The implementation of selected technologies to enhance the restoration of indigenous tree species in the deforested riparian areas in the Mapungubwe National Park, South Africa

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Abstract

Stretches of forest along the Limpopo and Shashe Rivers have been classified as a unique forest type in the vegetation of South Africa and are considered as being “critically endangered” by the South African Biodiversity Institute. Roughly 400 hectares of this riverine forest area inside the western section of the Mapungubwe National Park (MNP), a UNESCO World Heritage site, were deforested and therefore degraded due to previous agricultural cultivation practices. Given the extent of forest degradation that has occurred, the restoration of this area by means of the re-vegetation of indigenous trees to its former composition is one of the objectives of the MNP’s management plan. The successful establishment of tree seedlings, especially in semi-arid systems, is however presented with a wide range of constraints and limiting conditions, which often result in very high mortality rates during restoration projects. An experimental exclosure, as identified by South African National Parks (SANParks), was therefore fenced off inside the degraded old lands to act as a demonstration site for the restoration of indigenous trees.

A pilot study conducted in 2006, involved the transplantation of selected indigenous tree species with the aim of evaluating suitable re-vegetation technologies. The research contained in this dissertation was also conducted inside the experimental exclosure, where recommendations derived from the pilot study were evaluated, including the assessment of new re-vegetation technologies to enhance the establishment of the indigenous trees. This study was therefore a follow-up project which involved both field- and greenhouse trials. Seedlings of the following species were either transplanted into the experimental exclosure (field trial) or cultivated inside a controlled environment in the greenhouse at the North-West University: *Acacia xanthophloea* Benth. (fever tree), *Berchemia discolor* (Klotzsch) Hemsl. (brown-ivory), *Combretum imberbe* Wawra (leadwood), *Faidherbia albida* (Delile) A. Chev. (ana tree), *Philenoptera violacea* (Klotzsch) Schrire (apple-leaf), *Salvadora australis* Schweick. (narrow-leaved mustard tree) and *Xanthocercis zambesiaca* (Baker) Dumaz-le-Grand (nyala tree). During the follow-up study the effects of various enhancement treatments were tested regarding the survival, growth and physiological performance of seedlings in both the field- and greenhouse trials. The enhancement treatments consisted of the addition of compost and indigenous arbuscular mycorrhizal fungi (AMF). In addition, seedlings transplanted during the pilot study, which did not include enhancement treatments, were also monitored for establishment and growth. The potential use of established *Acacia tortilis* Hayne trees to facilitate growth and establishment and to act as “nursing plants”, was also assessed. In addition, various pre-sowing treatments were also applied to seeds of selected tree species in the greenhouse to assess the germination rate.

The survivorship and growth of seedlings in both the field- and greenhouse trial were determined by using three growth parameters, namely “stem diameter at the base”, “stem diameter 30 cm from the base", and "height of the tree in its natural growth form". Chlorophyll fluorescence induction (JIP test) was measured on seedlings in both trials, using the multi-parametric expression, namely performance index (PI_{ABS}), as a measure of the overall vitality of the plants of each species-treatment combination. Physical and chemical analyses were carried out on the soil inside the experimental exclosure. Basic descriptive statistics were used to analyse seedling survival and germination rates, and a two-way analysis of variance (ANOVA) was used to determine the statistical significant effects of the various enhancement treatments on diameter growth in each species ($p < 0.05$). Fluorescence data were processed using the Biolyzer software and significant effects in each species were determined using the Student's t-test ($p < 0.05$). Multivariate data ordinations using the CANOCO package were used to determine the differences in soil types inside the experimental exclosure.

Moisture stress due to transplantation shock, competition with dense grass cover and herbivory, resulted in an overall 55.8% seedling survival rate and negative stem diameter growth for transplanted seedlings in the field. In comparison, seedlings cultivated in the greenhouse had much higher survival rates and showed positive stem diameter growth. Most species in the greenhouse showed higher growth rates and significantly higher vitality values when planted with enhancement treatments. The responses of transplanted seedlings to the enhancement treatments were very species-specific in the field trials. Based on these results, it was concluded that the enhancement treatments were beneficial with regard to the establishment and growth of most of the species. The beneficial effect was however cancelled out by the various abiotic and biotic factors encountered in the natural environment. Seedlings transplanted in the understory of established pioneer *A. tortilis* trees had much lower survival rates as the extensive root system of *A. tortilis* most likely out-competed the transplanted seedlings for moisture and nutrients. Many seedlings were also predated by insects or small mammals which reduced the growing potential. The germination trials recorded the highest germination rates for most species when germinated in the compost-containing treatments. These trials also indicated that all of the investigated species showed higher survival rates when pre-sowing treatments, such as soaking, mechanical scarification and removing the seed from fruit, were applied. Various recommendations emphasising long-term monitoring, proper maintenance and after-care of future restoration efforts are made. These include experimental layout of exclosure plots and pre-transplantation treatments of seedlings while cultivated in the nursery. During this study, the experimental exclosure was also used as a demonstration site for training and capacity building for SANParks personnel and students from academic institutions.

Keywords: Restoration; deforestation; indigenous trees; transplantation; compost; arbuscular mycorrhiza fungi (AMF); performance index (PI_{ABS})

Opsomming

Die oewerbos-area wat langs die Limpopo en Shashe Riviere voorkom, word geklassifiseer as 'n unieke bos tipe in die plantegroei van Suid-Afrika en word ook deur die Suid-Afrikaanse Biodiversiteits Instituut as "krities bedreig" beskou. Ongeveer 400 hektaar van hierdie oewerbos-area, wat binne die westelike gedeelte van die Mapungubwe Nasionale Park (MNP) voorkom, is ontbos en gevolglik gedegradeer weens vorige verbouïngspraktyke. Die omvang van degradasie binne die oewerbos-area het daartoe gelei dat die restorasie van hierdie gebied tot sy vorige samestelling deur middel van die hervestiging van inheemse bome, as een van die doelwitte van die MNP se bestuursplan aangewys is. Die suksesvolle vestiging van boomsaailinge, veral in semi-ariede sisteme, word egter deur 'n verskeidenheid stremminge en beperkende omgewingsfaktore bemoeilik. Gevolglik is baie hoë saailingmortaliteite gedurende restorasie projekte 'n algemene bevinding. Daar is dus besluit om 'n eksperimentele uitsluitperseel op te rig wat deur die Suid-Afrikaanse Nasionale Parke (SANParke) geïdentifiseer is. Hierdie perseel is geleë binne 'n area van voorheen bewerkte lande binne die MNP. Die doel van hierdie uitsluitperseel was om as 'n demonstrasieterrein vir die restorasie van inheemse bome te dien.

Tydens 'n loodsstudie in 2006, is geselekteerde inheemse boomspesies binne die uitsluitperseel oorgeplant met die doel om geskikte hervestigings tegnologië te evalueer. Die navorsing wat in hierdie verhandeling vervat word, het die oorplanting van inheemse bome binne dieselfde uitsluitperseel behels. Aanbevelings wat deur die loodsstudie gemaak is, asook nuwe hervestigingstegnologië vir gebruik tydens die restorasie van inheemse bome, is tydens hierdie opvolgstudie geëvalueer. Hierdie opvolgstudie het uit 'n veld- en kweekhuisproef bestaan. Saailinge van die volgende spesies was óf binne die eksperimentele uitsluitperseel oorgeplant (veldproef), en gekweek onder gekontroleerde kondisies binne ‘n kweekhuis van die Noordwes Universiteit gekweek (kweekhuisproef): *Acacia xanthophloea* Benth. (koorsboom), *Berchemia discolor* (Klotzsch) Hemsl. (bruin-ivoor), *Combretum imberbe* Wawra (hardekool), *Faidherbia albida* (Delile) A. Chev. (ana boom), *Philenoptera violacea* (Klotzsch) Schrire (appel-blaar), *Salvadora australis* Schweick. (nou-blare mosterd boom) en *Xanthocercis zambesiaca* (Baker) Dumaz-le-Grand (njala boom). Hierdie opvolgstudie het die effek van verskeie verrykingsbehandelinge op saailingvestiging en -groei, asook fisiologiese werking binne beide die veld- en kweekhuisproef geëvalueer. Die verrykingsbehandelinge het uit ‘n kombinasie van kompos en inheemse arbuskulêre mycorrhiza fungi (AMF) bestaan. Die vestiging en groei van saailinge wat tydens die loodsstudie sonder verrykingsbehandelinge oorgeplant is, is ook gedurende die opvolgstudie gemoniteer. Die potensiële gebruik van gevestigde *Acacia tortilis* Hayne bome as "pleegplante" deurdat die bome vestiging en groei van oorgeplante saailinge fasiliteer en bevorder, is ook ondersoek. Die effek van verskeie voor-saaïngsbehandelinge op die ontkiemingskoers van geselekteerde boomspesies is ook binne die kweekhuisproef ondersoek. Drie parameters, naamlik "stamdeursneë by die basis", "stamdeursneë 30 cm vanaf die basis", en "hoogte van die boom in sy natuurlike groeivorm" is gemeet om die vestiging en groei van saailinge binne die veld- en kweekhuisproef te bepaal. Chlorofil fluoressensie is ook op saailinge in beide proewe gemeet - die multi-parametriese uitdrukking, naamlik die prestasie-indeks (PI_{ABS}), is as 'n maatstaf vir die vitaliteit van die plante van elke behandelingskombinasie gebruik. Fisiese en chemiese analises van grondmonsters van die verskillende blokke van die eksperimentele uitsluitperseel is uitgevoer. Basiese beskrywende statistiek is gebruik om saailingoorlewing en ontkiemingskoerse te bepaal. 'n Tweerigting variansie-analise (ANOVA) is gebruik om die statistiese betekenisvolheid van die uitwerking van die verskillende verrykingsbehandelinge op stamdeursneë binne elke spesie te bepaal ($p < 0.05$). Die fluoressensiedata is verwerk met die Biolyzer sagteware en statistiese verskille in elke spesie is met die Student’s-*t*-toets ($p < 0.05$) bepaal. Die CANOCO pakket is gebruik om die verskillende grondtipes binne die eksperimentele uitsluitperseel te bepaal.

Vogstremming as gevolg van oorplantingskok, kompetisie met digte gras en herbivorie, het tot 'n algehele 55,8% saailingoorlewing en negatiewe stam deursneë groei vir oorgeplante saailinge in die veld gelei. Die saailinge wat binne die kweekhuis gekweek is, het egter baie hoër saailingoorlewing en positiewe stamdeursneëgroei getoon. Tydens die kweekhuisproef is die hoogste groeikoerse en vitaliteitswaardes aangeteken vir spesies wat binne verrykingsbehandelinge gekweek is. Die uitwerking van die verrykingsbehandelinge op saailinge wat in die oorgeplant is, was egter baie spesie-spesifiek. Die resultate het getoon dat die verrykingsbehandelinge wel voordelig is vir saailingvestiging en groei. Die voordelige effek van hierdie behandelinge word egter deur verskillende abiotiese en biotiese faktore binne die natuurlike omgewing uitgekompeteer. Die laagste oorlewingssyfers is aangeteken vir saailinge wat rondom die gevestigde pionier *A. tortilis* bome geplant is. Die uitgebreide wortelstelsel van hierdie bome het waarskynlik die oorgeplante saailinge vir vog en voedingstowwe uitgekompeteer. ‘n Hoë saailingmortaliteit weens insekpredasie of skade deur klein soogdiere is ook rondom hierdie bome aangeteken. Die ontkiemingsproef het getoon dat die hoogste ontkiemingskoerse vir die meeste spesies aangeteken is indien saad binne 'n kompos-bevattende behandeling gekweek word. Tesame hiermee het al die spesies hoër oorlewingskoerse getoon wanneer voor-saaïngsbehandelingspraktyke, soos bv. week in water, meganiese insnyding en verwydering van vanuit die vrug, toegepas is. Verskeie aanbevelings het tydens hierdie studie na vore gekom. Die klem was egter op langtermyn-monitering, behoorlike instandhouding en na-sorg van oorgeplante saailinge tydens toekomstige restorasie projekte geplaas. Ander aspekte soos die eksperimentele uitleg van uitsluitpersele en die toediening van verrykingsbehandelinge binne die kwekery, is ook aangespreek. Die uitsluitperseel het tydens hierdie studie as 'n demonstrasie terrein vir opleiding en kapasiteitsbou vir SANParke personeel en studente van akademiese instellings gedien.

Sleutelwoorde: Restorasie; ontbossing; inheemse bome; oorplanting; kompos; arbuskulêre mycorrhiza fungi (AMF); prestasie-indeks (PI_{ABS})

CHAPTER 1

INTRODUCTION

1.1 Background

As stated by Cramer *et al.* (2007), “all cultivation leaves a legacy”. Whether it be biomass alteration, tillage, fertilisation or a change in hydrology, cultivation alters ecosystem processes in such a way that its legacy can be seen in vegetation composition and structure hundreds of years later (McLauchlan, 2006; Foster *et al.*, 2003). Such vegetation composition and structure alterations has occurred in an area of roughly 400 ha located in the western section of the Mapungubwe National Park (MNP) due to clearing and cultivation practices since the early 1980’s. This degraded and deforested area once formed part of the now “critically endangered” riparian forest area (SANParks, 2008) which is a unique and protected forest type occurring along the Limpopo and Shashe Rivers (Mucina & Rutherford, 2006). Once supporting majestic tree species, such as fever trees (*Acacia xanthophloea* Benth.), lead woods (*Combretum imberbe* Wawra) and apple leaves (*Philenoptera violacea* (Klotzsch) Schrire), the old lands are now dominated by competitive grass species and patches of thick *Acacia tortilis* Hayne stands depending on the period of abandonment (Götze, 2002 and personal observation).

This study finds its relevance in the MNP’s Rehabilitation Programme aimed at incorporating widespread rehabilitation within the park, and as stated in its Management Plan, the “rehabilitation of old lands, with particular emphasis (where appropriate as judged from historical aerial photos) on re-establishment of riparian woodland” which is to continue unabated for the following five years (SANParks, 2008). Various restoration studies around the world have incorporated approaches assisting natural regeneration by protecting the site from further disturbances thereby allowing successional processes to take place, as well as accelerating natural colonisation through artificial establishment of seedlings (Holl *et al.*, 2000; Lamb *et al.*, 1997). None such studies have however been conducted on the use of indigenous trees in semi-arid South African ecosystems. This includes the MNP which is a relative young park (formalised only in 2004), having received very little (if any) attention regarding the restoration of its riparian forest area. As a result the use of indigenous tree species for the restoration its riparian areas has been constrained by a lack of knowledge regarding the species’ requirements for propagation, survival and growth as well as suitable transplantation techniques.

A pilot study was consequently launched in 2006 during which a demonstration site was established for the evaluation of suitable restoration methods and techniques to be used in the park’s deforested riparian areas (Scholtz, 2008). The pilot study delivered valuable results regarding the use of indigenous tree seedlings and also made recommendations for future restoration endeavours. Recognising the need to implement these recommendations made by Scholtz (2008) and supported by a research grant from the National Geographic Conservation Trust (NGCT), this follow-up study was initiated in 2008.

1.2 Aims of this study

The general aim of this study was to evaluate and determine suitable technologies to be used for the restoration of degraded riparian forest ecosystems not only in the MNP, but in the surrounding conservation areas also.

The specific objectives were to:

- Assess seedling survival, growth and physiological performance of selected indigenous tree species in response to compost and AMF containing treatments when transplanted into the natural environment, and cultivated under controlled greenhouse conditions.
- Assess the potential use of established *Acacia tortilis* trees to facilitate growth and establishment of transplanted indigenous tree seedlings.
- Assess seedling survival and growth of selected indigenous tree species transplanted into the natural environment during a pilot study without the addition of any enhancement treatments.
- Assess the germination of selected indigenous tree species in response to various pre-sowing treatments under controlled conditions.
- Use the experimental exclosure as a demonstration site for training and capacity building within SANParks, as well as for students from other academic institutions.
- Make recommendations regarding future restoration efforts in the MNP.

1.3 Format of the dissertation

This dissertation is divided into six chapters. Chapter one contains a brief background regarding the instigation of this study in the MNP, as well as the specific objectives aimed to be reached. Chapter two contains a comprehensive literature review regarding various relevant aspects, such as the importance and state of semi-arid riparian ecosystems in the world and in Africa, and the various biotic and abiotic factors to be considered when attempting to restore these systems. Chapter three describes the historical and cultural context of the study area, including its natural and bio-physical characteristics. Chapter four describes the experimental layout and materials used in both the field-

(natural environment) and greenhouse (controlled environment) trials. This chapter also discusses the various methods used for monitoring and data analysis. Chapter five presents all the results collected from the natural- and controlled environment regarding the germination, survival, growth and physiological performance of the investigated species. These results are discussed and compared to previous research in each section. Finally chapter six brings it all together with concluding remarks and detailed recommendations regarding future restoration efforts in the MNP’s deforested riparian areas.

CHAPTER 2

LITERATURE REVIEW

2.1 Land degradation

Land degradation is a common feature in both developed and underdeveloped areas under all types of management and land use tenure systems in Southern Africa (Kellner, 1999). It has become synonymous with the problems of arid and semi-arid areas, leading to the reduction of productive potential of land and water resources (Wangari, 1996). The United Nations Convention to Combat Desertification (UNCCD, 1995) provides official definitions for the term land degradation: "...land degradation means the reduction or loss, in arid, semi-arid and dry sub-humid areas, of the biological or economic productivity and complexity of rainfed cropland, irrigated cropland, or range, pasture, forest and woodlands resulting from land uses or from a process or combination of processes, including processes arising from human activities and habitation patterns such as: soil erosion caused by wind and/or water; deterioration of the physical, chemical and biological or economic properties of soil; and long-term loss of natural vegetation..."

Land degradation can happen on a local scale, or over vast areas and is recognized as a forerunner of desertification, which is viewed as a gradual process rather than quick transformation from vegetated areas to deserts (UNCCD, 2009; UNCCD; 2006). The cost of land degradation in South Africa is estimated to be more than R2 billion per annum (SANBI, 2009) due to various problems associated with it, such as loss of vegetation cover, alien plants, bush encroachment and deforestation. The combating of degradation and desertification, thereby improving the production potential of degraded lands by reclamation and restoration, has therefore become a priority in large parts of Southern Africa (Kellner, 1999).

Two thirds of Africa are either desert of drylands and Hoffman and Ashwell (2001) report that 73% of Africa's agricultural drylands are already degraded. Many Southern African Developing Countries (SADC) are experiencing the ecologically destructive effects of poor land management practices, such as overcultivation, overgrazing and deforestation, leading to land degradation. Land degradation is often aggravated by climatic factors, such as drought (Hassan, 2000; Hoffman & Ashwell, 2001; Wangari, 1996). Current climate predictions entails that the next century will be characterized by shifts in global weather patterns (McCarthy *et al.*, 2001; Easterling *et al.*, 2000; Swetnam & Betancourt, 1998). These climate changes are expected to influence soil properties, nutrient recycling and vegetation growth in numerous ways. The International Panel on Climate Change (IPCC) concluded in 2001 that Africa is highly vulnerable to the various manifestations of climate change, and desertfication was one of the six areas of concern emphasized (Pittock, 2005). The potential increases in the frequency and intensity of the expected droughts across subhumid Africa are likely to increase desertification, and will therefore make any attempts to reclaim land lost to past degradation very challenging (Pittock, 2005).

2.2 Deforestation of riparian areas

Deforestation can be defined as the complete removal of tree cover or the substantial reduction of canopy cover (below 30%) over large areas, being one of biggest causes of land degradation world wide (Angelsen & Kaimowitz, 1999). In addition to the rapid depletion of natural woodlands and forest resources, deforestation leads to increased desertification and destruction of the ecosystem (Hassan *et al.*, 2009). Deforestation is often linked to riparian areas. The vegetation of riparian areas are often altered by human interventions (Sweeney & Czapka, 2004), as agriculture is highly dependent on water and clearing for cultivation regularly takes place in riparian zones. The restoration of species-rich communities in these riparian areas has therefore become a very serious issue, especially in countries with intensive farming systems (Bakker & Barendse, 1999).

Riparian areas are seen as ecotones where interaction takes place between the river and the landscape (Ivits *et al.*, 2009). According to Naiman *et al.* (1993) riparian corridors act as interfaces between terrestrial and aquatic systems where they encompass sharp environmental gradients, ecological processes, and vegetation communities. These areas often have higher species richness compared to their surrounding vegetation due to the heterogeneous environment created by flooding, sediment deposition and lateral channel migration (Naiman *et al.*, 1993). These areas play important roles as major forest resources, even in desert systems (Yang *et al.*, 2008). Riparian vegetation forms an integral and important part of any river ecosystem due to its role in the geomorphological, ecological and social attributes which contribute to the ecosystem function and services of the system (Kemper, 2001; Arthington *et al.*, 1993; Naiman *et al.*, 1993). Additionally these areas also provide important breeding and overwintering grounds for birds, and act as migration stopover areas and corridors for dispersal (Smiley *et al.*, 2007).

2.3 Land abandonment

In addition to deforestation, land abandonment also leads to land degradation. “Old lands” resulting from abandonment, display a variety of dynamics and has been cited as one of the leading causes of loss of biodiversity worldwide (Vitousek *et al.*, 1986). The causes of abandonment vary and include productivity loss due to depletion of soil nutrients, topsoil erosion, depleted water tables, and socioeconomic factors affecting the viability of some agricultural practices (Richter *et al.*, 2002).

Van der Wal *et al.* (2009) states that the most notable differences between undisturbed areas and recently abandoned lands, are the absence of an organic layer, relatively high pH and high nutrient availability, in particular phosphate due to fertilizer applications during cultivation. The higher soil pH of abandoned lands, as well as high levels of extractable phosphorous, favours fast-growing plant species such as pioneers and weeds. These fast-growing species will out-compete, overgrow, or prevent establishment of slow-growing plant species with nutrient-conserving strategies (Fenner & Thompson, 2005). Richter *et al.* (2002) states that the recovery of natural vegetation in arid and semi-arid areas is much slower when compared to areas with a higher rainfall. According to Wishnie *et al.* (2007), woody species can be slow to re-establish in degraded pastures (Gerhardt, 1993) and the processes of natural succession can be severely impaired by continued soil degradation (Nepstad *et al.*, 1991), dominance of invasive grasses (Hooper *et al.*, 2004; Jones *et al.*, 2004), lack of seed dispersal (Holl *et al.*, 2000) and poor micro-site conditions for seed germination (Aide & Cavelier, 1994). In a study done by Jackson (1991), little recruitment of perennial grass species or any vegetation was found in abandoned lands in the western United States, even after as many as 35 and 40 years after abandonment. In order to speed up the rate of natural successional processes, various studies have recommended the introduction of climax species seeds or seedlings immediately after abanddonement (van der Wal et al., 2009; Palmer *et al.*, 1997).

According to Palmer *et al.* (1997) and Parker (1997), often the first step in restoration is the re-establishment of the local species pool by actively planting pre-disturbance species. The various hazards faced by seedlings are magnified during transplantation efforts due to transplantation shock, during which a seedling is exposed to water stress conditions brought on by the temporary impairment of seedling root function and poor root-soil contact due to disturbance to the root system during lifting, transportation and planting (Oliet *et al.*, 2005; Kavanagh & Zaerr, 1997; Harris *et al.*, 1996). Over the past few years, land managers have preferentially used late-successional species in restoration projects, especially trees and large shrubs based on the idea that these will accelerate succession and improve ecosystem resilience (Bonet, 2004). In arid environments, however, the success of community restoration is especially at risk due to very stressful ecological conditions (Padilla *et al.*, 2009). Drought, together with high temperatures, high irradiance, grazing, and infertile soils, threaten the survival of planted seedlings (García-Fayos & Verdú, 1998).

2.4 Restoration of degraded environments

The restoration of degraded ecosystems are recognised as an essential component in stemming the global loss of biodiversity (Hobbs & Harris, 2001). The Society for Ecological Restoration’s (SER) International Primer on Ecological Restoration (2004) states that ecological restoration is “the intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity and sustainability.” It further states that the ecosystem in need of restoration has often been degraded, damaged, transformed or entirely destroyed as the direct or indirect result of human activities. The general goal of ecological restoration is therefore to emulate the structure, function, diversity and dynamics of a specified “reference ecosystem”.

Typically the reference represents a point of advanced development that lies somewhere along the intended trajectory of the restoration. In other words, the restored ecosystem is eventually expected to imitate the attributes of the reference, and project goals and strategies are developed in light of that expectation (SER Primer, 2004). An ecological trajectory is one that describes the development pathway of an ecosystem through time. In restoration, the trajectory begins with the unrestored ecosystem and progresses towards the desired state of recovery that is expressed in the goals of a restoration project and embodied in the reference system. The trajectory embraces all ecological attributes – biotic and abiotic – of an ecosystem, and in theory can be monitored by the sequential measurement of coherent suites of ecological parameters (SER Primer, 2004). In summary, restoration projects need to determine the nature and extent of intervention necessary, based on an assessment of what has led to ecosystem degradation or what is preventing system recovery.

The practice of ecological restoration, and the science of restoration ecology, has developed rapidly over the past few decades to such an extent that a cohesive body of theory is beginning to emerge involving a variety of increasingly sophisticated restoration practices (e.g. van Andel & Aronson, 2006; Higgs, 2003). Natural and semi-natural habitats are becoming scarcer and scarcer, and therefore one of the biggest challenges of restoration ecology lies particularly in abandoned lands (Padilla *et al.*, 2009; Cramer *et al.*, 2007; Hobbs & Harris, 2001; van Diggelen *et al*., 2001; Young, 2000).

Ecological restoration can be conducted at a wide variety of scales and may involve active or passive intervensions (Milton & Dean, 1995). Where ecosystem re-development proceeds along a path that leads to a desirable outcome, then there is little need for active intervention (Hobbs & Cramer, 2007). In such an environment ‘self-recovery’ or ‘autogenic’ restoration is both desireable and cost-effective. Active restoration is, however, required where the developmental pathway after abandonment is inappropriate from either a conservation or land use perspective.

2.4.1 Hardening of seedlings

As mentioned earlier, re-established seedlings are faced with an array of abiotic and biotic challenges in the natural environment. To enhance seedling survival during re-establishment practices, research has focused on developing new procedures aimed at protecting seedlings against limiting conditions (Padilla & Pugnaire, 2009; Jiménez *et al.*, 2005). One method used to promote the survival of transplanted seedlings during restoration practices entails the ‘hardening’ of seedlings before transplantation. “Hardening” refers to a process of acclimation by a plant to certain environmental stresses, such as drought and heat (Hopkins & Hüner, 2004). Sánchez-Blanco *et al.* (2006) evaluated the degree of hardening which resulted from different irrigation and air humidity conditioning in *Rosmarinus offincinalis* seedlings. Results indicated that seedlings subjected to deficit irrigation and low humidity showed a better water status after transplantation and during the establishment period. The hardening process therefore provides a seedling with a greater ability to withstand and adjust to adverse environmental conditions (Bañon *et al.*, 2006). “Hardening” results in a lower shoot:root ratio, which entails the loss of leaf surface to reduce transpiration, as well as a more thickened root growth to assist in the accumulation of reserves in the roots and enlarge the storage capacity of the roots (Bañon *et al.*, 2006).

2.4.2 Facilitation

Facilitation has also been long recognized as one of the main mechanisms that underlies the process of ecological succession (Walker & del Moral, 2003; Connell & Slayter, 1977). Especially in primary succession, each stage may create the conditions that promote the regeneration of a new set of species (Padilla *et al.*, 2009). The occurence of facilitation has been recorded in a wide variety of habitats, including deserts, alpine sites, sand dune and salt marshes. Generally, one species facilitates another species, but cases of co-specific facilitation are known. “Nurse” plants are typically other perennial species that provide benefits to the “nursed” individual through the modification of a sub-canopy micro-climate, providing shade (Valiente-Banuet & Ezcurra, 1991; Turner *et al.*, 1966), reducing daytime and summer high temperatures (Franco & Nobel, 1989), reducing soil surface temperatures (Franco & Nobel, 1989), reducing wind (Parker, 1989), protecting seedlings from browsing animals (Niering *et al.*, 1963), and adding nutrients to the soil (Franco & Nobel, 1989). This type of positive interaction is especially frequent in harsh environments, such as deserts, alpine sites and salt marshes (Brooker & Callaghan, 1998; Valiente-Banuet & Ezcurra, 1991).

Facilitation has a well-established place in forestry practice where so-called “nurse” trees are often planted in association with seedlings of a more valuable species (Fenner & Thompson, 2005). Experiments in the artificial regeneration of tropical trees show that in many cases, late successional species can be readily established by the provision of “nurse” trees in the early stages of growth (Fenner & Thompson, 2005). A study by Sweeney and Czapka (2004) demonstrated that the impact of nursing species on growth varies significantly among species. Some species provide better spaces for regenerating seedlings than others (Nunez *et al.*, 1999), and facilitation therefore seems to be dependent upon species attributes and habitat conditions (Tewksbury & Lloyd, 2001; McAuliffe, 1988).

Acacia tortilis is an example of an indigenous leguminous plant, able to form symbiotic relationships with nitrogen bacteria called *Rhizobium* and thus being able to contribute to the overall nutrient cycle in the environment (Van Wyk & Van Wyk, 2007). Additionally, Ludwig *et al.* (2003) conducted a study where it was determined that *A. tortilis* had the ability to hydraulically lift water from deeper soil aquifers to the surface. This species could therefore potentially be used to “nurse” other seedlings. The relationship between the facilitator species and its beneficiery changes with time however. The uneasy balance between facilitation and competition can also shift from place to place between the same species. In some cases the seedlings facilitated by adults early in life frequently become unsuccessful competitors against much larger “nurses” in time (Miriti *et al.*, 2007). In a study by Miriti *et al.* (2007) it was also observed that the roles of facilitation and competition change with extreme drought. In desert communities, facilitation is expected to increase and competition decrease with decreasing habitat quality (Goldberg & Novoplansky, 1997), but apparently extreme stress such as drought may compromise this trade-off (Maestre *et al.*, 2005; Tielbörger & Kadmon, 2000).

2.4.3 Arbuscular mycorrhizal fungi (AMF) and compost

According to Fenner & Thompson (2005), the formation of arbuscular mycorrhizal fungi (AMF) effectively enables seedlings with small root extensions (which may have limited access to an external phosphorous supply) to form a sufficiently extensive root system adequate for accessing external supplies of soil phosphorous. Allsopp & Stock (1995) stated that mycorrhizal infection is probably essential in many cases for the seedling to progress beyond its initial germination stages, especially in poor soils. The abundance and activity of the AMF is however greatly affected by the soil environment (Yang *et al.*, 2008).

Compost is widely used as an amendment during forest restoration practices, especially in the semi-arid Mediterranean (Larchevêque *et al.*, 2006). The use of compost during tree restoration projects has been shown to increase soil fertility and plant biomass (FFTC, 2010; Brady & Weil, 2008; Martinez et al., 2003; Caravaca et al., 2002).Woodchip compost can also ameliorate the physical properties of a growth medium by increasing water holding capacity and stimulate biological activity (Brady & Weil, 2008; Logan, 1992).

2.5 Abiotic and biotic stressors on seedling survival and growth

According to Hughes (1994), the regeneration of many arid and semi-arid riverine trees are dependent on overbank floods which provide soil moisture for seed germination and seedling establishment. Various other sources also state that flood pulses in riparian corridors play a crucial role in seed dispersal, plant establishment, nutrient cycling, scouring, sediment deposition, and maintenance of species richness (Friedman & Auble, 1999; Nilsson *et al.*, 1997; Stromberg *et al.*, 1993). Temperature, light, oxygen, carbon dioxide, and factors influencing the availability of water constitute to the main environmental factors controlling seed germination (Desai, 2004). Any of these factors can favour or inhibit germination in the natural environment. The emerging seedling also faces a vast and new set of hazards. Whereas a lack of light, water or nutrients has little or no effect on seed survival, these become major causes of seedling mortalities. The predators and pathogens that target seed are replaced by a different set at the seedling stage. A number of local ecological factors can possibly negatively effect seedling regeneration, namely drought, seed predation, browsing by rodents and ungulates, trampling, and competition particularly with grassses.

One of the main causes of mortality in seedlings is competition from other seedlings or from surrounding vegetation. A newly germinated seedling is at great disadvantage with established plants in capturing resources before the formation of its roots and expansion of its leaves. In savanna systems, competition from the existing vegetation has been identified as a major limitation for the establishment of climax tree species (Child *et al.*, 2009; Sharam *et al.*, 2006; Fetene, 2003).

Herbivory is another major cause of seedling mortality in many communities (Sharam *et al.*, 2006; Fenner & Thompson, 2005; Alvarez-Aquino *et al.*, 2004; Pedraza & Williams-Linera, 2003). The herbivores may be vertebrate (often rodents) or invertebrate (usually insects or molluscs). The removal of even a small part of a seedling can have fatal consequences, especially if the root is attacked at ground level. The risk of herbivory is probably greatest in the very early stages of establishment (Fenner & Thompson, 2005). The level of herbivory suffered by seedlings in the field are influenced by a range of ecological factors, such as the density of the seedlings and the presence of vegetation that provides suitable habitat for rodents.

In addition to the biotic factors already mentioned, seedlings also face a number of abiotic hazards that limit recruitment. One of these is the occurrence of physical damage due to branch falls and other disturbance (Zida *et al.*, 2008; Athy *et al.*, 2006; Fenner & Thompson, 2005).

Another common mortality factor is the lack of moisture. Important to note is that seed germination rates are usually sufficient to sustain recruitment, but seedling establishment is challenged by the ability of their roots to trace the capillary fringe of the declining water table as the floodwater recedes (Stave *et al.*, 2005; Mahoney & Rood, 1998). The uptake of water into the plant takes place almost entirely through the roots so that to make use of the soil water, roots must either be present in the particular zone where the water is held or the water must move from that zone to the place where the roots are (Winter, 1974). The most efficient means by which a plant can utilise the soil water is by having an extensive root system. Thus the efficiency of exploitation of soil available water depends on the size and rate of expansion of the root system rather than on the movement of water through the soil towards the roots. There is evidence that the failure of roots of tree seedlings to grow into dry soil was associated with the physical impedance of the hard dry soil to penetration by the comparitively soft root tips rather than with lack of water itself (Winter, 1974).

According to Stave *et al.* (2005), after a flood event, the water table declines in conjunction with the river stage and the rate of decline may have species-specific effects on the regeneration of riverine trees. Their experiment was conducted on *Acacia tortilis* and *Faidherbia albida* (Delile) A. Chev. seedlings, and their results showed that *F. albida* attained larger shoot growth but shorter root lengths than *A. tortilis*, while water table decline promoted root elongation in both species. However, *F. albida* seedlings were adversely affected by moisture deficits under the rapid rate of water table decline and rainfall treatments. In contrast, *A. tortilis* seedlings were sustained under all treatments, suggesting that *A. tortilis* is more drought-tolerant compared to *F. albida* (Stave *et al.*, 2005). The apparent drought tolerance of *A. tortilis* seedlings are consistent with earlier experimental studies (Otieno *et al.*, 2001), as well as with the widespread occurrence of adult *A. tortilis* trees in arid environments (Kennenni, 1991; Kennenni & Van der Maarel, 1990). This study concluded that the regeneration of strictly riverine trees such as *F. albida* depends on slow rates of water table decline in the post-flood period. The survival of *F. albida* seedlings was severely limited by drought stress.

CHAPTER 3

STUDY AREA

3.1 Location

3.1.1 The Mapungubwe National Park

The Mapungubwe National park (MNP) is a relatively recently established park in South Africa, situated on the South African side of the confluence of the Shashe and Limpopo Rivers in the Limpopo Province (Robinson, 1996) (Fig. 3.1). The park constitutes a key cultural holding of South African National Parks (SANParks), forming the core area of the Mapungubwe Cultural Landscape which was added to the list of the UNESCO World Heritage sites in July 2003 (UNESCO World Heritage Centre, 2008; Carruthers, 2006).

The wealth of Mapungubwe was realised in the 1930s when extensive archaeological research uncovered valuable artefacts on the sacred Mapungubwe hill. Numerous rock paintings provide additional evidence of an earlier occupation of Mapungubwe by San and hunter-gatherer inhabitants. Archaeological research spanning from the 1930’s, has indicated that the Mapungubwe Cultural Landscape was the centre of the first known powerful indigenous kingdom in southern Africa. There are more than four hundred documented archaeological sites in the vicinity of Mapungubwe (Carruthers, 2006; Götze *et al.*, 2002; Robinson, 1996). Wealth accrued by the leaders, through trade from the Indian Ocean network, resulted in social organisation changing to a situation in which the ruling elite lived separately from commoners. The kingdom dispersed toward the end of the thirteenth century, owing to rapid change in climate as the effects of the ‘Little Ice Age’ began to manifest, probably due to climate change, resulting in a shift of the regional power to Great Zimbabwe, north of the Limpopo River (O’Connor, 2010a; SANParks, 2008). Further archaeological work at several related sites spanned right into the early 2000’s, and the extensive historical importance of the wider region was discovered.

The creation of the MNP has been an objective of SANParks for many years (Robinson, 1996) and a long history preludes its formalisation in 2004. A summarised timeline of the key events leading to the establishment of the MNP are listed in Table 3.1. During the 1920’s South African botanists drew attention to the Mapungubwe area when a number of botanical reserves were set aside in different ecosystems of South Africa (Carruthers, 2006). One of these reserves was a block of nine farms established in the Mapungubwe area in 1922 - the Dongola Botanical Reserve. Following much controversy and lobbying, not only based on account of the wildlife contained, but on the basis of the scientific value of a natural research station, the reserve was declared as a wildlife sanctuary in 1947 (Carruthers, 2006). In the following year a political shift occurred when Jan Smuts’ United Party was defeated by The National Party, and consequently the park was repealed - the first time ever in South African history that a National park has been de-proclaimed. The park was divided into farms and allocated to white Afrikaner Nationalist farmers (SANParks, 2008). By 1967 there was a renewed lobby for the re-establishment of the Dongola Sanctuary, now also including the important archaeological values which had become apparent.

During the 1970s and 1980s South Africa was involved in a war with its neighbouring states and the region had a significant military presence which has left a legacy of fences and other infrastructure within the park (SANParks, 2008). The military presence sparked renewed attention to the archaeological sites in the area and resulted in two of the most prominent archaeological features, Mapungubwe Hill and K2, to be declared as National monuments (Carruthers, 2006). By 1986 interest aroused again in pursuing national status of the park, a move which was later supported by De Beers following the establishment of the Venetia Diamond Mine to the south of the park in 1990. At the end of the apartheid era, a shift in the political grounds within South Africa took place. Eventually provincial and national authorities in the new South African democracy reached a consensus regarding the park by way of an agreement signed on 9 June 1995, committing the area to National park status (SANParks, 2008). The Vhembe-Dongola National Park was consequently established through an interdepartmental government transfer and a number of acquisitions commencing in 1996, leading to a declaration on 9 April 1998.

In 1997 the Peace Parks Foundation (PPF) was founded to assist in the facilitation of the creation of transfrontier parks, also sometimes referred to as peace parks, in southern Africa. The PPF bought much-needed land for the creation of the new National park and initiated the process by purchasing a number of critical properties in the core area where the park was to be proclaimed (Berry & Cadman, 2007) (Fig. 3.1). The Mapungubwe Cultural Landscape was added to the list of UNESCO World Heritage sites in July 2003. Based on the rich biodiversity of the area, its great scenic beauty and the cultural importance of the archaeological treasures of Mapungubwe, the Mapungubwe National Park was officially opened in 2004 (Berry & Cadman, 2007).

Table 3.1. A timeline of events leading to the establishment of the Mapungubwe National Park and UNESCO World Heritage Site (adapted from Berry & Cadman, 2007).

Date	Event
1922	Dongola Botanical Reserve established.
1932	Mapungubwe rediscovered.
1947	Dongola Wildlife Sanctuary proclaimed.
1949	Dongola Wildlife Sanctuary de-proclaimed.
1968	Attempt to revive Dongola Wildlife Sanctuary.
1997	Peace Parks Foundation formed.
2003	Mapungubwe World Heritage Site declared.
2004	Mapungubwe National Park formalised.
2006	South Africa, Botswana, Zimbabwe Transfrontier park agreement.

Figure 3.1. A map of the Mapungubwe National Park, indicating the different ownership of land as well as the experimental exclosure in the western section of the park where this study took place. (Adapted from a map provided by the Peace Parks Foundation, see www.peaceparks.org).

3.1.2 The Greater Mapungubwe Transfrontier Conservation Area

As in many other places in the world, the Southern African Developing Community (SADC) contains various protected areas which cross international boundaries (Hanks, 2003). These are referred to as Transfrontier Conservation Areas (TFCAs), of which the Ai-Ais/Richterveld Transfortier Park and the Kgalagadi Transfortier Park are some examples (Fig. 3.2).

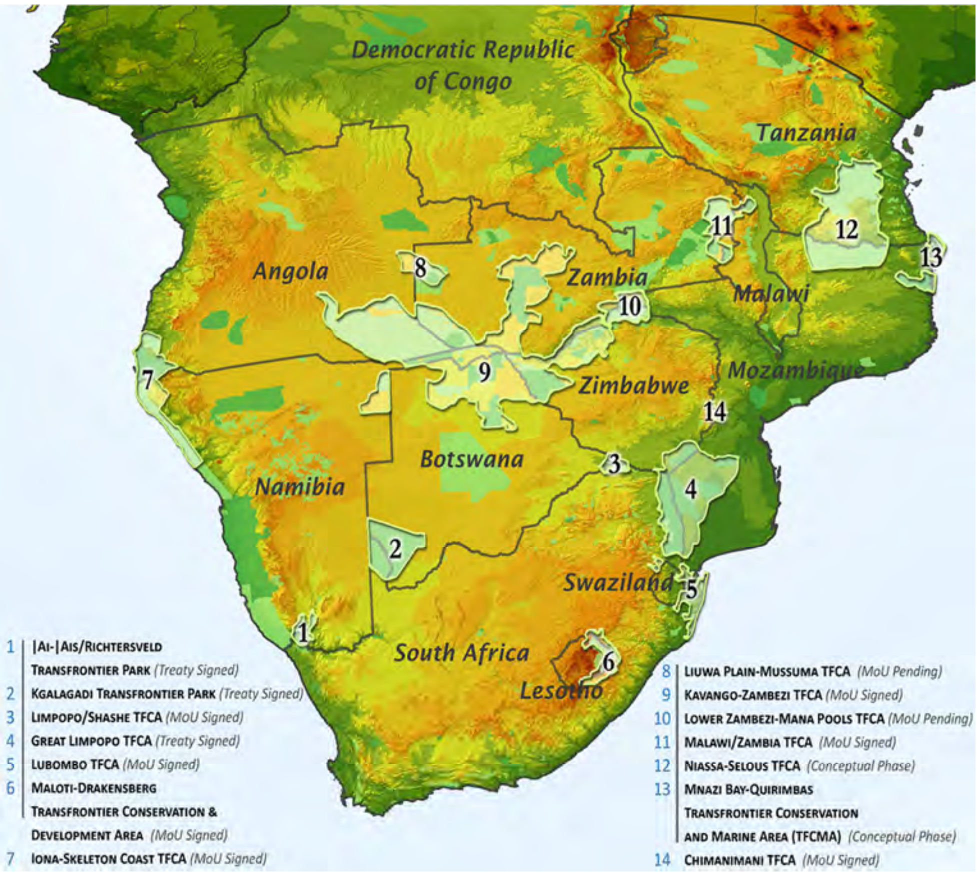

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Figure 3.2. The various Transfrontier Conservation Areas (TFCAs) situated within the Southern African Developing Community (SADC). The Greater Mapungubwe TFCA (previously known as the Limpopo-Shashe TFCA), is indicated by nr 3. (Map provided by the Peace Parks Foundation, see www.peaceparks.org).

The MNP also forms part of such a TFCA, namely the Greater Mapungubwe TFCA, which encompasses areas in three countries – Botswana, South Africa and Zimbabwe. Initially named the Limpopo-Shashe TFCA (the new name was adopted on the 19th June 2009), it was officially agreed upon when a Memorandum of Understanding (MoU) was signed by the environmental ministers of the participating countries in June 2006 (Berry & Cadman, 2007; Treasure, 2006). TFCAs have become the focus of the international conservation community during recent years (Stern *et al.*, 2003). There has been a rapid growth in the number of TFCAs over the last 18 years – from 59 in 1988, mainly in Europe and North America, to 169 in 2001, with examples from every continent (Zbicz, 2001). The establishment of TFCAs in southern Africa was facilitated by post-apartheid political, socio-economic and historical circumstances (Ramutsindela, 2004). Modise (2002) claims that the establishment of TFCAs in the southern African sub-region is an extension of goodwill and equitable sharing of the benefits derived from the conservation of natural resources. There are currently 14 TFCAs in SADC countries in Africa in various stages of development (Duffy, 2006) – this includes the Greater Mapungubwe TFCA (Fig. 3.2). TFCAs in southern Africa has been well promoted and publicised and attract huge foreign donor funding, national prestige and international recognition – all of which is much needed in the southern African region (Treasure, 2006).

The selection of the Greater Mapungubwe TFCA (GM-TFCA) was based upon the rich biodiversity of the area, its scenic beauty and the importance of cultural heritage in the region (De la Harpe & De la Harpe, 2004; Modise, 2002). The specific objectives of the GM-TFCA entail the following (Treasure, 2006):

- fostering trans-national collaboration and co-operation between Botswana, South Africa and Zimbabwe;
- promoting alliances in the management of biological and cultural resources; enhance ecosystem integrity and natural ecological processes;
- developing frameworks and strategies whereby local communities can participate in, and benefit from, the management and sustainable use of natural and cultural resources; and,
- promoting cross-border tourism as a means of fostering regional socio-economic development.

The GM-TFCA, once established, will cover an area of approximately 4 800 km^2 including the Tuli Circle, portions of the Maramani communal lands and some privately-owned farms in Zimbabwe, the privately-owned Northern Tuli Game Reserve in Botswana and South Africa, the MNP and the adjacent privately-owned conservancies such as Venetia Private Game Reserve. South Africa will contribute the largest portion of area to the GM-TFCA – 53%, with Botswana contributing 28% and Zimbabwe 19% (Purchase & Wilson, 2004).

3.1.3 Natural vegetation

The MNP falls within southern Africa’s largest Biome - the Savanna Biome (Mucina & Rutherford, 2006; Low & Rebelo, 1998; Rutherford & Westfall, 1986). This Biome is typically dominated by a clearly defined grassy ground layer followed by a distinct upper layer of woody plants. Insufficient rainfall is the main limiting factor which prevent the woody layer from dominating, where wild fires and grazing prevents the grassy layer from dominating (Low & Rebelo, 1998). According to the most recent vegetation map of South Africa, Lesotho and Swaziland (Mucina & Rutherford, 2006), the MNP consists of three vegetation types, namely the Limpopo Ridge Bushveld, Musina Mopane

Bushveld and Subtropical Alluvial Vegetation which all form part of the Mopane Bioregion which is the smallest subdivision of the Savanna Biome (Fig. 3.3).

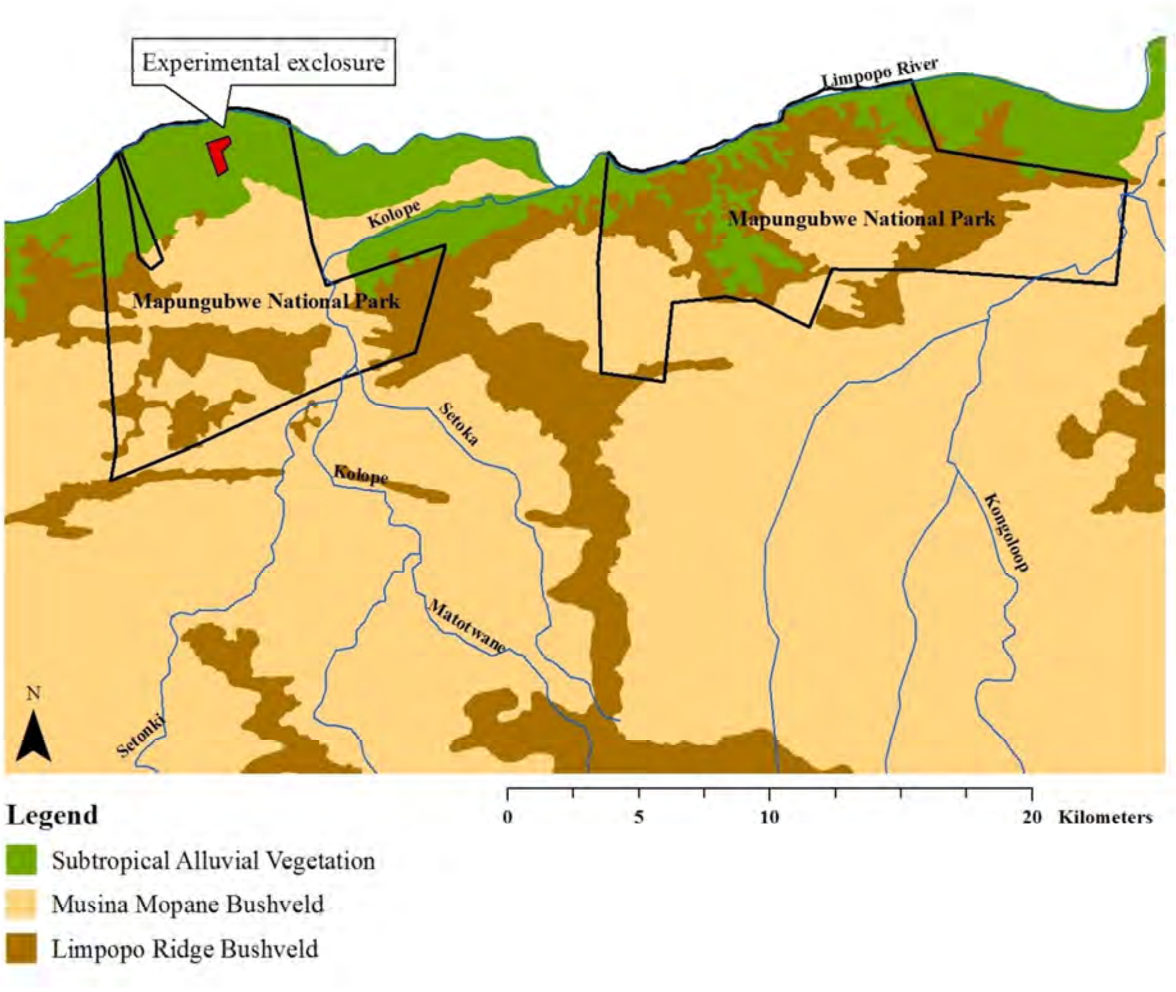

Figure 3.3. The different vegetation types of the Mapungubwe National Park (Mucina & Rutherford, 2006). The experimental exclosure falls within the Subtropical Alluvial Vegetation type. (Map designed by Marié du Toit[1]).

The area where the experimental exclosure is located falls within the Subtropical Alluvial Vegetation type which is distinguished from the Musina Mopane Bushveld by the flat alluvial riverine terraces, supporting the intricate collection of macrophytic vegetation, minor reed belts, flooded grasslands, temporary herblands and riverine thickets (Mucina & Rutherford, 2006). Stretches of forest along the Limpopo and Shashe Rivers support majestic sycamore figs (*Ficus sycomorus* subsp. *sycomorus*), ana trees (*Faidherbia albida*), nyala trees (*Xanthocercis zambesiaca*), fever trees (*Acacia xanthophloea*), marulas (*Sclerocarya birrea* subsp. *caffra)* and many other species, standing in contrast with the surrounding semi-arid savanna landscape. Many of the savanna trees in the lowveld support open

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riverine woodland, but in their classification of the vegetation of South Africa, Mucina and Rutherford (2006) recognise the Limpopo riverine forests of this region as a distinct forest type. Even more so, these stretches of riparian vegetation in MNP appears to be under considerable threat as it has been classified as “critically endangered” by the South African National Biodiversity Institute (SANBI) (SANParks, 2008). The alluvial vegetation band around the forest area is however classified as “least threatened”. Large patches of the Subtropical Alluvial Vegetation are conserved in the Kruger and Mapungubwe National Parks, as well as various other game and nature reserves in South Africa.

According to an extensive vegetation classification of the MNP conducted by Götze (2002), the experimental exclosure is situated in the most disturbed land type inside the MNP, namely the Ia155 land type. *Acacia stuhlmanni* and *A. tortilis* communities occur on the old lands with few riparian species such as *Combretum imberbe* regenerating naturally.

3.1.4 Climate

Mucina & Rutherford (2006) classifies the study area’s climate as semi-arid, with a long-term mean annual rainfall of 419 mm per annum. Rainfall is highly variable and usually falls during the summer months between October and March (Mucina & Rutherford, 2006). According to Robinson (1996) extended periods of below average rainfall can occur. Evaporation from free water surfaces is in excess of 2 500 mm per year over the most of the year (Mucina & Rutherford, 2006; Robinson, 1996). According to Mucina & Rutherford (2006), the mean annual temperature for the region is 21.6 °C with summer temperatures often reaching 45 °C (Robinson, 1996). The winters are generally mild, with the mean annual frost days amounting to a maximum of 4 days per year.

During this study climate (rainfall and temperature) data spanning from 1990 to 2009 were collected from Mr Stefan Cilliers[2] who is the head section ranger at the MNP, the South African Weather Service[3] and the ZZ2® Pontdrift tomato farm[4]. Rainfall data for 1990 to 2002 were collected at Pontdrift weather station (15 km to the west of the exclosure), rainfall data from 2003 to 2008 were collected at Rhodesdrift Lodge (5 km to the west of the study site; the location of the nursery used during this study as well), and rainfall data for 2009 were collected at a weather station situated on the ZZ2® Pontdrift tomato farm (8 km to the west of the study site) (see Fig. 3.1). The temperature data for 1990 to 2007 were collected at the Alldays weather station (60 km to the south of the study site), and the temperature data for 2008 to 2009 were collected at a weather station on the ZZ2® Pontdrift tomato farms. A combination of various locations had to be utilised due to incomplete data basis at the various weather stations.

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[3] South African Weather Service, Tel: 012 367 6000, Website: www.weathersa.co.za
[4] ZZ2® Pontdrift, Danie Nortje, Tel: 015 575 9942

The collected data were compiled and the short- and long-term means for the study area were determined. Figure 3.5 indicates that the mean annual rainfall in the study area over the last 19 years (1990 – 2009), has been approximately 340 mm per annum. During the early nineties, with the exception of 1991, below-average rainfall was recorded for the study area (Fig. 3.5). During this time South Africa experienced one of the worst droughts on record during which flow of the Limpopo River ceased completely during the summer – something that had never been recorded before (O'Connor, 2010a). In 2000, one of the largest floods on historical records occurred, with 907.7 mm rain pouring down onto the study area. The four years during which the pilot- and current study has taken place (2006 – 2009), received above average annual rainfall (Fig. 3.4).

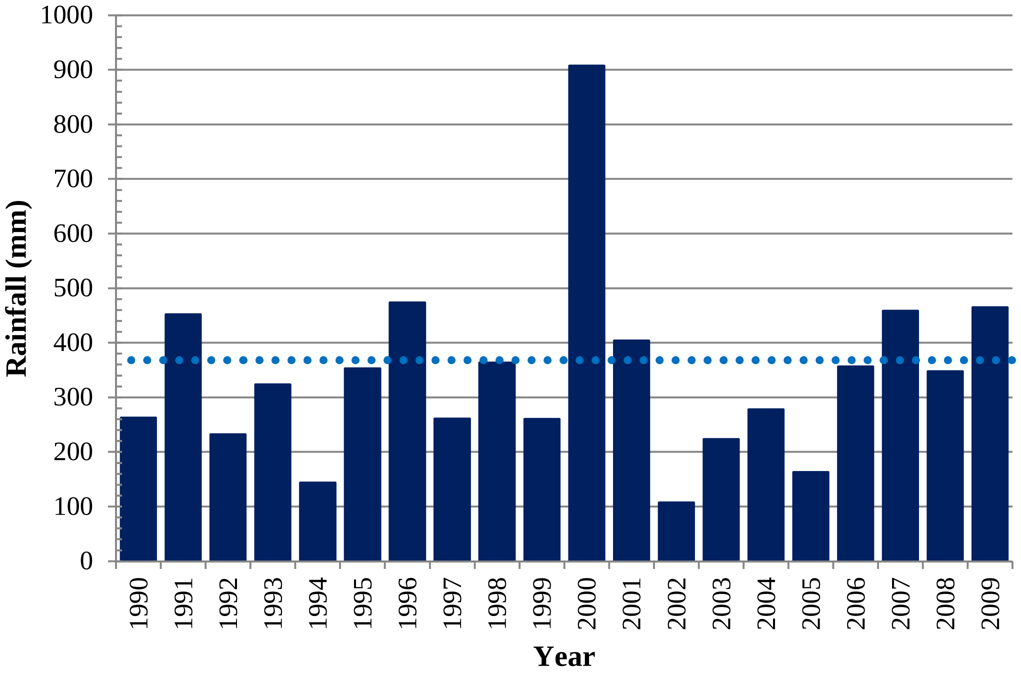

Figure 3.4. The long-term annual rainfall occurring in the study area over a period of nineteen years (1990-2009). The mean long-term rainfall experienced is indicated with the dotted line.

Figure 3.5 indicate that the 2008/9 season experienced the highest rainfall in the study area compared to the other seasons during the study period, with 346 mm of rain falling in January 2009. This period of high rainfall occurred shortly after tree seedlings were transplanted into the experimental exclosure at the end of October 2008 (refer to section 4.5 for more information). Maximum temperatures regularly exceeded 30 °C, with minimum temperatures never falling below 6 °C.

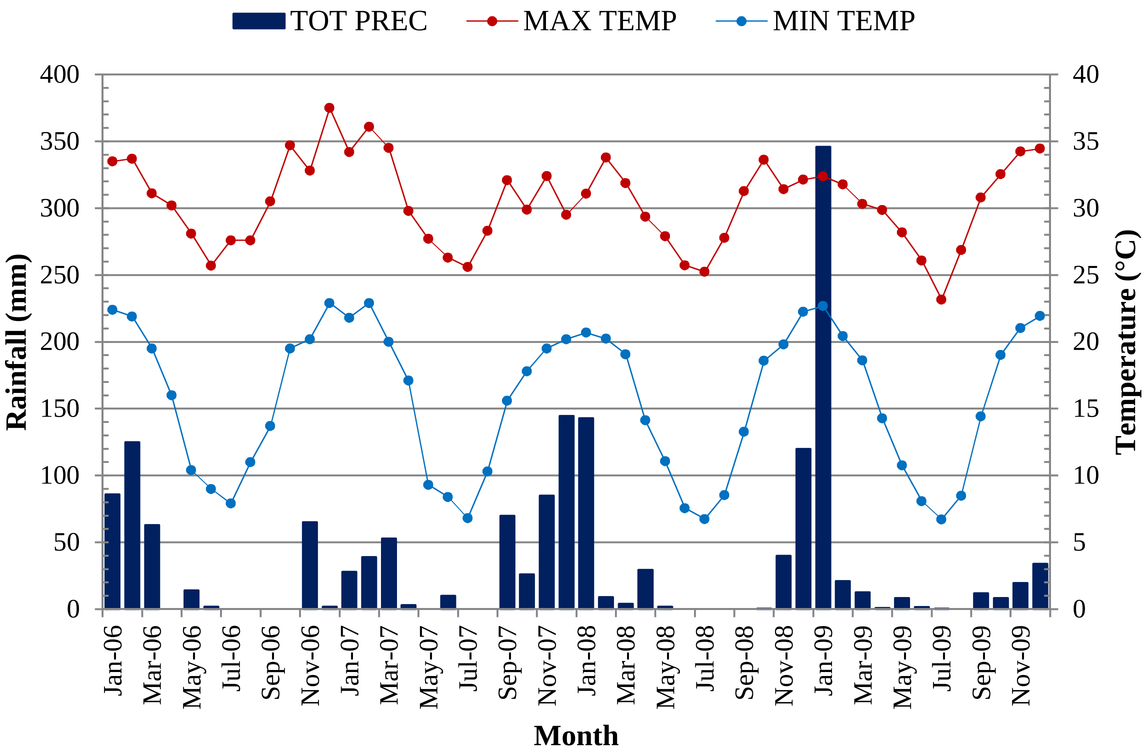

Figure 3.5. The monthly rainfall and minimum and maximum temperatures occurring in the study area over four years (2006-2009).

3.1.5 Geology, geomorphology and soils

Mapungubwe is an attractive semi-arid landscape with varied geology, including extremely old archean rocks, metamorphics of intermediate age, karoo sandstone/conglomerate uplands of 200 million years age (Huffman, 2005), as well as recent alluvium and sands (Mucina & Rutherford, 2006). Mapungubwe lies within the Limpopo Mobile Belt that joins two ancient continents known as the Zimbabwe and Kaapvaal cratons (McCarthy & Rubidge, 2005). Granite forms the two cratons to the north and south, while millions of years of erosion are responsible for the sandstones currently present in the basin. When the continents experienced movement, magma was pushed through the cracks and appeared on the earth's crust to form basalt sheets and dolerite dykes (Huffman, 2005) which is evident today in mostly the eastern section of the park. South of the Limpopo River, the topography tends to be flat with sandstone and conglomerate ridges and koppies. Nearer to the Limpopo River, the plains give way to a rugged, hilly terrain (Götze, 2002).

The whole park lies between 300 m and 780 m above sea level, with a variety of soils present (Transvaalse Provinsiale Administrasie, 1989). Large areas are characterised by sandy, lime-rich soils generally deeper than 750 mm and other areas are characterized by brown to dark brown clays with high silt content (Götze, 2002; Robinson, 1996). Soils generally have low agricultural potential, with the irrigated alluvium tending to become brackish as a result of the accumulation of salts due to the strong evaporation (Mucina & Rutherford, 2006). This poor agricultural soil is also easily degraded by overgrazing (Transvaalse Provinsiale Administrasie, 1989).

3.1.6 Hydrology

Surface drainage occurs in mostly a northerly direction towards the Limpopo River (Götze, 2002; Robinson, 1996). The confluence of the seasonally-flowing Shashe and Limpopo Rivers is a dominant hydrological feature, as is the large ephemeral Kalopi/Maloutswa wetland upstream of the confluence (SANParks, 2008) (Fig. 3.1). None of the rivers in the area, including the Limpopo, are perennial. However, hydrological data indicate that the Limpopo River would be permanent if it were not for the numerous dams and boreholes throughout the catchment area (personal communication with AquaTech Consultants in 1999, as quoted by Huffman, 2008). The area along the Limpopo River under irrigation was 329 034 ha in 1995, which corresponds to 0.79% of the entire Limpopo River Basin (Amaral & Sommerhalder, 2004). Irrigation withdrawals up- and downstream of the MNP are large relative to water supplies (SANParks, 2008), and the groundwater supplies in the area are generally poor except along fault lines (Van den Heever, 1983). These withdrawals are due to water that is being abstracted from the Limpopo aquifer on the farm Greefswald (refer to Fig. 3.1) which supply the intermediate requirement of 0.6 million m^3 of water per year to Venetia Diamond Mine to the south of the park, as well as the long-term requirement of 4.2 million m^3 water per year (Klapwijk, 1990).

Studies such as Le Maitre *et al.* (1999), report that the lowering of the water table has direct effects on regional riparian vegetation in general. A long-term study was recently completed where the objective was to determine the main cause of mortalities of forest trees on the Greefswald farm (O’Connor, 2010b). It could not be determined directly whether the trees had succumbed to natural drought or deprivation of water supply by abstraction, but tree mortality within the area of abstraction was one and a half times that of what it was without. Another article by O’Connor (2010a) reports that diamond mines does not use as much water compared to other land uses - the amount is equivalent to two centre-pivot irrigation schemes. By the 1950’s almost all land along the Limpopo River had been committed to agriculture (O’Connor, 2010a), with most of the farmers using centre-pivot irrigations schemes – some of which are still active today. The evidence of these pivot-irrigation systems which has been removed on the land now part of the MNP, is still visible in the areas adjacent to the experimental exclosure (Fig. 3.3).

At this time, a large water impoundment on the Shashe River is close to completion, and increasing amounts of water will be abstracted from the Limpopo catchment for power generation (O’Connor, 2010a). A change in the hydrological flows of the Limpopo valley are therefore anticipated in the near future, and organisations such as the South African Earth Observation Network (SAEON) have taken it upon themselves to assess the anticipated impacts on the riparian habitats (O'Connor, 2010a).

3.2 Experimental exclosure

The site used during this study entails a 70 ha fenced off area, located in the western section of the MNP (Latitude: 22º11'43.2" South and Longitude: 29º12'53.9" East), on the farm previously known as Den Staat (Fig. 3.1 and 3.6). Based on the recommendation made by Aerts *et al.* (2009), this site will be referred to as an "exclosure", as exclosures are "areas from which unwanted animals are excluded" and the main purpose is to keep things (animals) out of the given area. Experimental exclosures have been widely used as treatments to exclude (or statistically control for) the effects of predation or herbivory on species richness and recruitment in plant and animal communities (Jacobs & Naiman, 2008; Levick & Rogers, 2008). Fencing off areas in this way is common practice for forest management throughout the world, because high tree seedling mortality is often related to high browsing pressure by large and small herbivores (Coop & Givnish, 2008; Negussie *et al.*, 2008).

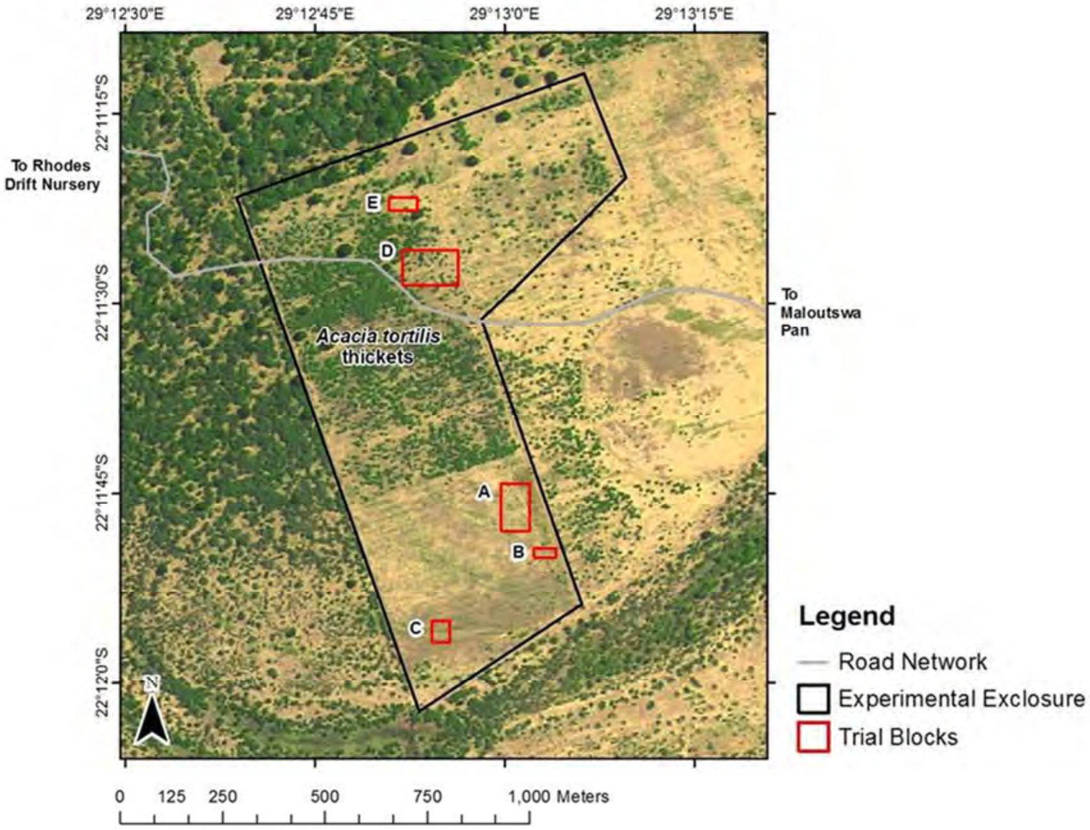

Figure 3.6. The layout of the experimental exclosure situated in the western section of the Mapungubwe National Park (Latitude: 22º11'43.2" South and Longitude: 29º12'53.9" East), also indicating the five blocks where indigenous tree seedlings were transplanted into. (Map designed by Marié du Toit).

The exclosure is located about 500 m south of the Limpopo River and falls within an area which has been degraded and deforested for agricultural purposes (Fig. 3.6). The past and present management practices followed in this area are discussed in section 3.3. The experimental exclosure in MNP was erected with an electrified game fence in January 2006 with funds and labour provided by the Poverty Relief Program (funded by the Department of Agriculture and Tourism) (Scholtz, 2008). The Poverty Relief Program again provided funds in June 2008 to electrify the cattle-grid entrance to the exclosure also, as elephants still managed to enter the site consequently trampling the vegetation and transplanted seedlings inside. The experimental layout within the exclosure is discussed in section 4.3.

3.3 Management practices

3.3.1 Former management practices

Due to a change in Mapungubwe's climate to its semi-arid status coupled with erratic flooding patterns, the more recent white farmers which started settling toward the late 1800s were rendered highly dependent on irrigation for cultivation. Both the white farmers and the indigenous Bantu farmers contributed to the degradation of the region through deforestation of mainly indigenous trees, especially in the area where the experimental exclosure is located (Robinson, 1996). According to De Beer (2006), severe pressure on the Limpopo River riparian forest and associated plant communities due to extensive agricultural and infrastructure developments has taken place only since the 1978. Much of the riparian woodland which has been cleared through deforestation for agricultural purposes is still in the process of being purchased by SANParks to enlarge the MNP and GM-TFCA (SANParks, 2008) (Fig. 3.1).

An intensive irrigation scheme was erected on the farm Den Staat where the exclosure is situated in the 1980s, leading to the deforestation of a large area of riparian vegetation in this area. During the pilot study a thorough interview was conducted with Mr Wim Neethling[5], who rented the farm Den Staat from 1995-1998, to get some background information of the area where the exclosure is situated. The following is a summary of the most important aspects mentioned (Scholtz, 2008):

- The farm Den Staat covers 1900 ha and the Wim Neethlings' farming activities ended in 1998. Other lease farmers did however continue with cultivation on a small area of Den Staat until 2004 when the farm was bought by SANParks and all farming activities ended.
- Most parts of the farm were deforested in 1982. The farms' pre-deforestation state entailed a natural plain consisting of native grasses, narrow-leaved mustard trees (*Salvadora australis* Schweick.), and ilala palms (*Hyphaene natalensis*) as well as the occasional leadwood (*Combretum imberbe*) and nyala tree (*Xanthocercis zambesiaca* (Baker) Dumaz-le-Grand).

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[5] Wim Neethling, cell: 083 283 9461

- A big drought occurred from 1980 to 1990. The mean annual rainfall of the area between 1990 and 2000 was estimated at a mere 250 – 300 mm per annum, compared to the long term mean of 419 mm per annum as reported by Mucina and Rutherford (2006).
- Rotational cropping was the method of cultivation practiced on Den Staat. Various vegetables, such as potatoes and tomatoes as well as runner crops were planted on a rotational three year base.
- Regular fertilizer and insecticide applications took place.
- Large quantities of natural game consisting of mostly impala, zebra and blue wildebeest occurred in the area.
- Boreholes situated along the Limpopo River were used for three different irrigation methods, namely centre-pivot irrigation, drip irrigation, and dragline sprinkle irrigation.

3.3.2 Current management practices

The establishment of MNP in 2004 resulted in the termination of all agricultural activities within park boundaries (Fig. 3.2). Currently the mission of the MNP, as stated in its management plan (SANParks, 2008), is as follows: “Mapungubwe National Park and Mapungubwe Cultural Landscape will be developed by SANParks to maintain the faunal and floral assemblages, ecological processes, cultural resources and landscape characteristics representative of the area, to foster international co-operation for the establishment of a transfrontier conservation area, and offer long-term benefit to the people of the area.”

In order to reach its mission, the MNP follows an adaptive management approach where the park aims to set up a park desired state through the adaptive planning process (Rogers, 2003). The desired state of a park is the parks’ long-term vision (30-50 years) translated into sensible and appropriate objectives through broad statements of desired outcomes (SANParks, 2008), and rather refers to a ‘desired set of varying conditions’ than a static state. Adaptive management is not restricted to natural resource management, but is also relevant to management issues dominated by economic and social factors (Sabine *et al.*, 2004). Many conservation organisations have adopted an adaptive management approach for their projects (Biggs & Rogers, 2003). The management of natural resources is often conducted under great uncertainty regarding future condition, relationships among components, user response to management, management objectives, and even abundance of the resource itself (Johnson, 1999). Adaptive management provides a solution to these uncertainties as it consists of the following four elements: “trial and error”; “focussed on experimentation”; “research”; and, “learning by doing” (Meffe *et al.*, 2002; Walters & Holling, 1990). More specifically, SANParks is following a Strategic Adaptive Management (SAM) process where, in contrast with the conventional adaptive management, the stronger emphasis is on operating in a proactive rather than reactive mode (Biggs & Rogers, 2003).

Within the adaptive management of ongoing changing systems, thresholds of concern are the upper and/or lower limits of flux allowed, literally specifying the boundaries of the dynamic desired state (SANParks, 2006). Thresholds of potential concern (TPCs) specify the measurable ‘boundaries’ of the desired state, flowing out of the objectives developed for the park (SANParks, 2008). When monitoring (also in combination with predictive modeling in the case of SAM) indicates certain or very likely exceedences beyond these limits, then mandatory management options of the adaptive cycle are prompted for evaluation and consideration. Based on a parks’ biophysical objective, certain thresholds of concern are therefore set up. In order to reach the park’s desired state, certain programmes are set in place which make up the approaches to certain issues, and leads to the actions on the ground.

This project finds its relevance in one of the park’s programmes, namely the Rehabilitation Programme. This programme incorporates widespread rehabilitation taking place within the park, and as stated in the 2008 Management Plan for the MNP, the “rehabilitation of old lands, with particular emphasis (where appropriate as judged from historical aerial photos) on re-establishment of riparian woodland” which is to continue unabated for the following five years. The importance of this project is however linked to the parks’ Gallery Forest Programme as well. This programme is aimed at preventing the conversion of riparian forest to woodland on a widespread scale as this conversion will significantly alter the character of Mapungubwe and have obvious biodiversity consequences (SANParks, 2008). As already mentioned, a long-term study conducted by O’Connor (2010b) found that the riparian forest in the Greefswald area, situated upstream from the experimental exclosure, has been already been transformed to woodland during the period from 1990 to 2007. The anticipation of more such conversions in Mapungubwe’s riparian vegetation due to expected hydrological changes (section 3.5) in the near future, emphasizes the importance of information gained during this study regarding the restoration of indigenous riparian tree species.

CHAPTER 4

MATERIAL AND METHODS

4.1 Introduction

This study was comprised of seedlings transplanted into the natural environment inside an experimental exclosure within the MNP (field trial) and seedlings cultivated under controlled conditions inside a greenhouse at the North-West University (NWU), Potchefstroom (greenhouse trial). The same species and enhancement treatments were evaluated in both trials. The experimental design, materials and methods used in each of the trials are discussed in this chapter.

4.2 Species and enhancement treatments

A total of seven indigenous tree species were selected for cultivation and transplantation in both the field- and greenhouse trials. Species selection was based on a study conducted by Götze (2002) in the MNP, historical aerial photography and results from the pilot study (Scholtz, 2008). As part of a complete phytosociological study of the MNP, Götze (2002) also evaluated restoration technologies in the different land-use areas of the park. He recommended ten specific indigenous tree species to be used for the restoration of the park's riparian woodlands. Based upon the survival rates of these ten species during the pilot project (Scholtz, 2008), as well as the availability of seedlings in the Rhodesdrift nursery of the MNP, only seven of these species were used for this study: *Acacia xanthophloea* (fever tree), *Berchemia discolor* (Klotzsch) Hemsl. (brown-Ivory), *Combretum imberbe* (leadwood), *Faidherbia albida* (ana tree), *Philenoptera violacea* (apple-leaf), *Salvadora australis* (narrow-leaved mustard tree), and *Xanthocercis zambesiaca* (nyala tree). Table 4.1 provides a useful summary of the general characteristics, habitat preferences, distribution, propagation and uses of the investigated species.

Table 4.1. The general characteristics and uses of the investigated species (Liu *et al*., 2008; Van Wyk & Van Wyk, 2007; Van Wyk *et al*., 2000; Venter & Venter, 2007).

Species and common name	Tree number	Growth form	Habitat/ecology	Distribution	Propagation	Use
Acacia xanthophloea (Fever tree)	189	Tree, up to 30 m	Riverbanks, low lying swampy areas, depressions and shallow pans	From Kwa-Zulu Natal in the south to the whole of Kenya	Fast grower (1-1.5 m per year);1000 seed weight=34 g; contains nitrogen fixing root nodules; frost sensitive	Browsed by game, timber, medicine, very popular for horticultural use
Berchemia discolor (Brown-ivory)	449	Tree, up to 20 m	Along riverbeds, low-altitude and well-drained bushveld soils	Throughout Africa, from Kwa-Zulu Natal in the south to Ethiopia in the north	Moderate grower (0.6-0.8 m per year); 1000 seed weight=303 g; drought- and frost sensitive	Browsed by game, furniture, food
**Combretum imberbe* (Leadwood)	539	Tree, up to 20 m	In alluvial soils along river and dry watercourses	From Kwa-Zulu Natal in the south to the whole of Tanzania	Moderate grower (0.5-1 m per year); 1000 seed weight=66.11 g; long lived (>1000 years); frost sensitive	Browsed by game, outstanding fuel wood, furniture, food, medicine
**Faidherbia albida* (Ana tree)	159	Tree, up to 30 m	Along rivers (deep alluvial soils), marshes, floodplains, riverbeds	Throughout Africa, from Kwa-Zulu Natal in the south to Israel in the north	Fast grower (1-2 m per year); 1000 seed weight=92 g; fairly drought resistant	Browsed extensively by stock/game, medicine, food
**Philenoptera violacea* (Apple-leaf)	238	Tree, up to 12 m	Along riverbeds, low-altitude bushveld and woodlands, sandy soils	From Kwa-Zulu Natal in the south to the Democratic Republic of the Congo and Tanzania	Moderately fast grower; 1000 seed weight=302.4 g; moderately frost sensitive; drought resistant	Browsed by stock/game, furniture, medicine
Salvadora australis (Narrow-leaved mustard tree)	621	Shrub/ tree, 1-4 m	Hot, arid bushveld soils; particularly on floodplains and brackish flats	From the northern parts of Limpopo and Mpumalanga in South Africa to Zimbabwe and Mosambique	Slow grower; 1000 seed weight=not available	Browsed by stock/game, medicine
Xanthocercis zambesiaca (Nyala berry)	241	Tree, up to 30 m	Hot, arid bushveld soils; deep alluvial soils near rivers, clay soils	From the northern parts of Limpopo in South Africa to Zambia and Malawi	Slow grower; 1000 seed weight=1265.9 g; frost- and drought sensitive	Browsed by game, food

*Protected tree in terms of South Africa’s National Forests Act (84/1998)

Four enhancement treatments were evaluated. The enhancement treatments used in both the field- and greenhouse trial, were mixed with soil from inside the experimental exclosure and prepared in the same ratios for both trials (Table 4.2). They consisted of the following: a mixture of indigenous AMF in the form of Mycoroot^{TM} Super Booster and compost (MYCCOM), indigenous AMF only (MYC), compost only (COM), and the control (CONTROL).

Table 4.2. The composition of the various enhancement treatments evaluated in both the field- and greenhouse trials (Enhancement treatment names are explained in Appendix A).

Treatment	Composition
MYCCOM	50% Compost, 50% soil and 10 ml Mycoroot^{TM} Super Booster
MYC	100% Soil and 10 ml Mycoroot^{TM} Super Booster
COM	50% Compost and 50% soil
CONTROL	100% Soil

The compost used in the enhancement treatments was woodchip compost obtained from Monontsha (Pty) Ltd[1] - a black empowerment company working in conjunction with the Impala Platinum Company located in Rustenburg, North West Province, South Africa (IMPLATS, 2007). The woodchips are a waste product created during the extraction of platinum and arises from underground blasting in the vicinity of wooden buttresses (van Rensburg & Morgenthal, 2004). The woodchips are taken from the mining operations and combined with sewage sludge from the company's water treatment works to form compost which is used in the rehabilitation of the slopes of tailings dams. Due to the blasting, the woodchips contain concentrations of nitrate and has been reported to be well suited for use in the re-establishment of vegetation after being composted (van Rensburg & Morgenthal, 2004). Various sources indicate that the use of woodchip compost have a range of beneficial effects such as supplying additional organic material to growth mediums (FFTC, 2009; Brady & Weil, 2008), ameliorating the physical properties of the growth medium by increasing its water holding capacity and stimulating biological activity (Brady &Weil, 2008), which is essential for nutrient recycling (Logan, 1992).

The indigenous AMF was added in the form of a product called Mycoroot^{TM} Super Booster developed by MYCOROOT^{TM} (Pty) Ltd[2]. This is a commercial product developed with Southern African isolates of AMF and specifically recommended for use during the transplantation of trees (MYCOROOT^{TM}, 2008). The production of Mycoroot^{TM} Super Booster is based upon a soil-less culture and is pathogen free. The fungal isolates alone and the carrier have no function as it is the combination of the host

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[1] Monontsha (Pty) Ltd, Tel: 014 569 1145, Website: www.implats.co.za/woodchips

[2] MYCOROOT^{TM} (Pty) Ltd, Tel: 046 603 8443, Website: www.mycoroot.com

plant and the fungus which forms the symbiotic relationship and enhances soil microbial functioning and plant growth (MYCOROOT^{TM}, 2008).

4.3 Experimental design

4.3.1 Natural environment (field trial)

The experimental exclosure was originally divided into four blocks (block A, B, C and D) during the pilot study. The layout was based upon discussions held with the various stakeholders involved in the project (especially previous land-owners), historical aerial photographs dating back to 1955 and the current adjacent vegetation within the park (Scholtz, 2008). Using the historical aerial photographs and adjacent vegetation as a “reference site” with regard to composition and distribution, seedlings of the same species that occurred in the area before deforestation, were transplanted into the allocated blocks. The same blocks, with the exception of block D, were used for transplantations during this follow-up study. The original block D was discarded as this area experienced the highest seedling mortality rates during the pilot trials (approximately 100%) (Scholtz, 2008). Blocks A, B and C were therefore retained and two new blocks, namely a Nursing block (the “new” block D) and a Nursing Control block (block E), were added to the exclosure layout during this follow-up study. The new blocks were used to investigate the “nursing” effect that established *Acacia tortilis* trees might have on transplanted seedlings (Fig. 3.3). Block D enclosed an area with sparsely distributed *A. tortilis* trees of which the canopies did not touch, thereby minimising the possible effect of competition for light and nutrients on seedling establishment and growth. Block E was set up in an open canopy area adjacent to block D in order to evaluate the effect of “nursing” within the same soil type.

4.3.2 Controlled environment (greenhouse trial)

Pot trials were conducted in a greenhouse at the NWU, Potchefstroom, during the warmer months (January to May). Due to a lack in a heating system at the university’s greenhouse, the trial was moved to a nearby greenhouse belonging to the Agricultural Research Council (ARC) during the colder months (June to September). Nocturnal temperature in the ARC’s greenhouse was controlled by a hot-air blower to keep temperature at above 5 ºC. Both greenhouses received natural light (40% shade-cloth in the NWU greenhouse and transparent roof panels in the ARC greenhouse) and no other artificial modifications were made. The potted seedlings were well-watered throughout the study period (January to September 2009), with the exception of a two-week drought simulation period initiated in June 2009, during which the seedlings did not receive any water. Regular watering continued thereafter. The drought simulation period was carried out to monitor the effect of drought on the various species’ survival, growth and physiological performance. Potted seedlings were kept clear from weeds and other vegetation, and pesticide was applied when necessary.

4.4 Seedling cultivation and transplantation

4.4.1 Natural environment (field trial)

The seedlings transplanted into the experimental exclosure were germinated from seed collected by field rangers in the western section of the MNP. The seed collection method differed from species to species. The seeds were either harvested from the tree itself or collected from the ground underneath the tree. For certain species, seedlings were also removed from underneath the big trees and transplanted into nursery bags. The different collection methods used for each species are listed in Table 4.3. The seeds were collected in bulk and subsequently stored at room temperature (15-25 ºC) until planting. The seedlings were cultivated inside the Rhodesdrift nursery which is situated approximately 5 km to the west of the experimental exclosure (Latitude: 22º 12’ 10.2’’ South and Longitude: 29º 10’ 28.8’’ East). The nursery was covered with a 20% shade cloth and the seeds were sown into two big seedbeds (13 m long x 1 m wide x 0.3 m high) which were filled with a growth medium consisting of a 1:1 ratio of compost (decomposed humus collected in the vicinity of the nursery) and river sand.

Table 4.3. The different seed collection methods used for each of the seven investigated species.

Species	Collection method
Acacia xanthophloeae	Harvested from the ground; Naturally regenerating seedlings removed and transplanted into nursery bags
Berchemia discolor	Harvested from the ground
Combretum imberbe	Harvested from the ground; Naturally regenerating seedlings removed and transplanted into nursery bags
Faidherbia albida	Harvested from the ground
Philenoptera violacea	Harvested from the ground
Salvadora australis	Harvested from the trees; Naturally regenerating seedlings removed and transplanted into nursery bags
Xanthocercis zambesiaca	Harvested from the ground; Naturally regenerating seedlings removed and transplanted into nursery bags

Seeds were sown directly into the seedbeds at a depth of approximately 10 mm and drip irrigation was used to keep the growth medium wet. After about two weeks the seedlings were transplanted into 10 L nursery bags. These seedlings were drip irrigated daily in the summer months and every second day in the winter months. Pre-sowing treatments such as scarification or soaking were not applied to the seeds germinated in the Rhodesdrift nursery. Some of these treatments were, however, performed on seeds germinated during the greenhouse trials for this study – refer to section 4.4.2.

Two weeks before transplantation, the seedlings were subjected to a “hardening” process. This process involved the removal of the seedlings from inside the Rhodesdrift nursery to outside in full sun exposure, whilst watering previously well-watered seedlings only once a week (Fig. 4.1). The "hardening" process is a widely used practice in nurseries prior to transplantation and has been shown to increase seedling establishment by reducing the "shock" experienced by seedlings when having to adapt to the new environmental conditions (Oliet *et al.*, 2005; Hopkins & Hüner, 2002). The hardening of seedlings therefore entails the gradual exposure of seedlings cultivated in favourable greenhouse conditions to the harsh environmental conditions expected in the field.

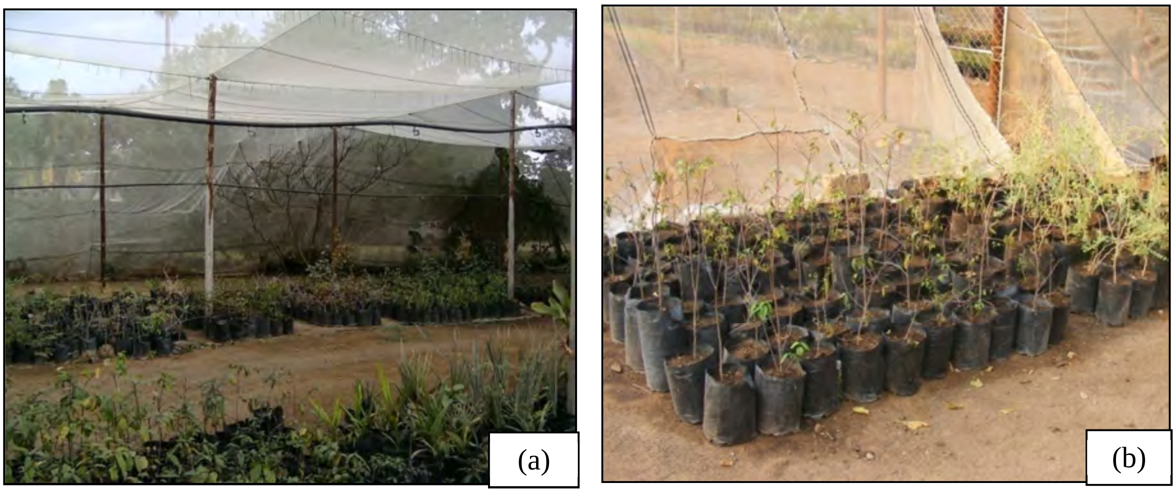

Figure 4.1. (a) Seedlings inside the Rhodesdrift nursery in September 2008, and (b) seedlings undergoing a "hardening" process outside the nursery two weeks before transplantation in October 2008.

In October 2008 a total of 258 seedlings were transplanted into the experimental exclosure inside the MNP. Most of the seedlings were approximately eighteen months old during transplantation. A mixture of five species (*B. discolor*, *C. imbere*, *F. albida*, *P. violacea* and *X. zambesiaca*) were transplanted into block A, with only *S. australis* transplanted into block B and a mixture of *A. xanthophloea* and *C. imberbe* transplanted into block C (Fig. 3.3). In blocks D and E a mixture of four species (*B. discolor*, *C. imberbe*, *F. albida* and *X. zambesiaca*) were transplanted. The type and amount of seedlings transplanted into each block, as well as relevant enhancement treatments added, are presented in Table 4.4

During the pilot study "activity lines" and "non-activity lines" were identified inside the various blocks, and the subsequent results of this study indicated that a combination of watering and transplantation on "activity lines" ensured the highest survival and establishment of transplanted seedlings (Scholtz, 2008). During this follow-up study, all the seedlings were therefore planted into pits dug on so called "activity-lines" as pointed out during the pilot-study (Scholtz, 2008), with the exception of the nursing blocks. Eight new pits were dug around each of the 16 selected established *A. tortilis* trees in block D. Two pits were dug at each aspect (N, S, E and W) around the tree, just underneath the tree's dripline. In block D new pits were also dug with a spacing of 1.5 m between pits within rows, and 2 m between rows. The pits for blocks D and E were thus not dug on "activity lines". A map of the layout for transplantations inside each block is available in Appendix C.

Table 4.4. The type and amount of seedlings transplanted per enhancement treatment into the experimental exclosure in October 2008 (see Appendix A for enhancement treatment abbreviations).

		Amount of seedlings per enhancement treatment				
		MYCCOM	MYC	COM	CONTROL	Total
Block A	*Berchemia discolor*	2	2	2	2	8
	Combretum imberbe	3	2	3	2	10
	Faidherbia albida	3	2	3	2	10
	Philenoptera violacea	3	2	3	2	10
	Xanthocercis zambesiaca	3	2	3	2	10
Block B	*Salvadora australis*	4	5	4	4	17
Block C	*Acacia xanthophloea*	4	3	3	4	14
	Combretum imberbe	4	3	4	4	15
Block D	*Berchemia discolor*	8	8	8	8	32
	Combretum imberbe	8	8	8	8	32
	Faidherbia albida	8	8	8	8	32
	Xanthocercis zambesiaca	8	8	8	8	32
Block E	*Berchemia discolor*	2	3	2	2	9
	Combretum imberbe	2	3	2	2	9
	Faidherbia albida	2	3	2	2	9
	Xanthocercis zambesiaca	2	3	2	2	9
Total amount of seedlings transplanted						258

All the pits were square and at least 50 cm wide x 50 cm long x 50 cm deep (a total volume of 0.125 m^3). Complying with restoration recommendations made during the pilot study (Scholtz, 2008), the following transplantation procedure was followed:

- Firstly, the pits were half filled with loose soil and wetted with 20 L of water. This step was aimed at creating a growth medium which will be easily penetrated by the roots of the growing seedlings.
- The plastic nursery bags were carefully removed and the seedlings positioned into the pit ensuring that the stem flares - which is situated where the roots and the stem bark meets, were exposed to the air after the pits were filled with the growth medium.
- The pits were then wetted with 20 L water and filled with the different enhancement treatments ensuring that the Mycoroot^{TM} Super Booster granules came into contact with the roots of the seedlings.

- After the pits were filled, the soil around the seedlings were compacted and wetted with 25 L water again.
- A layer of mulch consisting of dead plant material (mostly grass litter) collected inside the exclosure were then applied around the seedlings with the aim of regulating soil temperature and limiting evaporation.
- Seedlings were then labelled according to species and specific treatment added. Colour codes were added to the labels for easy identification in the field (Yellow = Mycoroot^{TM} Super Booster; red = compost) (Fig. 4.2d).

All the transplanted seedlings were watered once weekly for a total of ten weeks after transplantation. All seedlings received 20 L of water once weekly for the first two weeks after which the amount of water was reduced with 5 L every two weeks until the seedlings received no water at all. The seedlings therefore received no artificially added water from December 2008 onward.

One year later, in October 2009, another batch of seedlings (356 in total) were transplanted into the experimental exclosure (a map of the layout of these transplantations is available in Appendix C). At that time the preliminary results from the greenhouse trial indicated that the combination of AMF and compost as an enhancement treatment had the most positive influence on the growth and physiological performance of seedlings. The MYCCOM treatment was therefore added to all the seedlings transplanted in 2009. The majority of the seedlings were transplanted into the north-eastern corner of the experimental exclosure around sparsely distributed *A. tortilis* trees (Appendix C). Due to time constraints and limited funding, this dissertation will only discuss data collected from seedlings which were planted during the pilot study in 2006 and the follow-up study 2008. SANParks will continue with the long-term monitoring and analysis of the 2009 transplanted seedlings.

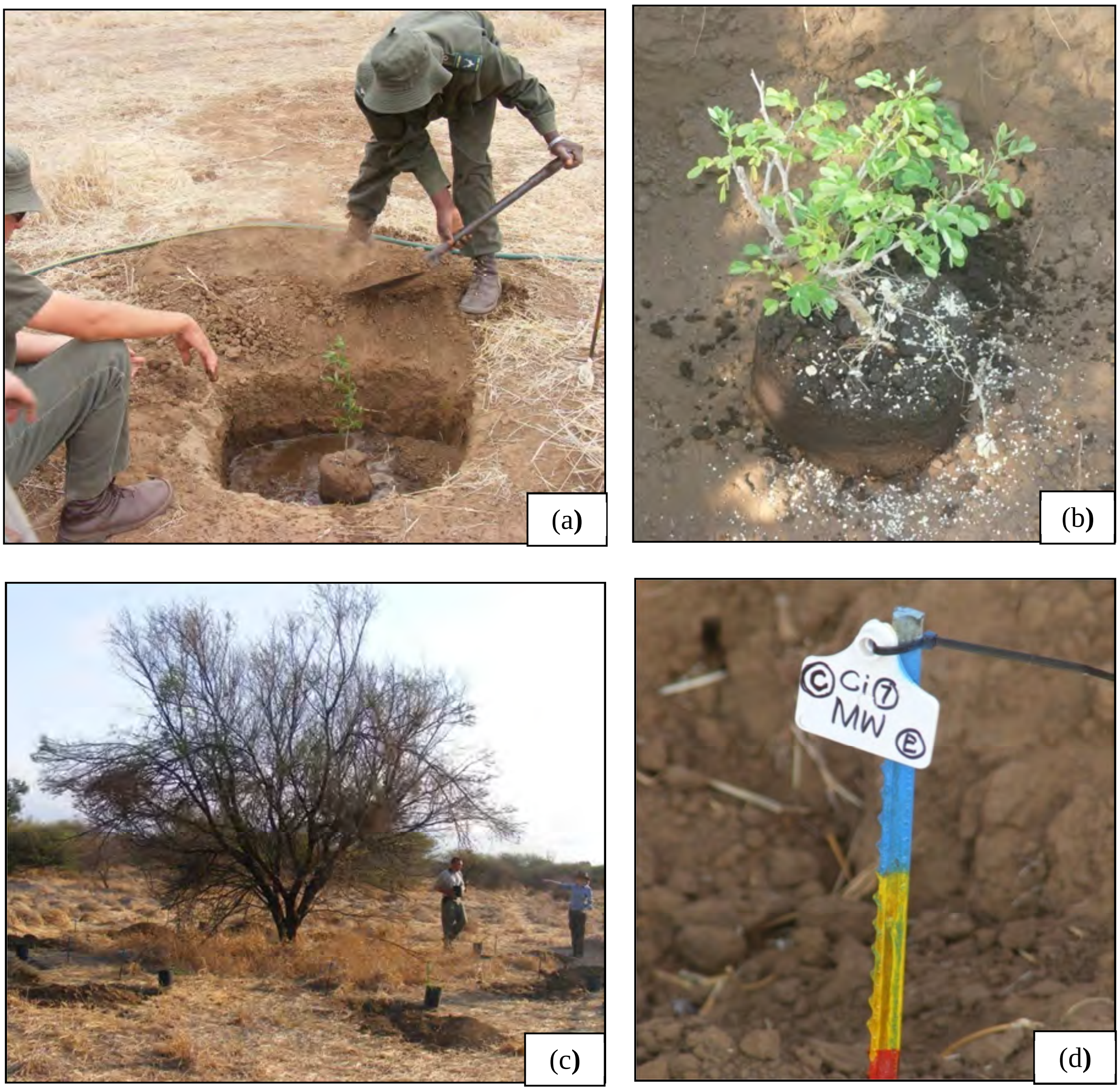

Figure 4.2. (a) A pit with a *Xanthocercis zambesiaca* seedling positioned inside after initial watering; (b) a seedling inside a pit with the Mycoroot^{TM} Super Booster granules clearly visible; (c) an established *Acacia tortilis* tree inside block D where seedlings were transplanted into pits dug around the tree; and (d) an iron dropper with a colour-coded label to indicate species and enhancement treatment applied.

4.4.2 Controlled environment (greenhouse trial)

A germination trial was initiated in the NWU greenhouse in January 2009 for five of the seven species used for transplantation during the field trial (*B. discolor*, *C. imberbe*, *F. albida*, *P. violacea* and *X. zambesiaca*). Between five and six seeds (depending on seed size) were planted into 25 L pots filled with the same enhancement treatments evaluated in the field. The soil used for the pot trials were collected from the different blocks inside the experimental exclosure in the MNP. The soil of the various blocks inside the exclosure consisted of three different soil textural classes, namely (1) a sandy loam, (2) a clay loam and (3) a loam (see section 5.2.4). The pot-trials inside the greenhouse were divided into blocks representing these three textural classes and three different indigenous tree species were germinated in block. The type of species germinated per block was chosen in accordance to the species transplanted in the representative blocks inside the experimental exclosure (Fig. 4.4.1). Due to a shortage in the availability of seeds, certain species such as *A. xanthophloea* and *S. australis* could not be germinated. Five different species were therefore used for the germination trial.

The seeds sown into the pots were treated with pre-sowing treatments according to guidelines by Venter and Venter (2007) and Mr Willem Basson[3], an employee of the NWU with years of experience in the cultivation of indigenous trees. All of the seeds, with the exception of *C. imberbe*, were collected in the MNP. The *C. imberbe* seeds were collected in the northern Limpopo area close to the MNP by members of the South African Department of Water Affairs and Forestry (DWAF).

Four repetitions per species for each enhancement and pre-sowing treatment in each of the three soil textural classes were therefore germinated during the greenhouse trial. The pre-sowing treatments that were performed on the different seeds, included:

- *Berchemia discolor* (brown-Ivory): Healthy seeds were soaked in warm water for 12 hours after which six seeds were planted per pot and covered with a 1 cm layer of growth medium. According to Venter and Venter (2007) a high germination rate of 80 - 100% were to be expected.
- *Combretum imberbe* (leadwood): The seeds of the leadwood are contained within a four-winged fruit which colours yellowish-brown when dried out. For the germination trial, six seeds were planted per pot, where half of the seeds were carefully removed from the fruit (exposed) and then planted, and the other half were planted still inside the fruit. All the seeds, whether exposed or still inside the fruit, were soaked in warm water for 12 hours before planting. Seeds/fruit were covered with a 1 cm layer of growth medium after planting. A relatively low germination rate was to be expected (Venter & Venter, 2007).
- *Faidherbia albida* (ana tree): Healthy seeds were soaked in warm water for 12 hours after which six seeds were planted per pot. Before soaking, however, half of the seeds were mechanically scarified using a set of small pliers, whilst the other half were left unscarified. After planting, the seeds were covered with a 1 cm layer of growth medium. A high germination rate was to be expected (Venter & Venter, 2007).
- *Philenoptera violacea* (apple-leaf): Healthy seeds were soaked in warm water for 12 hours after which six seeds were planted per pot. Before soaking, however, half of the seeds were mechanically scarified using a set of small pliers, whilst the other half were left unscarified. After planting, the seeds were covered with a 1 cm layer of growth medium. A relatively high germination rate was expected for this species (Venter & Venter, 2007).
- *Xanthocercis zambesiaca* (nyala tree): Very few information regarding the cultivation of this species was available. Healthy seeds were soaked in warm water for 12 hours after which

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[3] Willem Basson, Tel: 018 299 2255, E-mail: willem.basson@nwu.ac.za

only five seeds were planted per pot due to the large seed size. Seeds were then covered with a 1 cm layer of growth medium.

The germination experiments were carried out for a total of 90 days (January to March 2009). The amount of germinated seeds were counted and recorded every 10 days for the first three months, after which the seedlings inside each pot were thinned out leaving only a single seedling per pot in order to analyse the growth and physiological performance throughout the rest of the study period.

4.5 Data collection and analysis

4.5.1 Vegetation sampling

The same morphological and physiological measurements were performed on seedlings transplanted into the field and seedlings cultivated in the greenhouse. All the vegetation measurements were non-destructive in order to minimise any potential negative impact upon seedling growth and establishment.

Figure 4.3 presents a time-line of the various monitoring and transplantation events taking place in the field during this follow-up study. During the field trial morphological measurements were conducted on all the seedlings still alive from the 2008 transplantation, as well as on seedlings still alive from the pilot transplantation in 2006. Only *C. imberbe* and *S. australis* were measured from the 2006 transplantation. The decision was made to discontinue future monitoring of *A. tortilis*, as literature and field observations clearly states that this is a pioneer species, naturally colonizing the old lands (Götze, 2002). In March 2008 the experimental exclosure was visited for the first time during this follow-up study and the surviving seedlings from the pilot transplantation were labelled and monitored throughout 2008/2009. New seedlings with the addition of the various enhancement treatments were transplanted in October 2008. The first morphological monitoring of these seedlings was conducted in November 2008. Morphological monitoring commenced again in March 2009 just after the rainy season (Fig. 4.3). During this time physiological measurements were also performed, but only on the seedlings transplanted in 2008. During May 2009 the mortality of the 2008 transplanted seedlings was determined. In June 2009 physiological measurements were conducted on the 2008 transplanted seedlings. Finally, in September 2009, both morphological measurements were conducted on seedlings transplanted during both transplantations, with physiological monitoring conducted again on only the 2008 transplanted seedlings. The establishment, growth and physiological performance of the 2008 transplanted seedlings were thus determined for one complete growth year (October 2008 to September 2009). The establishment and growth of the 2006 transplanted seedlings were determined over a period of almost three and a half years (April 2006 to September 2009).

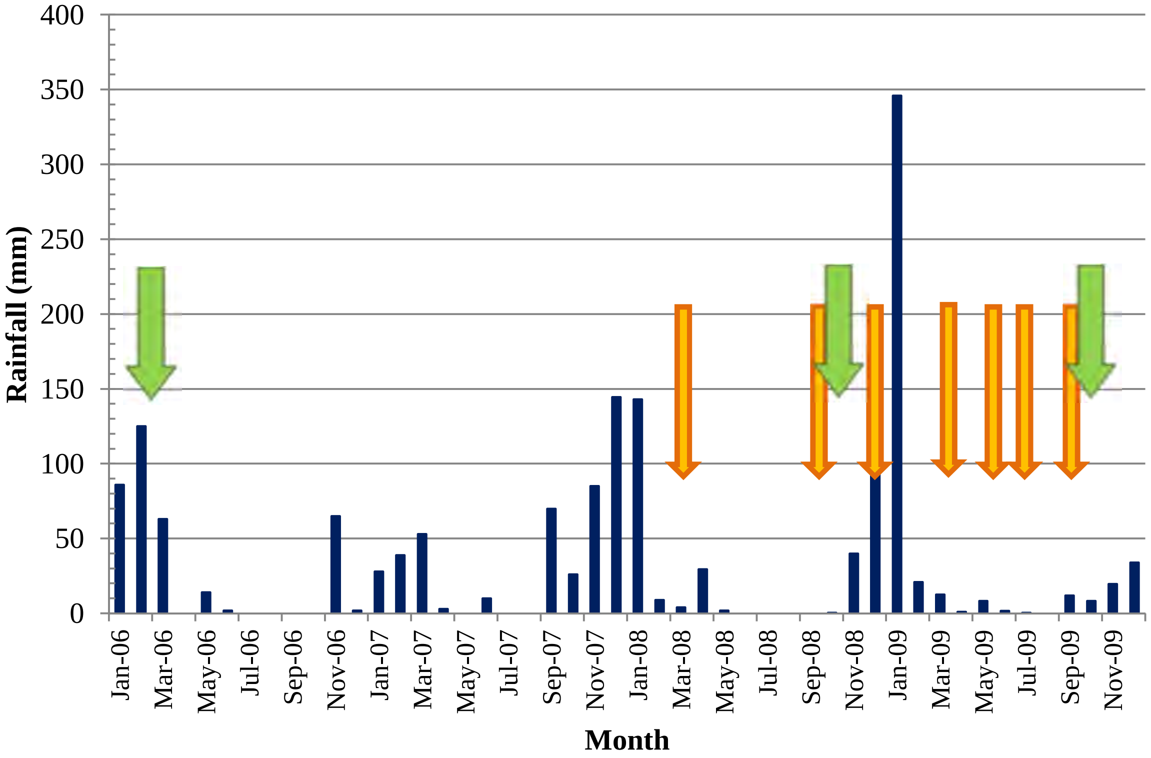

Figure 4.3. Mean monthly rainfall (mm) of the study area from January 2008 to December 2009, also indicating the various monitoring and transplantation events. The green arrows indicate the three transplantation events (2006, 2008 and 2009) and the yellow arrows indicate the various data collection occasions during this follow-up study.

Morphological and physiological monitoring of measurable seedlings in the greenhouse took place once monthly from April to September 2009.

4.5.1.1 Morphological measurements

Morphological measurements were conducted in order to record survivorship and growth of seedlings in the field and greenhouse trials. During each survey the seedling, whether dead or alive, was recorded by location, species and enhancement treatment added.

An array of articles has been published with regard to the different parameters to be used for monitoring tree growth (McIver & Ottmar, 2007; McElhinny *et al.*, 2006; Mengistu *et al.*, 2005; Argaw *et al.*, 1999; Geldenhuys & Von dem Bussche, 1997). It appears that the two predominantly used parameters are stem diameter and height of the tree. Three parameters as recommended by Scholtz (2008) were measured on seedlings in both trials: (1) stem diameter at the base, (2) stem diameter 30 cm from the base, and (3) the height of the seedling in its natural growth form (Fig. 4.4).

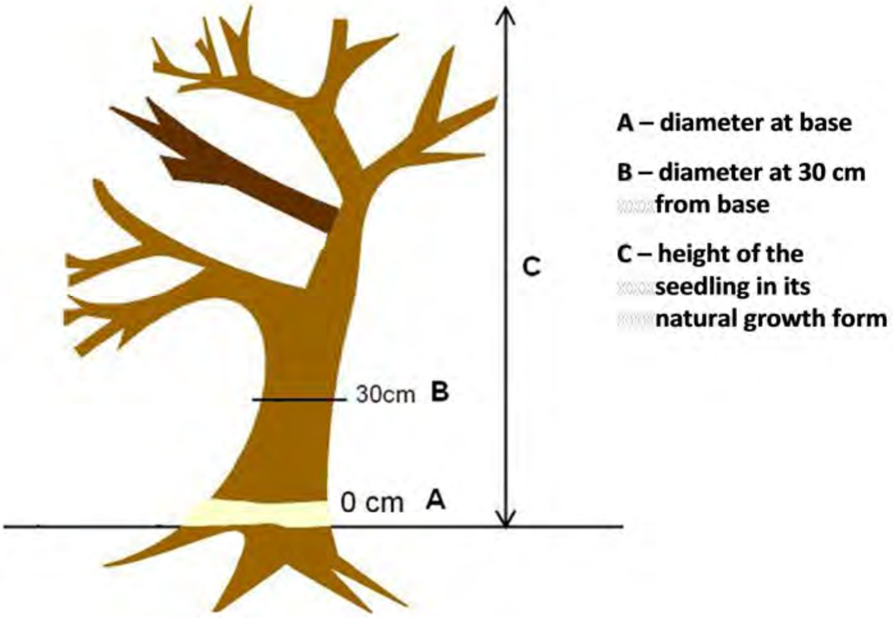

Figure 4.4. The three parameters measured during morphological monitoring of seedlings in the field- and greenhouse trials (adapted from Scholtz, 2008).

The stem diameter measurements were conducted with a digital calliper (mm). During the "stem diameter at base" measurements, the calliper was positioned on a strip of white paint applied at root collar height of each seedling to ensure the accuracy of morphological measurements (Fig. 4.5a). Aluminium measuring sticks (cm) were used for the height measurements (Fig. 4.5b). Seedling predation/herbivory was recorded by visual observation.

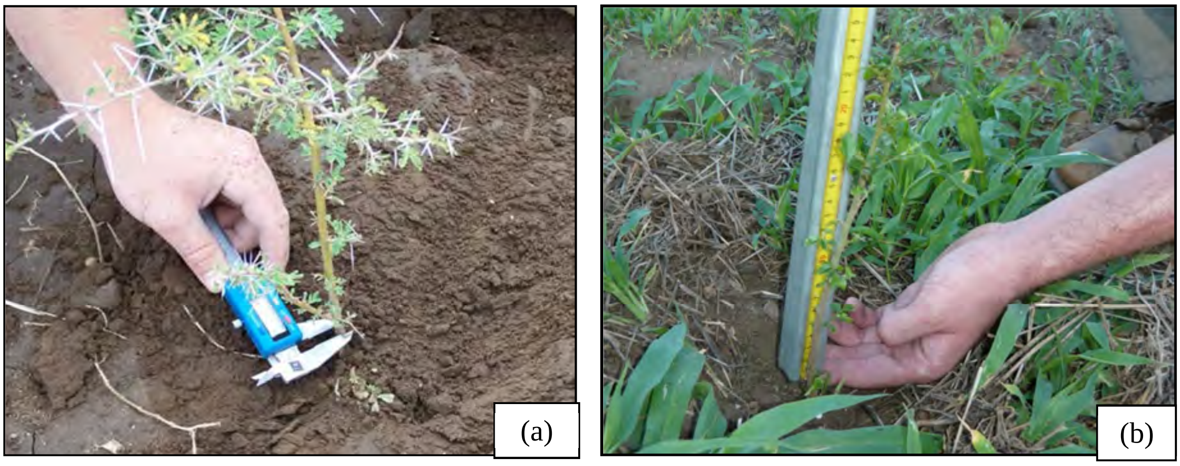

Figure 4.5. (a) The digital calliper (mm) and (b) aluminium measuring sticks (cm) used for morphological monitoring in both the field- and greenhouse trials.

The morphological data was organised in basic Microsoft Excel spreadsheets (Appendix E). Basic descriptive statistics regarding survival and growth were performed within the Microsoft Excel package. Two-way analysis of variance (ANOVA) was used to test for variations in mean growth between species and enhancement treatments using SAS statistical package (SAS Institute Inc., Cary, NC) – this was only determined for seedlings transplanted in 2008. Significant differences ($p = 0.05$) of means were determined using the Kruskal-Wallis non-parametric test.

4.5.1.2 Physiological measurements

Physiological measurements were conducted only on seedlings to which enhancement treatments were added. A non-invasive technique was employed to measure the effect of the various enhancement treatments on photosynthesis via chlorophyll fluorescence of the seedlings (Schulze *et al.*, 2005; Lichtenthaler & Miehé, 1997). Strasser *et al.* (1995) states that the analysis of changes in chlorophyll *a* fluorescence kinetics provides detailed information on the structure and function of the photosynthetic apparatus – especially Photosystem II (PSII). By quantifying the energy flow through PSII, stress-induced alterations caused by several environmental influences in the biophysical parameters of a plant, can be observed (Strauss *et al.*, 2006).

Chlorophyll fluorescence techniques are an easy tool to use for screening the photosynthetic vitality of plants (Strasser *et al.*, 1995). This technique was used in this study to determine whether there were any stress-induced alterations in the photosynthetic apparatus of the transplanted seedlings. By quantifying these alterations, general observations could be made regarding the physiological performance of the various tree species in relation to the different enhancement treatments applied. This quantitative analysis of the fluorescence transient, called the JIP-test, can be used to explain the stepwise flow of energy through PSII at the reaction centre (Strasser & Strasser, 1995). The JIP-test is named after the basic steps in the fluorescence transient when plotted on a logarithmic scale. The fluorescence rise exhibits the steps J and I between initial O (F_O) and maximum P (F_M) levels. Different environmental conditions cause changes in the shape of the O-J-I-P fluorescence transient, making it suitable for in vivo studies of the behaviour of PSII.

Among the constellation of the JIP expressions, one of the most sensitive namely the performance index (PI_{ABS}) (Hermans *et al.*, 2003), was used during this study. This parameter encompasses fluorescence transient changes associated with changes in antenna conformation and energy fluctuations. It is a multi-parametric expression that combines the three functional steps of photosynthetic activity by a PSII reaction centre complex, i.e. light energy absorption, trapping of excitation energy and the conversion of trapped energy to electron transport.

In order to characterise the investigated species' photosynthetic vitality, fast fluorescence transients were recorded on the leaves of all seedlings from which sufficient data could be obtained (at least 3 leaves had to be available for a measurement to be made). All the chlorophyll *a* fluorescence measurements were conducted on dark-adapted leaves by using a portable fluorimeter (Handy Plant Efficiency Analyser (PEA), built by Hansatech Instruments Ltd. King's Lynn Norfolk, UK) with high time resolution (µs). During the measurement, the leaf sample was shielded from ambient light by a clip system to reach a dark adapted state (at least 60 min adaptation to darkness) (Fig. 4.6). The leaf-clip itself has a small shutter plate which is closed over the leaf when the clip is attached allowing sufficient dark adaptation to take place. After dark adaptation the measuring head of the Handy PEA was fitted directly on the leaf clip and the fluorescence measurement was initiated. Thereby continuous light excitation (at 3000 µmol/m^{2}s) was provided by an array of three light-emitting diodes focused on the leaf surface to provide homogeneous irradiation over a 4 mm diameter leaf surface (the so-called cross-section). The single strong 1 s light pulse (600 W m^{-2}) had an excitation intensity sufficient to ensure closure of all PSII reaction centres. The chlorophyll *a* fluorescence emission induced by the strong light pulses was measured and digitised between 10 µs and 1 s by the instrument.

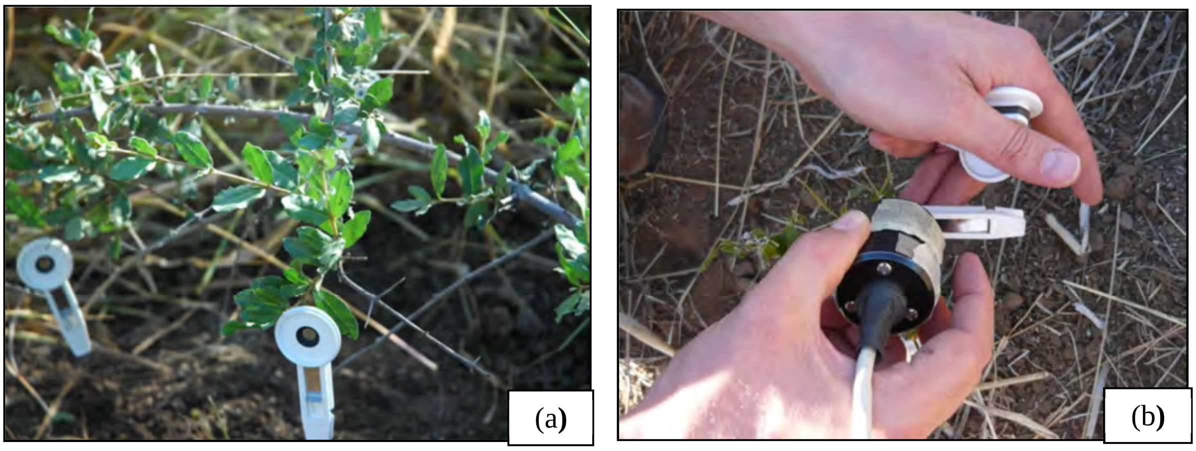

Figure 4.6. (a) Clips attached to the leaves of measured seedlings, and (b) chlorophyll *a* fluorescence measurements taken with the Handy PEA.

The fluorescence data was processed using Biolyzer software (Maldonado-Rodriguez, 2002). The difference between the mean performance index (PI_{ABS}) of seedlings planted into the various enhancement treatments for each species was determined with the Student's *t*-test

4.5.2 Soil sampling

4.5.2.1 Soil chemical analysis

Various soil samples were collected and analysed in order to determine the soil physical and chemical characteristics of the experimental exclosure. For this follow-up study samples were collected inside the exclosure and sent for analysis at the NWU's soil analysis laboratory, Eco Analytica[4]. In September 2008 composite soil samples consisting of three individual samples were collected within each block (blocks A, B, C, D and E) inside the experimental exclosure. A composite sample was also collected in the remnant riparian forest area about 100 m to the north of the exclosure in order to compare the soil structure and properties of the experimental exclosure (degraded old lands) to that of the remnant forest area (representing the reference site).

A full chemical analysis was carried out on the soil samples including a 1:2 water extraction, pH (KCl) and the pH (water) of the soil, as well as the presence of macro- and micro-elements and heavy metals. The particle size (percent sand, silt, and clay), nutritional value, and amount of exchangeable cations of the soil were also determined. The soil textural classes of the various samples were determined using the well known textural class triangle (Appendix B). The texture of a soil is not readily subject to change, and is therefore considered as a basic property of a soil (Brady & Weil, 2008). The pH was measured using a 1:2 soil/water suspension extract on a mass basis. This is not the 'true' pH of the soil, but the most useful in soil-plant relations. It is an indication of the readily available cations, and therefore, an understanding of the short-term functional processes in the soil such as leaching of mobile cations (Gobat *et al.*, 2004). The electrical conductivity (EC) was measured in a saturated extract of de-ionized water. The EC is an indication of the total dissolved solids in the extract and therefore the dissolved solids in the soil. The EC is a measure of salinity of the soil, and the higher the EC values the more saline the soil (Brady & Weil, 2008). The sodium adsorption ratio (SAR) was also calculated to determine salinity, as the SAR gives information on the comparative concentrations of Na^+, Ca^{2+}, and Mg^{2+} in the soil solution (Brady & Weil, 2008). The various symbols and ionic names, as well as other chemical variables measured, are listed in Appendix B.

Multivariate analysis was used to analyse the soil data. Multivariate analysis is the branch of mathematics which deals with the examination of numerous variables simultaneously: "The need for multivariate analyses arises whenever more than one characteristic is measured on a number of individuals, and relationships among the characteristics make it necessary for them to be studied simultaneously" (Krzanowski, 1972). The soil data obtained from the analytical laboratory was analyzed using the CANOCO-package in which the principal component analysis (PCA), the detrended correspondence analysis (DCA) and the canonical correspondence analysis (CCA) ordinations could be used, depending on the requirements of data set. These multivariate data analyses

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[4] EcoAnalytica Laboratories, Potchefstroom, Tel: 018 293 3900

techniques are used to “synthesise environmental data and to produce an ordination of quadrats based on environmental variables alone” (Kent & Coker, 1992).

4.5.2.2 Soil water content

In addition to the physical and chemical analysis, the soil water content (Θ_m) of the soil inside each block was also determined. The Θ_m was determined in order to establish whether the “nursing” *A. tortilis* trees created favourable micro-habitats which were more conducive to seedling establishment and growth compared to open areas with no tree canopy present. The Gravimetric method, a destructive and direct method, was used to determine the Θ_m (Brady & Weil, 2008). This method determines the water associated with a given mass of dry soil solids. To determine the ratio between the water and dry solids, the Θ_m had to be determined by drying the soil to a constant weight and measuring the soil sample mass before and after drying. The Θ_m is the difference between weights of the wet and oven dried samples. The samples for each block were weighed in Petri dishes and then dried in an oven at 88 ºC overnight. After drying the samples were weighed once more and again dried overnight at the same temperature. This process was repeated five time until a constant weight for the soil sample was achieved.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Introduction

This chapter presents all the relevant data in a similar sequence as presented in the previous chapter (Chapter 4 – Material and methods). The morphological and physiological aspects of the seedlings are discussed separately for the natural environment (field trials) and the controlled environment (greenhouse trials) where after selective data are collectively compared between the two trial environments. In this chapter various analytical techniques were implemented for the respective data are discussed in the following order and outline:

- Natural environment (field trials): survival, growth, physiology and soil.
- Controlled environment (greenhouse trials): germination, survival, growth and physiology.

5.2 Natural environment (field trial)

5.2.1 Survival

Survival data were obtained from a total of 436 seedlings (including seedlings transplanted during the pilot study conducted by Scholtz, 2008) of seven species planted into the five blocks within the experimental exclosure (see Fig. 3.3 for a layout of the experimental exclosure). The survival rate of the seedlings in all the blocks was surveyed from 2006 until 2009. The monitoring was conducted on seedlings transplanted during the pilot study in 2006, as well as on seedlings more recently transplanted in 2008. The survival rates of seedlings for the two transplantations are discussed separately.

5.2.1.1 Seedlings transplanted in 2008

The survival of a total of 258 seedlings transplanted in 2008 was noted on four occasions (refer to Fig. 4.4 for a detailed timeline of the monitoring events):

- November 2008 (a month after planting),
- March 2009 (just after the rainy season),
- May 2009, and
- September 2009 (at the end of the dry season).

Refer to Figure 3.3 for the layout of the experimental exclosure and Table 4.3 for the list of species transplanted. The total survival rate measured in September 2009 for all the species evaluated within all five blocks were 55.8%, which means that a total of 144 of the 258 seedlings transplanted were still alive one year after transplantation (Table 5.1). *B. discolor* (75.5%) and *S. australis* (70.6%) had the highest total survival rates, with *F. albida* (17.6%) scoring the lowest when all blocks in which species were planted are compared (Table 5.1). Different combinations of species were planted into each block. Within each block, the survival rates varied according to species, with block A and B having the highest average survival rate of >70%. Block E showed the highest mortalities with an average survival rate of only 33.4%. *B. discolor* had the highest survival rate in block D (78.1%) and E (66.7%), *A. xanthophloea* had the highest survival rate in block C (64.3%), and *X. zambesiaca* the highest survival rate in block A (90.0%)(Table 5.1). *S. australis* was the only species planted into block B with a relatively high survival rate of 70.6%.

Table 5.1. The total survival rate (%) of seven species, irrespective of enhancement treatment, transplanted into the various blocks allocated within the experimental exclosure and measured from November 2008 to September 2009.

	Survival (%)					
Species	**Block A**	**Block B**	**Block C**	**Block D**	**Block E**	**Total**
Acacia xanthophloea	-	-	64.3	-	-	64.3
Berchemia discolor	75.0	-	-	78.1	66.7	75.5
Combretum imberbe	50.0	-	56.3	46.9	55.6	50.7
Faidherbia albida	70.0	-	-	6.3	0.0	17.6
Philenoptera violacea	66.7	-	-	-	-	66.7
Salvadora australis	-	70.6	-	-	-	70.6
Xanthocercis zambesiaca	90.0	-	--	40.6	11.1	45.1
Average survival (%)	**70.4**	**70.6**	**60.3**	**43.0**	**33.4**	**55.8**

(- indicates that seedlings of the specific species were not transplanted into that block)

One of the main objectives of this study was to determine the effect of various enhancement treatments on the survival of transplanted seedlings (refer to Chapter 1 for the study's specific objectives). Seedlings were planted into four treatments, including the control, during the 2008 transplantation event (refer to Table 4.4). These treatments consisted of (1) Mycorrhiza and compost (MYCCOM), (2) Mycorrhiza only (MYC), (3) Compost only (COM), and (4) the Control. In Figure 5.1 the survival rates as determined in September 2009 for each of the seven species are consolidated for all the blocks inside the exclosure, and compared across treatments for each species. It seemed that the effect of the treatments on survival was rather species-dependent as no general trend for all the species was observed. The compost treatment resulted in the highest survival of *A. xanthophloea* (100%) and *F. albida* (28%) seedlings, whilst the mycorrhiza-only treatment resulted in the highest survival of *B. discolor* (84%) and *X. zambesiaca* (60%) seedlings. Both the mycorrhiza-only and control treatments resulted in the highest survival of *C. imberbe* (56%) and *P. violacea* (100%)

seedlings. For *S. australis*, both the mycorrhiza-compost and compost-only treatment resulted in the highest survival rate (80%). In general the compost containing treatments were beneficial with regard to survival for four species, namely *A. xanthophloea*, *F. albida*, *S. australis* and *X. zambesiaca*. The control treatment which consisted only of soil from inside the exclosure, resulted in higher seedling survival rates compared to the mycorrhiza and compost treatments (MYCCOM) in three species, namely *B. discolor*, *C. imberbe*, and *P. violacea*.

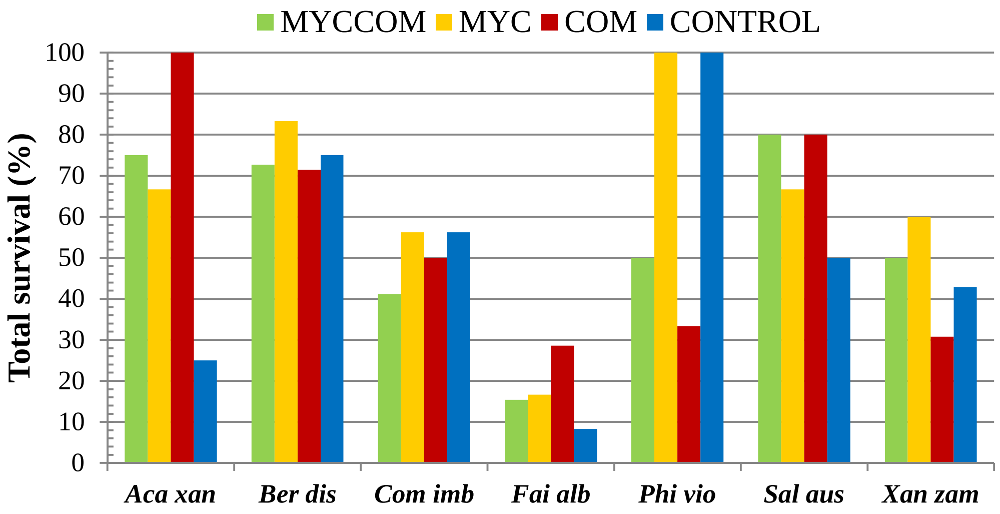

Figure 5.1. The total survival rate (%) of seven species planted into four enhancement treatments inside the experimental exclosure, and measured over one year (November 2008 - October 2009). The enhancement treatment names and abbreviations are explained in Appendix A.

Data from the pilot study carried out by Scholtz (2008) showed that the survival rate of transplanted seedlings for all species declined over time. The pilot study attributed this decline in survival to biotic and abiotic factors, such as drought stress, predation and grass-seedling competition. Intensive radiation on the ground (especially true in a semi-arid environment such as Mapungubwe) and competition by grasses are well known to hinder the initial phase of seedling survival in reforestation projects. Data from the more recent 2008 transplantation also showed a decline in seedlings survival over time, with certain species such as *F. albida* experiencing much higher mortalities compared to the other species (Fig. 5.1). The trends in the cumulative survival rate of each species transplanted in 2008 are presented in Figure 5.2, also differentiating between the various enhancement treatments. In general Figure 5.2 shows that there were a decline in survival for all the species and treatments over time. The trend in cumulative survival of each species transplanted in 2008 will now be discussed separately.

Acacia xanthophloea

A. xanthophloea seedlings planted in the mycorrhiza-compost and control treatments already showed mortalities one month after transplantation. The survival rate of seedlings in the control showed a steep decline in survival throughout the study period (Fig. 5.2a). The survival rate of seedlings in the mycorrhiza-compost and compost-only treatment remained stable at 75% and 100% respectively, throughout the study period. Seedlings in the mycorrhiza-only treatment showed a steep decline in survival from March to May 2009. The total survival rate for *A. xanthophloea* was 64.3% and the compost containing treatments resulted in the highest and most stable survival rates (Fig. 5.1-2 & Table 5.1).

Berchemia discolor

All the *B. discolor* seedlings were still alive when monitored for the first time in November 2008, one month after transplantation. The seedlings showed a synchronized decline in survival rate for all the treatments throughout the study period. The overall survival rate of this species was higher for all the treatments when compared to the other species, with a total survival rate of 75.5% (Table 5.1 & Fig. 5.2b). None of the treatments stood out as being beneficial with regard to survival, with the seedlings in the mycorrhiza-only treatment showing the most stable survival rate over time.

Combretum imberbe

All the *C. imberbe* seedlings were still alive when monitored for the first time in November 2008. The survival rate of these seedlings declined for all treatments but especially for seedlings in the mycorriza-compost treatment during the rainy months (Fig. 5.2c). Only 50.7% of this species were still alive on the last measuring date in September 2009. None of the treatments stood out a being beneficial with regard to survival, with the seedlings in the mycorrhiza-only treatment showing the smallest decline in survival rate.

Faidherbia albida

F. albida already showed a few mortalities when measured one month after transplantation, resulting in the highest mortality rates of all the species evaluated. Only 17.6% were still alive on the last measuring date in September 2009 (Fig. 5.2d). Seedlings showed a synchronized decline in survival rate for all the treatment throughout the study period. The steepest decline in survival, irrespective of treatments, occurred during the rainy months, with seedlings in the control treatment showing the steepest decline. Seedlings in the compost-only treatment had the highest survival rate throughout the study period (Fig 5.2).

Philenoptera violacea

The seedlings in the control and mycorrhiza-only treatments had a 100% survival rate throughout the study period (Fig. 5.2e). Seedlings in the compost-only treatment already had a relatively low survival (66.7%) when monitored for the first time in November 2008, and showed a steep decline in survival during the dry months (May - September 2009). For this species, both the mycorrhiza-only and the control treatment were most beneficial with regard to survival.

Salvadora australis

S. australis showed a continuous decline in survival rate for all the treatments throughout the study period (Fig. 5.2f). The rainy months (November 2008 – March 2009) accounted for most of the mortalities. This species had a total survival of 70.6% one year after transplantation, irrespective of treatment. The compost-only treatment showed to be most beneficial with regard to survival.

Xanthocercis zambesiaca

The *X. zambesiaca* seedlings showed a steady decline in survival rate for all the treatments throughout the one year monitoring period (Fig. 5.2g). None of the treatments had a substantially beneficial effect on seedling survival, with the mycorrhiza-only treatment resulting in the highest and most stable survival rates.

The highest mortalities for most of the species occurred from November 2008 to March 2009. The cause of mortalities during this time can most probably be ascribed to the “shock” effect of transplantation and also competition with grasses and herbs which showed a drastic increase in cover within the exclosure, especially after the summer rains (see discussion in 5.2.1.3). The latter could have also lead to the further decline in survival of most seedlings just after the rainy season (March – May 2009). As with the total survival rate discussed previously, the cumulative survival trends also clearly indicate that the effect of the various enhancement treatments were species specific, and recommendations will therefore be made accordingly. See section 5.2.1.3 for a further discussion of the survival results.

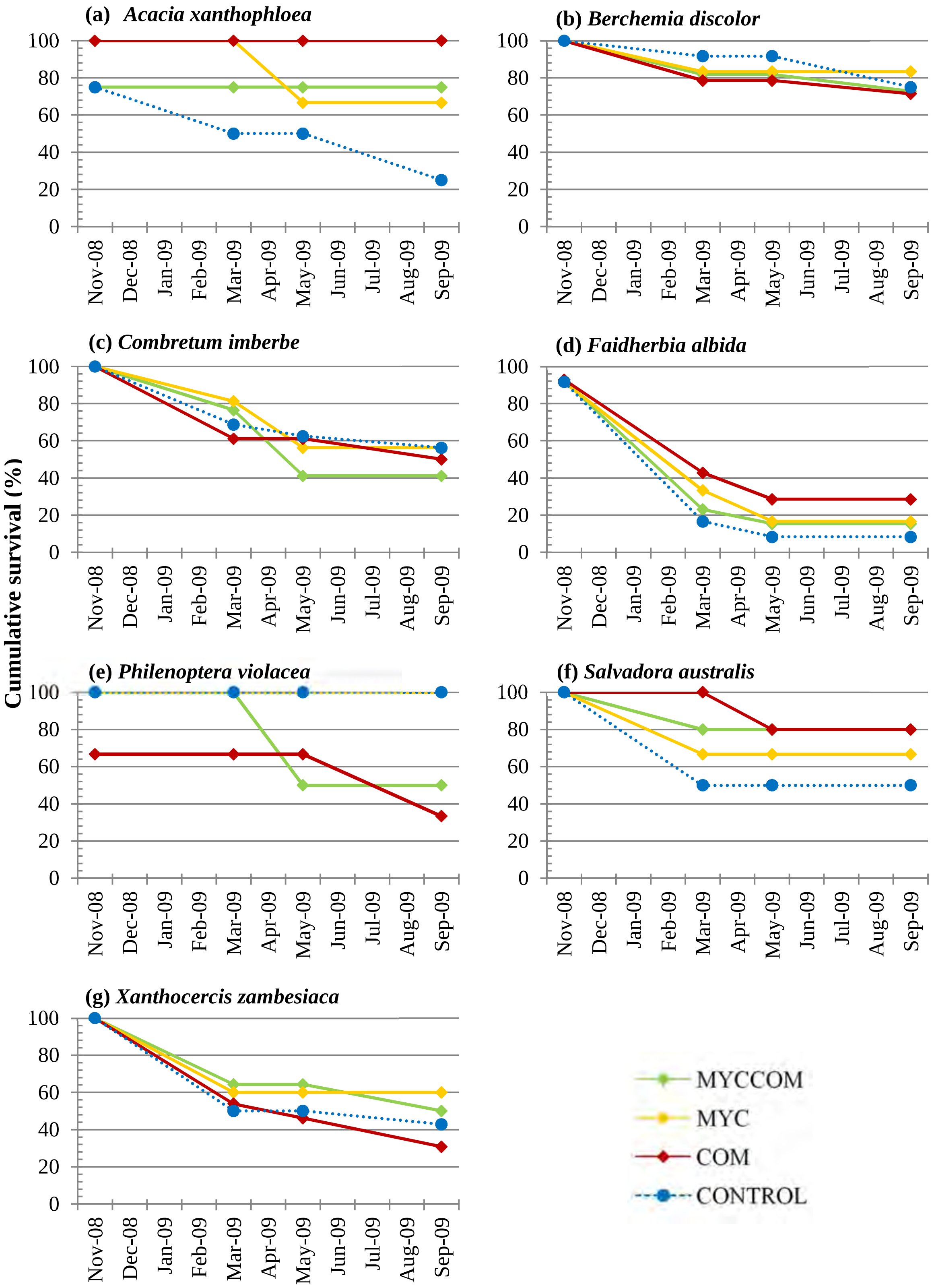

Figure 5.2. The cumulative survival percentage for the seven species at four different enhancement treatments when transplanted into the experimental exclosure, measured over one year (November 2008 - October 2009). (a) *Acacia xanthophloea*, (b) *Berchemia discolor*, (c) *Combretum imberbe*, (d) *Faidherbia albida*, (e) *Philenoptera violacea*, (f) *Salvadora australis*, and (g) *Xanthocercis zambesiaca*. The enhancement treatment names and abbreviations are explained in Appendix A.

5.2.1.2 Seedlings transplanted in 2006

The seedlings transplanted during the pilot study in 2006 by Scholtz (2008) were continually monitored until September 2009. These seedlings were however planted into only three of the five blocks used during the current study, namely block A, B and C. During the pilot study a total of 386 seedlings of ten species were transplanted. At the end of the pilot study (February 2007) only 142 individuals survived, with a survival rate of 37%. The two species that recorded the lowest mortality rates during the pilot study until 2007 were *A. tortilis* and *C. imberbe* (Scholtz, 2008). The latter two species and also *S. australis* were the only species out of ten species evaluated, to produce sufficient data for analysis during the pilot study. It was decided to continue monitoring on only *C. imberbe* and S. *australis* seedlings during this follow-up study. *A. tortilis* was discarded for further monitoring because it was regarded as a pioneer species which had already recruited quite vigorously in areas in and around the exclosure (Götze, 2002, and personal observation). During the current study survival data were collected from a total of 178 saplings, of which 99 were *C. imberbe* and 79 *S. australis*. The pilot study carried out by Scholtz (2008) did not take different enhancement treatments into consideration. The survival of the species planted during the pilot study can therefore not be directly compared to the survival of species planted in 2008. It is however still of interest to measure the survival rate of the trees planted during the pilot study, as they were transplanted two years before and therefore provide valuable information regarding the long-term performance of these species. Please refer to section 4.1.1.3 or Scholtz (2008) for further information regarding the specific aspects investigated during the pilot study.

Figure 5.3 show that *C. imberbe* had a higher survival rate compared to *S. australis* throughout the entire study period for both the pilot and the current study. *C. imberbe* seedlings experienced no mortalities during the first six months after transplantation. Most of the die-offs occurred from February 2007 to March 2008. The survival rate stayed relatively constant thereafter. Figure 5.3 also show that *S. australis* already experienced a large amount of seedling die-offs during the first month after transplantation (77.5% survival rate). The survival of this species continued to show a drastic decline until March 2008. The transplantation technique used during the pilot study differed from the technique used in 2008, and could have contributed to seedling die-off (refer to the planting technique implemented during the current study in section 4.1.1.3). Mortalities due to poor planting technique might have played a role during the initial phase after transplantation, but other factors such as competition with especially the surrounding grass cover and nutrient deficiencies could also have contributed.

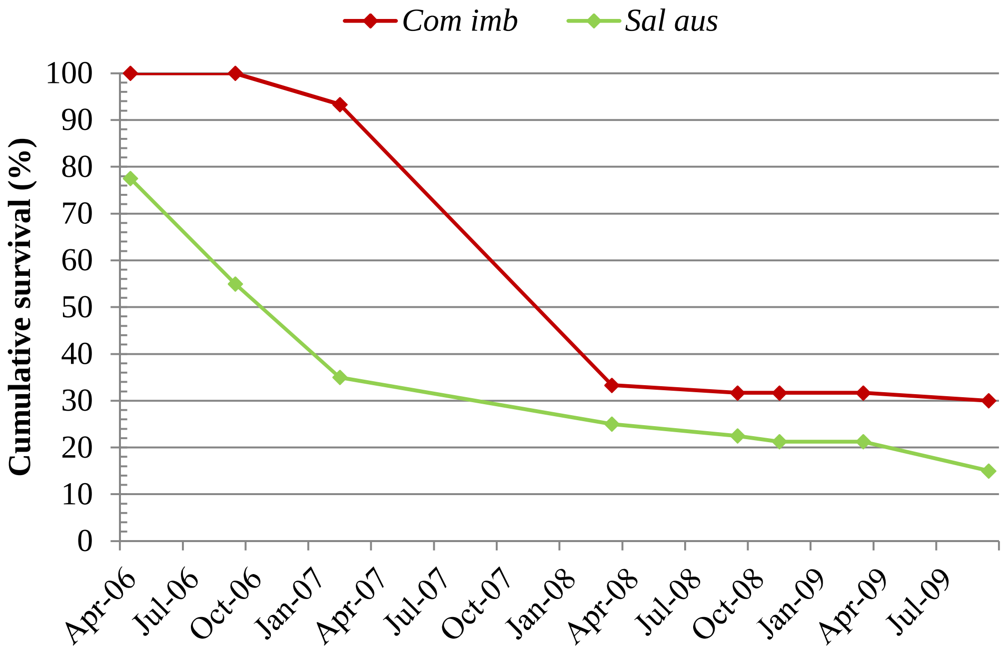

Figure 5.3. The cumulative survival (%) of *Combretum imberbe* and *Salvadora australis* which were transplanted into the experimental exclosure during the pilot study by Scholtz (2008) and monitored from April 2006 to September 2009.

Scholtz (2008) discontinued monitoring in February 2007 at which time the survival rate of the transplanted *C. imberbe* and *S. australis* seedlings were still relatively high at 93.3% and moderate low 35.0% respectively (Fig. 5.3). Monitoring of these seedlings resumed in March 2008 revealing a significant drop in survival rate down to 33.3% and 25.0% for *C. imberbe* and *S. australis* respectively. The drop in survival could most probably be ascribed to the low rainfall occurring between February 2007 and March 2008 (Fig. 4.4). In September 2009 only 25% of the *C. imberbe* seedlings and 7.7% of the *S. australis* seedlings planted during the pilot study, were still alive within the exclosure (Table 5.2).

Table 5.2. The total survival (%) of *Combretum imberbe* and *Salvadora australis* which were transplanted into three of the five blocks inside the experimental exclosure during the pilot study and monitored from April 2006 to September 2009.

	Total survival (%)			
Species	Block A	Block B	Block C	Total
Combretum imberbe	10.0	-	40.0	25.0
Salvadora australis	0.0	23.1	0.0	7.7
Average survival (%)	**5.0**	**23.1**	**20.0**	**16.4**

(- indicates that seedlings of the specific species were not transplanted into that block)

5.2.1.3 Discussion

Scholtz (2008) recommended the addition of organic matter during transplantation in the form of compost aimed at increasing the survival and growth of seedlings. Having incorporated this recommendation as an enhancement treatment during the current study, the issue remains whether the addition of compost did indeed increase survival.

Figure 5.4 shows the average survival rate of seedlings one year after transplantation in 2006 (pilot study) and 2008 for *C. imberbe* and *S. australis* respectively. It is important to note that these two species were transplanted into different blocks with different soil types (see section 4.1.1.3). *C. imberbe* seedlings transplanted during the pilot study had a higher survival rate one year after transplantation (93.3%) compared to seedlings of the same species transplanted in 2008 (50.7%) (Fig. 5.4a). The addition of enhancement treatments therefore did not lead to an increase survival of *C. imberbe* when measured over one year. The majority of the *C. imberbe* seedlings were transplanted into block C, and the cause of the high mortalities may be a result of various factors including competition with grasses and herbs, predation and drought stress. In contrast, *S. australis* seedlings transplanted during the pilot study had a lower survival rate (35%) compared to the same species transplanted in 2008 (70.6%) (Fig. 5.4b). The increased survival rate of the 2008 transplanted *S. australis* seedlings can be contributed to a combination of effects, namely the effect of the enhancement treatments, a better transplantation technique followed, different soil types and the fact that a much higher annual rainfall was experienced in 2009 compared to the previous years (Fig. 3.6). *S. australis* seedlings were planted into block B which was classified as a sandy loam soil. The higher sand content makes this soil more dynamic in which organic treatments may be more favourable toward plant growth. When considering only *C. imberbe* and *S. australis*, the average survival for seedlings transplanted in 2008 was 60.7% compared to 64.2% survival of seedlings planted during the pilot study. The survival of seedlings transplanted during the pilot study was therefore slightly higher within the first year after planting compared to seedlings from the current study. The two species are, however, not directly comparable as they were transplanted into different blocks within the exclosure consisting of different soil types.

In summary, the proportion of seedlings that survived to the end of the field trial varied among the blocks, treatments and species. The nursing blocks (blocks D and E) experienced the most seedling mortalities when compared to the other blocks. This is contradictory to other studies which found that seedlings re-established in forest environments showed higher survival rates when planted inside sites with remnant trees compared to sites without any tree canopy (Alvarez-Aquino *et al.*, 2004; Pedraza & Williams-Linera, 2003; Holl *et al.*, 2000). The latter studies were however conducted in forest environments (e.g. the Mexican Cloud Forest and tropical forests in Costa Rica) where abundant micro-habitats are much more conducive for seedling survival compared to the harsh conditions in the Mapungubwe site. Also, the remnant trees were used in these studies were climax forest species compared to the pioneer *A. tortilis* in the Mapungubwe site. The results of the above mentioned studies also indicate that seedling mortality is the highest in the first year after transplantation for all tree species evaluated. Similar results have been found in other studies concluding that seedlings planted for restoration purposes usually display high mortality during the first growing season (Athy *et al.*, 2006; De Steven, 1991).

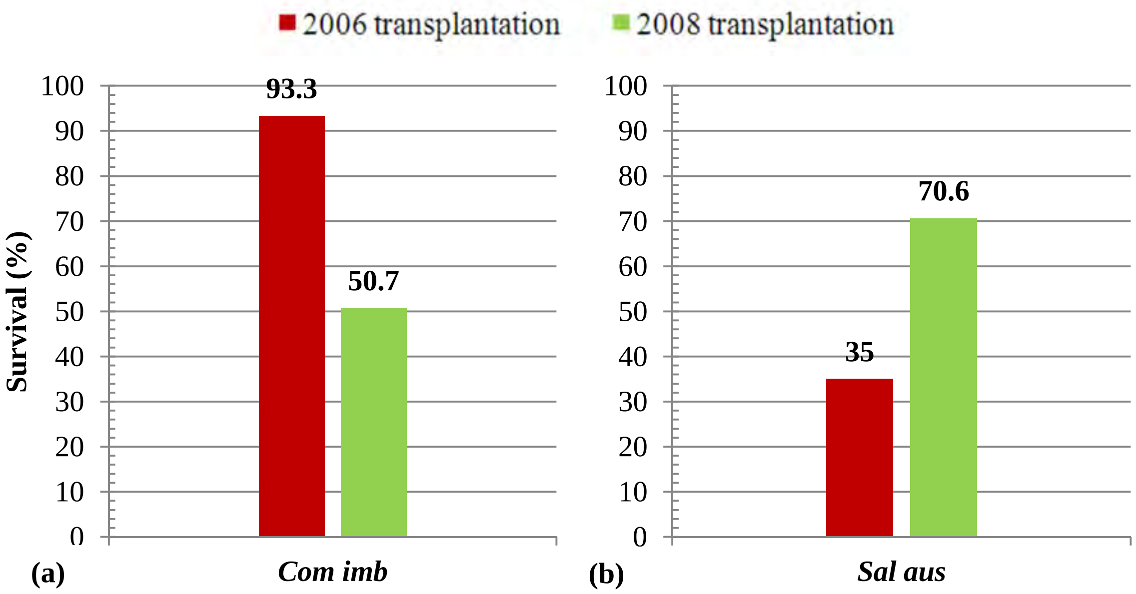

Figure 5.4. The total survival rate (%) for (a) *Combretum imberbe* and (b) *Salvadora australis* seedlings transplanted into the experimental exclosure in 2006 and 2008 respectively, and measured one year after transplantation.

In general, the overall survival of seedlings transplanted in 2008 (55.8%, Table 5.1) was higher compared to seedlings transplanted during the pilot study. The first monitoring event of the current study (March 2008) revealed that only 37% of all the species planted during the pilot study was still alive. This indicates that either the enhancement treatments or improved transplantation techniques (including specific planting date) used during the follow-up study had a positive effect on the survival of the trees.

In general, species appropriate for planting in reforestation programs are selected on the basis of their high growth rates and utility to wildlife (Lamb *et al.*, 1997). For the purpose of this study however, and in accordance with the fundamentals of restoration 5.18logy (SER Primer, 2002), the selection of species used for this study were based on a reference system compiled by historical aerial photographic records, adjacent vegetation and interviews with previous land managers (Scholtz, 2008). The selection of species for transplantation was therefore not purely reforestation by any type of species, but was aimed at biodiversity conservation and establishing species that occurred there previously. Much information is still to be acquired regarding the autoecology of the species evaluated during this study, as well as other constituents of Mapungubwe's riparian forest flora if the species' establishment requirements are to be defined with more precision. In particular, more research and additional analysis are required of the factors responsible for the low survival records in some of the experimental blocks. Recommendations regarding further research and analysis are discussed in the following chapter. The pursuit of higher survival rates for tree species transplanted into Mapungubwe's old fields is not an idealistic trajectory, as various studies have indicated that forest tree species can be successfully established on old agricultural lands (Alvarez-Aquino *et al.*, 2004). The key factor, however, is where these old agricultural lands occur, including the environmental conditions and historical background of each. The old fields of the Mapungubwe exclosure was established in the early 1980s and only abandoned in 2004 (refer to Chapter 3 for further information regarding the history of the study site). These fields were heavily cultivated and fertilized in the past with high impacts of mechanical disturbances which have been shown to be unfavourable for mycorrhiza growth (Kurle & Pfleger, 1994; Johnson & Pfleger, 1992).

The high rainfall (Fig. 3.6) and lack of grazing inside the exclosure during the trial period created the ideal growing conditions for grasses and herbs, as these increased in cover, density and abundance inside the exclosure (Fig. 5.5). Many studies have shown that grasses easily out-compete seedlings during the initial growth phase (Fenner & Thompson, 2005). As mentioned in Chapter 3, the experimental exclosure was established on old lands which were previously cultivated with fertilizer applications and then abandoned. Through the process of natural succession, a number of different pioneer grasses, forbs and tree species have since established and are growing well. The grass species *Aristida congesta* subsp. *congesta*, *Enneapogon cenchroides*, *Eragrostis lehmaniana*, and pioneer tree species like *Acacia tortilis* were the first to colonise the old lands over a short period of time, and became the dominant vegetation inside the exclosure (Götze, 2002 and personal observation). The stand of high vegetation cover also bears evidence of a rich soil seed bank still present after abandonment, even though no-reseeding has taken place from 2006-2009. Based on a visual on-site estimation, *E. cenchroides* is the dominant grass species inside the exclosure. This grass species is classified as a pioneer to sub-climax species (van Oudtshoorn, 2006), often occurring in thick stands in the veld after drought periods and able to rapidly cover and protect degraded areas. *E. cenchroides* is a quick growing grass with a large root system and high seed production, which makes this grass a strong competitor. Fast-growing grasses have been shown to out-compete any recruited seedling that may try to establish (Athy *et al*., 2006). Figure 5.5 illustrates the notable increase of *E. cenchroides* and other grasses inside the exclosure from the start of the pilot transplantations in 2006 until the end of the current study in 2009.

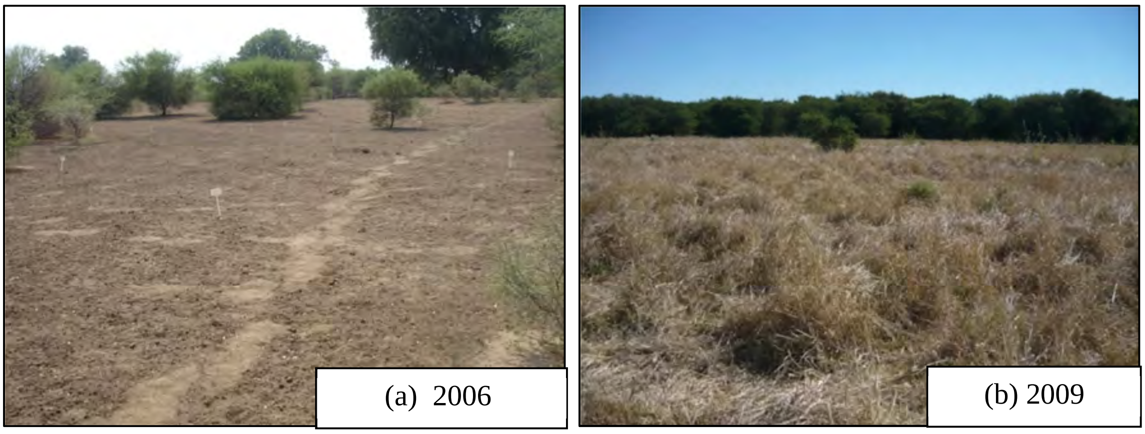

Figure 5.5. (a) The experimental exclosure with almost no grass cover in November 2006 when the pilot study's trials were started, and (b) the significant increase in grass cover and density in September 2009.

As previously mentioned, many of the seedling die-offs occurring inside the exclosure were also related to predation, especially by porcupines (*Hystrix africaeaustralis*), warthogs (*Phacochoerus africanus*) and insects (Fig. 5.6b-d) (Scholtz, 2008). The exclosure is fenced with an electrified game fence but warthogs still manage to find their way inside (Fig. 5.6a). Warthogs and porcupines are known to excavate roots, tubers and bulbs (Fey, 2010) and both occur abundantly enough inside the exclosure to have had a significant effect on seedling survival. According to observational data, seedlings transplanted into the nursing blocks (blocks D and E) experienced the highest occurrence of predation as burrows and stem damage evidence were regularly encountered in these blocks during field visits (Fig. 5.6b). Seedlings planted in the vicinity of the nursing blocks during the pilot study also showed the highest mortalities due to predation (Scholtz, 2008). The nursing blocks are situated in the northern side of the exclosure and are covered with a dense stand of *A. tortilis* trees (Fig. 3.3). The dense vegetation in this area creates the ideal habitat preferred by warthogs and other smaller herbivores, as shade, food resources (e.g. fallen *A. tortilis* pods) and protection is provided.

Other studies by Alvarez-Aquino *et al.* (2004), Goheen *et al.* (2004) and Pedraza and Williams-Linera (2003) also recognise that high tree seedling mortality can result from the herbivory effects by mammals and insects. The occurrence of insect predation was repeatedly observed during field visits and when monitoring was carried out. Ants and termites, but especially corn crickets (*Eugaster longipes*) were found on seedlings in large numbers during the March 2008 and March 2009 measuring events (Fig. 5.6c-d). A study conducted in the Mexican Cloud Forest on transplanted tree seedlings also found high mortalities of certain species due to cricket predation (Alvarez-Aquino *et al.*, 2004). Woody seedling establishment is therefore highly negatively affected by insect predation.

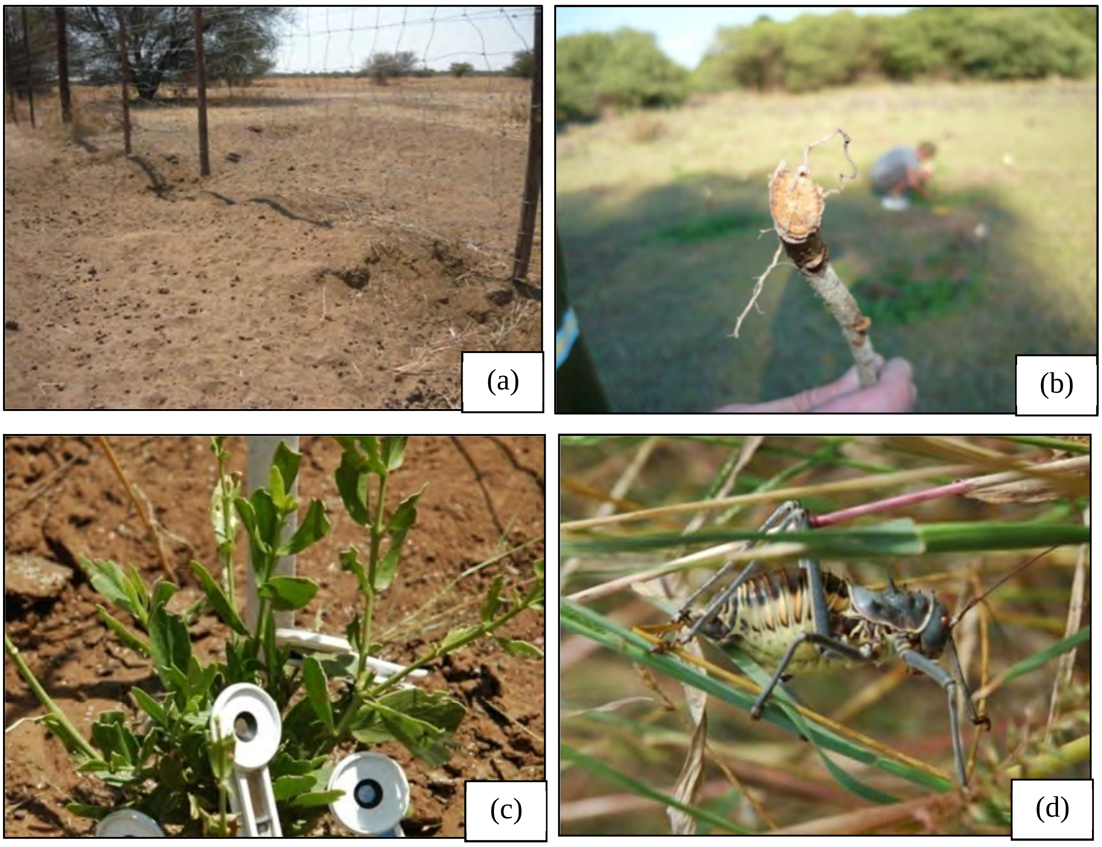

Figure 5.6. (a) The evidence left by warthogs digging their way underneath the electrified fence into the experimental exclosure, (b) a seedling mortality due to severe stem damage caused by porcupines, (c) a *Salvadora australis* seedling showing evidence of insect predation, and (d) corn crickets (*Eugaster longipes*) which were regularly encountered feeding on the transplanted seedlings.

An important factor also possibly contributing to the survival of transplanted seedlings is the distance of the various blocks towards the neighbouring riparian zone. Athy *et al.* (2006) found that the distance to the neighbouring riparian zone positively affected the survival of certain species. These results are consistent with plant survival and abundance relationships found in other literature (Meiners *et al.*, 2000; Lawson *et al.*, 1999). The latter studies concluded that the increased survival is attributed to mycorrhiza distribution. Boerner *et al.* (1996) found that the presence and infectivity success of soil mycorrhiza in tree roots were related to temporal disturbance. Mycorrhizae grow slowly through the soil and develop a patchy distribution in disturbed areas. This pattern is associated with rodent activity, as they collect food from forested areas and store this food elsewhere, thus dispersing mychorrizal spores. It is estimated that it will take at least 30 years for mycorrhizae to re-establish in disturbed soil (Boerner *et al.*, 1996). Until that time, mycorrhizal-independent plants dominate the landscape as is currently evident inside the exclosure. Trees that are dependent on this association with the fungus – like most climax forest species, will therefore have a difficult time establishing. Thus, survival will be highest for seedlings transplanted closest to forest-field margins where mycorrhizae distribution is more homogenous. The true benefit of erecting of an exclosure in order to protect seedlings from browsing can therefore be debated, unless a part of the neighbouring riparian forest forms part of the exclosure. The inclusion of the forest area will ensure the more rapid spread of mycorrhiza by small mammals. The exclosure for this trial was erected to keep out herbivores that could browse on the tree seedlings, but did not enclose a part of the riparian forest. This is an aspect which is further discussed in Chapter 6.

5.2.2 Growth

For the field trials, growth data were obtained from the same amount of seedlings (n = 436) and species (n = 7) as used to calculate survival in the previous sections. When referring to growth in general, it can be implicitly assumed that tree dimensions, such as stem diameter at base, increases for an individual tree over a period of time. Three growth parameters were measured for each seedling transplanted (stem diameter at base, stem diameter at 30cm from base and height of the tree in its natural growth form). Any damage, such as browsing or insect predation was also noted. The three parameters to be measured were recommended by the pilot study as being the most reliable and presentable in terms of growth (Scholtz, 2008) (see section 4.1.2).

During the field trials two of the growth parameters measured, namely ‘diameter at 30cm from base’ and ‘height of the tree in its natural growth form’, resulted in some inconsistencies within the data. The accurate representivity of these two parameters were continually influenced by factors such as browsing. Browsing and breaking of stems by small ungulates were evident in a large proportion of seedlings, especially during the first month after transplantation. Seedlings were transplanted in October 2008, just before the onset of the rainy season, and therefore stood out as the only available greenage in the veld during that time. The average seedling heights at the inception of the field trial were 80 cm for *A. xanthophloea*, *B. discolor*, *F. albida* and *X. zambesiaca*, 60 cm for *C. imberbe*, 50 cm for *P. violacea*, and 30 cm for *S. australis*. Most of the seedlings therefore exceeded 30 cm in height when transplanted in October 2008, but due to the ungulate damage, few values for the ‘diameter at 30 cm’ parameter could be collected. Additionally, height growth in trees decreased over time due to die-back or breaking of branches due to wind or other disturbances, as confirmed by Pastur *et al.*, 2006. Also, the grass cover inside the exclosure increased considerably over the 2008/2009 rainy season which resulted in an unnatural growth form of some seedlings covered in a thick grass layer. The latter was especially true for *C. imberbe* seedlings. Based on the inconsistencies just mentioned, the decision was made to present and analyze data collected only from one growth parameter, namely the “stem diameter at the base”.

5.2.2.1 Seedlings transplanted in 2008

Growth data for the 2008 transplantation were collected from 258 seedlings of seven species measured on three monitoring events over almost one year (November 2008 – September 2009) (refer to Fig. 4.4 for a timeline of the monitoring events). The diameter growth rate at the base for each species planted in each of the enhancement treatments were calculated by the difference in growth between the first (November 2008) and last (September 2009) measuring dates. Only parameter values from seedlings still alive in September 2009 were used. The dataset for each block was however considerably reduced due to a high amount of mortalities experienced, especially during the first six months after transplantation (section 5.2.1). The correlation between the affect of the blocks on the growth of the species were proven to be non-significant ($p < 0.05$). Growth data from all the blocks were therefore consolidated into one dataset in order to provide a bigger sample size. The stem diameter growth data were tested for Gaussian distribution but failed, showing an exceptionally skew Gaussian distribution (Shapiro Wilks test, $p < 0.05$). Therefore, a non-parametric test, the Kruskal-Wallis test, was conducted to establish whether significant differences existed, and to identify the treatments in which these significant differences occurred. The Kruskal-Wallis test is a non-parametric analogue to ANOVA based on rank-transformed data. This test revealed that the field growth data had no significant differences in median diameter growth values between any of the treatments for any species existed, and no post-hoc test was therefore needed to determine the statistical difference ($p < 0.05$) (Table 5.3).

Table 5.3. Statistical output of the Kruskal-Wallis test, where the mean diameter growth at the base of the seven species planted in the different enhancement treatments are compared to the control treatment for each species. Note that there were no significant differences present ($p < 0.05$) (N is the number of plants per sample and $\chi^2_{\text{K-W}}$ is the chi-square value).

Species	N	$\chi^2_{\text{K-W}}$	Sig. (*p*-value)
Acacia xanthophloea	14	0.169	0.982
Berchemia discolor	49	1.621	0.655
Combretum imberbe	67	2.970	0.396
Faidherbia albida	51	2.164	0.539
Philenoptera violacea	9	3.311	0.346
Salvadora australis	17	1.968	0.579
Xanthocercis zambesiaca	51	4.866	0.182

The means of the diameter growth rates at the base for each species and treatment were then compared through a one-way analysis of variance (ANOVA). All tests were evaluated for significance at $p < 0.05$. High variation and small and unequal sample size in the growth dataset resulted in quite big standard errors for all the treatments and species (Fig. 5.7). The overall diameter growth rate at the base for the various species during the period from November 2008 to September 2009, was ranked in the following manner: *P. violacea* (-0.2 mm/year) > *B. discolor* (-1.5 mm/year) > *A. xanthophloea* (-

2.4 mm/year) > *C. imberbe* (-2.5 mm/year) > *S. australis* (-2.8 mm/year) > *X. zambesiaca* (-3.7 mm/year) > *F. albida* (-5.0 mm/year) (Fig. 5.7). Figure 5.7 shows that the mean growth of the stem diameters at the base was negative for all the species and treatments, with the exception of *P. violacea* seedlings in the mycorrhiza-only and control treatments and *S. australis* seedlings in the mycorrhiza-compost treatment. No significant differences were found and the effect of the various treatments on diameter growth at the base was found to be species-specific. Taking into account that most of the stem diameter at base measurements were negative, the least negative diameters were measured for *A. xanthophloea* (-2.5 mm/year), *C. imberbe* (-1.4 mm/year), *F. albida* (-3.5 mm/year), and *X. zambesiaca* (-2.4 mm/year) in the compost-only treatment. The control treatment resulted in the least negative diameters at base for *B. discolor* (-1.1 mm/year) and *P. violacea* (1.6 mm/year), and the mycorrhiza-compost treatment showed the highest mean diameters at base in *S. australis* (0.5 mm/year). In general the diameter growth rate at the base for each species was highest when compost was added.

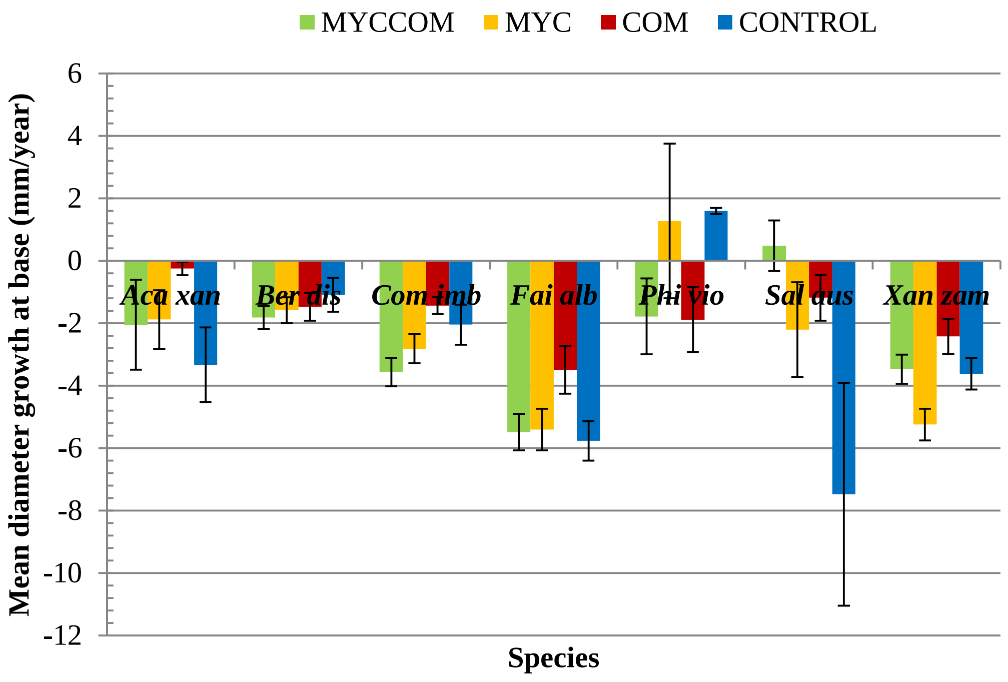

Figure 5.7. A comparison of the mean diameter growth at the base of seven species planted in four different enhancement treatments, and monitored from November 2008 to September 2009. Note the high standard errors, and that no significant differences were present ($p < 0.05$). The enhancement treatment names and abbreviations are explained in Appendix A. (The bars represent standard errors).

Growth rate is a species-specific trait and can therefore not be compared between species, but rather for treatments *within* a species, as demonstrated in Figure 5.7 which showed that the effect of the treatments was species specific also. The means of the diameter at base measurements for each species were plotted over time in Figure 5.8, thereby presenting the seasonal growth trend separately for each species. Again only measurements collected from seedlings still alive when measured in September 2009 were used.

Acacia xanthophloea

A. xanthophloea seedlings in the compost-only treatment showed no change in mean stem diameter at base for the duration of the study period (Fig. 5.8a). Seedlings in the mycorrhiza-compost and mycorrhiza-only treatment however, showed a slight decrease in stem diameter during the rainy months (November 2008 – March 2009). The seedlings in the control treatment showed a notably larger decrease in stem diameter at base during the dry months when compared to the other treatments. In general the *A. xanthophloea* seedlings followed a synchronized diameter growth trend for all the treatments, with a very small increase in stem diameter over the one year of measurements. None of the enhancement treatments stood out as being more beneficial with regard to growth when compared to the control.

Berchemia discolor

The mean stem diameter growth at base followed a synchronized trend in *B. discolor* seedlings, irrespective of specific treatment, when measured over one year (Fig. 5.8b). All the seedlings and treatments showed a slight increase in mean stem diameter at base during the high rainfall months (November 2008–March 2009), and a slight decrease in stem diameter during the dry months (April 2009–September 2009). None of the enhancement treatments stood out as being more beneficial with regard to growth when compared to the control.

Combretum imberbe

All the *C. imberbe* seedlings, irrespective of treatment, showed a minor increase in mean stem diameter at base during the high rainfall months (November 2008–March 2009) (Fig. 5.8c). After March 2009, only seedlings in the control treatment increased in stem diameter at the base. The compost treatment therefore stood out as being more beneficial with regard to growth when compared to other treatments, including the control.

Faidherbia albida

The *F. albida* seedlings in all of the treatments, with the exception of the seedlings in the compost-only treatment, resulted in an overall decrease in mean stem diameter at base throughout the one year of measurement. Seedlings in the compost-only treatment continuously increased in mean stem diameter growth at the base (Fig. 5.8d). The seedlings in the control treatment resulted in a notably bigger decrease in diameter at base, especially during the dry months (April 2009–September 2009) when compared to seedlings in the enhancement treatments. The compost-only treatment therefore stood out as being more beneficial with regard to growth when compared to the other treatments, including the control.

Philenoptera violacea

All the *P. violacea* seedlings, irrespective of treatment, resulted in an overall increase in mean stem diameter at base during the one year of measurements (Fig. 5.8e). All the seedlings showed a continuous increase in stem diameter during both the high and low rainfall months, with the exception of seedlings in the mycorrhiza-only treatment which decrease in stem diameter during the rainy months. In general the compost-only and control treatments stood out as being more beneficial with regard to growth when compared to the other treatments.

Salvadora australis

The *S. australis* seedlings in the mycorrhiza-compost and mycorrhiza-only treatments resulted in a fairly constant increase in mean stem diameter at base during the one year of measurements (Fig. 5.8f). The seedlings in the control treatment showed a notable decrease in stem diameter during the high rainfall months (November 2008–March 2009) and an increase again during the dry months (April 2009–September 2009). The seedlings in the compost treatment did not show any increase in stem diameter growth throughout the study period. In general the mycorrhiza-compost treatment showed to be more beneficial with regard to growth when compared to the other treatments, including the control.

Xanthocercis zambesiaca

All the *X. zambesiaca* seedlings, irrespective of treatment added, resulted in a continuous slight decrease in mean stem diameter at base during the one year of measurements, with the exception of seedlings in the mycorrhiza-only treatment which resulted in a larger decrease in stem diameter during the dry months (April 2009-September 2009) (Fig. 5.8g). None of the enhancement treatments stood out as being more beneficial with regard to growth when compared to the control.

Based on the seasonal stem diameter growth trends presented in Figure 5.8, it is clear that the effect of the enhancement treatments were species-specific. The overall growth, irrespective of treatment added, was remarkably low within each species, with *P. violacea* and *F. albida* having the highest (-0.201 mm/year) and lowest (-5.040 mm/year) diameter growth rates respectively. The majority of species showed an increase in stem diameter for all treatments during the high rainfall months. In general no pattern for diameter growth at base could be observed across any of the treatments and species combinations. These growth trends may however provide a clearer indication of the effect of the various treatments when monitoring is continued on a long-term basis.

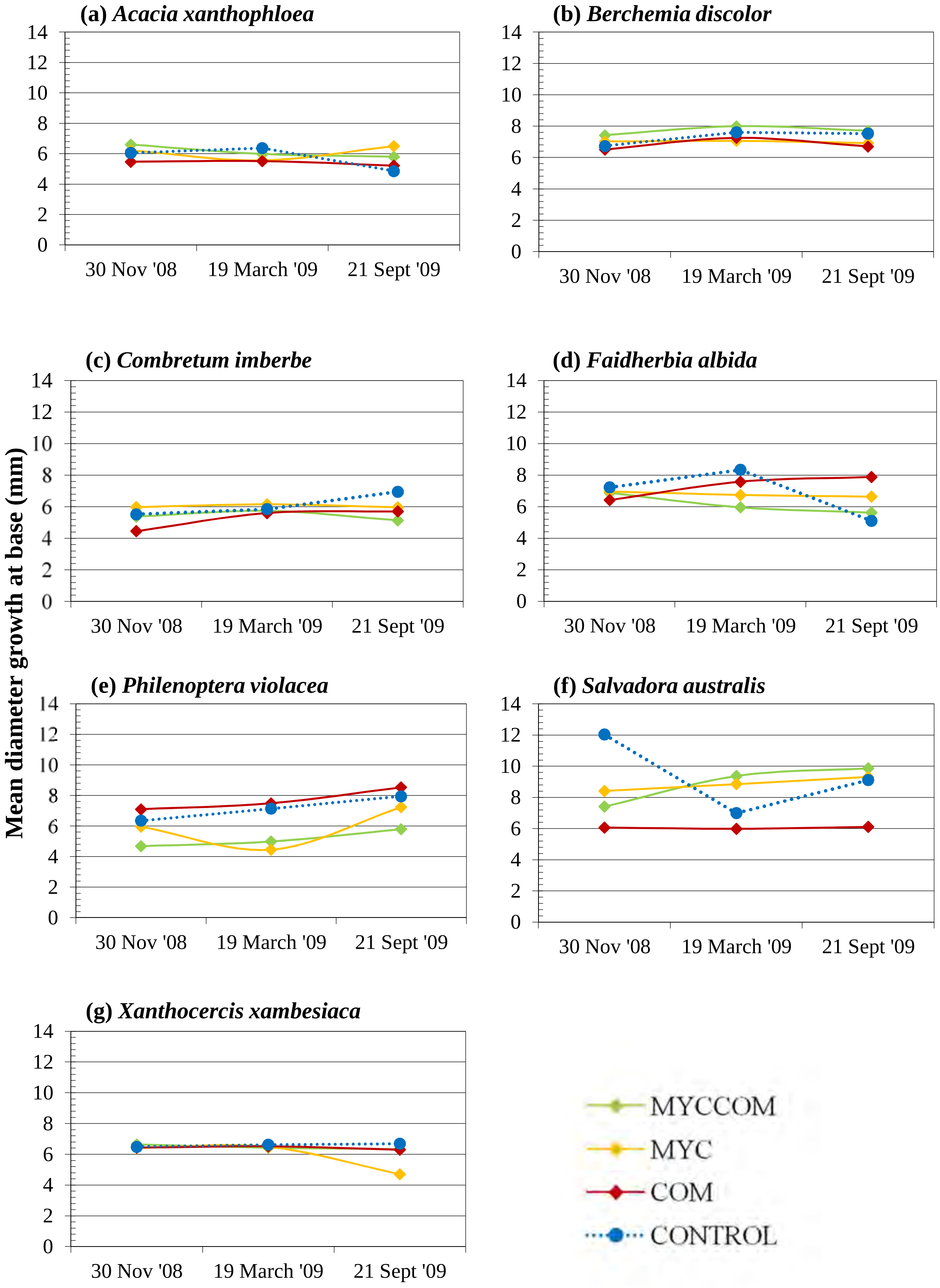

Figure 5.8. The trends in mean diameter growth increments at the base of seven species planted into the various enhancement treatments inside the experimental exclosure, and measured on three monitoring events over one year (November 2008 – September 2009), where (a) *Acacia xanthophloea*, (b) *Berchemia discolor*, (c) *Combretum imberbe*, (d) *Faidherbia albida*, (e) *Philenoptera violacea*, (f) *Salvadora australis*, and (g) *Xanthocercis zambesiaca*. The enhancement treatment names and abbreviations are explained in Appendix A.

5.2.2.2 Seedlings transplanted in 2006

As mentioned in section 5.2.1.2, the seedlings transplanted during the pilot study were continually monitored from 2006 until 2009. These seedlings did not receive any of the enhancement treatments that were evaluated during the current study. The growth data analysed for the pilot transplantation consisted of data collected for *C. imberbe* in block A and C, and *S. australis* in block B (see Fig. 3.3 for a layout of the experimental exclosure). Because the pilot transplantation took place in 2006, most of the *C. imberbe* and *S. australis* trees exceeded 30 cm in height when measured on the last measuring date in September 2009. It was therefore decided to present growth data collected from both the 'stem diameter at the base' and the 'height of the tree in its natural growth form' parameters.

The seasonal growth trend in the mean stem diameter at base for *C. imberbe* and *S. australis* trees are presented in Figure 5.9. This figure shows that there was a continuous increase in diameter growth during the four years of measurements (April 2006−September 2009). *C. imberbe* did however show a minor decrease in stem diameter during the summer of 2006/2007 and again during the winter of 2008. The highest increase in stem diameter growth for both species occurred from February 2007 to March 2008. This period however also accounted for the highest mortalities in the two species (Fig. 5.3). The individuals that did not die during this period continued to show a steady increase in stem diameter growth throughout the four year period, from 13.6 mm to 23.3 mm (increment by 9.7 mm) for *C. imberbe* and 2.9 to 19.8 mm (increment by 16.9 mm) for *S. australis* (Fig. 5.9).

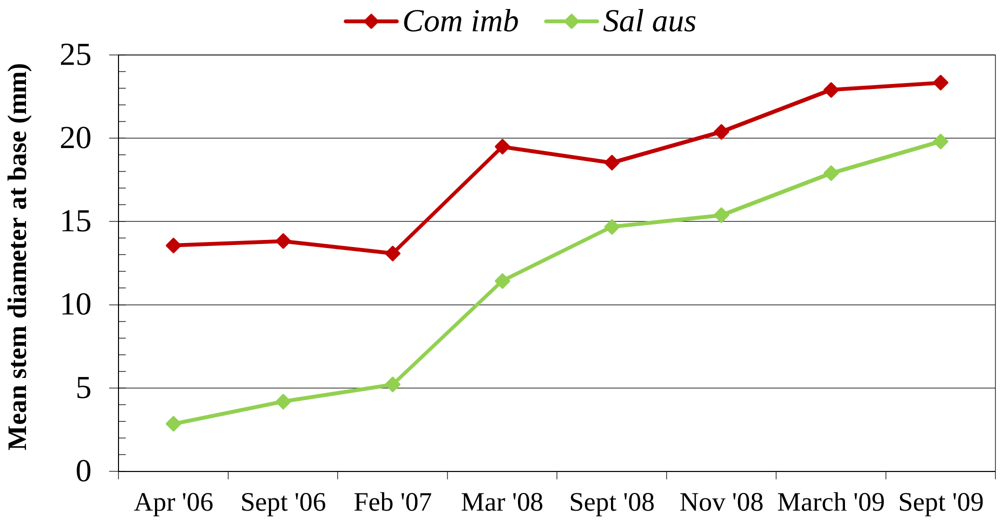

Figure 5.9. Trends in mean diameter growth at the base of *Combretum imberbe* and *Salvadora australis* trees transplanted into the experimental exclosure during the pilot study, and measured from April 2006 to September 2009.

In September 2009 the average height of the *C. imberbe* and *S. australis* trees which were transplanted during the pilot study, exceeded 60 cm and 30 cm respectively - an indication that these trees have grown past the most vulnerable seedlings phase and were therefore far less susceptible to damage caused by predation and herbivory compared to the juvenile status of the trees transplanted during the current study in 2008. Lawson *et al.* (1999) determined that trees between 20 cm and 30 cm in height are the preferred forage height for small ungulates, as trees beyond 30 cm decreased in mortality due to less ungulate browsing. In another study by De Steven (1991), five tree species were planted and it was also found that ungulate activity was significantly reduced at taller heights. Based on the mean heights of both *C. imberbe* and *S. australis*, it can therefore be predicted that the chance of future mortalities within these two species are very low.

Figure 5.10 shows that both species had an overall increase in height during the four years of measurement (*C. imberbe* = 84.2 mm; *S. australis* = 288.9 mm). *C. imberbe* however, showed a slight decrease in height during the summer of 2006/2007 (93.6 mm), and an even more notable decrease during the winter of 2008 (198.7 mm). The initial decrease in height could be attributed to the branches growing more sideways due to a thick stand of grass that established inside the exclosure, causing pressure on the branches (refer to section 5.2.1.3 where the grass stand is discussed). The decrease in height during the winter of 2008 can however not be adequately explained, as large herbivores which might have led to branch damage were excluded from the exclosure. As shown in Figure 5.11, *S. australis* also showed a slight decrease in height during the winter of 2008. This decrease was likely due to predation by corn crickets (*Eugaster longipes*) often encountered on these seedlings during this time (Fig. 5.6c-d). The *S. australis* trees has a more shrub-like growth form and it is therefore expected to flatten out at around 2 m in height. *C. imberbe* however is classified as a tree and can grow up to 20 m in height (Venter & Venter, 2007). In September 2009 some of the *C. imberbe* trees which were transplanted during the pilot study, had already reached heights of up to 2 m (Fig. 5.11).

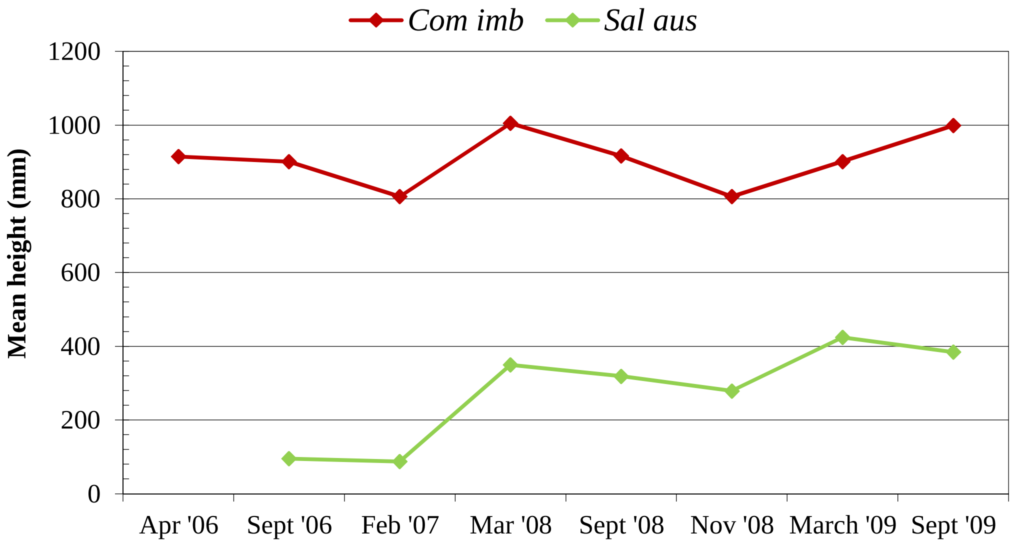

Figure 5.10. Trends in the mean growth in height for *Combretum imberbe* and *Salvadora australis* planted into the experimental exclosure during the pilot study, and monitored from April 2006 to September 2009.

Figure 5.11. One of the *Combretum imberbe* trees which were transplanted during the pilot study, and almost exceeded 2 m in height when last measured in September 2009.

Table 5.4 shows the average growth rate calculated for the ‘stem diameter at base’ and the ‘height of the tree in its natural growth form’ parameter measurements for *C. imberbe* and *S. australis* trees for each year after transplantation in 2006. *C. imberbe* displayed a negative stem diameter growth rate during the first year after transplantation and a negative growth in height within year 3. In general however, positive growth rates for both parameters were shown in most of the years, resulting in a total positive growth rate for both parameters at the end of the four years of measurements. The biggest stem diameter and height growth for *C. imberbe* took place in the second year after transplantation (diameter growth = 0.64 cm/year; height growth = 19.86 cm/year) (also see Fig. 5.10). Table 5.4 shows that *S. australis* trees experienced overall positive growth rates in both parameters measured throughout the four years of measurements, with the exception of a decrease in height during the first and fourth year. The biggest increase in stem diameter occurred in the second and third year after transplantation (0.65 cm/year), and the biggest increase in height growth occurred in the second year after transplantation (26.20 cm/year). At the end of the four year monitoring period, *S. australis* showed an overall positive total growth rate for both growth parameters.

Table 5.4. The average growth rate in base diameter and height of *Combretum imberbe* and *Salvadora australis* seedlings calculated per year for four years after transplantation in 2006. (DB = stem diameter at base, and H = height of the tree in its natural growth form).

	Year 1		Year 2		Year 3		Year 4		Total growth	
Species	DB	H	DB	H	DB	H	DB	H	DB	H
				(cm/year)					(cm/4 years)	
Combretum imberbe	-0.05	-10.82	0.64	19.86	0.34	-10.39	0.04	9.77	0.97	8.42
Salvadora australis	0.23	-0.78	0.62	26.20	0.65	7.45	0.19	-3.99	1.69	28.9

In total *C. imberbe* has therefore shown a mean growth of 0.97 cm in stem diameter at base and 8.42 cm in height over the four years following transplantation (Table 5.4). Under ideal greenhouse conditions, *C. imberbe* could grow 50−100 cm/year (Venter & Venter, 2007), which means that the growth in the field was slowed down due to many unfavourable conditions encountered in the natural environment. In total, *S. australis* has shown a mean growth of 1.69 cm in stem diameter at base and 28.89 cm in height over the four years after transplantation (Table 5.4). *S. australis* thus had a larger increase in mean height growth compared to *C. imberbe* (Fig. 5.11 & Table 5.4). The height structure of the two species at the time of the last measuring date in September 2009 was a reflection of their growth potential and respective classification as a tree and a shrub species under natural conditions (Table 4.1).

The overall positive growth of the two species from the pilot transplantation which was continually monitored, compared to the overall negative growth of the species transplanted in 2008 (section 5.2.2.1), supports and emphasizes the value of long-term monitoring.

5.2.2.3 Discussion

Studies by Lesser and Kalsbeek (1999) and Pastur *et al.* (2006) has found that negative growth rates for stem diameter at base measurements could be predominantly accredited to human error. In this study it is highly likely that human error has also contributed to the negative growth rates presented in the previous sections. The use of different people to do the monitoring on the various monitoring dates as well as not always positioning the calliper on the exact position and orientation at the base of the stem could have contributed to human error during this study. Water stress has however also been found to be responsible for negative growth diameter values (Sevanto, 2003; Vesala *et al.*, 2000; Zürcher *et al.*, 1998). Trees tend to recover their negative growth after the unfavourable conditions encountered have seized. Pastur *et al.* (2006) investigated the decrease in annual diameter growth of tree stems in Argentinean forests and found that some trees did present negative annual diameter increments associated with a decrease in water content within stem trunks. Pastur *et al.* (2006) also found that most of the dead trees decreased in stem diameter during the preceding years. They attributed die-back to moisture loss in the stem due to an inability of the trees to absorb water via their roots. Die-back was thus mostly related to root-competition, which led to detrimental water loss in the stems. A threshold therefore exists for stem moisture in trees, and when the water levels drop beyond this threshold it will lead to imminent death. Such a threshold for stem moisture might also exist for the seven species investigated during this study. Taking into account the strong competition for moisture presented by the roots of grasses, forbs and *A. tortilis* trees inside the experimental exclosure, it can be assumed that the died-off seedlings were outcompeted rendering their roots unable to absorb the limited water in the soil. The moisture content inside the stems of the transplanted seedlings is unfortunately a parameter which was not measured during this study. It requires further investigation as it can be used as a valuable tool when predicting and understanding die-back in transplanted trees.

Some of the species also showed a negative growth rate in height during the monitoring period. A reduction in height can mostly be accredited to shoot die-back which has also been encountered in a study by Zida *et al.* (2008) on other savanna tree species. The same observation was made by Athy *et al.* (2006), who also found that certain species actually became shorter as the seasons progressed. Browsing by duikers and steenbok were encountered within the experimental exclosure, which could have influenced the growth of seedlings negatively. Although the exclosure should have kept out small ungulate species, they did enter the exclosure from time to time. Continuous browsing by small ungulates will either kill the plant or keep it at shrub level. The only way for trees to overcome this fate, is by maintaining a positive growth rate and growing out of reach of these herbivores - this is possible over longer periods of growth as proven in Table 5.4. Insect predation could also have had the same effect as continuous browsing on the growth and structure of the transplanted trees.

5.2.3 Plant vitality

As discussed in Chapter 4, the multi-parametric expression performance index (PI_{ABS}) was used as a metric for the photosynthetic vitality of each species-treatment combination. This parameter was therefore used to determine whether the trees' performance was influenced, and to what extent, by the different enhancement treatments these trees were planted into. In addition to the effect of the enhancement treatments, the effect of the water-stress or drought conditions present in the semi-arid Mapungubwe landscape were also expected to be reflected in the trees' photosynthetic parameters. The pilot study did not evaluate any of the enhancement treatments and therefore plant vitality measurements were conducted only on the seedlings transplanted in 2008.

5.2.3.1 Seedlings transplanted in 2008

Physiological monitoring took place on three occasions during the study period: March 2009, June 2009 and September 2009. In general chlorophyll fluorescence was measured on five leaves of a total of 153 seedlings of the seven species transplanted. At first leaves were dark-adapted for at least one hour using leaf clips (see section 4.2.3), whereupon chlorophyll fluorescence was measured. The fast phase fluorescence transients were quantified by means of the JIP-test (Strasser & Strasser, 1995) and Biolyzer software (Strasser *et al.,* 2004).

During field measurements most of the transplanted seedlings were without leaves due to various factors such as die-back, browsing and senescence, and therefore only few measurements could be made on seedlings which still had some foliage left. Moreover, there were very few repetitions of the same species-treatment combinations suited for measurements throughout the study period. The lack of samples therefore made it impossible to present the temporal trend in vitality of the various species-treatment combinations. The fluorescence data collected on the three measuring dates were however consolidated and analysed in order to present the mean PI_{ABS} values for each species, irrespective of treatment, and also for each species-treatment combination.

Table 5.5 presents the mean PI_{ABS} values of the seven species which were transplanted in 2008, irrespective of the enhancement treatment planted into. As growth rate is a characteristic property within a species, so it is with physiological performance also, with each species having a unique range for each parameter. The PI_{ABS} values of the various species can therefore not be compared over species. Taking this into consideration, the field samples showed that *F. albida* has much higher mean PI_{ABS} values (PI_{ABS} = 93.10) when compared to the other species, such as *P. violacea* (PI_{ABS} = 20.78).

Table 5.5. The mean performance index (PI_{ABS}) and standard error (±S.E.) values of the seven species transplanted into the experimental exclosure in 2008, irrespective of enhancement treatment planted into, and measured on three occasions throughout 2009.

Species	Mean performance index (PI_{ABS})	±S.E.
Acacia xanthophloea	38.20	6.38
Berchemia discolor	26.52	2.89
Combretum imberbe	29.84	2.29
Faidherbia albida	92.10	21.46
Philenoptera violacea	20.78	4.13
Salvadora australis	30.22	4.96
Xanthocercis zambesiaca	57.79	4.46

Figure 5.12 compares the mean PI_{ABS} values of each species for each treatment planted into. Note that due to the lack of leaves for various reasons mentioned earlier and also high mortalities within the experimental exclosure (section 5.2.1), no data could be collected for *A. xanthophloea* planted in the mycorrhiza-compost and compost-only treatments, as well as for *F. albida* seedlings planted in the control treatment. This is also reflected in the big standard errors present in especially *F. albida* of which very few repetitions for each treatment could be sampled due to this species' low survival rate (Table 5.1). The highest PI_{ABS} values for control plants were measured for *C. imberbe* (PI_{ABS} = 41.35), *P. violacea* (PI_{ABS} = 36.64), *S. australis* (PI_{ABS} = 38.41) and *X. zambesiaca* (PI_{ABS} = 90.22), being significantly higher than the other treatments in *X. zambesiaca* ($p < 0.05$). *B. discolor* showed the highest vitality, although not significant, when planted into the compost-only treatment (PI_{ABS} = 37.34). The mycorrhiza-only treatment resulted in significantly higher vitality of *A. xanthophloea* when compared to the control treatment (PI_{ABS} = 63.89). The mycorrhiza-compost treatment resulted in the highest vitality of *F. albida* seedlings when compared to the mycorrhiza-only and compost-only treatments (PI_{ABS} = 120.98). The effect of the enhancement treatments on the vitality of the various species indicated that the absolute PI_{ABS} values of different species cannot be compared directly, and recommendations for future transplantations therefore need to be made accordingly.

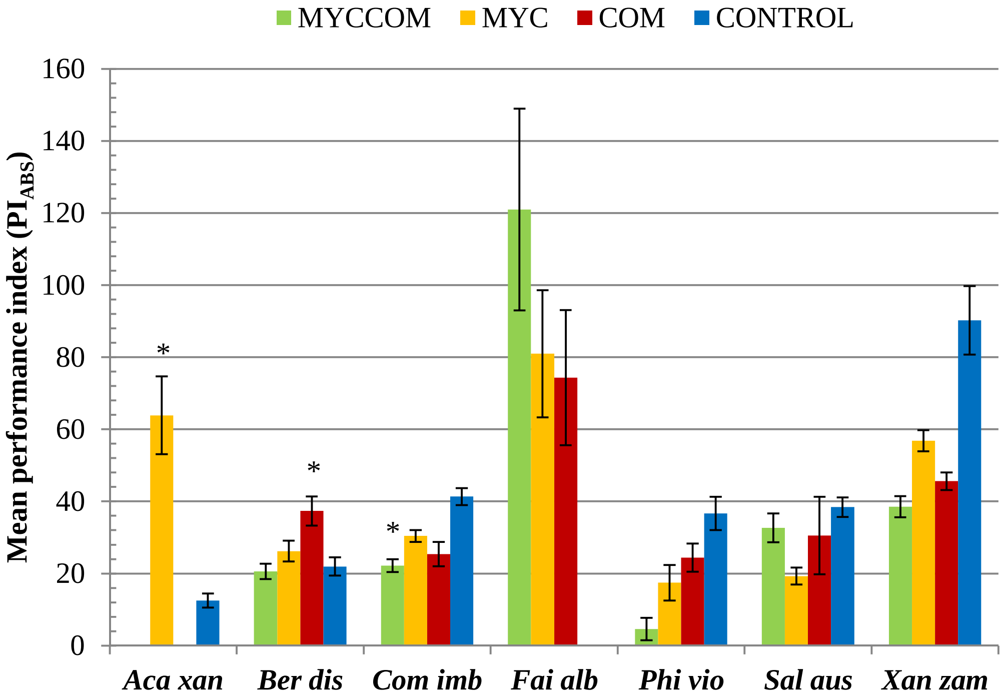

Figure 5.12. The mean performance index (PI$_{ABS}$) values of the seven species (*Acacia xanthophloea*, *Berchemia discolor*, *Combretum imberbe*, *Faidherbia albida*, *Philenoptera violacea*, *Salvadora australis* and *Xanthocercis zambesiaca*) transplanted into the different enhancement treatments inside the experimental exclosure. The treatment names and abbreviations are explained in Appendix A. (The bars represent standard errors and findings significantly different from the control are indicated with an asterisk at a 95% probability level according to the Student's t-test).

In Figure 5.13, the mean performance index (PI$_{ABS}$) of the five species planted into the various enhancement treatments are compared to the corresponding means of seedlings transplanted into the control treatment. This graph represents only the species for which sufficient fluorescence data could be collected, therefore excluding *A. xanthophloea* and *F. albida*. It is clearly indicated that *B. discolor* was the only species to show a positive deviation in mean performance index (PI$_{ABS}$) values compared to when planted in the control treatment. *X. zambesiaca* showed the most negative deviation from the control treatment. The majority of the species transplanted into the field therefore did not benefit from the various enhancement treatments with regard to overall vitality.

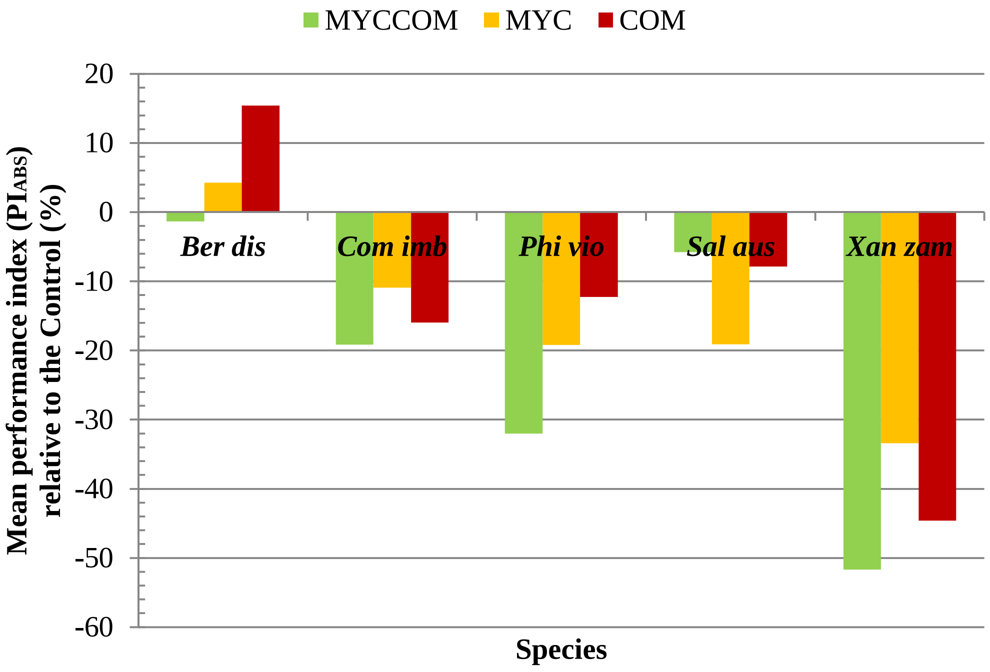

Figure 5.13. The deviation in mean performance index (PI_{ABS}) values relative to the control of five species (*Berchemia discolor*, *Combretum imberbe*, *Philenoptera violacea*, *Salvadora australis* and *Xanthocercis zambesiaca*) transplanted into the different enhancement treatments inside the experimental exclosure. *Acacia xanthophloea* and *Faidherbia albida* has been excluded due to insufficient data. The treatment names and abbreviations are explained in Appendix A.

5.2.3.2 Discussion

A species' ability to maintain photosynthetic functionality under water stress conditions such as those present in the semi-arid Mapungubwe landscape is of major importance for its survival. The Performance Index (PI_{ABS}) is regarded as one of the most useful chlorophyll fluorescence expressions, providing quantitative information about the state of plants and their vitality (Hermans *et al.*, 2003). This index encompass fluorescence transient changes associated with changes in antenna formation and energy fluctuations, thereby estimating tree vitality with high resolution. In particular, it aids in understanding how stresses damage photosynthesis (Quiles & Lopez, 2004; Maxwell & Johnson, 2000).

It is unfortunate that so few data could be collected from the seedlings transplanted into the field. The majority of the physiological data were collected on the first measuring date in March 2009. The other two measuring dates (May and September 2009) produced few data as seedling either died-off or had no leaves due to senescence or browsing and insect predation. Even when the seedlings still had leaves, attaching the leafclips and doing the measurements were quite tricky due to the specific leaf-forms of the various species, e.g. the very small leaves of *X. zambesiaca* (Fig. 5.14a). Also, a lot of the leaves measured were near the point of falling off due to senescence, rendering the leaves very fragile to work with (Fig 5.14b).

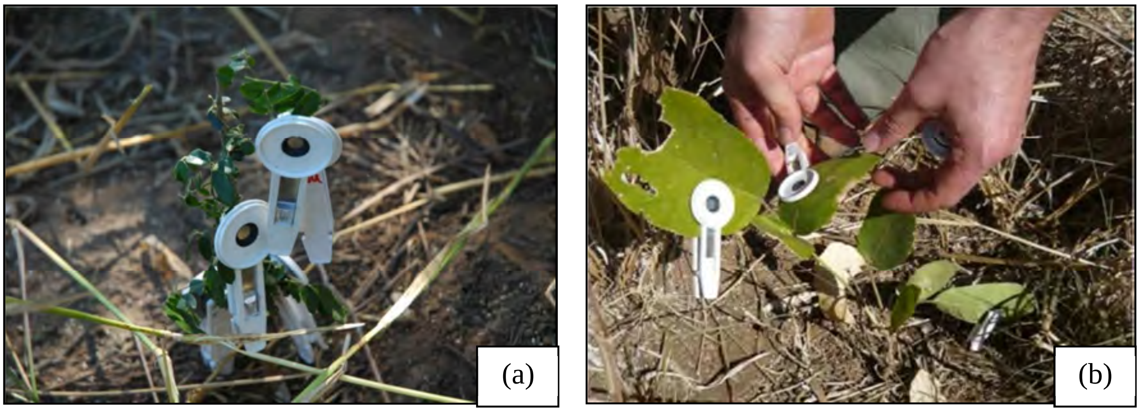

Figure 5.14. Leafclips attached to (a) the small leaves of a *Xanthocercis zambesiaca* seedling, and (b) the fragile leaves of a *Philenoptera violacea* seedling which has been damaged due to insect predation and were close to falling off due to senescence.

Because it was not possible to present the trend in vitality of the relevant species throughout the study period, no assumptions could be made about the seasonal effect on the vitality of the trees. Assumptions could only be made regarding the beneficial effects of the enhancement treatments on the vitality of each species. The control treatment produced the highest vitality values in most of the species (Fig. 5.12), indicating the enhancement treatments were not beneficial with regard to physiological performance in general. Physiological performance is however a species-specific characteristic, as mentioned in the previous section, and treatment recommendations need to be made accordingly (these recommendations are discussed in Chapter 6).

5.2.4 Soil

5.2.4.1 Soil chemical analysis

As discussed in Chapter 4, soil samples were collected inside and outside the experimental exclosure in order to characterise the soils' edaphic properties, such as pH and nutrient content. With the aim of keeping the soil sampling method and design consistent with that of the pilot study, the same accredited laboratory, Eco Analytica[1], was used to analyse soil samples collected during this study. Only the results from the soil chemical analysis conducted for the current study are discussed in this dissertation. The reason for excluding the pilot study's results is that two new blocks, namely block D and E (the nursing blocks), were demarcated within the experimental exclosure in 2008. It was also

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[1] Eco Analytica, Potchefstroom, Tel: 018 293 3900

impossible to collect the samples in the exact locations where samples were collected in 2006 as no geographical referencing for the sample locations was made during the pilot study.

In October 2008 composite samples were collected inside each of the blocks at a depth of 0−30 cm, as well as in the adjacent riparian forest area situated ± 100 m to the north of the exclosure (refer to Fig. 4.1). Due to the close proximity and consequent homogeneity of block A and B, and block D and E, their composite samples were consolidated for the analysis. The results from the soil chemical analysis of the following samples are therefore discussed in this section: block A and B compiled (AB), block C (C), block D and E compiled (NUR), and the riparian forest area (RIPFOR). These results are presented in Tables 5.6 and 5.7, as well as Figure 5.15.

The particle size distribution of the various samples is presented in Table 5.6. The texture classes of the various samples were determined by using the well-known textural class triangle (Brady & Weil, 2008) (Appendix B). Blocks A and B were classified as a sandy loam, block C as a clay loam, and both the nursing blocks and the riparian forest area as a loam. The higher clay content in block C is explained by the semi-wetland it is situated upon in the southern section of the exclosure. The similar textural classification of the nursing blocks and riparian forest area can be explained by their close proximity to each other. Another factor possibly contributing to their similarity is that the nursing blocks has been abandoned for the longest time and therefore functions in an more advanced successional state (also evident by the presence of a dense *A. tortilis* stand) compared to blocks A and B which was most recently abandoned.

Table 5.6. The particle size distribution (%) and consequent textural classification of the soils within each block inside the experimental exclosure (also including the riparian forest area outside the exclosure). The block names and abbreviations are explained in Appendix B.

Location	Coarse fragments fraction (> 2 mm) (%)	Fine earth fragments (< 2 mm) Sand	Silt (%)	Clay	Textural classification
AB	0.2	53.80	31.40	14.80	Sandy loam
C	0.5	42.83	26.87	30.30	Clay loam
NUR	0.1	39.00	39.30	21.70	Loam
RIPFOR	0.1	54.90	29.30	15.80	Loam

A Principal Component Analysis (PCA) ordination displaying the similarity of the soils within the various blocks with regard to their particle size distribution is presented in Figure 5.15. The first ordination axis had an eigenvalue of 0.708 and thus accounted for 70.8% of the variance in the dataset. The eigenvalue represents the highest possible degree of correlation of all variables with the principal axes and thus is a measure of the amount of variation in the dataset accounted for by the first axis (Kent & Coker, 1992). The nursing blocks (NUR) were positively correlated with silt particles. The riparian forest samples (RIPFOR) which were collected outside the exclosure, showed a positive correlation with sand particles. Blocks A and B showed to have sandier soils based on the positive correlation with sand particles also reflected in their textural classification as a sandy loam. The PCA in Figure 5.15 indicates that blocks A and B actually share the most similarity in physical properties with that of the adjacent riparian forest area. Block C's strong correlation with clay is due to its location on a semi-wetland as mentioned previously.

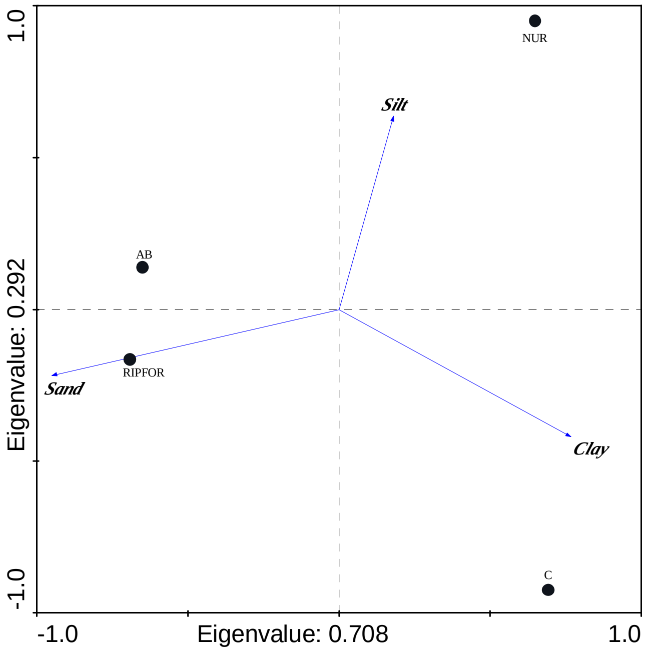


Figure 5.15. Principle Component Analysis (PCA) of the particle size distribution of the soils within each block inside the experimental exclosure (also including the riparian forest area outside the exclosure).

The particle distribution results therefore indicate that the soils within the exclosure are fairly heterogeneous with regard to texture. The specific vegetation which will regenerate naturally within the exclosure is correlated to the physical attributes of the soils within each block. All the species used for transplantation during this study were indigenous riparian species with a preference habitat close to rivers and on floodplains (Table 4.1). It is therefore essential to incorporate the various species and their habitat preferences, especially with regard to soil physical properties, when making recommendations for future restoration practices within the experimental exclosure and surrounding area (this aspect is further discussed in Chapter 6).

Table 5.7 contains the results from the soil chemical analysis for the various composite samples taken from each of the blocks inside the exclosure, as well as in the adjacent riparian forest area. The pH of the southerly situated blocks (AB and C) was more alkaline compared to the nursing blocks and riparian forest area. As mentioned earlier, the southern part of the exclosure has been most recently abandoned and these soils' alkalinity can most probably be attributed to the effect of irrigation during the many years of cultivation. The previously cultivated crops were irrigated with a center pivot irrigation system which pumped water from boreholes located in the nearby Limpopo Riverbed. The evidence of these pivot systems is still clearly visible in the more recently abandoned lands surrounding the experimental exclosure (Fig. 4.1). The Limpopo River has been reported to contain high levels of mineralization due to its drainage over an arid zone (Amaral & Sommerhalder, 2004). In addition to the river's mineralization content, large amounts of water was needed for irrigation due to the high levels of evaporation associated with Mapungubwe's semi-arid climate. During the years of irrigation the pure water evaporated leaving the salts behind to accumulate. Gypsum (calcium sulphate) was also found in the southerly blocks. Gypsum is generally applied in high-sodium soils in semi-arid regions with the aim of improving the physical condition of these soils (Brady & Weil, 2008). Although the southerly blocks' soils were alkaline, their sodium adsorption ratios (SAR) as well as those of the rest of the samples, were within a normal range not exceeding levels which could negatively affect plant growth (SAR > 13 will start showing a reduction in plant growth according to Brady & Weil, 2008). In the future, it is possible that the high pH in blocks A, B and C may become more buffered as leaf litter and other organic material accumulates (accumulation of humic and fulvic acids) with the establishment of large trees (Muthaiya & Felker, 1997).

According to the relationships existing between pH and the availability of plant nutrients (see Appendix 4 for the pH ranges effecting nutrient availability), all the macro-nutrients (Ca, Mg, K, P, N and S) will be amply available for plant uptake. The exception however is the availability of phosphorus, as this nutrient's availability is likely to be reduced in the moderately alkaline soils of block A, B and C (pH > 7.2). Between a pH of 6.8 and 7.2 (as present in the riparian forest area) the predominant form of phosphorus is HPO_4^-, which is moderately absorbed by plant roots. In alkaline soils however the predominant form is the trivalent PO_4^{3-}, which is essentially not available for uptake by plants (Hopkins & Hüner, 2004). The addition of nitrogen, particularly in the form of ammonium (NH_4^+) can increase this nutrient's uptake (Barber, 1984). The pH range of 5.5 to 7.0 provides the most satisfactory plant nutrients levels (Brady & Weil, 2008). Nutrient uptake will therefore not be constrained in the nursing blocks (NUR = pH 7.9) or the riparian forest area (RIPFOR = pH 6.8). Block C showed the highest concentrations of potassium (K= 399.0 mg/kg) compared to the other blocks within the exclosure due to its high clay fraction (Mengel & Kirkby, 2001). Calcium had the highest concentrations compared to the other macro-nutrients in all of samples. This is normal as calcium is the nutrient required by plants in the largest amounts (Hopkins & Hüner, 2004). The cation exchange capacity (CEC) is the sum total of exchangeable cations that a soil can absorb (Ashman & Puri, 2005) which means it directly impacts the nutrient supply of a soil. A higher CEC therefore indicates higher concentrations of nutrients such as calcium, magnesium and potassium. The riparian forest area had the highest CEC compared to the blocks inside the exclosure. The lower nutrient concentrations inside the exclosure were to be expected due to many years of nutrient depletion associated with previous agricultural practices. None of the macro-nutrient concentrations in any of the samples (Ca, Mg, K, Na and P) exceeded ranges which will be detrimental to plant growth (Brady & Weil, 2008; Marschner, 1995).

Table 5.7. The nutritional status of the soils within experimental exclosure (also including a composite sample from the riparian area outside the exclosure) when sampled in October 2008. The chemical symbols are explained in Appendix B.

Location	Ca	Mg	K	Na	P-Bray 1 (P)	SAR	CEC	Base saturation	$pH(H_2O)$
		(mg/kg)			(ppm)		(cmol(+)/kg)	(%)	
AB	3905.0	568.0	311.0	76.5	23.9	0.095	22.9	110.5	8.1
C	3339.5	1134.0	399.0	249.0	28.2	0.076	26.6	105.6	8.0
NUR	2814.5	787.5	55.0	142.0	17.6	0.194	20.9	104.4	7.9
RIPFOR	3235.0	876.0	1387.5	92.0	105.8	2.350	32.1	85.0	6.8

5.2.4.2 Soil water content

The mass soil water content (Θ_m) is measured by the ratio of the mass of water to dry soil, and was determined using the Gravimetric method (Brady & Weil, 2008) (refer to section 4.2 for the equation used in this method). The Θ_m was determined for composite samples collected in October 2008 in each block inside the exclosure. The Θ_m was determined in order to establish whether the "nursing" *A. tortilis* trees created favourable micro-habitats which will be more conducive for seedlings growth compared to open areas with no tree canopy present. Generally Θ_m ranges between 0.05 g/g and 0.50 g/g (gram of water per gram of dry soil) and soils with high organic matter or high clay contents can hold large amounts of water up to 0.50 g/g. The Θ_m for the relevant samples are presented in Table 5.8. Block C had the highest (0.10 g/g) and block D the lowest (0.06 g/g) Θ_m values respectively. The higher water content in block C is attributed to its higher clay fraction when compared to the other blocks. As mentioned previously, block C is situated in a semi-wetland area and swelling and crimping ("cracking") characteristics were observed during the field visits. The low water content in block D indicates that the presence of the thick *A. tortilis* stand did not create favourable micro-climate conditions. Instead of aiding the growth and establishment of the transplanted seedlings, its extensive root systems competed with seedlings for moisture and nutrients. Seedlings were transplanted at the edge of the tree's canopies where the "dripping effect" from the leaves and branches were expected to result in higher soil moisture contents when compared to open areas without any tree canopy. The *A. tortilis*' wide canopy could have a favourable shade function during hot days, which might have resulted in reduced transpiration and a lower water consumption of seedlings. The water potential or transpiration rates of seedlings were however not measured during this study. It is important to note that quite weak assumptions are derived from the Θ_m results in Table

5.8 as samples were collected only once in October 2008. It is strongly recommended that the number of sampling times should be increased if more valuable insight into the soil moisture patterns present inside the exclosure is to be gained.

Table 5.8. The mass soil water content (Θ_m) determined for composite samples collected in the various blocks inside the experimental exclosure.

Location inside the experimental exclosure	Θ_m (g/g)
Block A	0.09
Block B	0.09
Block C	0.10
Block D	0.06
Block E	0.09

5.2.4.3 Discussion

The soil within the exclosure is of a relatively good condition. The increased grass cover and presence of *A. tortilis* stand, are slowly but surely restoring important nutrient cycles, such as the carbon- and nitrogen cycle. It will however take a long time before all cycles are restored to that of the reference riparian forest area. This is true for the natural recovery of vegetation also as the thick grass stand is actually arresting successional progression by inhibiting germination of later successional species.

The soil moisture results were contrary to what was expected. Seedlings were transplanted around the *A. tortilis* trees with the aim of benefiting from the micro-environment and increased moisture levels created under the canopy of these trees. The results, however, showed that soil moisture was lower in the *A. tortilis* understory compared to the open blocks (Table 5.8). The survival data also indicated the highest occurrence of die-back within the nursing block. These results are contradicting to other studies which have found that the ability of *A. tortilis* to hydraulically lift water from deeper soil levels to surface soil, stimulating the growth of understory vegetation in an *A. tortilis* savannah (Labidi *et al.*, 2007; Ludwig *et al.*, 2003). This ability of *A. tortilis* is reported to enhance soil moisture around shallow fine roots, thus forming a suitable micro-environment around tree roots for mycorrhiza survival and growth. Previous research conducted explains the hydraulic lift effect that can take place in a typical savannah landscape with similar climate and soil types as the study site in Mapungubwe. The stand of *A. tortilis* trees inside the experimental exclosure however is much more densely distributed compared to a typical savannah. The roots of *Acacia* seedlings can reach a depth of 1.2 m after two months in dry conditions as a strategy to efficiently reach the fluctuating alluvial water table (Stave *et al.*, 2007). Due to the rigorous root system of *A. tortilis*, it is possible that its dense distribution inside the exclosure substantially increased root-competition between individual trees, thereby eliminating any hydraulic lift capabilities.

An additional effect on the low soil moisture content could be the distance of each block to the nearest remnant forest area that serves as a reference site. A study conducted by Pareliussen *et al.* (2006) on native Madagascan tree species, determined a significantly lower survival with an increase in distance from the reference site. The distance from existing forest fragments must therefore be taken into consideration when analysing the survival and growth of transplanted seedlings, as the rate of successful tree establishment should be higher in areas closer to forest fragments. Various other studies have also found that soil moisture was significantly lower in open areas than in closed forest gaps (Yohannes, 1999; Nepstad *et al.*, 1996).

5.3 Controlled environment (greenhouse trial)

5.3.1 Germination

The germination trial was conducted in pots in the greenhouse at the North-West University, Potchefstroom (section 4.1.2). Seeds from only five of the seven species used for transplantation during the field trial were germinated in the same enhancement treatments evaluated in the field. The amount of germinated seeds were counted and recorded every ten days over a period of four months (January 2009−April 2009), after which the seedlings inside each pot were thinned out leaving only a single seedling per pot in order to further analyze their growth and physiological performance. These seedlings from the greenhouse trials in Potchefstroom were not transplanted into the experimental exclosure in the Mapungubwe National Park.

5.3.1.1 Pre-sowing treatments

Various pre-sowing treatments, as described in section 4.1.2.2, were applied to seeds of five species (*B. discolor*, *C. imberbe*, *F. albida*, *P. violacea* and *X. zambesiaca*). An overview of the germination rates for all the species receiving the pre-sowing treatments and planted into the different enhancement treatments are presented in Table 5.9. To follow is a summary of the germination rates for each species with regard to pre-sowing treatments applied.

Berchemia discolor

All the seeds of *B. discolor* were soaked in hot water overnight and left unscarified. Seed in the mycorrhiza-compost treatment had the highest (62.5%) and fastest germination rate, as most of these seeds (41%) had already germinated after 30 days. The seeds in the control treatment had the lowest (37.5%) and slowest germination rate, with only 20.8% germinated after 30 days.

Combretum imberbe

All the seeds of *C. imberbe* were soaked in hot water overnight and either retained inside the four-winged fruit or removed (exposed). Seeds in the mycorrhiza-compost treatment which were left inside the fruit had the highest (25%) germination rate. Seeds in the mycorrhiza-compost treatment but exposed, had the fastest germination rate with 22.2% and all of the seeds germinated after 20 days. The seeds in the control treatment and left inside the fruit had the lowest (5.6%) and slowest germination rate with 2.8% of the seeds germinated after the first 20 days.

Faidherbia albida

All the seeds of *F. albida* were soaked in hot water overnight and either scarified or left unscarified. Seeds in the mycorrhiza-compost scarified and the compost-only scarified treatments had the highest germination rate (58.3%). Seeds in the mycorrhiza-compost scarified treatment also had the fastest germination rate, with 50% of the seeds germinated after the first ten days. Seeds in the mycorrhiza-only unscarified treatment had the lowest (25%) and slowest germination rate, with 8.3% of the seeds germinated after the first 20 days.

Philenoptera violacea

All the seeds of *P. violacea* were soaked in hot water overnight and either scarified or left unscarified. Seed in three treatments, namely compost-only scarified, compost-only unscarified and in the control unscarified, had the highest germination rate (83.3%). The seeds in the control unscarified treatment also had the fastest germination rate with 83.3% of the seeds germinated after 20 days. The seeds in the control scarified treatment had the lowest (33.3%) and slowest germination rate, with 33.3% of the seeds germinated after 20 days.

Xanthocercis zambesiaca

All the seeds of *X. zambesiaca* were soaked in hot water overnight and left unscarified due to this species' hard seed coat. Seeds in the mycorrhiza-compost unscarified treatment had the highest germination rate (8.3%). Seeds in the mycorrhiza-only unscarified treatment had the fastest germination rate, with 3.3% of the seeds germinated after 30 days. The seeds in the control unscarified treatment had the lowest (1.7%) and the slowest germination rate, with 1.7% of the seeds germinated after 30 days.

Table 5.9. An overview of the germination rates of five species (*Berchemia discolor*, *Combretum imberbe*, *Faidherbia albida*, *Philenoptera violacea* and *Xanthocercis zambesiaca*) receiving different pre-sowing treatments and planted into different enhancement treatments. Germination was monitored over a 90 day period. The species and treatment abbreviations are explained in Appendix A.

	Time (days)	10	20	30	40	50	60	70	80	90
Species	Treatment	Germination percentage (%)								
Berchemia discolor	MYCCOM, unscarified	0.0	0.0	41.7	58.3	62.5	62.5	62.5	62.5	62.5
	MYC, unscarified	0.0	0.0	29.2	33.3	41.7	45.8	50.0	54.2	54.2
	COM, unscarified	0.0	0.0	37.5	37.5	37.5	37.5	37.5	37.5	37.5
	CONTROL, unscarified	0.0	0.0	20.8	20.8	29.2	33.3	37.5	37.5	37.5
Combretum imberbe	MYCCOM, seed in fruit	0.0	13.9	25.0	25.0	25.0	25.0	25.0	25.0	25.0
	MYC, seed in fruit	0.0	5.6	8.3	8.3	8.3	8.3	8.3	8.3	8.3
	COM, seed in fruit	0.0	11.1	16.7	16.7	16.7	16.7	16.7	16.7	16.7
	CONTROL, seed in fruit	0.0	2.8	5.6	5.6	5.6	5.6	5.6	5.6	5.6
	MYCCOM, seed exposed	8.3	22.2	22.2	22.2	22.2	22.2	22.2	22.2	22.2
	MYC, seed exposed	2.8	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.9
	COM, seed exposed	5.6	16.7	19.4	19.4	19.4	19.4	19.4	19.4	19.4
	CONTROL, seed exposed	2.8	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.9
Faidherbia albida	MYCCOM, scarified	50.0	58.3	58.3	58.3	58.3	58.3	58.3	58.3	58.3
	MYC, scarified	25.0	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
	COM, scarified	33.3	58.3	58.3	58.3	58.3	58.3	58.3	58.3	58.3
	CONTROL, scarified	25.0	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
	MYCCOM, unscarified	0.0	8.3	16.7	25.0	25.0	33.3	33.3	33.3	33.3
	MYC, unscarified	0.0	0.0	8.3	25.0	25.0	25.0	25.0	25.0	25.0
	COM, unscarified	0.0	0.0	16.7	25.0	25.0	25.0	25.0	25.0	25.0
	CONTROL, unscarified	0.0	0.0	16.7	25.0	25.0	33.3	33.3	33.3	33.3
Philenoptera violacea	MYCCOM, scarified	8.3	58.3	66.7	75.0	75.0	75.0	75.0	75.0	75.0
	MYC, scarified	16.7	41.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7
	COM, scarified	16.7	50.0	75.0	75.0	75.0	75.0	83.3	83.3	83.3
	CONTROL, scarified	0.0	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
	MYCCOM, unscarified	8.3	66.7	66.7	75.0	75.0	75.0	75.0	75.0	75.0
	MYC, unscarified	8.3	58.3	58.3	58.3	58.3	58.3	58.3	58.3	58.3
	COM, unscarified	25.0	83.3	83.3	83.3	83.3	83.3	83.3	83.3	83.3
	CONTROL, unscarified	33.3	83.3	83.3	83.3	83.3	83.3	83.3	83.3	83.3
Xanthocercis zambesiaca	MYCCOM, unscarified	1.7	1.7	1.7	3.3	3.3	6.7	6.7	6.7	8.3
	MYC, unscarified	0.0	0.0	3.3	3.3	5.0	5.0	5.0	5.0	5.0
	COM, unscarified	0.0	0.0	1.7	1.7	1.7	1.7	1.7	3.3	5.0
	CONTROL, unscarified	0.0	0.0	1.7	1.7	1.7	1.7	1.7	1.7	1.7

P. violacea therefore had the highest and *X. zambesiaca* the lowest germination success, with 83.3% and 1.7% germination rates respectively. The mycorrhiza-compost treatment resulted in the highest germination rates in four of the species evaluated. For *P. violacea*, seeds in the compost-only treatment had the highest germination rate. More importantly is that the seeds in the control treatment had the lowest germination rates for most of the species, clearly indicating that the mycorrhiza-compost treatment were beneficial with regard to germination. As the arbuscular mycorrhiza fungi interact with the rhizophere of a plant (Braby & Weil, 2008; Larcher, 2003), it can be assumed that it is rather the addition of compost which was beneficial toward germination. The effect of the various treatments is further discussed in section 5.3.1.3.

5.3.1.2 Overall germination

The results in this section presented the germination rates of the five species cultivated in the greenhouse without differentiating between pre-sowing and enhancement treatments. Figure 5.16 shows that *P. violacea* had the highest (69.9%) and fastest overall germination rate compared to the other species, with 59.4% of its seeds germinated after 20 days. *X. zambesiaca* had the lowest overall germination percentage (5%) and slowest overall germination rate as well, with 2.1% of the seeds germinated after 30 days. A staggering 95% of the *X. zambesiaca* seeds therefore did not develop into seedlings. With the exception of *P. violacea*, all the other species had a germination rate below 50% after the 90 days monitoring period. The greenhouse provided ideal growing conditions, such as adequate watering and light, for the germinating seeds also excluding any stressors such as competition from other plants. The germination success of all the species investigated during the greenhouse trial is therefore expected to be much lower in the field where much harsher growing conditions are present.

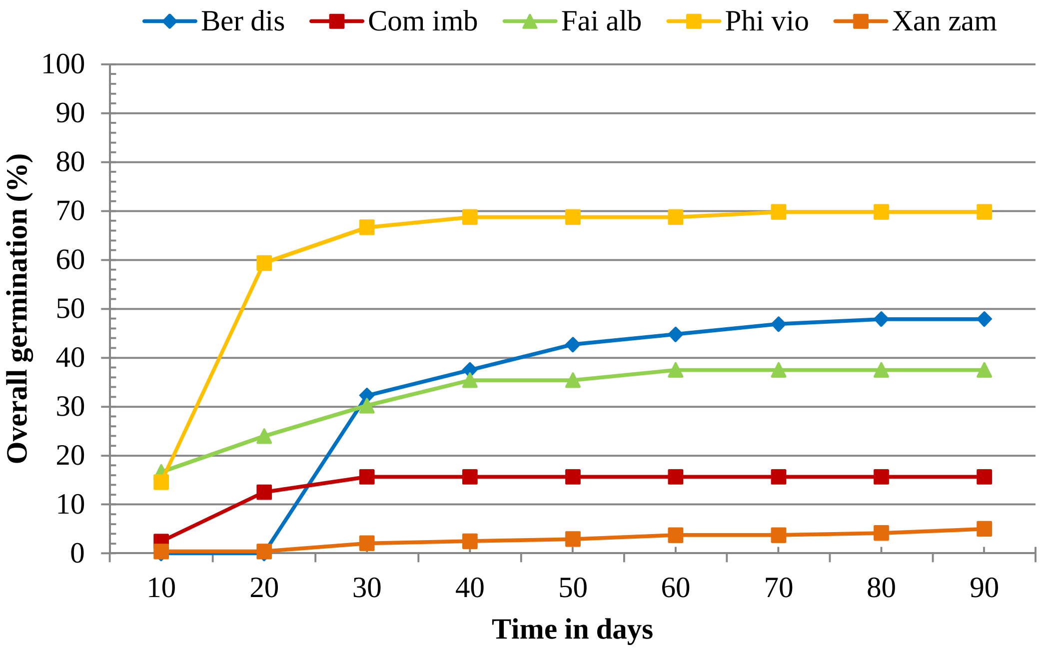


Figure 5.16. The overall germination percentage of five species (*Berchemia discolor*, *Combretum imberbe*, *Faidherbia albida*, *Philenoptera violacea* and *Xanthocercis zambesiaca*) cultivated in the greenhouse at Potchefstroom, irrespective of pre-sowing or enhancement treatment. Germination was monitored over a 90 day period. Species abbreviations are explained in Appendix A.

5.3.1.3 Different soil textural classes

The germination data discussed in the previous two sections were recorded in the greenhouse from seeds sown into pots in the greenhouse which were filled with soil collected inside the experimental exclosure at Mapungubwe (section 4.1.1.2). As discussed in section 5.2.4, the blocks inside the exclosure were classified into three different soil textural classes, namely a sandy loam in blocks A and B, a clay loam in block C, and a loam in blocks D and E (Table 5.6). In order to ensure that the layout of the greenhouse trial resembled that of the field trial as much as possible, the greenhouse trial was divided into three blocks representing the various soil textural classes as well. It was also part of the original layout to collect seeds of the same species used for transplantation into the field, and then cultivating the same species into the coordinating soil classes in the greenhouse as transplanted into the experimental exclosure. Unfortunately seeds of all the species used during the field trial were either not available, or not enough seeds of each species were available. Seeds of *S. australis* were not available and *A. xantophloea* seeds were collected inside the Mapungubwe National Park but suffered parasitic infection shortly before the start of the greenhouse trial. Consequently only three species, namely *C. imberbe*, *F. albida* and *X. zambesiaca*, were planted into correlating soil classes within the greenhouse, with *B. discolor* planted in the clay loam and *P. violacea* planted in the loam soil. The germination rates of these species within each soil class are presented in Figure 5.17a-c. *C. imberbe* had relatively similar germination ratios of less than 20% in all three soil types (sandy loam = 15.6%, clay loam = 14.6%, loam = 16.7%), and *X. zambesiaca* very low germination rates in all the soil classes - the highest (8.8%) were recorded in the sandy loam soil. *B. discolor* seeds were planted into only the clay loam soil, and also recorded the highest germination rate (47.9%) compared to the other species planted in this soil class. A similar trend was observed for *F. albida* and *P. violacea*, which recorded the highest germination rates of 37.5% and 69.8% in the sandy loam and loam soil respectively. *X. zambesiaca* had the lowest germination rate in all three soil classes.

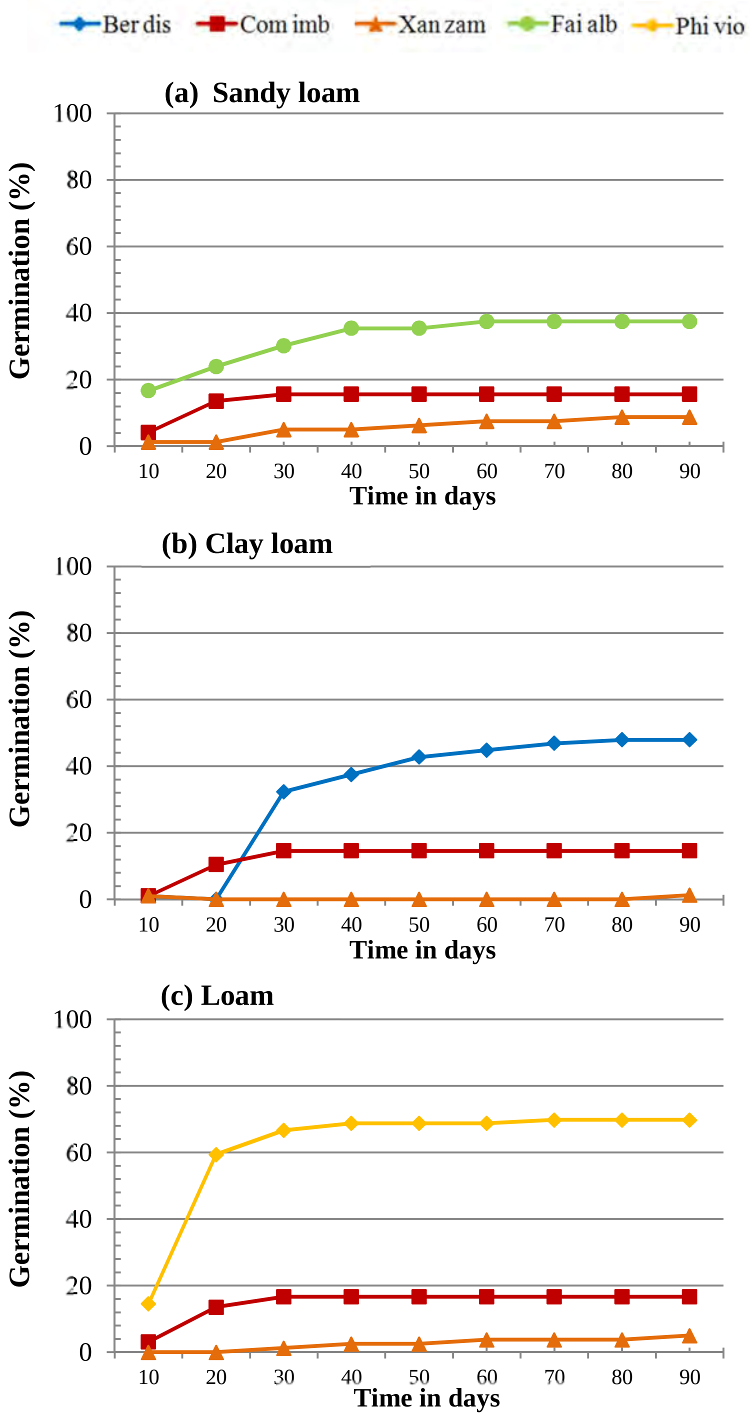


Figure 5.17. Germination rates of five species (*Berchemia discolor*, *Combretum imberbe*, *Faidherbia albida*, *Philenoptera violacea* and *Xanthocercis zambesiaca*), irrespective of enhancement or pre-sowing treatments, germinated in a (a) sandy loam, (b) clay loam and (c) loam soil in the greenhouse at Potchefstroom.

By comparing the germination rates of the various species to the same species' survival rates when transplanted into the correlating blocks inside the experimental exclosure, some conclusions can be made regarding the feasibility of using direct seeding as a possible restoration action to be taken in the MNP. *C. imberbe* had much lower germination rates in all three soil classes (sandy loam = 15.6%; clay loam = 14.6%; loam = 16.7%) in the greenhouse trial compared to its survival rates in the correlating blocks inside the exclosure (block A = 50%; block C = 56.3%; block D and E = 51.3%)(Table 5.1). This was the case for *X. zambesiaca* also, with much lower germination rates (sandy loam = 8.8%; clay loam = 1.3%; loam = 5.0%) compared to its survival rates in the correlating blocks inside the exclosure (block A = 90.0%; blocks D and E = 25.0%) (Table 5.1). *F. albida* also had a much higher germination rate (sandy loam = 37.5%) compared to its survival rate in the correlating block inside the exclosure (block A = 70.0%). *B. discolor* and *P. violacea* could not be compared to their survival rates in the field as these two species were not planted into corresponding soil classes in greenhouse. All of the comparable species therefore recorded much higher survival rates in the field compared to germination rates in the greenhouse. As mentioned earlier, due to the exclusion of many factors such as drought stress and competition in the greenhouse, all the investigated species' germination rates will be much lower in natural field conditions. Direct seeding should therefore not be considered as a restoration action in the MNP.

5.3.1.4 Discussion

As seeds were germinated in soil collected from inside the experimental exclosure at Mapungubwe, the pot-trials served therefore as a simulation of real germination rates which can be expected if seeds of the five species were sown into the experimental exclosure. Influences such as moisture stress, competition and seed predation were however eliminated in the greenhouse. *P. violacea* had the fastest and highest germination rate compared to the other four species. This species' high germination rates are reflected by its high establishment rate in the natural environment, as it is one of the dominant species present in the riparian forest zone (Götze, 2002). The high germination and establishment of this species are related to its drought-tolerant properties (Table 4.1). *X. zambesiaca* had the lowest and slowest germination rate, mostly related to its hard seed coat. Establishment of this species under natural conditions is low, based on its scarce distribution in the riparian forest zone (Götze, 2002).The hard seed coats of *X. zambesiaca* were not scarified due to labour and time constraints. Seeds of *X. zambesiaca* had the hardest seed coat of all five species evaluated. The permeability to water was therefore of an imminent greater challenge for this species. In nature, hard seed coats are made permeable to water through mechanisms such as the ingestion by animals (Gardener *et al.*, 1993; Russi *et al.*, 1992; Lamprey *et al.*, 1974), abrasion in unstable soil (Gutterman, 1993), fire (Sabitii & Wein, 1987), and soil acids and micro-organisms (Bewley & Black, 1994). The protected *C. imberbe* also showed very low germination rates, emphasizing the conservation of remnant *C. imberbe* trees, as well as the establishment of suitable habitat for this species.

Various studies have found an increase in the germination of certain savannah tree species when soaked in warm water (Argaw *et al.*, 1999; Demel, 1996). This pre-sowing treatment also worked best during this study's greenhouse trials, even though no comparisons were made to a "no soaking" control. Scarified and unscarified seeds of *F. albida* and *P. violacea*, including both exposed and unexposed seeds of *F. albida*, showed no significant differences in germination rates. These results can be viewed as an indication that these two species are able to readily germinate in a soil medium in a relatively short period of time without any pre-sowing treatment applied, provided the presence of appropriate environmental conditions such as adequate moisture and light. Although *F. albida* scarified seeds showed higher germination rates, it was not statistically significant. Scarification, whether mechanical or chemical, have been shown to improve germination in legume seeds (Argaw *et al.*, 1999; Demel, 1996; Tybirk, 1991; Doran *et al.*, 1983). As *F. albida*, often named *Acacia albida* (Van Wyk & Van Wyk, 2007), is a member of the legume family, it was expected that scarified seeds will have a higher germination rate compared to unscarified seeds. The seedlings used for transplantation into the field were cultivated in the Rhodesdrift Nursery situated inside the MNP (section 4.1.1.2). These seeds were germinated in river sand and no pre-sowing treatments were applied. Unfortunately no records are available of the germination success of the various species in the nursery. In order to ensure higher germination rates during cultivation, the appropriate pre-sowing treatments as determined during the greenhouse trials are recommended to be applied in future cultivation practices inside the Rhodesdrift nursery. These recommendations are further discussed in Chapter 6.

The mycorrhiza-compost treatment resulted in the highest germination rates of most of the species evaluated during the greenhouse trials. This enhancement treatment therefore created the most favourable conditions for seed germination. As mentioned earlier, the mycorrhiza interacts with the rhizosphere of a plant (Brady & Weil, 2008; Larcher, 2003). As the germinating seeds have yet to develop a rhizophere, it can be assumed that the compost most likely played a bigger role in enhancing germination. The presence of compost increased porosity and infiltration in the pot soils, thereby creating minimum resistance for a germinating seed compared to a dense and compacted soil with a tendency to form surface crusts which hinders movement of the seedling during germination (Brady & Weil, 2008). It seems that the increased infiltration caused by the compost also increased soil moisture which is one of the most important triggers for seed germination. These findings are supported by various studies which found that the addition of compost improved degraded soils within semi-arid areas, also having beneficial effects on the growth of indigenous arbuscular mycorrhizal fungi in the nutrient-limited soils (Caravaca *et al.*, 2002; Gaur & Adholeya, 2002).

Based on the investigated species’ low germination rates, it can be concluded that the transplantation of seedlings will be more effective than direct re-seeding when attempting to restore these species in the deforested areas along the Limpopo River. Similar conclusions are made by a study conducted by Dayamba (2010) in the woodlands of Burkina Faso which found that direct seeding frequently failed due to high mortality of both seeds and seedlings, especially when exposed to drought, fire and herbivory. The low germination rates of the climax forest species found during the greenhouse trial explains the slow rate of natural succession inside the exclosure. The low germination results are supported by Moe *et al.* (2009) which found that species such as *F. albida*, *C. imberbe* and *P. violacea* show little signs of regeneration over the long-term, suggesting either a lack of viable seeds or low seedling survival. The introduction of seeds should, however, not be totally discarded as even a small regeneration percentage could still contribute to local diversity and soil enhancement processes inside an exclosure.

5.3.2 Survival

5.3.2.1 Seedlings cultivated in pots

As discussed in the previous sections, only five (*B. discolor*, *C. imberbe*, *F. albida*, *P. violacea* and *X. zambesiaca*) of the seven species used for transplantation during the field trials, were cultivated from seeds in the greenhouse of the North-West University, Potchefstroom. Five or six seeds were sown per pot depending on seed size (refer to Chapter 4). After the germination trial was completed, seedlings were thinned out to a single seedling per pot in order to avoid competition for moisture and nutrients between seedlings. Seeds were originally sown into a total of 164 pots representing the three soil textural classes present in the exclosure (sandy loam, clay loam and loam) (section 5.2.4 and 5.3.1.3). Due to the low germination rates of all the species (Table 5.9), only 87 seedlings were available to monitor throughout the rest of the study period. Seventy one of these seedlings (81.6%) were still alive on the last measuring date in September 2009 (Table 5.10).

As mentioned in Chapter 4, a two week drought simulation period was initiated in early June 2009, during which the previously well-watered seedlings did not receive any water. Regular watering continued thereafter. The drought simulation period was carried out to monitor the effect of drought on the various species' survival, growth and physiological performance. Both *P. violacea* and *X. zambesiaca* had a 100% survival rate throughout the study period (Table 5.10). *C. imberbe* had the lowest survival rate of 63.3% on the last measuring date in September 2009. Seedlings planted in the clay loam recorded the lowest survival rate (65.2%) compared to seedlings planted in the other two soil classes.

Table 5.10. The total survival rate (%) of five species cultivated in pots at the greenhouse in Potchefstroom, irrespective of enhancement or pre-sowing treatments applied. The seedlings were monitored over a six month period (April – September 2009). The species abbreviations are explained in Appendix A.

	Survival (%)			
Species	**Sandy loam**	**Clay loam**	**Loam**	**Total**
Berchemia discolor	-	75.0	-	75.0
Combretum imberbe	70.0	50.0	70.0	63.3
Faidherbia albida	87.5	-	-	87.5
Philenoptera violacea	-	-	100.0	100.0
Xanthocercis zambesiaca	100.0	100.0	100.0	100.0
Average survival (%)	**84.4**	**65.2**	**90.6**	**81.6**

(- indicates that seedlings of the specific species were not planted into that soil class)

The survival rate of each species in the various enhancement treatments are presented in Figure 5.18. This figure indicates that there was no trend with regard to a specific enhancement treatment ensuring higher survival rates when compared over species. *B. discolor* and *F. albida* seedlings planted into the control treatment had the lowest survival rates (50.0% and 75.0% respectively), with the lowest survival rate recorded for *C. imberbe* in the mycorrhiza-compost treatment (30.0%).

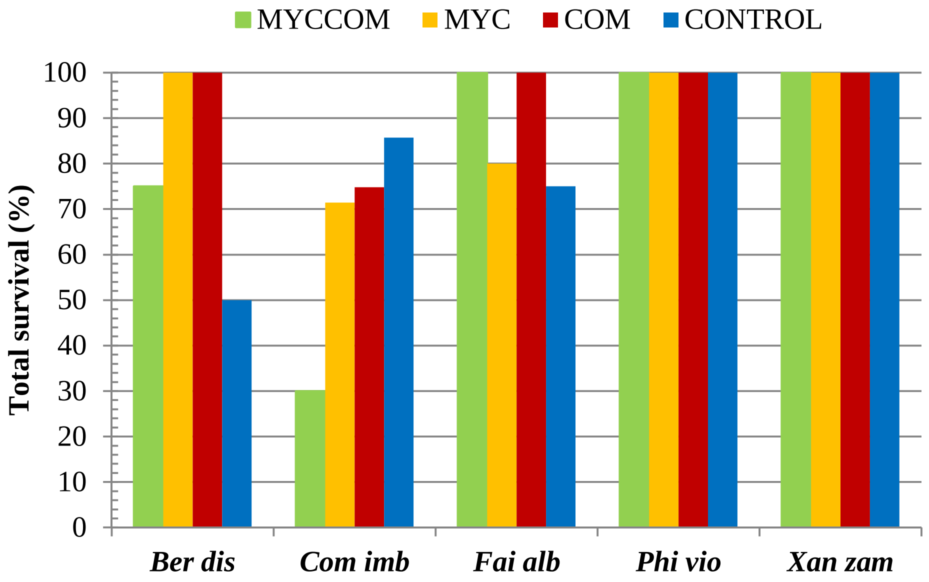


Figure 5.18. The total survival rate (%) of five species planted into different enhancement treatments in pots at the greenhouse in Potchefstroom. Seedlings were monitored over a period of six months (April – September 2009). The species and enhancement treatment abbreviations are explained in Appendix A.

The mortalities recorded in the pot-trials were most likely due to water-stress experienced by the seedlings during the two week drought simulation period. Any other factors which could possibly negatively influence survival were eliminated (e.g. pots were kept clean from any weeds or other vegetation to ensure seedlings did not compete with other plants for moisture or nutrients). The drought-induced mortalities are confirmed in the cumulative survival trends of the various species which is presented in Figure 5.19a-c. The survival trends of *P. violacea* and *X. zambesiaca* are not shown, as these two species had a 100% survival rate throughout the study period. The two-week drought period was initiated in early June 2009. *B. discolor*, *C. imberbe* and *F. albida* started showing a decrease in cumulative survival only after the drought period. The leaves of all the died-off seedlings were recorded to wilt and dry out in the second week of the drought period. All the seedlings of *B. discolor*, *C. imberbe* and *F. albida* senesced their leaves during the month following the drought period. New leaves were however recorded on the surviving seedlings in September 2009. *P. violacea* and *X. zambesiaca* seedlings did not senesce any leaves nor did these seedlings push out new leaves during the study period.

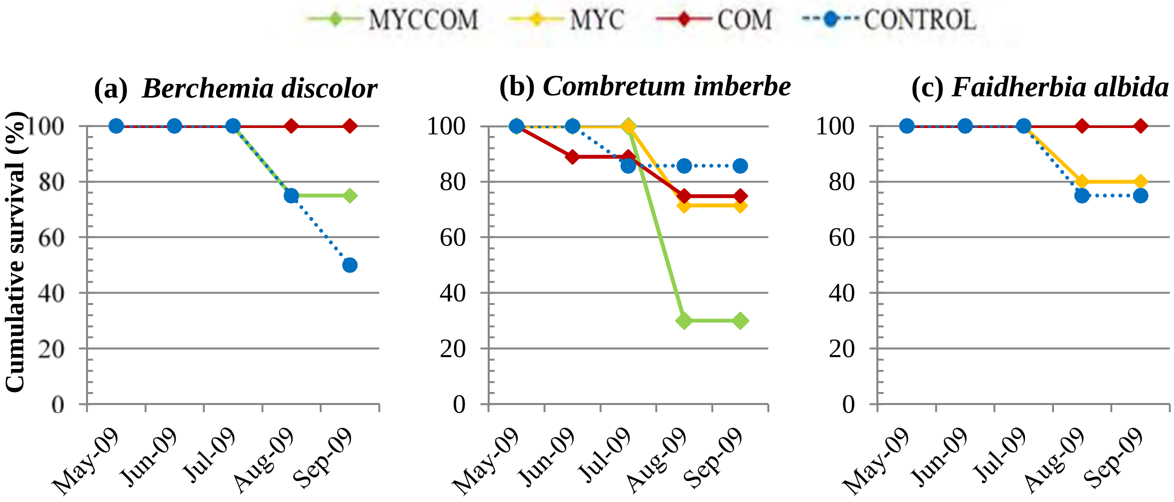


Figure 5.19. The cumulative survival percentage three species cultivated in different enhancement treatments in pots at the greenhouse in Potchefstroom. (a) *Berchemia discolor*, (b) *Combretum imberbe* and (c) *Faidherbia albida*. Seedlings were monitored over a period of six months (April – September 2009). The survival trends of *Philenoptera violacea* and *Xanthocercis zambesiaca* are not shown as these two species had a 100% survival rate throughout the study period. The enhancement treatment names and abbreviations are explained in Appendix A.

5.3.2.2 Discussion

The much higher overall survival rates of the seedlings cultivated in the greenhouse (81.6%) compared to the seedlings transplanted into the experimental exclosure (55.5%), indicate that the greenhouse conditions were much more conducive for growth compared to the harsh conditions in the field (Table 5.1 and 5.10). None of the enhancement treatments stood out as being beneficial with regard to survival in any of the species evaluated when compared to the control treatments. What stood out however is the drought-resistant characteristic of *P. violacea* and *X. zambesiaca*, which recorded a

100% survival rate throughout the study period despite the two week drought period. The six month monitoring period was however too short and long-term trials are recommended to confirm the drought-resistant characteristics of the various species as well as the possible beneficial effects of the enhancement treatments on their survival.

5.3.3 Growth

5.3.3.1 Seedlings cultivated in pots

Growth data were collected from the same amount of seedlings (n = 71) and species as used to calculate survival in the previous section. These seedlings came from the seeds used for the germination trials discussed in section 5.3.1 and growth measurements were conducted once every month for a period of six months (April – September 2009). The same growth parameters measured during the field trial were also measured on the greenhouse seedlings (section 5.3.1). The majority of the seedlings cultivated in the pots did not reach 30 cm in height and therefore only growth data from the ‘stem diameter at the base’ parameter are presented in this section. The seedlings in the greenhouse were cultivated under optimum growing conditions with the exception of a two week drought simulation period during which the previously well-watered seedlings did not receive any water (Chapter 4). Regular watering continued thereafter. Growth data collected from seedlings planted into the three soil classes (sandy loam, clay loam and loam – see section 5.3.1.3) were compiled into one dataset in order to increase the sample size. The diameter growth rate at the base for each of the five species planted into the various enhancement treatments, were calculated by the difference in growth between the first (April 2009) and last (September 2009) monitoring dates. As with the field data, the growth data of the potted seedlings were tested for Gaussian distribution, and failed (Shapiro Wilks test, $p < 0.05$). The non-parametric Kruskal-Wallis test was conducted with Dunn’s procedure for group comparisons with unequal sample sizes in order to establish whether significant differences exist, and to identify the treatments in which these significant differences occur. The Kruskal-Wallis test revealed that only *F. albida* had a possible significant difference ($p = 0.031$) in its diameter growth median values between the different enhancement treatments ($p < 0.05$), but Dunn’s post-hoc test determined that there were no significant difference (Table 5.11). The p-values for the five species evaluated in the greenhouse were much smaller (with the exception of *X. zambesiaca* seedlings) compared to the p-values for the field trial which is explained by the smaller variation within the pot-trials’ dataset (Table 5.3).

Table 5.11. Statistical output given by the Kruskal-Wallis test, where the mean diameter growth rate at the base for five species over a period of seven months are compared to the control for each species. (significance $p < 0.05$) (N is the number of plants per sample and $\chi^2_{K\text{-}W}$ is the chi-square value).

Species	N	$\chi^2_{K\text{-}W}$	Sig. (*p*-value)
Berchemia discolor	18	3.505	0.320
Combretum imberbe	54	3.711	0.294
Faidherbia albida	18	8.902	**0.031**
Philenoptera violacea	18	6.211	0.102
Xanthocercis zambesiaca	53	0.913	0.822

As with the field data, an ANOVA was used to compare the means of the diameter growth rates at the base for each species and treatment. High variation and small and unequal sample size in the dataset resulted in big standard errors for all five species-treatments combinations (Fig. 5.20). The overall diameter growth rate at the base for species cultivated in the greenhouse was ranked in the following manner: *B. discolor* (0.881 mm/6 months) > *C. imberbe* (0.520 mm/6 months) > *F. albida* (0.518 mm/6 months) > *P. violacea* (0.489 mm/6 months) > *X. zambesiaca* (0.105 mm/6 months) (Fig. 5.20). As mentioned in section 5.2.2, growth rate is a species-specific trait. The growth rates can therefore not be compared across species but should rather be compared between the various enhancement treatments within each species. All the species-treatment combinations showed a positive mean diameter growth rate at base during the six month study period, with the exception of *P. violacea* seedlings in the mycorrhiza-only treatment. *B. discolor* and *C. imberbe* seedlings showed the highest diameter growth rate in the compost treatment (1.76 mm/6 months and 0.94 mm/6 months respectively). The mycorrhiza-compost treatment resulted in the highest diameter growth rates for *F. albida* (1.07 mm/6 months) and *P. violacea* (1.25 mm/6 months). *X. zambesiaca* had the overall lowest diameter growth rate at the base, with the mycorrhiza-only treatment resulting in slightly higher growth rates compared to the other treatments (0.2 mm/6 months). The seedlings in either the mycorrhiza-compost or the compost-only treatments showed the highest diameter growth rates at the base for most of the species – the exception being *X. zambesiaca* in the mycorrhiza-only treatment. This indicated that the enhancement treatments, especially the compost containing treatments, were beneficial with regard to growth for most of the species investigated.

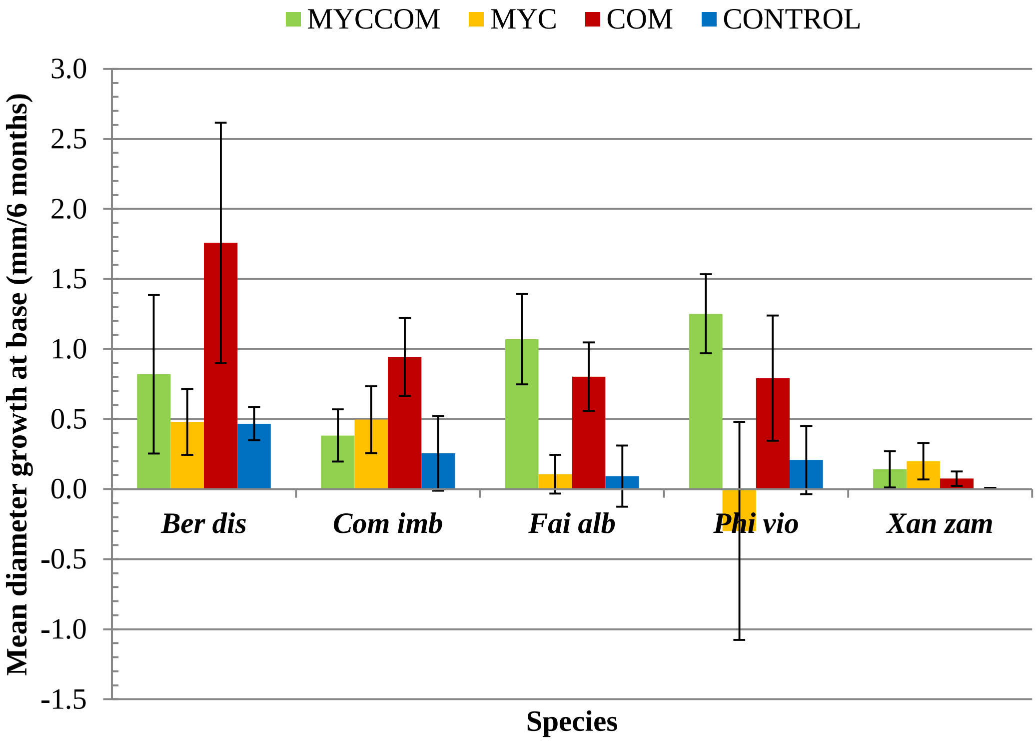
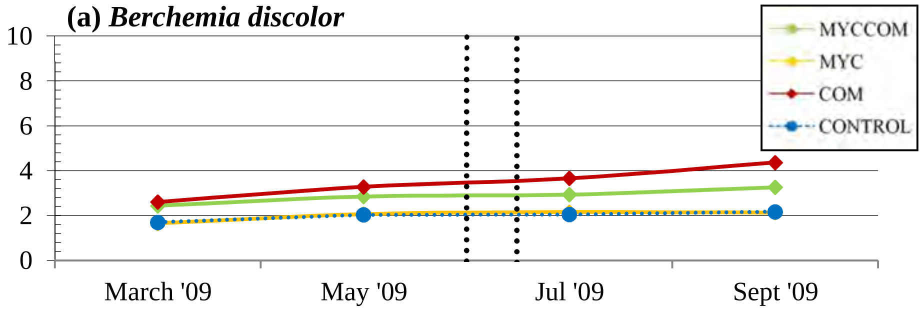


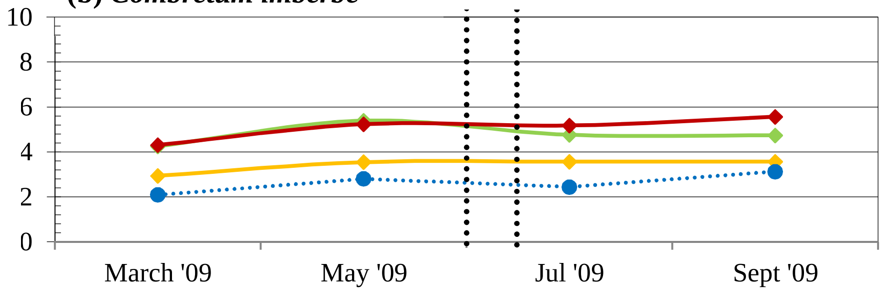
Figure 5.20. A comparison of the mean diameter growth rates at the base for five species (*Berchemia discolor*, *Combretum imberbe*, *Faidherbia albida*, *Philenoptera violacea* and *Xanthocercis zambesiaca*) planted into four different enhancement treatments in the greenhouse at Potchefstroom. Growth was measured over a six month period (April − September 2009). Note that there were no significant differences between the enhancement treatments ($p < 0.05$). The species and enhancement treatment names and abbreviations are explained in Appendix A. (The bars represent standard errors).

The temporal trends in diameter growth at the base for the five species cultivated in the greenhouse are presented in Figure 5.21. The two week drought period which took place in early June 2009 is also indicated on the growth trend graphs. All the species, irrespective of treatment, showed an increase, although very slight, in stem diameter at the base during the months before the onset of the two week drought period. Three of the species, namely *B. dicolor*, *C. imberbe* and *P. violacea*, showed an increase in stem diameter increments during the months following the drought period but only when planted into the compost containing treatments. *C. imberbe* seedlings in the compost-only treatment also showed a diameter increase during this period, but the biggest increase for this species occurred when planted into the control treatment. *X. zambesiaca* showed a continual increase in diameter at the base when planted into the mycorrhiza-only treatment. The results therefore indicate that the addition of compost might have lead to a higher drought-tolerance within most of the species, as most species in the control treatment had the lowest diameter at base increments throughout monitoring period.

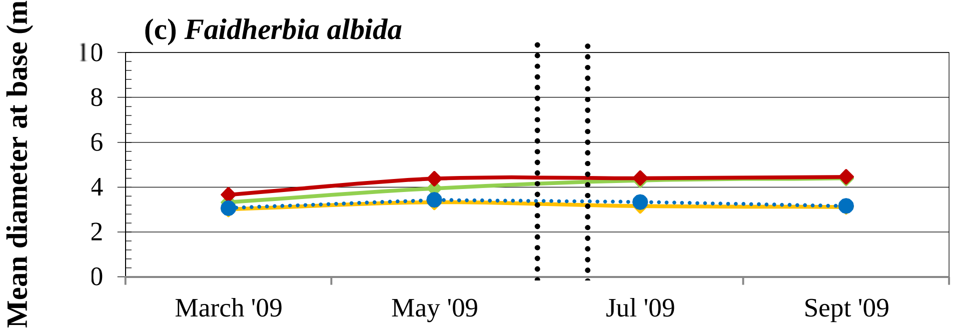
(a) ***Berchemia discolor***



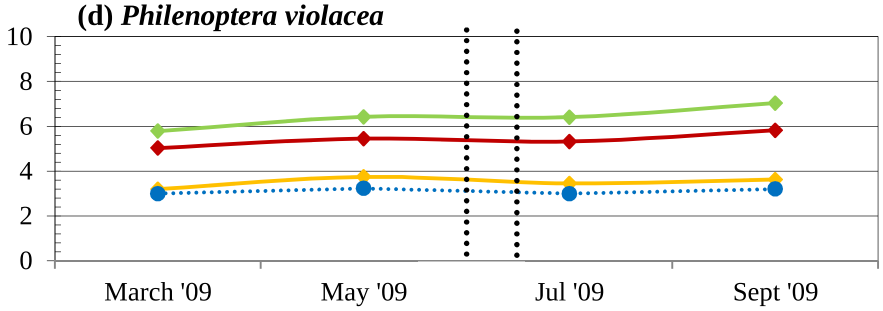
(b) ***Combretum imberbe***



(c) ***Faidherbia albida***



(d) ***Philenoptera violacea***



(e) ***Xanthocercis zambesiaca***

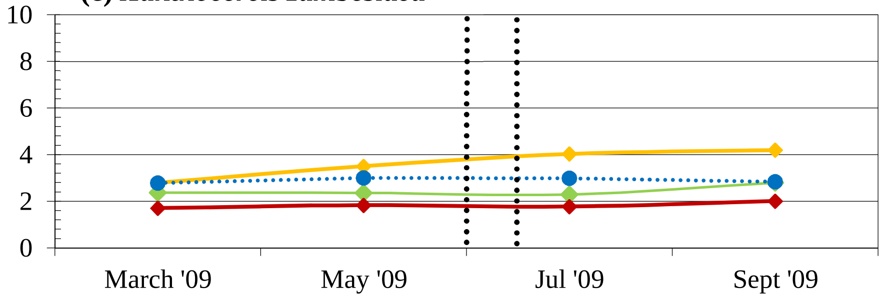


Figure 5.21. Trends in the mean diameter at the base increments of five species cultivated in the various enhancement treatments inside the greenhouse at Potchefstroom, where (a) *Berchemia discolor*, (b) *Combretum imberbe*, (c) *Faidherbia albida*, (d) *Philenoptera violacea* and (e) *Xanthocercis zambesiaca*. The seedlings were monitored over a six month period (April – September 2009). The dotted lines indicate the onset of the two week drought simulation period on 4 June 2009 and the commencement of re-watering on 18 June 2009. The enhancement treatment names and abbreviations are explained in Appendix A.

5.3.3.2 Discussion

The mean stem diameter growth rates at the base were highest for all the species when planted into the compost containing treatments, with the exception of *X. zambesiaca* which responded best to the mycorrhiza-only treatment (Fig. 5.20). The mean diameter growth rates were also positive for all the species-treatment combinations, with the exception of *P. violacea* in the mycorrhiza-only treatment. The temporal trends in stem diameter increments at the base also indicated that all the species planted into the compost-containing treatments, with the exception of *X. zambesiaca*, increased in stem diameter throughout the monitoring period even after being exposed to the two week drought period. Most of the species cultivated in the greenhouse therefore experienced higher and more rapid growth when planted in the compost containing treatments. The sewage sludge component of the woodchip compost used during this study (see section 4.1.1.3) have been shown to be a source of organic matter and plant nutrients (Martinez *et al.*, 2003; Brockway, 1983) The organic matter provided by the compost most likely increased the water-holding capacity of the pot soils (Brady & Weil, 2008; Logan, 1992), making seedlings more resilient to water stress during the two week drought period. The added arbuscular mycorrhiza resulted in higher growth rates only when applied in combination with compost. This result indicates a possible interaction between the mycorrhiza fungi and the nutrients provided by the compost. The water-holding capacity and nutrient status of the pot soils were unfortunately not analysed due to time and economic constraints.

The growth rates of the various species cultivated in the greenhouse are supported by literature regarding the propagation of these species (Table 4.1). An example is *X. zambesiaca* which is classified as a slow grower in literature and also recorded the lowest growth rates during the greenhouse trial (Van Wyk & Van Wyk, 2007). *F. albida* however was an exception as this species is classified as a fast grower and actually recorded lower growth rates compared *B. discolor* and *C. imberbe* during the greenhouse trials. *F. albida* has a deep root system and prefers deep alluvial soils with a high water table (Table 4.1). It is therefore likely that the root growth of this species was inhibited in the greenhouse by the container size used (25 L pots, see section 4.1.2.2) as well as the two week drought period. Stave *et al.* (2005) conducted a comparative study between *A. tortilis* and *F. albida* seedlings and found that both species elongated their roots under simulated drought conditions, but that only *A. tortilis* could withstand the prolonged drought simulation.

The six months study period was however much too short to adequately evaluate the effect of the various treatments on the growth of the five species, as well as the treatments' benefit with regard to drought tolerance.

5.3.4 Plant vitality

5.3.4.1 Seedlings cultivated in pots

Chlorophyll fluorescence was measured on the same seedlings and species used to monitor survival and growth in the greenhouse, as discussed in the previous sections. Physiological monitoring took place once a month from May to September 2009. In June 2009, however, physiological monitoring occurred twice in order to evaluate the effect of the two week drought simulation period on the physiological performance of the seedlings (refer to section 5.3.2 and 5.3.3 for further information regarding the objectives of the simulated drought conditions). The monitoring therefore coincided with the initiation of the drought period on 4 June 2009 and then again at the end of the drought period on 15 June 2009. As discussed in section 5.2.3, the multi-parametric performance index (PI_{ABS}) expression was used to quantify PSII behaviour and served as a metric for the overall vitality of each species-treatment combination. The fluorescence data were processed by means of Biolyzer software as discussed in section 4.1.1, and the mean PI_{ABS} values for each species-treatment combination were determined (Fig. 5.22). Temporal trends in PI_{ABS} for each species-treatment combination were also determined (Fig. 5.24).

The mean performance index values for each species, irrespective of treatment, are presented in Table 5.12. As mentioned in section 5.2.3, performance index values are species-specific and can therefore not be compared across species. Bearing this in mind, *X. zambesiaca* had the highest (PI_{ABS} = 80.15) and *F. albida* the lowest (PI_{ABS} = 36.62) mean performance index values respectively.

Table 5.12. The mean performance index (PI_{ABS}) and standard error (±S.E.) values of the five species cultivated in pots in the greenhouse, irrespective of enhancement treatment planted into, measured over a five month period (May – September 2009).

Species	Mean performance index (PI_{ABS})	±S.E.
Berchemia discolor	43.38	6.98
Combretum imberbe	61.31	5.70
Faidherbia albida	36.62	5.97
Philenoptera violacea	48.49	5.03
Xanthocercis zambesiaca	80.15	16.30

Figure 5.22 presents the mean PI_{ABS} values for each species-treatment combination during the five month monitoring period. Significant differences between the enhancement treatments compared to the control were determined with the Student t-test at a 95% probability level ($p < 0.05$). Seedlings of three species, namely *B. discolor*, *F. albida* and *P. violacea*, had significantly higher PI_{ABS} values when planted into an enhancement treatment compared to the control. *F. albida* and *P. violacea* were the only two species to show significantly higher PI_{ABS} values for all three enhancement treatments. In contrast, *C. imberbe* seedlings in all three enhancement treatments had significantly lower PI_{ABS} values

when compared to the control. For *B. discolor*, only the seedlings planted into the mycorrhiza-compost treatment showed significantly higher PI_{ABS} values compared to the control – for this species the seedlings in the control had significantly higher PI_{ABS} values compared to the mycorrhiza-only treated seedlings. *X. zambesiaca* seedlings had significantly lower PI_{ABS} values compared to the control only when planted into the mycorrhiza-compost treatment. The vitality of the greenhouse seedlings therefore indicate that the beneficial effect of the various enhancement treatments varied across species, with most of the species functioning at higher vitality levels when planted into the enhancement treatments.

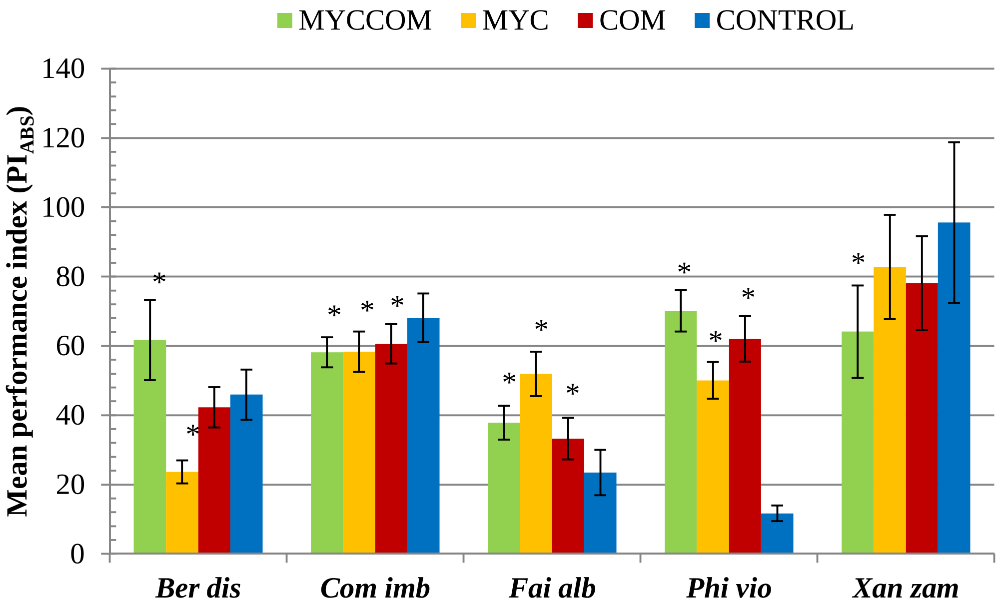


Figure 5.22. The mean performance index (PI_{ABS}) of five species (*Berchemia discolor*, *Combretum imberbe*, *Faidherbia albida*, *Philenoptera violacea* and *Xanthocercis zambesiaca*) planted into four different enhancement treatments inside the greenhouse at Potchefstroom, measured from May to September 2009. The enhancement treatment names and abbreviations are explained in Appendix A. (The bars represent standard errors and findings significantly different from the control are indicated with an asterisk at a 95% probability level according the Student's t-test).

In Figure 5.23, the mean performance index (PI_{ABS}) of the five species cultivated in the various enhancement treatments are compared to the corresponding means of species cultivated in the control treatment. This direct comparison of the response of the different species to the enhancement treatments clearly indicates that *F. albida* and *P. violacea* were the only species to show an overall positive deviation in performance index values (PI_{ABS}) compared to the control treatment. *B. discolor* was the only species to show both a positive and negative deviation for the various enhancement treatments relative to the control treatment. *F. albida* and *P. violacea* are therefore the only two species to have truly benefited from the various enhancement treatments with regard to overall vitality.

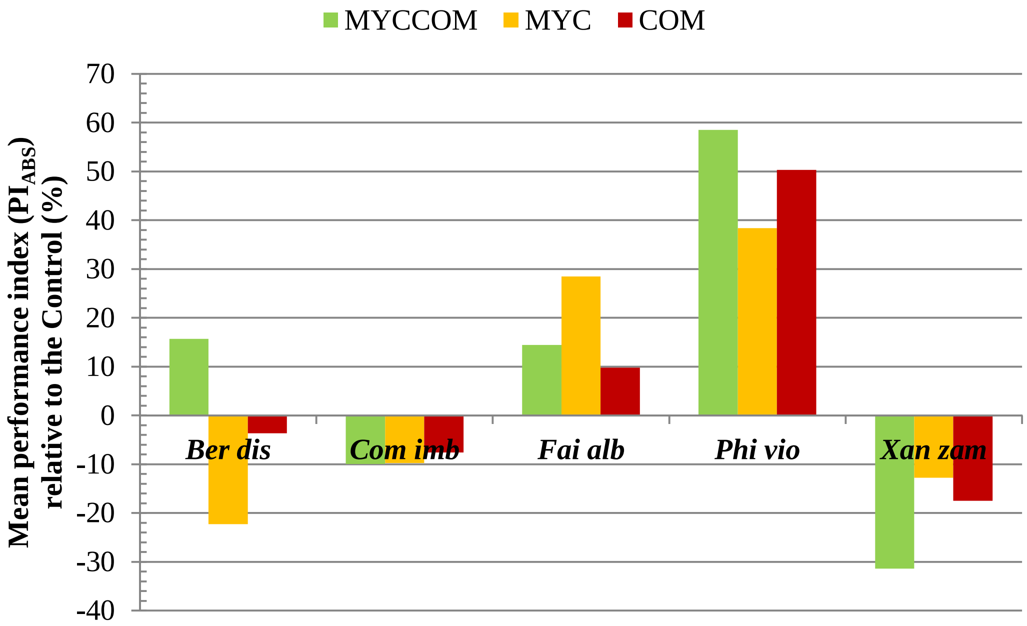


Figure 5.23. The deviation in mean performance index (PI_{ABS}) values relative to the control of five species (*Berchemia discolor*, *Combretum imberbe*, *Philenoptera violacea*, *Salvadora australis* and *Xanthocercis zambesiaca*) cultivated in the various enhancement treatments in pots at the greenhouse in Potchefstroom. The treatment names and abbreviations are explained in Appendix A.

The two-week drought simulation period may have influenced the responsiveness of certain species to the effect of the enhancement treatments. The effect of the drought period on the relative performance index in the leaves of the various species, are presented in Figure 5.24. The temporal trend for each species will now be discussed separately.

Berchemia discolor

Seedlings in all of the treatments, with the exception of the compost-only treatment, showed a gradual increase in PI_{ABS} during the months prior to the two week drought period (Fig. 5.24a). PI_{ABS} values dropped for seedlings in all of the enhancement treatments during the months following the drought period, with only seedlings in the control showing an increase during this time. *B. discolor* seedlings in the control treatment were therefore the only seedlings not negatively affected by the drought period.

Combretum imberbe

The *C. imberbe* seedlings followed a synchronized trend in PI_{ABS} values for all four treatments evaluated (Fig. 5.24b). Seedlings in all of the treatments increased in PI_{ABS} during the months prior to the two week drought period. All the seedlings, irrespective of treatment, dropped in PI_{ABS} during the months following the drought period with seedlings in the control treatment showed the smallest decrease during this time. The synchronized drop in PI_{ABS} for all the treatments indicate that *C.*

imberbe is quite drought-sensitive compared to the other species, with none of the enhancement treatments being beneficial with regard to vitality during the drought period.

Faidherbia albida

Seedlings in all of the treatments, with the exception of the control treatment, increased in PI_{ABS} values during the months following the two week drought period (Fig. 5.24c). Seedlings in the mycorrhiza-only treatment showed a notably bigger increase compared to the other treatments. Seedlings in the control were the only seedlings to show a slight drop in PI_{ABS} during the first two months following the drought period, increasing in vitality again to the end of the monitoring period. The overall trend therefore indicates that *F. albida* is quite drought-resistant compared to the other species, with the mycorrhiza-only treatment benefiting seedlings with regard to vitality.

Philenoptera violacea

All the *P. violacea* seedlings, irrespective of treatment, followed a synchronized trend in PI_{ABS} throughout the monitoring period (Fig. 5.24d). All the seedlings in all of the treatments dropped in PI_{ABS} during the month prior to the drought period. All the seedlings increased in PI_{ABS} during the two week drought period, where after seedlings dropped in PI_{ABS} again. Seedlings planted into the compost-only treatment were the only seedlings to increase in PI_{ABS} towards the end of the monitoring period. The control treatment resulted in the lowest PI_{ABS} values throughout the entire monitoring period. The trends in vitality therefore indicate that *P. violacea* seedlings benefited with regard to vitality from the enhancement treatments.

Xanthocercis zambesiaca

The *X. zambesiaca* seedlings planted into all four of the treatments showed a greatly unsynchronized trend in vitality throughout the five month monitoring period (Fig. 5.24e). All the seedlings dropped in PI_{ABS} values during the months prior to the two week drought period, with the exception of seedlings in the compost-only treatment which continually increased in PI_{ABS} until the end of the monitoring period. The vitality of seedlings in the other treatments dropped during the months following the drought period. The trends in vitality therefore indicate that *X. zambesiaca* is more drought-tolerant when planted into the compost-only treatment.

As encountered in the survival and growth analysis, the vitality trends also indicated that the beneficial effects of the enhancement treatments varied across species. Vitality increased and then decreased again following the two week drought period in *C. imberbe*, *P. violacea* and *X. zambesiaca*, indicating that the PSII of these species acclimatized to the drought-stress conditions. Changes in PSII behaviour were more pronounced during the first two months following the drought period in most of the species. The drought period therefore influenced the normal functioning of PSII in most of the seedlings.

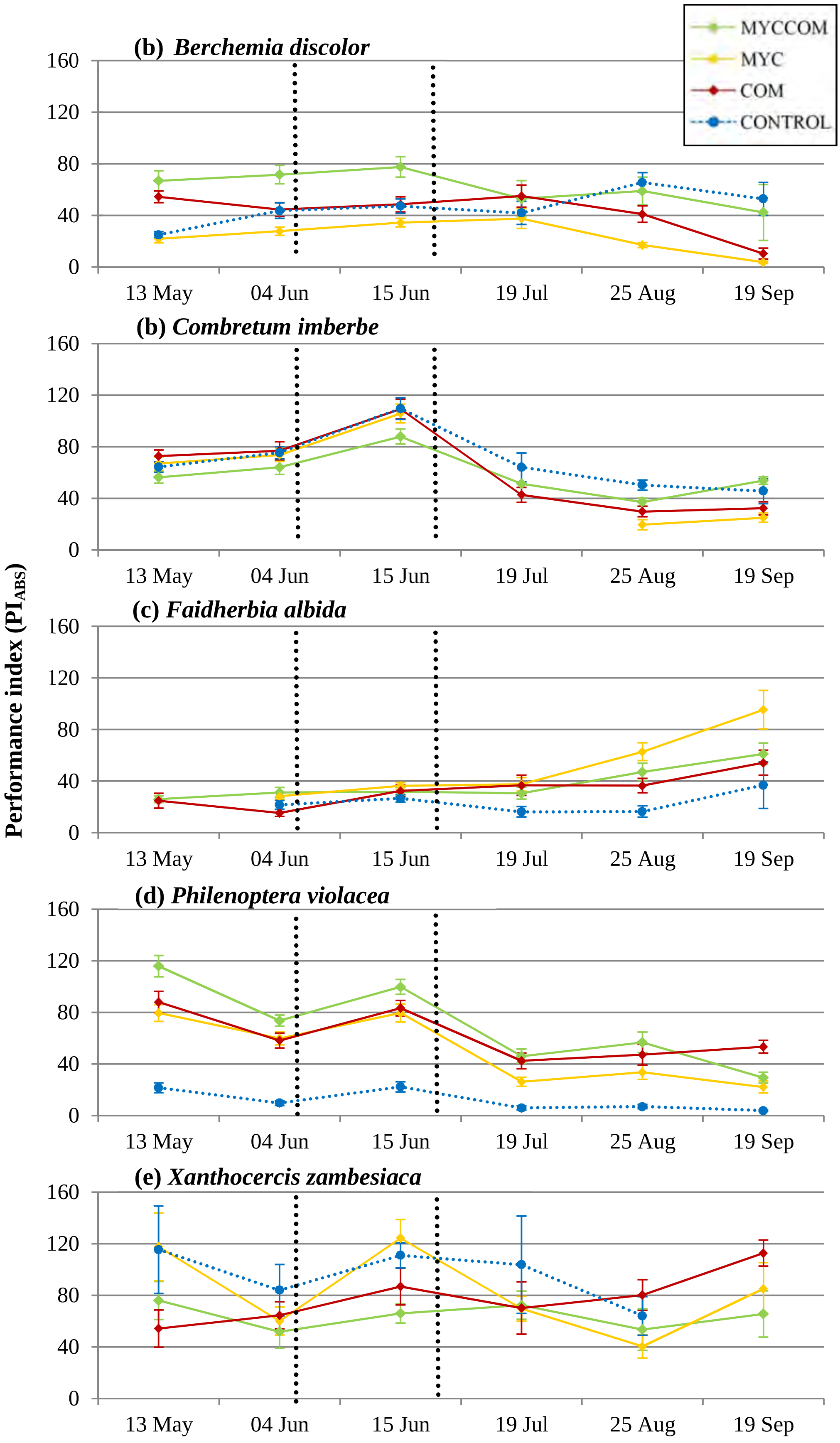


Figure 5.24. The temporal trends in performance index (PI_{ABS}) for the five species cultivated in the greenhouse, where (a) *Berchemia discolor*, (b) *Combretum imberbe*, (c) *Faidherbia albida*, (d) *Philenoptera violacea* and (e) *Xanthocercis zambesiaca*, measured from May to September 2009. The dotted lines indicate the onset of the two week drought simulation period on 4 June 2009 and the commencement of re-watering on 18 June 2009. The enhancement treatment names and abbreviations are explained in Appendix A.

5.3.4.2 Discussion

In the greenhouse trial, five tree species were subjected to drought stress and re-watering. Various studies have used the performance index to estimate plant vitality in response to environmental stressors (Oukarroum *et al.*, 2007; Van Heerden *et al.*, 2004; Hermans *et al.*, 2003; Clark *et al.*, 2000). The performance index used during this study as a measure of plant performance, revealed differences in the response to the enhancement treatments and drought simulation period in the studied tree species. The performance index was created out of three expressions: the concentration of reaction centres per chlorophyll, an expression related to primary photochemistry and an expression related to electron transport (see section 4.1.1). This means that performance index is sensitive to changes in either antenna properties, trapping efficiency or electron transport beyond QA (Oukarroum *et al.*, 2007; Strasser *et al.*, 1995).

The mean performance index observed during the entire study period in the greenhouse differed greatly between the studied species. *C. imberbe* and *X. zambesiaca* in the control treatment functioned at a significantly higher performance index compared to the enhancement treatments, with the other species maintaining higher performance index when planted into one of the enhancement treatments.

The decrease in performance index of *C. imberbe* and *P. violacea* in response to drought stress during the month following the two week drought simulation period was probably due to a decrease of the photochemical efficiency of photosynthetic electron transport. As with the mean performance index, the recovery observed during the months following the drought period also differed strongly between the studied species. *F. albida* and to a lesser extent *X. zambesiaca*, stood out as the only species to continually increase in performance index after re-watering took place. The ability of plants to show high performance index after a period of re-watering can be interpreted as a compensation for their lower photosynthetic capacity during drought (Oukarroum *et al.*, 2007). The smaller recovery in performance index observed in *B. discolor*, *C. imberbe* and *P. violacea* suggests the recovery of photosynthesis in these species to have been less complete.

5.4 Natural versus controlled environment

A summary of the various treatments' effect on survival, growth and vitality measurements of the species transplanted into the experimental exclosure in 2008 and cultivated in the greenhouse at Potchefstroom in 2009, are presented in Table 5.23. The overall survival rate of the species cultivated in the greenhouse were considerably higher (81.6%; Fig. 5.10) compared to that of the field trial (55.8%; Fig. 5.1). The optimum treatment with regard to survival rates differed greatly between the studied species in both the field and greenhouse trial (Table 5.23). With the exception of *X. zambesiaca*, survival was however highest for most of the species when planted into compost-containing treatments. The species cultivated in the greenhouse showed an overall positive diameter growth rate at the base, compared to the overall negative growth rate recorded for the same species transplanted into the field (Table 5.23). As mentioned in previous sections, the greenhouse trial was conducted in order to eliminate some of the environmental variables associated with field trials. Greenhouse seedlings were therefore cultivated in an ‘enemy-free’ environment with sufficient moisture and without the presence of inter- or intra-specific competition. Although the greenhouse seedlings were subjected to a two week drought period, the drought stress conditions present in the experimental exclosure far exceeded that of the greenhouse. As discussed in the previous sections, many studies have found that drought stress is the major cause of tree seedling mortalities and negative growth in semi-arid areas. Intensive competition with grasses (Child *et al.*, 2009; Sharam *et al.*, 2006; Fetene, 2003) as well as browsing and insect predation (Sharam *et al.*, 2006) has also been reported to negatively influence the survival and growth of tree seedlings in semi-arid environments.

One of the most detrimental factors absent in the greenhouse however was transplantation shock, which is considered as the most frequent cause of reforestation failure (Oliet *et al.*, 2005) and most likely the major cause of seedling mortalities during this study. Transplantation shock occurs when the normal physiology of a seedling becomes interrupted due to being transplanted. The transplanted seedlings are exposed to water stress conditions brought on by the temporary impairment of seedling root function or poor root-soil contact (Oliet *et al.*, 2005; Kavanagh & Zaerr, 1997; Folk *et al.*, 1996). These water stress conditions are magnified in semi-arid environments with limited soil moisture.

The vitality responses of the seedlings to the various treatments also differed greatly among species in both the field and the greenhouse trials (Table 5.23). Although the vitality of three species in the field trial showed significant differences, the greenhouse trial showed significant differences between the treatments in all of the species. Fluorescence data collected in the greenhouse were also much more reliable with higher repetitions of species-treatment combinations (browsing and senescence rendered many of the field seedlings without foliage – see section 5.2.3) and much more controlled conditions during measurements.

Table 5.13. A summary of the survival rate, mean diameter growth at the base and performance index of the species transplanted into the experimental exclosure (natural environment) and cultivated in the greenhouse at Potchefstroom, also listing the enhancement treatment responsible for the highest values of each. The enhancement treatment names and abbreviations are explained in Appendix A.

	Natural environment (experimental exclosure)						Controlled environment (greenhouse)					
Species	Survival (%)	Treatment	Mean diameter growth at the base (mm/year)	Treatment	Mean performance index (PI_{ABS})	Treatment	Survival (%)	Treatment	Mean diameter growth at the base (mm/6 months)	Treatment	Mean performance index (PI_{ABS})	Treatment
Acacia xanthophloea	100	COM	-2.5	COM	38.20	*MYC	-	-	-	-	-	-
Berchemia discolor	84	MYC	-1.09	CONTROL	26.52	*COM	100	MYC/COM	1.76	COM	43.38	*MYCCOM
Combretum imberbe	56	MYC/CONTROL	-1.43	COM	29.84	*CONTROL	86	CONTROL	0.94	COM	61.31	*CONTROL
Faidherbia albida	28	COM	-3.50	COM	92.10	MYCCOM	100	MYCCOM/COM	1.07	MYCCOM	36.62	*MYC
Philenoptera violacea	100	MYC/CONTROL	1.60	CONTROL	20.78	CONTROL	100	ALL	1.25	MYCCOM	48.49	*MYCCOM
Salvadora australis	80	MYCCOM/COM	0.48	MYCCOM	30.22	CONTROL	-	-	-	-	-	-
Xanthocercis zambesiaca	60	MYC	-2.42	COM	57.79	CONTROL	100	ALL	0.20	MYC	80.15	*CONTROL

(- indicates that seedlings of the specific species were cultivated during the greenhouse trial and significant differences ($p < 0.05$) compared to the control are indicated with an asterisk)

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Introduction

The findings and recommendations of this study will be a valuable contribution to research as very few restoration studies have been conducted on indigenous savanna tree species in Southern Africa. In the following sections, the outcomes of the monitoring of indigenous tree species will be briefly summarized for seedlings transplanted into an experimental exclosure located in the degraded and deforested old lands of the MNP (field trials). These outcomes include both the seedlings transplanted during this follow-up study in October 2008 (with the addition of enhancement treatments) as well as seedlings transplanted during a pilot-study in April 2006 by Scholtz (2008) (without the addition of enhancement treatments). In addition to the field trials, outcomes from monitoring seedlings of the same tree species cultivated in a greenhouse at the NWU (with the addition of the same enhancement treatments as evaluated in the field) will also be discussed (greenhouse trials). The greenhouse trials' outcomes include the germination rates of the different species in response to various pre-sowing treatments. The contribution of this follow-up study towards capacity building regarding tree restoration methods and awareness will also be discussed. Then site-specific recommendations will be made for future restoration- and research efforts regarding the re-establishment of indigenous savanna tree species in the degraded and deforested areas along the Limpopo River. These recommendations include aspects regarding experimental layout and monitoring methodology proposed for incorporation into the relevant management plan for the MNP. The shortcomings of this study will also be addressed by these recommendations.

6.2 General performance of seedlings in the natural and controlled environment

6.2.1 Survival

Morphological measurements were conducted to record survivorship of seedlings transplanted into the experimental exclosure (seedlings transplanted during the pilot- and current study) as well as seedlings cultivated inside the greenhouse. At the end of the field trial in September 2009, survival for the 2008 transplanted seedlings varied among blocks, treatments and species. Although the overall total survival rate of 55.8% was lower compared to other studies conducted on savanna tree species (Zida *et al.*, 2008), it is not uncommon to encounter these low survival rates in restoration projects. Mortality levels can be high in the extreme conditions often encountered in degraded sites that have to be restored, and that is why survival rates as low as 50% are often deemed acceptable (Sweeney *et al.*, 2002).

Transplantation shock is often considered as the most common cause for high mortality rates of transplanted seedlings, frequently leading to the failure of tree re-establishment projects (Oliet *et al.*, 2005). This was most likely also one of the biggest factors leading to seedling mortality in this study. During transplantation, seedlings are exposed to water stress conditions brought on by the temporary impairment of seedling root function and poor root-soil contact due to disturbance to the root system during lifting, transportation and planting (Oliet *et al.*, 2005; Kavanagh & Zaerr, 1997; Folk *et al*., 1996). These water stress conditions were magnified in the already moisture limited semi-arid environment of the exclosure. The cumulative survival rates of seedlings transplanted during both the pilot- and follow-up study showed that most seedling mortalities occurred during the rainy months which coincided with the significant increase in grass- and herbaceous biomass inside the exclosure, contributing towards high competition between tree seedlings and herbaceous cover. It can therefore be assumed that the grasses out-competed the transplanted seedlings for moisture, light and nutrients. These findings are supported by many other studies (Child *et al.*, 2009; Sharam *et al.*, 2006; Fenner & Thompson, 2005; Fetene, 2003). Browsing and insect predation also contributed to seedling mortality, especially in blocks D and E, in which a high density of *A. tortilis* trees occurred. Animal activity was higher in these blocks as more resources (food and shelter) were available for browser and predator species. Various other studies reported on high seedling mortality resulting from the herbivory effects by mammals and insects (Sharam *et al.*, 2006; Alvarez-Aquino *et al.*, 2004; Goheen *et al.*, 2004; Pedraza & Williams-Linera, 2003). The mortality of transplanted seedlings therefore occurred due to biotic stressors such as herbivory and competition which were likely to be exacerbated by the water-limited semi-arid environment of the study area (Scholtz, 2008; Engelbrecht *et al.*, 2005; Paoletti, 2005; Ffolliott *et al.*, 2003; Wilson & Witkowski, 1998).

During this follow-up study, seedlings were either transplanted (field) or cultivated (greenhouse) in four enhancement treatments which consisted of a combination of indigenous AMF and compost, AMF only, compost only, and the control consisting only of soil from inside the experimental exclosure. The effect of the various enhancement treatments on seedling survival for the 2008 transplantation was species-specific. The same species-specific survival responses to the treatments were recorded in the greenhouse trial, where the most limiting factors present in the field (transplantation shock, moisture stress, competition and herbivory) was excluded. The greenhouse trial did however have much higher survival rates (81.6%) compared to the field trial (55.5%). This leads to the conclusion that the limiting factors present in the natural environment possibly cancelled out by the beneficial effects of the enhancement treatments. Similar finding were reported by Stave *et al.* (2005). Long-term monitoring will be more useful to assess the true effect of the enhancement treatments as other studies have shown that seedling survival rate only started to respond in the second year after planting in trials that also evaluated enhancement treatments such as compost (Larchevêque *et al.*, 2006).

The continued monitoring of only two of the ten species transplanted during the pilot-study was possible. The cumulative survival rates of these two species, namely *C. imberbe* and *S. australis*, showed that most seedling mortalities occurred during the first two years after transplantation with mortality rates stabilising from April 2008 until the end of the study period in September 2009. Seedling mortality was therefore most severe during the first year after transplantation for both the pilot- and follow-up transplantation. Similar results have been found in other studies which also recorded that seedlings planted for restoration purposes usually display highest mortality during the first growing season (Athy *et al.*, 2006; Alvarez-Aquino *et al.*, 2004; Pedraza & Williams-Linera, 2003; Holl *et al.*, 2000; De Steven, 1991). These results emphasize the necessity and value of long-term monitoring.

During this follow-up study, seedlings were also transplanted in the understory of established *A. tortilis* trees, with the aim of evaluating the “nursing” effect that these trees might have on seedling establishment. Seedling mortality was however highest when transplanted in the understory of these trees, and the soil water content around these trees were determined to be lower compared to that of the blocks without tree cover. It seems therefore, that the pioneer *A. tortilis* trees did not create favourable micro-habitats that more conducive for seedling survival. It is likely that the competitive root system of this species out-competed the transplanted seedlings for moisture and nutrients. Established *A. tortilis* trees should therefore not be used as “nurse” trees for future restoration efforts under these environmental conditions. It is therefore recommended to rather re-establish seedlings around established climax tree species from the remnant forest area adjacent to the open and degraded old lands. This recommendation is discussed in more detail in section 6.2.4.

6.2.2 Growth

Three morphological parameters were measured on seedlings in both the field- and greenhouse trials. These parameters included “stem diameter at the base”, “stem diameter 30 cm from the base”, and “height of the tree in its natural growth form”. After one year of monitoring, all the species transplanted into the exclosure during this follow-up study in October 2008, showed an overall negative stem diameter growth rate at the base. This was in contradiction with the overall positive stem diameter growth rates recorded for the same species cultivated in the greenhouse. The same factors leading to seedling mortality in the field, such as transplantation shock and competition with other vegetation, are believed to be the cause of negative growth rates for these seedlings also. This finding is supported by a study conducted by Sharam *et al.* (2006) in the Serengeti, Tanzania, which found that competition with grass produced negative growth rates in the natural environment and that the highest growth rates were recorded when grass was removed from around seedlings. Moisture stress, whether caused by transplantation shock or the water limited semi-arid environmental conditions, has also been found to be responsible for negative stem diameter growth values in many other studies (Sevanto, 2003; Vesale *et al.*, 2000; Zürcher *et al.*, 1998).

Browsing and insect predation were also found to reduce the height and cover of the canopy of the seedlings transplanted during both the pilot- and follow-up study, leading to much inconsistencies in the “stem diameter at 30 cm” and “height of the tree in its natural growth form” parameters. The decreases in height were attributed to browsing and shoot die-back – findings also encountered in savanna trees by Zida *et al.* (2008) and Athy *et al.* (2006). This means that “stem diameter at the base” was the only reliable morphological parameter used to measure the growth of seedlings in the field. In addition to the biotic and abiotic stressors, human error in measuring growth, especially if different individuals are involved between monitoring events, could also have influenced the accuracy of growth data (Pastur *et al.*, 2006; Lesser & Kalsbeek, 1999). Clark and Clark (1999) also reported that a decline in diameter growth can result from errors in the measurement, from shrink and swell with changing stem moisture, or from changes in bark thickness. The positive diameter growth rates recorded for the greenhouse seedlings emphasize that many stressors affect transplanted seedlings (as well as naturally regenerating seedlings) in the natural environment. The two species monitored from the pilot study’s transplantation (*C. imberbe* and *S. australis*) showed a significant increase in stem diameter growth from the second year after transplantation, indicating that these trees have grown past the most vulnerable phase and were therefore less susceptible to damage caused by environmental stressors. These results suggest that more than two seasons’ monitoring will provide a more accurate dataset and reliable results when assessing the effects of the various restoration technologies and treatments on responses following planting. These two species did however decrease in mean height on occasions throughout the study period.

The seedlings transplanted during both the pilot- and follow-up transplantation, showed the highest stem diameter growth rates during the summer rainfall months (November to April). The seedlings cultivated in the greenhouse were regularly watered, with the exception of a two-week simulated drought-period, during which the previously well-watered seedlings did not receive any water. The greenhouse seedlings also showed the highest stem diameter growth rates during the well-watered months before the onset of the simulated drought-period. The high growth rates of both field- and greenhouse seedlings coinciding with well-watered periods confirm that growth is dependent on the availability of water.

The growth responses of seedlings transplanted into the field towards the enhancement treatments were shown to be species-specific, with none of the treatments standing out as being more beneficial for growth. In the greenhouse, however, most of the species showed highest growth rates when planted into the compost-containing treatments. This was true for *B. discolor*, *C. imberbe*, *F. albida* and *P.*

violacea. *X. zambesiaca* was the only species not benefiting from the compost-containing treatments in terms of growth rate. It can therefore be assumed that the compost-containing treatments are generally beneficial for growth of indigenous trees, but that its beneficial effects (increased permeability and water retension, added nutrients, etc.) were out-competed by the various biotic and abiotic stressors (competition with other vegetation and herbivory) present inside the exclosure.

The cumulative growth rate data from this study provided valuable empirical data regarding the growth strategies of riparian savanna species during restoration activities. In the past, very few studies have recorded the growth rates of savanna trees, especially taking seasonal patterns of growth of more than one species from the same location into consideration (February *et al.*, 2007; Shackleton, 2002; Higgins *et al.*, 1999). Although the growth rates monitored during this study were slow for all of the investigated species, it still provided valuable results. The growth rates were higher due to above-average rainfall during the study period. These growth rates are therefore expected to be much slower during drought conditions.

6.2.3 Plant vitality

Chlorophyll fluorescence was measured on seedlings transplanted and cultivated during this follow-up study, with the aim of determining the effect of the various enhancement treatments on overall vitality. The multi-parametric expression, namely the performance index (PI_{ABS}), was used to quantify PSII behaviour and serve as a measure for the overall vitality of each species-treatment combination. The greenhouse seedlings were also exposed to two-week drought simulation period, during which the previously well-watered seedlings did not receive any water.

The vitality of the various species in response to the enhancement treatments differed greatly between the field- and greenhouse seedlings. Both trials showed that the responses varied across each species for the various enhancement treatments. Most of the seedlings transplanted into the field did however show high vitality values when planted into the control treatments (consisting of only soil from inside the experimental exclosure), indicating that the enhancement treatments did not benefit the vitality of species planted. These findings should however be treated with caution as limited repetitions of measurements throughout the season were made due to senescence of the seedlings and herbivory, which caused that insufficient amount of leaves were available on the seedlings for proper measurements. The data collected in the field and in the greenhouse also differed considerably. Due to financial and time constraints resulting from the vast distance between the NWU and the MNP, monthly fluorescence measurement could only be made on seedlings in the greenhouse. The fluorescence data collected in the greenhouse was therefore much more reliable. *C. imberbe* and *X. zambesiaca* in the control functioned at vitality levels statistically higher ($p < 0.05$) compared to when planted in the enhancement treatments. The opposite was true for the other three species where *B.*

discolor, *F. albida* and *P. violacea*, functioned at vitality levels statistically higher ($p < 0.05$) when cultivated in enhancement treatments compared to when cultivated in the control.

The temporal trends in vitality (PSII activity) indicated that only *C. imberbe, P. violacea* and *X. zambesiaca* acclimatized to the drought-stress conditions simulated during the greenhouse trial. These findings confirm the drought-resistant characteristics of *P. violacea* and *X. zambesiaca* which is also reflected by the growth and survival data as discussed above. All the species did however show a change in PSII behaviour following the two week drought simulation period, thereby indicating that normal PSII functioning was interrupted by drought stress. This interruption in PSII function was reflected in other morphological symptoms such as the de-foliation of a number of the potted seedlings as well as a halt in stem diameter growth during the months following the drought simulation period.

6.2.4 Germination

Seeds of five of the seven species used for transplantation during this follow-up study were sown into pots filled with soil collected from inside the experimental exclosure situated inside the MNP. This soil was used to prepare the same enhancement treatments as evaluated during the field trials for evaluation during the greenhouse trials also. Overall germination rates of < 50% was recorded for most of the species cultivated in the greenhouse. *P. violacea* had the highest (69.9%) and *X. zambesiaca* the lowest (5%) overall germination rates. The greenhouse excluded most limiting factors present in the natural environment (moisture stress, competition, predation, etc.) and it can therefore be assumed that the germination rates of the investigated species will be even lower under natural conditions. This assumption is supported by Moe *et al.* (2009) which found that species such as *F. albida*, *C. imberbe* and *P. violacea* show little signs of regeneration over the long-term, suggesting either a lack of viable seed or low seedling survival. These low germination rates can therefore be one of the factors leading to the slow rate of natural succession inside the experimental exclosure.

The combination mycorrhiza-compost treatment resulted in the highest germination rates for all the species. The organic matter provided by the compost increased the infiltration and porosity of the pot-soils, thereby increasing the soil moisture and aeration (Brady & Weil, 2008), which are some of the important triggers for the germination of seed (Fenner & Thompson, 2005). Although not directly measured during this study, it was assumed that the mycorrhiza treatment had a limited affect on germination as the growth of AMF requires interaction with the rhizophere of a plant (Brady & Weil, 2008; Larcher, 2003) which only starts to develop after the germination phase during the period of seedling establishment.

Pre-sowing treatments, including soaking, mechanical scarification and removing the seed from its fruit, were applied to the seeds before sowing. The response of germination to the pre-sowing treatments revealed that legume species such as *F. albida* have higher germination rates when mechanically scarified. Scarification, whether mechanical or chemical, have been shown to improve germination in legume seed type by many other studies, such as Argaw *et al.* (1999), Demel (1996) and Tybirk (1991). *P. violacea* has a very thin seed coat. The germination rates for these seeds were therefore very higher whether scarified or not. The high germination rates of these seeds lead to the "easy establishment" of this species without the addition of enhancement treatments or scarification, also shown by the high abundance of this species in Mapungubwe's riparian woodlands (Götze, 2002). The very low germination rates recorded for *X. zambesiaca* indicate that this species will need some pre-sowing treatments, such as acid or mechanical scarification, to make the hard seed coat permeable to water and improve germination. The germination trial also indicated that *C. imberbe* seed does not need to be removed from its fruit to ensure higher germination rates. Despite the fact that no comparisons were made to "non-soaking" pre-sowing treatments, it was assumed that soaking the seeds in warm water did result in higher germination rates as germination was triggered by moisture (Fenner & Thompson, 2005). This finding is supported by various other studies, such as Argaw *et al.* (1999) and Demel (1996).

The germination rates of seedlings cultivated in the greenhouse were therefore very low for most of the species evaluated. As mentioned previously, germination rates are expected to be even lower under natural conditions – even with the application of pre-sowing treatments. From these results it can therefore be concluded that the use of seedlings for re-vegetation during the restoration of these specific species, will be a more effective compared to direct seeding. Dayamba (2010) support this conclusion as this study have found that direct seeding frequently failed due to the high mortality of seeds and newly germinated seedlings, especially when exposed to drought and herbivory.

6.2.5 Capacity building

Successful and sustainable restoration will only be possible in the MNP with the co-operation and 'buy-in' from land managers and other stakeholders. This study succeeded to build partnerships with various participants including SANParks staff, researchers and students from academic institutions, farmers and volunteers from the private sector. Good collaboration existed in many activities of this project. SANParks staff and students were provided with pre-planting practical training on how to dig holes, proper planting and monitoring techniques, as well as some maintenance aspects, such as watering, clearing of vegetation around seedlings, etc (Fig. 6.1).

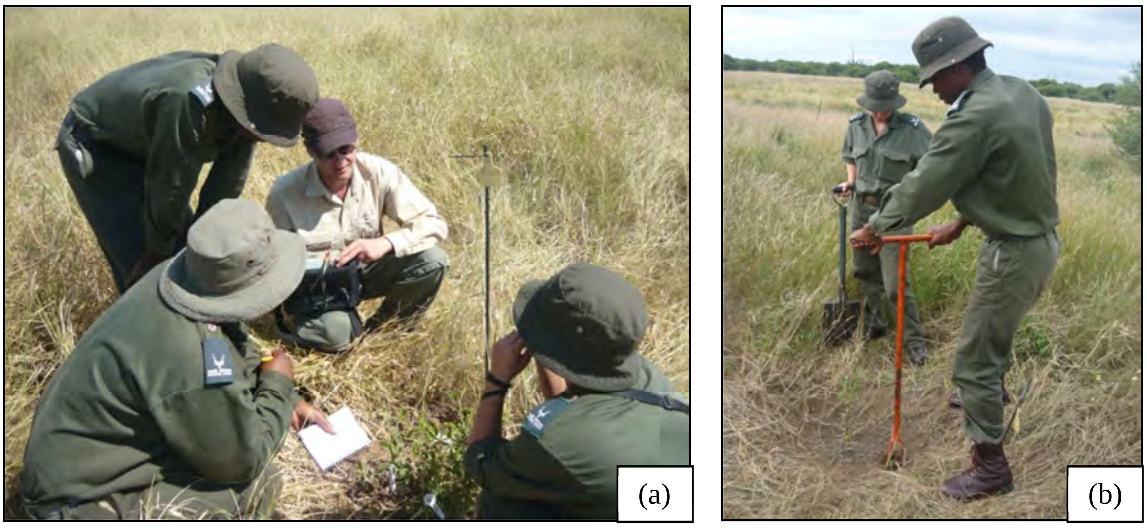


Figure 6.1. SANParks staff and students were trained in various aspects regarding (a) monitoring methodology and (b) survey techniques.

A big planting effort, in collaboration with the West Rand Honorary Rangers Association[1], SANParks staff, researchers, university students and international volunteers, took place in October 2009 (Fig. 6.2). Funds for labour and compost were graciously provided by the West Rand Honorary Rangers Association enabling the transplantation of more than 400 tree seedlings into the experimental exclosure. Labourers from the local community were employed to dig pits in the north-eastern side of the exclosure and compost was purchased at a local nursery. Seedlings were transplanted according to recommendations made during this study and were labelled and geo-referenced for future monitoring (Appendix E). This and previous transplantation events (including training provided to SANParks staff and students on each monitoring event during the study period) contributed to the awareness and capacity building activities for this study.

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[1] West Rand Honorary Rangers Association, Johanna van der Merwe, Tel: 011 660 5521, Website: www.westrandhr.co.za

Figure 6.2. The group of participants involved in a tree transplantation event sponsored by the West Rand Honorary Rangers Association in October 2009. Participants involved SANParks staff, researchers, university students, international volunteers and members of the West Rand Honorary Rangers Association.

6.3 Recommendations

6.3.1 Long-term monitoring

The conclusions drawn from the data collected for the 2008 transplanted seedlings must be treated with caution, as they are based on just the first 11 months of survival and growth data. This is also true for data collected from the greenhouse trial which is based on only six months of growth. The data collected from the continued monitoring of seedlings transplanted during the pilot study was of greater importance, indicating that most growth occurred in the second year after planting, therefore emphasizing the value and necessity of long-term monitoring. It is therefore strongly recommended to continue with long-term monitoring of seedlings that are transplanted during restoration projects that include re-vegetation using indigenous tree species. The survival and growth of the transplanted trees, whether it be in response to environmental stressors or the possible benefit gained from enhancement treatments, will be more accurate if evaluated in the long-term.

The MNP falls within a summer rainfall area (Mucina & Rutherford, 2006). From the results of this study, it is therefore advised that morphological monitoring should only be conducted once a year at the end of the growing season (April - May).

The method used during this study for calculating growth rate, where repeated measurements over time are subtracted from each other, is still one of the most commonly used (Clark *et al.*, 2007). It is therefore recommended to use this calculation for future analysis of growth date when monitoring transplanted trees. It is further recommended to measure only two of the growth parameters during future monitoring. These include "stem diameter at the base" and "height of the tree in its natural growth form". Only when the transplanted trees start exceeding a height of 1.5 m, it is then advised to include the "diameter at breast height" parameter. These three parameters were also most commonly used to measure tree growth in other studies (Walker *et al.*, 2008; Zida *et al.*, 2008; Pastur *et al.*, 2007; Sharam *et al.*, 2006; Alvarez-Aquino *et al.*, 2004) and will therefore enable comparison with other studies. These measurements are non-destructive and more cost-effective in terms of equipment needed. This approach has another advantage that diameters at base can be measured over a short study period and it can be used where trees do not produce increase in height – as was the case with transplanted seedlings due to the effect of herbivory during this study. Stem diameter at base measurements do however have some disadvantages which have to be taken into consideration, such as (1) sufficient time has to elapse between observation to allow for a confident estimate of the increment, and (2) long intervals are needed, because diameter at base measurements may have substantial error due various factors such as human error mentioned previously (Biondi, 1999; Gregoire *et al.*, 1990; Biging & Wensel, 1988).

SANParks staff and nature conservation students were trained in the relevant monitoring techniques on each monitoring occasion during the field trial surveys. The general feedback and observations were that these monitoring techniques are simple and easy to use. Future monitoring activities can therefore be carried out by SANParks staff and intern students from other academic institutions to build up a long-term data base, which will be of use for both SANParks' Scientific Services and other scientists.

Shoot-root ratio measurements and determining biomass production should be additional considerations in the short-term monitoring of transplanted tree species in future. Many studies use these methods to determine the effect of specific factors, such as enhancement treatments, on the morphological characteristics of a species (Rolando & Little, 2008; Zida *et al.*, 2008; Fetene, 2003). Plants normally use more energy for root development to ensure stability and steadiness, and to acquire efficient water and nutrients for optimal growth, especially in the short-term just after transplantion. Plants will therefore transfer more energy towards the below-ground part of the plant to increase its biomass (Hopkins & Hüner, 2004). These are however destructive sampling methods and future restoration efforts will have to increase sample size if it is to be used.

The success of a restoration projects can only be determined if continuously evaluated according to the objectives identified for the restoration project as a whole. Long-term monitoring is necessary for these objectives, especially when transplanting tree seedlings. The latter is often neglected in restoration projects due to various factors such as a lack of funding, a lack of expertise and knowledge, land managers not implementing monitoring protocol because they do not agree on the principles, or the lack of "buy in" from senior management to comply with specific objectives.

6.3.2 Maintenance and after-care

Maintenance of seedlings for the first three years after transplantation is essential to ensure optimum establishment and survival rates. Restoration activities are expensive endeavours and the proper maintenance and management needs to support the financial inputs.

The results from this study indicated that drought-stress was the major cause of seedling mortality and it is therefore strongly recommended that transplanted seedlings be regularly watered for the first two to three years after transplantation. If seedlings do not receive water during the period of new root development, its internal water deficiency will increase considerably (Burdett, 1990). Accessibility of many trial areas by vehicle, such as the experimental exclosure in this study, is often impossible especially during the rainy season. Watering during these times will therefore not be possible. Watering is however not necessary during high rainfall months as the rain water will be sufficient. This study have shown that each seedling needs to be watered (at least 25 L per of water per tree) during the drier months for at least once every second week.

Equally important is the clearing of grasses and any herbaceous growth in a 50 cm radius around the base of transplanted seedlings as these plants normally have very strong root system and can easily out-compete seedlings for moisture and nutrients (Child *et al.*, 2009; Sharam *et al.*, 2006; Fenner & Thompson, 2005; Fetene, 2003). This maintenance activity needs to occur more regularly during the growing high rainfall season (November to March), but can coincide with watering events to keep disturbance by vehicles and other movements to a minimum.

In addition to watering and clearing of vegetation, transplanted seedlings need to be protected from browsing by small ungulates such as impala and duiker which still managed to enter the experimental exclosure during this study. The use of dead *A. tortilis* branches with spines, if available, will be a cost-effective (although labour-intensive) solution to protect seedlings from browsing. The branches can also contribute to the organic material after decomposition. Sharam *et al.* (2006) built similar thorn fences around seedlings in the Serengeti using *Dichrostachys cinerea* branches. These branches were also shown to provide more light to reach seedlings than allowed by grass neighbours. As with the other maintenance activities, branches should be replaced when necessary for the first two to three years after transplantation.

It is easy to assume that the re-colonisation of old lands by the appropriate species is just a matter of time if the adequate soil seed bank is present. In certain cases this might be true, but considering the low germination and regeneration rates of especially climax savanna tree species (results from this study and Moe *et al.*, 2009) as well as the highly competitive life strategy of the currently dominating pioneer *A. tortilis* trees, it can be assumed that the re-colonisation of Mapungubwe's old lands with previously existing indigenous species, will only occur within a very long time-span. The re-establishment of climax tree species will accelerate the successional rate within these old lands towards the target trajectory, if suitable conditions prevail and vegetation material, such as seed and/or seedlings, is available. The active restoration action of seedling re-establishment is advised for other degraded and deforested areas along the Limpopo River as well. Before embarking on any restoration action, an appropriate reference system with regard to species composition and distribution need to be determined in order to assess the restoration successes over time. This can be done by various methods, such as the use of aerial photographs and remote sensing images, as well as interviews with previous land-owners.

6.3.3 Experimental layout

For future restoration efforts it is recommended that tree seedlings are to be transplanted around remnant forest areas in the direction of the old lands, and not the other way around. Based on previous studies, this approach will ensure higher seedling survival and growth rates due to the micro-habitats created by the already established climax species acting as "nursing" trees (Alvarez-Aquino *et al.*, 2004; Pedraza & Williams-Linera, 2003; Holl *et al.*, 2000). The placing of an exclosure is therefore very important as it should not only be used to protect transplanted seedlings from herbivory and trampling damage caused by large mammals, but also include remnants of the existing forest or woodland area. The implication of this recommendation therefore entails that the current exclosure at the MNP used for this study, need to be expanded in order to include parts of the remnant forest area. Seedlings transplanted in this way will also be exposed to indigenous AMF which have been linked to higher seedling survival after re-establishment (Meiners *et al.*, 2000; Lawson *et al.*, 1999). The exclosure is currently dominated by mycorrhizae-independent *A. tortilis* trees, making survival very difficult for other indigenous and climax tree species that are dependent on the association with this fungus. Indigenous AMF can take up to 30 years to re-establish in the exclosure's disturbed soil (Boerner *et al.*, 1996), and the inclusion of remnant forest area inside the exclosure will speed up this process.

6.3.4 Seedlings cultivated under optimum nursery conditions prior to transplantation

Seedlings need to be cultivated under optimum nursery conditions prior to transplantation as studies have found that the morphological and physiological conditions of seedlings inside the nursery will determine their ability to survive and grow subsequently in the field (Paliwal & Kannan, 1999). Keeping cost-effectiveness in mind, it is realised that temperature control inside the nursery will not be possible but other infra-structure such as shade-cover and a sufficient irrigation system should be in place. A fully equipped and well managed nursery will not only be of use for future restoration programmes inside the park, but can also be of economic benefit to the park. Many other parks, such as the Kruger National Park (KNP), have fully operational nurseries that cultivate indigenous trees that are used for both research and income generation. The upgrading and restoration of MNP's Rhodesdrift nursery is therefore strongly advised.

Seeds of the selected species should be collected inside the park by either collecting fallen seed from the ground or harvesting seed directly from the tree. The regular collection of seed need not be a time-consuming or labour-intensive activity, as it can be incorporated into the park ranger's daily patrols or maintenance activities. Seeds should of course not be over-harvested, ensuring that the natural seed bank retain sufficient supplies. Seeds should be sown into seedbeds filled with a combination of river sand and compost as the results from this study showed that the organic material provided by the compost increased germination of all the investigated species. Seedbeds are simple rectangular brick structures filled with growth medium, and are already constructed in the Rhodesdrift nursery (section 4.1.1.2).

In order to reach optimum germination rates, seeds are to be treated with pre-sowing treatments before being sown into the seedbeds. Unfortunately the pre-sowing experiment of this study evaluated only five species and further small-scale germination experiments are encouraged to investigate the germination requirements of other riparian savanna species to be used. Seeds of all the species are to be soaked in warm water for 12 hours before sowing as the moisture will trigger germination (Fenner & Thompson, 2005) and therefore ensure higher germination rates (Argaw *et al.*, 1999; Demel, 1996). Further experiments should however be conducted to determine the optimum time of soaking for the selected species. Acid or mechanical scarification is advised for legume seeds and other species with hard seed coats, whilst species such as *C. imberbe* can be sown whilst still contained inside the fruit.

About two weeks after germination, seedlings should be carefully removed and transplanted into bigger 50 L nursery bags filled with a combination of river sand and fertilizer. The reforestation community view the practice of fertilizing prior to transplantation as one of the most important practices to ensure high quality of transplanted seedlings (Oliet *et al.*, 2005). The beneficial effects of fertilizer includes the increase in infiltration and porosity of the growth medium, the improvement of post-transplant rooting and growth capacity and increase resistance to water stress and diseases (Brady & Weil, 2008; Shaw *et al.*, 1998). Also, the remobilisation of internal nutrient reserves will enable the transplanted seedlings to be partly independent from external nutrient availability (Cherbuy *et al.*, 2001). These mineral reserves can thus play an important role after planting when nutrient uptake is limited by poor root-soil contact after transplantation (Kavanagh & Zaerr, 1997; Folk *et al.*, 1996). If fertilizer is not a viable option due to budget constraints, then it is recommended to mix river sand with soil collected from around climax tree species inside the MNP, thereby still incorporated nutrients as well as indigenous AMF. Long-term monitoring will however be required to assess the effect of mineral nutrition in the nursery on the survival and growth of transplanted seedlings, as many studies have shown that growth only started to respond within the second year after transplantation (Oliet *et al.*, 2005).

The use of 50 L nursery bags is recommended in order to grow seedlings of > 30 cm in height before being transplanted. Special elongated nursery bags, which can accommodate root growth of more than 1 m, should be used for species such as *F. albida* which grows extremely deep tap roots (van Wyk & van Wyk, 2007). Many seedlings with deep root systems consequently die whilst still in the nursery due to spatial constraints of the containers sizes (personal communication with Michelle Hofmeyer[2], manager at the Skukuza Indigenous Nursery of the KNP). Seedlings of > 30 cm in height are advised to be used for transplantation not because bigger seedling size will ensure higher survival and growth after planting, but because browsing by small ungulates will be reduced at taller seedling heights (Lawson *et al.*, 1999; De Steven, 1991). No conclusive evidence have suggested that bigger sizes of transplanted savanna seedlings will affect survival and growth post transplantation or make them more drought tolerable (Zida *et al.*, 2008).

The condition or quality of seedlings from the nursery determines their ability to establish quickly and survive and grow satisfactory post-transplantation (Ward *et al.*, 2000; Noland *et al.*, 2001). Studies such as Zida *et al.* (2007) therefore recommend that savanna seedlings be of "high quality", i.e. well-watered and fertilized, to ensure higher survival and growth after transplantation.

Seedlings transplanted into the Mapungubwe experimental exclosure were "hardened" inside the nursery prior to transplantation. This process entails the gradual exposure or "acclimation" of seedlings to full sunlight and less water for a certain period before transplantation. "Hardening" is widely practiced in nurseries and has been shown to provide seedlings with a greater ability to withstand and adjust to adverse environmental conditions after transplantation (Bañon *et al.*, 2006; Sánchez-Blanco *et al.*, 2006; Hopkins & Hüner, 2004). The effect of hardening on seedling survival and growth post-transplantation was unfortunately not directly measured during this study. It is therefore uncertain whether "high quality" or "hardened" seedlings from the nursery will ensure higher survival and growth rates post-transplantation. This is an aspect recommended for further research as very few such studies, especially on savanna species, have been conducted. More specifically, the appropriate duration of hardening should be further investigated. It is likely that the two week hardening period to which seedlings were subjected in the Rhodesdrift Nursery was too short to properly result in thickened root growth in order to adequately assist in the accumulation of

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[2] Michelle Hofmeyer, Manager:Skukuza Indigenous Nursery, Tel: 013 735 4312, E-mail: michelleh@sanparks.org

root reserves and storage after transplantation. Although not measured, it is sure that the seedlings experienced physiological stress when exposed to the increased sunlight and reduced water conditions of the hardening process. It is therefore also likely that two weeks were not a sufficient period of time for the seedlings to recover from this physiological stress, therefore resulting in the transplantation of physiologically 'poor' seedlings possibly resulting in the high mortality rates in the field, especially under the canopies of the *A. tortilis* trees. Taking into consideration that the findings of this study contradicted findings of previous researchers who identified *A. tortilis* as a potentially good nursing tree (Ludwig *et al.*, 2003), it is recommended that further studies regarding the true effect of these trees on seedling survival should also be ensued.

Whether "high quality" or "hardened" seedlings are to be used, the objective of tree restoration projects remain the attainment of optimum survival and growth rates after transplantation. This objective will reduce site maintenance costs and improve the confidence of land managers in artificial regeneration by means of re-establishment. It is therefore recommended that seedlings cultivated in the nursery should be regularly watered (at least once a week during the winter months and twice weekly during the summer months), fertilized and kept clear from weeds and grasses whilst in the greenhouse. The use of insecticides is also strongly recommended inside the nursery when needed to ensure optimum morphological and physiological conditions of seedlings prior to transplantation.

6.3.5 Transplantation technique

The MNP falls within a summer rainfall area and as the availability of water is a function of rainfall, it is important that planting be carried out during the summer months. Ideally planting should occur at the beginning of the first rains (October – November) thereby coinciding with natural regeneration events which typically occurs with episodic events in semi-arid environments (Rolando & Little, 2008; Viero & Little, 2006).

Poor transplantation technique, such as excessive disturbance to seedlings during transportation and planting as well as inadequate watering, can often lead to increased seedling mortality after transplantation. The proper transplantation technique is recommended as follows:

- Seedlings should be planted into pits of at least 50 cm wide x 50 cm long x 50 cm deep (a total volume of 0.125 m^2) and with a 1.5 m x 1.5 m spacing (Scholtz, 2008; Zida *et al.*, 2008; Oliet *et al.*, 2005).
- Pits should be half filled with loose soil before adding any soil treatments to ensure a growth medium which will be easily penetrated by the growing roots of transplanted seedlings.

- Seedling should be handled very carefully during positioning into the pit, ensuring that the stem flare (which is positioned where the roots and stem bark meet) is exposed above to the air after the pit has been filled with the growth medium.
- Seedlings should be watered before and directly after transplantation as this will insure a wet root zone at the time of planting which will minimise immediate post-planting stress (Rolando & Little, 2008).
- The soil around planted seedlings should be compacted and watered again after the pits have been filled in order to secure the transplanted seedling.

After planting it essential to label and geo-reference the location of transplanted seedlings for re-location purposes during future monitoring events.

CHAPTER 7

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Appendix A.

Species and enhancement treatment codes used during this study.

Species	Family	Code	Status
Acacia xanthophloea	Fabaceae	Aca xan	Indigenous tree
Berchemia discolor	Rhamnaceae	Ber dis	Indigenous tree
Combretum imberbe	Combretaceae	Com imb	Indigenous tree
Faidherbia albida	Fabaceae	Fai alb	Indigenous tree
Philenoptera violacea	Fabaceae	Phi vio	Indigenous tree
Salvadora australis	Salvadoracea	Sal aus	Indigenous shrub/tree
Xanthocercis zambesiaca	Fabaceae	Xan zam	Indigenous tree

Treatments	Code
Soil, Mycoroot^{TM} Super Booster and compost	MYCCOM
Soil and Mycoroot^{TM} Super Booster	MYC
Soil and compost	COM
Soil only	CONTROL

Appendix B.

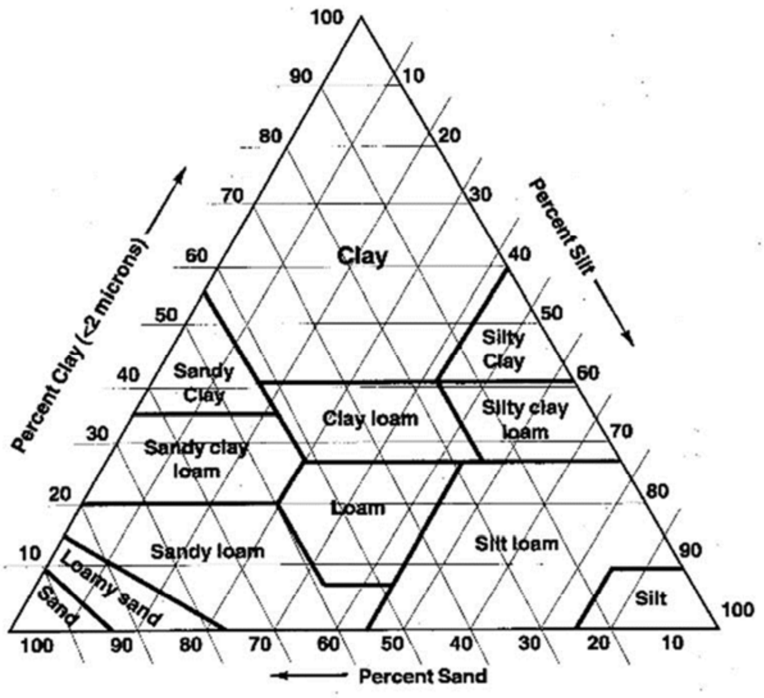
Chemical symbols with ionic names and forms.

Element	Symbol	Ionic form	Ion name
Base cations			
Potassium	K	K^+	Potassium
Calcium	Ca	Ca^{2+}	Calcium
Magnesium	Mg	Mg^{2+}	Magnesium
Sodium	Na	Na^+	Sodium
Phosphorus	P	HPO_4^{2-}	Hydrogen Phosphate
	P	$H2PO_4^-$	Dihydrogen Phosphate
Other cations and anions			
Nitrogen	N	NO_3^-	Nitrate
	N	NH_4^+	Ammonium
Sulfur	S	SO_4^{2-}	Sulfate
Carbon	C	CO_3^{2-}	Carbonate

Other chemical variables measured

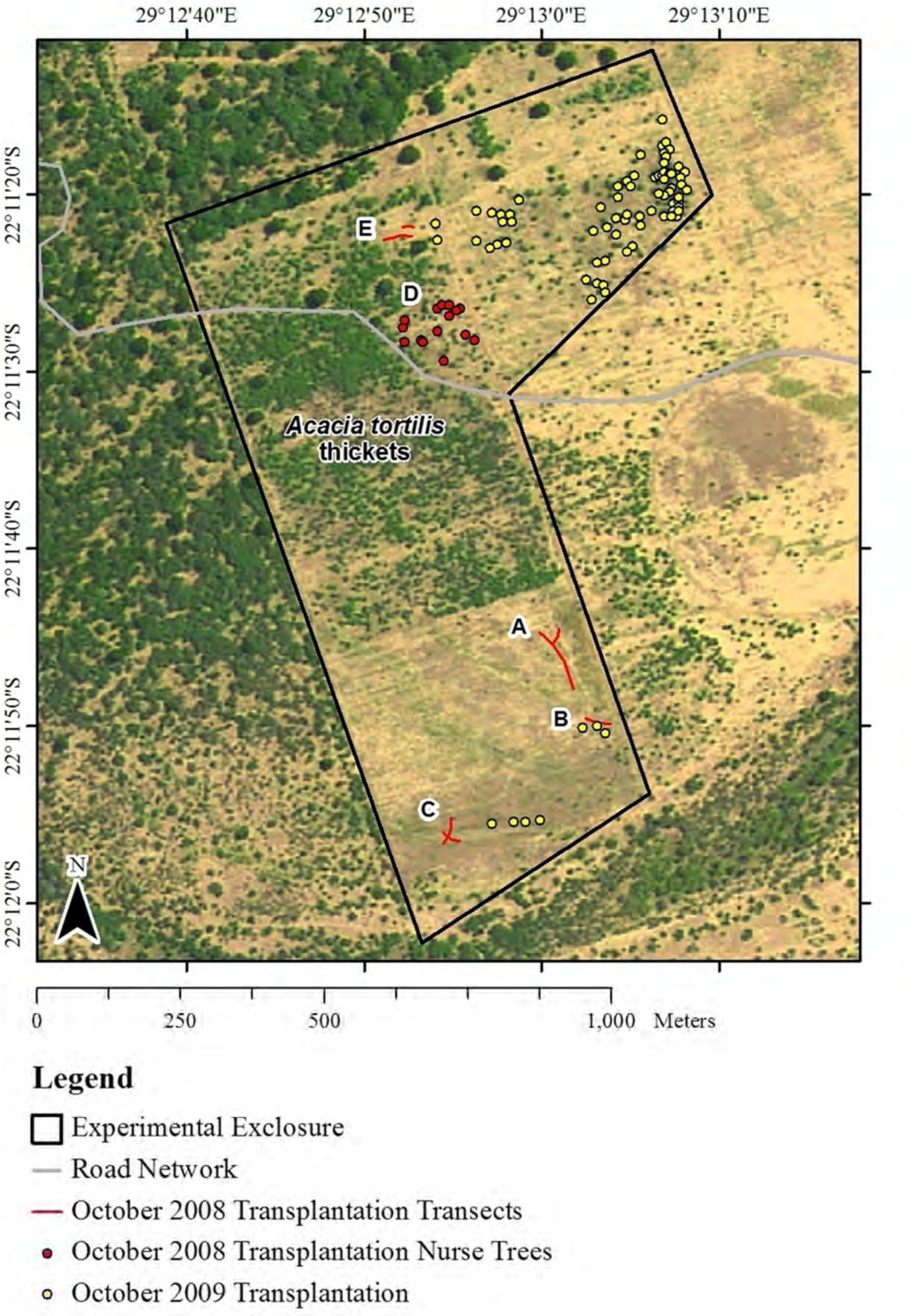
Variable	Explanation
CEC	Cation exchange capacity
EC	Electrical conductivity
pH (KCl)	Acidity/alkalinity using ammonium-chloride extraction
SAR	Sodium adsorption ratio

Soil textural triangle presenting the major soil textural classes by the percentages of sand, silt, and clay according to the heavy boundary layers on the triangle (adapted from Brady & Weil, 2008).



Appendix C.

The layout and location of seedlings transplanted in October 2008 and October 2009 (see coordinates in Appendix D).



Appendix D.

The location, layout and labels of seedlings transplanted in October 2008 and October 2009.

Year of transplantation_ block inside the experimental exclosure	Type of layout	Seedling labels	GPS Coordinates	
			Latitude: South	Longitude: East
October 2008_A1	Transect	Bd1-Bd8; Ci1-Ci10; Fa1-Fa10; Pv1-Pv8; Xz1-Xz10	22º 11’ 47.9’’	29 º 13’ 01.8’’
October 2008_A2	Transect		22 º 11’ 45.4’’	29 º 13’ 00.6’’
October 2008_A3	Transect		22 º 11’ 44.7’’	29 º 12’ 59.9’’
October 2008_A4	Transect		22 º 11’ 44.5’’	29 º 13’ 01.0’’
October 2008_B1	Transect	Sa1-Sa17	22 º 11’ 49.9’’	29 º 13’ 03.9’’
October 2008_B2	Transect		22 º 11’ 49.6’’	29 º 13’ 02.5’’
October 2008_C1	Transect	Ax1-Ax12; Ci1-Ci18	22 º 11’ 55.2’’	29 º 12’ 54.9’’
October 2008_C2	Transect		22 º 11’ 56.0’’	29 º 12’ 54.5’’
October 2008_C3	Transect		22 º 11’ 56.6’’	29 º 12’ 54.5’’
October 2008_C4	Transect		22 º 11’ 56.5’’	29 º 12’ 55.4’’
October 2008_D1	Around a "nurse" tree	Ci1-Ci8	22 º 11’ 28.3’’	29 º 12’ 52.3’’
October 2008_D2	Around a "nurse" tree	Fa1-Fa8	22 º 11’ 27.5’’	29 º 12’ 52.2’’
October 2008_D3	Around a "nurse" tree	Xz1-Xz8	22 º 11’ 27.1’’	29 º 12’ 52.3’’
October 2008_D4	Around a "nurse" tree	Ci9-Ci16	22 º 11’ 26.9’’	29 º 12’ 63.1’’
October 2008_D5	Around a "nurse" tree	Ci17-Ci24	22 º 11’ 26.4’’	29 º 12’ 54.1’’
October 2008_D6	Around a "nurse" tree	Bd17-Bd24	22 º 11’ 26.2’’	29 º 12’ 54.4’’
October 2008_D7	Around a "nurse" tree	Fa17-Fa24	22 º 11’ 26.2’’	29 º 12’ 54.8’’
October 2008_D8	Around a "nurse" tree	Ci25-Ci32	22 º 11’ 26.4’’	29 º 12’ 55.4’’
October 2008_D9	Around a "nurse" tree	Xz17-Xz24	22 º 11’ 26.5’’	29 º 12’ 55.2’’
October 2008_D10	Around a "nurse" tree	Bd9-Bd16	22 º 11’ 29.4’’	29 º 12’ 54.5’’
October 2008_D11	Around a "nurse" tree	Xz9-Xz16	22 º 11’ 27.7’’	29 º 12’ 54.1’’
October 2008_D12	Around a "nurse" tree	Bd1-Bd8	22 º 11’ 28.2’’	29 º 12’ 53.2’’
October 2008_D13	Around a "nurse" tree	Fa9-Fa16	22 º 11’ 28.3’’	29 º 12’ 53.3’’
October 2008_D14	Around a "nurse" tree	Fa25-Fa32	22 º 11’ 27.9’’	29 º 12’ 55.7’’
October 2008_D15	Around a "nurse" tree	Xz25-Xz32	22 º 11’ 26.8’’	29 º 12’ 54.8’’
October 2008_D16	Around a "nurse" tree	Bd25-Bd32	22 º 11’ 28.2’’	29 º 12’ 56.2’’
October 2008_E1	Transect	Ci1-Ci9; Bd1-8; Fa1-Fa9; Xz1-Xz9	22 º 11’ 22.5’’	29 º 12’ 51.1’’
October 2008_E2	Transect		22 º 11’ 22.3’’	29 º 12’ 52.7’’
October 2008_E3	Transect		22 º 11’ 21.8’’	29 º 12’ 52.8’’
October 2008_E4	Transect		22 º 11’ 21.8’’	29 º 12’ 52.2’’
October 2009_B1	Cluster	032-036	22 º 11’ 50.1’’	29 º 13’ 02.3’’
October 2009_B2	Cluster	037-041	22 º 11’ 50.0’’	29 º 13’ 03.1’’
October 2009_B3	Cluster	042-046	22 º 11’ 50.4’’	29 º 13’ 03.6’’
October 2009_C1	Cluster	001-008	22 º 11’ 55.5’’	29 º 12’ 57.2’’
October 2009_C2	Cluster	009-016	22 º 11’ 55.4’’	29 º 12’ 58.4’’
October 2009_C3	Cluster	017-021	22 º 11’ 55.4’’	29 º 12’ 59.1’’
October 2009_C4	Cluster	022-026	22 º 11’ 55.3’’	29 º 12’ 59.9’’
October 2009_C5	Cluster	027-031	22 º 11’ 55.3’’	18 º 13’ 00.6’’

October 2009_1	Around a "nurse" tree	047-050	22 º 11’ 21.6’’	29 º 12’ 54.0’’
October 2009_2	Around a "nurse" tree	051-054	22 º 11’ 22.5’’	29 º 12’ 54.1’’
October 2009_3	Around a "nurse" tree	055-058	22 º 11’ 20.9’’	29 º 12’ 56.3’’
October 2009_4	Around a "nurse" tree	05-062	22 º 11’ 21.0’’	29 º 12’ 57.2’’
October 2009_5	Around a "nurse" tree	063-066	22 º 11’ 21.1’’	29 º 12’ 57.7’’
October 2009_6	Around a "nurse" tree	067-069	22 º 11’ 21.1’’	29 º 12’ 58.2’’
October 2009_7	Around a "nurse" tree	070-073	22 º 11’ 20.3’’	29 º 12’ 58.7’’
October 2009_8	Around a "nurse" tree	074-075	22 º 11’ 21.5’’	29 º 12’ 58.3’’
October 2009_9	Around a "nurse" tree	076-077	22 º 11’ 21.5’’	29 º 12’ 57.8’’
October 2009_10	Around a "nurse" tree	078-081	22 º 11’ 22.7’’	29 º 12’ 58.0’’
October 2009_11	Around a "nurse" tree	082-085	22 º 11’ 22.8’’	29 º 12’ 57.5’’
October 2009_12	Around a "nurse" tree	086-090	22 º 11’ 23.0’’	29 º 12’ 57.1’’
October 2009_13	Around a "nurse" tree	091-094	22 º 11’ 22.6’’	29 º 12’ 56.3’’
October 2009_14	Around a "nurse" tree	095-100	22 º 11’ 15.7’’	29 º 13’ 06.8’’
October 2009_15	Around a "nurse" tree	101-103	29 º 11’ 17.6’’	29 º 13’ 04.8’’
October 2009_16	Around a "nurse" tree	104-108	27 º 11’ 18.1’’	29 º 13’ 05.5’’
October 2009_17	Around a "nurse" tree	109-112	22 º 11’ 17.7’’	29 º 13’ 05.6’’
October 2009_18	Around a "nurse" tree	113-115	22 º 11’ 17.2’’	29 º 13’ 06.8’’
October 2009_19	Around a "nurse" tree	1169-119	22 º 11’ 17.0’’	29 º 13’ 07.0’’
October 2009_20	Around a "nurse" tree	120-126	22 º 11’ 17.4’’	29 º 13’ 07.2’’
October 2009_21	Around a "nurse" tree	127-130	22 º 11’ 17.6’’	29 º 13’ 06.9’’
October 2009_22	Around a "nurse" tree	131-134	22 º 11’ 17.8’’	29 º 13’ 07.0’’
October 2009_23	Around a "nurse" tree	135-138	22 º 11’ 18.2’’	29 º 13’ 06.9’’
October 2009_24	Around a "nurse" tree	139-142	22 º 11’ 18.9’’	29 º 13’ 05.2’’
October 2009_25	Around a "nurse" tree	143-144	22 º 11’ 19.2’’	29 º 13’ 04.8’’
October 2009_26	Around a "nurse" tree	145-148	22 º 11’ 19.5’’	29 º 13’ 04.3’’
October 2009_27	Around a "nurse" tree	149-150	22 º 11’ 20.1’’	29 º 13’ 04.3’’
October 2009_28	Around a "nurse" tree	151-154	22 º 11’ 19.5’’	29 º 13’ 05.0’’
October 2009_29	Around a "nurse" tree	155-157	22 º 11’ 19.0’’	29 º 13’ 06.4’’
October 2009_30	Around a "nurse" tree	158-161	22 º 11’ 18.9’’	29 º 13’ 06.6’’
October 2009_31	Around a "nurse" tree	163-165	22 º 11’ 18.9’’	29 º 13’ 06.8’’
October 2009_32	Around a "nurse" tree	166-169	22 º 11’ 18.7’’	29 º 13’ 06.9’’
October 2009_33	Around a "nurse" tree	170-175	22 º 11’ 18.8’’	29 º 13’ 07.2’’
October 2009_34	Around a "nurse" tree	176-179	22 º 11’ 18.4’’	29 º 13’ 07.7’’
October 2009_35	Around a "nurse" tree	180-184	22 º 11’ 18.8’’	29 º 13’ 07.3’’
October 2009_36	Around a "nurse" tree	185-188	22 º 11’ 18.7’’	29 º 13’ 08.1’’
October 2009_37	Around a "nurse" tree	189-192	22 º 11’ 19.0’’	29 º 13’ 07.8’’
October 2009_38	Around a "nurse" tree	193-196	22 º 11’ 19.4’’	29 º 13’ 07.9’’
October 2009_39	Around a "nurse" tree	197-200	22 º 11’ 19.6’’	29 º 13’ 07.3’’
October 2009_40	Around a "nurse" tree	201-202	22 º 11’ 19.8’’	29 º 13’ 07.2’’
October 2009_41	Around a "nurse" tree	203-204	22 º 11’ 20.0’’	29 º 13’ 06.9’’
October 2009_42	Around a "nurse" tree	205-208	22 º 11’ 19.1’’	29 º 13’ 06.9’’
October 2009_43	Around a "nurse" tree	209-212	22 º 11’ 19.9’’	29 º 13’ 06.6’’
October 2009_44	Around a "nurse" tree	213-214	22 º 11’ 20.7’’	29 º 13’ 03.3’’
October 2009_45	Around a "nurse" tree	215-218	22 º 11’ 22.0’’	29 º 13’ 02.9’’
October 2009_46	Around a "nurse" tree	219-226	22 º 11’ 21.8’’	29 º 13’ 03.7’’
October 2009_47	Around a "nurse" tree	227-230	22 º 11’ 21.3’’	29 º 13’ 04.2’’
October 2009_48	Around a "nurse" tree	231-234	22 º 11’ 21.4’’	29 º 13’ 04.7’’

October 2009_49	Around a "nurse" tree	235-238	22 º 11’ 21.1’’	29 º 13’ 04.8’’
October 2009_50	Around a "nurse" tree	239-246	22 º 11’ 22.2’’	29 º 13’ 04.2’’
October 2009_51	Around a "nurse" tree	247-250	22 º 11’ 21.7’’	29 º 13’ 05.6’’
October 2009_52	Around a "nurse" tree	251-258	22 º 11’ 21.2’’	29 º 13’ 05.5’’
October 2009_53	Around a "nurse" tree	259-262	22 º 11’ 20.9’’	29 º 13’ 06.2’’
October 2009_54	Around a "nurse" tree	263-270	22 º 11’ 21.2’’	29 º 13’ 06.9’’
October 2009_55	Around a "nurse" tree	271-274	22 º 11’ 21.2’’	29 º 13’ 07.6’’
October 2009_56	Around a "nurse" tree	275-281	22 º 11’ 20.5’’	29 º 13’ 07.4’’
October 2009_57	Around a "nurse" tree	282-284	22 º 11’ 20.4’’	29 º 13’ 07.7’’
October 2009_58	Around a "nurse" tree	285-288	22 º 11’ 20.1’’	29 º 13’ 07.7’’
October 2009_59	Around a "nurse" tree	289	22 º 11’ 20.7’’	29 º 13’ 07.7’’
October 2009_60	Around a "nurse" tree	290-293	22 º 11’ 20.9’’	29 º 13’ 07.4’’
October 2009_61	Around a "nurse" tree	294-296; 298	22 º 11’ 21.2’’	29 º 13’ 07.3’’
October 2009_62	Around a "nurse" tree	297; 299-300	22 º 11’ 20.9’’	29 º 13’ 07.7’’
October 2009_63	Around a "nurse" tree	301-304	22 º 11’ 19.7’’	29 º 13’ 08.2’’
October 2009_64	Around a "nurse" tree	305-315	22 º 11’ 22.9’’	29 º 13’ 05.1’’
October 2009_65	Around a "nurse" tree	316-319	22 º 11’ 23.2’’	29 º 13’ 04.8’’
October 2009_66	Around a "nurse" tree	320-323	22 º 11’ 23.7’’	29 º 13’ 03.6’’
October 2009_67	Around a "nurse" tree	324-327	22 º 11’ 23.8’’	29 º 13’ 03.1’’
October 2009_68	Around a "nurse" tree	328	22 º 11’ 24.8’’	29 º 13’ 02.5’’
October 2009_69	Around a "nurse" tree	329-332	22 º 11’ 25.0’’	29 º 13’ 03.1’’
October 2009_70	Around a "nurse" tree	333-335	22 º 11’ 25.1’’	29 º 13’ 03.5’’
October 2009_71	Around a "nurse" tree	336-339	22 º 11’ 25.5’’	29 º 13’ 03.6’’
October 2009_72	Around a "nurse" tree	340-342	22 º 11’ 25.9’’	29 º 13’ 02.8’’

Appendix E.

The spreadsheet used for morphological monitoring.

<table>
<tr><th colspan="12">Plant morphological measurements</th></tr>
<tr><td colspan="4">Date:</td><td colspan="8">Location:</td></tr>
<tr><td colspan="4">Observer:</td><td colspan="8">GPS coordinates:</td></tr>
<tr><td rowspan="2">Species code</td><td colspan="5">Treatment</td><td colspan="3">Growth parameters measured</td><td></td><td></td><td></td></tr>
<tr><td>MYCCOM</td><td>COM</td><td>MYC</td><td>CONTROL</td><td>Nursing</td><td>Diam at base (mm)</td><td>Diam at 30cm (mm)</td><td>Height (cm)</td><td>Mortality</td><td>Browsing</td><td>Notes</td></tr>
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