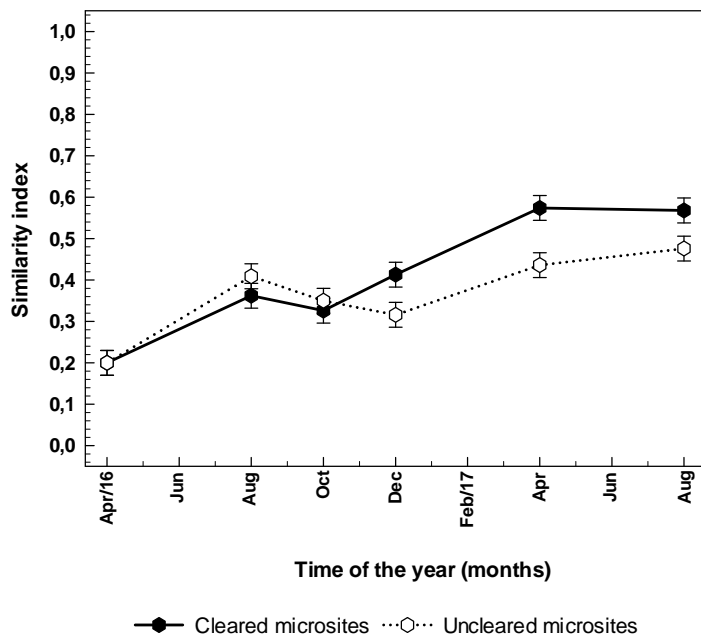

Figure 2 The similarity between the soil seed bank and above ground vegetation in bush cleared and uncleared microsites at Radi from April 2016 to August 2017.

Key: Apr = April, Jun = June, Aug = August, Oct = October, Dec = December, Feb = February

4.3. Similarity between SSB and AGV

The similarity between the soil seed bank and above ground vegetation in cleared and uncleared microsites at Radi is presented in **Figure 2.** Generally, the similarity between the SSB and AGV was low, ranging from 0.20 to 0.59 and 0.20 to 0.45 in cleared and uncleared microsites, respectively. However, treatments and time had a significant interaction ($p = 0.012$) on the similarity between SSB and AGV. Similarity increased linearly with time in both cleared ($r^2 = 0.91$, $p = 0.003$) and uncleared ($r^2 = 0.69$, $p = 0.042$) microsites. In August and October 2016,

similarity was relatively low (Sørensen's coefficient <0.40) and statistically similar ($p > 0.05$) between cleared and uncleared microsites. In December 2016, seed bank slightly mirrored the above ground vegetation with cleared microsites having higher similarity than uncleared microsites. From December to April 2017, similarity increased linearly in both microsites, however; cleared microsites exhibited higher similarity than uncleared microsites. From April to August 2017, similarity was similar ($p > 0.05$) in cleared microsites.

The increase in similarity in cleared microsites could be ascribed to increase in species richness, with *Bidens pilosa* (L.) Timeroy*, Rhynconsia minima* (L.) DC*, Hibiscus trionum* (L.), *Blepharis intergrifolia* (Schinz.) Oberm and *Aristida conjesta subsp. barbicolis* (Roen & Schult.) emerging for the first time in the SSB in April 2017 (**Table 1**). The emergence of these species increased the number of species shared between the SSB and AGV to 14 species in cleared microsites relative to 10 species in uncleared microsites. Similar to our study, Kemeny et al. (2005) found higher similarity between AGV and SSB in autumn. However, more than 50% of species found in AGV did not appear in the SSB at each sampling time of the year in this study. Some species, perennials in particular produce transient seeds and they rely more on vegetative reproduction (Mitlacher et al., 2002; Snyman, 2010; Tedder et al., 2012). Moreover, perennial species are less likely to contribute to the soil seed bank due to grazing which reduces their seed production (Veenendal et al., 1996). However, due to the fact that microsites were protected from grazing, livestock could have only had an effect on the SSB before bush clearance in this study. In cleared and adjacent uncleared microsites, vegetation did not change suggesting that there was no introduction of new species into cleared microsites. Although we accounted for seasonal variability and vertical distribution of seed bank, seeds of rare species that lack long distance dispersal could be omitted due to small spatial area of sampling. This has been reported in various seed bank studies (e.g. Kellerman et al., 2007).
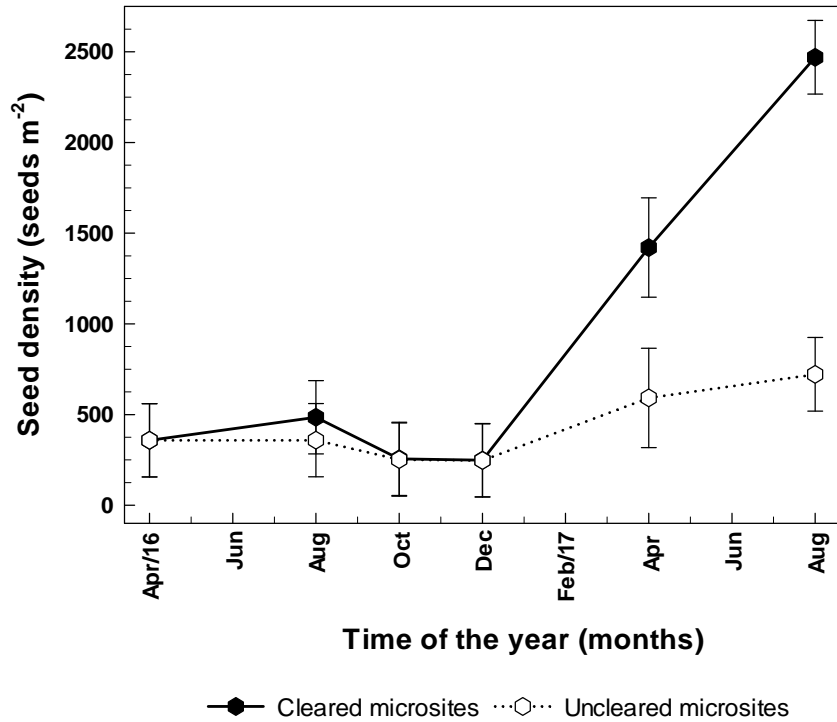
Figure 3 Total seed density over time in bush cleared and uncleared microsites at Radi from April 2016 to August 2017.

Key: Apr = April, Jun = June, Aug = August, Oct = October, Dec = December, Feb = February.

4.5. *Total seed bank density over time*

The total seed density over time in cleared and uncleared microsites at Radi rangelands is shown in **Figure 3**. The treatment and time had a significant effect ($p < 0.05$) and together they interacted significantly ($p = 0.001$) on total seed density. Seed bank density in cleared and uncleared microsites showed differential responses to time ($p < 0.0001$), suggesting that the response to bush clearance is largely dependent on seasonality. The seed bank density in cleared microsites was comparable ($p > 0.05$) to uncleared microsites until December 2016, after which seed densities increased linearly to 1 421 and 2 470 seeds m^{-2} in April and August 2017, respectively in cleared microsites. The total seed density was 2 to 3-fold higher in cleared than uncleared microsites in April and August 2017, respectively. Given high fecundity of

approximately 4000 seeds m^{-2} (McGillion and Storie, 2006) and high seedling emergence (Werth et al., 2008), *B. eruciformis* could have colonised vigorously in bare patches in cleared microsites. Even when rain falls later in the season, this species grows very fast and completes its cycle within 4-6 weeks and produce large number of seeds (McGillion and Storrie, 2006). The seed densities in uncleared microsites were not significantly different ($p > 0.05$) across all sampling times of the year. This indicated that woody plants restrained seed production of herbaceous species in uncleared microsites. In bushlands, the shade imposed by woody plants restrain germination and seed production of heliophytic species, with most plants disappearing even before seed set (Van Calster et al., 2008; Tessema et al., 2017). Similarly, in Ponderosa and Juniper woodland ecosystems, thinned microsites had 2 times greater seed densities than unthinned microsites (Faist et al., 2015). The total seed densities in our study were lower than the 4 700 seeds m^{-2} reported by Mitlacher et al. (2002) in bush cleared and continuously grazed rangelands in Oland, Sweden.

4.6. Total seed bank density by depth over time

Total seed density at various burial depths in cleared and uncleared microsites at Radi is shown in **Figure 4**. The burial depth, treatments and time had a significant ($p < 0.0001$) interaction on the total seed density. The soil seed density varied significantly with burial depth over time in cleared microsites, with the highest density recorded in the upper 0-10 cm followed by >10-20 cm and >20-30 cm. Similar seed density distribution patterns were reported by Sharma et al. (2018) in thinned forest of the Southeastern United States of America. However, seed bank density at 0 - 10 cm was depleted to levels comparable to >10 - 20 and >20 - 30 cm in October and December 2016 in cleared microsites, suggesting that more seeds germinated in the field due to increased temperatures and soil moisture during the rainy season. The proportion of seeds buried at >10 - 20 and >20 - 30 cm in cleared microsites, collectively were 40 and 42% in April and August 2017, respectively. These results indicated that the seed burial beyond 10 cm depth

depended largely on the total amount of seeds constituting the soil seed bank at that point in time. Seed bank density did not show any significant ($p > 0.05$) differences between depths over time in uncleared microsites and all sampling times remained statistically similar and comparable to pre-treatment density.
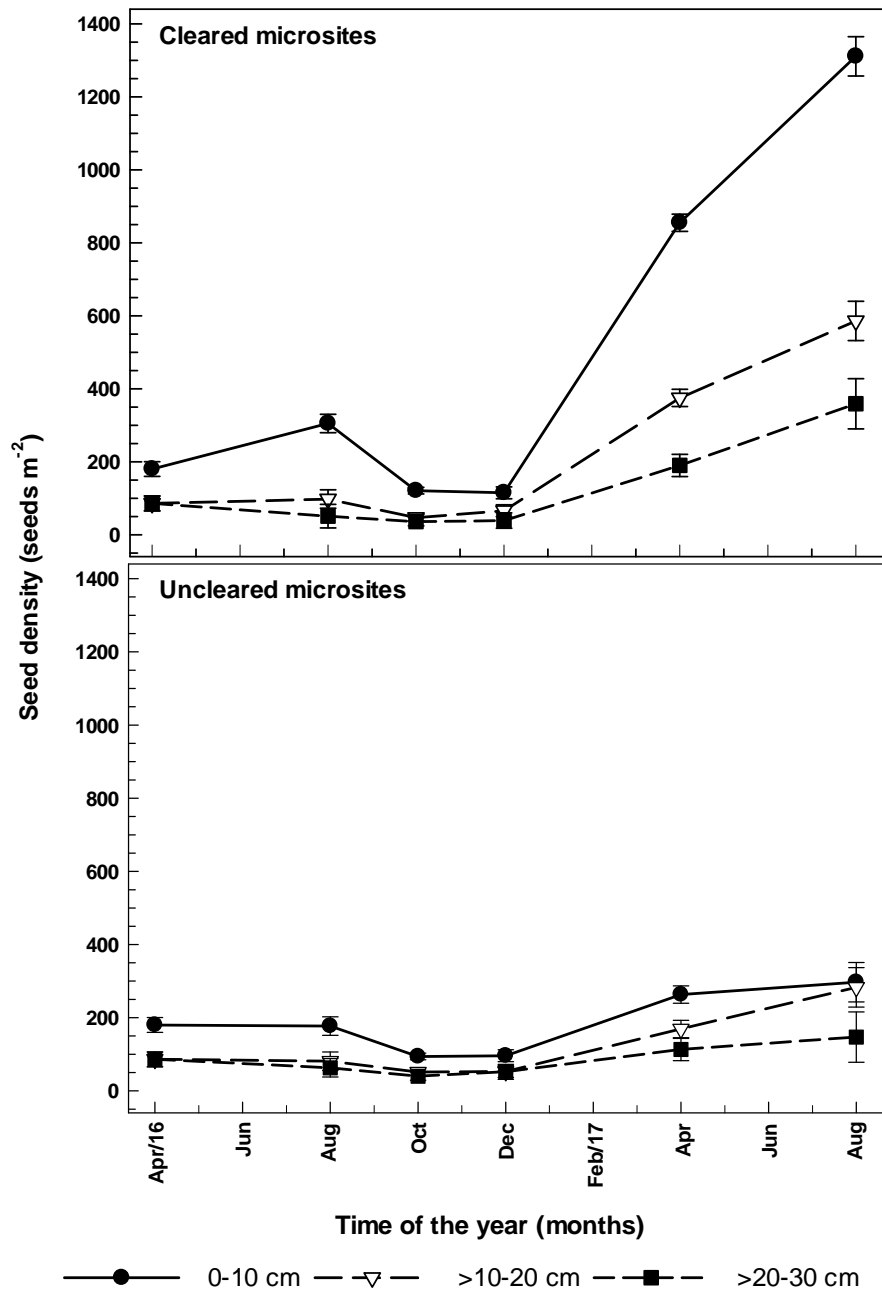

Figure 4 Total seed density at various burial depths in bush cleared and uncleared microsites at Radi from April 2016 to August 2017.

Key: Apr = April, Jun = June, Aug = August, Oct = October, Dec = December, Feb = February.

4.7. Seed bank densities by functional groups over time

Soil seed density of forbs and grasses at various soil depths in bush cleared and uncleared microsites at Radi is presented in **Figure 5**. The interaction between time, treatments and functional groups was significant (p = 0.0003) for seed densities of grasses and forbs. Seed densities varied vertically with burial depth over time in cleared microsites and no significant variation was detected ($p > 0.05$) in uncleared microsites. Seed density of grasses was highest at 0-10 cm, with the densities increasing linearly with time from 55 to 1 243 seeds m^{-2} from December 2016 to August 2017 in cleared microsites. At deeper depths (>10-20 and >20 – 30 cm), the significant increase in seed densities of grasses was most apparent in August 2017 in cleared microsites. In August, almost all herbaceous plants are likely to have dropped seeds because in semi-arid Southern Africa, annual species produce seeds very early around December while perennials drop most of seeds at the end of the growing season around April/May (Veenendal et al., 1996). Furthermore, the time between seed set and the beginning of the rainy season is long enough for seed dormancy of most grasses to break in semi-arid Southern Africa (Veenendal et al., 1996). The seed density of forbs in both cleared and uncleared microsites was similar ($p > 0.05$) at all burial depths across all sampling times of the year, indicating that seed bank size of forbs does not depend entirely on bush clearance. The results of this study concur with Figueroa et al. (2004), who recorded high seed densities of annual grasses than forbs and legumes in open spaces compared to under woody canopies. Contrary, Korb et al. (2005) found no significant effect of bush clearance, burial depth and time since bush clearance on functional groups, 18 months after thinning of Pinus Ponderosa forest.
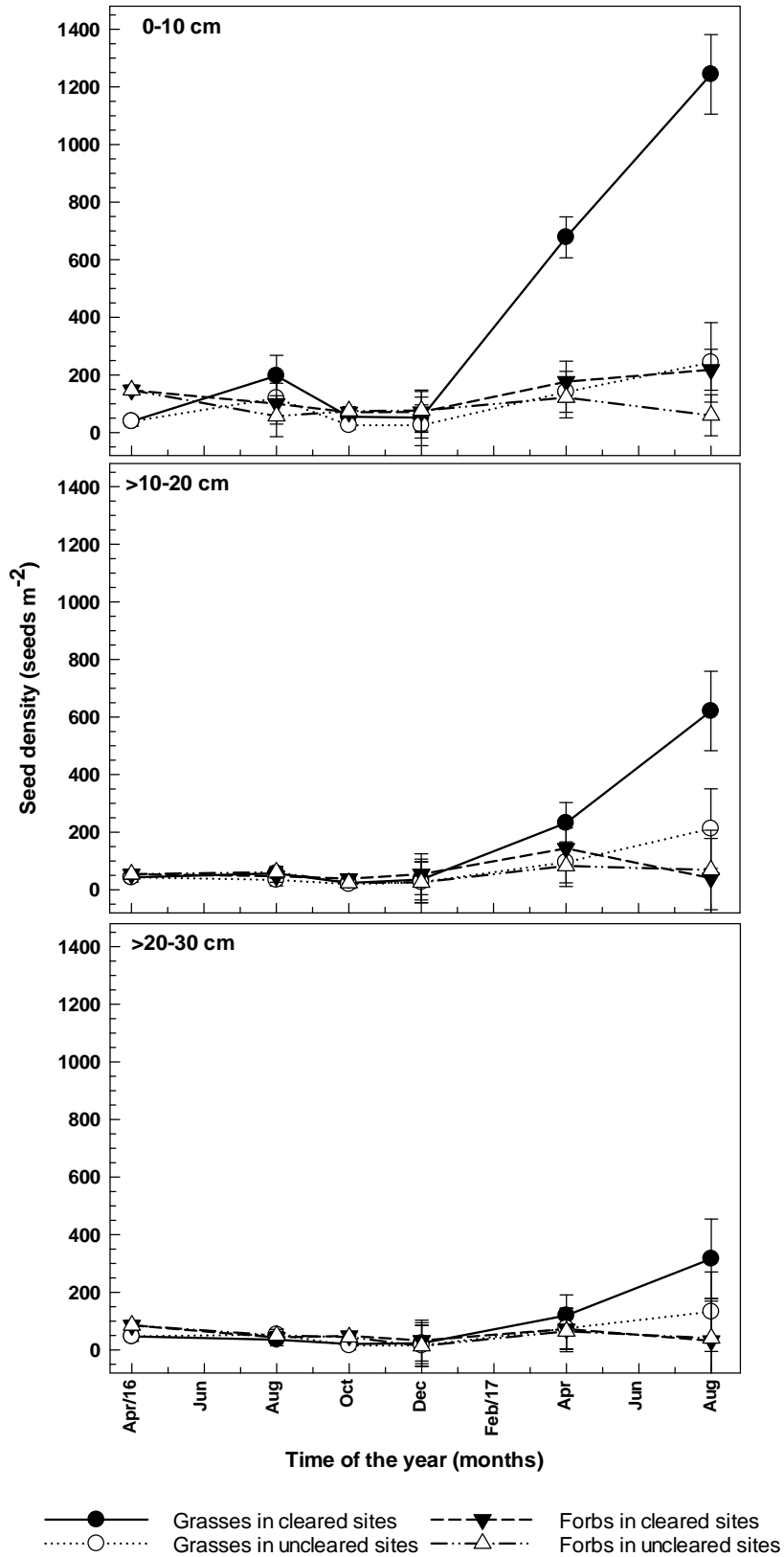
Figure 5 Soil seed density of forbs and grasses at various soil depths in bush cleared and uncleared microsites at Radi from April 2016 to August 2017.

Key: Apr = April, Jun = June, Aug = August, Oct = October, Dec = December, Feb = February

Table 2 Mean soil seed bank density of plant species at various soil burial depths in bush cleared and uncleared microsites at Radi from April 2016 to August 2017.

Species	Plant class	LF[†]	Depth	Cleared microsites 2016 Apr	Aug	Oct	Dec	2017 Apr	Aug	Uncleared microsites 2016 Aug	Oct	Dec	2017 Apr	Aug
V. tenuispina	Shrub	D	10				4			8	9		1	12
			20		4		12			6	3		6	14
			30			8			14				8	
A. conjesta subsp. barbicolis	Grass	WP	10					39	62				71	114
			20					14	29				41	35
			30					8	31				15	
A. bipartita	Grass	P	10	3	18	19	8	52	8	13	9	6		6
			20	19	14	3	18	8	23	7	6	10		
			30	15	14	3	12	6			6	8		
B. eruciformis	Grass	A	10		70	12	20	406	647	50	26	4	64	77
			20		23			212	408	11			31	50
			30					87	187	19			19	
B. insulpta	Grass	P	10		13	6	6	21	44	10				
			20		4			5	14	4				12
			30						9					
C. asplenifolius	Forb	P	10	3	12	8	8	79	23		8	18	42	6
			20		14	3	12	64	17	7		6	31	
			30		11			52	6	11	7		23	
C. decumbens	Forb	A	10		8	8	6	5		13	10	5		
			20			6		6			6			
			30			14		6			6			
D. eriantha	Grass	P	10		15			8	234	7	6		8	
			20		5				52	7			22	6
			30						23					
E. lehmaniana	Grass	P	10	27	95	15	33	39	34	23	33	12	50	21
			20	27	8	17	10	7	12	13	15	5	6	14
			30	5				13	20	15	10		11	
J. aurantiaca	Forb	P	10	19	17	8	17	15	6	5		17	12	
			20	21	17		21	9		13		6	9	
			30	10			7			13		5		
Kohautia spp	Forb	A	10	1	21	22	17	17		24	27	8	8	
			20		10	13	6	9		11	19	12		
			30			8				4	6			
P. maderaspatensis	Forb	P	10		10	5	6	41	9	8	8	11	15	6
			20		4		8	15	6	7	12	12	8	6
			30				6		6	6		10	11	
S. sinensis	Forb	A	10	1	12			14	6	12	3	5	10	
			20				13	4	0	13			14	6
			30					1			6		14	
S. pinnata	Forb	A	10	9					122	4	8		14	17
			20					11	15					12
			30							7				
T. beteroniunus	Grass	A	10		4	14	5	42	6	8	8		17	23
			20					17	12				5	9
			30				7	5					6	

Key: Apr = April, Jun = June, Aug = August, Oct = October, Dec = December, Feb = February.

[†]LF (Life form): D = Deciduous, WP = Weak perennial, A = Annual and P = Perennial

4.8. Seed bank densities by species over time

Mean soil seed density of plant species over time at various soil depths in bush cleared and uncleared microsites at Radi is presented in **Table 2**. The treatment, depth and species had a significant interaction ($p < 0.05$) on the seed densities. The species showed inter-specific variation over time since bush clearance. Three grasses (*B. eruciformis, A. bipartita*, *E. lehmaniana*) and four forbs species (*Chochorous asplenifolius* (Burch.)*, J. aurantiaca, Phyllanthus maderaspatensis* L. and *Kohautia virgata* (Willd.) emerged frequently and their densities fluctuated over time in both cleared and uncleared microsites. This might be due to inter-specific variation in seed dormancy of these species. The dormancy of some species may be broken immediately after seed set in semi-arid ecosystems whereas for others it requires prolonged seed exposure to increased soil temperatures (Tessema et al., 2016). The seeds of *Bothriochloa insulpta* (Hochst.) ex A. Rich*, Digitaria eriantha* (Stent.) Steud (both perennials) and *A. conjesta subsp. barbicolis* (weak perennial) were very rare (≤ 6 seeds m^{-2}) in October and December 2016 both in cleared and uncleared microsites. These species are all large seeded and according to Snyman (2010), species that exhibit these characteristics produce short-lived seeds.

The seed densities of *B. eruciformis* increased significantly to 402 and 647 seeds m^{-2} in April and August 2017, respectively at 0-10 cm depth in cleared microsites. These seed densities were 6 and 8-fold greater than seed densities recorded at 0-10 cm in uncleared microsites. The seed densities of *B. eruciformis* declined with an increase in burial depth in cleared and uncleared microsites, respectively. Nearly half (42 - 48%) of *B. eruciformis* seeds were buried deeper than 10 cm in April and August 2017, respectively in cleared microsites. It appears that deeper burial of *B. eruciformis* seeds was a function of the total seeds constituting the SSB at a particular sampling time rather than dispersal by any vector. This is not surprising as *B. eruciformis* seeds are very small (Werth et al., 2008) and able to fall directly into the soil cracks of vertisols. The seeds that are buried deeper have high probability to escape predation and remain dormant for

extended periods (Snyman, 2010). However, the deep burial of these seeds implies that they are unlikely to contribute in restoration after bush clearance because according to Werth and Osten (2008), large proportion (39%) of *B. eruciformis* seeds germinate when buried 2-5 cm deep in the soil.

Digitaria eriantha had the second largest total seed density (309 seeds m^{-2}) which varied significantly with depth ($p < 0.05$) in August 2017 in cleared microsites. Of the total seed density of *D. eriantha*, 76% of seeds were retained at 0-10 cm, indicating that *D. eriantha* would drive the succession to more perennial species community. In uncleared microsites, *A. conjesta* subsp. *barbicolis* had a significantly ($p < 0.05$) high seed density and its densities declined with an increase in burial depth in April and August 2017. Although most previous studies sampled down to 15 cm depth e.g. Sassaki et al. (1999), they reported similar seed distribution patterns. Interestingly, the seeds of all species were not buried deep to >20-30 cm in uncleared microsites in August 2017, owing to the lack of dispersal vectors to transport seeds down the soil profile.

4.9. Seed bank communities

A two-way cluster dendrogram showing presence and absence of the soil seed bank species in cleared and uncleared microsites at Radi is presented in **Figure 6.** Three distinct seed bank communities were identified. The largest community was Community 1 with five forbs and grasses, a legume, shrub and a succulent species. Community 2 was completely different from the other two communities, having six forbs and a grass species. The former was largely associated with both cleared and uncleared microsites while the latter was associated with cleared microsites only. The third community was less species-rich (**Figure 6**), but the species constituting this community had high seed bank densities in August 2017 (**Table 2**). Usually, high seed production in communities of low species diversity is a response to reduced interspecific competition (Hillerislambers et al., 2009). Therefore, our results suggested that the fewer the species in a community, the lower the competition and the higher the seed production.

The species from Community 2 emerged in cleared microsites only, suggesting that these species are opportunistic colonisers following clearance of woody plants.
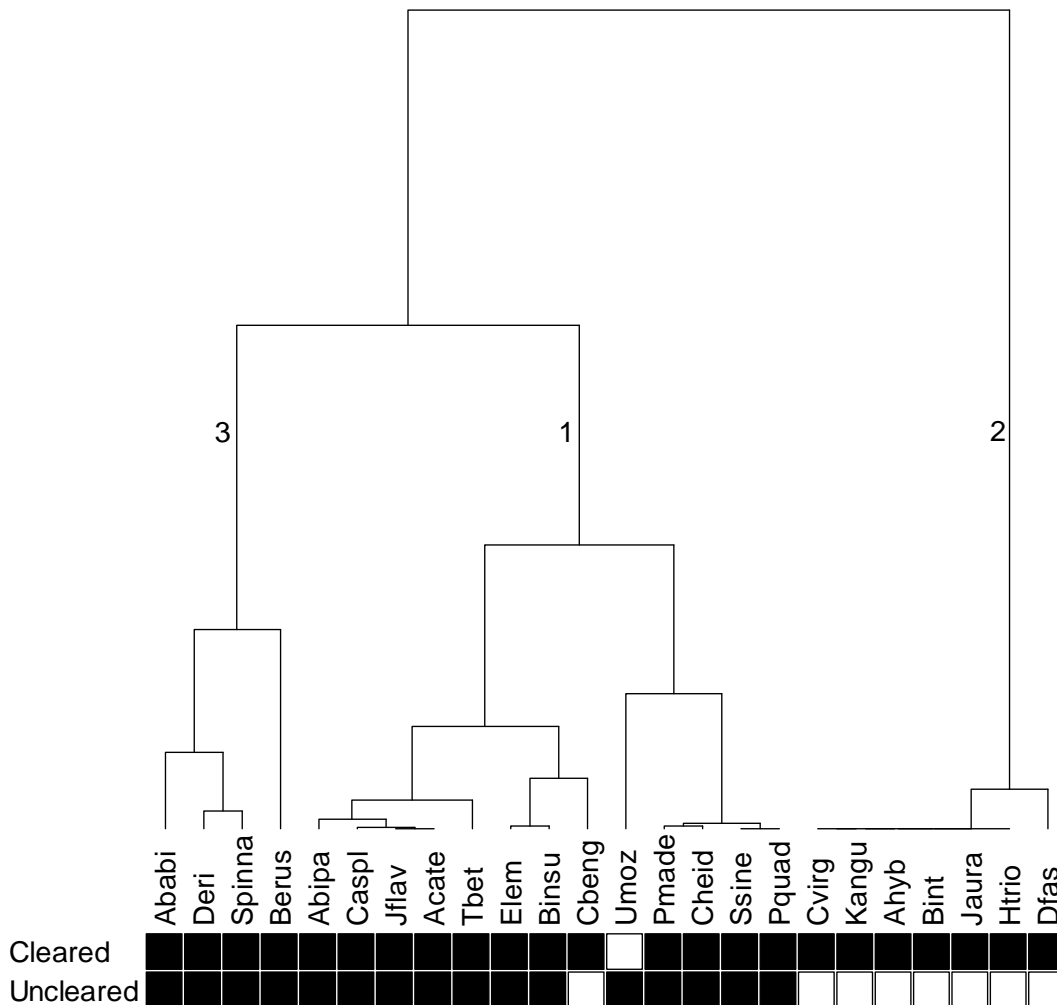

Figure 6 A two-way cluster dendrogram showing presence (■) and absence (□) of the soil seed bank species in cleared and uncleared microsites at Radi.

Key to species: *Ababi = Aristida conjesta subsp. barbicolis* (weak perennial grass), *Pmade = Phyllanthus maderaspatensis* (perennial forb), *Elem = Eragrostis lehmaniana* (perennial grass), *Tbet = Tragus beteronianus* (annual grass), *Cheid = Crotalaria heidmanii* (annual legume), *Pquad = Portulaca quadrifida* (annual succulent), *Umoz = Urochloa mosambicensis* (annual grass), *Berus = Brachiaria eruciformis* (annual grass), *Casple = Chochorous asplenifolius* (perennial forb), *Cbeng = Commelina benghalensis* (annual forb), *Dfas = Datura fastuosa* (annual forb), *Deri = Digitaria eriantha* (perennial grass), *Spinna = Schkuhria pinnata* (annual forb), *Binsu = Bothriochloa insulpta* (perennial grass), *Abipa = Aristida bipartita* (perennial grass), *Htrio = Hibiscus trionum* (annual forb), *Cvirg = Chloris virgata* (annual grass), *Kangu = Kyphocarpa angustifolia* (annual forb), *Bint = Blepharis intergrifolia* (perennial forb), *Jaura = Jamesbrittenia aurantiaca* (perennial forb), *Jflav = Justicia flava* (perennial forb), *Acate = Vachellia tenuispina* (deciduous shrub), *Ssine = Spermacoce sinensis* (annual forb), *Ahyb = Amaranthus hybridus* (annual forb).
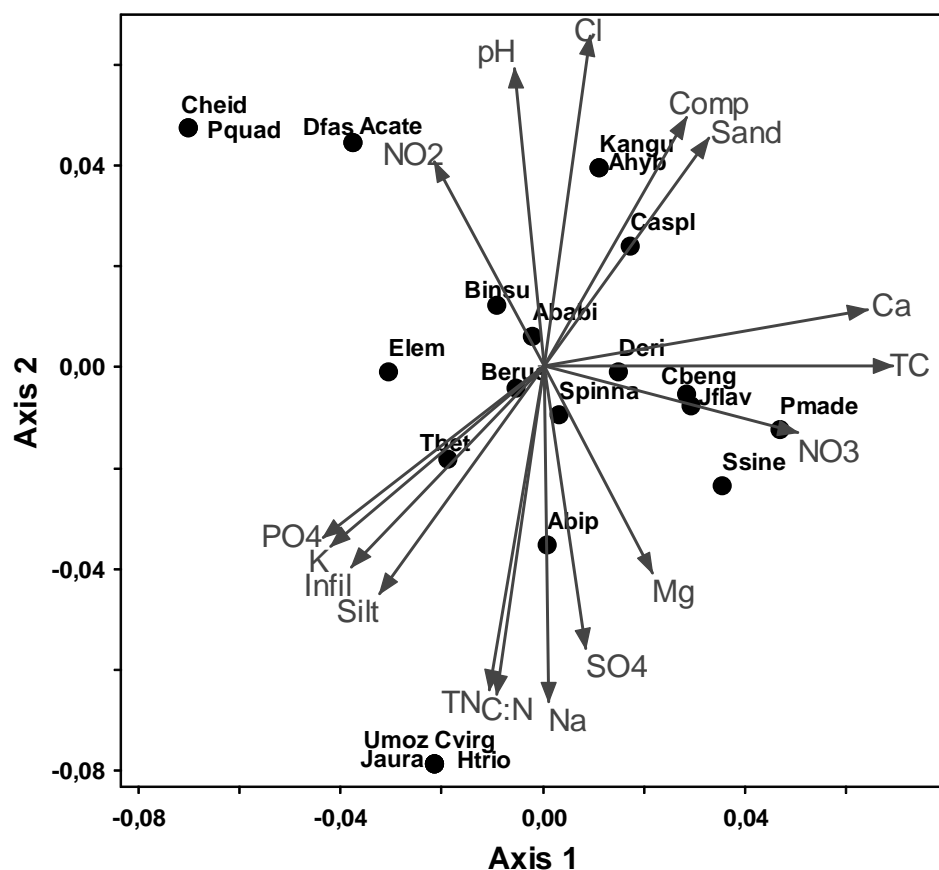
Figure 7 PCA plot showing relationship between soil seed bank species and soil properties at Radi.

Key to symbols: Black dots (●) indicate species and the vectors (→) denote direction of the relationship between species and soil properties. The longer the vector radiating from the centre the stronger the effects of the soil properties and the closer the dot to the vector the stronger the relationship between species and soil property.

Key to soil properties: Mg = magnesium, SO4 = sulphate (SO$_4$), Na = sodium, TN = total nitrogen, TC = total carbon, Infil = infiltration, PO4 = phosphate (PO$_4$), K = potassium, NO2 = nitrite (NO$_2$), NO3 = nitrate (NO$_3$), Cl = chloride, Comp = compaction, Ca = calcium.

Key to species: *Ababi = Aristida conjesta subsp. barbicolis* (weak perennial grass), *Pmade = Phyllanthus maderaspatensis* (perennial forb), *Elem = Eragrostis lehmaniana* (perennial grass), *Tbet = Tragus beteronianus* (annual grass), *Cheid = Crotalaria heidmanii* (annual legume), *Pquad = Portulaca quadrifida* (annual succulent), *Umoz = Urochloa mosambicensis* (annual grass), *Berus = Brachiaria eruciformis* (annual grass), *Casple = Chochorous asplenifolius* (perennial forb), *Cbeng = Commelina benghalensis* (annual forb), *Dfas = Datura fastuosa* (annual forb), *Deri = Digitaria eriantha* (perennial grass), *Spinna = Schuria pinnata* (annual forb), *Binsu = Bothriochloa insulpta* (perennial grass), *Abipa = Aristida bipartita* (perennial grass), *Htrio = Hibiscus trionum* (annual forb), *Cvirg = Chloris virgata* (annual grass), *Kangu = Kyphocarpa angustifolia* (annual forb), *Bint = Blepharis intergrifolia* (perennial forb), *Jaura = Jamesbrittenia aurantiaca* (perennial forb), *Jflav = Justicia flava* (perennial forb), *Acate = Vachellia tenuispina* (deciduous shrub), *Ssine = Spermacoce sinensis* (annual forb), *Ahyb = Amaranthus hybridus* (annual forb).

4.10. Soil-species relationships

The principal component analysis (PCA) with axis 1 and 2 explaining 82.6 and 17.4% of variance respectively is presented in **Figure 7**. *Eragrostis lehmaniana*, *Tragus beteronianus*, *Brachiaria eruciformis*, *Digitaria eriantha*, *Justicia flava*, *Phyllanthus maderaspatensis* and *Commelina benghalensis* correlated with PCA axis 1. The rest of species correlated with Axis 2 of the PCA. As depicted in **Figure 7**, three species from community 1 (*Chroris virgata, J. aurantiaca* and *Hibiscus trionum*) showed a strong positive association with total nitrogen (TN) and carbon-nitrogen ratio (C:N). Similarly, *A. bipartita* and *Urochloa mosambicensis* correlated with TN and C:N. Further, *A. bipartita* also correlated with sodium (Na). On the other hand, a suite of forbs from different communities namely; *S. sinensis, P. maderaspatensis, J. flava* and *C. benghalensis* were associated with soils high in nitrates (NO_3^-). The distribution of these species in different communities might be due to the fact that soil nutrients are heterogeneous and spatially distributed allowing plants to coexist at various spatial scales in different communities (Gilliam and Dick, 2010). John et al. (2007) reported that the effect of soil nutrients on plant communities is largely dependent on the magnitude by which those nutrients vary and also on plant specific requirements for a particular soil nutrient.

The annual grasses (*T. beteronianus* and *B. eruciformis*) correlated with silt content, infiltration rate, phosphates and potassium (K). *Amaranthus hybridus* and *Kyphocarpa angustifolia* were strongly associated with chloride (Cl) and compacted soils, whereas *Chochorous aspleninifolius* was associated with sandy soils. *Vachellia tenuispina*, *Bothriochloa insulpta* and *Datura fastuosa* were associated with soils high in nitrites. The PCA revealed no clear associations between soil seed bank species and soil pH, total carbon (TC), magnesium (Mg) and sulphates. Overall, the PCA showed that species differ in nutrient requirements and soil condition preferences, with total nitrogen being the most important soil nutrient influencing the distribution of most seed bank species. Soil nitrate (NO_3) was shown to be the most vital source

of N for most forb species. These results concur with Falkengren-Grerup and Schottelndreier (2004) who found that a large number of forbs were indicators of soils with high mineralised NO_3^-. The species richness of native species was negatively associated with total nitrogen but increased with C:N ratio (Hrivnak et al., 2015).

5. Conclusion

Bush clearance increased the soil seed bank density, species richness and the relationship between seed bank and above ground vegetation over time. The highly abundant life form was annual species, with grasses, particularly *B. eruciformis* being the most abundant species. Large seed bank size of grasses in cleared microsites indicated a high potential for passive recovery of herbaceous vegetation following bush clearance. However, follow-up management to foster vegetation progression to perennial species dominated state is necessary. One of possible options is the integration of bush clearance and brush packing to ensure that seedlings emerging from the soil seed bank are protected from grazing and trampling. We recommend research that will monitor the long-term changes of the soil seed bank and above ground vegetation over time. Such research will provide more knowledge on the long-term vegetation dynamics and will help in planning further rangeland management strategies.

Acknowledgements

Conflict of interest

We have no competing interest.

References

AOAC., 1990. Association of official analytical chemists (13th edition). Official methods of analysis, Washington, DC.

Bakker, J.P., Hoffmann, F., Ozinga, W.A., Rosen E., 2014. Shading results in depletion of the soil seed bank. *Nordic Journal of Botany* 32, 674 – 679.

Beretta, A.N., Silbermann, A.V., Paladino, L., Torres, D., Basahun, D., Musseli, R., Garcia-lamohte, A., 2014. Soil texture analysis using a hydrometer: Modification of the Bouyoucos method. *Cientiae Investigacion Agraria.* 41, 263 – 271.

Bisteau, E., Mahy, G., 2005. Vegetation and seed bank in calcareous grasslands restored from a Pinus forest. *Applied Vegetation Science.* 8, 167 – 174.

Caballero, I., Olano, J.M., Loidi, J., Escudero, A., 2008. A model for small-scale seed bank and standing vegetation connection along time. *Oikos* 177, 1788 – 1795.

Chao, A., Chazdon, R.L., Colwell, R.K., Shen, T., 2006. Abundance-Based Similarity Indices and Their Estimation When There Are Unseen Species in Samples. *Biometrics* 62, 361 – 371.

D'Souza, L.E., Barnes, P.W., 2008. Woody Plant Effects on Soil Seed Banks in a Central Texas Savanna. *The Southwest Nataturalist* 53, 495 – 506.

DIGES., 2012. Proposed construction of 100MW solar power plant on portion 6 and 7 of farm Bezuiidenhoutskraal 96 JR within Moretele Local Municipality of Bojanala Platinum District Municipality, North West Province. Draft scoping report.

Eager, E.A., Haridas, C.V., Pilson, D., Rebarber, R., Tenhunberg, B., 2013. Disturbance frequency and vertical distribution of seeds affect long-term population dynamics, A mechanistic seed bank model. *The American Nataturalist* 182, 180 – 190.

Faist, A.M., Stone, H., Tripp, E.A., 2015. Impacts of Mastication, Soil Seed Bank Responses to a Forest Thinning Treatment in Three Colorado (USA) Conifer Forest Types. *Forests* 6, 3060 – 3074.

Falkengren-Grerup, U., Schottelndeier, M., 2004. Vascular plants as indicators of nitrogen enrichment in soils. *Plant Ecology* 172, 51 – 62.

Farnsworth, E.J., Plotkin, A.A.B., Ellison, A.M., 2012. The relative contributions of seed bank, seed rain, and understory vegetation dynamics to the reorganization of Tsuga canadensis forests after loss due to logging or simulated attack by Adelges tsugae. *Canadian Journal Forest Restoration* 42, 2090 – 2105.

Figueroa, J., Teillier, S., Jaksic, F.A., 2004. Composition, size and dynamics of the seed bank in a mediterranean shrubland of Chile. *Austral Ecology* 29, 574 – 584.

Fourie, S., 2007. Composition of the soil seed bank in alien-invaded grassy fynbos, Potential for recovery after clearing. *South African Journal of Botany* 74, 445 – 453.

Gabay, O., Perevolotsky, A., Sashak, M., 2012. How landscape modulators function, woody plant impact on seed dispersal and abiotic filtering. *Plant Ecology* 213, 685 – 693.

Gelviz-Gelvez, S.M., Sanchez-Montano, L.R., Lopez-Toledo, L., Barragan, F., 2016. The Andean forest soil seed bank in two successional stages in northern Colombia. *Botanical Sciences* 94, 1 – 15.

Gilliam, F.S., Dick, D.A., 2010. Spatial heterogeneity of soil nutrients and plant species in herb-dominated communities of contrasting land use. *Plant Ecology.* 209, 83 – 94.

He, M., Lv, L., Li, H., Meng, W., Zhao, N., 2016. Analysis on soil seed bank diversity characteristics and chemical properties after substrate addition. *Plos one.* 11, e0147439.doi:10.1371/journal.pone.

Heras, M.M.L., Turnbul, L., Wainright, J., 2016. Seed-bank structure and plant-recruitment conditions regulate the dynamics of a grassland-shrubland Chihuahuan ecotone. *Ecology* 97, 2303 – 2318.

Hillerislambers, J., Harpole, W.S., Schnitzer, S., Tilman, D., Reich, P.B., 2009. CO_2, nitrogen, and diversity differentially affect seed production of prairie plants. *Ecology* 90, 1810 – 1820.

Holmes, P.M., 2002. Depth distribution and composition of seed-banks in alien-invaded and uninvaded fynbos vegetation. *Austral Ecology.* 27, 110 – 120.

Hrivnak, R., Slezak, M., Jarcuska, B., Jarolimek, I., Kochjarova, J., 2015. Native and alien plant species richness response to soil nitrogen and phosphorous in temperate Floodplain and swamp forest. *Forests* 6, 3501 – 3513.

Ismail, A.M.A., 1985. A comparative study of the effects of clumping, substrate, light, depth of burial and soil moisture upon the germination of seeds of weeds and crops grown in the Sudan Gezira. *Qata University Science Bulletin* 5, 153 – 167.

John, R., Daling, J.W., Harms, K.E., Yavitt, J.B., Stallard, R.F., Mirabello, B., Hubbell, S.P., Vallencia, R., Navarrete, H., Vallejo, M., Foster, R.B., 2007. Soil nutrients influence spatial distributions of tropical tree species. *PNAS* 104, 864 – 869.

Kellerman, M.J.S., van Rooyen, M.W., 2007. Seasonal variation in soil seed bank size and species composition of selected habitat types in Maputaland, South Africa. *Bothalia* 37, 249 – 258.

Kemeny, G., Nagy, Z., Tuba, Z., 2005. Seed bank dynamics in a semiarid sandy grassland in Hungary. *Ekologia (Bratislava)* 24, 1 – 13.

Korb, J.E., Springer, J.D., Powers, S.R., Moore, M.M., 2005. Soil seed banks in Pinus Ponderosa forests in Arizona, Clues to site history and restoration potential. *Applied Vegetation Science* 8, 103 – 112.

Lang, N.L., Halpern, C.B., 2007. The seed bank of a Montane meadow, Consequences of a conifer encroachment and implications for restoration. *Canadian Journal of Botany* 85, 557 – 569.

Ma, M., Zhou, X., Qi, W., Liu, K., Jia, P., Du, G., 2013. Seasonal dynamics of the plant community and soil seed bank along a successional gradient in a subalpine meadow on the Tibetan Plateau. *PloS one* 8, e80220. doi:10.1371/journal.pone.0080220.

Maguran, A., 1988. Ecological diversity and its measurement. Princeton University Press, New Jersey.

Marchante, H., Freitas, H., Hoffmann, J.H., 2011. Post-clearing recovery of coastal dunes invaded by Acacia longifolia: is duration of invasion relevant for management success? *Journal of Applied Ecology* 48, 1295 – 1304.

McCune, B., Mefford, M .J., 2011. PC-ORD. Multivariate analysis of ecological data. Version 6. MjM Software, Gleneden Beach, Oregon, U.S.A.

McDonald, S.J., Frank, D.S., Ramirez, J.A., Wang, B., Brooks, J.M., 2006. Ancillary Methods of the National Status and Trends Program: 2000-2006 Update. Silver Springs, MD. NOAA Technical Memorandums NOS NCCOS 28. 17 pp.

McGillion, T., Storrie, A (eds)., 2006. Integrated weed management in Australian cropping systems – A training resource for farm advisors. CRC for Australian Weed Management, Adelaide, South Australia.

Mckinney, A.M., Goodell, K., 2010. Shading by invasive shrub reduces seed production and pollinator services in a native herb. *Biological Invasions* 12, 2751 – 2763.

Metlen, K.L, Fiedler, C.E., 2006. Restoration treatment effects on the understory of Ponderosa pine/Douglas-fir forests in western Montana, USA. *Forest Ecology and Management* 222, 355 – 369.

Mitlacher, K., Poschold, P., Rosen, P., Bakker, J.P., 2002. Restoration of wooded meadows – a comparative analysis along a chronosequence on Oland, Sweden. *Applied Vegetation Science* 5, 63 – 73.

Moerane, R., 2013. The impact of training using a structured primary animal health care model on the skills of rural small scale farmers. Master's thesis. University of Pretoria, Pretoria, South Africa.

Mucina, L., Rutherfored, M.C., Palmer, A.R., Milton, S.J., Scott, L., Lloyd, J.W., van der Merve, B., Hoare, D.B., Bezuidenhout, H., Vlok, J.H.J., Euston-Brown, D.I.G., Powrie, L.W., Dold, A.P., 2006. Nama-karoo Biome. Strelitzia 19. South African Biodiversity Institute, Pretoria, South Africa.

Olano, J.M., Caballero, I., Escudero A., 2012. Soil seed bank recovery occurs more rapidly than expected in semi-arid Mediterranean gypsum vegetation. *Annals of Botany* 109, 299 – 307.

Plue, J., Verheyen, K., van Calster, H., Marage, D., Thompson, K., Kalamees, R., Jankowska-Blaszcuk, M., Bossuyt, B., Hermy, M., 2010. Seed banks of temperate deciduous forests during secondary succession. *Journal of Vegetation Science* 21, 965 – 978.

Price, J.N., Morgan, J.W., 2008. Woody plant encroachment reduces species richness of herb-rich woodlands in southern Australia. *Austral Ecology.* 33, 278 – 289.

Reeuwijk, L.P., 2002. "Procedures for Soil Analysis," 6th Edition, International Soil and Reference Information Centre, Food and Agriculture Organisation, Wageningen.

Richardson, D.M., Kluge, R.L., 2008. Seed banks of invasive Australian Acacia species in South Africa, role in invasiveness and options for management. *Perspectives in Plant Ecology, Evolution and Systematics* 10, 161 – 177.

SAS Institute., 2009. Users guide, Statistics, SAS Institute Inc., Cary, NC, USA.

Sassaki, R.M., Rondon, J.N., Zaidan, L.B.P., Felippe, G.M., 1999. Number of buried seeds and seedlings emergence in cerradão, cerrado and gallery forest soils at Pedregulho, Itirapina .SP., Brazil. *Revista Brasileira de Botanica* 22, 147 – 152.

Scott, K.A., Setterfield, S.A., Douglas, M.M., Anderson, A.N., 2010. Environmental factors influencing the establishment, height and fecundity of the annual grass *Sorghum intrans* in an Austalian tropical savana. *Journal of Tropical Ecology* 26, 313 – 322.

Sharma, A., Bohn, K.K., Jose, S., Miller, D.L., 2018. Seed Bank—Vegetation dynamics along a restoration management gradient in pine flatwoods ecosystems of the florida gulf coast. *Natural Areas Journal* 38, 26 – 43.

Smith, G.F., Crouch, N.R., Figuereido, E., 2017. Field guide to succulents in Southern Africa (1st edition). Struik Nature, Cape Town, South Africa. pp 427 – 429.

Snyman, H.A., 2010. Longevity of grass seeds in a semi-arid grassland. Grassroots. The Grassland Society of Southern Africa.

Tedder, M.J., Kirkman, K.P., Morris, C.D., Trollope, W.S.W., Bonyongo, M.C., 2012. The influence of *Pechuel-Loeschea leubnitziae* (wild sage) on grass sward and soil seed bank composition. *African Journal of Range and Forage Science* 29, 101 – 107.

Tessema, K.Z., de Boer, W.F., Prins, H.H.T., 2016. Changes in grass plant populations and temporal soil seed bank dynamics in a semi-arid African savanna, Implications for restoration. *Journal of Environmental Management* 182, 166 – 175.

Tessema, K.Z., Ejigu, B., Nigatu, L., 2017. Tree species determine soil seed bank composition and its similarity with understory vegetation in a semi-arid African savanna. *Ecological Processes* 6, DOI 10.1186/s13717-017-0075-7.

Van Calster, H., Chevalier, R., van Wyngene, B., Archaux, F., Verheyen, K., Hermy, M., 2008. Long-term seed bank dynamics in a temperate forest under conversion from coppice-with-standards to high forest management. *Applied Vegetation Science* 11, 251 – 260.

Van der Walt, R., 2009. Wild flowers of the Limpopo valley including Mapungubwe National Park .1st edition.. Musina, Buisness print. pp 11 – 335.

Van Oudtshoorn, F., 1999. Guide to grasses of southern Africa. Pretoria, Briza Publications.

Van Wilgen, B.W., Cowling, R.M., Marais, C., Esler, K.J., McConnachie, M., Sharp, D., 2012. Challenges in invasive alien plant control in South Africa. *South African Journal of Science* 108, 1 – 3.

Van Wyk, B., Van Wyk, P., 2013. Field guide to trees of Southern Africa. 2nd edition. Struik Nature .Pty. Ltd., Wembley Square, Cape Town, South Africa. pp 136 – 596.

Veenendaal, E.M., Ernst, W.H.O., Modise, G.S., 1996. Effect of seasonal rainfall pattern on seedling emergence and establishment of grasses in a savanna in southeastern Botswana. *Journal of Arid Environment* 32, 305 – 317.

Werth, J.W., Widderick, M., Osten, V., Walker, S., 2008. Prolonging glyphosate effectiveness on difficult to control summer weeds. Sixteenth Australian Weeds Conference.

Werth, J., Osten, V., 2008. Improving management of summer weeds in dryland cropping systems with cotton. Part 3 – Final report. pp 4 – 5.

Witkowski, E.T.F., Garner, R.D., 2008. Seed production, seed bank dynamics, resprouting and long-term response to clearing of the alien invasive Solanum mauritianum in a temperate to subtropical riparian ecosystem. *South African Journal of Botany* 74, 476 – 484.