Potential eco-physiological and phytosociological impacts of fracking on the vegetation of the Karoo, Eastern Cape, South Africa.

By

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ABSTRACT

Hydraulic fracturing or fracking is a technique that is used to extract gas from low permeable rocks. Large volumes of fluids (typically water combined with chemicals and sand) are injected at high pressure into rock formations to fracture them, allowing the gas to be released. A number of criticisms have come to light regarding the potential environmental impacts of this process. One concern is that there will be contamination of groundwater due to the toxicity of the chemicals used in the fracking process. There have been limited studies on the effects of fracking fluid on vegetation and no studies on South African vegetation specifically. The effects of fracking chemicals on the germination success and photosynthetic efficiency of plants was investigated for species common in areas earmarked for possible future hydraulic fracturing in the Karoo, South Africa. Germination of seeds was unaffected by these fracking fluids at application concentration in most species, but dwarf shrub and grass seeds were found to be sensitive to contamination. A single application treatment of plants with fracking fluid resulted in mortality in 50% of the species with reduced photosynthetic efficiency and growth in some of the surviving species. Long term continual treatment with diluted fracking fluids had an even greater effect on mortality and photosynthetic efficiency than a single high dose. The major vegetation types of the proposed fracking footprint were surveyed and analyses of the species, communities and their physiognomy were used to predict the tolerance of the Karoo vegetation to degradation resulting from shale gas development. An understanding of the sensitivity of vegetation was obtained from impacts of livestock on the vegetation. The results indicated that Grassland communities are least tolerant to degradation, Albany Thicket communities more tolerant and Nama-Karoo communities most tolerant. Escarpment Thickets were shown to be Nama-Karoo rather than Albany Thicket elements, and should be grouped with the former when considering the impacts of fracking.

Key words: Hydraulic Fracturing, Karoo, germination, photosynthetic efficiency, phytosociology, Grassland, Nama-Karoo, Albany Thicket

DECLARATION

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In accordance with Rule G5.6.3, I hereby declare that the above-mentioned treatise/ dissertation/ thesis is my own work and that it has not previously been submitted for assessment to another University or for another qualification.

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CHAPTER 1. INTRODUCTION

Extraction of natural gas may be conventional or unconventional depending on its geophysical location and extraction method (Peduzzi & Harding Rohr Reis, 2013). Conventional gas is located in permeable rocks from which gas escapes freely once drilling has taken place. Unconventional gas is located in less permeable rocks such as shale, tight sands and coal beds. These formations must be fractured to release commercial quantities of gas (Peduzzi & Harding Rohr Reis, 2013). Hydraulic fracturing or fracking (also referred to as unconventional gas production) is an induced extraction technique that is used to extract gas from low permeable rocks (Peduzzi & Harding Rohr Reis, 2013). Large volumes of fluids (typically water combined with chemicals and sand) are injected at high pressure into rock formations to fracture them, allowing the gas to be released (IEA, 2012).

At first shale gas was welcomed as an alternative to coal power (Ridley, 2011). It stimulated interest by offering economic and energy security benefits (Peduzzi & Harding Rohr Reis, 2013). However, a number of criticisms have come to light regarding the safety and environmental impacts of extracting shale gas. Groundwater contamination from some of the toxic chemicals used in the fracking process, gases escaping into aquifers, polluted waste water tainting local streams and surface spills of fracking fluids are some of the environmental concerns (EU, 2011). About a third of the water that is pumped down the fracking well returns to the surface with the gas during production. This is known as flowback water. Depending on the shale type, this water may be sufficiently saline to create additional environmental concerns including the inhibition of seed germination and plant growth (Takaki & Wolf, 2011; Wolf & Brye, 2012). However, flowback water can be reused for the fracking process, reducing the water requirements from other water sources and also reducing the volume of flowback water requiring disposal (ASSAF, 2016). Land application of flowback fluids is one way to dispose of these fluids (Adams, 2011). The soil may be affected by excessive sodium, resulting in poor drainage and reduced crop yield (IEA, 2012). In most cases the gas bearing layers and groundwater bearing resources are hundreds of metres apart lowering the risk of aquifer contamination (World Energy Council, 2010). However, contamination of subterranean water sources

has been recorded in Wyoming (EPA, 2011a), Pennsylvania and New York (Osborn et al., 2011). At first, it appeared that unwanted environmental impacts of the exploitation of unconventional gases may be unavoidable, regardless of the adequate application of technology to control them (EU, 2011). More recent studies have shown that if best practice engineering procedures are followed in combination with geological analyses and monitoring, shale gas can be safely exploited and aquifers protected (Hannover Declaration, 2013; ACOLA, 2013).

Although there are potentially hundreds of chemicals that could be used during fracking only a limited number are routinely used (FracFocus, 2014). The fracking fluid that is pumped into the fracking well typically contains water (>90% of the total volume), sand, friction reducers (polyacrylamide), antimicrobial agents (bromine, methanol and naphthalene), hydrochloric acid, scale inhibitors (hydrochloric acid and ethylene glycol) and surfactants (butanol and ethylene glycol monobutyl ether) (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013). Chemical additives typically form a small proportion of the fracking fluid but due to the large quantities of water required for the fracking process, significant amounts of chemicals may be used (ASSAF, 2016).

Organic rich shales are formed by marine or lake deposits of mud, silt, clay and organic matter that form into sedimentary rock and are a potential source of natural gas. Certain layers within the Karoo Basin have been formed from concentrations of organic matter, making them potential methane gas sources. The most promising are the Lower and Upper Ecca Group sequences with estimates of 10-50 tcf and 100-400 tcf respectively (Chere et al., 2017). These rocks outcrop in the Southern Karoo and are found at depths of 3000-4000 m. Recent geological and geochemical evaluations have shown that the gas reserves in the Karoo are potentially recoverable (ASSAF, 2016).

This study forms part of the AEON-ESSRI Baseline Research Program at Nelson Mandela University, which is undertaking a technical evaluation and socio-economic analyses of the Central Karoo region, to document the status quo before shale gas development. Researchers from various disciplines such as Geophysics, Hydrochemistry, Geohydrology, Geology, Chemistry, Ecology, Conservation, Development Studies, Economics and Energy Engineering are collaborating on this

research program. This study adds the Botanical element of the baseline research program.

The AEON-ESSRI group is considering three study areas (Fig. 1.1) that roughly coincide with the three precincts of the Shell exploration areas. Although the exploration areas include many of the South African biomes (Fig. 3.2), the most affected will be the Nama-Karoo, Grassland and Thicket biomes. This study focuses on the Aberdeen and Cradock Study Areas (Fig. 1.1).

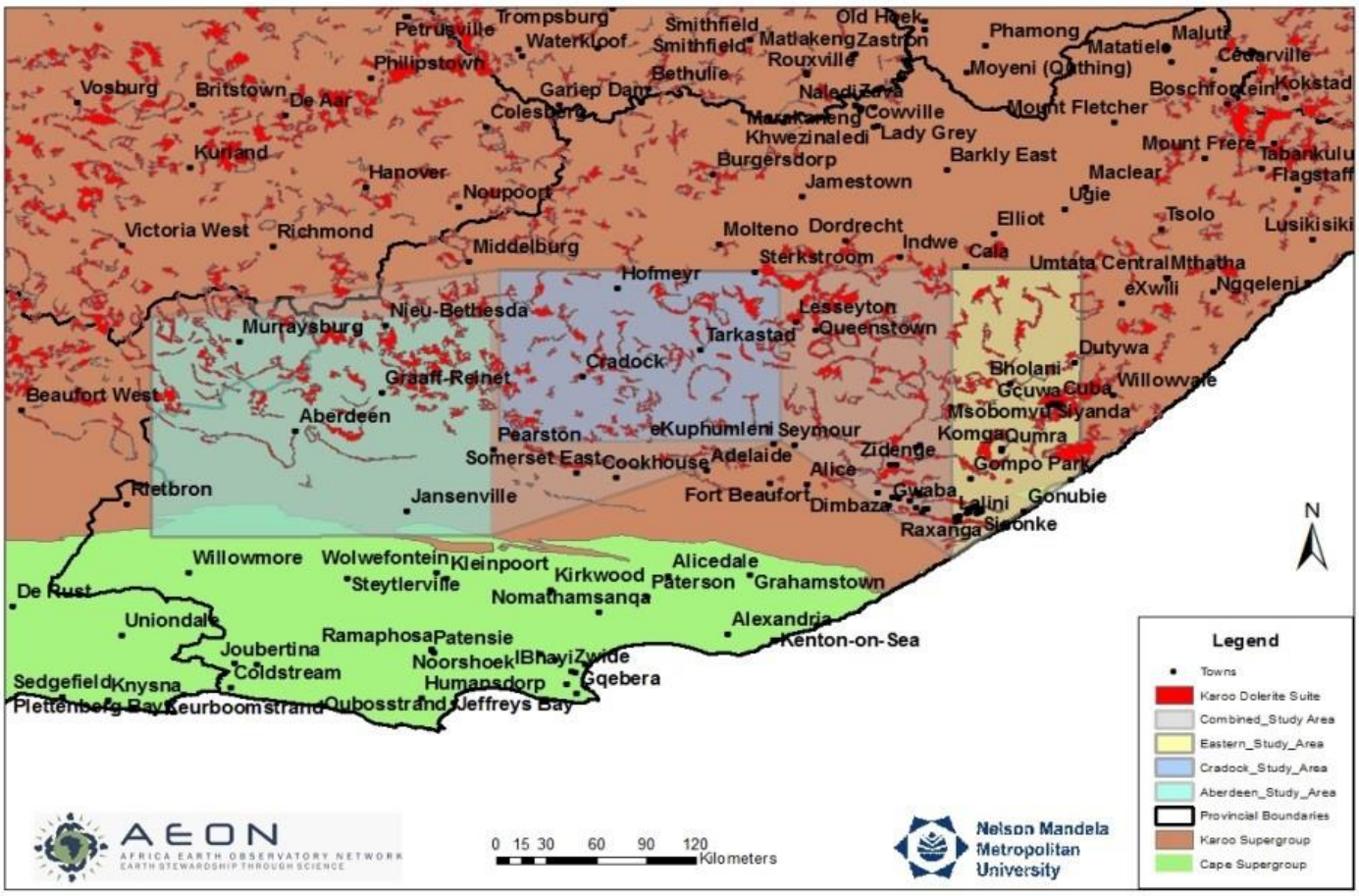


Figure 1.1. Study areas considered for the AEON-ESSRI Nelson Mandela University Shale Gas Baseline Research Program, Eastern Cape, South Africa.

The Nama-Karoo is an extensive biome which is flanked by six other biomes (Mucina et al., 2006a). The flora of the Nama-Karoo biome is not particularly rich and does not contain any centres of endemism (Van Wyk & Smith, 2001; Mucina et al., 2006a). The vegetation is dominated by dwarf shrubs that co-occur with grasses (C_3 and C_4), succulents, geophytes and annual forbs. Small trees are only found on rocky outcrops or along drainage lines (Mucina et al., 2006a).

Originally described as Valley Bushveld, the Albany Thicket Biome has since been recognised as a biome due to unique climate and growth forms as well as high regional levels of endemism (Low & Rebelo, 1996; Robertson & Palmer, 2002b; Vlok & Euston-Brown, 2002). Thicket vegetation is characterized by dense formations of evergreen and weakly deciduous shrubs and low trees (maximum height of 5 m), often spiny and overgrown with vines. It is almost impenetrable in its natural state (Acocks, 1953; Everard, 1987). The Grassland Biome vegetation is dominated by graminoids, especially of the family Poaceae. There are a number of endemic grass taxa and orchid taxa (Mucina et al., 2006b).

1.1 Project motivation

Mining, agriculture and urban and rural developments currently place pressure on the environment, highlighting the need for proper planning to identify ecologically sensitive areas before any developments take place. In terms of the *National Environmental Management: Biodiversity Act* (Act No. 10 of 2004) these studies are compulsory in South Africa (South African Government, 2004). Species of Conservation Concern (SCCs) are those species that would benefit the most from conservation efforts. This includes threatened species (according to IUCN Red List categories), species with internationally important populations and species which have become rare through the process of decline rather than those that are historically rare (Keller & Bollman, 2004). The first aim of this study is

- to compile an annotated plant species list for the areas most likely to be affected by shale gas exploration, particularly highlighting any SCCs and an assessment of any extinction risks associated with the impacts of fracking.

Scientifically sound environmental decisions on wildlife management and nature conservation are based on vegetation surveys, classifications and maps (Brown et al., 2013). A thorough knowledge of the ecosystems present in an area is essential for effective conservation (Brown et al., 2013). An understanding of their functioning and dynamics as well as the phytosociology of plant communities is also an essential part of endangered ecosystem monitoring programs (Brown et al., 2013). The second aim of this study is

- to contextualise poorly understood vegetation types of the fracking footprint in contribution to informed decision-making and management of shale gas development in the Karoo.

There have been a limited number of studies on the effects of fracking fluids on vegetation and specifically plant growth, survivorship and seed germination. In a study by Adams (2011), fracking fluid sourced from a local gas well was applied directly to vegetation in the Fernow Experimental Forest in West Virginia, USA. Here, ground vegetation was worst affected with damage and mortality evident soon after application while trees suffered premature leaf drop approximately 10 days after application. Takaki and Wolf (2011) and Wolf and Brye (2012) investigated the effects of drilling mud applied to plants and both found reduced growth and increased mortality in treated plants. Although effects on plant growth have been investigated to a limited extent, none of these studies considered how seed germination would be affected, or made use of South African examples when investigating plant resilience to fracking fluid contamination. The third aim of this study is

- to investigate how the local vegetation might react to contamination by fracking fluids.

1.2 Project objectives and hypotheses

The primary objectives of this study are to survey the vegetation of the proposed fracking areas, highlighting Species of Conservation Concern, contribute to an understanding of poorly researched vegetation, and to determine the eco-physiological responses of plants

to fracking fluids. The structure of the study, general objectives and hypotheses are provided below.

Chapter 2: Literature Review.

This chapter provides general background on the fracking process, vegetation in the proposed fracking footprint, and the potential eco-physiological responses of plants to fracking-related stresses.

Chapter 3: Phytosociology of selected Karoo areas earmarked for fracking.

This chapter considers the plant community composition of sites within the areas proposed for fracking in the Karoo, with emphasis on the presence of Species of Conservation Concern and the generation of an annotated baseline species list. Plant community composition of reserve sites and farmed/degraded sites are compared, and the condition of the soil is described. A special study into the relationship between the Karoo thicket elements (Camdebo and Escarpment Thicket) and core Thicket elements (Sundays and Great Fish Thicket) is also included.

Chapter 4: Effect of the application of fracking fluids on the germination of selected Karoo and Thicket species.

This chapter investigates the effect of the application of fracking fluids on the germination success and rate of a number of representative species from the areas earmarked for fracking. The hypothesis to be addressed in this chapter is that

- application of fracking fluids will negatively affect the germination rate and germination success of Karoo and Thicket species.

Chapter 5: Effect of the application of fracking fluids on the photosynthetic efficiency and condition of selected Karoo and Thicket species.

This chapter considers the effect of the application of fracking fluids on the photosynthetic efficiency and growth of number of plant species from the areas earmarked for fracking. The parameters measured are chlorophyll fluorescence (Fv/Fm ratio), growth rate and biomass allocation (root:shoot ratio). The hypotheses to be addressed in this chapter include:

- The application of fracking fluids will negatively affect the growth and survivorship of Karoo and Thicket species.
- Phreatophytes will be particularly sensitive to treatment with fracking fluids.
- Species treated with fracking fluids will exhibit signs of environmental stress in the form of lowered photosynthetic efficiency (reduction in Fv/Fm ratio).
- Root:shoot ratios will be higher in those plants treated with fracking fluids.

Chapter 6: General discussion.

This chapter provides a synthesis of the key findings of this study. Project limitations and further research opportunities are also discussed.

CHAPTER 2. LITERATURE REVIEW

2.1 The shale gas extraction process

Fossil fuels account for more than 80% of global energy use (IEA, 2011) and there is ever increasing pressure to exploit unconventional energy sources as energy demand rises and conventional fossil fuels reserves are depleted (UNEP, 2011a; UNEP, 2011b). In South Africa the current use of natural gas is small and relatively recent (Morkel & De Wit, 2017). South Africa's energy needs are mainly served by coal, heavy fuel oil and liquefied petroleum gas (ASSAF, 2016). The severe electricity constraints in South Africa since 2007 highlight the need for alternative energy sources.

Hydraulic fracturing or fracking (also referred to as unconventional gas production) is an extraction technique that is used to extract gas from low permeable rocks (Peduzzi & Harding Rohr Reis, 2013). Large amounts of fluids (typically water combined with chemicals and sand) are injected at high pressure into rock formations to fracture them, allowing the gas to be released (IEA, 2012). At first shale gas was welcomed as an alternative to polluting coal power generation (Ridley, 2011) but, as with most mining operations, concern has been expressed regarding the safety and environmental impacts of the process of extracting shale gas. Groundwater contamination from the chemicals used in the fracking process, escaping of gases into aquifers, and polluted waste water tainting local streams are some of the environmental concerns (EU, 2011). The hydraulic fracturing process itself is seldom the source of contamination, but rather poor well construction, drilling practices or improper waste management (ASSAF, 2016).

There are several major impacts that may result from the fracking process:

1. Chemicals used in the fracking process may contaminate groundwater. The fracking fluid (also known as slick water) that is pumped into the fracking well typically contains 1 – 2 % chemical additives by volume but due to the large volumes of water required for the fracking process, significant amounts of these chemicals may be present and pose an environmental risk (ASSAF, 2016).
2. Gas may escape from poorly cased wells into underground aquifers (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013). There is however conflicting evidence on the

relationship between stray gas contamination and gas well drilling, and gas migration into groundwater can also occur naturally (Soeder, 2017).

3. Soil contamination may occur from surface leaks of fracking fluid (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013). Soils may also be contaminated by flowback water that typically contains salt, selenium, arsenic, NORMs (Natural Occurring Radioactive Materials) and deep formation bacteria (ASSAF, 2016).
4. Streams may be polluted by flowback water (contaminated with salt and radon) (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013; ASSAF, 2016).
5. The fracking process uses a large volume of water (an issue in a water scarce area such as the Karoo) (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013). The typical volume of water required for the hydraulic fracturing of a well is 6 800 m^3 but ranges from 3 800 – 26 3000 m^3 required per well (ASSAF, 2016).
6. Air pollution may occur from volatile contaminants (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013). The Strategic Environmental Assessment (SEA) for shale gas development in the Karoo found a high risk of air pollution, both to shale gas workers and to the climate (from unintended methane leaks) and a moderate risk to local communities but stated that the risks could be reduced to moderate or low through mitigation (Lochner et al., 2016).
7. During the drilling and fracking process, there will be noise pollution (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013). The Karoo is in general a quiet landscape (Lochner et al., 2016) and the SEA identified noise as a risk, particularly during the exploration phase of shale gas development and also increased noise from traffic on newly built roads.
8. The clearing of land has a negative impact on landscapes and biodiversity and can lead to soil erosion and sediment disposition (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013). Vegetation removal and soil disturbance resulting from shale gas development may also facilitate the spread of invasive plants (Barlow et al., 2017).
9. There will be damage to the amenity and landscape value of the area (Ridley, 2011;

Peduzzi & Harding Rohr Reis, 2013). The fragmentation of Karoo landscapes and transformation of the landscape to a more industrial one is likely without mitigation (Lochner et al., 2016). The SEA suggested the identification of scenic Karoo "hotspots" to avoid.

Unconventional gas extraction leaves a more significant 'footprint' on the landscape in comparison to conventional gas exploration. More wells are required for unconventional gas extraction and up to two hectares of land, apart from the road networks, is required for the placement of a fracking well (Belvalkar & Oyewole, 2010). Land must be cleared for drilling to take place, which impacts local biodiversity and may also result in soil erosion and sediment disposition (Adams et al., 2011). The presence of the fracking equipment is a temporary feature on the landscape, but the surface infrastructures such as well pads, roads and pipelines will have long term impacts on the landscape (Soeder, 2017). These more permanent structures may affect infiltration of rainfall into soil which in turn affects groundwater infiltration and aquifer recharge (Soeder, 2017). Although well sites may be restored after drilling ceases, this restoration often fails due to toxicity problems in the soil (Cook & Johnson, 2002).

An association between shale gas development and increased seismicity has been suggested. Peduzzi and Harding Rohr Reis (2013) stated that a link between fracking and earthquakes cannot be completely ruled out. For example, fifty small earthquakes occurred within 3.5 km of fracking sites in the Eola Field, Garvin County, Oklahoma, within seven hours of the first fracking explosion (Holland, 2011). The injection of fluids increases the pore pressure that may cause seismicity by affecting the stress on pre-existing faults (ASSAF, 2016). The actual fracking process is unlikely to trigger significant earthquakes as the pressure increase only affects a small volume of rock for a short time period (Ellsworth, 2013). Significant earthquakes are more likely to be induced by wastewater injection wells (McGarr, 2014; Hornbach et al., 2015; Rubinstein & Mahani, 2015; Witze, 2015). The disposal of saline flowback by injecting waters into deep, permeable geological formations is a common disposal method. High pumping rates of flowback waters is thought to have resulted in induced seismicity in Oklahoma, USA (ASSAF, 2016). The Karoo SEA found that the likelihood of fracking-related earthquakes

in the study area is very low (Lochner et al., 2016)

Fracking has been considered as a means to reduce greenhouse gas emissions (Burnham et al., 2011). However, further studies have refuted this claim (Howarth et al., 2011; Hultman et al., 2011; Wigley, 2011;) and its effect on greenhouse gas emissions is therefore still unresolved (Peduzzi & Harding Rohr Reis, 2013). In the Karoo Strategic Environmental Assessment (SEA), it was found that shale gas provides both an opportunity to reduce greenhouse gas emissions and a risk of increased greenhouse gas emissions (Lochner et al., 2016). Emissions are only likely to be reduced under the scenario of gas replacing coal as an energy source, rather than gas being used in addition to coal (Lochner et al., 2016).

The impact of fracking on water resources is a major concern. In a recent review paper by Vengosh et al. (2014) the risks to water resources from shale gas exploration were investigated. The four major risks to water resources were found to be:

1. Stray gas contamination, which is fugitive hydrocarbon gas contamination of shallow aquifers. Stray gas contamination may also lead to the salinization of shallow groundwater due to subsurface flow or leaking gas wells (Vengosh et al., 2014). Leakage of either gas or fracking chemicals into shallow aquifers is possible but the probability is low (Peduzzi & Harding Rohr Reis, 2013). The man-made fractures in the shale are usually several kilometres below the groundwater aquifers (Fisher & Warpinski, 2012). Despite these assurances, water contamination has been recorded – for example in Wyoming (EPA, 2011a), Pennsylvania and New York (Osborn et al., 2011). Should the well casings of the cement columns not be properly sealed, fracking fluids may leak into the water table. This can lead to explosions and contamination of the groundwater as reported from several sites in the USA (Myers, 2011; Zoback et al., 2010). King (2012) states however that neither the volumes of fracking fluid nor the pressure at which they are pumped is sufficient to contaminate shallow aquifers from below.
2. Spills, leaks or improper disposal of wastewater could contaminate both surface and shallow groundwater (Vengosh et al., 2014). Waste water is one of the biggest concerns in the process with about a third of the water that is pumped down the well

returning to the surface with the gas during production (flowback water). Depending on the shale type, the water exiting the well may have become saline. Plant germination and growth may be inhibited by saline water and the soil may be affected by excessive sodium, resulting in poor drainage and reduced crop yield (IEA, 2012). Pools that have been double-lined with heavy-duty polythene are used to collect the water once it has been separated from the gas. The water is either disposed of or re-used. Spills have been recorded from these pools, often from the improper lining of the pools (Gilliland, 2010). If the waste water is improperly disposed of, seepage may occur into groundwater (Peduzzi & Harding Rohr Reis, 2013). In the USA, the majority of flowback water is disposed of in deep wells. This may not be an option in South Africa, making the treatment of flowback water necessary (ASSAF, 2016). In South Africa, it is expected that the waste water produced from the fracking process will be of poor quality and have a high salinity (ASSAF, 2016). On-site surface spills or spills along transport networks were found to be the most likely source of water contamination during shale gas development in the Karoo (Lochner et al., 2016).

3. Toxic or radioactive elements may accumulate in the soil or stream sediments near disposal or spill sites (Vengosh et al., 2014). Naturally occurring toxic substances such as mercury, lead or arsenic may be more likely to migrate to the surface after the surrounding rocks have been fractured (EPA, 2011b; EU, 2011). Gas shales often have higher natural background radioactivity than other strata and these radioactive materials may be extracted in the flowback water. There are numerous sandstone lenses in the Karoo Basin's sedimentary formations that contain Natural Occurring Radioactive Materials (NORMs) (ASSAF, 2016).

4. Regional water shortages may result from over-extraction of water for the fracking process, particularly in water-scarce areas (Vengosh et al., 2014). Shale gas extraction is a water-heavy process; the amount of water required depends on the size of the exploited area, the well depth and the geological characteristics of the formation (Harper, 2008; Brownell, 2008). Some argue that this water consumption is not great in comparison to other water users. For instance, a golf course uses up the same amount of water in one shale gas well in only three weeks (Ridley, 2011). Excessive

water usage may negatively impact the local biodiversity and ecosystems, may lower the water table and as a result reduce the availability of water for local communities and agriculture (IEA, 2012). Depletion of aquifers due to coal bed methane extraction is well documented (IEA, 2012). Areas of water scarcity or those close to densely populated areas are not suggested for unconventional gas exploration. The safest sites would be those deep below the water table (IEA, 2012). In a review by Kondash and Vengosh (2015) the authors concluded that while fracking has increased water use and the production of wastewater in the USA, this volume of water use is still lower than other energy extraction methods.

The semi-arid and arid central and western regions of South Africa are largely dependent on groundwater as there are limited rivers or other surface sources of water (Botha et al., 1998). A large proportion of these arid areas are underlain by the Karoo Supergroup formations, making the aquifers of the main Karoo Basin a significant water source. The complex and unpredictable nature of Karoo aquifers affect their reliability as water sources.

A number of water sources for the fracking process have been suggested for the Karoo. Water could be sourced from shallow boreholes and piped to the fracking site under a low water requirement scenario. Should greater volumes of water be required, it could be sourced from a well field of Karoo boreholes, though there are doubts as to the feasibility of this option (ASSAF, 2016). However, utilizing shallow boreholes would impact the yield existing shallow boreholes in the Karoo. Mauter et al. (2014) suggested rather that water be sourced from deep saline aquifers. Weckman et al. (2012) report that sufficient quantities of saline water are available from productive aquifers in the Karoo. It may however be necessary to increase the volume of additives in the fracking fluid when using saline water and this would increase the production cost (ASSAF, 2016). As mentioned, flowback water can also be recycled to reduce the volume of water required for fracking.

The Strategic Environmental Assessment (Lochner et al., 2016) found that existing local resources in the Karoo do not have the capacity to supply water for shale gas development. This is due to low surface water availability and existing heavy demand on groundwater resources. Low and sporadic groundwater recharge in the area is also an

issue. The assessment did however propose brackish groundwater as a potential water source, but at a limited scale.

2.2 Fracking Fluid

Initially the industry refused to reveal the components of the fracking fluids, fueling the fears of environmentalists. Following pressure from industry regulators, shale gas companies are now divulging these details (Ridley, 2011). Although there are potentially hundreds of chemicals which could be used during fracking (Table 2.1) only a limited number are routinely used (FracFocus, 2014). The chemicals used are common in other industrial and domestic applications and are highly diluted (Ridley, 2011; ASSAF, 2016).

Proponents of unconventional gas exploration suggest numerous advantages over other fossil fuel energy sources:

1. Adding natural gas to the global resource base (World Energy Council, 2010).

2. Shorter production time over that of conventional gas (World Energy Council, 2010).

3. Cleaner energy resource (World Energy Council, 2010). Theoretically gas burns up to 50% cleaner than coal. South Africa's target of reducing its carbon footprint in line with the 2020 UN carbon-emission targets would be made more attainable if we had access to local shale gas (de Wit, 2011).

4. Availability of new drilling technologies (World Energy Council, 2010). Technology and drilling techniques have improved much over the past few years, with more casings being used in the wells and the use of more environmentally friendly chemicals in the fracking process (Vermeulen, 2012), reducing environmental impacts.

5. Improve supply security for those countries that import gas (World Energy Council, 2010) and improve the energy deficit should shale gas resources be sufficient (Vermeulen, 2012).

Table 2.1. Typical components of fracking fluid (Ridley, 2011; FracFocus, 2014; Range Resources (2014). The approximate contribution (%) is given where available.

Chemical	**%**	**Function**
Water	94	Carrier
Sand	5	Proppant
Polyacrylamide, Ethylene Glycol, Ammonium Acetate, Petroleum Distillate, Hydrotreated Light Petroleum Distillate, Methanol, Ethylene Glycol	>0.05	Friction reducer
Bromine, Methanol, Naphthalene, Dimethylaxazolidine, Trimethylaxazolidine, Gluteraldehyde, Quaternary Ammonium Chloride, Tetrakis Hydroxymethyl-Phosphonium Sulfate	>0.05	Antimicrobial agent
Hydrochloric Acid, Ethylene Glycol, Copolymer of Acrylamide and Sodium Acrylate, Sodium Polycarboxylate, Phosphonic Acid Salt	0.01	Scale inhibitor
Butanol, Ethylene Glycol Monobutyl Ether, Lauryl Sulfate, Ethanol, Naphthalene, Methanol, Isopropyl Alcohol, 2-Butoxyethanol		Surfactant (increase viscosity of the fracture fluid, product stabilizer, winterizing agent)
Ammonium Persulfate, Sodium Chloride, Magnesium Peroxide, Magnesium Oxide, Calcium Chloride		Breaker (stabilizes product and prevents delayed breakdown of gel)
Choline Chloride, Tetramethyl Ammonium Chloride, Sodium Chloride		Clay stabilizer
Isopropanol, Methanol, Formic Acid, Acetaldehyde		Corrosion inhibitor
Petroleum Distillate, Hydrotreated Light Petroleum Distillate, Potassium Metaborate, Triethanolamine Zirconate, Sodium Tetraborate, Boric Acid, Zirconium Complex, Borate Salts, Ethylene Glycol, Methanol		Crosslinker (carrier fluids, maintenance of fluid viscosity, product stabilizers, winterizing agents)
Guar Gum, Petroleum Distillate, Hydrotreated Light Petroleum Distillate, Methanol, Polysaccharide Blend, Ethylene Glycol		Gelling agent (thickens water to suspend sand, carrier fluid for guar gum, product stabilizer, winterizing agents)
Citric Acid, Acetic Acid, Thioglycolic Acid, Sodium Erythorbate		Iron control (prevention of precipitation of metal oxides)
Lauryl Sulfate, Isopropanol, Ethylene Glycol		Non-emulsifier
Sodium Hydroxide, Potassium Hydroxide, Acetic Acid, Sodium Carbonate, Potassium Carbonate		pH Adjusting Agent (maintain effectiveness of other components e.g. crosslinkers)
Hydrochloric Acid	>0.05	Cleans perforation, protects casing, prevent emulsions, iron chelator, dissolves minerals and initiates cracks in rock

6. Increase local employment. In the South African setting this is unlikely as most of the employment opportunities will be in the upstream activities and these skills are rare or non-existent in South Africa (ASSAF, 2016; Morkel & de Wit, 2017).

7. An assessment of the opportunities and risks associated with shale gas development in the Karoo found that the addition of natural gas to the current energy resource in South Africa would make the energy system more resilient, efficient, cheaper and more reliable (Lochner et al., 2016).

The World Energy Council (2010), however, admits that there are drawbacks to shale gas exploration. Affordability and costs are uncertain, uncertainty over the environmental acceptability of the technology; unknown rates of decline of reserves (impacting reserve estimates) and local opposition to fracking are some of the disadvantages listed.

2.3 Gas drilling steps

The basic shale gas drilling steps are:

1. Seismic exploration: Sound waves and 3D reconstruction is used to map underground rock formations to identify the depth and thickness of shales. The data may be collected by a desktop study of old data, aerial or ground surveys (Ridley, 2011).

2. Pad construction: A drilling rig platform is levelled and hard-cored (Ridley, 2011).

3. Vertical drilling: Up to 12 holes are drilled down into the shale using a small drilling derrick. The depth at which suitable shales occur ranges from 1 200 to 3 700 m below the surface (Ridley, 2011).

4. Horizontal drilling: A large drilling derrick (45 m high) is assembled at the site and is used to slant-drill each well into the shale for a maximum of 1 200 m in various directions. Gas sensors are used to ensure the drilling stays within the seam of shale. After 30 to 40 days the derrick is removed and the wellhead is capped (Ridley, 2011).

5. Fracking (fracturing): Small explosive charges are used to perforate the concrete casing of the horizontal pipe. Water mixed with sand is pumped through the

resulting holes at a pressure of 35 mpa which fractures the rock with hairline cracks up to 300 m from the horizontal pipe. The fracking stage takes between 3 and 10 days (Ridley, 2011).

6. Waste disposal: Water flowing back out of the well is collected in tanks and either re-used for fracking or desalinated and disposed of through sewerage systems (Ridley, 2011).
7. Production: Gas and small quantities of oil are collected on site by a 'Christmas tree' valve assembly and a set of small tanks. The collected gas flows through underground pipes to a compressor station. The compressor station serves a large number of wellheads on the site. The gas then flows onwards to trunk pipelines (Ridley, 2011).
8. Decommissioning: Should a shale layer not be encountered or if no gas is present in the shale, the well site will be decommissioned. Cement plugs will be placed in the reservoir and the well head removed (Golder Associates Africa, 2011).

2.4 Karoo Basin Geology

The Karoo Basin covers half of South Africa, an area of approximately 600 000 km^2. The basin is made up of mid-Paleozoic to Lower Jurassic sedimentary rock sequences that are 3 - 6 km thick (Linol et al., 2016). The Main Karoo Basin is a large erosional remnant of the Karoo Supergroup which was deposited 300 - 183 million years ago on Gondwanaland (Johnson et al., 1996). The Karoo Basin flanks the Cape Mountains to the south and in the north the sequences are intruded by dolerite sills and capped by basalts (Linol et al., 2016).

The controlling and shaping of the Karoo Basin can be attributed to four major geodynamic events: 1) the deposition of Karoo sediments and uplift of the Cape Fold Belt (Carboniferous-Triassic); 2) the extrusion of abundant Karoo basalts and dolerite intrusions (Jurassic); 3) kimberlite intrusions (mostly Cretaceous); and 4) the deposition of recent sediments (Cenozoic), uplift and the cessation of regional tectonics (Woodford & Chevallier, 2002). The Karoo Basin preserves several major lithostratigraphic units, 1 to 5 km thick, named the Dwyka, Ecca, Beaufort and Stormberg Groups, capped by a unit

of basaltic lavas (the Drakensberg Group) (Linol & de Wit, 2016). These strata act as caps for shale gas reservoirs, which may be found at a depth of between 1 400 and 1 500 m below surface (Chere et al., 2017). The strata may also isolate the overlying aquifers containing surface groundwater (Woodford & Chevallier, 2002).

The Karoo Supergroup at the base is formed by Dwyka tillites (Carboniferous) and then fossil-rich sediments of the Ecca and Beaufort Groups (Permian-Triassic). The intrusion of dolerite sills and dykes into these formations is the result of emplacement of the Karoo Large Igneous Province at 180 Ma (Meadows & Watkeys, 1999). Dolerite intrusions in the Karoo Basin consist of an interconnected network of dykes and sills (Woodford & Chevallier, 2002). These dolerite dykes are vertical discontinuities with higher permeability than the surrounding rock, allowing them to act as conduits for groundwater flow. Dolerite sills and ring-complexes are one of the most recognizable features of the Karoo landscape as they form near-circular outcrops.

2.5 Shale gas in the Karoo

Through re-analysis of borehole cores drilled by SOEKOR in the 1960s, the stratigraphy and structure of the southern Karoo Basin has been reconstructed. In the southern Karoo Basin, the Karoo Supergroup is 750 - 5540 m thick; 79 - 569 m of this sequence is made up of thick black shales of the Lower Ecca Group (Linol et al., 2016). These black shales are rich in organic matter and are potential shale gas reservoirs (Linol et al., 2016). Potential gas content of these shales is affected by the total organic carbon (TOC) content (a direct relationship exists between total organic carbon and gas content) and the thickness of the black shale (the majority of gas is produced from shale layers of between 90 and 180 m thick; Steyl et al., 2012). Total organic carbon contents in the Karoo that are comparable to those of producing shales in other countries (e.g. USA) are mostly found in the lower Ecca Group. Rowsell and De Swardt (1976) used the results of desorbed gas analysis using core samples from SOEKOR boreholes to delineate this shale gas potential distribution (Steyl et al., 2012). The Whitehill Formation most likely has the greatest dry gas generating potential due to this formation containing the highest total organic carbon content. Rowsell and De Swardt (1976) measured organic content values of between 3 and 7% here, but values over 14% have been recorded in other

areas of the Karoo (Cole & McLachlan, 1994). Research has shown that features favourable for the occurrence of shale gas occur in some regions within the southern Karoo Basin (Decker & Marot, 2012; Geel et al., 2013; Geel, 2014; Geel et al., 2015; Chere, 2015). The potential of sedimentary basins to harbour shale gas varies depending on the structure and thermal history of the basin. In the south of the Karoo where the basins flank the Cape Fold Mountains, the folding and faulting of the shales may have naturally “squeezed out” their hydrocarbons. If basins have been tectonically buried (as happens in the southern margin of the Karoo Basin), the excessive temperatures may have degraded the hydrocarbons, making them over-mature. Basins that have not been heated or have remained at shallow depths may never have generated thermogenic gases, which is likely along the western and northern margins of the Karoo Basin. This indicates the importance of ideal depositional and post-depositional conditions to form gas in shale-bearing basins. As such, only limited areas in the southern Karoo Basin in the central and eastern sections of the basin meet these conditions (ASSAF, 2016). Due to favourable porosity and permeability of the Karoo gas-shales and a shale layer thick and widespread enough to host shale gas reservoirs, the shale gas in the Karoo is potentially recoverable (ASSAF, 2016). Jackson et al. (2015) and DiGuilio and Jackson (2016) suggested avoiding shallow reserves i.e. 1 - 1.5 km below the surface, until fracking technologies and geological understanding of these deposits improve. If these shale gas reserves are avoided, a substantial portion of the initial shale gas exploitation target is eliminated (ASSAF, 2016).

Though the presence of shale gas in the Karoo Basin is not refuted, the size of the shale gas reserve is debated due to the limited knowledge of the 3-D shape of the basin (ASSAF, 2016; Chere et al., 2017). There have been a number of studies attempting to estimate the potential Karoo shale gas resource. Kuuskraa et al. (2011) estimated a 485 tcf (trillion cubic feet) reserve, which would make it the fourth largest reserve in the world. Subsequent estimates have been more conservative – 50 tcf (Decker & Marot, 2012), 72.5 tcf (Cole, 2014) and 19 – 23 tcf (Geel et al., 2015). In a recent study by de Kock et al. (2017), it was suggested that the lowest estimate of the existing resource estimates is the most realistic (13 tcf). However, Chere et al. (2017) estimated 10 – 50 tcf in the Lower

Ecca sequences and additional 100 – 400 tcf in the Upper Ecca sequences. While the exact size of the shale gas resource in the Karoo Basin is unknown, even at the lowest estimate it would still represent a large gas resource potential for South Africa (de Kock et al., 2017).

The potential of the Karoo for hydrocarbon reserves was first investigated by SOEKOR (South African National Oil and Gas Agency, Southern Oil Exploration Corporation) in the 1960s. They found deep gas-bearing formations, but their prime interest was in oil, and as such there were no further investigations. From 2008 onwards, as natural gas emerged as a globally significant resource, commercial interest in shale gas reserves in the Karoo was renewed. Bundu Oil and Gas, Falcon Oil and Gas and (Royal) Dutch Shell International were the first to apply for exploration rights in the southern Karoo. In 2011, a moratorium was placed by the South African government on exploration, following opposition from environmental organisations and local Karoo farmers and landowners. This moratorium was due to be lifted in September 2012, but to date the exploration rights have not yet been approved for the above applicants. In October 2013 new draft regulations for shale gas exploration in South Africa were published and the moratorium was extended by a further two years. The applicants were instructed to review their environmental management plans (EMPs) and resubmit them to the Petroleum Agency of South Africa (PASA) by February 2015. Bundu Oil and Gas and Falcon Oil and Gas submitted revised EMPs while and (Royal) Dutch Shell International did not (ASSAF, 2016; Morkel & de Wit, 2017). In March 2016 the South African government announced that shale gas exploration was expected to commence in 2017 (Morkel & de Wit, 2017).

2.6 Karoo flora and vegetation in the proposed fracking area

Phytosociology (phyto = plant; sociology = groupings of species) is a branch of vegetation science that describes plant assemblages or communities (Jörg, 2003; Dengler, 2017). Plant species co-occurrences are studied to describe compositional patterns and gradients within vegetation communities (Jörg, 2003). Species composition is used to delimit and characterize vegetation types (Dengler, 2017). Phytosociological classification, also known as syntaxonomy, uses degrees of floristic similarity to place vegetation units into a hierarchical system (Dengler, 2017).

Dengler et al. (2008) list three goals of classifying vegetation, which we will also use:

1. To enable communication about the vegetation by delimiting and naming parts of the vegetation continuum.
2. To allow the prediction of ecosystem attributes such as species composition, site conditions and ecological processes.
3. Use descriptions, maps and diagrams to represent multispecies co-occurrences.

2.6.1 Nama-Karoo Biome

Much of the proposed fracking area falls within the Nama-Karoo biome in the western half of South Africa. It is an extensive biome which is flanked by six others: Succulent Karoo, Desert, Savanna, Grassland, Albany Thicket and Fynbos (Mucina & Rutherford, 2006a).

The flora of the Nama-Karoo biome is not particularly rich and does not contain any centres of endemism (Van Wyk & Smith, 2001; Mucina & Rutherford, 2006a). The dominant families are Asteraceae, Fabaceae and Poaceae. In the south and west of the biome, the flora includes elements of the Succulent Karoo and Fynbos biomes (Aizoaceae and Asteraceae). Elements of the tropical summer rainfall floras are included in the northern and eastern sections of the biome (Acanthaceae, Capparaceae and Cucurbitaceae) as well as Poaceae and Fabaceae. The contribution of succulent genera (Aizoaceae, Crassulaceae, Euphorbiaceae and Apocynaceae) in terms of cover and species diversity decreases towards the north-west of the biome. However, cover and diversity of grasses increases in this region (Mucina & Rutherford, 2006a). Hilton-Taylor (1987) suggested that the flora of the Nama-Karoo appears to be a filtered subset of the flanking biomes.

Though floristic diversity is low and biome-level endemism is extremely low, the vegetation has a high diversity of life forms. The vegetation is dominated by dwarf shrubs that co-occur with grasses (C_3 and C_4), succulents, geophytes and annual forbs. Small trees are only found on rocky outcrops or along drainage lines (Mucina & Rutherford, 2006a).

2.6.2 Albany Thicket Biome

Thicket has long puzzled plant ecologists and biogeographers, and was only recognized as a Biome in its own right in the 1990s (Low & Rebelo, 1996). Originally described as Valley Bushveld, the Albany Thicket Biome has since been recognised as a biome, due to unique climate and growth forms as well as high regional levels of endemism (Robertson & Palmer, 2002b; Vlok & Euston-Brown, 2002). The biome has posed a challenge to researchers due to its various constituent vegetation types and wide variety of plant communities with differing structure and species composition (Hoare et al., 2006).

Thicket vegetation is characterized by dense formations of evergreen and weakly deciduous shrubs and low trees (maximum height of 5 m), often spiny and overgrown with vines. It is almost impenetrable in its natural state (Acocks, 1953; Everard, 1987). The flora of thicket is rich with approximately 20% of the species being endemic to the Biome (Vlok et al., 2003). There is an over-representation of range-restricted succulents and geophytes (Cowling et al., 2005), strongly associated with a limited number of families, namely Aizoaceae, Asphodelaceae, Crassulaceae, Euphorbiaceae and Apocynaceae (Cowling, 1983; Cowling and Holmes 1991; Hoffman & Cowling 1991; Johnson et al., 1999; Vlok et al., 2003). In contrast, most of the shrub and tree species have relatively wide distributions.

2.6.3 Grassland Biome

The Grassland biome is located centrally in Southern Africa. Grassland vegetation is generally uniform in structure but due to the wide environmental variation across the biome there is variation in the floristic composition, vegetation dynamics and ecosystem functioning (O'Connor & Bredenkamp, 1997). The vegetation is herbaceous, short and simple and is dominated by graminoids (especially the family Poaceae). Grasslands are divided in to 'sweetveld' and 'sourveld' based on the rainfall regime and species composition. 'Sourveld' is dominated by andropogonoid grasses whereas 'sweetveld' is dominated by chloridoid grasses (Mucina et al., 2006b).

The Grassland biome has a number of endemic grass taxa (from the subfamilies Arundinoideae and Pooideae (Steenkamp et al., 2002) and a high proportion of endemic

orchid taxa (Linder et al., 2005). Local species richness is high even though there is low richness of growth forms (Mucina et al., 2006b).

2.7 The effects of fracking fluids on plants

Esterhuyse et al. (2016) investigated the biophysical and socio-economic effects that unconventional gas extraction may have specifically in South Africa (Table 2.2).

Table 2.2. Potential impacts of various phases of shale gas extraction on the vegetation of South Africa (Esterhuyse et al., 2016).

Phase	**Impact**	**Reference**
Exploration	Surface spills of hazardous materials on to the vegetation	Adams, 2011
	Clearance of land for well pads and roads may cause invasive species to encroach, habitat fragmentation and biodiversity loss	Milton & Dean, 2012; Kiviat, 2013; Balow et al., 2017
Extraction	Spills of hazardous materials may occur on a larger scale than during the exploration phase	De Rijke et al., 2013
	Vegetation may die back due to water pollution in the rooting zone (resulting from the migration of contaminated ground or surface water)	Steyl et al., 2012
Post extraction	Poor maintenance of infrastructure or invasive controls may cause continued habitat fragmentation	Steyl et al., 2012 O'Conner, Kuyler, 2006 & Kiviat, 2013
	Continued loss of ecosystem services and increased encroachment of invasive species may lead to continued loss of biodiversity	Northrup & Wittemyer, 2013 Lovegrove, 1993
	More access roads could enable trade of succulents previously not accessible	Lovegrove, 1993

The Strategic Environmental Assessment highlighted the high levels of biodiversity and the presence of sensitive and unique ecosystems in the proposed fracking footprint. The

Karoo is particularly sensitive to disturbance and recovery and rehabilitation is slow and often unsuccessful (Holness et al., 2016). The SEA found that fragmentation of the landscape through the construction of roads, pipelines and powerlines is of major concern, potentially resulting in loss of connectivity and the disruption of ecological processes. Mitigation of environmental impacts of shale gas development at a landscape level was suggested as was rigorous monitoring to detect unforeseen and cumulative impacts of shale gas development on biodiversity (Holness et al., 2016). Species with restricted ranges that occur within shale gas development areas are particularly vulnerable to the environmental impacts of fracking (Gillen & Kiviat, 2012). The authors reviewed the ecological requirements of 15 species with small geographic ranges and a large overlap with the Marcellus and Utica shale-gas region in the USA. Most species studied were found to be sensitive to landscape fragmentation and water quality degradation.

To date, only one published study has investigated the effect of applying fracking fluids to plants (Adams, 2011). In this, 303 000 L of fracking fluid sourced from a local gas well was applied directly to 0.2 ha of vegetation in the Fernow Experimental Forest in West Virginia, USA. Several states in the USA consider land application of waste fracking fluids as an acceptable form of disposal, although it is regulated through a permit system (Adams, 2011). The hydrofracturing fluid was applied to a deciduous forest stand in an area of mixed hardwood forest in the experimental area. Ground vegetation was worst affected with damage and mortality evident soon after application. Almost all the ground cover had died within just a few days. Overstory trees suffered premature leaf drop approximately 10 days after application. All tree species showed symptoms of damage and 56% of all the trees in the fluid application area were dead within two years of the treatment. The author does however state that due to the small area over which the fracking fluid was applied, ‘loading’ occurred, i.e. a very large dose was applied over a small area (Adams, 2011). As a result, while this study provides some insight into the reaction of plants to direct contact with high doses of fracking fluid, it does not provide insight as to the possible effects of surface spills that may occur over large areas or the

effect of groundwater contamination on vegetation, as the fracking fluid would be greatly diluted in these instances.

In a similar study by Takaki and Wolf (2011), the effect of applying drilling mud to plants was investigated. In addition to the production of drilling fluids, natural gas extraction also generates drilling mud that has high salt concentrations. Sudangrass (*Sorghum sudanense* (Piper) Stapf) and bermudagrass (*Cynodon dactylon* L.) were grown in a mixture of soil and drilling mud. In both species, root and shoot biomass production was significantly reduced by the addition of the drilling mud. The authors found that Ca and Mg levels of the soil increased with the addition of drilling mud and concluded that increased salinity was the most likely cause of the decreased plant growth (Takaki & Wolf, 2011).

Wolf and Brye (2012) hypothesized that plant growth in drilling-fluid-contaminated soil could be increased by deep plowing and the addition of nutrients. Bermudagrass sprigs were planted in drilling-fluid-contaminated soil collected from a natural gas drilling site in Arkansas. During the nine week study, 11 of the 64 plants died, despite the addition of various fertilizers (Wolf & Brye, 2012). The study concluded that mortality observed was as a result of increased soil salinity due to excess application of drilling fluids (Wolf & Brye, 2012). Increased soil salinity has been widely shown to inhibit seed germination and negatively affect plant growth and survivorship (e.g. Brady & Weil, 2002; Zvomuya et al., 2009).

2.7.1 Phreatophytes

Phreatophytes are typically riparian plants that obtain water directly from the saturated zone of the soil (Le Maitre et al., 1999). Obligate phreatophytes are dependent on access to groundwater whereas facultative phreatophytes are able to use available shallow soil water while their deep root systems have access to groundwater (Le Maitre et al., 1999). Phreatophytes often grow where precipitation is insufficient for long-term survival and access to groundwater in that specific environment is essential for survival (Naumberg et al., 2005).

The significance of deep rooting systems and their impacts on ecosystem processes was

until relatively recently poorly understood. Deep-rooting plants have since been shown to play an important role in ecosystem water fluxes as well as carbon and nutrient cycling (Nepstad et al., 1994; Fisher et al., 1994; Richter & Markewitz, 1995; Trumbore et al., 1995; Dawson & Pate, 1996; Schulze et al., 1996).

In arid and semiarid regions, groundwater is also an important water source for plants (Naumberg et al., 2005). Plants occurring in arid zones or areas with long dry seasons were shown to have the deepest rooting habits (Canadell et al., 2006). The roots of woody species have been recorded at exceptional depth in the soil. For instance, *Vachellia erioloba* roots were found at 60 m and roots of *Boscia albitrunca* at 68 m below the surface (Jennings, 1974). Tap roots, and to a lesser extent, sinker roots and obliquely descending lateral roots are the most common root types found in phreatophytes, enabling them to access deep soil layers (Schulze et al., 1996). Because of their competitive advantage, phreatophytes occasionally become invasive in arid regions. Some examples include *Tamarix* spp. (trees), *Nicotiana glauca* (shrub), *Salsola kali* (annual forb) and *Bromus* spp. (grasses) (Loope et al., 1988).

In arid and semi-arid regions, groundwater is an important water source for vegetation and humans. Shallow water tables in these regions support a greater density of plant life by the provision of additional water for plant growth (Naumberg et al., 2005). Groundwater extraction for human needs may decrease groundwater levels, negatively affecting groundwater-dependant vegetation. Gradual plant community changes may result, or in extreme cases, there may be extensive mortality of vegetation (Groom et al., 2000). An increase in groundwater level may also negatively affect vegetation; anoxic conditions resulting from flooding also lead to water stress. Fractured aquifers occur across approximately 90% of the surface of South Africa (Vegter, 1995) and the quality of the water recharging these aquifers is influenced by plant decomposition dynamics and nutrient uptake (Le Maitre et al., 1999). Due to their direct use of groundwater as a water source, the foliar chemistry of phreatophytes has been shown to be a reliable indicator of groundwater contamination (Erdman & Christensen, 2000).

Applicable lessons to be learnt for the Karoo may come from studies on Acid Mine Drainage. Acid Mine Drainage is associated with coal and gold deposits in South Africa

(Weiersbye & Witkowski, 2007). When pyrite comes into contact with oxygenated water oxidation occurs, producing sulphuric acid, ferrous sulphate and ferric hydroxide. The extensive fragmentation of the rock mass during mining dramatically increases the rate of acid production. As a result, large quantities of acidic water are released into groundwater and consequently streams and rivers. Should there be aluminium or heavy metals present in the polluted area, the acidic water increases the solubility of these elements. This results in water of varying degrees of toxicity; dilution and reaction with river sediments or minerals in soils may neutralize the water, but constituents such as sulphates that have high solubility remain in the water (McCarthy, 2011). Furthermore, acid mine drainage water generally has elevated concentrations of calcium sulphate and various chlorides (Weiersbye & Witkowski, 2007). Fracking fluids may also contain chlorides (Quaternary Ammonium Chloride, Sodium Chloride, Calcium Chloride, Choline Chloride, Tetramethyl Ammonium Chloride, see Table 2.1). Although the effect of fracking fluids on vegetation has not been extensively studied, a report by Weiersbye and Witkowski (2007) investigated the effects of acid mine drainage on vegetation. Regeneration of both shallow and deep-rooted species was shown to be severely impaired by exposure to waste water and the viability and germination of seeds was also compromised. A high proportion of the exposed seeds had lethal developmental abnormalities (Weiersbye & Witkowski, 2007). Contaminated groundwater could therefore have a major impact on the germination, regeneration, growth and survivorship of phreatophytes (and other life forms) in the Karoo.

CHAPTER 3 DIVERSITY AND PHYTOSOCIOLOGY OF THE VEGETATION IN THE PROPOSED KAROO FRACKING FOOTPRINT.

3.1 Introduction

The energy shortfall in South Africa has encouraged the search for new energy sources. Over the last decade this search has been focused on the availability and feasibility of extracting shale gas from Karoo type formations (Steyl et al., 2012; ASSAF, 2016; Lochner et al., 2016). A number of companies have applied for exploration rights to investigate shale gas in the Karoo in six of the nine South African provinces. This study focuses on the Shell exploration areas in the western parts of the Eastern Cape Karoo (Fig. 3.1).

The Eastern Cape is the meeting point of five phytochoria as described by White (1983) - the Cape Region, the Karoo-Namib Region, the Maputaland-Pondoland Regional Mosaic, the Afromontane Region and the Kalahari-Highveld Regional Transition Zone - resulting in a complex and transitional flora (Cowling, 1983; Cowling & Campbell, 1983).

The environment is physically represented *inter alia* in the vegetation and as a result changes in environment are observed in the vegetation (Kent, 2012). This makes assessment of the vegetation one of the most widely used tools for interpreting and understanding complex ecosystems (Doing, 1970).

Anthropogenic disturbances such as urbanization, mining, deforestation and commercial farming are responsible for many environmental changes, including biodiversity loss. The clearing of land for the construction of fracking well pads and roads connecting them may result in the loss of local Karoo species. Species loss affects ecosystem processes (Pimm et al., 1995) and its prevention is critical for the sustaining of ecosystem services (Carpenter et al., 2009). There have been many studies that indicate a positive relationship between biodiversity and ecosystem functioning (e.g. Hooper et al., 2005; Balvanera et al., 2006; Cardinale et al., 2006). Biodiverse environments have been shown to maintain and increase the resilience of communities to degradation or disturbance (Reid et al., 2009). The concept of resilience was originally described by Holling (1973) as “the capacity of an ecosystem to tolerate disturbance without collapsing into a

qualitatively different state that is controlled by a different set of processes". More recently Chapin et al. (2009) defined natural system resilience as "that property that enables ecosystems to absorb both expected and unforeseen change". Though diversity is important to maintain ecosystem services, higher species richness in a community does not necessarily imply greater resilience in that community (Elmqvist et al., 2003). Even though the relationship between diversity and stability of an ecosystem is neither linear nor random (Elmqvist et al., 2003), biodiversity does appear to play a substantial role in resilience (Peterson et al., 1998). The diversity of functional groups of species are contributing factors in this role (Walker, 1992; Walker, 1997; Norberg et al., 2001). Although not a strictly linear relationship, some authors (Tilman & Downing, 1994; Tilman et al., 1996) have demonstrated that the efficiency and stability of some ecosystem functions are increased by an increase in species number. This can be explained by the myriad of ecological functions that different species may perform, including regulation of biogeochemical cycles, alteration of disturbance regimes, modification of the physical environment or regulation of ecological processes through predation, parasitism, pollination or seed dispersal (Gunderson, 2000). Tilman et al. (1996) stated that each species can only perform a certain number of these above-mentioned functions and as a result increasing the species richness should also increase the functional diversity of the community, which in turn would increase its ecological stability.

In terms of conservation, both biodiversity pattern and process should be conserved. "Pattern" is the structure and composition of biodiversity, including genetic variability, and the number, spatial and temporal distribution of species, communities, ecosystems and landscapes. The 'process' element is the interaction between populations, species, and communities allowing the persistence of biodiversity in a landscape. Ecosystem services are underpinned by both pattern and process, highlighting the importance of conserving both elements (Brownlie, 2005). This is particularly important when considering the landscape fragmentation that would result from the construction of well pads for fracking. Fragmentation often significantly changes species richness (MacArthur & Wilson, 1967; Diamond, 1975; Fahrig, 2003; Marcantonio et al., 2013). Loss of habitat changes the amount of habitat as well as the configuration of the habitat. Responses at the population,

community and ecosystem levels may include decreased nutrient retention, decreased movement among fragments (important for animals) and reduced species richness across taxonomic groups (Haddad et al., 2015). As biodiversity and ecosystem functioning are linked, fragmentation affects both – i.e. functions may be lost should biodiversity be lost (Kiviat, 2013; Haddad et al., 2015). The construction of roads between well pads will fragment the Karoo habitat. Roads divide natural habitats into “islands” and these islands will be exposed to “edge effects”. These edges may change the biotic and abiotic conditions affecting the organisms in the islands (Marcantonio et al., 2013). Road edges have been shown to contain few locally rare species and even if the plant species richness might be high, the vegetation is often dominated by exotics and species tolerant to disturbance (Forman & Alexander, 1998; Tyser & Worley, 1992). Large portions of the South Coast Renosterveld have been transformed by agricultural activities, resulting in small fragments of this vegetation type scattered amongst agricultural lands. Kemper et al. (1999) found that invasive species, especially graminoid invasives, increased in the smaller fragments when compared to larger, intact patches of South Coast Renosterveld. The reproductive success of plants is also affected by habitat fragmentation. Donaldson et al. (2002) found that small isolated fragments of Renosterveld were more prone to pollination failure than large or well-connected fragments. The grassland biome in South Africa is particularly prone to transformation from forestry. Armstrong et al. (1998) reported extinctions and near –extinctions of plants and animal species in the Midlands Mistbelt Grassland as a result of fragmentation due to commercial tree farming.

In South Africa, species’ risk of extinction is measured using the International Union for the Conservation of Nature’s (IUCN) Red List (Raimondo, 2011). The Red List categories used in South Africa vary slightly from those used on the current IUCN Red List (Raimondo, 2011), but are based on the results of regional and national assessments in South Africa. The Red List categories are Extinct (EX), Extinct in the Wild (EW), Regionally Extinct (RE), Possibly Extinct (CR PE), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Critically Rare, Rare, Least Concern, Data Deficient - Insufficient Information (DDD), Data Deficient - Taxonomically Problematic (DDT) and Not Evaluated (NE) (SANBI, 2017). Species that face a high risk

of extinction are considered threatened species, including those species in the Critically Endangered, Endangered or Vulnerable categories. Species with high conservation value are those that are important for preserving the high floristic diversity in South Africa (often referred to as Species of Conservation Concern or SCCs). Red Listed species (Extinct in the Wild (EW), Regionally Extinct (RE), Near Threatened (NT), Critically Rare, Rare, Declining and Data Deficient - Insufficient Information (DDD) categories) as well as protected species (SANBI, 2017). Keller and Bollman (2004) define Species of Conservation Concern as those that conservation efforts would benefit the most.

Veld (veld = open rural landscape) degradation can affect species diversity and composition. Hoffman et al. (1999b) listed the most important types of veld degradation as change in veld condition (loss of vegetation cover and change in species composition), bush encroachment, alien plant infestation and deforestation.

Bush encroachment is particularly problematic in the sub-humid areas of South Africa where it may either be the invasion of woody elements into grasslands or invasion of woodlands into savannas – resulting in an increase in woody biomass (Meadows & Hoffman, 2002). Some of the negative impacts of bush encroachment include decrease in species richness (Báez & Collins, 2008; Knapp et al., 2008), reduction in grazing capacity, and reduction of the economic viability of rangelands (Smit et al., 1999).

Infestation by alien plants causes significant changes in catchment hydrology and may also result in the loss of diversity (Meadows & Hoffman, 2002).

Unlike bush encroachment, deforestation is the loss of woody elements in the veld. This is a relatively recent environmental problem in South Africa (Meadows & Hoffman, 2002).

One of the biggest causes of veld degradation, particularly in South Africa, is herbivory by domestic animals (Van Auken, 2000). The responses of vegetation to grazing are mixed. In a study by Rutherford et al. (2012a) the canopy cover and height of woody shrubs were shown to increase with heavy grazing whereas cover of graminoids decreased. These authors reported that species richness was maintained but species diversity and evenness was increased by grazing. They hypothesized that this was caused by the suppression of dominant species. Their study sites in the Nama-Karoo

showed significant increases in species richness, possibly indicating that arid species may have a higher resistance to grazing. Increased species richness with heavy grazing has also been reported in Nama-Karoo vegetation in Namibia (Dreber & Esler, 2011). Todd & Hoffmann (1999) suggested that an increase in species richness with grazing may be as a result of reduced competition. Species diversity may also decrease with increased grazing intensity, as was found in North American (Milchunas et al., 1988 & 1998) and southern Australian grasslands (Dorrough et al., 2007). Grazing and trampling by livestock was found to result in an increase in species diversity by Todd (2006). Grazing of palatable species often causes the vegetation to shift towards a non-palatable dominated assemblage of plants, including toxic and spinescent woody plants (Westoby et al., 1989; Milton & Hoffman, 1994). Todd and Hoffmann (1999) found that the responses of plants to grazing differed for different growth forms. Growth forms likely to increase due to grazing included annuals and geophytes and those likely to decrease included large shrubs and leaf succulents.

In savannas livestock-induced degradation causes shrub encroachment, whereas in subtropical thickets heavy grazing causes the loss of the shrub canopy (O'Connor, 1994; Higgins et al., 1999; Lechmere-Oertel et al., 2005a). Closed canopy thicket that has been heavily browsed by goats is transformed into scattered clumps of thicket in a matrix of ephemeral herbs (Hoffman & Cowling, 1990; Stuart-Hill, 1992; Moolman & Cowling, 1994; Lechmere-Oertel et al., 2005b, 2005a). In South Africa, subtropical thickets are one of the most extensively livestock-degraded ecosystems (Hoffman & Cowling, 1990; Kerley et al., 1995; Mills & Fey, 2004). Subtropical thicket is more vulnerable to browsing by domestic goats than by indigenous herbivores (Stuart-Hill, 1992). Forms of thicket dominated by spekboom *Portulacaria afra* are particularly vulnerable, as spekboom does not regenerate once browsing ceases and may be completely eliminated in severely degraded areas (Lechmere-Oertel et al., 2005a). Spekboom thicket provides a number of valuable ecosystem services such as carbon sequestration and forage for domestic and indigenous herbivores (Lechmere-Oertel et al., 2005a) and as such, degradation of these thickets is particularly detrimental. In a study by Rutherford et al. (2012b) heavy grazing and browsing in subtropical thicket was shown to result in a decrease in species

richness. Species turnover and beta diversity were high in degraded areas where thicket species were mostly replaced by weedy annual grasses and alien forbs.

The aim of this chapter is to describe the vegetation of the major vegetation types within the proposed fracking footprint (Fig. 3.3). Emphasis is placed on Species of Conservation Concern and endemic taxa in the vegetation types as these are likely to be threatened by shale gas development. Furthermore, several of the Karoo vegetation types have been poorly researched, and their affiliations are elucidated. In particular, differences between the plant community composition and soil conditions of conserved and degraded sites are considered, as this provides a clue as to what the effects of fracking will be on the vegetation. In particular, the relationship between the core Thicket elements (Camdebo and Eastern Cape Escarpment Thicket) and Karoo thicket elements (Sundays and Great Fish thicket) are considered, as Thicket vegetation is likely to be less affected by fracking than the less resilient Karoo vegetation.

3.2 Study Area

The study area, based on the AEON-ESSRI Nelson Mandela University Shale Gas Baseline Research Program study area covers a large area (47 082 km^2) in the Eastern Cape section of the Karoo (Fig. 3.1). The study area roughly coincides with the three precincts of the Shell exploration areas. The biomes included in the study area are the Nama-Karoo, Grassland and Albany Thicket (Fig. 3.2). Figure 3.3 shows the vegetation types included in the entire study AEON-ESSRI area. In this study, eleven of those vegetation types were included.

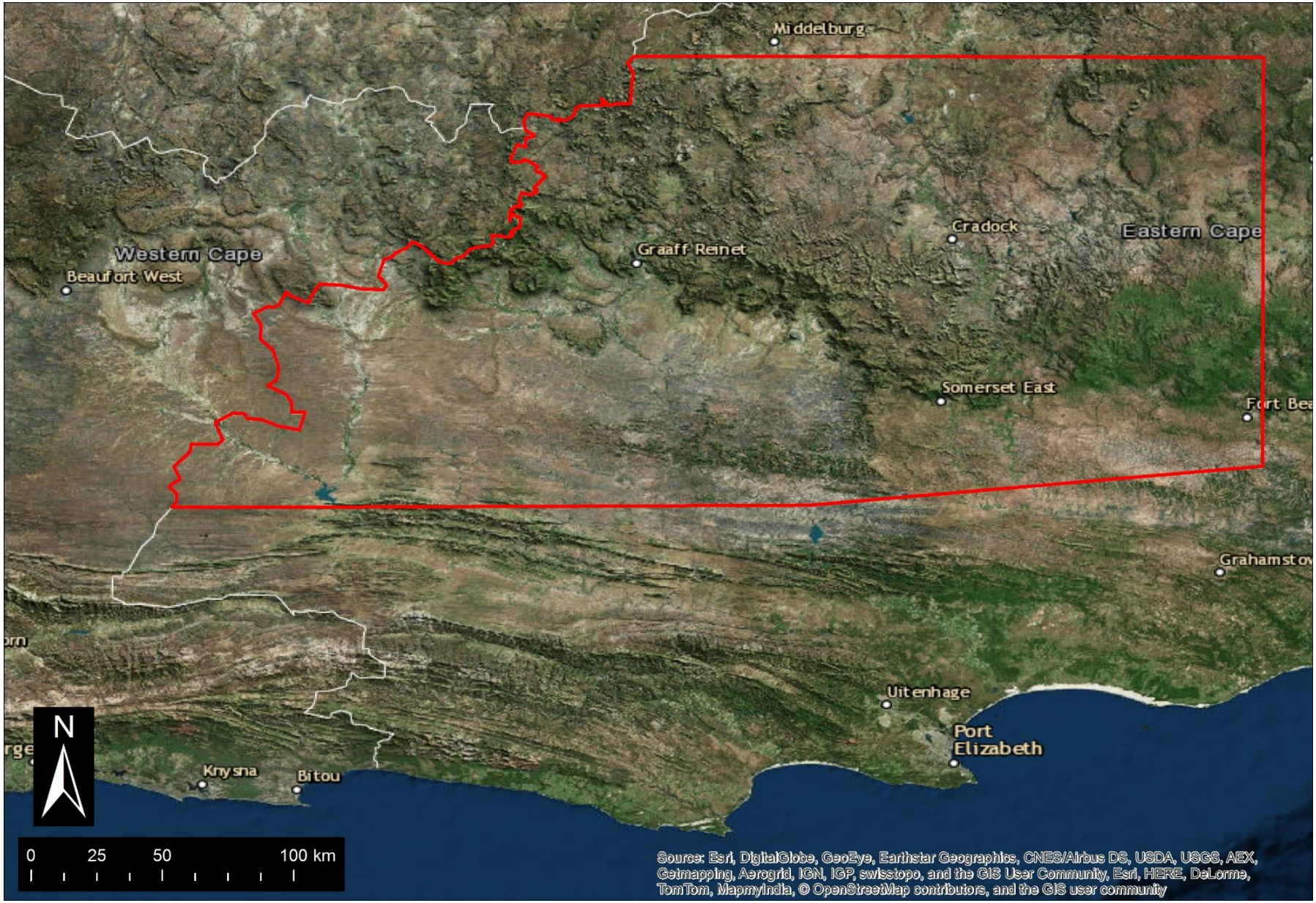


Figure 3.1. The AEON-ESSRI Nelson Mandela University Shale Gas Baseline Research Program study area (47 082 km^2).

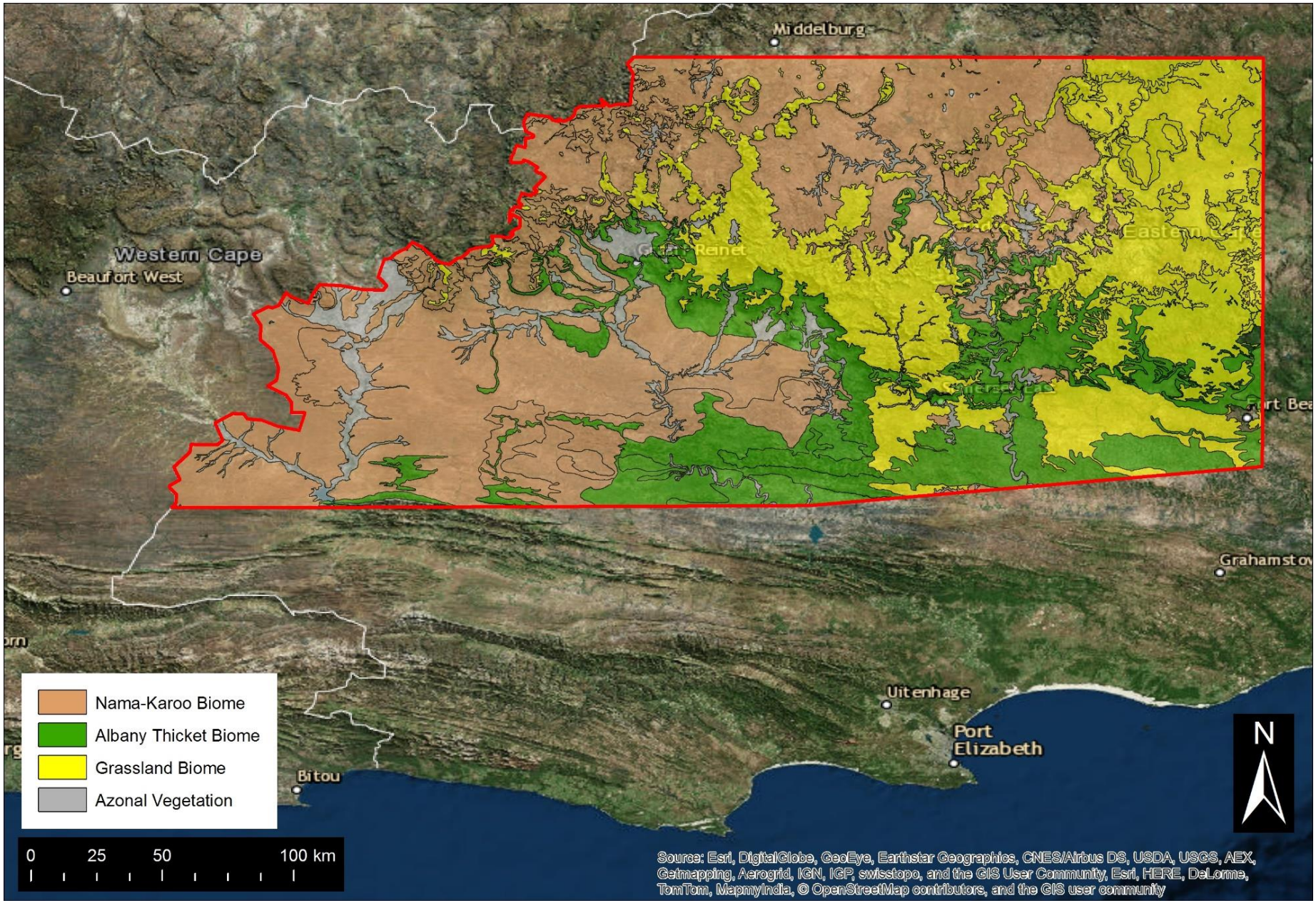


Figure 3.2. Biomes included in the AEON-ESSRI Baseline study area (within the red boundary line; area = 47 082 km^2).

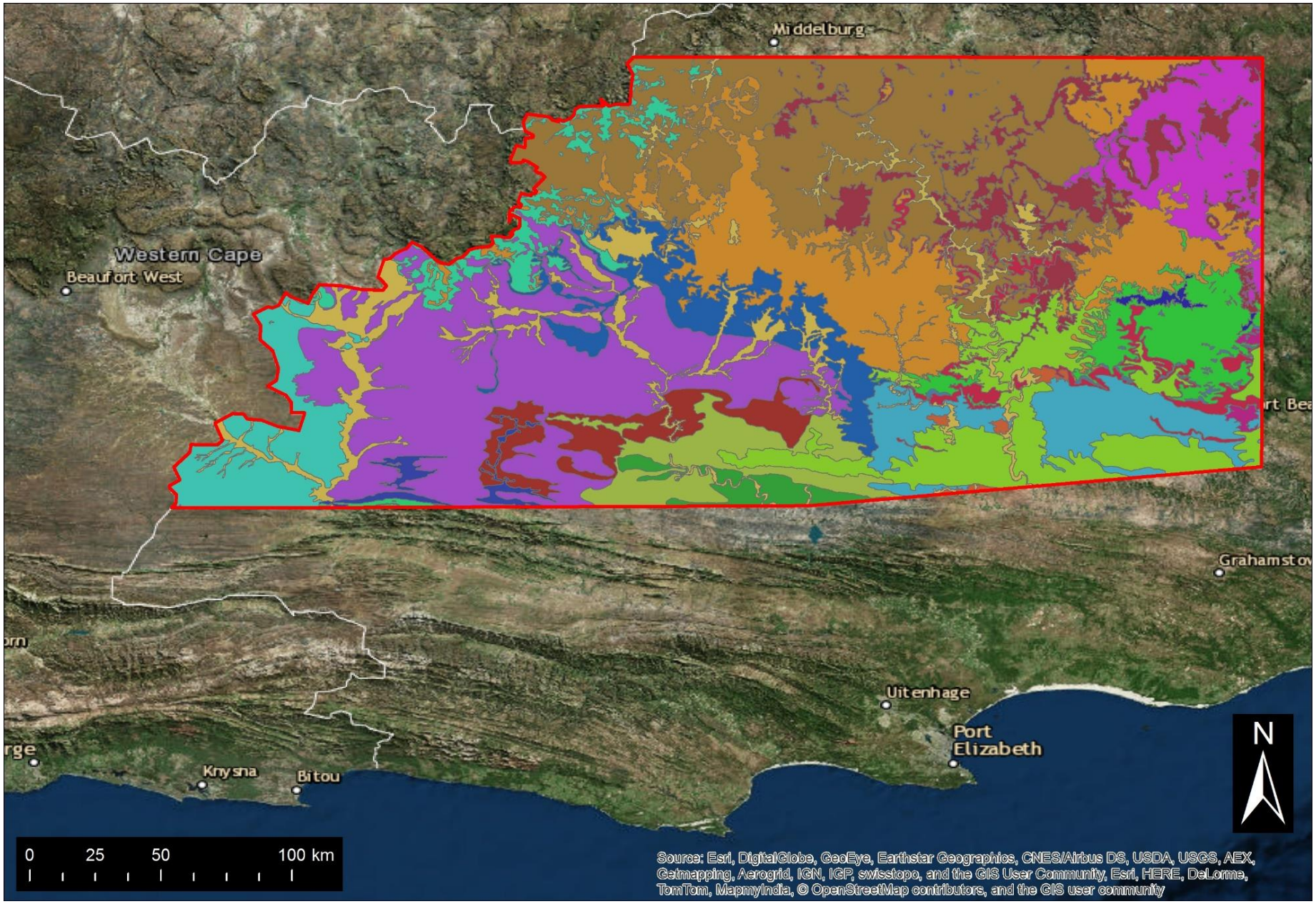


Figure 3.3. Vegetation types included in the AEON-ESSRI Baseline study area. Legend on next page.


Albany Broken Veld
Amathole Mistbelt Grassland
Amathole Montane Grassland
Bedford Dry Grassland
Besemkaree Koppies Shrubland
Bhisho Thornveld
Camdebo Escarpment Thicket
Cape Inland Salt Pans
Eastern Cape Escarpment Thicket
Eastern Lower Karoo
Eastern Temperate Freshwater Wetlands
Eastern Upper Karoo
Gamka Karoo
Great Fish Thicket
Groot Thicket
Grootrivier Quartzite Fynbos
Highveld Salt Pans
Karoo Escarpment Grassland
Lower Karoo Gwarrieveld
Prince Albert Succulent Karoo
Queenstown Thornveld
Southern Karoo Riviere
Southern Mistbelt Forest
Sundays Noorsveld
Sundays Thicket
Tarkastad Montane Shrubland
Tsomo Grassland
Uniondale Shale Renosterveld
Upper Karoo Hardeveld

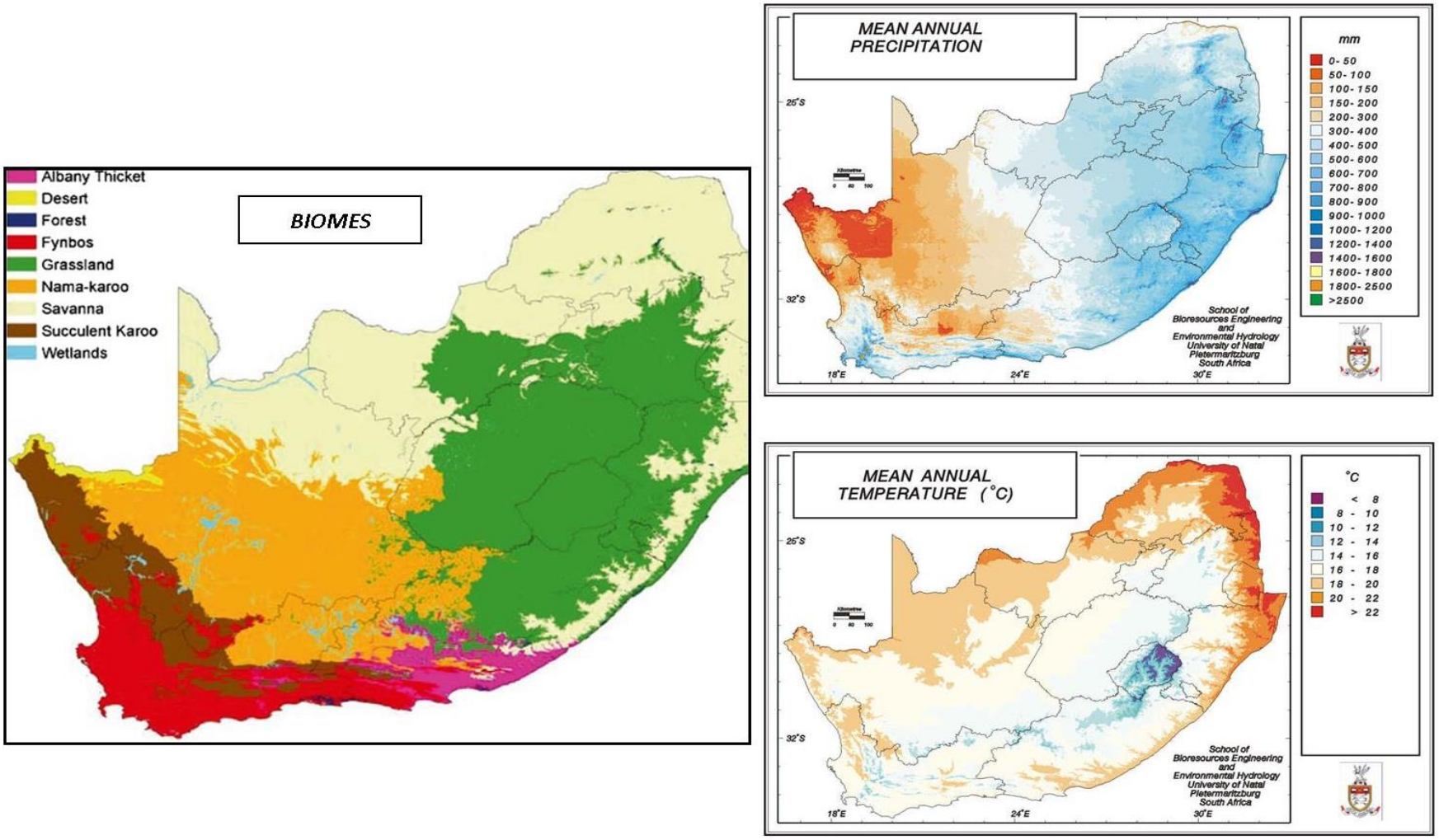


Figure 3.4. The biomes of South Africa, Lesotho and Swaziland (Driver et al., 2005) and the mean annual rainfall and mean annual temperature of the biomes (Shulze, 2007).

3.3 Nama-Karoo Biome

The Nama-Karoo is a large, landlocked biome (Mucina et al., 2006a) flanked by six other biomes (Succulent Karoo, Desert, Savanna, Grassland, Albany Thicket and Fynbos; Fig. 3.4). It is found on the central plateau of the western half of South Africa and extends into Namibia.

The climate of the biome is arid and continental with mostly summer rain (Mucina et al., 2006a). Summers are hot and winters are cold (Fig. 3.4), with frost being common in most of the biome.

In terms of geology, the Nama Karoo is underlain by a series of sedimentary and igneous rocks (Mucina et al., 2006a): the Cape Supergroup, followed by Dwyka tillites then sediments of the Karoo Supergroup including Ecca and Beaufort Groups. The Karoo sedimentary rocks are also intruded by dolerite sills and dykes. Soils in the biome are base-rich, skeletal and weakly structured in general.

The origins of the Karoo flora are not well understood (Mucina et al., 2006a). It has been described as transitional by Werger (1978a, b) who suggested Sudano-Zambezian affinities towards the northern and eastern boundaries of the biome and Cape affinities towards the southwest of the biome. Coetzee (1978) proposed that karroid plant communities are likely to have developed due to an increase in aridity during the late Miocene. During the Cretaceous period the Nama-Karoo plateau was raised by continental uplift (Tinker et al., 2008). This would have resulted in cooling and a rainshadow effect that may have resulted in asteraceous shrublands replacing dry woodlands (Mucina et al., 2006a). In the absence of a well-dated and continuous fossil record, the timing of the evolution of the Nama-Karoo vegetation cannot be clearly established (Mucina et al., 2006a). The Quaternary period saw widespread fluctuations in climate in the Nama-Karoo region. Vegetation in the area has been shown to have alternated between grassland and arid Karoo vegetation in response to this climatic variation (Van Zinderen Bakker, 1989; Grün et al., 1996). This alternation of states may have resulted in the expansion of the Karoo vegetation in “pulses” to cover an area larger than its present range (Mucina et al., 2006a). Sensitivity to changes in quantity or seasonality of precipitation appears to have been the driving force in shifts between grass and shrubs during the Holocene period (Bousman et al., 1988; Meadows & Watkeys, 1999). Increased stock farming was

proposed to have caused an increase in shrub elements at the expense of grass elements during the 20th century (Acocks, 1953; Acocks, 1988). This has been disputed by Bousman and Scott (1994) in a pollen composition study that suggested the spread of Asteraceae started before the intensification of stock farming. According to available pollen records, even though the Asteraceae increased during certain events in the last 200 000 years the vegetation has generally remained grassy rather than karroid (Mucina et al., 2006a).

The flora of the Nama-Karoo is neither species rich nor does it contain any centres of endemism (Cowling et al., 1998; Van Wyk & Smith, 2001). Local endemism is very low in general, with the Upper Karoo Hardeveld having the highest concentration of endemics (Mucina et al., 2006a). According to Cowling and Hilton-Taylor (1999) the species richness at the biome level may be affected by the reliability of rainfall and the length of the rainfall quality gradient. Rapid diversification such as is seen in the Succulent Karoo may have been prevented in the Nama-Karoo by high extinction rates as a result of rainfall unreliability.

The dominant families in the biome are the Asteraceae, Fabaceae and Poaceae (Mucina et al., 2006a). Elements of the flanking biomes dominate in areas close to these biomes. Succulent Karoo and Fynbos elements are included in the south and west of the Nama-Karoo and elements of the tropical summer-rainfall floras are included in the north and east. In the north and east the cover of grasses increases as the number of succulent genera decreases. The flow of species between the Nama-Karoo and the surrounding biomes indicates that the Nama-Karoo flora is subset of the floras of the flanking biomes (Hilton-Taylor, 1987). There have been various biogeographical and vegetation subdivisions proposed for the Nama-Karoo biome (Table 3.1).

Table 3.1. The various vegetation subdivisions proposed for the Nama-Karoo biome in chronological order.

Authors	**Proposed subdivions**
Acocks (1953)	Five of Acocks' veld types fall almost entirely in the biome (Central Upper Karoo, Central Lower Karoo, Orange River Broken Veld, Arid Karoo and False Arid Karoo) and a further five fall mostly in the biome (False Upper Karoo, Karroid Broken Veld, Namaqualand Broken Veld, False Karroid Broken Veld, False Succulent Karoo and Karroid *Danthonia* Mountain Veld).
White (1976, 1983) Werger (1978a, 1978b)	Defined the Nama-Karoo as one phytochorion called the Karoo-Namib Region
Jürgens (1991)	Recognised two regions – Succulent Karoo and Nama-Karoo. Also suggested the Succulent Karoo should form part of the Cape Floristic Kingdom.
Low & Rebelo (1996)	Regrouped Acock's veld types to form six vegetation types in the biome
Palmer & Hoffman (1997)	Defined three biome subdivisions - the Griqualand West and Bushmanland (highest annual rainfall and higher mean temperature), the Great Karoo and Central Lower Karoo (largest bioregion with more reliable rainfall and lowest mean temperature) and the Upper Karoo and Eastern Cape Midlands (smallest bioregion, climatically intermediate between the other two bioregions).

The vegetation of the Nama-Karoo is dominated by low dwarf shrubs growing in a mixture of grasses, succulents, geophytes and forbs (Mucina et al., 2006a). Small trees can be found, but only along drainage lines or on rocky outcrops. Although the floristic diversity is relatively low in the biome, there is a high diversity of life forms including annuals, geophytes, succulents, chamaephytes, trees and both C_3 and C_4 grasses (Mucina et al., 2006a). Cowling et al. (1994) suggested this high diversity of life forms is most likely the result of the ecotonal and climatically unstable nature of the biome region. The variation in rainfall amount, seasonality, frequency and timing drives the variation in vegetation appearance and structure (Hoffman et al., 1990; Kellner & Booysen, 1999).

Vegetation dynamics may also be affected by grazing (both domestic and wild animals), fire, rainfall (including erosion caused by run off) and other climatic events such as hailstorms (Mucina et al., 2006a). High rainfall intensity, low vegetation cover, aridity and grazing all contribute to a high soil erosion potential in the region. Semi-arid regions are particularly vulnerable to nutrient loss through soil erosion, as most of the nutrients are located near the soil surface (Snyman, 1999). The various life forms of the vegetation can also affect erosion. Roux and Opperman (1986) stated that grass cover is more resistant to erosion than shrub cover. Fire is rare in the biome, with only localised burns occurring (Edwards, 1984). Grasses may increase temporarily where fires have occurred (Roux & Vorster, 1983). In terms of herbivory, animals found in the biome include herbivores such as ostrich and springbok, nomadic granivorous birds and invertebrates such as brown locusts and the Karoo caterpillar (Mucina et al., 2006a). Opportunistic insect predators such as storks, bustards, starlings and kestrels are also common (Barber, 1880; Dean, 2000). Large herbivores and predators are mostly confined to nature reserves and game farms (Acocks, 1979; Dean & Milton, 2003). Plant defence mechanisms or adaptations for mammalian seed dispersal are not common except near water sources where herbivores are found for longer periods of time (Milton et al., 1990; Milton, 1991). Domestic stock such as sheep and goats have almost completely replaced the migratory ungulates indigenous to the biome (Roux & Opperman, 1986; Roux & Theron, 1987). This change in the grazing regime of the biome resulted in major changes in species composition (Roux & Theron, 1987). Roux and Theron (1987) found that intensified grazing in addition to drought was responsible for the loss of many palatable plants in the Nama-Karoo flora. Game farming in the area was historically predominantly with springbok (Jooste, 1983) but farming with nyala, buffalo and sable antelope has become more popular in recent years (Bezuidenhout, 2017). The veld in the Nama-Karoo is considered “sweet” as the grasses remain palatable even as they age and browsing in winter is made possible by the presence of evergreen and woody shrubs (Vorster, 1999).

The Nama-Karoo has not been largely transformed by other land uses that generally threaten natural diversity (Hoffman et al., 1999a). Ranching of small stock, cattle and game farming are the dominant land uses. A small percentage of the land is protected in national and provincial reserves but local authorities and private land owners also contribute to the proportion of protected areas in the biome (Hilton- Taylor & Le Roux,

1989). The current conservation network is not adequately designed for the conservation of vegetation or fauna and as such the conservation in the biome rests heavily on the privately owned land.

One of the biggest threats to vegetation in the biome is over-grazing, particularly in conjunction with drought conditions. In the 20th century, alien drought-hardy forage plants were introduced in an attempt to deal with drought. This led to invasions of these species throughout the region. Multiple species of *Prosopis*, Australian *Atriplex* and Cactaceae have invaded the biome (Milton et al., 1999; Dean & Milton, 2000). The value and productivity of the land has been decreased by the firm establishment of these unpalatable and/or poisonous alien herbs.

The Nama-Karoo vegetation types included in this study are described below.

3.3.1 Upper Karoo Hardeveld

The Upper Karoo Hardeveld is found in the Northern, Western and Eastern Cape Provinces (Fig. 3.3). It is found on steep slopes and ridges at altitudes of 1 000 to 1 900 m.a.s.l. In terms of conservation it is considered **not threatened**. The Camdeboo National Park and the Karoo Nature Reserve conserve 3% of the vegetation type with a further small percentage protected in private reserves (Palmer, 1990).

A number of taxa are endemic to the vegetation type (Table 3.2), many of which occur along the Great Escarpment. The vegetation of the Upper Karoo Hardeveld is pictured in Plate 3.1.

Plate 3.1. Upper Karoo Hardveld vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

Table 3.2. Taxa endemic to Upper Karoo Hardeveld (Palmer, 1990).

Life form	Species	Life form	Species
Succulent shrub	*Aloe chlorantha* *Crassula barbata* subsp. *broomii* *Delosperma robustum* *Sceletium expansum* *Stomatium suaveolens*	Herb	*Cineraria arctotidea* *Vellereophyton niveum*
Low shrub	*Cineraria polycephala* *Euryops petraeus* *Lotononis azureoides* *Selago magnakarooica*	Succulent herb	*Adromischus fallax* *Aloe humilis*
Tall shrub	*Anisodontea malvastroides*	Geophytic herb	*Gethyllis longistyla* *Lachenalia auriolae* *Ornithogalum paucifolium* subsp. *karooparkense*

Mean annual precipitation is low, ranging from 150 mm to 350 mm and frost is common (Fig. 3.5).

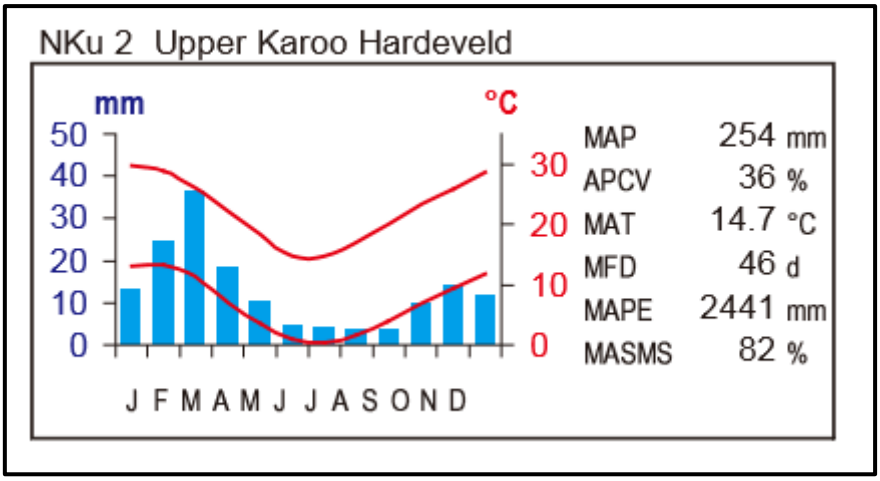


Figure 3.5. Climate diagram for Upper Karoo Hardeveld. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Mucina et al., 2006a).

The Upper Karoo Hardeveld is considered one of the richer floras of the Nama-Karoo. The unit also contains a number of Nama-Karoo diagnostic species including *Asparagus mucronatus*, *A. striatus*, *Cissampelos capensis*, *Pachypodium succulentum*, *Rhigozum obovatum* and *Cenchrus ciliaris.*

3.3.2 Eastern Upper Karoo

The Eastern Upper Karoo is also found in the Northern, Western and Eastern Cape Provinces on gently sloping plains and hills at altitudes of between 1 000 and 1 700 m.a.s.l. (Mucina et al., 2006a; Fig. 3.3). It is dominated by dwarf microphyllous shrubs and the grass genera *Aristida* and *Eragrostis* (Plate 3.2). There is an increase in grass cover along a gradient from the southwest to the northeast. In terms of conservation the vegetation type is considered **not threatened**. It is formally conserved in the Mountain Zebra National Park and the Camdeboo National Park as well as in the Oviston, Commando Drift, Rolfontein and Gariep Dam nature reserves. A number of dams have been constructed within the Eastern Upper Karoo resulting in approximately 2% being transformed. Other threats are erosion and invasion by the alien *Medicago laciniata.*

Plate 3.2. Eastern Upper Karoo vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

Rainfall in the Eastern Upper Karoo falls mainly in autumn and summer. Frost is common but ranges from less than 30 days per year in the Cradock area to more than 80 days per year in the mountains in the west of the area (Fig. 3.6).

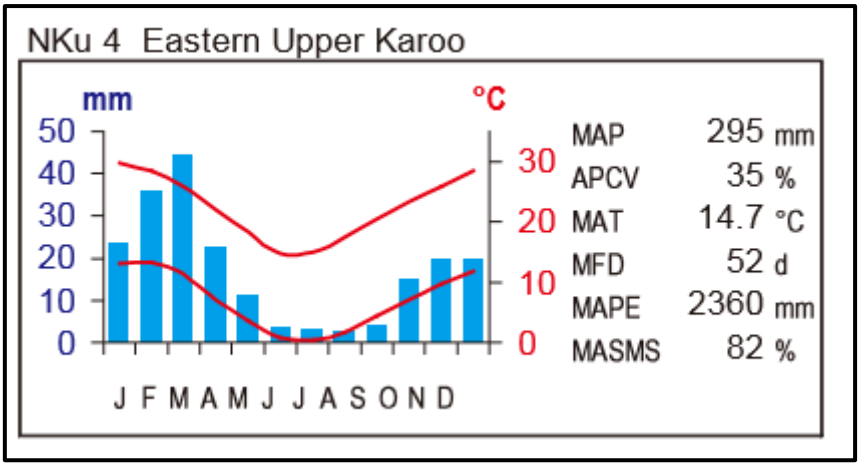


Figure 3.6. Climate diagram for Eastern Upper Karoo. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Mucina et al., 2006a).

The Eastern Upper Karoo has fewer endemic taxa compared to the Upper Karoo Hardeveld (Table 3.3).

Table 3.3. Taxa endemic to Eastern Upper Karoo (Mucina et al., 2006a).

Succulent shrub	*Chasmatophyllum rouxii* *Hertia cluytiifolia* *Rabiea albinota* *Salsola tetrandra*
Low shrub	*Aspalathus acicularis* subsp. *planifolia* *Selago persimilis* *S. walpersii*
Tall shrub	*Phymaspermum scoparium*

3.3.3 Eastern Lower Karoo

The Eastern Lower Karoo is found only in the Western and Eastern Cape provinces (Mucina et al., 2006a; Fig. 3.3). It is found on plains, sometimes interrupted by dolerite dykes, buttes and mesas, at altitudes of between 500 and 1 100 m.a.s.l. Some higher elevation “islands” of Camdebo Escarpment Thicket, Groot Thicket and Lower Karoo

Gwarrieveld may occur within the Eastern Lower Karoo plains. In terms of conservation, the vegetation type is considered **not threatened**. Patches of Eastern Lower Karoo are formally protected in the Aberdeen and Karoo Nature Reserves and also in some private reserves in the area. Threats include minor alien infestation (1-2%) and erosion.

Rainfall in the Eastern Lower Karoo is mostly in late summer and early autumn. The region experiences high summer temperatures and low winter temperatures (Fig. 3.7)

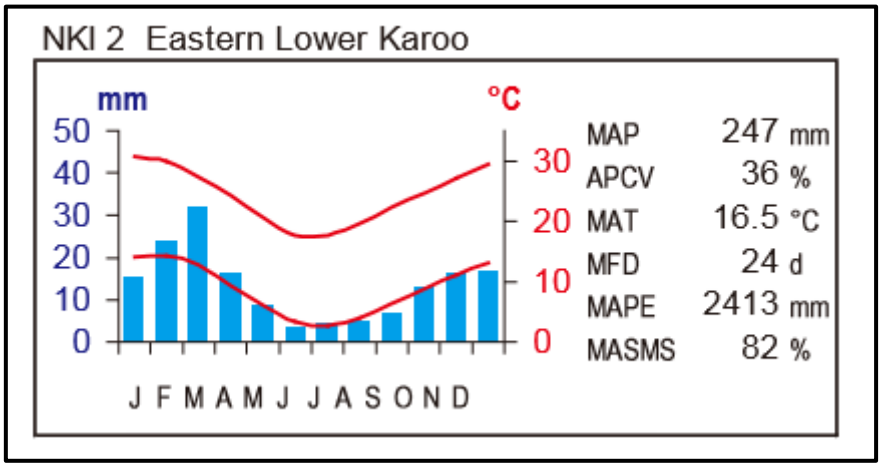


Figure 3.7. Climate diagram for Eastern Lower Karoo. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Mucina et al., 2006a).

The vegetation is dominated by low to medium height microphyllous shrubs and 'white' grass genera such as *Aristida* and *Eragrostis* (Mucina et al., 2006a; Plate 3.3). Members of the leaf-succulent families Aizoaceae and Crassulaceae are also common. Eastern Lower Karoo differs from the Gamka Karoo in that it has a higher proportion of dwarf succulent shrubs e.g. *Ruschia* spp. and more large woody shrubs such as species of *Diospyros, Lycium, Euclea* and *Searsia*. Endemic taxa found in the Eastern Lower Karoo are listed in Table 3.4.

Plate 3.3. Eastern Lower Karoo vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

Table 3.4. Taxa endemic to Eastern Lower Karoo (Mucina et al., 2006a).

Succulent shrub	*Aloinopsis rubrolineata* *Chasmatophyllum nelii* *Cylindrophyllum calamiforme* *Euphorbia coerulans* *Ruschia vanderbergiae*
Succulent herb	*Haworthia decipiens* var. *cyanea* *H. greenii*

3.4 Albany Thicket Biome

Albany Thicket vegetation is structurally unusual and found in the semi-arid river valleys on the eastern seaboard of South Africa. It was originally described as “Valley Bushveld” by Acocks (1953). This term probably arose from the local agriculturalists who found Albany Thicket vegetation to be impenetrable and in need of “opening up” to allow their livestock through. Acocks (1953) described Valley Bushveld as “semi-succulent thorny scrub 2–3 metres in height”.

This biome occurs across Eastern and Western Cape in semi-arid areas receiving between 200 and 900 mm mean annual precipitation (Vlok & Euston-Brown, 2002; Fig. 3.4). The region experiences all-year rainfall with spring and autumn maxima

(Aucamp & Tainton, 1984). In the northeast there is an increase in summer rainfall that results in an increase in grassland and thorn-tree savannah vegetation. A gradual replacement by fynbos occurs in the southwest in response to an increase in winter rainfall. Rainfall in the biome is unreliable and drought is common. High temperatures in summer and low winter temperature with frost are common in the inland regions of the biome (Fig. 3.4). The harsh climate is reflected in the life forms and strategies of the dominant plants of these inland regions. Succulence, sclerophylly, slow growth, deep rooting and storage organs are common features (Hoare et al., 2006). Coastal areas of the biome experience milder climatic conditions resulting in less succulent vegetation with lower levels of sclerophylly and higher growth rates.

The east-west trending Cape Mountains are the dominant geological feature of the biome (Hoare et al., 2006). The sandstones and quartzites of the Table Mountain and Witteberg Groups are biogeographically important in the biome as they support fynbos and renosterveld species in thicket matrices (Gibbs Russell & Robinson, 1981). Karoo Supergroup sedimentary rocks of the Dwyka and Ecca Group sediments (shales) and fine-grained Beaufort Group also feature. The afore-mentioned rocks are sometimes intruded by dolerite dykes and sills. High sea levels during the Tertiary Period caused peneplanation of the areas between the coast and the mountains. During the subsequent drop in sea level, plains were dissected by a series of large river valleys. Thicket vegetation is restricted to the slopes and floors of these large valleys, giving rise to the name “Valley Bushveld”. Soils found in the biome are dependent on the underlying geology: deep, well-structured soils originate from Karoo Supergroup rocks whereas coarse, unstructured soils that are shallow and nutrient poor originate from rocks of the Witteberg and Table Mountain Groups (Hoare et al., 2006).

The Albany Thicket biome is characterised by subtropical, semixeric conditions, such as were important during the Eocene. It is therefore expected that most thicket plant lineages would have originated during this period. Cowling et al. (2005) showed that many characteristic thicket vegetation plant groups are of Eocene age, supporting the idea that thicket lineages originated in this era. The theory is further supported by palynological evidence (Boureau et al., 1983; Salard-Cheboldaeff & Dejax, 1991). Cowling et al. (2005) listed the Ebenaceae, parts of Celastraceae, Sapindaceae, Didiereaceae and Crassulaceae families as most likely to have diversified in the Eocene. These taxa are well represented in the biome and in general are either

endemic to, or most diverse in semixeric African vegetation. Certain taxa of Mesozoic age (*Encephalartos*, *Cussonia* and *Strelitzia*) may have adopted a semixerophytic habit during the Eocene. Several clades within the Aizoaceae and Asteraceae, centered in the arid southwest (Nama-Karoo and Succulent Karoo Biomes), are of more recent origin (Klak et al., 2004; Procheş et al., 2006). This is thought to indicate retrocolonisation of a semixeric environment from the xeric conditions of the Nama-Karoo and Succulent Karoo Biomes.

The paucity of fossils sites of appropriate age in the biome has resulted in poor understanding of the geographical origins of the Albany Thicket vegetation. Woody plants that are associated with the present-day taxa in the biome, such as Ebenaceae, Celastraceae and Oleaceae, occur mostly in Eocene to Miocene deposits from East Africa (Dupéron-Laudoueneix & Dupéron, 1995). A Mediterranean origin during the Eocene has been proposed for the 'succulent biome' plant forms by Schrire et al. (2005). The idea of a northern hemisphere origin is also supported in studies by Gottschling et al. (2002) and Ingrouille et al. (2002). These studies found Tertiary records of *Ehretia* and *Rhoicissus* from Europe. All the above evidence suggests that the Albany Thicket Biome may be a relict formation rooted in the Eocene (Hoare et al., 2006).

There are several African xeric and semixeric centres of plant diversity (Cowling et al., 1999). The Succulent Karoo Region, the Eastern Cape and western Madagascar are of most importance as they appear to have been the points of origin for the earliest branches in semixeric lineages. It is proposed that dispersal from Madagascar occurred through wind and bird seed dispersal well after the separation of Madagascar from Africa (Grubb, 2003; Pell, 2004).

Due to the unique flora and number of local endemics, it is suggested that the existence of the Albany Thicket in its current distribution has been uninterrupted. The size of the biome may have however fluctuated over time. Cowling et al. (1999) suggested that Pleistocene glacial cycles may have been the cause of biome size. The expansion of the biome was likely due to the establishment of non-seasonal rainfall and fire-protected areas in the Eastern Cape. Palmer (1990) suggested that browsing pressure and unreliable climate in the region could be the evolutionary driving forces in the biome since the Last Glacial Maximum.

In the Albany area alone, 21 of Acocks' (1953) 70 Veld Types are represented. Climate and land use are the primary drivers for the distributions of the various phytochoria, resulting in a mosaic of plant communities with mixed chorological affinities (Cowling, 1983). The Albany Thicket biome supports the highest number of endemic taxa of any of the Eastern Cape biomes. The Albany Centre of Endemism has its core in this biome (Van Wyk & Smith, 2001), with Cowling and Hilton-Taylor (1994) reporting at least 2 000 taxa in the Albany hotspot. The biome is a centre of endemism for karroid succulents in particular (Hoffman & Cowling, 1991; Van Wyk & Smith, 2001). The Asclepiadaceae, Crassulaceae and Euphorbiaceae families and certain taxa in the Asparagales have high levels of endemism in the region (Smith & Marx, 1990). There have been a number of estimates of endemism in the biome ranging from 10% to 20% (Lubke et al., 1986; Van Wyk & Smith, 2001).

The earliest classification of the Albany Thicket biome was alluded to by Rutherford and Westfall (1986) based on a dominant life-form combination that did not match any of the then classified biomes. Rutherford and Westfall (1986) placed Thicket in the Savanna biome due to the dominance of phanerophytes and "co-dominance of hemicryptophytes". Scholes (1997) used the same classification system and mapped the region as part of a broad-leaved Savannah. Evidence for the region to be classified as a distinct floristic unit was presented by White and Moll (1978) and Cowling (1983) resulting in the recognition of the Thicket Biome by Low and Rebelo (1996). Rutherford and Westfall (1986) suggested that Thicket was in essence, a 'missing biome' in that Spekboomveld, Fish River Scrub, Addo Bush and Sundays River scrub had vegetation that was phanerophyte and chamaephyte co-dominant (rather phanerophytes dominant and hemicryptophyte co-dominant as in Savanna). Analyses within the STEP project (Cowling et al., 2003) confirmed the climatic uniqueness of the region, the peculiar vegetation structure and high regional endemism as justification for the recognition of the area as a biome in its own right (Robertson & Palmer, 2002b; Vlok & Euston-Brown, 2002). The vegetation units as described by Hoare et al. (2006) are compared to the equivalent STEP vegetation units in Table 3.5 below. The currently accepted delimitation of the biome closely follows the STEP domain of Vlok and Euston-Brown (2002).

Table 3.5. Vegetation types (Hoare et al., 2006) used in this study and the corresponding STEP units (Vlok & Euston-Brown, 2002). The STEP units included have at least 80% of their area within the corresponding vegetation type.

Vegetation units	**STEP units**	**Vegetation units**	**STEP units**
Southern Cape Valley Thicket	Gouritz Valley Thicket	Kowie Thicket	Albany Spekboom Thicket Albany Spekboomveld Albany Thicket Albany Valley Thicket Ecca Bontveld Salem Karroid Thicket Shamwari Grassland Thicket Thorndale Forest Thicket
Gamka Thicket	Gamka Arid Spekboomveld Gamka Spekboom Thicket Oudtshoorn Karroid Thicket	Albany Coastal Belt	Geluk Grassland Thicket Hamburg Dune Thicket Kiwane Dune Thicket Nanaga Savanna Thicket Paterson Savanna Thicket Zuney Strandveld
Groot Thicket	Baviaans Spekboom Thicket Baviaans Valley Thicket Bethelsdorp Bontveld Groot Arid Spekboomveld Kleinpoort Karroid Thicket	Great Fish Noorsveld	Fish Noorsveld
Gamtoos Thicket	Gamtoos Arid Spekboomveld Gamtoos Bontveld Gamtoos Thicket Gamtoos Valley Thicket Kromme Forest Thicket Otterford Forest Thicket Vanstadens Forest Thicket	Great Fish Thicket	Crossroads Grassland Thicket Doubledrift Karroid Thicket Fish Spekboom Thicket Fish Thicket Fish Valley Thicket Hartebeest Karroid Thicket
Sundays Noorsveld	Sundays Noorsveld	Buffels Thicket	Buffels Thicket Buffels Valley Thicket Kei Thicket Mountcoke Grassland Thicket
Sundays Thicket	Elands Forest Thicket Koedoeskloof Karroid Thicket Kremlin Grassland Thicket Motherwell Karroid Thicket Sundays Spekboom Thicket Sundays Spekboomveld Sundays Thicket Sundays Valley Thicket Zuurberg Fynbos Thicket	Eastern Cape Escarpment Thicket	Escarpment Thicket
Coega Bontveld	Grass Ridge Bontveld	Camdebo Escarpment Thicket	Escarpment Spekboom Thicket

The Albany Thicket vegetation is considered to be transitional between subtropical regions of the eastern seaboard and the Nama-Karoo biome (Hoare et al., 2006), including elements of forests, shrublands, Karoo and grasslands (Cowling, 1984; Palmer, 1990; Everard, 1991; Kerley et al., 1995; Vlok & Euston-Brown, 2002). This transitional nature is reflected in the wide variety of growth forms found in the Albany Thicket. In general terms the vegetation of the Albany Thicket Biome is dense, woody, semi-succulent and thorny with an average height of 2 to 3 m (Acocks, 1953; Everard, 1987). In an unaltered condition it is relatively impenetrable. Gradients in climate, geology, soil and herbivory result in a broad range of physiognomic types. One of the most distinguishing features is the clumping of the vegetation in this biome, thought to be facilitated by below-ground animal activity (Palmer et al., 1988).

The vegetation has high species diversity and a wide range of growth forms (Cowling, 1983). Locally endemic and rare dwarf succulent shrubs and forbs are found in the understorey while perennial grasses (such as *Panicum* and *Eragrostis*) are prevalent outside the bushclumps (Cowling, 1983; Johnson et al., 1999; Vlok & Euston-Brown 2002; Vlok et al., 2003).

The impenetrable nature of the thicket is a result of a guild of spinescent woody plants that have recurved branches. As adjacent plants mature they become entwined in these woody species. Vines in particular become interwoven making the bush increasingly impenetrable (Vlok & Euston-Brown, 2002).

Variations in annual precipitation appear to have little effect on the dynamics of the vegetation as many of the life forms have drought resistant strategies such as below-ground storage organs, sclerophylly, CAM photosynthesis and succulence. Fire is also not an important driver in the biome as fuel availability is low and there is a high degree of succulence (Kerley et al., 1995).

Historically this biome supported both small and large herbivores, and Kerley et al. (1999) showed that herbivory has played an important role in the structure of the vegetation. A case in point is the evolutionary selection of plant species that are well defended against browsing (Everard, 1987; Haschick, 2002). The structure of thicket vegetation is affected by megaherbivores in particular (Stuart-Hill, 1992). Elephants have been shown to maintain vegetation structure, promote asexual recruitment of e.g. *Portulacaria afra* and encourage coppicing in woody shrubs (Stuart-Hill, 1992).

The vegetation of the Albany Thicket is widely transformed and degraded (Lloyd et al., 2002; Palmer et al., 2004; Rutherford et al., 2012b). The major causes of degradation include clearing for cultivation, herbivory by livestock and urban settlement (Lloyd et al., 2002). Individual species are also under threat due to illegal collecting, industrial and residential development, alien plants, agriculture and medicinal harvesting (Victor & Dold, 2003). The vegetation has also been shown to be at risk of a significant loss in areas due to climate change (Rutherford et al., 1999; WWF, 2001). Individual species would also potentially be affected, with predictions that 20% of the area in which *Portulacaria afra* is currently found would become unsuitable due to changes in climate (Robertson & Palmer, 2002a).

There are a number of large reserves in which Albany Thicket is formally protected, such as the Addo Elephant National Park, but a number of Albany Thicket vegetation types remain unprotected outside of these reserves.

Large areas of degraded thicket in the region require restoration and rehabilitation, but these processes are labour-intensive and resource-heavy (Todkill et al., 2006).

The Albany Thicket vegetation types included in this study are described below.

3.4.1 Sundays Noorsveld

Sundays Noorsveld is found only in the Eastern Cape, at altitudes of 100 to 600 m.a.s.l., from the Klein Winterhoek Mountains towards Jansenville with some patches in the Sundays River Valley (Fig. 3.3). In the lowlands where the vegetation is dense, the thicket is 1 to 2 m tall and contains a mosaic of *Euphorbia caerulescens* (noors) clumps interspersed with low karoo shrubs such as *Pentzia incana* and solitary trees and shrubs such as *Pappea capensis, Euclea undulata, Searsia longispina* and *Gymnosporia polyacantha* (Plate 3.4).

Rainfall is non-seasonal but with a maximum in late summer. Frost occurs for an average of 5 days a year (Fig. 3.8).

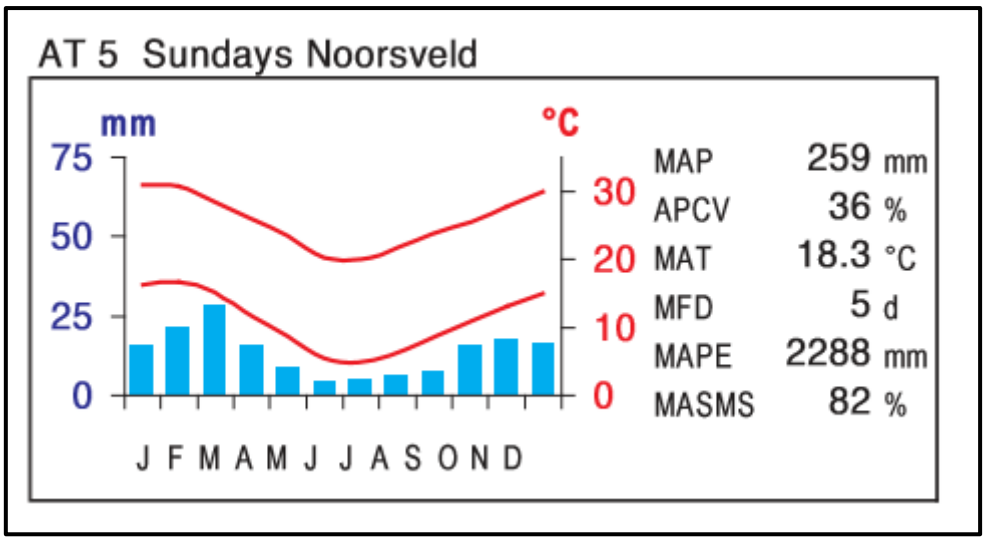


Figure 3.8. Climate diagram for Sundays Noorsveld. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Hoare et al., 2006a).

In terms of conservation the vegetation type is considered **not threatened**. About 15% of Sundays Noorsveld is formally conserved in the Greater Addo Elephant National Park and a further 3% is in private game ranches. Cultivation is the biggest cause of transformation. Erosion in this vegetation type is moderate to very low (Hoare et al., 2006).

Plate 3.4. Sundays Noorsveld vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat). Species composition was similar in the conserved and degraded quadrats, hence the similarity.

3.4.2 Sundays Thicket

Sundays Thicket is also limited to the Eastern Cape, occurring at altitudes of 0 to 800 m.a.s.l., extending from Port Elizabeth and Uitenhage to the Sundays River Valley in the east and north towards the Zuurberg Mountains (Fig. 3.3). Sundays Thicket is also found north of the Klein Winterhoek Mountains as far as Jansenville, Pearston and Somerset East districts (Hoare et al., 2006).

Sundays Thicket can be found on plains and low mountains. It tends to be dense and tall, consisting of trees, shrubs, lianas and succulents (Plate 3.5). Many species found in this vegetation unit are spinescent. The relative abundance of *Portulacaria afra* increases while woody species decrease along a gradient of increasing aridity. Soils are predominantly loamy to clayey, but sandier soils are found in the region of the Zuurberg Mountains (Hoare et al., 2006).

Rainfall is non-seasonal with slight peaks in March and October (Aucamp & Tainton 1984). Mean annual precipitation increases across the vegetation unit in a south-easterly direction, peaking near Port Elizabeth. Frost is more frequent in the inland sites than near the coast, averaging 8 days of frost per year (Hoare et al., 2006; Fig. 3.9).

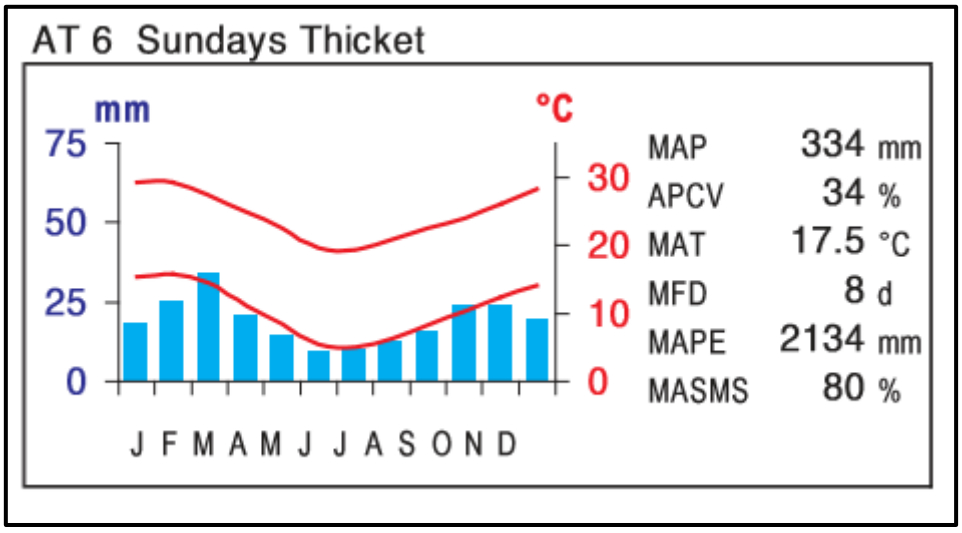


Figure 3.9. Climate diagram for Sundays Thicket. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Hoare et al., 2006a).

Plate 3.5. Sundays Thicket vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

In terms of conservation, Sundays Thicket is considered **not threatened** (Hoare et al., 2006). It is protected in a number of formal and private conservation areas, including the Greater Addo Elephant National Park and Groendal Wilderness Area. More than 6% of this vegetation type is transformed, primarily through cultivation, grazing and urbanization. Degraded Sundays thicket is dominated by invasive weeds and resembles secondary thornveld or grassland. Erosion in this unit is moderate to very low.

The Sundays Thicket is home to a number of endemic and biogeographically important taxa (Table 3.6).

3.4.3 Great Fish Thicket

The Great Fish Thicket is found in the Eastern Cape Province at altitudes up to 1000 m.a.s.l. It occurs mainly in the valleys of the Great Fish and Keiskamma Rivers and extends inland as far as Cookhouse and Cradock (Fig. 3.3). Great Fish Thicket grows along the steep slopes of deeply dissected rivers. This vegetation type is the easternmost unit in the Albany Thicket (Hoare et al., 2006).

Table 3.6. Endemic and biogeographically important taxa in Sundays Thicket (Hoare et al., 2006).

	Life form	**Species**
Biogeographically important taxa (southern limit of their distribution)	Succulent climber	*Ceropegia ampliata* var. *ampliata*
	Herbaceous climber	*Fockea sinuata*
Biogeographically important taxa (other)	Epiphytic parasitic herb	*Cuscuta bifurcata*
	Geophytic herb	*Pelargonium campestre*
Endemic taxa	Small tree	*Encephalartos horridus*
	Succulent shrub	*Aloe bowiea* *A. gracilis* *Bergeranthus addoensis* *Glottiphyllum grandiflorum* *Orthopterum coegana* *Ruschia aristata* *Trichodiadema rupicola*
	Succulent climber	*Aptenia haeckeliana* *Ceropegia dubia*
	Succulent herb	*Haworthia arachnoidea* var. *xiphiophylla* *H. aristata* *Huernia longii* subsp. *longii*
	Geophytic herb	*Brachystelma cummingii* *B. schoenlandianum* *B. tabularium* *Pelargonium ochroleucum* *Strelitzia juncea* *Tritonia dubia*
	Herb	*Arctotis hispidula* *Argyrolobium crassifolium* *Lessertia carnosa* *Lotononis monophylla* *Senecio scaposus* var. *addoensis* *Wahlenbergia oocarpa*

Soils of Great Fish Thicket are mostly shallow clay soils.

Thickets may be short, medium or tall (Palmer, 1981; Palmer et al., 1988; Evans et al., 1997). Spinescent shrubs are common, along with woody trees and succulents (Plate 3.6). As the aridity increases, the locally dominant *Portulacaria afra* is replaced by *Euphorbia bothae*. In areas with increased moisture, *Portulacaria afra* is replaced by *Euphorbia tetragona* and *E. triangularis.* The vegetation may be clumped as a result of zoogenic mounds, and these nutrient rich mounds are occupied by *Pappea capensis* and *Boscia oleoides* as well as endemic geophytes. One of the distinctive features of this vegetation type is the closed canopy of the *Portulacaria afra*-dominated thicket.

Rainfall is non-seasonal with slight maxima in March and October (Aucamp & Tainton, 1984). Mean annual precipitation is higher in the coastal areas (600 mm) compared to the inland ones (300 mm). Incidence of frost varies widely, with frost being much more common in the upper reaches of valleys in the vegetation type (Fig. 3.10).

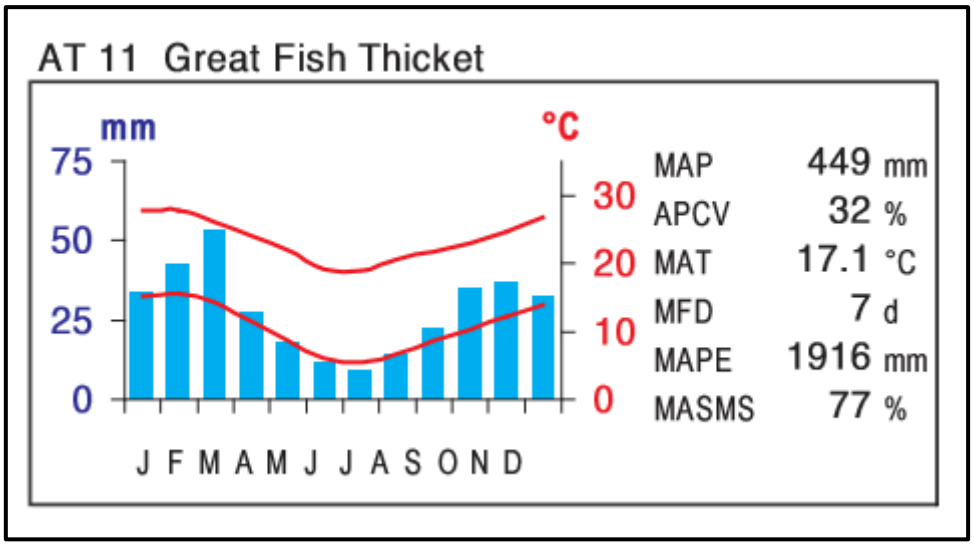


Figure 3.10. Climate diagram for Great Fish Thicket. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Hoare et al., 2006a).

In terms of conservation status, Great Fish Thicket is considered **not threatened**. Of the 6% conserved area in this unit, the majority is protected within the Great Fish River Complex Nature Reserve and further areas are conserved on private land. This vegetation type has not been greatly transformed, although cultivation and urbanisation have altered the vegetation to some degree (Hoare et al., 2006).

Plate 3.6. Great Fish Thicket vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

A number of endemic species are found in the Great Fish Thicket (Table 3.7).

Table 3.7. Species endemic to the Great Fish Thicket (Hoare et al., 2006).

Life form	**Species**
Succulent shrub	*Euphorbia cumulata*
Low shrub	*Euryops gracilipes*
Succulent herb	*Haworthia angustifolia* var. *paucifolia* *H. cummingii* *H. cymbiformis* var. *incurvula* *H. cymbiformis var. ramosa*
Herb	*Zaluzianskya vallispiscis*

3.4.4 Eastern Cape Escarpment Thicket

Also found only in the Eastern Cape, this vegetation type occurs at altitudes of 450 to 1 250 m (Hoare et al., 2006). It is found in the foothills of the steep Amathole, Winterberg and Swaershoek Mountains as well as the mountainous regions surrounding Cradock (Fig. 3.3).

Eastern Cape Escarpment Thicket grows on steep slopes as well as the lowlands of the escarpment. This thicket, dominated by *Olea europaea* subsp. *africana* and *Acacia natalitia,* is of medium height (3 to 7 m tall). Lower on the slopes it grades into thrornveld and higher up on the slopes it grades into forest (Plate 3.7). It is floristically closely related to Camdebo Escarpment Thicket and has a similar structure. To the north it is closely related to Tarkastad Montane Shrubland and similar to mesic Buffels Thicket in the east. Soils in the area range from fine-grained, nutrient-poor silts to nutrient-rich red clays (Hoare et al, 2006).

Rainfall is non-seasonal with March and November maxima (Aucamp & Tainton, 1984). The mean annual precipitation tends to be higher on the southern side of the escarpment (400–700 mm) as opposed to the northern side (310–400 mm). Frost is more common on the slopes, where snow may also fall in winter (Fig. 3.11).

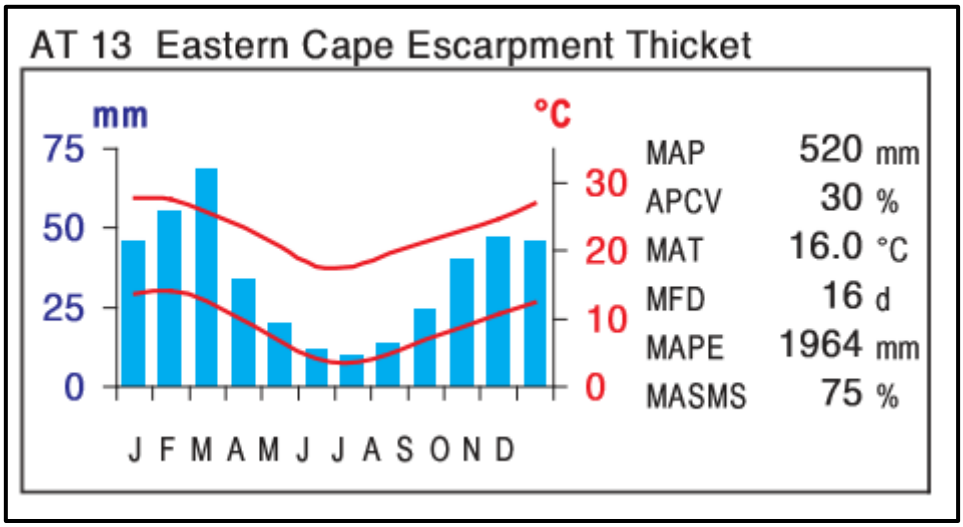


Figure 3.11. Climate diagram for Eastern Cape Escarpment Thicket. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Hoare et al., 2006a).

This vegetation type has been altered through cultivation and urbanisation (Hoare et al, 2006). Local-authority reserves, such as Bosberg, conserve 5% of the Eastern Cape Escarpment Thicket with a further 2% protected in private conservation areas. Erosion in this unit is variable, ranging from very low to high.

Eastern Cape Escarpment Thicket has no vegetation type endemics.

Plate 3.7. Eastern Cape Escarpment Thicket vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

3.4.5 Camdebo Escarpment Thicket

Camdebo Escarpment Thicket is found only in the Eastern Cape at altitudes of 570 to 1600 m (Hoare et al, 2006). It is found on the south-facing slope of the Great Escarpment from Bruintjieshoogte in the east via Graaff-Reinet to Aberdeen in the west (Fig. 3.3).

The vegetation occurs on steeply sloping, rugged mountain slopes (Hoare et al, 2006). The thicket is generally 2 to 3 m tall and dominated by *Portulacaria afra* (Plate 3.8). In areas where there has been heavy goat browsing, *P. afra* is replaced by low trees such as *Pappea capensis* and *Boscia oleoides.*

Camdebo Escarpment Thicket is floristically more related to Nama-Karoo vegetation (Palmer, 1988; 1991a, b). The linkage of this unit to the Albany Thicket is due to the dominance of *Portulacaria afra*, but it is most likely controlled by regional geomorphology and microclimate.

Soils are generally shallow and skeletal Mispah soils (Hoare et al, 2006).

Rainfall is non-seasonal with March and November maxima (Hoare et al, 2006). Mean annual precipitation increases with elevation, ranging from 270 to 550 mm. Frost is

much more common higher up on the escarpment slopes where it may also snow in winter (Fig. 3.12).

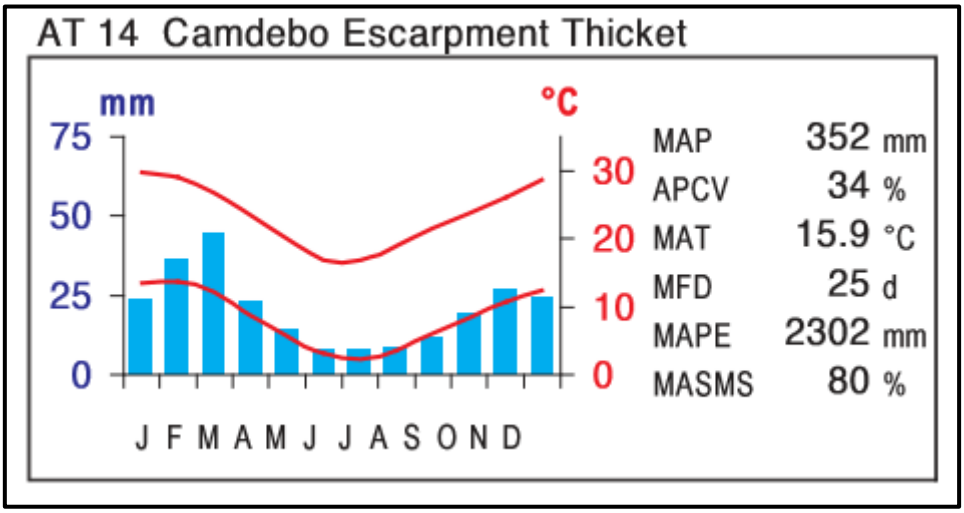


Figure 3.12. Climate diagram for Camdebo Escarpment Thicket. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Hoare et al., 2006a).

In terms of conservation, Camdebo Escarpment Thicket is considered **not threatened**. The Karoo Nature Reserve protects approximately 5% and a further 15% is protected in private conservation areas. Camdebo Escarpment Thicket has not been greatly transformed, but cultivation and grazing by goats are threats. Erosion is moderate to high (Hoare et al., 2006).

Plate 3.8. Camdebo Escarpment Thicket vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

A number of endemic species are found in the Camdebo Escarpment Thicket – these are listed in Table 3.8.

Table 3.8. Species endemic to the Camdebo Escarpment Thicket (Hoare et al., 2006).

Life form	**Species**
Succulent shrubs	*Astroloba corrugata* *Bergeranthus* sp. nov. (‘*nanus*’ A.P. Dold ined.) *Delosperma karrooicum,* *Trichodiadema olivaceum*
Succulent herb	*Haworthia marumiana* var. *batesiana* *Huernia kennedyana*
Geophytic herb	*Apodolirion bolusii* *Dierama grandiflorum*

3.5 Grassland Biome

Grassland vegetation is herbaceous, short and of simple structure (Mucina et al., 2006b). Graminoids, especially the family Poaceae are dominant, while woody plants are rare. The extent of South African grasslands is defined based on the structure of the vegetation and climatic factors such as summer rainfall and winter temperatures.

Grasslands experience cool, dry conditions due to the high elevation (Mucina et al., 2006b; Fig. 3.4). Temperate grasslands in South Africa experience summer rainfall and winter drought (Fig. 3.4). Frost and fog are common throughout the biome. Lightning-induced fire is common due to the high lightning flash densities.

The biome covers a significant proportion of the Karoo Supergroup. Dolerite dykes, which are a characteristic feature of the Karoo in general, are also common here (Mucina et al., 2006b).

Fossil pollen records indicate that grasses and related families such as restios most likely developed in the Late Cretaceous (Jardine & Magloire, 1965; Muller, 1981; Scott & Srivastava, 1984; Grass Phylogeny Working Group, 2001). Marine isotope records indicate cooling of global ocean temperatures during the late Miocene (Shackleton & Kennet, 1975; Edwards et al., 2010: Fig. 3.13). The development of the Nama-Karoo,

Fynbos and possibly Grassland appears to coincide with these cooler ocean temperatures (Scott et al., 1997; Linder, 2003). Fossil pollen evidence has shown that during the glacial and interglacial periods there were shifts in grassland composition and boundaries with the Nama-Karoo, Savanna and Afromontane fynbos (Scott et al., 1997). The Late Quaternary period saw more extensive grasslands but grassland distributions were more similar to present patterns during interglacial periods (Scott & Vogel, 1983; Bond et al., 2003a).

The evolution of C_4 metabolism was a major event in grassland evolution (Cerling et al., 1997; Ehleringer et al., 1997; Keeley & Rundel, 2003) that evolved during the Cenozoic. The worldwide expansion of C_4 grasses during the late Miocene and Pliocene (3 to 8 million years ago) has been documented using stable carbon isotope data ($\delta^{13}C$). The spread of C_4 grasses was at first diagnosed by the $\delta^{13}C$ of fossil carbonates (Quade et al., 1989). Subsequent studies have provided further evidence for the Late Miocene–to–Pliocene explosion of C_4 grasses through $\delta^{13}C$ of n-alkanes and marine sediments (Tipple et al., 2007; Huang et al., 2007) and $\delta^{13}C$ records of ungulate teeth (Cerling et al., 1997; Passey et al., 2009).

Two other major phytochoria (White, 1983) coincide with the Grassland biome: the Kalahari-Highveld Regional Transition Zone (linked to the largest part of the biome boundary with the Savanna Biome and with more tropical affinities) and the Afromontane and Afroalpine Region (mountainous landscapes along the Northern Escarpment, containing temperate components, fall into this region).

The Grassland and Savanna Biomes share many similarities in rainfall seasonality, patterns and amounts, but summer aridity and cooler minimum winter temperatures in the Grassland biome result in the absence of major woody components that is the biggest difference between these two biomes.

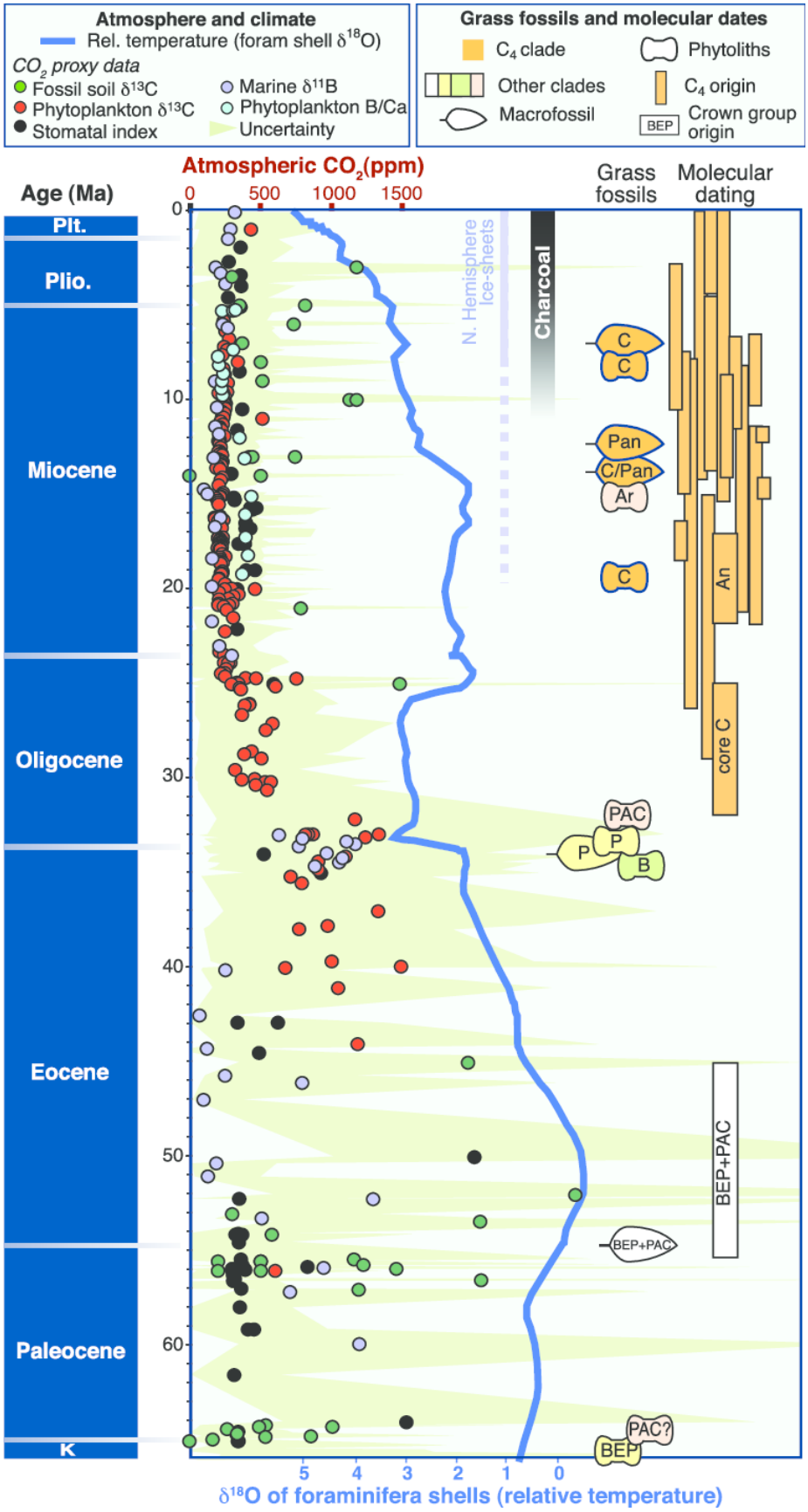


Figure 3.13. Record of CO^2 and temperature change in the Cenozoic, including evidence for grass evolution (Edwards et al., 2010).

There are five centres of plant endemism within the Grassland biome, two of which fall completely within it (Drakensberg Alpine and Wolkberg) and the other three are shared with the Savanna biome (Barberton, Sekhukhune and Soutpansberg) (Van Wyk & Smith, 2001). Of these the Drakensberg Alpine centre is most extensive and most endemic-rich of these five centres, with 13% endemism (Van Wyk & Smith, 2001; Carbutt & Edwards, 2004; Carbutt & Edwards, 2006). The centres of endemism are linked either with high altitude or special substrates such as quartzites and all occur in the so-called Grassland-Savanna 'tension' zone'. There are no centres of endemism in the core of the biome, where C_4 grasses dominate.

Steenkamp et al. (2002) report 34 grass taxa endemic to the biome; 13 from the subfamily Arundinoideae and 11 from the Pooideae. The winter-rainfall regions in the biome are dominated by the former (Gibbs Russell, 1986). A high proportion (67%) of the orchid taxa found in the Grassland biome are endemic (Linder et al., 2005), but endemism is not high in other herbs of the biome compared to winter-rainfall biomes. Those herb genera that are endemic are found mostly in the Drakensberg.

In terms of diversity, grasslands have high alpha diversity and medium gamma diversity (Cowling et al., 1989). Even though there is limited growth form richness, high local species richness does occur. This may be as a result of differential responses of various species in the guilds to grazing, fire and variable climate (Cowling et al., 1989). Beta diversity (the ratio between regional and local species diversity; Whittaker, 1972) is high where steep topographical and environmental gradients exist in the biome (Hoare, 2003). Management regimes of grasslands can also affect species richness. Poorly managed areas have more exotic species and tend to be dominated by forbs rather than grasses (Hoare, 2002).

Grasslands are divided into dry and moist types based on annual rainfall (the boundary is 500 to 700 mm rainfall). This corresponds to the division of South African grasslands into 'sweetveld' and 'sourveld' (Huntley, 1984; Ellery et al., 1995; Bond, 1997). Andropogonoid grasses dominate the grassland above 600 mm rainfall whereas sweet chloridoid grasses dominate below 600 mm. This rainfall threshold also controls soil factors – in moist grasslands soils are usually nutrient limited whereas in dry grasslands soils are generally eutrophic and water-limited. Climatic and ecological factors influence patterns and diversity relationships found in grasslands. The

subdivisions of the biome in this study follows that of Mucina et al. (2006b) and are based on gradients of altitude and moisture and correlating floristic factors.

Fire is essential in the grassland biome for the maintenance of structural and textural patterns (Edwards, 1961; Granger, 1976; Tainton, 1981; Everson, 1985; Bainbridge, 1993; O'Connor & Bredenkamp, 1997). Bond et al. (2003b) postulates that most of the eastern half of South Africa would be tree-covered without fire. Frequency, seasonality and intensity are the most important components of the fire regime (Gill, 1975). Fire occurs on average every 1 to 4 years in grasslands (Le Maitre & Midgley, 1992). Fuel moisture, air temperature and wind speed control the fire intensity and the primary source of ignition is lightning. High incidence of lightning strikes in the Grassland biome ensures that natural fires maintain grassland dominance over woody components.

In the sourveld, soils are dystrophic and leached, canopy cover and plant production are high and fire is frequent. In the sweetveld, soils are not as leached and are eutrophic. Canopy cover, plant production and fire frequency is lower than in sourveld. Sourveld is usually found at higher altitudes (high water supply) and sweetveld at lower altitudes (lower water supply) (Mucina et al., 2006b).

Canopy structure and species composition in grasslands are greatly influenced by grazing. Although grasses are well adapted to defoliation, excessive or frequent defoliation can eventually have adverse effects (Rutherford & Westfall, 1986). The size and density of plants, plant longevity, community composition, species diversity, response of plants to climate patterns or abiotic factors and grass seed production can all be affected by grazing. Grassland structure is determined by herbivory, rainfall, soil nutrient availability and fire (Walker, 1993).

High stocking rates of domestic livestock in commercial farm areas have been blamed for the degradation of South African grasslands (Owen-Smith & Danckwerts, 1997). However, indigenous herbivores can also affect species cover and composition. The major difference is that domestic livestock numbers are kept relatively constant during times of drought through the provision of supplementary fodder. Wild herbivores found in the biome include black wildebeest, blesbok, springbok and eland. Small herbivores include porcupine, several species of hare, tortoises and insects such as grasshoppers and harvester termites (Owen-Smith & Danckwerts, 1997).

Fairbanks et al. (2000) found that nearly 30% of the Grassland biome had been transformed by cultivation, forestry, erosion, urbanisation and mining. Untransformed grassland is highly fragmented, further threatening the biome. Many areas are suitable for farming, and the transformation of the grassland for economic activities and due to climate change are the greatest threats to the biome. Highveld grasslands are most at risk for transformation into farming land and the escarpment areas are threatened by afforestation with exotic *Pinus* and *Eucalyptus* species.

Although there are numerous small reserves in the biome, they protect only a small fraction of the biome and are not evenly distributed. The Highveld region in particular is poorly conserved (Mucina et al., 2006b).

The Grassland vegetation types included in this study are described below.

3.5.1 Amathole Montane Grassland

Amathole Montane Grassland is found in the Bosberg, Amathole, Winterberg and Kologha mountains in the Eastern Cape, at altitudes between 650 and 1 500 m (Mucina et al., 2006b; Fig. 3.3). Outside of the mountainous areas, it is also found on broken veld between Stutterheim and Komga.

This is a short grassland characterised by high species richness of forbs, especially Asteraceae (Mucina et al., 2006b). Grasses that are dominant include *Themeda triandra, Elionurus muticus, Sporobolus africanus, Eragrostis chloromelas, E. curvula, Heteropogon contortus, Alloteropsis semialata* and *Tristachya leucothrix* (Plate 3.9). In the northern watershed areas, the vegetation forms a mosaic with Karoo Escarpment Grassland and is gradually replaced by this vegetation type in mountainous areas.

Sedimentary rocks (sandstones and shales) of the Beaufort Group in the Karoo supergroup dominate (Hartmann, 1988). Soils are deep, freely drained and highly weathered.

Rainfall is bimodal with spring and late summer peaks (Hoare & Bredenkamp, 1999). High altitude areas receive the most rainfall (1 000 mm in places). Frost is frequent in the western and north-western regions (Fig. 3.14).

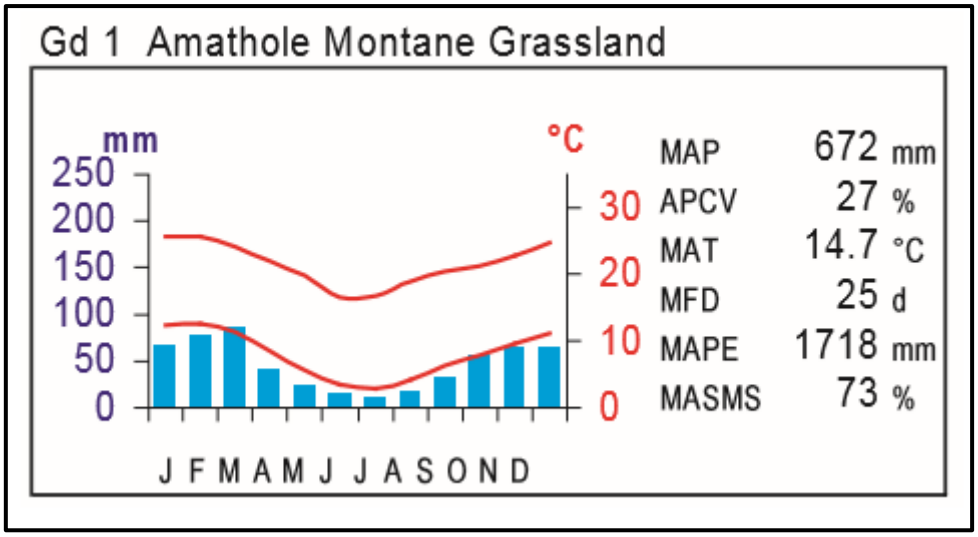


Figure 3.14. Climate diagram for Amathole Montane Grassland. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Mucina et al., 2006b).

Amathole Montane Grassland is considered **not threatened**. Statutory conservation areas such as the Mpofu Game Reserve and the Boschberg conserve 5% and a further small percentage is conserved in private reserves. It has been heavily transformed by plantation, cultivation and heavy grazing by domestic livestock. There has been some invasion by exotics including *Acacia mearnsii* and *A. dealbata* but erosion is generally low (Mucina et al., 2006b).

Plate 3.9. Amathole Montane Grassland vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

A number of biogeographically important taxa and endemic species are found in Amathole Montane Grassland (Tables 3.9 and 3.10 respectively) (Mucina et al., 2006b).

Table 3.9. Biogeographically important taxa (Drakensberg endemics) found in the Amathole Montane Grassland (Mucina et al., 2006b).

Life form	**Species**
Graminoid	*Bromus speciosus* *Helictotrichon galpinii* *Pentaschistis airoides* subsp. *jugorum*
Herb	*Helichrysum aureum* var. *serotinum* *Psammotropha mucronata* var. *marginata*
Geophytic herb	*Disa stricta*

Table 3.10. Endemic taxa found in the Amathole Montane Grassland (Mucina et al., 2006b).

Life form	**Species**
Herb	*Alchemilla bolusii* *Alepidea macowani* *Cineraria vagans* *Diascia ramosa* *Helichrysum isolepis* *Heliophila katbergensis* *Hermannia violacea* *Wahlenbergia laxiflora*
Geophytic herb	*Aspidoglossum uncinatum* *Nerine filamentosa* *Pachycarpus linearis* *Watsonia amatolae*
Succulent shrub	*Delosperma katbergense*
Semiparasitic herb	*Thesium orientale*
Low shrub	*Abutilon flanaganii* *Arrowsmithia styphelioides* *Erica amatolensis* *Euryops ciliatus* *Garuleum tanacetifolium* *Indigofera cuneifolia* var. *angustifolia* *Lotononis trichodes* *Macowania revoluta* *Muraltia rara* *Phylica galpinii* *P. simii* *Tephrosia polystachya* var. *longidens*

3.5.2 Bedford Dry Grassland

Bedford Dry Grassland is found in the Eastern Cape Province at altitudes of 480 to 990 m (Mucina et al., 2006b). It extends from Somerset East in the west to Fort Beaufort in the east, north of the Great Fish River Valley (Fig. 3.3).

This grassland is found interspersed with *Vachellia karroo* woodlands on gently undulating plains (Hoare, 1997). The vegetation contains Kowie Thicket and Albany Broken Veld elements in the incised river valleys in the south. The maximum height of the vegetation is 100 cm and it is dominated by the grasses *Digitaria argyrograpta, Tragus koelerioides, Eragrostis curvula* and *Cymbopogon caesius* (Plate 3.10).

Plate 3.10. Bedford Dry Grassland vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

Bedford Dry Grassland soils are mostly loamy or clay (Hoare, 1997).

Rainfall in the region is bimodal, occurring in spring and late summer (Mucina et al., 2006b). Mean annual precipitation is relatively even across the region, but may be higher closer to mountains. Frost is more common in the west than in the east (Fig. 3.15).

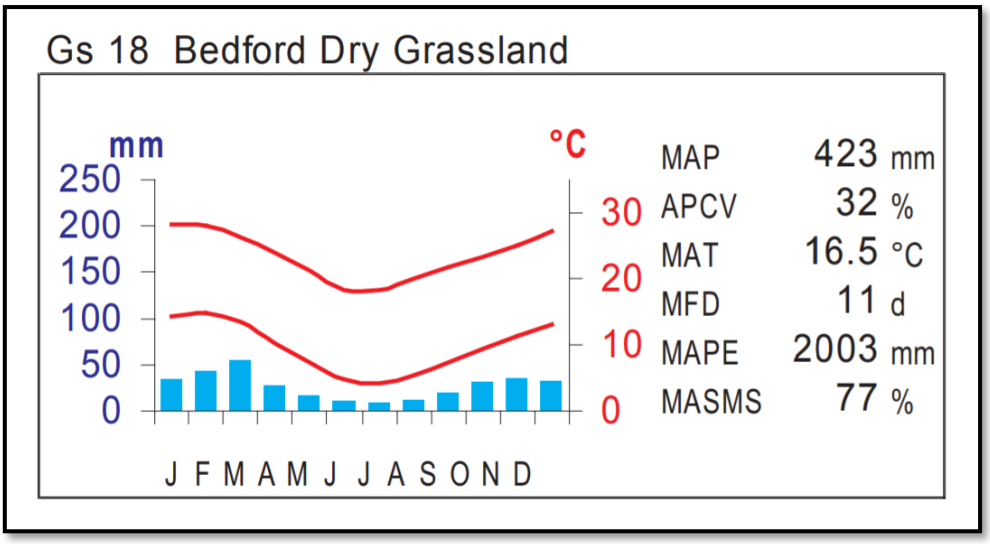


Figure 3.15. Climate diagram for Bedford Dry Grassland. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Mucina et al., 2006b).

Bedford Dry Grassland is considered **not threatened** (Mucina et al., 2006b). There are no formal conservation areas protecting this vegetation unit and only 1% is conserved in private reserves. Cultivation has transformed approximately 3% of the unit. Erosion varies from high to very low.

Bedford Dry grassland falls within the Albany centre of endemism (Mucina et al., 2006b), but contains no vegetation type endemics.

3.5.3 Karoo Escarpment Grassland

Karoo Escarpment Grassland occurs in the Eastern, Northern and Western Cape Provinces at an altitude range of 1 100 to 2 502 m.a.s.l. (Mucina et al., 2006b). It is found, as the name suggests, along the Karoo Escarpment – the east-west extent is from Molteno to Noupoort and the north-west extent is from Somerset East to Nieu-Bethesda (Fig. 3.3). These grasslands are also found in the Winterberg Mountains near Tarkastad. It is generally found on mountain summits, low mountains and hills. These are generally tussock grasslands dominated by *Merxmuellera disticha.* Grasses typical of dry grasslands, such as *Eragrostis, Karroochloa, Melica, Elionurus* and *Aristida* genera are also common (Plate 3.11). Low shrubs may be an important component. There is a high floristic variability, with both Karoo and Grassland elements being represented. Inclusion in the grassland biome is due to the presence

and dominance of C_3 and C_4 grasses as well as many fynbos elements (Mucina et al., 2006b). One of the centres of diversification of the genus *Euryops* can be found in mountains of the Karoo Escarpment Grassland (Nordenstam, 1968).

Mudstones and sandstones of the Beaufort Group (Karoo Supergroup) are overlain by shallow soils (Hoare & Bredenkamp, 1999). Dolerite intrusions are also common, sometimes forming ridges across the area.

Winters are very dry and rainfall peaks slightly in March and November/December (Hoare & Bredenkamp, 1999). Mean annual precipitation increases from the west to the east and also as elevation increases. Frost may occur for up to 100 days, with an increase in frequency at higher elevations. Snow may also occur for a number of days per year, particularly at higher elevations and near the edge of the Great Escarpment (Fig. 3.16).

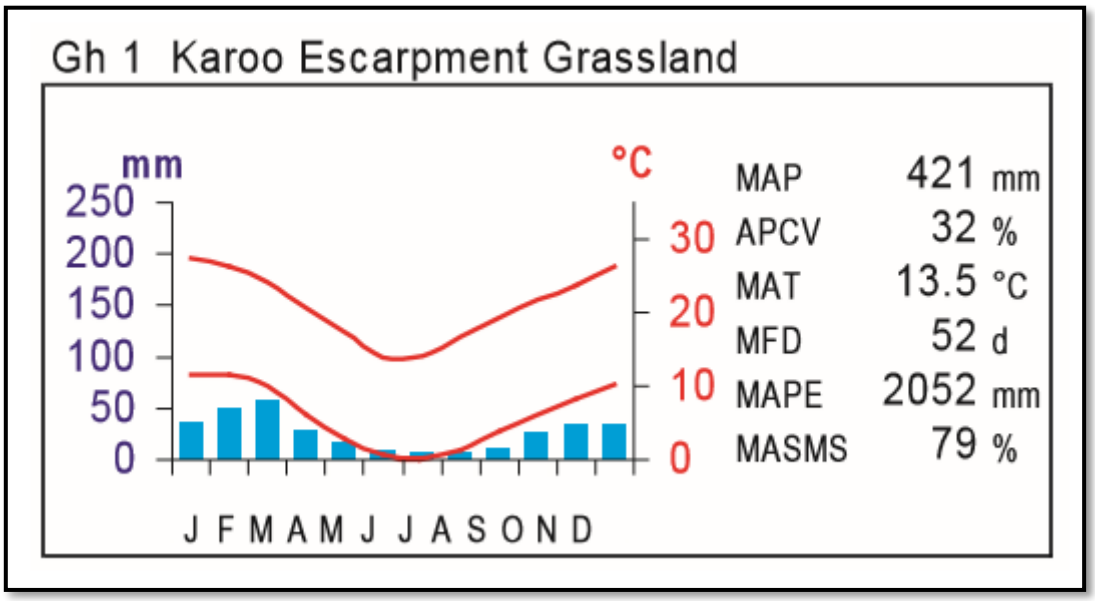


Figure 3.16. Climate diagram for Karoo Escarpment Grassland. Blue bars = median monthly precipitation. Upper and lower red lines = mean daily maximum and minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (Mucina et al., 2006b).

Plate 3.11. Bedford Dry Grassland vegetation in conserved (right) and degraded (left) areas (white tape demarcates the 25 m^2 quadrat).

Karoo Escarpment Grassland is considered **least threatened**. Approximately 3% is conserved in formal conservation areas, including the Mountain Zebra and Camdeboo National parks. Game farms and private reserves conserve an even higher percentage of this unit. Erosion is moderate to high (Mucina et al., 2006b).

The biogeographically important taxa (Table 3.11) and vegetation type endemics (Table 3.12) of the Karoo Escarpment Grassland are taken from Mucina et al. (2006b).

Table 3.11. Biogeographically important taxa found in the Karoo Escarpment Grassland (Mucina et al., 2006b).

Biogeographical importance	**Life form**	**Species**
Camdebo endemic	Succulent herb	*Duvalia modesta*
Link to Drakensberg Alpine Centre of Endemism	Graminoid	*Pentaschistis cirrhulosa* *P. microphylla*
	Low shrub	*Helichrysum sessile* *Pentzia cooperi*
	Succulent shrub	*Delosperma congestum*

Table 3.12. Endemic taxa found in the Karoo Escarpment Grassland (Mucina et al., 2006b).

Life form	**Species**
Graminoid	*Schoenoxiphium rufum* var. *dregeanum*
Herb	*Lithospermum diversifolium* *Wahlenbergia sphaerica*
Geophytic herb	*Kniphofia acraea* *Syringodea pulchella*
Low shrub	*Euryops dentatus* *E. trilobus* *Helichrysum scitulum* *Selago bolusii*
Succulent shrub	*Delosperma gramineum*

3.6 Materials and Methods

3.6.1 Study sites

Study areas were chosen based on potential fracking hotspots predicted through the research of the AEON-ESSRI Baseline Research Program at NMU (Figure 3.17). Within those areas, study sites were chosen to represent important vegetation types within the major biomes likely to be affected by shale gas development (Figure 3.2).

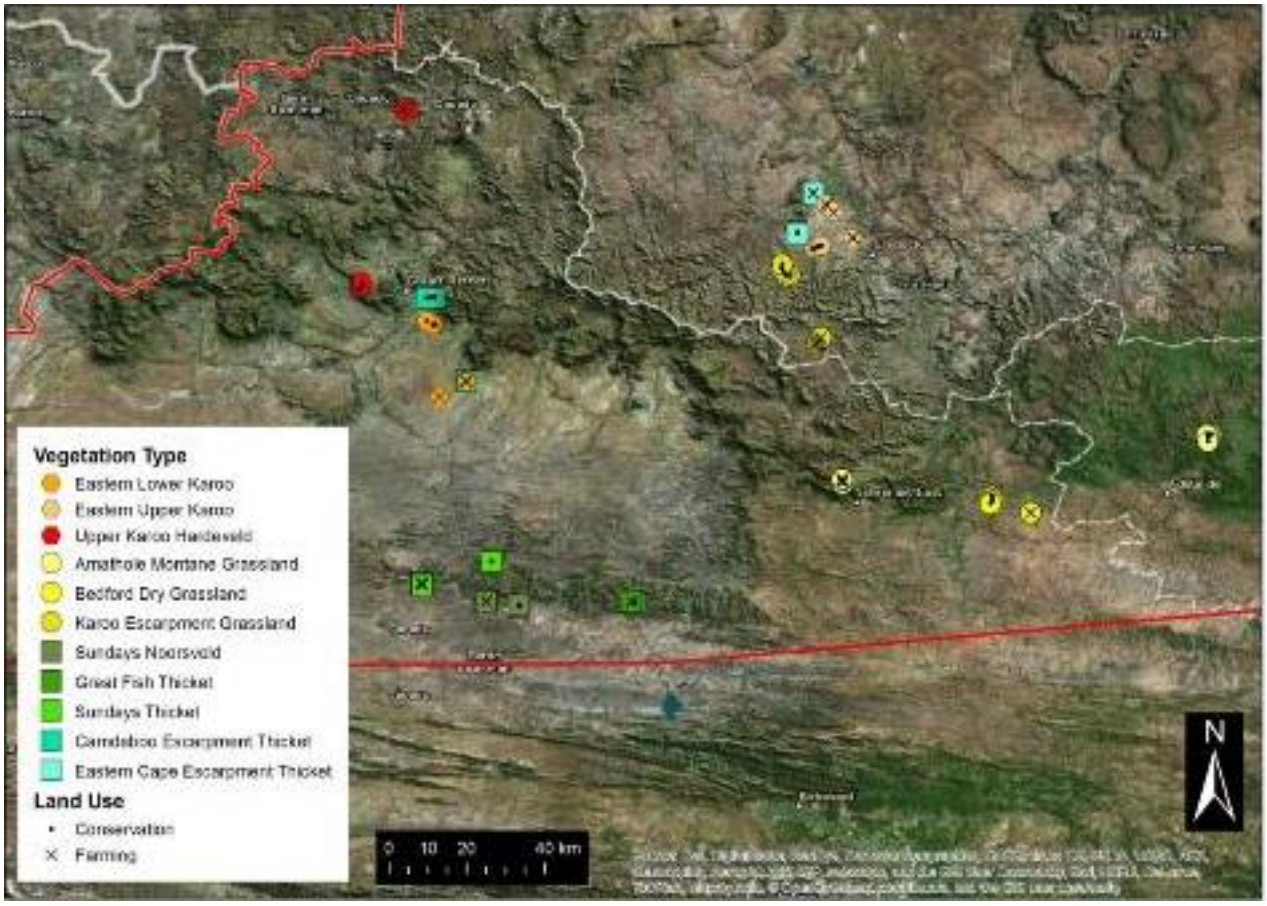


Figure 3.17. Study sites in the AEON-ESSRI Baseline study area.

Within each vegetation type two sites were chosen – one area with conservation land use (game effects) and one farm (livestock – identified through the presence of droppings). Currently, the only impacts on the vegetation are due to livestock farming activities. Permission to collect samples on the various farms was obtained by the Shale Gas Group who had already visited these farms to collect borehole water samples (Stroebel, pers. comm, 2014). The Mountain Zebra National Park and the Camdeboo National Park are SANParks owned and managed reserves. The appropriate collection permits are provided in Appendix A. The general Eastern Cape collection permit is also attached in Appendix A. Verbal permission was obtained from the Cookhouse Wind Farm.

The **Mountain Zebra National Park** is situated on the Northern Slopes of the Bankberg mountain range in the Eastern Cape (SANPARKS, 2008). The park was proclaimed in 1937 initially for the protection of a remnant population of the Cape Mountain Zebra, but it now serves the larger purpose of conserving the regional

biodiversity as a whole. The climate of the park is cool and arid with mostly summer rain, averaging 400 mm a year. The geology is dominated by sandstones, siltstones and mudstones of the Beaufort Series. In some areas doleritic intrusions are prevalent (SANPARKS, 2008). The park is a transitional area between the Grassland, Nama Karoo and Thicket biomes. The transition between these biomes results in wide variation in plant and animal species. The vegetation types protected within the Mountain Zebra Park are poorly protected elsewhere in South Africa (Driver et al., 2005), highlighting the park's vital role in the long term preservation of the local biodiversity. Pond et al. (2002) recorded a total of 680 plant species in the park, representing 333 genera and 87 families. Of these, thirteen species are Red Data species (Pond et al., 2002). The biggest threats to the park are climate change, development of conflicting land uses, mismanagement of large herbivores and fire (SANPARKS, 2008).

The **Camdeboo National Park** surrounds the town of Graaff-Reinet in the Eastern Cape (SANPARKS, 2013). The park had its beginnings in the Karoo Nature Park that had the Valley of Desolation as its core. It was proclaimed a provincial nature reserve in 1976. In 1993 the Karoo National Park and the Graaff-Reinet golf course were consolidated and the land was held in abeyance for a number of years. The park was officially proclaimed as Camdeboo National Park in 2005.

The climate of the park is semi-arid with rain falling mostly between February and April (SANPARKS, 2013). The average annual rainfall is 336 mm. Fog is common from February to April, and frost from April to September. The mountains in the park contain dolerite intrusions which affect the surrounding mudstones, siltstones and sandstones. The characteristic Karoo landscape of buttes and mesas is as a result of the erosion of these intrusions.

Three physiognomic classes of vegetation have been described in the park – Shrubland, Succulent Thicket and Dwarf Shrubland (SANPARKS, 2013). A total of 336 species representing 71 families have been recorded to date.

The **Boschberg Nature Reserve** is a local authority reserve owned by the Somerset East municipality. It is found on the slopes of the Boschberg Mountains above Somerset East (Clark et al., 2011). The reserve covers an area of ca. 2 050 ha and was established in 1937. It has been declared a water catchment area for Somerset

East since 1885, with numerous reservoirs on the reserve (Van der Walt, 1972). Sandstones and shales of the Beaufort Group dominate the geology (Clark et al., 2011) and the reserve receives 700 to 800 mm of rain annually, mostly in summer (Van der Walt, 1972; Clark et al., 2011). Agriculture in the form of sheep and cattle farming as well as infestations of alien species such as pine and wattle trees are the major threats to this reserve (Clark et al., 2011).

The **Cookhouse Wind Farm** is one of the largest wind farms in the country. It is situated near Cookhouse in the Eastern Cape, approximately 150 km from Port Elizabeth (Szewczuk, 2014). It is surrounded by Great Fish Thicket and Bedford Dry Grassland.

3.6.2 Methods

Five quadrats of 5 x 5 m (Rutherford & Westfall, 1986) were sampled within either conservation or farm sites, therefore a total of 10 quadrats were sampled per vegetation type. Quadrats were placed at least 100 m apart close to major roads to ensure easy access in order to monitor the plots at a later stage, should fracking commence.

In each quadrat, samples of all species were collected, and the percentage cover of each species visually estimated. All species found in the area surrounding the quadrat were also collected and they were recorded as present, but with no (0.01%) cover. Samples were collected during peak grass flowering times to ensure inflorescences were present for identification purposes.

Plant specimens were pressed and dried and identified in the Ria Olivier Herbarium, Nelson Mandela University, Port Elizabeth and the Selmar Schonland Herbarium, Grahamstown. Voucher specimens were deposited at the Ria Olivier Herbarium and voucher specimens collected in the SANParks were deposited at the Kimberley South African National Parks Herbarium. Nomenclature was after the Plants of South Africa Annotated Checklist (POSA, 2017). Red Data List status was determined using the SANBI Red List of South African Plants (SANBI, 2017). Those species that had approximately 80% or more of their distribution range within the bounds of the study area were considered to be Species of Conservation Concern (Holness et al., 2016; POSA, 2017).

Five soil samples were also collected in each of the quadrats using a hand-held spade. Soil was analysed for organic content and water holding capacity. Organic content was calculated using the loss on ignition method. An adaptation of Abella and Zimmer's (2007) method was used with ashing occurring at 550℃.

Water holding capacity was calculated using the Naeth et al. (1991) method.

3.6.3 Statistical analyses

Life form composition of the various plant communities was calculated by using Raunkiær's biological spectrum (Raunkiær, 1934).

Diversity was assessed using the following indices (Magurran, 2004):

Shannon-Weaver (*H'*) $H' = -\, pi \sum lnpi$

Pielou's evenness (*J*) $J = H'/ln(S)$

Beta diversity (β) $\beta = \gamma/\alpha$

where γ = gamma diversity and α = alpha diversity

The means of the diversity indices, soil organic content and water holding capacity were compared using either paired T tests or Wilcoxon paired-sample tests in R version 3.3.2 (R Core Team, 2016) and R-Studio (R Studio Team, 2015).

Phytosociological analysis was done using Detrended Correspondence Analysis (DCA) in R version 3.3.2 (R Core Team, 2016) and R-Studio (R Studio Team, 2015) using the package "Vegan" (Oksanen et al., 2017).

3.7 Results

3.7.1 Life form composition

When combining the conserved and degraded vegetation to compare the biomes, the observed life form compositions were similar to the expected life form compositions but did differ (Fig. 3.18). Nama-Karoo was dominated by chamaephytes and phanerophytes but hemicryptophytes and succulents also contributed substantially to the life form composition. Grassland was dominated by chamaephytes and to a lesser extent hemicryptophytes and forbs. Albany Thicket more or less equally dominated by succulents, chamaephytes, phanerophytes and hemicryptophytes.

Though life form composition was similar in the conserved and the degraded vegetation in all three of the biomes (Fig. 3.19), the richness of the taxa in the various life form categories changed (Fig. 3.20). In Nama-Karoo chamaephytes and phanerophytes dominated both the conserved and degraded vegetation but there was a decrease in the number of chamaephytic taxa in the degraded vegetation. Chamaephytes and hemicryptophytes were dominant in both degraded and conserved vegetation in Grassland. The number of taxa in the dominant life forms (chamaephytes and hemicryptophytes) dropped drastically (Fig. 3.20) with degradation and the reduction in the number of forb taxa with degradation was also large. In Albany Thicket there was a shift in the life form composition with degradation – phanerophytes, chamaephytes and succulents dominated the conserved vegetation whereas degraded thicket vegetation was dominated by succulents and chamaephytes. Succulence is however a problematic life form category as succulents may either be phanerophytes or chamaephytes so there might not be an actual shift in life form composition with degradation in Albany Thicket.

Life form composition was different when comparing “Core Thicket” and “Escarpment Thicket” (Fig. 3.21). “Core Thicket” was dominated by succulents and phanerophytes (similar to the expected life form composition of Thicket) whereas “Escarpment Thicket” was dominated by chamaephytes and hemicryptophytes which is the expected life form composition of Nama-Karoo vegetation.

3.7.2 Species richness

When combining conserved and degraded vegetation, the Grassland biome was found to have the highest species richness (Fig. 3.22 A, 18.7 ± 5.3 S.D.) and species richness was similar in Albany Thicket (17 ± 5.8 S.D.). Nama-Karoo had significantly lower species richness than the other two biomes (15.7 ± 4.2 S.D.; $t = 2.719$, $df = 9$, $p = 0.024$).

Degradation affected species richness in the biomes, with conserved vegetation in Nama-Karoo, Grassland and Albany Thicket being more species rich than degraded vegetation in these biomes (Fig. 3.22 B). Species richness did not differ significantly between the conserved and degraded vegetation in any of the biomes (Nama-Karoo: $t = 0.41$, $df = 4$, $p = 0.703$; Grassland: $t = 1.803$, $df = 4$, $p = 0.146$; Albany Thicket: $t = 1.007$, $df = 4$, $p = 0.371$). Nama-Karoo was not greatly affected by degradation, but

species richness decreased noticeably in Albany Thicket and even more so in Grassland.

“Core Thicket” and “Escarpment Thicket” did not have similar species richness (Fig. 3.22 C). Species richness was significantly higher in “Core Thicket” (18.1 ± 7 S.D.) than “Escarpment Thicket” (15.3 ± 3.8 S.D.; t = 4.045, df = 5, p = 0.01). The species richness of the “Core Thicket” was similar to the richness of Albany Thicket whereas “Escarpment Thicket” richness was closer to that of Nama-Karoo vegetation (Fig. 3.22 A).

3.7.3 Diversity and evenness

Nama-Karoo, Grassland and Albany Thicket vegetation was similar in diversity and evenness (Fig. 3.23 A) with no significant differences in diversity or evenness measures for the biomes.

Degradation had no effect on diversity in all three biomes (Fig. 3.23 B; Nama-Karoo: V = 0, p = 0.181; Grassland: V = 3, p = 0.999; Albany Thicket: V = 5, p = 0.625) and also had no effect on evenness of the vegetation (Nama-Karoo: V = 0, p = 0.181; Grassland: V = 3, p = 1; Albany Thicket: V = 5, p = 0.625).

Diversity and evenness was similar in the two thicket groups (Fig. 3.23 C; diversity: V = 14, p = 0.563; evenness: V = 11, p = 0.999).

3.7.4 Beta Diversity

Beta diversity was generally low in Albany Thicket. Nama-Karoo and Grassland had higher beta diversity. There were no statistically significant differences in beta diversity between the biomes (Grassland vs Nama-Karoo: V = 6, p = 0.402; Grassland vs Albany Thicket: V = 8, p = 0.049; Nama-Karoo vs Albany Thicket: V = 14, p = 0.193). Degradation also did not show a significant influence on beta diversity (Nama-Karoo: t = 0.025, df = 4, p = 0.982; “Core Thicket”: t = 0.701, df = 5, p = 0.515; Grassland: t = 2.086, df = 4, p = 0.105; “Escarpment Thicket”: t = 0.701, df = 5, p = 0.515).

3.7.5 Soil Organic Matter Content and Water Holding Capacity

Differences among biomes in soil organic matter content were not observable when conserved and degraded soils were combined (Fig. 3.25 A). All three biomes had very similar mean soil organic matter content and water holding capacity (Fig. 3.26A) and there were no statistically significant differences between the biomes (Grassland and

Nama-Karoo: V = 9, p = 0.834; Grassland and Albany Thicket: V = 14, p = 0.193; Nama-Karoo and Albany Thicket: V = 9, p = 0.064)..

In all three of the biomes degradation of the vegetation resulted in the soil having a lower organic content (Fig. 3.25 B). Though not statistically significant due to high variability (t = 1.119, df = 4, p = 0.326), this was most noticeable in the Nama-Karoo sites where the soil organic content in the conserved areas was 7.1% (± 5.8 S.D.) compared to 4.1% (± 1.4 S.D.) in the degraded areas in the biome. The water holding capacity became more variable under degradation (Fig. 3.26 B) and as a result did not differ significantly between the conserved and degraded soils (Nama-Karoo: t = -1.26, df = 4, p = 0.276; Grassland: t = 0.674, df = 4, p = 0.537; Albany Thicket: t = 0.356, df = 4, p = 0.74).

Soil organic content was similar in “Core Thicket” and “Escarpment Thicket” (Fig. 3.25 C). The “Escarpment Thicket” soils (6.1% ± 0.8 S.D.) were not significantly different from the “Core Thicket” soils (5.3% ± 4.4 S.D.) (V = 9, p = 0.844). Water holding capacity was also similar in the soils of the two thicket elements (Fig. 3.26 C) with “Core Thicket” at 54.1% ± 3.9 S.D. and the “Escarpment Thicket” soils 54.6% ± 9 S.D.) (V = 17, p = 0.219).

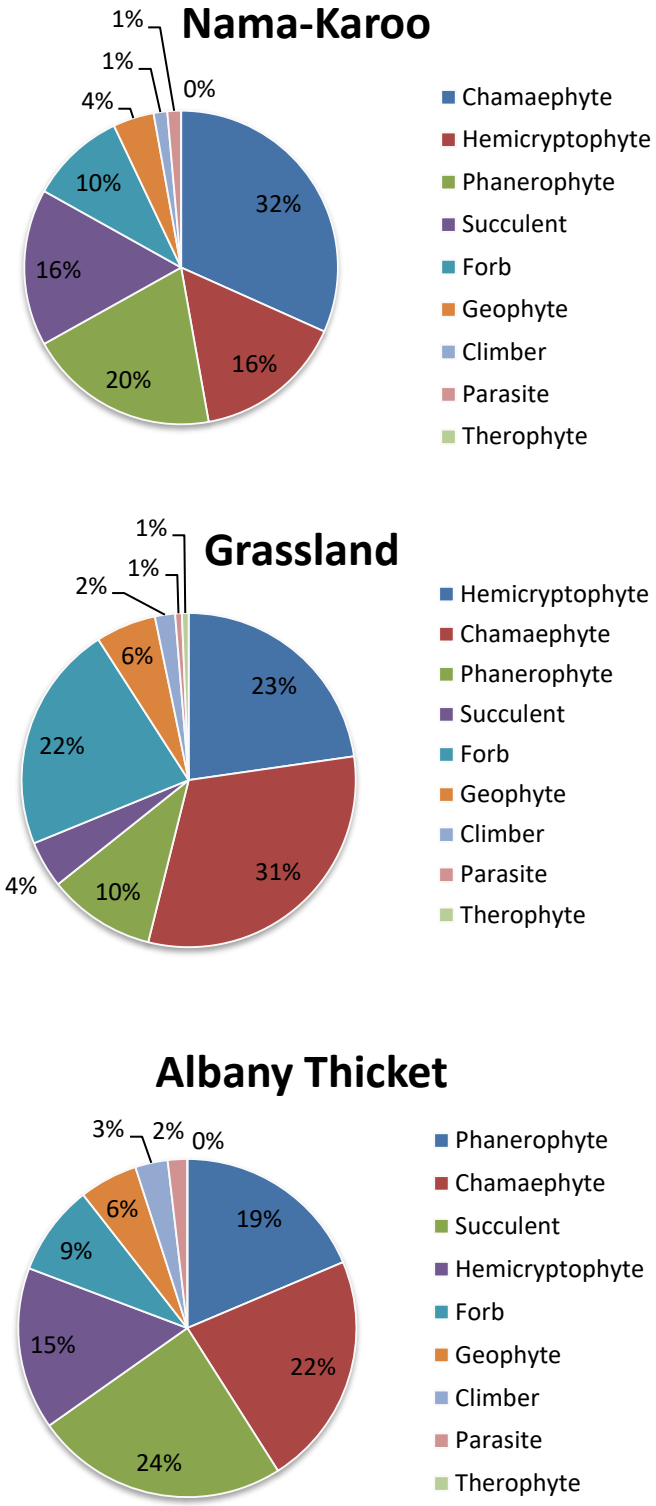


Figure 3.18. Life form composition (conserved and degraded vegetation combined) of Nama-Karoo (top), Grassland (middle) and Albany Thicket (bottom).

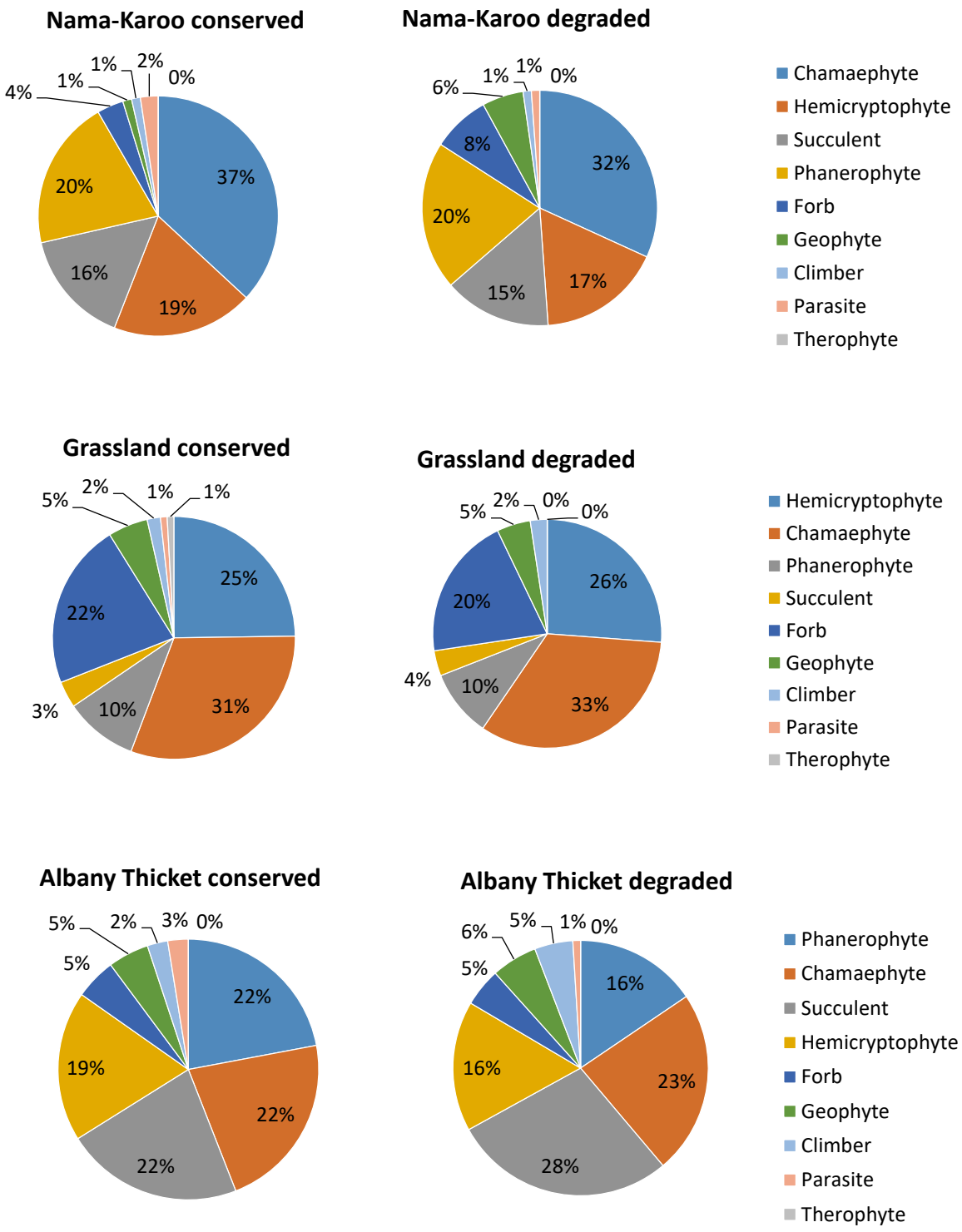


Figure 3.19. Life form composition differences in conserved and degraded vegetation in Nama-Karoo (top), Grassland (middle) and Albany Thicket (bottom).

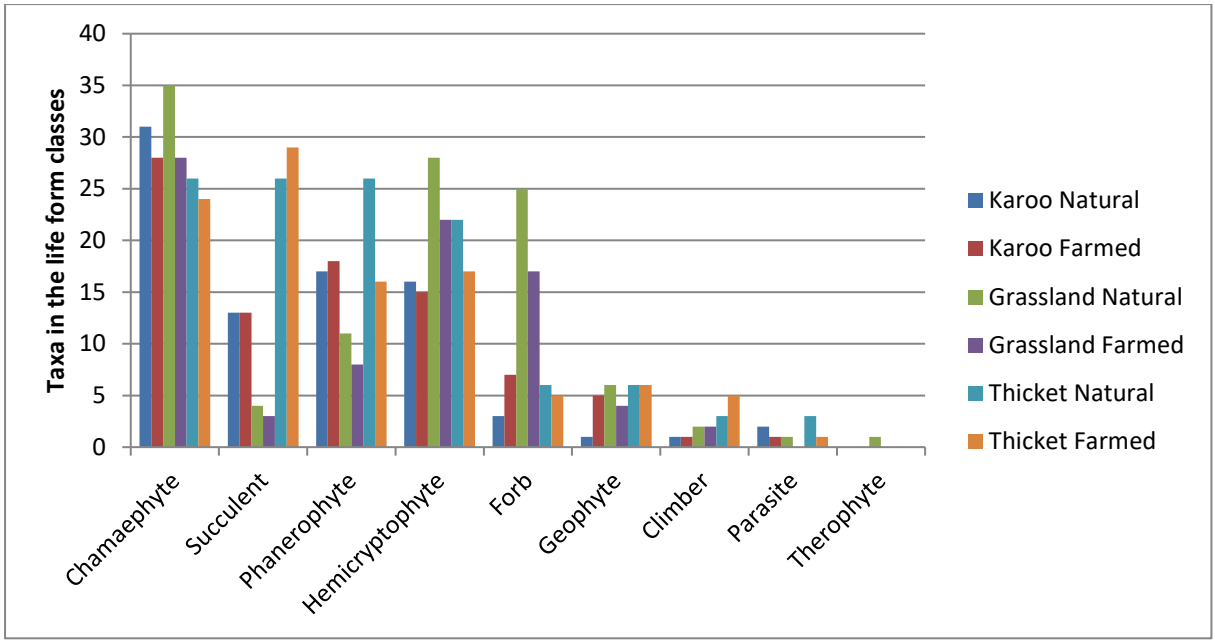


Figure 3.20. Differences in number of taxa in the life form classes in the biomes showing the effect of disturbance on species richness within the life form classes.

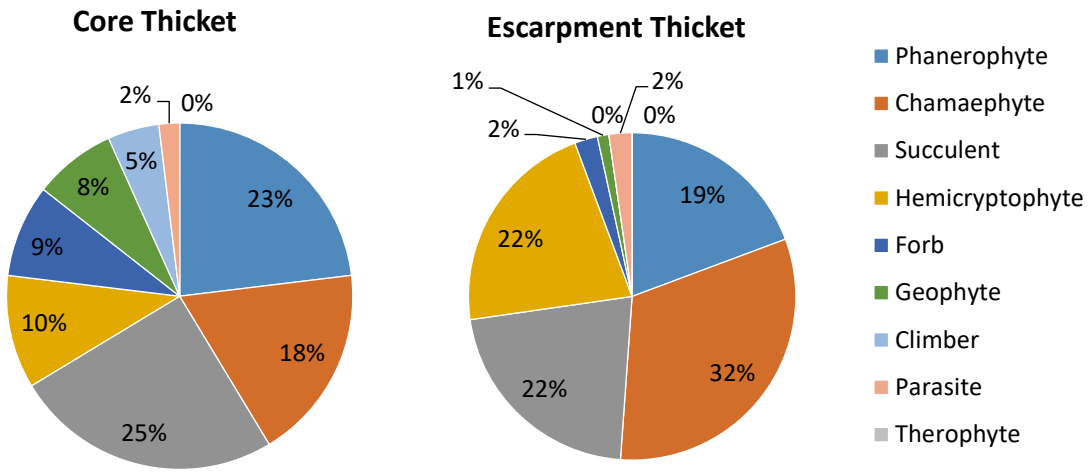
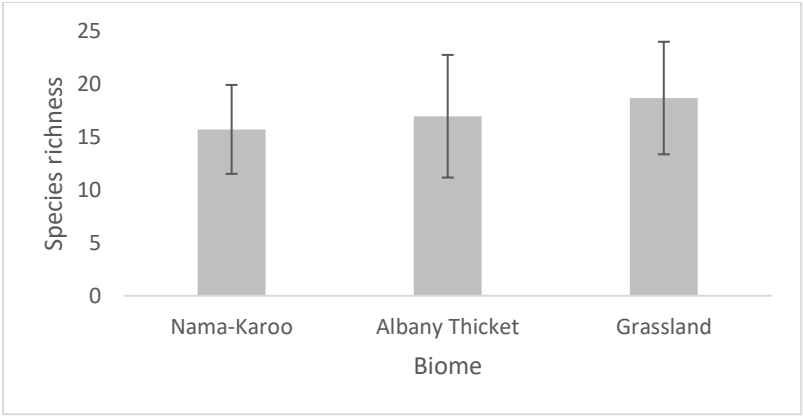
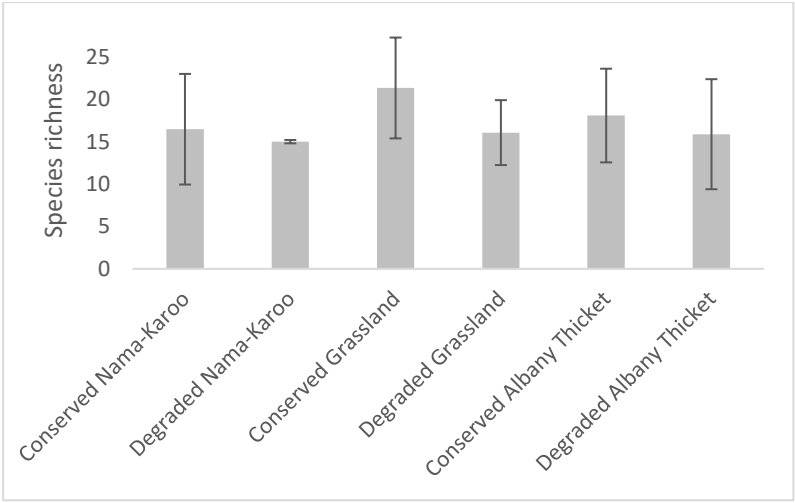


Figure 3.21. Life form composition of “Core Thicket” vegetation (Sundays Noorsveld, Sundays Thicket, and Great Fish Thicket; top) compared to “Escarpment Thicket” vegetation (Eastern Cape Escarpment Thicket and Camdebo Escarpment Thicket; bottom).

A



B



C

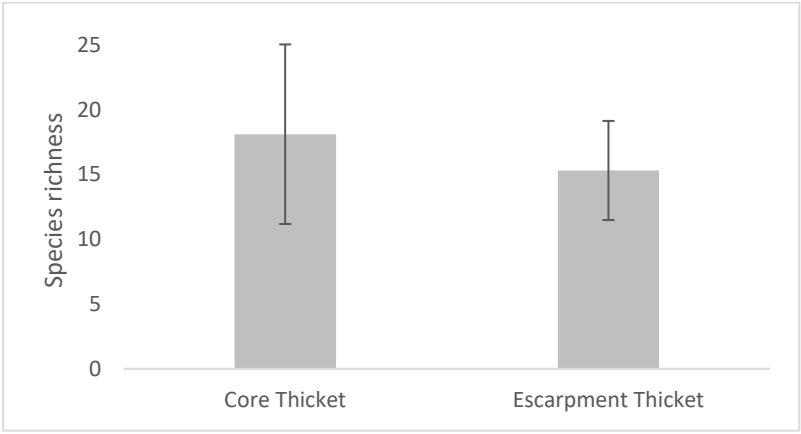
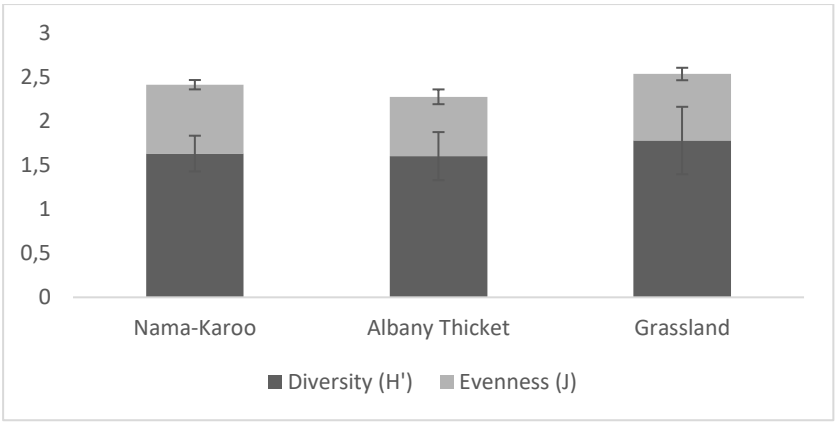
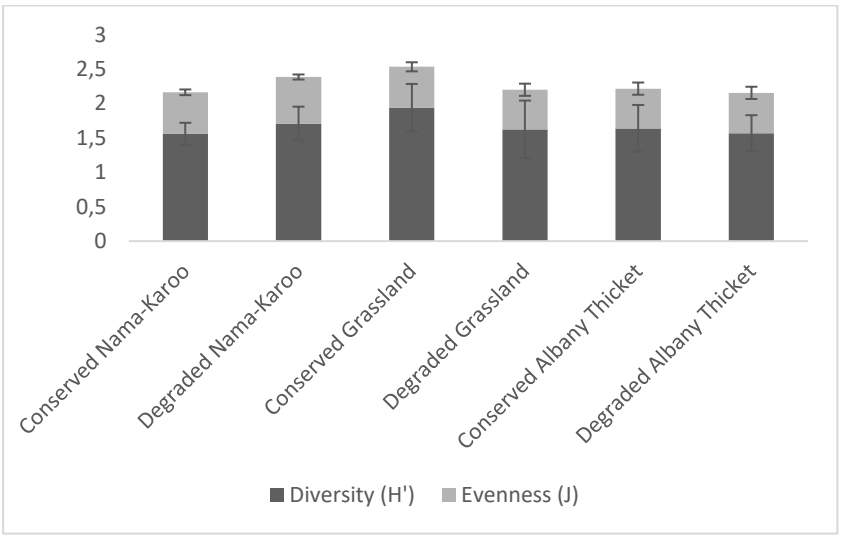


Figure 3.22. Comparison of species richness at the biome level (conserved and degraded vegetation combined; A), differences in species richness between conserved and degraded vegetation (B) and comparison of species richness in “Core Thicket” and “Escarpment Thicket” (C) (Vertical lines represent ± 1 S.D.).

A



B



C

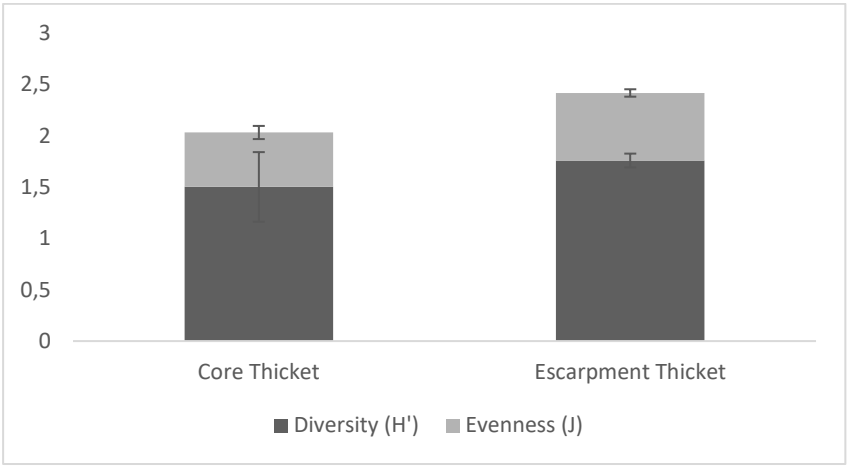


Figure 3.23. Comparison of diversity and evenness at the biome level (conserved and degraded vegetation combined; A), differences in diversity and evenness between conserved and degraded vegetation (B) and comparison of diversity and evenness in “Core Thicket” and “Escarpment Thicket” (C) (Vertical lines represent ± 1 S.D.).

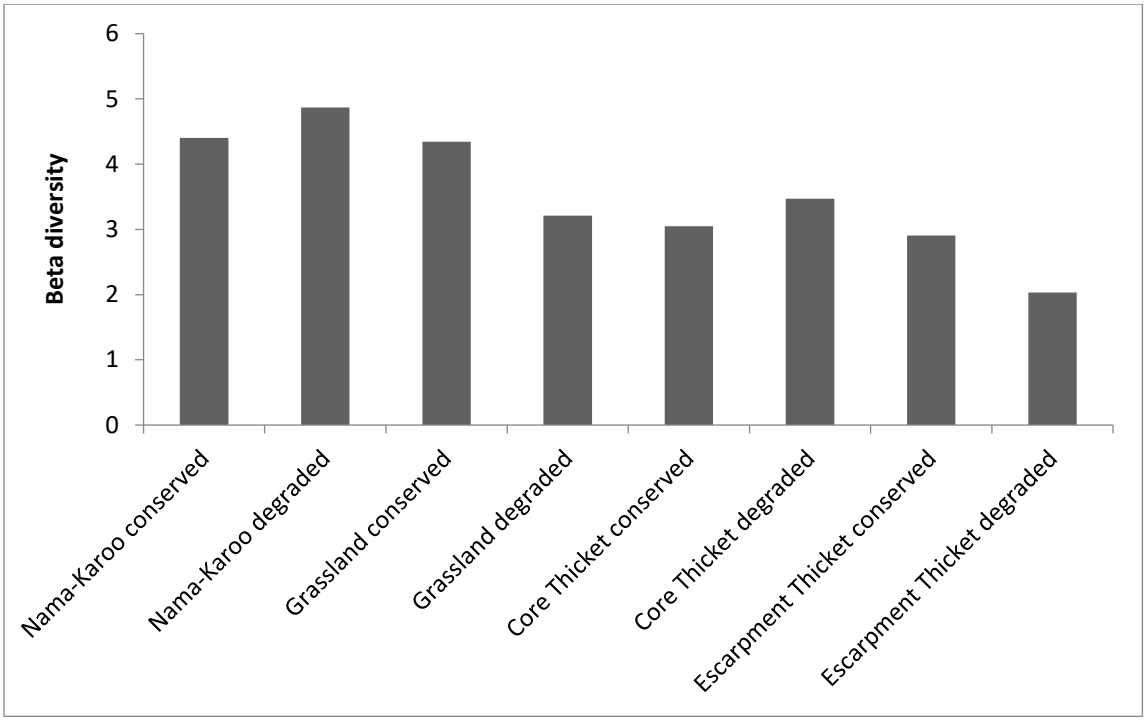
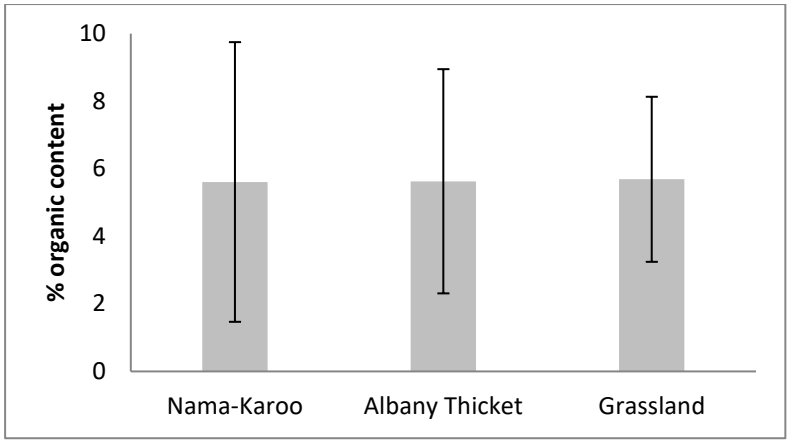
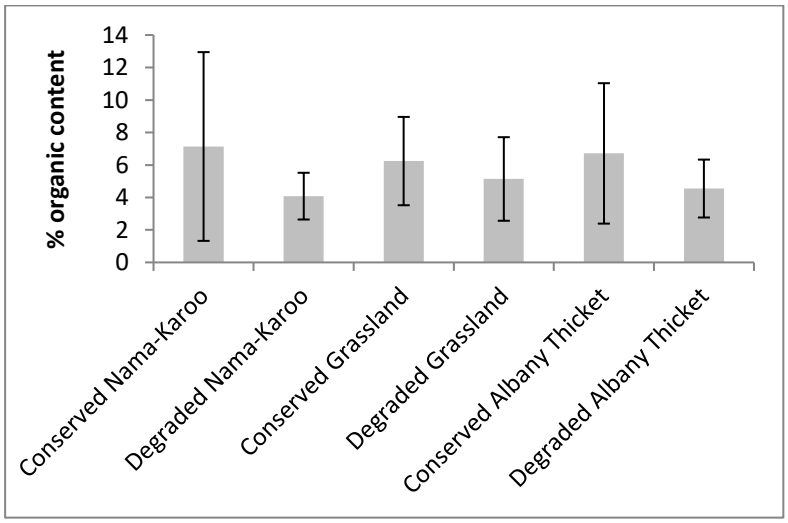


Figure 3.24. Comparison of beta diversity of conserved and degraded vegetation. "Core Thicket" and "Escarpment Thicket" were analysed separately, but both represent Albany Thicket.

A



B



C

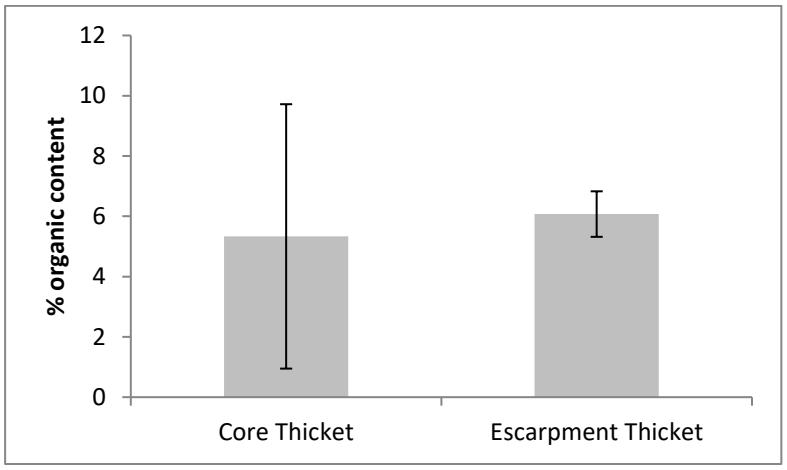
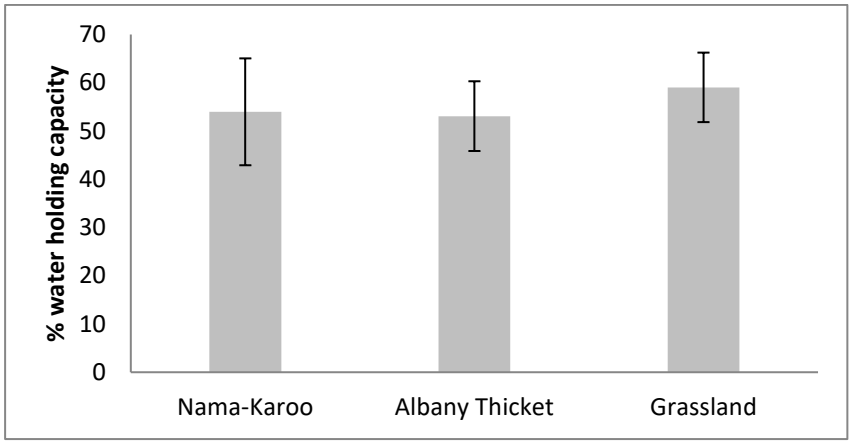
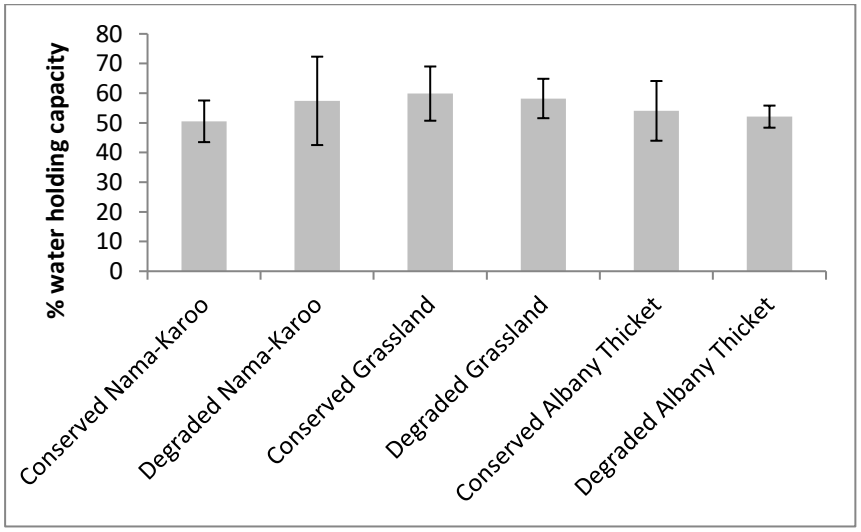


Figure 3.25. Soil organic matter (%) in the biomes (conserved and degraded vegetation combined; A), differences in soil organic matter (%) between conserved and degraded vegetation (B) and comparison of soil organic matter (%) in “Core Thicket” and “Escarpment Thicket” (C) (Vertical lines represent ± 1 S.D.).

A



B



C

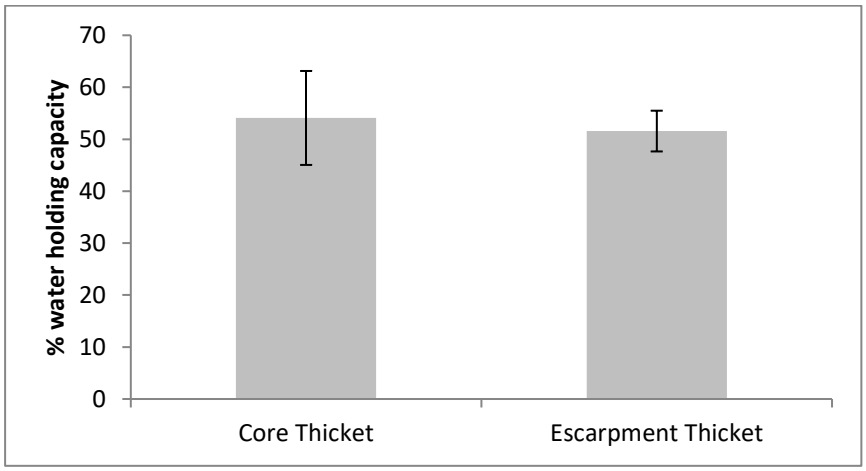


Figure 3.26. Water holding capacity (%) of the soils in the biomes (conserved and degraded vegetation combined; A), differences in water holding capacity (%) between conserved and degraded vegetation (B) and comparison of water holding capacity (%) in “Core Thicket” and “Escarpment Thicket” (C) (Vertical lines represent ± 1 S.D.).

3.7.6 Species of Conservation Concern

A full species list is given in Appendix B. A total of 320 species were collected in the study area. Only two Species of Conservation Concern (SCC) were identified in this study. *Ruschia vanderbergiae* L.Bolus was classified as a SCC based on 80% of its distribution range being within the study area. It is endemic to the Eastern Lower Karoo in the Nama-Karoo biome. Though it is considered Least Concern and its population is considered stable (according to the most recent Red List of South African Plants assessment by Burgoyne, 2006) it has a restricted range, warranting its designation as a Species of Conservation concern.

In terms of the Red Data List category, only one species was identified as a Species of Conservation concern. *Pelargonium reniforme* Curtis is classified as Near Threatened A4bd (Raimondo et al., 2012). Taxa in this category are at risk of extinction in the near future (SANBI, 2017). *P. reniforme* is a slow growing geophyte that is declining due to medicinal harvesting. *P. reniforme* is often harvested with *Pelargonium sidoides* DC. as the latter is targeted for medicinal use. These two species grow sympatrically in the Eastern Cape and *P. reniforme* is collected together with *P. sidoides*. *P. sidoides* looks almost identical to *P. reniforme*, hence the confusion, but *P. sidoides* is a common and widespread species (Raimondo et al., 2012).

Though our sampling only identified two SCCs, there are likely to be many more. Traditional sampling approaches, as used in this study, are not sufficient to find rare species (Guisan et al., 2006). The Red List of South African Plants (SANBI, 2017) does however list the following Species of Conservation for each of the vegetation types included in this study (Table 3.13).

Of the 320 species identified, the majority (282) are categorized as Least Concern (see Appendix B for details). One species was Not Evaluated (*Amaranthus dinteri* Schinz). Taxa that are either naturalized exotics, hybrids (natural or cultivated), or synonyms do not qualify for assessment (SANBI, 2017). Exotics and/or invasives made up the remainder of the species list (16 species).

Table 3.13. Species of Conservation Concern found in the study area listed for the vegetation types included in this study (SANBI, 2017).

Species	**Family**	**Status**	**Vegetation type & Biome**
Alepidea amatymbica Eckl. & Zeyh.	Apiaceae	Endangered A2d	Amathole Montane Grassland (Grassland)
Alepidea macowani Dummer	Apiaceae	Vulnerable A2ad; B1ab(v)	Amathole Montane Grassland (Grassland)
Bergeranthus albomarginatus A.P.Dold & S.A.Hammer	Aizoaceae	Vulnerable D2	Eastern Cape Escarpment Thicket (Albany Thicket)
Bergeranthus nanus A.P.Dold & S.A.Hammer	Aizoaceae	Vulnerable D2	Upper Karoo Hardveld, Eastern Upper Karoo (Nama-Karoo) and Karoo Escarpment Grassland (Grassland)
Bowiea volubilis Harv. ex Hook.f. subsp. *volubilis*	Hyacinthaceae	Vulnerable A2ad	Amathole Montane Grassland (Grassland)
Diascia ramosa Scott-Elliot	Scrophulariaceae	Vulnerable D2	Amathole Montane Grassland (Grassland)
Dierama grandiflorum G.J.Lewis	Iridaceae	Endangered B1ab(iii)	Amathole Montane Grassland and Karoo Escarpment Grassland (Grassland)
Disa lugens Bolus var. *lugens*	Orchidaceae	Vulnerable C2a(i)	Amathole Montane Grassland (Grassland)
Euphorbia jansenvillensis Nel	Euphorbiaceae	Vulnerable D2	Sundays Thicket
Euphorbia obesa Hook.f.	Euphorbiaceae	Endangered B1ab(iv,v)+2ab(iv,v); C2a(i)	Eastern Lower Karoo (Nama-Karoo)
Euphorbia polycephala Marloth	Euphorbiaceae	Vulnerable A2ac	Great Fish Thicket (Albany Thicket), Eastern Upper Karoo, Eastern Lower Karoo (Nama-Karoo) and Bedford Dry Grassland (Grassland)
Euryops dentatus B.Nord.	Asteraceae	Vulnerable D2	Eastern Upper Karoo (Nama-Karoo) and Karoo Escarpment Grassland (Grassland)
Haworthia aristata Haw.	Asphodelaceae	Endangered B1ab(i,ii,iii,iv,v)	Sundays Thicket, Eastern Cape Escarpment Thicket and Camdebo Escarpment Thicket (Albany Thicket)
Hesperantha helmei Goldblatt & J.C.Manning	Iridaceae	Rare	Karoo Escarpment Grassland (Grassland)
Indigofera asantasanensis Schrire & V.R.Clark	Fabaceae	Vulnerable D2	Karoo Escarpment Grassland (Grassland)
Nerine huttoniae Schönland	Amaryllidaceae	Vulnerable B1ab(iii,v)	Eastern Upper Karoo (Nama-Karoo)
Rhinephyllum inaequale L.Bolus	Aizoaceae	Endangered 1ab(iii,v)+2ab(iii,v)	Eastern Lower Karoo (Nama-Karoo)

3.7.7 Vegetation community analysis

For the broad scale analysis of the plant communities in the fracking footprint, only conserved vegetation was taken into account. Figure 3.27 below shows the Detrended Correspondence Analysis of the "natural" Karoo plant communities. Three communities are evident – Grassland (comprising Amathole Montane Grassland and Bedford Dry Grassland), Nama-Karoo (comprising the expected Nama-Karoo vegetation types Upper Karoo Hardveld and Eastern Upper Karoo but also including the Karoo Escarpment Grassland and the "Escarpment Thicket" elements Camdebo Escarpment Thicket and Eastern Cape Escarpment Thicket) and the "Core Thicket" community (comprising Great Fish Thicket, Sundays Thicket and Sundays Noorsveld). The Eastern Lower Karoo (a Nama-Karoo unit) formed part of the Nama-Karoo community but was not as closely clustered.

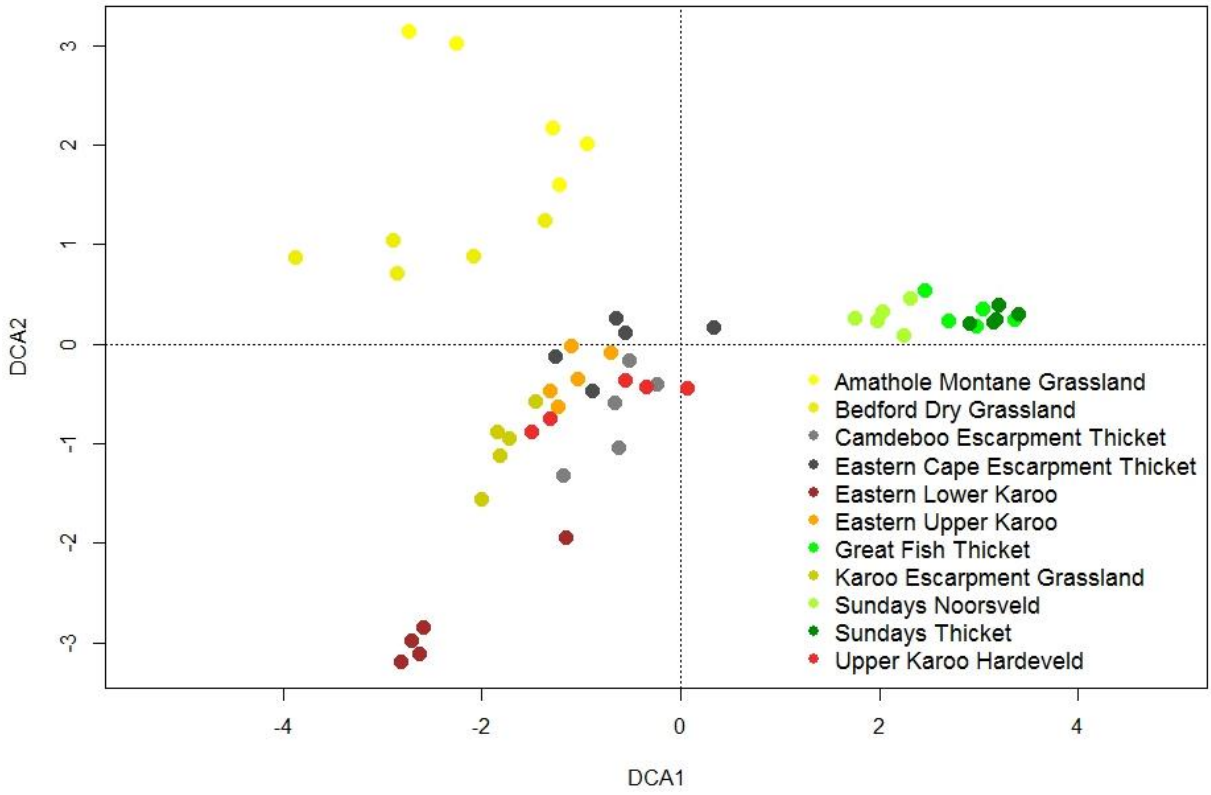


Figure 3.27. Detrended Correspondence Analysis (DCA) of plant communities within the study site. The sum of the eigenvalues is 1.609 and the cumulative percentage variance is 12.76.

In the Nama-Karoo, the conserved and degraded communities were similar to each other in all of the vegetation types (Fig. 3.28). There was no overlap in the domains of the vegetation types

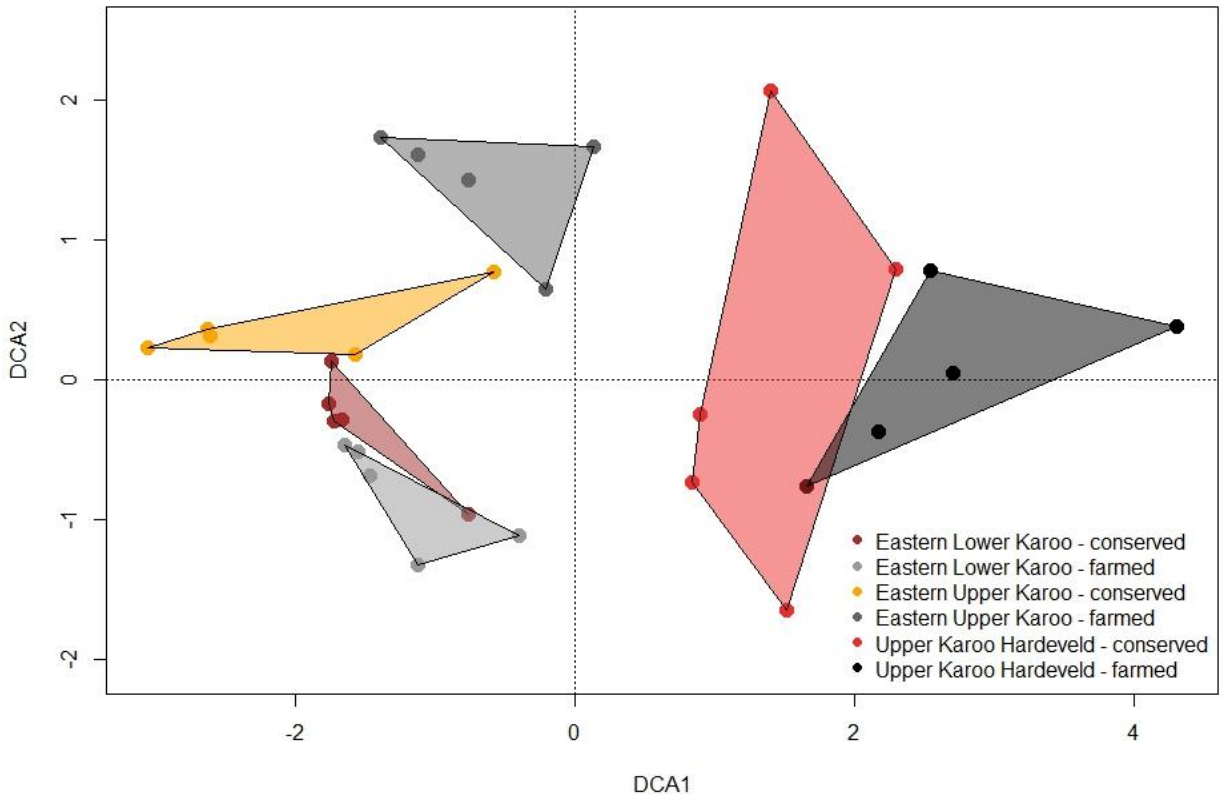


Figure 3.28. Detrended Correspondence Analysis (DCA) of Nama-Karoo communities within the study site. The sum of the eigenvalues is 1.379 and the cumulative percentage variance is 19.81.

In the Grassland vegetation types communities responded differently to degradation (Fig. 3.29). The conserved and degraded communities were very similar in Bedford Dry Grassland with some domain overlap, less similar in Karoo Escarpment Grassland and dissimilar in Amathole Montane Grassland.

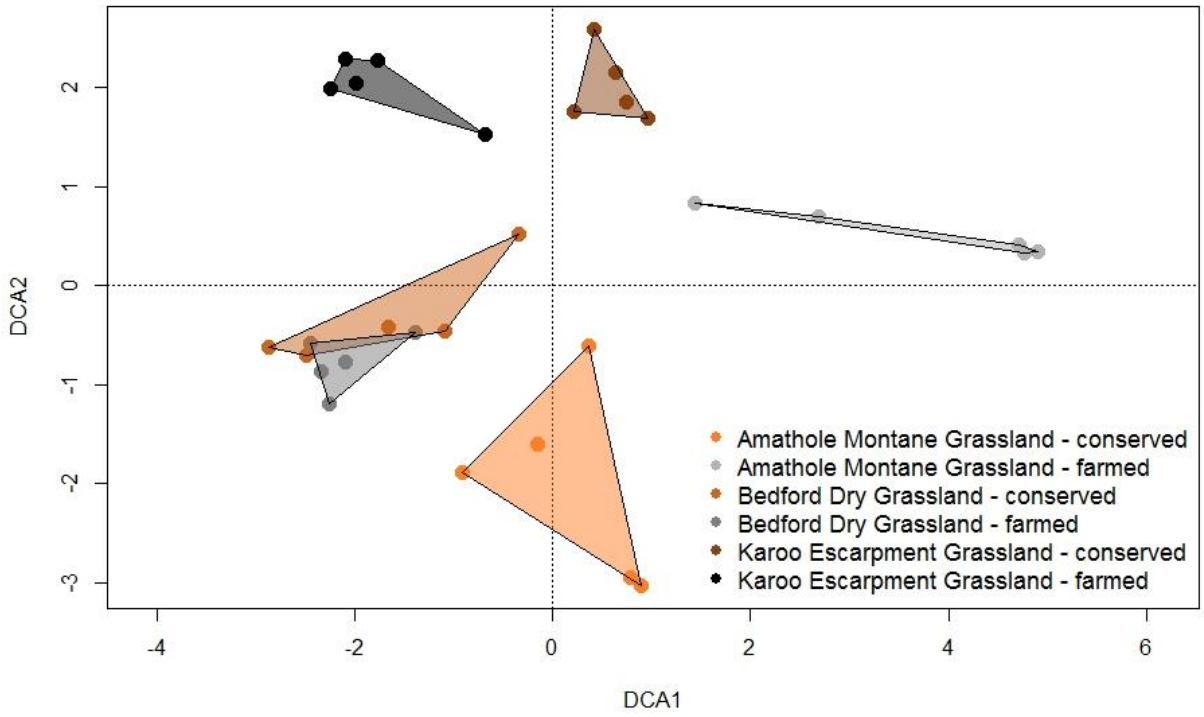


Figure 3.29. Detrended Correspondence Analysis (DCA) of Grassland communities within the study site. The sum of the eigenvalues is 1.648 and the cumulative percentage variance is 21.4.

In “Core Thicket”, the degraded communities of the Sundays Noorsveld were similar to the conserved communities. For Great Fish Thicket and Sundays Thicket the degraded and conserved communities were very dissimilar. The conserved communities of the Great Fish Thicket and Sundays Thicket were similar, as were the degraded communities (Fig. 3.30).

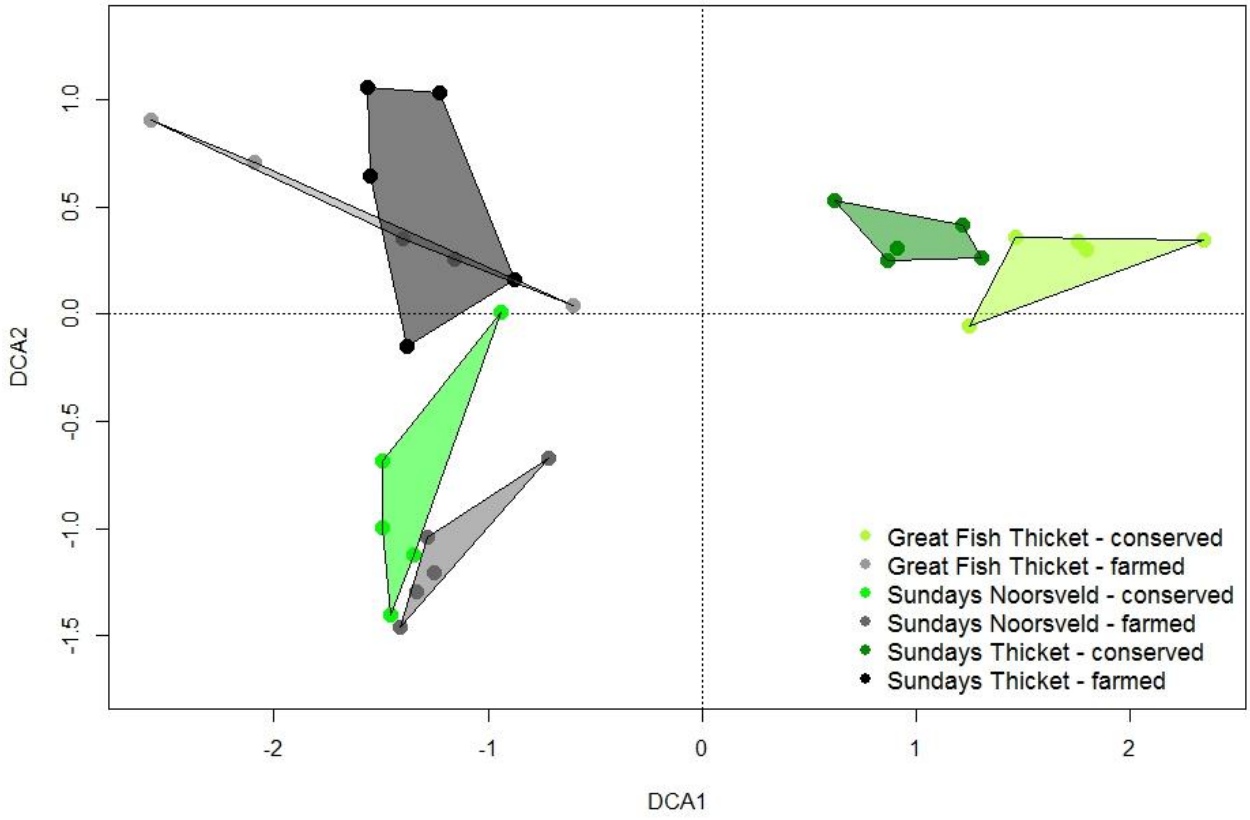


Figure 3.30. Detrended Correspondence Analysis (DCA) of “Core Thicket” communities within the study site. The sum of the eigenvalues is 1.232 and the cumulative percentage variance is 31.73.

In the “Escarpment Thicket”, there was clear separation of the conserved and degraded communities in the Camdebo Escarpment Thicket; whereas the conserved and degraded communities were very similar in the Eastern Cape Escarpment Thicket (Fig. 3.31).

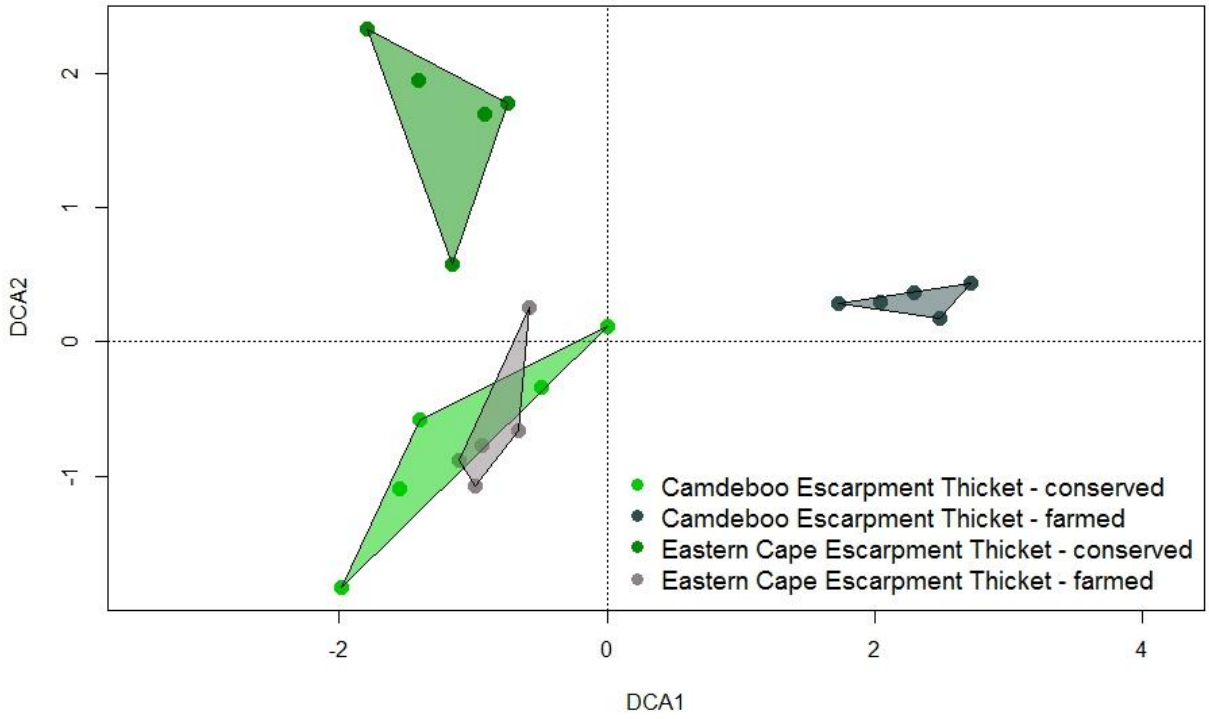


Figure 3.31. Detrended Correspondence Analysis (DCA) of “Escarpment Thicket” communities within the study site. The sum of the eigenvalues is 0.695 and the cumulative percentage variance is 27.87.

3.8 Discussion

Life form composition

Life form composition of the vegetation within the fracking footprint differed slightly from what was expected. The dominant life forms in Thicket are phanerophytes and chamaephytes (Rutherford & Westfall, 1986; Everard, 1991), hemicryptophytes in Grassland, and chamaephytes and hemicryptophytes in Nama-Karoo (Gibbs Russel, 1987). The dominant life form in the Nama-Karoo vegetation was indeed chamaephytes but phanerophytes were slightly more important than hemicryptophytes. The vegetation of the Nama-Karoo exhibits a high diversity of life forms most likely as a result of varied ecotonal and climatic conditions across the region (Cowling et al., 1994). Rainfall also appears to have a considerable impact on the vegetation structure across the region (Hoffmann et al., 1990; Cowling et al., 1994; Kellner & Booysen, 1999). Succulents, chamaephytes and C_3 grasses dominate after winter rains whereas C_4 grasses are more dominant after summer rains. The rainfall regime in the year that the samples were collected is therefore also likely to have affected the life form composition. Life form composition was quite different from expected in Grassland. Hemicryptophytes did not dominate, but did contribute considerably to the life form composition. The vegetation was dominated by chamaephytes; and forbs became more dominant in Grassland than in the other biomes. Bush encroachment (more common in degraded vegetation) could explain a greater low woody shrub component in the Grassland vegetation (Meadows & Hoffman, 2002). The life form composition of Grassland may also be affected by rainfall, grazing and fire (Mucina et al., 2006b). Phanerophytes and chamaephytes were expected to dominate the Albany Thicket vegetation. This was partly true; succulents and chamaephytes were slightly more dominant than phanerophytes and hemicryptophytes but the latter did contribute substantially to the overall life form composition of Albany Thicket. The succulent life form category can be misleading when considering total life form composition; succulents are in fact either phanerophytes or chamaephytes, depending on their size. A wide range of life forms and a combination of succulent and non-succulent plants was expected in the Albany Thicket (Hoare et al., 2006). Life form composition was expected to differ between degraded sites and control sites. An increase in annuals and geophytes and a decrease in shrubs was anticipated in the degraded sites (Todd & Hoffmann, 1999).

In this study, grazing was used as a measure of degradation, but the degradation from shale gas development is expected to have an even greater impact on the vegetation (complete loss of vegetation in certain areas vs. damage to plants from grazing). While degradation did not alter life form composition of the biomes in terms of dominance, there was a decrease in the number of taxa within those life form categories. Degradation resulted in a reduction in the diversity of life forms, particularly in Albany Thicket and even more so in Grassland. These results suggest that the life form composition of Nama-Karoo is less likely to be affected by fracking disturbance, Albany Thicket is likely to be affected and Grassland life form composition would be the most affected by degradation. The life form composition of Camdebo Escarpment Thicket and Eastern Cape Escarpment Thicket was more similar to that of Nama-Karoo vegetation than that of Albany Thicket vegetation. It has been suggested that Camdebo Escarpment Thicket is a marginal Nama-Karoo vegetation type rather than an Albany Thicket element (Hoare et al., 2006) and these results support that notion. This implies that “Escarpment Thicket” will behave more like Nama-Karoo, and “Core Thicket” will behave as Albany Thicket under the shale gas development degradation scenario.

Species richness

Species richness was similar in the three biomes, but Nama-Karoo had the lowest number of species. Species richness was not expected to be particularly high in the Nama-Karoo sites (Mucina et al., 2006a) but higher richness was expected in the Grassland (Cowling et al., 1989; Mucina et al., 2006b) and Albany Thicket sites (Cowling, 1983). Greater species richness may increase the stability of ecosystems (Tilman & Downing, 1994; Tilman et al., 1996). The Nama-Karoo with its low species richness may therefore be susceptible to loss of ecosystem function should shale gas development go ahead in these areas. Higher species richness was observed in the conserved vegetation than in the degraded vegetation in all three biomes. Degradation may either increase or decrease species richness; studies have shown that either may occur (as discussed in the introduction) so mixed results were expected. Lower species richness with degradation has been shown for Albany Thicket (Rutherford et al., 2012b) and Grassland (Milchunas et al., 1988 & 1998; Dorrough et al., 2007) while studies have shown an increase of species richness in degraded Nama-Karoo (Dreber & Esler, 2011). The difference in species richness between conserved and degraded

vegetation was most noticeable in the Albany Thicket and even more so in the Grassland vegetation whereas species richness did not change much with degradation in the Nama-Karoo. This implies that Grasslands in particular and Albany Thicket to a lesser degree would be less resilient to degradation from shale gas development than Nama-Karoo. Species richness was lower in the “Escarpment Thicket” than the “Core Thicket” and closer to the species richness observed in the Nama-Karoo vegetation. This provides more evidence that the “Escarpment Thicket” is a Nama-Karoo vegetation type that belongs to the Albany Thicket biome.

Diversity and evenness

Diversity and evenness were similar between the three biomes. Grassland and Albany Thicket are considered to have high diversity (Mucina et al., 2006b and Hoare et al., 2006 respectively) while the Nama-Karoo is not considered to be particularly diverse (Mucina et al., 2006a). Diversity was similar in the three biomes and Nama-Karoo diversity was comparable with the other two biomes. The inclusion of the highly diverse Upper Karoo Hardeveld (Mucina et al., 2006a) perhaps explains the higher than expected diversity of the Nama-Karoo. Diversity and evenness was slightly higher in the “Escarpment Thicket” than in the “Core Thicket”.

Degradation was expected to affect the diversity of the vegetation either increasing (Dreber & Esler, 2011; Rutherford et al., 2012a) or decreasing the diversity (Milchunas et al., 1988 & 1998; Todd, 2006; Dorrough et al., 2007). Under degraded conditions, there was a reduction in diversity in Grasslands and Albany Thicket but an increase in Nama-Karoo. These results imply that degradation is most likely to have the greatest effect on diversity of Grassland vegetation. Albany Thicket is also susceptible to a reduction in diversity with degradation, but Nama-Karoo vegetation appears to be less effected by degradation.

Beta diversity

Beta diversity is generally higher in Grassland (Mucina et al., 2006b) and Nama-Karoo (Mucina et al., 2006a) than in Albany Thicket (Vass, 2005). Beta diversity may increase or decrease in response to farming, species invasions and urbanization (Socolar et al., 2016). Degradation in the Nama-Karoo and “Core Thicket” increased the beta diversity in this study. The increase in beta diversity may be due to the replacement of vegetation type-specific species with more generalized weedy species

(Rutherford et al., 2012b). In both Grassland and “Escarpment” Thicket, beta diversity decreased with degradation.

Species of Conservation Concern

Only two Species of Conservation Concern were identified in the study area. There are most likely many more (see Table 3.13) but traditional sampling methods are not likely to detect them in such vast landscapes with many microhabitats. Large, randomly chosen sampling sites are unlikely to have rare species, and targeted, specialized sampling will be required when looking for rare species (Guisan et al., 2006). These species are at high risk should fracking go ahead. The clearing of land for fracking well pads may reduce or eliminate populations of range-restricted or rare species.

Soil organic content

At a biome level, soil organic content was similar, with no significant differences between the three biomes. Soil organic content was expected to be higher in Albany Thicket. Lechmere-Oertel et al. (2008) and Mills and Fey (2004) attributed the high soil organic matter found in succulent thicket to the high litter production of *Portulacaria afra*. Soil organic content is strongly influenced by the rate of litter production, decomposition and incorporation into the soil (Lechmere-Oertel et al., 2008). *Portulacaria afra* is locally dominant in the Sundays Thicket (Hoare et al., 2006) explaining the higher organic content in Albany Thicket. High organic content levels were not expected for the Grassland and Nama-Karoo soils as arid and semi-arid regions generally have low soil organic contents (Carr et al., 2013). Vegetation in the Nama-Karoo tends to be sparse in comparison to other biomes and this lower input of leaf litter may be the cause of the lower organic content observed. Higher soil organic content was observed in the conserved soil which was expected; the removal of vegetation through the process of degradation is known to reduce the soil organic matter content (Lechmere-Oertel et al., 2008; Mills & Fey, 2003). Lower levels of leaf litter due to browsing/grazing or the clearing of land has detrimental effects as litter modifies the soil microclimate (Whitford, 2002). Leaf litter and soil organic matter are vital for the maintenance of productivity (Lechmere-Oertel et al., 2008), particularly in semi-arid ecosystems such as the Karoo. The construction of well pads and roads for

fracking will result in vegetation loss and therefore lower soil organic content in the degraded areas. These areas are likely to be less productive than non-fracked areas.

Soil water holding capacity

The water holding capacity of the soils of the three biomes was very similar. Water holding capacity and soil organic matter are closely related. Soil organic matter influences soil properties such as water retention (Mills & Fey, 2003 & 2004), directly affecting the water holding capacity of the soil. A reduction in soil organic matter is likely to result in a reduction in soil quality, including the water holding capacity (Smith et al., 1990; Mills & Fey, 2003). Due to the close relationship between soil organic matter and water-holding capacity, the water holding capacity of the soils of the three biomes was expected to be similar, due to their similar organic matter content. As with the effect of degradation of soil organic matter, the clearing of land for fracking is likely to reduce the water holding capacity of the soil with the resulting loss in ecosystem productivity.

Vegetation community analysis

The effect of degradation on plant communities was further elucidated through community analysis of the vegetation in the study site. In the majority of the vegetation types, the degraded community was dissimilar to the conserved community. This provides further evidence for the effect that shale gas development degradation might have on affected communities – not only will species richness and diversity be affected, but the species composition of communities is likely to change.

In a scientific assessment of the potential risks of shale gas development in the Karoo, Holness et al. (2016) also alluded to the variable response of the vegetation types to shale gas development disturbance. They also concluded that Nama-Karoo is likely to be more tolerant than Albany Thicket to degradation, but that the Karoo in general (taking all biomes into consideration) is sensitive to disturbance (Holness et al., 2016). Many of the Karoo shrub species are long-lived with infrequent recruitment and low rehabilitation success (Carrick & Kruger, 2007; Visser et al., 2004). Therefore, should these shrubs be cleared for the construction of roads or well pads, the rehabilitation of that landscape post-fracking is likely to be unsuccessful.

Another outcome of the community analysis is that Camdebo Escarpment Thicket and Eastern Cape Escarpment Thicket were shown to form part of the Nama-Karoo

community, rather than the “Core” Thicket community. Camdebo Escarpment Thicket and Eastern Cape Escarpment Thicket are closely related floristically and have similar vegetation structure (Hoare et al., 2006) so their grouping together was expected. Hoare et al. (2006) suggested that Camdebo Escarpment Thicket is rather a marginal Nama-Karoo unit than an Albany Thicket unit based on its overall floristic composition. Palmer et al. (1988) and Palmer (1991a, b) suggested that the dominance of *Portulacaria afra* in the Camdebo Escarpment Thicket was the main link to the Albany Thicket biome. Our data, however, suggest that the Camdebo Escarpment Thicket and the closely related Eastern Cape Escarpment are indeed rather Nama-Karoo elements rather than Albany Thicket elements. Hoare et al. (2006) attribute the thicket structure of the Camdebo Escarpment Thicket and the Eastern Cape Escarpment to adaptations related to geomorphology and associated microclimate. In terms of the linkage to the Albany Thicket through the presence of *P. afra*, its distribution has been linked to its ability to switch between C_3 and CAM photosynthesis during the extreme temperature and moisture variations present on the south-facing slopes in the Camdebo Escarpment Thicket and the Eastern Cape Escarpment (Guralnick & Ting, 1987).

3.9 Conclusion

The flora of the proposed fracking footprint considered in this study is diverse, spanning three biomes and eleven vegetation types. Though the biomes did not differ greatly from each other in terms of the diversity metrics, the differences between the conserved and degraded vegetation within the biomes gave an indication of what may be expected on degradation through shale gas development.

Although the dominant life forms in the respective biomes stayed more or less the same, the number of contributing taxa in the life form classes was reduced by degradation. The life form composition study did however indicate that Camdebo Escarpment Thicket and Eastern Cape Escarpment Thicket are Nama-Karoo rather than Albany Thicket elements. The life form composition of Albany Thicket is expected to change under the fracking scenario.

On a broad scale the biomes did not differ greatly in terms of diversity, but degradation resulted in a change in diversity. Overall, species richness, diversity and evenness were higher in vegetation within conservation areas.

Though not a direct relationship, there is some evidence that ecosystems with greater diversity and higher species richness may tolerate disturbance better than those with lower diversity and species richness (Elmqvist et al., 2003). One of the potential forms of degradation from shale gas development will be through complete loss of vegetation due to land clearing and the fragmentation of vegetation by the construction of roads. Many of the vegetation types were shown to be species rich and diverse, and as such a complete loss of this vegetation would be detrimental. Special attention needs to be paid to sites in which Species of Conservation Concern are found, particularly those sites where range restricted species occur. Though only two Species of Conservation Concern were found during sampling it is likely that many more occur within the fracking footprint.

The relationship between soil conditions and species diversity observed in this study has important implications for the vegetation under fracking conditions. The clearing of land for the construction of well pads will have cascading ecosystem effects; even once the site is decommissioned the soil will have reduced organic content, negatively affecting the diversity of plant life that may be able to establish on that soil.

The key findings of this chapter include the greater understanding of poorly understood vegetation in the fracking footprint and the potential effects that degradation through shale gas development (habitat loss through land clearing, loss of biodiversity and fragmentation of the landscape due to road construction) may have on the biodiversity and ecosystem functioning of the Karoo vegetation. Grasslands in particular appear to be least resilient to degradation. Albany Thicket would also be affected but not to the same extent as Grassland. Nama-Karoo vegetation is likely to be the least affected by degradation from shale gas development. The notion that Eastern Cape Escarpment Thicket and Camdebo Escarpment Thicket are Nama-Karoo elements rather than Albany Thicket elements is supported by the data in this study.

CHAPTER 4. THE EFFECT OF FRACKING FLUID ON THE GERMINATION OF KAROO AND THICKET SPECIES

4.1 Introduction

In a broad sense, germination is a term applied to seeds, spores and pollen indicating when these structures cease to be quiescent and reinitiate growth (Nonogaki et al., 2007). The survival of the embryo during the time from seed maturation and seedling establishment is made possible by the structure of the seed. Seeds are the structures that ensure the initiation of the next sporophyte generation of the parent plant (Koorneef et al., 2002). Physiologists, seed analysts and seed growers all have differing definitions of what they consider germination to be. Germination *sensu stricto* can be defined as “the period from the start of imbibition of water into the dry seed until the embryo (usually the radicle) first emerges from any tissues surrounding it” (Perino & Côme, 1991). Bewley and Black (1994) state that “germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis”. Both of these definitions highlight the importance of the process of imbibition for germination to occur. The emergence of the radicle is taken as a visual indication that germination is complete. The processes that follow visible germination, such as the mobilization of storage reserves, are associated with seedling growth rather than germination (Bewley, 1997).

Imbibition is required for seeds to germinate as the uptake of water during imbibition activates metabolic processes necessary for germination. Those seeds that have hard seed coats will remain quiescent until weathering of the seed coat allows for water penetration (Nonogaki et al., 2007). Seed germination is regulated by certain physiological properties of the seed. Seeds are enclosed in a testa that provides protection from environmental damage. However, the impermeability of the testa to water may delay imbibition and its mechanical strength may hinder radicle protrusion. Another tissue component of the seed that may restrict radicle penetration is the pericarp, an embryo-covering tissue (Ogawa & Iwabuchi, 2001). Seeds containing pericarps are commonly found in grasses and the Asteraceae and Apiaceae families (Nonogaki et al., 2007).

Seed dormancy is a means by which dry seeds can survive extended periods of unfavourable environmental conditions, to ensure germination under certain

conditions (Koorneef et al., 2002). Dormancy can be defined as “the failure of an intact viable seed to complete germination under favourable conditions and is controlled by several environmental factors such as light, temperature and the duration of seed storage” (Koorneef et al., 2002). There are two types of dormancy: coat enhanced dormancy and embryo dormancy. In coat enhanced dormancy the seeds do not germinate due to the constraints of its surrounding structures. Embryo dormancy is where the embryo itself is dormant within the seed (Bewley, 1997). Both dormancy and germination are complex processes, affected by numerous variables including both developmental and environmental factors. The seed structures and the factors that affect the growth of the embryo both affect germination and dormancy. Plant hormones have been shown to play a crucial role in both seed dormancy and germination, with abscisic acid being essential for inducing dormancy and gibberellin for germination (Koorneef et al. 2002). For germination to be complete, the embryo must receive environmental stimuli to signal the transduction chain resulting in metabolic and hormonal changes in the embryo. These events result in the emergence of the embryonic axis from the seed, which heralds the completion of germination (Bewley, 1997).

Growth of the embryo is essential for successful germination to occur. Embryo morphology varies widely at seed maturity ranging from rudimental to fully developed embryos (Nonogaki et al., 2007). Cell expansion due to water uptake is essential for the initiation of embryo growth (Nonogaki et al., 2007) and subsequent development into a mature plant.

There have been a limited number of studies on the effects of fracking fluid on vegetation. In a study by Adams (2011), concentrated hydrofracturing fluid sourced from a local gas well was applied directly to a stand of deciduous forest in West Virginia, USA. In this study, overdosing over a small area caused the understorey to die within days. The overstory trees lost their leaves approximately 10 days after application and 56% died within two years of the application. Due to the difference in vegetation, this experiment cannot be used to represent the effects of an accidental spill on Karoo and Thicket vegetation.

Application of drilling mud to plants was also tested (Takaki & Wolf, 2011), indicating that increased salinity was the most likely cause of the limitation of plant growth. Wolf & Brye (2012) hypothesized that plant growth in drilling-fluid-contaminated soil could

be increased by deep plowing and the addition of nutrients. However, in this study that the 20% mortality observed was considered to be as a result of increased soil salinity due to excess application of drilling fluids (Wolf & Brye, 2012). Although effects on plant growth have been investigated to some extent, none of these studies considered how seed germination would be affected. Various authors have reported a significant decrease in germination success in seeds grown in soils contaminated with heavy metals and other hazardous waste compounds (Chang et al., 1992; Maila & Cloete, 2002; Adam & Duncan, 2002).

This study aims to investigate the effect that contamination with fracking fluids would have on the germination rate and success in species commonly found within the areas earmarked for shale gas development in the Karoo.

4.2 Materials and Methods

Germination experiments are short-term studies that are useful in assessing toxicity effects in plants (Banks & Schultz, 2005). Germination bioassays are used to detect the presence of toxic substances by documenting the changes caused in an organism after exposure (Valerio et al., 2007). Bioassays are considered reliable, simple and cost-effective tests (Gustavson et al., 2000) and are often used to measure potential environmental risks (Gopalan, 1999). Lettuce seeds (*Lactuca sativa* L.) are routinely used in these bioassays (Gopalan, 1999).

Species for the germination trials were chosen based on the most common and important taxa listed in the study areas (Fig. 3.1) according to Mucina et al. (2006a) and Hoare et al. (2006). Representatives of each life form were included and lettuce seeds were used as a bioassay (Table 4.1). The species list was cross-checked on the SIBIS website (The South African National Biodiversity Institute's Integrated Biodiversity Information System) to ensure that the species were found in the focus areas of the study sites (SIBIS, 2014). Decisions were also affected by the commercial availability of seeds (seeds were not wild harvested but purchased from various seed suppliers).

Table 4.1. Life form categories with their species representatives chosen for germination trials.

Life form	**Species**
Tree / phreatophyte	*Vachellia karoo* (Hayne) Banfi & Gallaso
Low shrub	*Chrysocoma ciliata* L.
Succulent shrub	*Drosanthemum lique* (N.E.Br.) Schwantes
Graminoid	*Eragrostis curvula* (Schrad.) Nees
Herb	*Gazania krebsiana* Less.
Succulent tree	*Aloe ferox* Mill.
Bioassay	*Lactuca sativa* L.

As part of the germination trials, seeds were treated with fracking fluids of differing dilutions. The chemicals used in fracking fluids are tailored to the specific well to be fracked and the fracking fluid composition varies between different geologic formations (Steyl et al., 2012; FracFocus, 2014). A solution containing the most commonly used fracking chemicals (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013) was used to treat the seeds (Table 4.2).

Table 4.2. Chemical constituents of fracking fluids used in the germination trials. Their functions and volumes used for the various treatments are also given.

Chemical	**Function**	**% volume**	**Volume added to 10 L - neat**	**Volume added to 10 L - dilute**
Polyacrylamide	Friction reducer	0.05	5	0.0005
Methanol	Anti-microbial, corrosion inhibitor, stabilizer, friction reducer, surfactant	0.05	5	0.0005
Gluteraldehyde	Anti-microbial	0.05	5	0.0005
Ethylene Glycol	Scale inhibitor, stabilizer, friction reducer	0.05	5	0.0005
Sodium Chloride	Breaker	0.05	5	0.0005
Petroleum distillate	Carrier fluid	0.05	5	0.0005
Acetic Acid	Iron control, pH adjusting agent	0.05	5	0.0005
Ethanol	Surfactant	0.05	5	0.0005
Lauryl Sulfate	Surfactant	0.05	5	0.0005
Hydrochloric Acid	Helps dissolve minerals and initiate cracks in the rock	0.03	3	0.0003
Water	Carrier	94.52	9452	9999.4952
Sand	Proppant	5	500	0.5
Total volume		100	10000	10000

Two scenarios were simulated (Table 4.2) – a surface spill (concentrated/neat fracking fluids) and groundwater contamination (diluted fracking fluids). For the surface spill treatment, undiluted fracking fluids were applied to the dormant seeds. For the groundwater contamination treatment diluted fracking fluids were applied. The degree to which the fluids should be diluted was estimated based on studies of contaminated wells (Fontenot et al., 2013). The maximum methanol levels found in those contaminated wells was used as an indicator of how much of the contaminant could potentially be present in groundwater once the fracking fluid has been diluted in the aquifer and the groundwater has been contaminated. Calculating the dispersivity of contaminants in groundwater is problematic and requires detailed knowledge of the aquifer material, average aquifer thickness, hydraulic conductivity, porosity and flow configuration (Gelhar et al., 1992). This is likely to differ greatly over the entire Karoo basin, giving different contaminant dispersivity rates for different sites, hence the choice to base the dilution rate on published data from existing sampled wells. The control samples were watered with tap water. The salinity and pH of the various solutions were measured using a calibrated YSI Multiprobe.

Seeds were germinated according to each species' optimal germination instructions from the suppliers (taking into consideration imbibition requirements, soil and temperature preferences). *Vachellia karoo* seeds were soaked in hot water overnight, left to swell up, then sowed the following morning. The hairy fruits of *Gazania krebsiana* were mixed with sand before sowing. Seedling trays were set up in a glasshouse, with three seeds of each treatment in each tray. The position of the trays was shifted daily for randomization. The seeds were grown in the optimal medium as suggested by the seed suppliers. Samples being treated with neat fracking fluids were watered with the fluid at the appropriate solutions once only, to simulate a surface spill, and then watered with tap water subsequently. Samples treated with dilute fracking fluids were watered with the dilute fracking solution throughout the experiment, to simulate groundwater contamination.

Rate of germination was recorded (measured in days) as well as the percentage of seeds successfully germinated. The emergence of at least 1 mm of the radicle was taken as successful germination (Weiersbye & Witkowski, 2007).

The significance of treatment with fracking fluids on percentage germination and germination rate were tested using either a One-way Analysis of Variance followed by a TukeyHSD post hoc test or Kruskal Wallis Analysis of Variance followed by a Dunn post hoc test (Dinno, 2017) for each species. The differences between the means of salinity and pH were determined using a Student's t-test. All statistical analyses were done using R version 3.3.2 (R Core Team, 2016) and R-Studio (R Studio Team, 2015).

4.3 Results

Percentage germination was relatively good in all treatments and controls with the exception of *Chrysocoma ciliata* and *Eragrostis curvula* (Fig. 4.1). In *E. curvula* and *C. ciliata*, the seeds treated with neat fracking fluids did not germinate at all. Germination success was low in those species even in the control.

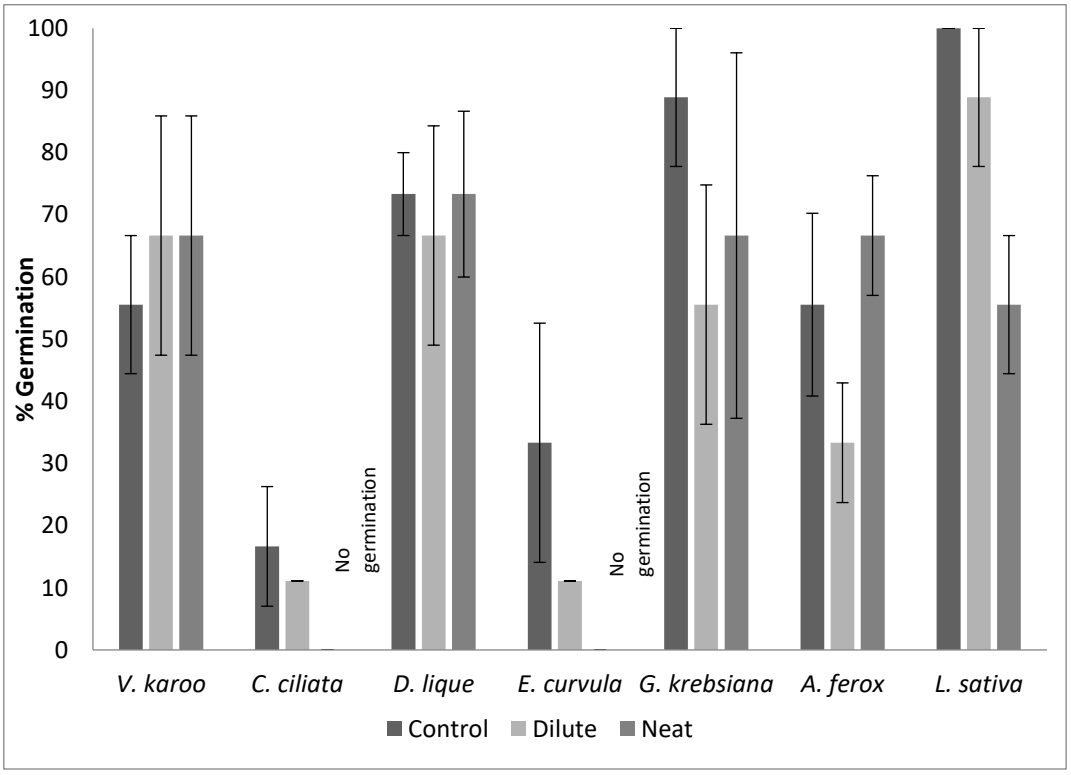


Figure 4.1. The average percentage germination of treated and non-treated seeds of *Vachellia karroo*, *Chrysocoma ciliata*, *Drosanthemum lique*, *Eragrostis curvula*, *Gazania krebsiana*, *Aloe ferox*, and the bioassay species, *Lactuca sativa* (Vertical lines represent ± 1 S.E.).

In the low shrub *Chrysocoma ciliata,* the grass *Eragrostis curvula,* the herb *Gazania krebsiana* and the bioassay species, *Lactuca sativa*, germination success was higher

in the control seeds though this difference was not significant (*C. ciliata*: H = 2.318, df = 2, p = 0.314; *E. curvula*: H = 2.889, df = 2, p = 0.236; *G. krebsiana*: H = 1.233, df = 2, p = 0.540; *L. sativa*: H = 2.154, df = 2, p = 0.056) . The germination success of the seeds of the tree, *Vachellia karroo,* was the same in seeds treated with dilute or neat fracking fluids and higher than in the seeds treated with water, but not significantly so (H = 0.305, df = 2, p = 0.859). Germination success in the succulent shrub, *Drosanthemum lique,* was the same in the control and neat treatments but lower in the dilute treatment though this difference was not significant (H = 0.321, df = 2, p = 0.852). For the succulent tree, *Aloe ferox*, seeds treated with neat fracking fluids had the highest germination success but not significantly so (F = 2.154, df = 2, p = 0.197).

For *Chrysocoma ciliata* and *Eragrostis curvula* germination was significantly slower when treated with fracking fluids (*C. ciliata*: H = 7.056. df = 2, p = 0.029; *E. curvula*: H = 6.054, df = 2, p = 0.050; Fig. 4.2). However, treatment with fracking fluids made no significant difference to the rate of germination in *Vachellia karroo* (H = 0.667, df = 2, p = 0.717)*, Drosanthemum lique* (F = 0.048, df = 2, p = 0.954)*, Gazania krebsiana* (F = 0.036, df = 2, p = 0.965), *Aloe ferox* (F = 0.142, df = 2, p = 0.871), and *Lactuca sativa* (H = 2.889, df = 2, p = 0.236).

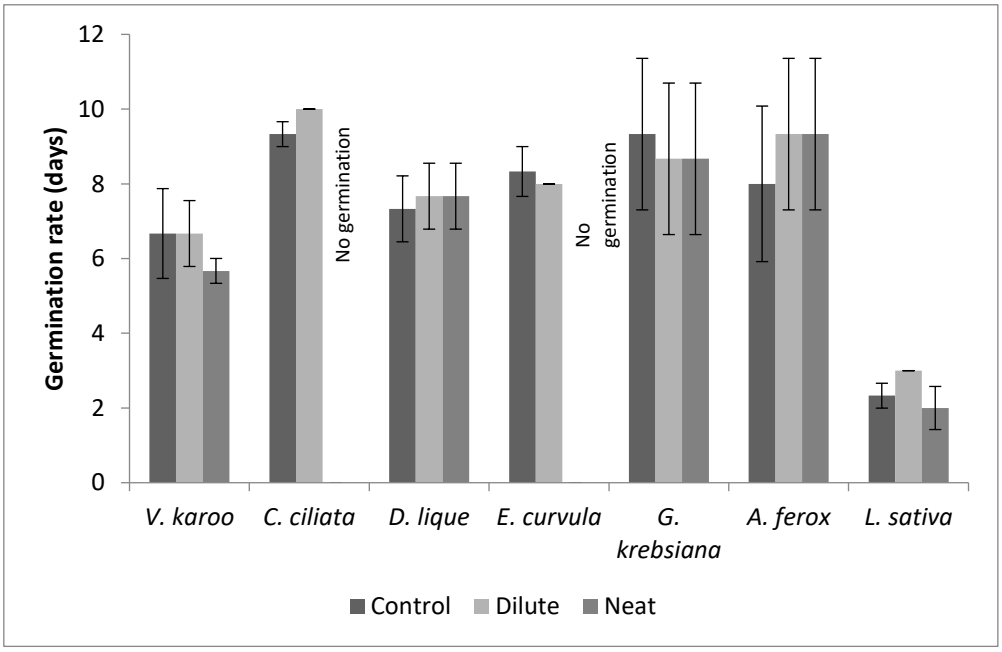


Figure 4.2. The average germination rate (in days ± 1 S.E.) of treated and non-treated seeds of *Vachellia karroo*, *Chrysocoma ciliata*, *Drosanthemum lique*, *Eragrostis curvula*, *Gazania krebsiana*, *Aloe ferox*, and the bioassay species, *Lactuca sativa*.

Salinity was high in the neat fracking fluids and these fluids are considered brackish. Tap water and the dilute fracking fluids had freshwater salinity levels. There was no significant difference in the salinity of tap water and the dilute fracking fluids ($t = 0.378$, $df = 2$, $p = 0.742$) or the tap water and the neat fracking fluids ($t = -0.989$, $df = 2$, $p = 0.427$). The pH of the tap water was slightly alkaline (Table 4.3) and fracking fluids reduced the pH to acidic (dilute fluid) and strongly acidic (neat fluid). The neat fluids were significantly more acidic than the dilute fluids ($t = 7.221$, $df = 2$, $p = 0.019$) and the tap water ($t = 152.88$. $df = 2$, $p < 0.05$). The dilute fluids were significantly more acidic than the tap water ($t = 11.806$, $df = 2$, $p = 0.007$).

Table 4.3. Average pH and salinity values for the various water treatments used.

	Control	Neat fracking fluids	Dilute fracking fluids
Salinity	0.007 ± 0.009	0.84 ± 0.589	0.003 ± 0.005
pH	8.76 ± 0.03	1.25 ± 0.1	4.32 ± 0.5

4.4 Discussion

In this study, fracking fluids had little influence on the majority of the species, implying that germination was initiated by imbibition of water irrespective of salinity, pH or toxic chemicals (Nonogaki et al., 2007).

The seeds of the low shrub (*Chrysocoma ciliata*) and the grass (*Eragrostis curvula*) generally had poor germination (in the control) and treatment with fracking fluids reduced germination success to the extent that, in the neat treatment, seeds did not germinate at all. The seeds of these two species also germinated at a significantly slower rate. The majority of Karoo vegetation is dominated by low shrubs and grasses (Mucina et al., 2006a) so these results have implications for the germination success of these plants or life forms in the fracking footprint. Though the neat fracking fluids were more much acidic and more saline than the tap water, the treatment was once off and therefore unlikely to explain the difference in germination success. A more likely cause is the contents of the fracking fluids. Ethylene glycol and lauryl sulphate have been shown to negatively affect germination (Endo et al., 1969; Bose & Datta,

1973; Bose & Bandyopadhyay, 1975; Bose & Naskar, 1975; Edwards et al., 2011). This could indicate that the seeds of low shrubs and grasses in particular are sensitive to the contents of the fracking fluids.

For the other life forms i.e. tree (*Vachellia karoo)*, succulent shrub (*Drosanthemum lique)*, herb (*Gazania krebsiana)*, succulent tree (*Aloe ferox)* and the bioassay species (*Lactuca sativa* L.) germination appears to be unaffected by the application of fracking fluids at these concentrations. Cell expansion due to water uptake is essential for the growth potential of the embryo (Nonogaki et al., 2007). As such, the high water content of the fracking fluids (approximately 95%) may be the reason that germination of seeds is not significantly affected during treatment. The seeds have sufficient water to allow imbibition and subsequently germination to occur.

4.5 Conclusion

The hypothesis that application of fracking fluids will negatively affect the germination rate and germination success of Karoo and Thicket species does not apply to all species. This study shows a variable response in terms of germination of Karoo vegetation to the application of fracking fluids. The results indicate that low shrubs and grasses in the fracking footprint are at risk of reduced germination success should contamination with fracking fluids occur.

CHAPTER 5. THE EFFECT OF FRACKING FLUID ON THE PHOTOSYNTHETIC EFFICIENCY AND CONDITION OF KAROO AND THICKET SPECIES

5.1 Introduction

Photosynthesis is the process by which plants use the energy of sunlight to create metabolic changes in biochemical reactions through photochemical processes (Harbinson & Rosenqvist, 2003). In this way light is captured and converted into chemical energy by plants and other photosynthetic organisms. In higher plants this light is captured by pigments such as chlorophylls, carotenoids and anthocyanins that form light-harvesting centres (Rosenqvist & Van Kooten, 2003). Photosynthesis exploits the region of 400 to 700 nm of the electromagnetic spectrum (Harbinson & Rosenqvist, 2003).

The measurement of photosynthetic efficiency can be used as an indicator of environmental stress as the physiological state of the plant can be represented by the inhibition of photosynthesis or altered biochemical processes linked to photosynthesis (Popovic et al., 2003). The processes involved in photosynthesis, particularly the functioning of the photosystem II (PSII) protein complex, are extremely sensitive to environmental stresses (Mohammed et al., 2003). Photosynthetic plant tissues emit red and far-red light in response to active radiation. This is known as chlorophyll fluorescence (Mohammed et al., 2003). The amount of chlorophyll fluorescence is typically less than 5% of the total light absorbed. It is however easily quantified through the use of sensitive equipment (Mohammed et al., 2003). The analysis of chlorophyll fluorescence has become a widely used technique for measuring stress responses in plants (Maxwell & Johnson, 2000; Popovic et al., 2003) and is considered a rapid and non-destructive measurement of photosynthetic efficiency (Mohammed et al., 2003). Chlorophyll fluorescence can also be used as a “pre-visual” indicator of stress in plants (Mohammed et al., 2003) and is inversely related to photosynthetic efficiency (high levels of stress result in low photosynthetic efficiency and high levels of chlorophyll fluorescence). However, when under stress, plant tissues dissipate excess energy by increasing heat production. The increase in heat production usually results in a decrease in chlorophyll fluorescence. Therefore, the actual response pattern of chlorophyll fluorescence is determined by the relative balance of photosynthesis, heat production and chlorophyll fluorescence emission by the plant (Mohammed et al., 2003).

The parameter most commonly used in stress studies that use changes in chlorophyll fluorescence is the Fv/Fm value (Zarco-Tejada et al., 2002). This term is used to quantify the maximum efficiency of photon capture by PSII reaction centres. It is calculated by the following equation:

$$\frac{Fv}{Fm} = \frac{Fm - Fo}{Fm}$$

where Fv = total amount of variable fluorescence
Fm = maximal fluorescence yield of a dark adapted sample (all PSII reaction centres closed)
Fo = minimum fluorescence yield of a dark adapted sample (all PSII reaction centres open)

The measurement of chlorophyll fluorescence can indicate the ability of plants to tolerate certain environmental stresses and also provides an insight into extent of damage to the photosynthetic apparatus caused by these stresses (Maxwell & Johnson, 2000). The average Fv/Fm value of most healthy plant species is 0.83 (Bjorkman & Demmig, 1987). A decrease in the Fv/Fm value may be observed during periods of increased light intensities, the onset of dormancy or when the plants are under stress (Mohammed et al., 2003). Mohammed et al. (2003) suggest a scale of Fv/Fm values to indicate levels of stress (Table 5.1). Although this scale was based on a study of northern hemisphere temperate tree species, Fv/Fm values are relatively consistent across plant species in general (Mohammed et al., 2003).

Table 5.1. Ranges of Fv/Fm value proposed to represent different levels of plant stress (Mohammed et al., 2003).

Plant condition	**Fv/Fm**
Excellent	0.83 – 0.76
Good	0.75 – 0.70
Fair	0.69 – 0.66
Minor strain	0.65 – 0.60
Moderate strain	0.59 – 0.50
Severe strain	≤ 0.49

The ability of plants to recover from the above-mentioned levels of strain depends on the environmental conditions following the stress event, the vigour of the plants and their capacity for repair as well as the quality of the site on which the plants are growing (Mohammed et al., 2003).

Biomass allocation in plants varies over time, across different environments and from species to species (Poorter et al., 2012). If the growth limiting factor is below ground, such as nutrients or water, plants will generally allocate more biomass to the roots. If the growth limiting factor is above ground, such as light or CO_2, more biomass will generally be allocated to the shoots (Poorter et al., 2012). By shifting biomass allocation under limiting conditions, plant growth can be increased by adjusting to accommodate the limiting factor (Poorter et al., 2012). One measure of biomass allocation is the root:shoot ratio. Plants found in low-resource environments or polluted environments often have a high root:shoot ratio (Chapin et al., 1993; Nie et al., 2010). This suggests that plants may respond to environmental stresses by increasing the root:shoot ratio. The same approach has been used to consider the effect of environmental stresses such as pollution on plant resource allocation. In a study by Fiorentino et al. (2017), above ground biomass of giant reed *Arundo donax* L. was found to be higher in plants grown in unpolluted soils than those grown in polluted industrial soil and sludge. Although there is no equivalent research on the effects of fracking fluid on plants, fracking fluid has a low pH making studies on the effects of acid rain on plants applicable. Neufeld et al. (1985) investigated the effect of acid rain (low pH) on *Platanus occidentalis* L. They recorded foliar damage and reduced growth under conditions of low pH. They suggested that the reduction in growth may have been as a result of reduced photosynthetic rates (Neufeld et al., 1985).

This study aims to investigate the effect that contamination of soils with fracking fluids would have on the photosynthetic efficiency and plant condition in species commonly found in the proposed Karoo shale gas development footprint.

5.2 Materials and Methods

Species for the photosynthetic efficiency experiment were chosen based on the most common and important taxa of the fracking study area (Fig. 3.1) listed in Mucina et al (2006a) and Hoare et al. (2006). This list was cross-checked on the SIBIS website to ensure the species were found in the focus areas of the study sites (SIBIS, 2014).

Selections were also based on the commercial availability of seedlings. Representatives of each life form were chosen (Table 5.2).

Table 5.2. Karoo species representing different life forms selected for the photosynthetic efficiency trials.

Life form	**Species**
Tree / phreatophyte	*Vachellia karroo* (Hayne) Banfi & Gallaso
Tall shrub	*Euclea undulata* Thunb.
Low shrub	*Chrysocoma ciliata* L.
Succulent shrub	*Portulacaria afra* Jacq.
Graminoid	*Themeda triandra* Forssk.
Herb	*Gazania krebsiana* Less.
Geophytic herb	*Ammocharis coranica* (Ker Gawl.) Herb.
Succulent herb	*Crassula expansa* Dryand.
Succulent tree	*Aloe ferox* Mill.
Climber	*Sarcostemma viminale* (L.) R.Br.

Vachellia karroo (Hayne) Banfi & Gallaso is a perennial tree or shrub found throughout South Africa (Germishuizen et al., 2006). Many of the *Acacia* spp. (now *Vachellia* for many taxa) are phreatophytic (Jennings, 1974) and *V. karroo* has also been observed rooting at great depth in the Karoo (Milton-Dean, pers. comm., 2014). The common name of *V. karroo* is “sweet thorn”. Shrubs or trees of this species are between 5 and 15 m tall with long paired spines protruding from the stem. The flowers are deep yellow in colour and held in spherical inflorescences.

Euclea undulata is an evergreen shrub or small tree that is densely leafy. It is found in bushveld, grassland and semi-desert areas in South Africa. The common name of *E.*

undulata is “common guarri”. This shrub has tough durable wood. It is also commonly browsed by game and livestock (Van Wyk & Van Wyk, 2013).

Chrysocoma ciliata L. is a perennial shrub that occurs throughout South Africa (Germishuizen et al., 2006). Also known as “bitterbos”, this plant forms bushes of up to 0.6 m in height with yellow rayless inflorescences in a hemispherical head (Vanderplank, 1998).

Portulacaria afra Jacq. is a perennial succulent plant that may be a tree or shrub (Germishuizen et al., 2006). It is a robust, common species found throughout most of South Africa (Court, 2010). The stems are thick and supple with opposite branchlets. The leaves are small, fleshy and sessile. The plant is densely foliate and it produces tiny pink flowers, usually after rain (Court, 2010). The common name of *Portulacaria afra* is “spekboom” as it is a valuable food source for stock and wild animals, especially during periods of drought (Court, 2010).

Themeda triandra Forssk. is a perennial, palatable C_4 grass found throughout South Africa. It grows to a height of 2 m (Germishuizen et al., 2006).

Gazania krebsiana Less. is a perennial herb found in the Eastern, Western and Northern Cape, Free State, KwaZulu-Natal and Limpopo (Germishuizen et al., 2006). The ray florets of the flowers are orange with small dark spots near the base (Vanderplank, 1998).

Ammocharis coranica (Ker Gawl.) Herb. is a perennial geophyte found in most of the provinces in South Africa (Germishuizen et al., 2006). Also known as the “Bible flower” this plant may grow to 250 mm in height when in flower. The pale pink flowers are borne on an inclining stem. The leaves may reach a length of 200 mm and only appear after the peduncle of the inflorescence has withered (Vanderplank, 1998).

Crassula expansa Dryand. is a small, perennial, succulent herb (Vanderplank, 1998; Germishuizen et al., 2006). The flowers have white petals and red sepals. The peduncle elongates as the seeds form. It has succulent leaves and prostrate stems that root as they grow. This much branched herb often forms large patches (Vanderplank, 1998). It is widespread in Southern Africa, occurring from southwestern Namibia to KwaZulu-Natal, Swaziland, and into tropical Africa, but is common in the eastern provinces of South Africa (Court, 2010).

Aloe ferox Mill. is a perennial succulent tree found in the Eastern and Western Cape, Limpopo and KwaZulu-Natal (Germishuizen et al., 2006). It is a robust single-stemmed aloe growing up to 5 m tall. The succulent leaves are broad with spines along the edges and lower surfaces of the leaves. The flowers are generally bright orange-red. This species is very adaptable across rainfall regimes, flourishing in both the dry Karoo and the moister parts of the Eastern Cape. *Aloe ferox* is commonly grown in gardens and has medicinal uses (Van Wyk & Smith, 2003).

Sarcostemma viminale (L.) R.Br. is a perennial succulent scrambler common in most South African provinces (Germishuizen et al., 2006). It is leafless with rounded stems and a scrambling growth habit. The flowers have a white corona and greenish yellow petals (Vanderplank, 1998).

Well established, healthy seedlings were purchased from a nursery. The seedlings were in pots or growing bags and growing in the medium most suited to the species. Three plants of each species were placed in a glasshouse to enable the watering regime to be controlled without any input from rainfall. A solution containing the most commonly used fracking chemicals (Ridley, 2011; Peduzzi & Harding Rohr Reis, 2013) was used to treat the seedlings (see Table 4.2 in Chapter 4) while the control was tap water.

Two scenarios were simulated: a surface spill (neat or concentrated fracking fluids) and groundwater contamination (diluted fracking fluids). For the surface spill treatment, undiluted fracking fluids were applied to the seedlings while for the groundwater contamination treatment, diluted fracking fluids were applied. The control samples were watered with tap water. The seedlings were watered once a week for four months. Plants used to simulate a surface spill were watered with neat fracking fluids once only, after which they were watered once a week with tap water. Samples treated with dilute fracking fluids were watered with the dilute fracking solution throughout the experiment. The salinity and pH of the various fluids were measured using a YSI Multiprobe (Refer to Table 4.3 in Chapter 4).

The chlorophyll fluorescence of the seedlings was measured every day for the first week. Based on the findings of Adams (2011) a quick response to the fracking fluid was expected. A Hansatech Plant Efficiency Analyzer was used to measure the Fv/Fm ratio of two random leaves per seedling (three seedlings per species per treatment).

After the first week, measurements were taken once a week and thereafter once a month. Photographs of the seedlings were taken on a weekly basis to document the deterioration in plant condition, leaf colour and flowering status.

After four months the seedlings that had died were removed from the experiment and the remaining more tolerant seedlings were used to investigate at which fracking fluid application concentration plant condition was affected. Those seedlings initially treated once-off with neat fracking fluids were then treated on a weekly basis with neat fracking fluids, with the number of doses needed to make an observable change recorded. The Fv/Fm ratio was measured on a weekly basis and photographs taken as in the first experiment. The second phase of the experiment continued for a further 10 months (8 months for *Crassula expansa* as the plants died) but had to be terminated as access to the glasshouse was limited and the treatments could not continue until all plants had received a lethal dose.

At the end of the experiment the plants were harvested to measure shoot and root biomass and root:shoot ratio according to the method of Williams et al. (2013). At the time of this experiment too many of the *Crassula expansa* seedlings had died to be included in this study. Plants were separated into root and shoot material and the roots were rinsed to remove soil. The plant material was oven dried at 60℃ for 24 hours and weighed to the nearest 0.001 g.

Plant height was measured for the tall shrub (*Euclea undulata*) and the succulent shrub (*Portulacaria afra*) at the start of the experiment and final measurements were taken before the plants were harvested. In these two species, a noticeable difference in shoot height between the different treatments was observed during the monthly assessments. The other species in the experiment were difficult to measure accurately due to their growth form.

A Two-Way Analysis of Variance followed by a TukeyHSD post hoc test or a Friedman rank sum test was used to determine the significance of differences in the Fv/Fm measurements for the different treatments. Paired t-tests were used to compare the means of growth, shoot biomass and root:shoot ratios. Tests were done using R version 3.3.2 (R Core Team, 2016) and R-Studio (R Studio Team, 2015).

5.3 Results

After an initial period of four months, half of the plants in the study that were treated with fracking fluids had either died or the treatment plants were in too poor a condition to continue the experiment (see Plate 5.1). Based on these results, the species were grouped into two categories: less tolerant species and more tolerant species (Table 5.3).

Table 5.3. Grouping of Karoo species (and their life forms) based on their tolerance to fracking fluid application.

Less tolerant	**More tolerant**
Vachellia karroo (tree/phreatophyte)	*Euclea undulata* (tall shrub)
Chrysocoma ciliata (low shrub)	*Portulacaria afra* (succulent shrub)
Themeda triandra (graminoid)	*Gazania krebsiana* (herb)
Ammocharis coranica (geophytic herb)	*Crassula expansa* (succulent herb)
Aloe ferox (succulent tree)	*Sarcostemma viminale* (climber)

A

B

Plate 5.1. Deterioration of plant condition in *Vachellia karroo* before (A) and after (B) four months of treatment with diluted fracking fluid.

The Fv/Fm ratios were measured over four months for the less tolerant species (Table 5.3) while for the more tolerant species the Fv/Fm ratios were measured for fourteen months (Table 5.3).

The control and dilute treatments are considered to be fresh but the neat fluids are equivalent to brackish water. The pH of the tap water was slightly alkaline (Table 4.3 in Chapter 4) and fracking fluids reduced the pH to acidic (dilute fluids) and strongly acidic (neat fluids).

In those species with thick leaves/stems (*Aloe ferox* and *Sarcostemma viminale*) the Fv/Fm ratios were generally lower than expected, due to the difficulty of fitting the

Hansatech leaf clips to the leaves with no leakage. However, the results were still useful, given this constraint.

5.3.1 Stress responses of less tolerant species

In the *Vachellia karroo* plants (representing tree life forms and found to be relatively intolerant to fracking fluids), control plants had an average Fv/Fm ratio 0.739 ± 0.099 S.D. over the four months of the experiment (Fig. 5.1). Despite high variability as the plants became stressed, by the end of the experiment, the control plants were still unstressed (Fv/Fm of >0.7 is considered to be unstressed, Mohammed et al., 2003). with a mean Fv/Fm ratio of 0.696 ± 0.140 S.D. At this stage, plants treated with the single application of concentrated fracking fluids, followed by watering with tap water showed less stress (Fv/Fm mean of 0.459 ± 0.352 S.D.) than the continual treatment with diluted fracking fluids (a mean Fv/Fm of 0.3 ± 0.2 S.D.). After four months, the treated plants were significantly more stressed than at the outset at a 90% confidence threshold (Fig. 5.1; Friedman chi-squared = 6.6, df = 3, p = 0.086).

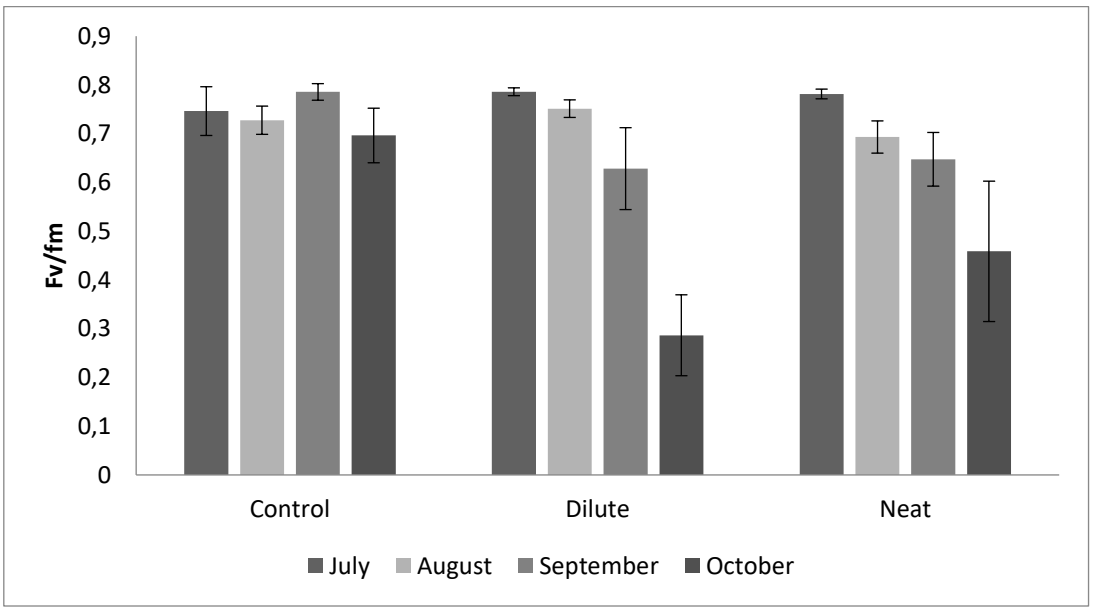


Figure 5.1. The average Fv/Fm ratio of *Vachellia karroo* plants (life-form: tree) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). (Vertical lines represent ± 1 S.E.).

Chrysocoma ciliata control plants (representing low shrubs) became stressed after three months in the glass house (the Fv/Fm ratio dropped from 0.798 ± 0.023 S.D. to 0.515 ± 0.106 S.D.; Fig. 5.2). The plants treated with the single application of concentrated fracking fluids, followed the same pattern as the control (no significant difference: Friedman chi-squared = 3.5, df = 2, p = 0.174). After four months, the continual treatment with fracking fluids resulted in significantly lower Fv/Fm ratio in the low shrub (Friedman chi-squared = 7.4, df = 3, p = 0.060, 90% confidence threshold).

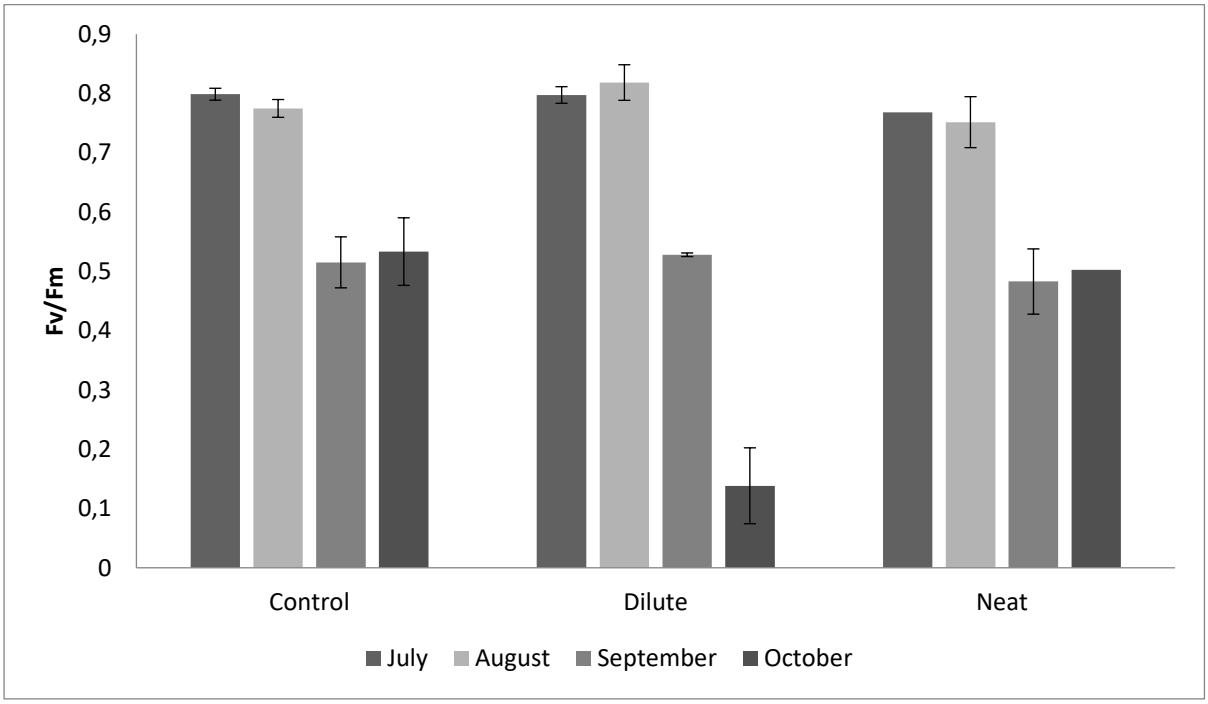


Figure 5.2. The average Fv/Fm ratio of *Chrysocoma ciliata* plants (life-form: low shrub) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). (Vertical lines represent ± 1 S.E.).

In the *Themeda triandra* plants (representing graminoids) all treatments became gradually more stressed (Fig. 5.3). After four months this effect was noticeable in the plants treated with dilute and neat fracking fluids. Fv/Fm had dropped from 0.697 ± 0.046 to 0.392 ± 0.225 in the grasses treated with dilute fluids and from 0.722 ± 0.033 to 0.372 ± 0.260 in the plants treated with the single application of concentrated fracking fluids, followed by watering with tap water. For the treated plants, the Fv/Fm ratio at the start of the experiment was significantly lower to higher than the Fv/Fm after four months of treatment with fracking fluids (Friedman chi-squared = 8.2, df = 3,

p = 0.042). By the end of the experiment, the control grasses were still unstressed (a mean Fv/Fm of 0.669 ± 0.047) whereas the treated grasses were considered to be under severe strain (Fv/Fm ≤ 0.49, Mohammed et al., 2003) and had significantly lower photosynthetic efficiency than the control plants (Friedman chi-squared = 6, df = 2, p = 0.05).

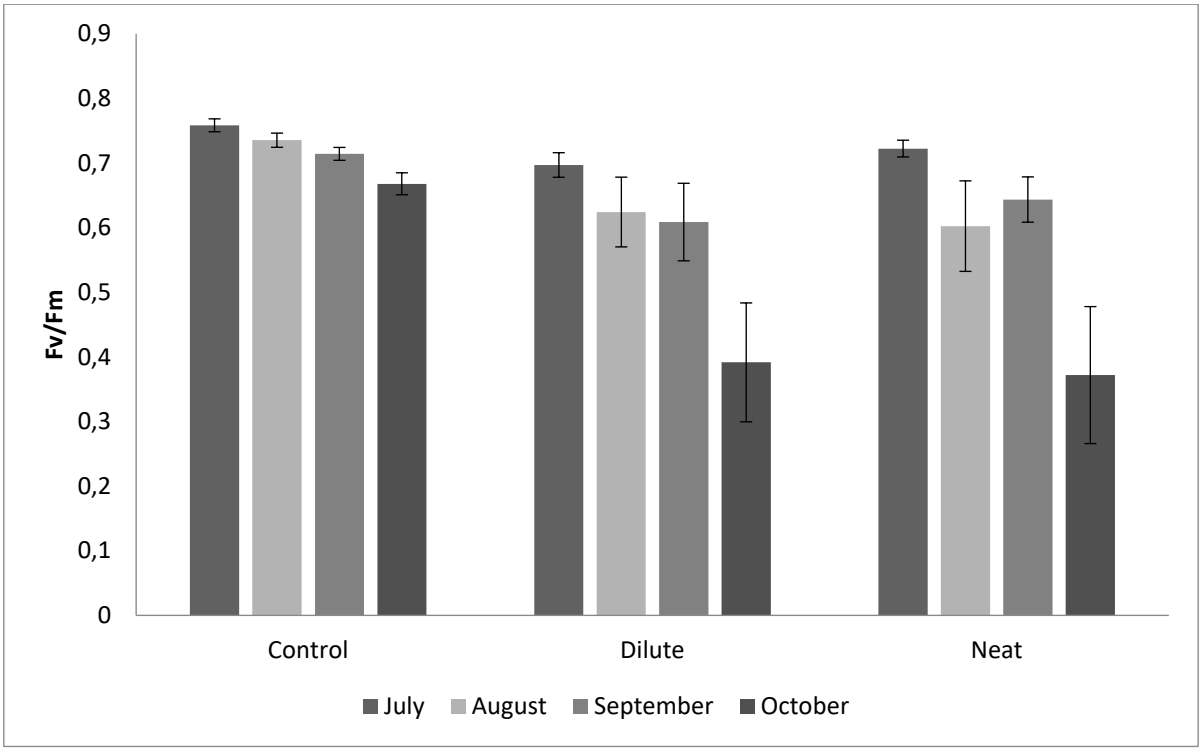


Figure 5.3. The average Fv/Fm ratio of *Themeda triandra* plants (life form: grass) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). (Vertical lines represent ± 1 S.E.).

The geophytic herb *Ammocharis coranica* remained unstressed for the first three months of the experiment (Fig. 5.4). Only in the fourth month did Fv/Fm drop dramatically in the treated plants. The control plants remained unstressed (a mean Fv/Fm of 0.778 ± 0.021) for the duration of the experiment. Plants treated with dilute or neat fracking fluids had significantly lower Fv/Fm by the end of the four months (Friedman chi-squared = 7.4, df = 3, p = 0.06 at 90% confidence threshold). Fv/Fm decreased in *Ammocharis coranica* plants treated repeatedly with dilute fluids from 0.797 ± .0.015 to 0.16 ± 0.28 and plants treated once off with neat fracking fluids then

watered with tap water saw a decrease in Fv/Fm from 0.78 ± 0.023 down to 0.291 ± 0.401.

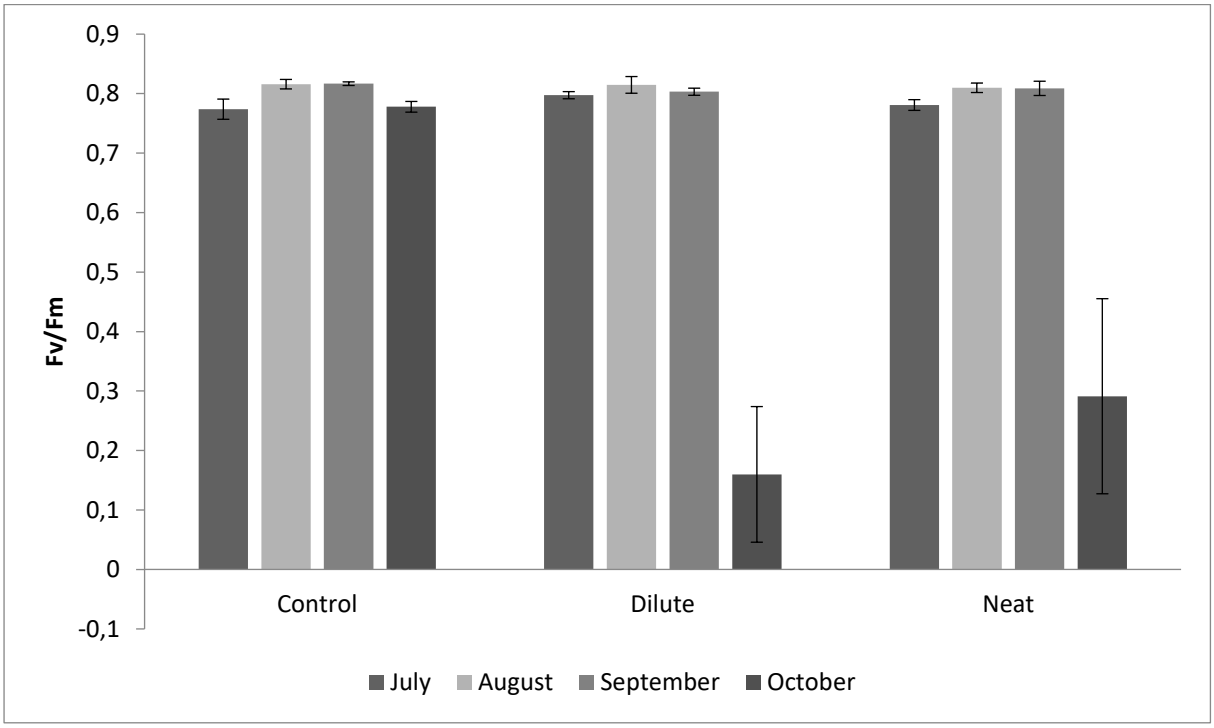


Figure 5.4. The average Fv/Fm ratio of *Ammocharis coranica* plants (life form: geophytic herb) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). (Vertical lines represent ± 1 S.E.).

In the *Aloe ferox* (representing succulent tree life forms) issues with attaching the leaf clips to the thick leaves resulted in variable results. Regardless of treatment, the plants became gradually more stressed over the course of the experiment with a significant decrease in Fv/Fm from the start of the experiment to the end of the four month period (Fig. 5.5; $F = 9.490$, $df = 3$, $p < 0.05$). Though Fv/Fm was similar in all three treatments by the end of the experiment (Control plants – mean Fv/Fm of 0.272 ± 0.194; Dilute treated plants – mean Fv/Fm of 0.231 ± 0.092; Neat treated plants – mean Fv/Fm of 0.187 ± 0.165) those *Aloe ferox* plants treated with fracking fluids at either dilution were in such poor condition they were removed from the experiment.

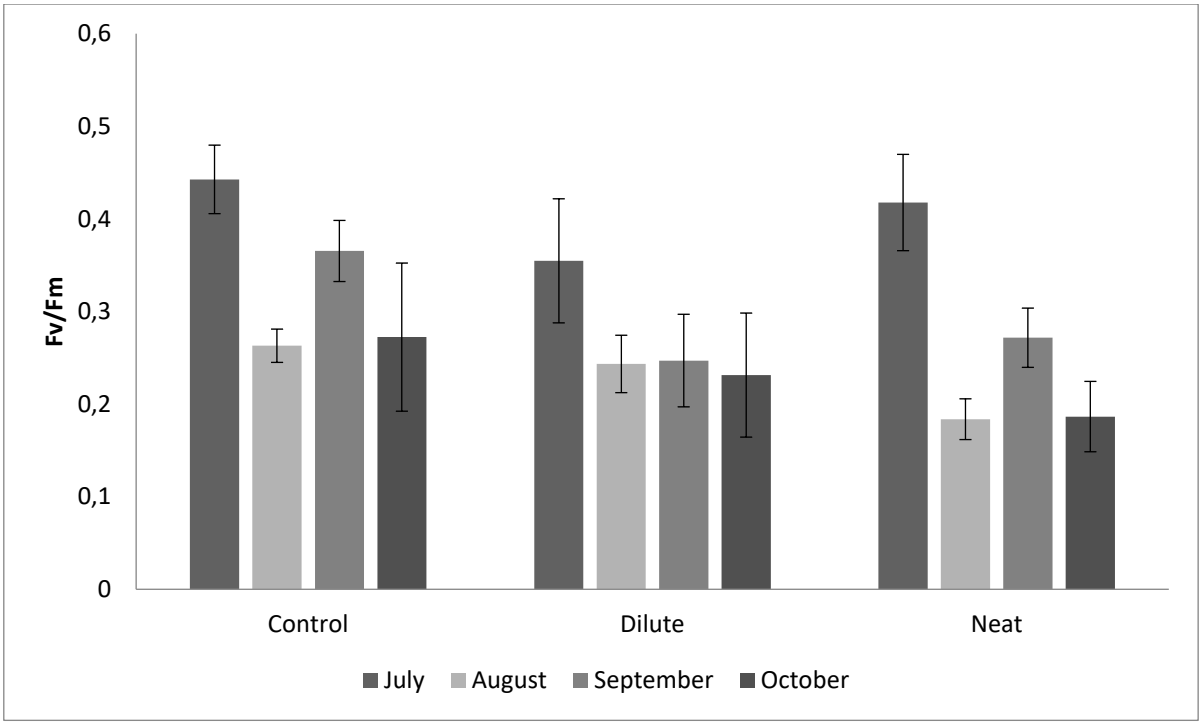


Figure 5.5. The average Fv/Fm ratio of *Aloe ferox* plants (life form: succulent tree) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). (Vertical lines represent ± 1 S.E.).

5.3.2 Stress responses of more tolerant species

Euclea undulata (representing tall shrub life forms) remained unstressed (Fv/Fm > 0.6, Mohammed et al., 2003) throughout the duration of the experiment (Fig. 5.6). All three treatments showed some heat stress over the summer months, with lower Fv/Fm ratios in those months. There was no significant difference in Fv/Fm in the *Euclea undulata* plants under the different treatment scenarios (Friedman chi-squared = 1.3846, df = 2, p = 0.5).

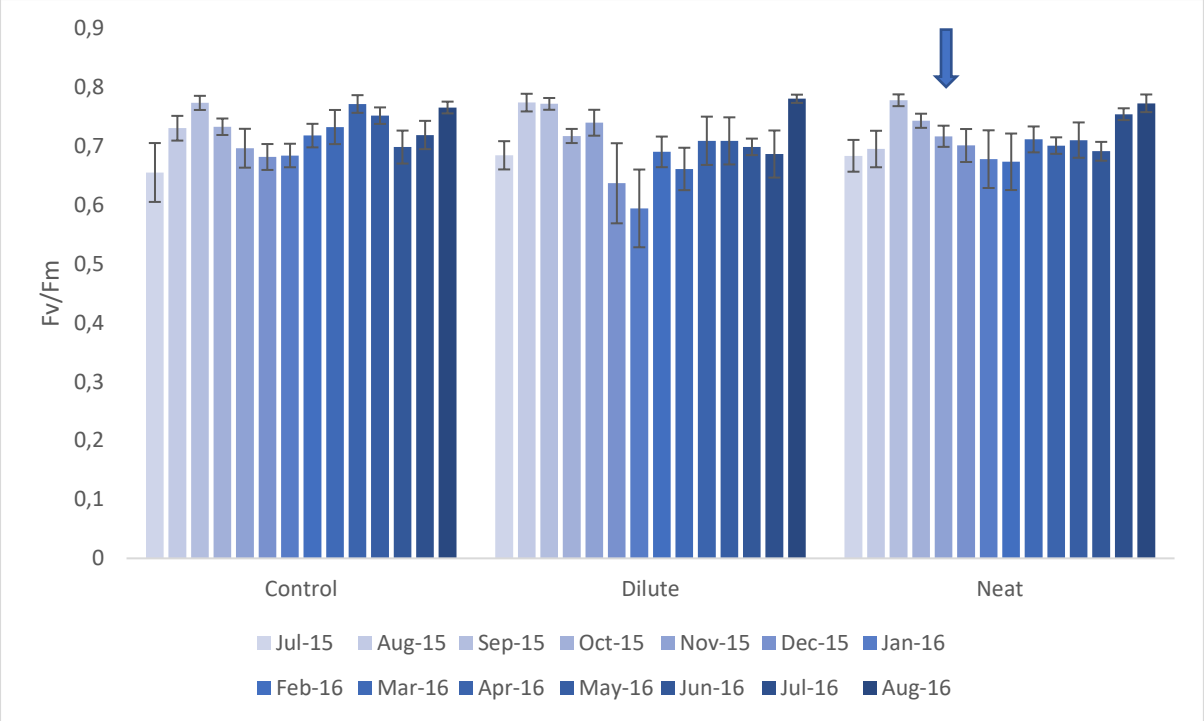


Figure 5.6. The average Fv/Fm ratio of *Euclea undulata* plants (life-form: tall shrub) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). After four months (at arrow) the plants of the Neat treatment were watered weekly with concentrated fracking fluids (Vertical lines ± 1 S.E.).

The *Portulacaria afra* plants (representing the succulent shrub life forms) had fluctuating Fv/Fm ratios, with the plants exhibiting heat stress over the summer months (observed as lowered Fv/Fm) but recovering during the cooler months, with all treatments being unstressed by the end of the experiment (Fv/Fm ratio > 0.7 in all treatments, Fig. 5.7). The application of either dilute or neat fracking fluids appeared to have little effect on the photosynthetic efficiency of the succulent shrub; the only significant difference was found in the drop in Fv/Fm ratio during summer when compared to the Fv/Fm ratio of the *Portulacaria afra* plants at the end of the experiment (Friedman chi-squared = 29.495, df = 12, p = 0.003) across all treatments. In the plants treated with neat fracking fluids, the Fv/Fm was significantly higher at the end of the experiment (0.76 ± 0.04) than at the beginning of the experiment (0.649 ± 0.236; Friedman chi-squared = 6.615, df = 2, p = 0.037).

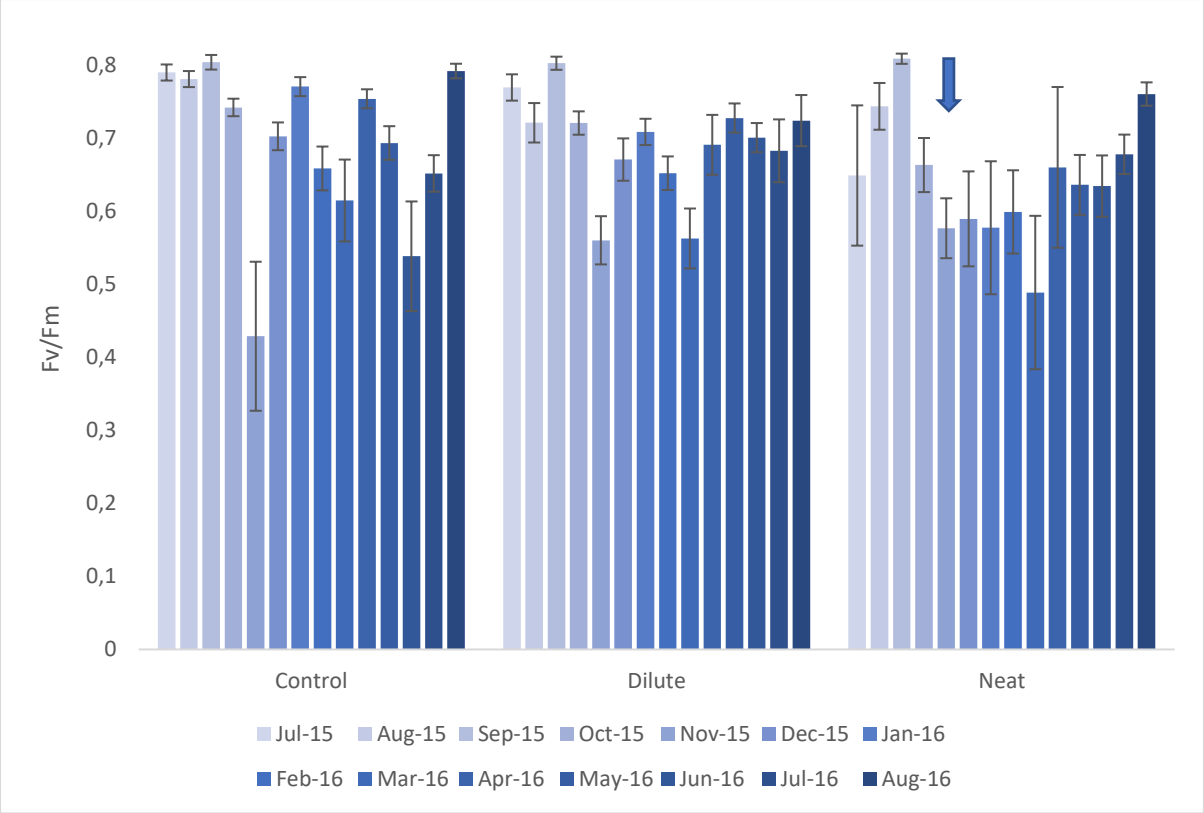


Figure 5.7. The average Fv/Fm ratio of *Portulacaria afra* plants (life-form: succulent shrub) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). After four months (at arrow) the plants of the Neat treatment were watered weekly with concentrated fracking fluids (Vertical lines ± 1 S.E.).

The application of fracking fluids did not appear to affect the *Gazania krebsiana* plants (representing the herb life forms, Fig. 5.8). In all treatments the Fv/Fm fluctuated in the unstressed range, with the exception of a large drop in the summer months due to heat stress. However, by the end of the experiment, all the *G. krebsiana* plants had recovered to an unstressed Fv/Fm range. A significant difference was found in the Fv/Fm ratio during summer when compared to the Fv/Fm ratio of the *G. krebsiana* plants at the end of the experiment (Friedman chi-squared = 28.176, df = 12, p = 0.005) across all treatments.

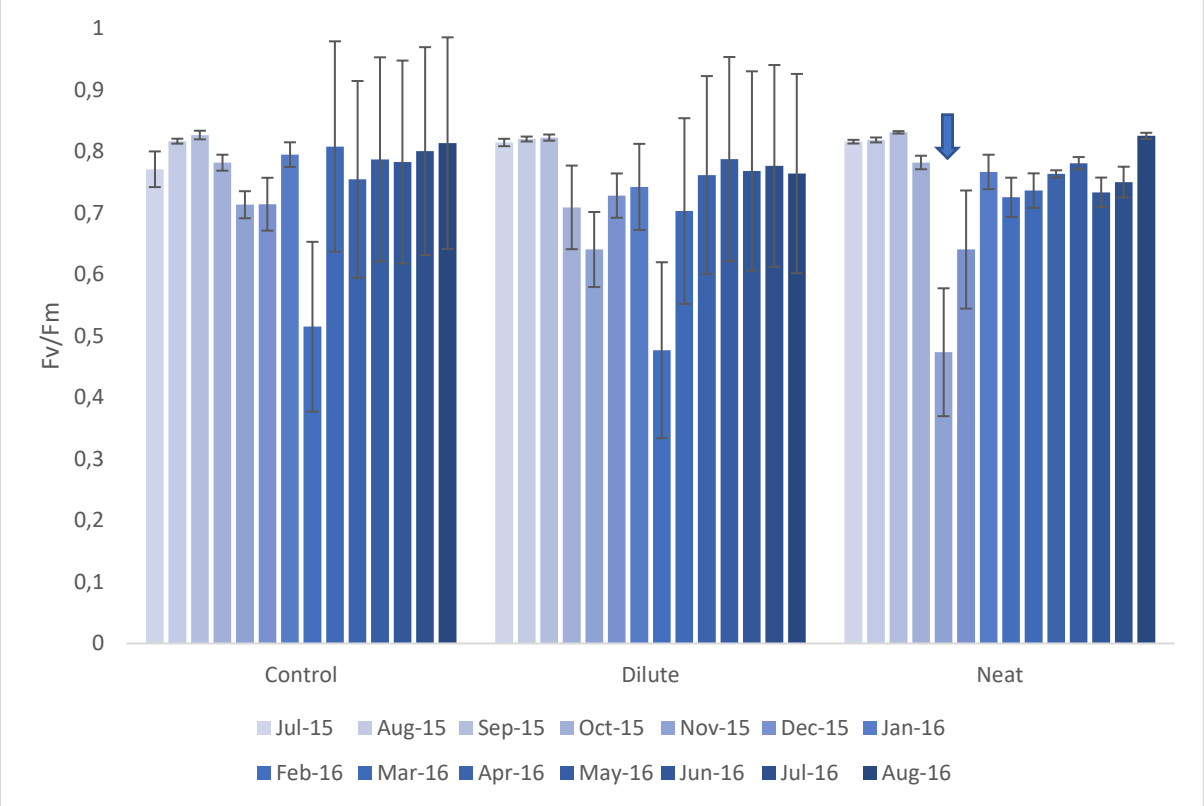


Figure 5.8. The average Fv/Fm ratio of *Gazania krebsiana* plants (life-form: herb) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). After four months (at arrow) the plants of the Neat treatment were watered weekly with concentrated fracking fluids (Vertical lines ± 1 S.E.).

Photosynthetic efficiency fluctuated in the *Crassula expansa* plants (representing the succulent herb life forms) in all the treatments (Fig. 5.9). Heat stress during the summer months was also evident in the succulent herbs. At the end of the experiment the plants treated with tap water and those treated with dilute fracking fluids were in a fair to moderately strained state (Fv/Fm 0.6-0.69, Mohammed et al., 2003). The *C. expansa* plants treated with concentrated fracking fluids were stressed (Fv/Fm = 0.399 ± 0.185; severely strained according to Mohammed et al., 2003) and the Fv/Fm ratio was significantly lower for the plants at the end of the experiment compared to the starting Fv/Fm (Friedman chi-squared = 23.821, df = 11, p = 0.014).

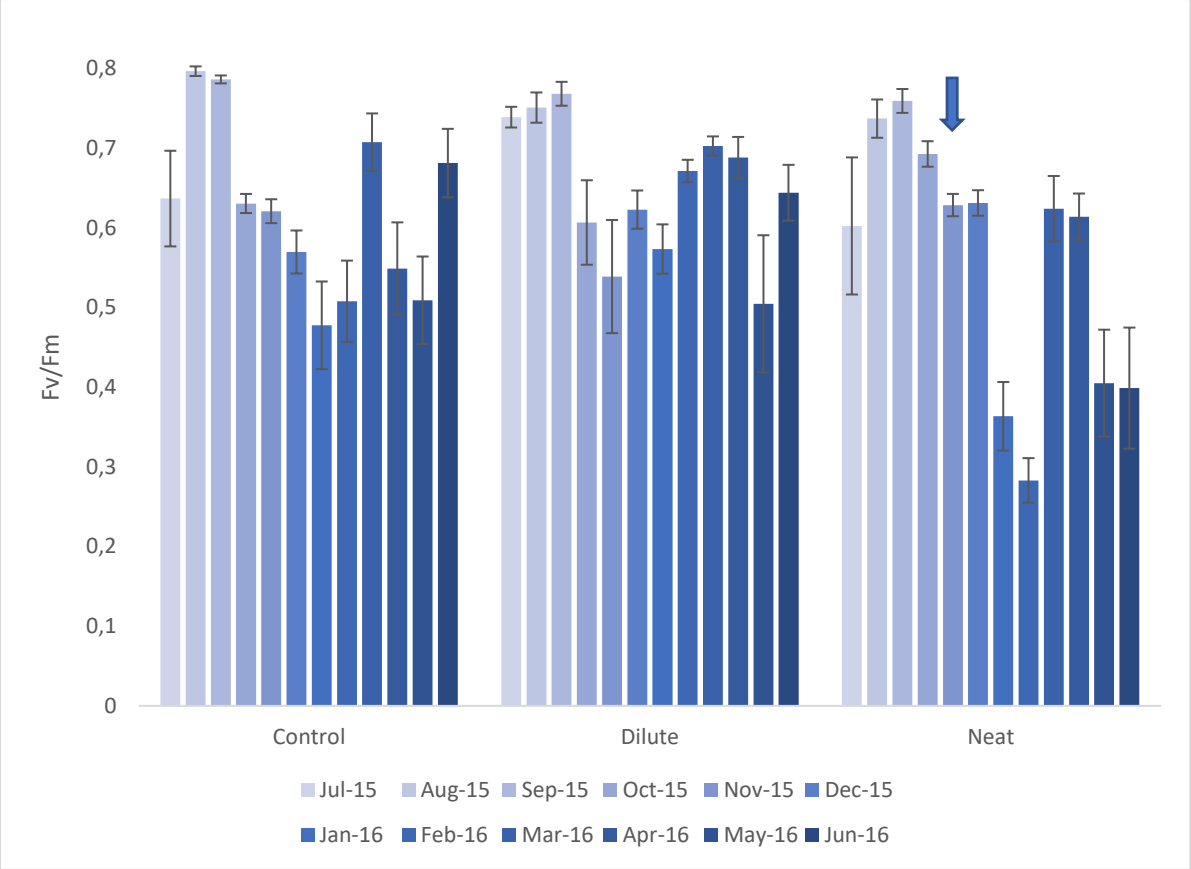


Figure 5.9. The average Fv/Fm ratio of *Crassula expansa* plants (life-form: succulent herb) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). After four months (at arrow) the plants of the Neat treatment were watered weekly with concentrated fracking fluids (Vertical lines ± 1 S.E.).

As with the *Aloe ferox* plants, issues with attaching the leaf clips to the photosynthetic stems of *Sarcostemma viminale* (representing the climber life forms) resulted in variable and low readings. There appeared to temperature stress in the *S. viminale* plants evident in both summer and winter (Fig. 5.10). Plants treated with either dilute or neat fracking fluids had similar Fv/Fm ratios after the fourteen months but the control plants were significantly less stressed than the treated plants (Fv/Fm = 0.636 ± 0.096; Friedman chi-squared = 23.209, df = 12, p = 0.026).

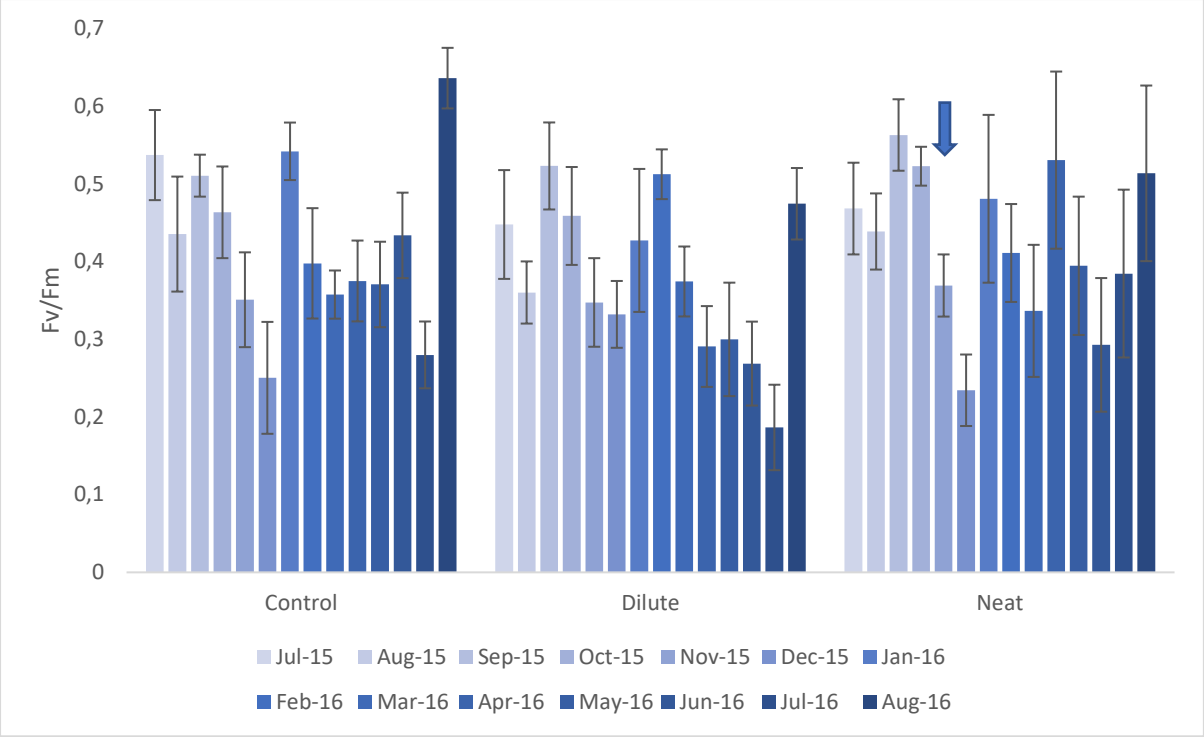


Figure 5.10. The average Fv/Fm ratio of *Sarcostemma viminale* (life-form: climber) measured monthly after watering with tap water (Control), watering weekly with dilute fracking fluids (Dilute), and watering with tap water weekly after a single application of concentrated fracking fluids (Neat). After four months (at arrow) the plants of the Neat treatment were watered weekly with concentrated fracking fluids (Vertical lines ± 1 S.E.).

5.3.3 Plant height

Initially all plants were of similar size. However, after treatment with fracking fluids, growth became stunted in *Portulacaria afra* and *Euclea undulata* (both neat and dilute fracking fluids; Plate 5.2). Over the course of the fourteen month experiment control plants grew tall, while treating with neat fracking fluids resulted in reduced growth and in plants treated weekly with dilute fracking fluids, plants remained small.

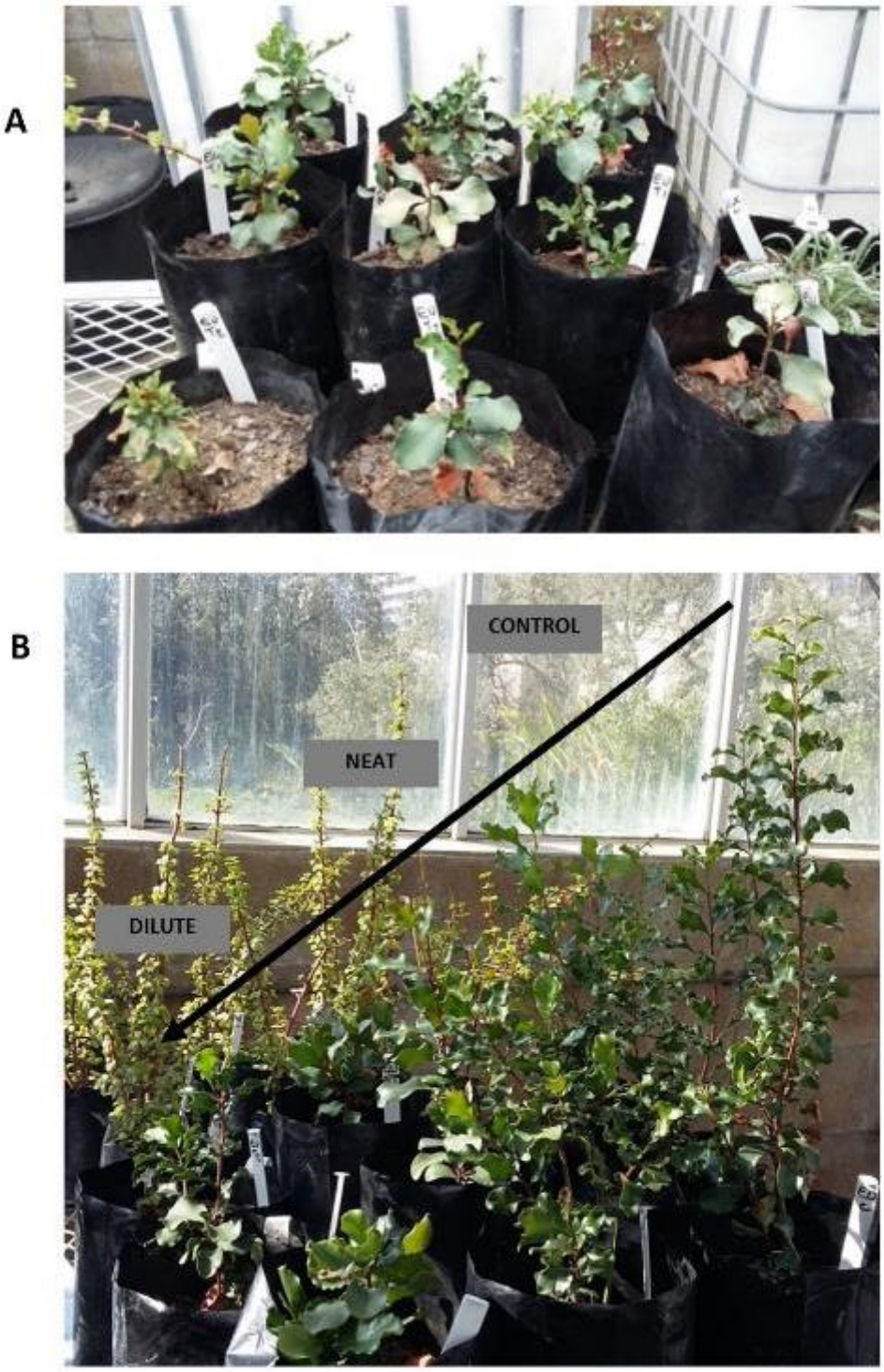


Plate 5.2. *Euclea undulata* plants at the start of the experiment (A) and at the end of the experiment (after 14 months).

Even though *Euclea undulata* and *Portulacaria afra* showed no stress due to application of fracking fluids (Fig. 5.6 and 5.7), plant growth rates were affected (Fig. 5.11 and 5.12).

The control plants of *Euclea undulata* grew at an average rate of 3.5 cm/month (± 1 S.D.). The application of neat fracking fluids weekly reduced the growth rate of the plants to 1.7 cm/month (± 1.3 S.D.). The *E. undulata* plants treated with dilute fracking fluids weekly for fourteen months had the most stunted growth (average growth rate of 1 cm/month ± 0.6 S.D.; Fig. 5.11). The application of dilute fracking fluids reduced the growth rate of the *E. undulata* plants by 28.6%, while the application of neat fracking fluids decreased the growth rate by 48.5%. The growth rate of *E. undulata* treated with tap water was significantly higher than the growth rate of *E. undulata* plants treated with dilute fracking fluids (t = 3.24, df = 2, p = 0.084, 90% confidence threshold).

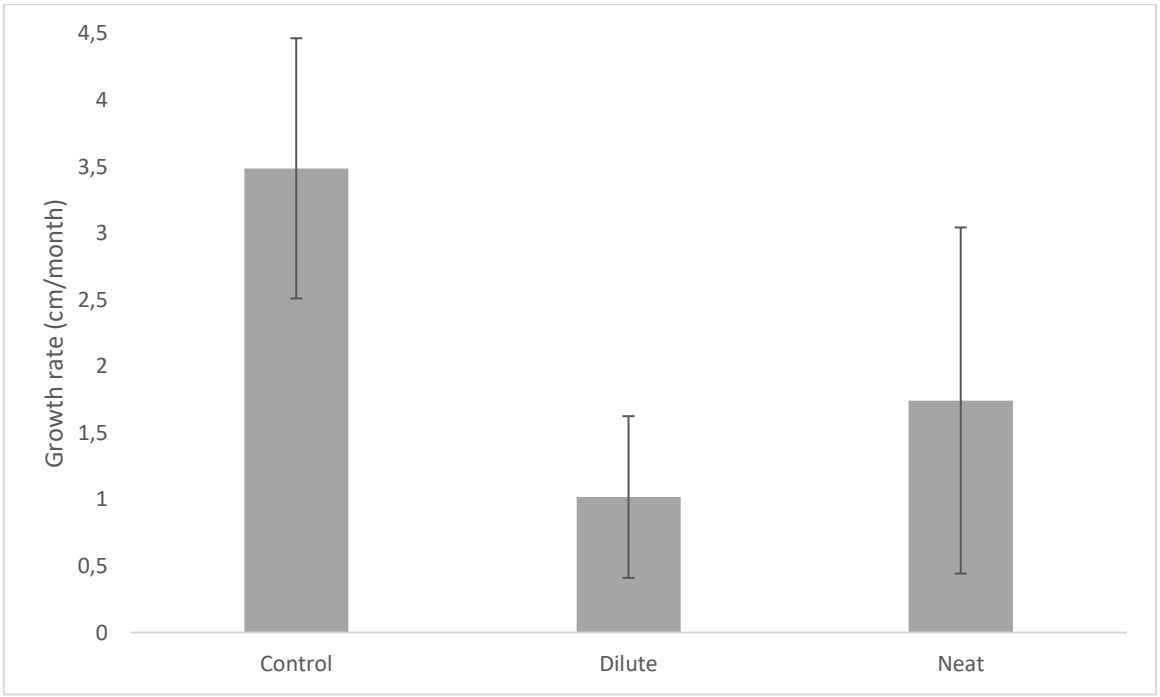


Figure 5.11. The average growth rate (cm/month) of *Euclea undulata* plants measured over 14 months (vertical lines represent ± 1 S.D.). The control was watered weekly with tap water and the dilute treatment was watered weekly with dilute fracking fluids. The neat treatment was watered with tap water weekly after a single application of concentrated fracking fluids for the first three months, after which plants were watered weekly with concentrated fracking fluid.

The control plants of *P. afra* grew at an average rate of 1.4 cm/month (± 0.6 S.D.). The application of diluted fracking fluids reduced this growth rate to 1.0 cm/month (± 0.2 S.D.). Growth was most stunted when neat fracking fluids were applied weekly (0.5 ± 0.4 cm/month). The application of dilute fracking fluids reduced the growth rate of the

P. afra plants by 71.4%, while the application of neat fracking fluids decreased the growth rate by 35.7%. This treatment was more variable than the others due to the change in application regime after four months (Fig. 5.12). There were no significant differences in growth rate for *P. afra* between the treatments.

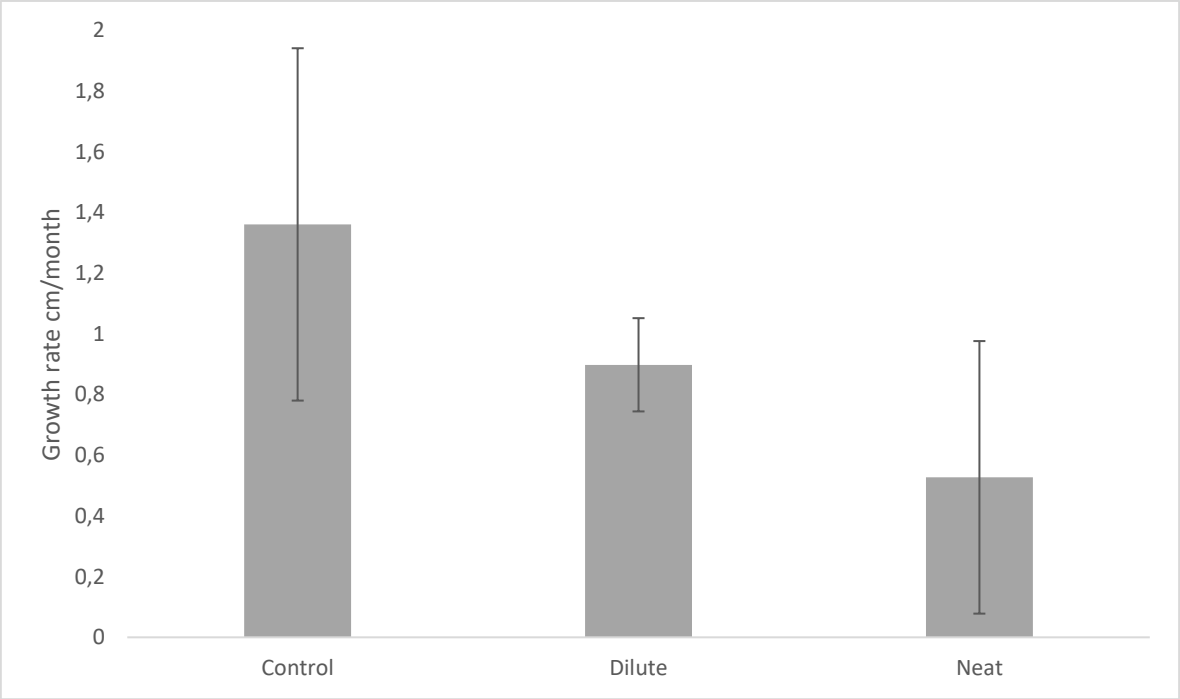


Figure 5.12. The average growth rate (cm/month) of *Portulacaria afra* plants measured over 14 months (vertical lines represent ± 1 S.D.). The control was watered weekly with tap water and the dilute treatment was watered weekly with dilute fracking fluids. The neat treatment was watered with tap water weekly after a single application of concentrated fracking fluids for the first three months, after which plants were watered weekly with concentrated fracking fluids.

5.3.4 Plant root:shoot ratio

Some of the plants showed different resource allocation responses to fracking fluids (Fig. 5.13 to 5.16).

Euclea undulata plants showed no statistically significant change in root:shoot ratio (Fig. 5.13), however there was a significant decrease in shoot biomass in the plants watered with the dilute fracking fluids ($t = 8.347$, $df = 2$, $p = 0.014$) and those watered with the neat fracking fluids ($t = 3.687$, $df = 2$, $p = 0.066$, 90% confidence threshold). Root biomass was also significantly lower in the plants watered with the dilute fracking

fluids (t = 4.954, df = 2, p = 0.038) and those watered with the neat fracking fluids (t = 3.212, df = 2, p = 0.085, 90% confidence threshold).

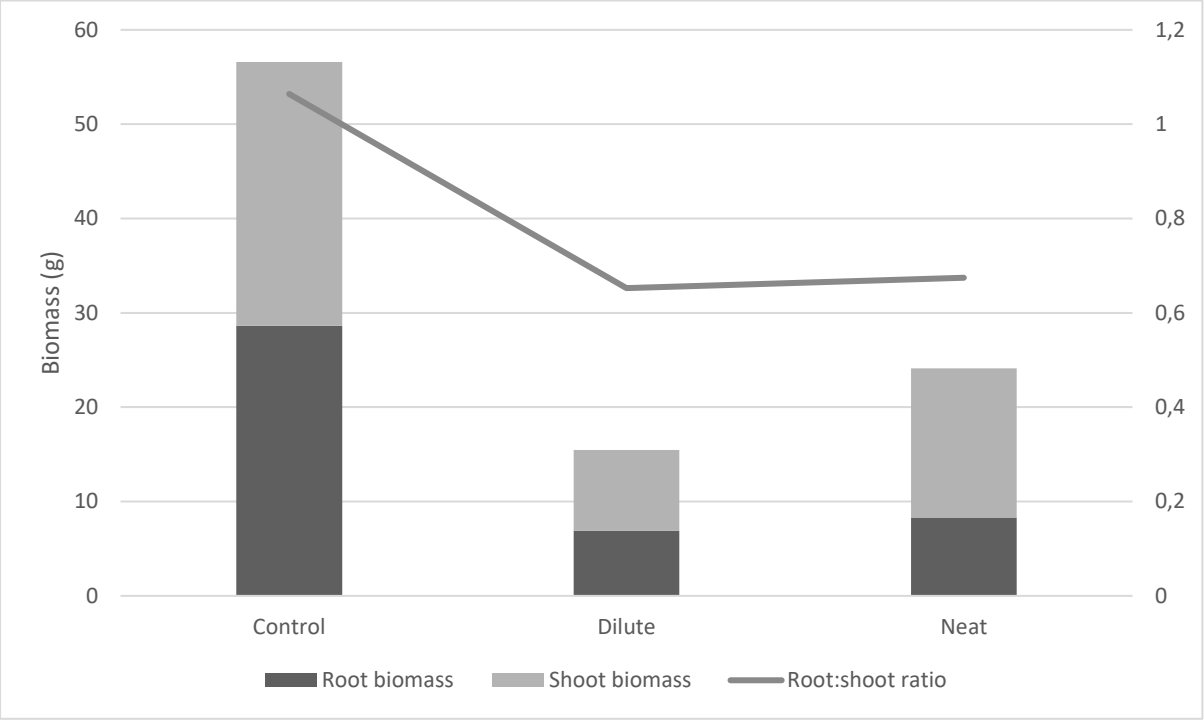


Figure 5.13. The average root biomass, shoot biomass and root:shoot ratio of *Euclea undulata* plants harvested after 14 months. The control was watered weekly with tap water and the dilute treatment was watered weekly with dilute fracking fluids. The neat treatment was watered with tap water weekly after a single application of concentrated fracking fluids for the first four months, after which plants were watered weekly with concentrated fracking fluids.

Root:shoot ratio did not change significantly in the *Portulacaria afra* plants (Fig. 5.14). Root biomass was also unaffected by the application of fracking fluids in these plants. However, the control plants (watered with tap water only) had significantly higher shoot biomass than the plants watered with dilute fracking fluids (t = 4.954, df = 2, p = 0.038) and those watered with concentrated fracking fluids (t = 3.212, df = 2, p = 0.085, 90% confidence threshold).

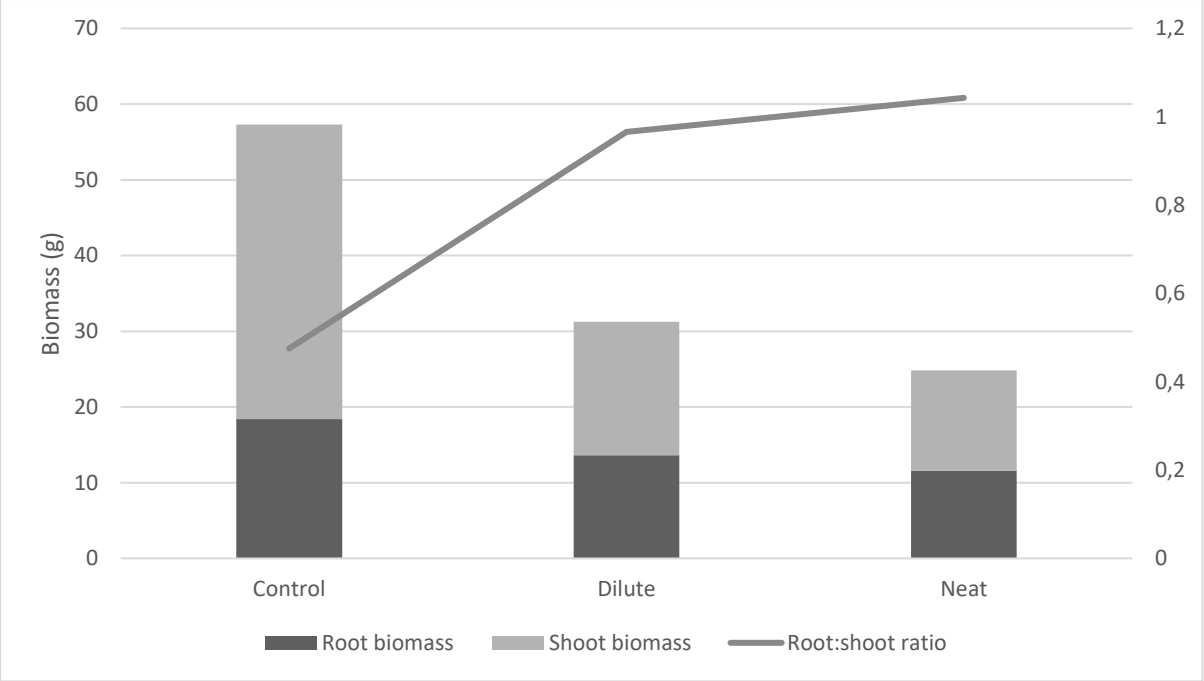


Figure 5.14. The average root biomass, shoot biomass and root:shoot ratio of *Portulacaria afra* plants harvested after 14 months. The control was watered weekly with tap water and the dilute treatment was watered weekly with dilute fracking fluids. The neat treatment was watered with tap water weekly after a single application of concentrated fracking fluids for the first four months, after which plants were watered weekly with concentrated fracking fluids.

In the *Gazania krebsiana* plants, those watered with tap water had a significantly higher root:shoot ratio than those plants watered with neat fracking fluids ($t = 12.202$, $df = 1$, $p = 0.05$). Though the root:shoot ratio was lower in those *G. krebsiana* plants watered with dilute fracking fluids, the difference was not significant ($t = 1.623$, $df = 1$, $p = 0.352$). Both root and shoot biomass of *G. krebsiana* was not significantly affected by the application of dilute or neat fracking fluids (Fig. 5.15).

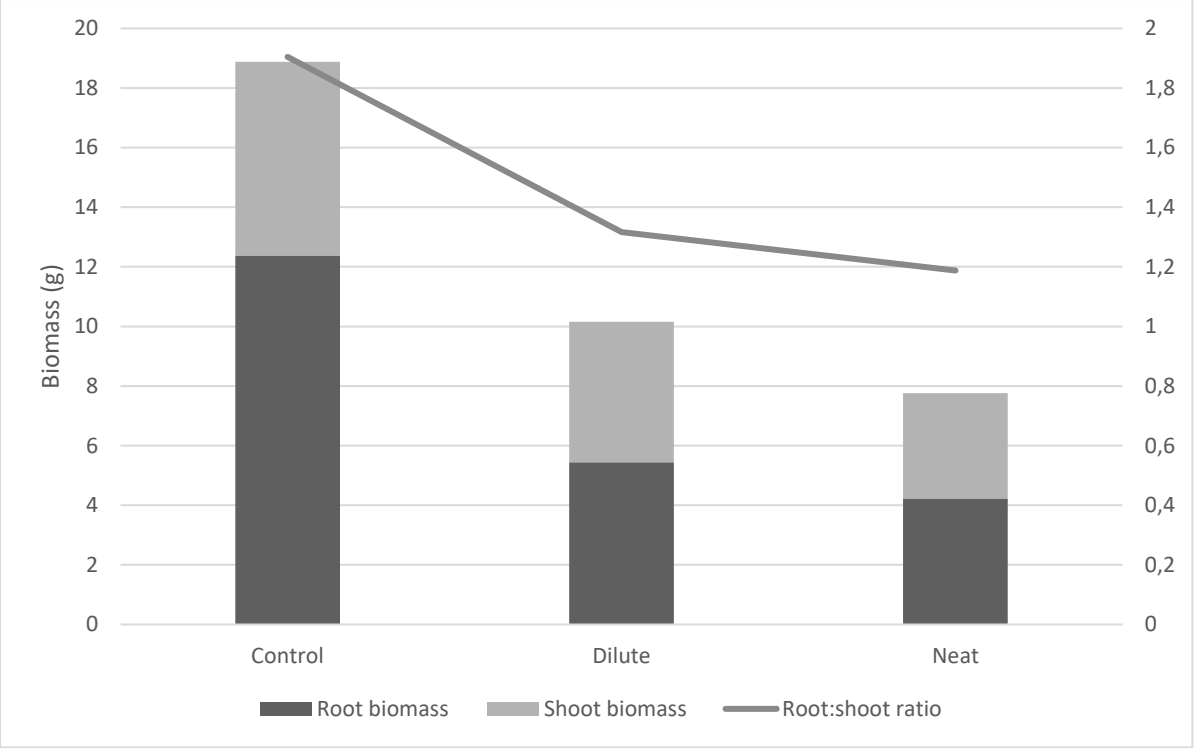


Figure 5.15. The average root biomass, shoot biomass and root:shoot ratio of *Gazania krebsiana* plants harvested after 14 months. The control was watered weekly with tap water and the dilute treatment was watered weekly with dilute fracking fluids. The neat treatment was watered with tap water weekly after a single application of concentrated fracking fluids for the first four months, after which plants were watered weekly with concentrated fracking fluids.

The *Sarcostemma viminale* plants were unaffected by the application of fracking fluids at the various dilutions (Fig. 5.16). Root:shoot ratio was less in the dilute and neat treated plants than in the control plants, but not significantly so. There were also no significant differences in the root or shoot biomass of the plants treated with either tap water or fracking fluids.

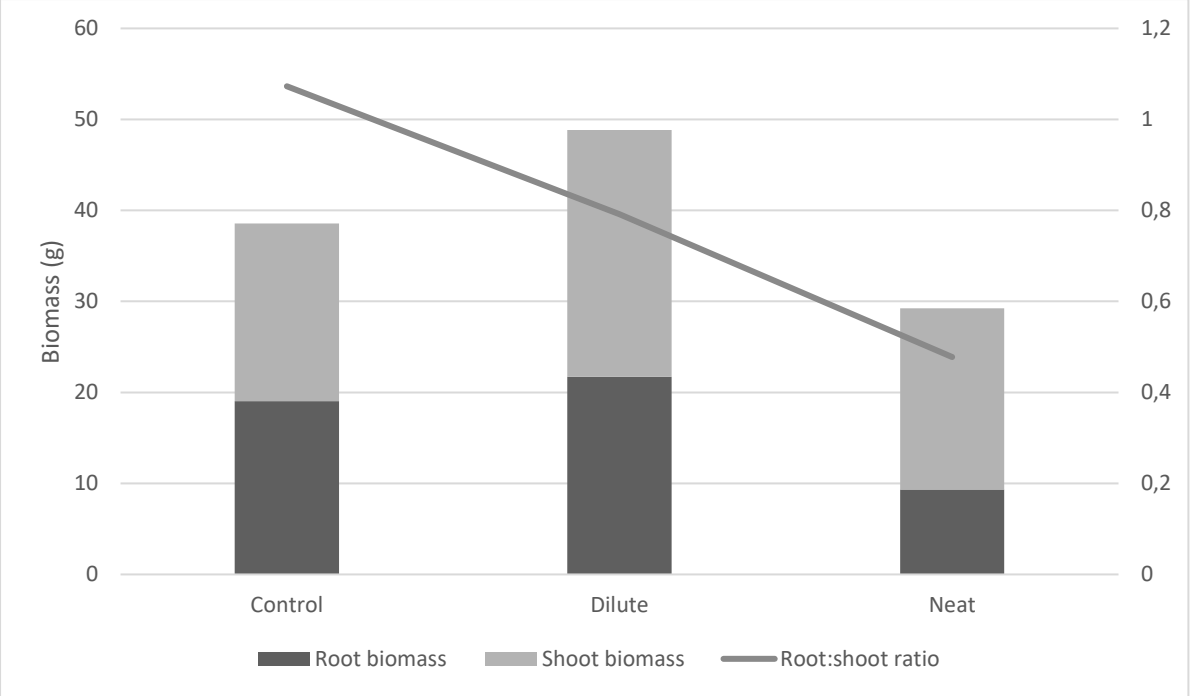


Figure 5.16. The average root biomass, shoot biomass and root:shoot ratio of *Sarcostemma viminale* plants harvested after 14 months. The control was watered weekly with tap water and the dilute treatment was watered weekly with dilute fracking fluids. The neat treatment was watered with tap water weekly after a single application of concentrated fracking fluids for the first four months, after which plants were watered weekly with concentrated fracking fluids.

5.4 Discussion

Within four months of the start of the experiment, 50% of the species treated with fracking fluids had died. The control plants of the less tolerant species all had Fv/Fm ratios still within the healthy range whereas the plants treated with either neat or dilute fracking fluids had ratios indicating minor to major strain.

The low shrub *Chrysocoma ciliata*, the grass *Themeda triandra* and the phreatophytic tree *Vachellia karroo* were amongst the species found to be intolerant to contamination with fracking fluid. In a landscape such as the Karoo where low shrubs and grasses often dominate, and trees such as *V. karoo* are also common, contamination with fracking fluids is likely to change the dominant life form dynamics and species composition of large areas in the fracking footprint. In a study by Adams (2011) herbaceous vegetation, small trees and shrubs were the first to show signs of damage of the application of fracking fluid. Browning, wilting and curling of leaves was observed in these plants. The physiological responses observed in this study are

similar to those found by Adams (2011) but the response time was longer in this current study. Adams (2011) did however admit that the volume of fracking fluids applied was very high for the volume of land used, so her results were likely an over-estimation.

Taking all species into consideration, the Fv/Fm ratio was higher in the control plants than in the treated plants in 80% of the species studied. Of that 80%, the Fv/Fm ratio was higher in the neat treatment compared to the dilute treatment. This could be due to the plants being treated with dilute fluid regularly from the start of the experiment whereas the neat treated plants were initially treated with fluid once off. Only after the four month period were the plants treated with neat fluid twice weekly. This may indicate that consistent exposure to low doses of fracking fluids, such as with groundwater contamination, may be more harmful than a single high dose contamination.

In 50% of the species studied the control plants Fv/Fm remained in the healthy range (as described by Mohammed et al., 2003) while both neat and dilute treatments ratios fell in the stressed range. In 30% of the species the control and both treatment ratios remained in the healthy range.

In terms of plant condition, growth rate and shoot and root biomass were higher in the control plants of *Portulacaria afra* and *Euclea undulata* than in those treated with fracking fluids. Popovic et al. (2003) states that environmental stresses such as pollutants may have deleterious effects on the photosynthesis of the affected plants, which could negatively affect plant growth. Higher shoot biomass was expected in the plants not treated with fracking fluids as studies have shown that above-ground biomass tends to be higher in plants not exposed to pollutants (Takaki & Wolf, 2011; Fiorentino et al., 2017). Root:shoot ratio was higher in the control plants of *Euclea undulata, Sarcostemma viminale* and *Gazania krebsiana* compared to the plants treated with fracking fluids. This was not as expected; plants under stress generally have a higher root:shoot ratio than non-stressed plants. Root:shoot ratios are also influenced by many other factors aside from environmental stresses, such as inherent species characteristics, soil moisture, nutrient availability and light levels (Mokany et al., 2006). These results indicate that even though certain life forms appear to be more tolerant of contamination with fracking fluids in terms of photosynthetic responses, plant growth may still be affected. *Portulacaria afra* and *Euclea undulata* are common

in many of the vegetation types included in the fracking footprint. Stunting of growth through the contamination of the vegetation by fracking fluids is likely to change the vegetation structure of those habitats.

In the study by Adams (2011) the trees were the most resilient life form treated with fracking fluids, so the mortality of *V. karroo* so early in the study was not expected. It was however hypothesized that phreatophytes would be more susceptible to contamination due to the large volumes of water taken up by these trees, and this appears to be supported by the data. The *V. karroo* trees showed visible signs of stress (as seen in Plate 5.1) with the leaves turning brown and becoming brittle and desiccated. The visual damage to the trees coupled with the Fv/Fm ratio findings indicates that these trees were highly susceptible to contamination from fracking fluids.

In similar studies increased salinity was found to be the cause of mortality or reduced plant growth (Brady & Weil, 2002; Zvomuya et al., 2009; Wolf & Brye, 2012). Initially, increased salinity inhibits plant growth due to osmotic effects of decreased water availability. Thereafter, the accumulation of salt in leaves can lead to leaf senescence or necrosis, which in turn reduces photosynthesis and limits growth (Munns et al., 1995). Only the neat fracking fluids had salinity high enough to negatively affect the plants so this does not explain the more pronounced results in the plants treated with dilute fluids. A possible explanation is that those plants treated with dilute fluids were treated regularly with the fluid from the beginning of the experiment, whereas those treated with the neat fluids were treated only once then watered with standard water. Only after the initial four month period were the plants dosed regularly with the neat fluids. Hence the dilute treatment plants had longer term exposure to the fracking fluids, indicating that even at low doses continuous exposure to fracking fluids may affect photosynthetic efficiency and vegetative growth in some species.

While the effects of air pollution and heavy metal contamination on plants are well documented, the effects of fracking fluids have not been studied in detail. Comparable studies are not readily available. The closest comparison may be studies on the effect of acid rain on plants. Both the neat and the dilute fluids are acidic; fluids with a pH under five are considered acidic, comparable to acid rain (Lal, 2016). Previous studies have shown that exposure to low pH fluids can lower photosynthetic rates resulting in reduced plant growth and visible damage to the leaves (Neufeld et al., 1985). Application with simulated acid rain (SAR) has been shown to decrease shoot height,

root length, leaf area and above and below ground biomass (Singh & Agrawal, 1996 & 2004; Tyagi et al., 2004; Dhaka, 2006; Kausar et al., 2010). Kausar et al. (2010) also found that photosynthetic pigments (Chl a, Chl b, total Chl and carotenoids), seed carbohydrates, seed proteins and leaf epidermal parameters (number of stomata, stomatal aperture and trichomes) decreased significantly with SAR treatment. The effects of the acidic fluid on the photosynthetic apparatus would explain the lower photosynthetic rates in contaminated plants, and the subsequent reduction in growth rates.

The specific chemicals used in the fracking fluid mixture may also be the cause of the apparent reduction in photosynthetic efficiency and resultant reduction of plant growth. Most toxicology studies on the chemicals used focus on human/mammal health and/or aquatic systems rather than terrestrial plants. Methanol has been shown to increase photosynthetic efficiency and growth (Mortensen, 1995; Zheng et al., 2008). Glutaraldehyde is not considered a risk to the terrestrial environment as it biodegrades in soil and does not bioaccumulate. It also biodegrades rapidly so it is unlikely to contaminate surface or groundwater (IPCS, 1998). Polyacrylamide is used commercially as a soil stabilizer. It has been shown to have no effect on crop yield and is non-toxic to plants (Seybold, 1994). Ethylene glycol has been shown to negatively affect germination and plant growth (Bose & Datta, 1973; Bose & Bandyopadhyay, 1975; Bose & Naskar, 1975; Edwards et al., 2011) but the application concentrations were much higher than the concentrations found in fracking fluids. As such, the level of ethylene glycol found in fracking fluid is unlikely to negatively affect plants. Low doses of ethanol may be beneficial to plants. Rowe et al. (1994) found increased leaf and stem dry weights on application of ethanol. For ornamental plants, ethanol increased bougainvillea flower longevity and delayed senescence in cut flower storage conditions. Those bougainvillea plants treated with low dose ethanol also exhibited higher Fv/Fm ratios (Hossain et al., 2008). Petroleum distillates such as the hydrotreated kerosene used in this experiment have been shown to be phytotoxic (Odjegba & Sadiq, 2002; Anienye et al., 2015) but at much higher concentrations than those found in fracking fluids. Acetic acid may have negative effects on the roots of exposed plants. Studies on the effects of acetic acid on certain wheat cultivars found that seedling development was reduced with exposure to acetic acid mainly due to a reduction in the length of the radicles (De Tunes et al., 2012). Acetic acid may also

cause cell membranes to leak (Van Overbeek & Blondeau, 1954; Jackson & Taylor, 1970). Armstrong and Armstrong (2001) concluded that root damage observed in common reed *Phragmites australis* (Cav.) Steud. and rice *Oryza sativa* L. was due to cell membrane damage caused by acetic acid. Therefore, acetic acid may damage the roots of exposed plants, but it is unlikely at the low concentrations found in fracking fluids. Though lauryl sulphate has been shown to affect germination and shoot and root growth in a number of monocots, the surfactants were more toxic in solution than once they were absorbed by the soil (Endo et al., 1969). A similar study by Luxmoore et al. (1974) also showed that the adsorption of the surfactants reduced phytotoxity in barley roots. As such, low concentrations of lauryl sulphate or contaminated water which runs off into soils and is absorbed poses little threat to terrestrial vegetation. The presence of hydrochloric acid would lower the pH of the soil should it be contaminated with fracking fluids. As discussed, low pH growing conditions may result in lowered photosynthetic ability, reduced plant growth and leaf damage (Neufeld et al., 1985). Most of the individual constituents of fracking fluids are not phytotoxic and those that have been shown to cause damage to terrestrial plants do so at concentrations way above what is found in fracking fluids. It is therefore unlikely that the specific chemical composition is responsible for any negative effects observed in the treated plants. Rather the cumulative effect of the combination of ingredients resulting in the solution being mildly acidic (dilute treatment) to very acidic (neat treatment) is more likely to be the cause of the reduced photosynthetic efficiency and growth observed in some of the species.

In many of the species photosynthetic responses to temperature stress were observed. These were mostly related to heat stress due to high temperatures in the glasshouse during summer. Inhibition of photosynthesis has been shown to be caused by both high temperatures (Berry & Björkman, 1980; Haldimann & Feller, 2004) and low temperatures (Huner et al., 1993). The geophyte *Ammocharis coranica* appeared unaffected by heat stress, which was expected as most of the plant is underground (not exposed to the heat stress).

5.5 Conclusions

The application of fracking fluids at various dilutions resulted in a 50% mortality rate in this study. Growth was somewhat stunted in those species that were more tolerant to the fracking fluids. This suggests that even if species are tolerant to contamination

with fracking fluids, the growth rate of those species may still be affected. The hypothesis that the application of fracking fluids will negatively affect the growth and survivorship of Karoo and Thicket species is accepted.

Most of the species studied showed a decrease in photosynthetic efficiency after exposure to fracking fluids. The hypothesis that species treated with fracking fluids will exhibit signs of environmental stress in the form of lowered photosynthetic efficiency (reduction in Fv/Fm ratio) is therefore accepted.

Biomass allocation seemed to be unaffected by treatment with fracking fluids at these concentrations and as such the hypothesis that root:shoot ratios will be higher in those plants treated with fracking fluids is rejected.

Those plants treated with dilute fracking fluids over a longer period of time performed more poorly than those treated with neat fluids over a short period. This suggests that low dose long term exposure (groundwater contamination) may be more detrimental than once off high dose exposure (surface spill of fracking fluids).

The phreatophyte *Vachellia karroo* was found to be intolerant to contamination with fracking fluids and died within four months of contamination. The hypothesis that phreatophytes will be particularly sensitive to treatment with fracking fluids is therefore accepted. Low growing shrubs such as *Chrysocoma ciliata* and grasses such as *Themeda triandra* appear to be intolerant to contamination with fracking fluids. This has important implications for the vegetation of the fracking footprint, as these life forms tend to be dominant. Large-scale land application of fracking fluids to Karoo and Thicket vegetation is suggested to further expand the knowledge of how species and various life forms may be affected by contamination. Long term monitoring would also be advantageous to investigate how the various life stages of the plants are affected.

CHAPTER 6 GENERAL DISCUSSION

6.1 Introduction

The potential fracking footprint in the Karoo, Eastern Cape, spans a large, diverse area encompassing three biomes and many different vegetation types. Should fracking go ahead, the various environmental risks associated with the fracking process may affect the various vegetation types in the Karoo differently. To elucidate the potential effects of fracking, the effects of surface and groundwater contamination on the germination and growth of Karoo plants was investigated. A baseline description of the flora at risk in the fracking footprint was also completed, including an investigation of the responses of the various vegetation types to degradation. This chapter is a synthesis of the findings of this study.

6.2 Chapter synopses

Chapter 3: Diversity and phytosociology of the vegetation in the proposed Karoo fracking footprint.

The three biomes in the fracking footprint were similar in measures of biodiversity. The effect of degradation on life form composition, biodiversity and soil properties in the three biomes was observable. Community analysis of the vegetation in the fracking footprint also provided an understanding of previously poorly understood vegetation.

The key findings of this chapter are:

- Degradation will affect life form composition and biodiversity.
- Though the dominant life forms in each biome may not change, the contribution of the number of taxa to each life form is reduced with degradation.
- Degradation results in reduced species richness and diversity and a change in community composition.
- Soil organic content and water holding capacity are reduced with degradation.
- Grassland vegetation is most sensitive to degradation, while Albany Thicket is less sensitive and Nama-Karoo the least sensitive to degradation.
- Eastern Cape Escarpment Thicket and Camdebo Escarpment Thicket are low diversity forms of Nama-Karoo vegetation, not Albany Thicket vegetation types.

Chapter 4: The effect of fracking fluid on the germination of Karoo and Thicket species

For the majority of species in this study, fracking fluids had little influence on seed germination implying that germination was initiated by imbibition of water irrespective of salinity, pH or toxic chemicals.

The key findings of this chapter are:

- In general, seed germination is unaffected by the application of a single high dose or a continual low dose of fracking fluids.
- Low shrubs and grasses in the fracking footprint are at greatest risk of reduced germination success should contamination with fracking fluids occur.

Chapter 5: The effect of fracking fluid on the photosynthetic efficiency and condition of Karoo and Thicket species

In this study the application of fracking fluids at two different concentrations resulted in a 50% mortality rate. Most of the species studied showed a decrease in photosynthetic efficiency after exposure to fracking fluid. Growth was somewhat stunted in those species that were more tolerant to the fracking fluids. The phreatophyte *Vachellia karroo* was found to be intolerant to contamination with fracking fluids and died within four months of contamination. Low growing shrubs such as *Chrysocoma ciliata* and grasses such as *Themeda triandra* were also intolerant to contamination with fracking fluids.

The key findings of this chapter are:

- Contamination of groundwater (continual, low dose treatment) or a surface spill (single, high dose treatment) with fracking fluids is likely to result in substantial plant mortality.
- Continual low dose contamination with fracking fluids is more detrimental to the photosynthetic efficiency and growth of plants than a single high dose.
- In plants exposed to fracking fluids, growth is likely to be stunted.
- Contamination by fracking fluids reduces the photosynthetic efficiency of plants, as indicated by an increase in plant stress.
- Phreatophytes, low shrubs and grasses are particularly at risk of stress and/or mortality should contamination occur.

6.3 General discussion

The results of this study have shown the potential eco-physiological effects of fracking contamination (surface spill of fracking fluids or contamination of groundwater with fracking fluids) on Karoo plants but also the potential effects of fracking related degradation on the plant communities within the fracking footprint. The degraded sites (farmlands) are degraded by grazing, not pollution, but should shale gas development proceed on those farm sites, there will be further degradation from the currently degraded state. The first concern addressed was how fracking related degradation – land clearing, construction of roads and well pads (habitat fragmentation), and a potential increase in exotic species – might affect Karoo vegetation communities in the fracking footprint. In this study, the degraded communities assessed had been impacted by livestock grazing. The degradation from fracking activities is expected to be more intense. The various plant communities in the fracking footprint showed variable reaction to degradation but in general these communities changed in terms of biodiversity, for the most part negatively. Loss of species richness, diversity and changes in community composition as well as changes in soil properties which directly affect ecosystem functioning were observed. At a broad scale the biomes differed in their potential tolerance to degradation, with Grassland emerging as the most at risk of the impacts of degradation, Albany Thicket less at risk and Nama-Karoo low risk. Regardless of the extent of the impact, the clearing of land for fracking is bound to result in a loss of biodiversity through the loss of species. Such loss of biodiversity affects the functioning of ecosystems which in turn affects the ability of ecosystems to deliver services (Cadman et al., 2010). From a landscape ecology point of view, it is more desirable to maintain as much diversity as possible at a larger scale (biome wide, regardless of the expected tolerance of degradation) (Cadman et al., 2010).

The next concern addressed was the potential contamination of surface or groundwater by fracking fluid, and subsequent uptake of the contaminated water by plants. Although the phytosociological results indicate that Nama-Karoo is likely to be more resilient than Albany Thicket and Grassland, the findings of the eco-physiological experiments must also be taken into account. Low shrubs, grasses and phreatophytes were shown to be most affected by contamination with fracking fluid. The Nama-Karoo vegetation is in general dominated by grasses and low shrubs, so even though phytosociological analysis puts the Nama-Karoo vegetation at low risk, the potential

impacts may be much higher under a surface or groundwater spill of fracking fluid. *Vachellia karroo* is common throughout the Karoo and was shown to be sensitive to contamination with fracking fluid. Therefore, regardless of the resilience of the biome as a whole, communities in which *V. karroo* is common are likely to be more highly impacted by the mortality of these phreatophytes under a contamination scenario.

6.4 Significant contributions

This study is the first to investigate the potential eco-physiological effects on fracking fluid contamination on plants within a South African setting. Even at an international scale, little research is available on the potential responses of vegetation to fracking. The study by Adams (2011) investigated the response of vegetation to contamination with fracking fluids, but to date no studies have investigated the response of South African vegetation. This is the first study to show that germination of Karoo species is not likely to be affected by surface spills or groundwater contamination by fracking fluids. It is also the first study known to provide an empirical assessment of stress in plants contaminated with fracking fluid, through measurement of photosynthetic efficiency. This is also the first study to measure changes in plant growth after fracking fluid contamination. These experiments were also used to evaluate how the various vegetation communities, particularly the currently degraded ones, in the fracking footprint may respond to further degradation due to fracking. A predicted level of risk is assigned to each biome (i.e. Grassland high risk, Albany Thicket lower risk and Nama-Karoo lowest risk) based on the findings of this study. Though it was suspected that Eastern Cape Escarpment Thicket and Camdebo Escarpment Thicket were marginal Nama-Karoo elements rather than Albany Thicket elements, this study provides phytosociological evidence to support this notion. The effects of fracking on vegetation types previously classified as Thicket should be considered as similar to the Nama-Karoo vegetation, but with less resilience due to lower biodiversity.

6.5 Project limitations

As with most studies based on projected impacts, there were limitations in this one. The fracking fluid used was formulated according to available literature and available chemicals, and is not necessarily the same as the fluid that will be used should fracking proceed in the Karoo. The addition or removal of chemicals from the formulation may change the outcomes of the experiments presented here and may also affect the

environmental risk that the fluid has on the environment (i.e. the removal of certain chemicals may make the fluid less toxic).

The germination and photosynthetic efficiency experiments were carried out in controlled environments and on selected species. Responses of vegetation *in situ* may be different from these results and other taxa may respond differently from those chosen for experimentation.

While the vegetation surveys were extensive they only covered part of the potential fracking footprint. In particular, the quadrat-based surveys are acknowledged as being not particularly successful in finding rare species or Species of Conservation Concern.

6.6 Future research

A vegetation-scale application of fracking fluid on natural vegetation in the Karoo is suggested to test the validity of the findings of the eco-physiological glasshouse-based experiments presented here. The only comparable study to date is that of Adams (2011) which was a vegetation-scale application of hydraulic fracturing fluids in a forest in the USA. Vegetation-scale application of fracking fluids in the Karoo would make the results of the two studies more comparable. This would also allow long term monitoring of the effects of the pollution on the vegetation to assess morphological effects of contamination and the changes in community composition over time. In particular, longer-term mortality rates of certain species could be determined. Further botanical surveys are also suggested, particularly as continued geological evidence indicates where in the Karoo the shale gas “hotspots” may be. This would allow the focus of the surveys on the vegetation that is most at risk. More detailed soil studies are also recommended. Only simple soil analyses were included in this study, but more robust soil analyses could be undertaken during the vegetation-scale trials, including chemical and physical characterisation of the impact of the fracking fluids on the soils and the subsequent impacts on the vegetation.

LITERATURE CITED

ABELLA, S.R. & ZIMMER, B.W. 2007. Estimating organic carbon from loss-on-ignition in Northern Arizona forest soils. *Soil Sci. Soc. Am. J.* 71: 545–550.

ACOCKS, J.P.H. 1953. Veld types of South Africa. *Mem. Bot. Surv. S. Afr*. 28: 1–192.

ACOCKS, J.P.H. 1979. The flora that matched the fauna. *Bothalia* 12: 673–709.

ACOCKS, J.P.H. 1988. Veld types of South Africa. 3rd ed. *Mem. Bot. Surv. S. Afr*. 57: 1–146.

ACOLA. 2013. *Engineering Energy: Unconventional Gas Production. A study of shale gas in Australia*. Australian Council of Learned Academies, Melbourne Victoria. Available from: http://www.acola.org.au/index.php/projects/securing-australia-s-future/project-6.

ADAMS, M.B. 2011. Land application of hydrofracturing fluids damages a deciduous forest stand in West Virginia. *J. Environ. Qual.* 40: 1340–1344.

ADAM, G. & DUNCAN, H. 2002. Influence of diesel fuel on seed germination. *Environ. Poll.* 120: 363–370.

ADAMS, M., FORD, W., SCHULER, T. & THOMAS-VAN GUNDY, M. 2011. Effects of natural gas development on forest ecosystems. In: *Proceedings of the 17th Central Hardwood Forest Conference*, pp. 219–226.

ANIENYE, P.N., IJAH, U.J.J. & NNAMDI, N. 2015. Assessment of the effect of kerosene spill on the physicochemical properties of soil ten years after spill at Maikunkele, Niger State Nigeria*. IJHSRP*. Volume 5, Issue 8: 1-8.

ARMSTRONG, J. & ARMSTRONG, W. 2001. Rice and *Phragmites*: effects of organic acids on growth, root permeability, and radial oxygen loss to the rhizosphere. *Am. J. Bot*. 88: 1359–1370.

ARMSTRONG, A.J., BENN, G., BOWLAND, A.E., GOODMAN, P.S., JOHNSON, D.N., MADDOCK, A.H. & SCOTT-SHAW, C.R. 1998. Plantation forestry in South Africa and its impact on biodiversity. *Southern African Forestry Journal* 182: 59-65.

ACADEMY OF SCIENCE OF SOUTH AFRICA (ASSAF). 2016. *South Africa's Technical Readiness to Support the Shale Gas Industry*. doi: http://dx.doi.org/10.17159/assaf.2016/0003.

AUCAMP, A.J. & TAINTON, N.M. 1984. *Veld Management in the Valley Bushveld of the Eastern Cape*. Department of Agriculture, Pretoria, 34 pp.

BÁEZ, S. & COLLINS, S.L. 2008. Shrub invasion decreases diversity and alters community stability in northern Chihuahuan Desert plant communities. *PLoS ONE* 3: e2332. doi:10.1371/ journal.pone.0002332.

BAINBRIDGE, W.R. 1993. Management of mountain catchment grassland with special reference to the Natal Drakensberg. In: Van der Sijde, H.A. (ed.) *South African Forestry Handbook.* Southern African Institute of Forestry, Pretoria. pp. 675–690.

BALVANERA, P., PFISTERER, A.B., BUCHMANN, N., HE, J.-S., NAKASHIZUKA, T., RAFFAELLI, D. & SCHMID, B. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecol. Lett.* 9: 1146–56.

BANKS, M.K. & SCHULTZ, K.E. 2005. Comparison of plants for germination toxicity tests in petroleum-contaminated soils. *Water Air Soil Poll.* 167: 211-219.

BARBER, M.E. 1880. Locusts and locust birds. *Trans. Phil. Soc.* 3: 193–218.

BARLOW, K.M., MORTENSEN, D.A., DROHAN, P.J. & AVERILL, K.M. 2017. Unconventional gas development facilitates plant invasions. *J. Environ. Manage.* 202: 208-216.

BELVALKAR, R. & OYEWOLE, S. 2010. Development of Marcellus shale in Pennsylvania. In: Proceedings of the 2010 SPE Annual Technical Conference and Exhibition (ATCE), Florence, Italy. September 20-22, 2010.

BERRY, J.A. & BJÖRKMAN, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Ann. Rev. Plant Phys.* 31: 491–543.

BEWLEY, J.D. 1997. Seed germination and dormancy. *Plant Cell* 9: 1055-1066.

BEWLEY, J.D. & BLACK, M. 1994. *Seeds: Physiology of Development and Germination.* Plenum Press, New York. 445 pp.

BEZUIDENHOUT, R. 2017. The many rewards of game farming in the Karoo. *Farmer's Weekly* 17008: 42-44.

BJÖRKMAN, O. & DEMMIG, B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* 170: 489-504.

BOND, W.J. 1997. Fire. In: Cowling, R.M., Richardson, D. & Pierce, S.M. (eds.) *Vegetation of Southern Africa.* Cambridge Univ. Press, Cambridge. pp. 421–446.

BOND, W.J., MIDGLEY, G.F. & WOODWARD, F.I. 2003a. The importance of low atmospheric CO_2 and fire in promoting the spread of grasslands and savannas. Glob. *Change Biol.* 9: 973–982.

BOND, W.J., MIDGLEY, G.F. & WOODWARD, F.I. 2003b. What controls South African vegetation – climate or fire? *S. Afr. J. Bot.*, 69: 79–91.

BOSE, S. & BANDYOPADHYAY, M. 1975. Effect of dimethyl sulfoxide, ethylene glycol and hydroxylamine on tomato (*Lycopersicon esculentum* Mill.). *Sci. Cult.* 41:240–241.

BOSE, S. & DATTA, G.C. 1973. Effect of treatments of colchicine, dimethylsulphoxide, ethylene glycol, hydroxylamine and triethanolamine in jute (*Corchorus capsularis* L.). *Bangladesh J. Botany*. 2:1–6.

BOSE, S. & NASKAR, S.K. 1975. Effect of dimethyl sulfoxide, ethylene glycol, hydroxylamine and triethanolamine in M1 generation in cluster bean. *Bulletin of the Botanical Society of Bengal* 29:49–52.

BOTHA, J.F., VERWEY, J.P., VAN DER VOORT, I., VIVIER, J.J.P., COLLISTON, W.P. & LOOCK, J.C. 1998. *Karoo Aquifers. Their Geology, Geometry and Physical Behaviour.* WRC Report No 487/1/98. Water Research Commission.

BOUREAU, E., SALARD-CHEBOLDAEFF, M., KOENINGEUR, J.-C. & LOUVET, P. 1983. Evolution des flores et de la végétation Tertiaires en Afrique, au nord de l'Equateur. *Bothalia* 14: 355–367.

BOUSMAN, C.B., PARTRIDGE, T.C., SCOTT, L., METCALFE, S.E., VOGEL, J.C., SEAMAN, M. & BRINK, J.S. 1988. Palaeoenvironmental implications of Late

Pleistocene and Holocene valley fills in Blydefontein basin, Noupoort, C.P., South Africa. *Palaeoecology of Africa* 19: 43–67.

BOUSMAN, B. & SCOTT, L. 1994. Climate or overgrazing? The palynological evidence for vegetation change in the eastern Karoo. *S. Afr. J. Sci.* 90: 575–578.

BRADY, N.C. & WEIL, R.R. 2002. *The Nature and Properties of Soils*. 13th ed. Pearson Education, Upper Saddle River, NJ. 960 pp.

BROWN, L.R., DU PREEZ, P.J., BEZUIDENHOUT, H., BREDENKAMP, G.J., MOSTERT, T.H.C. & COLLINS, N.B. 2013. Guidelines for phytosociological classifications and descriptions of vegetation in southern Africa. *Koedoe* 55: 1-10.

BROWNLIE, S. 2005. *Guideline for Involving Biodiversity Specialists in EIA Processes*. CSIR Report No ENV-S-C 2005 053 C. Republic of South Africa, Provincial Government of the Western Cape, Department of Environmental Affairs & Development Planning, Cape Town.

BURNHAM, A., HAN, J., CLARK, C., WANG, M., DUNN, J. & PALOU-RIVERA, I. 2011. Life-cycle greenhouse gas emissions of shale gas, natural gas, coal, and petroleum. *Environ. Sci. Tech.* 46: 619–627.

CANADELL, J., JACKSON, R.B., EHLERINGER, J.R., MOONEY, H.A., SALA, O.E. & SCHULZE, E.D. 2006. Maximum rooting depth of vegetation types at the global scale. *Oecologia* 108: 583–595.

CARBUTT, C. & EDWARDS, T.J. 2004. The flora of the Drakensberg Alpine Centre. *Edinb. J. Bot.* 60: 581–607.

CARBUTT, C. & EDWARDS, T.J. 2006. The endemic and near-endemic angiosperms of the Drakensberg Alpine Centre. *S. Afr. J. Bot.,* 72: 105– 132.

CARDINALE, B.J., SRIVASTAVA, D.S., DUFFY, J.E., WRIGHT, J.P., DOWNING, A.L., SANKARAN, M. & JOUSEAU, C. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* 443:989–92.

CARPENTER, S.R., MOONEY, H.A., AGARD, J., CAPISTRANO, D., DE FRIES, R.S., DI´AZ, S., DIETZ, T., DURAIAPPAH, A.K., OTENG-YEBOAH, A., PEREIRA, H.M., PERRINGS, C., REID, W.V., SARUKHAN, J., SCHOLES,

R.J. & WHYTE, A. 2009. Science for managing ecosystem services: beyond the millennium ecosystem assessment. *Proc. Natl. Acad. Sci. USA* 106:1305–1312.

CARR, A.S., BOOM, A., CHASE, B.M., MEADOWS, M.E., ROBERTS, Z.E., BRITTON, M.N. & CUMMING, A.M. 2013. Biome-scale characterisation and differentiation of semi-arid and arid zone soil organic matter compositions using pyrolysis–GC/MS analysis. *Geoderma* 200: 189-201.

CARRICK, P.J. & KRÜGER, R. 2007. Restoring degraded landscapes in lowland Namaqualand: Lessons from the mining experience and from regional ecological dynamics. *J. Arid Env.* 70: 767-781.

CERLING, T.U., HARRIS, J.M., MACFADDEN, B.J., LEAKEY, M.G., QUADE, J., EISENMAN, V. & EHLERINGER, J.R. 1997. Global vegetation change through the Miocene/Pliocene boundary. *Nature* 389: 153–158.

CHANG, A.C., GRANATO, T.C. & PAGE, A.L. 1992. A methodology for establishing phytotoxicity criteria for chromium, copper, nickel, and zinc in agricultural land application of municipal sewage sludges. *J. Environ. Qual.* 21: 521–536.

CHAPIN, F.S. III, AUTUMN, K. & PUGNAIRE, F. 1993. Evolution of suites of traits in response to environmental stress. *Am. Nat.* 142: S78-S92.

CHAPIN, S.C., KOFFINAS, G.P. & FOLKE, C. 2009. *Principles of Ecosystem Stewardship: Resilience-based Natural Resource Management in a Changing World.* Springer, New York.

CHERE, N. 2015. *Sedimentological and Geochemical Investigations on Borehole Cores of the Lower Ecca Group Black Shales, for Their Gas Potential - Karoo Basin, South Africa.* MSc Thesis, Nelson Mandela Metropolitan University, Port Elizabeth. 175 pp.

CHERE, N., LINOL, B., SCHULZ, H-M. & DE WIT, M.J. 2017. Lateral and temporal variations of black shales across the southern Karoo Basin - Implications for shale gas exploration. *S. Afr. J. Geol.* (in press).

CLARK, V.R., BARKER, N.P., MCMASTER, C. & MUCINA, L. 2011. The Boschberg (Somerset East, Eastern Cape) – A floristic cross-roads of the southern Great Escarpment. *S. Afr. J. Bot.* 77: 94-104.

COETZEE, J.A. 1978. Climatic and biological changes in southwestern Africa during the Late Cainozoic. *Palaeoecol. Afr.* 10: 13–29.

COLE, D.I. 2014. *Geology of Karoo Shale Gas and How This Can Influence Economic Gas Recovery*. Presentation, Gas – The Game Changer for Southern Africa? Fossil Fuel Foundation, Glen Hove, Johannesburg.

COLE, D.I. & MCLACHLAN, I.R. 1994. *Oil shale potential and depositional environment of the Whitehill Formation in the main Karoo Basin*. SOEKOR, now PASA, South African Petroleum Agency, Cape Town. Report No 213.

COOK, J. & JOHNSON, M. 2002. Ecological restoration of land with particular reference to mining metals and industrial minerals: A review of theory and practice. *Environ. Rev.* 10: 41–71.

COURT, D. 2010. *Succulent flora of Southern Africa*. Struik Nature, Cape Town. 336 pp.

COWLING, R.M. 1983. Phytochorology and vegetation history in the south-eastern Cape, South Africa. *J. Biogeogr.* 10: 393-419.

COWLING, R.M. 1984. A syntaxonomic and synecological study in the Humansdorp region of the fynbos biome. *Bothalia* 15: 175–227.

COWLING, R.M. & CAMPBELL, B.M. 1983. A comparison of fynbos and nonfynbos coenoclines in the lower Gamtoos River Valley, southeastern Cape, South Africa. *Vegetatio* 53: 161–178.

COWLING, R.M., CARTWRIGHT, C.R., PARKINGTON, J.E. & ALLSOPP, J. 1999. Fossil wood charcoal assemblages from Elands Bay Cave, South Africa: implications for Late Quaternary vegetation and climates in the winter-rainfall fynbos biome. *J. Biogeogr.* 26: 367–378.

COWLING, R.M., ESLER, K.J., MIDGLEY, G.F. & HONIG, M.A. 1994. Plant functional diversity and climate in arid and semi-arid southern Africa. *J. Arid Environ.* 27: 141–158.

COWLING, R.M., GIBBS RUSSELL, G.E., HOFFMAN, M.T. & HILTON-TAYLOR, C. 1989. Patterns of plant species diversity in southern Africa. In: Huntley, B.J. (ed.) *Biotic diversity in southern Africa. Concepts and conservation*. Oxford University Press, Cape Town. pp. 19–50.

COWLING, R.M. & HILTON-TAYLOR, C. 1994. Patterns of plant diversity and endemism in southern Africa: an overview. In: Huntley, B.J. (ed.), *Botanical diversity in southern Africa.* Oxford University Press, Cape Town. pp. 31–52.

COWLING, R.M. & HILTON-TAYLOR, C.I. 1999. Plant geography, endemism and diversity. In: R.W. Dean, S.J. Milton (eds.) *The Karoo: ecological patterns and processes*. Cambridge University Press, Cambridge, pp 42-56.

COWLING, R.M. & HOLMES, P.M. 1991. Subtropical thicket in the southeastern Cape: a biogeographical perspective. In: Zacharias, P.J.K., Stuart-Hill, G.C. and Midgley, J.J. (eds): *Proceedings of the First Valley Bushveld/Subtropical Thicket Symposium* Howick, Kwazulu-Natal: Grassland Society of Southern Africa. pp. 3-4.

COWLING, R.M., LOMBARD, A.T., ROUGET, M., KERLEY, G.I.H., WOLF, T., SIMS-CASTLEY, R., KNIGHT, A., VLOK, J.H.J., PIERCE, S.M., BOSHOFF, A.F. & WILSON, S.L. 2003. A conservation assessment for the Subtropical Thicket Biome. Terrestrial Ecology Research Unit Report 43, University of Port Elizabeth.

COWLING, R.M., PROCHEŞ, Ş. & VLOK, J.H.J. 2005. On the origin of southern African subtropical thicket vegetation. *S. Afr. J. Bot.* 71: 1-23.

COWLING, R.M., RUNDEL, P.W., DESMET, P.G. & ESLER, K.J. 1998. Extraordinary high regional-scale plant diversity in southern African arid lands: subcontinental and global comparisons. *Divers. Distrib.* 4: 27–36.

DAWSON, T.E. & PATE, J.S. 1996. Seasonal water uptake and movement in root systems of Australian phreatophytic plants of dimorphic root morphology: A stable isotope investigation. *Oecologia* 107:13–20.

DE KOCK, M.O., BEUKES, N.J., ADENIYI, E.O., COLE, D., GÖTZ, A.E., GEEL, C. & OSSA, F.G. 2017. Deflating the shale gas potential of South Africa's Main Karoo basin. *S. Afr. J. Sci.* 113: 87-98.

DE RIJKE, K. 2013. The Agri-gas fields of Australia: black soil, food, and unconventional gas. *Cult. Agric. Food. Environ.* 35: 41-53.

DE TUNES, L.M., AVELAR, S.A.G., BARROS, A.C.S.A., PEDROSO, D.C., MUNIZ, M.F.B. & DE MENEZES, N.L. 2012. Critical levels of organic acids on seed germination and seedling growth of wheat. *Rev. Bras. Sem.* 34: 366-372.

DE WIT, M.J. 2011. The great shale debate in the Karoo. *S. Afr. J. Sci.* 107: 1-9.

DEAN, W.R.J. 2000. Factors affecting bird diversity patterns in the Karoo, South Africa. *S. Afr. J. Sci.* 96: 609–616.

DEAN, W.R.J. & MILTON, S.J. 2000. Directed dispersal of *Opuntia* species in the Karoo, South Africa: are crows the responsible agents? *J. Arid Environ.* 45: 305–314.

DEAN, W.R.J. & MILTON, S.J. 2003. Did the flora match the fauna? Acocks and historical changes in Karoo biota. *S. Afr. J. Bot.* 69: 68–78.

DECKER, J. & MAROT, J. 2012. Investigation of hydraulic fracturing in the Karoo of South Africa. Annexure A, Resource Assessment, Petroleum Agency SA. Available at: http://www.dmr.gov.za/publications/viewdownload/182/854.html.

DENGLER, J. 2017. Phytosociology. In: Richardson, D., Castree, N., Goodchild, M.F., Kobayashi, A., Liu, W. & Marston, R.A. (eds.) *The International Encyclopedia of Geography*. John Wiley & Sons, Ltd.

DENGLER, J., CHYTRÝ, M. & EWALD, J. 2008. Phytosociology. In: Jørgensen, S.E. & Fath, B.D. (eds.) *Encyclopedia of Ecology.* Elsevier, Oxford. pp. 2767–2779.

DHAKA, T.S. 2006. Effect of acid rain upon growth parameters of *Zinnia elegans*. *Adv. Plant Sci*. 19: 435-438.

DIAMOND, J.M. 1975. The island dilemma: lessons of modern biogeographic studies for the design of natural reserves. *Biol. Conserv*. 7:129–146.

DIGIULIO, D.C. & JACKSON, R.B. 2016. Impact to Underground Sources of Drinking Water and Domestic Wells from Production Well Stimulation and Completion Practices in the Pavillion, Wyoming, Field. *Env. Sci. Tech.* 50: 4524-4536.

DINNO, A. 2017. Dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package version 1.3.4. http://CRAN.R-project.org/package=dunn.test.

DOING, H. 1970. The use of phytosociological methods in various parts of the world. *Belmontia* 4: 53–61.

DONALDSON, J., NÄNNI, I., ZACHARIADES, C. & KEMPER, J. 2002. Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. *Conserv. Biol.* 16: 1267-1276.

DORROUGH, J.W., ASH, J.E., BRUCE, S. & MCINTYRE, S. 2007. From plant neighbourhood to landscape scales: how grazing modifies native and exotic plant species richness in grassland. *Plant Ecol.* 191: 185–198.

DREBER, N. & ESLER, K.J. 2011. Spatio-temporal variation in soil seed banks under contrasting grazing regimes following low and high seasonal rainfall in arid Namibia. *J. Arid Env.* 75: 174–184.

DRIVER, A., MAZE, K., ROUGET, M., LOMBARD, A.T., NEL, J., TURPIE, J.K., COWLING, R.M., DESMET, P., GOODMAN, P., HARRIS, J., JONAS, Z., REYERS, B., SINK, K. & STRAUSS, T. 2005. National Spatial Biodiversity Assessment 2004: Priorities for biodiversity in South Africa. *Strelitzia* 17. South African National Biodiversity Institute, Pretoria.

DUPÉRON-LAUDOUENEIX, M. & DUPÉRON, J. 1995. Inventory of Mesozoic and Cenozoic woods from Equatorial and North Equatorial Africa. *Rev. Palaeobot. Palynol.* 84: 439-480.

EDWARDS, P.J. 1961. *Studies on veld burning and mowing in the Tall Grassveld of Natal*. M.Sc. thesis, University of Natal, Pietermaritzburg.

EDWARDS, D. 1984. Fire regimes in the biomes of South Africa. In: Booysen, P. de V. & Tainton, N.M. (eds), *Ecological effects of fire in South African ecosystems.* Springer, Berlin. pp. 19–37.

EDWARDS, M.R., HETU, M.F., COLUMBUS, M., SILVA, A. & LEFEBVRE, D.D. 2011. The effect of ethylene glycol on the phytovolatilization of 1,4-dioxane. *Int. J. Phytoremediat.* 13: 702-716.

EDWARDS, E.J., OSBORNE, C.P., STRÖMBERG, C.A., SMITH, S.A. & C_4 GRASSES CONSORTIUM. 2010. The origins of C_4 grasslands: integrating evolutionary and ecosystem science. *Science* 328: 587-591.

EHLERINGER, J.R., CERLING, T.E. & HELIKER, B.R. 1997. C_4 photosynthesis, atmospheric CO_2, and climate. *Oecologia* 112: 285–299.

ELLERY, W.N., SCHOLES, R.J. & SCHOLES, M.C. 1995. The distribution of sweetveld and sourveld in South Africa's grassland biome in relation to environmental factors. *Afr. J. Range Forage Sci.* 12: 38–45.

ELLSWORTH, W.L. 2013. Injection-induced earthquakes. *Science* 341: 1-7.

ELMQVIST, T., FOLKE, C., NYSTROM, M., PETERSON, G., BENGTSSON, J., WALKER, B. & NORBERG, J. 2003. Response diversity, ecosystem change, and resilience. *Front Ecol. Environ.* 1: 488–94.

ENDO, R.M., LETEY, J., VALORAS, N. & OSBORN, J.F. 1969. Effects of nonionic surfactants on monocots. *Agron. J. 61*: 850-854.

ERDMAN, J.A. & CHRISTENSON, S. 2000. Elements in cottonwood trees as an indicator of ground water contaminated by landfill leachate. *Groundwater Monitoring & Remediation* 20: 120-126.

ESTERHUYSE, S., AVENANT, M., REDELINGHUYS, N., KIJKO, A., GLAZEWSKI, J., PLIT, L., KEMP, M., SMIT, A., TASCHA VOS, A. & WILLIAMSON, R. 2016. A review of biophysical and socio-economic effects of unconventional oil and gas extraction – Implications for South Africa. *J. Env. Man.* 184: 419-430.

EU, 2011. *Impacts of Shale Gas and Shale Oil Extraction on the Environment and on Human Health, Environment, Public Health and Food Safety.* Policy Department Economic and Scientific Policy. Directorate-General for Internal Policies, Brussels, European Parliament, pp. 91.

EVANS, N., AVIS, T. & PALMER, A.R. 1997. Changes to the vegetation of the mid-Fish River valley, Eastern Cape, South Africa, in response to land-use, as revealed by a direct gradient analysis. *Afr. J. Range Forage Sci.* 14: 68–74.

EVERARD, D.A. 1987. A classification of subtropical transitional thicket in the eastern Cape, based on synecological and structural attributes. *S. Afr. J. Bot.* 53: 320-340.

EVERARD, D.A. 1991. Is Subtropical Thicket the missing biome of South Africa? In: Zacharias, P.J.K., Stuart-Hill, G.C. & Midgley, J.J. (eds), Proceedings of the

first Valley Bushveld/Subtropical Thicket Symposium. Grassland Society of Southern Africa, Howick. pp. 1–3.

EVERSON, C.S. 1985. *Ecological Effects of Fire in the Montane Grasslands of Natal.* Ph.D. thesis, University of Natal, Pietermaritzburg.

FAHRIG, L. 2003. Effects of habitat fragmentation on biodiversity. *Annu. Rev. Ecol. Evol. Syst.* 34: 487–515.

FAIRBANKS, D.H.K., THOMPSON, M.W., VINK, D.E., NEWBY, T.S., VAN DEN BERG, H.M. & EVERARD, D.A. 2000. The South African land-cover characteristics database: a synopsis of the landscape. *S. Afr. J. Sci.* 96: 69–82.

FIORENTINO, N., VENTORINO, V., ROCCO, C., CENVINZO, V., AGRELLI, D., GIOIA, I., DI MOLA, I., ADAMO, P., PEPE, O. & FAGNANO, M. 2017. Giant reed growth and effects on soil biological fertility in assisted phytoremediation of an industrial polluted soil. *Sci. Total Env.* 575: 1375–1383.

FISHER, M.J., RAO, I.M., AYARZA, M.A., LASCANO, C.E., SANZ, J.I., THOMAS, R.J. & VERA, R.R. 1994. Carbon storage by introduced deep-rooted grasses in the South American savannas. *Nature* 371: 236-238.

FISHER, M.K. & WARPINSKI, N.R. 2012. Hydraulic-fracture-height growth: Real data. *SPE Production & Operations* 27: 8–19.

FONTENOT, B.E., HUNT, L.R., HILDENBRAND, Z.L., CARLTON JR., D.D., OKA, H., WALTON, J.L., HOPKINS, D., OSORIO, A., BJORNDAL, B., HU, Q.H. & SCHUG, K.A. 2013. An evaluation of water quality in private drinking wells near natural gas extraction sites in the Barnett Shale Formation. *Environ. Sci. Technol*. 7: 10032–10040.

FORMAN, R.T. & ALEXANDER, L.E. 1998. Roads and their major ecological effects. *Ann. Rev. Ecol. Syst.* 29: 207-231.

GEEL,C. 2014. *Shale gas characteristics of Permian black shales in the Ecca Group, near Jansenville, Eastern Cape, South Africa*. MSc thesis. Nelson Mandela Metropolitan University, Port Elizabeth.

GEEL, C., DE WIT, M., BOOTH, P., SCHULZ, H.M. & HORSFIELD, B. 2015. Palaeo-environment, diagenesis and characteristics of Permian black shales

in the Lower Karoo Supergroup flanking the Cape Fold Belt near Jansenville, eastern Cape, South Africa: Implications for the shale gas potential of the Karoo Basin. *S. Afr. J. Geol.* 118: 249-274.

GEEL, C., SCHULZ, H.M., BOOTH, P., DE WIT, M. & HORSFIELD, B. 2013. Shale gas characteristics of Permian black shales in South Africa: results from recent drilling in the Ecca Group (Eastern Cape). *Energy Procedia* 40: 256-265.

GELHAR, L.W., WELTY, C. & REHFELDT, K.R. 1992. A critical review of data on field-scale dispersion in aquifers. *Wat. Res. Res.* 28: 1955-1974.

GERMISHUIZEN, G., MEYER, N.L., STEENKAMP, Y. & KEITH, M. (eds). 2006. *A Checklist of South African Plants*. Southern African Botanical Diversity Network Report No. 41. Sabonet, Pretoria: 1126 pp.

GIBBS RUSSELL, G.E. 1986. Significance of different centres of diversity in subfamilies of Poaceae in southern Africa. *Palaeoecol. Afr.* 17: 183–191.

GIBBS RUSSELL, G.E. 1987. Preliminary floristic analysis of the major biomes in southern Africa. *Bothalia* 17: 213–227.

GIBBS RUSSELL, G.E. & ROBINSON, E.R. 1981. Phytogeography and speciation in the vegetation of the eastern Cape. *Bothalia* 13: 467–472.

GILL, A.M. 1975. Fire and the Australia flora: a review. *Australian Forestry*, 38: 4–25.

GILLEN, J.L. and KIVIAT, E. 2012. Environmental reviews and case studies: hydraulic fracturing threats to species with restricted geographic ranges in the Eastern United States. *Environ. Prac.* 14: 320–331.

GOLDER ASSOCIATES AFRICA, 2011. *Volume 1: Environmental Management Plan, South Western Karoo Basin Gas Exploration Application: Central Precinct.* Shell Exploration Company B.V. PASA Reference No. 12/3/220. For submission to the Petroleum Agency of South Africa. Golder Report Number: 12800-10534-29. 243pp.

GOPALAN, H.N.B. 1999. Ecosystem health and human well being: the mission of the international programme on plant bioassays. *Mutat. Res. 426*: 99–102.

GOTTSCHLING, M., MAI, D.H. & HILGER, H.H. 2002. The systematic position of *Ehretia* fossils (Ehretiaceae, Boraginales) from the European Tertiary and implications for character evolution. *Rev. Paleobot. Palynol*. 121: 149–156.

GRANGER, J.E. 1976. *The vegetation changes, some related factors and changes in the water balance following twenty years of fire exclusion in catchment IX, Cathedral Peak Forestry Research Station*. Ph.D. thesis, University of Natal, Pietermaritzburg.

GRASS PHYLOGENY WORKING GROUP. 2001. Phylogeny and subfamiliar classification of the grasses (Poaceae). *Ann. Missouri Bot. Gard*. 88: 373–457.

GROOM, P.K., FROEND, R.H. & MATTISKE, E.M. 2000. Impact of groundwater abstraction on a *Banksia* woodland, Swan Coastal Plain, Western Australia. *Ecol. Manage. Restor.* 1: 117-124.

GRUBB, P.J. 2003. Interpreting some outstanding features of the flora and vegetation of Madagascar. Persp. *Plant Ecol. Evol. Syst*. 6: 125–146.

GRÜN, R., BRINK, J.S., SPOONER, N.A., TAYLOR, L., STRINGER, C.B., FRANCISCUS, R.G. & MURRAY, A.S. 1996. Direct dating of Florisbad hominid. *Nature* 382: 500–501.

GUISAN, A., BROENNIMANN, O., ENGLER, R., VUST, M., YOCCOZ, N.G., LEHMANN, A. & ZIMMERMANN, N.E. 2006. Using niche-based models to improve the sampling of rare species. *Cons. Biol.* 20: 501–511.

GUNDERSON, L.H. 2000. Ecological resilience - in theory and application. *Ann. Rev. Ecol. Syst.* 31: 425–439.

GURALNICK, L.J. & TING, I.P. 1987. Physiological changes in *Portulacaria afra* (L.) Jacq. during a summer drought and rewatering. *Plant Physiol*. 85: 481–486.

GUSTAVSON, K.E., SONSTHAGEN, S.A., CRUNKILTON, R.A. & HARKIN, J.M. 2000. Groundwater toxicity assessment using bioassay, chemical, and toxicity identification evaluation analysis. *Environm. Toxicol.* 15: 421–430.

HADDAD, N.M., BRUDVIG, L.A., CLOBERT, J., DAVIES, K.F., GONZALEZ, A., HOLT, R.D., LOVEJOY, T.E., SEXTON, J.O., AUSTIN, M.P., COLLINS, C.D., COOK, W.M., DAMSCHEN, E.I., EWERS, R.M., FOSTER, B.L., JENKINS,

C.N., KING, A.J., LAURANCE, W.F., LEVEY, D.J., MARGULES, C.R., MELBOURNE, B.A., NICHOLLS, A.O., ORROCK, J.L., SONG, D.X. & TOWNSHEND, J.R. 2015. Habitat fragmentation and its lasting impact on Earth's ecosystems. *Sci. Adv.* 1: e1500052.

HALDIMANN, P. & FELLER, U. 2004. Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1, 5-bisphosphate carboxylase/oxygenase. *Plant Cell Env.* 27: 1169–1183.

HARBINSON, J. & ROSENQVIST, E. 2003. An introduction to chlorophyll fluorescence. In: *Practical applications of chlorophyll fluorescence in plant biology*. DeEll, J.R. & Toivonen, P.M.A. (eds.). Kluwer Academic Publishers, USA: 1–29.

HARPER, J. 2008. The Marcellus Shale - an Old ''New'' gas reservoir in Pennsylvania. *Penn. Geol.* 38: 2–12.

HARTMANN, M.O. 1988. The soils of the Eastern Cape. In: Bruton, M.N. & Gess, F.W. (eds) *Towards an environmental plan for the Eastern Cape.* Rhodes Univ., Grahamstown. pp. 37–43.

HASCHICK, S.H. 2002. *Body size influences on foraging behaviour of thicket biome browsers.* Ph.D. thesis, University of Port Elizabeth.

HIGGINS, S.I., SHACKLETON, C.M. & ROBINSON, E.R. 1999. Changes in woody community structure and composition under constrasting landuse systems in a semi-arid savanna, South Africa. *J. Biogeogr.* 26: 619–627.

HILTON-TAYLOR, C. 1987. Phytogeography and origins of the Karoo flora. In: Cowling, R.M. & Roux, P.W. (eds), *The Karoo biome: a preliminary synthesis. Part 2 – Vegetation and history.* FRD, Pretoria. pp. 70-95.

HILTON-TAYLOR, C. & LE ROUX, A. 1989. Conservation status of the Fynbos and Karoo biomes. In: Huntley, B.J. (ed), *Biotic diversity in southern Africa: concepts and conservation.* Oxford University Press, Cape Town. pp. 202–223.

HOARE, D.B. 1997. *Syntaxonomy and synecology of the grasslands of the southern parts of the Eastern Cape*. M.Sc. thesis, Department of Botany, University of Pretoria.

HOARE, D.B. 2002. Biodiversity and performance of grassland ecosystems in communal and commercial farming systems in South Africa. In: *Proceedings of the FAO's Biodiversity and Ecosystem Approach in Agriculture, Forestry and Fisheries Event*: 12–13 October, 2002. FAO, Rome. pp. 10–27.

HOARE, D.B. 2003. Species diversity patterns in moist temperate grasslands of South Africa. *Afr. J. Range Forage Sci.* 20: 84.

HOARE, D.B. & BREDENKAMP, G.J. 1999. Grassland communities of the Amatola/Winterberg mountain region of the Eastern Cape, South Africa. *S. Afr. J. Bot. 65*: 75–82.

HOARE, D.B., MUCINA, L., RUTHERFORD, M.C., VLOK, J.H., EUSTON-BROWN, D.I., PALMER, A.R., POWRIE, L.W., LECHMERE-OERTEL, R.G., PROCHES, S.M., DOLD, A.P. & WARD, R.A. 2006. Albany thicket biome. In: *The vegetation of South Africa, Lesotho and Swaziland.* (eds L. Mucina and M.C. Rutherford). South African National Biodiversity Institute, Pretoria.

HOFFMAN, M.T., BARR, G.D. & COWLING, R.M. 1990. Vegetation dynamics in the semi-arid eastern Karoo, South Africa: the effect of seasonal rainfall and competition on grass and shrub basal cover. *S. Afr. J. Sci*. 86: 462–463.

HOFFMAN, M.T., COUSINS, B., MEYER, T., PETERSEN, A. & HENDRICKS, H. 1999a. Historical and contemporary landuse and the desertification of the Karoo. In: Dean, W.R.J. & Milton, S.J. (eds), *The Karoo: ecological patterns and processes.* Cambridge Univ. Press, Cambridge. pp. 257–273.

HOFFMAN, M.T. & COWLING, R.M. 1990. Desertification in the lower Sundays river valley, South Africa. *J. Arid Env.* 19: 105–117.

HOFFMAN, M.T. & COWLING, R.M. 1991. Phytochorology and endemism along aridity and grazing gradients in the lower Sundays River Valley, South Africa: implications for vegetation history. *J. Biogeogr.* 18: 189–201.

HOFFMAN, M.T., TODD, S., NTSHONA, Z. & TURNER, S. 1999b. *Land degradation in South Africa*. Department of Environment Affairs and Tourism, Pretoria.

HOLLAND, A. 2011. *Examination of possibly induced seismicity from hydraulic fracturing in the Eola Field*. Garvin County, Oklahoma, Oklahama Geological Survey. Open-File Report OF1-2011.

HOLLING, C.S. 1973. Resilience and stability of ecological systems. *Ann. Rev. Ecol. Syst.* 4: 1-23.

HOLNESS, S., DRIVER, A., TODD, S., SNADDON, K., HAMER, M., RAIMONDO, D., DANIELS, F., ALEXANDER, G., BAZELET, C., BILLS, R., BRAGG, C., BRANCH, B., BRUYNS, P., CHAKONA, A., CHILD, M., CLARKE, R.V., COETZER, A., COETZER, W., COLVILLE, J., CONRADIE, W., DEAN, R., EARDLEY, C., EBRAHIM, I., EDGE, D., GAYNOR, D., GEAR, S., HERBERT, D., KGATLA, M., LAMULA, K., LEBALLO, G., LYLE, R., MALATJI, N., MANSELL, M., MECENERO, S., MIDGLEY, J., MLAMBO, M., MTSHALI, H., SIMAIKA, J., SKOWNO, A., STAUDE, H., TOLLEY, K., UNDERHILL, L., VAN DER COLFF, D., VAN NOORT, S. & VON STADEN, L. 2016. Biodiversity and Ecological Impacts: Landscape Processes, Ecosystems and Species. In Scholes, R., Lochner, P., Schreiner, G., Snyman-Van der Walt, L. and de Jager, M. (eds.). 2016. *Shale Gas Development in the Central Karoo: A Scientific Assessment of the Opportunities and Risks*. CSIR/IU/021MH/EXP/2016/003/A, ISBN 978-0-7988-5631-7. CSIR, Pretoria.

HOOPER, D.U., CHAPIN, F.S., EWEL, J.J., HECTOR, A., INCHAUSTI, P., LAVOREL, S., LAWTON, J.H., LODGE, D.M., LOREAU, M., NAEEM, S., SCHMID, B., SETÄLÄ, H., SYMSTAD, A.J., VANDERMEER, J. & WARDLE, D.A. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* 75: 3–35.

HORNBACH, M.J., DESHON, H.R., ELLSWORTH, W.L., STUMP, B.W., HAYWARD, C., FROHLICH, C., OLDHAM, H.R., OLSON, J.E., MAGNANI, M.B., BROKAW, C. & LUETGERT, J.H. 2015. Causal factors for seismicity near Azle, Texas. Nature Communications. 6. [Online] Available from: http://www.nature.com/ncomms/2015/150421/ ncomms7728/full/ncomms7728.html. doi:10.1038/ncomms7728.

HOSSAIN, A.B.M.S., BOYCE, A.N. & MAJID, H.M.A. 2008. Vase life extension and chlorophyll fluorescence yield of bougainvillea flower as influenced by ethanol

to attain maximum environmental beautification as ornamental components. *Am. J. Env. Sci.* 4: 625–630.

HOWARTH, R., SANTORO, R. & INGRAFFEA, A. 2011. Methane and the greenhouse-gas footprint of natural gas from shale formations. *Climatic Change* 106: 679–690.

HUANG, Y., CLEMENS, S.C., LIU, W., WANG, Y. & PRELL, W.L. 2007. Large-scale hydrological change drove the late Miocene C_4 plant expansion in the Himalayan foreland and Arabian Peninsula. *Geology* 35: 531-534.

HULTMAN, N., REBOIS, D., SCHOLTEN, M. & RAMIG, C. 2011. The greenhouse impact of unconventional gas for electricity generation. *Env. Res. Let.* 6: 1–9.

HUNER, N.P., ÖQUIST, G., HURRY, V.M., KROL, M., FALK, S. & GRIFFITH, M. 1993. Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. *Photosynth. Res.* 37: 19-39.

HUNTLEY, B.J. 1984. Characteristics of South African biomes. In: Booysen, P. de V. & Tainton, N.M. (eds), *Ecological Effects of Fire in South African Ecosystems*, Springer, Berlin. pp. 1–18.

IEA, 2011. *World Energy Outlook*. International Energy Agency, 2011.

IEA, 2012. *Golden Rules of a Golden Age of Gas.* World Energy Outlook. Special Report on Unconventional Gas. International Energy Agency, Paris, France, pp. 143.

INGROUILLE, M.J., CHASE, M.W., FAY, M.F., BOWMAN, D., VAN DER BANK, M. & BRUIJN, A.D.E. 2002. Systematics of Vitaceae from the viewpoint of plastid rbcL sequence data. *Bot. J. Linn. Soc*. 138: 421–432.

JACKSON, R.B., LOWRY, E.R., PICKLE, A., KANG, M., DIGIULIO, D.C. & ZHAO, K. 2015. The Depths of Hydraulic Fracturing and Accompanying Water Use across the United States. *Environ. Sci. Tech.* 49: 8969-8976.

JACKSON, P.C. & TAYLOR, J.M. 1970. Effects of organic acids on ion uptake and retention in barley roots. *Plant Physiol.* 46: 538–542.

JARDINE S. & MAGLOIRE, L. 1965. Palynologie, stratigraphy du Crétacé des basins du Sénégal et de la Côte d'Ivoire. *Mém. Bur. Rech. Geol. Miner*. 32: 187–245.

JENNINGS, C.M.H. 1974. *The hydrology of Botswana*. PhD thesis, University of Natal, South Africa.

JOHNSON, C.F., COWLING, R.M. & PHILLIPSON, P.B. 1999. The flora of the Addo Elephant National Park, South Africa: are threatened species vulnerable to elephant damage? *Biodiv. Conserv.* 8: 1447-1456.

JOHNSON, M.R., VAN VUUREN, C.J., HEGENBERGER, W.F., KEY, R. & SHOKO, U. 1996. Stratigraphy of the Karoo Supergroup in southern Africa: An overview. *J Afr Earth Sci.*, 23(1): 3–15. http://dx.doi.org/10.1016/S0899-5362(96)00048-6.

JOOSTE, J.F. 1983. Game farming as a supplementary farming activity in the Karoo. *Proc. Grassl. Soc. S. Afr*, 18: 46–49.

JÖRG, E. 2003. Forum: A critique for phytosociology. *J. Veg. Sci.* 14: 291-296.

JÜRGENS, N. 1991. A new approach to the Namib Region. I: Phytogeographic subdivision. *Vegetatio* 97: 21–38.

KAUSAR, S., HUSSAIN, M.A. & KHAN, A.A. 2010. Response of simulated acid rain on morphological, biochemical and leaf characteristics of wheat. *Trends Biosci.* 3: 34-36.

KEELEY, J.E. & RUNDEL, P.W. 2003. Evolution of CAM and C_4 carbon-concentrated mechanisms. *Int. J. Plant Sci.* 164: S55–S77.

KELLER, V. & BOLLMANN, K. 2004. From red lists to species of conservation concern. *Cons. Biol.* 18: 1636-1644.

KELLNER, K. & BOOYSEN, J. 1999. Modelling populations and community dynamics in Karoo ecosystems. In: Dean, W.R.J. & Milton, S.J. (eds), *The Karoo: ecological patterns and processes.* Cambridge Univ. Press, Cambridge. pp. 224–230.

KEMPER, J., COWLING, R.M. & RICHARDSON, D.M. 1999. Fragmentation of South African renosterveld shrublands: effects on plant community structure and conservation implications. *Biol. Cons.* 90: 103-111.

KENT, M. 2012. *Vegetation Description and Analysis: A Practical Approach*. Wiley Blackwell, Oxford.

KERLEY, G.I.H., KNIGHT, M.H. & DE KOCK, M. 1995. Desertification of subtropical thicket in the Eastern Cape, South Africa: are there alternatives? *Environ. Monit. Assess.* 37: 211–230.

KERLEY, G.I.H., TONGWAY, D. & LUDWIG, J.A. 1999. Effects of goat and elephant browsing on soil resources in Succulent Thicket, Eastern Cape, South Africa. In: Proceedings of the VIth International Rangeland Congress 1, pp. 116, 117. Townsville, AU.

KING, G.E. 2012. Hydraulic fracturing 101: what every representative, environmentalist, regulator, reporter, investor, university researcher, neighbor, and engineer should know about hydraulic fracturing risk. *J. Petro.Tech.* 64: 34–42.

KIVIAT, E. 2013. Risks to biodiversity from hydraulic fracturing for natural gas in the Marcellus and Utica shales. *Ann. NY Acad. Sci.* 1286: 1–14.

KLAK, C., REEVES, G. & HEDDERSON, T. 2004. Unmatched tempo of evolution in southern African semi-desert ice plants. *Nature* 427: 63–65.

KNAPP, A.K., BRIGGS, J.M., COLLINS, S.L., ARCHER, S.R., BRET-HARTE, M.S., EWERS, B.E., PETERS, D.P., YOUNG, D.R., SHAVER, G.R., PENDALL, E. & CLEARY, M.B. 2008. Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology*, 14: 615–623.

KONDASH, A. and VENGOSH, A. 2015. Water footprint of hydraulic fracturing. Environmental Science & Technology Letters, 2(10), pp. 276–280.

KOORNNEEF, M., BENTSINK, L. & HILHORST, H. 2002. Seed dormancy and germination. *Current Opinion in Plant Biology* 5: 33–36.

KUUSKRAA, V., STEVENS, S., VAN LEEUWEN, T. & MOODHE, K. 2011. World shale gas resources: An initial assessment of 14 regions outside the United

States. Washington DC: United States Energy Information Administration. Available from: http:// www.eia.doe.gov/analysis/studies/worldshalegas.

LAL, N. 2016. Effects of acid rain on plant growth and development. *e-Journal of Science & Technology (e-JST)* 11: 85–101.

LE MAITRE, D.C. & MIDGLEY, J.J. 1992. Plant reproductive ecology. In: Cowling, R.M. (ed.) *The ecology of fynbos: nutrients, fire and diversity*, pp. 135–174. Oxford Univ. Press, Cape Town.

LE MAITRE, D.C., SCOTT, D.F. & COLVIN, C. 1999. A review of information on interactions between vegetation and groundwater. *Water SA* 25: 137–152.

LECHMERE-OERTEL, R.G., KERLEY, G.I.H. & COWLING, R.M. 2005a. Patterns and implications of transformation in semi-arid succulent thicket, South Africa. *J. Arid Environ.* 62: 459–474.

LECHMERE-OERTEL, R.G., KERLEY, G.I.H. & COWLING, R.M. 2005b. Landscape dysfunction and reduced spatial heterogeneity in soil resources and fertility in semi-arid succulent thicket, South Africa. *Austral Ecology* 30: 615–624.

LECHMERE-OERTEL, R.G., KERLEY, G.I.H., MILLS, A.J. & COWLING, R.M. 2008. Litter dynamics across browsing-induced fenceline contrasts in succulent thicket, South Africa. *S. Afr. J. Bot.* 74: 651–659.

LINDER, H.P. 2003. The radiation of the Cape flora, southern Africa. *Biol. Rev.* 78: 597–638.

LINDER, H.P., KURZWEIL, H. & JOHNSON, S.D. 2005. The southern African orchid flora: composition, sources and endemism. *J. Biogeogr.* 32: 29–47.

LINOL, B., CHERE, N., MUEDI, T., NENGOVHELA, V. & DE WIT, M.J. 2016. Deep Borehole Lithostratigraphy and Basin Structure of the Southern Karoo Basin Re-Visited. In: Linol, B. & de Wit, M.J. (eds), *Origin and Evolution of the Cape Mountains and Karoo Basin*, Regional Geology Reviews. Heidelberg, Springer. pp. 3-16.

LINOL, B. & DE WIT, M.J. 2016. (eds.) *Origin and Evolution of the Cape Mountains and Karoo Basin*, Regional Geology Reviews, (Regional Geology Reviews). Heidelberg, Springer, 242 pp.

LLOYD, J.W., VAN DEN BERG, E., VAN WYK, E. & PALMER, A.R. 2002. *Patterns of degradation and degradation in the Thicket Biome*. Terrestrial Ecology Research Unit Report 39, Univ. of Port Elizabeth, South Africa.

LOCHNER, P., SCHREINER, G. & DE JAGER, M. 2016. Shale gas Development in the Central Karoo: A Scientific Assessment of the Opportunities and Risks. Summaries for Policy makers. In: Scholes, R., Lochner, P., Schreiner, G., Snyman-Van der Walt, L. & de Jager, M. (eds.). *Shale Gas Development in the Central Karoo: A Scientific Assessment of the Opportunities and Risks.* CSIR/IU/021MH/EXP/2016/003/A, ISBN 978-0-7988-5631-7.

LOOPE, L.L., SANCHEZ, P.G., TARR, P.W., LOOPE, W.L. & ANDERSON, R.L. 1988. Biological invasions of arid land nature reserves. *Biol. Conserv*. 44: 95-118.

LOVEGROVE, B. 1993. *The Living Deserts of Southern Africa*. Fernwood Press, Vlaeberg, South Africa.

LOW, A.B. & REBELO, A.G. (eds.) 1996. *Vegetation of South Africa, Lesotho and Swaziland*. A companion to the vegetation map of South Africa, Lesotho and Swaziland. Dept of Environmental Affairs & Tourism, Pretoria.

LUBKE, R.A., EVERARD, D.A. & JACKSON, S. 1986. The biomes of the Eastern Cape with emphasis on their conservation. *Bothalia* 16: 251–261.

LUXMOORE, R.J., VALORAS, N. & LETEY, J. 1974. Nonionic surfactant effects on growth and porosity of barley roots. *Agron J.*: 66: 673–675.

MACARTHUR, R.H. & WILSON, E.O. 1967. *The Theory of Island Biogeography*. Princeton University Press, Princeton.

MAGURRAN, A.E. 2004. *Meausuring Biological Diversity*. Blackwell, Ontario.

MAILA, M.P. & CLOETE, T.A. 2002. Germination of *Lepidium sativum* as a method to evaluate polycyclic aromatic hydrocarbons (PAHs) removal from contaminated soil. *Internat. Biodet. Biodegrad.* 50: 107–113.

MARCANTONIO, M., ROCCHINI, D., GERI, F., BACARO, G. & AMICI, V. 2013. Biodiversity, roads, & landscape fragmentation: Two Mediterranean cases. *Applied Geography* 42: 63–72.

MAUTER, M.S., ALVAREZ, P.J., BURTON, A., CAFARO, D.C., CHEN, W., GREGORY, K.B., JIANG, G., LI, Q., PITTOCK, J., REIBLE, D. & SCHNOOR, J.L. 2014. Regional variation in water-related impacts of shale gas development and implications for emerging international plays. *Environ. Sci. Technol.* 48: 8298–8306.

MAXWELL, K. & JOHNSON, G.N. 2000. Chlorophyll fluorescence – a practical guide. *J. Exp. Bot.* 51: 659–668.

MCCARTHY, T.S. 2011. Impact of acid mine drainage in South Africa. *S. Afr. J. Sci.* 107, Art. #712, 7pp.

MCGARR, A. 2014. Maximum magnitude earthquakes induced by fluid injection. Journal Geophysical Research. *Solid Earth*, 119: 1008–1019.

MEADOWS, M.E. & HOFFMAN, M.T. 2002. The nature, extent and causes of land degradation in South Africa: legacy of the past, lessons for the future? *Area* 34: 428–437.

MEADOWS, M.E. & WATKEYS, M.K. 1999. Palaeoenvironments. In: Dean, W.R.J. & Milton, S.J. (eds), *The Karoo: ecological patterns and processes*, pp. 27–41. Cambridge University Press, Cambridge.

MILCHUNAS, D.G., LAUENROTH, W.K. & BURKE, I.C. 1998. Livestock grazing: animal and plant biodiversity of shortgrass steppe and the relationship to ecosystem function. *Oikos* 83: 65–74.

MILCHUNAS, D.G., SALA, O.E. & LAUENROTH, W.K. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. *Am. Nat.* 132: 87–106.

MILLS, A.J. & FEY, M.V. 2003. Declining soil quality in South Africa: effects of land use on soil organic matter and surface crusting. *S. Afr. J. Sci.* 99: 429–436.

MILLS, A.J. & FEY, M.V. 2004. Transformation of thicket to savanna reduces soil quality in the Eastern Cape, South Africa. *Plant Soil* 265: 153–163.

MILTON, S.J. 1991. Plant spinescence in arid southern Africa: does moisture mediate selection by mammals? *Oecologia* 87: 279–287.

MILTON, S.J. & DEAN, W.R.J. 2012. Potential environmental risks and impacts of mining in the Karoo. Paper presented at the Karoo Development Conference, Beaufort Wast, October 2012.

MILTON, S.J. & HOFFMAN, M.T. 1994. The application of state and-transition models to rangeland research and management in arid succulent and semi-arid grassy Karoo, South Africa. *Afr. J. Range Forage Sci.* 11: 18–26.

MILTON, S.J., SIEGFRIED, W.R. & DEAN, W.R. 1990. The distribution of epizoochoric plant species: a clue to the prehistoric use of arid Karoo rangelands by large herbivores. *J. Biogeogr*. 17: 25–34.

MILTON, S.J., ZIMMERMANN, H.G. & HOFFMANN, J.H. 1999. Alien plant invaders of the karoo: attributes, impacts and control. In: Dean, W.R.J. & Milton, S.J. (eds.) *The Karoo: Ecological Patterns and Processes*, pp. 274–287. Cambridge University Press, Cambridge.

MOHAMMED, G.H., ZARCO-TEJADA, P. & MILLER, J.R. 2003. Applications of chlorophyll fluorescence in forestry and ecophysiology. In: *Practical Applications of Chlorophyll Fluorescence in Plant Biology*. DeEll, J.R. & Toivonen, P.M.A. (eds). Kluwer Academic Publishers, USA: 79–124.

MOKANY, K., RAISON, R.J. & PROKUSHKIN, A.S. 2006. Critical analysis of root: shoot ratios in terrestrial biomes. *Global Change Biol.* 12: 84–96.

MOOLMAN, H.J. & COWLING, R.M. 1994. The impact of elephant and goat grazing on the endemic flora of South African succulent thicket. *Biol. Cons.* 68: 53–61.

MORKEL, B. & DE WIT, M. 2017. Trying Not to Repeat History: Are Shale Gas Development and Broad-Based Economic Development Compatible? In: *The Shale Dilemma: A Global Perspective on Fracking and Shale Development* Gamper-Rabindran. S. (ed.) University of Pittsburgh University Press, USA.

MORTENSEN, L.M. 1995. Short Communication: Effects of foliar sprays of methanol on growth of some greenhouse plants. *Scientia Hortic.* 64: 187–191.

MUCINA, L., HOARE, D.B., LÖTTER, M.C., DU PREEZ, P.J., RUTHERFORD, M.C., SCOTT-SHAW, C.R., BREDENKAMP, G.J., POWRIE, L.W., SCOTT, L., CAMP, K.G.T., CILLIERS, S.S., BEZUIDENHOUT, H., MOSTERT, T.H., SIEBERT, S.J., WINTER, P.J.D., BURROWS, J.E., DOBSON, L., WARD,

R.A., STALMANS, M., OLIVER, E.G.H., SIEBERT, F., SCHMIDT, E., KOBISI, K. & KOSE, L. 2006b. Grassland biome. In: *The vegetation of South Africa, Lesotho and Swaziland* (eds L. Mucina and M. C. Rutherford). South African National Biodiversity Institute, Pretoria.

MUCINA, L., RUTHERFORD, M.C., PALMER, A.R., MILTON, S.J., SCOTT, L., LLOYD, J.W., VAN DER MERWE, B., HOARE, D.B., BEZUIDENHOUT, H., VLOK, J.H.J. & EUSTON-BROWN, D.I.W. 2006a. Nama-Karoo Biome. In: *The vegetation of South Africa, Lesotho and Swaziland* (eds L. Mucina and M. C. Rutherford). South African National Biodiversity Institute, Pretoria.

MULLER, J. 1981. Fossil pollen records of extant angiosperms. *Bot. Rev*. 47: 1–142.

MUNNS, R., SCHACHTMAN, D.P. & CONDON, A.G. 1995. The significance of a two-phase growth response to salinity in wheat and barley. *Aust. J. Plant Physiol*. 22: 561–569.

NAETH, M.A., BAILEY, A.W., CHANASYK, D.S. & PLUTH, D.J. 1991. Water holding capacity of litter and soil organic matter in mixed prairie and fescue grassland ecosystems of Alberta. *J. Range Manag.* 44: 13–17.

NAUMBERG, E., MATA-GONZALEZ, R., HUNTER, R.G., MCLENDON, T. & MARTIN, D.W. 2005. Phreatophytic vegetation and groundwater fluctuations: A review of current research and application of ecosystem response modeling with an emphasis on Great Basin Vegetation. *Environ. Manage.* 35: 726–740.

NEPSTAD, D.C., DE CARVALHO, C.R., DAVIDSON, E.A., JIPP, P.H., LEFEBVRE, P.A., NEGREIROS, G.H., DA SILVA, E.D., STONE, T.A., TRUMBORE, S.E. & VIEIRA, S. 1994. The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. *Nature* 372: 666–669.

NEUFELD, H.S., JERNSTEDT, J.A. & HAINES, B.L. 1985. Direct foliar effects of simulated acid rain I. Damage, growth and gas exchange. *New Phytol.* 99: 389–405.

NIE, M., YANG, Q., JIANG, L.-F., FANG, C.-M., CHEN, J.-K. & LI, B. 2010. Do plants modulate biomass allocation in response to petroleum pollution? *Biol. Lett.* 6: 811–814.

NONOGAKI, H., CHEN, F. & BRADFORD, K.J. 2007. Mechanisms and genes involved in germination *sensu stricto*. In: *Seed development, dormancy and germination*. Bradford, K. & Nonogaki, H. (eds.) Blackwell Publishing, Ontario. pp. 264-304.

NORBERG, J., SWANEY, D.P. & DUSHOFF, J. 2001. Phenotypic diversity and ecosystem functioning in changing environments: a theoretical framework. *Proc. Natl. Acad. Sci. USA* 98: 11376–11381.

NORDENSTAM, B. 1968. The genus *Euryops*. Part 1. Taxonomy. *Opera Bot.* 20: 7–409.

NORTHRUP, J.M. & WITTEMYER, G. 2013. Characterizing the impacts of emerging energy development on wildlife, with an eye towards mitigation. *Ecol. Lett.* 16: 112-125.

O'CONNOR, T.G. 1994. Composition and population responses of an African savanna grassland to rainfall and grazing. *J. App. Ecol.* 31: 155–171.

O'CONNOR, T.G. & BREDENKAMP, G.J. 1997. Grassland. In: Cowling, R.M., Richardson, D.M. & Pierce, S.M. (eds), *Vegetation of southern Africa*, pp. 215–257. Cambridge University Press, Cambridge.

ODJEGBA, V.J. & SADIQ, A.O. 2002. Effect of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amarathus hybridus* L. *The Environmentalist* 22:23 – 28.

OGAWA, K. & IWABUCHI, M. 2001. A mechanism for promoting the germination of *Zinnia elegans* seeds by hydrogen peroxide. *Plant Cell Physiol.* 42: 286–291.

OKSANEN, J., BLANCHET, F.G., FRIENDLY, M., KINDT, R., LEGENDRE, P., MCGLINN, D., MINCHIN, P.R., O'HARA, R.B., SIMPSON, G.L., SOLYMOS, P., STEVENS, M.H.H., SZOECS, E. & WAGNER, H. 2017. Vegan: Community Ecology Package. R package version 2.4-3. http://CRAN.R-project.org/package=vegan.

OSBORN, S., VENGOSH, A., WARNER, N. & JACKSON, R. 2011. Methane contamination of drinking water accompanying gas-well drilling and hydraulic fracturing. *P. Natl. Acad. Sci.* 108: 8172–8176.

OWEN-SMITH, N. & DANCKWERTS, J.E. 1997. Herbivory. In: Cowling, R.M., Richardson, D.M. & Pierce, S.M. (eds), *Vegetation of southern Africa*, Cambridge Univ. Press, Cambridge. pp. 397–420.

PALMER, A.R. 1981*. A study of vegetation of the Andries Vosloo Kudu Reserve, Cape Province*. M.Sc. thesis, Dept of Botany, Rhodes University, Grahamstown.

PALMER, A.R. 1990. *Vegetation survey of the Carnarvon Experimental Station, Cape Province*. Report, Agricultural Research Council, Grassland Institute, Pretoria.

PALMER, A.R. 1991a. A syntaxanomic and synecological account of the vegetation of the eastern Cape midlands. *S. Afr. J. Bot.* 57: 76–94.

PALMER, A.R. 1991b. Vegetation/environment relationships in the central area of the Cape Midlands, South Africa. *Coenoses* 6: 29–38.

PALMER, A.R., CROOK, B.J.S. & LUBKE, R.A. 1988. Aspects of the vegetation and soil relationships in the Andries Vosloo Kudu Reserve, Cape Province. *S. Afr. J. Bot.* 54: 309–314.

PALMER, A.R. & HOFFMAN, M.T. 1997. Nama-Karoo. In: Cowling, R.M., Richardson, D.M. & Pierce, S.M. (eds), *Vegetation of southern Africa*, pp.167–188. Cambridge Univ. Press, Cambridge.

PALMER, A.R., KAKEMBO, V., LLOYD, W. & AINSLIE, A. 2004. Degradation patterns and trends in the Succulent Thicket. In: Proceedings of the Thicket Forum held at Zuurberg Inn, Addo Elephant National Park, May 2004, pp. 1–10.

PASSEY, B.H., AYLIFFE, L.K., KAAKINEN, A., ZHANG, Z., ERONEN, J.T., ZHU, Y., ZHOU, L., CERLING, T.E. & FORTELIUS, M. 2009. Strengthened East Asian summer monsoons during a period of high-latitude warmth? Isotopic evidence from Mio-Pliocene fossil mammals and soil carbonates from northern China. *Earth Planet. Sc. Lett.* 277: 443-452.

PEDUZZI, P. & HARDING ROHR REIS, R. 2013. Gas fracking: can we safely squeeze the rocks? *Environmental Development* 6: 86–99.

PELL, S.K. 2004. *Molecular systematics of the cashew family (Anacardiaceae).* Ph.D. thesis, Louisiana State Univ., Baton Rouge, LA.

PERINO, C. & CÔME, D. 1991. Physiological and metabolic study of the germination phases in apple embryo. *Seed Sci. Technol.* 19: 1–14.

PETERSON, G., ALLEN, C.R. & HOLLING, C.S. 1998. Ecological resilience, biodiversity, and scale. *Ecosystems* 1: 6–18.

PIMM, S.L., RUSSELL, G.J., GITTLEMAN, J.L. & BROOKS, T.M. 1995. The future of biodiversity. *Science* 269: 347–350.

POND, U., BEESLEY, B.B., BROWN, L.R. & BEZUIDENHOUT, H. 2002. Floristic analysis of the Mountain Zebra National Park, Eastern Cape. *Koedoe* 45: 35–58.

POORTER, H., NIKLAS, K.J., REICH, P.B., OLEKSYN, J., POOT, P. & MOMMER, L. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.* 193: 30–50.

POPOVIC, R., DEWEZ, D. & JUNEAU, P. 2003. Applications of chlorophyll fluorescence in ecotoxicology: heavy metals, herbicides, and air pollutants. In: *Practical applications of chlorophyll fluorescence in plant biology*. DeEll, J.R. & Toivonen, P.M.A. (eds.). Kluwer Academic Publishers, USA: pp. 151-184.

PROCHEŞ, Ş., COWLING, R.M., GOLDBLATT, P., MANNING, J. & SNIJMAN, D.A. 2006. An overview of the Cape geophytes. *Biol. J. Linn. Soc.* 87: 27–43.

QUADE, J., CERLING, T.E. & BOWMAN, J.R. 1989. Development of Asian monsoon revealed by marked ecological shift during the latest Miocene in northern Pakistan. *Nature* 342: 163–166.

R CORE TEAM. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

R STUDIO TEAM. 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

RAIMONDO, D. 2011. The Red List of South African plants – A global first. *S. Afr. J. Sci.*:107(3/4), Art. #653, 2 pages. DOI: 10.4102/sajs. v107i3/4.653.

RAUNKIÆR, C. 1934. *Life form of Plants and Statistical Plant Geography*. Clarendon Press, Oxford.

REID, H., PHILLIPS, J. & HEATH, M. 2009. *Natural Resilience: healthy ecosystems as climate shock insurance*. International Institute for Environment and Development (IIED) Briefing, December 2009.

RICHTER, D.D. & MAKEWITZ, D. 1995. How deep is soil? *BioScience* 45: 600–609.

RIDLEY, M. 2011. **The Shale Gas Shock**. The Global Warming Policy Foundation Report 2: 34 pp.

ROBERTSON, M.P. & PALMER, A.R. 2002a. Predicting the extent of succulent thicket under current and future climate scenarios. *Afr. J. Range Forage Sci.* 19: 21–28.

ROBERTSON, M.P. & PALMER, A.R. 2002b. Predicting the potential historic extent of thicket vegetation using *Portulacaria afra* distribution records and a multivariate correlative modelling approach. *Afr. J. Range Forage Sci.* 19: 21-28.

ROSENQVIST, E. & VAN KOOTEN, O. 2003. Chlorophyll fluorescence: a general description and nomenclature. In: *Practical applications of chlorophyll fluorescence in plant biology*. DeEll, J.R. & Toivonen, P.M.A. (eds.). Kluwer Academic Publishers, USA. pp. 31-77.

ROUX, P.W. & OPPERMAN, D.P.J. 1986. Soil erosion. In: Cowling, R.M., Roux, P.W. & Pieterse, A.J.H. (eds), *The Karoo biome: a preliminary synthesis. Part 1—Physical environment, pp. 92–111.* FRD, Pretoria.

ROUX, P.W. & THERON, G.K. 1987. Vegetation change in the Karoo Biome. In: Cowling, R.M. & Roux, P.W. (eds), *The Karoo biome: a preliminary synthesis. Part 2—Vegetation and history*, pp. 50–69. FRD, Pretoria.

ROUX, P.W. & VORSTER, M. 1983. Development of veld management research in the Karoo region. *Proc. Grassl. Soc. S. Afr*. 18: 30–34.

ROWE, R.N., FARR, J. & RICHARDS, B.A.J. 1994. Short communication: Effects of foliar and root applications of methanol or ethanol on the growth of tomato plants (*Lycopersicon esculentum* Mill). *New Zeal. J. Crop Hort.* 22: 335–337.

ROWSELL, D.M. & DE SWARDT, A.M.J. 1976. Diagenesis in Cape and Karoo sediments, South Africa, and its bearing on their hydrocarbon potential. *Transactions, Geological Society of South Africa* 79: 81–145.

RUBENSTEIN, J.L. & MAHANI, A.B. 2015. Myths and facts on wastewater injection, hydraulic fracturing, enhanced oil recovery, and induced seismicity. *Seismol. Res. Lett.* 86: 1060–1067.

RUTHERFORD, M.C., MIDGLEY, G.F., BOND, W.J., POWRIE, L.W., ROBERTS, R. & ALLSOPP, J. 1999. *South African country study on climate change. Plant biodiversity: vulnerability and adaptation assessment.* Report, National Botanical Institute, Kirstenbosch.

RUTHERFORD, M.C., POWRIE, L.W. & HUSTED, L.B. 2012a. Plant diversity consequences of a herbivore-driven biome switch from Grassland to Nama-Karoo shrub steppe in South Africa. *Appl. Veg. Sci.* 15: 14-25.

RUTHERFORD, M.C., POWRIE, L.W. & HUSTED, L.B. 2012b. Herbivore-driven land degradation: consequences for plant diversity and soil in arid subtropical thicket in south-eastern Africa. *Land Degrad. Dev.* 25: 541–553.

RUTHERFORD, M.C. & WESTFALL, R.H. 1986. Biomes of southern Africa: an objective categorization. *Mem. Bot. Surv. S. Afr.* 63: 1–94.

SALARD-CHEBOLDAEFF, M. & DEJAX, J. 1991. Evidence of Cretaceous to recent West African intertropical vegetation from continental sediment spore-pollen analysis. *J. Afr. Earth Sci.* 12: 353–361.

SCHOLES, R.J. 1997. Savanna. In: Cowling, R.M., Richardson, D.M. & Pierce, S.M. (eds), *Vegetation of southern Africa*. Cambridge University Press, Cambridge. pp. 258–277.

SCHRIRE, B.D., LAVIN, M. & LEWIS, G.P. 2005. Global distribution patterns of the Leguminosae: insights from recent phylogenies. *Biol. Skrif*. 55: 375–422.

SCHULZE, R.E. 1997. South African Atlas of Agrohydrology and Climatology: Contribution Towards a Final Report to the Water Research Commission on Project 492: *Modelling Impacts of the Agricultural Environment on Water Resources: TT82-96*. Water Research Commission (WRC).

SCHULZE, E.-D., BAUER, G., BUCHMANN, N., CANADELL, J., EHLERINGER, J.R., JACKSON, R.B., JOBBAGY, E., LORETI, J., MOONEY, H.A., OESTERHELD, M. & SALA, O. 1996. Water availability, rooting depth, and vegetation zones along an aridity gradient in Patagonia. *Oecologia* 108: 503–511.

SCOTT, L., ANDERSON, H.M. & ANDERSON, J.M. 1997. Vegetation history. In: Cowling, R.M., Richardson, D.M. & Pierce, S.M. (eds), *Vegetation of southern Africa*, pp. 62–84. Cambridge Univ. Press, Cambridge.

SCOTT, L. & SRIVASTAVA, S.K. 1984. Reworked Cretaceous palynomorphs in Late Quaternary deposits from central Colorado, USA. *Pollen et Spores* 26: 227–240.

SCOTT, L. & VOGEL, J.C. 1983. Late Quaternary pollen profile from the Transvaal highveld, South Africa. *S. Afr. J. Sci.,* 79: 266–272.

SEYBOLD, C.A. 1994. Polyacrylamide review: soil conditioning and environmental fate. *Commun. Soil Sci. Plan.* 25: 2171–2185.

SHACKLETON, N.J. & KENNET, J.P. 1975. Palaeotemperature history of the Cenozoic and the initiation of the Antarctic glaciation: oxygen and carbon isotope analyses in DSDP sites 277, 279 and 281. *Initial Reports of the Deep Sea Drilling Project* 29: 743–755.

SINGH, A. & AGRAWAL, M. 1996. Responses of two cultivars of *Triticum aestivum* L. to simulated acid rain. *Environ. Pollu.* 91: 161–167.

SINGH, A. & AGRAWAL, M. 2004. Impact of simulated acid rain on growth and yield of two cultivars of wheat. *Water Air Soil Poll.* 152: 71–80.

SMIT, G.N., RICHTER, C.G.F. & AUCAMP, A.J. 1999. Bush encroachment: an approach to understanding and managing the problem. In: Tainton, N.M. (ed.) *Veld management in South Africa*. University of Natal Press, Pietermaritzburg. pp. 246 –260.

SMITH, H.J.C., LEVY, G.J. & SHAINBERG, I. 1990. Water-droplet energy and soil amendments: Effect on infiltration and erosion. *Soil Sci. Soc. Am. J.* 54: 1084–1087.

SMITH, G.E. & MARX, G. 1990. Notes on the vegetation and succulent flora of the Eastern Cape. *Aloe* 27: 56–66.

SNYMAN, H.A. 1999. Soil erosion and conservation. In: Tainton, N.M. (ed.), *Veld management in South Africa*. University of Natal Press, Pietermaritzburg. pp. 355–380.

SOCOLAR, J.B., GILROY, J.J., KUNIN, W.E. & EDWARDS, D.P. 2016. How should beta-diversity inform biodiversity conservation? *Trends Ecol. Evol.* 31: 67–80.

SOEDER, D.J. 2017. *Unconventional: The Development of Natural Gas from the Marcellus Shale.* The Geological Society of America Special Paper 527. The Geological Society of America, USA: 143 pp.

SOUTH AFRICAN GOVERNMENT, 2004. *National Environmental Management: Biodiversity Act No. 10*, South African Government, Pretoria.

STEENKAMP, Y., STEYN, H., SMITH, G., ARNOLD, T. & VAN WYK, B. 2002. Does the Grassland Biome of South Africa have endemic grass species and genera? *SABONET News* 7: 197–199.

STEYL, G., VAN TONDER, G.J. & CHEVALIER, L. 2012. *State of the art: fracking for shale gas exploration in South Africa and the impact on water resources.* WRC Report. KV 294-11. Water Research Commision, Pretoria.

STUART-HILL, G.C. 1992. Effects of elephants and goats on the Kaffrarian succulent thicket of the eastern Cape, South Africa. *J. Appl. Ecol.* 29: 699–710.

SZEWCZUK, S. 2014. *Review of the wind energy activities in South Africa.* Green Building. CSIR report, Pretoria.

TAINTON, N.M. 1981. *Veld and pasture management in South Africa*. Shuter & Shooter, Pietermaritzburg.

TAKAKI, S. & WOLF, D.C. 2011. Plant growth in soil amended with drilling mud. *Discovery* 12: 74–79.

TILMAN, D. & DOWNING, J.A. 1994. Biodiversity and stability in grasslands. *Nature* 367: 363–365.

TILMAN, D., WEDIN, D. & KNOPS, J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379: 718–720.

TINKER, J., DE WIT, M. & BROWN, R. 2008. Mesozoic exhumation of the southern Cape, South Africa, quantified using apatite fission track thermochronology. *Tectonophysics* 455: 77-93.

TIPPLE, B.J. & PAGANI, M. 2007. The early origins of terrestrial C_4 photosynthesis. *Annu. Rev. Earth Planet. Sci.* 35: 435–461.

TODD, S.W. 2006. Gradients in vegetation cover, structure and species richness of Nama-Karoo shrublands in relation to distance from livestock watering points. *J. App. Ecol.* 43: 293–304.

TODD, S.W. & HOFFMAN, M.T. 1999. A fence-line contrast reveals effects of heavy grazing on plant diversity and community composition in Namaqualand, South Africa. *Plant Ecol.* 142: 169–178.

TODKILL, W.B., KERLEY, G.I.H. & CAMPBELL, E.E. 2006. Brushpiles and dung as rehabilitation patches: effect on soil resources in degraded succulent thicket, Eastern Cape, South Africa. *Afr. J. Range Forage Sci.* 23: 39–48.

TRUMBORE, S.E., DAVIDSON, E.A., BARBOSA, P.C., NEPSTAD, D.C. & MARTINELLI, L.A. 1995. Belowground cycling of carbon in forests and pastures of Eastern Amazonia. *Global Biogeochem. Cy.* 9: 515–528.

TYAGI, K., MEENAKSHI, K., CHAUDHARY, M., JOSHI, R. & PRAKASH, G. 2004. Assessment of seedling growth of *Pisum sativum* subjected to simulated acid rain. *Progress. Agr.* 4: 83-84.

TYSER, R.W. & WORLEY, C.A. 1992. Alien flora in grasslands adjacent to road and trail corridors in Glacier National Park, Montana (USA). *Cons. Biol.* 6: 253-262.

UNEP, 2011a. United Nations Environment Programme. Athabasca oil sands, require massive investments and energy and produce massive amounts of oil and CO2 - Alberta, Canada. Global Environment Alert Service. *United Nations Environment Programme*, January 2011, 54, pp. 1–5.

UNEP, 2011b. United Nations Environment Programme. Oil palm plantations: threats and opportunities for tropical ecosystems. Global Environment Alert Service. *United Nations Environment Programme*, December 2011, 73, pp. 1–10.

VALERIO, M.E., GARCÍA, J.F. & PEINADO, F.M. 2007. Determination of phytotoxicity of soluble elements in soils, based on a bioassay with lettuce (*Lactuca sativa* L.). *Sci. Total Environ.* 378: 63-66.

VAN AUKEN, O.W. 2000. Shrub invasions of North American semiarid grasslands. *Ann. Rev. Ecol. Syst.* 31: 197–215.

VAN DER WALT, P.T. 1972. *'n Plantekologiese plantopname van Boschberg en sy omringende gebiede met spesiale verwysing na die weidingsfaktor.* PhD Thesis, Faculty of Natural Science, University of Natal, Pietermaritzberg.

VAN OVERBEEK, J. & BLONDEAU, R. 1954. Mode of action of phytotoxic oils. *Weeds* 3: 55–65.

VAN WYK, A.E. & SMITH, G.F. 2001. *Regions of floristic endemism in southern Africa: a review with emphasis on succulents*. Umdaus Press, Pretoria.

VAN WYK, B.-E. & SMITH, G.F. 2003. *Guide to the aloes of South Africa*. 2nd ed. Briza Publications, Pretoria: 304 pp.

VAN WYK, B. & VAN WYK, P. 2013. *Field Guide to Trees of Southern Africa*. 2nd ed. Struik, South Africa Pages, 732 pp.

VAN ZINDEREN BAKKER, E.M. 1989. Middle Stone Age palaeoenvironments at Florisbad (South Africa). *Palaeoecol. Afr.* 20: 133-154.

VANDERPLANK, H.J. 1998. *Wildflowers of the Port Elizabeth area. Swartkops to Sundays River*s. Bluecliff Publishing, Port Elizabeth. 160 pp.

VASS, P.A. 2005. *Plant diversity and spatial discontinuities of the Albany Centre in the south-eastern Cape, South Africa*. Ph.D dissertation, School of Oriental and African Studies, University of London.

VEGTER, J.R. 1995. *An Explanation of a Set of National Groundwater Maps*. Report No. TT 74/95, Water Research Commission, Pretoria.

VENGOSH, A., JACKSON, R.B., WARNER, N., DARRAH, T.H. & KONDASH, A. 2014. A Critical Review of the Risks to Water Resources from Unconventional

Shale Gas Development and Hydraulic Fracturing in the United States. *Environ. Sci. Technol.* Special Issue: Understanding the Risks of Unconventional Shale Gas Development (dx.doi.org/10.1021/es405118y).

VERMEULEN, P.D. 2012. A South African perspective on shale gas hydraulic fracturing. International Mine Water Assocation Annual Conference (eds. McCullough, Lund & Wyse): 149-156.

VICTOR, J.E. & DOLD, A.P. 2003. Threatened plants of the Albany Centre of Floristic Endemism, South Africa. *S. Afr. J. Sci.* 99: 437–446.

VISSER, N., BOTHA, J.C. & HARDY, M.B. 2004. Re-establishing vegetation on bare patches in the Nama Karoo, South Africa. *J. Arid Env.* 57: 155-177.

VLOK, J.H.J. & EUSTON-BROWN, D.I.W. 2002. *Subtropical Thicket Ecosystem Planning Project (STEP). Biological Survey Report (plants and birds).* Report, Terrestrial Ecology Research Unit, University of Port Elizabeth.

VLOK, J.H.J., EUSTON-BROWN, D.I.W. & COWLING, R.M. 2003. Acock's Valley Bushveld 50 years on: a new perspective on the delimitation, characterization and origin of subtropical thicket vegetation. *S. Afr. J. Bot.* 69: 27-51.

VORSTER, M. 1999. Karoo and associated sweet grassveld. In: Tainton, N.M. (ed.), *Veld management in South Africa.* University of Natal Press, Pietermaritzburg. pp. 293–312.

WALKER, B. 1992. Biological diversity and ecological redundancy. *Conserv. Biol.* 6: 18–23.

WALKER, B.H. 1993. Rangeland ecology: understanding and managing change. *Ambio* 22: 80–87.

WALKER, B.H. 1997. Functional types in non-equilibrium ecosystems. In: Smith, T.M., Shugart, H.H., and Woodward, F.I. (Eds). *Plant Functional Types*. Cambridge, UK: Cambridge University Press.

WECKMANN, U., RITTER,O., CHEN, X., TIETZE, K. & DE WIT, M. 2012. Magnetotelluric image linked to surface geology across the Cape Fold Belt, South Africa. *Terra Nova* 24: 207-212.

WEIERSBYE, I.M. & WITKOWSKI, E.T.F. 2007. Impacts of acid mine drainage (AMD) on the regeneration potential of highveld phreatophyte plants. In J.J. Bester, A.H.W. Seydack, Vorster, T., Van der Merwe, I.J. and Dzivhani, S. (eds). *Multiple Use Management of Natural Forests and Woodlands: Policy Refinements and Scientific Progress* IV. Department of Water Affairs and Forestry of South Africa. www.dwaf.gov.za/forestry, pp 224-237.

WERGER, M.J.A. 1978a. The Karoo-Namib Region. In: Werger, M.J.A. & Van Bruggen, A.C. (eds), *Biogeography and ecology of southern Africa.* Dr W. Junk, The Hague. pp. 231–302.

WERGER, M.J.A. 1978b. Vegetation structure in the southern Kalahari*. J. Ecol*. 66: 933–941.

WESTOBY, M., WALKER, B. & NOY-MEIR, I. 1989. Opportunistic management for rangelands not at equilibrium. *J. Range Manage*. 42: 266-274.

WHITE, F. 1976. The vegetation map of Africa—the history of a completed project. *Boissiera* 24: 659–666.

WHITE, F. 1983. *Vegetation of Africa: a descriptive memoir to accompany the UNESCO/AETFAT/UNSO vegetation map of Africa*. UNESCO, Paris.

WHITE, F. & MOLL, E.J. 1978. The Indian Ocean coastal belt. In: Werger, M.J.A. & Van Bruggen, A.C. (eds). *Biogeography and ecology of southern Africa.* Dr W. Junk, The Hague. pp. 561–598.

WHITFORD, W.G. 2002. *Ecology of Desert Systems*. Academic Press, San Diego.

WHITTAKER, R.H. 1972. Evolution and measurement of species diversity. *Taxon* 21: 213-251.

WIGLEY, T. 2011. Coal to gas: the influence of methane leakage. *Climatic Change* 108: 601–608.

WILLIAMS, J.D., MCCOOL, O.K., REARDON, C.L., DOUGLAS, JR., C.L., ALBRECHT, S.L. & RICKMAN, R.W. 2013. Root:shoot ratios and belowground biomass distribution for Pacific Northwest dryland crops. *J. Soil Water Conserv.* 68: 349-360.

WITZE, A. 2015. Race to Unravel Oklahoma's artificial quakes. *Nature* 520: 418–419.

WOLF, D.C. & BRYE, K.R. 2012. *Bermudagrass Growth in Hydraulic-Fracturing-Drilling-Fluid-Contaminated Soil.* American Society of Agronomy poster number 119 Symposium-- National Student Research Symposium Poster Contest.

WOODFORD, A.C. & CHEVALLIER, L. 2002. *Hydrogeology of the Main Karoo Basin: Current Knowledge and Research Needs*. Water Research Commission WRC Report No TT 197.

WORLD ENERGY COUNCIL, 2010. *Survey of Energy Resources: Focus on Shale Gas*. Regency House, London.

WORLD WILDLIFE FUND, 2001. *The heat is on… Impacts of climate change on plant diversity in South Africa.* National Botanical Institute, Kirstenbosch.

ZARCO-TEJADA, P.J., MILLER, J.R., MOHAMMED, G.H., NOLAND, T.L. & SAMPSON, P.H. 2002. Vegetation stress detection through chlorophyll *a* + *b* estimation and fluorescence effects on hyperspectral imagery. *J. Environ. Qual.* 31: 1433–1441.

ZHENG, Y-J., YANG, Y-Q., LIANG, S-S. & XIAN-FENG, Y. 2008. Effect of methanol on photosynthesis and chlorophyll fluorescence of flag leaves of winter wheat. *Agr. Sci. China.* 714: 432–443.

ZOBACK, M., KITASEI, S. & COPITHORNE, B. 2010. *Addressing the Environmental Risks from Shale Gas*. Development Briefing Paper 1 Worldwatch Institute Natural Gas and Sustainable Energy Initiative.

ZVOMUYA, F., F.J. LARNEY, P.R. DEMAERE, & OLSON, A.F. 2009. Hydraulic properties of a sandy loam soil following spent drilling mud application on native prairie. *Soil Sci. Soc. Am. J*. 73: 1108–1112.

Websites

BROWNELL, M. 2008. Gas well drilling and development: Marcellus shale. Susquehanna River Basin Commission. Available online: http://www.srbc.net/whatsnew/docs/Marcellusshale61208ppt.PDF (accessed 15/07/2014).

BURGOYNE, P.M. 2006. *Ruschia vanderbergiae* L.Bolus. National Assessment: Red List of South African Plants version 2017.1. http://redlist.sanbi.org/species.php?species=163-354 (Accessed on 2017/10/11).

EPA, 2011a. Investigation of Ground Water Contaminatoin near Pavillion, Wyoming. /http://www.epa.gov/region8/superfund/wy/pavillion/EPA_ReportOnPavillion_Dec-8-2011.pdfS (accessed 15/07/2014).

EPA, 2011b. Plan to Study the Potential Impacts of Hydraulic Fracturing on Drinking WaterResources./http://water.epa.gov/type/groundwater/uic/class2/hydraulicfracturing/upload/FINAL-STUDY-PLAN-HF_Web_2.pdfS (accessed 15/07/2014).

FRACFOCUS, 2014. http://fracfocus.org/chemical-use/what-chemicals-are-used. Accessed 15/07/2014.

GILLILAND, D., 2010. Marcellus Shale gas drillers committed 1435 violations in 2.5 years, report says, The Patriot-news, 2 August, 2010. http://www.pennlive.com/midstate/index.ssf/2010/08/marcellus_shale_gas_drillers_c.htmlS (accessed on 25.11.12.).

HANNOVER DECLARATION, 2013. Abschlusserklärung zur Konferenz Umweltverträgliches Fracking? 24 and 25 June 2013 in Hannover. Available from:http://www.bgr.bund.de/DE/Gemeinsames/Nachrichten/Veranstaltungen/2013/GZH-Veranst/Fracking/fracking_kongress_node.html.

IPCS, 1998. Glutaradehyde. OECD Screening information dataset (SIDS) high production volume chemicals. International Programme on Chemical Safety. http://www.inchem.org/documents/sids/sids/111308.pdf. (Accessed on 22 June, 2017).

MYERS, R., 2011. The Environmental Dangers of Hydro-Fracturing the Marcellus Shale Lock Haven University Environmental Studies site. /http://www.lhup.edu/rmyers3/marcellus.htms (Accessed 25/11/2014).

POSA, 2017. South African National Biodiversity Institute. Botanical Database of Southern Africa (BODATSA). http://newposa.sanbi.org/sanbi/Explore. (Accessed 2014 – 2017).

RAIMONDO, D., VICTOR, J.E., DOLD, A.P. & DE CASTRO, A. 2012. *Pelargonium reniforme* Curtis. National Assessment: Red List of South African Plants version 2017.1. http://redlist.sanbi.org/species.php?species=1976-4012 (Accessed on 2017/10/11).

RANGE RESOURCES, 2014. http://www.rangeresources.com/. (Accessed 27/04/2014).

SANBI, 2017. SANBI Red List of South African Plants. http://redlist.sanbi.org/index.php. (Accessed August, 2017).

SANPARKS, 2008. Mountain Zebra National Park Management Plan. https://www.sanparks.org/assets/docs/conservation/park_man/mznp_approvedplans.pdf (Accessed March, 2017).

SANPARKS, 2013. Camdeboo National Park Park Management Plan. https://www.sanparks.org/assets/docs/conservation/park_man/camdebooapprovedplan.pdf (Accessed March, 2017).

SIBIS, 2014. Biodiversity data provided by: *South African National Biodiversity Institute* (Accessed through the SIBIS portal, sibis.sanbi.org, 15/07/2014).

Personal Communication

MILTON-DEAN, S. 2014. Research Associate at the Percy FitzPatrick Institute of African Ornithology.

STROEBEL, D. 2014. Associate Researcher, AEON-ESSRI, Nelson Mandela University, Port Elizabeth.

APPENDIX A

South African
NATIONAL PARKS

RESEARCH AGREEMENT

BETWEEN

SOUTH AFRICAN NATIONAL PARKS

herein represented by **Dr. S. Freitag-Ronaldson**

in her capacity as **GM: Savanna & Arid Research Unit, Scientific Services**

(hereinafter referred to as “SANParks”)

AND

Ms K.Ellis

_____

8008160014085

Id no. _____

(hereinafter referred to as “the Researcher”)

WHEREAS the Researcher submitted a research application to SANParks to conduct a research on **“Impacts of fracking on the vegetation of the Karoo”** (“Research”) and to obtain a sample of a biological resource (“Material”) in the “**Multipark (Camdeboo National Park, Mountain Zebra National Park)”** (“the Park”);

AND WHEREAS SANParks accepted the Researcher’s application to conduct Research and obtain the Material in the Park subject to the terms and conditions as stipulated hereunder:

THE PARTIES AGREE AS FOLLOWS

1. DEFINITIONS

1.1 The definitions of the words used in this Agreement are attached hereto as Annexure A.

2. PERIOD OF AGREEMENT

2.1 This Agreement shall commence on the date of the last signature hereto and shall expire on 31 December 2017

2.2 Either party may terminate this agreement by giving the other party at least 2 (two) months written notice.

2.3 SANParks reserves the right to terminate this Agreement with immediate effect should the Researcher of any employee or agent of the Researcher assisting with the Research be charged with transgression of any statutory provision relating to the conservation asset or non-compliance with a statutory provision relating to the conservation asset.

2.4 Should credible information arise implicating the Researcher or any employee or agent of the Researcher in activities deemed detrimental to the conservation asset by SANParks, SANParks may terminate this Agreement after consultation with the Researcher and allowing the Researcher to provide reasons why this Agreement should not be terminated.

3. THE RESEARCH

This study forms part of the AEON-ESSRI Baseline Research Program at NMMU, which is undertaking a technical evaluation and socio-economic analyses of shale gas in the Eastern Cape. Researchers from various departments such as Geophysics, Hydrochemistry, Geohydrology, Geology, Chemistry, Ecology, Conservation, Development studies, Economics and Energy Engineering are collaborating on this research program. This study forms the Botanical element of the baseline research program. The aim of this study is to survey the proposed fracking areas and compile a complete species list, highlighting Species of Conservation Concern and to describe ecosystem patterns and processes for the fracking area. The National Parks will be used as control sites to give us an indication of the species composition of the area in its natural state.

4. PERMIT TO COLLECT NATURAL RESOURCES MATERIAL

4.1 In compliance with regulation 4(1) of the regulations under the *National Environmental Management: Protected Areas Act* No. 57 of 2003, permission is hereby granted to the Researcher to collect the following natural resources Material in the Park:

3.1.1 The Material to be collected: plant material

3.1.2 The reason for collection permit: To determine the eco-physiological responses of plants (in particular phreatophytes) to exposure to fracking fluid.

5. THE RESEARCHER'S OBLIGATION

5.1 The Researcher acknowledges that he (assistance or team included) will conduct the Research in the Park entirely at own risk.

5.2 The Researcher will obtain the prior written permission from SANParks to take out of the Park any Material and restricted to the total number that will give sufficient results of the Research. Furthermore, the Researcher shall obtain any other necessary permits from relevant authorities for the possession and transportation of such Material.

5.3 The Researcher shall sign both this Agreement and the indemnity form before Research can begin and shall ensure that all co-workers sign the indemnity form before commencing the Research in the Park.

5.4 The Researcher shall carry a signed copy of the research authorization when working in the Park.

5.5 The Researcher shall contact SANParks to arrange their visit to the Park, well in advance.

5.6 The Researcher shall adhere to *Protected Areas Act* 57 of 2003 and the regulations under that Act as well as the tourist traveling times and park rules and regulations when doing fieldwork in the Park. Where sampling has to be done at night, the Researcher shall obtain relevant permission from SANParks.

5.7 Where necessary, the Researcher shall be accompanied by a game guard during their fieldwork within the Park, and they will pay for use of game guard (including a daily fee, overtime and subsistence & travel costs) in accordance with SANParks' standard tariffs.

5.8 The Researcher shall submit an annual report to SANParks in accordance with SANParks' standard format. Where biological Material was collected, the Researcher shall submit duplicate samples to SANParks' Scientific Services Biological Reference Museum unless specified otherwise under item 4.

5.9 The Researcher will provide a well-organized documented electronic copy of data sets generated from this study on an annual basis, with the prescribed metadata files. The data and metadata requirements are attached hereto as Annexure B. SANParks has the right to use data for further research purposes.

5.10 It is agreed between the Parties that issues relating to benefit sharing of the proceeds of the Intellectual Property developed from the Research will be discussed as they arise, and appropriate sharing proportions will be formalized in addenda to this Agreement.

5.11 The Researcher shall make available copies of publications, reports or theses arising from this study to SANParks.

5.12 The Researcher shall acknowledge SANParks as the source of the Material in any publication ensuing from such data. In the case of significant assistance, due consideration to co-authorship should be given.

5.13 The Researcher shall not disclose the details of the Research project to the media (including social media on any platform), until it has provided SANParks with a copy of any proposed press release. SANParks shall provide comment on any proposed release within 21 days of receipt. However, SANParks shall not have the right to prohibit academic publications.

OBLIGATIONS OF SANParks

SANParks shall afford the Researcher (and his assistant or team) free park entry.

SANParks shall provide discounted accommodation (when available) to the Researcher (and his assistant or team) at the research camps (where available) while doing Research in the Park.

Any other accommodation required in the Park for the purpose of sample collection will have to be booked and paid for by the Researcher at normal tourist rates.

Where deemed necessary (such as parks that contain dangerous animals), SANParks shall provide a game guard to accompany the Researcher and his assistant (team) during field work, provided SANParks is notified well in advance and subject to availability.

Where required, SANParks will supply the Researcher with a SANParks vehicle decals (at a refundable cost of R100 per pair after approval) if fieldwork will be in view of tourists.

SANParks shall initially inform in advance the local Rangers of the activities of the Researcher in their sections. However, the Researcher must contact the relevant Section Ranger at least one day before they go out into the field.

Where available, SANParks shall provide basic laboratory facilities which shall not be exclusive to The Researcher.

Where no conflict of interest arises, SANParks shall make available existing datasets (including GIS data layers) subject to the Researcher signing a data user agreement form. These datasets should not be distributed to other parties. Some datasets including lead-time and copyright protected datasets will not be available to the Researcher.

OBLIGATIONS PERTAINING TO COLLECTED MATERIAL

7.1 The Researcher agrees that the Material will be used for research purposes only and not for commercial, industrial or bio-prospecting purposes.

7.2 The Researcher shall take every reasonable precaution that the Material is not in the possession of unauthorized third party. Should there be a need to transfer the Material to a third party a prior written consent must be obtained from SANParks.

7.3 The Researcher agrees that all information disclosed by SANParks will remain confidential and should not be divulged without the prior written consent of SANParks.

7.4 The Researcher shall ensure that the importation, transport, use, maintenance and disposition of the Material will be conducted in strict accordance with all appropriate local, national and international laws as well as guidelines and regulations.

7.5 Once the Material has been used for the agreed purpose, or at the termination of this Agreement the Researcher agrees to return the Material to SANParks or dispose of the Material in the manner agreed with SANParks and will provide SANParks with the necessary proof.

7.6 SANParks undertakes to make Material available to the Researcher in accordance with the terms of this Agreement.

7.7 SANParks shall provide the necessary written consent after a request by the Researcher to provide the Material or to disclose information to a third party has been assessed and it was concluded that it poses no danger or disadvantage to SANParks.

BREACH OF AGREEMENT

Should any Party commit a breach of any of the provisions of this Agreement and fail to remedy the breach within a period of 7 (seven) business days after receipt of the notice by the injured Party to remedy the breach, the injured Party shall at its discretion and without prejudice to any other rights be entitled to terminate the Agreement.

INDEMNITY

9.1 SANParks, its Board, directors, employees and agents are not liable for any loss or damage:

9.1.1 to the property or possession of any Researcher or his assistant/team, whether such damage is caused by the negligent, or grossly negligent, act or omission of SANParks;

9.1.2 arising from death or any bodily injuries of whatsoever nature sustained by a Researcher whether such injuries are caused by the negligent, or grossly negligent, act or omission by SANParks, and/or by the defective functioning of any apparatus.

9.2 The Researcher and his assistant/team will conduct the Research in the Park at their own risk and hereby indemnifies SANParks against any damage, loss, injury or death suffered by any person resulting from the Research in the Park.

9.3 The Researcher shall be liable for any loss incurred by SANParks as a result of a negligent act or omission by the Researcher while conducting the Research in the Park and/or as a result of the willful breach of the terms of this Agreement.

AMENDMENTThis document constitutes the entire Agreement between two Parties and no amendment thereof shall have any effect unless reduced to writing and signed by both Parties.

No indulgence on the part of either Party shall constitute a waiver of rights in terms of this Agreement.

The Researcher shall not be entitled to cede or assign this Agreement, nor in any other way transfer any of its rights or obligations under this Agreement.

DOMICILIUM CITANDI ET EXECUTANDI

The parties choose as their *domicilium citandi et executandi* for all purposes under this Agreement the following addresses:

SANParks	**The Researcher**
Manager: Legal Services	Ms K. Ellis
643 Lleyds Street	2 Canterbury Gardens
	Prestwick Crescent,
MUCKLENEUK	Greenshields Park
PRETORIA	Port Elizabeth,
0001	6070
	Tel: 041 5042084
Tel: (012) 426-5000	Cell: 076 7114329
Fax: (012) 343-0155	Email:

Any notice or communication required or permitted to be given in terms of this Agreement shall be valid and effective only if in writing.

Either party may by written notice to the other party change the physical address chosen as its *domicilium citandi et executandi* to another physical address where postal delivery occurs, provided that the change shall become effective on the seventh business day from the deemed receipt of the notice by the other Party.

Any notice to a Party –

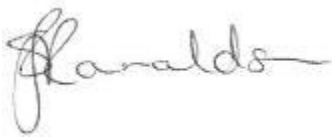
Sent by prepaid registered post (by airmail if appropriate) in a correctly addressed envelope to it at the address chosen as its *domicilium citandi et executandi* to which post is delivered shall be deemed to have been received on the fifth business day after posting (unless the contrary is proved);

Delivered by hand to a responsible person during ordinary business hours at the physical address at is *domicilium citandi et executandi* shall be deemed to have been received on the day of the delivery.

Notwithstanding anything to the contrary herein contained a written notice of communication actually received by a Party shall be adequate written notice of communication to it notwithstanding that it was not sent to or delivered at its chosen *domicilium citandi et executandi*.

SANPARKS

SIGNED AT _Skukuza__ ON THIS _9th_ DAY OF _June 2015_____



_____

Dr. S. Freitag-Ronaldson

AS WITNESS

_____  _____ 2. _____  _____

RESEARCHER

SIGNED AT _____ ON THIS _____ DAY OF _____

_____

XXXXXX

AS WITNESS

_____ 2. _____

Appendix A

DEFINITIONS and intepretation

In this Agreement, unless the context clearly indicates a contrary intention, the following terms shall have their meanings assigned to them hereunder, namely:

“**Agreement**” means this Agreement together with all annexures hereto;

“**Annexure A”** means additional information to this biological material agreement made available to SANParks;

“**Background Intellectual Property**” means intellectual property rights belonging to the Recipient and/or a third party associated with the Biological Material Agreement, that existed prior to the Effective Date;

"**Intellectual Property**" means any and all rights vesting in technical information, any inventions, processes, information and/or know-how, improvements, copyrightable works, designs and trade secrets**,** including, but not limited to, records of confidential information generated or maintained, data, test results, bibliographies, research findings, organisms, cells, DNA sequences, and other biological materials**,** whether in a written or electronic form, raw or derived, in the form of text, multimedia, computer programmes, spreadsheets, formatted fields in records, forms within files, databases, graphics, digital images, compositions and/or executions of processes, developed by the Recipient within the scope of the Biological Material Agreement;

“**Material**” means biological resources consisting of -

a living or dead animal, plant or other organism of an indigenous species;

a derivative of such an animal, plant or other organism, as defined in section 1 of the Biodiversity Act; or

any genetic material of such animal, plant or other organism, as defined in section 1 of the Biodiversity Act; wherever the term genetic resources is mentioned, it shall be taken as subset of biological resources in a holistic interpretation of all the provisions of the Convention on Biological Diversity as well as to include a reproductive resource, its functional units of heredity or other components which are expressed by such unit(s), excluding commodities marketed as such rather than as a means for developing such units;

“**Recipient**” means ___ _____, a natural or juristic person with ID/passport number _____, with its principal place of business at_____and includes the faculty, staff, and other persons employed or contracted by the Recipient, or the Recipient himself/herself, whether full- or part-time; and/or any other persons, including a student, a student employee, a graduate student, a post-doctoral fellow, and a non-employee (including visiting faculty, affiliate and adjunct faculty, industrial personnel, fellow, etc.) who participates in the creation or generation of applicable knowledge and/or Intellectual Property in the scope of the Agreement;

“Effective Date” means the date of signing of this agreement;

“Park” means the _____ National Park under the management of SANParks in terms of the National Environmental Management: Protected Areas Act 57 of 2003;

“**SANParks**” means South African National Parks, a statutory body established in terms of the National Parks Act No. 57 of 1976 and continuing to exist in terms of section 54(1) of the National Environmental Management: Protected Areas Act 57 of

2003 (as amended), with its principle place of administration at 643 Leyds Street, Muckleneuk, Pretoria, Gauteng;

"**Party/ies**" means SANParks and the Researcher, individually or collectively, as the case may be;

"**Signature Date**" means the date of signature of this Agreement by the last signing Party;

References to this Agreement shall include the annexures to this Agreement.

The headings to the clauses in this Agreement are for reference purposes only and shall not be used in the interpretation of this Agreement.

Words and phrases defined in this Agreement shall also apply in the interpretation of the same words and phrases in annexures to this Agreement, save where specifically indicated to the contrary in such annexure.

Unless the context otherwise require:

the singular shall import and include the plural and vice versa;

words indicating a gender shall import and include other genders;

words indicating natural persons shall include juristic persons.

This Agreement shall be construed and interpreted in accordance with the Laws of the Republic of South Africa.

Appendix B - Data and Metadata requirements

We are busy establishing a data catalogue that will be available through the internet. We have already added the KNP datasets and would like to add the research datasets as the projects are completed. For us to be able to do this efficiently could you please submit the original unprocessed data and metadata in the following way

General metadata required for the whole studies data:

The final report needs to be completed as requested.

Abstract for the dataset.

Geographic coverage. Area of the study needs to be stipulated e.g. Entire KNP or where you are working with transects the beginning and end point coordinates need to be given. If points are used then a GPS point for each should be given.

Temporal coverage. The dates that the data was collected

Keywords

Taxonomic coverage of the dataset. Please provide the genus and specie name of the individuals that were sampled in your dataset. This can be provided in a table format.

Data Usage rights. Enter a paragraph that describes the intended usage rights of the data. Specifically include any restrictions (scientific, technical, and/or ethical) to sharing your data within the public scientific domain. If your dataset is lead time protected please include the length of this period.

Access control .If you do want to restrict the dataset but have certain people that you would like to be able to access this data they should be mentioned here

Methods. The methods of the study should be discussed here. If you already have them in your project proposal please just copy and paste them.

People and organizations. Please supply the contact details of the people that you would like to be associated with the dataset and also the role that they played on the dataset e.g. metadata provider, principal investigator.

The metadata needed for each dataset is as follows

GIS data and Imagery

Each shape file needs to be submitted with a FGDC xml metadata document that can be made via the metadata tool of Arc catalogue.

Any imagery needs to be accompanied by a text file that indicates the level of processing of the image.

Spreadsheet or column data

Excel spreadsheet and any other column data (e.g. Access tables) need to be exported as text files. For each column in the text file the following information is needed.

` 1. Column heading

2. Column description

3. Type of variable i.e. numeric, date/time, enumerated (i.e. if you have codes you need to describe all the codes used. This description may be in another text file then just indicate that here.

4 Measurement unit e.g. mm, parts per million (ppm) etc.

5. Precision of the measurement i.e. if your measurements are in meters and your precision is 1 it means that your measurement is accurate to the nearest meter.

6. Bounds if the variable that you measured can only take on certain values stipulate them e.g. if a value can only be between 0 1 and 1 say min =0 max = 1.

This data and metadata need to be submitted to Judith.botha@sanparks.org. If your data does not fit in any of the above categories please contact judith.botha@sanparks.org for help.

Province of the Eastern Cape

ECONOMIC DEVELOPMENT, ENVIRONMENTAL AFFAIRS AND TOURISM

Chief Directorate: Environmental Affairs
SARAH Baartman Region

Collegiate Building
Cnr Belmont Terrace and Castle Hill
Private Bag X5001, Greenacres, Port Elizabeth 6057
South Africa

Collegiategebou
H/v Belmont Terrace en Castle Hill
Privaatsak X5001, Greenacres, Port Elizabeth 6057
Suid-Afrika

Miss Kristen Ellis,
2 Canterbury Place,
Prestwick Crescent,
Greenshields Park,
PORT ELIZABETH,
6025

Tel/Itoni: (041) 508 5813
Fax/Ifax: (041) 508 5850
Enquiries/Imibuzo: A. Southwood

Ref/Ireferensi: CRO 62/17CR
Date: 24 March 2017

Dear Miss Ellis,

RE: PERMIT TO TAKE SAMPLES OF VARIOUS PLANT SPECIES ON THE BASELINE VEGETATION SURVEY OF KAROO AREAS THAT MAY BE AFFECTED BY FRACKING: PRIVATE LAND IN THE EASTERN CAPE PROVINCE: PERMIT NO. CRO 62/17CR

In terms of Section 63 of the Nature and Environmental Conservation Ordinance, 1974 (Ordinance 19 of 1974), Sections 24 and 25 of Environmental Conservation Decree, 1992 (Decree No. 9 of 1992, former Transkei) and sections 20 and 21 of the Nature Conservation Act 1987 (Act No.10 of 1987, former Ciskei) you are hereby granted permission to take samples of various plant species in Karoo areas for research purposes as per your application:

Note the following conditions:

1. Written permission of the landowner must be obtained before entering the property to take samples of various plants. The written permission must reflect: -
1.1 the full names and address of the owner of the land concerned or the person authorised to grant such permission;
1.2 the full names and address of the person to whom permission is granted;
1.3 the number and the land in respect of which permission is granted; and
1.4 is signed and dated by such owner or person authorised by him.

2. This permit is valid until 24 March 2018 and it must be returned to this office within fourteen days of the expiry date.

Alan Southwood

3. After the expiry date of this permit, you must submit copies of all reports / publications resulting from this research, to this office.

4. The Department must be acknowledged in any publications that result from the taking of samples resulting from this research.

5. Failure to adhere to the above conditions may lead to this permit being cancelled with immediate effect. Issuing of further permits depend upon the fulfilling of the above conditions.

Yours faithfully,

pp **DAYALAN GOVENDER**
REGIONAL MANAGER: ENVIRONMENTAL AFFAIRS
SARAH BAARTMAN REGION

N40 **No. CRO 62/17CR** 331538

PROVINSIALE ADMINISTRASIE VAN DIE OOS-KAAP PROVINSIE
HOOF DIREKTORAAT OMGEWINGSAKE

PERMIT OM *BESKERMDE FLORA/FLORA TE PLUK

Ordonnansie op Natuur- en Omgewingsbewaring, 1974 (Ordonnansie 19 van 1974) (Artikel 63), Environmental Conservation Decree, 1992 (Decree No. 9 of 1992, former Transkei) (Sections 24 and 25) and Nature Conservation Act 1987 (Act No. 10 of 1987, former Ciskei) (Sections 20 and 21)

PROVINCIAL ADMINISTRATION OF THE EASTERN CAPE PROVINCE
CHIEF DIRECTORATE ENVIRONMENTAL AFFAIRS

PERMIT TO PLUCK *PROTECTED FLORA/FLORA

Nature and Environmental Conservation Ordinance, 1974 (Ordinance 19 of 1974) (Section 63), Environmental Conservation Decree, 1992 (Decree No. 9 of 1992, former Transkei) (Sections 24 and 25) and Nature Conservation Act 1987 (Act No. 10 of 1987, former Ciskei) (Sections 20 and 21)

NIE OORDRAAGBAAR /NOT TRANSFERABLE

'n Permit word hierby ingevolge Artikel 63 van die Ordonnansie op Natuur- en Omgewingsbewaring, 1974 (Ordonnansie 19 van 1974), Environmental Conservation Decree, 1992 (Decree No. 9 of 1992, former Transkei) (Sections 24 and 25) and Nature Conservation Act, 1987 (Act No. 10 of 1987, former Ciskei) (Sections 20 and 21) uitgereik aan -

In terms of section 63 of the Nature and Environmental Conservation Ordinance, 1974 (Ordinance 19 of 1974), Environmental Conservation Decree, 1992 (Decree No. 9 of 1992, former Transkei) (Sections 24 and 25) and Nature Conservation Act, 1987 (Act No. 10 of 1987, former Ciskei) (Sections 20 and 21) a permit is hereby issued to -

Miss Kristen Ellis
2 Canterbury Place
Prestwick Crescent, Greenshields Park
Port Elizabeth
6025

om die ondergemelde getal en spesies *beskermde flora/flora, uitgesonderd bedreigde flora, te pluk, nl. -

to pluck the hereinafter mentioned number and species *protected flora/flora, excluding endangered flora, viz. -

Spesie/Species	Getal / Number
All species encountered in the sampling area will be collected	

* op die eiendom / in die -

* on the property / in the

Middelburg, Somerset East, Cradock, Aberdeen, Graaff-Reinet and Paterson

VOORWAARDES

1. Hierdie permit is geldig tot **24 March 2018**

2. Die houer van hierdie permit moet dit binne 14 dae vanaf die vervaldatum daarvan aan die Streeksdirekteur van Ekonomiese Sake, Omgewing & Toerisme, Privaatsak X5001, Greenacres 6057, terugstuur tesame met 'n opgawe van die spesies flora en getal van elke spesie wat hy daarkragtens gepluk het.

3. Die skriftelike toestemming van die Grondeienaar moet verkry word, alvorens die eiendom betree word.

CONDITIONS

1. This permit is valid until **24 March 2018**

2. The holder of this permit shall return it together with a return of the species flora and the number of each species which he pluck thereunder, to the Regional Director of Economic Development, Environmental Affairs and Tourism, Private Bag X5001, Greenacres 6057, within 14 days of the date of expiry thereof.

3. The written permission of the Landowner must be obtained before entering the property.

Senior Bestuurder van Omgewingsake/Senior Manager of Environmental Affairs

24 March 2017
Datum/Date

*Skrap wat nie van toepassing is nie

*Delete whichever is not applicable

APPENDIX B

Species	Family	Life form after Rutherford & Westfall (?)	Status	Endemism
Barleria stimulans E.Mey. ex Nees	Acanthaceae	C	LC	SA
Blepharis mitrata C.B.Clarke	Acanthaceae	C	LC	
Monechma spartioides (T.Anderson) C.B.Clarke	Acanthaceae	P	LC	
Delosperma multiflorum L.Bolus	Aizoaceae	S	LC	EC
Delosperma peersii Lavis	Aizoaceae	S	LC	EC
Delosperma robustum L.Bolus	Aizoaceae	S	LC	EC
Drosanthemum lique (N.E.Br.) Schwantes	Aizoaceae	S	LC	EC
Galenia procumbens L.f.	Aizoaceae	C	LC	SA
Glottiphyllum depressum (Haw.) N.E.Br.	Aizoaceae	S	LC	SA
Lampranthus productus (Haw.) N.E.Br.	Aizoaceae	S	LC	SA
Malephora lutea (Haw.) Schwantes	Aizoaceae	S	LC	SA
Mesembryanthemum guerichianum Pax	Aizoaceae	S	LC	
Phyllobolus splendens (L.) Gerbaulet subsp. *pentagonus* (L.Bolus) Gerbaulet	Aizoaceae	S	LC	SA
Plinthus karooicus I.Verd.	Aizoaceae	C	LC	
Psilocaulon coriarium (Burch. ex N.E.Br.) N.E.Br.	Aizoaceae	S	LC	
Ruschia divaricata L.Bolus	Aizoaceae	S	LC	
Ruschia intricata (N.E.Br.) H.E.K.Hartmann & Stüber	Aizoaceae	S	LC	SA
Ruschia perfoliata (Mill.) Schwantes	Aizoaceae	S	LC	SA
Ruschia spinosa (L.) Dehn	Aizoaceae	S	LC	
Ruschia vanderbergiae L.Bolus	Aizoaceae	S	LC	EC

Tetragonia arbuscula Fenzl	Aizoaceae	S	LC	
Trichodiadema barbatum (L.) Schwantes	Aizoaceae	S	LC	EC
Trichodiadema densum (Haw.) Schwantes	Aizoaceae	S	LC	EC
Trichodiadema pomeridianum L.Bolus	Aizoaceae	S	LC	
Trichodiadema setuliferum (N.E.Br.) Schwantes	Aizoaceae	S	LC	EC
Alternanthera pungens Kunth	Amaranthaceae	F	Naturalized exotic	
Amaranthus dinteri Schinz	Amaranthaceae	T	NE	
Exomis microphylla (Thunb.) Aellen *var. axyrioides* (Fenzl) Aellen.	Amaranthaceae	C	LC	SA
Atriplex lindleyi Moq. subsp. *inflata* (F.Muell.) Paul G.Wilson	Amaranthaceae	C	Naturalized exotic; Invasive	
Atriplex semibaccata R.Br. var. *appendiculata* Aellen	Amaranthaceae	C	LC	
Rhigozum obovatum Burch.	Anacardiaceae	P	LC	
Searsia burchellii (Sond. ex Engl.) Moffett	Anacardiaceae	P	LC	
Searsia dregeana (Sond.) Moffett	Anacardiaceae	P	LC	
Searsia erosa (Thunb.) Moffett	Anacardiaceae	P	LC	
Searsia longispina (Eckl. & Zeyh.) Moffett	Anacardiaceae	P	LC	SA
Searsia pallens (Eckl. & Zeyh.) Moffett	Anacardiaceae	P	LC	
Cyclospermum leptophyllum (Pers.) Sprague ex Britton & P. Wilson	Apiaceae	F	Naturalized exotic	
Carissa bispinosa (L.) Desf. ex Brenan	Apocynaceae	P	LC	
Pachypodium succulentum (L.f.) Sweet	Apocynaceae	S	LC	SA
Cussonia spicata Thunb.	Araliaceae	P	LC	
Asparagus aethiopicus L.	Asparagaceae	C	LC	SA
Asparagus burchellii Baker	Asparagaceae	C	LC	SA
Asparagus crassicladus Jessop.	Asparagaceae	C	LC	SA

Asparagus racemosus Willd.	Asparagaceae	C	LC	
Asparagus retrofractus L.	Asparagaceae	C	LC	
Asparagus striatus (L.f.) Thunb.	Asparagaceae	C	LC	SA
Asparagus suaveolens Burch.	Asparagaceae	C	LC	
Asparagus subulatus Thunb.	Asparagaceae	C	LC	EC
Aloe ferox Mill.	Asphodelaceae	S	LC	
Aloe striata Haw.	Asphodelaceae	S	LC	SA
Bulbine favosa (Thunb.) Schult. & Schult.f.	Asphodelaceae	S	LC	SA
Bulbine frutescens (L.) Willd.	Asphodelaceae	S	LC	
Trachyandra asperata Kunth	Asphodelaceae	G	LC	
Arctotheca calendula (L.) Levyns	Asteraceae	F	LC	
Arctotis arctotoides (L.f.) O.Hoffm.	Asteraceae	F	LC	
Arctotis microcephala (DC.) Beauverdla	Asteraceae	C	LC	
Artemisia afra Jacq. ex Willd. var. *afra*	Asteraceae	C	LC	
Berkheya carlinifolia (DC.) Roessler subsp. *carlinifolia*	Asteraceae	C	LC	SA
Berkheya heterophylla (Thunb.) O.Hoffm. var. *radiata* (DC.) Roessler	Asteraceae	C	LC	SA
Chrysocoma ciliata L.	Asteraceae	C	LC	
Chrysocoma rigidula (DC.) Ehr. Bayer	Asteraceae	C	LC	EC
Cirsium arvense (L.) Scop.	Asteraceae	H		
Cirsium vulgare (Savi) Ten.	Asteraceae	H	Naturalized exotic; Invasive	
Conyza podocephala DC.	Asteraceae	F	LC	
Cotula heterocarpa DC.	Asteraceae	C	LC	SA
Curio radicans (L.) P.V.Heath	Asteraceae	S	LC	
Cuspidia cernua (L.f.) B.L.Burtt subsp. *cernua*	Asteraceae	F	LC	EC
Dicerothamnus rhinocerotis (L.f.) Koekemoer	Asteraceae	P	LC	

Dimorphotheca cuneata (Thunb.) Less.	Asteraceae	C	LC	
Dimorphotheca zeyheri Sond.	Asteraceae	F	LC	
Eriocephalus ericoides (L.f.) Druce subsp. *ericoides*	Asteraceae	C	LC	
Felicia fascicularis DC.	Asteraceae	C	LC	SA
Felicia filifolia (Vent.) Burtt Davy subsp. *filifolia*	Asteraceae	C	LC	
Felicia muricata (Thunb.) Nees subsp. *muricata*	Asteraceae	C	LC	
Felicia ovata (Thunb.) Compton	Asteraceae	C	LC	SA
Gazania krebsiana Less.	Asteraceae	F	LC	
Gazania linearis (Thunb.) Druce var. *linearis*	Asteraceae	F	LC	
Gazania sp.	Asteraceae	F		
Gnaphalium confine Harv.	Asteraceae	F	LC	
Helichrysum anomalum Less.	Asteraceae	C	LC	
Helichrysum hamulosum E.Mey. ex DC.	Asteraceae	C	LC	SA
Helichrysum nudifolium (L.) Less.	Asteraceae	F	LC	
Helichrysum odoratissimum (L.) Sweet	Asteraceae	P	LC	
Helichrysum rosum (P.J.Bergius) Less. var. *arcuatum*	Asteraceae	C	LC	SA
Helichrysum rosum (P.J.Bergius) Less. var. *rosum*	Asteraceae	P	LC	SA
Helichrysum teretifolium (L.) D.Don.	Asteraceae	C	LC	SA
Helichrysum zeyheri Less.	Asteraceae	C	LC	
Hypochaeris radicata L.	Asteraceae		Naturalized exotic	
Ifloga glomerata (Harv.) Schltr.	Asteraceae	F	LC	
Lactuca inermis Forssk.	Asteraceae	F	LC	
Macledium spinosum (L.) S.Ortíz	Asteraceae	C	LC	SA
Pegolettia retrofracta (Thunb.) Kies	Asteraceae	C	LC	

Pentzia globosa Less.	Asteraceae	C	LC	
Pentzia incana (Thunb.) Kuntze	Asteraceae	C	LC	
Pentzia sphaerocephala DC.	Asteraceae	C	LC	
Phymaspermum parvifolium (DC.) Benth. & Hook. ex B.D.Jacks.	Asteraceae	C	LC	SA
Pseudognaphalium luteo-album (L.) Hilliard & B.L.Burtt	Asteraceae	P	LC	
Pseudognaphalium undulatum (L.) Hilliard & B.L.Burtt	Asteraceae	C	LC	
Pteronia paniculata Thunb.	Asteraceae	P	LC	
Rosenia humilis (Less.) K.Bremer	Asteraceae	C	LC	
Schkuhria pinnata (Lam.) Kuntze ex Thell.	Asteraceae		Naturalized exotic	
Senecio acutifolius DC.	Asteraceae	C	LC	SA
Senecio juniperinus L.f.	Asteraceae	C	LC	SA
Senecio leptophyllus DC.	Asteraceae	C	LC	SA
Senecio macrocephalus DC.	Asteraceae	F	LC	
Senecio polyanthemoides Sch.Bip.	Asteraceae	P	LC	
Senecio ruwenzoriensis S.Moore	Asteraceae	C	LC	
Tagetes minuta L.	Asteraceae	F	Naturalized exotic	
Ursinia sericea (Thunb.) N.E.Br.	Asteraceae	C	LC	SA
Rhigozum trichotomum Burch.	Bignoniaceae	P	LC	
Anchusa capensis Thunb.	Boraginaceae	F	LC	
Ehretia rigida (Thunb.) Druce subsp. *rigida*	Boraginaceae	P	LC	SA
Heliophila suavissima Burch. ex DC.	Brassicaceae	F	LC	
Lepidium africanum (Burm.f.) DC. subsp. *africanum*	Brassicaceae	C	LC	
Buddleja glomerata H.L.Wendl.	Buddlejadaceae	P	LC	SA
Buddleja saligna Willd.	Buddlejadaceae	P	LC	

Opuntia aurantiaca Lind.	Cactaceae	S	Naturalized exotic; Invasive	
Cyphia linarioides C. Presl	Campanulaceae	L	LC	SA
Wahlenbergia albens (Spreng. ex A.DC.) Lammers	Campanulaceae	C	LC	
Wahlenbergia nodosa (H.Buek) Lammers	Campanulaceae	C	LC	SA
Wahlenbergia undulata (L.f.) A.DC.	Campanulaceae	F	LC	
Cadaba aphylla (Thunb.) Willd.	Capparaceae	P	LC	
Dianthus micropetalus Ser.	Caryophyllaceae	F	LC	SA
Gymnosporia heterophylla (Eckl. & Zeyh.) Loes.	Celastraceae	P	LC	
Gymnosporia szyszylowiczii (Kuntze) M.Jordaan	Celastraceae	P	LC	
Putterlickia pyracantha (L.) Szyszyl.	Celastraceae	P	LC	SA
Chenopodium phillipsianum Aellen	Chenopodiaceae	F	Not assessed	
Commelina africana L.	Commelinaceae	F	LC	
Dichondra micrantha Urb.	Convolvulaceae	L	Naturalized exotic	
Cotyledon orbiculata L.	Crassulaceae	S	LC	
Crassula capitella Thunb.	Crassulaceae	S	LC	SA
Crassula ericoides Haw.	Crassulaceae	S	LC	SA
Crassula expansa Dryand. subsp. *expansa*	Crassulaceae	S	LC	
*Crassula mesembryanthoides (*Haw.) D.Dietr. subsp. *mesembryanthoides*	Crassulaceae	S	LC	EC
Crassula muscosa L.	Crassulaceae	S	LC	
Crassula orbicularis L.	Crassulaceae	S	LC	SA
Crassula ovata (Mill.) Druce	Crassulaceae	S	LC	SA
Crassula perfoliata L. var. *coccinea* (Sweet) G.D.Rowley	Crassulaceae	S	LC	EC
Crassula perforata Thunb.	Crassulaceae	S	LC	SA

Crassula rogersii Schönland	Crassulaceae	S	LC	SA
Crassula rubricaulis Eckl. & Zeyh.	Crassulaceae	S	LC	SA
Crassula rupestris Thunb. subsp. *rupestris*	Crassulaceae	S	LC	SA
Crassula tetragona L. subsp. *acutifolia* (Lam.) Tölken	Crassulaceae	S	LC	SA
Crassula tetragona L. subsp. *tetragona*	Crassulaceae	S	LC	SA
Kalanchoe rotundifolia (Haw.) Haw.	Crassulaceae	S	LC	
Kedrostis africana (L.) Cogn.	Cucurbitaceae	L	LC	
Pilogyne scabra (L.f.) W.J.de Wilde & Duyfjes	Cucurbitaceae		LC	
Pilogyne scabra (L.f.) W.J.de Wilde & Duyfjes	Cucurbitaceae	L		
Cyperus usitatus Burch.	Cyperaceae	H	LC	
Ficinia sp.	Cyperaceae	H		
Kyllinga pulchella Kunth	Cyperaceae	H	LC	
Diospyros austro-africana De Winter var. *austro-africana*	Ebenaceae	P	LC	SA
Diospyros lycioides Desf. subsp. *lycioides*	Ebenaceae	P	LC	
Diospyros scabrida (Harv. ex Hiern) De Winter	Ebenaceae	P	LC	SA
Euclea undulata Thunb.	Ebenaceae	P	LC	
Clutia daphnoides Lam.	Euphorbiaceae	P	LC	SA
Euphorbia gorgonis A.Berger	Euphorbiaceae	S	LC	SA
Euphorbia mammilaris L.	Euphorbiaceae	S	LC	
Euphorbia mauritanica L. var. *mauritanica*	Euphorbiaceae	S	LC	EC
Euphorbia radyeri Bruyns	Euphorbiaceae	S	LC	EC
Euphorbia rhombifolium Boiss.	Euphorbiaceae	S	LC	
Euphorbia tetragona Haw.	Euphorbiaceae	S	LC	SA
Argyrolobium collinum Eckl. & Zeyh.	Fabaceae	C	LC	SA
Aspalathus frankenioides DC.	Fabaceae	C	LC	SA

Calobota spinescens (Harv.) Boatwr. & B.-E.van Wyk	Fabaceae	P	LC	
Crotalaria obscura DC.	Fabaceae	F	LC	SA
Sp.	Fabaceae	C		
Indigofera disticha Eckl. & Zeyh.	Fabaceae	F	LC	EC
Indigofera sessilifolia DC.	Fabaceae	C	LC	
Lessertia depressa Harv.	Fabaceae	C	LC	
Melolobium candicans (E.Mey.) Eckl. & Zeyh.	Fabaceae	C	LC	
Rhynchosia caribaea (Jacq.) DC.	Fabaceae	F	LC	
Schotia afra (L.) Thunb. var. *afra*	Fabaceae	P	LC	SA
Vachellia karroo (Hayne) Banfi & Gallaso	Fabaceae	P	LC	
Sebaea grisebachiana Schinz	Gentianaceae	C	LC	SA
Geranium schlechteri R.Knuth	Geraniaceae	F	LC	
Pelargonium abrotanifolium (L.f.) Jacq.	Geraniaceae	F	LC	SA
Pelargonium althaeoides (L.) L'Hér.	Geraniaceae	F	LC	
Pelargonium aridum R.A.Dyer	Geraniaceae	F	LC	
Pelargonium patulum Jacq.	Geraniaceae	F	LC	
Pelargonium peltatum (L.) L'Hér.	Geraniaceae	L	LC	SA
Pelargonium reniforme Curtis	Geraniaceae	F	Near Threatened A4bd	SA
Pelargonium sidoides DC.	Geraniaceae	G	LC	
Sarcocaulon camdeboense Moffett	Geraniaceae	S	LC	SA
Albuca cf. *viscosa* L.f.	Hyacinthaceae	G	LC	
Albuca setosa Jacq.	Hyacinthaceae	G	LC	
Albuca shawii Baker	Hyacinthaceae	G	LC	
Albuca sp.	Hyacinthaceae	G		
Albuca spiralis L.f.	Hyacinthaceae	G	LC	SA

Albuca virens (Ker Gawl.) J.C.Manning & Goldblatt subsp. *arida* (Oberm.) J.C.Manning & Goldblatt	Hyacinthaceae	G	LC	
Dipcade ciliare (Zeyh. ex Harv.) Baker	Hyacinthaceae	G	LC	SA
Drimia intricata (Baker) J.C.Manning & Goldblatt	Hyacinthaceae	G	LC	
Ornithogalum dubium Houtt.	Hyacinthaceae	G	LC	SA
Sansevieria hyacinthoides (L.) Druce	Hyacinthaceae	G	LC	
Dietes iridioides (L.) Sweet ex Klatt	Iridaceae	G	LC	
Moraea polystachya (Thunb.) Ker-Gawl.	Iridaceae	G	LC	
Romulea macowanii Baker var. *macowanii*	Iridaceae	G	LC	
Syringodea bifurcata M.P.de Vos	Iridaceae	G	LC	SA
Lamium amplexicaule L.	Lamiaceae	F	Naturalized exotic; Invasive	
Mentha longifolia L.	Lamiaceae	F	LC	
Ocimum burchellianum Benth.	Lamiaceae	C	LC	EC
Salvia scabra L.f.	Lamiaceae	F	LC	EC
Salvia verbenaca L.	Lamiaceae	F	LC	
Sp.	Lamiaceae	P		
Stachys linearis Burch. ex Benth.	Lamiaceae	C	LC	
Teucrium trifidum Retz.	Lamiaceae	F	LC	
Lobelia cuneifolia Link & Otto var. *cuneifolia*	Lobeliaceae	F	LC	SA
Monopsis sp.	Lobeliaceae	F		
Grewia occidentalis L. var. *occidentalis*	Malvaceae	P	LC	
Grewia robusta Burch.	Malvaceae	P	LC	SA
Hermannia althaeoides Link	Malvaceae	C	LC	SA
Hermannia cernua Thunb.	Malvaceae	C	LC	
Hermannia coccocarpa (Eckl. & Zeyh.) Kuntze	Malvaceae	C	LC	

Hermannia cuneifolia subsp. c*uneifolia*	Malvaceae	C	LC	
Hermannia desertorum Eckl. & Zeyh.	Malvaceae	C	LC	
Hermannia flammea Jacq.	Malvaceae	C	LC	SA
Hermannia glabrata L.f.	Malvaceae	C	LC	SA
Hibiscus pusilus Thunb.	Malvaceae	C	LC	
Sida rhombifolia L. var. *rhombifolia*	Malvaceae	F	LC	
Melianthus comosus Vahl	Melianthaceae	P	LC	
Cissampelos capensis L.f.	Menispermaceae	P	LC	
Eriospermum sp.	Nolinoideae	G		
Olea europaea L. subsp. *africana* (Mill.) P.S.Green	Oleaceae	P	LC	
Oxalis commutata Sond.	Oxalidaceae	F	LC	WC
Oxalis sp.	Oxalidaceae	F		
Oxalis stricta L.	Oxalidaceae	H	Weed	
Plantago lanceolata L.	Plantaginaceae	F	LC	
Andropogon sp.	Poaceae	H		
Aristida congesta Roem. & Schult. subsp. *barbicollis* (Trin. & Rupr.) De Winter	Poaceae	H	LC	
Aristida congesta Roem. & Schult. subsp. *congesta*	Poaceae	H	LC	
Aristida diffusa Trin. subsp. *burkei* (Stapf) Melderis	Poaceae	H	LC	
Aristida diffusa Trin. subsp. *diffusa*	Poaceae	H	LC	SA
Brachiaria serrata (Thunb.) Stapf	Poaceae	H	LC	
Bromus hordaceus L. subsp. *molliformis* (J.Lloyd) Maire & Weiller	Poaceae	H	Naturalized exotic	
Cenchrus ciliatus L.	Poaceae	H	LC	
Cymbopogon marginatus (Steud.) Stapf ex Burtt Davy	Poaceae	H	LC	
Cymbopogon pospichilli (K.Schum.) C.E.Hubb.	Poaceae	H	Not in Red List	
Cynodon dactylon (L.) Pers.	Poaceae	H	LC	

Cynodon incompletus Nees	Poaceae	H	LC	SA
Digitaria eriantha Steud.	Poaceae	H	LC	
Ehrharta calycina Sm.	Poaceae	H	LC	
Elionurus muticus (Spreng.) Kunth	Poaceae	H	LC	
Enneapogon scoparius Stapf	Poaceae	H	LC	
Eragrostis capensis (Thunb.) Trin.	Poaceae	H	LC	
Eragrostis chloromelas Steud.	Poaceae	H	LC	
Eragrostis curvula (Schrad.) Nees	Poaceae	H	LC	
Eragrostis lehmanniana Nees var. *lehmanniana*	Poaceae	H	LC	
Eragrostis obtusa Munro ex Ficalho & Hiern	Poaceae	H	LC	
Eustachys paspaloides (Vahl) Lanza & Mattei	Poaceae	H	LC	
Fingerhuthia africana Lehm.	Poaceae	H	LC	
Sp.	Poaceae	H		
Helictotrichon turgidulum (Stapf) Schweick.	Poaceae	H	LC	
Heteropogon contortus (L.) Roem. & Schult.	Poaceae	H	LC	
Hyparrhenia hirta (L.) Stapf	Poaceae	H	LC	
Koeleria capensis (Steud.) Nees	Poaceae	H	LC	
Melica decumbens Thunb.	Poaceae	H	LC	
Melica racemosa Thunb.	Poaceae	H	LC	
Melinis nerviglumis (Franch.) Zizka	Poaceae	H	LC	
Merxmuellera disticha (Nees) Conert	Poaceae	H		
Microchloa caffra Nees	Poaceae	H	LC	
Panicum deustum Thunb.	Poaceae	H	LC	
Panicum maximum Jacq.	Poaceae	H	LC	
Paspalum dilatatum Poir.	Poaceae	H	Naturalized exotic	

Pentameris setifolia (Thunb.) Galley & H.P.Linder	Poaceae	H	LC	
Setaria sphacelata (Schumach.) Stapf & C.E.Hubb. ex M.B.Moss	Poaceae	H	LC	
Sporobolus africanus (Poir.) Robyns & Tournay	Poaceae	H	LC	
Sporobolus fimbriatus (Trin.) Nees	Poaceae	H	LC	
Tenaxia disticha (Nees) N.P.Barker & H.P.Linder	Poaceae	H	LC	
Themeda triandra Forssk.	Poaceae	H	LC	
Tragus koelerioides Asch.	Poaceae	H	LC	
Tribolium curvum (Nees) Verboom & H.P.Linder	Poaceae	H	LC	SA
Polygala leptophylla Burch. var. *leptophylla*	Polygalaceae	C	LC	
Polygala uncinata E.Mey. ex Meisn.	Polygalaceae	C	LC	
Rumex acetosella L. subsp. *angiocarpus* (Murb.) Murb.	Polygonaceae	F	Naturalized exotic	
Portulacaria afra Jacq.	Portulacaceae	S	LC	
Cheilanthes eckloniana (Kunze) Mett.	Pteridaceae	Fern	LC	
Cheilanthes hirta Sw.	Pteridaceae	Fern	LC	
Cheilanthes viridis (Forssk.) Sw. var. *glauca* (Sim) Schelpe & N.C.Anthony	Pteridaceae	Fern	LC	
Leucosidea sericea Eckl. & Zeyh.	Rosaceae	P	LC	
Rubus rigidus Sm.	Rosaceae	P	LC	
Sp.	Rosaceae	P		
Anthospermum galioides Rchb.f.	Rubiaceae	C	LC	SA
Nenax microphylla (Sond.) Salter	Rubiaceae	C	LC	
Azima tetracantha Lam.	Salvadoraceae	P	LC	
Osyris lanceolata Hochst. & Steud.	Santalaceae	P	LC	
Thesium galioides A.DC.	Santalaceae	C	LC	SA
Thesium hystrix A.W.Hill	Santalaceae	R	LC	
Thesium junceum Bernh.	Santalaceae	R	LC	SA

Dodonaea viscosa Jacq. var. *angustifolia* (L.f.) Benth.	Sapindaceae	P	LC	
Pappea capensis Eckl. & Zeyh.	Sapindaceae	P	LC	
Aptosimum procumbens (Lehm.) Steud.	Scrophulariaceae	C	LC	
Chaenostoma campanulatum Benth.	Scrophulariaceae	F	LC	EC
Jamesbrittenia atropurpurea (Benth.) Hilliard	Scrophulariaceae	C	LC	
Jamesbrittenia pinnatifida (L.f.) Hilliard	Scrophulariaceae	F	LC	SA
Jamesbrittenia tysonii (Hiern) Hilliard	Scrophulariaceae	C	LC	SA
Nemesia affinis Benth.	Scrophulariaceae	C	LC	SA
Selago albida Choisy	Scrophulariaceae	C	LC	SA
Selago corymbosa L.	Scrophulariaceae	C	LC	SA
Selago decipiens E.Mey.	Scrophulariaceae	F	LC	EC
Selago geniculata L.f.	Scrophulariaceae	C	LC	SA
Selago gracilis (Rolfe) Hilliard	Scrophulariaceae	C	LC	SA
Chaenostoma halimifolium Benth.	Scrophulariaceae	C	LC	
Pellaea calomelanos (Sw.) Link var. *calomelanos*	Sinopteridaceae	Fern	LC	
Lycium cinereum Thunb.	Solanaceae	P	LC	
Lycium ferocissimum Miers	Solanaceae	P	LC	
Lycium oxycarpum Dunal	Solanaceae	P	LC	SA
Solanum rigescens Jacq.	Solanaceae	C	Weed	
Solanum sp.	Solanaceae	C		
Solanum tomentosum L.	Solanaceae	P	LC	
Lasiosiphon capitatus (L.f.) Burtt Davy	Thymelaeaceae	P	LC	
Lantana rugosa Thunb.	Verbenaceae	C	LC	
Verbena bonariensis L.	Verbenaceae	F	Naturalised exotic; Invasive	
Viscum obovatum Harv.	Viscaceae	R	LC	
Viscum rotundifolium L.f.	Viscaceae	R	LC	

Rhoicissus tridentata (L.f.) Wild & R.B.Drumm. subsp. *tridentata*	Vitaceae	L	LC	
Zygophyllum microcarpum Licht. ex Cham. & Schltdl.	Zygophyllaceae	S	LC	
Geophyte 1		G		
Geophyte 2		G		
Geophyte 3		G		

Life form codes:

C Chamaephyte

F Forb

G Geophyte

H Hemicryptophyte

L Climber

P Phanerophyte

R Parasite

S Succulent

T Therophyte