

an integral part of the environment in many areas, contributing to the evolution and maintenance of plant communities". A new stage in ecosystem fire was initiated when man used fire to modify their environment. The dominance of man and fire in some ecosystems may compromise the sustainability of landscapes in terms of overuse and damage (Pausas and Keeley 2009).

1.2. Fire as an ecosystem engineer

Fire is of global importance in natural and man-managed ecosystems both as a destructive force and management tool (Raison 1979). Despite the key role fire plays in ecosystem composition, its significance in the distribution of ecosystems has been overlooked (Bond et al. 2005; Pausas and Keeley 2009). The spread of C4 grasses during the late Tertiary is attributed to the opening of woodlands by fire, resulting in the shift of these grasses to more mesic environments (Pausas and Keeley 2009). Thus, a feedback process is postulated to have developed amongst fire activity and the highly flammable C4 grasses, which in turn maintained the grassland dominated landscape, similar to savannah ecosystems (Pausas and Keeley 2009).

Mediterranean ecosystems are synonymous with fire and high species diversity (Cowling et al. 1996). This is attributed to a suite of factors, including the active role of fire in both equilibrium and non-equilibrium dominated ecosystems. These systems provide a unique opportunity to evaluate the effects of Mediterranean type climates and recurrent fire on coexistence and diversification in floras (Cowling et al. 1996). However, the impact of fire in arid to semi-arid systems is still lacking.

Fire regimes can be thought of as a spatially variable template of fire intensity, severity, type, frequency, spatial scale and seasonality, within which biotas have co-evolved (Pausas and Keeley 2009). These regimes are susceptible to shifts due to land-use and climate change (Pausas et al. 2004).

1.2.1. Fire and community structure

It is generally assumed that temperature and rainfall are the main factors determining the distribution of vegetation, as argued by the “green world” hypothesis (Bond et al. 2003; Bond and Keeley 2005; Bond et al. 2005). This is because the occurrence of biomes is largely being predicted by temperature and precipitation (Bond et al. 2005). However, models based on these climatic parameters emphasised the significance of consumer control like fire and grazing on plant community distribution and composition (Bond and Keeley 2005).

Fire and grazing are critical processes in the development of grassland ecosystems and the evolution of species within these environments (Fuhlendorf et al. 2009). Plant communities subjected to disturbance, are transformed to those tolerant thereof rather than those best able to compete for resources (Bond and Keeley 2005). Currently, fire is an important disturbance of global vegetation, affecting ecosystems that are adapted to, tolerant of, dependent on or susceptible to either natural or human-caused fires (Pausas and Keeley 2009).

The importance of fire is exemplified by the Fynbos / Succulent Karoo boundary, in which fire is an abiotic determinant which inhibits the encroachment of the Succulent Karoo into the Fynbos biome (Lechmere-Oertel and Manning 2001).

1.2.2. Plants adapted to fire

The likelihood of fire is influenced by vegetation composition, which provides fuel for the fire, whilst the composition of the vegetation is, in turn, affected by fire frequency (Beckage and Stout 2000). Plant species adapted to the occurrence of fire increase in abundance after fire whilst fire intolerant plant species decline (Platt et al. 1988; Beckage and Stout 2000).

Mediterranean plant communities have high resilience after fire. Two regeneration strategies are followed by plants after a fire, they are: resprouting or reseeding (Verdu 2000; Xavier et al. 2007). Seeder taxa depend on the seeds released for recruitment, as the progenitor of these seeds is destroyed by fire. By contrast, resprouter taxa have buds in underground organs or in stems that allow them to resprout after fire (Verdu 2000). Obligate seeders are most abundant post-fire, but are subject to high mortality after the burn (Xavier et al. 2007). Both adaptation strategies are threatened by anthropogenically altered fire regimes often increasing or decreasing fire frequency (Keeley et al. 2011). Not all plants are able to employ these strategies, often resulting in their disappearance after fire (Xavier et al. 2007).

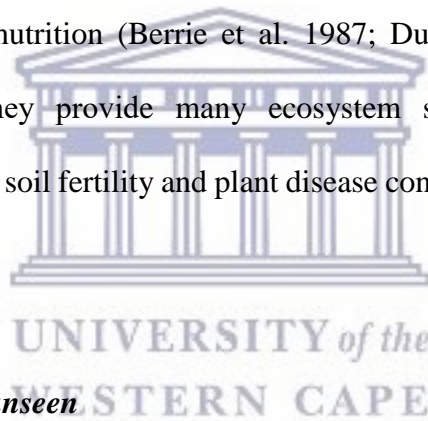
The fire-prone Fynbos displays a greater growth rate than Succulent Karoo plant species, providing the former with a competitive advantage over the latter (Lechmere-Oertel and Manning 2001). However, the fast growing Fynbos plant species are more limited by water than Succulent plant species, therefore, the role of edaphic factors should not be neglected.

1.3. Soil, fire and bacteria

Soil is an important factor in determining the distribution and productivity of plant communities. However, limited data is available on the effects of fire on the bulk soil, particularly on soil biological processes, related nutrient cycling and soil fertility (Raison 1979; Choromanska and De Luca 2002). This significant gap in knowledge contributes to the poor understanding of the role of fire on many plant communities (Raison 1979). Fire can have three effects on plant communities: (a) the direct action of heat on plants and soils, (b) removal of the litter and standing crop, creating new microclimates, and (c) the redistribution and changed availability of nutrient elements. Soil properties can be altered immediately by a fire, or can be changed by subsequent modifications of vegetation and the activities of soil macro- and micro-organisms (Bias et al. 2006; Raison 1979). Plant communities vary and are therefore controlled by different ecological processes. This results in their response to environmental drivers, such as climate and fire, to vary as well (Peters et al. 2006). Thus fire impacts both microbial communities, the nutrient availability within the rhizosphere as well as the vegetation composition thereof.

Bacteria are the most abundant group of microbes present in the soil, the presence of streptomycete bacteria are responsible for the earthy smell of soil (Gerber and Lechevalier 1965; Dutta 2004; Jiang et al. 2007). Both fire and microbes are able to alter soil properties. Fire promotes the free available N within the rhizosphere, whilst microbes are able to liberate phosphorus (P) through chelation. In addition, the heat generated by fire induces chemical oxidation of soil organic matter, altering N transformations (Choromanska and De Luca 2002). Thus fire may promote microbial diversity, which in turn promotes botanical diversity, eliciting a change in the response of the community to future disturbance (Hughes et al. 2007).

Bacteria, like other organisms require energy to function, using various modes of nutrition, but can be divided into autotrophic and heterotrophic organisms. Autotrophic bacteria may be photosynthetic or chemosynthetic (Berrie et al. 1987; Dutta 2004). Chemotaxis allows bacteria to synthesise carbohydrates without light as a source of energy, relying on the oxidation of inorganic compounds (e.g. sulphur, iron and nitrogen compounds) in their environment (Berrie et al. 1987; Dutta 2004). Most bacteria however, are heterotrophic and are either saprophytic or parasitic in habit. Parasites infect plants and animals, absorbing food from their host. Saprobes are commonly found in soil and water containing the organic compounds of plants or animals and secrete enzymes which breakdown complex organic compounds into simpler ones which they absorb for nutrition (Berrie et al. 1987; Dutta 2004). Many bacteria are nature's scavengers, thus they provide many ecosystem services like decomposition, nitrification, nitrogen fixation, soil fertility and plant disease control (Sinnot et al. 1963; Berrie et al. 1987; Dutta 2004).



1.3.1. Plants and the unseen

All plant roots are able to modify their surrounding rhizosphere (Shane and Lambers 2005; Bias et al. 2006). Plant roots are able to communicate with microbes by producing signals that are recognized by soil microbes, enabling the recruitment of desired symbiotes from the bulk soil into the rhizosphere (Bais et al. 2006). Most higher plants are able to enter into symbiosis with fungi, allowing for the trade of plant carbon for fungal phosphate (Stracke et al. 2002). In contrast, the more recently evolved symbiosis between nitrogen-fixing root nodules, are phylogenetically restricted and therefore not able to associate with most plants (Stracke et al. 2002). Both symbioses rely on partially overlapping genetic cues (Stracke et al. 2002). Mycorrhizal fungi extensively invade host root tissues upon perceiving a chemical

signal from the host roots. Similarly, bacteria are able to locate plant roots through cues exuded from the root, and root exudates such as carbohydrates and amino acids stimulate plant growth promoting bacteria chemotaxis on root surfaces (Bais et al. 2006).

1.3.1.1. Mycorrhizal associations

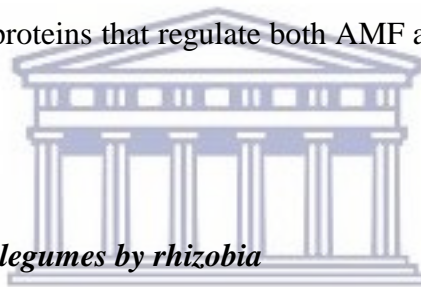
Root exudates play a key role during the pre-symbiotic growth phase and have been shown to stimulate hyphal branching and the catabolic metabolism of arbuscular mycorrhizal (AM) fungal spores (Buckling et al. 2008). Once the arbuscular mycorrhizal fungi (AMF) are well established, changes in the flavonoid pattern play a regulatory role in the uptake of fungi by the plant (Steinkellner et al. 2007).

The most common fungi forming endomycorrhizal mutualisms with vascular plants are AMF. These fungi are also the most ancient endomycorrhizal fungi known (Bidartondo et al. 2010). Similar to the rhizobium-legume interaction in the AMF - host interaction, root exudates play an important role at the beginning of a signal exchange chain between the host plant and the AMF. As flavonoids are present in root exudates, their involvement in the signaling of the host plant-AMF interaction has been suggested (Steinkellner et al. 2007). Unlike the selective legume-rhizobial associations, AMF and plant roots form associations with approximately 80 % of terrestrial plants. This symbiotic relationship increases nutrient uptake, improving plant fitness, and in turn, the associated fungi extract lipids and carbohydrates from the host root (Bais et al. 2006).

The critical developmental step in the life cycle of mycorrhizal fungi is hyphal branching, which ensures contact with the host root and establishment of symbiosis. Flavonoids exhibit a clear stimulatory effect on AMF hyphal growth and this effect seems to depend on the chemical

structure of the compound (Steinkellner et al. 2007). These stimulatory effects of flavonoids have been shown to be exacerbated in the presence of CO₂ at concentrations similar to those found in the rhizosphere (Steinkellner et al. 2007). Branch-inducing factors are present in root exudates of all the mycotrophic plants, but absent in those of non-host plants. Root exudates from phosphate (P)-limited plants are more active than those from plants with sufficient P, suggesting that the production and/or exudation of branching factor in roots is regulated by P availability (Bais et al. 2006).

Both AMF and rhizobial associations with plants derive from a common ancestral plant-microbe interaction, likely of fungal origin. This position is supported by the fact that AMF and rhizobia share conserved proteins that regulate both AMF and rhizobial associations with plants (Bais et al. 2006).



1.3.1.2. Nodulation of legumes by rhizobia

Rhizobia form symbiotic relationships with leguminous plants by fixing atmospheric nitrogen in root nodules. These symbiotic associations are very specific, restricting rhizobial strains to nodulate with specific host legumes (Bais et al. 2006). These host associations are mediated by flavonoids. Approximately 4000 flavonoids have been identified in vascular plants of which isoflavonoids are restricted to the legume family (Bais et al. 2006). This subset of flavonoids not only induces nodulating gene expression, but also rhizobial chemotaxis (Bais et al. 2006).

Plants with symbiotic relationships with rhizobia are better able to acquire nutrients and therefore persist in nutrient limited environments. This is exemplified through the utilisation of cluster roots by proteoid plants and subsequent chelating root exudates which modify their surroundings at a significant carbon cost to the plant (Cordell et al. 2009). This allows these

pecies to acquire more P from soils that are low in available P when compared with non-cluster root species (Shane and Lambers 2005).

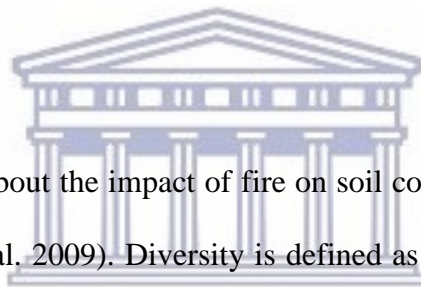


1.4. Rangelands under fire

South African rangelands largely comprise of the grassland, arid savannah, semi-arid savannah, thicket, nama karoo, succulent karoo, desert and fynbos biomes (Naidoo et al. 2013). Due to either climatic, topographic or edaphic factors these systems are often not suited for intensive agriculture but can be productively used as rangelands (Holechek et al. 2004). Fire is a tool, one which man has used to conquer his environment. This was achieved by altering landscapes and today it is still used to increase the productivity of rangelands, as animals prefer burnt rangelands over mechanically defoliated rangelands and are considered to be more nutritious (Snyman 2003).

However, rangelands have their own unique evolutionary history with disturbance, which does not always include the occurrence of fire, such as the arid Succulent Karoo biome of South Africa. The occurrence of unplanned fires however is a natural phenomenon of South African rangelands, with a notable increase in fire frequency observed today (Snyman 2003; DEA 2010). This is attributed to the occurrence of wildfire being strongly dependant on climatic parameters such as drought (Keeley and Zedler 2009; Pausas and and Fernández-Muñoz 2012). This may result in the encroachment of fire into areas in which it shared no previous historical occurrence. South African rangelands are currently degraded by unsustainable agricultural practices, such as overgrazing (Darkoh 2009; Rutherford and Powrie 2013). This, in addition to the intense drought South Africa has endured since 2015 affects both vegetation productivity and composition. In particular succulent plant species, which have displayed sensitivity to drought and appear to be susceptible to daytime warming-induced mortality (Midgley and Van der Heyden 1999; Musil et al. 2005).

The response of vegetation to fire, drought and grazing are often sporadic and irreversible. This suggestion lead to the inference that thresholds exist between different rangeland states, described in state and transition models. These models incorporate the following: multiple successional pathways, multiple stable states, thresholds of change, and discontinuous and irreversible transitions. Rangelands could also exist in alternative vegetation states, characterised by the dominance of functional groups (i.e. shrubs and herbs) and usually the structure, biomass and percent cover of vegetation differs between states. These states are separated by what is known as critical thresholds, drought can lower the threshold to a different state such that a particular level of disturbance is sufficient to trigger a transition. These thresholds are difficult to detect in arid rangelands due to variability in both vegetation and rainfall (Vetter 2009).



Furthermore, little is known about the impact of fire on soil communities (Neary et al. 1999; Snyman 2003; Fuhlendorf et al. 2009). Diversity is defined as the ability to adapt to change (Frankham et al. 2017). The more diverse a population is, the better it is able to contend with disturbance or fluxes within their system. Therefore, microbial diversity is an important factor when seeking to promote, sustain or manage both biodiversity and rangeland productivity. The study of soil microbiology was severely limited in the past due to the inability to culture soil microbes. Today bacterial communities can be characterised through an assortment of culture-independent approaches, consisting mostly of information derived from 16S rRNA gene sequences (Kirk et al. 2004; Spencer and Ragout de Spencer 2004; Rettedal et al. 2010). Amongst the techniques for characterizing microbial communities denaturing gradient gel electrophoresis (DGGE) has proven to be one of the most popular methods for determination of microbial diversity (Rettedal et al. 2010). This is because it is less expensive than high throughput sequencing (Kirk et al. 2004; Rettedal et al. 2010).

1.5. Research aim

The overall aim of this study is to establish a baseline understanding of the impact of fire within Succulent Karoo. This was achieved by determining the effect of fire on the plant and soil-microbial communities of the Succulent Karoo.

1.6. Outcome

The main practical outcome of this study was to investigate the composition and evolution of communities after the passage of fire in the Succulent Karoo.

1.7. Research questions

1) How does fire influence plant community assemblages, species richness, diversity and cover in the Succulent Karoo?



Fire is a ubiquitous functional herbivore, consuming both living and inanimate objects and reducing them to organic and mineral compounds. This results in the promotion of microbial diversity, thus altering microbial communities, which in turn may promote botanical diversity and increase the environmental resistance of systems to change.

2) Does fire alter the microbial profile of bulk soil in the Succulent Karoo rangelands?

Fire in any environment results in a cascade of dynamic fluxes. This includes heat induced mortality which reduces microbial abundance, directly influencing the microbial communities

within the bulk soil. Indirectly, fire may also influence root exudates, altering soil pH and composition of the microbial communities present.

1.8. Thesis layout

Chapter 1 provides the rationale, motivation and importance of this study within the context of today. The implications of this study are also presented in this section, highlighting the lack of knowledge presently with regards to impact of fire on ecological service providers not often noticed. Focus is then given to microbial diversity and the possible influence disturbance may have on vegetation diversity. The focus of this study is given to the impact on fire on communities both above and below-ground.

Chapter 2 provides a general context with regards to the area in which this study will take place. Providing the early land-use history of the area, the vegetation types present as well as the importance of research within this area.

Chapter 3 focuses on vegetation dynamics post fire in a region of the Hantam-Tanqua-Karoo that displayed high relative diversity amongst the vegetation types present.

Chapter 4 focuses on the effect of fire on microbial communities in a region of the Hantam-Tanqua-Karoo that displayed high relative diversity amongst the vegetation types present.

Chapter 5 reconciles the data obtained within main findings of this study and will pose critique that will expose gaps in knowledge not currently addressed and provide opportunities for future studies.

Chapter 2: Study Area

The South African flora is rich in species and high in endemism. These are grouped within the sub-continent's floristically distinct biomes (Figure 1) (Cowling et al. 2004). The Cape Floristic Region (CFR) comprises approximately 90 000 km² of South Africa and for its size, is amongst the world's richest areas of plant diversity, of which 22 % are under some form of protection (Goldblatt and Manning 2002; Cowling et al. 2004). This region is home to over 1 406 Red Data Book plant species and has been identified as a hotspot for diversity due to its high levels of endemism and fragmentation (Cowling et al. 2004; Egoh 2009).

Today, conservation systems which are able to sustain biodiversity patterns and process are essential (Cowling et al. 2004). The most susceptible plant species to the projected climate change scenarios are those within the winter rainfall biomes. The winter-rainfall region of South Africa is characterised by the fynbos and succulent karoo biomes (Hoffman et al. 2009). Both these biomes will be exposed to novel climatic conditions, including rainfall variability, by 2050 the Fynbos and Succulent Karoo, (DEA 2010). This is considered as being one of the most serious problems facing rangelands, negatively impacting on the productivity of rangelands and exacerbates pre-existing vulnerabilities (DEA 2010).

This study took place in the succulent Karoo and focused on its fire ecology.

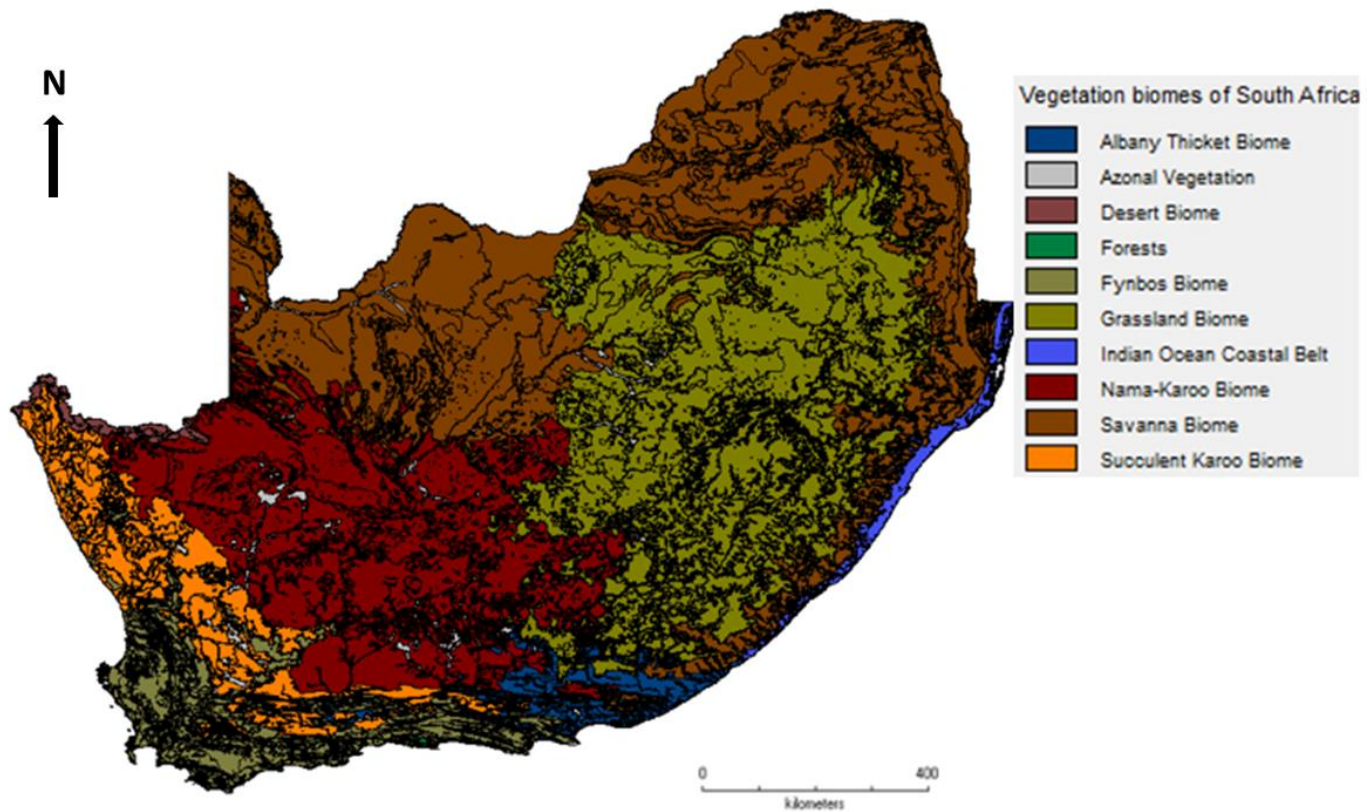


Figure 1: Biomes of South Africa 2009 (data sourced from: <http://bgis.sanbi.org/>)



2.1. Succulent Karoo Biome

Approximately 21 000 years ago (Later Stone Age), indigenous people first began occupying the Succulent Karoo in larger numbers, subsequent the waning of the previous cooler glacial conditions. The loss of large grazing herbivores, such as the hartebeest, is attributed to this early encroachment of man. The introduction of domestic livestock (cattle, sheep and goats) by Khoikhoi herders facilitated the expansion of the human population. The impact of these introductions are unknown, but are considered significant with an estimated human population of 50 000, between 250 000 - 500 000 cattle and up to 1 million sheep and goats (Jonas 2004).

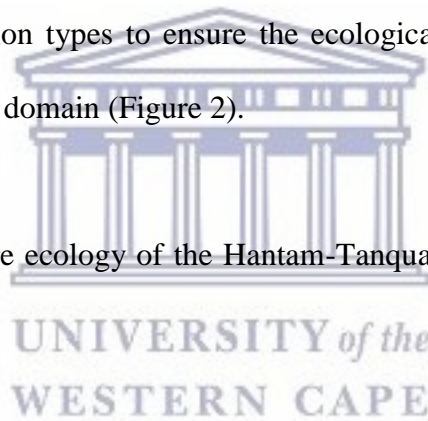
Today the Succulent Karoo is of great ecological significance, stretching along Namibia and the western side of South Africa (Van der Merwe et al. 2008; DEA 2010). The annual rainfall for this region is less than 400 mm, occurring predominantly in winter, and during summer it is subjected to temperatures in excess of 40 °C. The topography of the Succulent Karoo is variable, with the flat to gently undulating plains of the Knersvlakte and Tanqua Karoo, and the more tumultuous topography occurring in Namaqualand, Little Karoo and Robertson. Due to this variation in topography the altitudinal ranges between 800 meters above sea level (m.a.s.l.) and 1500 m.a.s.l. The majority of river courses within this biome are seasonal and are derived from the Orange river, Gouritz river and the Tanqua-Doring-Olifants river. The geology of the Succulent Karoo includes granite, limestone, quartz and shale. The soils present are lime-rich, weakly developed soils on rock (Jonas 2004).

This biome is characterised by its rich geophyte flora and high diversity of succulents (Mucina et al. 2006; Van der Merwe et al. 2008). It is recognised as a global diversity hotspot, due to its high levels of biodiversity and endemism despite being completely arid, it has been an area of

great interest for researchers and conservationists (DEA 2010). Despite the high ecological value of this biome, it is poorly conserved, as less than 5 % of it is formally protected. The need to protect this biome is further emphasized by the fact that only 26.8 % of its original extent remains, thus, a loss of approximately 82 000 km² (Myers et al. 2000).

In order to curb this loss and to better manage the natural capital of this area, a project was launched in 2002, entitled the Succulent Karoo Ecosystem Plan (SKEP) and funded by Critical Ecosystem Partnership Fund (CEPF) (Driver et al. 2003; Conservation International-website 2016). This project delineated areas of conservation importance and enabled the prioritisation of resources, resulting in what today is known as the SKEP planning domain. The domain included surrounding vegetation types to ensure the ecological viability thereof. Four sub-regions are present within this domain (Figure 2).

This study focused on the fire ecology of the Hantam-Tanqua-Roggeveld regions botanical and microbial communities.



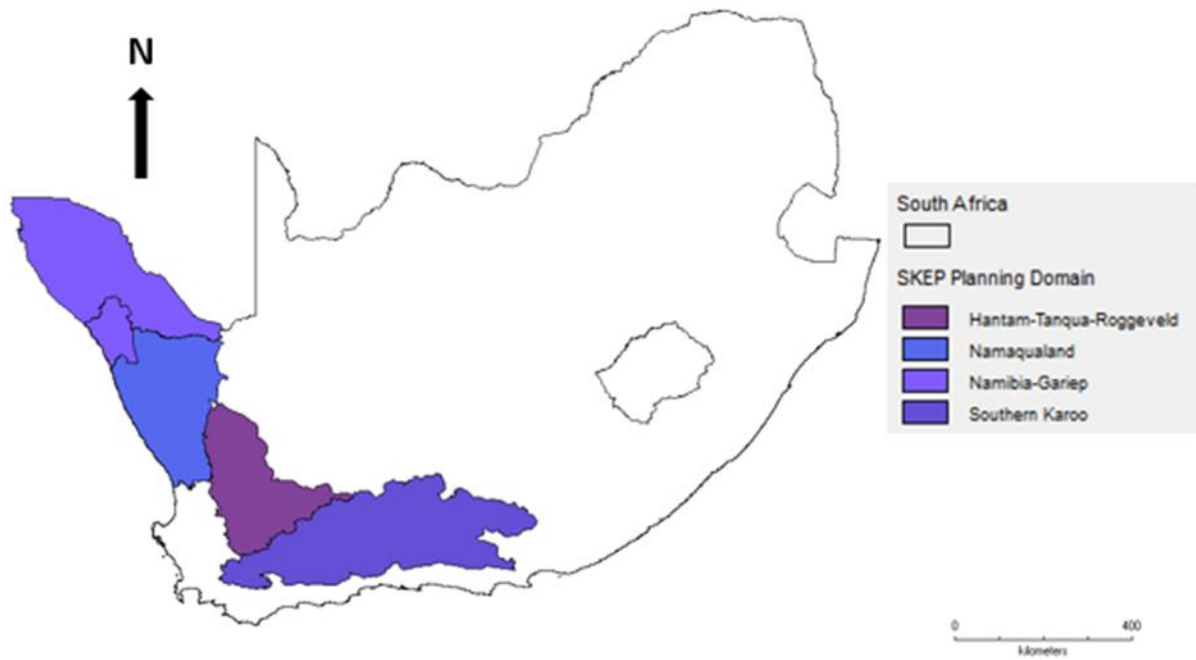


Figure 2: SKEP planning domain sub-regions (data sourced from: <http://bgis.sanbi.org>)

2.1.1. *Hantam-Tanqua-Roggeveld*

This study was conducted in the Hantam-Tanqua-Roggeveld subregion, characterised by undulating to steeply rolling topography, with peaks reaching 1800 m.a.s.l. (Van der Merwe et al. 2008). This subregion hosts mainly the Succulent Karoo vegetation but also elements of Fynbos and Nama karoo biomes (Figure 3).

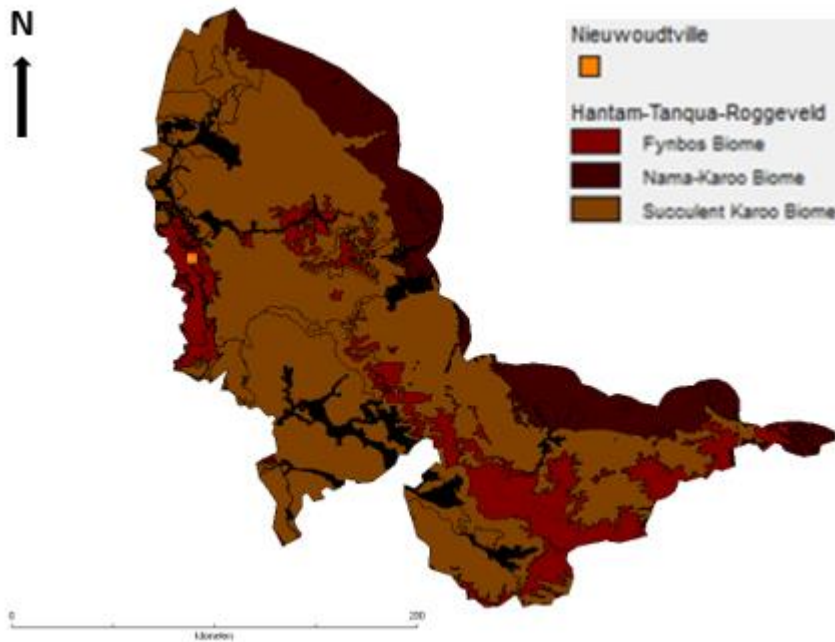


Figure 3: SKEP planning domain of the Hantam-Tanqua-Roggeveld sub-region (data sourced from: <http://bgis.sanbi.org>)

The geology of this region is dominated by an amalgamation of sediments collectively known as the Ecca Group, consisting of: shale, mud-rock, siltstone and silt (Johnson et al. 2006; Van der Merwe 2009). In addition, the dolerite intrusions are easily recognised by their dark colouration. The Tanqua Karoo is dominated by lithosols (including desert pavement), the Hantam is dominated by lithosols and duplex soils, whilst the Hantam-mountain range is dominated by stony shallow lithosols (Francis et al. 2007).

2.1.2. Nieuwoudtville

Nieuwoudtville is recognised for its biodiversity and is surrounded by the Bokkeveld Escarpment which hosts more than 1 600 plant species, of which approximately 5% are endemic (Todd et al. 2010). This town is colloquially known as the bulb capital of the world due to the high representation of geophytes. This town is characterised by cold-wet winters and hot, dry-summers, with an annual rainfall of 350 mm. Many of the plant species become dormant during November-March (dry season), whilst the plant biomass and flowering peaks during August-October (winter/early spring) (Bragg et al. 2005). Farming is the main contributor to the economy and most households practise animal husbandry (Stats SA 2012).

In 2007 the Hantam National Botanical Garden was established and is managed by the South African National Biodiversity Institute. It allows for the continued and long-term ecological research and environmental monitoring of different farming practices (Jürgens et al. 2010).

2.1.3. Sampling sites

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Sampling occurred within the Hantam National Botanical Gardens, close to Nieuwoudtville, in which two unplanned fires had occurred during February 2008 and December 2013. The subsequent sampling within this study was restricted within these treatments (Figure 4).

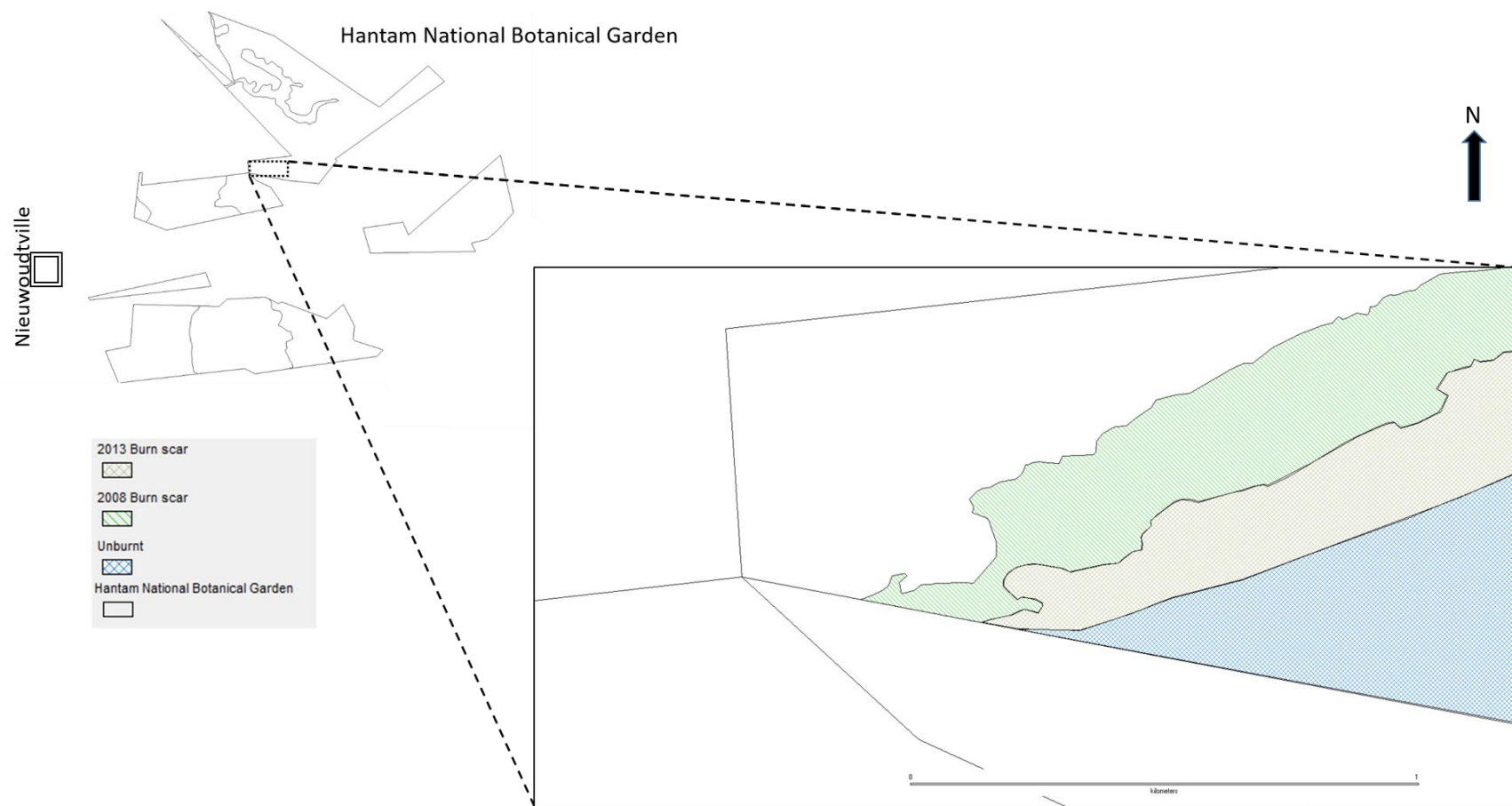


Figure 4: The Hantam National Botanical Garden burn scars (unburnt treatment, 2008 burn treatment and 2013 burn treatment) investigated (data sourced from: <http://bgis.sanbi.org>).

Chapter 3: Post fire response of botanical communities of the Hantam-Tanqua-Roggeveld subregion in the Succulent Karoo

3.1. Introduction

Changes in landuse attributed to changes in society changed the fire regime of many landscapes throughout history. The use of fire to promote the growth of fodder in rangelands is actively employed by land users and is known as pyric herbivory. This practice is commonly adopted as burnt rangeland is more palatable for grazing animals than rangeland which has been defoliated by either grazing or mowing. These burnt rangelands are more palatable because they have higher protein and mineral content (Snyman 2003).

The occurrence of wildfires is strongly dependant on climatic parameters such as drought, therefore given projected climate change the occurrence of these fires in South Africa may increase. However, the relative role of fuel and climate on current fire regimes has been debated (Keeley and Zedler 2009). There have been recent changes in both human pressure and climate, so fire regime shifts concomitant with changes in the relative role of these two drivers are expected (Pausas and Fernández-Muñoz 2012).

The Succulent Karoo is characterized by an open dwarf non-succulent shrubland dominated by leaf-succulents, here fire is a destructive disturbance. A study conducted by Lechmere-Oertel and Manning (2001), showed that one of the factors that limited the expansion of the succulent karoo biome into the fynbos biome is the occurrence of fire. No species is 'fire adapted' but rather is adapted to a particular fire regime, which, among other things, includes fire frequency, fire intensity and patterns of fuel consumption (Keeley et al. 2011).

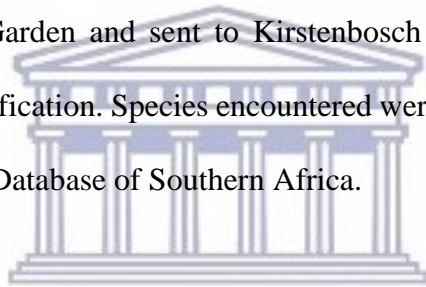
Fire as a management tool should be well planned, or unplanned fires well managed due to importance in maintaining soil and productivity in semi-arid and arid rangelands (Snyman 2003). Since the Succulent Karoo, a biome not subjected to a fire regime within its evolutionary history it is assumed that fire would have a devastating impact on its diversity, especially the succulent vegetation present. In addition, considering future climate change and human population growth, the demand on rangelands like the Succulent Karoo is set to increase, this study will provide a baseline understanding required for the efficient management of these reangelands. This study explored the impact of unplanned fires within these rangelands. This was achieved by assessing the impact of fire (unburnt, 2013 burn (2 years post-fire) and 2008 burn (7 years post-fire) on botanical diversity, which can serve as guidelines in management planning of semi-arid rangeland areas of South Africa. To reduce the bias which may arise due to ephemeral flushes (mostly characterised by opportunistic species) which may occur, only perennial plant species were accounted for in this study.



3.2. Materials and methods

3.2.1. Vegetation survey

Vegetation sampling occurred during September 2015, the flowering season, this allowed for the rapid identification of species in the field and also provided the maximum representation of diversity. In addition, this sampling time is congruent with those published in Van der Merwe and Van Rooyen (2011), which occurred in September 2005. Plants were identified with the aid of those employed by the South African Biodiversity Institute, in tandem with the use of wild flower guides such as the *Nieuwoudtville: Bokkeveld Plateau & Hantam* (Manning and Goldblatt, 1997). Plants that could not be identified were collected in field herbaria for the Hantam National Botanical Garden and sent to Kirstenbosch National Botanical Gardens-Compton Herbarium for identification. Species encountered were classified into life forms and life cycle using the Botanical Database of Southern Africa.



3.2.1.1. Plant diversity and species richness

Three different vegetation states were studied, unburnt, medium-term (7 years post-fire) post-fire and short-term (2 years post-fire) post-fire. Six 5x20 m quadrats were erected randomly in each state, unburnt, 2008 burn (medium-term) and 2013 burn (short-term) (Figure 5), at approximately 50 m from each other. The species composition, percent cover and number of species (richness and abundance) was determined in each quadrat. Percent vegetation cover was determined using the descending point method (Roux 1963; Du Toit 1995, 1998). This method employed the use of a 50 m line transect directly adjacent to the quadrats erected and the species which occurred every metre was recorded.

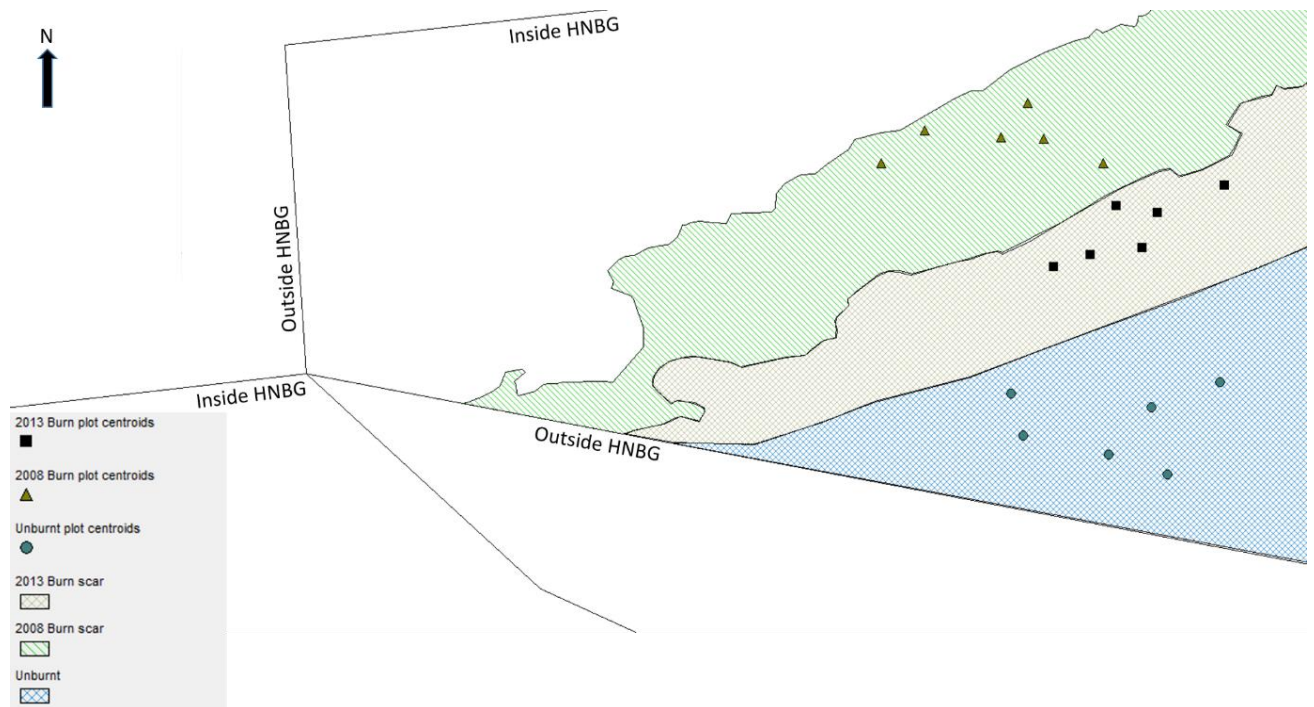


Figure 5: Sample site centroids and treatments investigated in the Hantam National Botanical Garden (data sourced from: <http://bgis.sanbi.org>).

The Shannon-Weiner diversity index assumes that all species have been accounted for, therefore the values presented must be regarded as the lower bound of the number of species present. This diversity index was used in this study and calculated for each sampled quadrat as ascribed in Krebs (1999).

$$H' = - \sum_i^s i \log p_i$$

[Eqn 1]

Diversity indices were compared across states using Analysis of variance (ANOVA) and Bray-Curtis dissimilarity index. Statistical analysis was conducted using Statistical Package for the Social Sciences Version 22 (SPSS Inc., Chicago IL). A Shapiro-Wilk's test was conducted to test for normality, as the sample size did not exceed 2000 but was greater than 50. Subsequent to these findings either a One-Way ANOVA or Kruskal-Wallis analyses was conducted.

Diversity, richness and percent vegetation cover were determined based on perennial plant cover.

The pooled information from the each of the treatments was used to calculate association between plant species in each treatment using equation 2 as ascribed in Goldsmith and Harrison (1976). These indicate positive or negative associations, depending on whether the observed number of co-occurrences was greater than the expected or not.

$$X^2 = \frac{(ad-bc)^2n}{(a+b)(b+d)(c+d)(a+c)}$$

In which:

a = number of co-occurrence of both species

b = occurrence of only species 1

c = occurrence of only species 2

d = neither species present

n = number of plots



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[Eqn 2]

3.2.1.2. Effect of fire on community assemblages

The impact of fire on the community assemblages present in each state was conducted using **Primer Premier5** statistical software (as shown below, Figure 6).

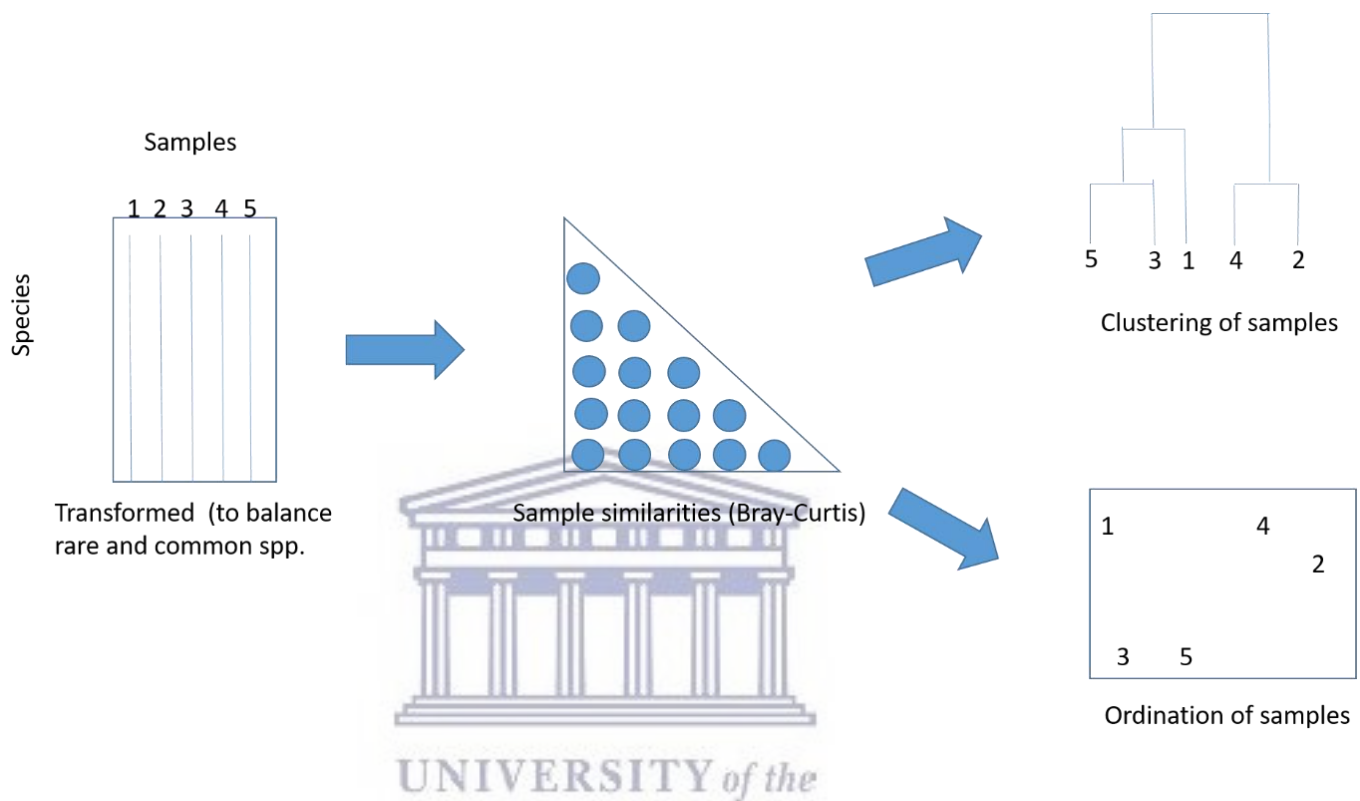


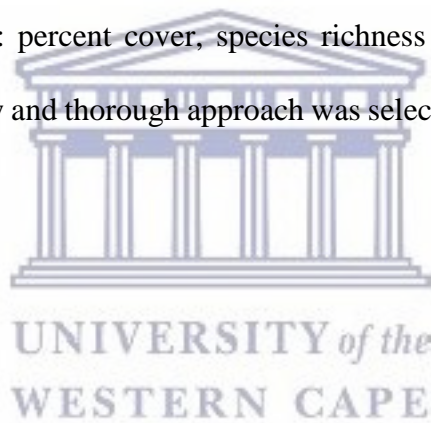
Figure 6: Flow diagram of analyses conducted (adapted from: Clarke and Warwick 1994).

Abundance data was used as the primary matrix in the data input, the Bray-Curtis dissimilarity index was applied to this data after undergoing a double root ($\sqrt{\sqrt{\cdot}}$) transformation. This allows all species to contribute to the definition of dissimilarity/similarity eliminating further bias that may occur.

Cluster analysis was used to elucidate natural grouping across treatments. This analysis visually displayed samples within treatments that are more similar to each other than samples in another group. The Bray-Curtis dissimilarity matrix previously derived was used as input data for this analysis. It clearly delineated groups of sites with distinct community composition. However,

cluster analysis is less useful and may be misleading where there is a gradation in community structure across sites (this is attributed to environmental forcing-edaphic factors such as differences in soil). Cluster analysis is best used in conjunction with ordination, therefore a 2 dimensional Multidimensional scaling (MDS) analysis was conducted. This allowed for the relationships amongst groups to be more informatively displayed and strengthened the adequacy of both approaches.

For more descriptive data regarding the groupings displayed by the analyses above a Non-Metric Multidimensional Scaling (NMDS) was constructed in the statistical package PCORD version 5.0. The relationship between treatments and community assemblage were evaluated using the following variables: percent cover, species richness and diversity. To ensure the integrity of this analysis a slow and thorough approach was selected and allowed to run for 500 iterations.



3.3. Results

3.3.1. Effect of fire on plant diversity

The Shannon index takes into account the number of species present and generates a proportionate measure of diversity. In ecological studies this measure is rarely larger than 4, but often between 1.5 and 3.5 (Magurran 2004). The plant diversity amongst the different burns did not differ significantly ($F_{(2,15)}=2.026; p=0.166$). The range of Shannon index values ranged from 1.36-1.91 (Unburnt), 1.09-2.42 (2008 burn) and 1.27-2.33 (2013 burn) (Figure 7). Plant diversity was influenced by the occurrence of fire, as the greatest mean diversity occurred in burn sites, albeit not significantly.

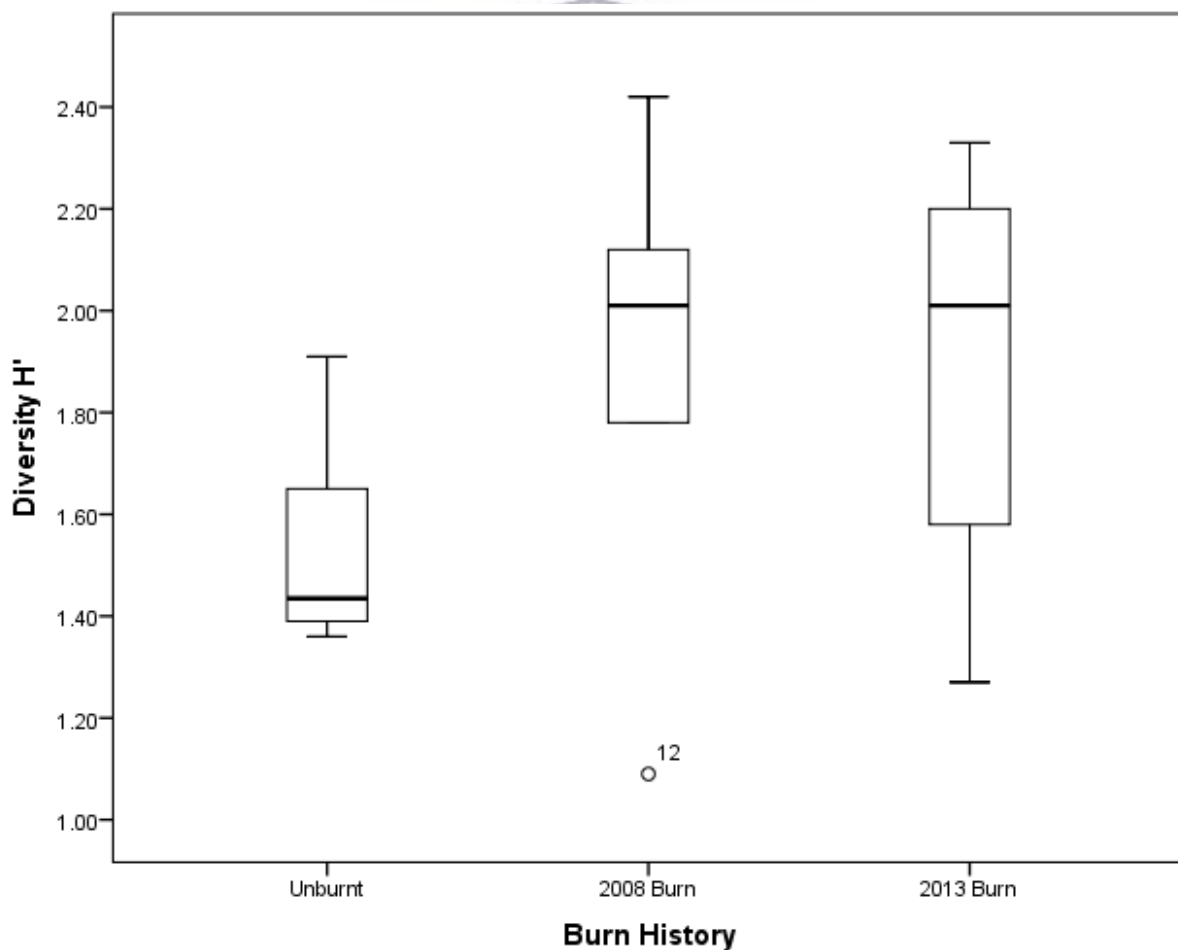


Figure 7: Comparison of Shannon-Weiner diversity index across treatments (burn history) investigated in the Hantam National Botanical Garden.

3.3.2. Effect of fire on species richness

Richness is a simple measure of species occurrence, disregarding the species abundance/number of individuals present (Tuomisto 2010). The species richness amongst the different burns did not differ significantly ($F_{(2,15)}=1.675; p=0.221$). The range of species richness values ranged from 15-19 (Unburnt), 7-15 (2008 burn) and 8-29 (2013 burn) in this study (Figure 8).

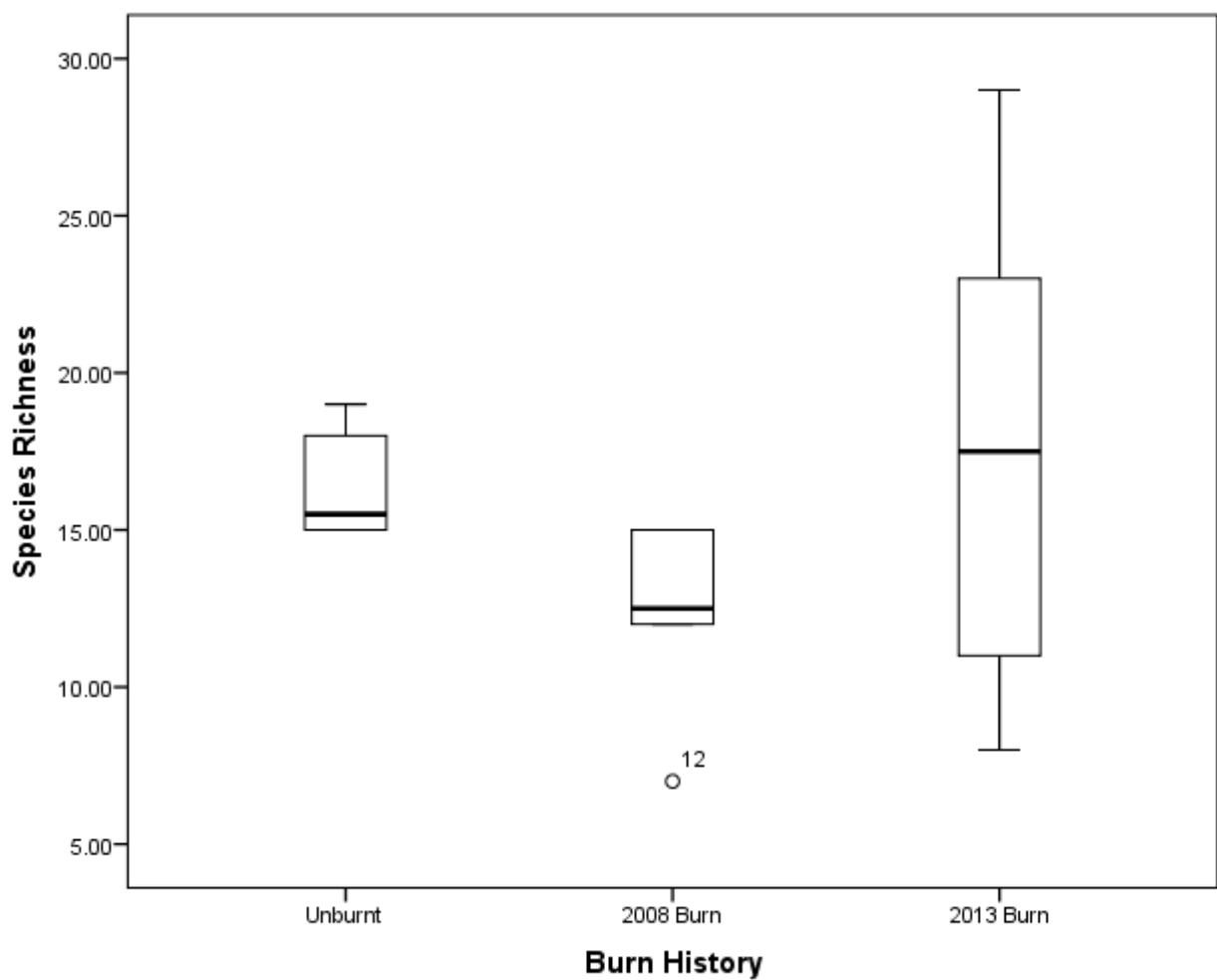


Figure 8: Comparison of species richness across treatments (burn history) investigated in the Hantam National Botanical Garden.

3.3.2.1. Plant species association

Plant species association was used as a measure of the similarity of occurrence of two species. This analysis revealed that plant associations varied across treatments in response to disturbance.

The complete Chi-square matrix for the unburnt treatment (Figure 9) showed that 8 species demonstrated positive occurrence associations with each other. Positive occurrence associations was present amongst 20 species for the 2008 burn treatment (Figure 10). Whilst the 2013 burn treatment (Figure 11) revealed that 29 species demonstrated positive occurrence associations with one another.



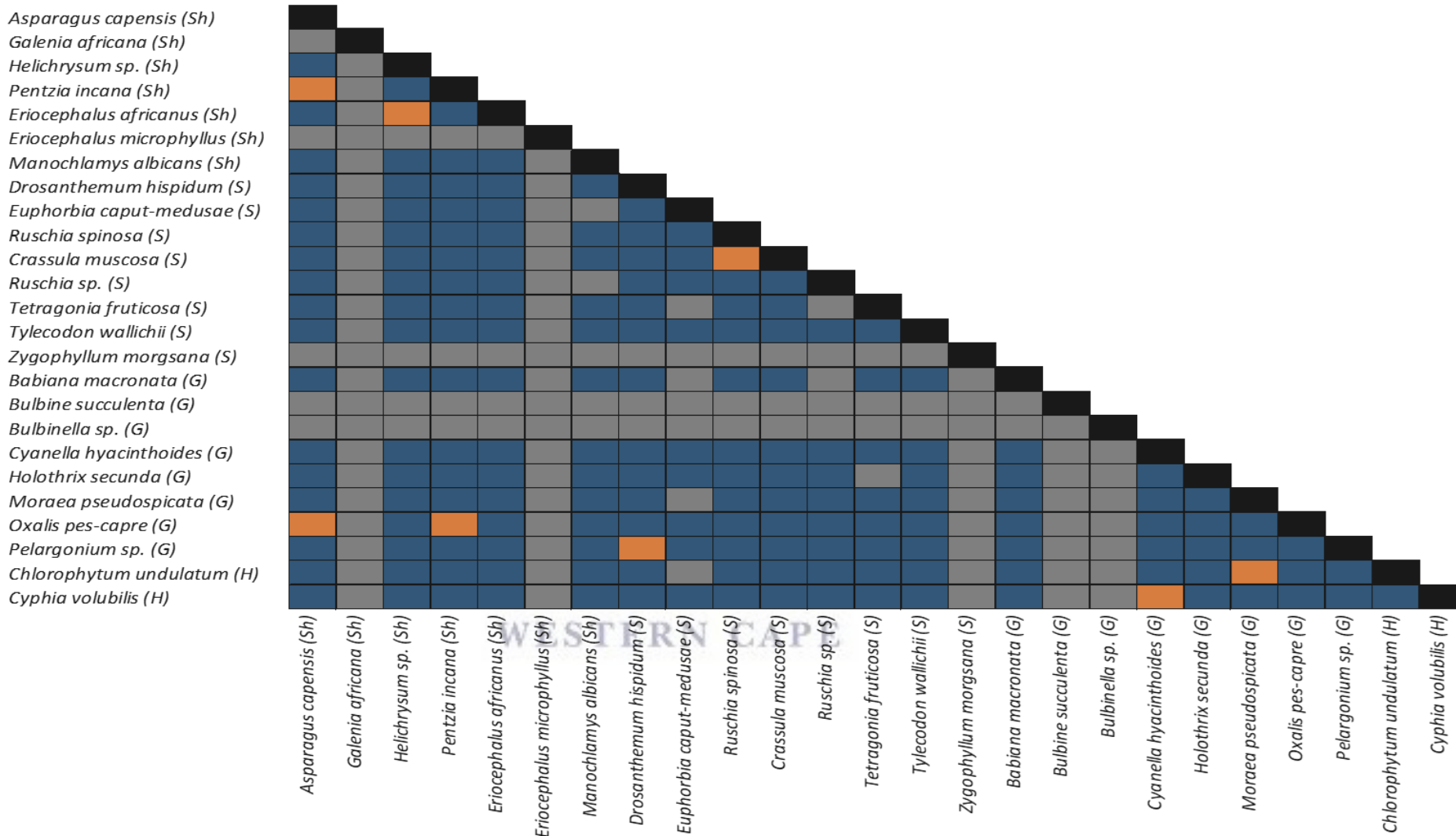


Figure 9: Complete chi-square matrix for unburnt treatments showing positive (orange) and negative (blue) species relationships present. †Life-forms: Geophyte (G), Non-succulent shrub (Sh), Herb (H) and Succulent (S).

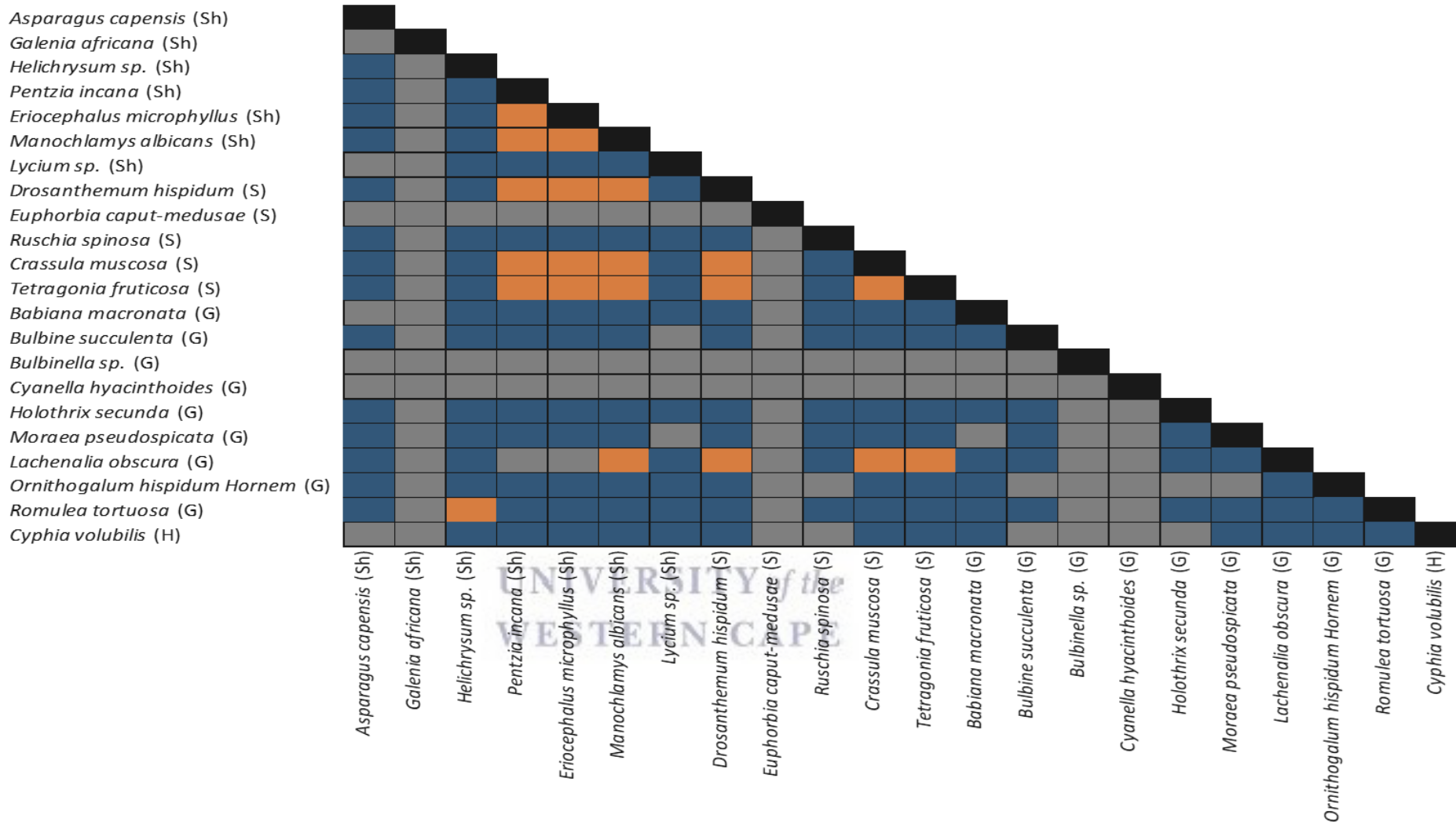


Figure 10: Complete chi-square matrix for 2008 treatments showing positive (orange) and negative (blue) species relationships present. †Life-forms: Geophyte (G), Non-succulent shrub (Sh), Herb (H) and Succulent (S).

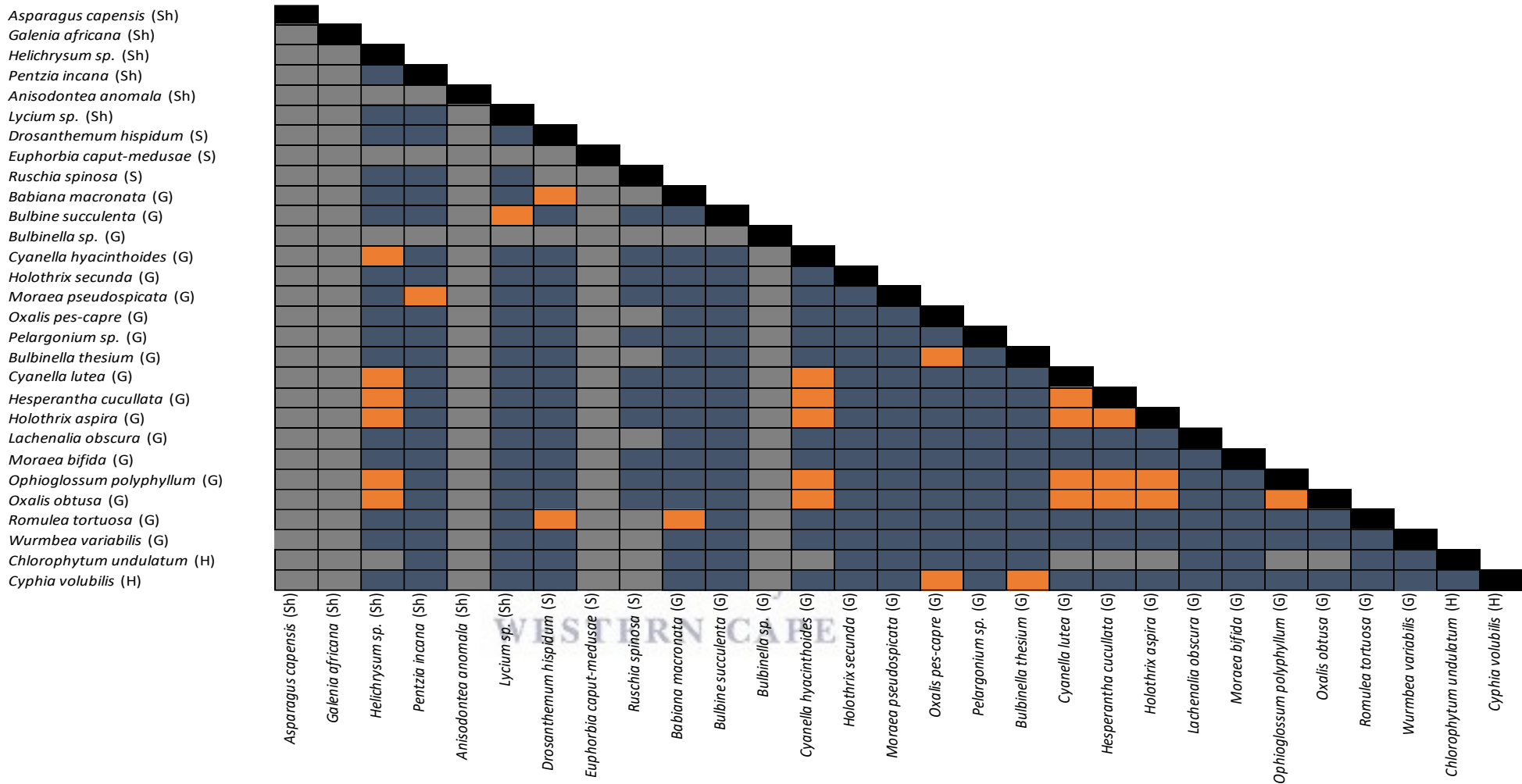


Figure 11: Complete chi-square matrix for 2013 treatments showing positive (orange) and negative (blue) species relationships present.
 †Life-forms: Geophyte (G), Non-succulent shrub (Sh), Herb (H) and Succulent (S).

Despite no significant difference being present amongst species across treatments, the species present were affected by the occurrence of fire, as displayed in Appendix 1. Fire resulted in the loss of 8 species, consisting of succulent and non-succulent shrub species, In contrast, 13 geophyte and non-succulent shrub species only occurred post-fire. However, the occurrence of 17 species was not affected by the occurrence of fire, the greatest number of which were geophyte (47 %) and non-succulent shrub (23 %) species. Whilst succulent (18 %) and herbaceous (12 %) plant species constituted the lowest proportion of species able to persist post fire. Therefore, whilst fire does not affect diversity and species richness, it does influence the community assemblages present. Further analyses was thus conducted on the community assemblages in Section 3.3.3. which elucidated the effect of fire on the communities present.



3.3.3. Effect of fire on community assemblages

Three distinct community assemblages were delineated at 55 % dissimilarity (Figure 12). The dendrogram depicted is appropriate for delineating groups of sites with distinct community assemblages, thus clades are arranged on the inherent characteristic patterns of abundance found consistently in different groups. Clade I represents three of the 2013 burn sites (13 1, 13 2 and 13 3), Clade II all unburnt sites and Clade III all 2008 sites (08 1, 08 2, 08 3, 08 4, 08 5 and 08 6), as well as three 2013 sites (13 4, 13 5 and 13 6).



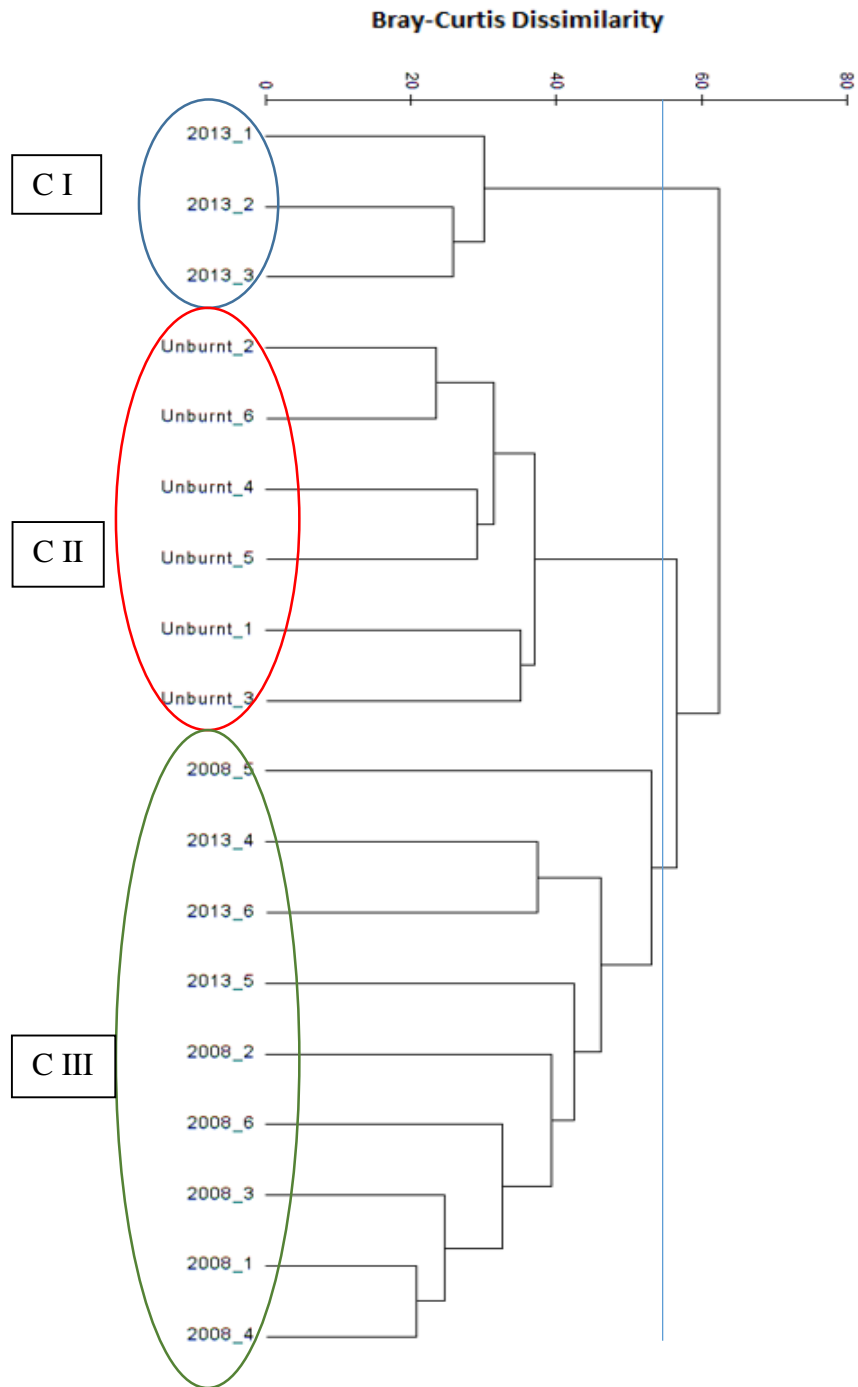


Figure 12: Dendrogram for hierarchical clustering of 18 sites, using group-average linking of Bray-Curtis dissimilarities at 55 % dissimilarity across treatments (unburnt, 2008 burn and 2013 burn) in the Hantam National Botanical Garden.

These clusters have been superimposed on an ordination plot to allow the relationship between groups to be more informatively displayed (Figure 13). The congruence present between the dendrogram and the 2 dimensional Multidimensional scaling (MDS) strengthens the adequacy of both

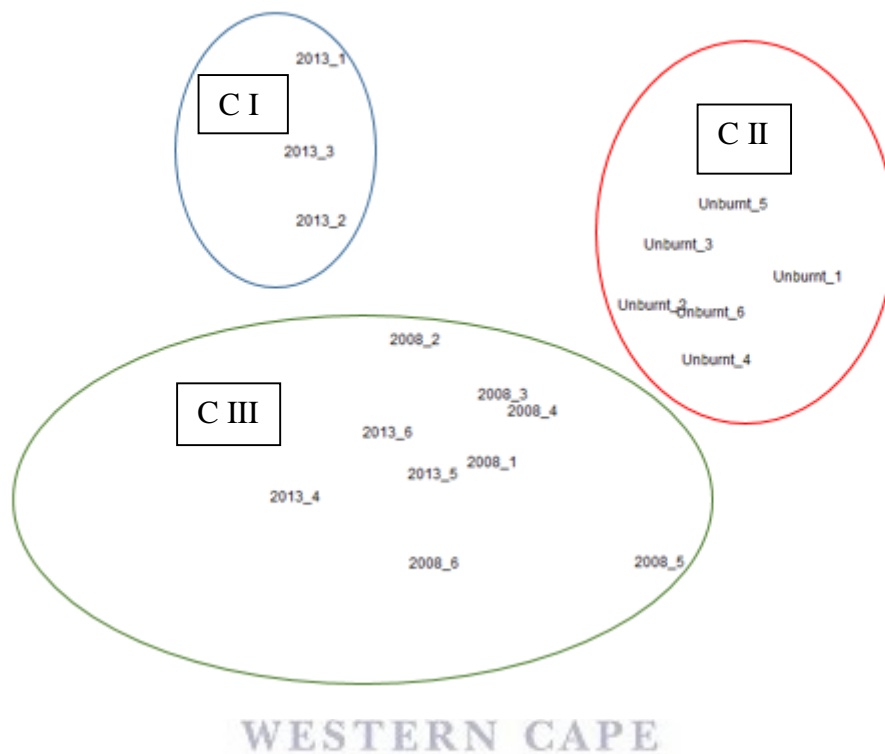


Figure 13: Group-averaged clustering from Bray-Curtis dissimilarities at 55 % dissimilarity superimposed on the 2-dimensional MDS (stress=0.14) across treatments (unburnt, 2008 burn and 2013 burn) in the Hantam National Botanical Garden.

The clades previously delineated were further assessed based on species composition and response (Figure 14). Clade II was composed of only unburnt sites, 32 % of the species present within this clade were absent post-fire (both 2008 and 2013 treatments), the remaining species present were able to persist despite the occurrence of fire. This clade is clearly delineated and is representative of the unburnt community. Clade I was composed of mainly new species recruited post-fire (52 %), the remaining species present were those able to persist (48 %) post-

fire. This Clade is representative of the early response of the plant community post-fire. Clade III was a combination of all the 2008 burn sites, as well as the remaining three 2013 burn sites. Within this Clade 62 % of the species which were present were those able to persist, 21 % were species recruited post-fire and the remaining 17 % consisted of species which were previously lost due to fire and have now re-established. This clade is representative of an early transitional stage, sharing community characteristics of both the 2013 burn treatments and unburnt treatments.

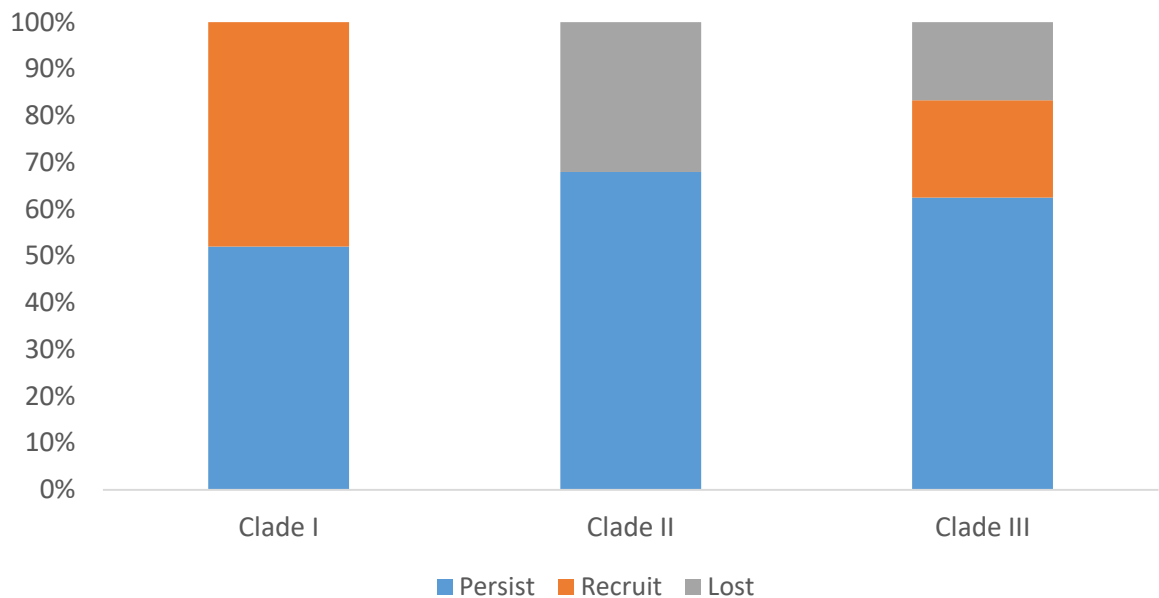


Figure 14: The compositional response (persist-present before and after fire, lost – species absent post-fire and recruit- species which only occurred post-fire) amongst delimited Clades.

A strong negative relationship was displayed by the Non-Metric Multidimensional Scaling (NMDS) of variables (Figure 15) for vegetation cover (tau value=0.135). The grouping of sites generated via NMDS based on diversity, species richness and percent cover are congruent with those displayed previously (Figure 12 and Figure 13). In addition, the vegetation cover of non-succulent shrubs and succulent lifeforms also displayed a strong negative relationship with the groupings present. These factors have a significant effect on the groupings present, as they

have tau values < 0.3 . Vegetation cover had a greater impact on the groupings present as the metrics of both the diversity index and species richness had tau values > 0.3 . An acceptable final stress value for the 2D solution of 15.13536 was achieved. .

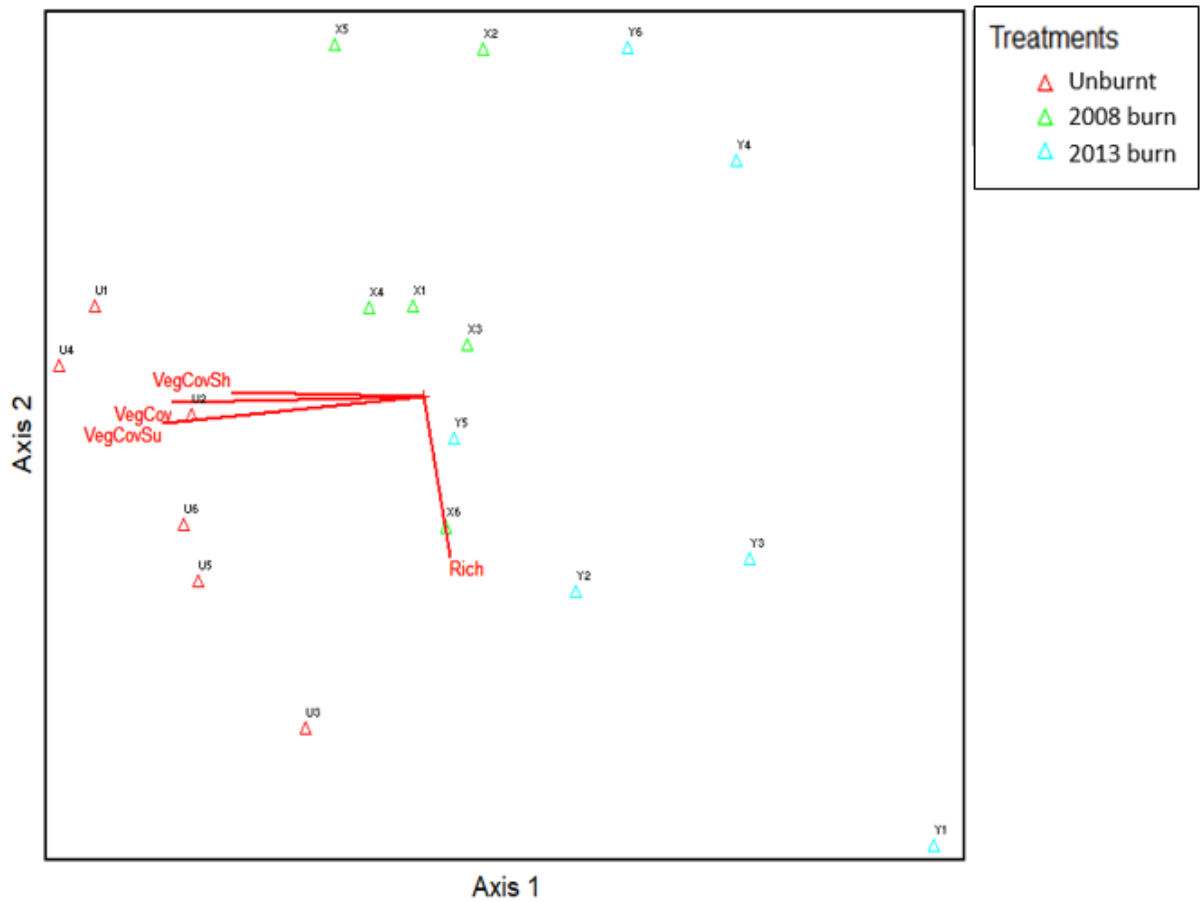


Figure 15: Non-metric Multidimensional Scaling of variables obtained from plant diversity plots and line transects between treatments (U-unburnt, X- 2008 burn, Y- 2013 burn; numbers displayed represent corresponding plots).

3.3.4. Effect of fire on vegetation cover

The impact of fire is more pronounced when assessing vegetation cover, altering vegetation composition within each treatment (Figure 16).

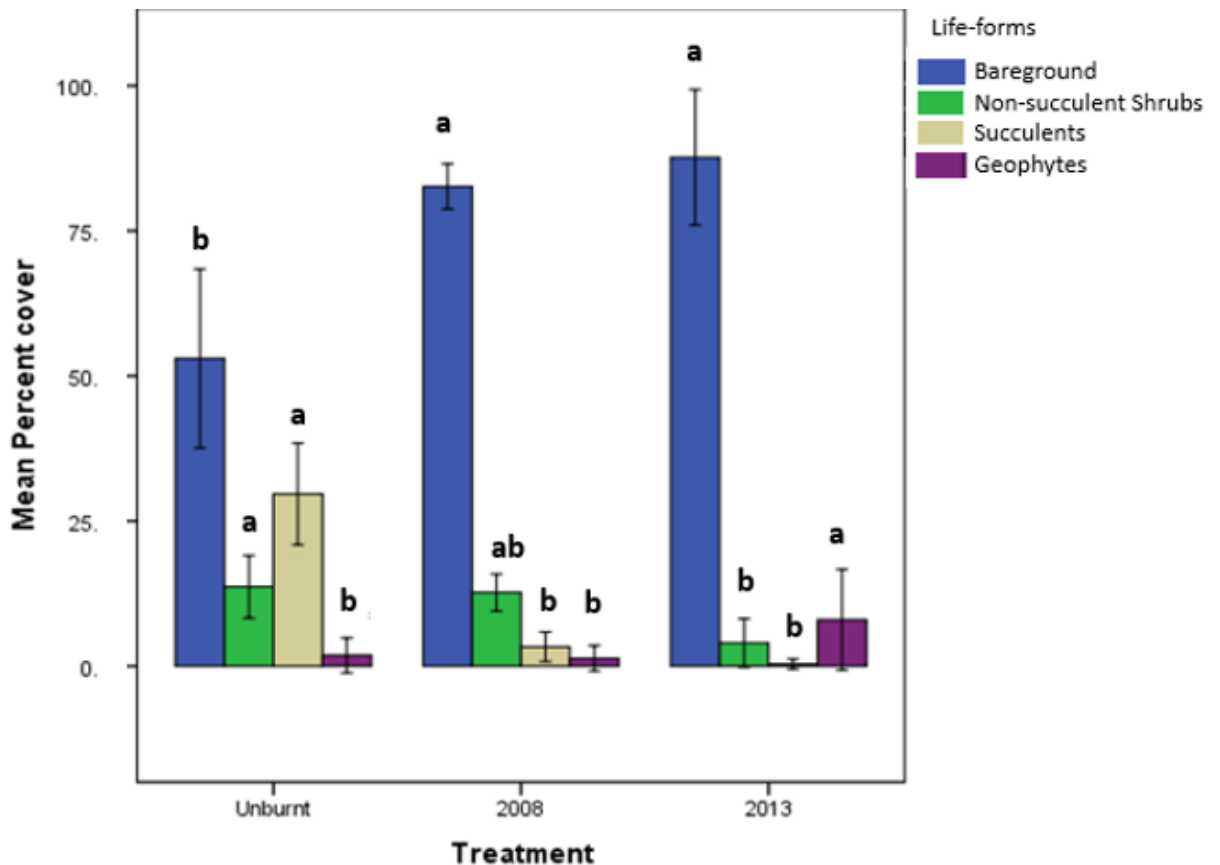


Figure 16: Percentage cover of Lifeforms across treatments (unburnt, 2008 burn and 2013 burn) with error bars at 95 % CI in the Hantam National Botanical Garden.

*Comparisons were made within Life-forms between different treatments.

Fire had a significant impact on bare ground percentage cover, being significantly higher in the burn treatments than the unburnt ($p < 0.05$). The cover of non-succulent shrubs in the unburnt and 2013 sites were also significantly reduced by fire, with unburnt hosting a significantly greater percent cover than 2013 ($p < 0.05$). There was no significant difference in the non-succulent shrub cover of the two burn treatments, 2008 and 2013 respectively ($p > 0.05$). Succulents occupied a significantly larger percent cover in the unburnt sites than in the burn treatments ($p < 0.05$), with no significant difference in cover amongst the 2008 and 2013 burn

treatments ($p>0.05$). In addition, a significant increase in the percent cover of geophytes was recorded shortly after the occurrence of fire (2 years-2013 burn) and a significant reduction in the percent cover was observed between the 2013 and 2008 burn treatments ($p<0.05$).



3.4. Discussion

The mean Shannon-Weiner Index and species richness values calculated in this study for all treatments did not exceed the average index obtained by Van der Merwe and Van Rooyen (2011). This was expected as the values obtained in this study are assumed to be the lower bound of the number of species present and did not account for the occurrence of annual plants. The increase in diversity and species richness was not significant after burn treatments, however, the trend displayed may support anecdotal evidence offered by land users indicating changes in the veld post-fire.

3.4.1. Effect of fire on botanical diversity

The low level of impact that fire displayed in this study was unexpected, as within this particular biome fire is considered as destructive and limiting disturbance (Lechmere-Oertel and Cowling 2001). This resulted in the expected loss of non-fire tolerant species from the community. Studies have shown that disturbance is an important driver of diversity (Bond and Keeley 2005), however, as systems are dynamic and open to fluxes the impact thereof is often complex. High levels of disturbance reduce diversity as the environment is too stochastic to allow for the establishment and acclimation of many species, therefore opportunistic species tolerant or resistant to disturbance will dominate the area. Low levels of disturbance result in higher levels of competition amongst species present for resources, with some species engaging in allelopathy to reduce competition as well as the recruitment of other species. The intermediate level of disturbance is a hypothesised ideal level of disturbance at which seedbanks may accrue, competition amongst species is reduced and the opportunity for recruitment from the surrounding environment is facilitated (Wilkinson 1999). Regarding this trend, the results obtained in this study should be considered with caution due to the slow

recovery of the Succulent Karoo and the short time frame post-fire assessed. The statistically insignificant difference observed may be attributed to possible mitigating factors such as the occurrence of low flammability plants, the microbial communities present, seed bank reserves or the aeolean deposition of nutrient carrying soot.

3.4.2. Effect of fire on plant species richness, association and community assemblages

Species richness is predicted to be highest at intermediate levels of disturbance (Beckage and Stout 2000), however this trend is not displayed above (Figure 8.). This may support the supposition that disturbance may either negatively or positively influence species richness depending on the productivity of the system in which it occurs (Kondoh 2001). A study by Proulx and Mazmuder (1998) demonstrated that plant species richness increased as grazing increased in a nutrient rich environment, but decreased in a nutrient poor environment as grazing increased. The loss observed in species richness post fire, is attributed to rainfall and the role of productivity within these systems. As the role of competition between perennials has a negligible influence on structuring the Succulent Karoo (Yeaton and Esler 1990; Esler and Cowling 1993; Milton et al. 1997; Esler et al. 1999 and Carrick 2003).

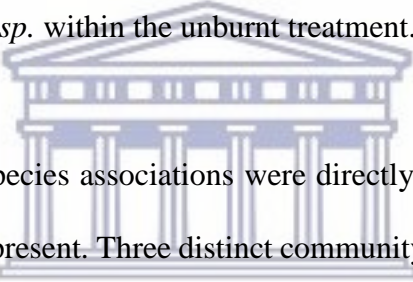
In the unburnt treatment, the majority of the species amongst which positive associations occurred were geophytic species, this may be attributed to local edaphic factors which allow for their emergence, such as bareground, due to the sparse patchy habit of the Succulent Karoo, which accounted for approximately 55 % cover in unburnt areas. This in turn, allowed for the coexistence of geophytic species despite the absence of fire. Both *Asparagus capensis* and *Pentzia incana*, as well as *Eriocephalus africanus* and *Helichrysum sp.* also displayed a positive occurrence with one another in the unburnt treatment. This co-occurrence displayed

may be attributed to competitive dominance, as these life forms are better able compete for resources after establishment in an environment absent disturbance(Connell and Slatyer 1977; Whittaker 1993).

The greatest number of positive associations present (25 %) amongst the 2008 burn treatment was with the non-succulent shrub species *Pentzia incana*, of which 40 % was non-succulent shrub species and 60 % succulent plant species. In addition, the succulent plant species *Drosanthamum hispidum*, which only shared a single positive association with the geophyte *Pelargonium sp*, in the unburnt plots, was positively associated with the occurrence of the following three non-succulent shrub species: *Eriocephalus microphyllus*, *Manochlamys albicans* and *Pentzia incana* in the 2008 burn. This may be the result of these non-succulent shrub species providing refuge from environmental stressors, by acting as islands for sediment, nutrient and moisture accumulation. In addition, within this treatment there were no positive species association present amongst *Asparagus capensis* and any other species. Only negative associations were present in this treatment amongst *Asparagus capensis* and other species present. This may be attributed to the early stages of this non-succulent shrub re-establishing dominance post-fire.

The plant species association present in the 2013 burn treatment was greatest amongst treatments. However, no association was present post-fire amongst *Asparagus capensis* and other species. In addition, the plant species association present is greatest amongst treatments, this may be attributed to the exacerbation of interspecies interactions such as facilitation during succession post-disturbance. This interaction is exemplified by the plant associations present with the non-succulent shrub species *Helichrysum sp.*, this non-succulent shrub species was only associated with *Eriocephalus africanus* during the unburnt treatment, during the

subsequent burn treatments this species was positively associated with the occurrence of geophyte species. In the 2013 burn treatment 21 % of the positive species occurrence associations present was amongst *Helichrysum sp.* and the following geophyte plant species: *Cyanella hyacinthoides*, *Cyanella lutea*, *Hesperantha cucullata*, *Holothrix aspera*, *Ophioglossum polyphyllum* and *Oxalis obtusa*. This high representation of positive associations amongst a non-succulent shrub species and geophyte species during the 2013 burn treatment is attributed to the significant increase in the bareground percentage cover in addition to the reduction of the non-succulent shrub percentage cover. However, the loss of non-succulent shrub percentage cover appears to play a greater role than that of bareground percentage cover as the occurrence of *Cyanella hyacinthoides* was negatively associated with the occurrence of *Helichrysum sp.* within the unburnt treatment.



Both species occurrence and species associations were directly influenced by fire, impacting upon community assemblages present. Three distinct community assemblages were identified. The community assemblage present in Clade I forms geophyte dominated non-succulent shrubland with low representation of succulents and herbaceous plant species. It hosts a high percentage of species recruited after fire and the remaining species consisted of persistent plant species. This visually distinct Clade was formed by the sites most recently disturbed and therefore is associated with early response of the vegetation post fire. The low representation of succulent plant species within this clade is to be expected as the fire occurred in 2013 and succulent plant species are not adapted to the occurrence of fire, with fire being considered as being destructive in the Succulent Karoo. In addition, Succulent karoo plants display a conservative growth rate indicative of the harsh environment in which they occur (Lechmere-Oertel and Cowling 2001). The recovery of succulent plant species is therefore predicted to take longer to recover from disturbance.

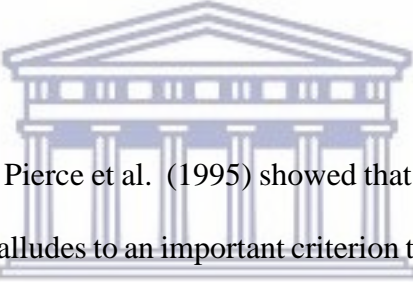
The community assemblages resulting from the occurrence of fire are further exemplified by Clade II, which comprised of only unburnt sites and represented succulent-geophyte dominated vegetation with a low representation of herbaceous species. The species within this Clade were largely composed of persisting species and the remaining species were those that were excluded post-fire.

The community assemblage displayed in Clade III also formed a geophyte dominated non-succulent shrubland with low a representation of succulent and herbaceous plant species. In addition, this was the largest Clade derived, an amalgamation of the 2008 and 2013 burn treatments. Thus it may be a successional stage post-fire, recruiting from both disturbed sites and hosts species able to persist post-fire, species recruited post-fire, as well as species previously lost post-fire (Figure 14). This Clade hosts a greater representation of non-succulent shrub species than what was present in Clade I, but lower than what was present in Clade II. The Succulent component displays a similar trend, with a high representation in Clade II, followed by Clade III and lastly Clade I. This may be indicative of gradual recovery taking place. Whilst geophyte species displayed a trend not congruent with this, with a high representation of species in Clade I, followed by Clade III and lastly in Clade I. This suggests that fire favours the occurrence of geophyte species in the Succulent Karoo, whilst reducing non-succulent shrub and succulent species (Appendix 2).

The occurrence of fire therefore influenced the community assemblages present within each treatment. Within semi-arid and arid regions, the role of perennial plants (shrubs in particular) are recognised for their importance in acting as resource islands. Shrubs are able to accumulate more favourable microclimates around themselves by trapping sediment, debris and intercepting water (Noy-Meir 1985; Schlesinger and Pilmanis, 1998).

3.4.3. Effect of fire on plant percent cover

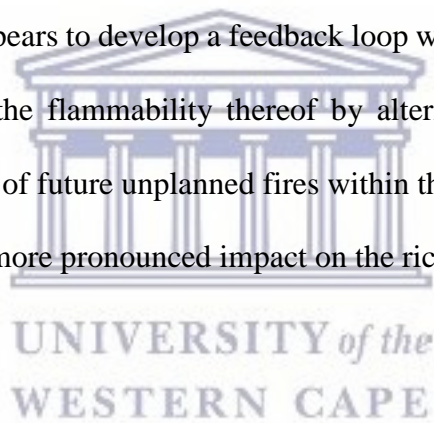
The impact of fire on succulents appears to be detrimental, reducing the percent cover of these plants significantly. A study conducted by Calitz et al. (2005) investigated the species-level flammability across five biomes and found that succulents had low flammability. Therefore, the reduction of these succulent plant species which are less flammable, may in turn increase the flammability of the veld, resulting in more intense fires. This is attributed to the occupation of vacant gaps generated by this loss allowing for the increase or recruitment of more flammable plant species, such as fine leaved non-succulent shrubs. The rise of plant flammability and its role in overall fitness has been a quagmire in fire ecology. In fire prone systems such as the Fynbos biome it is argued that this trait enhances reproductive success and reduces competition.



However, a study conducted by Pierce et al. (1995) showed that fire promoted the germination of succulent plant species. This alludes to an important criterion that is lacking in fire ecological studies, fire intensity. As the fires investigated within this study were not reflected within the records obtained from the NASA fire database and assumed to be of low intensity. This may be attributed to the community present during these burns, as the unburnt treatment has a higher percent cover of succulents and the 2008 burn is predominantly covered by non-succulent shrubs, in contrast the most recent 2013 burn percent cover is dominated by geophytes. This high representation of geophytes may be attributed to a mechanism for post burn seedling recruitment, known as fire stimulated flowering. This flowering usually occurs in the first year post-fire, borne from the resprouts of rhizomes or bulbs, followed by an abundant seedling recruitment in the subsequent year (Bond and Keeley 2005). This results in a higher representation of geophytes as most species continue to flower sporadically in subsequent years with no obligate dependence on fire for flowering.

This displays that systems are dynamic in their response to disturbance and that successional stages do not necessarily result in the return to an unburnt state. However, the influence of this work on supporting the state and transition model cannot be demonstrated, as a time series analysis will be required that incorporates spatial and temporal fluxes demonstrated by communities in response to fire.

The results of this study display that fire does not significantly impact botanical diversity. However, it does change community assemblages and plant cover, this change in turn may result in the increased flammability of this community. Thus making it more vulnerable to future disturbance, such as fire which may reduce its overall resilience through the loss of succulent plant cover. This appears to develop a feedback loop within the system, in which the occurrence of fire increases the flammability thereof by altering the community structure present. Therefore, the impact of future unplanned fires within the Hantam National Botanical Garden is expected to have a more pronounced impact on the richness and diversity present.



Chapter 4: Post-fire response of microbial communities in the Succulent Karoo

4.1. Introduction

Soil systems act as a microhabitat in which 80-90 % of its processes are mediated by microbes (Nannipieri et al. 2003). This substrate forms a complex and dynamic biological system, however, it is still difficult to determine the composition of microbial communities present. In addition, these communities are associated with plant health, growth and the promotion of biodiversity (Lugtenberg et al. 2002; Morrissey et al. 2004)

Interest in the effect of disturbance on these communities has been piqued in recent years, such as Wang et al. 2015, who demonstrated that fire alters both vegetation and microbial communities. Earlier studies by Hupwayi et al. (1998) and Inbar et al. (2005) demonstrated that these communities are sensitive to both physical disturbance (tillage) and chemical disturbance (soil enrichment using fertilisers) respectively. The techniques currently available however, have to be carefully selected as the misuse thereof may occur (Frostgard et al. 2011).

Microbes act as indicators of disturbance and are sensitive to environmental changes (Wurst et al. 2012). In addition, microbial communities correlate well with the chemical and physical parameters of the substrate (Mijangos et al. 2006). Studying these communities may provide consistent and effective indicators of change, acting as indicators of the negative or positive impact of a particular disturbance (Pankhurst et al. 1996; Sharma et al. 1998; Bending et al. 2004). Community-level molecular techniques are used in comparative microbial ecology to assess the diversity of microbial communities and their response to change. These include: temperature gradient gel electrophoresis and denaturing gradient gel electrophoresis

(TGGE/DGGE), singlestrand conformation polymorphism (SSCP), terminal-restriction fragment length polymorphism (tRFLP), length heterogeneity-PCR (LH-PCR) and 16S rRNA gene clone libraries (Marzorati et al. 2008).

One of the most well established tools in microbial ecology is DGGE, this fingerprinting technique generates a profile representing the genetic structure and diversity of microbial communities from a specific environment, with a high versatility, reliability and reproducibility (Marzorati et al. 2008; Green et al. 2009). This technique entails the separation of double-stranded DNA by electrophoresis through a gradient of increasing denaturant. The DNA molecules that differ by only one nucleotide will have slightly different denaturing points, resulting in the DNA molecule being retarded and forming into single strands.

This study will employ the use of DGGE to assess the impact of fire on microbial communities present in the botanically diverse winter-rainfall region of the Succulent Karoo (Van der Merwe and Van Rooyen 2011).



4.2. Materials and methods

4.2.1. Microbial survey

Soils were sampled during both the wet (July) and dry season (February) of 2016.

4.2.1.1. Soil sampling

Composite soil samples were obtained from each plot (as depicted in Figure 5), approximately 0-25 cm (Fierer et al. 2003) of soil was collected at 1 m from each corner and placed within a zip-lock bag. These zip-lock bags were immediately placed within a black container. Sampling utensils were sterilised before obtaining each composite sample using 95 % ethanol and bleach.

4.2.1.2. Lab work

Samples were stored at 4 °C in a cold room and sieved at 2 mm mesh size before commencing with extraction. The successive steps engaged are displayed below (Figure 17).



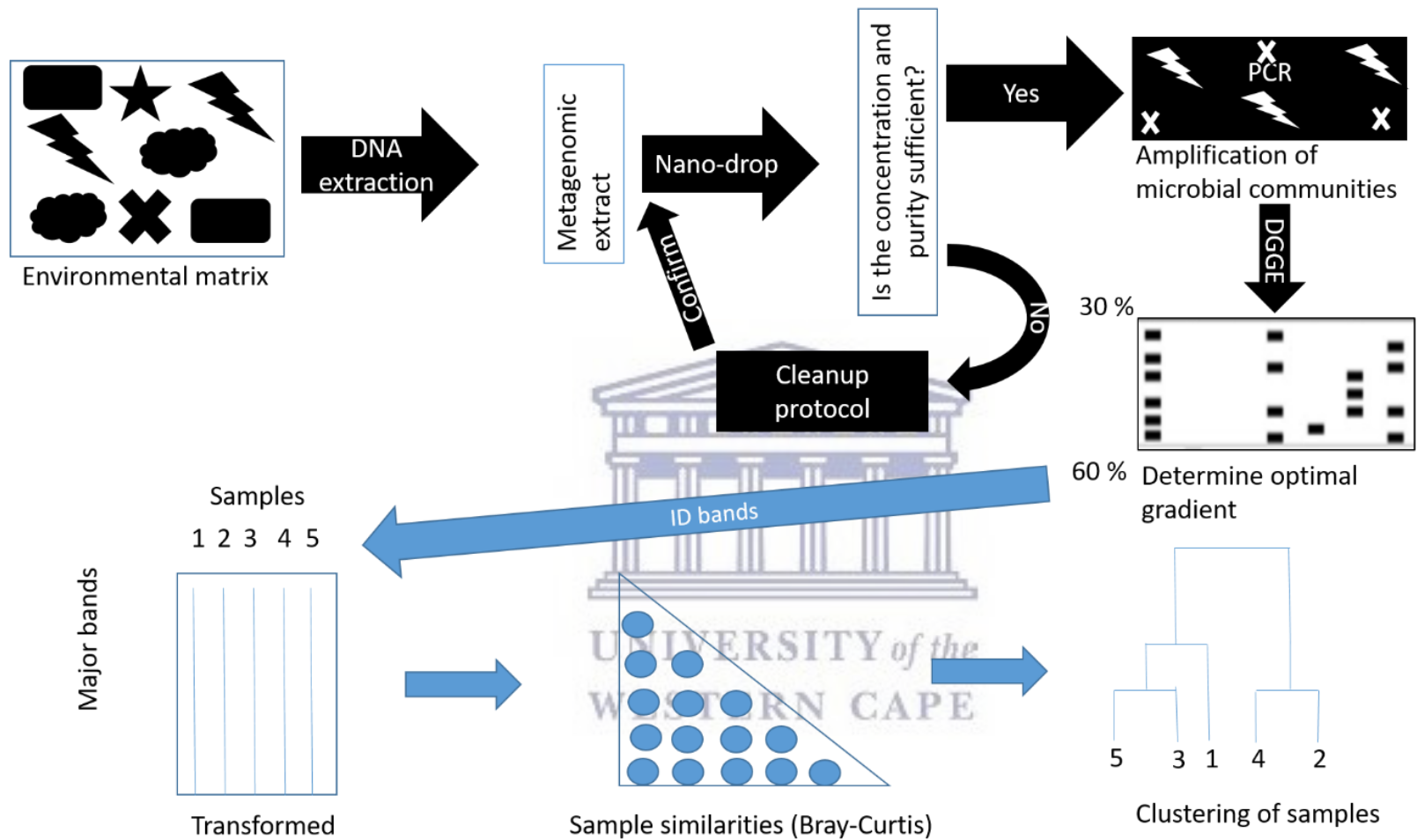


Figure 17: Flow diagram of successive steps required to assess microbial communities (adapted from: Clarke and Warwick 1994; Spencer and Ragout de Spencer 2004).

Metagenomic extraction (DNA isolation from soil) - Modified Miller method

The metagenomic extraction of DNA was conducted based on the Miller protocol (Miller et al. 1999). Approximately 0.5 g of soil was placed within a sterile 2 ml Eppendorf tube. To this 300 µl of 0.1 M Potassium phosphate was added, 300 µl lysis buffer (pH 8: 0.5 M Tris-HCl, 10 % SDS [v/v], 100 mM NaCl and 300 µl chloroform/isoamyl alcohol (24:1) was added. This mixture was incubated at 80 °C in an AccuBlock™ Digital Dry bath (Labnet International, Inc) for 20 minutes and cooled on ice for 15 minutes before being vortexed for 2 minutes at maximum speed. Subsequently, this mixture was then centrifuged at 13 000 rpm for 10 minutes and approximately 300 µl of supernatant was aliquoted into sterile 1.5 ml Eppendorf tubes. Potassium acetate was then added up to a final concentration of 2.5 M, these tubes were manually inverted until white flocculates appeared. These tubes were then centrifuged at 13 000 rpm for 10 minutes. Approximately 300 µl of supernatant was then transferred to sterile 1.5 ml tubes, 0.6 volumes of isopropanol was added and tubes were manually inverted. Incubation at room temperature then commenced for 30 minutes, after which DNA was pelleted by centrifugation at 13 000 rpm for 30 min and washed twice with 70 % ethanol. After allowing the pellet to air dry nuclease free water was used to resuspend it and was stored at -20 °C for further analysis.

Nano-drop ND-1000 was used per manufacturer's instructions to check purity and concentration of DNA, 2 µl of extract was used for analysis.

Purification

The Polyvinylpyrrolidane (PVPP) clean-up protocol was followed as described by Menking et al. (1999), with minor adjustment through the construction of spin columns using filter-tips instead of using shredder tubes. Two columns were prepared per sample, 300 µl of 10 % PVPP

was added to each column. These columns were then centrifuged until dry at 13 000 rpm and 100 µl of extract was added. It was then centrifuged for 1 minute at 13 000 rpm and stored at -20 °C.

Subsequent purification, Nano-drop ND-1000 was used per manufacturer's instructions to check purity and concentration of DNA, 2 µl of extract was used for analysis. Extracts with a minimum concentration of 20 ng/µl and 260/280 purity ratio of 1.3 were used for subsequent analyses.

Amplification

The purified extract was used as template DNA for the polymerase chain reaction (PCR). One primer set was used to amplify partial regions of bacterial 16S rRNA gene: 357F-GC (5'-cgcccg ccg cgc gcg gcg ggc ggg gcg ggg gca cgg gggg cct acg gga ggc agc ag-3') and 517R (5'-att acc gcg gct gg-3'; Muyzer et al. 1993). The target DNA was amplified using sterilised 0.2 ml thin walled tubes in Gene Amp®, Eppendorf Master cycler gradient or Thermo Hybrid PCR Sprint machines. Each PCR reaction (25 – 50 µl) consisted of more than 5ng of template DNA, 1X OneTaq standard reaction buffer (from 5 X buffer consisting of 20 mM Tris-HCl [pH 8.9], 22 mM KCl, 22 mM NH₄Cl, 1.8 mM MgCl₂, 0.06 % IGEPAL® CA-630 and 0.05 % Tween® 20), 0.2 µM of both reverse and forward primers, 200 µM dNTP mixture (dATP, dCTP, dGTP and dTTP), and 1.25 µl OneTaq DNA polymerase. These reactions were made up to the appropriate final volume using nuclease free water. These PCR tubes were then transferred to a PCR machine and thermocycling was initiated within the parameters stated in Table 1.

Table 1: Thermocycler setting for amplification protocol.

Stages	Temperature	Time
Initial denaturation	94 °C	30 seconds
Denaturing	94 °C	30 seconds
Annealing	55 °C	60 seconds
Extension	68 °C	60 seconds
Final extension	68 °C	5 minutes
Hold	10 °C	∞

*run for 36 cycles

Agarose gel electrophoresis

An aliquot of the PCR reaction mixture was analysed by gel electrophoresis. This resulted in the separation and visualisation of DNA fragments (Sambrook and Russell, 2001). The visualisation of DNA was conducted using an AlphaImager 2000 digital imaging system (Alpha Innotech, San Leandro, CA). The appropriate percentage (v/w) TAE agarose gels were cast and set to run for 90 minutes at 90 V in 1X TAE buffer (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA [pH 8.0]). The size of DNA fragments was determined by comparing those visualised to standard molecular markers (100 bp ladder).

Denaturing gradient gel electrophoresis (DGGE)

The DGGE was conducted using the protocol of Muyzer et al. (1993) using the Dcode™ universal mutation detection system (Bio-Rad) according to the manufacturer's instructions. DGGE was performed using a 16 X 16 cm, 10 % polyacrylamide gel (Acrylamide/bis 37.5:1) containing a linear chemical gradient. The optimum gradient determined ranged from 30-60 %, 20 ml of both high and low denaturing solutions were synthesised as stipulated in Table 2

and filtered through a 0.45u filter. These solutions were stored at 4 °C in brown bottles. The polymerisation of the acrylamide gel was initiated by adding 160 µl of 10 % APS (ammonium persulfate) and 14 µl of TEMED (Tetramethylethylenediamine) to each denaturing solution. This was then cast between the assembly plates using a gradient former and allowed to polymerise. As the gel polymerises, 7 L of 1X TAE (40 mM Tris–HCl, 20 220 mM acetic acid, 1 mM EDTA at pH 8.3) was heated to 60 °C. Approximately 44 µl of PCR product was loaded into each well before being electrophoresed in 1X TAE buffer at a constant voltage of 60 for 16 hours. After electrophoresis the gel was stained in 1X GelRed™ for 20 minutes and visualized using the AlphaImager 2000 digital imaging system (Alpha Innotech, San Leandro, CA).

Table 2: Denaturing solution reagents.

Reagent	30 % Denaturing solution	60 % Denaturing solution
40 % Bis/Acrylamide (ml)	25	25
50X TAE (ml)	2	2
Formamide (ml)	12	24
Urea (g)	12.6	25.2
dH ₂ O	Up to 100ml	

The effect of fire on the microbial communities present was investigated as stipulated in above. However, abundance data was not used as presence/absence data of major bands identified was used as input.

4.3. Results

4.3.1. Metagenomic extraction and purification

Metagenomic DNA was extracted for both the dry and wet season as ascribed in Section 4.2.1, and the DNA yields obtained are displayed in Figure 18 and Figure 19.

During the dry season the average purity of the direct metagenomic extract (DPurity) before purification was recorded at 1.33. This ratio of absorbance at 260 nm and 280 nm is relatively low, with a ratio of 1.8 accepted as pure for DNA and 2.0 for RNA. After following the PVPP purification protocol the average purified metagenomic extract (PPurity) was record at 1.53 (Figure 18).

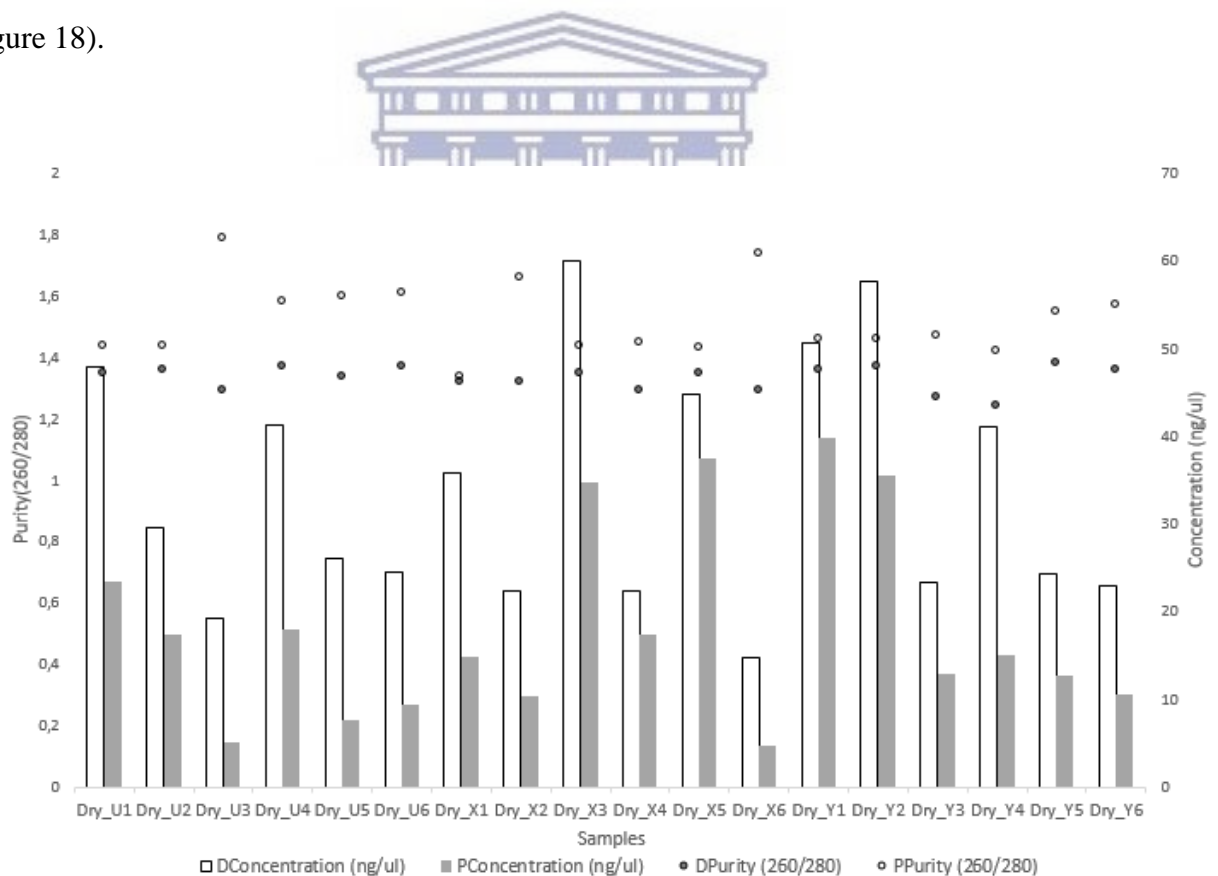
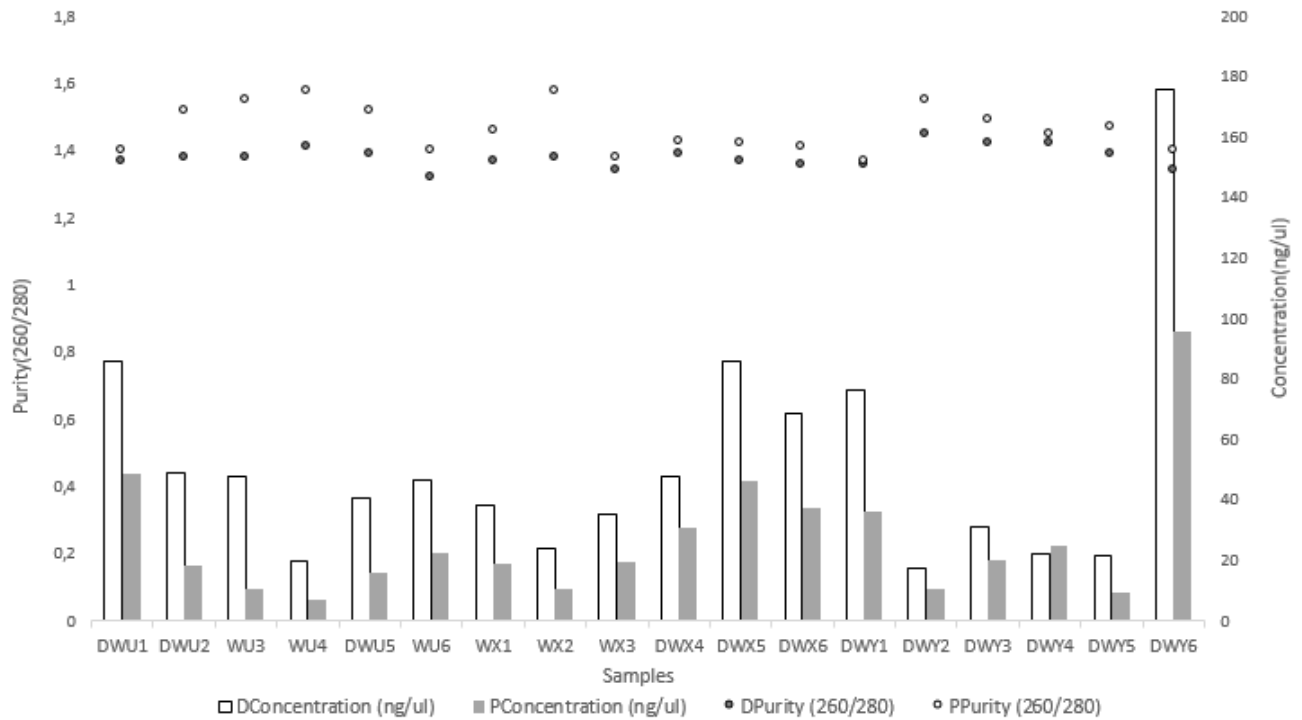


Figure 18: Dry season metagenomic extraction purity and concentration.

The average direct metagenomic extract had a greater mean concentration during the wet season (59.18 ng/ μ l) than during the dry season. (33.86 ng/ μ l). The mean DPurity was recorded as 1.38, whilst the mean PPurity was recorded as 1.47 (Figure 19).



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 Figure 19: Wet season metagenomic extraction purity and concentration.

These results show that the purification protocol was successful, however, a reduction in the concentration of the metagenomic extract was noted (DConcentration and PConcentration, respectively).

4.3.2. 16S rRNA amplification

The amplicons required for the subsequent DGGE analysis was prepared by employing the PCR-DGGE strategy as ascribed in Section 4.2.1. Despite the employment of the PVPP cleanup protocol amplicons could not be generated for all samples, this was attributed to low yields and the presence of PCR-inhibiting properties within samples.

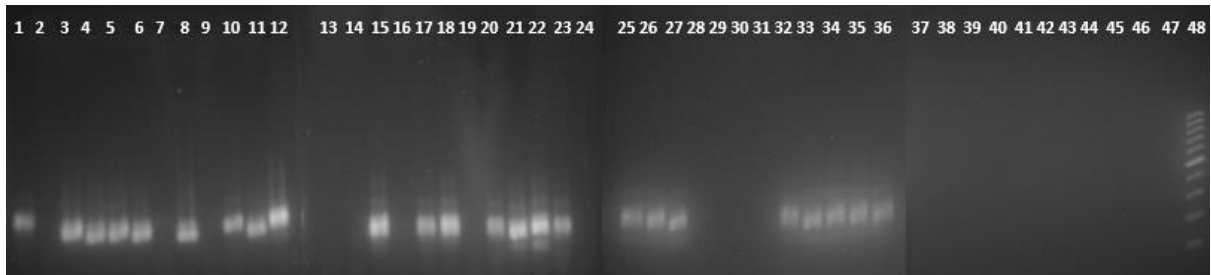


Figure 20: Agarose gel (2 %) showing amplicons obtained following 16S rRNA amplification using primer pair 357F-GC and 517R. Lanes 1-6, PCR reactions of unburnt samples during the dry season; Lanes 7-12, PCR reactions of the 2008 burn samples during the dry season; Lanes 13-18, PCR reactions of the 2013 burn samples during the dry season; Lanes 19-24, PCR reactions of the unburnt samples during the wet season; Lanes 25-30, PCR reactions of the unburnt samples during the wet season; Lanes 31-36, PCR reactions of the 2008 samples during the wet season; Lanes 37 and 48, contains the blank (negative control) and DNA ladder (100 bp), respectively. Those PCR amplicons that rendered the best images after electrophoresis were then selected and used for further analyses.

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The amplicons generated (Figure 20) were separated by DGGE to analyze the microbial communities across treatments. PCR allowed for the amplification of the microbial communities present, whilst microbial community profiles were generated via DGGE. The most intense band types are indicated on the gels representing the microbial community profiles.

The dry season microbial community profile generated through DGGE across treatments (Figure 21). The profiles generated appeared to vary across treatments. Eleven distinct major band types were identified (as displayed below), 55 % of which occurred in the 2013 burn samples, 82 % in the 2008 samples and 64 % in the unburnt samples. .

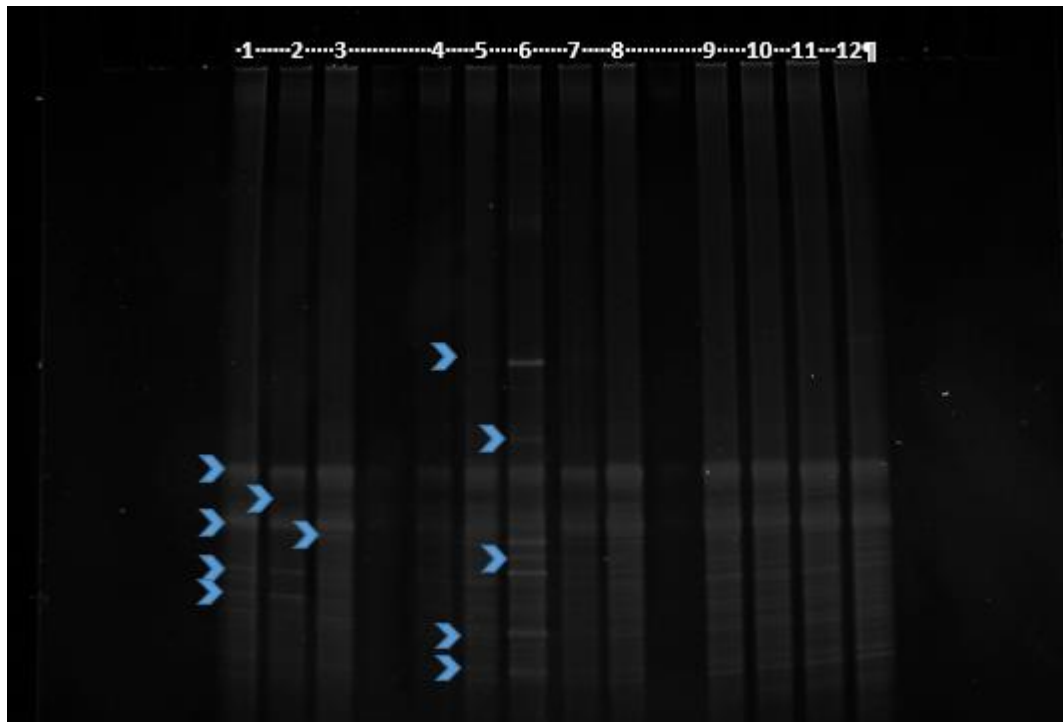


Figure 21: Dry season DGGE profile (30-60 % gradient) of soil samples under different treatments. Lanes 1-3, microbial profile of 2013 burn samples plots 6, 5 and 3; Lanes 4-8, microbial profile of the 2008 burn samples plots 6, 6, 5, 4 and 2; Lanes 9-12, microbial profile of the unburnt samples plots 6, 5, 4 and 3.

The wet season microbial community profile generated through DGGE across treatments (Figure 22). The microbial profile generated for the wet season displayed a greater representation of bands than the profile obtained during the dry season. This densely packed profile is attributed to a greater density of microbial populations being present during this season. Eleven distinct major band types were also identified within the wet season's microbial profile (as displayed below), of which 91 % occurred in the 2013 burn samples, 82 % in the 2008 samples and 82 % in the unburnt samples.

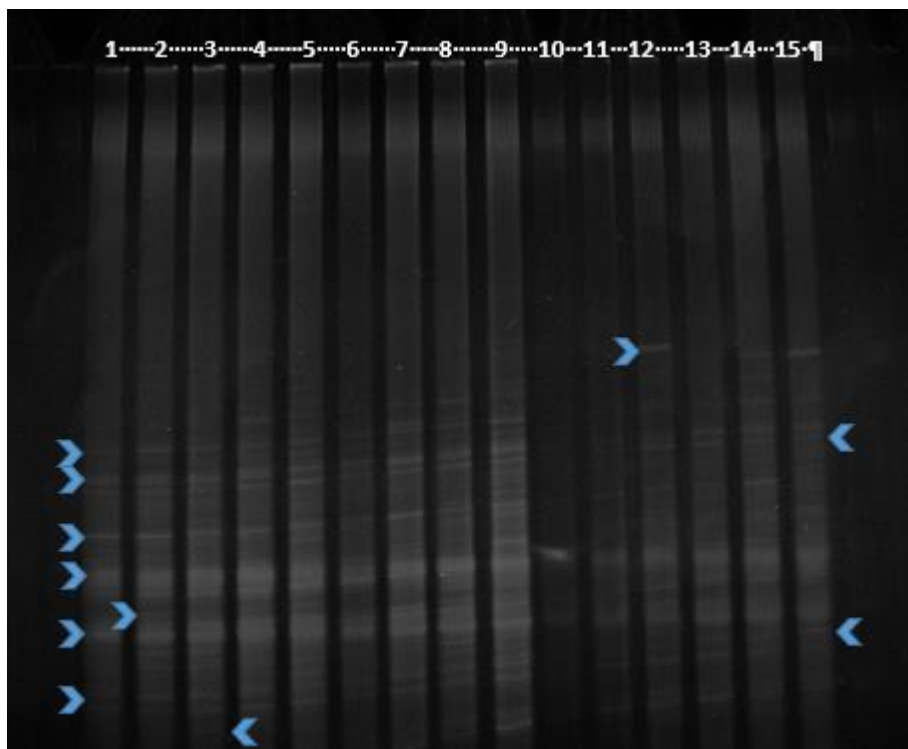


Figure 22: Wet season DGGE profile (30-60 % gradient) of soil samples under different treatments. Lanes 1-6, microbial profile of unburnt samples plots 1,2,3,4, 5 and 6; Lanes 7-10, microbial profile of the 2008 burn samples plots 1,2,3 and 4; Lanes 9-12, microbial profile of the 2013 burn samples plots 1, 2, 3, 4 and 5.

4.3.3. Effect of fire on community assemblages

The dendograms depicted below delineated groups of sites which shared distinct community structure with one another as per DGGE, clades were therefore arranged on the inherent characteristic patterns of occurrence consistently found in different groups.

The dry season dendogram delineated five distinct community assemblages (Figure 23). Clades were delineated as follows at a 15 % dissimilarity: Clade I (two 2013 burn treatments shared a similar profile), Clade II (hosted a single 2008 burn treatment), Clade III (consisted of two 2008 burn treatments), Clade IV (hosted a single 2013 burn treatment) and Clade V (comprised of all the unburnt treatments).



Bray-Curtis Dissimilarity

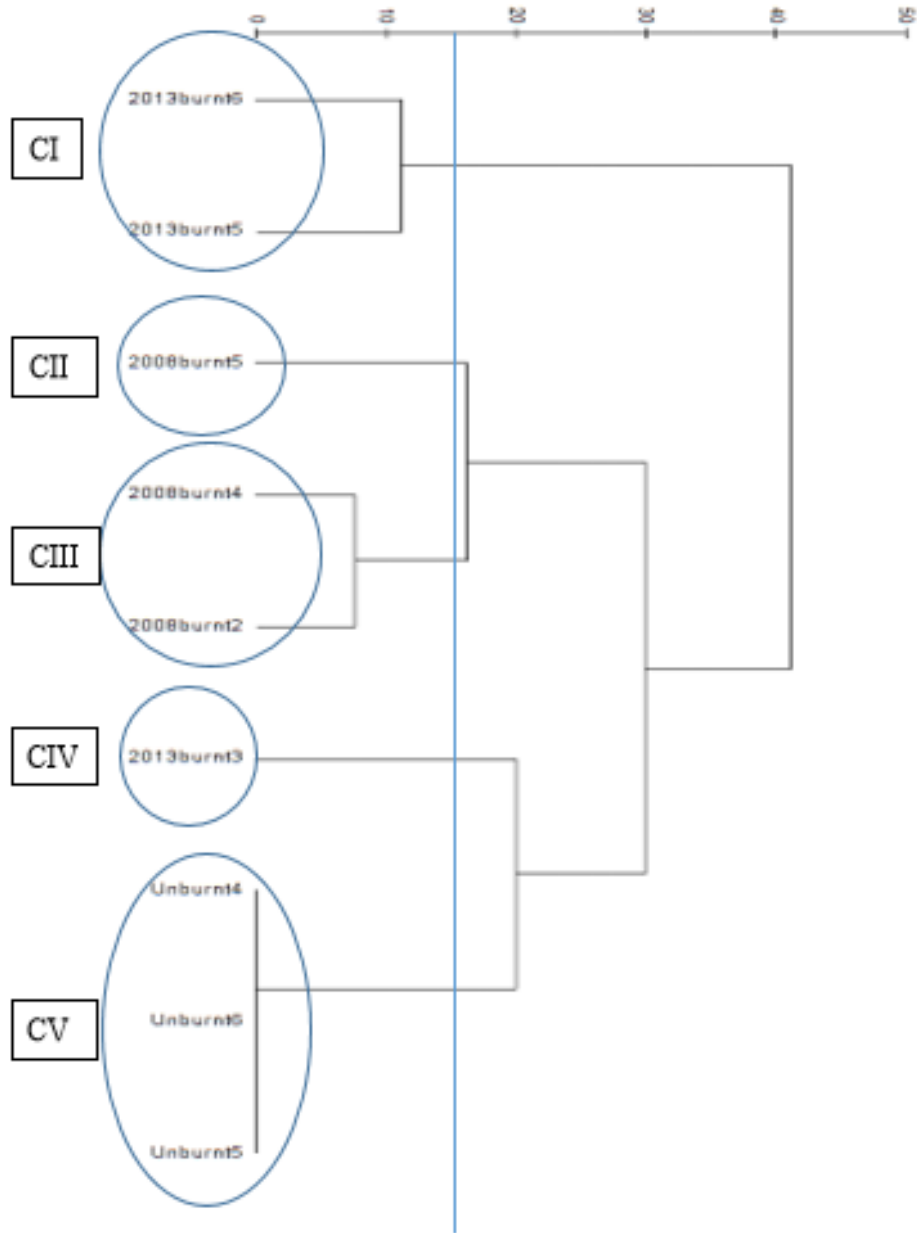


Figure 23: Dendrogram for hierarchical clustering of major bands within sites during the dry season, using group-average linking of Bray-Curtis dissimilarities at 15% dissimilarity calculated on $\sqrt{\sqrt{}}$ transformed presence/absence data.

The dendrogram generated for the wet season delineated three distinct community assemblages (Figure 24). Congruent with the dry season clades were also delineated at a 15 % dissimilarity: Clade I (hosted a single 2008 burn treatment), Clade II (comprised of all the 2013 burn treatments) and Clade III (consisted of all the unburnt treatments as well as two 2008 burn treatments).



Bray-Curtis Dissimilarity

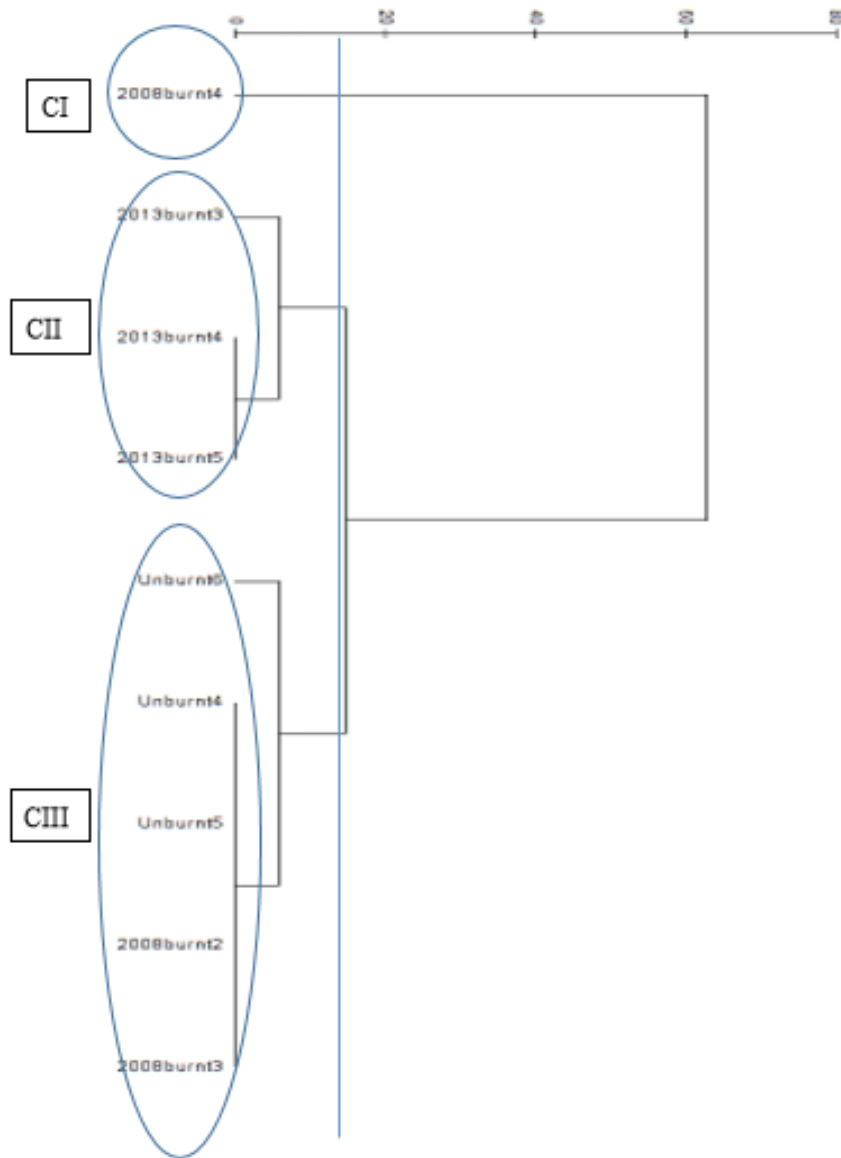


Figure 24: Dendrogram for hierarchical clustering of major bands within sites during the wet season, using group-average linking of Bray-Curtis dissimilarities at 15 % dissimilarity calculated on $\sqrt{\lambda}$ transformed presence/absence data.

4.4. Discussion

In this work, the microbial community profiles during the wet and dry season from a region of relatively low productivity in the winter-rainfall Succulent Karoo (Braschler et al. 2012) was studied. The impact of fire on microbial communities is of particular importance in understanding the role of fire in ecosystem functioning. Several studies have provided information about the total microbial biomass and activity in soils affected by fire, however, little has been rendered regarding the recruitment and composition of the microbial populations after the occurrence of fire.

4.4.1. Metagenomic extraction and purification

The metagenomic extraction is fundamental in analysing the total microbial pool in any environment, as the subsequent analyses will be dependent on the purity of this extract. In this study, the initial metagenomic extract for both the wet and dry season was high, recorded at an average of 51.98 ng/μl and 33.86 ng/μl respectively. However, the corresponding purity of this extract was relatively low, obtaining an average 260/280 ratio of 1.33 and 1.38, respectively. Initial attempts to directly amplify from these extracts resulted in no yield. This was attributed to the presence of PCR inhibiting compounds present within the soils sampled, such as polysaccharides and humic acids (Tebbe and Vahien 1993; Von Wintzingerode 1997, La Montagne et al. 2002; Inbar et al. 2005). Therefore, in order to deal with these environmental contaminants, the PVPP protocol was employed, this resulted in the reduction of concentration and the increase in purity for samples (Figure 18 and Figure 19). The overall concentration as well as the purity of the metagenomic extract was greatest during the wet season than during the dry season. Indicative of environmental-forcing attributed to the increased moisture

availability of the wet season in comparison to the dry season (Figure 18 and Figure 19).- seasonal no info on fire chnges in terms of purity, but is seasonal.

After successfully employing the PVPP protocol, PCR amplification was once again implemented, this resulted in the successful amplification of some of the samples (Figure 20). Those that were successfully amplified were used in the subsequent analyses. This amplification was achieved by utilising universal PCR primers to amplify bacterial 16S rRNA genes. The PCR products generated for DGGE contained a GC-rich region and was designed to prevent self-annealing. This clamp was incorporated during the PCR amplification and was initially attached to the forward primer.

4.4.2. Denaturing Gradient Gel Electrophoresis community assemblages

DGGE provided a visual depiction of the microbial communities present through the separation of double stranded DNA fragments. These fragments, generated as PCR product, are identical in length but differ in sequence. Fragments richer in GC are more resilient to denaturation, thus they will remain stable at lower denaturant concentrations. After denaturation the molecule is retarded and larger than the initial fragments (Fischer and Lerman 1983; Muyzer et al. 1993; Miller et al. 1999; Ercolini 2004). This resulted in the pattern/fingerprint displayed (Figure 21 and Figure 22), and from the gels 11 major bands were identified in each season, of which three major band groupings were selected for each treatment.

These profiles were visually distinct from one another, alluding to the impact of seasonal fluxes on microbial communities. The wet season profiles generated displayed a more densely packed profile, the denaturing gradient was congruent for both seasonal profiles generated. A study conducted by Williams 2007 states that soilborne bacteria are adapted to water stress which

enable them to survive unfavourable conditions. Gram positive bacteria is recognised as being more desiccation resistant than gram negative bacteria, this may elucidate the seasonal variation displayed (Williams 2007).

Communities assemblages were visually displayed using cluster analysis, generating two distinct dendograms for each season (Figure 23 and Figure 24). This was expected as the microbial profiles generated previously were visually distinct (Figure 21 and Figure 22). As expected seasonal variation plays an important role in soil microbial communities. We hypothesize that the influx of gram-negative bacteria results in a more homogeneous community assemblage within the wet season and the selective pressure present during the dry season results in a more heterogeneous community assemblage within the microbial population. In addition, the unburnt treatments appear to host community assemblages which are more resilient to these seasonal fluxes, clustering together during both the wet and dry season. The presence of gram-negative or gram-positive bacteria are not indicative of ecosystem health, as strains belonging to either group may result in either negative or positive plant-microbe interactions. Bais et al. (2004) found that the gram-positive bacteria, *Bacillus subtilis* 6051 strain, assists in avoiding infection from the gram-negative bacteria *Pseudomonas syringae*. In a separate study conducted by Grinchko and Glick (2001), the gram-negative bacteria *Pseudomonas putida* was used to inoculate tomato seeds resulting in an increased resistance to environmental stress.

In future, in order to limit seasonal biases which may occur one should restrict sampling to during the dry season in order to obtain a snapshot of the communities present. This is of particular importance given the context in which this study occurred, as the Succulent Karoo is predicted to be negatively impacted by the predicted future occurrences of drought and

rainfall variability. Therefore, this study provides evidence that microbial variation occurs during dry and wet seasons (with or without burns) will be an important factor to incorporate in ecosystem control in the Succulent Karoo in the near future.



Chapter 5: Conclusion and future prospects

The Succulent Karoo is threatened by drought and rainfall variability (IPCC 2007; Vetter 2009). In addition, the occurrence of unplanned fires within South Africa is set to increase due to environmental and anthropogenic influences (Snyman 2003; DEA 2010). Drought has been associated with the occurrence of flammability-enhancing traits within plant species (Lavorel and Garnier 2002). Recent climate change projections suggest that there will be an increase in the frequency of extreme climatic events, such as drought. As lower rainfall and increased temperature, increases vegetation flammability it may result in a shift in species composition (Rouault and Richard 2003; Hoffman et al. 2009; DEA 2010; Sangeda and Malole 2014). Under these future scenarios the way in which conservation is practised will have to be altered. Despite this, the implications of fire-vegetation feedbacks in the dynamics of this vulnerable biome are still poorly characterised (Batlorri et al. 2015), with limited understanding the efficient management of natural resources is constrained. This study revealed that while literature illustrates that several edaphic factors are impacted upon by fire, little has been done to assess the vulnerability/resilience of ecosystems in which the occurrence of fire does not share an evolutionary history. Fire in the Succulent Karoo did not significantly impact both species richness and diversity, as stated in Chapter 4. This was an unexpected, but elucidating result, as fire regimes act as a proxy in which biotas have co-evolved and these metrics form the crux of many ecological studies and theories, such as the Intermediate Disturbance Hypothesis (Connell 1978; Stevens 1989).

Whilst investigating communities in which change is expected to occur, metrics such as diversity and richness may be misleading. Upon further interrogation of the data, changes in species association, species occurrence and community assemblages was revealed. Cluster

analyses showed that the occurrence of fire did in fact alter the community assemblages present. Succulents, considered as one of the least flammable life forms (Calitz et al. 2005), were significantly greater in the unburnt sites. With climatic parameters set to increase the occurrence of fire, this may cause a critical shift, resulting in fire-facilitating communities replacing fire-inhibiting plant communities. This generates a positive feedback which increases fire frequency and forces the ecosystem to a state in which fire would occur more often than predicted by climate change alone. This change renders a system which may not return to its original state despite mitigation measures as the system now promotes fire.

Fire-adapted plant species, such as small-leaved shrubs with densely packed, fine twigs may facilitate the spread and initiation of fire (Beckage and Ellingwood 2008; Calitz et al. 2015). In addition, these species increase in abundance post-fire, whilst species that are fire-intolerant decline. Fire-intolerant species which are susceptible to fire damage may in turn inhibit fire (Beckage and Ellingwood 2008). Lechmere-Oertel and Cowling (2001) and Esler et al. (2015) both state that Succulent Karoo species are intolerant of fire and inhibits its encroachment into the fire-prone Fynbos. Calitz et al. (2015) revealed that large leaves were weakly associated with high flammability and succulent plants had low flammability overall. This appears to be congruent with the findings presented in Chapter 4, as further investigation revealed that fire resulted in the significant decrease of succulent plant percent cover. These changes, whilst not previously detected will affect the resilience of the Succulent Karoo to future unplanned fires.

Several authors have provided data about total microbial biomass and activity in soils affected by fires. However, little information about the composition and recruitment of soil microbial populations after the passage of fire is available. Whilst assessing the impact of fire on the microbial communities it was found that these soils contained PCR inhibiting properties (Tebbe

and Vahien 1993; Von Wintzingerode 1997, La Montagne et al. 2002; Inbar et al. 2005), as well as low purity and concentration yield. The impact of fire on the microbial communities was not as pronounced as expected, this is attributed to seasonality.

Microbes have a relatively faster life turn over than plants therefore the fluxes induced by fires might be short lived. The seasonal variation present within these communities was visually distinct. Presenting a more densely packed profile during the wet season than the dry season. This was attributed to the seasonal fluxes of microbes in response to water availability (Williams 2007). The unburnt community assemblages identified appeared to be more resilient to change, refer to Chapter 5, in regards to season and treatments. This may be attributed to the plant percentage cover, as the bare ground percentage cover was significantly lower in the unburnt treatments (refer to Chapter 4). This cover might limit the impact of environmental forcing due to seasonal and disturbance driven fluxes.

This study revealed that the implications of fire-vegetation feedbacks should not be measured by relying on the orthodox metrics of diversity and richness. Subtle changes in the community assemblages better reflect the feedback dynamic present. In addition, fire directly impacted upon the botanical communities present, whilst indirectly influencing microbial communities.

The increase in future fire intensities and changing fire regimes will result in vegetation shifts, similar to the feedback process between fire activity and highly flammable C4 grasses (Bond et al. 2005; Pausas and Keeley 2009). Fire adapted species may host characteristics which facilitate and aid in the spread of fire, whilst fire intolerant species decline (Bond et al. 2005; Pausas and Keeley 2009). This feedback was explicated by the occurrence of the high flammability shrub species *Asparagus capensis* (Calitz et al. 2015) which persisted across

treatments and the loss of low flammability succulent plant species such as *Tylecodon wallichii*, colloquially known as ‘krimsiek’, a poisonous plant. This may support anecdotal information provided by land users that fire increases the forage quality of the veld. However, this was not the focus of this study, but may be assessed in future studies through the use of control burns and exclusion plots.

This study provided a baseline understanding of the implications of fire on vegetation feedbacks in the dynamics of the Succulent Karoo. In future the fluxes represented within the microbial profiles needs to be further quantified through molecular sequencing/ phylogenetics. In addition, a snapshot of these communities may be easier to quantify. Spatio-temporal factors are important and therefore DGGE should be employed in future as preliminary tool to decide when would be the optimal time to sample. In addition, instead of seasonal variation, before and after rainfall may display sufficient interactions amongst the communities present and water availability. This could also be proven true when attempting to assess the impact of fire, elucidating whether the changes caused will facilitate negative or positive plant microbe relationships.

The outcome of this study supports De Bano et al. (1998) who illustrated that fire influenced the soil microbial composition and activity in the short-term. Future studies should incorporate direct plant-microbe interactions, in particular those present within the rhizosphere of plant species which persist despite the occurrence of fire (i.e. *Asparagus capensis*). The microbes associated with this persisting high flammability plant species may form a biofilm which protects their root systems from the loss of moisture in addition to altering the surrounding bulk soil and influencing both recruitment and regeneration. The application of these microbes

to agricultural systems through inoculation may mitigate the impact of future predicted drought and unplanned fires.

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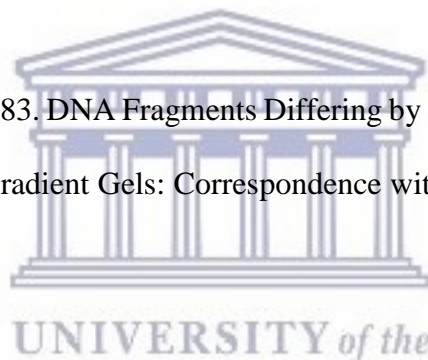
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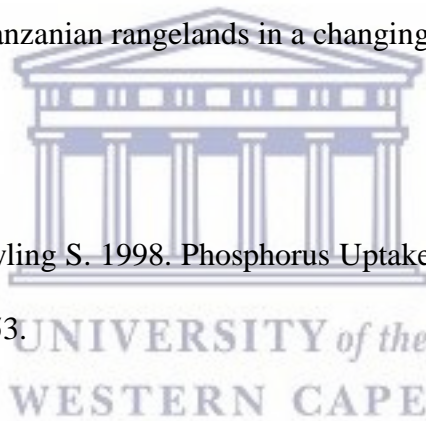
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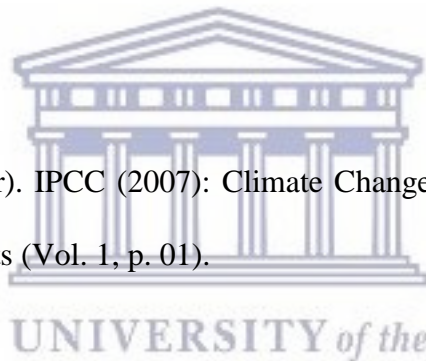
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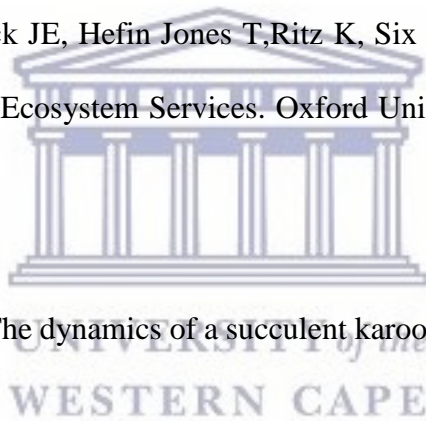
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Appendix 1: Comparison of species occurrence and response across all treatments.

Species	Life form	Response	Unburnt 1	Unburnt 2	Unburnt 3	Unburnt 4	Unburnt 5	Unburnt 6	2008 1	2008 2	2008 3	2008 4	2008 5	2008 6	2013 1	2013 2	2013 3	2013 4	2013 5	2013 6
<i>Anisodonte anomala</i>	S	R	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
<i>Asparagus capensis</i>	S	P	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1
<i>Eriocephalus africanus</i>	S	L	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eriocephalus microphyllus</i>	S	L	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
<i>Galenia Africana</i>	S	P	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Helichrysum sp.</i>	S	P	0	0	0	0	1	0	0	1	0	0	0	0	1	1	1	0	0	0
<i>Lycium sp</i>	S	R	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0
<i>Manochlamys albicans</i>	S	L	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0
<i>Pentzia incana</i>	S	P	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
<i>Crassula muscosa</i>	S u	L	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Drosanthemum hipsidum</i>	S u	P	0	0	0	1	0	0	1	1	1	1	0	1	1	1	1	0	1	0
<i>Euphorbia caput-medusae</i>	S u	P	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
<i>Ruschia sp.</i>	S u	L	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ruschia spinose</i>	S u	P	1	1	0	1	1	1	1	0	1	1	0	1	0	0	1	0	1	1
<i>Tetragonia fruticose</i>	S u	L	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Tylecodon wallichii</i>	S u	L	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Zygophyllum morgsana</i>	S u	L	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Babiana macronata</i>	G	P	0	1	0	1	1	1	0	1	1	0	1	0	1	1	1	0	1	0
<i>Bulbine succulenta</i>	G	P	1	1	1	1	1	1	0	1	1	1	1	0	0	1	0	0	0	0
<i>Bulbinella sp.</i>	G	P	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Bulbinella thesium</i>	G	R	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
<i>Cyanella hyacinthoides</i>	G	P	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	1	1	1
<i>Cyanella lutea</i>	G	R	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
<i>Hesperantha cucullata</i>	G	R	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
<i>Holothrix aspera</i>	G	R	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
<i>Holothrix secunda</i>	G	P	1	1	1	0	0	0	1	1	1	0	1	0	0	0	0	0	1	1
<i>Lachenalia obscura</i>	G	R	0	0	0	0	0	0	1	1	1	1	0	1	1	1	1	0	0	1
<i>Moraea bifida</i>	G	R	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
<i>Moraea pseudospicata</i>	G	P	1	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	1

*Table continues on next page

<i>Ophioglossum polyphyllum</i>	G	R	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
<i>Ornithogalum hispidum</i> Hornem	G	R	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0
<i>Oxalis obtusa</i>	G	R	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
<i>Oxalis pes-caprae</i>	G	P	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
<i>Pelargonium sp.</i>	G	P	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Romulea tortuosa</i>	G	R	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	1	0
<i>Wurmbea variabilis</i>	G	R	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
<i>Chlorophytum undulatum</i>	H	P	1	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1
<i>Cyphia volubilis</i>	H	P	1	1	1	1	1	0	1	1	0	1	0	0	1	0	1	0	0	0

*Response was defined as : Persist-species which occurred in both burnt and unburnt plots; Lost-species which did not occur post-fire; Recruit-species which only occurred post-fire.

†Life-forms: Geophyte (G), Non-succulent shrub (S), Herb (H) and Succulent (Su).

†Response: Persist (P), Recruit (R) and Lost (L).



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Appendix 2: Major life-forms and community assemblages.

