SOIL NITROGEN AND PHOSPHORUS DEPLETION AS A MEANS OF RESTORING DEGRADED LOWLAND FYNBOS ECOSYSTEMS INVADED BY ALIEN GRASSES

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Abstract

Much of South African lowland fynbos vegetation has been transformed by agriculture and invasive alien grass species. The artificial reduction of plant available N and P levels in soils, through the addition of carbon and calcium respectively, may provide a means of retarding the growth of alien grasses stimulated by soil nutrient enrichment. Furthermore, the competitive advantage of native lowland fynbos species adapted to nutrient impoverished soils may be increased by these additions.

The above premise was tested in both field- and greenhouse-based trials by applying systemic and contact herbicides to reduce the large alien invasive grass biomass. This was followed by the addition of C as sucrose and Ca as gypsum to reduce plant available N and P respectively in the soils. The effects of these combined herbicide and soil nutrient amendment treatments on plant physiology and growth were examined in both resident alien and indigenous species and in several herbaceous and woody native species introduced as seeds and seedlings. Also, soils sampled from the different treatments in both trials were chemically analyzed. There was a total absence of seedling recruitment from seeds of all 9 indigenous species sown into soils in the field-based trial while introduced juveniles of another 9 indigenous species displayed a high mortality during the dry summer season. These detrimental effects were less severe in the greenhouse-based trial which received more regular watering and where successful seedling recruitment from seeds sown occurred in four indigenous species. Sucrose additions, both exclusively and in combination with gypsum, caused significant reductions in foliar chlorophyll, photosystem II (PSII) function and above-ground biomass of most resident and introduced alien and indigenous species. These reductions were less prominent where herbicides were applied, a possible consequence of N and P supplementation of soils by the decomposing plant biomass. This was supported by the elevated soil K, Na and N concentrations measured in soils where contact and systemic herbicides were applied. However, no significant changes in soil N or P were apparent following sucrose and gypsum additions respectively, the latter attributed to the acidic soils which precluded the formation of insoluble P complexes.

A second study tested the hypothesis that exogenous sucrose addition to soils inhibits plant growth by stimulating soil microbial biomass which accumulates soil nitrogen rendering it unavailable to plants. Two native, early seral species (*Dimorphotheca pluvialus* (L.) Moench and *Ursinia anthemoides* (L) Poir. *subsp anthemoides*) were cultured in heat sterilized (220^0C for 72 hours) and non-sterilized soils in a greenhouse under four different levels of sucrose (0, 100, 200 and 300 g m^{-2}) supplied monthly over a four-month active growing period. Foliar chlorophyll

contents, photosystem II (PSII) efficiencies, shoot and root lengths and dry mass, inflorescence numbers and N and P contents were measured in the plants, and N and P contents and bacterial cell and coliform numbers analyzed in the soils. Both *D. pluvialis* and *U. anthemoides* displayed significant reductions in PSII efficiency, chlorophyll content, accumulation of biomass and N and P in response increased levels of sucrose, which initially seemingly supported the hypothesis as these reductions were of substantially greater magnitude in plants cultivated in non-sterilized than sterilized soils. Despite this, there was no evidence of any significant increases in bacterial and coliform cell numbers in response to increased levels of sucrose supplied or any significant reductions in soil N and P contents following sucrose additions in both sterile and non-sterilized soils. Greater numbers of bacteria and coliforms were measured in sterilized than non-sterilized soils which corresponded with reduced soil N contents but these were not reflected in like changes in plant PSII efficiency and growth and total amounts of N taken up by plants which displayed massive increases in sterilized soils. The findings did not support the hypothesis and pointed to an abiotic mechanism of sucrose inhibition of plant photosynthesis and growth.

The study concludes that the suitability of adding sucrose and gypsum to degraded renosterveld ecosystem soils to promote the competitiveness of native taxa against alien grasses is dubious. Other restoration alternatives such as natural re-colonization, transfer of soils containing viable seeds from pristine communities and top soil removal should be considered.

Opsomming

Baie van Suid Afrika se laagland fynbos plantegroei is verander deur landbou en uitheemse indringer grasspesies. Die kunsmatige reduksie van plantbeskikbare N en P vlakke in die grond deur die toevoeging van koolstof en kalsium onderskeidelik, kan 'n metode wees om die groei van indringer grasse te vertraag, wat gestimuleer word deur grondvoedingstofverryking. Die kompeterende voordeel van die inheemse laagland fynbosspesies wat aangepas is tot voedingstofarme grond kan verhoog word deur die toevoegings.

Bogenoemde postulaat is in beide die veld- en die glashuis-gebaseerde eksperimente getoets deur die aanwending van sistemiese en kontak onkruiddoder om die groot indringer grasbiomassa te verminder gevolg deur die byvoeging van C as sukrose en Ca as gips om die plantbeskibare N en P onderskeidelik te verminder in die grond. Die effekte van die gekombineerde onkruiddoder en grondvoedingstof verbeteringsbehandelings op die fisiologie en groei van die plante is ondersoek in beide inheemse- en residente indringerspesies asook in verskeie kruidagtige- en houtagtige inheemse spesies wat aangeplant was as sade en saailinge. Grondmonsters van die verskillende behandelings in beide studies was versamel en was chemies geanaliseer. Daar was 'n definitiewe afwesigheid van nuwe saailinge van sade van al nege indringerspesies wat gesaai was in grond in die veldgebaseerde studie, en saailinge van nog nege inheemse spesies het 'n hoë mortaliteit getoon gedurende die droë somerseisoen. Hierdie skadelike effekte was minder ernstig in die glashuisgebaseerde studie wat meer benat was, en waar nuwe saailinge suksesvol geproduseer was deur sade in vier inheemse spesies. Sukrose byvoegings, beide uitgesluit en in kombinasie met gips, het 'n afname in blaarchlorofil, fotosisteem II en bogrondse biomassa van die meeste van die residente en aangeplante indringer- en inheemse spesies getoon. Hierdie afnames was minder prominent waar onkruiddoder aangewend was, 'n moontlke oorsaak van N en P aanvulling van grond deur die verrottende plantbiomassa. Dit word ondersteun deur verghoogde grond K, Na en N konsentrasies, gemeet in grond waar kontak en sistemiese onkruiddoder toegevoeg was. Geen noemenswaardige veranderinge in grond N of P was sigbaar na byvoeging van sukrose en gips onderskeidelik nie. Laasgenoemde het bygedra tot suuragtige grond wat die formasie van onoplosbare P komplekse verkom het.

'n Tweede studie het die hipotese getoets waar eksogene sukrose byvoeging tot grond plantegroei inhibeer deur die grond mikrobe biomassa te stimuleer wat akkumuleer wat in grond stikstof en dit nie beskikbaar maak vir plante nie.Twee inheemse vroeë intermediêre stadium spesies (*Dimorphotheca pluvialus* (L.) Moench en *Ursinia anthemoides* (L) Poir*. subsp*

anthemoides) was gekweek in hitte gesteriliseerde grond   (220^0 C vir 72 uur) en in nie-gesteriliseerde grond in 'n glashuis onder vier verskillende vlakke van van sukrose (0, 100, 200 en 300 g m^{-2}) bygevoeg maandeliks oor 'n 4 maande aktiewe groei periode. Blaarchlorofilinhoud, fotosisteem II (FS II) doeltreffendheid, groeipunt en wortel lengte en droë massa, blomgetalle en N en P inhoud was gemeet in die plante sowel as N en P inhoud en bakteriële sel en kolivorm getalle was geanaliseer in die grond. Beide *D. pluvialis* en *U . anthemoides* het 'n afname getoon in FS II doeltreffendheid, chlorofilinhoud, biomassa akkumulasie, N en P response op verhoogde vlakke van sukrose, wat aanvanklik aangetoon het dat dit die hipotese ondersteun want hierdie afnames wat heelwat groter in plante wat gekweek was in ongesteriliseerde grond as in gesteriliseerde grond. Daar was geen toename in baketriële en kolivorm sel getalle in rssponse tot verhoogde vlakke van sukrose byvoegings of enige noemenswaardige in grond N en P inhoud na byvoeging van sukrose in beide steriele en nie-steriele grond nie. Groot getalle bakterieë en kolivorme was gemeet in gesteriliseerde grond as in ongesteriliseerde grond. Dit korrespondeer met verminderde grond N inhoud maar dit was nie gereflekteer in veranderinge in plant FS II doeltreffendheid, groei en die totale hoeveelhede N wat opgeneem was deur plante wat 'n massiewe toename getoon het ongesteriliseerde grond nie. Hierdie bevindings het nie die hipotese ondersteun nie en het gewys na 'n abiotiese meganisme van sukrose inhibisie van plant fotosintese en groei.

Die studie lei dus af dat die geskiktheid om sukrose en gips by te voeg tot gedegradeerde renosterveld ekosisteemgrond om kompetisie tussen inheemse plante en indringer grasse te promoveer, twyfelagtig is. Ander restorasie alternatiewe soos natuurlike herkolonisasie, oordrag van grond wat lewensvatbare sade bevat van onbeskadigde gemeenskappe en bogrond verwydering word oorweeg.

Acknowledgements

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Chapter 1

Study ecosystem and site, research objectives, and thesis structure

1.1. Background and rationale

The research presented in this thesis was undertaken in the field of ecological restoration, a discipline that deals with recovery of an ecosystem that has been degraded, damaged, transformed or entirely destroyed by direct or indirect human activities (SER 2004). As defined by SER (2004), restoration attempts to return an ecosystem to its historic trajectory, namely a state where biotic and abiotic resources within an ecosystem can sufficiently develop structurally and functionally without further assistance. An ecosystem is said to have been restored if it demonstrates resilience to normal environmental stress and disturbances.

As outlined in the definition above, restoration is a recovery response to ecological degradation. One main cause of ecosystem degradation is biological invasion (Mack et al. 2000; Krupa 2006; Milton 2004; Suding et al. 2004). This poses a serious threat to biodiversity due to the ability of invasive species to modify native communities by either out-competing native plants for nutrients, water and sunlight, or by modifying ecosystem trophic structure, ecosystem productivity, fire frequency and intensity (Corbin & D'Antonio 2004; Mack et al. 2000; Blignaut et al. 2007).

As a result, biological invaders have seriously impacted pattern and process in native ecosystems with the most notable example being the invasion of the entire ecosystem of California grasslands in the United States of America with annual Mediterranean grasses of *Schismus barbatus* and *Bromus tectorum* (Krupa 2006). These grasses have transformed nearly 10 million hectares of native grasslands, thereby impacting negatively on the country's economy (Corbin et al. 2004; Krupa 2006). There are also examples in Africa of large-scale invasions by alien grasses from other continents, or from other areas within the continent. These include the establishment of several European annual grasses in Mediterranean climate regions of South Africa and the recent spread of perennial grasses of South American, Central and North African origin in southern Africa (Milton 2004). In southern Africa, invasive grasses are especially prevalent in natural ecosystems along the West Coast of South Africa, including wetlands (Bromilow 2001) and along roadsides (Milton & Dean 1998; Milton et al. 1998) which can be viewed as conduits for invasion. This is a cause for concern, especially in terms of the wildflower diversity, which forms the basis of a growing, lucrative nature-based tourist industry in a

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Mediterranean-climate region unique in terms of its rich floristic diversity and endemism (Goldblatt & Manning 2000).

Apart from the much documented ecological consequences of biological invaders (D'Antonio & Vitousek 1992; Bluthmenthal et al. 2003; Aronson et al. 2007; Miller & Hobbs 2007), social and economic effects are also a cause of concern in dealing with alien invaders. When these modify ecosystem processes and impact local ecosystem services, considerable costs are incurred because of the loss of direct economic benefits, notably income generated from tourism as well as resultant costs associated with damage to and restoration of such ecosystems (Blignaut et al. 2007; Clewell & Aronson 2007). For example alien invasive species have cost South Africa millions of Rands in clearing operations (Nel et al. 2004) while concurrently depriving the country of revenue derived from nature based ecotourism and the lucrative wildflower industry in the Cape Floristic Region (Turpie 2004).

In this regard restoration of invaded areas should be made a priority by all governments so as to reduce biodiversity loss. The Convention on Biological Diversity (CBD) has acknowledged that invasive species are a threat to ecosystems and as a result there is need to control and eradicate them. Therefore this thesis is centered on managing alien grass infestations through nutrient manipulation as a means of restoring natural biodiversity.

1.2. Study region

The Cape Floristic Region (CFR) is one of the most biologically diverse regions on earth (Goldblatt & Manning 2002). Considered as one of six global Floral Kingdoms, this relatively small region of approximately 90 000km^2, situated at the southwestern tip of Africa, is home to about 9 000 vascular plant species, 69 percent of which are endemic (Cowling et al. 1996; Holmes 2001). The CFR covers the Mediterranean climate region of South Africa's Western Cape Province, and extends eastward into the Eastern Cape Province, a transitional zone between the winter-rainfall region to the west and the summer-rainfall region to the east (Cowling et al. 1996).

The major plant cover in the Cape Floristic Region is a shrubland known as fynbos, which comes from an Afrikaans word meaning "fine bush". Fynbos is made up of four growth forms namely the proteoids, ericoids, restoids and geophytes (Cowling et al. 1996; Rebelo 1996). Fynbos occurs predominantly on well leached, infertile soils (Rebelo 1996; Cowling & Holmes 1992), but in areas of high rainfall (600 to 800 mm/year) granites and even shales become sufficiently leached to support Asteraceous fynbos (Cowling & Richardson 1995). Below 200 mm fynbos is replaced by arid Succulent Karoo vegetation (Rebelo 1996). Fire is a major influence

on fynbos community processes. Fire frequencies range between 6 and 45 years and are necessary to sustain plant species diversity (Moll & Bossi 1983; Moll et al. 1984; Cowling & Richardson 1995). In the absence of fire, fynbos becomes senescent and forest and thicket elements begin invading.

Moll & Bossi (1983) described four distinctive fynbos vegetation types, namely renosterveld, western strandveld, mesic mountain fynbos and sandplain fynbos, though more recently fynbos has been reclassified into more detailed vegetation units based on their floristic composition and underlying geology (Mucina & Rutherford 2006).

Generally the whole CFR is seriously threatened by human activities, especially agriculture that has seen renosterveld and sandplain fynbos reduced by 48% and 83% of their original extent, respectively (Rouget & Richardson 2003a). Even in the mountains, where impoverished soils previously limited agriculture, farming based on indigenous crops such as rooibos tea (*Aspalathus linearis*), honeybush tea (*Cyclopia* spp.), and cut flowers (mainly Proteaceae) is rapidly encroaching on natural habitat. Nonetheless, the greatest threat to the Cape Flora overall is undoubtedly its invasion by alien plants (Richardson et al. 1992), mainly originating from other Mediterranean-type climate regions such as southern Australia (wattles [*Acacia* spp.], myrtle [*Leptospermum laevigatum*]), the Mediterranean Basin (*Pinus halepensis*, *P. pinaster*), and California (*P. radiata*). Originally introduced to supplement the meager Cape tree flora, these fire-adapted woody perennials have invaded about 70% of both mountain and lowland fynbos (Rouget & Richardson 2003a). Apart from these species, several invasive perennial grasses like thatching grass (*Hyparrhenia hirta*), kikuyu (*Pennisetum clandestinum*) and weeping love grass (*Eragrostis curvula*) as well as annual invasive grasses of wild oats (*Avena fatua*), rye grass (*Lolium sp*), quaking grass (*Briza sp*), ripgut/predikantsluis (*Bromus diandrus*), and rats tail (*Vulpia myuros*) have also invaded the region especially ex-arable lands (Milton 2004). All in all, some 33% of the Cape Floristic Region has been transformed by agriculture, urbanization, and dense stands of alien plants (Rouget & Richardson 2003a).

1.3. Study ecosystem

The study ecosystem was an area currently classified as Swartland Alluvium fynbos by Mucina & Rutherford (2006), however previously classified as renosterveld by Moll et al. (1984) and West Coast renosterveld by Rebelo (1996). The vegetation of Swartland Alluvium fynbos is dominated by a matrix of low, evergreen shrubland with emergent sparse, moderately tall shrubs and a conspicuous graminoid layer (Mucina & Rutherford 2006). Proteoid, Restioid and Asteraceous fynbos types are dominant, with closed-scrub fynbos common along the river

courses. With a mean annual rainfall of 655 mm, varying from 350-980 mm, this high rainfall has resulted in alluvial gravel soils dominating the area (Mucina & Rutherford 2006). Renosterveld as the areas was previously classified (Moll et al. 1984; Rebelo 1996) is dominated by an evergreen, fire-prone low shrubland dominated by small, cupressoid-leaved, evergreen Asteraceous shrubs (principally renosterbos) with an understorey of grasses (Poaceae) and a high biomass and diversity of geophytes (Mucina & Rutherford 2006). Apart from the Asteraceae, other important shrub families include Fabaceae, Malvaceae, Poaceae and Iridaceae (Von Hase et al. 2003).

Fine textured, shale-derived soils characterize renosterveld (Rebelo 1996; Mucina & Rutherford 2006). Rainfall in this vegetation type is moderate, between 350 to 650 mm/year (Cowling & Holmes 1992). At a rainfall exceeding 800 mm/year, renosterveld in general is replaced by mesic mountain fynbos (Walton 2006; Holmes 2001) whilst at less than 250 mm/year Succulent Karoo vegetation dominates (Holmes & Cowling 1997; Holmes & Richardson 1999; Holmes 2001). Mature renosterveld comprises an upper stratum of Asteraceous shrubs, a middle stratum of dwarf shrubs, and a lower stratum of grasses, forbs, geophytes and annuals (Adamson 1938; Boucher & Moll 1981).

Moll et al. (1984) initially distinguished four renosterveld classes, namely renosterveld of the West Coast centre, renosterveld of the South Coast centre, inland renosterveld of the Mountain centre and renosterveld of the Eastern centre. However, the more recent revisions have created and added new vegetation units to the existing renosterveld classes. The original classification of West Coast renosterveld by Moll et al. (1984) was subsequently reclassified by Rebelo (1996) as Swartland renosterveld whilst Mucina & Rutherford (2006) re-classified Swartland renosterveld into two vegetation units, namely Swartland Shale renosterveld and Swartland Alluvium fynbos vegetation. Therefore our study site was directly adjacent to an alluvium fynbos vegetation community making the study ecosystem more of an ecotone or transitional zone.

West Coast lowland vegetation types, particularly the Swartland Alluvium fynbos and Swartland Shale renosterveld, are highly fragmented due to land transformation for development of crops, vineyards and orchards (Von Hase et al. 2003). This has reduced the existence of both vegetation types to fewer than 35% and 10% of their original areas respectively (Rebelo et al. 2006). The remaining extent of Swartland Alluvium fynbos and Swartland Shale renosterveld are nearly 18 000 isolated patches (von Hase et al. 2003). Most of those fragments are small patches (von Hase et al. 2003; Rouget et al. 2004), but eight of these have areas of more than 1 000 ha (von Hase et al. 2003), the largest being approximately 7 400 ha in a West Coast

lowland vegetation types mosaic (Krug et al. 2004b). Fragmentation, nutrient enrichment from the surrounding farmlands, invasions by alien plants particularly European winter-growing annual grasses (Milton 2004; van Rooyen 2004; Shiponeni & Milton 2006) and overgrazing are cited as the main drivers of West Coast lowland degradation.

1.4. Study site

The study site was an area with the geographic coordinates (33°44"67' S to 33°44"72' S; 19°03"13' E to 19°03"17' E) situated in the Elandsberg Private Nature Reserve. The reserve occurs on Bartholomew's Klip farm near Bo-Hermon, approximately 25 km north of Wellington in the Cape winelands district of Western Cape Province of South Africa (Figure 1.1). It was proclaimed a protected area in 1973, initially to protect the endangered geometric tortoise (*Psammobates geometricus*) and in 1988 it was declared a natural Heritage site (Midoko-Iponga et al. 2005). The reserve is registered as a private nature conservation area with CapeNature and is fenced to prevent large game movement to the surrounding fields, however it is open to the Elandskloof mountains on the eastern side of the reserve.

The reserve currently covers 3 900 ha of natural veld, which comprises Swartland Shale renosterveld and Swartland Alluvium fynbos (Figure 1.1). Swartland Alluvium fynbos is dominant on finer sediments in areas adjacent to the Elandskloof mountains. It occurs mainly on alluvial gravel and is critically endangered with more than 70% already transformed for vineyards, olive orchards, pine plantations and urban settlements (Mucina & Rutherford 2006). Mucina & Rutherford (2006) described Alluvium fynbos as a high rainfall version of Alluvium renosterveld, the major difference being the coarser nature of the sediments and greater leaching due a higher rainfall. Dominant plant forms are Asteraceous and Proteoid shrubs, such as *Leucadendron chamaelea* and *L. corymbosum,* and Restioids. Clay soils derived from Malmesbury group shales (specifically the Porterville formation in the north and east and the Moorreesburg formation in the west) underlie Swartland Shale renosterveld (Mucina & Rutherford 2006) which is found at lower elevation and also rated as critically endangered with more than 90% of its area totally transformed. The disturbed areas (an abandoned agricultural field) adjacent to Swartland Shale renosterveld are dominated by *Athanasia trifurcata* and *Otholobium hirtum* with perennial grass *Cynodon dactylon* occurring in abundance.
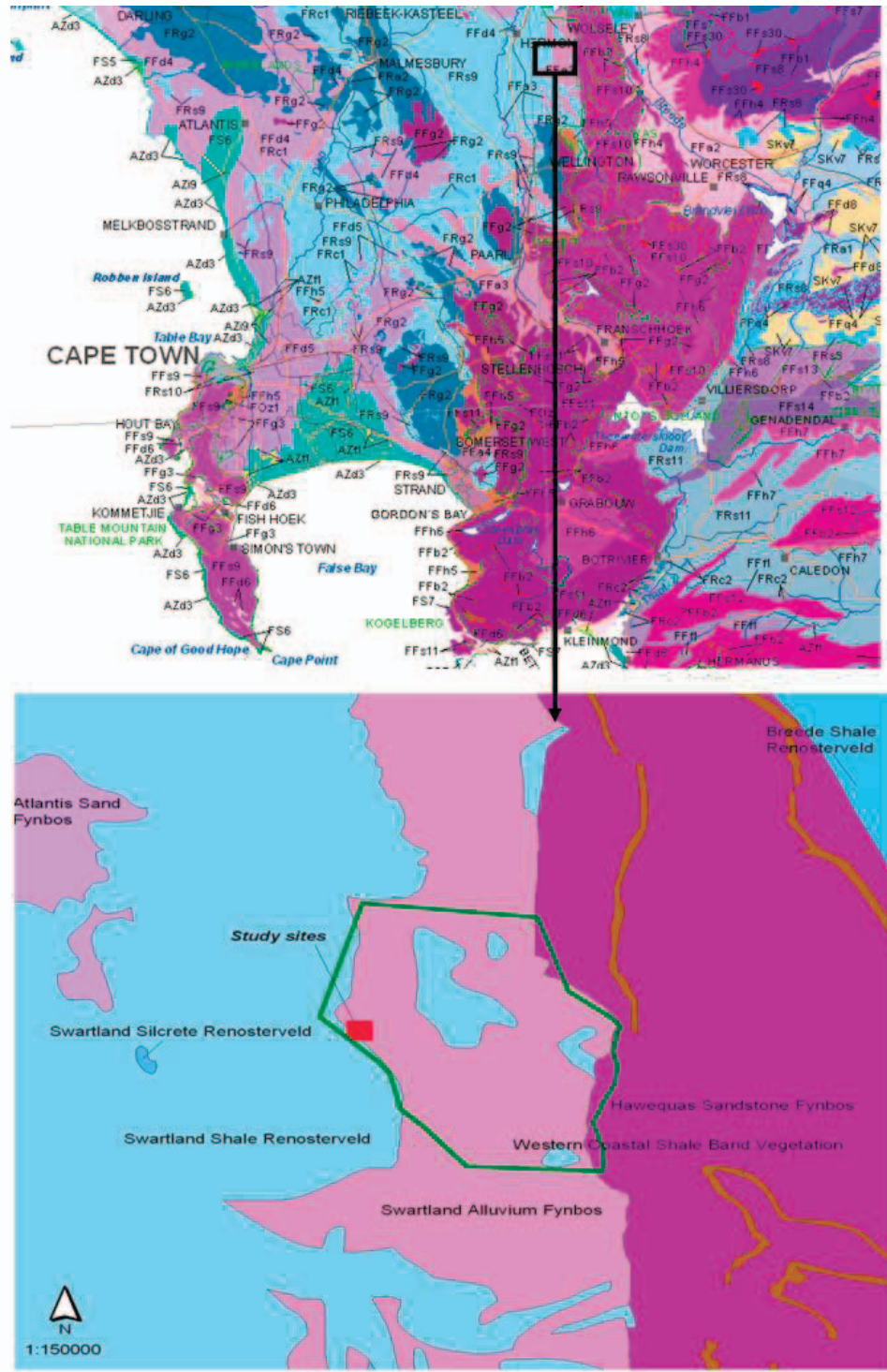
Figure 1.1. Location of experimental field site in the Elandsberg Private Nature Reserve and associated fynbos and renosterveld vegetation units (Mucina & Rutherford 2006).

Some 2 600 ha adjacent to the reserve is used for farming which include sheep and cattle rearing, wheat, oats, barley and other feeding crops. Fertilizer runoff from surrounding agricultural areas could explain the proliferation of invasive grasses at the study site. The most prominent alien grasses at the site include the European winter-growing annual grasses, *Briza maxima* and *B. minor* (Midoko-iponga et al. 2005; Milton 2004), which are believed to have been introduced into South Africa from Italy's Mediterranean climate as ornamentals (Bromilow 2001) for use in flower arrangements (Van Oudtshoorn 2002). Other common invasive grasses like *Lolium multiflorum* and *Avena fatua*, introduced as contaminants of wheat seed, are also present at the site. These highly competitive species are difficult to control as their seed can remain dormant for up to nine years (Milton 2004). *L. multiflorum* is also susceptible to infection by toxic nematodes such as *Anguina agrostis* and bacteria such as *Corynebacterium rathayi* which poison livestock (Milton 2004). *Cynodon Dactylon,* also present at the site, is referred to as a cosmopolitan weed of the world (Harlan and de Wet 1969), and is believed to have originated from Eurasia (Harlan and de Wet 1969; Harlan et al. 1970d), with its center of evolution being the geographic area extending from western Pakistan to Turkey (Wu et al. 2004). However Chippindall (1955), Clayton & Renvoize (1986) and Van Oudtshoorn (1992) considered *C. dactylon* to have originated from East Africa.

1.5. Thesis objective and structure

The thesis examines the practicality of applying chemicals that reduce available nitrogen and phosphorus levels in soils in combination with herbicides to limit the growth of alien grasses. The broader objective is to promote the competitiveness of native taxa adapted to nutrient impoverished soils, thereby restoring natural biodiversity. The thesis comprises four chapters, namely a literature review (Chapter 2), two analytical studies (Chapters 3 and 4), each presented in condensed forms for publication in scientific Journal of Restoration Ecology, and a general discussion and conclusions (Chapter 5). All references in this thesis are cited according to Journal of Restoration Ecology format.

1.6. Overview of thesis Chapters

1.6.1. Literature review

Ecologists and restoration practitioners have become increasingly interested in the effects that invading species have on ecosystem processes so as to develop best-practice restoration guidelines in such ecosystems (Corbin & D'Antonio 2004). Therefore, chapter 2 reviews existing

published information (125 articles appearing in scientific journals, congress proceedings and book chapters) on the causes, effects and spread of invasive species and means of restoring degraded ecosystems.

1.6.2. Soil nutrient depletion as a restoration aid in a South African lowland fynbos ecosystem: an empirical appraisal

Chapter 3 examines whether the addition of sucrose and gypsum to soils to reduce soil nitrogen and phosphorus availability have greater negative impacts on the physiology and growth of alien grasses than indigenous species. It also examines whether the additions of these chemicals cause a depletion in soil N and P content and alterations in other elemental concentrations in soils. Experiments were conducted under natural conditions in the field and also at a reduced scale in a greenhouse to ascertain whether the latter could be used as a reliable and less costly surrogate of the former.

1.6.3. Do sucrose additions inhibit plant growth by depleting soil N?

Chapter 4 examines whether the inhibition of plant growth by additions of carbon as sucrose to soils do result from increased microbial activity (Blumenthal et al. 2003) that lead to decreased rates of net N mineralization (Johnson & Edwards 1979) and nitrification (Gilliam et al. 2005) and reduced concentrations of ammonium and nitrate in soils (Török et al. 2000).

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CHAPTER 2

A review of alien grass invasions and restoration options

2.1. Biological invasion and global change

Biological invasions are a leading cause of species extinctions and biotic homogenization worldwide (Sala et al. 2000; Perrings et al. 2000). Serious ecological, economic and social consequences result from invasion of natural ecosystems by foreign biological organisms (Perrings et al. 2000; McNeely et al. 2001). In several countries, the costs caused by biological invasions are enormous. For instance, the costs of invasive species' impacts are estimated to amount to about 1% of New Zealand's GDP (Bertram 1999). Although *Acacia* species have generated income through use as timber and firewood in South Africa (Turpie & Heydenrych 2000; Wit et al. 2001), they have negatively affected water supplies, justifying government expenditures of US$40 million per year for manual and chemical control. Globally, costs of alien invasive species on natural ecosystems have been estimated to exceed the total economic output of the entire African continent (Mooney & Hobbs 2000). With global temperatures on the increase, predictions are that biological invasions will intensify (Mooney & Hobbs 2000). Therefore an integrated approach that amalgamates the best available scientific management policy and strategies is needed to minimize impacts of biological invaders.

The main reason why biological invasions cause wide-range impacts lies in their ability to traverse natural barriers that formerly limited their dispersal (McNeely 2005). This has been aided by human movement across the globe with the advance of aerial, maritime and road travel. It is from such human movement and their products that alien invasive plants have been transported into novel environments (McNeely 2005). North American nurseries, for example, offer nearly 60 000 plant species to a global market each year (Mitchell et al. 2006; McNeely 2005) with a consequent side effect of species being transported into new habitats.

Biological invasion together with climate change, habitat destruction, overexploitation and pollution have been identified as the major drivers of biodiversity loss at the global scale, and are a source of concern for human well-being (Chown & Gaston 2008). This shows that understanding how these drivers operate and interact and how they might be mitigated are among the most pressing questions facing humanity. Mediterranean climate ecosystems are especially sensitive to biodiversity loss induced by biological invasions (Sala et al. 2000) which in turn have the potential to transform entire ecosystem processes through feed-back effects on

other ecosystem components (Dukes & Mooney 1999), this making them a particularly important threat to biodiversity loss (Chown & Gaston 2008; Richardson et al. 2000).

2.2. Invasive grasses as a global and regional problem

Grasses have been identified as a particular set of invasive species that collectively threaten regional and even global aspects of ecosystem function (D'Antonio & Vitousek 1992; Knapp 1996). They are exceptionally successful world travelers, particularly in livestock-based economies. For example, of the 580 species of alien grasses present in the British Isles, 430 are thought to have been brought there in imported wool, 95 in imported grains and seeds, and 55 as horticultural introductions (Ryves et al. 1996).

Numerous examples of alien grass invasions are found on all continents. Examples include the infestation of northern Australian savanna by Gamba grass (*Andropogon gayanus*), a tall perennial grass originating from Africa (Rossiter et al. 2003). Gamba grass has fuel loads which are seven times higher than that of Australian native grasses (Rossiter et al. 2003), making it a serious threat to Australia's northern savanna. This alien grass also has the potential to alter vegetation structure and initiate a grass-fire cycle that promotes its further expansion. Most perennial grasses of African origin were introduced into Australia as livestock forage. Typical examples include *Pennisetum polystachyon* (Mission grass) found in northern Australia and *Ehrharta longiflora* (African veldt grass) found in coastal and southern Australia (Rossiter et al. 2003).

In North America, *Centaurea diffusa* (Diffuse knapweed) and *Bromus tectorum* (Cheatgrass) are among the most widespread invaders of the prairies (Suding et al. 2004; Krupa 2006). When dominant, both *C. diffusa* and *B. tectorum* are able to shift plant-to-plant interactions through allelochemical, microbial and resource depletion mechanisms (Callaway & Aschehoug 2000). D'Antonio & Vitousek (1992) identified *B. mollis*, *B. arborea*, *B. tectorum* and *B. diandrus* as dominant invaders of grasslands in California where they have replaced grazing intolerant native bunchgrasses. Other European annual grasses like *Poa pratensis* (Kentucky bluegrass) and *B. inermis* are also common invaders of the disturbed Great Plains region including Canada (D'Antonio & Vitousek 1992). In the Hawaiian woodlands of the Hawaii Volcano National Park, exotic grasses like *Milinis minutifora* which have a high fuel load have altered plant species composition resulting in both structural and functional changes to plant communities in the area (Mack & D'Antonio 2003).

In Central and South America, particularly in Brazil, Colombia and Venezuela, African grasses introduced as forage have successfully established themselves, particularly as climatic

conditions in these areas are similar to those in Africa (Pivello et al. 1999). Their success is due mainly to them having developed perennial organs near or below-ground that provides them with a competitive advantage over native South American grasses for water and nutrient acquisition (Pivello et al. 1999; D'Antonio & Vitousek 1992). Other African grasses that have invaded natural ecosystems in South America include *Hyparrahenia rufa* (Jaragua grass) and *M. minutiflora* (Molasses grass), both of which have displaced native grasses like *Trachypodon plumosus* in Brazil (D'Antonio & Vitousek 1992; Pivello et al. 1999).

In Europe, particularly in Britain and Germany, the smooth cord-grass *Spartina alterniflora* was introduced around 1870 from North America causing huge ecological, social and economic effects to these countries (D'Antonio & Vitousek 1992) whilst in France's Mont Saint-Michel Bay, salt marshes have been invaded by the alien species *Elymus athericus* (Pétillon et al. 2005).

In southern Africa, the giant reed (*Arundo donax*) is the most widespread and abundant invasive grass (Milton 2004). South American tussock grasses *Cortaderia selloana* and *Cortaderia jubata* (Pampas grasses) which were originally planted for decoration and mine dump stabilization as well as several other perennial grasses like *Nassella tenuissima, Pennisetum setaceum* and *Pharadendron villosum* are also currently classified as serious threats to southern African ecosystems especially in the Cape Floristic Region (Milton 2004).

2.3. Hypotheses explaining success of alien invasive grasses

The most straightforward explanation for the establishment and proliferation of alien invasive grasses is the enemy release hypothesis (Wolfe 2002; Mitchell et al. 2006). This postulates that the impact of an alien invasive species, measured as individual size, population abundance, or propensity to displace native species, is related to the scarcity of its natural enemies in the new habitat (Keane and Crawley 2002; Mitchell & Power 2003). Current studies supporting this hypothesis indicate that introduced species are exposed to fewer pathogens and disease causing insects in novel habitats (Mitchell & Power 2003).

Beside this hypothesis, several other ecological hypotheses have been postulated to explain why alien invasive grasses succeed in novel environments. An evolutionary corollary of the enemy release hypothesis is the evolution of increased competitive ability hypothesis (EICA). This proposes that when few or no natural enemies of an alien plant species are present, it will direct less energy towards defence mechanisms and more to growth and propagation thereby improving its competitive ability (Mitchell & Power 2003; Mitchell et al. 2006). Another hypothesis is the so called biotic resistant hypothesis which states that reduced competition from native taxa in disturbed natural communities allows the establishment of the alien invasive species (Keane &

Crawley 2002). The mutualist facilitation hypothesis argues that replacement of lost mutualists from a plant's native range with new mutualists in the introduced range is key to an invaders establishment and spread (Keane & Crawley 2002; Mitchell & Power 2003). Shifts in mutualist species composition from plants native to introduced ranges are pervasive, and the acquisition of new mutualists is often essential for the establishment of introduced populations (Keane & Crawley 2002; Mitchell & Power 2003).

The empty niche and novel hypotheses argue that invaders succeed because they are able to acquire resources by avoiding competition. The empty niche hypothesis states that invader success is greater when species are introduced into communities where they can take advantage of under utilized resources (Mitchell & Power 2003). This implies that species are limited by competitors that do utilize these resources and in absence of competitors invaders will succeed. Thus, introduced species benefit from a difference in the species composition of competitors relative to that in their native range (Mack et al. 2000). Finally, the novel weapon hypothesis argues that some invaders are more successful in novel areas than in their native range because competitors in their native range have evolved to tolerate allelopathic compounds while competitors in the introduced range lack such tolerance (Mitchell et al. 2006). Allelopathy is considered as a negative effect of one plant on another through the release of chemical compounds into the environment (Mitchell et al. 2006).

2.4. Spread and impacts of invasive grasses

The spread and dominance of invasive grasses can be attributed to a number of mechanisms, with humans being the principal cause for the rapid spread of alien invasive species (McNeely et al. 2001). Apart from humans being the main facilitator for movement of invasive seeds, be it accidentally or deliberate (Knapp 1996), seeds can also be transported on the hide of grazing animals (Shmida & Ellner 1983; Knapp 1996), and in the dung of domestic livestock and wildlife (Davidse 1986; Malo & Suarez 1995; Shiponeni 2003) as well as in forage/hay and other plant products. Also, rangeland deterioration caused by ploughing, vegetation clearing and burning, soil nutrient enrichment from fertilizer run-off and nitrogen-fixing leguminous species (Milton 2004), and grazing that tends to be more intensive in small habitat fragments (Kemper et al. 1999; Van Rooyen 2003) exacerbate the spread of invasive grasses into new areas.

Alien grasses are known to impact on ecosystem structure, function and resources by accelerating wild fires, decreasing floral and faunal diversity and forage stability, altering soil food webs, soil water dynamics and decomposition cycles (Vila et al. 2000; Hobbs 2001; Lenz et

al. 2003). For example, in the Great Plains of USA, alien annual grasses are one of the major causes of poor oak recruitment because they rapidly draw down soil moisture thereby suppressing growth of oak seedlings (Lambrinos 2000). In Hawaiian woodlands, it has also been observed that soils beneath exotic grasses have lower nitrogen levels than soils under native grasses (Dyer & Rice 1997; Mack & D'Antonio 2003). This feature is exemplified by the alien invasive grass *Agropyron desertorum* which is a more efficient competitor for nitrogen than the native grass *A. spicatum* in the Hawaiian woodlands (Mack & D'Antonio 2003). Also, alien invasive grasses respond to elevated soil nitrogen levels by investing more energy into shoot than root production (Dyer & Rice 1997), thereby out-competing native species for aboveground space (Carlsen et al. 2000). A consequence of the high shoot production is the reduction of light to native species thereby limiting their photosynthesis and growth (Dyer & Rice 1997). For example, the alien invasive grass *Miscanthus sinensis* in its colonization of abandoned fields in Japan has reduced light availability to oak seedlings and as a consequence their rate of carbon assimilation thus slowing the rate of encroachment of oak trees into grassland (Miyawaki & Washitani 2004). Similarly, alien grasses in Texas (USA) reduce the growth rates of seedlings of woody species such as *Baccharis neglecta* and *Prosopis glandulosa* by reducing light availability (D'Antonio & Vitousek 1992). Also, the increased litter resulting from the high shoot production by alien grasses alters soil surface temperatures and diminishes light penetration to the soil surface that hampers the germination and recruitment of native species (D'Antonio & Vitousek 1992; Carlsen et al. 2000). This build-up of litter also decreases evaporation from the soil surface which favors the germination and growth of some alien grasses such as *B. japonicus* (D'Antonio & Vitousek 1992).

The high shoot and litter production by alien grasses enhances landscape flammability resulting in more frequent and hotter fires (D'Antonio & Vitousek 1992). Intense fires may lead to the loss of indigenous annuals and geophytes whose seeds cannot survive hot fires, whereas fire intervals shorter than those experienced by native plants (Brooks & Pyke 2001) diminishes their ability to recover with each successive fire. In contrast, the recovery of alien invasive grasses that are fuelling the fires is often promoted. This alien-grass-fire cycle has converted native communities into alien invasive annual grasslands that appear to persist indefinitely in most ex-arable lands where perennial grasses such as *C. dactylon* dominate (Brooks & Pyke 2001; D'Antonio & Vitousek 1992).

At a broader scale, alien invasive grasses affect geomorphologic processes which in turn influence erosion patterns and plant diversity (D'Antonio & Vitousek 1992; Mack et al. 2000). For example, *Ammophila arenaria* (European beach grass) alters dune formation patterns where it is

planted. Its ability to bind sand is greater than that of native species, and dunes formed by *A. arenaria* tend to be steeper and taller than those formed by native species, a condition that promotes soil erosion (D'Antonio & Vitousek 1992).

2.5. Control of invasive grasses

The best way for managing and controlling alien grasses is undoubtedly to prevent their initial introduction (Van Wilgen & Richardson 2004; Reichard & Hamilton 1997). However, because it is often too late for such preventative action, the only means of mitigating alien invasions is either through mechanical, chemical, or biological measures (Milton 2004). In most cases, control and preventative methods are meant to reduce growth and expansion of alien invasive grasses but do not help in restoration of indigenous species.

Although fire is commonly applied as a mechanical means of controlling alien invasive grasses, its effectiveness is often temporary. For example, fire was initially proposed as a method of controlling the invasive annual grass *Taeniatherum caput-medusae* (Furbish 1953), but later studies indicated that its effectiveness was incomplete (Torell et al. 1961) as it failed to clear them completely. Follow-up treatments with herbicides may be necessary to control individuals that escape initial fire treatments (Torell et al. 1961). Fire is only an effective tool for controlling alien invasive grasses where it kills all adult plants including their vegetative propagating organs and destroys their seed banks (Whelan 1995). However, the application of fire may promote the establishment of other fire-tolerant alien invasive species. In Mojave Desert, for example, Brooks & Pyke (2001) observed that as the biomass of the alien invasive grass species *B. rubens* (red brome) was reduced by fire, the biomass of *Erodium cicutarium* (red-stemmed filaree) subsequently increased. In most cases where alien invasive grasses are targeted for control by fire, the potential for other alien invasive species to fill their void must be considered.

Another mechanical control method includes removal of the alien populations by hand clearing which is more effective if plants are isolated and in small patches. Removal by hand is easiest when soils are moist however it is laborious and capital intensive as much human effort is needed (Milton 2004). Cutting of aliens using a mower, brush cutter, weed eater, tractor-drawn mower or machete only eradicates the plants temporally (Richburg & Patterson 2003b). Studies show that mowing some alien species actually stimulates vegetative production, though repeated mowing coupled with subsequent herbicide application can yield successful results (Wilson & Clark 2001).

Grazing is another method used for controlling alien invasive species (Olson 1999; Olson & Wallander 1998). In Montana in the USA, sheep have been effectively used to control *Eurphorbia esula* (leafy spurge) in pastures and along rivers (Olson & Lacey 1994; Olson 1999). However, grazing rarely eradicates aliens unless combined with other control techniques, such as chemical or biological methods. Animals used in grazing have the potential to break up soils and incorporate seeds of desirable native plants (Olson & Wallander 1998), although they equally have the same potential to disperse invasive alien seeds (Shmida & Ellner 1983; Knapp 1996). In cases where grazing is not properly applied it may cause significant damage to ecosystems and promote the spread of alien invasive grasses (Olson & Lacey 1994; Olson 1999). For example, overgrazing can also reduce native plant cover, disturb soils, and weaken native communities allowing exotic grasses to expand.

Herbicides that have proven effective for controlling alien invasive grasses include glyphosate (Roundup), imazapic, sulfometuron and grass-specific herbicides such as fluazifop-p-butyl and paraquat. Although herbicides have been referred to as the most effective method of controlling alien grasses (Nielsen et al. 2005), particularly Roundup which effectively kills annual grasses in 3 days and perennial grasses in 5 days, most herbicides are not ecologically friendly. Chemicals can contaminate waterways or kill other plants in the immediate area or negatively affect humans and other animals if not used properly. In a study conducted by Midoko-Iponga et al. (2005) in Elandsberg Private Nature Reserve, results indicate that herbicide application using *Gallant Super* (a systematic herbicide) proved to be most effective in reducing the cover of alien grasses on the old field than burning and mowing. However the effectiveness of herbicides varies with species as some herbicides are less damaging to native species as well as to soil micro-organisms (Nielsen et al. 2005). Herbicidal application is best when it is applied during early stages of plant growth (Krug 2004) as this will facilitate eradication of seedlings. Research has shown that, glyphosate is effective at eradicating *B. tectorum* (cheat grass) during early vegetative stages (Nielsen et al. 2005). However, of particular concern to herbicide use is the reported rapid development of multiple herbicide resistance among especially annual hybrids of the invasive alien grass *Lolium* in South Africa, which indicates that chemical control measures may become less cost effective with repeated herbicide use (Gill 1996; Cairns & Eksteen 2001).

2.6. Restoration overview

Invasion by alien species over the past century have resulted in dramatic habitat transformation and reduced native species numbers, which have resulted in loss of ecosystem services that benefit human beings (MA 2005; SER 2004; Holl et al. 2007). For example the

direct economic costs of alien plants and alien plant control in the United States by 1994 amounted to more than $15 billion annually (Bridges 1994). In developing countries, an estimated 25% of food production is lost due to the interference of invasive weeds with crops (Koch 1992) making the cost of invasive plants even higher than in developed countries. Also, in South Africa and many other parts of Africa, alien plants impact negatively on natural resources, and particularly on water (Milton 2004; Le Maitre et al. 2002; Versveld et al. 1998). The 'Working for Water' programme which recognizes the threat that alien plant invasions pose to the water resource aims at bringing the problem of plant invasions under control (Versveld et al. 1998), through biological, chemical and mechanical weed control practices. Given these problems ecological restoration is now regarded as an effective response to reduce and reverse these negative effects of habitat loss, fragmentation on native biological diversity and ecological processes upon invasion (Clewell & Aronson 2006; Aronson et al. 2007).

As defined by SER (2004), Suding et al. (2004), Miller & Hobbs (2007), Krug (2004), ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed. It is an intentional activity that initiates or accelerates an ecological pathway—or trajectory through time—towards a reference state (SER 2004). Recovery is normally taken to imply the revival of the indigenous species assemblages, biophysical structure, and ecological functions characteristic of the desired target state (Blignaut et al. 2007; SER 2004). In its simplest form, restoration consists of removing or modifying a specific disturbance, thereby allowing ecological processes to bring about an independent recovery (SER 2004). In this form a successional model for restoration is used (Suding et al. 2004; Holmes 2008), which assumes that, once the historical physical environment is re-established, natural successional processes will return the biotic system to its original condition (Dobson et al. 1997; Young et al. 2001). This simple restoration option or successional model, has worked in many degraded systems, for example, degradation of prairie pothole communities is often related to changes in the natural flooding regime. If hydrology can be restored and the seed bank has persisted, the original plant assemblage can re-establish by themselves (Mitsch & Wilson 1996). However, restoration relying on successional recovery has been unpredictable (Suding et al. 2004), for example, Anderson et al. (2000) found that re-introduction of fire to prairie barrens in southern Illinois after a 25-year hiatus shifted community composition did not return it to a trajectory community. In such cases a more complex approach of deliberately reintroducing native species that have been lost, whilst eliminating or controlling harmful invasive exotic species is needed (Casburn et al. 2006). For example intense grazing often removes drought-tolerant grasses in semi-arid rangelands, which can facilitate the growth of woody

species and soil degradation (Suding et al. 2004). Following these secondary changes, reduction of grazing intensity does not restore the grass dominance (Noy-Meir 1975) meaning that innovative mechanisms that address constraints and potential recovery from degradation are required.

Ecological restoration has goals (Clewell & Aronson 2007; Clewell et al. 2005), the broader goal being to create an ecosystem that is resilient and self-sustaining with respect to structure, species composition and function. Restoration goals are derived from a complex mix of ecological, social, historical, and philosophical viewpoints (Miller & Hobbs 2007; Clewell & Aronson 2007) but, in many cases, they are not formulated in such a way that guide effective habitat restoration due to social and economic constants (Miller & Hobbs 2007). Identifying a focal or target species or group of species must be the first step in all restoration initiatives because the requirements of these target species will serve to guide the process (Miller & Hobbs 2007; Scott et al. 2001). Once an appropriate focal species or group has been identified, the next step is to identify the biotic and abiotic resources that are required by the species to persist (Miller & Hobbs 2007). In some instances, identifying resources must be preceded by a decision on the life stage or process that the restoration is intended to accommodate. Ensuring availability of resources through time must also be an issue to be considered (Miller & Hobbs 2007). Resource availability will depend on landscape connectivity for species requiring multiple habitats, and this also becomes a key issue, especially in areas dominated by human activities (Debinski & Holt 2000; Hobbs 2002). As a result additional resources may also be required especially where dispersal capability of the focal species is limited relative to the distance that must be covered.

Although restoration initiatives are guided by goals, constraints be they ecological, economic or social always hinder restoration projects. Ecological constraints which include climate, soils, biotic community and landscape changes set limits on what is possible based on the biophysical realities of the site and its surroundings (Bailey 1998). On the other hand both financial and social constrains always question how realistic and acceptable a restoration project is based on funding availability, cost and benefits, ongoing maintenance, public understanding and community values (Miller & Hobbs 2007). All these constraints need to be considered in all restoration projects and must be evaluated first so as to build good goals.

2.7. Restoration of old fields and grass invaded areas

Cultivation leaves a legacy (Cramer et al. 2008), of biomass alteration, tillage, fertilization and changed hydrology which then alters ecosystem processes such that the legacy of

cultivation can be seen in vegetation composition and structure years later (McLauchlan 2006; Cramer & Hobbs 2007). Cramer et al. (2008) have concluded that the assembly of plant communities in many old fields occurs along a broadly repeatable pathway which resembles the composition, structure and function of the historical vegetation state that existed before clearing. This conclusion supports the succession theory (Miller & Hobbs 2007; Cramer et al. 2008), however this repeatable trajectory is not evident in some old fields (Cramer & Hobbs 2007), where vegetation can persist for decades in a degraded state dominated by invasive exotic species. Therefore where succession has failed, intervention mechanisms to assist old field recovery have been used.

Before physical restoration intervention processes can begin, it is essential to think about what is to be achieved with the old-field restoration (Krug 2004; Cramer & Hobbs 2007). For example, is the goal to restore the old-field for purely aesthetic reasons, to return native species to an area where they occurred before, or will the area be used for other activities like game or livestock farming (Krug 2004)? The reason for understanding these objectives is that each goal needs a different approach to reach the intended objective. The current state of the area to be restored has also to be taken into consideration, for example, is the site mainly covered by alien plants or not? (Krug 2004).

Upon evaluating these questions Krug (2004) concluded that successful old-field restoration (including other disturbed areas), needs a two-way approach. Firstly, any undesired vegetation must be removed, these are mainly alien grasses and weeds that sprout soon after land abandonment due to there ability to out-compete natives for high nutrients caused by fertilization (Suding et al. 2004). To remove unwanted vegetation, the application of herbicides has been the most effective option compared to mechanical methods like burning which promote after fire invasion (Motooka et al. 2002; Gill 1996; Whelan 1995), and biological methods which are expensive (Van Wilgen & Richardson 2004). However herbicides must be carefully selected, as they should be specific to the targeted plants at the same time destroying the seeds of the invasive species. Apart from that, application of herbicides must be carefully timed. Ideally, spraying should be conducted just before the germination of the seeds, so as to kill all sprouting propagules (Krug 2004).

Once the alien vegetation has been removed, seeds of indigenous species must be brought onto the area to be restored. Since this might or cannot happen naturally, especially if the restoration site is isolated from natural vegetation, introduction of native seeds is important. Introduction of native seeds after removal of aliens is mainly done by broadcasting; however this depends on the species being introduced (Krug 2004). In some cases seedlings and seed-

bearing soils taken from natural areas can be introduced (Hölzel & Otte 2003; McDonald 1993). Advantages of introducing seed-bearing soils are that the entire species-pool of a plant community may be covered by soil transfer, including rare species whose seed is often not easily collected. Secondly the genetic variability of locally adapted ecotypes and races is preserved and maintained (Hölzel & Otte 2003). However negative factors like unfavorable target site conditions such as soil nutrient status and moisture regime, coupled with poor conditions for seedling recruitment may hamper soil transfer processes (Aerts et al. 1995).

In areas where natural vegetation persists nearby, methods that promote natural re-colonization must be facilitated (Milton 2001; Holmes 2005). Methods that promote capture of dispersed seeds from nearby natural forests, for example roughening the soil surface to trap wind-blown indigenous seeds can be done in most old fields (Milton 2001). This may be done through soil pitting (Van der Merwe & Kellner 1999) or the placement of dead branches or shrubs on the soil surface (Whisenant 1999) so as to capture seeds.

In as much as these methods can result in restoration of old-fields, important issues like landscape and ecological factors that may influence recovery potential must be considered (Holmes 2005). For example topographical and soil changes, grazing pressures where animals are present, floods and fires need to be considered before restoration starts. With respect to landscape differences, Holmes (2005) recommended that large ungulates should be avoided in all restoration initiatives so as to allow plants to mature and produce seeds for dispersal into neighbouring areas of the field. Considering that cultivation may have changed the soil texture and soil nutrient capacity, addition of fertilizer especially carbon-rich mulch (Zink & Allen 1998; Cione et al. 2002) such as reeds or wood chips is important (Holmes 2005). Mulch helps in increasing soil moisture and seedling establishment (Cione et al. 2002) and it serves as a carbon source for bacteria, allowing them to immobilize nutrients such as nitrogen, and more quickly return disturbed, nutrient-rich soil to pre-disturbance conditions with lower nutrient availability (Suding et al. 2004; Blumenthal et al. 2003).

2.8. Other restoration practices

Examples of successful restoration projects range from the reinstatement of vegetation on mined lands, restoring corridors to connect forest fragments, renovating river systems, inland water bodies, marshes and stream-side vegetation (Aronson et al. 2007), decommissioning of dams, restoring coastal ecosystems and coral reefs, recontouring of hill roads through forests, and restoring natural vegetation in alien invaded areas (Clewell & Aronson 2006).

2.8.1 Mine dumps

Any successful restoration on mine dumps and previously mined areas must first establish a stable nutrient supply adequate for plant growth and microbial processes before the introduction of vegetation (Singh et al. 2002). Beside fertilizer addition to improve soil fertility, modern restoration techniques recognize the replacement of removed topsoils as the best restoration method following decommissioning of mines (Harris et al. 1996). An example where topsoil additions were successfully applied was on the north-east coast of South Africa near Richards Bay where dredge mining for heavy minerals in coastal dunes had taken place since 1977 (Camp & Weisser 1991). Restoration at this mine involved removal of the topsoil in a prescribed mining path through out the dunes. The removed topsoil was then stored and subsequently used in the restoration process that relies heavily on succession and natural re-establishment of the ecosystem (Mentis & Ellery 1998). In this restoration initiative, the topsoil seed bank was the major source of seed for the developing ecosystem with an estimated 75% of the native species originating from the soil seed bank (Mentis & Ellery 1998). At Bauxite mining in the northern Jarrah forest in Western Australia (Baker et al. 1995b), the top 5–15 cm of topsoil was stripped to maintain the seed-bank. However the lack of adequate seed numbers in the topsoil required mixing of the topsoil with seeds of 60 different native species, including the important under-storey legumes (Baker et al. 1995b). Fertilizer was also added at 500 kg ammonium phosphate per hectare (trace elements included) with a dual purpose of alleviating initial deficiencies to achieve satisfactory plant establishment, and at the same time replacing nutrients removed from the soil vegetation system during the mining process (Baker et al. 1995b).

Where topsoil has been lost or is unusable, planting of metal tolerant plants, such as the temperate grasses *Agrostis capillaris* and *Festuca rubra* can assist in restoring dump sites (Tordoff et al. 2000). Some species, (e.g., *Minuartia verna*), are described as "hyperaccumulators" in recognition of their ability to accumulate elements that are usually present in trace concentrations in plants (Johnson et al. 1994). For highly toxic metal mine wastes, it has been suggested that such species could be manipulated to clean-up or 'bioremediate' soils and at the same time both stabilize and restore land for other purposes (Johnson et al. 1994).

2.8.2. Degraded rangelands

Restoration of degraded rangelands which have been overgrazed required prioritization of grazing management strategies before the introduction of seeds of native species (Casburn et

al. 2006). Grazing management strategies assist in reducing the risk of further degradation, with set-stocking or continuous stocking being the most common management strategy applied in the past (Dowling et al. 2001). However, at high stock densities, continuous stocking (set-stocking) due to use of one grazing paddock for long periods of time results in a decline in more palatable perennial species causing an increase in less edible species. Therefore, timely, "tactical grazing" can have a positive impact on rangeland restoration. Tactical grazing, which involves adjusting stock numbers in accordance with changes in seasonal and climatic conditions and plant growth, is a concept which combines positive outcomes for both vegetation and animal production (Jones & Dowling 2004). One of the objectives of tactical grazing is to encourage palatable, productive perennials to re-germinate in paddocks, since they are good for environmental sustainability and animals (Jones & Dowling 2004). This can be achieved by controlled grazing which allows productive perennials to restore by natural processes (Dowling et al. 2001). However, in dysfunctional landscapes, the introduction of seeds of palatable, productive perennials may be required. Re-sowing of native perennial grasses is normally done after herbicidal application which acts to control undesirable weed species (Casburn et al. 2006; Krug 2004). Under extreme cases further action such as improving soil fertility may be required to ensure long-term restoration (Casburn et al. 2006). In such cases, soil fertility levels are enhanced through the application of phosphorus and nitrogen fertilizers, as well as lime; this alters competitive interactions in favour of the desired species. A good example of the successful restoration of a degraded rangeland was a program conducted at Carcoar in central New South Wales from 1997-2001 (Michalk et al. 2003). Addition of phosphorus fertilizer was accompanied by strategic grazing of resting paddocks and de-stocking in summer (Michalk et al. 2003). This treatment improved the composition of perennials grasses in almost all paddocks thereby restoring the degraded rangeland.

2.9. Restoration through resource manipulation

One of the most critical factors determining the success of invasive alien grasses on abandoned agricultural land is the high nutrient availability resulting from previous land use (Marrs 1993). In most abandoned farms, a rapid establishment of annual and fast-growing perennial and early seral plants (invasive grasses mainly) often characterizes the initial phase of vegetation development (Hansson & Fogelfors 1998; Baer et al. 2004). These continue to dominate as long as the nutrient availability remains high, and thus impede the establishment of late seral plant species (natives in most cases), even when such species are introduced by sowing (Kindscher & Tieszen 1998).

Therefore any restoration on nutrient rich soils should consider lowering soil nitrogen as well as phosphorus levels to shift community composition back towards dominance by native species (Blumenthal et al. 2003; Suding et al. 2004; Corbin et al. 2004). Reducing nitrogen and phosphorus concentrations in soils by adding carbon and calcium respectively has been considered as a practical means of restoring natural biodiversity in ex-arable areas invaded by alien grasses (Blumenthal et al. 2003; Suding et al. 2004). The addition of carbon promotes soil microbial immobilization of nitrogen (Corbin et al. 2004; Corbin & D'Antonio 2004; Suding et al. 2004; Krupa 2006; Blumenthal et al. 2003; Schimel & Bennett 2004) whilst the addition of Calcium enhances the fixation of available phosphorus into unavailable inorganic forms through the formation of dibasic calcium phosphate dihydrate, octocalcium phosphate, and hydroxyapatite, the latter most prevalent at soil pH greater than 7 (Barrow 1983; Busman et al. 2002). The formation of each product results in a decrease in solubility and availability of phosphate to plants. Therefore both C and Ca addition to reduce N and P prevents rapid growth of alien grasses thereby providing native species with a better chance of competing for available nutrients as well as water resources depleted by the alien species (Morgan 1994).

Several studies have concluded that carbon addition leads to decreased rates of net nitrogen mineralization (Corbin & D'Antonio 2004; Blumenthal et al. 2003; Perkins 2006) and nitrification (Gilliam et al. 2005) resulting in diminished concentrations of ammonium and nitrate in soils (Blumenthal et al. 2003). Carbon may be applied to soils as sucrose (Reever Morghan & Seastedt 1999; Blumenthal et al. 2003; Perkins 2006) sawdust or chopped wood (Corbin & D'Antonio 2004) with effects on soil N content differing with the carbon source. For example, readily available forms of carbon such as sucrose may stimulate microbial activity within hours (Mack et al. 2000; Corbin et al. 2004) while other carbon sources, such as sawdust or wood chippings that consist of structurally more complex molecules, take longer to degrade (Mack et al. 2000; Corbin et al. 2004; Perkins 2006). The decay of sawdust is slower than that of sugar but presumably faster than that of wood-chips making sawdust a potential substitute for more expensive sugar as a carbon source. On the other hand, studies that have added Ca in the form of gypsum ($CaSO_4$) have concluded that resin-extractable phosphorus was reduced by an average of 34% however Ca addition did not influence inorganic N availability (Suding et al. 2004).

All in all, studies have shown that carbon addition, and in combination with calcium, reduce above-ground biomass of both alien invasive grasses and native species (Michelsen et al. 1999; Alpert & Maron 2000; Blumenthal et al. 2003). However, the increase in microbial nitrogen immobilization through carbon addition may be a temporary phenomenon as little is known

about how the soil microbial community responds to cessation of carbon addition, and how these effects will translate to a shift in vegetation composition (Perkins 2006). In fact, once carbon addition is stopped, the inorganic nitrogen concentration in soils may rise again as a result of an increased release from decaying microbial biomass (Mack et al. 2000; Corbin et al. 2004; Perkins 2006). The same also applies to Ca additions, since the conversion of phosphorus into unavailable inorganic forms is also a transient phenomenon (Barrow 1983; Busman et al. 2002). Ongoing mineralization of organic substrates by micro-organisms providing plants with a constant source of available P (Busman et al. 2002). Micro-organisms mineralize phosphorus are highly influenced by soil temperature, soil moisture and suitable soil pH (Busman et al. 2002). This process is more efficient in warm and moist but well drained soils at neutral pH (range: 5.5 to 7.0) levels (Busman et al. 2002).

The general effect of manipulating resources to decrease soil fertility is a reduction in plant growth (Blumenthal et al. 2003) but its effectiveness as tool for managing alien invaded areas is uncertain. In a greenhouse experiment which tested the effect of carbon additions on the growth of early- and late-seral species in the absence of competition, Eschen et al. (2007) and Suding et al. (2004) observed that carbon additions reduced the growth of annual plants to a greater extent than that of perennial plants. These findings suggest that carbon combined with calcium additions may provide a useful tool for restoring natural vegetation in nutrient impoverished Mediterranean-climate ecosystems, especially on nitrogen rich ex-arable lands invaded by annual grasses.

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Chapter 3

Soil nutrient depletion as a restoration aid in a South African lowland fynbos ecosystem: an empirical appraisal

Abstract

Over 95% of South African lowland fynbos vegetation has been transformed by agriculture and invasive alien grass species. The artificial reduction of plant available N and P levels in soils through the addition of carbon and other elements may provide a means of retarding the growth of alien grasses stimulated by soil nutrient enrichment and increasing the competitive advantage of native lowland fynbos species adapted to nutrient impoverished soils of renosterveld. This premise was tested in both field- and greenhouse-based trials. Systemic and contact herbicides were applied to reduce the large alien invasive grass biomass followed by the addition of C as sucrose and Ca as gypsum to reduce plant available N and P respectively in the soils. The effects of these combined herbicide and soil nutrient amendment treatments on plant physiology and growth were examined in both resident alien and indigenous species and in several herbaceous and woody native species introduced as seeds and seedlings. Also, soils sampled from the different treatments in both trials were chemically analyzed. There was a definitive absence of seedling recruitment from seeds of all nine indigenous species sown into soils in the field-based trial and introduced juveniles of another nine indigenous species displayed a high mortality during the dry summer season. These detrimental effects were less severe in the greenhouse-based trial which received more regular water input and where successful seedling recruitment from seeds sown occurred in four indigenous species. Sucrose additions, both exclusively and in combination with gypsum, caused significant reductions in foliar chlorophyll, photosystem II (PSII) function and above-ground biomass of most resident and introduced alien and indigenous species. These reductions were less prominent where herbicides were applied, a possible consequence of N and P supplementation of soils by the decomposing plant biomass. This was supported by the elevated soil K, Na and N concentrations measured in soils where contact and systemic herbicides were applied. However, no significant changes in soil N or P were apparent following sucrose and gypsum additions, the latter attributed to the acidic soils which precluded the formation of insoluble P complexes, and the former requiring further investigation. The observed inhibition of photosynthesis and growth of both alien and native species by sucrose additions and the poor seedling recruitment and high

mortalities observed among introduced native species, present severe challenges for restoring ex-arable lands in the renosterveld. Alternative options are considered.

Key words: Sucrose addition, restoration, microbial immobilization, abandoned agricultural lands, and herbicide application.

3.1. Introduction

South Africa has a long history of problems with alien invasive species (Milton 2004; van Wilgen & Richardson 2004). Of significance is the proliferation of alien grasses of temperate and subtropical origin in highly fragmented natural ecosystems in the Mediterranean-climate region of South Africa (Vlok 1988; Steinschen et al. 1996) which is unique in terms of its rich floristic diversity and endemism and which is listed among 34 global biodiversity hot spots (Mittermeier et al. 2004). Alien grasses are known to impact on ecosystem structure, function and resources (D'Antonio & Vitousek 1992; Knapp 1996). Their recent increase in abundance on bottomlands and plains in the South African Mediterranean climate region (Vlok 1988; Steinschen et al. 1996) have resulted in the displacement of wildflowers which form the basis of a growing, lucrative nature-based tourist industry (Goldblatt & Manning 2000). Invasion by these alien grasses is facilitated by both human and animal movements (Schmida & Ellner 1983) and exacerbated by habitat deterioration caused by ploughing, vegetation clearing and burning, soil nutrient enrichment from surrounding agricultural areas and grazing by herbivores which disperse the grass seeds on their hides and in their dung (Kemper et al. 1999; van Rooyen 2003).

Alien grasses possess a broad spectrum of physiological features that promote their establishment (Kemper et al. 1999) and are well suited to disturbed and fragmented habitats (D'Antonio & Vitousek 1992). As a consequence they threaten heavily fragmented communities such as renosterveld in the South African Mediterranean climate region, 95% of which has already been transformed for development of field crops, vineyards and orchards (Kemper et al. 1999; Von Hase et al. 2003). Several studies have examined potential options for restoring degraded renosterveld communities invaded by alien grasses. Midoko-Iponga (2004) observed that competition by alien grasses had a greater negative impact on seedlings of native species than grazing by large herbivores and concluded that removal of alien grasses was a prerequisite for successful re-establishment of renosterveld in old fields. Shiponeni & Milton (2006) subsequently found that the dung of large indigenous herbivores such as antelopes contained a greater abundance of viable seeds of alien annual and perennial grasses than indigenous ephemeral and shrub species that characterize renosterveld which led them to conclude that grazing by these animals could essentially

retard the recovery of renosterveld. An empirical appraisal of the cost-effectiveness of different labour-intensive strategies, viz: grass mowing, hand-clearing, light and intense burning and pre-emergent herbicide application, linked to a national poverty relief program for controlling invasive annual grasses in renosterveld to promote natural biodiversity was conducted by Musil et al. (2005). They found that intense burning, the cheapest strategy overall, was ineffective as it promoted recruitment of both alien invasive annual and perennial grasses and inhibited recruitment of native geophytes. They concluded that mowing of grass-infested renosterveld prior to grass seed maturation, and the removal of the cut grass biomass for use as fodder in restricted feed lots to offset clearing costs, provided the most credible strategy for controlling the annual grass populations to conserve native floral diversity over the short term. They also proposed that effective long-term control of invasive grasses in renosterveld would likely be realized by an integrated management approach, which would seek to limit factors that promote their success, e.g. soil N enrichment and intensive disturbance, and apply strategies that reduce seed production and seed accumulation. In this regard, Holmes (2008) examined the effects of tilling, herbicide application and carbon-rich mulching on weed cover and shrub establishment in a sand plain lowland fynbos community. Herbicide application reduced weed cover for significantly longer periods than tilling, but the effects of mulching on soil N content and mineralization rates and establishment of native plants were inconclusive (Holmes 2008).

Numerous studies have reported that the success of invasive grasses in novel environments is due to soil nutrient enrichment and the reduction of plant available N and P in soils may provide a means enhancing the competitive advantage of native species (Marrs 1993; Tilman 1993). Indeed, research has shown that abandoned agricultural fields have higher soil nutrient levels than natural areas in the Cape lowlands. Results from a study conducted by Memiaghe (2008) indicate that abandoned agricultural fields in the renosterveld area have higher soil nitrogen and phosphorus levels as compared to natural areas. Soil N and P levels did, however, decrease with time from agricultural abandonment, with higher levels in recently abandoned fields and lower levels in older fields. Certainly, the suggestion is that such high soil nutrient levels have hindered the recovery of native species in these fields (Milton 2004; Memiaghe 2008; Krug et al. 2004b). As a result a variety of different techniques have been proposed to reduce levels of growth limiting nutrients in soils, including topsoil removal and addition of organic matter (Eschen et al. 2007) which comprises a rich source of carbon that stimulates soil microbial activity leading to a depletion of soil N available for plant uptake (Hunt et al. 1988; Redent 1992; Reever Morghan & Seastedt 1999; Eschen et al. 2007). Carbon has been applied to soils as sucrose (Reever & Seastedt 1999; Blumenthal et al. 2003; Perkins 2006), straw, sawdust and chopped wood (Corbin & D'Antonio 2004) with effects on soil N content differing with the carbon source.

Also, the addition of minerals such as Ca, Al and Fe to soils may assist in reducing levels of plant available P in soils through formation of amorphous Ca, Al and Fe phosphates which gradually change into insoluble compounds (Barrow 1983; Busman et al. 2002). However, studies that have examined the feasibility of applying such soil nutrient amendment techniques in improving the competitive advantage of native species have yielded conflicting results (Reever Morghan & Seastedt 1999; Blumenthal et al. 2003; Eschen et al. 2007; Corbin & D'Antonio 2004).

In view of these findings, this study's objective is to examine the practicality of applying chemicals that reduce available nitrogen and phosphorus levels in soils in combination with herbicides to limit the growth of alien grasses as a means of promoting the competitiveness of native taxa in restoring natural biodiversity.

3.2. Methods and materials

3.2.1. Study area and site

The study area was the Elandsberg Private Nature Reserve situated on Bartholomew's Klip farm near Bo-Hermon, approximately 25 km north of Wellington in the Cape winelands district of South Africa. The reserve was proclaimed in 1973 (Parker 1982) and declared a natural heritage site in 1988 (Farley pers com cited in Midoko-Iponga 2004). The study site comprised an area of degraded natural vegetation situated in a transition zone (33°44"67' S to 33°44"72' S; 19°03"13' E to 19°03"17' E) between Swartland Shale renosterveld and Swartland Alluvium fynbos (Mucina & Rutherford 2006) transformed for cultivation of oats between 1960 and 1965 and then used for cultivation of European pasture grasses for livestock grazing between 1965 and 1987 (Gregor pers. com. cited in Midoko-Iponga 2004). The vegetation at the site comprised the dominant perennial grass *Cynodon dactylon* of tropical African origin, the winter growing annual grasses B*riza maxima, Bromus pectinatus* and *Paspalum dilatatum* of European origin (Shiponeni 2003), and some renosterveld remnants, namely the renosterveld shrub *Elytropappus rhinocerotis* and the geophyte *Oxalis purpurea.*

3.2.2. Experimental design and treatments

Three parallel transects each measuring 40 m in length and 5 m in breadth were located 12m apart on a level plain at the study site. Two of these transects were treated with herbicides to reduce alien grass biomass and the third served as a control. The contact herbicide Paraquat (Dimethyl bipyridynium dichloride-CH$_3$ (C$_5$H$_4$N) 2CH3.2Cl)) and the systemic herbicide Roundup (Isopropylamine salt of N-(phosphonomethyl glycine) were applied to the second and third transects. Four weeks after herbicide application, each of the

three transects were subdivided to 24 x 1.5 m x 1.5 m square plots, spaced 1.5 m apart, with each plot subdivided further into nine 0.5 m x 0.5 m sub-plots (Appendix 3.1 & 3.2). Three soil nutrient amendment treatments and one control, each replicated six times, were distributed sequentially among the 24 plots in each transect. The treatments comprised: 1. the addition of carbon as sucrose at a concentration of 200 g sucrose m^{-2} of soil to reduce soil N content, 2. the addition of Ca as gypsum ($CaSO_4.2H_2O$) at a concentration of 10 g Ca m^{-2} of soil to reduce soil P content, and 3. the addition of both sucrose and gypsum to reduce both soil N and P content. Sucrose (450 g/plot) and gypsum (101.25 g/plot) additions (in dry form) were performed monthly over a four-month rainy season extending from late autumn (May 2007) to late winter (August 2007).

Field-based soil nutrient amendment treatments were replicated at a reduced scale in a surrogate greenhouse-based study. Following field herbicide applications, 72 x 28 cm wide x 30 cm long x 10 cm deep soil cores were excavated from areas between the delineated plots in each of the 3 transects. The 216 soil cores excavated in total were placed into plastic trays of similar dimension and transported to a passively ventilated greenhouse where air temperatures closely approximated those outdoors. The experimental layout comprised 6 blocks located at different positions in the greenhouse. The 72 soil cores excavated from each of the three field transects were randomly assigned to two of the six blocks, each block comprising 36 soil cores arranged into nine rows with four soil cores each (Appendix 3.1 & 3.3). The three soil nutrient amendment treatments and control, each replicated nine times in each block, were applied sequentially to the four soil cores in each row.

3.2.3. Introduction of indigenous taxa

Indigenous species common in Swartland Shale Renosterveld and Swartland Alluvium Fynbos were introduced into the differentially treated soils in the field-based and greenhouse-based trials both as seed, sourced from a commercial seed supplier (Silverhill Seeds Pty Ltd), and as juvenile plants sourced from the Kirstenbosch National Botanical Gardens.

The species introduced as seeds comprised the six shrub species *Athanasia trifuricuta* (L.) L., *Elytropappus rhinocerotis* (L. F.) *Less*, *Olea europea L. subsp. africana* (Mill.) P. S. Green, *Eriocephalus africanus* L. *var africanus*, *Rhus laevigata* L. *var villosa* (L.F) R. Fern, and *Felicia filifolia* (Vent) Burtt Davy *subsp filifolia* and the three herbaceous species *Arctotis acaulis* L., *Dimorphotheca pluvialis* (L.) Moench and *Ursinia anthemoides* (L) Poir. *subsp anthemoides*. In the field study, 20 seeds of each of the nine species were sown at depths of 5 – 10 mm during late autumn (May 2007) into one of the nine subplots present in each of the 24 main plots in each transect. In the surrogate greenhouse study, 10 seeds of each

species were similarly sown during late autumn into each of the four soil cores present in one of the nine rows in each block.

The species introduced as juvenile plants comprised the five renosterveld shrub species *Olea europea L. subsp africana* (Mill) P. S. Green, *Salvia africana-lutea* L., *Rhus lucida* L. forma *lucida*, *Eriocephalus africanus* L. var *africanus* and *Pelargonium cordifolium* (cav.) Curtis and the four fynbos species *Leucadandron xanthoconus* (Kuntze) K. Schum, *Leucospermum praecox* Rourke, *Protea scolymocephala* (L) Reichard and *Phylica pubescens* Aiton var. *pubescens*. In the field study, one juvenile of each of the nine species was transplanted during early winter (June 2007) into one of the nine subplots present in each of the 24 main plots in each transect. In the surrogate greenhouse study, no juvenile plants were transplanted into the soil cores due to their shallow depth.

Introduced and resident species in the field-based soil nutrient amendment treatments received only natural precipitation (averaging 5 mm per day over the four-month rainy season) whereas those in the greenhouse received 4 mm of water daily supplied by an automated irrigation system over the entire experimental period.

3.2.4. Plant photochemical efficiency, pigment composition, and growth

The numbers of seedlings that emerged from plots and soil cores in the field- and greenhouse-based trials were recorded at weekly intervals over a three-month period following seed sowing. Field plots were re-examined at the end of the second winter season for evidence of any residual seedling recruitment.

A chlorophyll content meter (Model CCM-200, Opti-Sciences Inc., Hudson, NH, USA) measured foliar chlorophyll contents and a modulated chlorophyll fluorometer (Model OSI-FI, Opti-Sciences Inc., Hudson, NH, USA) measured yields of quantum efficiency at a steady state (Y_s) in the introduced and resident species in the field- and greenhouse-based trials. Measurements were conducted on fully expanded apical leaves during the active growing period in early spring (September 2007) and confined to clear sky conditions between 1100 and 1300 SAST (solar noon).

At the end of the rainy season in mid spring (October 2007) and again at the end of the dry summer season in March 2008, the numbers of introduced species that had deceased in the plots and soil cores in the field-based trials were counted and expressed as percentages of the total numbers of individuals introduced. The above-ground parts of all annual species were harvested from the plots and soil cores in the field- and greenhouse-based trials in late spring (November 2008) as they had attained full reproductive maturity. Those of perennial species were harvested in late summer (March 2008). The above ground parts of each species collected from each plot and soil core were bagged, dried in forced draft oven at 65^0C, and weighed.

3.2.5. Soil physical and chemical analyses

For soil chemical assays, 200 cm^2 samples of soil were collected to a depth of 50 mm from each of the field-based sub-plots and greenhouse-based soil cores after plants had been harvested. Soil samples were dried at 30°C in a forced draft oven, sieved to $\leq$ 2 mm particle size, and 5.0 g $\pm$ 0.05 g samples placed into extraction bottles. Electrical conductivity was measured in a 1:2.5 soil-water extract and pH in 1:5 soil-KCl extract (Rhoades 1982). Cations were extracted in a 1:10 ammonium acetate solution using the centrifuge procedure described by Thomas (1982), filtered and analyzed by atomic absorption spectrometry (SP428, LECO Corporation, USA). Water soluble anions in a 1:5 soil-water extract were analyzed by ion chromatography; extractable P using a Bray-2 extract as described by Bray & Kurtis (1945); total C and N by complete combustion using a Eurovector Euro EA Elemental Analyser and labile C (readily oxidized C) using a modified Walkley-Black method as described by Chan et al. (2001).

3.2.6. Statistical analyses

A two-factor analysis of variance (generalized linear model) tested the effects of the different herbicide and soil nutrient amendment treatments and their interaction on soil chemical composition, plant pigment content, photochemical efficiency and growth in the field- and greenhouse-based trials. Significantly different nutrient amendment treatment means within each herbicide application were separated with a Duncan's multiple range tests.

3.3. Results

3.3.1. Seedling recruitment

No seedlings of any of the 9 species emerged from the 12 960 seeds in total sown into the 72 field-based plots. In contrast, seedlings of four of the nine species, namely the introduced indigenes *A. acaulis*, *D. pluvialis*, *U. anthemoides* and *R. laevaegata* emerged from seeds sown into the soil cores in the greenhouse-based study. In these soil cores, significantly (P ≤ 0.001) lower seedling recruitment was observed where sucrose was applied both exclusively and in combination with gypsum in all but one of the introduced indigenes, namely *R. laevaegata* (Figure 3.1). These reduced levels of seedling recruitment occurred both in soil cores taken from plots where herbicides were excluded and where these were applied. In *R. laevaegata,* a significant (P ≤ 0.05) interaction between herbicide application and nutrient amendment was observed. This species displayed significantly (P ≤ 0.05) better seedling recruitment where sucrose was applied, both exclusively and in combination with

gypsum, but only in those greenhouse-based soil cores taken from plots where no herbicide was applied.

3.3.2. Foliar chlorophyll contents

Significantly (P ≤ 0.05) increased foliar chlorophyll contents were observed in eight species where herbicides were applied, namely the resident alien *B. maxima*, the introduced indigenes *O. europea*, *L praecox*, *P. cordifolium*, *R. lucida*, and *S. africana* in the field-based trial and the resident alien *C. dactylon* and the resident indigene *O. purpurea* in the greenhouse-based trial (Table 3.1). All species exhibited elevated chlorophyll contents in plots where the contact herbicide was applied with the exception of *C. dactylon* and *O. europea* which only displayed elevated chlorophyll contents in those plots where the systemic herbicide was applied. In contrast, the addition of sucrose, both exclusively and in combination with gypsum, caused significant (P ≤ 0.01) reductions in foliar chlorophyll contents in all but one of the 14 resident and introduced alien and indigenous species on which measurements were performed in the field- and greenhouse-based trials (Table 3.1). These reduced foliar chlorophyll contents following sucrose addition occurred both where herbicides were excluded and where these were applied, except in three species, namely in the introduced indigene *R. lucida* in the field-based trial and the resident alien *C. dactylon* and resident indigene *O. purpurea* in the greenhouse-based trial where significant (P ≤ 0.05) interactions between herbicide application and soil nutrient amendment treatment were apparent (Table 3.1). In *R. lucida*, sucrose additions resulted in decreased foliar chlorophyll contents in those plots where no herbicide was applied as well as in those plots where only the systemic herbicide was applied (Appendix 3.4). In *C. dactylon* sucrose additions only resulted in decreased chlorophyll contents in those soil cores taken from plots where the systemic herbicide was applied (Appendix 3.5). In *O. purpurea*, a significantly (P ≤ 0.05) increased foliar chlorophyll content was observed following gypsum addition but this only occurred in those soil cores taken from plots where the contact herbicide was applied (Appendix 3.6).

3.3.3. Steady-state fluorescence yield

Significantly (P ≤ 0.05) increased steady-state fluorescence yields were observed in five species where the contact herbicide was applied, namely the introduced indigenes *L. praecox*, *R. lucida* and *S. africana*, in the field-based trial, the resident alien *B. maxima* and the resident indigene *O. purpurea* in the greenhouse-based trial (Table 3.2). The resident alien *B. maxima* in the greenhouse-based trial also exhibited an elevated steady-state fluorescence yield where the systemic herbicide was applied. In contrast, the addition of sucrose, both exclusively and in combination with gypsum, caused significant (P ≤ 0.001)

reductions in all but three of the 13 resident and introduced alien and indigenous species on which measurements were performed in the field- and greenhouse-based trials (Table 3.2). These reduced steady-state fluorescence yields following sucrose addition occurred in both herbicide excluded and herbicide treated plots in all except three species, namely the introduced indigene *L. praecox* in the field-based trial, the resident alien *C. dactylon* and the resident indigene *B. maxima* in the greenhouse-based trial where significant (P ≤ 0.05) interactions between herbicide application and soil nutrient amendment treatment were apparent (Table 3.2). In *L. praecox*, sucrose additions in those field plots where no herbicide was applied resulted on much greater reductions in steady-state fluorescence yield (121% decrease) than in those where the contact herbicide was applied (54% decrease) as well as those where the systemic herbicide (19% decrease) was applied (Appendix 3.7). In *C. dactylon* and *B. maxima* sucrose additions only resulted in significant (P ≤ 0.05) reductions in steady-state fluorescence yield in those soil cores taken from plots where no herbicide was applied (Appendix 3.8 & 3.9).

3.3.4. Plant growth and mortality

In both field-based and greenhouse-based trials, the resident aliens *B. maxima* and *C. dactylon* and the resident indigene *O. purpurea* all displayed significantly (P ≤ 0.05) reduced above-ground biomass in those plots where the contact herbicide was applied (Table 3.3). These significantly (P ≤ 0.05) reduced above-ground biomass were also evident in those plots where the systemic herbicide was applied, except in *C. dactylon* in the field-based trial and in *O. purpurea* in the greenhouse-based trial (Table 3.3). In contrast, the four introduced indigenes *A. acualis*, *D. pluvialis*, *R. laevaegata* and *U. anthemoides*, in the greenhouse-based trial all exhibited significantly (P ≤ 0.001) increased above-ground biomass in those soil cores taken from plots where the contact herbicide was applied. These significantly (P ≤ 0.001) increased above-ground biomass were also apparent in *A. acualis* and *U. anthemoides* cultivated in soil cores taken from plots where the systemic herbicide was applied (Table 3.3). In the field-based trial, the addition of sucrose, both exclusively and in combination with gypsum, significant (P ≤ 0.05) reduced above-ground biomass of the resident aliens *B. maxima* and *C. dactylon* and the resident indigene *O. purpurea* (Table 3.3). However, in the greenhouse-based trial, significant (P ≤ 0.001) interactions between herbicide application and soil nutrient amendment treatment were apparent in these three resident and alien species. The addition of sucrose resulted in significantly (P ≤ 0.05) decreased above-ground biomass in *B. maxima* and *O. purpurea*, but only in those soil cores taken from plots where no herbicide was applied (Appendix 3.10 & 3.11). Conversely, in *C. dactylon*, significantly (P ≤ 0.05) increased above-ground biomass were observed following addition of gypsum, but these increases were apparent only in those soil cores taken from

plots where no herbicide was applied as well as those where the systemic herbicide was applied (Appendix 3.12). Also, significant (P ≤ 0.05) interactions between herbicide application and soil nutrient amendment treatment were observed in the two introduced indigenes *A. acaulis* and *R. laevigata* in the greenhouse-based trial. The observed reductions in above-ground biomass in these two species following sucrose additions were only apparent in those soil cores taken from plots where no herbicide was applied (Appendix 3.13 & 3.14). No clear effects of herbicide application or soil nutrient amendment treatment on above-ground biomass of the six introduced species were apparent in the field-based trials as mass measurements were convoluted by the exceptionally high plant mortalities (up to 100%) recorded for these species (Table 3.3). Indeed, the significantly (P ≤ 0.05) higher above-ground biomass measured for *P. cordifolium* in those plots where the systemic herbicide was applied corresponded with a substantially lower recorded mortality.

3.3.5. Soil physical and chemical properties

Significantly (P ≤ 0.05) elevated K, Na, N and C concentrations were recorded in soils taken from plots in the field-based trials but only in those where the contact and systemic herbicides were applied (Table 3.4). In the greenhouse-based trials, only soils cores taken from plots where the contact herbicide was applied displayed significantly (P ≤ 0.05) increased C and also soluble P concentrations. Also, a significant interaction between herbicide application and soil nutrient amendment treatment was apparent for soil C concentration in the field-based trial (Appendix 3.15). Here soil C concentrations displayed greater increases following sucrose additions in those field plots where the contact and systemic herbicide (13% to 18% increase) were applied than in those plots where no herbicide was applied (3% decrease). Soil pH displayed a slight yet significant (P ≤ 0.05) increase in soils cores taken from plots where the systemic herbicide was applied in the field-based trial with the converse apparent in the greenhouse-based trial. In both the field- and greenhouse-based trials, significantly (P ≤ 0.001) increased Ca concentrations but significantly (P ≤ 0.01) decreased Mg concentrations were apparent in soils where gypsum was added both exclusively and in combination with sucrose.

3.4. Discussion

The addition of sucrose, both exclusively and in combination with gypsum did not facilitate the growth of native species. Sucrose applications resulted in decreased foliar chlorophyll contents, steady-state fluorescence yields and above-ground plant biomass in almost all resident and introduced aliens and indigenes in both the field- and greenhouse-based trials. These findings contrast with previous reports that the addition of C, either as sucrose, sawdust or a mixture of sucrose and sawdust, stimulates growth of native species

(Reever Morghan & Seastedt 1999; Blumenthal et al. 2003; Eschen et al. 2007), implying that C addition is a useful management method to restore alien grass invaded communities. However, this study's findings did concur with those reported by Corbin & D'Antonio (2004) who concluded that the addition of C as a tool in restoring native species diversity and biomass provided no significant benefit over a 2 year time period.

Although it is still fundamentally unclear as to how C addition to soils detrimentally affects plant growth and photosystem II (PSII) function, there is considerable evidence that C additions do cause significant reductions in inorganic N availability (Blumenthal et al. 2003; Eschen et al. 2007). Indeed, plants need nitrogen for the production of leaf area which in combination with the rate of photosynthesis per unit area determines total plant productivity (Field & Mooney 1986). It is this reduction in N after sucrose addition that could explain the measured decreases in foliar chlorophyll content observed in this study, a feature also apparent in some crop plants under nitrogen limiting conditions (Shangguan et al. 2000), as well as the observed decline in steady state fluorescence yield, a sensitive indicator of plant response to various environmental stresses including nutrient deficiency (Schreiber & Lange 1987; Őquist 1987; Baker & Horton 1988). Nevertheless, the observed negative impact of sucrose additions on resident alien grasses also suggests that sucrose additions could supplement other alien grass control methods like fire (Whelan 1995), grazing (Matthews 1996), herbicide application and mowing (Mathews 1996; Gill 1996) over the short term due to its greater cost relative to other C sources such as sawdust (Eschen et al. 2007). However, over the medium term the application of sawdust makes it a potential C substitute for sugar due its relative short decay rate whereas over the long term the addition of wood chips may exert a slower but longer-lasting effect on alien grasses (Eschen et al. 2007; Török et al. 2000).

Several studies have reported reduced inorganic N and P levels in soils following sucrose and gypsum additions (Zink & Allen 1998; Reever Morghan & Seastedt 1999; Blumenthal et al. 2003), the magnitude of reduction dependent on initial soil fertility, quality of C added and the form (sucrose versus sawdust) of C added (Blumenthal et al. 2003). However, in both the field- and greenhouse-based trials, no significant reductions in soil inorganic N and P levels were observed in this study following sucrose and gypsum additions, even though these additions were reflected in increased soil C and Ca concentrations. These seemingly unaltered soil N and P concentrations were possibly a consequence of soil N and P supplementation by decomposing plant biomass originating from alien and indigenous species exterminated by herbicide application. Also, heterotrophic decomposition of organic matter dictates that substrate supply rate, including energy and nutrients, can limit soil microbial activity and thereby soil N and P immobilization (Kelliher et al. 2005). Both suggestions were supported by the elevated soil N and P levels observed in

especially the field-based plots where contact and systemic herbicides were applied. Conversely, there was no clear evidence that the soil physical and chemical environment in this study was unsuitable for growth of micro-organisms that immobilize N and P in soils (Blumenthal et al. 2003). This microbial immobilization process optimum in warm and moist but well drained slightly acidic soils with pH's ranging between 5.5 to 7.0 (Busman et al. 2002), the soil pH's (range: 4.8 to 5.6) recorded in this study were only slightly below this optimum range. Also, in these acidic soils the reaction between soil P and added Ca would have precluded the formation of dibasic calcium phosphate dihydrate, octocalcium phosphate, and hydroxyapatite (Busman et al. 2002), that result in a decrease in the solubility and availability of phosphate as these reactions occur mostly in alkaline soils with a soil pH > 7.3. In acidic soils, especially with a soil pH less than 5.5, the addition of Fe and Al would potentially provide a better option in reducing P availability as these react with soil P by first forming amorphous Al and Fe phosphates which gradually change into insoluble compounds of phosphate generally unavailable to plants that resemble crystalline variscite, an Al phosphate, and strengite, an Fe phosphate (Busman et al. 2002).

No seedlings emerged from the vast quantity of seeds (12 960) of the nine different species, that also included *E. africanus*, sown into soils at the study site, which contrasted with reported seedling recruitment from *E. africanus* seeds sown into soils of brush cut, burnt and herbicide treated plots at the same study site in a previous study (Midoko-Iponga 2004). The observed recruitment of seedlings from seeds in four of the nine species sown into soil cores in the greenhouse-based trial which received more regular precipitation supplied by an automated irrigation system suggested that the observed absence of seedling recruitment in the field-based plots may have resulted from insufficient water resources or allelopathy. Persistent, creeping perennial grasses, such as *C. dactylon* are known to exclude the establishment of native shrubs (Midoko-Iponga 2004), particularly where grazers and browsers are present (Van Auken 1994). Their dense root systems intensify below ground competition which inhibits nutrient and water acquisition and consequent growth of native species (Cabin et al. 2002; Yoder & Caldwell 2002). Also, it has been reported that *C. dactylon* produces inhibitory chemical substances that hinder cotton seed germination and growth (Vasilakoglou et al. 2005) and their release into soils at the study site may well have prevented seedling recruitment and growth of introduced native species (Callaway 2002), the latter partly supported by the high mortalities observed also among those native species transplanted as established juveniles into the field-based plots.

3.5. Conclusions and recommendations

The observed inhibition of photosynthesis and growth of both alien and native species by sucrose additions and the poor seedling recruitment and high mortalities observed among

introduced native species, present severe challenges for restoring disturbed renosterveld communities. The application of herbicides to remove alien grasses prior to native species introduction is considered a priority (Midoko-Iponga 2004). It does provide an effective means of reducing annual alien grasses if applied prior to seed maturation but has only a limited impact on creeping perennial grasses which rapidly regenerate from underground rhizomes (Musil et al. 2005). Transfer of soils containing seeds and other propagules of native species from pristine communities to disturbed areas has proved a more effective means than sowing or broadcasting of seeds in restoring native species diversity (Hölzel & Otte 2003; McDonald 1993). The advantages associated with such soil transfers are that the entire species-complement (especially those stored as seed bank) is introduced including rare species whose seed is often not easily collected and that the genetic variability of locally adapted ecotypes and races is preserved and maintained (Hölzel & Otte 2003). Also at sites where a pristine community borders a disturbed area, methods that promote natural re-colonization require testing (Milton 2001; Holmes 2005). These would include the capture of dispersed seeds from adjacent pristine communities by, for example, roughening (pitting) of the soil surface or the placement of dead branches or shrubs on the soil surface to trap wind-blown indigenous seeds (Van der Merwe & Kellner 1999; Milton 2001). At the same time, the natural colonization process at the disturbed site could be assisted by the application of mulches (Cione et al. 2002) or reeds or wood chips (Holmes 2005) which are rich in carbon to promote bacterial immobilization of N as well as the erection of fences or enclosures to assist native species survival by restricting herbivory (Midoko-Iponga 2004). Further studies are required to identify the mechanisms of sucrose inhibition of plant photosynthesis and growth.

3.6. References

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Table 3.1. Effects of different herbicide applications and soil nutrient amendment treatments and their interaction on foliar chlorophyll contents of resident and introduced species in field-based and greenhouse-based trials. Values presented in bold type within each column followed by different letters significantly different at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

TRIALS/ SPECIES	HERBICIDE APPLICATION			SOIL NUTRIENT AMENDMENT TREATMENT				ANALYSIS OF VARIANCE		
	NONE	CONTACT	SYSTEMIC	NONE	GYPSUM	SUCROSE	GYPSUM + SUCROSE	HERBICIDE	NUTRIENT AMENDMENT	HERBICIDE X NUTRIENT AMENDMENT
FIELD-BASED TRIALS										
Resident aliens										
B. maxima	**2.3a**	**4.3b**	**2.4a**	**4.5a**	**3.9a**	**1.7b**	**1.6b**	$F_{2,255} = 4.4^*$	$F_{3,255} = 6.1^{***}$	$F_{6,255} = 1.4$
C. dactylon	1.8a	1.8a	1.9a	**2.3a**	**2.4a**	**1.4b**	**1.3b**	$F_{2,235} = 0.1$	$F_{3,235} = 8.8^{***}$	$F_{6,235} = 0.9$
Resident indigenes										
O. purpurea	-	-	-	-	-	-	-	-	-	-
Introduced indigenes										
L. praecox	**14.5a**	**22.3b**	**20.9b**	**28.1a**	**23.5a**	**12.0b**	**10.9b**	$F_{2,167} = 11.7^{***}$	$F_{3,167} = 28.0^{***}$	$F_{6,167} = 0.8$
L. xanthoconus	2.5a	3.7a	3.1a	**4.8a**	**3.9a**	**2.0b**	**1.7b**	$F_{2,200} = 2.3$	$F_{3,200} = 12.1^{***}$	$F_{6,200} = 0.6$
O. europea	**2.1a**	**2.0a**	**2.8b**	**2.9a**	**3.3a**	**1.6b**	**1.6b**	$F_{2,200} = 6.4^{**}$	$F_{3,200} = 15.7^{***}$	$F_{6,200} = 1.9$
P. cordifolium	**3.0a**	**8.1b**	**6.2ab**	**8.9a**	**7.8a**	**2.7b**	**1.9b**	$F_{2,107} = 7.5^{***}$	$F_{3,107} = 10.9^{***}$	$F_{6,107} = 1.9$
R. lucida	**16.0a**	**27.9b**	**18.5a**	**27.9a**	**31.5a**	**12.9b**	**10.2b**	$F_{2,194} = 28.2^{***}$	$F_{3,194} = 58.6^{***}$	$F_{6,194} = 3.3^{**}$
S. africana lutea	**4.2a**	**11.1b**	**6.2a**	**11.0a**	**10.7a**	**3.4b**	**3.7b**	$F_{2,194} = 20.9^{***}$	$F_{3,194} = 22.2^{***}$	$F_{6,194} = 1.7$
GREENHOUSE-BASED TRIALS										
Resident aliens										
B. maxima	3.3a	3.5a	3.4a	**4.4a**	**4.5a**	**2.0b**	**1.9b**	$F_{2,202} = 0.04$	$F_{3,202} = 23.7^{***}$	$F_{6,202} = 0.3$
C. dactylon	**1.4a**	**1.3a**	**1.6b**	1.5a	1.5a	1.5a	1.3a	$F_{2,175} = 4.0^*$	$F_{3,175} = 0.7$	$F_{6,175} = 3.7^{**}$
Resident indigenes										
O. purpurea	**7.1a**	**9.5b**	**7.9ab**	**11.5a**	**12.5a**	**4.3b**	**4.5b**	$F_{2,217} = 5.0^*$	$F_{3,217} = 45.9^{***}$	$F_{6,217} = 2.4^*$
Introduced indigenes										
A. acaulis	4.5a	6.9b	6.1ab	**8.2a**	**8.2a**	**2.3b**	**2.6b**	$F_{2,244} = 2.7$	$F_{3,244} = 19.7^{***}$	$F_{6,244} = 0.7$
D. pluvialis	5.0a	6.2a	6.0a	**7.1a**	**8.7a**	**3.2b**	**2.6b**	$F_{2,284} = 1.9$	$F_{3,284} = 22.1^{***}$	$F_{6,284} = 1.3$
R. laevaegata	-	-	-	-	-	-	-	-	-	-
U. anthemoides	1.3a	1.3a	1.2a	**1.3a**	**1.4a**	**1.1b**	**1.2b**	$F_{2,223} = 0.7$	$F_{3,223} = 33.0^{***}$	$F_{6,223} = 1.4$

Table 3.2. Effects of different herbicide applications and soil nutrient amendment treatments and their interaction on steady-state fluorescence yields of resident and introduced species in field-based and greenhouse-based trials. Values presented in bold type within each column followed by different letters significantly different at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

TRIALS/ SPECIES	HERBICIDE APPLICATION			SOIL NUTRIENT AMENDMENT TREATMENT				ANALYSIS OF VARIANCE		
	NONE	CONTACT	SYSTEMIC	NONE	GYPSUM	SUCROSE	GYPSUM + SUCROSE	HERBICIDE	NUTRIENT AMENDMENT	HERBICIDE X NUTRIENT AMENDMENT
FIELD-BASED TRIALS										
Resident aliens										
B. maxima	-	-	-	-	-	-	-	-	-	-
C. dactylon	-	-	-	-	-	-	-	-	-	-
Resident indigenes										
O. purpurea	0.681a	0.706a	0.679a	0.687ab	0.695ab	0.701a	0.668c	$F_{2,217} = 2.4$	$F_{3,217} = 1.9$	$F_{6,217} = 0.5$
Introduced indigenes										
L. praecox	**0.448a**	**0.514b**	**0.505b**	**0.610a**	**0.536b**	**0.381c**	**0.404c**	**$F_{2,157} = 6.9***$**	**$F_{3,157} = 25.5***$**	**$F_{6,157} = 3.3***$**
L. xanthoconus	0.586a	0.598a	0.577a	**0.650a**	**0.667a**	**0.513b**	**0.512b**	$F_{2,200} = 1.3$	**$F_{3,200} = 40.8***$**	$F_{6,200} = 0.9$
O. europea	0.429a	0.471a	0.449a	**0.536a**	**0.572a**	**0.338b**	**0.343b**	$F_{2,199} = 1.9$	**$F_{3,199} = 48.9***$**	$F_{6,199} = 1.5$
P. cordifolium	-	-	-	-	-	-	-	-	-	-
R. lucida	**0.417a**	**0.490b**	**0.428a**	**0.534a**	**0.562a**	**0.359b**	**0.332b**	**$F_{2,194} = 4.0*$**	**$F_{3,194} = 41.7***$**	$F_{6,194} = 1.1$
S. africana lutea	**0.554a**	**0.680b**	**0.578a**	**0.667a**	**0.693a**	**0.518b**	**0.542b**	**$F_{2,194} = 24.8***$**	**$F_{3,194} = 32.5***$**	$F_{6,194} = 1.1$
GREENHOUSE-BASED TRIALS										
Resident aliens										
B. maxima	**0.557a**	**0.597b**	**0.592b**	0.595a	0.600a	0.550b	0.573ab	**$F_{2,202} = 4.6*$**	$F_{3,202} = 1.6$	**$F_{6,202} = 3.0**$**
C. dactylon	0.569a	0.556a	0.574a	0.559ab	0.594a	0.566ab	0.554b	$F_{2,159} = 0.3$	$F_{3,159} = 2.1$	**$F_{6,159} = 2.8*$**
Resident indigenes										
O. purpurea	**0.532ab**	**0.571b**	**0.501a**	**0.608a**	**0.614a**	**0.474 b**	**0.454b**	**$F_{2,215} = 7.6***$**	**$F_{3,215} = 40.7***$**	$F_{2,215} = 0.4$
Introduced indigenes										
A. acaulis	0.595a	0.595a	0.585a	**0.659a**	**0.675a**	**0.488b**	**0.516b**	$F_{2,196} = 0.2$	**$F_{3,196} = 53.7***$**	$F_{6,196} = 2.0$
D. pluvialis	0.573a	0.570a	0.561a	**0.627a**	**0.624a**	**0.512b**	**0.505b**	$F_{2,217} = 0.3$	**$F_{3,217} = 27.2***$**	$F_{6,217} = 1.0$
R. laevaegata	0.515a	0.539a	0.544a	**0.494b**	**0.518ab**	**0.571a**	**0.546ab**	$F_{2,171} = 2.9$	**$F_{3,171} = 7.9***$**	$F_{6,171} = 0.8$
U. anthemoides	0.550a	0.555a	0.542a	**0.613a**	**0.624a**	**0.462b**	**0.479b**	$F_{2,204} = 0.2$	**$F_{3,204} = 33.3***$**	$F_{6,204} = 1.0$

Table 3.3. Effects of different herbicide applications and soil nutrient amendment treatments and their interaction on above-ground dry biomass of resident and introduced species in field-based and greenhouse-based trials. Values presented in bold type within each column followed by different letters significantly different at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. Values in parenthesis give percentage mortalities.

TRIALS/ SPECIES	HERBICIDE APPLICATION			SOIL NUTRIENT AMENDMENT TREATMENT				ANALYSIS OF VARIANCE		
	NONE	CONTACT	SYSTEMIC	NONE	GYPSUM	SUCROSE	SUCROSE + GYPSUM	HERBICIDE	NUTRIENT AMENDMENT	HERBICIDE X NUTRIENT AMENDMENT
FIELD-BASED TRIALS										
Resident aliens										
B. maxima	**3.14a**	**0.96b**	**0.68b**	**2.05ab**	**2.18a**	**1.20bc**	**0.97c**	$F_{2,71} = 114.6$***	$F_{3,71} = 17.4$***	$F_{6,71} = 1.3$
C. dactylon	**10.84a**	**2.28b**	**9.59a**	**8.78a**	**8.42a**	**6.27b**	**6.81b**	$F_{2,71} = 78.5$***	$F_{3,71} = 4.1$*	$F_{6,71} = 0.7$
Resident indigenes										
O. purpurea	**7.76a**	**1.76c**	**5.81b**	**7.23a**	**6.71a**	**3.33b**	**3.92b**	$F_{2,71} = 70.4$***	$F_{3,71} = 26.0$***	$F_{6,71} = 2.9$
Introduced indigenes										
L. praecox	2.67a(100.0)	3.41a(87.5)	2.32a(100.0)	3.00a(100.0)	3.07a(100.0)	2.74a(88.9)	2.41a(94.4)	$F_{2,49} = 2.0$	$F_{3,49} = 0.2$	$F_{6,49} = 3.7$**
L. xanthoconus	1.75a(87.5)	5.96a(66.7)	0.81a(79.2)	3.71a(100.0)	6.16a(77.8)	4.06a(61.1)	1.23a(72.2)	$F_{2,36} = 2.1$	$F_{3,36} = 0.5$	$F_{6,36} = 0.3$
O. europea	4.91a(100.0)	5.36a(95.8)	5.49a(100.0)	5.35a(100.0)	5.18a(94.4)	5.25a(100.0)	5.19a(100.0)	$F_{2,62} = 0.7$	$F_{3,62} = 0.0$	$F_{6,62} = 0.7$
P. cordifolium	**1.26a(100.0)**	**1.43a(100.0)**	**2.31b(58.3)**	2.26a(100.0)	1.67a(100.0)	1.68a(100.0)	1.26a(100.0)	$F_{2,24} = 4.3$*	$F_{3,24} = 1.7$	$F_{6,24} = 1.5$
R. lucida	9.91a(58.4)	11.37a(62.5)	8.18a(75.0)	9.29a(88.9)	9.28a(61.1)	9.19a(61.1)	10.99a(50.0)	$F_{2,53} = 0.9$	$F_{3,53} = 0.6$	$F_{6,53} = 1.9$
S. africana lutea	6.49a(100.0)	11.72b(75.0)	5.77a(100.0)	7.39a(88.9)	7.83a(88.9)	7.21a(100.0)	10.57a(88.9)	$F_{2,46} = 2.1$	$F_{3,46} = 0.5$	$F_{6,46} = 1.1$
GREENHOUSE-BASED TRIALS										
Resident aliens										
B. maxima	**3.20a**	**0.47b**	**0.60b**	**1.72a**	**1.98a**	**1.16a**	**0.82a**	$F_{2,59} = 155.2$***	$F_{3,59} = 13.8$***	$F_{6,59} = 9.4$***
C. dactylon	**13.03a**	**1.18c**	**6.93b**	**6.48a**	**5.09a**	**7.74a**	**8.89a**	$F_{2,59} = 162.7$***	$F_{3,59} = 8.2$***	$F_{6,59} = 3.0$*
Resident indigenes										
O. purpurea	**6.83a**	**2.10b**	**6.17a**	**6.26a**	**7.75a**	**3.33b**	**2.76b**	$F_{2,71} = 21.0$***	$F_{3,71} = 13.0$***	$F_{6,71} = 8.8$***
Introduced indigenes										
A. acaulis	**0.06a**	**0.19b**	**0.13b**	**0.17a**	**0.20a**	**0.07b**	**0.06b**	$F_{2,68} = 23.1$***	$F_{3,68} = 19.2$***	$F_{6,68} = 2.6$*
D. pluvialis	**0.13a**	**0.30b**	**0.20ab**	**0.35a**	**0.36a**	**0.05b**	**0.07b**	$F_{2,70} = 9.8$***	$F_{3,70} = 34.6$***	$F_{6,70} = 1.3$
R. laevaegata	**0.04a**	**0.30b**	**0.06a**	**0.09a**	**0.13ab**	**0.18b**	**0.10a**	$F_{2,283} = 86.9$***	$F_{3,283} = 11.4$***	$F_{6,283} = 14.2$***
U. anthemoides	**0.10a**	**0.31b**	**0.18b**	**0.32a**	**0.33a**	**0.06b**	**0.08b**	$F_{2,70} = 13.4$***	$F_{3,70} = 15.3$***	$F_{6,70} = 2.2$

Table 3.4. Effects of different herbicide applications and soil nutrient amendment treatments and their interaction on soil physical and chemical properties in field-based and greenhouse-based trials. Values presented in bold type within each column followed by different letters significantly different at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

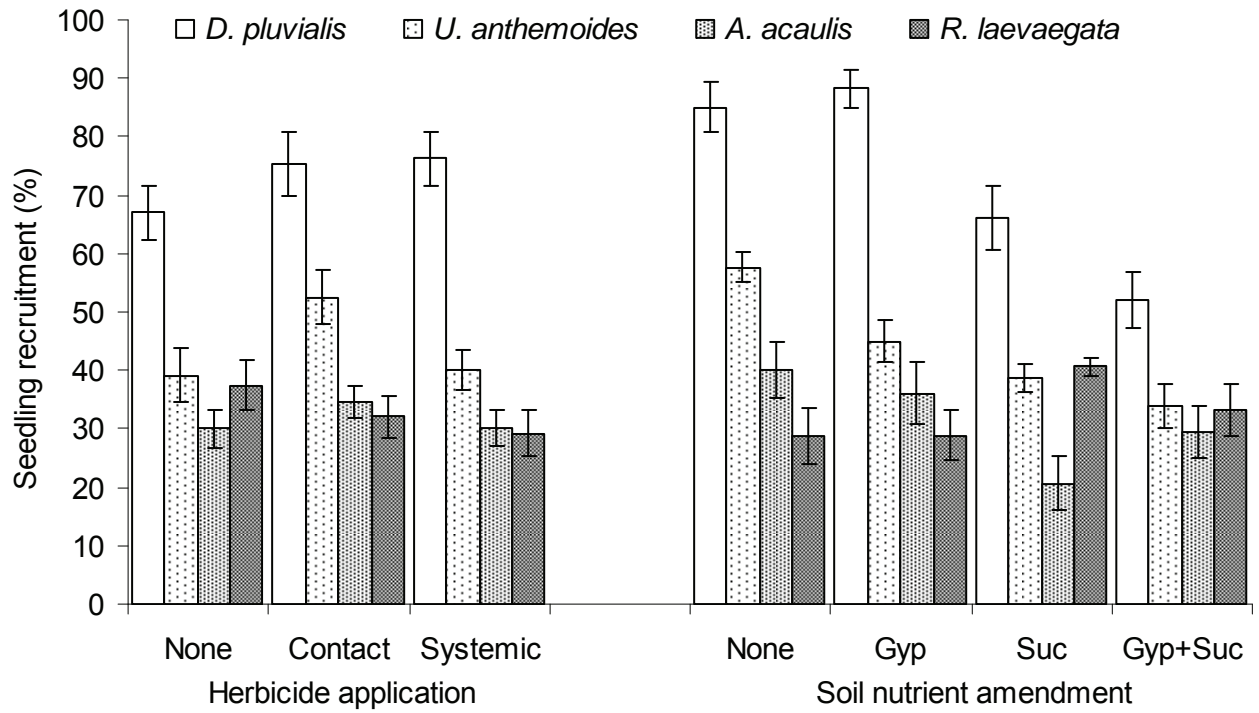|	HERBICIDE APPLICATION			SOIL NUTRIENT AMENDMENT TREATMENT				ANALYSIS OF VARIANCE		
	NONE	CONTACT	SYSTEMIC	NONE	GYPSUM	SUCROSE	SUCROSE + GYPSUM	HERBICIDE	NUTRIENT AMENDMENT	HERBICIDE X NUTRIENT AMENDMENT
FIELD-BASED TRIALS										
pH (KCl)	**4.77a**	**4.75a**	**4.95b**	4.86a	4.84a	4.83a	4.76a	**$F_{2,71}$ = 3.4***	$F_{3,71}$ = 0.4	$F_{6,71}$ = 0.3
K (mmol kg^{-1})	**1.99a**	**2.45b**	**2.41b**	2.17a	2.20a	2.50a	2.26a	**$F_{2,71}$ = 7.4*****	$F_{3,71}$ = 1.8	$F_{6,71}$ = 0.4
Na (mmol kg^{-1})	**0.51a**	**0.66b**	**0.60b**	0.59a	0.57a	0.60a	0.60a	**$F_{2,71}$ = 7.7*****	$F_{3,71}$ = 0.2	$F_{6,71}$ = 0.7
Ca (mmol kg^{-1})	25.94a	26.99a	32.75a	**21.34a**	**38.02b**	**20.77a**	**34.12b**	$F_{2,71}$ = 2.5	**$F_{3,71}$ = 10.9*****	$F_{6,71}$ = 0.2
Mg (mmol kg^{-1})	4.56a	5.19a	5.18a	**5.69a**	**4.98b**	**5.68a**	**4.14b**	$F_{2,71}$ = 1.4	**$F_{3,71}$ = 5.5****	$F_{6,71}$ = 0.8
C (cmol kg^{-1})	**64.93a**	**83.75b**	**83.93b**	**72.45a**	**75.78a**	**81.34b**	**80.56b**	**$F_{2,71}$ = 6.6****	**$F_{3,71}$ = 0.7***	**$F_{6,71}$ = 2.2***
N (mmol kg^{-1})	**38.84a**	**59.40c**	**51.48b**	48.55a	49.33a	51.26a	50.40a	**$F_{2,71}$ = 31.0*****	$F_{3,71}$ = 0.3	$F_{6,71}$ = 0.3
P Bray II (mmol kg^{-1})	0.51a	0.76a	0.78a	0.65a	0.73a	0.78a	0.58a	$F_{2,71}$ = 2.4	$F_{3,71}$ = 0.6	$F_{6,71}$ = 0.7
GREENHOUSE-BASED TRIALS										
pH (KCl)	**5.55a**	**5.22b**	**5.29b**	5.42a	5.45a	5.37a	5.37a	**$F_{2,68}$ = 5.6****	$F_{3,68}$ = 0.5	$F_{6,68}$ = 0.5
K (mmol kg^{-1})	1.19a	1.26a	1.21a	1.31a	1.19a	1.22a	1.17a	$F_{2,68}$ = 0.3	$F_{3,68}$ = 0.7	$F_{6,68}$ = 1.7
Na (mmol kg^{-1})	0.97a	1.01a	0.96a	1.08a	0.89a	1.02a	0.95a	$F_{2,68}$ = 0.1	$F_{3,68}$ = 0.6	$F_{6,68}$ = 1.0
Ca (mmol kg^{-1})	31.12a	26.67b	29.16ab	**24.69a**	**32.50b**	**24.84a**	**33.61b**	$F_{2,68}$ = 2.5	**$F_{3,68}$ = 10.0*****	$F_{6,68}$ = 1.1
Mg (mmol kg^{-1})	**3.11a**	**2.64a**	**2.61a**	**3.55a**	**1.95b**	**3.51a**	**2.16b**	**$F_{2,68}$ = 4.2***	**$F_{3,68}$ = 21.3*****	$F_{6,68}$ = 1.6
C (cmol kg^{-1})	**73.08a**	**86.62b**	**83.26ab**	78.47a	82.77a	84.27a	79.01a	**$F_{2,68}$ = 3.5***	$F_{3,68}$ = 0.6	$F_{6,68}$ = 0.7
N (mmol kg^{-1})	62.97a	64.18a	61.61a	61.83a	66.54a	59.90a	63.68a	$F_{2,68}$ = 0.2	$F_{3,68}$ = 0.7	$F_{6,68}$ = 0.9
P Bray II (mmol kg^{-1})	**0.15a**	**0.18b**	**0.15ab**	0.16a	0.16a	0.16a	0.14a	**$F_{2,68}$ = 5.6*****	$F_{3,68}$ = 0.7	$F_{6,68}$ = 0.3

Figure 3.1. Effect of different herbicide and soil nutrient amendment treatments on seedling recruitment of 4 different species in a greenhouse-based experimental trial.

Chapter 4

Does sucrose addition inhibit plant growth by depleting soil N?

Abstract

The hypothesis that exogenous sucrose addition to soils inhibits plant growth by stimulating soil microbial biomass, which accumulates soil nitrogen rendering it unavailable to plants, was tested. Two native, early seral species (*Dimorphotheca pluvialus* (L.) Moench and *Ursinia anthemoides* (L) Poir*. subsp anthemoides*) were cultured in heat sterilized (220^0C for 72 hours) and non-sterilized soils in a greenhouse under four different levels of sucrose (0, 100, 200 and 300 g m^{-2}) supplied monthly over a 4-month active growing period. Foliar chlorophyll contents, photosystem II (PSII) efficiencies, shoot and root lengths and dry masses, inflorescence numbers and N and P contents were measured in the plants, and N and P contents and bacterial cell and coliform numbers analyzed in the soils. Both *D. pluvialis* and *U. anthemoides* displayed significant reductions in PSII efficiency, chlorophyll content, accumulation of biomass and N and P in response to increased levels of sucrose. These findings initially seemingly supported the hypothesis since these reductions were of substantially greater magnitude in plants cultivated in non-sterilized than sterilized soils. Despite this, there was no evidence of any significant increases in bacterial and coliform cell numbers in response to increased levels of sucrose supplied or any significant reductions in soil N and P contents following sucrose additions in both sterile and non-sterilized soils. Greater numbers of bacteria and coliforms were measured in sterilized than non-sterilized soils. This corresponded with reduced soil N contents but these were not reflected in changes in plant PSII efficiency and growth and total amounts of N taken up by plants which displayed massive increases in sterilized soils. This study's findings did not support the hypothesis and pointed to an abiotic mechanism of sucrose inhibition of plant photosynthesis and growth.

Key words: Sucrose addition, soil sterilization, bacterial immobilization and soil nitrogen depletion.

4.1. Introduction

Addition of carbon sources to soils, such as sucrose, sawdust, straw, grain hulls or chopped wood that are high in carbon and low in nitrogen (Morgan 1994) have been

proposed as a means of reducing the availability of essential nutrients, especially nitrogen to plants (Corbin & D'Antonio 2004; Blumenthal et al. 2003; Eschen et al. 2007). These proposals are supported by reported increased rates of net N mineralization (Johnson & Edwards 1979; Gilliam et al. 2005) and nitrification (Gilliam et al. 2005) and reduced ammonium (Hopkins 1998) and nitrate (Blumenthal et al. 2003) concentrations in soils following carbon additions. The addition of carbon stimulates the growth and activity of soil microbes which accumulate soil nitrogen in their biomass rendering nitrogen unavailable to plants (Morgan 2004; Alpert & Maron 2000; Corbin & D'Antonio 2004; Blumenthal et al. 2003). As a consequence, native plants and late seral species are more able to out-compete invasive species and early seral species whose growth is restricted in the resulting low nitrogen environment (Redente et al. 1992; Wedin & Tilman 1990; Reever Morghan & Seastedt 1999; Alpert & Maron 2000; Blumenthal et al. 2003). Also, the lowering of soil nitrogen by adding carbon is believed to prevent invasion by weedy species (Willems & van Nieuwstadt 1996), since previous studies have shown that communities with high nutrient levels and low species diversity are more prone to alien invasion (Lodge 1993; Cowie & Werner 1992).

Conflicting reports exist as to the efficacy of carbon additions in reducing soil nitrogen levels and consequent plant growth. Both reduced (Blumenthal et al. 2003; Eschen et al. 2007) and unaltered (Corbin & D'Antonio 2004) soil N concentrations have been reported following carbon additions. Also, in a recent field and surrogate greenhouse trial, diminished biomass accumulation and decreased photosynthetic efficiencies were observed among both alien and native species following sucrose additions without corresponding decreases in soil N content (Chapter 3). This anomaly was attributed to transient increases in soil microbial populations induced by the more rapidly available source of carbon in sucrose (Dalenberg & Jager 1981), the observed effects of which last for periods of less than one month (Török et al. 2000). This contrasts with the less rapidly available sources of carbon in structurally more complex compounds such as wood chips and sawdust that take longer to degrade (Magill & Aber 2000), the effects of which last up to 4 months (Reever Morghan & Seastedt 1999; Cione et al. 2002).

There do exist alternate explanations for the observed inhibition of plant growth and photosynthetic performance following exogenous sucrose additions apart from an increased accumulation of available soil nitrogen in microbial biomass. Volatile metabolites produced by a number of rhizosphere pseudomonads, one of which identified as cyanide, have been shown to inhibit lettuce root growth in a seedling bioassay (Alström & Burns 1989). Also, high sugar concentrations in growth media are known to negatively affect plant photosynthetic capacity (Santamaría et al. 1999), this effect less pronounced at low irradiances (Fuentes et al. 2005) and rationalized by a source-sink equilibrium (Van Quy et al. 2001).

In view of these findings, the objectives of this study were to test whether the inhibition of plant growth and photosynthesis by exogenous addition of sucrose to soils has a biotic or abiotic influence. It was hypothesized that if a biotic component was involved, namely that the addition of exogenous sucrose stimulates soil microbial biomass which accumulates soil nitrogen rendering it unavailable to plants, then the effects of sucrose additions on soil N content, microbial abundance, plant growth and photosynthesis would be greater in non-sterilized than sterilized soils.

4.2. Methods and materials

4.2.1. Soil source

Soils for the experiment were sourced from an area of degraded natural vegetation situated in a transition zone (33°44"67' S to 33°44"72' S; 19°03"13' E to 19°03"17' E) between Swartland Shale Renosterveld and Swartland Alluvium Fynbos (Mucina & Rutherford 2006) in the Elandsberg Private Nature Reserve located 25 km north of Wellington in the Cape winelands district of South Africa (Parker 1982).

4.2.2. Experimental design and treatments

Ninety-six soil cores (20 cm diameter x 20 cm deep) collected from the reserve were sieved through a 2 mm mesh. Half of the collected soil volume was sterilized in forced draft oven at 220^0C for 72 hours and the other half retained as non-sterilized controls. The experimental design comprised 6 blocks located at different positions in a passively ventilated greenhouse disinfected with sodium hypochlorite (NaClO). Each block contained 16 pots also disinfected with sodium hypochlorite (NaClO). Half the pots in each block were filled with equivalent volumes (4.6 kg dry mass) of sterilized soil and the remaining pots with the same volumes of non sterilized soil (Appendix 4.1). Seeds of two target species, namely *Dimorphotheca pluvialus* (L.) Moench and *Ursinia anthemoides* (L) Poir*. subsp anthemoides*, common ephemerals in Swartland Shale renosterveld and Swartland Alluvium fynbos, were individually sown at depths of 5 mm into 4 of the sterilized and 4 of the non-sterilized potted soils in each block (8 pots per species per block). To the sterilized and non-sterilized potted soils containing each of the two target species in each block, 4 different levels of sucrose, namely 0 (control), 100 (S-level1), 200 (S-level2) and 300 (S-Level3) g m^{-2} were applied to the pots at monthly intervals over a four-month active growing period extending from late autumn (May 2008) to late winter (August 2008). During this period, pots were irrigated daily with an automated irrigation sprinkler system that supplied 4 mm of water per day. Seedlings of the two target species were thinned to equivalent densities (four seedlings per pot) three-weeks after germination and the pots weeded weekly to remove superfluous plant biomass.

Blocks and pots within each block were rotated monthly to account for minor variations in air temperature, light intensity and amounts of water dispensed within the greenhouse.

4.2.3. Plant photochemical efficiency and pigment composition

In fully expanded apical leaves of the two target species, foliar chlorophyll contents (CCI) were measured with a chlorophyll content meter (Model CCM-200, Opti-Sciences Inc., Hudson, NH, USA) and yields of quantum efficiency at a steady state (Y_s) measured with a modulated fluorometer (Model OSI-FI, Opti-Sciences Inc., Hudson, NH, USA). Measurements were confined to clear sky conditions between 1100 and 1300 SAST (solar noon) and conducted on mature plants prior to the commencement of flowering.

4.2.4. Plant growth responses

After termination of flowering 5 months after seed sowing, above- and below-ground parts of the two target species were harvested from the pots. Plant heights and root lengths were measured and the numbers of inflorescences on each plant counted. Above- and below-ground plant parts were dried in a forced draft oven at 60^0C and weighed. The dry plant samples were milled and stored in labeled containers for subsequent chemical analysis.

4.2.5. N and P concentrations in plant tissues and total uptake

N concentrations in dry plant tissue samples were determined with an elemental analyser (FP-528, LECO Corporation, USA) at a combustion temperature of 900°C (Horneck & Miller 1998). P was extracted from the dry plant tissue samples with concentrated hydrochloric acid (Bray & Kurtz 1945) and concentrations determined with an Inductive Coupled Plasma Optical Emission Spectrometer (700-ES, Varian Inc. Melbourne, Australia) at a wavelength of 213 nm (Isaac & Johnson 1998). The total amounts of N and P taken up by plants in each pot were computed from the products of their total dry mass (roots plus shoots) and their measured tissue concentrations (% dry mass/100).

4.2.6. Soil N and P contents

125 cm^3 samples of soil were collected from each pot after plants were harvested. The soil samples were dried at 30ºC in a forced draft oven and sieved through a 2 mm mesh. P concentrations in 5 g soil sub samples were analyzed according to the method of Bray & Kurtz (1945) following extraction of soil soluble P using a Bray-II extract. N concentrations in 5 g soil sub samples were measured with an Auto-Analyser (SP428, Nitrogen Analyser LECO Corporation, USA) following soil combustion as described by Clesceri et al. (1998). The total amounts of N and P remaining in the soils in each pot were computed from the

product of the initial soil dry mass and the measured fractional soil N and P contents (mg kg^{-1} P/1000; % N/100).

4.2.7. Soil microbial abundance

125 cm^3 samples of fresh soil were collected from each pot immediately after plants were harvested. Total numbers of bacterial cells present in 10 g sub samples of soil diluted 10-fold were assayed applying the membrane plate streak method and the numbers of coliforms present in the diluted soil sub samples assayed applying the membrane filter method (Finney et al. 2003).

4.2.8. Statistical analyses

A two-factor analysis of variance (generalized linear model) using UNISTAT version 5.5.06 tested the effects of the soil sterilization and different sucrose levels and their interactions on measured plant and soil parameters. Significantly different treatment means were separated with a Duncan's multiple range test.

4.3. Results

4.3.1. Plant photochemical efficiency and pigment composition

Significantly (P ≤ 0.001) higher foliar chlorophyll contents and steady state fluorescence yields (Y$_s$) were observed in both *D. pluvialis* and *U. anthemoides* grown in sterilized soils (Table 4.1). In contrast, the addition of sucrose at all three levels caused significant (P ≤ 0.001) reductions in foliar chlorophyll content and photosystem II (PSII) in both *D. pluvialis* and *U. anthemoides* (Table 4.1). However, there was a significant (P ≤ 0.01) interaction between soil sterilization and level of sucrose applied for foliar chlorophyll content in *D. pluvialis* and for Y$_s$ in both *D. pluvialis* and *U. anthemoides* (Table 4.1). Reductions in foliar chlorophyll levels in *D. pluvialis* following sucrose additions were of greater magnitude in non-sterilized soils than in sterilized soils (Appendix 4.4). Similarly decreases in Y$_s$ following sucrose additions were of greater magnitude in both *D. pluvialis* and *U. anthemoides* in non-sterilized soils than sterilized soils (Appendix 4.5 & 4.6).

4.3.2. Plant growth and reproduction

Significantly (P ≤ 0.001) increased plant heights, root lengths, inflorescence numbers, shoot and root dry masses were observed in both *D. pluvialis* and *U. anthemoides* grown in sterilized soils (Table 4.1). In contrast, the addition of sucrose at all three levels caused significant (P ≤ 0.001) reductions in plant heights, root lengths, inflorescence numbers and shoot dry masses in both species, but root dry masses in *U. anthemoides* only (Table 4.1).

However, in both species there were significant (P ≤ 0.05) interactions between soil sterilization and level of sucrose applied for all measured growth and reproductive parameters with the exception of inflorescence numbers in *D. pluvialis* and root lengths and root dry masses in *U. anthemoides* (Table 4.1). Again, reductions following sucrose additions were of greater magnitude in non-sterilized than sterilized soils with respect to plant height (Appendix 4.7 & 4.8) and shoot dry mass (Appendix 4.9 & 4.10) in both species, inflorescence numbers in *U. anthemoides*, and root lengths and root dry masses in *D. pluvialis* (Table 4.2).

4.3.3. N and P concentrations in plant tissues and total uptake

Soil sterilization resulted in significantly (P ≤ 0.01) increased tissue N concentrations in *D. pluvialis,* but significantly (P ≤ 0.01) decreased tissue N concentrations in *U. anthemoides* (Table 4.1). However, total amounts of N and P taken up by both *D. pluvialis* and *U. anthemoides* were significantly (P ≤ 0.001) increased in sterilized soils (Table 4.1). Also, there were significant (P ≤ 0.01) interactions between soil sterilization and level of sucrose applied for tissue P concentrations in *D. pluvialis* as well as for total amounts of N and P taken up by both *D. pluvialis* and *U. anthemoides* (Table 4.1). With respect to tissue P concentrations in *D. pluvialis*, these displayed decreases following sucrose additions were of much greater magnitude in non-sterilized than sterilized soils (Appendix 4.11). With respect to total amounts of N and P taken up by both *D. pluvialis* and *U. anthemoides* following sucrose additions, these also showed reductions of much greater magnitude in the non-sterilized than sterilized soils (Appendix 4.12 & 13).

4.3.4. Soil N and P contents

Significantly (P ≤ 0.05) diminished quantities of N were measured in sterilized soils in which both *D. pluvialis* and *U. anthemoides* were cultivated but total quantities of P were not-significantly (P ≥ 0.05) affected by soil sterilization (Table 4.1). Also, N and P contents of soils in which both *D. pluvialis* and *U. anthemoides* were cultivated were not significantly (P ≥ 0.05) affected by sucrose additions both in sterilized and non-sterilized soils.

4.3.5. Soil microbial abundance

Significantly (P ≤ 0.01) greater numbers of bacteria and coliforms were present in sterilized than non sterilized soils in which both *D. pluvialis* and *U. anthemoides* were cultivated (Table 4.1). Also, there was a significant (P ≤ 0.01) interaction between soil sterilization and level of sucrose applied for bacterial numbers in soils in which *U. anthemoides* was cultivated (Appendix 4.14). In these soils, total bacterial numbers displayed

greater increases overall following sucrose additions in sterilized than non sterilized soils (Table 4.2).

4.4. Discussion

Both *D. pluvialis* and *U. anthemoides*, like other early seral species (Redente et al. 1992; Wedin & Tilman 1990), displayed significant reductions in PSII efficiency, chlorophyll content, accumulation of biomass and N and P in response to increased levels of sucrose (Appendix 4.2 & 4.3) which initially seemingly supported the hypothesis as these reductions were of substantially greater magnitude in plants cultivated in non-sterilized than sterilized soils. Despite this, there was no evidence of any significant increases in bacterial and coliform cell numbers in response to increased levels of sucrose supplied, a feature also observed in other studies (Blumenthal et al. 2003; Reever Morghan & Seastedt 1999). Furthermore, there were no significant reductions in soil N and P contents following sucrose additions in both sterile and non-sterilized soils. The significantly greater numbers of bacteria and coliforms measured in sterilized than non-sterilized soils did concur with previous reports that soil sterilization either by application of chemicals, steam, or dry heat reduces seedling mortality and increases plant growth, and availability of soil nutrients (Thulin et al. 1958; Will & Bassett 1962; Ingestad & Nilsson 1964), except in N-fixing plants (Rodríguez-Echeverri & Pérez-Fernández 2005). Strangely, greater numbers of bacteria and coliforms were measured in sterilized than non-sterilized soils, a possible consequence of reduced competition and predation from other soil micro-flora and fauna exterminated by heating of the soils. These increased bacterial and coliform numbers in sterilized soils corresponded with reduced soil N contents, the latter an unlikely consequence of only microbial immobilization of N, since heat sterilization probably also destroyed a high fraction of the decomposable organic matter present in the sterilized soils. Also, the reduced soil N contents in the sterilized soils were not reflected in like changes in plant PSII efficiency and growth and total amounts of N taken up by plants which displayed massive increases in sterilized soils, though these amounts represented less than 4% of the total amount present in the soils. The elevated plant PSII efficiencies and growth observed in the sterilized soils were likely indicative of a release from natural pathogens in compliance with the enemy release hypothesis (Mitchell & Power 2003; Keane & Crawley 2002). In this regard, nematodes, such as *Pratylenchus* and *Paratrichodorus* spp, have been implicated in inhibiting plant growth by extracting nutrients from and damaging plant root systems (Zahid et al. 2002).

In view of the above findings, an abiotic mechanism of sucrose inhibition of plant photosynthesis and growth seems more likely. Indeed, several studies have reported that exogenous sucrose additions reduce foliar concentrations of both chlorophyll *a* and *b* and net photosynthetic rates (Mosaleeyanon et al. 2004; Fuentes et al. 2005). This attributed to the

accumulation of hexose (Hdider & Desjardins 1994) and starch in chloroplasts (Capellades et al. 1991; Mosaleeyanon et al. 2004; Fuentes et al. 2005) causing feedback inhibition of photosynthesis and consequent decreased plant growth (Langford & Wainwright 1987). Addition of exogenous sucrose at high concentrations has also been shown to inhibit both root and shoot growth in rice (Thapa et al. 2007) hypocotyl elongation (Jang et al. 1997) and light-induced cotyledon opening (Dijkwel et al. 1997; Jang et al. 1997) of *Arabidopsis* seedlings (Ohto et al. 2001) and also causes flowering delays in *Arabidopsis* (Ohto et al. 2001; Zhou et al. 1998), the latter attributed to a metabolic rather than an osmotic effect (Ohto et al. 2001). The differential effects of exogenous sucrose additions on plant photosynthetic rates and growth under low and high photosynthetic photon flux densities (PPFD) respectively have been rationalized by an alteration in source-sink equilibrium (Van Quy et al. 2001). Under source-limitations of photosynthesis (low PPFD as under laboratory conditions and low atmospheric CO_2) the supply of sucrose results in increased plant growth and plant photosynthetic rates, foliar chlorophyll contents and quantum yields of PS II, as well as soluble sugar (hexose, sucrose) and starch contents and increased the activity of sucrose synthase (SS), sucrose phosphate synthase (SPS) acid invertase (INV) and ADP-glucose pyrophosphorylase (ADPGppase). In contrast, under sink-limitations of photosynthesis (high PPFD as under natural conditions and high atmospheric CO_2 concentration) the supply of sucrose results in decreased plant growth accompanied by larger accumulations of hexose and starch greater stimulation of INV but marked repression of SPS and ADPGppase activity (Van Quy et al. 2001).

In conclusion, this study's findings did not support the hypotheses that exogenous sucrose additions inhibit plant photosynthesis and growth by stimulating soil microbial biomass which accumulates soil nitrogen rendering it unavailable to plants.

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Table 4.1. Effects of soil sterilization and different added levels of sucrose on measured soil and plant parameters. Values presented in bold type within each soil sterilization treatment followed by different superscript letters significantly different at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Species/ Parameter	NON-STERILIZED SOILS				STERILIZED SOILS				ANALYSIS OF VARIANCE		
	Cont	Sucrose Level 1	Sucrose Level 2	Sucrose Level 3	Cont	Sucrose Level 1	Sucrose Level 2	Sucrose Level 3	Soil Sterilization	Sucrose Level	Soil Sterilization x Sucrose Level
Dimorphotheca pluvialis											
Chlorophyll content index	**15.75[a]**	**4.53[b]**	**5.27[b]**	**1.05[b]**	**40.40[a]**	**18.54[b]**	**15.85[bc]**	**10.40[c]**	**F$_{1,287}$ = 108.6*****	**F$_{3,287}$ = 48.0*****	**F$_{3,287}$ = 4.6****
Steady state fluorescence yield	**0.78[a]**	**0.54[b]**	**0.53[b]**	**0.28[c]**	**0.79[a]**	**0.75[ab]**	**0.72[b]**	**0.66[c]**	**F$_{1,289}$ = 57.4*****	**F$_{3,289}$ = 29.1*****	**F$_{3,289}$ = 11.7*****
Plant height cm	**13.95[a]**	**9.09[b]**	**5.92[c]**	**3.99[c]**	**20.71[ab]**	**24.71[a]**	**18.21[b]**	**12.30[c]**	**F$_{1,177}$ = 164.1*****	**F$_{3,177}$ = 24.9*****	**F$_{3,177}$ = 5.8*****
Root length cm	**5.56[a]**	**3.31[b]**	**2.15[c]**	**1.18[c]**	**4.87[ab]**	**5.13[a]**	**4.15[b]**	**2.00[c]**	**F$_{1,177}$ = 16.7*****	**F$_{3,177}$ = 38.5*****	**F$_{3,177}$ = 7.0*****
Inflorescence numbers	**1.71[a]**	**0.71[b]**	**0.27[c]**	**0.00[c]**	**4.96[a]**	**4.42[a]**	**2.33[b]**	**1.65[b]**	**F$_{1,170}$ = 77.4*****	**F$_{3,170}$ = 13.7*****	F$_{3,170}$ = 2.3
Total shoot dry mass mg pot^{-1}	**1503.80[a]**	**427.30[b]**	**200.00[bc]**	**20.50[c]**	**7700.00[a]**	**7793.30[a]**	**5392.20[ab]**	**1769.60[b]**	**F$_{1,47}$ = 65.1*****	**F$_{3,47}$ = 6.8*****	**F$_{3,47}$ = 3.6****
Total root dry mass mg pot^{-1}	**194.70[a]**	**59.90[b]**	**33.90[bc]**	**4.30[c]**	**424.00[a]**	**538.50[a]**	**401.00[a]**	**100.80[b]**	**F$_{1,47}$ = 120.2*****	**F$_{3,47}$ = 19.8*****	**F$_{3,47}$ = 9.7*****
Plant N content % dry mass	1.22[a]	0.96[a]	1.01[a]	1.06[a]	2.26[a]	1.82[a]	2.36[a]	1.96[a]	**F$_{1,47}$ = 18.9*****	F$_{3,47}$ = 0.4	F$_{3,47}$ = 0.2
Plant P content % dry mass	**0.27[a]**	**0.11[c]**	**0.18[bc]**	**0.20[ab]**	0.20[a]	0.27[a]	0.30[a]	0.23[a]	**F$_{1,47}$ = 8.6****	F$_{3,47}$ = 1.0	**F$_{3,47}$ = 6.1****
Total plant N mg pot^{-1}	**20.86[a]**	**4.74[b]**	**2.71[b]**	**0.26[b]**	**145.07[a]**	**132.30[a]**	**125.60[a]**	**35.33[c]**	**F$_{1,47}$ = 101.7*****	**F$_{3,47}$ = 7.7*****	**F$_{3,47}$ = 4.9****
Total plant P mg pot^{-1}	**4.41[a]**	**0.56[b]**	**0.44[b]**	**0.05[b]**	**17.55[ab]**	**22.46[a]**	**17.11[ab]**	**5.55[b]**	**F$_{1,47}$ = 53.9****	**F$_{3,47}$ = 4.2***	**F$_{3,47}$ = 3.1****
Total soil N mg pot^{-1}	3989.79[a]	4603.60[a]	5754.50[a]	5524.32[a]	3913.06[a]	4143.24[a]	4066.51[a]	3913.06[a]	**F$_{1,47}$ = 5.9***	F$_{3,47}$ = 1.1	F$_{3,47}$ = 1.1
Total soil P mg pot^{-1}	224.28[a]	237.23[a]	231.42[a]	229.91[a]	235.90[a]	230.13[a]	241.60[a]	245.08[a]	F$_{1,47}$ = 3.4	F$_{3,47}$ = 0.7	F$_{3,47}$ = 1.5
Total bacteria cells g^{-1} x 10^{-6}	2.63[a]	3.30[a]	15.07[a]	8.40[a]	18.98[a]	35.80[a]	20.03[a]	21.41[a]	**F$_{1,23}$ = 9.4****	F$_{3,23}$ = 0.5	F$_{3,23}$ = 1.1
Total coliform cells g^{-1} x 10^{-3}	**0.97[b]**	**2.83[ab]**	**3.73[a]**	**3.17[ab]**	3.37[a]	4.00[a]	4.00[a]	4.00[a]	**F$_{1,23}$ = 7.8****	**F$_{3,23}$ = 3.2****	F$_{3,23}$ = 1.2
Ursinia anthemoides											
Chlorophyll content index	**1.36[a]**	**1.04[b]**	**0.00[b]**	**1.00[b]**	**1.60[a]**	**1.32[b]**	**1.32[b]**	**1.17[b]**	**F$_{1,251}$ = 20.8*****	**F$_{2,251}$ = 17.0*****	F$_{2,251}$ = 0.2
Steady state fluorescence yield	**0.77[a]**	**0.60[b]**	**0.00[c]**	**0.20[c]**	**0.77[a]**	**0.73[b]**	**0.73[b]**	**0.73[b]**	**F$_{1,251}$ = 58.4*****	**F$_{3,251}$ = 34.5*****	**F$_{3,251}$ = 91.4*****
Plant height cm	**20.63[a]**	**4.53[b]**	**4.47[b]**	**3.36[b]**	**54.80[a]**	**45.54[b]**	**40.17[b]**	**25.40[c]**	**F$_{1,176}$ = 442.9*****	**F$_{3,176}$ = 39.4*****	**F$_{3,176}$ = 6.3*****
Root length cm	**3.24[a]**	**1.41[b]**	**1.60[b]**	**1.06[b]**	**6.17[a]**	**5.95[a]**	**4.97[ab]**	**4.32[b]**	**F$_{1,176}$ = 145.4*****	**F$_{3,176}$ = 8.9*****	F$_{3,176}$ = 1.5
Inflorescence numbers	**1.30[a]**	**0.00[b]**	**0.00[b]**	**0.00[b]**	**10.38[a]**	**6.29[b]**	**3.83[c]**	**1.45[d]**	**F$_{1,169}$ = 250.2*****	**F$_{3,169}$ = 45.8*****	**F$_{3,169}$ = 24.6*****
Total shoot dry mass mg pot^{-1}	**739.30[a]**	**37.10[b]**	**12.70[b]**	**9.40[b]**	**19461.70[a]**	**12765.00[b]**	**7938.30[b]**	**2918.40[c]**	**F$_{1,47}$ = 154.7*****	**F$_{3,47}$ = 18.7*****	**F$_{3,47}$ = 15.8*****
Total root dry mass mg pot^{-1}	**88.00[a]**	**9.60[b]**	**3.80[b]**	**2.70[b]**	**1355.6[ab]**	**1860.50[a]**	**887.00[ab]**	**489.80[c]**	**F$_{1,47}$ = 36.4*****	F$_{3,47}$ = 2.7	F$_{3,47}$ = 2.4
Plant N content % dry mass	**1.73[c]**	**1.62[c]**	**2.24[b]**	**2.80[a]**	0.69[a]	0.65[a]	0.90[a]	1.12[a]	**F$_{1,49}$ = 63.7*****	**F$_{3,47}$ = 5.8****	F$_{3,47}$ = 1.1
Plant P content % dry mass	0.21[a]	0.16[a]	0.16[a]	0.18[a]	0.16[a]	0.21[a]	0.22[a]	0.21[a]	F$_{1,47}$ = 2.3	F$_{3,47}$ = 0.1	F$_{3,47}$ = 2.1
Total plant N mg pot^{-1}	**13.23[a]**	**0.75[b]**	**0.37[b]**	**0.34[b]**	**134.72[a]**	**90.18[b]**	**67.14[b]**	**27.81[c]**	**F$_{1,47}$ = 148.0*****	**F$_{3,47}$ = 16.0*****	**F$_{3,47}$ = 10.0*****
Total plant P mg pot^{-1}	**1.78[a]**	**0.06[b]**	**0.02[b]**	**0.02[b]**	**34.51[a]**	**30.81[ab]**	**19.21[b]**	**5.62[c]**	**F$_{1,47}$ = 115.4*****	**F$_{3,47}$ = 11.0*****	**F$_{3,47}$ = 9.2*****
Total soil N mg pot^{-1}	5217.41[a]	4833.78[a]	5217.41[a]	5524.32[a]	4143.24[a]	3913.06[a]	3836.33[a]	4450.15[a]	**F$_{1,47}$ = 6.9***	F$_{3,47}$ = 0.4	F$_{3,47}$ = 0.1
Total soil P mg pot^{-1}	231.52[a]	224.79[a]	228.40[a]	225.09[a]	228.04[a]	218.56[a]	228.37[a]	239.52[a]	F$_{1,47}$ = 0.1	F$_{3,47}$ = 0.6	F$_{3,47}$ = 0.6
Total bacteria cells g^{-1} x 10^{-6}	4.38[a]	5.10[a]	2.46[a]	7.60[a]	**20.46[b]**	**40.00[a]**	**24.67[b]**	**13.83[b]**	**F$_{1,23}$ = 95.6*****	**F$_{3,23}$ = 6.8****	**F$_{3,23}$ = 8.7*****
Total coliform cells g^{-1} x 10^{-3}	**3.80[a]**	**3.50[ab]**	**2.17[c]**	**3.47[ab]**	3.00[a]	2.67[a]	4.00[a]	4.00[a]	F$_{1,23}$ = 0.2	F$_{3,23}$ = 0.4	F$_{3,23}$ = 1.9

Table 4.2. Percentage changes relative to controls of measured parameters in two target species and soils in which they were cultivated in response to added levels of sucrose within two soil sterilization treatments.

Target species/ Parameter	NON-STERILIZED SOILS			STERILIZED SOILS		
	Sucrose Level 1	Sucrose Level 2	Sucrose Level 3	Sucrose Level 1	Sucrose Level 2	Sucrose Level 3
Dimorphotheca pluvialis						
Chlorophyll content index	-71.2	-66.5	-93.3	-54.1	-60.8	-74.3
Steady state fluorescence yield	-30.8	-32.1	-64.1	-5.1	-8.9	-16.5
Plant height cm	-34.8	-57.6	-71.4	19.3	-12.1	-40.6
Root length cm	-40.5	-61.3	-78.8	5.3	-14.8	-58.9
Inflorescence numbers	-58.5	-84.2	-100.0	-10.9	-53.0	-66.7
Shoot dry mass mg pot^{-1}	-71.6	-86.7	-98.6	1.2	-30.0	-77.0
Root dry mass mg pot^{-1}	-69.2	-82.6	-97.8	27.0	-5.4	-76.2
Plant N % dry mass	-21.3	-17.2	-13.1	-19.5	4.4	-13.3
Plant P % dry mass	-59.3	-33.3	-25.9	35.0	50.0	15.0
Total plant mg pot^{-1}	-77.3	-87.0	-98.8	-8.8	-13.4	-75.6
Total plant P mg pot^{-1}	-87.3	-90.0	-98.9	28.0	-2.5	-68.4
Total soil N mg pot^{-1}	15.4	44.2	38.5	5.9	3.9	0.0
Total soil P mg pot^{-1}	5.8	3.2	2.5	-2.4	2.4	3.9
Total bacteria cells g^{-1} x 10^{-6}	25.5	473.0	219.4	88.6	5.5	12.8
Total coliform cells g^{-1} x 10^{-3}	191.8	284.5	226.8	18.7	18.7	18.7
Ursinia anthemoides						
Chlorophyll content index	-23.5	-	-26.5	-17.5	-17.5	-26.9
Steady state fluorescence yield	-22.1	-	-74.0	-5.2	-5.2	-5.2
Plant height cm	-78.0	-78.3	-83.7	-16.9	-26.7	-53.6
Root length cm	-56.5	-50.6	-67.3	-3.6	-19.4	-30.0
Inflorescence numbers	-100.0	-100.0	-100.0	-39.4	-63.1	-86.0
Shoot dry mass mg pot^{-1}	-95.0	-98.3	-98.7	-34.4	-59.2	-85.0
Root dry mass mg pot^{-1}	-89.1	-95.7	-96.9	37.2	-34.6	-63.9
Plant N % dry mass	-6.4	29.5	61.8	-5.8	30.4	62.3
Plant P % dry mass	-23.8	-23.8	-14.3	31.3	37.5	31.3
Total plant mg pot^{-1}	-94.3	-97.2	-97.4	-33.1	-50.2	-79.4
Total plant P mg pot^{-1}	-96.6	-98.9	-98.9	-10.7	-44.3	-83.7
Total soil N mg pot^{-1}	-7.4	0.0	5.9	-5.6	-7.4	7.4
Total soil P mg pot^{-1}	-2.9	-1.3	-2.8	-4.2	0.1	5.0
Total bacteria cells g^{-1} x 10^{-6}	16.4	-43.8	73.5	95.5	20.6	-32.4
Total coliform cells g^{-1} x 10^{-3}	-7.9	-42.9	-8.7	-11.0	33.3	33.3

Chapter 5

Conclusions and recommendations

5.1. Main conclusions

This thesis reports on field- and greenhouse-based experimental trials that examined the effects of adding carbon (as sucrose) and calcium (as gypsum) to soils of a degraded lowland fynbos ecosystem. The overall aim was to reduce soil N and P availability to promote the competitiveness of native taxa against alien invasive grasses. The main conclusions are as follows:

1. The addition of sucrose to soils reduces photosynthetic function and growth equally in both alien grass and indigenous species.

2. Reductions in photosynthetic function and growth of indigenous species following sucrose additions are of smaller magnitude where herbicides are first applied to remove excessive alien grass biomass.

3. The addition of gypsum to soils has a negligible effect on the photosynthetic function and growth of both alien grass and indigenous species.

4. Introduced seeds of indigenous species germinate poorly in soils from the degraded renosterveld ecosystem and seedlings of indigenous species introduced into the degraded ecosystem display high mortalities *in situ*.

5. Soil N and P concentrations are unaffected by sucrose and gypsum additions.

6. Plant photosynthetic function and growth are more severely inhibited by sucrose additions to non-sterilized than sterilized soils from the degraded renosterveld ecosystem but these are not matched by corresponding increases in microbial biomass and reductions in soil N and P concentrations.

5.2. Recommendations

The recommendations and restoration options arising from the main conclusions are as follows:

1. The feasibility of adding sucrose to a degraded renosterveld ecosystem to promote the competitiveness of native taxa against alien grasses is dubious, since equivalent reductions in photosynthetic function and growth occur among both alien and indigenous species. These findings concur with Corbin & D'Antonio (2004) who reported no negative effects of carbon additions on alien and native plant growth and soil N levels over a two year monitoring period. However, several other studies which applied different sources of carbon, such as sawdust and chopped wood, have

demonstrated significant soil N and alien grass growth reductions (Reever Morghan & Seastedt 1999; Blumenthal et al. 2003; Eschen et al. 2007). These findings imply that alternate C sources, such as sawdust, reeds, chopped wood or a mixture of these, which have been successfully applied in other degraded ecosystems (Török et al. 2000; Reever Morghan & Seastedt 1999; Blumenthal et al. 2003; Eschen et al. 2007), may provide a more efficient means of reducing soil N availability.

Where alternative C sources do not assist in restoring a degraded renosterveld ecosystem, additional techniques that promote the natural re-colonization of degraded areas by adjacent pristine communities may be considered (Milton 2001; Holmes 2005). These would include the capture of dispersed seeds from adjacent pristine communities by, for example, roughening (pitting) of the soil surface or the placement of dead branches or shrubs on the soil surface to trap wind-blown indigenous seeds (Van der Merwe & Kellner 1999; Milton 2001). At the same time, the natural colonization process in the degraded area could be further assisted by the application of mulches to limit soil moisture loss (Cione et al. 2002) and reeds or wood chips (Holmes 2005) which are rich in carbon to promote bacterial immobilization of N.

2. The application of herbicides to remove excessive alien grass biomass is not recommended as the decomposing grass biomass provides an additional source of nutrients for plant growth which reduces the efficacy of nutrient amendment treatments. This apparent from the smaller reductions in photosynthetic function and growth observed among introduced and resident indigenous species following sucrose additions. Other effective removal techniques such as grass mowing prior to seed maturation (Mathews 1996; Gill 1996; Musil et al. 2005) followed by the removal of the cut grass biomass for use as fodder in restricted feed lots to offset clearing costs could provide a more plausible grass removal strategy for use in combination with nutrient amendment treatments.

3. The observed inhibition by exogenous sucrose additions of photosynthetic function and growth in alien grasses suggest that sucrose applications may provide an environmentally friendly means of limiting their growth. The estimated costs of sucrose applications (R11 200 ha^{-1} based on the amount of sucrose we added) are more less equivalent to those reported (Musil et al. 2005) for application of pre-emergent herbicides (R13 380 ha^{-1}), but do exceed costs of hand clearing of grass infestations (R6 743 ha^{-1}), as well as clearing costs associated with intense burning of uncut grass (R415 ha^{-1}), grass mowing (R924 ha^{-1}) grass mowing and burning (R1338 ha^{-1}) and clearing of dense stands of woody aliens (up to R1 927 ha^{-1}).

4. The negligible effects of gypsum additions on photosynthetic function and growth of both alien and indigenous species is a likely consequence of the high acidity of fynbos soils that preclude the formation of insoluble P complexes such as dibasic calcium phosphate dihydrate, octocalcium phosphate and hydroxyapatite which occur mostly in alkaline soils with a soil pH > 7.3. In acidic soils, especially with a soil pH less than 5.5, the addition of Fe and Al would potentially provide a better option in reducing P availability as these elements react with soil P by first forming amorphous Al and Fe phosphates which gradually change into insoluble compounds of phosphate generally unavailable to plants that resemble crystalline variscite, an Al phosphate, and strengite, an Fe phosphate (Busman et al. 2002).

5. The transfer of seed bearing soils from pristine communities into adjacent degraded areas (Hölzel & Otte 2003; McDonald 1993) could provide a means of circumventing the poor *in situ* seed germination observed among seeds of indigenous species sown into soils of the degraded renosterveld ecosystem. The advantages associated with such soil transfers are that the entire species-complement is introduced, including rare species whose seeds are often not easily collected, and that the genetic variability of locally adapted ecotypes and races are preserved and maintained (Hölzel & Otte 2003) as well as addition of mutualist such as mycorrhizae which are known to improve the water and nutrient uptake of plants, especially of immobile nutrients such as phosphorus (Redman et al. 2001).

6. The mechanical removal of nutrient enriched top soils in degraded renosterveld ecosystems may provide a more reliable means of reducing soil N levels (Hölzel & Otte 2003; Aerts et al. 1995) in view of conflicting findings that C additions as sucrose increase microbial biomass and soil N immobilization (Zink & Allen 1998; Török et al. 2000; Reever Morghan and Seastedt 1999; Blumenthal et al. 2003; Eschen et al. 2007; Corbin & D'Antonio 2004). It has been shown that topsoil removal to depth of 30 cm leads to drastic reductions in organic matter and essential mineral nutrients (Aerts et al. 1995; Jansen & Roelofs 1996) as well as a 60 to 80% reduction in the alien grass seed bank (Hölzel & Otte 2003; Tallowin & Smith 2001; Verhagen et al. 2001). However, such topsoil removal needs to be combined with soil transfers from pristine communities in order to simultaneously introduce seeds and other propagules of native species.

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Appendices to Chapter 3

Appendix 3.1. Field- and greenhouse experimental designs with lists of species introduced as seeds and seedlings.
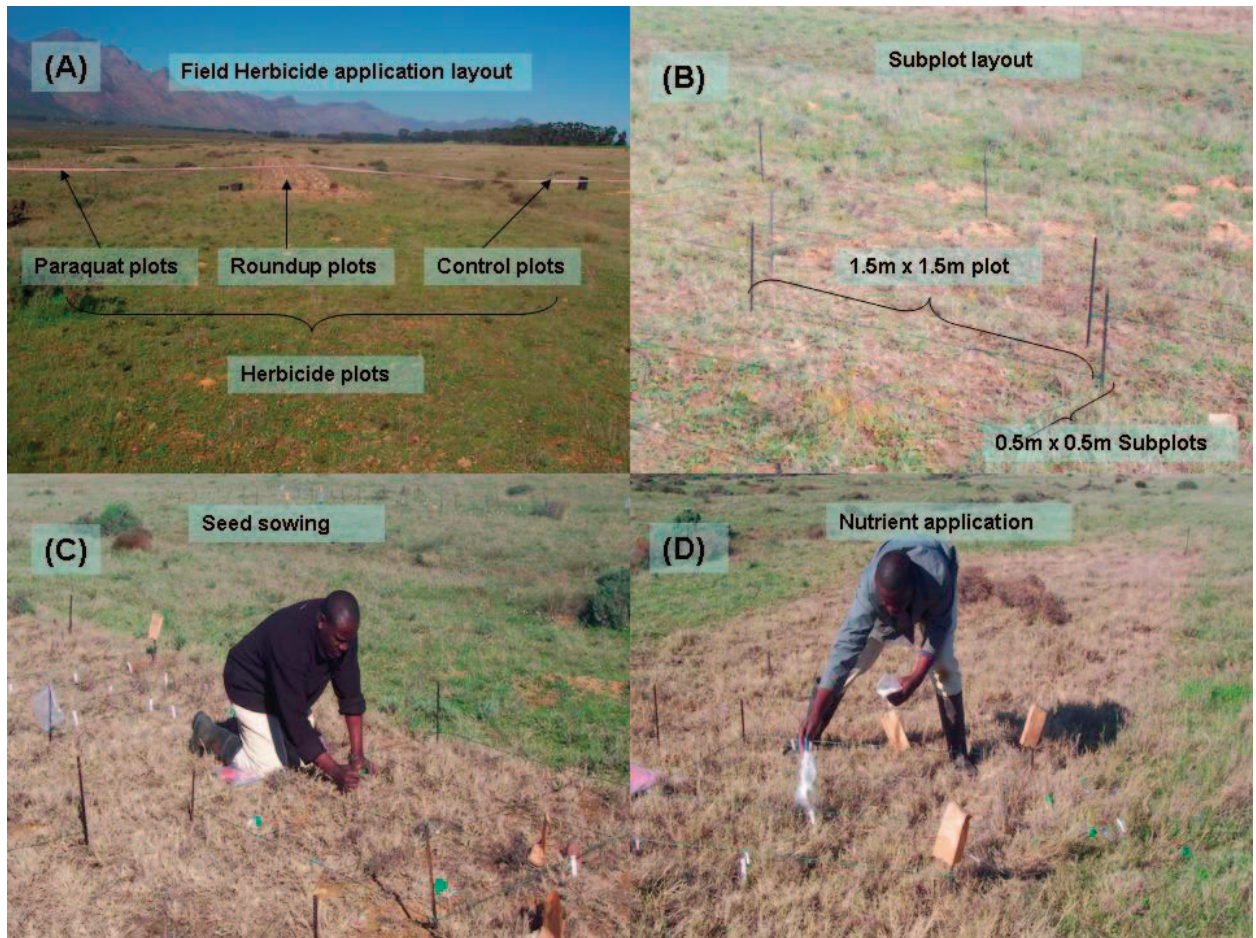
Appendix 3.2. Herbicide treated 40 m x 5 m field transects (a) experimental 1.5 m x 1.5 m plots and 0.5 m x 0.5 m subplots within each transect (b) seed sowing into sub-plots (c) and application of sucrose and gypsum into sub-plots (d).
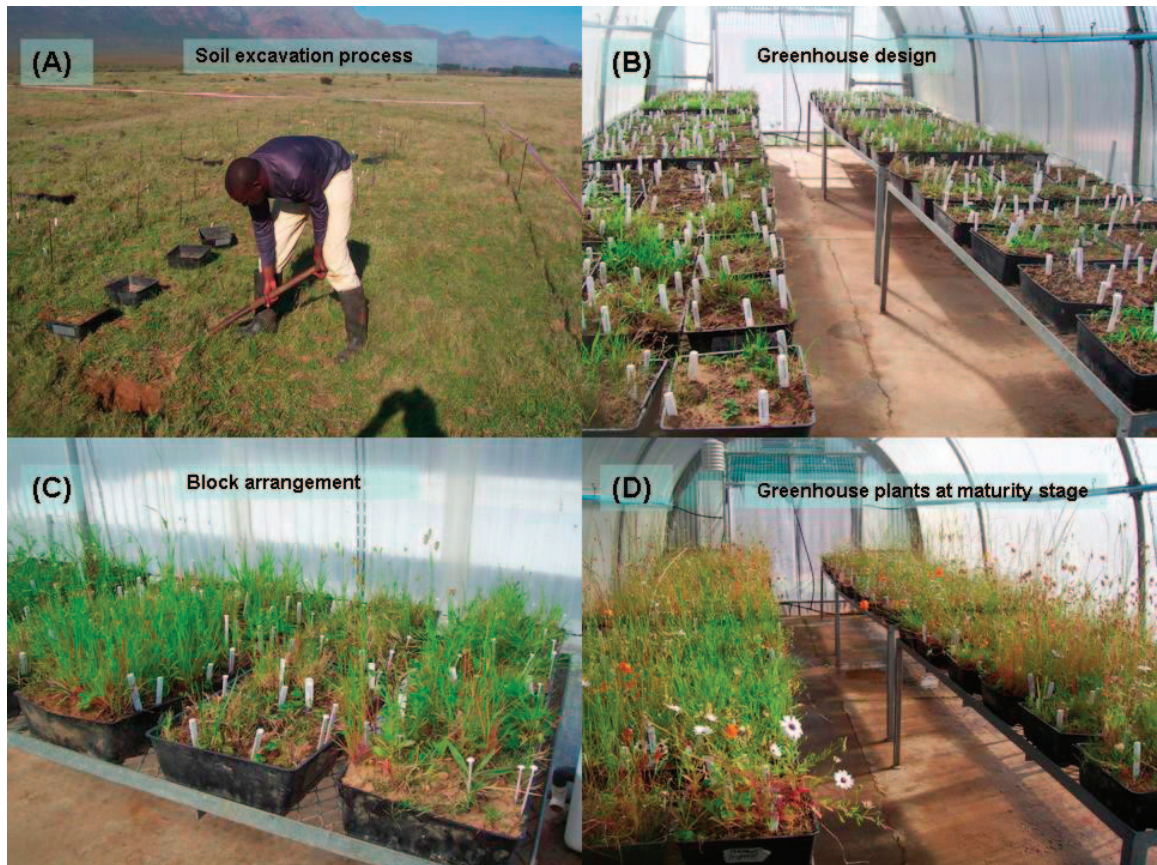
Appendix 3.3. Soil excavation into trays (a) and their layout in a randomized block design in a passively ventilated greenhouse (b, c & d).
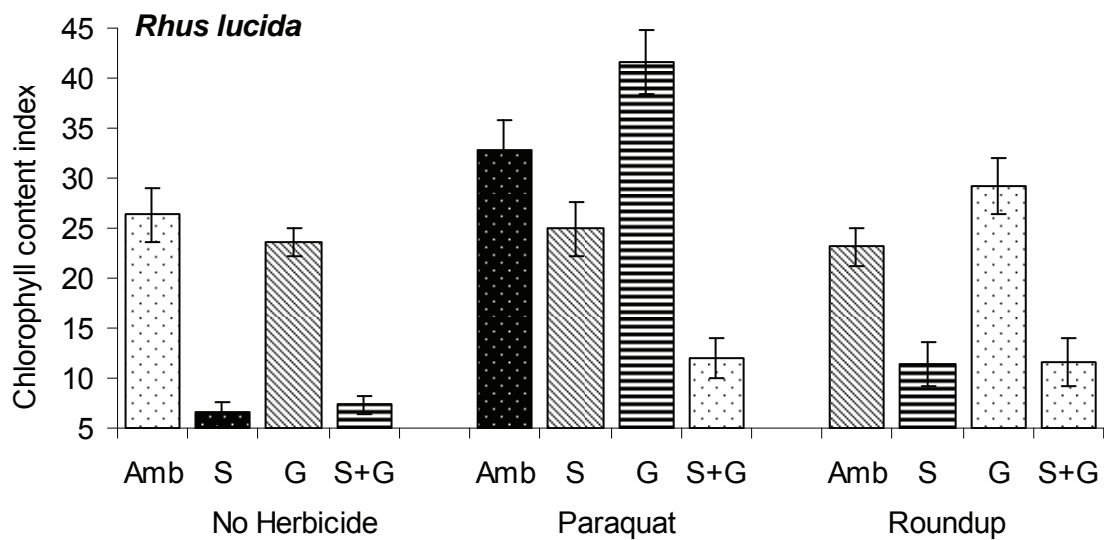
Appendix 3.4. Effect of different herbicide and soil nutrient amendment treatments on the chlorophyll content of *R. lucida* in the field based experimental trial.
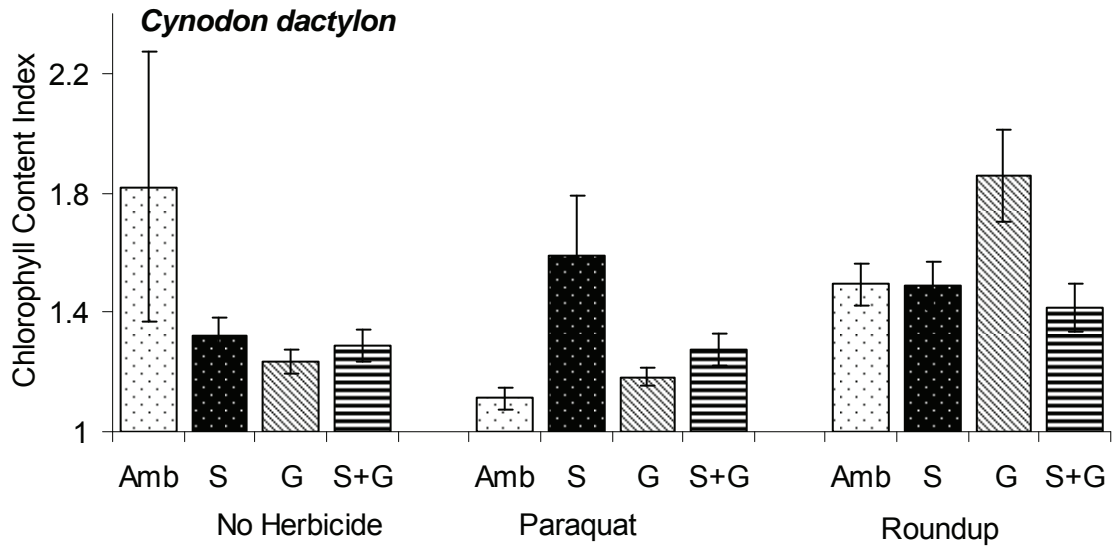
Appendix 3.5. Effect of different herbicide and soil nutrient amendment treatments on the chlorophyll content of *C. dactylon* in the greenhouse based experimental trial.
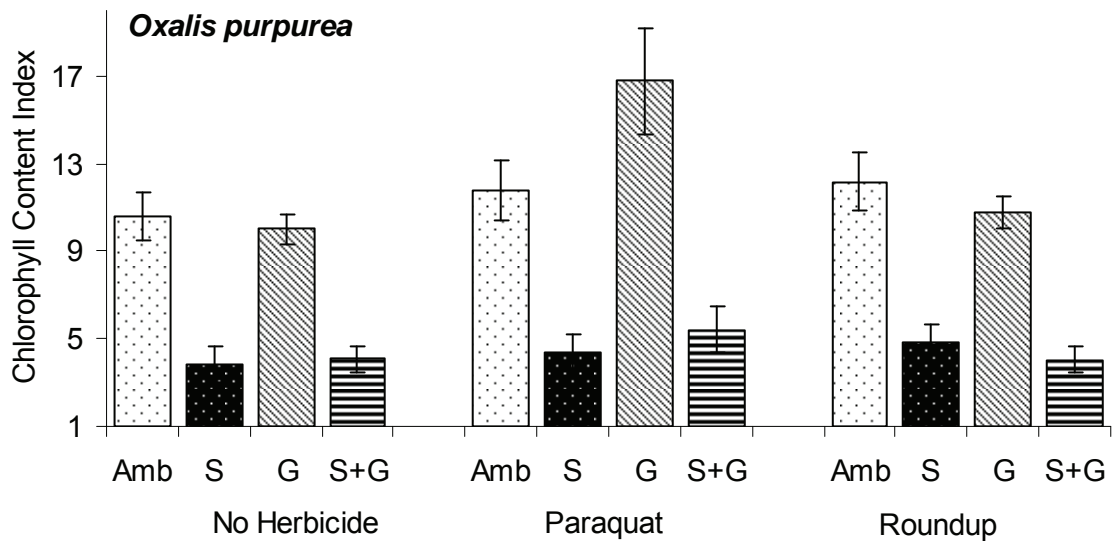

Appendix 3.6. Effect of different herbicide and soil nutrient amendment treatments on the chlorophyll content of *O. purpurea* in the greenhouse based experimental trial.
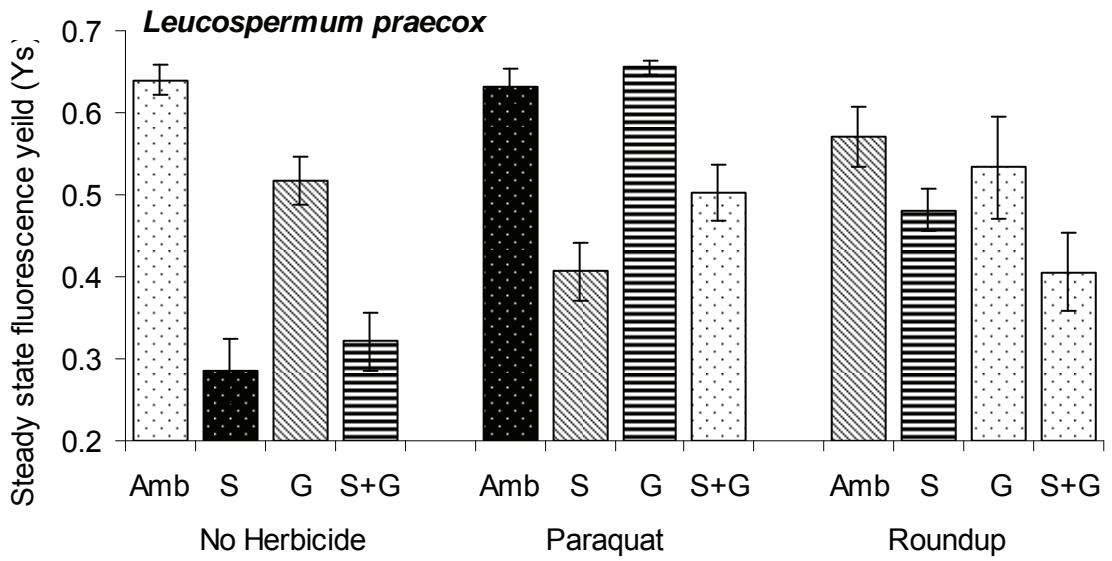
Appendix 3.7. Effect of different herbicide and soil nutrient amendment treatments on the steady state fluorescence yield of *L. praecox* in the field based experimental trial.
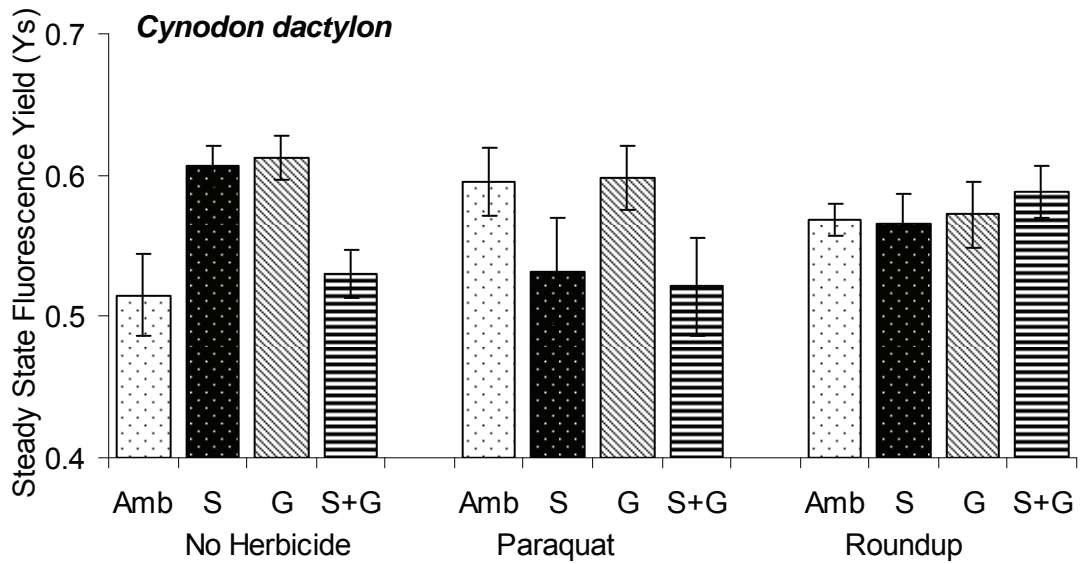
Appendix 3.8. Effect of different herbicide and soil nutrient amendment treatments on the steady state fluorescence yield of *C. dactylon* in the greenhouse based experimental trial.
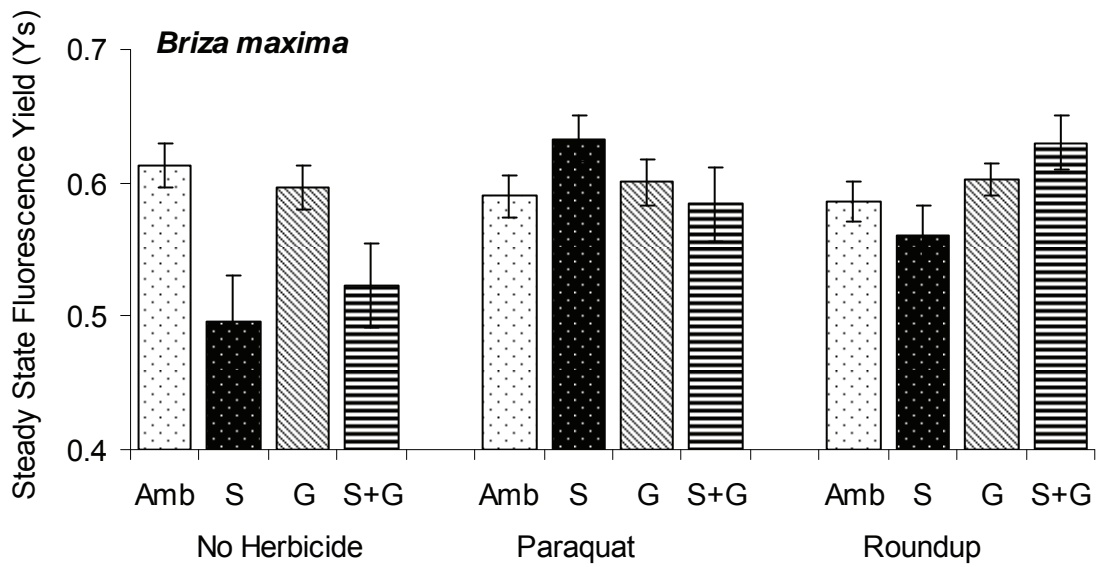
Appendix 3.9. Effect of different herbicide and soil nutrient amendment treatments on the steady state fluorescence yield of *B. maxima* in the greenhouse based experimental trial.
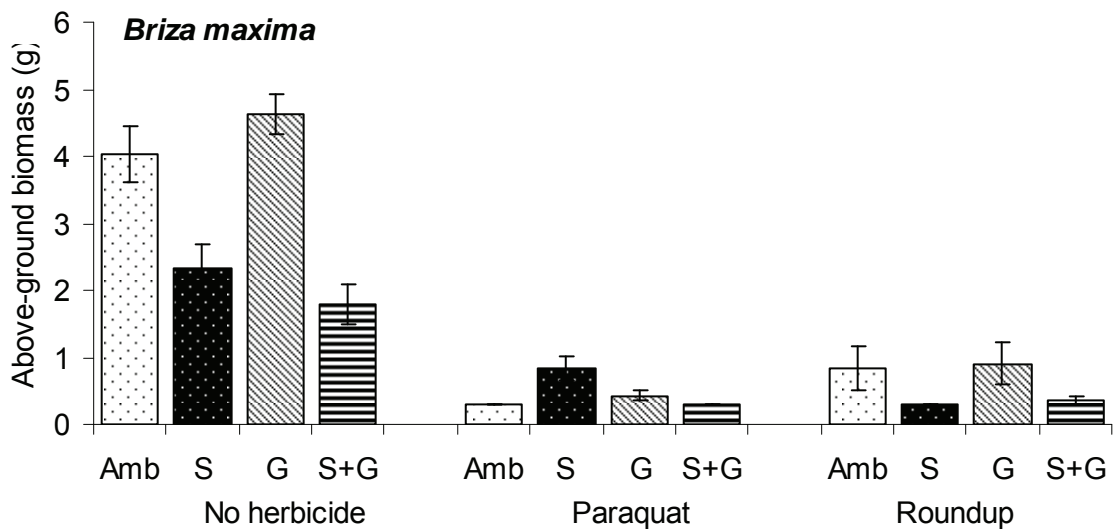
Appendix 3.10. Effect of different herbicide and soil nutrient amendment treatments on the above-ground biomass of *B. maxima* in the greenhouse based experimental trial.
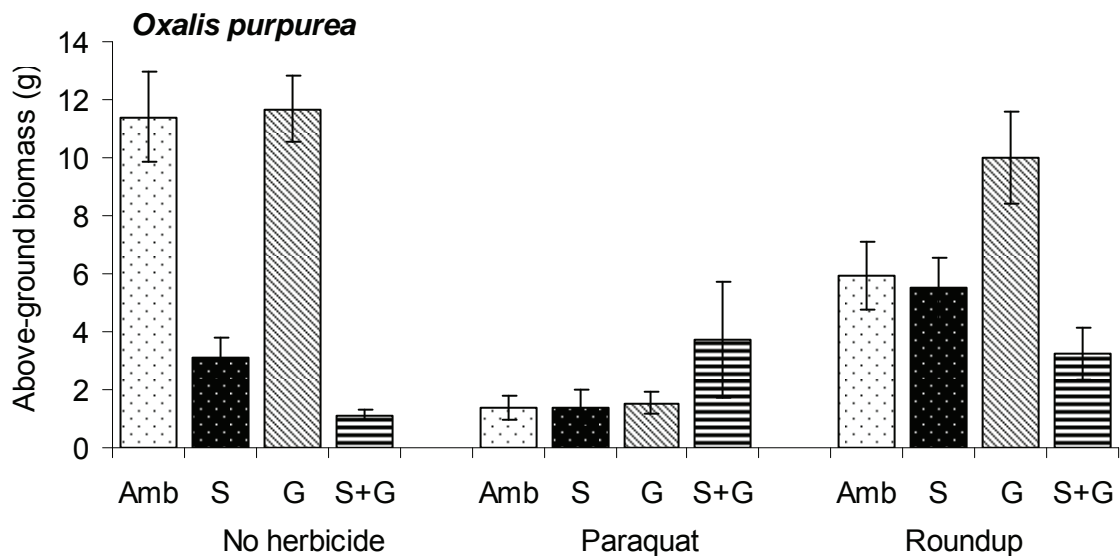
Appendix 3.11. Effect of different herbicide and soil nutrient amendment treatments on the above-ground biomass of *O. purpurea* in the greenhouse based experimental trial.
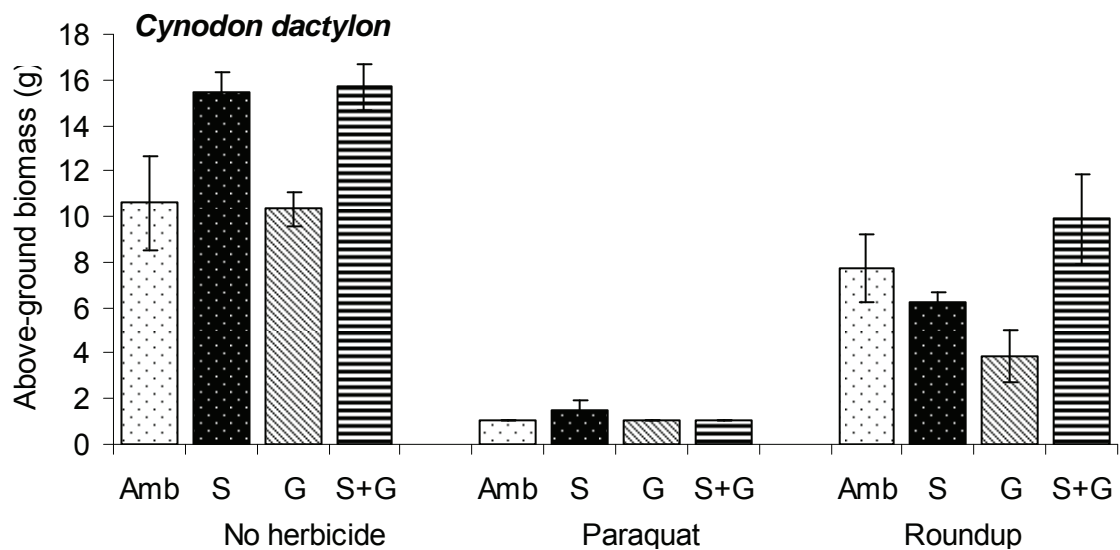
Appendix 3.12. Effect of different herbicide and soil nutrient amendment treatments on the above-ground biomass of *C. dactylon* in the greenhouse based experimental trial.
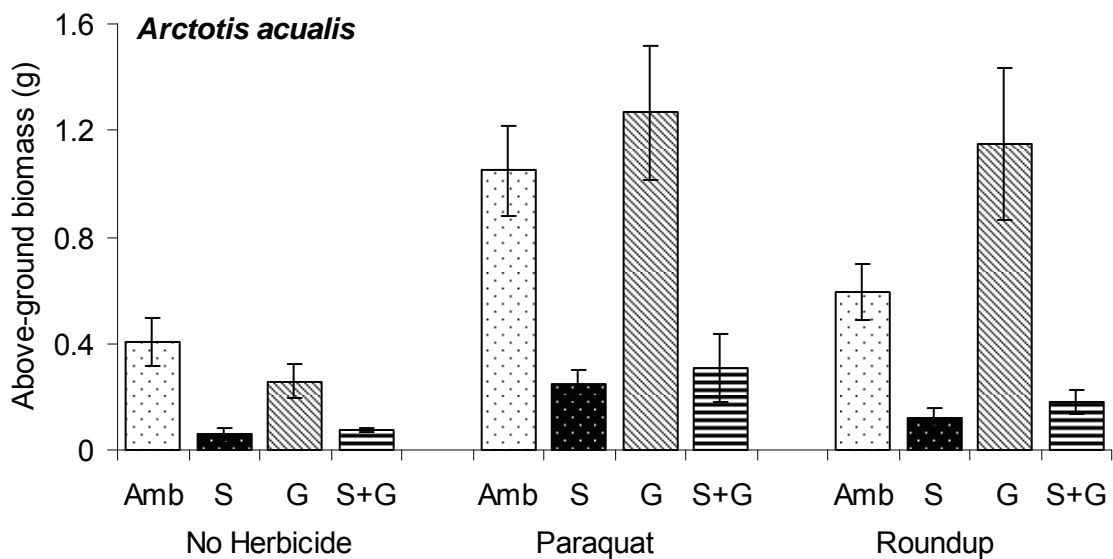
Appendix 3.13. Effect of different herbicide and soil nutrient amendment treatments on the above-ground biomass of *A. acaulis* in the greenhouse based experimental trial.
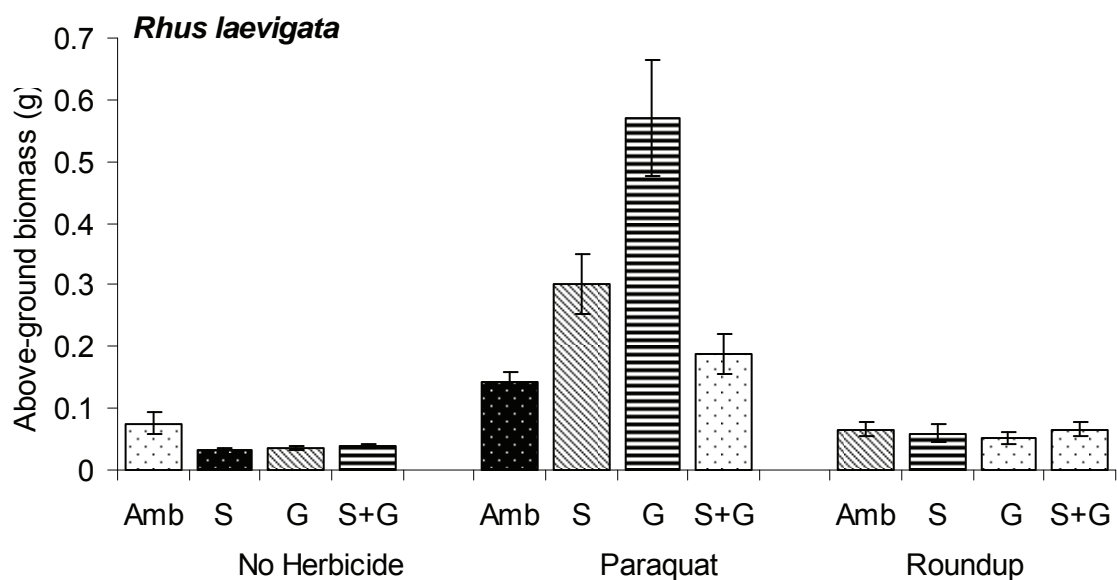
Appendix 3.14. Effect of different herbicide and soil nutrient amendment treatments on the above-ground biomass of *R. laevigata* in the greenhouse based experimental trial.
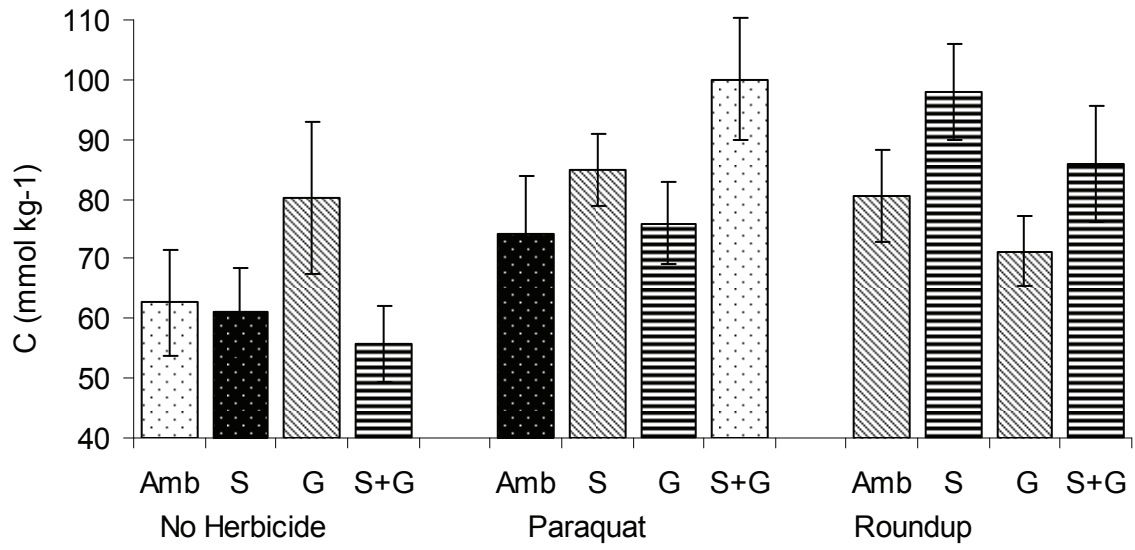
Appendix 3.15. Effect of different herbicide and soil nutrient amendment treatments on soil C concentrations in the field based experimental trial.

Appendices to Chapter 4
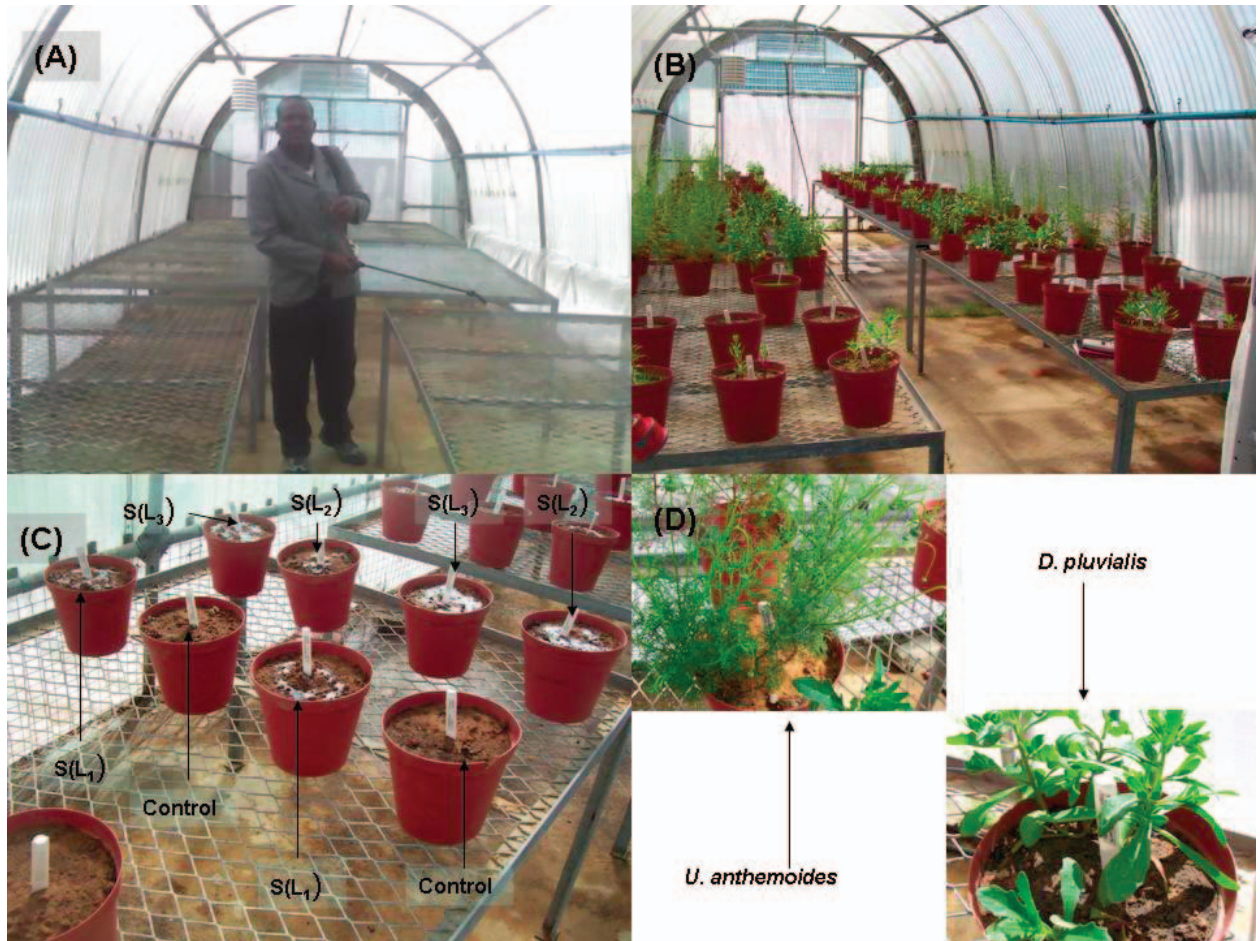
Appendix 4.1. Experimental set up (a) Disinfecting the greenhouse with sodium hypochlorite (NaClO) (b) experimental layout and (c) sucrose additions at 3 levels (L1 = 100g m-2, L2 = 200g m-2; L3 = 300g m-2) to pots in which (d) *D. pluvialus* and *U. anthemoides* were cultivated.

Appendix 4.2. Growth responses of *D. pluvialus* after soil sterilization (a & b) and sucrose additions (c & d).

Appendix 4.3. Growth responses of *U. anthemoides* after soil sterilization (a & b) and sucrose additions (c & d).
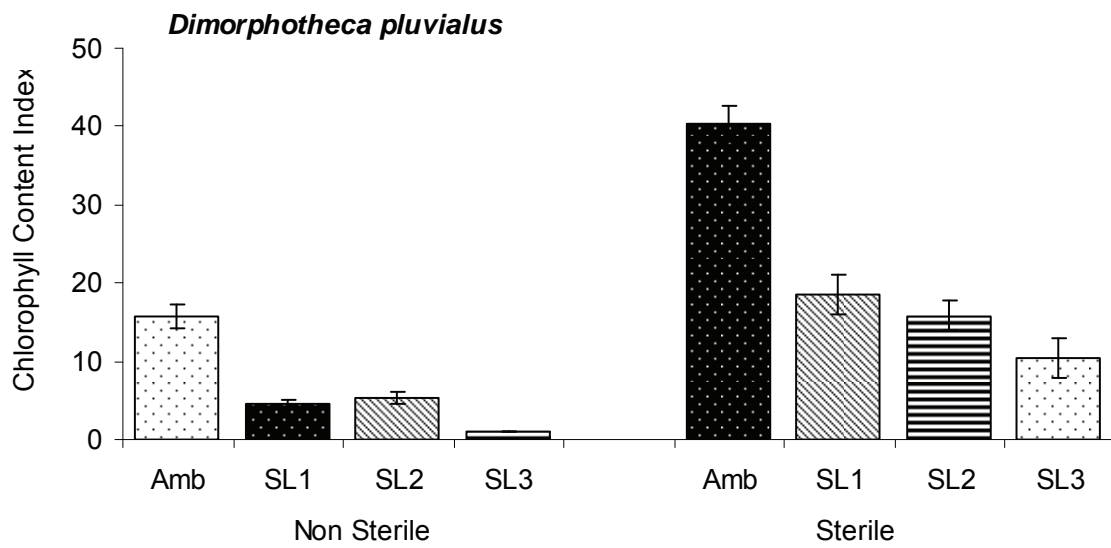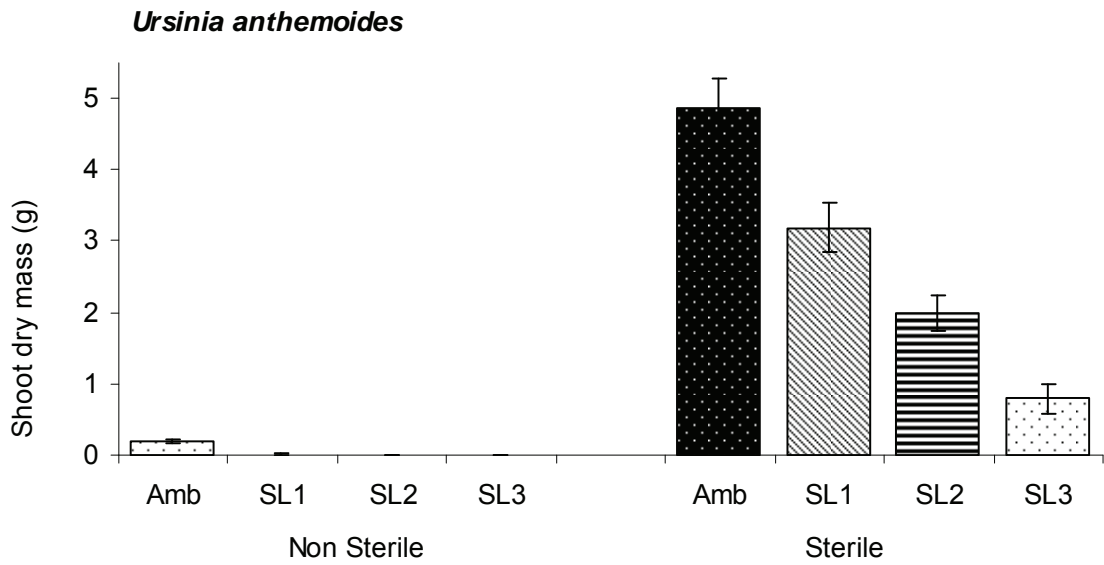
Appendix 4.4. Effect of soil sterilization and sucrose additions at 3 different levels on the chlorophyll content of *D. pluvialus*.
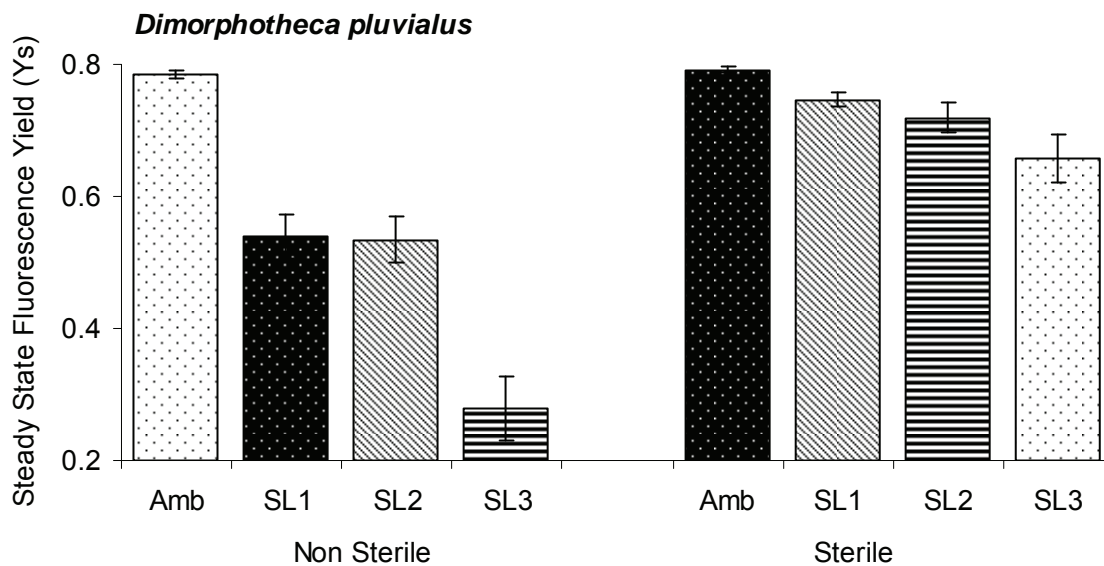
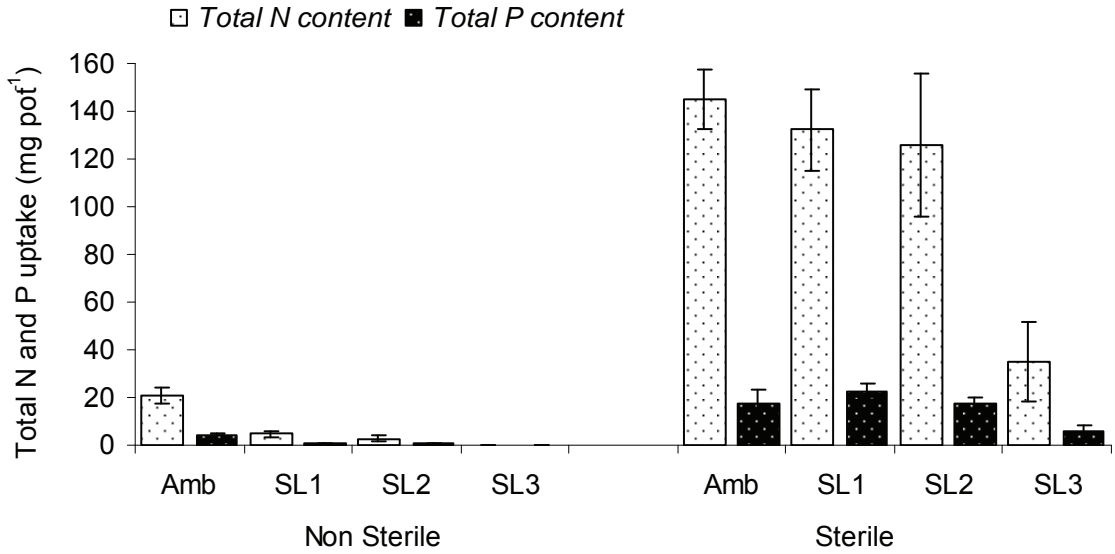Appendix 4.5. Effect of soil sterilization and sucrose additions at 3 different levels on the steady state fluorescence yield (Y_s) of *D. pluvialus*.
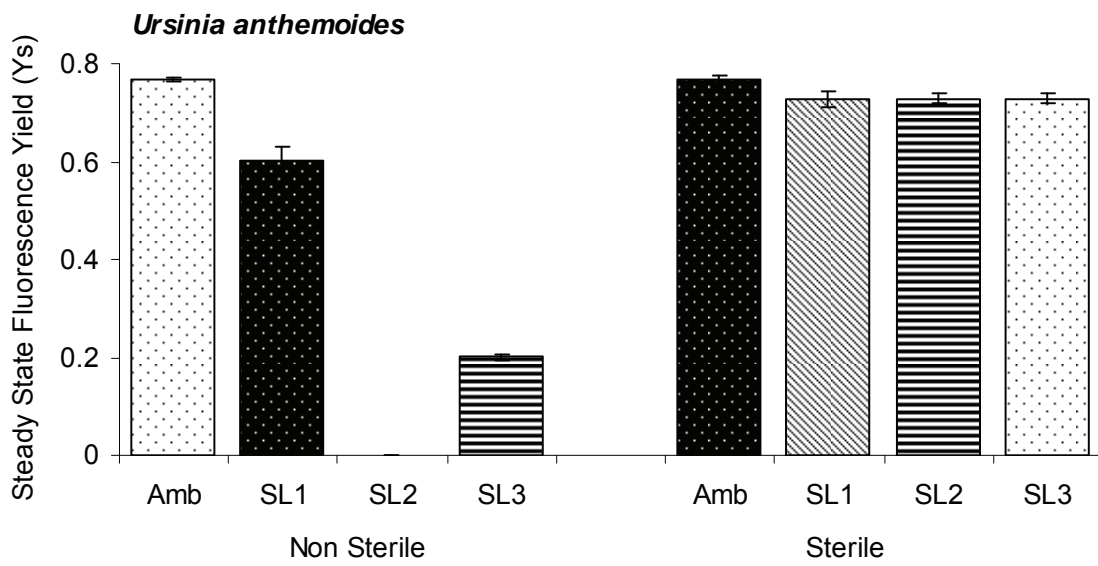
Appendix 4.6. Effect of soil sterilization and sucrose additions at 3 different levels on the steady state fluorescence yield (Y_s) of *U. anthemoides*.
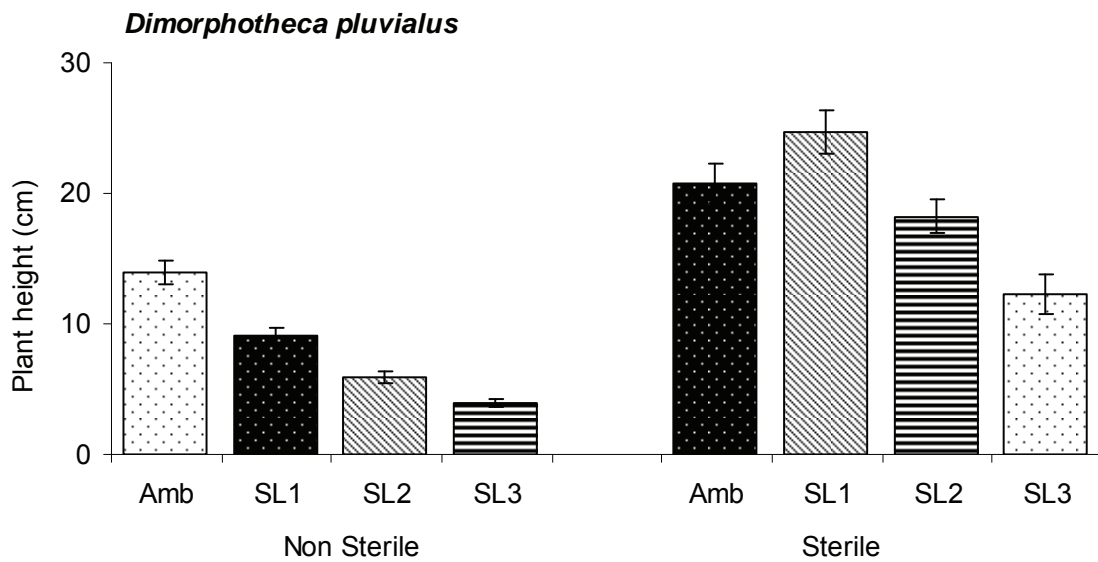
Appendix 4.7. Effect of soil sterilization and sucrose additions at 3 different levels on plant heights of *D. pluvialus*.

Ursinia anthemoides
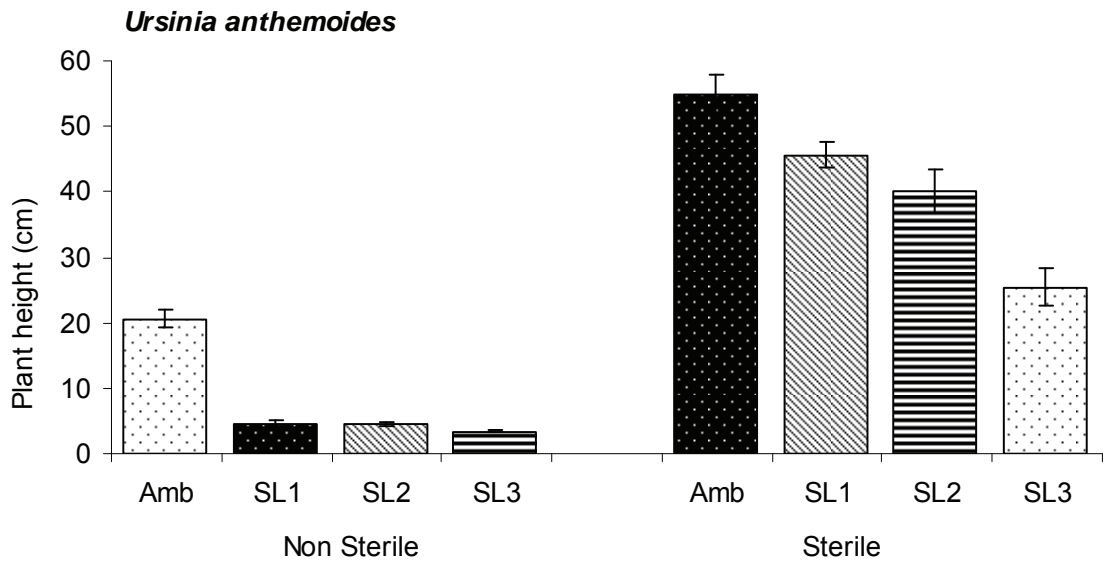
Appendix 4.8. Effect of soil sterilization and sucrose additions at 3 different levels on plant heights of *U. anthemoides*.

Dimorphotheca pluvialus
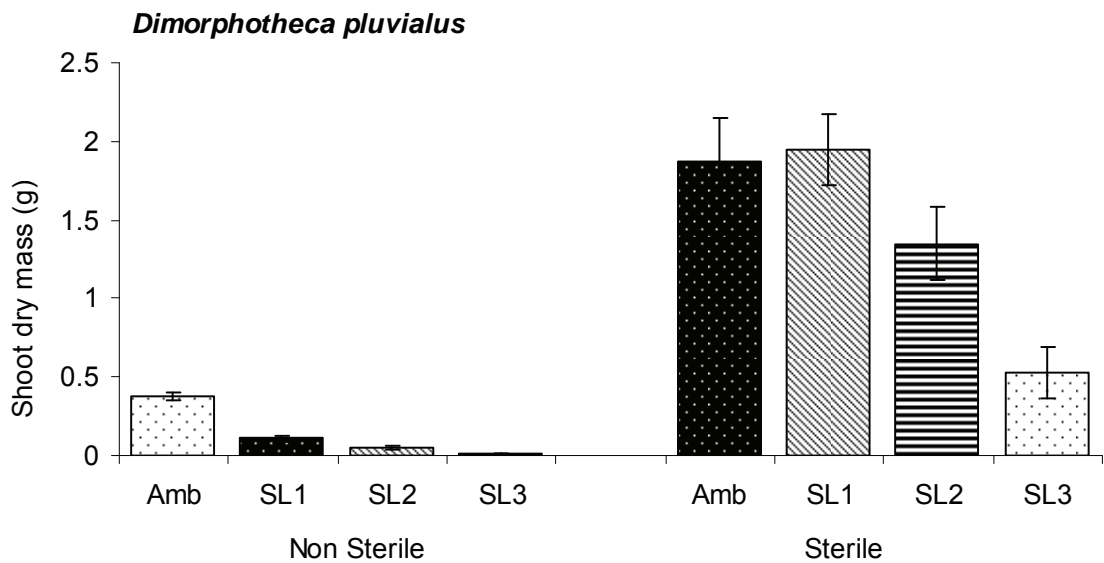
Appendix 4.9. Effect of soil sterilization and sucrose additions at 3 different levels on shoot dry masses of *D. pluvialus*.
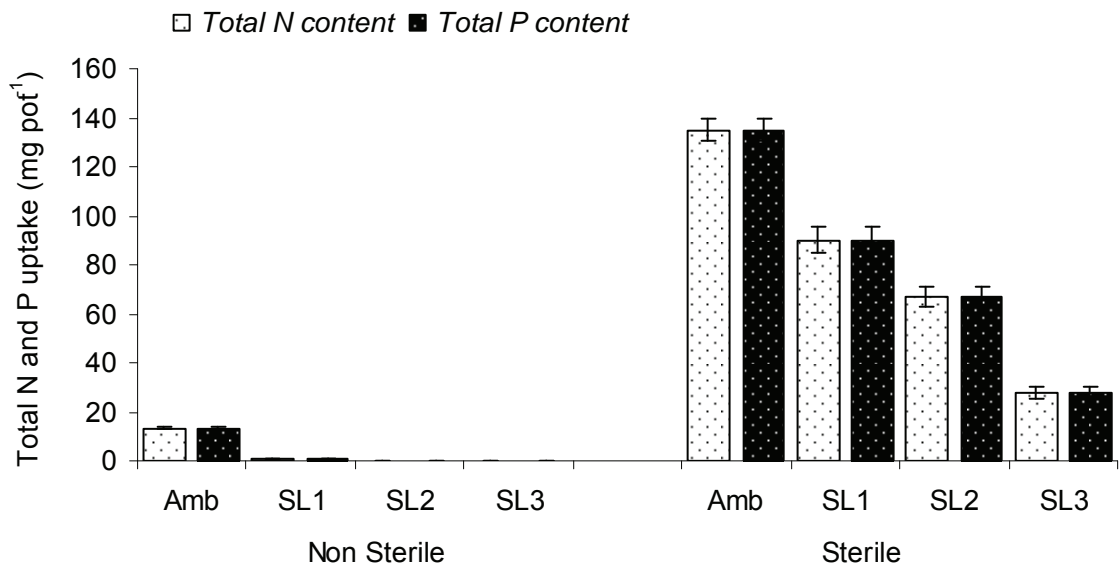
Appendix 4.10. Effect of soil sterilization and sucrose additions at 3 different levels on shoot dry masses of *U. anthemoides*.
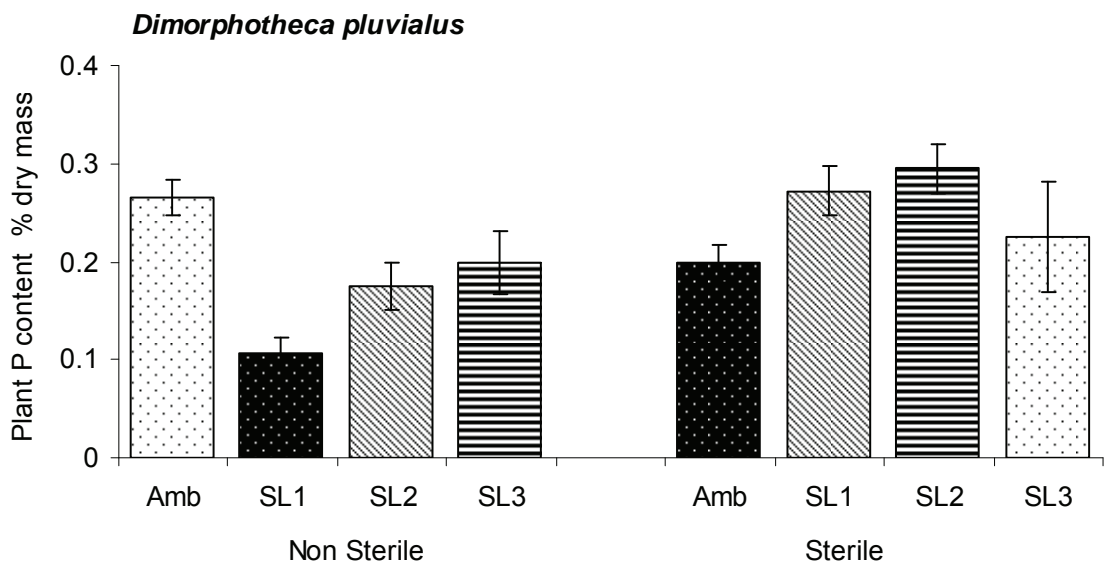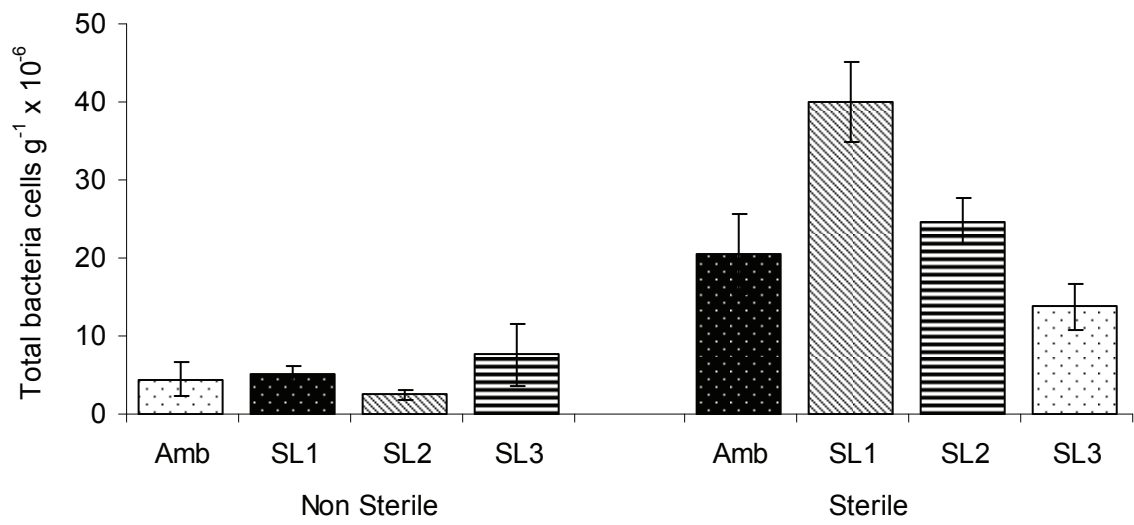

Appendix 4.11. Effect of soil sterilization and sucrose additions at 3 different levels on P contents of *D. pluvialus*.

Appendix 4.12. Effect of soil sterilization and sucrose additions at 3 different levels on total N and P uptake by *D. pluvialus* in sterilized and non-sterilized soils.

Appendix 4.13. Effect of soil sterilization and sucrose additions at 3 different levels on total N and P uptake by *U. anthemoides* in sterilized and non-sterilized soils

Appendix 4.14. Effect of soil sterilization and sucrose additions at 3 different levels on total bacterial cell numbers.