

**Determining the Potential drivers of invasive C₄ grasses at De Hoop
Nature Reserve, South Africa.**

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**A thesis presented to the Faculty of Science, University of Cape Town, for the
Degree of Master of Science in the Department of Biological Sciences.**

**Supervisor: Associate Professor Edmund Carl February
Co-supervisor- Emeritus Professor William John Bond**

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously, in its entirety or in part, submitted it at any university for a degree.

Signature.....

Date.....

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Abstract

Grassland and shrubland distribution in SA is associated with rainfall seasonality. In grassland the vegetation is exclusively C₄ while the shrub and tree component is generally C₃. Shrublands, in contrast, are predominantly C₃, for both woody and herbaceous species, though there can be a small C₄ sedge and grass component. The C₄ grassy biomes dominate the warm season rainfall region while C₃ grasses predominate in the shrublands of the cool season rainfall regions. The C₄ grasses are poorly competitive in cold climates.

There are however anomalous patches of C₄ grasses in cool season rainfall regions dominated by fynbos shrublands such as those at Potberg, De Hoop Nature Reserve, Overberg region, South Africa. Although the southern Overberg region receives rainfall year-round, more than half of the rain falls in the cooler months of the year. These C₄ grassland patches appear to be invading the fynbos even on the nutrient poor podsols. The main objective of this study was to gain a better understanding of the ecology of these anomalous C₄ grasslands in a fynbos-dominated region.

I first explored the role of roads as a conduit for grass invasion by sampling sites adjacent to and further away from the road verge. This was done by identifying all species in plots 5 m (Roadside pots) and 100 m (Adjacent plots) from the road verge every 500 m along a 14 km management road at Potberg. My results show that the most common C₄ grasses were *Cynodon dactylon* and *Eragrostis curvula* whilst the most common C₃ grass was *Merxmuellera disticha*. Both Roadside and Adjacent plots had more C₄ grass species than C₃ grasses. The frequency distribution of both C₃ and C₄ grass species was significantly higher on the Roadside plots than on the Adjacent plots. Although there was a higher incidence of C₄ grass occurrence on the Roadside plots I conclude that, roadsides are not the conduit for C₄ grass invasion into fynbos as several large patches of the C₄ grass *Eragrostis curvula* and *Imperata cylindrica* can be found several kilometres away from the roadside and there are fewer C₄ grasses away from the road verge.

I then explored the impacts of C₄ grasses on fynbos species diversity by identifying all the plants in 100 plots, half in fynbos-dominated vegetation and half in grassy patches. My results show that C₄ grasses had a negative effect on fynbos species richness. The areas invaded by the C₄ grass had fewer fynbos species (average of 7) compared to areas with C₃ grasses (average 17). Some C₄ grasses had more of an effect than others with some of the most aggressive the clonally spreading species *Imperata cylindrica*, *Setaria sphacelata* var. *torta* and *Cynodon dactylon*. C₄

grasses such as *Aristida diffusa* and *Themeda triandra* occur naturally in the fynbos with little impact on fynbos species richness. *Eragrostis curvula*, a tall bunch grass, was also locally dominant in places at the expense of fynbos species diversity.

To examine the drivers influencing colonisation of fynbos by C₄ grass, I set up a transplant experiment designed to examine the extent to which topographic position along a slope (moisture availability), an increase in nutrient availability and a decrease in competition for resources influences the establishment and growth of C₄ grass. To do this I transplanted tufts of *Setaria sphacelata* var. *torta* in three positions along a slope, with and without nutrient additions and with and without competition for soil resources. I removed competition for resources by trenching and inserting a plastic sheath around each grass tuft to a depth of 500 mm. My results show an increase in mortality in drier treatments at the top of the slope. Trenching and fertiliser addition had significant positive effects on grass biomass and negative effects on root shoot ratios. Slope position however only had significant effects on root shoot ratios. Plants at the top of the slope had significantly higher root/shoot ratios than those plants at the bottom ($P < 0.05$). Trenching did not have a significant effect on root shoot ratios ($P > 0.05$).

Based on the results of this research, it can be concluded that the grass patches have a negative effect on fynbos species diversity. These grass patches are not a remnant from when the reserve was still a farm but are relatively new and are not invading out from the roads. I examined rainfall seasonality from 1910 to 2015 and found no change. My results from trenching show that physical disturbance that breaks up fynbos root systems will help promote C₄ grass invasion. Thus, any activities that increases physical disturbance of the fynbos such as off-road vehicles and trampling by large mammals must be avoided to maintain intact fynbos. While this has not occurred at Potberg, I speculate that because of fire suppression, there has been a decline in species diversity and a reduction in competition for resources allowing the C₄ grasses to establish and dominate. The impact of fire on C₄ grass needs more research, to understand if too frequent or less frequent fire will disturb fynbos and allow invasion by C₄ grasses.

Chapter 1

General Introduction

The Greater Cape Floristic Region (GCFR) located in the Western Cape Province of South Africa is a biodiversity hotspot characterised by the unique Fynbos and Succulent Karoo biomes (Mucina and Rutherford 2006a, Valente et al. 2010). The diversity and endemism of plant species and genera in the Fynbos Biome are among the largest for any region of this size worldwide (Manning and Goldblatt 2012), while the Succulent Karoo Biome is one of only two entirely arid biodiversity hotspots (Cowling and Lombard 2002). A wide range of geologies, topographic variability and strong climatic gradients, all contribute to niche heterogeneity and potentially to the maintenance of the high species richness (Cramer and Hoffman 2015).

Fynbos is remarkable not only because of its high species diversity and endemism but also because it, and other shrubland biomes from the Mediterranean climate region, are so strikingly different from vegetation elsewhere in South Africa and the African continent (Bergh et al. 2014). Fynbos is dominated by shrubs while the warm season rainfall region of southern Africa is dominated by savanna vegetation characterised by the co-existence of trees and grasses (Mucina and Rutherford 2006, Scholes and Archer 1997). Major growth forms in fynbos are the grass like Restionaceae, small-leaved shrubs of which the Ericaceae are prime examples, and broad-leaved proteoid shrubs mostly belonging to the Proteaceae, as well as geophytes (Holmes and Richardson 1999). Among the graminoid growth forms other than Restionaceae, sedges (Cyperaceae) are sometimes abundant but grasses (Poaceae) are generally minor contributors to biomass (Taylor 1978; Kruger 1979; Rebelo et al. 2006) although moderately rich in species (Bergh et al. 2014).

Invasive alien plants have been identified as the second-most important threat to species diversity after humans (Foxcroft & Richardson, 2003). Of these invasive plants, grasses are among the most common and the most destructive, drastically altering fire regimes and impacting ecosystems by changing litter composition, microbial communities and soil nutrient status (D'Antonio and Vitousek 1992b, Gaertner et al. 2014). Several studies have also shown that invasive grasses may change the abiotic and biotic environment, as well as the ecological functionality of the ecosystem, often resulting in the exclusion of native species (Mack et al. 2000, Minchinton and Bertness 2003). In South Africa however, the impact of grasses is often overlooked in reviews of the effects of invasive alien plants because of major problems currently experienced with woody plant invasions

(Van Wilgen and Richardson 1985, Holmes et al. 2000, Le Maitre et al. 2002, Milton 2004, Holmes et al. 2005, Richardson and Kluge 2008). As many grass species are tolerant of fire and respond to burning with rapid growth, it is important to identify the dominant grass species in any ecosystem for optimum fire and vegetation management (D'Antonio and Vitousek 1992b). Grasses are categorized into either C₃ or C₄ photosynthetic pathways with major differences between the two being in response to atmospheric CO₂ and temperature. C₄ grasses differ from C₃ grasses in their light responses (Ehleringer et al. 1997), water and nitrogen use efficiencies (Boryslawski and Bentley 1985). As a result, the C₃ photosynthetic pathway is most efficient where the growing season is cool and wet, while the C₄ photosynthetic pathway is more efficient where the growing season is warm and wet with a crossover temperature between the two between 20° and 30° C (Ode et al. 1980, Ehleringer et al. 1997). This means that C₃ grasses dominate areas where temperatures in the growing season are below 20°C and C₄ grasses dominate where temperatures are above 30°C (Fig.1.2). In South Africa this means that C₄ grasses dominate throughout the warm season rainfall region whereas C₃ grasses dominate in the cool season rainfall region and on the high mountains (Vogel et al. 1978), (Fig. 1.1). Relatively common grass genera in fynbos are the C₃ grasses *Pentaschistis*, *Merxmuellera* and *Ehrharta*, while C₄ grasses such as *Andropogon*, *Cynodon* and *Eragrostis* also occur (McDonald and Morley 1988, Linder 1990). Even though the mechanisms explaining the lower performance of C₄ plants under cold conditions have not been clarified, studies have shown that C₄ species are poorly competitive against C₃ plants in cold climates (Sage and McKown 2005).

With increased temperatures plants with a C₄ photosynthetic pathway can maintain high photosynthetic rates even when stomatal conductance is low (Rawson et al. 1977, Kocacinar et al. 2008, Osborne and Sack 2012). Photorespiration, oxygen instead of CO₂ assimilation during photosynthesis, is a temperature-dependent process (Lambers et al 1998). The quantum yield of C₃ plants for a given CO₂ level declines as temperatures increase reflecting the negative impact of photorespiration on carbon gain (Ehleringer and Björkman 1977). Combined with this, plants with C₃ photosynthetic pathways undergo a large trade-off in water loss for carbon gain as several hundred molecules of water are lost from the leaf for each CO₂ molecule taken up (Raschke 1979, Schulze and Hall 1982). In contrast, low stomatal conductance, and thus low transpiration rates, do not affect photosynthetic rate in C₄ plants (Ghannoum 2008). As a result, photosynthetic water use efficiency (PWUE) is often higher in C₄ plants than in C₃ plants, that is, more carbon is fixed per unit transpired water (Ghannoum 2008, Taylor et al. 2010). This advantage in WUE gives C₄

plants an advantage over C₃ grasses in low rainfall, high-temperature environments such as in the arid warm season rainfall grasslands.

The two photosynthetic pathways also differ in the ability to assimilate CO₂ (carboxylation) in that C₃ plants use Rubisco to fix CO₂ to RuBp to form a three-carbon sugar. However, this process is not very efficient as Rubisco fixes both CO₂ and Oxygen. C₄ plants avoid the inefficiencies of Rubisco in a two-step process, firstly with PEP carboxylase fixing CO₂ to PEP in the outer mesophyll cell, and the resulting malate or aspartate, a four-carbon sugar, being transported to an inner mesophyll cell (bundle sheath cell) where the CO₂ is released for assimilation using Rubisco. This process gives the C₄ photosynthesis pathway significant advantages over the C₃ mode under conditions of high light intensities, high temperatures and low atmospheric CO₂ concentrations (Edwards et al. 1971, Osmond and Bjorkman 1975, Ehleringer and Björkman 1977).

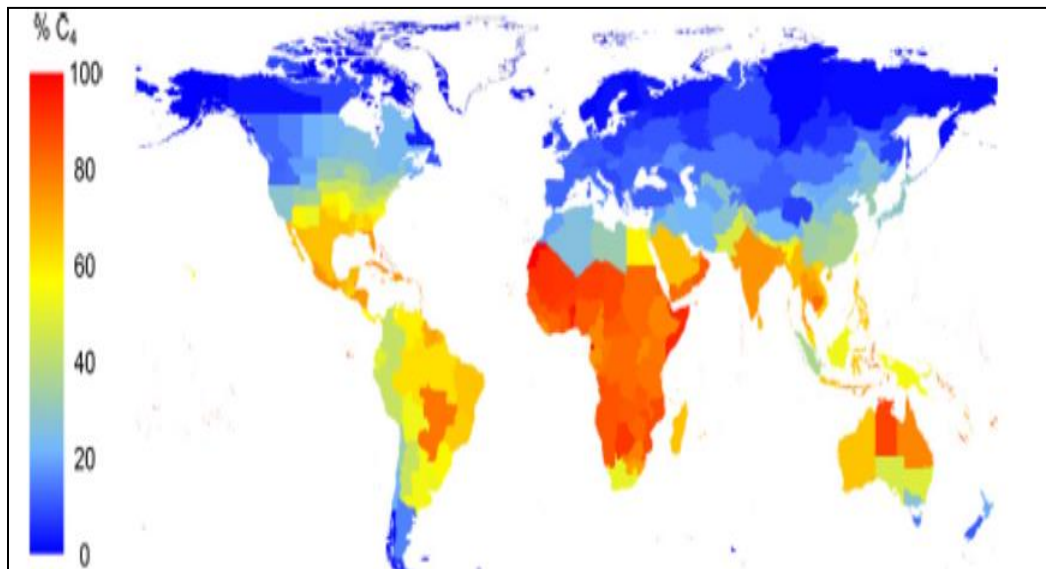


Figure 1.1: Global map of C₄ grass species distributions. The legend indicates the percentage of C₄ pathway grass species within each mapping unit (Osborne et al. 2014).

Atmospheric CO₂ levels have changed from ~180 ppm 20,000 years ago to 400 ppm today (Petit et al. 1999). This increase in CO₂ concentration will favour plants with the C₃ photosynthetic pathway as this would increase the efficiency of Rubisco (Ehleringer et al. 1997, Collatz et al. 1998, Ehleringer 2005). The accompanying increase in temperature related to an increase in CO₂ however, will tend to favour plants with a C₄ photosynthetic pathway (Wagner 1996, Christen and Matlack 2006, Tebaldi et al. 2006).

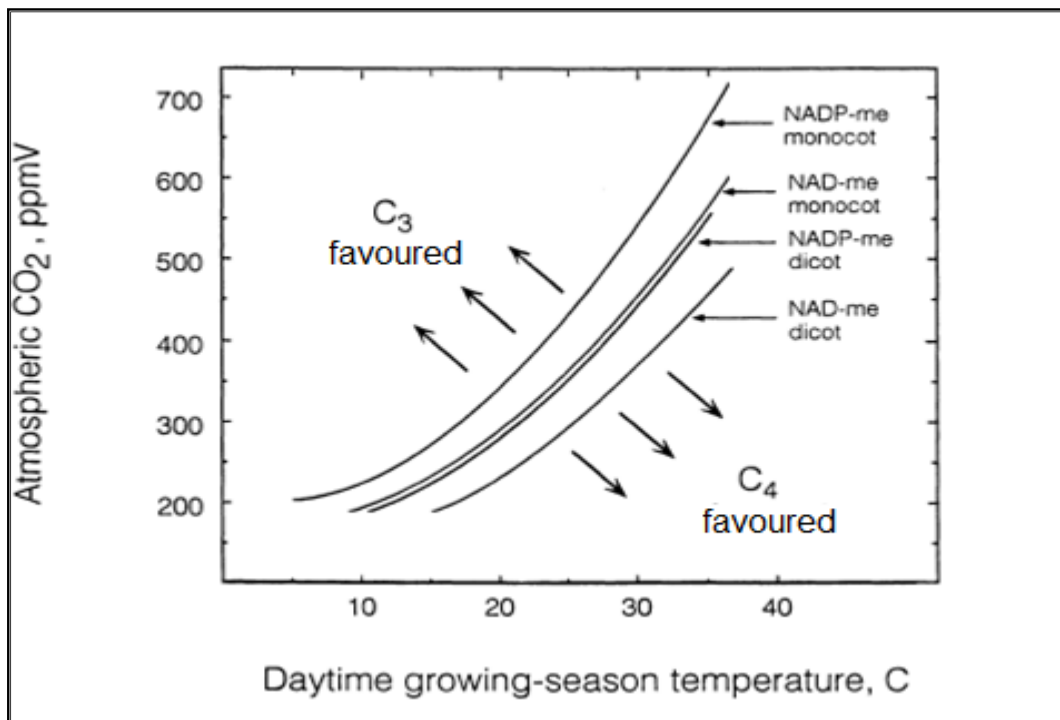


Figure: 1.2: Modelled crossover temperatures of the quantum yield for CO₂ uptake for monocots and dicots as a function of atmospheric CO₂ concentrations (from Ehleringer et al. 1997).

At Potberg in the De Hoop Nature Reserve in the Western Cape Province of South Africa grassy patches dominated by C₄ grasses are evident. This is despite the difference in physiology of the C₃ and C₄ grasses that influences their distribution as discussed above. The patches dominated by those C₄ grasses appear to exclude fynbos vegetation as there are few fynbos species within the plots relative to the surrounding fynbos-dominated areas. The aim of this study was to determine the potential drivers for the distribution of these C₄ grasses within this fynbos area and the relationships between C₄ grasses and fynbos diversity. Although the GCFR is characterised by a distinct cool, wet season and a hot, dry season (Cowling et al. 2005), several studies have demonstrated that global temperatures have increased between 1956 and 2005 by ± 0.13 °C per decade (Walther et al. 2002, Root et al. 2003). This increase in global temperature is also reflected in the GCFR where recent research has shown a significant increase in both maximum and minimum temperatures (Hoffman et al. 2011).

While there has been no trend either for a decrease or increase in rainfall in the last century a shift towards later precipitation into the warmer months has been demonstrated in South Africa (Hewitson and Crane 2006, MacKellar et al. 2007). With the availability of late rainfall and an increase in temperature it is possible for C₄ grasses to increase in dominance in the GCFR because, as discussed above, C₃ plants are at a disadvantage when temperatures increase. Possibility is that increasing atmospheric carbon dioxide has resulted in reduced stomatal conductance of fynbos plants, decreased water loss by transpiration and consequently increased soil moisture in the warmer months of the year (Morgan et al. 2004, Morgan et al. 2011, Higgins and Scheiter 2012).

Although climatic variables such as temperature and precipitation have been seen as the predominant determinants of C₃/C₄ grass distributions (Teeri and Stowe 1976, Epstein et al. 1997, Murphy and Bowman 2007, Fischer et al. 2008), various local environmental factors such as fire, herbivory and competition (Heckathorn et al. 1999) have not been incorporated into a single framework for predicting C₃/C₄ grass distributions despite the role they play (Pearson, 2005; Maestre *et al.*, 2009). C₄ grasses cure rapidly in the dry season changing both the fire regime (Bond 2008) and intensity of fires (D'Antonio and Vitousek 1992a). C₄ grasses also invest more carbon in below-ground biomass compartments that facilitate rapid re-growth after disturbances such as fire, allowing these grasses to outcompete the surrounding vegetation (Ripley et al. 2010). It is therefore extremely important to understand determinants of C₄ grass distribution in fynbos and how fire and other management tools might be used to influence their abundance.

To further understand C₃ - C₄ grass distribution and the potential consequences of C₄ grass movement into fynbos, the following research questions were explored: (1) Are C₄ grasses invading into fynbos? (2) If there is indeed an invasion what factors promote C₄ grasses within a generally fynbos-dominated landscape? (3) Do roadsides act as conduits for grass invasions? (4) What are the impacts of C₄ and C₃ grasses on fynbos species richness and diversity? My goal is to explore the interface between fynbos and the grassy biomes at the local scale and to stimulate interest in the potential impact of native invasive grasses on fynbos vegetation structure.

To address the issues identified above, the thesis is organised as follows:

Chapter 2: I provide a brief description of the study area including its location, vegetation, geology, soils and climate.

Chapter 3: Roadsides may act as corridors for the spread of invasive alien species into undisturbed ecosystems (Gelbard and Belnap 2003, Christen and Matlack 2006, Jodoin et al. 2008). I test the hypothesis that roadsides act as conduits for C₄ grass invasion.

Chapter 4: Potberg Reserve has several patches of C₄ grasses dominated by different species that include *Setaria sphacelata var torta*, *Eragrostis curvula* and *Imperata cylindrica*. In this chapter, I explore the effects of C₄ grass patches on fynbos species richness. The study helps indicate the potential effects of further invasion or expansion of existing C₄ grasses on fynbos communities.

Chapter 5: Here I explore factors influencing the distribution of the C₄ grass, *Setaria sphacelata var torta*. I do this using an experimental approach in which transplants of the grass were subjected to different treatments in the field including (1) with and without nutrient additions (2) different topographic positions with differing soil moisture status, and (3) trenched and not trenched treatments to test below-ground competition.

Chapter 6: I finally draw the thesis together in a synthesis and conclusions chapter.

Chapter 2

Study site

Location and topography

The study was conducted at De Hoop Nature Reserve (DHNR), in the Overberg region of the Western Cape Province, 47 km from Bredasdorp and 50 km from Cape Agulhas in South Africa (20°24'E, 34°27'S, Fig. 2.1). De Hoop Nature Reserve covers a land area of approximately 36000 ha. The reserve ranges in altitude from sea level to 611 m above sea level. The study area was situated in the western sector of the reserve known as Potberg.

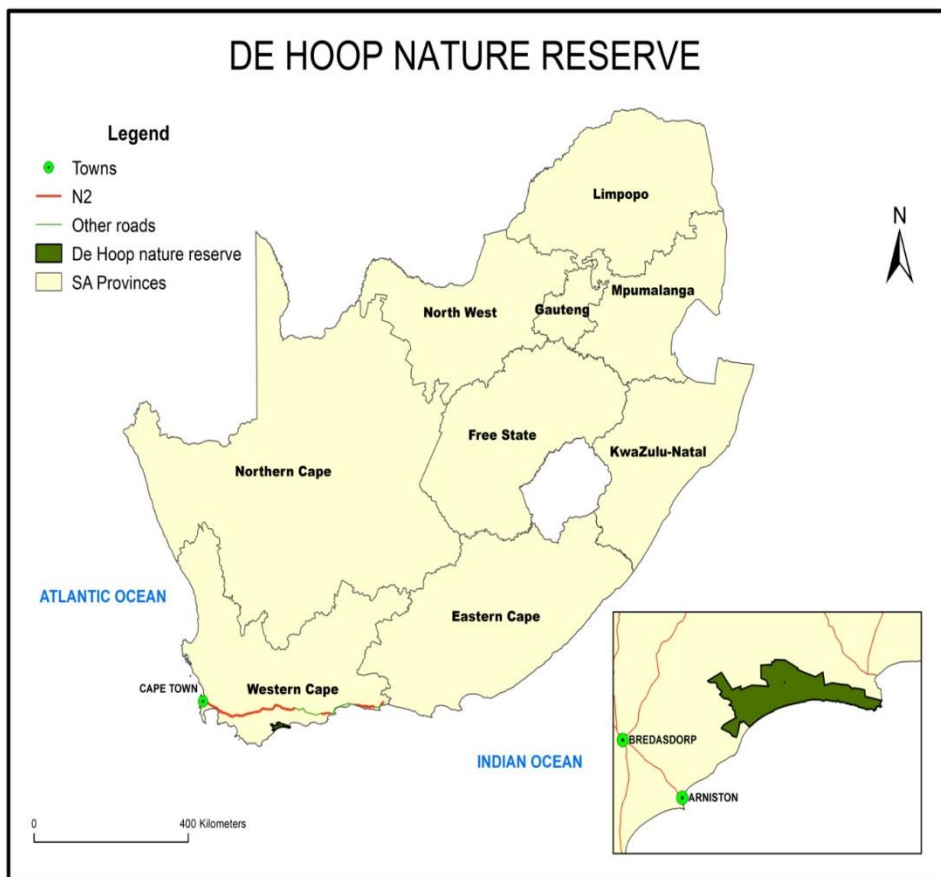


Figure 2.1: The location of the De Hoop Nature Reserve in South Africa.

Geology and soils

The geology of the reserve includes coastal limestone and aeolian sand, overlying shales of the Bokkeveld Group as well as sandstones and quartzites of the Table Mountain Group (Malan 1994). The Potberg area is dominated by dry, shallow loamy sand overlying a layer of ferricrete, silcrete or

sandstone of the Table Mountain Group, while shallow stony soils and sandy loams may occur on shales. A surface covering of rounded ferricrete stones, gravel and cobbles, locally called *Koffieklip* is considered to be the remains of an old African surface (Mucina and Rutherford 2006b). The soils over the eastern valley section of Potberg where much of the study was conducted are podsoles derived from aeolian sands. These soils are extremely infertile due to leaching of basic soil nutrients such as calcium, sodium, potassium and magnesium and are comprised of three distinct soil horizons: an A-horizon comprising of a dark, sandy top soil overlying a pale grey sandy E (= A2) horizon. Beneath this infertile horizon is a dark ferri-humic B-horizon, in which the leached nutrients of the A-horizon accumulate. Below the ferri-humic B-horizon are variously coloured (greyish brown, yellowish-brown) apedal sands and on lower slopes, seasonal waterlogging is not uncommon (Fey 2010b, Fey 2010a).

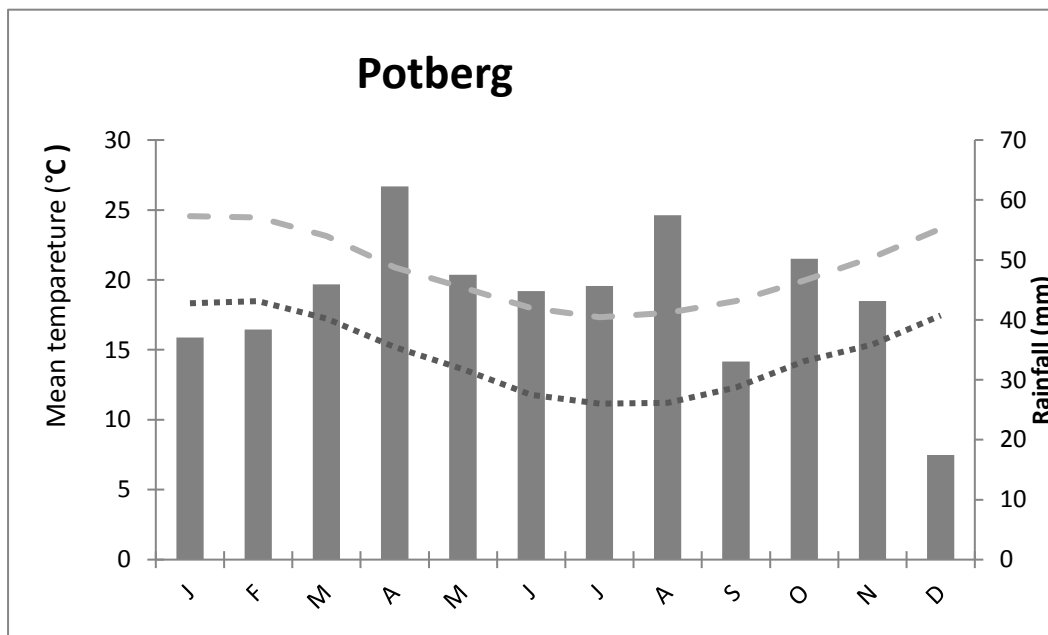


Figure 2.2: Average monthly rainfall (bars) and average maximum (--) and minimum (...) temperatures from 1960 to 2015 for Potberg at De Hoop Nature Reserve (Station No. 0000.3020 4; SAWS 2016).

Climate and Seasonality

Mean annual rainfall in the GCFR is under strong latitudinal control, decreasing towards the arid interior. The highest recorded rainfall is ~ 3000 mm per annum on the high mountains in the

southwest and the lowest rainfall is in Namaqualand in the northwest (Campbell et al. 1983). The mean annual precipitation for De Hoop Nature Reserve ranges from 230-730 mm, (mean 455mm) (Mucina and Rutherford 2006b). Unlike the more western GCFR the rainfall at Potberg is non-seasonal with slightly more rain in the cooler months than in the warmer months (Fig 2.2, Fig 2.3). The percentage rainfall for cooler and warmer months consecutively is 60.6% and 39.4%. July is the coldest month (mean Tmax = 17.3°C, mean Tmin = 11.15°C) and February the warmest month (mean Tmax = 24.4°C, mean Tmin = 18.4°C). The Potberg area experiences occasional frost on 10 to 20 days of the year. The area experiences strong south-easterly winds, regularly reaching speeds of 60 km/h or more (Cape Nature State of biodiversity report, 2012).

Has rainfall changed in amount or seasonality?

One of the most important factors determining dominant growth form distribution in South Africa is rainfall seasonality (Ehleringer 1978). The south-west coast, namely the Southern Overberg region, can also be thought of as a transitional region as it receives rainfall all year round (Mustart *et al.* 1997). Since C₄ grasses dominate in areas with rainfall in the warmer months, increases in the amount of soil moisture available in the warm season may have promoted their colonisation. To determine whether shifts in rainfall amount or seasonality, or both, had occurred, I obtained rainfall data from 1910 to 2016 from Zeekoevlei farm station which is located 14 km north of Bredasdorp (34.66015 S, 20.040635 E). This is the longest rainfall record available for the area (SAEON, Fynbos node, pers. comm. Margaret Koopman, Fig. 2.3). Foley's drought index is a technique that depicts the aggregate amount and duration of water surplus or deficit. Foley's approach normalizes each anomaly with respect to the annual average rainfall and is calculated as actual annual rainfall less mean annual rainfall divided by the mean annual rainfall. I used Foley's drought index to calculate 2 and 3 year time lag (Fensham et al. 2009) prior to and including each month (i.e. for September 1970 the running total is calculated from October 1967 to September 1970) (Foley 1957). The trends in rainfall were calculated through dividing the monthly cumulative rainfall by mean annual rainfall and dividing overall by mean annual rainfall. My results show multi-year dry periods alternating with wet periods and no trend of increasing rainfall from the early 1970's. (Fig 2.4)

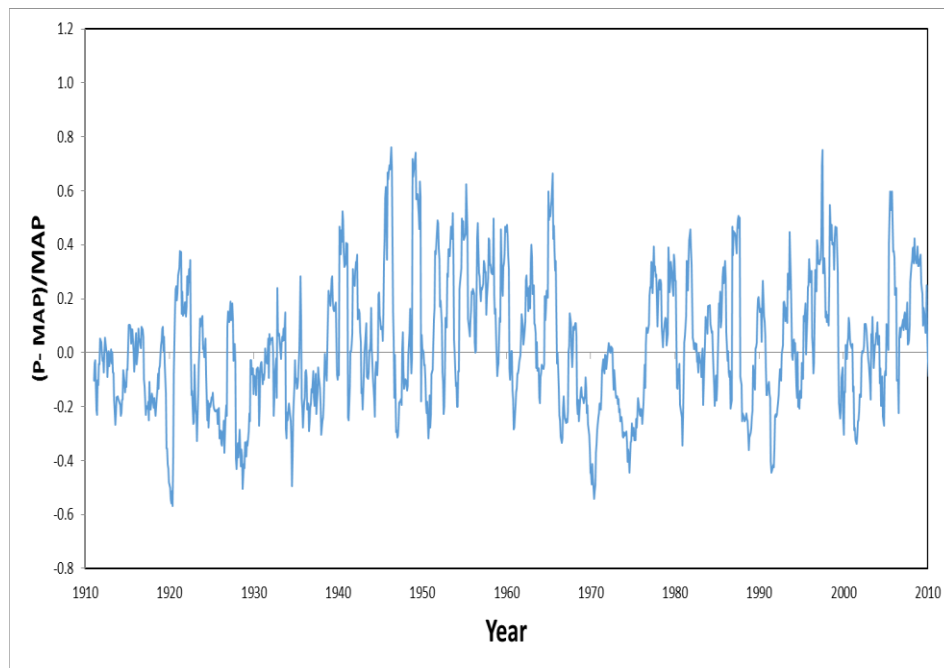


Figure 2.3: Trends in annual rainfall at Bredasdorp from 1950 to 2010 (Fensham et al. 2009).

P = 12-month cumulative rainfall and MAP = mean annual rainfall.

To explore trends in seasonality, I analysed the rain falling in the middle of the dry season (October, November, and December) as a proportion of the total rainfall over the record period. I also analysed rain falling in the beginning of the dry season (September, October, November) as a proportion of total rainfall for the hydrological year (July to June). C_4 grasses at Potberg are dormant in the cold wet season and only green up and begin to grow from late September or early October at the end of the wet season when temperatures begin to increase (personal observation). Thus, colonisation into fynbos may have been favoured by a shift to more rain in these months.

My results show that there is no increase in rainfall in the warmer months of the year since the 1970s (Fig 2.5 and Fig 2.6) suggesting that the increase in C_4 grass at Potberg cannot be attributed to changing rainfall amount or seasonality over the last century. Rather than an increase in rainfall in the warmer months, my drought index analysis has shown an increase in drought index from 1.2 to 1.5 between 1967 and 2017. Colonisation by C_4 grass patches may however have been due to factors such as disturbance of fynbos from land use practices and/or change in soil moisture regimes due to elevated CO_2 levels. High water use efficiency of plants growing at elevated CO_2 can alter the soil moisture regime (e.g. Morgan et al. 2004) and this mechanism may have acted to

increase water available in the early warm season favouring invading C₄ grasses over the last century (Masubelele et al. 2015). There is considerable practical interest in the interaction between soil moisture status, forage supply and increases in atmospheric CO₂ content as both theory and empirical data suggest that elevated CO₂ will result in more efficient use of water by plants (Eamus 1991, Morison 1993, Tyree and Alexander 1993). Nevertheless, the magnitude of the effect that elevated CO₂ may have on moisture availability in fynbos is unknown.

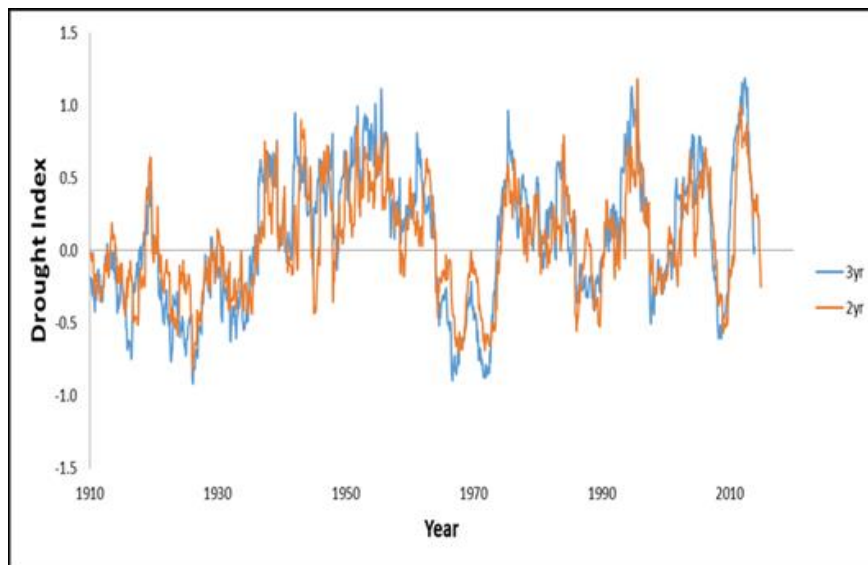


Figure 2.4: Foley's drought index for Bredasdorp for a 2 (orange line) and 3 (blue line) year time lag from 1950 to 2016.

Vegetation

The Potberg area has a complex vegetation system comprising several different vegetation types. According to Mucina and Rutherford (2006b), the vegetation type in the vicinity of the study area is a mixture of Agulhas Sand Fynbos and Albertinia Sand Fynbos. This vegetation is threatened by alien invasive trees such as *Acacia cyclops* and *Acacia saligna*. The shrubs include *Protea repens*, *Protea neriifolia*, *Erica quadrangularis*, *Leucadendron modestum*, *Leucadendron laureolum*, *Chrysanthemoides monilifera*, *Erica imbricata*, *Leucadendron salignum* and *Phyllica pupurea*. Restios include *Ischyrolepis capensis*, *Staberoha banksii*, *Thamnochortus spicigerus* and *Hypodiscus aristatus*. The grasses in the area include both C₃ and C₄ grasses. The C₄ grasses include *Aristida diffusa*, *Aristida junciformis*, *Andropogon eucomis*, *Cynodon dactylon*, *Eragrostis curvula*, *Imperata cylindrica*, *Setaria pallide fusca* and *Themeda triandra*, while the C₃ grasses

include *Pentameris scandens*, *Pentameris airoides*, *Pseudopentameris brachyphylla* and *Tribolium uniole*.

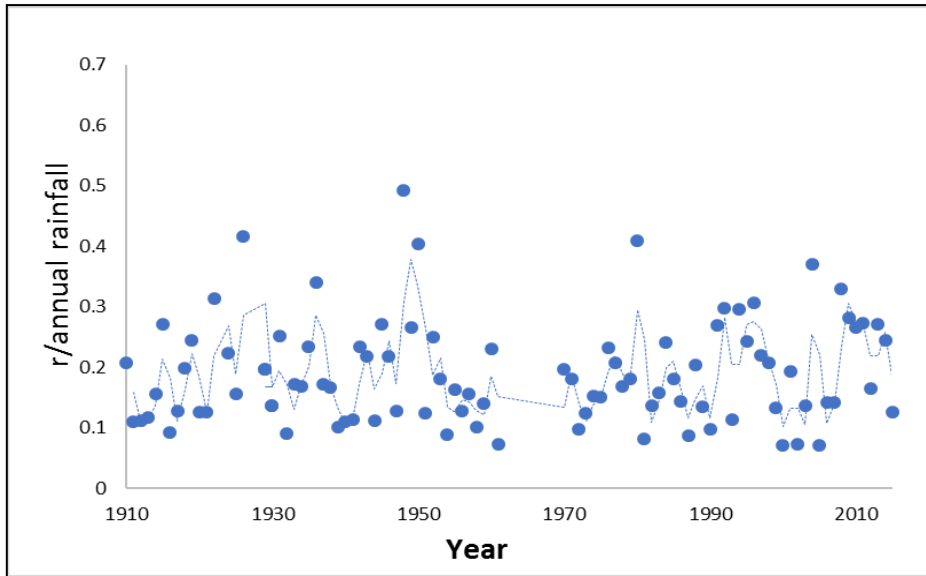


Figure 2.5: Proportion of annual rainfall in the middle of the dry season (October, November and December) from Zeekoevlei farm 14 km north of Potberg. ($r/\text{annual rainfall}$ is the dry season rainfall (r) divided by the annual rainfall).

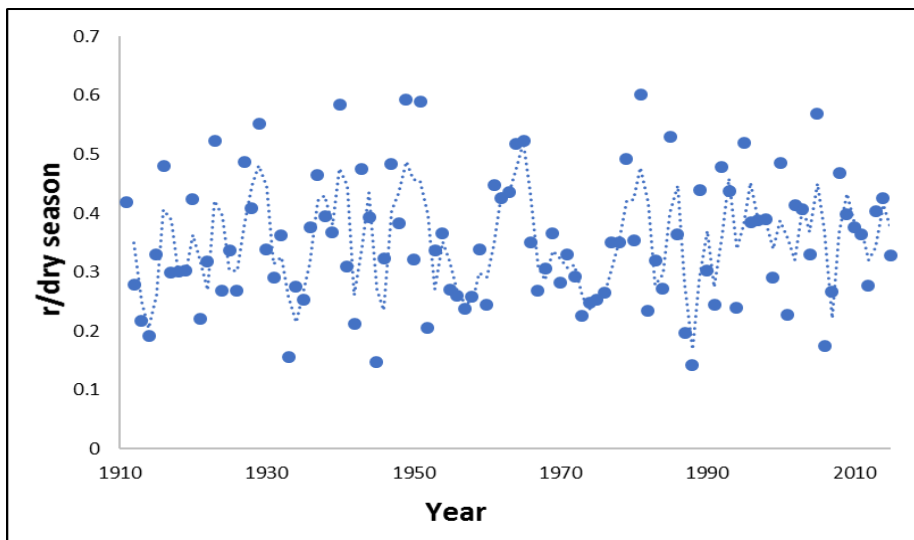


Figure 2.6: Proportion of annual rainfall at the beginning of the dry season (September, October and November), from Zeekoevlei farm 14 km north of Potberg. ($r/\text{dry season}$ is dry season rainfall (r) divided by the annual rainfall (Sept-Feb))

Fauna

The mammals in the reserve include Chacma baboons (*Papio cynocephalus ursinus*), Cape mountain zebra (*Equus zebra zebra*), eland (*Taurotragus oryx*), Honey badger (*Mellivora capensis*), African wild cat (*Felis silvestris lybica*) and Bontebok (*Damaliscus pygargus*) along with the largest and the oldest known Cape vulture (*Gyps coprotheres*) colony in Southern Africa (Cape Nature State of biodiversity reports 2012).

Land Use History

The C₄ grass patches at Potberg may be evidence of a Savanna/Grassland biome westward advancement into the Fynbos biome, potentially because of climate change or altered land use practices in the area. Biome shifts have been attributed to various factors such as differing physiological constraints to changing climate (Reu et al. 2011) loss of biodiversity, shifts in rainfall regimes (Huang et al. 2001), CO₂ elevation (Higgins and Scheiter 2012) and historical land use practices (Masubelele et al. 2014). In South Africa, extensive work has been done on biome shifts in the form of bush encroachment into the Savanna/Grassland biomes (Wigley et al. 2009, Buitenwerf et al. 2012), however very little work has been done on changing boundaries of the fynbos biome. The C₄ grass patches at Potberg might represent ancient outliers of the eastern grasslands in western shrublands or new colonisation as grassy biomes move westwards into the fynbos biome.

The results of carbon isotope analysis of these C₄ grass patches at Potberg have shown that this is not an old boundary between fynbos and grasslands but a recent one and that prior to this invasion C₃ species were the dominant vegetation in the area (Tucker 2016). The C₄ grass patches have not been present long enough to shift the soil organic matter isotope ratios to fall within the C₄ range (Fig 2.7, Tucker 2016). Several dense patches of C₄ dominated grasslands support old senescing proteas (Fig 2.8) that are >35 years old, based on node counts and the management fire report (De Hoop fire report 2017). It is highly unlikely that these proteas would have emerged in the presence of the current dense grass layers. It would therefore seem likely that the grass patches colonised Potberg after the 1950s or possibly even later (1970s from protea ages).

The C₄ grass patches may have occurred only recently with farming practices that may have induced disturbance and changed the fynbos structure allowing C₄ grasses to colonise. Between the years 1956-1969 one of the management objectives was protecting and raising antelope in fenced off areas that are still visible today (Tucker 2016, (Fig 2.9). Grass colonisation may have been

promoted through heavy disturbances such as trampling and overgrazing by heavy livestock densities in these fenced areas (Tucker 2016).

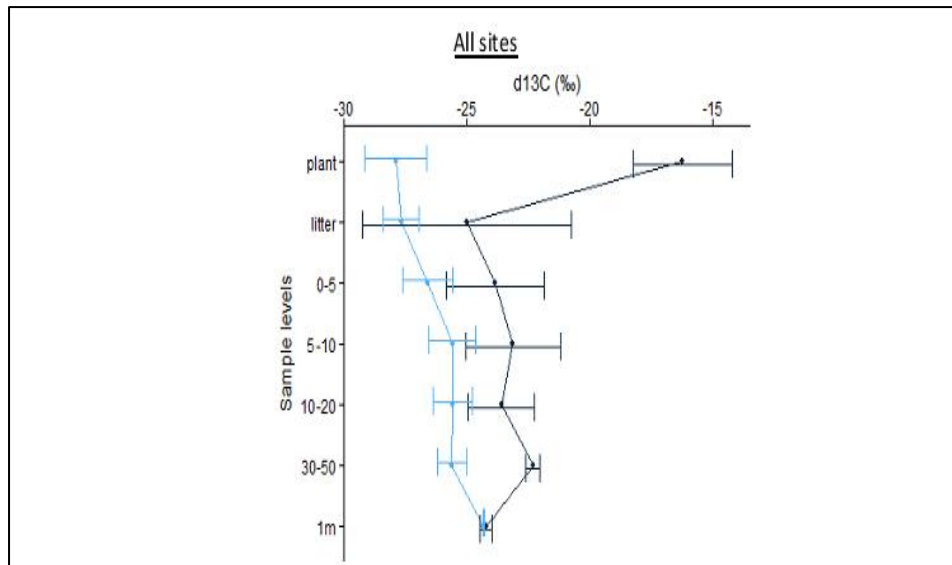


Figure 2.7: $\delta^{13}C$ values for samples of soil carbon in fynbos and adjacent grassland. Light blue lines indicate fynbos sites while dark blue lines indicate C_4 grass (Tucker 2016).



Figure 2.8: Senescent fynbos at the study site

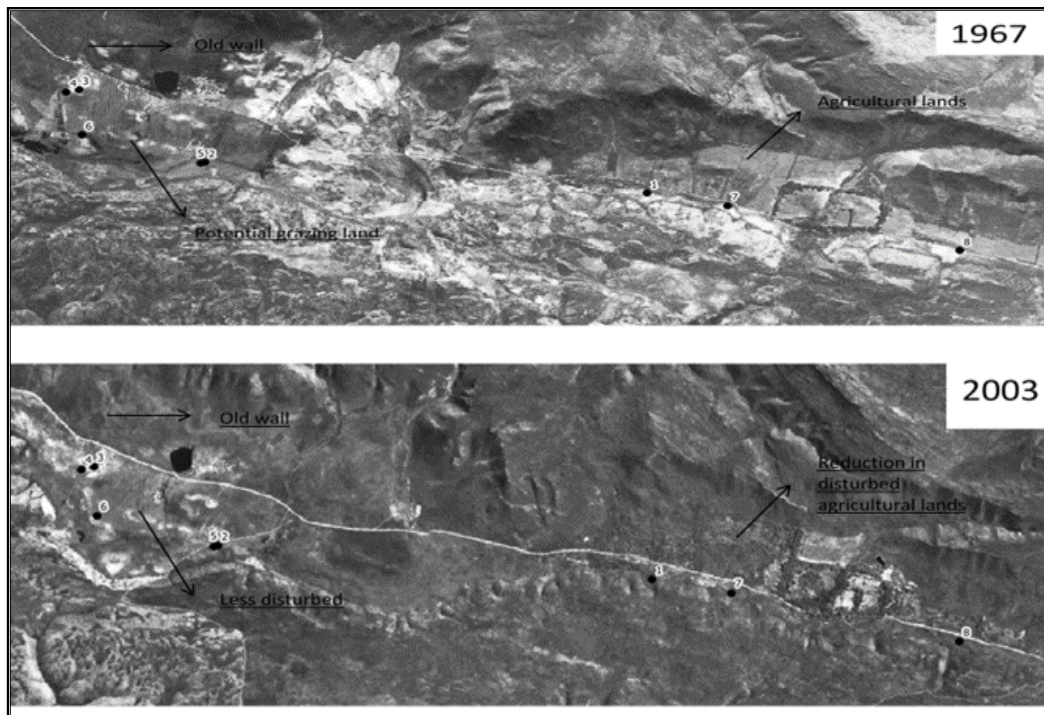


Figure 2.9: Repeat aerial photographs from 1967 and 2003. A reduction in disturbed agricultural land can be seen in the surrounding area when comparing the 1967 photograph with the 2003 photograph (Tucker 2016).

Summary

This study in the Potberg area was in a typical non-seasonal rainfall zone with fynbos as the dominant vegetation type. Warm season rainfall grasslands dominate vegetation to the north (<30° South) and east, hundreds of kilometres from my study site. The grass patches are not ancient but appear to have developed in the latter part of the last century. There are no obvious rainfall trends in the last century accounting for the apparent biome shift to warm season rainfall grasslands. The grasses occur on very low nutrient podsols but nutrient addition, coupled with trampling disturbance, may have promoted grass invasion when the area had high stocking densities of mammal herbivores. In the next chapter I determine the extent to which roads may have acted as conduits for these grasses to invade.

Chapter 3

Roadsides as conduits for C₄ grass invasion

Introduction

There is a strong positive relationship between C₄ grass abundance and growing season temperature with C₄ grasses dominant in parts of South Africa where rainfall is in the warmer months, and C₃ vegetation dominant where rainfall is in the cooler months (Teeri and Stowe 1976, Vogel et al. 1978, Hattersley 1983, Vogel et al. 1986). While some C₄ grasses occur in fynbos, the dominant plants such as the Ericaceae, Proteaceae and Restionaceae are C₃ as are grasses endemic to fynbos (e.g. *Ehrharta*, *Pentaschistis* and *Pentameris* (Mucina and Rutherford 2006b). Despite this general pattern, there is a high proportion of C₄ grasses growing among fynbos at Potberg (pers obs). Roads are considered as one of the anthropogenic corridors that may facilitate the spread of species beyond their native habitat and geographical boundaries (Forman and Alexander 1998, Gelbard and Belnap 2003, Christen and Matlack 2006, Benezeth et al. 2008). Vehicles and road-fill operations disperse the seeds of invading species into pristine areas (Trombulak and Frissell 2000). A decline in exotic species with distance from the road has been observed in the USA; in Glacier National Park, Montana (Christen and Matlack 2006), and in Putah and Cache Creek watersheds (Frenkel 1970, Gelbard and Harrison 2003) and, on the portion of the Los Angeles aqueduct system that cuts through the Mojave Desert mainly within Kern County (Johnson et al. 1975).

Also in Glacier National Park, a study by (Tyser and Worley 1992) found twice as many exotic species on roadside edges relative to 25 m from the roads in grassland. Several mechanisms have been proposed as explanations for these patterns including vehicle compaction and road-fill (Lonsdale and Lane 1994, Trombulak and Frissell 2000), vegetation clearing, top soil removal (Frenkel 1970, Trombulak and Frissell 2000) and mowing of the roadside vegetation (Forman and Alexander 1998, Benefield et al. 1999). Environmental conditions along road verges differ from those of the surrounding communities in availability of light, water, temperature and space (Greenberg et al. 1997, Trombulak and Frissell 2000, Foley et al. 2005, Mortensen et al. 2009). In the southern Karoo at Prince Albert in South Africa, runoff from roads increases the availability of water and nutrients on road verges, altering plant performance (O'farrell and Milton 2006). This increase in soil moisture and nutrients along roads can aid C₄ invasion through supplying moisture at the hottest and driest time of the year which is also the growing season for C₄ grasses. The higher temperatures of the roads may also favour C₄ grasses as these have evolved in high-temperature environments (Forman 2003, Karim and Mallik 2008). I therefore hypothesize that the high

frequency of disturbance along road verges combined with higher temperatures and an increase in nutrients and water allows the seedlings of C₄ grasses to become established along road verges in the Potberg Reserve (Frenkel 1970, Trombulak and Frissell 2000). This establishment reduces the vigour of native fynbos species allowing the more aggressive C₄ grasses to outcompete native species (Black et al. 1969, Bond 2008).

In this chapter, I determine if road verges may be facilitating the movement of C₄ grasses into fynbos at Potberg and the extent to which these grasses are spreading from the roadside into the surrounding fynbos. Although roads have been identified as a conduit for invasion by exotic plants, (Forman and Alexander 1998) there is very little understanding of the movement of invasive species away from road verges. I approached the problem by conducting a survey along a road in the Potberg Reserve. I predicted that C₄ grass species richness would decrease with increasing distance from the road verge and that the percentage cover of C₄ grasses would be higher in roadside plots rather than the plots in fynbos.

Methods

Study sites

I sampled a total of 100 plots along a 12.5 km section of the main management road in the Potberg Reserve (S 34.3748005; E 20.529611) to (S 3425100; E 2037440). While the vegetation in the reserve is primarily fynbos there are several patches of invasive tree and grass species. Invasive tree species include *Acacia cyclops*, *Acacia pycnantha*, *Acacia mearnsii* and *Acacia saligna*, while the invasive grasses *Cortaderia selloana* and *Pennisetum clandestinum* are also present.

Methodology

To determine the influence of the road on the spread of C₄ grasses into the reserve I established a series of 5 X 10 m plots located 5 m in from the very edge of the road (the Roadside plots) on both sides of the road. I also established a series of 10 X 10 m plots perpendicular to the road and 100 m from the edge of, and parallel to, the Roadside plots (the Adjacent plots) on both sides of the road. I did this at 500 m fixed intervals on both sides of the road for the entire length (12.5 km) of the road. A total of one hundred plots were sampled of which 50 were Roadside plots and 50 Adjacent plots (Fig. 3.1, Fig. 3.2). On each plot, I identified all grasses to species level. I also made a visual estimate of the percentage canopy cover of the grass relative to the fynbos (Mueller-Dombois and Ellenberg 1974). The fynbos and grasses were classified into six separate classes for percentage canopy cover 0 - 5, 5- 25, 25-50, 50-75, 75-95, 95-100 % (Daubenmire 1959). This was made

under the assumption that grass and fynbos species in the plots combined to make up 100% canopy cover. Each plot was georeferenced using a Garmin eTrex 10 GPS (Garmin, United States of America).

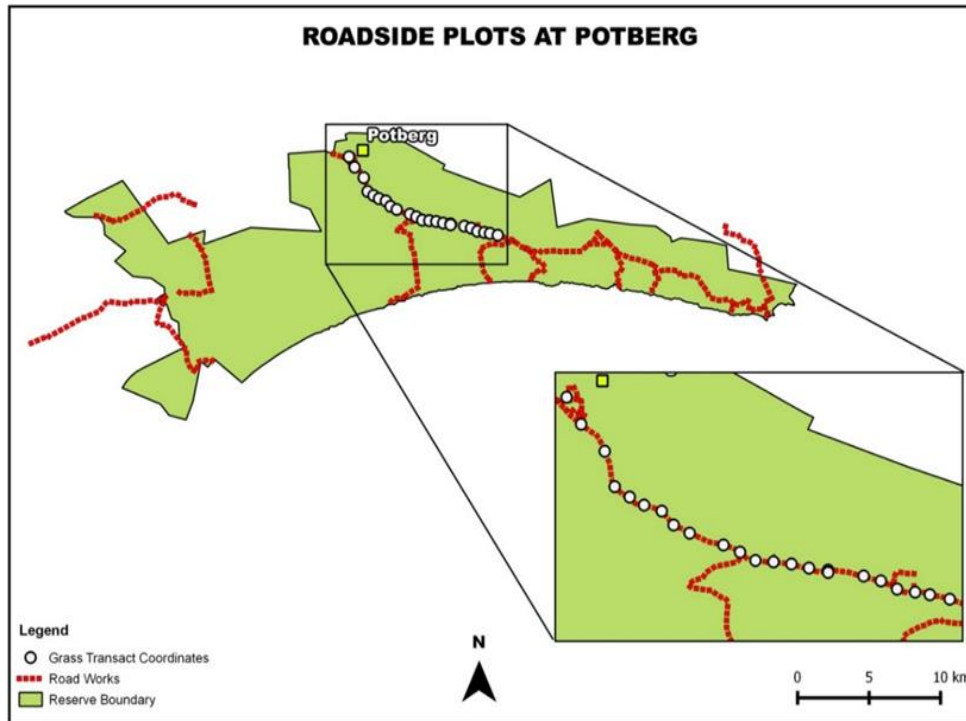


Figure 3.1: Site map, showing the location of the plots alongside the management road in the Potberg Reserve.

Data Analysis

The statistical analysis conducted includes both parametric and non-parametric tests. Chi-squared tests and T-tests were performed to determine if there were any significant differences in the distribution of C₃ and C₄ grasses on Roadside plots and Adjacent plots. A T-test was performed to determine if there was any significance difference in the percentage cover of fynbos and grass between the Roadside and Adjacent plots. I also used a T-test to determine the differences in relative distribution of fynbos and grass in Adjacent and Roadside plots. I used Venn diagrams to illustrate the relationship between the C₃ and C₄ grasses at the Adjacent and Roadside plots. These were created based on whether a certain grass type was observed at a plot. Visual and quantitative summaries of the data are presented in Tables and Figures. The data was organised in excel and all

tests on the data were assessed in R© v3.3.1 (R Core Development Team 2016) and a value of $p < 0.05$ was required for significance.

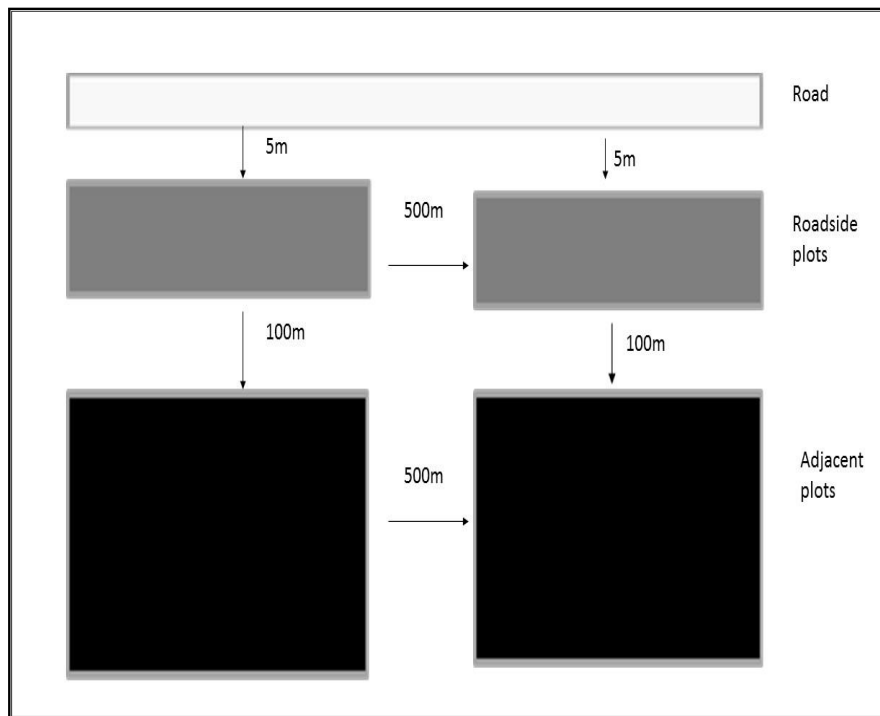


Figure 3.2: The Relative distribution of the Roadside and Adjacent plots along the management road in Potberg Reserve.

Results

Dominant and least dominant C₃ and C₄ species.

The dominant grass species on the Roadside plots were *Cynodon dactylon* (24%), *Eragrostis curvula* (21%) and *Tribolium uniolae* (17%). The first two of these are C₄ while the last one is C₃. The dominant grass species on the adjacent plots were *Pennisetum thunbergii*, *Aristida diffusa*, *Themeda triandra*, *Merxmuellera disticha*, *Tribolium uniolae* and *Cynodon dactylon*. Of the six species, three species were C₄ and three were C₃ (Fig.3.3).

In the Adjacent plots, *Merxmuellera disticha* was the most common C₃ grass, present in five of the 50 plots (10%) The least frequent C₃ grass *Pentameris pallida* was found at only two of the Adjacent plots (4%). On the Roadside plots, the most common C₃ grasses were *Merxmuellera disticha* and *Tribolium uniolae* present in 16% and 38% of the plots respectively. The most common C₄ grasses were *Cynodon dactylon* on 52% of the plots and *Eragrostis curvula* on 44%.

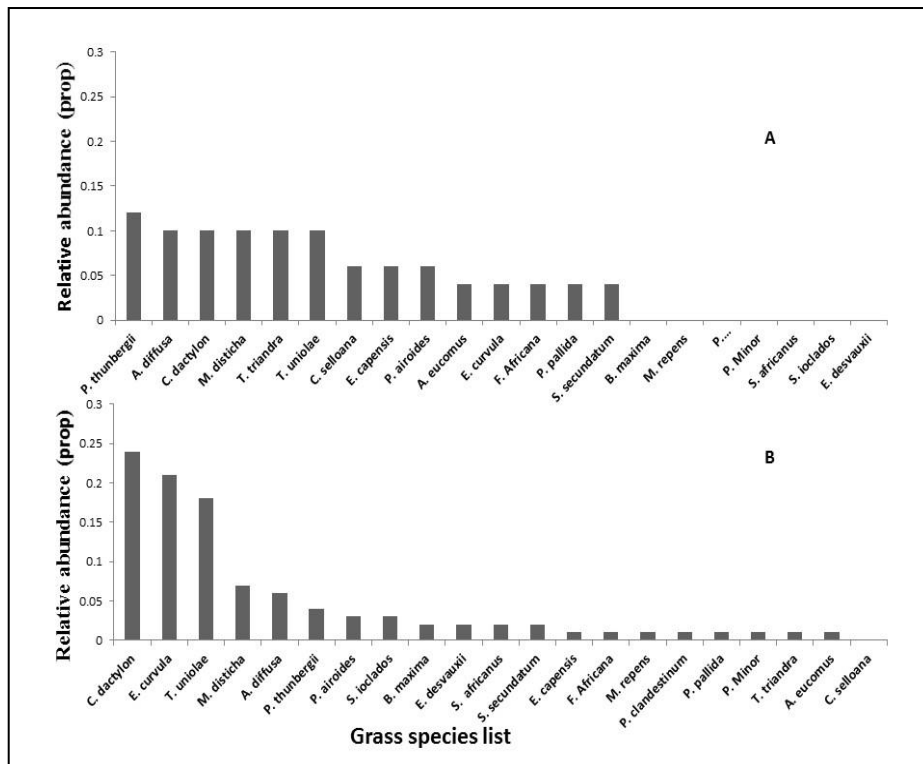


Figure 3.3: The ranked frequency of grass species in the Adjacent (A) and the Roadside (B) plots showing the significantly higher ($P < 0.001$) frequency of grasses in the Roadside plots. $N=50$ quadrats for each graph.

The relative distribution of C₃ and C₄ grasses in Adjacent and Roadside plots.

There were more grass species on the Roadside plots (20 species) than in the Adjacent plots (14 species). Thirteen (13 species) of the grasses in the Roadside plots and eight (8 species) on the Adjacent plots were C₄ species. Both Roadside and Adjacent plots had more C₄ grass species than C₃ grasses.

The frequency distribution of both C₃ and C₄ grass species was significantly higher on the Roadside plots than on the Adjacent plots ($P = 0.004$; T Statistic 7.18,3 degrees of freedom). There was however no significant difference in the relative proportion of C₃ and C₄ grass species in the Adjacent plots.

The results of a Chi square test show that there was a significant increase in C₃ grasses in the adjacent plots relative to the roadside plots ($P < 0.001$ Test Statistic 6.18, 2 degrees of freedom) and there was a significant difference in the relative abundance of C₄ grass species between roadside

and adjacent plots ($P = 0.0001$; Test Statistic 7.18,3 degrees of freedom). Twelve percent of the Roadside plots had only C_3 grass species, 42% had only C_4 grass species while 40% of the plots had both photosynthetic types co-occurring. In the Adjacent plots, C_3 grass species were exclusively observed on 18% of the plots, C_4 grass species on 34%, while both photosynthetic types coexisted on 14% of the plots. Generally, C_4 grasses were more abundant than C_3 grasses in both Adjacent and Roadside plots (Fig 3.4).

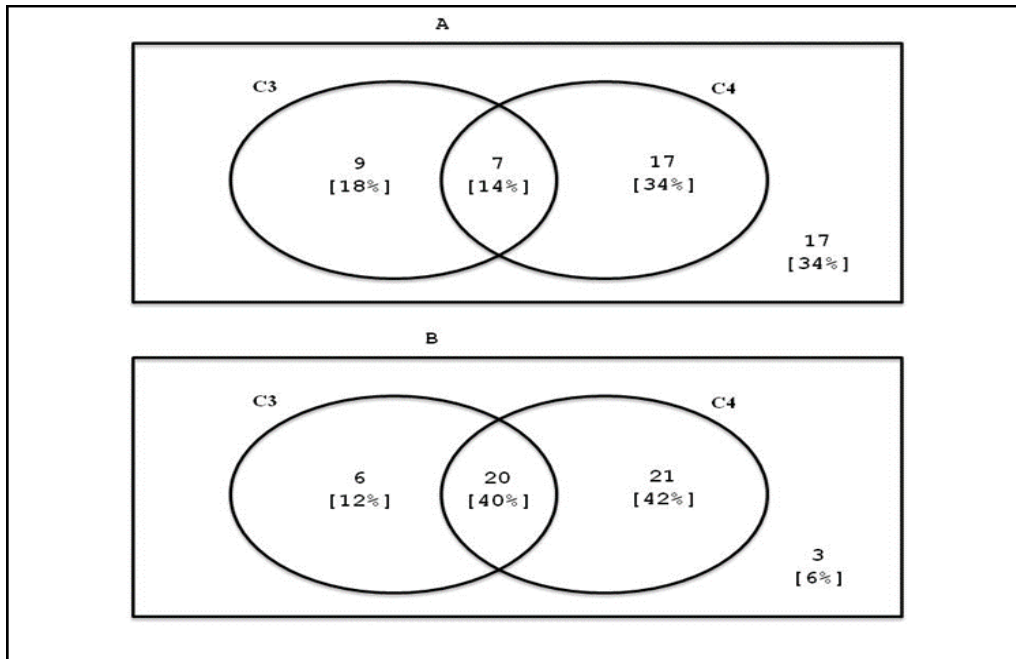


Figure 3.4: Venn diagrams illustrating the frequency distribution of C_3 grass and C_4 grasses in both the Adjacent (A) and Roadside (B) plots. Values outside the circles are number of plots with no grasses. Values in brackets are % of total plots in each habitat type.

Fynbos and grass percentage cover estimates in Roadside and Adjacent plots.

On the Adjacent plots fynbos had between 42% to 69% higher percentage canopy cover than on Roadside plots ($P < 0.05$). The Roadside plots however, had between 43% to 66% higher grass canopy cover than the Adjacent plots. The relative proportion of fynbos was higher on the Adjacent plots compared to Roadside ($P < 0.05$) (Fig.3.5).

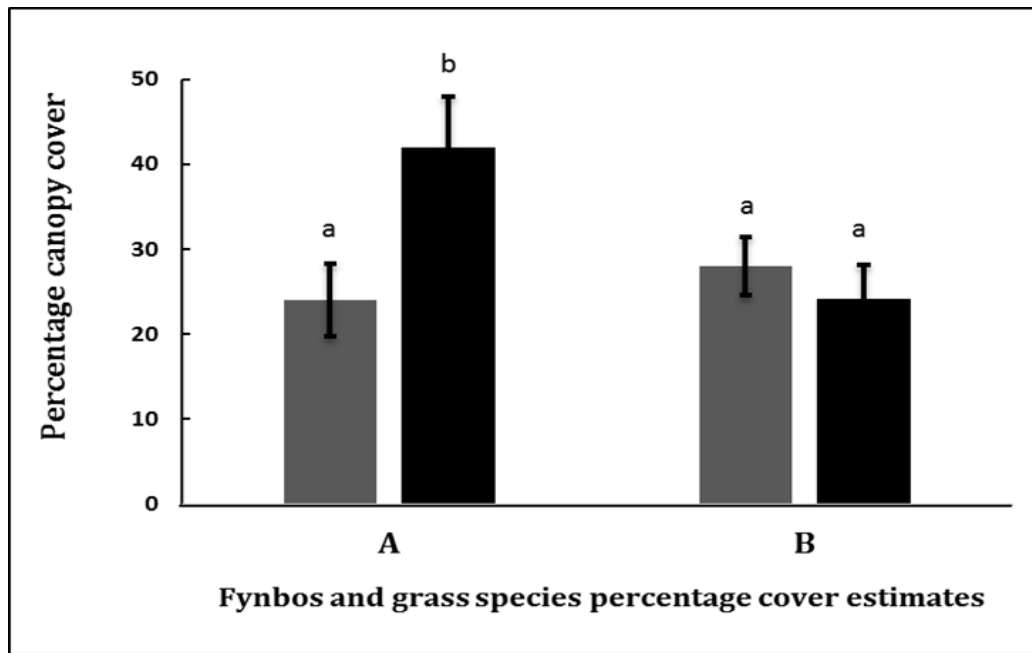


Figure 3.5: Percentage canopy cover of fynbos (light bars) and grass (dark bars) in Adjacent (A) and Roadside (B) plot. Bars are means and lines standard error. Different letters indicate significant differences at $p < 0.05$.

Discussion

The primary objective of this chapter was to determine the extent to which roads may be acting as conduits for the distribution of C_4 grasses into fynbos. My results show that there is a greater diversity of grass species in the Roadside plots with a great degree of species evenness in the Adjacent plots. On the Roadside plots 64 % of the plots were dominated by three species, the two C_4 species *Cynodon dactylon* and *Eragrostis curvula* and one C_3 species *Tribolium uniolae*. In the Adjacent plots 62 % of the plots were dominated by six species of which three were C_4 species *Aristida diffusa*, *Cynodon dactylon* and *Themeda triandra*, and three C_3 species *Pentameris thunbergii*, *Tribolium uniolae* and *Merxmuellera disticha*.

With two C_4 grasses dominating on the Roadside plots these results agree with my prediction that roads would be acting as a conduit for C_4 grasses to invade into fynbos. These results do not only show that roads are acting as a conduit for primarily C_4 grasses to colonise into fynbos but that roads are a conduit for grasses in general to invade into fynbos as there was higher grass species diversity in the Roadside plots relative to the Adjacent plots. All the dominant grasses on both series of plots are distributed widely throughout South Africa and could naturally occur at Potberg (Van Oudtshoorn 1992). The three dominant grasses on the Roadside plots however are all

associated with growing in disturbed areas such as in roadside reserves or overgrazed and trampled veld. Of the six grasses on the Adjacent plots, three species *Cynodon dactylon*, *Aristida diffusa* and *Tribolium uniolae*, prefer growing in disturbed areas while the remaining three grass species *Pennisetum thunbergii*, *Themeda triandra* and *Merxmuellera disticha* prefer undisturbed areas (Van Oudtshoorn 1992).

One possible mechanism to explain the high density of C₄ grass species along the roads is that the conditions on the Roadside plots such as disturbance, high light, water availability and increased nutrients may favour C₄ grass over C₃ grasses (Tyser and Worley 1992, Parendes and Jones 2000, Trombulak and Frissell 2000, Gelbard and Belnap 2003). In this regard, the two dominant grasses on the Roadside plots *Cynodon dactylon* and *Aristida diffusa* are primarily found in disturbed areas with *Cynodon dactylon* preferring both disturbance and high nutrient soils (Van Oudtshoorn 1992). While the wet season at Potberg may be in the cooler part of the year the increased radiation from the road would result in higher temperatures than on the Adjacent plots. Several studies have also shown that there is an increase in moisture along road verges (Johnson et al. 1975, Holzapfel and Schmidt 1990, Trombulak and Frissell 2000). The high temperature along the road verge combined with the increased runoff supplying moisture at the beginning of the dry season and even well into the dry season would favour C₄ grasses primarily because C₄ grasses have evolved in high-temperature regions with moisture during the hottest time of the year (Forman 2003, Karim and Mallik 2008). All the dominant C₄ grasses on the Roadside plots are long-lived perennials that can survive repeated disturbance such as fires or grazing because of stored resources in rhizomes and roots suggesting that the runoff from the road is sufficient to sustain these grasses during the dry season (Gautam et al. 2014) .

As I predicted fynbos percentage cover was high on the Adjacent plots relative to the Roadside plots while grass percentage cover was higher on the Roadside plots. Invasion by the C₄ grass *Cenchrus ciliaris* in Australia, the USA, Mexico and South America, has altered wildfire regimes, soil erosion rates, ground surface temperatures and supply of vital resources to surrounding life forms, compromising biodiversity (D'Antonio and Vitousek 1992a). Several studies have demonstrated a negative relationship between *Cenchrus ciliaris* occurrence and general species richness: in the southern Sonoran desert (Bestelmeyer and Schooley 1999), in Argentina (Blanco et al. 2005), in Central Queensland, Australia (Fairfax and Fensham 2000), in the plains of the Sonoran Desert in Mexico (Franklin et al. 2006) and on Dalrymple Shire, north-eastern Queensland, Australia (Jackson 2005). There are several ways in which grasses may impact the

diversity of fynbos species; this includes, decreasing the availability of light for seedlings and re-sprouts (Witkowski 1991), competition for nutrients (Pierce and Cowling 1984), increased fire intensity through increased litter from dead leaves (Van Wilgen and Viviers 1985) and alteration of nutrient cycles (D'Antonio and Vitousek 1992a).

In conclusion, my study shows that fynbos percentage cover was low on the Roadside plots relative to the Adjacent plots at my study site. These results also show that roads are acting as conduits for C₄ grass species and grasses in general to invade fynbos areas. Fynbos percentage cover on the Roadside plots is significantly lower than on the Adjacent plots, and I speculate that this is due to the high canopy cover of the grasses outcompeting fynbos seedlings for light and the more competitive below-ground root structure outcompeting the fynbos species for nutrients and water (Pierce and Cowling 1984, February et al. 2013). I suggest that roads should be considered important targets for both local and regional efforts to prevent and control grass invasions into fynbos (Gelbard and Belnap 2003). In Chapter Four I determine the impacts that these grasses are having on fynbos species richness, and species abundance.

Chapter 4

Impacts of C₄ grass on fynbos plant species richness and diversity.

Introduction

Alien invasive plants have been identified as the second most important threat to biodiversity after habitat loss (Foxcroft and Richardson 2003). Of these plants, grasses are particularly destructive ecosystem engineers, capable of changing the functioning of an ecosystem by impacting on both relative abundance and species diversity (Hobbs and Huenneke 1992). There are several ways in which grasses may impact species diversity, including decreasing the availability of light for seedlings and resprouts (Witkowski 1991), and by altering fire regimes and competition for nutrients and water (D'Antonio and Vitousek 1992b, Gaertner et al. 2014).

With an increase in plant canopy cover, the alteration of the quantity and the quality of light to understory plants may lead to a decline in species richness (Fenner 1989). Research in fynbos has shown that species richness is halved when an overstory shrub forms a dense canopy reducing the amount of light to the understory (Cowling and Gxaba 1990, Holmes and Cowling 1997). This would suggest that most fynbos species need light-stimulation for recruitment (Christensen and Muller 1975, Keeley 1987, Holmes and Cowling 1997).

With a return interval of between 10 to 30 years depending on rainfall and topography, fire is an essential driving force in fynbos dynamics, being both inevitable and crucial to maintain species diversity (Kruger and Bigalke 1984, Van Wilgen et al. 2010). Fynbos vegetation has evolved complex avoidance and tolerance mechanisms to survive fire (Kruger and Bigalke 1984), however the presence of C₄ grasses can alter both fire frequency and intensity (Rossiter-Rachor et al. 2009). Several studies have shown that invasion by the C₄ grasses *Andropogon gayanus* and *Cenchrus ciliaris* in Australia, the USA, Mexico and South America can drastically change an ecosystem by altering wildfire regimes, soil erosion rates, ground surface temperatures and supply of vital resources to surrounding life forms, thereby compromising biodiversity of invaded areas which, in turn, leads to fire-mediated nitrogen losses in invaded areas (D'Antonio and Vitousek 1992a, Rossiter-Rachor et al. 2009, Setterfield et al. 2010, Marshall et al. 2012). Several studies have also illustrated a negative relationship between *Cenchrus ciliaris* invasion and general species richness (Collins and Glenn 1991, Bestelmeyer and Schooley 1999, Fairfax and Fensham 2000, Blanco et al. 2005, Clarke et al. 2005, Jackson 2005, Flanders et al. 2006, Franklin et al. 2006). *Cenchrus ciliaris*

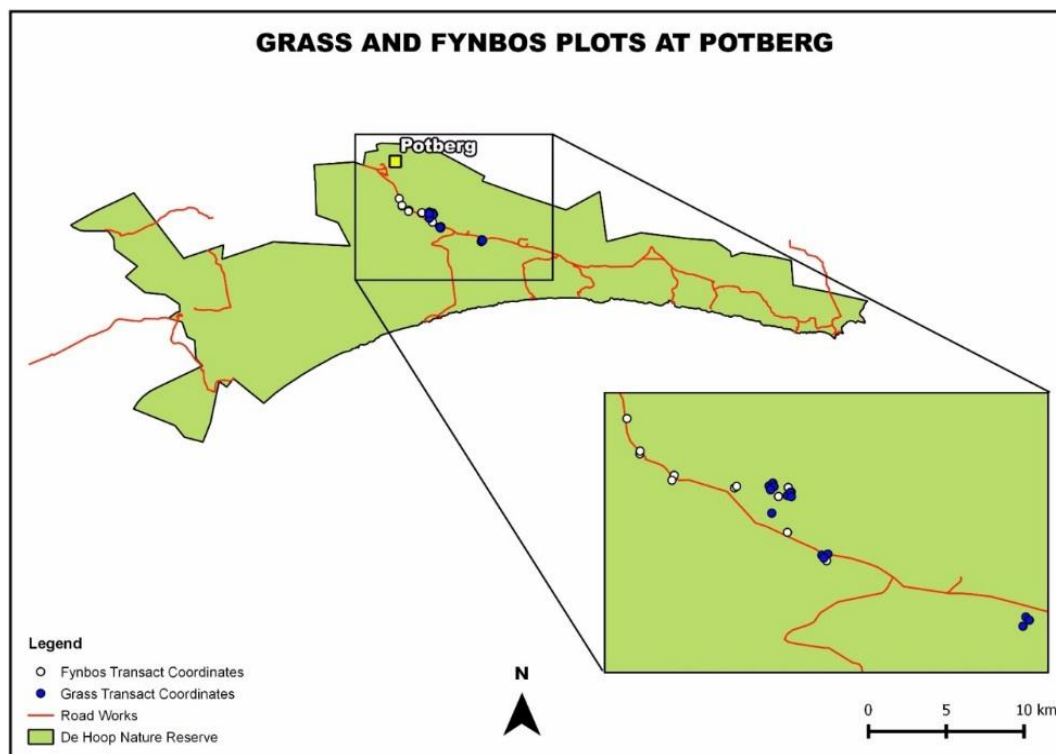
produces large volumes of biomass which burns hotter and faster than most grasses native to Australia and North America resulting in an increase in wildfire frequency and intensity (D'Antonio and Vitousek 1992a, Martin-R et al. 1999, Schiermeier 2005, Smyth et al. 2009). Additionally, *Cenchrus ciliaris* regenerates quicker than many native grasses creating a positive feedback loop that favours *Cenchrus ciliaris* regeneration (D'Antonio and Vitousek 1992a, Miller et al. 2010).

The CO₂ concentration mechanisms in C₄ plants enables these plants to have higher water-use efficiencies than C₃ plants as there is less water lost to the atmosphere with the same amount of carbon gain (Wand et al. 1999). Several studies have demonstrated that, under similar environmental conditions C₄ plants have photosynthetic water-use efficiencies (PWUE) that are typically 1.5 to 4 times greater than C₃ species (Morison and Gifford 1983, Huxman and Monson 2003, Larcher 2003, Vogan et al. 2007, Kocacinar et al. 2008). This allows C₄ plants to allocate a greater proportion of their biomass to root development (Long 1999, Ripley et al. 2008), with higher productivity and growth-rates (Percy and Ehleringer 1984) which in turn gives them competitive advantages over C₃ species (Knapp and Medina 1999, Sage and Kubien 2003).

The CO₂ concentrating mechanism also gives C₄ grasses enhanced nitrogen use efficiency (Vogan and Sage 2011). Several studies have shown that nitrogen use efficiency in C₄ plants is 50–100% greater than in C₃ plants (Bolton and Brown 1980, Sage and Percy 1987, Monson 1989, Ripley et al. 2008). The high nitrogen use efficiency of C₄ plants will enable these plants to outcompete C₃ species in low nitrogen environments (Ripley et al. 2008). This is supported by several studies which compared nitrogen use efficiency of C₃ and C₄ species belonging to the same genera such as *Flaveria* (Monson 1989) or *Panicum* (Bolton and Brown 1980). The combination of both nitrogen and water use efficiency in C₄ grasses would therefore allow these grasses to outcompete C₃ grasses in fynbos.

Several studies have identified the impact of C₄ invasive grass on species richness, fire regime and intensity (Clarke et al. 2005, Flanders et al. 2006, Franklin et al. 2006, Rossiter-Rachor et al. 2008, Foxcroft et al. 2010). Nevertheless, there have been no studies determining the impact of C₄ grasses on fynbos species richness and diversity. Fynbos species diversity and endemism is among the highest for any region of similar size worldwide and the most ecologically threatened of all plant kingdoms (Manning and Goldblatt 2012). It is therefore important to understand the potential effect that invasive C₄ grasses may have on fynbos species diversity.

In this chapter, I assess the impact that C₄ grasses may have on fynbos species richness. I approached the problem by determining the relationship between C₄ grass occurrence and fynbos species richness at my study site at Potberg. Although several studies have been done on the impacts that invasive alien trees may have on fynbos species diversity (Lambrinos 2000, Milton 2004, Musil et al. 2005) there is little or no understanding of the impacts that native C₄ grass



species may have on fynbos species richness.

Figure 4.1: The location of the fynbos (White) and grass (Blue) plots at my study site in the Potberg Reserve.

Methods

Study site

The vegetation at my study site is classified by Mucina & Rutherford (2006) as Agulhas Sand Fynbos and Albertinia Sand Fynbos with shrubs including *Protea repens*, *Protea neriifolia*, *Erica quadrangularis*, *Leucadendron modestum*, *Leucadendron laureolum*, *Chrysanthemoides monilifera*, *Erica imbricata*, *Leucadendron salignum* and *Phyllica purpurea*. Major species of Restionaceae include *Ischyrolepis capensis*, *Staberoha banksii*, *Thamnochortus spicigerus* and *Hypodiscus aristatus*. There are several alien invasive tree species such as *Acacia cyclops*, *Acacia mearnsii* and

Acacia pycnantha in the area. There are also patches of C₄ grasses such as *Imperata cylindrica*, *Eragrostis curvula*, *Setaria sphacelata var. torta* and *Cynodon dactylon*.

Experimental design

I established a total of 100, 10 m x 5 m plots, fifty (50) of which were classified as grass-dominated (GDPs) and (50) fynbos-dominated plots (FDPs) (Fig.4.1). The plots were selected based on the dominant vegetation, grass or fynbos, e.g., if the plot had more than 50% of grass cover it was identified as a grass dominated plot and if the plot had more than 50% fynbos cover the plot was identified as a fynbos dominated plot. The plots were at least 50 m apart. The plots were classified after a Braun Blanquet cover abundance estimate of the percentages of grass and fynbos (Mueller-Dombois and Ellenberg 1974). For this I classified percentage canopy cover into six separate classes 0 - 5, 5-25, 25-50, 50-75, 75-95, 95-100% (Daubenmire 1959). This was made under the assumption that grass and fynbos species in the plots combined to make up 100% canopy cover. In addition to canopy cover I also identified all the plant species in each plot while also recording the photosynthetic pathway of the grasses, and the GPS coordinates of each plot using a Garmin eTrex 10 GPS (Garmin, United States of America).

Data Analysis

Statistical analyses conducted included both parametric and non-parametric tests. Chi-squared tests were used to determine if there were any significance differences in the percentage canopy cover of the fynbos and grass dominated plots. I used a chi-squared test and T-tests to determine if there were significant differences in species diversity and species richness between the two treatments and I illustrated these differences using Venn diagrams. To quantify the degree of association between plant diversity and plant dominance in the plots, a logistic regression analysis was performed. Visual and quantitative summaries of the data are presented in Tables and Figures. The data was organised in excel and all tests on the data were assessed in R© v3.3.1 (R Core Development Team 2015) with a value of $p < 0.05$ required for significance.

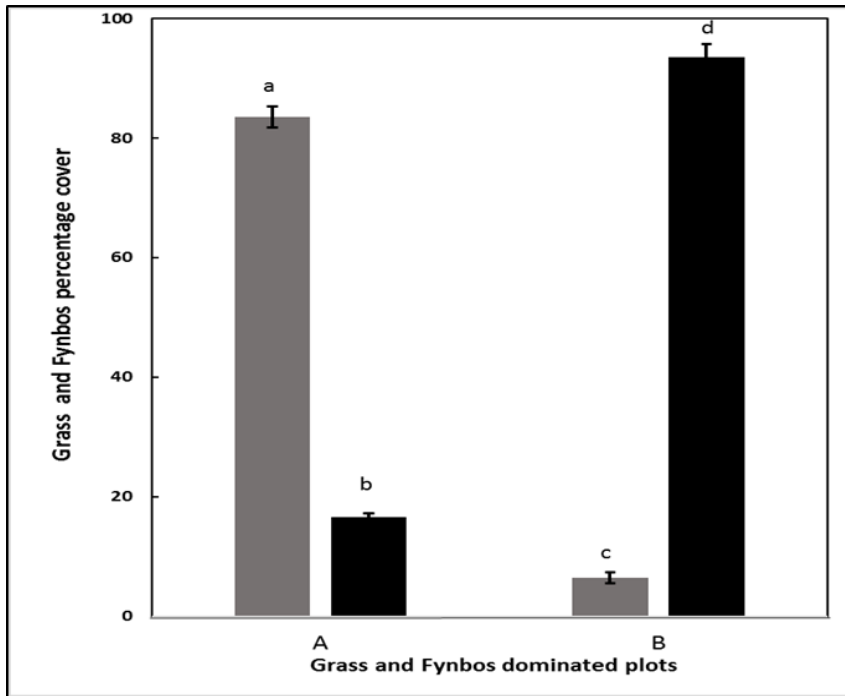


Figure 4.2: The mean percentage cover of grass (light bars) and fynbos (dark bars) on Grass dominated plots (A) and Fynbos dominated plots (B). Bars are means and lines standard error. Different letters indicate significant differences at ($p < 0.05$)

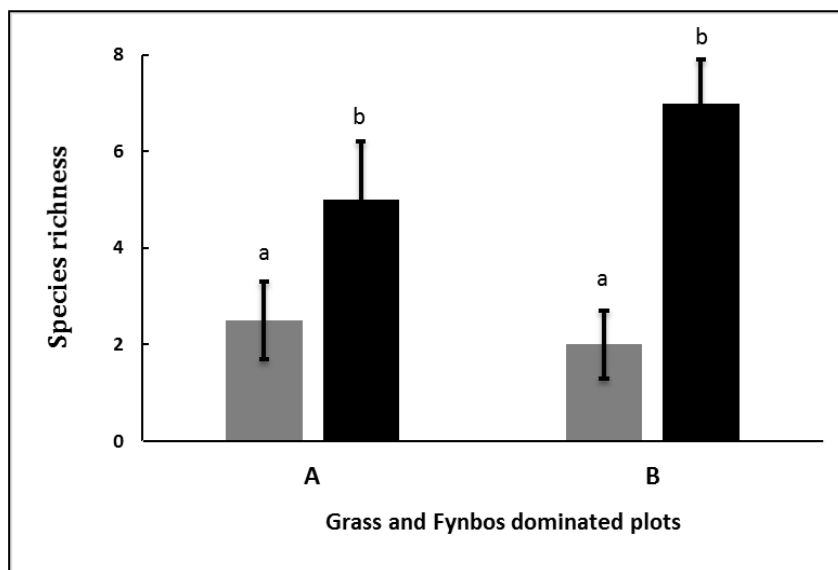


Figure 4.3: Grass (light bars) and Fynbos (dark bars) species diversity on Grass (A) and Fynbos (B) dominated plots. Bars are means and lines standard error. Different letters indicate significant differences at $p < 0.05$.

Results

Canopy cover was significantly higher for grass (83.5 %) on the grass dominated plots and higher for fynbos (93.6 %) on the fynbos dominated plots ($P < 0.0001$, Fig 4.2). There were more fynbos species on fynbos dominated plots (mean = 7.0 sd=2.89) than on the grass dominated plots (mean=3.5 sd=1.76) whilst the grass species numbers were significantly higher ($P < 0.0001$) in grass dominated plots (mean=2.4 sd=0.90) than on the fynbos dominated plots (mean= 0.9 sd=1.00, (Fig. 4.3). The fynbos dominated plots were dominated by four species; *Cliffortia stricta* (6,25%), *Leucadendron salignum* (5,01%), *Passerina rigida* (5,76%) and *Phylica purpurea* (4,76%) while the grass dominated plots were dominated by three grass species *Cynodon dactylon* (6,61%), *Merxmuellera disticha* (4,79%), *Setaria sphacelata var.torta* (4,79%) and one fynbos species *Helichrysum patulum* (4,1%). Fynbos species richness on the fynbos dominated plots was significantly higher ($P < 0.0001$) than on the grass dominated plots (Fig 4.4).

A Chi-Square (χ^2) test showed that a grass species diversity of zero or one is twenty-one (21) times more likely to be observed on fynbos dominated plots (FDPs) than on grass dominated plots (GDPs) ($P < 0.001$) and that it is 70% ($\approx 0.7037 = 1 - 0.2963$) less likely to observe two different grass species on a FDP than it is to observe these on a GDP ($P < 0.0025$). Of the fifty (50) sampled FDPs only four (4) plots had grass species diversity of three or more. It is five and a half times more likely to observe five or more different fynbos species on FDPs than on GDPs ($P < 0.00$). Of the 24-grass species recorded, 11 species were only recorded on the FDPs, six species were recorded on both FDPs and GDPs, while seven species were only recorded on GDPs. Of the 75-fynbos species recorded, 60 species were recorded on the FDPs only, while 13 species occurred on both FDPs and GDPs and 28 were only recorded on the GDPs (Fig. 4.5). Grass-dominated plots had slightly but not significantly higher percentages of C₄ grasses (69.33%) than FDPs (66.6%).

A chi-Square test showed that it is four times more likely to observe no fynbos species on plots with a high volume of C₄ to C₃ grasses compared to plots with at least the same number of C₃ to C₄ grasses. The most aggressive C₄ grass species were *Pennisetum thunbergii*, *Eustachys paspaloides* and *Imperata cylindrica* as these lie on the left of the vertical sample diversity line and are associated with low fynbos species richness, while the C₃ species lie on the right end of the vertical sample diversity line (Fig. 4.6).

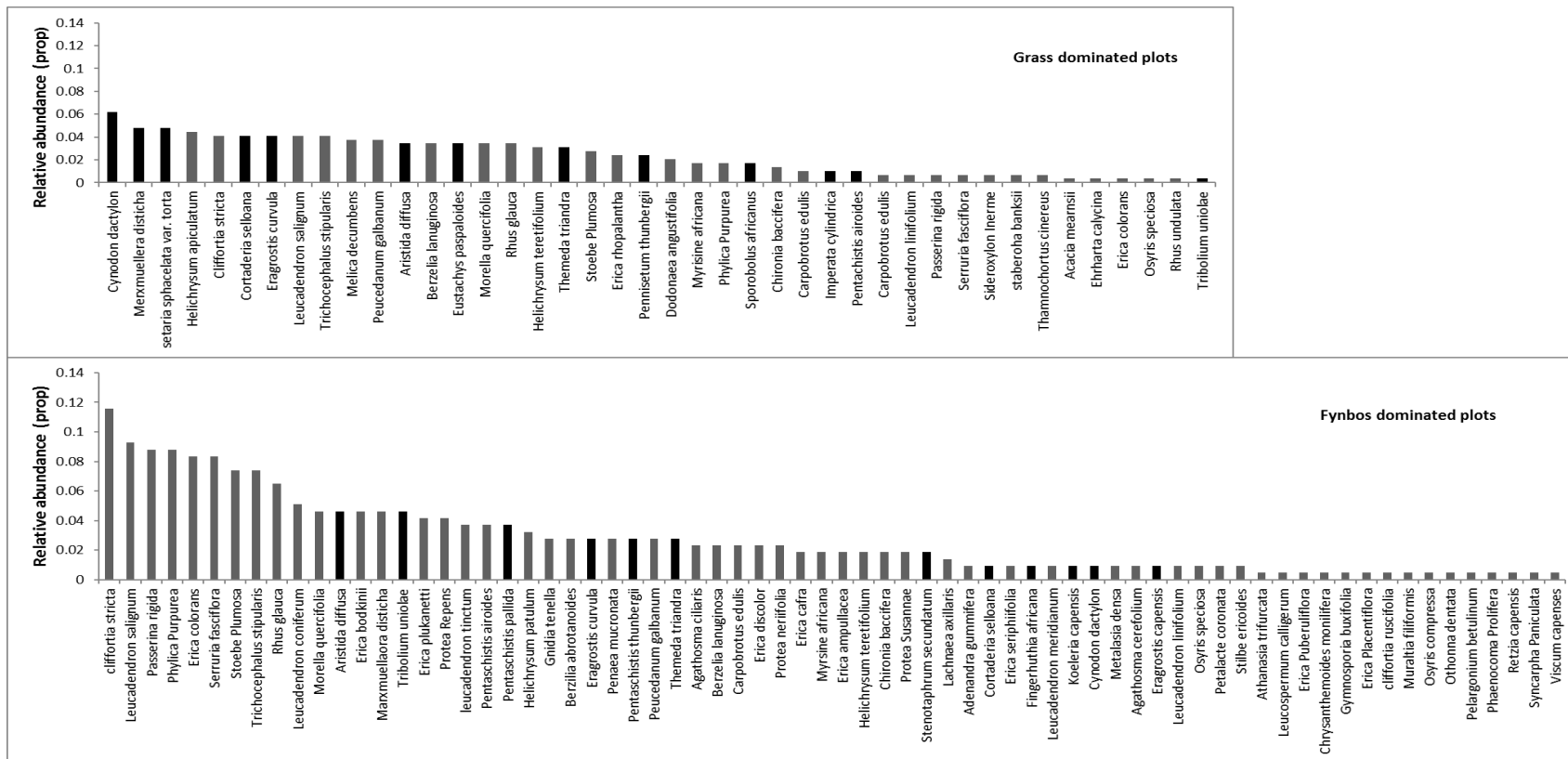


Figure: 4.4: The relative abundance of species at my study site showing the significantly higher ($P < 0.001$) proportion of the grasses *Cynodon dactylon*, *Merxmullera disricha* and *Setaria sphacelata*, and reduction in species diversity in the Grass-dominated plots. (Grass = Black and Fynbos = Grey).

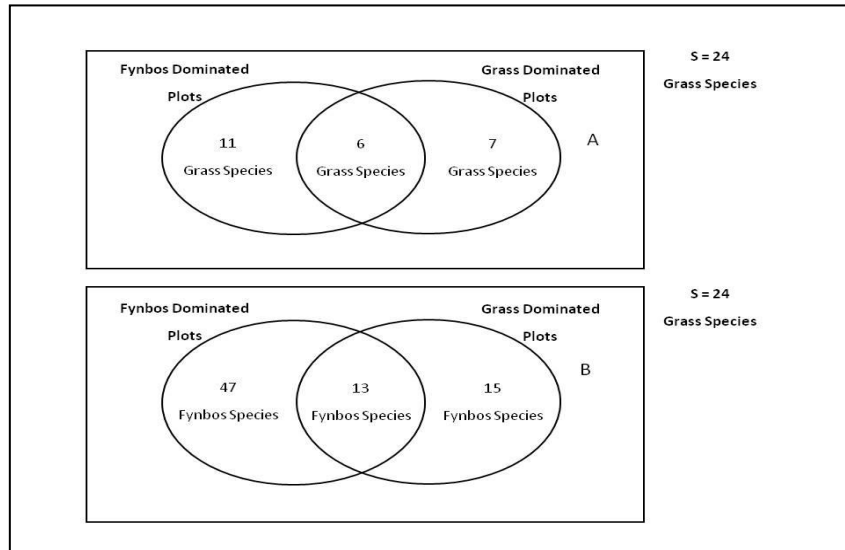


Figure 4.5: Venn diagrams illustrating the frequency distribution of grass species and fynbos species in both the Grass (A) and Fynbos-dominated (B) plots.

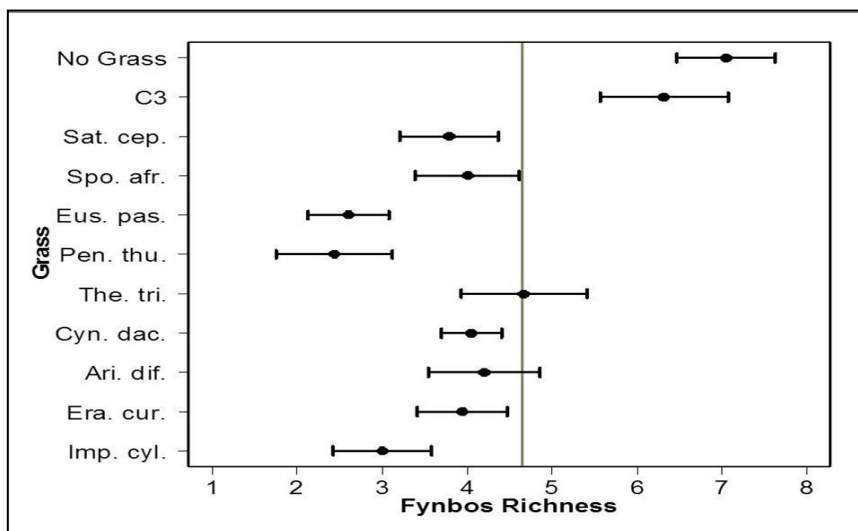


Figure 4.6: Fynbos species richness with no Grass, with C_3 grass and with 9 individual species of C_4 grass; *Imp.cyl* = *Imperata cylindrica*, *Era.cur* = *Eragrostis curvula*, *Ari.dif* = *Aristida diffusa*, *Cyn.dac* = *Cynodon dactylon*, *The.tri* = *Themeda triandra*, *Pen.thu* = *Pennisetum thunbergii*, *Eus.pas* = *Eustachys paspaloides*, *Spo.afr* = *Sporobolus africanus*, *Sat.cep* = *Setaria sphacelata var torta*.

In both fynbos and grass-dominated plots, the plots which had a high number of C₄ and low number of C₃ grass species had low fynbos species richness compared to plots with a high number of C₃ grass and low number of C₄ grass species (Fig. 4.7).

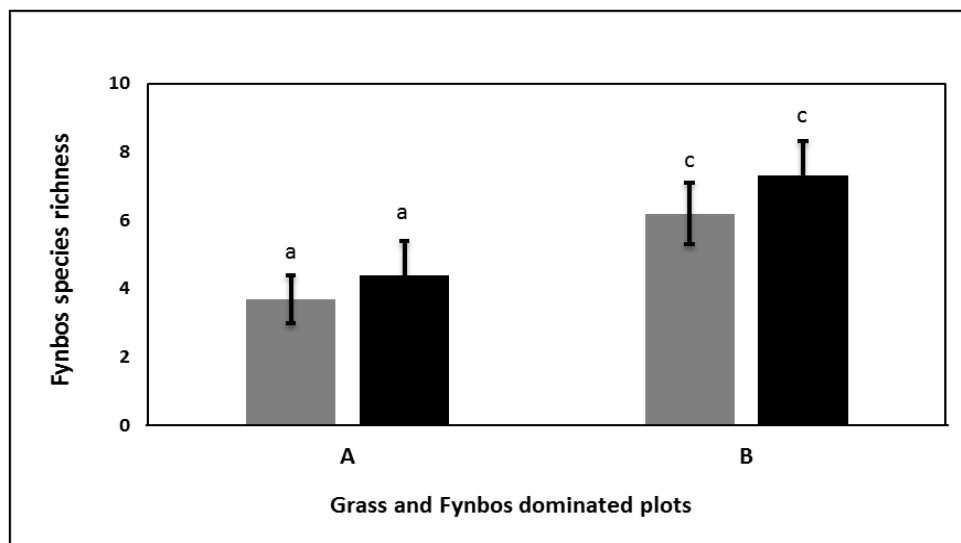


Figure 4.7: The effects of C₃ and C₄ grasses on fynbos species richness in both Grass Dominated plots (A) and Fynbos-Dominated Plots (B). Light bars are C₄ dominated plots and dark bars are C₃ dominated plots. Bars are means and lines standard error. Different letters indicate significant differences at $p < 0.05$. species.

Discussion

The primary objective of this chapter was to determine the impact of C₄ grass on fynbos species diversity and species richness. To achieve this objective I subjectively selected plots that were dominated by either grass or fynbos. My results show that there is a relationship between C₄ grass and fynbos species richness and that C₄ grasses do have a negative effect on fynbos plant species richness. Plots with a high relative abundance of C₄ grasses had low fynbos species richness. The most aggressive C₄ grasses were *Pennisetum thunbergii*, *Eustachys paspaloides* and *Imperata cylindrica* while the least aggressive was *Themeda triandra*. Of the three most aggressive grasses both *Pennisetum thunbergii* and *Imperata cylindrica* grow in wetlands or near rivers while *Eustachys paspaloides* grows in gravelly and sandy soil but sometimes also in clay soil (Van Oudtshoorn 1992). All these grasses are long-lived perennials (Van Oudtshoorn 1992), which enables them to survive repeated fires or grazing pressure by resprouting, and all can spread by vegetative reproduction as well as by seed (Milton 2004). C₄ grasses need water during the growing season at the warmest time of the year (Teeri and Stowe 1976, Vogel et al. 1978, Hattersley 1983, Vogel et al. 1986). Although Potberg receives most of its rain in the cooler months, there is still some rain in the warmer months, with a relative rainfall percentage

of 60% cool season and 40% warm season (Station No. 0000.3020 4; South African Weather Services, 2016). Considering the high nitrogen and water-use efficiency of C₄ species, this slight reduction in warm season rainfall might be enough to allow C₄ grasses to compete with fynbos species for nutrients and water (Ripley et al. 2008).

C₄ grasses do not only outcompete fynbos species for resources, but the high biomass of these grasses may also reduce or inhibit light from reaching the fynbos seeds and seedlings (Pierce and Cowling 1984), and as most of the fynbos species need light to regenerate, reduction or inhibition of light can reduce species richness (Vlok and Yeaton 2000). Invasion by C₄ grass may also result in increased wildfire frequency and intensity which may have a negative influence on fynbos regeneration (D'Antonio and Vitousek 1992a, Martin-R et al. 1999, Schiermeier 2005, Smyth et al. 2009). This however cannot be the case in Potberg as the vegetation had not burned for between 30-40 years prior to the 2016 fire (Cape Nature internal report, 2017).

Although there has been little or no research on the impacts of C₄ grass on fynbos, several studies have shown a negative relationship between the C₄ grass *Cenchrus ciliaris* invasion and general species richness (Blanco et al. 2005, Clarke et al. 2005, Flanders et al. 2006, Franklin et al. 2006). Invasion by the C₄ grasses *Andropogon gayanus* and *Cenchrus ciliaris* in Australia, the USA, Mexico and South America has compromised the biodiversity of invaded areas (D'Antonio and Vitousek 1992a, Rossiter et al. 2003, Rossiter-Rachor et al. 2009, Setterfield et al. 2010, Marshall et al. 2012). My study agrees with these studies showing that C₄ grass does indeed have an impact on fynbos species richness. These results also show that, although C₄ grasses in Potberg are indigenous these grasses are also aggressive competitors and their impacts on fynbos species diversity are comparable to those of alien invasive grasses such as *Andropogon gayanus* and *Cenchrus ciliaris* in Australia (Rossiter et al. 2003, Setterfield et al. 2010). I recommend that more studies be done on the impacts of C₄ grass on fynbos species diversity and richness in other areas to determine if the pattern demonstrated in my study is replicated elsewhere. Understanding the drivers for the expansion of C₄ grasses into fynbos will help conservation managers make informed decisions. In Chapter 2 I show that roads act as conduits for C₄ grass invasion into fynbos and in this chapter I demonstrate the impacts of this invasion on fynbos species diversity. While roads may indeed act as conduits for C₄ grass invasion into fynbos in the next chapter I ask if this is because of an increase in nutrients and water.

Chapter 5.

Factors influencing the colonisation of fynbos by C₄ grasses.

Introduction

The C₄ grass patches at Potberg described in this thesis are unusual and are invading into and replacing fynbos. These patches include *Cynodon dactylon*, *Eragrostis curvula*, *Imperata cylindrica* and *Setaria sphacelata var torta*. These dominant grasses are distributed widely throughout South Africa and may naturally occur at Potberg (Van Oudtshoorn 1992). Of the four most dominant grasses, three species *Cynodon dactylon*, *Setaria sphacelata var torta* and *Eragrostis curvula* are adapted to well drained, disturbed soils while one species *Imperata cylindrica* prefers poorly drained damp soil (Van Oudtshoorn 1992). These grasses are all native to the tropics but with a distribution range extending into the south-western Cape. The growing season for all four species is during the warmer months of the year characteristic of the grass land and savanna biomes (Van Oudtshoorn 1992).

Temporal niche separation between C₃ and C₄ species has long been suggested to be due to different optimum temperature requirements for photosynthesis (Kemp and Williams 1980, Monson et al. 1986). It is however difficult to extricate the roles of temperature, soil water and nitrogen availability in regulating the growth dynamics of C₃ and C₄ species under natural conditions, especially when there are seasonal fluctuations in temperature, soil water and nitrogen availability (Percy and Ehleringer 1984, Yuan et al. 2007). Several studies have, however, shown that seasonal water availability may potentially contribute to the distribution of C₃ and C₄ species (Winslow et al. 2003, Niu et al. 2005, Murphy and Bowman 2007).

In addition, seasonal changes in water regime can cause fluctuations in soil nutrient transformation and availability (Austin et al. 2004, Jankju-Borzelabad and Griffiths 2006). Mediterranean-ecosystem soils are generally nutrient poor with fynbos plants assumed to be particularly well adapted to these soils through specialised strategies such as carnivory, cluster roots, or associations with nitrogen fixing bacteria (Osmond et al. 1982, Lambers et al. 2008). While lacking in these nutritional strategies C₄ grasses are nevertheless both water use and nitrogen use efficient. The ability to use nitrogen more efficiently may allow these grasses to establish in low nutrient fynbos soils. Although most of the rain at Potberg is received during the cooler months of the year (60%), a significant fraction (40%) does fall in the warmer months. This warm season rainfall may be enough to allow for C₄ grasses to establish due to their high-

water use efficiency (Osmond et al. 1982, Brown and Wilson 1983, Long 1999). Neither available water during the warmer months of the year nor the low nutrient soils may be a deterrent to the establishment of these grasses in fynbos.

One of the important biotic factors regulating plant growth is competition for resources (Aarssen 1989, Aguiar et al. 2001). Fynbos has a remarkably high level of species diversity with the Cape Peninsula (471 km²) alone having 2351 native species (Kruger and Taylor 1980). Several studies have suggested that this high species diversity is only possible because there is no competition for resources with plants segregating along several niche axes including light, nutrients and water (Silvertown 2004). Alteration of resource availability in an ecosystem can shift the competitive balance of plant species (Wedin and Tilman 1993, Tielbörger and Kadmon 2000, Minchinton and Bertness 2003, Michalet 2007). Competition between C₃ fynbos plants and C₄ grasses will eventually result in altered species composition and a consequent change in biodiversity and carbon sequestration at the ecosystem scale (Still et al. 2003). Understanding the mechanism behind C₄ grass invasion into fynbos is therefore important for the maintenance of both biodiversity and ecosystem services. At the local level, an important question for conservation managers is to ask what might be contributing to the colonisation of fynbos by C₄ grasses. Identification of the key factors could lead to possible management measures being put in place to reduce or prevent further colonisation by grasses.

In this chapter I describe an experiment designed to examine the extent to which topographic position (moisture availability), an increase in nutrient availability and a decrease in competition for resources influences the establishment and growth of the C₄ grass, *Setaria sphacelata* var. *torta* at Potberg. In this experiment transplants of the grass were planted in three positions along a slope, with and without nutrient additions and with and without competition for soil resources. As the bottom of the slope tends to have higher available water, the specific question I ask is: how, relative to topographic position, soil moisture, nutrients and competition for soil resources affect the ability of *Setaria sphacelata* var. *torta* to establish in nutrient poor fynbos soil? As C₄ grasses are favoured in areas with a warm and wet growing season and are more water use efficient than fynbos plants (Vogel et al. 1978). I hypothesized that *Setaria sphacelata* var. *torta* would increase biomass at the bottom of the slope where soil moisture is highest. Fynbos soils are notoriously nutrient poor while C₄ grasses have high nutrient use efficiency (Osmond et al. 1982, Brown and Wilson 1983). I therefore hypothesised that *Setaria sphacelata* var. *torta* would be better able to utilise the increase in available nutrients and produce more biomass in the fertilized plots. I trenched to remove competition for resources from fynbos roots with the

hypothesis that C₄ grasses are highly competitive below-ground due to their large fine root system (Long 1999), and therefore trenching to remove competition from fynbos roots would not influence the growth of the competitively superior grasses.

Methods

I set up the experiment at the end of May 2015 along a soil catenal sequence at Potberg Nature Reserve where there was no C₄ grass. The dominant plant species were *Protea repens*, *Protea neriifolia*, *Erica quadrangularis*, *Leucadendron modestum*, *Leucadendron laureolum*, *Chrysanthemoides monilifera*, *Erica imbricata*, *Leucadendron salignum*, and *Phyllica purpurea*. Restios include *Ischyrolepis capensis*, *Staberoha banksii*, *Thamnochortus spicigerus* and *Hypodiscus aristatus*. Plots were laid out at three positions along a topographical gradient, bottom, middle and top. Moisture level was highest at the bottom of the slope, intermediate at mid-slope and driest at the top (Fig. 5.1). I tested moisture availability at these topographical positions using a soil auger in September 2015. The soil auger showed a water table within 80 cm of the soil surface at the bottom of the slope, at 1.2 m to 1.5 m mid-slope and with no water table to at least a depth of 2 m at the top of the slope.

Experimental layout

At each topographic position along the slope I transplanted a grass tuft into one of four (20 x 20 cm) treatments as follows: trench with fertiliser, trench with no fertiliser, not-trenched with fertiliser, not-trenched with no fertiliser (Fig 5.1 and Fig 5.2). Each group of four treatments was replicated five times at three locations at each topographic position with each replicate group of treatments 100 m apart from the next. This added to a total of 180 transplants (3 topographic positions x 3 locations at each position x 4 treatments x 5 replicates per treatment). I trenched by vertically inserting a 20 cm x 20 cm plastic sheet into the ground, on four sides of the targeted grass, with 1 cm of plastic protruding above the soil surface. The primary objective for trenching was to restrict root competition between the grass and the surrounding fynbos species. I applied 60 g of a slow-release granular fertilizer encapsulated in a multilayer polymeric coating, Multicote 4* urea-N (42-0-0, N:P:K, Haifa Group, South Africa), to plots where fertilizer was required. I specifically used a slow release fertilizer to prevent rapid volatilization of the nitrogen (Matimati *et al.*, 2013). Grass used for the transplant was collected in June 2015 from a single patch of *Setaria sphacelata var. torta* growing at the study site. All plots were established by June 2015. I measured grass height and mortality for a period of 3 months (until August 2015) at which time a wildfire burned through my study site. I then continued these measurements after the fire for a further 5 months (until January 2016). The experiment was

finally terminated in May 2017 (after two years) when I harvested the surviving grasses. I oven-dried the harvested samples at 70°C for 48 hours before weighing. The grasses were divided into above and below ground parts. This was done through gently shaking the sand off the roots and then separating the below and above ground parts with a pair of secateurs.

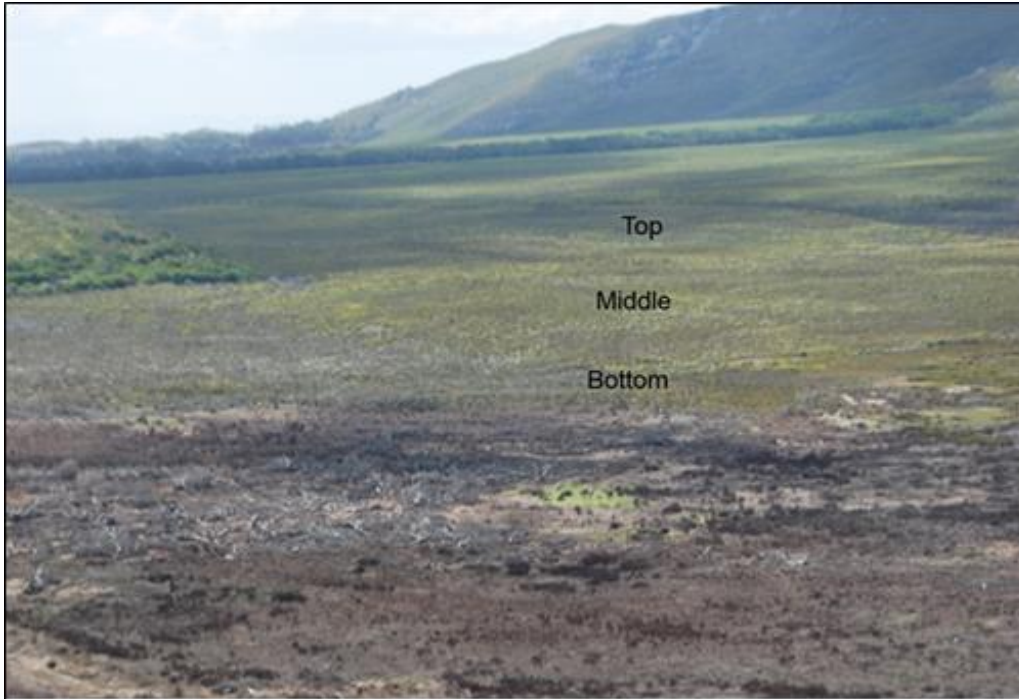


Figure 5.1: An Aerial view of my study site at Potberg showing the location of the topographical positions used in the transplant experiment Photo: Edmund February.

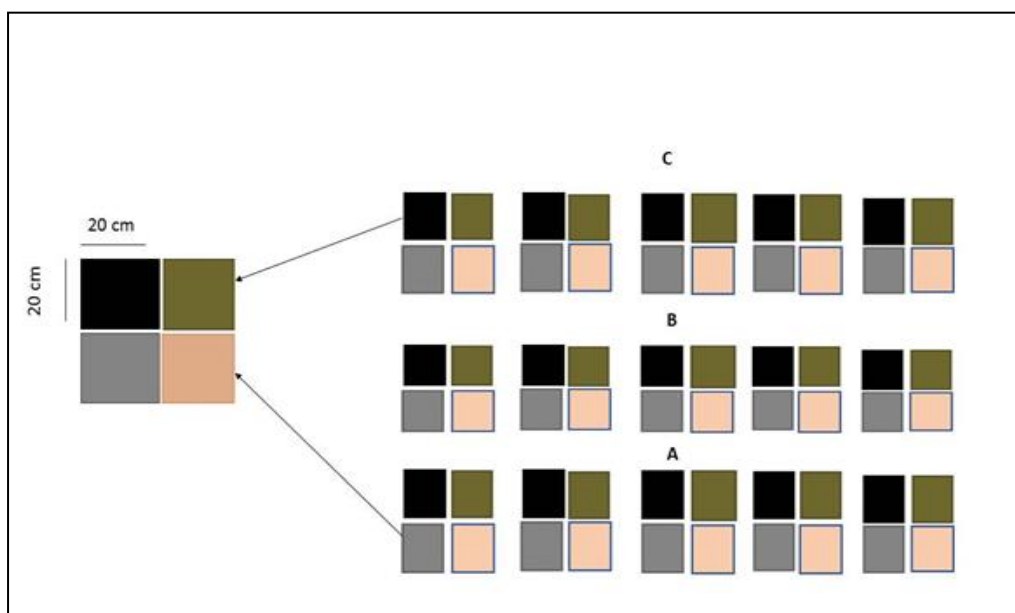


Figure 5.2: The experimental setup at my study site. This layout was replicated three times along each topographic position Bottom (A) Middle (B) and Top (C) and treatments, Trenched -fertilised ■ Trenched -unfertilised ■, Untrenched unfertilised and ■ Untrenched –unfertilised ■

Data Analysis

Grass mortality occurred, especially after the fire. Chi-squared was performed to determine if fire and the treatments had an impact on mortality. Data on growth was the final biomass recorded in May 2017. The data was split into two data sets: grass biomass and root/shoot ratio. Univariate ANOVA was used to determine if treatments (slope position, fertiliser and trenching) had a significant effect on grass biomass and root shoot ratio. Nested Anova was used to determine interaction effects of the treatments (slope position, fertiliser and trenching). Means and standard deviations (s.d.) were calculated. Visual and quantitative summaries of the data are presented in Tables and Figures. The data was organised in excel and all tests on the data were assessed in R© v3.3.1 (R Core Development Team 2016) and a value of $p < 0.05$ was required for significance.

Results

Although slope position did not have a significant effect on mortality, I recorded the highest mortality before the fire (3 months) on the top of the slope. Nineteen months after the fire I recorded significantly higher mortality at the top of the slope than in the middle or bottom of the

slope. Fertiliser had a significant effect on grass mortality, with fertilised plots having significantly higher mortality. Thirty nine grasses died in the fertilised plots and only nine died in the unfertilised plots ($P < 0.05$). (Table 5.1. Fig. 5.3, $P < 0.001$).

Table 5.1: Chi-squared test results for treatment and fire effect on Mortality. NS = not significant, ** = $P < 0.0001$.**

| | x-squared | Df | P-value |
|------------|-----------|----|---------|
| Fire | 29.447 | 1 | **** |
| Trenched | 0.60096 | 1 | NS |
| Fertiliser | 20.216 | 1 | **** |
| Site | 4.0385 | 2 | NS |

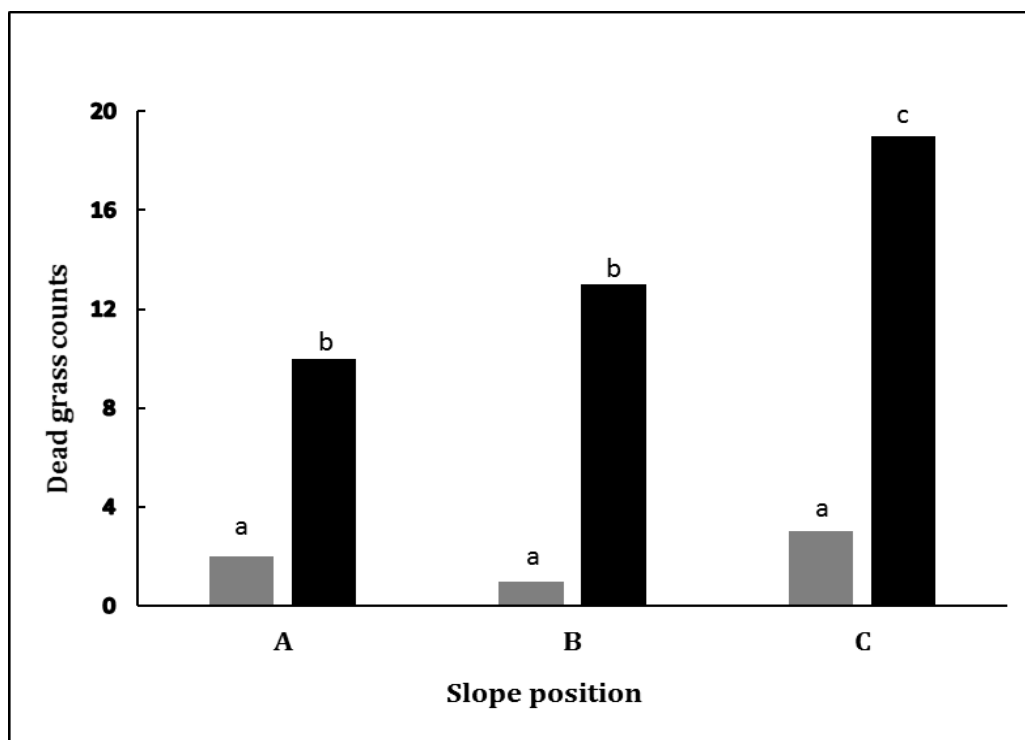


Figure 5.3: Total grass mortality counts both before and after fire across the slope positions, Bottom (A), Middle (B) and Top (C) before fire (light bars) and after fire (dark bars). Different letters indicate significant differences at $p < 0.05$.

While grass at the bottom of the slope had the highest above ground biomass ($11.5 \text{ g} \pm 0.8$), plants at the top of the slope had the lowest (mean= $7.9 \text{ g} \pm 0.7$), this was however not statistically significant. While trenching did not have a significant effect on mortality, trenching did have a significant effect on grass biomass with trenched plots having higher grass biomass (mean = $22.1 \text{ g} \pm 2.4$) than untrenched plots (mean = $16 \text{ g} \pm 1.2$) plots (Fig. 5.4, $P < 0.05$).

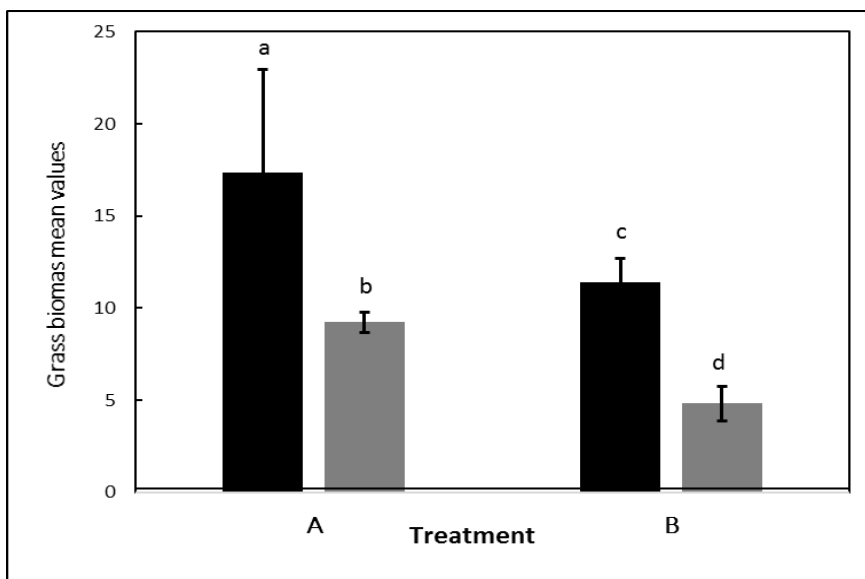


Figure 5.4: Total grass biomass in Trenched (A) and Untrenched (B), Fertilized (dark bars) and Unfertilized (light bars) plots. Different letters indicate significant differences at $p < 0.05$ (Wilcoxon Rank Sum Test, unpaired) between fertilised and unfertilised plots.

Root/shoot ratios were significantly influenced by slope position and fertiliser addition. Plants at the top of the slope had significantly higher root/shoot ratios than those plants in the middle and the bottom (Fig.5.5, $P < 0.05$). Fertilised plants also had significantly smaller root/shoot ratios (mean 0.79 ± 0.37) than unfertilised plants (mean 1.23 ± 0.5) (Fig. 5.6, $P < 0.001$) and trenching did not have a significant effect on root shoot ratios (Fig. 5.6).

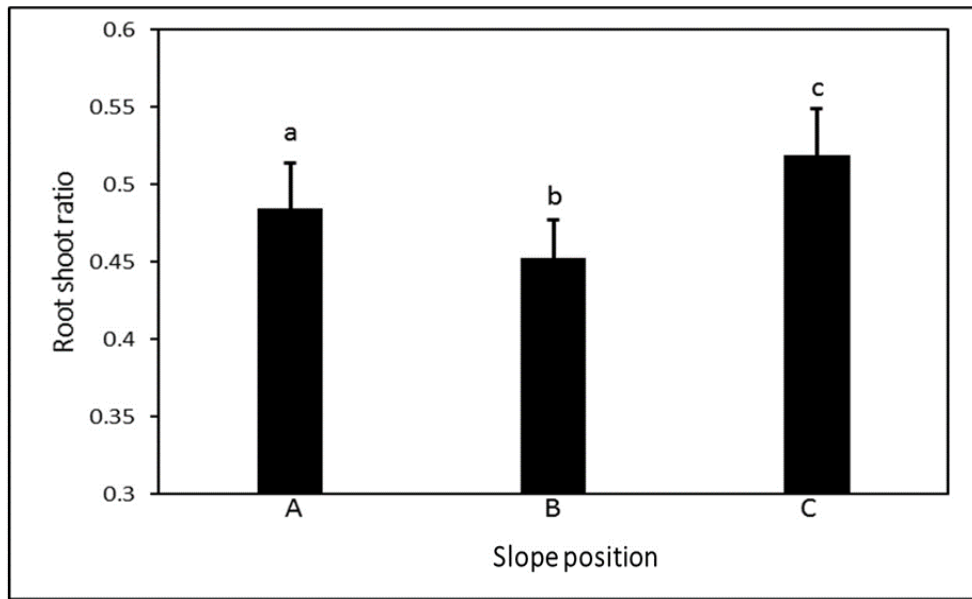


Figure 5.5: Mean values for Root/shoot ratio across the three slope positions, Bottom (A) Middle (B) and Top (C). Different letters indicate significant differences at $p < 0.05$ (Wilcoxon Rank Sum Test, unpaired).

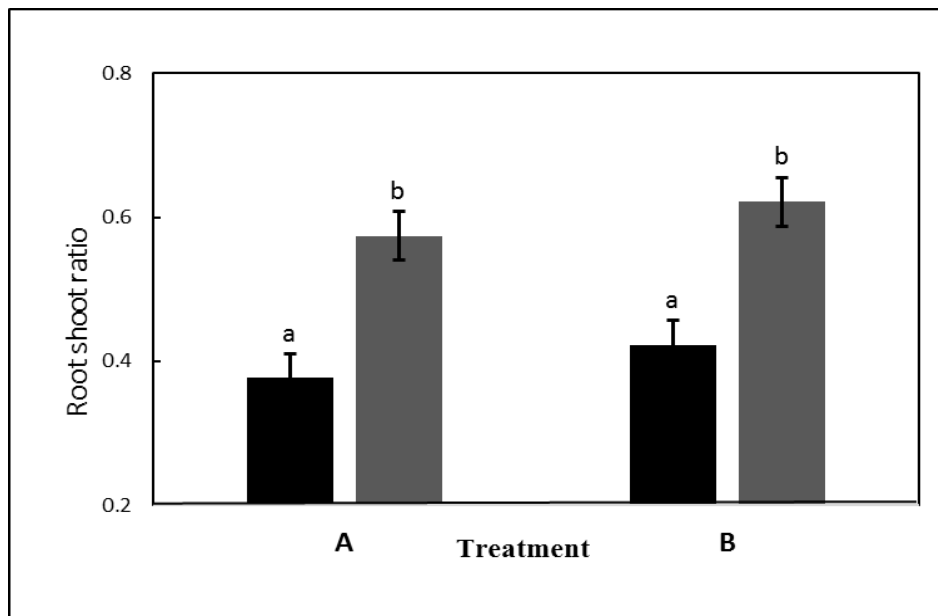


Figure 5.6: Mean values for Root/shoot ratios across the four treatments Trenched (A), and Untrenched (B), Fertilized (dark bars) and Unfertilized (light bars). Different

letters indicate significant differences at $p < 0.05$ (Wilcoxon Rank Sum Test, unpaired) between fertilised and unfertilised plots.

The interaction effects on biomass tested using nested Anova showed a significant interaction between slope position and fertiliser treatment and between trenching and fertilized treatments (Table.5.2, $P < 0.05$). Although the bottom slope had higher biomass compared to middle and top slope, the difference was not statistically significant when comparing the slope position alone, however looking at slope position and fertiliser in combination, there was statistically significant difference. Both trenching and fertilizer had a significant positive affect on grass biomass, there was also significant effect between trenched unfertilised and trenched fertilised plots (Table.5.3, $P < 0.05$).

Table 5.2: Analysis of Variance (Nested Anova) results for the effects of interaction between site and treatments in grass biomass. NS = not significant, * = $P < 0.05$, ** = $P < 0.01$, * = $P < 0.001$.**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---------------------|-----|--------|---------|---------|--------|
| Site | 2 | 211.3 | 105.67 | 2.087 | NS |
| Trenched | 1 | 336.20 | 336.17 | 6.641 | * |
| Fertilizer | 12 | 677.80 | 2677.82 | 52.901 | *** |
| Site:Trenched | 2 | 95.9 | 47.93 | 0.946 | NS |
| Site:Fertilizer | 2 | 333.70 | 166.86 | 3.296 | * |
| Trenched:Fertilizer | 1 | 385.10 | 385.14 | 7.608 | ** |
| Residuals | 131 | 6631.1 | 50.62 | | |

Table 5.3: Analysis of Variance (Nested Anova) results for the effects of the interaction between slope and fertilizer on grass biomass. NS = not significant, * = $P < 0.05$, ** = $P < 0.0001$.**

| | Df Sum Sq | Mean Sq | F value | Pr(>F) |
|---|-----------|---------|---------|--------|
| Middle:Fertilised- Bottom:Fertilised | -3.892 | -9.731 | 1.947 | NS |
| Top:Fertilised- Bottom:Fertilised | -6.893 | -12.666 | -1.12 | NS |
| Bottom:Unfertilised- Bottom:Fertilised | -12.646 | -18.3 | -6.992 | **** |
| Middle:Unfertilised- Bottom : Fertilised | -10.891 | -16.664 | -5.118 | **** |
| Top:Unfertilised- Bottom:Fertilised | -12.521 | -18.892 | -6.149 | **** |
| Top:Fertilised- Middle:Fertilised | -3.001 | -9.006 | 3.003 | NS |
| Bottom:Unfertilised- Middle:Fertilised | -8.754 | -14.645 | -2.863 | **** |
| Middle:Unfertilised- Middle:Fertilised | -6.999 | -13.003 | -0.994 | NS |
| Top:Unfertilised- Middle:Fertilised | -8.628 | -15.21 | -2.047 | * |
| Bottom:Unfertilised- Top:Fertilised | -5.753 | -11.578 | 0.071 | NS |
| Middle:Unfertilised- Top:Fertilised | -3.997 | -9.938 | 1.942 | NS |
| Top:Unfertilised- Top:Fertilised | -5.627 | -12.15 | 0.895 | NS |
| Middle:Unfertilised- Bottom:Unfertilised | 1.7554 | -4.069 | 7.58 | NS |
| Top:Unfertilised- Bottom:Unfertilised | 0.125 | -6.292 | 6.544 | NS |
| Top:Unfertilised- Middle:Unfertilised | -1.629 | -8.153 | 4.893 | NS |

Table 5.4: Analysis of Variance (Nested Anova) results for the effects of the interaction between trenching and fertilizer on grass biomass. NS = not significant, * = $P < 0.05$, ** = $P < 0.01$, * = $P < 0.001$.**

| | Df | Sum Sq | Mean Sq | F value | Pr (>F) |
|---|----|---------|---------|---------|---------|
| Trenched:Unfertilised- Trenched:Fertilised | | -11.989 | -16.384 | -7.594 | **** |
| Untrenched:Unfertilised- Trenched:Fertilised | | -11.907 | -16.405 | -7.409 | **** |
| Trenched:Unfertilised- Untrenched:Fertilised | | -5.445 | -9.782 | -1.107 | NS |
| Untrenched:Unfertilised- Untrenched:Fertilised | | -5.363 | -9.805 | -0.92 | ** |
| Untrenched:Unfertilised- Trenched:Unfertilised | | 0.082 | -4.446 | -4.61 | NS |

Discussion

The primary objective of this chapter was to determine the drivers that influence the establishment of C_4 grass patches, exemplified by *Setaria sphacelata var. torta*, into fynbos. As C_4 grasses have extensive root systems that allow these grasses to be competitive in most environments (Pierce and Cowling 1984, Long 1999, February et al. 2013), my hypothesis as outlined in the introduction to this chapter was that trenching would not have a significant effect on *Setaria sphacelata var. torta* biomass and root/shoot ratios. I also hypothesised that the grass would have high biomass at the bottom of the slope where moisture was highest primarily because these grasses depend on warm temperatures in the growing season, which means they will do better where the water table is nearer the surface during the warmer months (Ode et al. 1980, Ehleringer et al. 1997). My results show that, grass biomass was indeed significantly higher at the bottom of the slope. The bottom of the slope had, on average 10% higher grass biomass relative to the middle and top of the slope. I also hypothesised that there would be higher grass biomass in unfertilised plots due to the high nutrient use efficiency of the C_4 grass and in untrenched plots due to the competitive abilities of the C_4 grass. My results however do not agree with this hypothesis with significantly higher biomass in fertilised and trenched plots.

My results also show that after the fire at my study site and over a period of nineteen months there was an increase in mortality at the top of the slope in the drier treatment. Similarly, a study by Nippert et al (2012) at Konza Prairie Biological Station in north eastern Kansas, USA found

that grass biomass varied significantly by position, with the most biomass in the lowland position and the least biomass in the upland.

Several studies have shown that topographic position has a strong influence on surface soil moisture (Briggs and Knapp 1995) and nutrient availability (Schimel et al. 1991, Turner et al. 1997). Soil moisture has been identified as the most likely driver of the differences in grass biomass along topographic gradients in North American prairies (Briggs and Knapp 1995). At the beginning of my experiment I also showed higher soil moisture at the bottom of the slope. The high biomass at the bottom of the slope may therefore be, in part, attributed to the higher moisture content of the soil allowing the grass to grow in the warm dry season while also enabling easier transport of nutrients (Easton et al. 2005). Contrary to my expectation trenching did have a significant effect on grass biomass with trenched and fertilised plots having higher biomass than untrenched and unfertilised treatments. These results show that C₄ grasses are not more competitive than the surrounding fynbos vegetation. Rather, these results would suggest that the high biomass in trenched plots was due to competitive exclusion of the surrounding fynbos vegetation.

My results also demonstrate low root/shoot ratios at the bottom of the slope. This agrees with optimal biomass allocation models that predict decreasing root/shoot ratios with increasing nutrient availability (Shiponeni, Bloom et al. 1985, Watson et al. 2014). The dry sites tend to have higher root/shoot ratios whilst wet sites tend to have a reduction in root/shoot ratios (Monk 1966). Several studies have also shown that nutrients are leached from the top to the bottom of the slope (Khan et al. 2013) and that this increased nutrient concentration causes a decrease in root/shoot ratios (Ericsson 1995, Marschner et al. 1996, Watson et al. 2014).

These results suggest that C₄ grasses are only able to invade into fynbos where there is sufficient soil moisture in the warm dry season, where nutrients are higher and where there is less competition with fynbos species for resources. Given these results I speculate that there are two ways that *Setaria sphacelata var. torta* may have colonised at my study site. The first is through heavy grazing opening-up patches in the fynbos when De Hoop Nature Reserve was still a farm and before it was proclaimed as a nature reserve in 1957. The second is that fire has been actively suppressed at the reserve with the last fire more than 30 years ago (De Hoop fire report 2017). Fire is an integral part of fynbos ecology with, depending on rainfall, most fynbos fires occurring every 15 to 20 years (Van Wilgen et al. 2010, Kraaij et al. 2011). Fynbos becomes senescent with age and there is a decline in species diversity as the fire return interval increases

(Bond 1980, Kruger and Taylor 1980, Drake et al. 1989, van Wilgen 2009, Van Wilgen et al. 2010). I speculate that at Potberg, because of fire suppression, there has been a decline in species diversity and a reduction in competition for resources allowing the C₄ grasses to establish and dominate.

Chapter 6

Synthesis

The fynbos biome is diverse in plant species and genera with high endemism (Manning and Goldblatt 2012). This diversity and endemism is threatened primarily by alien invasive plants and habitat fragmentation (Richardson et al. 1996). Alien species are no longer the only category of biological organism establishing and rapidly spreading beyond historical boundaries, native species can also emulate the functional and structural changes associated with invasions by alien plant species (Nackley et al. 2017). There is evidence from ecosystems around the world that demonstrates that native invasions are widespread and large scale (Bowman and Panton 1995, Nackley et al. 2017). The effects of the native invasive C₄ grass at Potberg, have shared characteristics with alien invasive species, such as changes in population distributions (West et al. 2017) and ecosystem structure as well as impacts on biodiversity (Wigley et al. 2010) The impact of grass invasion in South Africa is overlooked primarily because of the problems currently experienced with woody plant invasions (Milton 2004).

The focus of both research as well as government agencies such as the Working for Water and Working on Fire programmes are primarily on alien invasive trees (Wintle et al. 2011). Relative to tree invasions C₄ grass patches are inconspicuous and easily overlooked and are therefore far more likely to cause damage to the ecosystem without managers being aware. In the absence of previous studies, this thesis explored several aspects of the C₄ grass patches at Potberg including, whether roads act as conduits for C₄ grass colonisation, whether grass patches have impacts on fynbos species diversity and finally what factors contribute to grass colonisation. The question of whether the grass colonisation is old or new was addressed in a companion study (Tucker, 2016, unpublished).

Although road side surveys have often been used to assess the extent of alien tree and grass invasion (Gelbard and Belnap 2003, Christen and Matlack 2006, Benezeth et al. 2008), there have been no South African studies looking at roadsides as conduits for C₄ grass colonising fynbos. I did this along a 12.5 km section of the road in Potberg to determine if roadside plots have higher C₄ grass species than plots away from the road and if an increase in C₄ grass biomass results in a decrease in fynbos species diversity. My results show that while roadside plots do have higher C₄ grass species richness, the Potberg road has not been the main conduit for C₄ grass invasion into the reserve. There are several patches of the C₄ grass at Potberg away from the roadsides including patches of *Cynodon dactylon*, *Eragrostis curvula* and *Setaria*

sphacelata var.torta. My results also show that the higher the biomass and diversity of C₄ grass species in a plot the lower the fynbos species richness demonstrating that C₄ grasses do have a negative effect on fynbos plant species richness. The increase in C₄ grasses demonstrated in my results would suggest that under the correct circumstances roadside could indeed act as conduits for grass invasion.

To determine the factors that influence C₄ grass colonisation into fynbos, a transplant experiment was laid out to examine the influence of slope position, nutrient addition and competition on the survival and growth of the C₄ grass *Setaria sphacelata var.torta*. My transplant experiment results show that there is a significant positive effect on both grass biomass and root shoot ratios with nutrient additions. My results also show that, the grass in unfertilised plots still managed to establish and grow in these infertile podsols consistent with the high water and nitrogen use efficiency of C₄ photosynthesis. Although slope position did not have significant effects on grass biomass, the bottom slope had the highest biomass implying that high moisture sites are most vulnerable to colonisation by *Setaria sphacelata var.torta*. Contrary to my expectation trenching also had a significant effect on grass biomass, *Setaria sphacelata var.torta* in untrenched plots (intact fynbos) had lower biomass than in trenched plots (competition exclusion). These results demonstrate that intact fynbos cannot be easily colonised by C₄ grass and that the patches of the C₄ grass dominant in the area came in when the fynbos vegetation was disturbed probably with farming activities (Tucker, 2016, unpublished). Potberg Nature Reserve was previously used as a farm and only proclaimed as a nature reserve in 1956 (PACGH, no.13.1956) when it was used to build up herds of game animals. Aerial photos indicate strong fence-line contrasts suggesting heavy grazing over most of my study area. Disturbance in the form of trampling and over grazing from the game may have opened spaces in the fynbos that allowed C₄ grasses to colonise (Tucker, 2016, unpublished).

The C₄ grass patches at Potberg may be evidence of a Savanna/Grassland biome westward advancement into the Fynbos biome, potentially as a result of climate change or altered land use practices in the area (Masubelele et al. 2014). Biome shifts have been attributed to various factors such as differing physiological constraints to changing climate (Reu et al. 2011), loss of biodiversity, shifts in rainfall regime (Huang et al. 2001), CO₂ elevation (Bond and Midgley 2000) and historical land use practices (Masubelele et al. 2014). In South Africa, extensive work has been done on biome shifts in the form of bush encroachment into the Savanna/Grassland biomes (Wigley et al. 2009), however very little work has been done on changing boundaries of the fynbos biome. The C₄ grass patches at Potberg might represent ancient outliers of the eastern

grasslands in western shrubland or new colonisation as grassy biomes move westwards into the fynbos biome. The results of a carbon isotope analysis of the C₄ grass patches at Potberg have shown that this is not an old boundary between fynbos and grasslands but a recent one and that C₃ species were the dominant vegetation in the area (Tucker, 2016, unpublished). The C₄ grass patches have not been present for long enough to shift the soil organic matter toward the C₄ isotopic range (Tucker, 2016, unpublished).

It has long been argued that C₄ grasses require warm temperatures during the growing season (Vogel et al. 1978), however temperature alone cannot encourage colonisation by C₄ grasses. The growing season in the Cape is restricted by a lack of rain in the hottest month restricting the establishment of C₄ grasses however; lowland habitats at Potberg have a high-water table throughout the hottest time of the year and may therefore maintain sufficient moisture to sustain C₄ growth. There is also a global increase in atmospheric CO₂ and several studies have shown that elevated CO₂ results in more efficient use of water by plants (Eamus 1991, Morison 1993, Tyree and Alexander 1993). High water and nitrogen use efficiency of C₄ grasses increases their efficiency at elevated CO₂ with low water availability. Reduced transpiration with elevated CO₂ have the additional advantage of reduced stomatal conductance so that each unit of dry matter costs less water to produce (Morison 1993). Thus, elevated CO₂ may be effectively reducing the severity for summer drought and thereby providing the climate conditions conducive for C₄ grasslands to spread westwards, shifts in rainfall seasonality to more summer rain would have the same effect but, as discussed in Chapter 2, there is no evidence for such a shift as yet.

As these grasses have a detrimental effect on fynbos species diversity, it is important to have suitable monitoring methods to minimise the spread of the grass into the surrounding fynbos. This includes manual removal of the grass or the application of herbicides where it threatens areas of particularly high conservation value. Unlike alien invasive trees, invasive grasses are difficult to remove, and the best management recommendation will be to minimise disturbance and keep fynbos as intact as possible to avoid colonisation by C₄ grasses and to inhibit further expansion of grass patches. Disturbance activities such as trampling by large mammals, tractors and vehicles that are used to clear the alien invasive plants must be minimised as any activities that opens up fynbos would encourage the C₄ grasses to colonise fynbos.

Fire is the main driving force of ecological processes in fynbos (Masson and Moll 1987, Cowling and Pierce 1988, Schwilk et al. 1997) and the frequency and intensity of fire is what drives fynbos species recruitment influencing biodiversity (Van Wilgen and Forsyth 1992).

While fynbos is a fire-adapted ecosystem it is crucial to maintain historical fire frequency and intensity. With a return interval of between 10 to 30 years depending on rainfall and topography, fire is an essential driving force in fynbos dynamics, being both inevitable and crucial to maintain species diversity (Kruger and Bigalke 1984, Van Wilgen et al. 2010). Too frequent fire may eliminate species that rely on seed to reproduce whilst less frequent fire will eliminate species with short lifespans that rely on fire to trigger seed release and germination (Van Wilgen and Forsyth 1992) and open spaces for C₄ grass colonisation.

As with alien invasive species, integrating native invasions as part of biodiversity threats is important for conservation in the midst of climate change (West et al. 2017). Suggestions for future research include; surveying areas along the rainfall gradient from warm season rain in the east to cool season rain in the west and; determining if there is a shift in rainfall seasonality with more rainfall at the beginning of the warm season which may be the reason for the C₄ grasses flourishing in fynbos.

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Appendix

Appendix 1: The relative distribution of C₃ and C₄ grass species on both the Roadside and Adjacent plots. N=50 for each plot.

| Grass species | Grass | | Adjacent plots | | Roadside plots | |
|--------------------------------|----------------|----------------|----------------|------------|----------------|------------|
| | C ₃ | C ₄ | Count | Proportion | Count | Proportion |
| <i>Andropogon eucomus</i> | | X | 2 | 0.04 | 1 | 0.01 |
| <i>Aristida diffusa</i> | | X | 5 | 0.1 | 6 | 0.06 |
| <i>Briza maxima</i> | X | | 0 | 0 | 2 | 0.02 |
| <i>Cortaderia selloana</i> | X | | 3 | 0.06 | 0 | 0 |
| <i>Cynodon dactylon</i> | | X | 5 | 0.1 | 26 | 0.24 |
| <i>Enneapogon desvauxii</i> | | X | 0 | 0 | 2 | 0.02 |
| <i>Eragrostis capensis</i> | | X | 3 | 0.06 | 1 | 0.01 |
| <i>Eragrostis curvula</i> | | X | 2 | 0.04 | 22 | 0.21 |
| <i>Fingerhuthia africana</i> | | X | 2 | 0.04 | 1 | 0.01 |
| <i>Merxmüllera disticha</i> | X | | 5 | 0.1 | 8 | 0.7 |
| <i>Melanis repens</i> | | X | 0 | 0 | 1 | 0.01 |
| <i>Pennisetum clandestinum</i> | | X | 0 | 0 | 1 | 0.01 |
| <i>Pentameris airoides</i> | X | | 3 | 0.06 | 3 | 0.03 |
| <i>Pentameris pallida</i> | X | | 2 | 0.04 | 1 | 0.01 |
| <i>Pentameris thunbergii</i> | X | | 6 | 0.12 | 4 | 0.04 |
| <i>Phalaris minor</i> | X | | 0 | 0 | 1 | 0.01 |
| <i>Sporobolus africanus</i> | | X | 0 | 0 | 2 | 0.02 |
| <i>Sporobolus ioclados</i> | | X | 0 | 0 | 3 | 0.03 |
| <i>Stenotaphrum secundatum</i> | | X | 2 | 0.04 | 2 | 0.02 |
| <i>Themeda triandra</i> | | X | 5 | 0.1 | 1 | 0.01 |
| <i>Tribolium uniolae</i> | X | | 5 | 0.1 | 19 | 0.18 |
| No grass | | | 17 | 0.34 | 3 | 0.06 |

Appendix 2: Species list in grass and fynbos plots with their growth forms.

| | |
|------------------------------------|-------|
| | |
| <i>Acacia mearnsii</i> | Tree |
| <i>Adenandra gummifera</i> | Shrub |
| <i>Agathosma cerefolium</i> | Shrub |
| <i>Agathosma ciliaris</i> | Shrub |
| <i>Aristida diffusa</i> | Grass |
| <i>Athanasia trifurcata</i> | Shrub |
| <i>Berzelia lanuginosa</i> | Shrub |
| <i>Berzelia abrotanoides</i> | Shrub |
| <i>Carpobrotus edulis</i> | Herb |
| <i>Chironia baccifera</i> | Shrub |
| <i>Chrysanthemoides monilifera</i> | Shrub |
| <i>cliffortia ruscifolia</i> | Shrub |
| <i>Cliffortia stricta</i> | Shrub |
| <i>Cortaderia selloana</i> | Grass |
| <i>Cynodon dactylon</i> | Grass |
| <i>Dodonaea angustifolia</i> | Shrub |
| <i>Ehrharta calycina</i> | Grass |
| <i>Eragrostis capensis</i> | Grass |
| <i>Eragrostis curvula</i> | Grass |
| <i>Erica ampullacea</i> | Shrub |
| <i>Erica bodkinii</i> | Shrub |
| <i>Erica cafra</i> | Shrub |
| <i>Erica colorans</i> | Shrub |
| <i>Erica colorans</i> | Shrub |
| <i>Erica discolor</i> | Shrub |
| <i>Erica Placentiflora</i> | Shrub |
| <i>Erica plukanetti</i> | Shrub |
| <i>Erica Puberuliflora</i> | Shrub |
| <i>Erica rhopalantha</i> | Shrub |
| <i>Erica seriphiifolia</i> | Shrub |
| <i>Eustachys paspaloides</i> | Shrub |
| <i>Fingerhuthia africana</i> | Grass |
| <i>Gnidia tenella</i> | Shrub |
| <i>Gymnosporia buxifolia</i> | Shrub |
| <i>Helichrysum patulum</i> | Herb |
| <i>Helichrysum teretifolium</i> | Herb |
| <i>Helichrysum apiculatum</i> | Herb |
| <i>Imperata cylindrica</i> | Grass |
| <i>Koeleria capensis</i> | Grass |
| <i>Lachnaea axillaris</i> | Shrub |
| <i>Leucadendron coniferum</i> | Shrub |
| <i>Leucadendron linifolium</i> | Shrub |
| <i>Leucadendron meridianum</i> | Shrub |

| | |
|--------------------------------------|--------|
| Leucadendron salignum | Shrub |
| leucadendron tinctum | Shrub |
| Leucospermum calligerum | Shrub |
| | |
| <i>Melica decumbens</i> | Grass |
| <i>Merxmuellera disticha</i> | Grass |
| <i>Metalasia densa</i> | Shrub |
| <i>Morella quercifolia</i> | Shrub |
| <i>Muraltia filiformis</i> | Shrub |
| <i>Myrsine africana</i> | Shrub |
| <i>Osyris compressa</i> | Shrub |
| <i>Osyris speciosa</i> | Shrub |
| <i>Othonna dentata</i> | Herb |
| <i>Passerina rigida</i> | Shrub |
| <i>Pelargonium betulinum</i> | Herb |
| <i>Penaea mucronata</i> | Shrub |
| <i>Pentaschistis airoides</i> | Grass |
| <i>Pentaschistis pallida</i> | Grass |
| <i>Pentaschistis thunbergii</i> | Grass |
| <i>Petalacte coronata</i> | Shrub |
| <i>Peucedanum galbanum</i> | Shrub |
| <i>Phaenocoma Prolifera</i> | Shrub |
| <i>Phylica Purpurea</i> | Shrub |
| <i>Protea neriifolia</i> | Shrub |
| <i>Protea Repens</i> | Shrub |
| <i>Protea Susannae</i> | Shrub |
| <i>Retzia capensis</i> | Shrub |
| <i>Rhus glauca</i> | Shrub |
| <i>Serruria fasciflora</i> | Shrub |
| <i>setaria sphacelata var. torta</i> | grass |
| <i>Sideroxylon Inerme</i> | Shrub |
| <i>Sporobolus africanus</i> | Grass |
| <i>staberoha banksii</i> | Restio |
| <i>Stenotaphrum secundatum</i> | Grass |
| <i>Stilbe ericoides</i> | Shrub |
| <i>Stoebe Plumosa</i> | Shrub |
| <i>Syncarpha Paniculata</i> | Shrub |
| <i>Thamnochortus cinereus</i> | Restio |
| <i>Themeda triandra</i> | Grass |
| <i>Tribolium uniolae</i> | Grass |
| <i>Trichocephalus stipularis</i> | Shrub |
| <i>Viscum capenses</i> | Shrub |