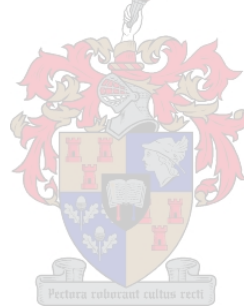


**Mycorrhiza re-establishment on post-mined rehabilitated areas of
the Brand se Baai Succulent Karoo vegetation.**

By

Albertina Ndeinoma



**A thesis presented in partial fulfillment of the requirement for the degree of
Master of Science in Environmental Impact Assessment**

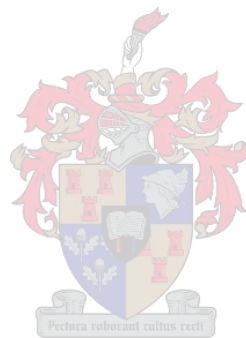
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June 2006

Declaration

I the undersigned hereby declare that I am the sole author of the work contained in this thesis which is my own in its original form and that it has not been previously entirely or in part submitted to any other university for a degree.



Signature *Andeumua* Date.... **14 JUNE 2006** ..

Abstract

Parts of the West Coast Strandveld and adjacent Succulent Karoo on the arid coast of Namakwaland in the Western Cape of South Africa are subject to surface mining. An understanding of mycorrhizal association of plants in the natural vegetation of this area could contribute to the improvement of post-mining re-vegetation of the area. This study investigated mycorrhizal association of plants in the West Coast Strandveld, and compared mycorrhizal infection rates of soils taken from natural vegetation to soils from post-mined rehabilitated vegetations. The study was divided into two components.

In the first component a pot experiment was conducted in the greenhouse to assess vesicular-arbuscular mycorrhiza (AM) infectivity of post-mined rehabilitated areas of Brand se Baai in Namakwa Sands mining areas. Rehabilitated areas used in this study included sites that has been strip mined for heavy minerals and then progressively backfilled with sub-soil sand remaining after mineral extraction (tailings), topsoil and translocated plants in an effort to restore the structure and functional aspects of the mined site to its original (pre-mining) ecosystem. Rehabilitated sites¹ assessed in this study included sites backfilled with: tailings + translocated plants (TP); tailing + topsoil + translocated plants (TSP) and tailings + topsoil only (TS). Natural sites (N) were also assessed to serve as reference points. AM infection was evaluated as percent root colonization on wheat planted as bioassay on sterilised sand and inoculum from rehabilitated sites in the ratio of 3:1 respectively. Results of this study component showed that mycorrhiza infectivity of rehabilitated soils was high on TSP and TS because mining disturbance has been remedied by topsoil with or without translocated plant replacement. The structural and chemical components of topsoil used as rehabilitation material favoured re-establishment of microbial activities. Infectivity was however low on soils rehabilitated with tailings and translocated plants (TP) because this treatment lacked topsoil which is a major source of infective mycorrhizal propagules. Infectivity was also low in soils from undisturbed sites (N) probably high phosphorus concentration or presence of perennial vegetation led to

¹translocated plants on tailing (TP), translocated plants on tailings and topsoil (TSP), tailings and topsoil only (TS), Natural site (N) and Control (C)

low mycorrhiza infection. Results showed that there was no significant effect of mycorrhiza on plant growth rate, nutrient uptake or carbon cost of mycorrhizal plants when related to non-mycorrhizal plants, instead the biomass production and nutrient contents of plants were determined by chemical properties of treatment soils.

The second component of the study investigated presence of mycorrhiza on randomly selected common indigenous species of Aizoaceae, Asparagaceae, Asteraceae, Chenopodiaceae, Fabaceae, Lamiaceae, Mesembryanthemaceae, Restionaceae, families growing on unmined areas of the study site. Total mycorrhiza infection was recorded on 85% of the assessed species with percent infection level ranging from 8% in *Atriplex lindleyi* and *Drosanthemum hispidum* to 98% in *Salvia lanceolata*. Functional mycorrhizal association with arbuscule structures were however only observed on 15% of all species assessed. Low arbuscules infection observed in indigenous species assessed in this study could be associated with the timing of mycorrhiza infection assessment and root competition in the soil. There was no infection observed on four species belonging to Chenopodiaceae, Zygophyllaceae, Sterculiaceae, and Asteraceae families, which represented 15% of all species assessed. Most species belonging to Chenopodiaceae and Zygophyllaceae have been reported as non-mycorrhizal in other studies, absence of mycorrhiza on the remaining three families species observed in this study require further confirmation.

Opsomming

Dele van die Weskus-Strandveld en die aangrensende Sukkulente Karoo aan die droë kus van Namakwaland in die Weskaap van Suid-Afrika word aan oppervlakmynbou onderwerp. Kennis van skimmelwortelassosiasie van plante in die natuurlike vegetasie van hierdie gebied kan bydra tot beter hervegetasie van die streek ná die mynbou-aktiwiteite. Hierdie ondersoek van skimmelwortelassosiasie van plante in die Weskus-Strandveld het ook die skimmelwortelinfeksietempo's van grondsoorte van die natuurlike plantegroei vergelyk met dié van grondsoorte van die gerehabiliteerde vegetasies ná die mynbou-aktiwiteite. Die ondersoek is in twee komponente verdeel.

In die eerste komponent is 'n poteksperiment in die kweekhuis uitgevoer om die vesikulêr-arbuskulêre skimmelwortel (VAS)-infektiwiteit van ná-mynbou-gerehabiliteerde areas van Brand se Baai in die Namakwa Sands-mynbougebiede te evalueer. Gerehabiliteerde areas wat in hierdie ondersoek gebruik is, het terreine ingesluit waar stroopmynbou vir swaar minerale plaasgevind het, wat dan weer progressief hervul is met ondergrondse sand wat na mineraalekstraksie oorgebly het (uitskot), bogrond en teruggeplaaste plante in 'n poging om die struktuur en funksionele aspekte van die gemynde terrein na sy oorspronklike (voor-mynbou-) ekosisteem te herstel. Gerehabiliteerde terreine wat in hierdie ondersoek geëvalueer is, sluit terreine in wat hervul is met: uitskot + teruggeplaaste plante (UP), uitskot + bogrond + teruggeplaaste plante (UBP) en slegs uitskot + bogrond (UB). Natuurlike terreine (N) is ook geëvalueer om as verwysingspunte te dien. VAS-infeksie is geëvalueer as persentasiewortelkolonisering op graan wat as biotoets geplant is op gesteriliseerde sand en innokulum van gerehabiliteerde terreine in die verhouding van onderskeidelik 3:1.

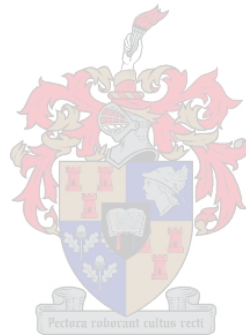
Resultate van hierdie studiekomponent het getoon dat skimmelwortelinfeksie van gerehabiliteerde grond hoog was by UBP en UB omdat die mynbouverstoring deur die hervulling met bogrond en teruggeplaaste plante herstel is. Die strukturele en chemiese komponente van bogrond wat as rehabiliteringsmateriaal gebruik is, is

gunstig vir skimmelwortelhervestiging van mikrobiese aktiwiteite. Infektiwiteit was egter laag by UP- en N-behandelings terwyl lae infeksie by UP geassosieer is met die afwesigheid van bogrond, wat 'n groot bron van infektiewe skimmelwortelpropagules is, terwyl lae infeksie by N-behandeling betrekking het op die effek van grondeienskappe en vegetasiedekkingstipe op skimmelwortelaktiwiteite. Hoë fosforkonsentrasie en die teenwoordigheid van meerjarige plantegroei op N-behandeling het tot lae skimmelwortelinfeksie gelei. Resultate in hierdie studie het getoon dat daar nie 'n beduidende effek op plantgroeitempo, nutriëntopname of koolstofkoste van skimmelwortelplante in verhouding tot nie-skimmelwortelplante was nie; die biomasseproduksie en nutriëntinhoud is eerder deur die chemiese eienskappe van behandelingsgrondsoorte geaffekteer.

Die tweede komponent van die studie het die teenwoordigheid ondersoek van skimmelwortel op ewekansig gekose algemene inheemse spesies van Asteraceae, Mesembryanthemaceae, Zygophyllaceae, Chenopodiaceae, Asparagaceae, Fabaceae, Aizoaceae, Sterculiaceae, Lamiaceae en Restionaceae families wat natuurlik in ongemynde areas van die studietrein groei. Totale skimmelwortelinfeksie is by 85% van die geëvalueerde inheemse spesies aangeteken met die persentasie-infeksiëvlak wisselend van 8% by *Atriplex lindleyi* en *Drosanthemum hispidum* tot 98% by *Salvia lanceolata*. Funksionele skimmelwortelassosiasie met arbuskulêre strukture is egter slegs by 15% van alle geëvalueerde spesies waargeneem. Tydsberekening van skimmelwortelinfeksie-evaluering en wortelwedywering in die grond was waarskynlik die rede waarom daar minder arbuskulêre ontwikkeling by geëvalueerde inheemse spesies was. Geen infeksie is waargeneem by vier spesies behorende tot die families Chenopodiaceae, Zygophyllaceae, Sterculiaceae en Asteraceae nie, wat 15% van al die geëvalueerde spesies verteenwoordig het. In ander studies is aangeteken dat die meeste spesies van Chenopodiaceae en Zygophyllaceae nie met skimmelwortel besmet is nie; die afwesigheid van skimmelwortel by die spesies van die ander drie families wat in hierdie ondersoek waargeneem is, vereis verdere bevestiging.

Dedications

To my parents, I appreciate your endless love and guidance that shaped both my social and academic determination.



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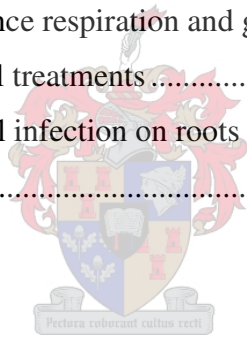
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1 INTRODUCTION

Coastal mining is carried out in several areas of South Africa like Namakwaland, Richards Bay (Mentis and Ellery, 1994) as well as in the coastal areas of Karas and Erongo Regions of Namibia (Burke, 2001). At Namakwaland coastal areas, the Anglo American company is mining an area of approximately 4700 ha mainly for titanium, zirconium, rutile and iron from sand on the west coast of Namakwaland (Anglo American Corporation, 2002). Before mining, the top soil to a depth of 5 cm is removed and stored later to be used for soil rehabilitation. The underlying sand to a depth of 1-3 m is then excavated (strip mining) and washed with sea water to extract the heavy minerals that have accumulated in the sand through the action of waves and wind. The topsoil is later replaced and indigenous seeds are sown or plants are translocated to these sites for vegetation rehabilitation. Strip mining in coastal areas causes damage to the ecosystem (soil and vegetation) as the topsoil and vegetation is removed. This changes micro-topography, salinity, as well as mycorrhiza network of strip mined soil surfaces (De Villiers *et. al.* 1999, Miller, 1978). Mining companies in South Africa are however, compelled by law to rehabilitate environments affected by their operations. Namakwa Sands Mine for instance, has a rehabilitation programme that will ultimately determine whether or not a closure certificate can be issued. Upon a successful rehabilitation, the company is freed from further responsibility of management of the rehabilitated land and can embark on further prospecting and mining activities.

About 80% plant species form mycorrhizal associations with fungi (Prescot *et al*, 1996; Smith and Read, 1997). In arid environments with infertile soils, such as the vegetation of the Strandveld Succulent Karoo, mycorrhiza contribution to plant nutrient uptake is significant. Studies in mycorrhiza research showed that mycorrhizal plant roots enhance uptake of nutrients like phosphorus, zinc and nitrogen in the form of NH_4^+ and NO_3^- which eventually leads to improved plant growth (Smith and Gianinazzi-Pearson, 1988). Disruption of the mycorrhizal network by surface mining can result in reduced infectivity of the soil and lowers the rate of nutrient uptake by plants translocated into these areas (Moorman and Reeves, 1979; Miller, 1978). Effective soil rehabilitation practices are therefore required to allow the mined landscapes to recover its microbial communities.

Topsoil replacement and translocation of indigenous plants to mined sites is one of the rehabilitation strategies that can be used to improve microbial activities of mined soils. Mycorrhiza quantification in these areas is therefore useful information in determining the progress in return of mycorrhiza to the mined site. This information will form the basis of recommendation as to whether mycorrhiza inoculation is required to enhance establishment of translocated plants on mined areas like Namaqua Sands.

1.1 Mycorrhiza symbiosis

Mycorrhiza is a mutual symbiotic relationship between plant roots and fungi characterized by a bi-directional movement of nutrients between plant roots and fungi (Jackson and Masson, 1984). Mycorrhizal fungus are heterotrophic, obtaining most of their energy in the form of carbon from plant roots while they supply the plant with inorganic minerals absorbed from the surrounding soil (rhizosphere) (Jackson and Mason, 1984). With few exceptions, mycorrhizae have been observed in most plants of economic importance to man. About 95% of the world plant species belongs to families that were studied and confirmed to form associations with mycorrhiza (Smith and Read, 1997; Malajczuk *et. al.*, 2000; Mauseth, 1995; Marschner, 1986). Two main types of mycorrhiza relationships are recognised based on the arrangement of fungal mycelium to the root structure, the ectomycorrhizae and the endomycorrhizae.

Ectomycorrhizal (ECM) relationships exist in nearly all woody plants and only in few herbaceous and graminaceous perennial plants. ECM symbiosis is common in tree families of Pinacea, Betulaceae, Fagaceae and Salicaceae of the northern hemisphere but they may also occur in some tropical and subtropical forest regions (Marschner, 1986). ECM symbiosis are characterised by a mantle of fungal hyphae formed around the root surface and a network of fungal mycelium, called a Hartig net formed between cortical cells of roots. With endomycorrhizae the fungi live within cortical cells and grow intercellularly. There are three well known types of endomycorrhizae, arbuscular mycorrhiza (AM); ericoid mycorrhiza and orchidaceous mycorrhiza whereby AM is by far most abundant (Mauseth, 1995; Marschner, 1986) hence the focus of this study.

AM is characterised by the formation of branched haustorial structures (arbuscules) within the cortex cells that serves as the main sites for mineral nutrients exchange with the host. Hyphae that grow externally to the surrounding soils are called mycelium. Other structures formed by many, but not all endomycorrhizal fungi, are vesicles that are lipid storage organs, hence the name sometimes called vesicular- arbuscular mycorrhiza.

1.1.1 Role of mycorrhizae in ecological rehabilitation

The primary benefit of mycorrhizal associations to plants is a positive growth response due to increased acquisition of nutrients such as P, Cu, and Zn that do not diffuse readily into plant roots in the absence of a symbiosis Brady, (1990) especially in dry soils of semi arid regions. Mycorrhiza applications gained popularity recently due to successful research on its application to pest and disease control, energy conservation, organic fertilizer as well as on reclamation of land disturbed by development industries (Jackson and Masson, 1984).

Considering the importance of mycorrhizal symbiosis, the absence or reduction of an effective mycorrhizal symbiosis in the soils is an important ecological factor that needs to be assessed and corrected for successful plant re-establishment in rehabilitation programmes (Moorman, 1979, Malajczuk *et. al.*, 2000). In strip mining industries for instance, the topsoil and vegetation is removed in order to access minerals. These practices disturb the mycorrhizal soil network as well as vegetation which serve as microhabitat for mycorrhizae. Large areas of waste lands are created with mine spoils, sand and other waste, each with peculiar problems related to pH, fertility, toxicity, erosion and instability. Decades of experimental work has shown the fundamental importance of mycorrhizal symbiosis in restoration and re-vegetation. Benefits of mycorrhizae to the plant community, especially in re-vegetation, are increased seedling survival, higher plant species diversity and improved soil structure (Adholeya *et. al.*, 1997). These benefits improve the tolerance of species to disturbances associated with mining activities and thereby leading to a successful land rehabilitation.

1.1.2 Factors affecting AM infection in plants

Roots of plants are infected with mycorrhizae either from spores, hyphal network, root residues or from a neighbouring root of the same or different plant species (Marschner, 1986). These propagules are the main source of AM inoculum to plant roots. Distribution and abundance of mycorrhizal infective propagules in the soil is affected by various factors including mycorrhiza biology, seasonal variation, soil properties, vegetation type and vegetation cover (Abbott and Robson, 1991; Brundrett, 1991).

1.1.2.1 Climatic and edaphic factors

Mycorrhizae exhibit little host-fungus specificity. This is the reason why mycorrhiza is present on many plants in nature. However, evidence is accumulating that AM adapt to specific edaphic and environmental conditions. This implies that AM fungi abundance and distribution is less influenced by the type of host plant but rather by soil and other environmental factors. There is therefore a considerable variation in symbiotic establishment in plants occurring in different levels of soil PH, nutrients, moisture, salinity and temperature (Brundrett, 1991; Malajczuk *et. al.*, 2000). At global level, generalisations have been made enabling recognition that specific suites of climatic and edaphic conditions have led to the selection of distinctive types of mycorrhiza each dominating a distinctive biome. In the absence of human disturbance, species with ericoid mycorrhiza predominate on non-humus soils of high latitude and altitude, ectomycorrhizal species predominate in forest ecosystems with surface litter accumulation and plants with arbuscular mycorrhizae dominate herbaceous and woody plant communities of low mineral soils at low altitude (Read, 1991).

1.1.2.2 Mineral nutrient level of the soil

Mineral nutrient supply may also suppress or enhance root infection and colonisation with mycorrhizae. Related studies show that extremely low levels of nutrients in the soil, leads to low root infection in both AM and ECM. Root infection however increases with

an increased level of nutrients until the optimum is achieved the level beyond which additional nutrients in the soil would inhibit root infection (Marschner, 1986; Claasen and Zasoski, 1993). This phenomenon is well demonstrated with the phosphorus level in soil (Amijee *et. al.*, 1989). At 50 mg kg⁻¹ phosphorus maximum infection was realised while beyond 100 mg kg⁻¹ infection is reduced and plant growth rate may also be reduced (Schubert and Hayman 1986; Amijee *et. al.*, 1989).

1.1.2.3 Season of active plant growth

Mycorrhizal status of plants also varies with season due to phenology of plant roots (Brundrett, 1991; Scheltema *et. al.* 1987). In plants with annual root development, mycorrhiza colonisation levels are high during season of active root growth. On plants with less or no root replacement per year (mainly perennials), mycorrhiza colonisation show less or no seasonal variation. Active associations with arbuscules on perennial vegetation only occur on their youngest roots which only comprises of a fraction of their entire root systems (Brundrett, 1991). The season in which active root growth occurs also affects the level of mycorrhiza colonisation. Plants with root development during warmer season have high colonisation while those with active root development in colder seasons tend to restrict microbial activities (Brundrett, 1991).

1.1.2.4 Vegetation cover type

The relative abundance of plant species and the level of infection on each species have an effect on mycorrhiza infectivity of the soil (Abbott and Robson, 1991). Generally most species form association with mycorrhiza, but there are also known species from families such as Brassicaceae, Chenopodiaceae, Cyperaceae, Juncaceae, Proteacea, Zygophyllaceae, that rarely form mycorrhizal associations (Brundrett, 1991). It has also been observed that there is generally a lower density of spores in natural ecosystems dominated by perennial shrubs than ecosystems dominated by annual grass or crops (Mosse and Bowen, 1968; Abbot and Robson, 1977a).

This therefore implies that, mycorrhiza infection would be high in soils where vegetation cover is dominated with mycorrhizal plants.

1.1.2.5 Soil disturbance

The pre-existing mycelial network in the soil promotes mycorrhiza infection. Soil disturbances (agricultural activities, mining and erosion) therefore reduce mycorrhiza distribution and abundance as it disrupts the network of mycorrhizal fungi in the soil.

1.1.3 Carbon cost of mycorrhizal associations

The cost on plants for maintaining mycorrhizal symbiosis is expressed in terms of organic carbon supplied to the fungi in return of nutrients (mainly P and N) supplied to the plant. In plants, carbon is lost mainly due to energy requirements for new tissues construction, and respiration (growth and maintenance) (Van Iersel and Seymour, 2000). In the presence of mycorrhiza association, the plant carbon requirement for root growth and maintenance respiration is elevated due to the additional sink effect of mycorrhiza symbiosis (Eissenstat *et. al.* 1993). AM fungi of a wide range of herbaceous and woody plants can receive up to 23% of the photosynthesized plant's daily carbon (Peng *et. al.* 1993, Douds *et. al.* 1988 Jakobsen and Rosendahl, 1990). In nutrient poor soils mycorrhiza can benefit the plant by increasing its nutrient uptake and eventually the rate of plant's photosynthesis. In these cases the fungi may use a considerable proportion of fixed carbon but this is compensated for by enhanced rate of photosynthesis, the process in which carbon is assimilated into the plant (Eissenstat *et. al.* 1993). Phosphorus and other forms of nitrogen like NH_4^+ and NO_3^- that are absorbed by plants are less mobile in the soil hence mycorrhiza benefits for uptake of these elements is often pronounced. AM can supply up to 80% of phosphorus and 25% of nitrogen to the host plant and this will directly or indirectly lead to increased growth rate on mycorrhizal plants (Peng *et al.* 1993, Smith and Read, 1997). However, in nutrient rich soils mycorrhizal associations can depress plant growth because the cost of root tissues construction in these soils is

lower than the cost of maintaining the fungal symbiosis (Peng *et. al.* 1993; Smith and Read, 1997). In these cases plants supply the fungi with carbon, in return of little or no nutritional benefit because nutrients are readily available in the soil and plants can easily access these nutrient without mycelia hyphae (Brundrett, 1991; Smith and Read, 1997). On the basis of the above background, it is therefore very important that mycorrhiza assessment studies also assess the cost to the plant toward maintaining the mycorrhiza association. This is important to determine whether the established mycorrhizal association is beneficial or parasitic to the plant. This is determined by establishing the amount of carbon released to the fungi in relation to the additional growth rate by additional nutrients by the fungi. Comparing mycorrhizal plants to non-mycorrhizal plant it can be determined whether mycorrhiza is beneficial to the plants in mineral rich soils or not. The accurate method to determine carbon cost is by measuring gas exchange during plant photosynthesis. The amount of carbon dioxide assimilated for photosynthesis is related to the amount of carbon produced and or lost by the plant to mycorrhizal symbiosis. In this study carbon was determined from whole plant dry matter analysis.

1.1.4 Review of methods used to assess AM soil infectivity

Two common methods have been used to quantify mycorrhiza communities in the soil. These include: isolation of spores and other mycorrhizal propagules from the soil by wet sieve count method and quantification of AM structure colonization in a host (plant assay) roots (Porter, 1979; Hendrix *et. al.* 1990). Having decided what method to use, it is important that the right sampling procedure is followed as the use of inappropriate method may not portray the true level of mycorrhiza infectivity in the soil.

Accuracy of the method used to quantify AM fungi depends on the intensity of sampling within a given study site. It is necessary to have sub-samples or more samples per site to analyse variation in soil infectivity or root colonisation in order to increase accuracy and reduce possible errors.

1.1.4.1 Wet sieve count of mycorrhizal spores in the soil

Spores of AM fungi are larger than most other fungi (ranging from 10-1000 μm in diameter) therefore they can easily be isolated from the soil and observed with a dissecting microscope (Sylvia, 1994). Spores can be isolated from the soil by different methods; wet sieving method is commonly used. For further details on the procedure of this method the reader is referred to (Hendrix *et. al.*, 1990; Douds, *et. al.*, 1999). With this method AM infectivity can not be accurately determined because dead or dormant spores can not be differentiated. Again colonized roots and hyphae that also serve as propagules for infection are not accounted for in the wet sieving spore isolation method because these particles are bigger and often removed by the coarse sieve used to remove debris and soil particles. The use of wet sieving to count spores in the soil is therefore not representative, mostly underestimate numbers of infective AM fungi in the soil (Sylvia, 1994). For this reason plant assays have been used to obtain an estimate of total propagules number in the soil.



1.1.4.2 Most probable number of infective propagules by assay plants

The “most probable number assay (MPN)” method for estimating the density of micro organisms in field soils, liquid culture and various forms of inocula have been found to be a solution to problems related to wet sieve counting method. This method relies on the detection of specific qualitative attributes of micro organisms of interest to enable population estimates. Assay hosts are planted in series of inoculum dilutions, each with replications, for a specified time limit. Inoculum dilution where microbial infection is accurately determined can be selected to form the experimental design. On assumption that propagules are uniformly distributed in the soil and that one or more propagules in the soil are capable of infecting the assay host, population estimates can be derived from MPN tables that have been calibrated based on given experimental designs (Woomer, 1994). A slight modification of this method was utilised for this study, where only one series of dilution was used and replicated three times on each treatment. Quantification of root colonisation on the host plants was not derived from calibrated tables but rather

expressed as observed under the microscope at regular intervals during plant growing period. Quantitative evaluation is carried out by gridline intersect method or slide method (Sylvia, 1994; Brundrett *et. al.*, 1994)

1.1.4.3 Evaluation of infective propagules by chemical assays

Quantification of AM can also be done by chemical assay methods whereby certain chemical component of fungi like chitin or fatty acids are detected and quantified (Sylvia, 1994). The use of chitin is however not preferred because chitin structures are common in nature, and they are found in cell walls of many non-mycorrhizal fungi like in exoskeleton of some insects. The use of other types of chemical components of fungi is therefore preferable.

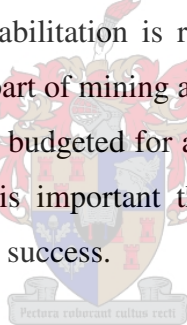
1.2 Ecological restoration of mined areas

A number of terminologies are used in relation to ecological restoration. It is important that terminologies are defined and appropriately used to avoid confusion among the readers. Ecological restoration is the process whereby ecological principles and research are applied in order to return the disturbed land to the original ecosystem in terms of its structure and functions (Cooke and Johnson, 2002). The progression of the disturbed land toward the original ecosystem is what is referred to as ecological rehabilitation. Other terms such as reclamation or replacement are frequently used whereby reclamation refers to the process of returning the land surface to some form of beneficial use not necessarily the original use and replacement is the creation of the original ecosystem with an alternative ecosystem. The word rehabilitation will therefore be used in this paper implying that efforts of either reclamation or rehabilitation are carried out with a definite aim of reinstating the original structure and functions of the ecosystem.

In strip mining, composition and structure of the soil and vegetation is destroyed, leaving a bare environment that will take a long time to recover. This type of mining is expanding in biologically diverse winter rainfall environments of South Africa and Namibia where growth is restricted by aridity, wind and nutrient poor soils (Burke, 2001; Milton, 2001). Restoration has a variety of benefits including improved aesthetics;

conservation of diversity, return of use values (such as grazing) to the land and reduced risk of sand and dust storms or floods (World Resource Institute, 2003; Adholeya *et. al.*, 1997).

In many countries, legislation requires that mined areas be rehabilitated sufficiently to stabilize the soil with vegetation and re-establish ecological functions so as to accelerate the succession process of landscape recovery. In South Africa for instance, post-mining rehabilitation is a legal requirement provided for by National Environmental Management Act (NEMA) No. 107 of 1998 and the Minerals and Petroleum Resources Development Act No. 28 of 2004. NEMA overrides all other legislation and it makes provision for integrated environmental management intended to ensure that environmental concerns are taken into account in developmental actions from planning phase of the project through to closure. In line with the requirement for closure and issue of new mining rights, effective rehabilitation is required in South Africa. Ecological rehabilitation has therefore become part of mining activities in areas like Namakwa Sands where mining is carried out and it is budgeted for accordingly. Because rehabilitation is costly and a legal requirement, it is important that research informed strategies and actions are carried out for restoration success.



In soil dynamics especially of sandy arid regions with limited nutrients, mycorrhizal associations are among the most important ecological processes that facilitate the capability of the land to capture and retain nutrient, water and species. They therefore need to be incorporated in ecological restoration programme objectives (Burke, 2001). Understanding of factors affecting the relative abundance and distribution of AM symbioses especially of disturbed ecosystem is required to enable us predict patterns of mycorrhiza development in soil rehabilitation programmes.

1.2.1 Conceptual ecological restoration planning

Ecological restoration consists of sets of broad activities, each appropriate to the type and extent of disturbance. In mining sites, this involves ecosystem reconstruction, whereby the soil and vegetation that was disturbed is introduced back to the site in order to re-establish its capability to capture and retain fundamental resources like energy, water, nutrient and species (Cooke and Johnson, 2002). This process is not governed by a holistic law but rather an adaptive environmental management process appropriate for a dynamic ecosystem. A well planned ecological rehabilitation programme should include goals, objectives and measurable criteria and indicators for monitoring rehabilitation success.

1.2.1.1 Ecological restoration goals and objectives

To monitor success of an ecological restoration programme, goals and objectives of rehabilitation should be developed. Restoration goals must be well defined, adapted to a specified site and also flexible to accommodate ecosystem perturbation. These objectives are however often broad such that some important structural measurements like soil microbial activities are not usually specified even though they provide important indications of long term land productivity and succession pathways. To achieve a given restoration goal, sets of objectives and strategic actions are therefore developed as operational guideline toward the achievement of a common goal. Restoration objectives may include measurable ecosystem attributes that are technically feasible, ecologically sound and socially relevant (Cooke and Johnson, 2002).

The restoration goal at Namakwa Sands mine for instance is “to rehabilitate impacted areas until a self sustaining indigenous vegetation cover is established in order to achieve vegetation cover and land productivity similar to small stock farming existed before mining” (Anglo American Corporation, 2002). With this objective in mind the environmental management unit at Namakwa Sands mine also focuses on rehabilitating detailed ecosystem attributes like soil process that facilitate uptake, storage and cycling of soil nutrients and eventually support vegetation re-establishment. The mined soil is

backfilled with tailings and topsoil. Salvaged indigenous species are also translocated to the mined sites. The landscapes are then re-shaped and graded to avoid soil movements by wind erosion, which is a prevailing form of erosion at the site. Barriers of polythene shade net windbreak are also erected perpendicular to wind direction around rehabilitated mined areas. These barriers are about 1 m in height and they are useful in reducing wind and wind blown sand damage to the establishing plants. Mulching with stockpiled vegetation and sometimes with fast growing crops like sorghum is also applied to facilitate water and nutrient retention of the soil at the site. These activities are carried out mainly to facilitate the return of indigenous species to the mined site in order to achieve measurable targets including vegetation cover and species richness within a specified period.

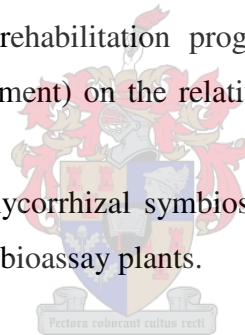
1.2.1.2 Monitoring criteria and indicators of rehabilitation success

Another important aspect of any restoration programme is monitoring the success of restoration. This is carried out by developing quantitative sets of criteria and indicators for a successful restoration. These should be measurable, and related to the management goals and objective. In general for a successful rehabilitation programme, the restored ecosystem should attain self regulation within a set period of time. In other words structural and functional attributes of the restored ecosystem should be able to sustain themselves in the absence of initial rehabilitation facilitating activities like fertilizing, seeding or watering. Initial goals and objectives established before restoration must also be attained with no observable adverse effect in the larger ecological landscape (Cooke and Johnson, 2002).

1.3 Objectives of the study

The aim of this study was to assess mycorrhiza density and infectivity of post-mined rehabilitated landscapes in comparison with natural sites in the vicinity of Brand se Baai mining area. The formation of mycorrhiza associations with common indigenous plant species in the natural areas of the site will also be assessed. Specific objectives of the study are to:

- Assess and quantify the presence of mycorrhiza on indigenous plants of various species growing on the Dwarf Shrub Strandveld and Tall Shrub Strandveld vegetation communities of Brand se Baai mining area in order to enable ascertain their dependency on mycorrhiza symbiosis for establishment and nutrient acquisition.
- Determine the effect of rehabilitation programme (topsoil and tailings with translocated plants replacement) on the relative density of infective mycorrhizal propagules.
- Determine the effect of mycorrhizal symbiosis on biomass production, nutrient uptake and carbon costs of bioassay plants.



Hypotheses of the study are that:

- Indigenous plant species differ in mycorrhizal infection level.
- Mycorrhizal density differs among soil treatment (natural and disturbed / rehabilitated soils).
- The presence of mycorrhiza on rehabilitated sites (with translocated plants) is associated with topsoil or with plants translocated to the site.
- The presence of mycorrhiza in the soil will have a positive effect on growth and nutrient content of wheat.

Field sampling of roots was used to test the first hypothesis. The remaining hypotheses were tested by using wheat as a test plant. Mycorrhizal infection on roots of the test plant grown under green house conditions was quantified. Growth and nutrient uptake in relation to mycorrhizal presence was analysed using shoot weights of the test plants.

1.4 Thesis structure

This study consists of two components. The first component assesses mycorrhiza infectivity of rehabilitated and undisturbed sites by means of assessing mycorrhiza infection on bioassay plants while the second component assessed mycorrhiza colonisation levels in various selected indigenous species in undisturbed areas at the study site. This thesis consists of five chapters. Chapter 1 is an introductory chapter that consists of concepts on ecology of mycorrhiza, methods used in microbial assessments as well as concepts of ecological rehabilitation of mined areas. Chapter 2 entails general materials and methods used for the two components of the study including topics on the location and biophysical factors of the study site and location of experimental areas used for the two components of the study. Chapter 3 presents the first component of this study which is the assessment of mycorrhiza infectivity of rehabilitated soils. This component also assessed the effect of mycorrhiza colonisation on biomass production, nutrient content and carbon cost of plant bioassays. In this chapter detailed materials and methods used for each aspect of the study are explained as well as results, discussions and conclusions of the chapter. Chapter 4 consists of the second component of the study which assessed mycorrhiza colonization of indigenous species in natural areas at the mine site. Chapter 5 provides general recommendations and conclusions as well as pointing out research gaps and further required research in rehabilitation of microbial activities at the mine site. Chapters 3 and 4 are written in the form of stand-alone journal papers. For this reason there is some repetition and overlap of information in the introductions of these chapters.

2 GENERAL MATERIALS AND METHODS

2.1 Study site

2.1.1 Geographical location

Namakwa Sands mine is situated on the West Coast of South Africa, about 400 km from Cape Town in the vicinity of Brand se Baai. The proposed mining areas include Graauwduinen, Haartebeeste Kom, Houtkraal and Rietfontein farms all of which forms part of the two mining areas illustrated in Figure 2.1 below. In these areas Namakwa Sands mining company extract deposits of heavy mineral (ilmenite, rutile, leucoxene and zircon) from the soil. These minerals are separated at the Mineral Separation Plant (MSP) located 7 km north of Koekenaap town. Therefore the region most affected by the mining developments extends from Vredendal in the south to Bitterfontein in the north and also including the coastal belt from Papendorp to the mouth of Soutrivier (Figure 2.1). The overall operational areas make up the total of 15516 ha (Environmental Evaluation Unit, 1990). On a wider perspective, Namakwa Sands mining area in Namakwaland falls in the strongly winter rainfall part of the Southern Africa's Succulent Karoo Biome vegetation type recognised as the Namakwaland Namib Domain (Cowling *et. al.* 1999)

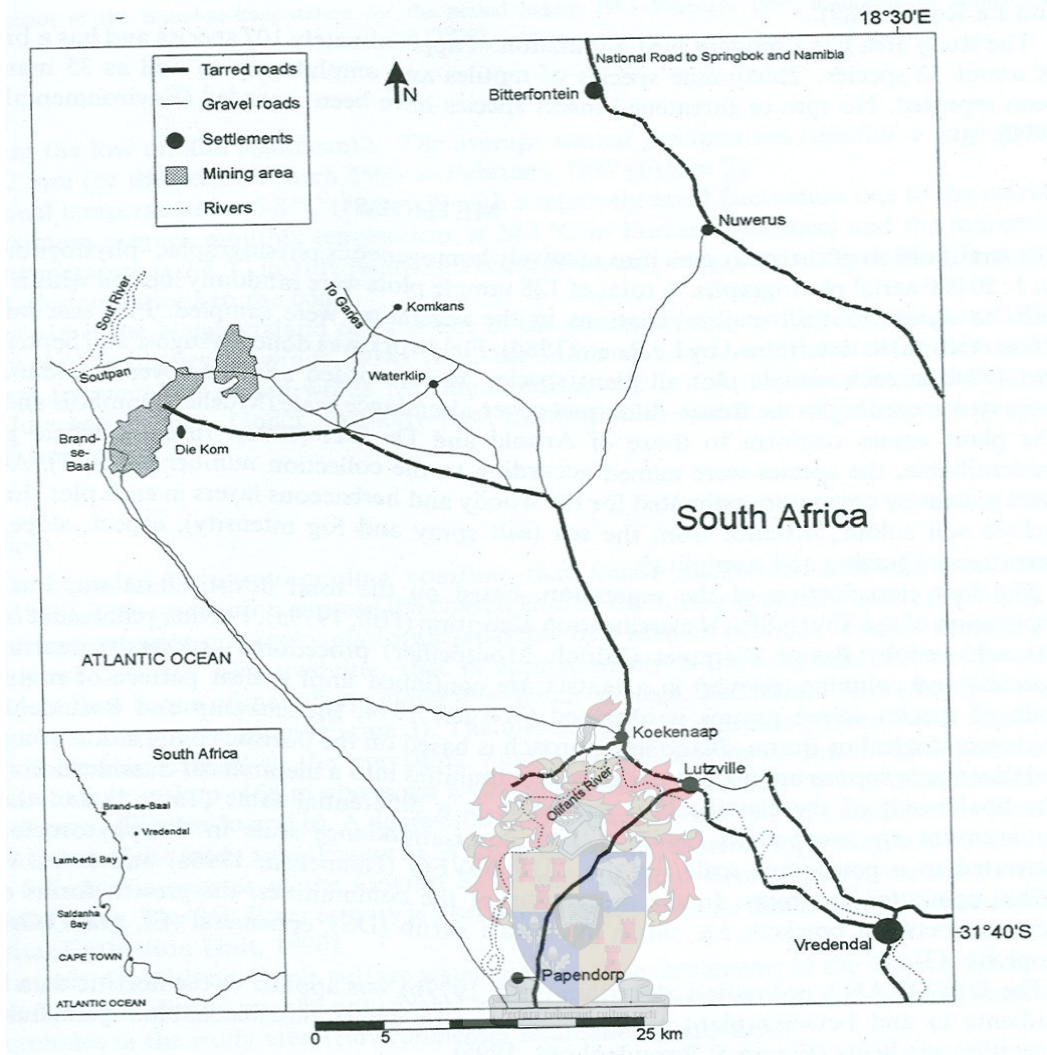


Figure 2.1 Location of Namakwa Sands mining areas at Brand se Baai (De Villiers, *et. al.* 1999).

The two mining areas shaded in figure 2.1 above consist of six different plant communities (Figure 2.2). Of these plant communities, only Dwarf Shrub Strandveld and the Tall Shrub Strandveld plant communities was used for this study. For the assessment of mycorrhiza soil infectivity of rehabilitated sites, inoculum soil was collected from post-mined rehabilitated sites within the Dwarf shrub strandveld plant community. To assess mycorrhiza infectivity of indigenous plants at the mining site, all plots were laid in a natural unmined sites within the Dwarf shrub and Tall shrub strandveld communities (figure 2.2 below).

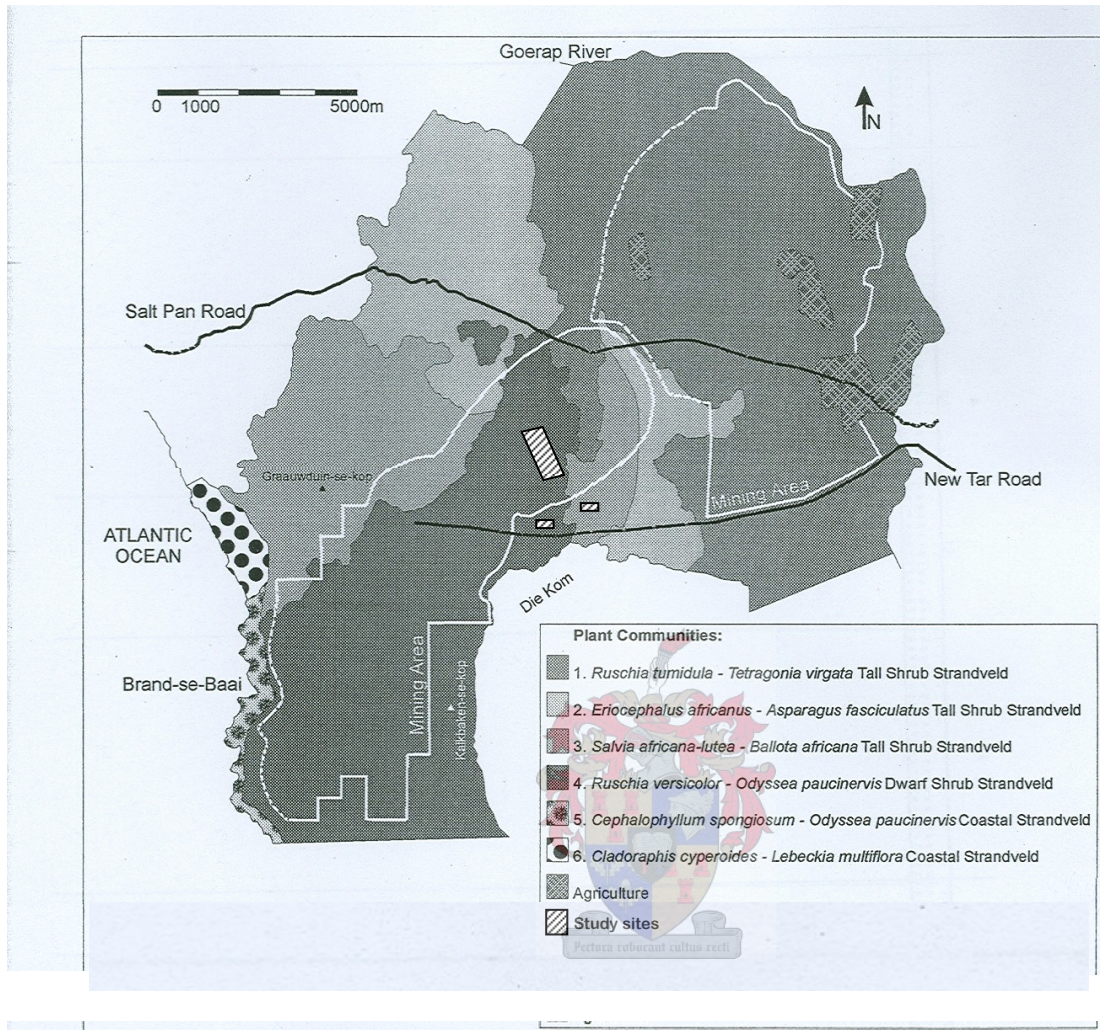


Figure 2.2 Location of sample plots (mined rehabilitated area and unmined natural areas) used for mycorrhiza infectivity adapted from (De Villiers, 1999; Mahood, 2003)

2.1.2 Physical environment

2.1.2.1 Climate

Namakwaland is a winter rainfall area, receiving an average rainfall in the range of 50 mm to 150 mm per annum with a rainfall increase from north to south. Rainfall in this dry region is highly predictable and prolonged droughts are rare, this is a unique phenomenon which is responsible for the unusual patterns and process of this region

(Cowling *et. al.* 1999). The climate is characterized by fog and dew falls that supplement the low rainfall of the area and leading to high humidity and relatively cool night temperatures (Cowling *et. al.* 1999). The wind regime on the Namakwaland coast is characterized by very strong and frequent southerly and south-south easterly winds in summer and by less frequent but strong winds from north and north-north easterly direction during winter months. Turbulent air known as “berg winds” from the high altitude plateau of southern Africa descent coastward leading to dynamic warming of the sea shore (Desmet and Cowling, 1999). These winds have a significant influence on plant life in Namakwaland.

2.1.2.2 Soils

The Namakwaland coastal area referred to as West Coast by Watkeys, (1999) is characterized by grey, regic calcareous sands of Post-African I association that show little evidence of pedogenesis. The Namakwaland coast north of Olifants river is included in the geomorphological subdivision of the Namib desert and it is referred to as the Namakwaland Sandy Namib (Environment Evaluation Unit, 1990). Generally the dunes along the coast are light coloured, becoming progressively red and yellow away from the coast. The lighter coloured dunes consist of unconsolidated quartz rich material while the red terrestrial deposits are derived from orange feldspathic sands. The terrestrial feldspathic deposits are the ones rich in heavy mineral deposits.

Generally these soils tend to be saline because of wind blown salt sprays from the sea. Soils at the mining site were found to have pH values exceeding 8 (Environment Evaluation Unit, 1990). However measurements by Mahood (2003) show lower pH of medium acidity on undisturbed sites. Salinity of mined soils (tailings) is increased (Mahood, 2003) probably as a result of the use of sea water during mineral extraction.

2.1.3 Biotic environment

2.1.3.1 Flora

Generally Namakwaland vegetation is characterised by the presence of dwarf leaf succulent shrubs, geophytes and annual plants that have specific life forms and functions allowing them to survive the low rainfall environment. Rainfall reliability and mild winters leads to a winter growth phenology of this area, where during autumn rain vegetative development of both annuals and perennial species begin and growth continue to reproductive maturity during winter (Cowling *et. al.* 1999). Namakwaland has a flora of about 3000 species distributed among 648 genera and 107 families. This is a very high number of species comparing to similar vegetations found in America, North Africa and Middle East. The region also has an extraordinary high level of endemism with about half of its plant life not found anywhere else in the world. Of the 107 families the common ones in a descending order include: Asteraceae, Mesembryanthemaceae, Poaceae, Scrophulanaceae, Fabaceae, Crassulaceae, Hyacinthaceae, Asclepiadaceae and Aizoaceae.

Boucher and Le Roux (1989) stratified the Strandveld Succulent Karoo vegetation into five zones on the basis of plant height that increases with soil depth. Five main types of vegetation zones identified on the basis of vegetation structure and floristic content are Strand communities, Strandveld communities, Succulent Karoo, Sand Plain Fynbos and River and Estuarine vegetation.

The *Strand community* classified by Boucher and Le Roux (1998) is a type of littoral vegetation which occurs as a transition zone between northern and southern Namakwaland strand communities. This littoral vegetation is different in the sense that most plant species that are found along the whole Namakwaland coast is represented in this transition zone. On the other hand the Strandveld vegetation consists of many drought deciduous and succulent species that are associated with the areas of calcareous sand. Height of plants is associated with soil depth, with the shortest vegetation growing on exposed calcrete and coastal rocks and the tallest growing where deep calcareous

sands occur. Small patches of Succulent Karoo vegetation occur and they are characterised by dwarf succulent leaved plants. Sand plain fynbos plant communities also occur in small patches of leached, acidic and low nutrient sands of the area. These vegetation types are sensitive to disturbance because they are subjected to heavy winds, salt sprays and drift sands. Disturbance to these areas leads to wind erosion, sand dune destabilisation and eventually decrease in soil depth.

2.1.3.2 Fauna

The distribution of animals in Namakwaland is poorly known and many invertebrate species are not described. In the Graauwduinen area approximately 107 bird species have been identified as being residents while 52 species are considered to maintain breeding populations in the area (Allan and Jenkins, 1990). Of these species, 33% are endemic to Southern or South Africa and 15% of the species were endemic to the Karoo biome. Environmental impact assessment studies confirmed an observation of Ludwig's Bustard (*Neotis ludwigii*) which is a Karoo endemic red data bird species breeding on the site. Other red data species including Martial eagle (*Polemaetus bellicosus*), and three species of terns are non-breeding visitors of the site.

About 38 reptile species and one amphibian species occurs in the Graauwduinen area in sparsely vegetated areas. The one amphibian *Breviceps namaquensis* and six reptiles *Pachidactylus austeni*, *Bradypodion ventrale occidentalle*, *Acontias litoralis*, *Typhlosauus caecus*, *Scelotes bipes sexlineatus* and *Cordylus macropholis* are endemic to the Western Cape (Environment Evaluation Unit, 1990).

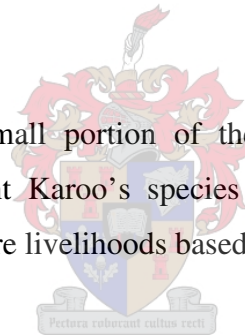
The area has a low diversity of mammals, where 35 species of mammals have been found including the African wild cat (*Felis lybica*), and Grant's golden mole (*Eremitalpa granti*), which are vulnerable and rare. A brief field visit by entomologists in 1990 did not reveal presence of any threatened or rare species of insect (Environment Evaluation Unit Environment Evaluation Unit, 1990), although the site may have importance for unspecified unique or localised insects. Benguela current system is associated with rich

fishing ground; therefore fishing is taking place on the entire west coast. Rock lobsters are abundant in the Brand se Baai coast. Seals, guano and kelps are exploited on a minor scale along the coast where mining activities is taking place.

2.1.4 Land uses

Namakwaland is mainly used for grazing, crop cultivation (dry and irrigated) and mining. Irrigated crops (mainly grapes) are cultivated in the floodplain of the Olifant's river valley areas while areas further from the river are used for dry land cultivation of cereal crops such as wheat. Namakwa Sand mining site lies on uncultivated, small stock grazing areas with the low grazing capacity of 10-20 ha per Small Stock Unit – the equivalent of one sheep or goat (Environment Evaluation Unit, 1990). Small stock grazing therefore constitutes an economic base of all larger settlements in the Brand se Baai area.

Although mining occurs in a small portion of the area, it poses a great threat to biodiversity because of Succulent Karoo's species richness and endemism. Mining activities also pose a threat to future livelihoods based on ranching and tourism.



2.2 Experimental areas

This study consists of two components. The first component assesses mycorrhiza infectivity of rehabilitated sites while the second component assesses mycorrhiza colonisation levels in various selected indigenous species in natural areas occurring at the study site. Experimental plots used for the first component of the study are located in the two sites (mined and natural) which fall in Dwarf shrub strandveld and Tall shrub strandveld plant communities. The second component also constituted plots in the two sites of Tall shrub strandveld and Dwarf shrub strandveld plant communities all which fall in the natural unmined site (Figure 2.2). Rehabilitation trial plots in the mined areas that were used for this study were established by Kirsten Mahood in 2001, (Mahood, 2003) while plots in the natural areas were established by Jeremy Blood, both Masters

students at the University of Stellenbosch. The two sites (mined and natural) are located in the vicinity of S31°16' E 17°56' and S31°15' E 17°58' respectively.



3 SOIL MYCORRHIZA INFECTIVITY OF POST-MINED REHABILITATED SOILS

3.1 Introduction

The occurrence of AM fungi is widely distributed in all soil types (Brundrett, 1991). The most important role of AM on plant growth is achieved through enhanced nutrient uptake as mycelia and hyphae of the fungi exploit a relatively larger soil surface area for mineral absorption. A well established mycorrhiza network is presumed to facilitate seedling establishment especially in arid environments like Succulent Karoo vegetation where water and nutrients are not readily available (Burke, 2001). Extensive soil disturbance by fires, agricultural activities, erosion or mining have an adverse effect on the distribution and abundance of fungi in the soil (Abbott and Robson, 1991). In most cases natural ecosystems are going through these extensive human impacts for landscape developments. In strip mining for instance, the soil and vegetation is removed. This has an adverse effect on various physical, chemical and biological properties of the soil. The network between soil and soil microbes that was established over a long period is broken, hence making the return of AM propagules to these sites a slow process. Microbial activities and symbioses with plants are an important factor regulating the cycling of nutrients in soils of the ecosystem. Rehabilitation of mined area is therefore required to hasten the process of AM return to mined sites. Currently there is little fundamental knowledge that can be applied on rehabilitation of Succulent Karoo vegetation. Given the requirement of rehabilitation of disturbed sites in environmental impact assessment, there is need for information about ecosystem dynamics of these vegetations. This study assessed the effect of rehabilitation on AM infectivity of post-mined areas of Succulent Karoo vegetation at Namakwa Sands mining areas. Infectivity of the soil was determined by plant root bioassays. The effect of AM infection on biomass production and nutrient (nitrogen and phosphorus) content of the test plant (bioassays) was analysed from whole plant dry matter recorded at intervals during plant growing season.

The carbon cost induced as a result of mycorrhiza infection was also analysed by equations provided by Williams *et. al.* (1987) and Peng *et. al.* (1993). It is documented that mycorrhiza colonization improves plant's nutrient acquisition, the effect which directly or indirectly increases the ability of the plant to fix CO₂ (Smith and Read, 1997). If the amount of carbon fixed as a result of fungal infection exceeds the amount of carbon required to maintain the symbiosis, then the carbon cost of the fungal association is offset and the association is beneficial.

It is therefore important to analyse the carbon cost of the fungal association as compared to carbon benefits in order to determine whether the mycorrhizal association is beneficial to the plant or not. It is also important to establish the efficiency of different combinations of plant and fungal associations because different plants respond differently to mycorrhiza associations. A non-beneficial association is the one in which the plant's dependency on the association is less than the fungal dependency on the plant for carbon. Similarly, the most efficient association is the one where the ratio of carbon cost to carbon benefits is low. For the purpose of ecological restoration, the use of beneficial and efficient combination of plants and fungi in re-vegetation of mined sites of the arid Succulent Karoo Biome is required to improve the availability of nutrients to plants. This would lead to improved plant growth hence accelerated process of vegetation establishment on these disturbed sites. This process therefore requires the use of appropriate species for the intended rehabilitation site. Further analysis of carbon cost will then provide information regarding effective strains of mycorrhizal fungi which could be utilized in ecological rehabilitation.

3.2 Materials and methods

3.2.1 Study site

A detailed description of location, biotic and abiotic factors of the study site are given in general study site description in chapter 2 of the document. Soil treatments (inoculum) were collected from the post-mined rehabilitated and undisturbed natural sites at S 31°16'

E 17°56' and S 31°15' E 17°58' respectively. For the post-mined rehabilitated sites, the soil was collected from experimental plots established by Mahood in 2001 (Mahood, 2003). Top soil had been replaced in these plots and mature plants translocated to the plots from natural vegetation, at 5 m spacing. For the undisturbed natural sites the soil was collected in the adjacent farm extension where mining activities did not take place. Two different natural sites were selected based on vegetation community type, the Tall shrub strandveld and Dwarf shrub strandveld plant community in the vicinity of S31°16' 063" E 17°56'129" and S31°15' 898"E 17°58'391" respectively (Figure 1.2).

3.2.2 Sampling design in the field

The landscape at Namakwa Sands in the vicinity of Brand se Baai comprises of natural (unmined) areas and mined areas at various stages of rehabilitation. In strip mining employed at Namakwa Sands, the topsoil (50 mm) is removed and stored in stockpile for about 3 months later to be spread on mine spoils as a rehabilitation strategy. The underlying soil is then mined to the depth of 1-5 m. Minerals are extracted from the sub-soil, the process through which sea water is used to wash the mined soil. The remaining sandy soil (tailings) is put back into the mined site while clay soils (slime) are dumped into slime dams. Topsoil is then spread on tailings to facilitate establishment of plants from seeds. In some cases plants salvaged from mined areas are also translocated into the rehabilitated landscape. Most plant recruitment on mined areas is derived from topsoil seed banks but sometimes seeds are collected from adjacent natural areas and broadcast on mined sites under rehabilitation.

The study assessed the establishment of mycorrhiza on seedlings of *Triticum aestivum* (wheat) growing on post-mined rehabilitated soil and natural soils (soil from undisturbed natural vegetation) of Namakwa Sands mining areas at Brand se Baai. Post-mined rehabilitated soils included three different soil treatments, namely soils rehabilitated with (1) tailings + translocated plants (TP), (2) tailings + topsoil + translocated plants (TSP) and (3) tailings + topsoil only (TS) (Table 3.1). Rehabilitation plots that had never been subjected to irrigation were used for this study. Each of the 50 m x 50 m plots were

divided into two sub-plots of 25 m x 50 m yielding two plots with different soil treatments of (TP) and (TSP). These plots yielded two different treatments because initially each half of the plot was treated differently. To acquire soil treatment of (TS), three 25 m x 50 m plots were established on the intervening matrix land between the existing experimental plots. This matrix had been treated with tailings and topsoil only no plants had been translocated to these areas.

Soils from undisturbed sites under natural vegetation (N) were obtained from plots in two sites of the natural vegetation. These were analysed differently as (N1) and (N2), but results were combined and presented as the average of the two sites therefore this treatment will be referred to (N) in this paper. There were two control treatments (control 1 and 2). Control 1 consisted of sterilized sand and sterilised mixture of all inoculum. While control 2 treatment consisted of sterilised sand only.



Table 3.1 Soil treatments tested for mycorrhiza infection

Treatment	ID Name
Tailings and translocated plants	TP
Tailings, topsoil and translocated plants	TSP
Tailings and topsoil only no translocated plants	TS
Natural site	N
Sandy soil with sterile mixture of inoculum	Control 1
Sterilized sand only	Control 2

3.2.3 Inoculum collection in the field

For each soil treatment, soil was collected on three plots of similar treatments to make up three sub-samples of each treatment. In total 12 plots were then sampled for the four treatments excluding control treatments. On each plot two 5 m wide strip transects were established enabling soil collection at the base of only four plants growing in the strip and making the total of 8 plants assessed per plot. Soil was collected at the base of plants using an auger of 14 cm long and 7 cm diameter. On the rehabilitated site, soil was collected at the base of translocated plants that were planted in clumps of *Ruschia versicolor*, *Othonna cylindrica*, *Lampranthus suavissimus*, *Zygophyllum morgsana* and *Asparagus* species. In most cases *Zygophyllum* and *Asparagus* species in these clumps were dead. Similarly, for the N and TS treatment soils were collected on any four plant species growing in the established transects. Figure 3.1 illustrates the schematic layout of three sub-plots of one soil treatment.

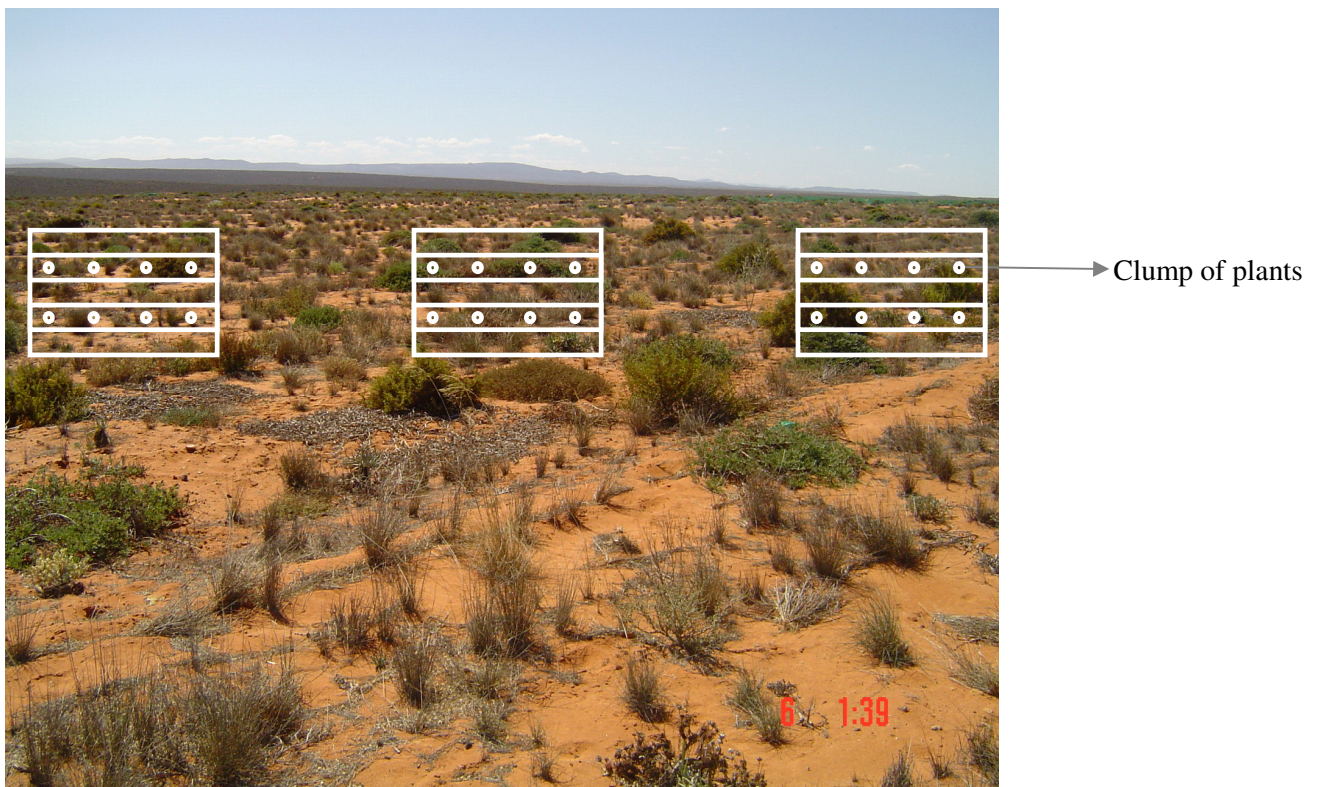


Figure 3.1 Illustration of sub-plots laid in the field for sampling of AM infectivity on rehabilitated and natural soils at Brand se Baai

3.2.4 Growing medium in the nursery

Bioassay plants were planted in polythene pots of 200 mm long and 100 mm in diameter (Figure 3.2). The growing medium was composed of sterilized Malmesbury river sand soil and inoculum soil in the ratio of 3:1. Sandy soil portion was autoclaved at 80°C for 30 minutes.

For control 1, both growing medium (sand and inoculum) components were autoclaved to kill fungal spores and other propagules which could be in the soil. The sandy soil portion was autoclaved at 80°C for 30 minutes and inoculum mixture at 80°C for 60 minutes. The inoculum mixture was autoclaved for a longer period because it was presumed to contain more mycorrhizal propagules. In order to maintain other non-mycorrhizal microbial community in the control 1 inoculum soil, this soil was first washed with distilled water before it is autoclaved. The soil was washed at the ratio of 1 g soil : 2 ml distilled water and then drained by sieving with a 36 µm sieve, which was considered small enough and can not allow mycorrhizal spores to pass through. Water filtrate

obtained from the soil wash, was then used to irrigate the control 1 plants in order to put back the minute non-mycorrhizal microbial community into the control 1 treatment.

Since inoculum soil for each treatment was collected from three sub-plots whereby in each sub-plot eight soil samples were collected and mixed. Soil from each sub-plot was therefore used as inoculum for ten seedlings in the nursery eventually making thirty (30) seedlings per treatment. For the six treatments a total of 180 seedlings were transplanted in the nursery.

Inoculum and sterilised sandy soil was placed in the polypot as illustrated in figure 3.2 below. Inoculum soil was placed in the middle as a core in which plants starts growing from. The soil type at the mining site is sandy and since it was dry season, it was impossible to get an intact inoculum core therefore hyphal network disturbance might have occurred during inoculum extraction and preparation.

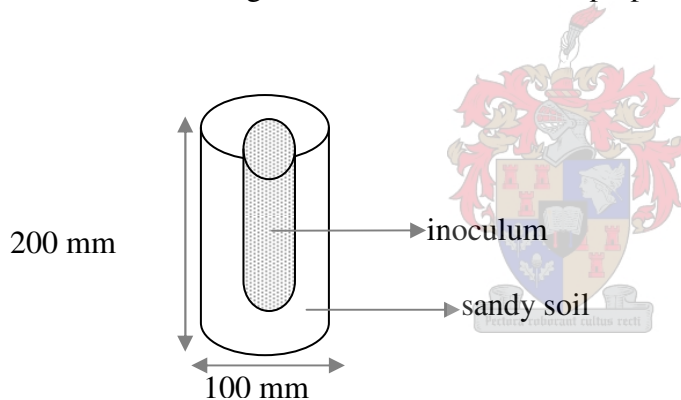


Figure 3.2 Illustration representing polythene plastic pot with two dilutions of growing medium used to assess soil infectivity.

3.2.5 Sowing, transplanting and fertilizer application

Wheat (*Triticum aestivum*) was used as a test plant. This species was selected because its mycorrhizal status has been determined as it has been extensively used in mycorrhizal studies before. Seeds from Brackenfell Agricol were sown on vermiculite in the laboratory (growth room) at 21 - 23 °C for 5 days. After five days seedlings were transferred into the planting pots in the greenhouse with temperatures of 30°C and 15°C

day and night respectively. Since river sand soil is low in nutrients, all plants were fertilized with the nutrient solution based on Long Ashton nutrient solution (Table 3.2). Field capacity of the soils after seven days was determined and formed the basis of irrigation requirements of plants per week. Plants were supplied with water and nutrient solution at 150 ml per pot per week as a source of sulphate, potassium, calcium, iron, nitrogen and micro nutrients.

Table 3.2 Standard Long Aston nutrient solutions applied to the bioassay plants

Solution type	Compound name	Chemical formula	Concentration (ml / l)
Macro nutrients	Magnesium Sulphate	MgSO ₄	10
	Potassium Sulphate	K ₂ SO ₄	10
	Calcium chloride	CaCl ₂	10
	Phosphate	PO ⁴⁻	0.25
Micro nutrients	Iron ethylene-diamine-tetra-acetic acid	Fe EDTA	1.25
	Sodium nitrate	NaNO ₃	2

3.2.6 Root and shoot harvesting

Root and shoot were harvested four times throughout the growing period at, 5, 21, 42 and 56 days after planting. At each harvesting, three plants from each replicate were harvested making the total of nine plants harvested per treatment and 54 plants per harvest.

A portion of thin roots was harvested from each plant and stored in a vial with 50% ethanol solution. Roots harvested from three plants of each replicate were collected in one vials and three slides were made out of the collected root sample.

Dry weight of shoots and roots were measured for growth response analysis. Whole plant dry weight biomass production was also analysed for nutrient content at each harvest.

3.2.7 Mycorrhiza infection analysis

The analysis of mycorrhizal infection in the roots was carried out based on the clearing and staining method proposed by Phillips and Hayman (1970). Roots were stored in 50% ethanol, washed with distilled water and cleared (delignified) in 10% potassium hydroxyl (KOH). Cleared roots were acidified with 1% hydrogen chloride (HCl) which enhance staining on root structures. Roots were then stained with 0.05% aniline blue. Excess stain remaining on roots was removed with a distaining solution of 95% lactic acid. Because of the delicate nature of wheat roots, cold clearing and staining was carried out to avoid root damage by heat. Roots remained in the clearing and staining solution for three and four days respectively.

The percentage infection of mycorrhizae along the root length was quantified by mounting 20 root segments of 1cm long on the microscopic slides (Caldwell and Virginia, 1989; Giovannetti and Mosse, 1980). Root segments on each slide were then examined with a light microscope at 40 X magnification for the presence of mycorrhizal structures. During microscope examination the hairline ruler visible under the microscope was used as the line of intersection, intersecting the root segment on a slide. Mycorrhiza was then observed only at intersections between the hairline and the root segments. At each intersection, observations were made for the following structures:

non = no fungal structure

a = arbuscules

v = mycorrhizal vesicles

a + v = arbuscules and mycorrhizal vesicles

mh= mycorrhizal hyphae (hyphae which is attached to arbuscules and or vesicles anywhere in the field of view)

h = hyphae (not seen attached to any mycorrhizal structures)

When the total of G (a + v+(a+v) + mh + h) intersections are observed the percent of root length infected by arbuscule, vesicle and hyphae is calculated as suggested in Brundrett *et. al.* 1994) as follows:

$$\text{Arbuscular colonization (AC)} = [a + (a+v) / G] 100 \dots\dots\dots(1)$$

$$\text{Vesicular colonization (VC)} = [v + (a+v) / G] 100 \dots\dots\dots(2)$$

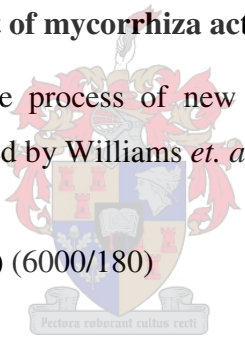
$$\text{Hyphal colonization (HC)} = [(G - \text{non}) / G] 100 \dots\dots\dots(3)$$

$$\text{Total fungal infection} = [(a+v+(a+v) + mh + h) / G] * 100 \dots\dots\dots(4)$$

3.2.8 Calculation of carbon cost of mycorrhiza activities on plants

The amount of carbon lost to the process of new tissue construction in plants was estimated by using equation (5) used by Williams *et. al.* (1987) and Peng *et. al.* (1993).

$$C_w = (C + kN/14 * 180/24) (1/0.89) (6000/180) \dots\dots\dots(5)$$



This equation assumes that there is a relationship between carbon concentration of the plant and plant size, therefore enabling determination of whole plant carbon lost to respiration. In the equation C_w represents the whole plant tissues construction carbon cost (mmolC gDW^{-1}), C is the carbon concentration in whole plant's tissues (mmolC gDW^{-1}), k is the reduction state of the N substrate (NO_3 was used therefore $k = +5$) and N is the organic nitrogen content of the whole plant tissues (g gDW^{-1}). The constant $1/0.89$ represents the proportion of reductants in construction cost which is not incorporated in biomass while $6000/180$ converts units of grams glucose gDW^{-1} to mmolC gDW^{-1} .

Carbon accumulation and depletion in plant depends in photosynthesis and respiration respectively. Growth and maintenance respiration of plants were therefore also determined. Maintenance respiration is given by subtracting change in whole plant

carbon content from daily carbon requirements for construction of new shoots mathematically expressed in equation (6)

$$R_m = C_t - \Delta W_c \dots\dots\dots(6)$$

where C_t ($\mu\text{mol CO}_2 \text{ day}^{-1}$) represent the carbon required for daily construction of new tissues and ΔW_c ($\mu\text{mol day}^{-1}$) is daily change in plant carbon content. C_t is calculated by multiplying the plant growth rate (g DWday^{-1}) by C_w and ΔW_c by multiplying plant carbon content with plant growth rate.

Growth respiration represent the proportion of carbon respired for biosynthesis of new tissues for the net gain of plant's biomass. Growth respiration ($\text{mmol CO}_2 \text{ g DW}^{-1}$) was also determined as proposed by Peng *et. al.* (1993) equation (7)

$$R_g = R_m / \text{plant gr} \dots\dots\dots(7)$$

where R_g represents growth respiration based on whole plant dry weight ($\text{mmol CO}_2 \text{ g DW}^{-1}$), plant gr is whole plant growth rate (g DWday^{-1}) and R_m is maintenance respiration which represent the daily growth respiration ($\mu\text{mol CO}_2 \text{ day}^{-1}$).

3.2.9 Experimental design and statistical analysis

A completely randomised design was established in the nursery. There were five mycorrhizal inoculum treatments comprising of inoculum from: natural sites or undisturbed areas (N), sites rehabilitated with tailings, topsoil and translocated plants (TSP), sites rehabilitated with tailings and translocated plants (TP), sites rehabilitated with tailing and topsoil only (TS) and control treatments (Control 1 and Control 2). Each treatment was divided into three sub-samples to make three replicates per treatment.

COSTAT 2.00 statistical computer programme was used for all statistical analysis. Analysis of variance was carried out to test for significance in differences between

mycorrhizal and non-mycorrhizal plants in terms of percentage infection, biomass production and nutrient content. The significance of the differences between treatments were separated using Student-Keuls Test at $P < 0.05$.



3.3 Results

3.3.1 Mycorrhizal infection on wheat

There was no mycorrhiza infection in test plants growing on Control 1 treatment at 21, and 42 days of growth (Figure 3.3). There was however an insignificant percent infection (1.1%) of due to hyphal infection on control 1 treatment at 56 days of growth (Figure 3.3c). Contrary to expectations mycorrhiza infection on roots of test plants growing on inoculum from natural unmined areas (N) had the lowest total colonisation at all three stages of plant growth. Few arbuscule structures encounter were observed only on soils rehabilitated with tailings + topsoil (TS) and in natural soil (N) (Table 3.3).



Table 3.3 Mycorrhiza colonisation on wheat bioassays planted with different types of inoculum from natural and post-mined rehabilitated areas of Brand se Baai Succulent Karoo vegetation.

Days after transplanting	Treatment	Fungal colonisation (%)			Total
		arbuscules	vesicle	hyphae	
21	Control 1	0	0	0	0 a
	Control 2	0	0	0	0 a
	N	0	2.1	7.3	7.6 a
	TS	5.4	17.8	47.6	49.5 b
	TSP	0	0	4.4	4.4 a
	TP	0	8.8	9.9	9.9 a
	Control 1	0	0	0	0 a
42	Control 2	0	0	0	0 a
	N	1.1	0	2.2	2.2 a
	TS	0	5.1	18.9	19.1 b
	TSP	0	13.3	15.4	15.4 b
	TP	0	0	0	0 a
	Control 1	0	0	1.1	1.1 a
	Control 2	0	0	0	0 a
56	N	0	4.5	4.5	4.5 a
	TS	0	0	0	0 a
	TSP	0	42.1	44.3	44 b
	TP	0	0	0	0 a
	Control 1	0	0	0	0 a

Percentage is the mean of three sub-samples, difference between percent infection determined by Student Newman Kuels at significance level $P \leq 0.05$. For total infection different letters in a column of each harvest indicates a significance differences.

With time there was an increase in mycorrhiza infection of plants growing on inocula from sites rehabilitated with tailings, topsoil and translocated plants (Figure 3.3a, b, c). Except at 21 days after planting, mycorrhiza infection was significantly high in TS and TSP and significantly low in control and TP treatments at all stages of plant growth (Figure 3.3 a, b,c).

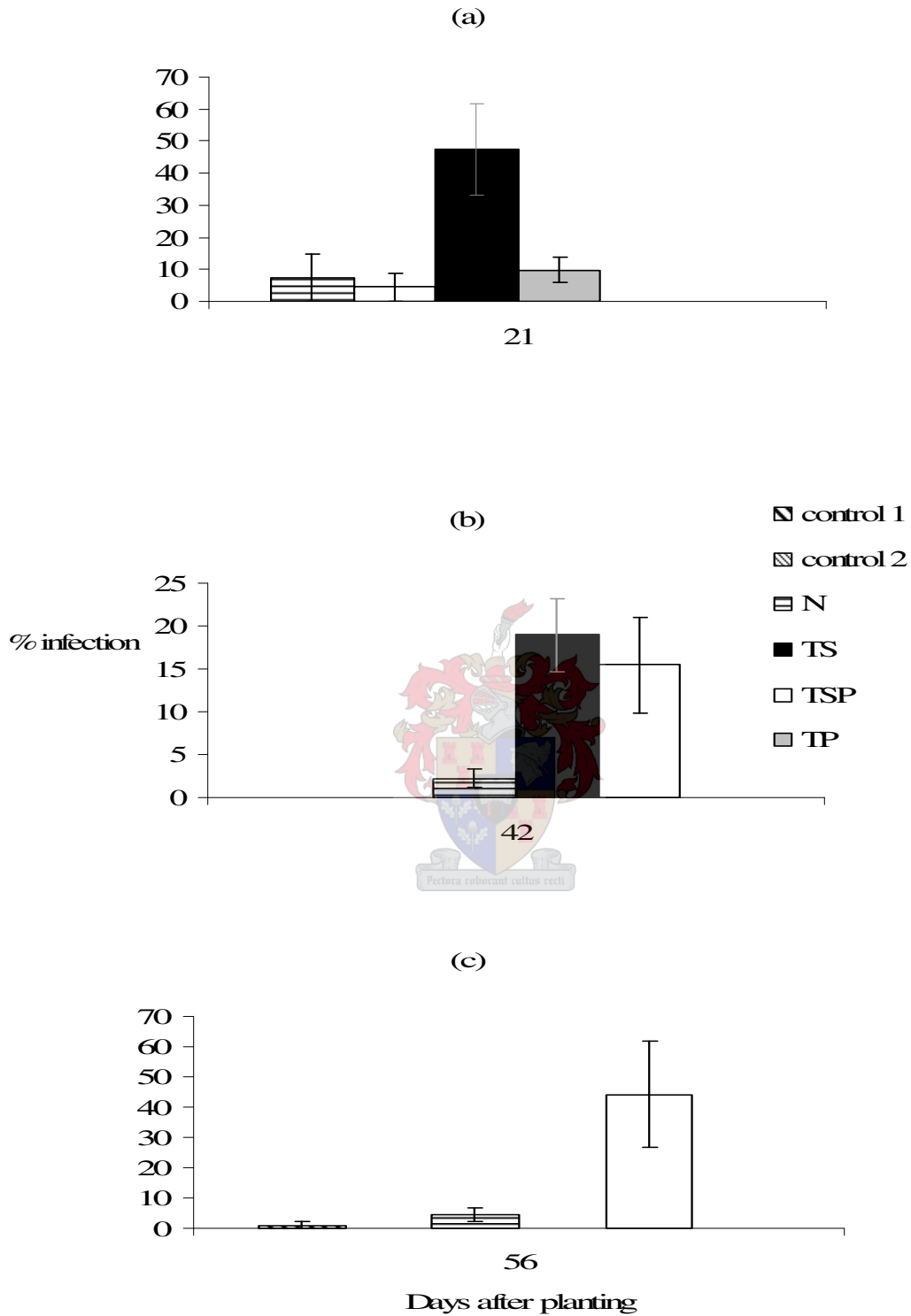


Figure 3.3 Mycorrhiza mean percent infection on wheat bioassays at (a) 21, (b) 42 and (c) 56 days after planting. Vertical bars indicate standard deviation on the mean.

3.3.2 Chemical composition of treatment soils

Nutrient content, resistance and cation exchange capacity of each soil treatment used was also analysed. Student Newman tests in Table 3.4 below shows that inoculum soil from undisturbed mine sites (N) had significantly lower pH and higher phosphorus, organic carbon and magnesium concentrations than soils from mined sites or the river sand (control 2) portion used as a growth medium. Phosphorus and organic carbon concentrations in soil from mined sites did not differ significantly from that of washed river sand.

Table 3.4 Chemical properties of the inoculum and sandy soil used as planting medium for bioassays.

Soil ID	pH (KCl)	Resistance (ohm)	P Bray II (mg/kg)	K (mg/kg)	Cation Exchange Capacity (cmol(+)/kg)			C (%)	
					Na	K	Ca		
Control 1	8.3 c	468.75 ab	15.94 a	167.50 a	0.85 b	0.43 a	1.46 a	0.9 b	0.41 a
Control 2	6.56 a	590 b	7.66 a	18.33 b	0.046 d	0.33 a	1.51a	0.14a	0.18 a
N	7.0 a	598.33 b	41.25 b	236.8 a	0.6 a	0.6 a	4.5 a	1.8 c	1.1 b
TSP	8.0 bc	253.33 a	8.8 a	188.3 a	1.4 c	0.5 a	1.5 a	1.0 b	0.3 a
TS	8.3 c	336.66 a	8.5 a	149.5 a	1.0 b	0.4 a	0.8 a	0.7 a	0.2 a
TP	7.4 b	686.66 b	5.2 a	155.7 a	0.6 a	0.4 a	0.6 a	0.7 a	0.2 a

Values in one column with different letters indicate a significant difference between treatments at the significance level $P \leq 0.05$ with a Student Keuls test.

3.3.3 Biomass yield production

There was no significant difference between treatments on whole plant dry weight (DW) production at 5 and 21 days after planting. However, whole plant biomass production was significantly different among treatments at 42 and 56 days of growth after planting. A significantly high DW biomass production was recorded on plants growing on inocula from natural areas (N), followed by those growing on soils rehabilitated with tailings,

topsoil and plants (TSP) at both 42 and 56 days after planting (Figure 3.4). Plants growing on control 1 treatment which represented sterilised mixture of all inoculum had a significantly low whole plant DW biomass production of all treatments then followed by TP and TS in an order which is not consistent (Figure 3.4). A similar pattern was also observed on root and shoot dry weight (results not shown).

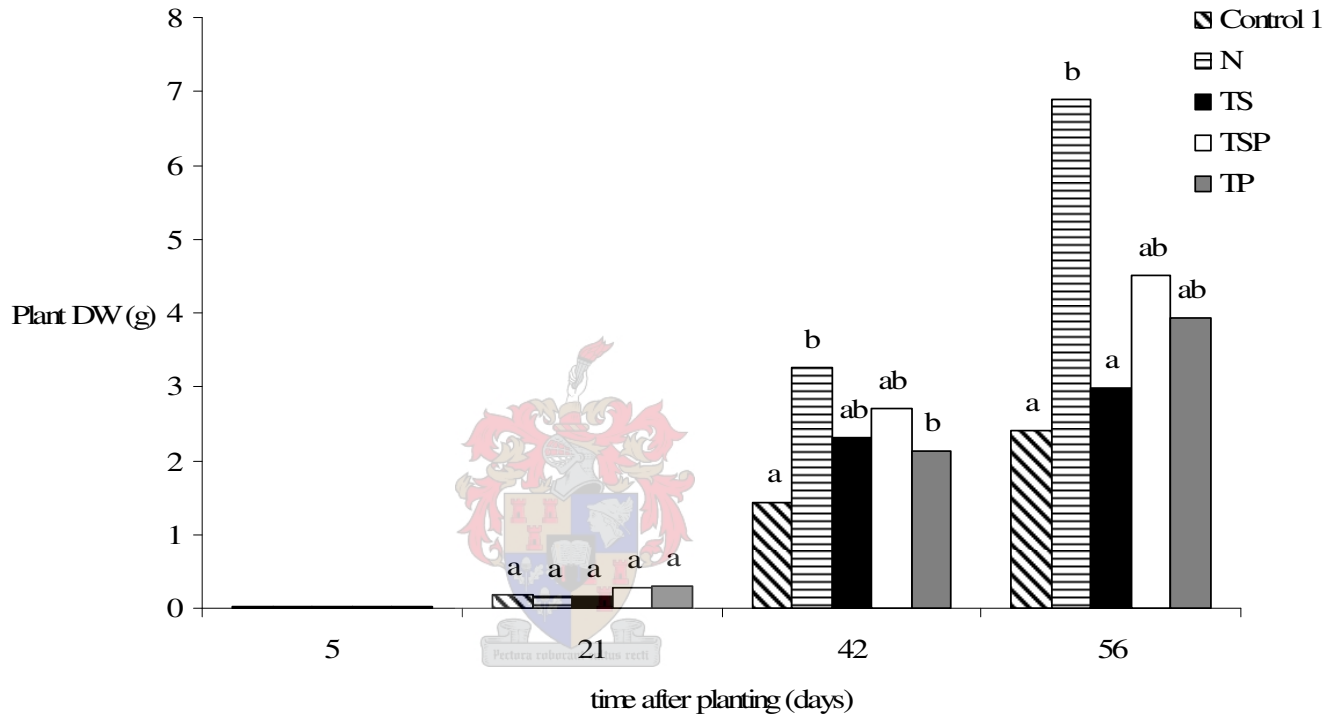


Figure 3.4 Whole plant dry weight at 5, 21, 42 and 56 days of growth after planting.

Differences between inoculum tested with a Student Keuls Test, significant differences ($P \leq 0.05$), $n = 3$. Different letters at a given stage of growth indicates significant differences between treatments.

There was however no relationship between mycorrhiza association and plant biomass production. (Figure 3.5).

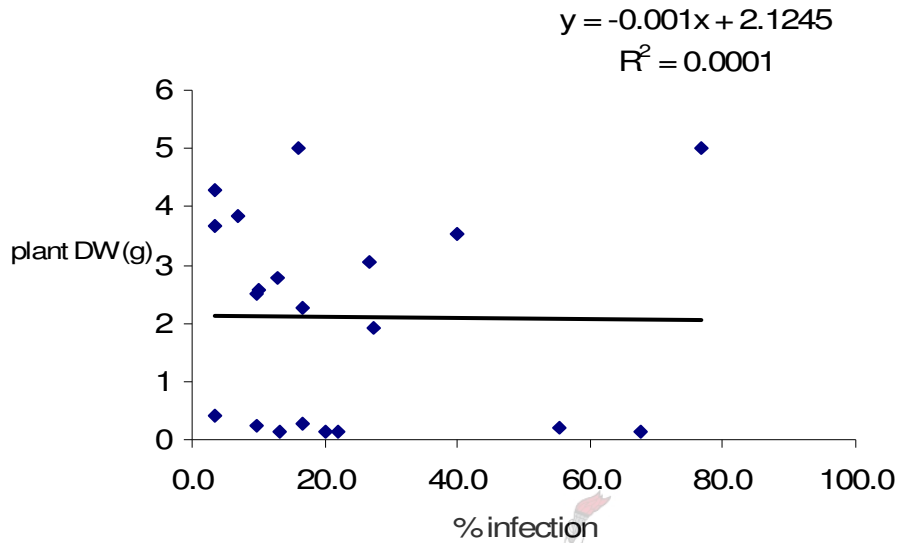


Figure 3.5 The relationship / correlation between mycorrhiza infection level and whole plant dry weight.

3.3.4 Nutrient content of plant dry matter

Except for carbon, all nutrients in plant dry weight decreased as plants ages (Table 3.5). Plant nutrient concentration (nitrogen and phosphorus) did not follow a consistent pattern at all stages of growth. However, there was a high level of nitrogen in plants treated with inocula from natural sites (N) at 21 and 42 (Table 3.5). Phosphorus was also significantly high at 21 and 56 days of growth on plants growing in control 1 (Table 3.5).

Table 3.5 Whole plant nutrient content of wheat bioassays treated with inoculum from different rehabilitated sites of Brand se Baai mining areas.

<i>Nutrient concentration (mol g⁻¹dry weight)</i>	<i>Growth period (days)</i>		
	21	42	56
Nitrogen			
Control 1	22.30 a	8.47 a	12.53 b
N	34.83 c	15.37 a	5.90 a
TS	29.33 bc	12.50 a	10.13 ab
TSP	30.83 bc	11.73 a	7.13 a
TP	28.20 b	11.30 a	7.03 a
Phosphorus			
Control 1	4.90 b	0.54 a	1.17 d
N	2.23 a	1.20 b	0.53 a
TS	2.07 a	0.85 ab	0.93 bc
TSP	2 a	0.94 ab	0.80 b
TP	1.67 a	1.03 b	0.77 b
Potassium			
Control 1	11.27 ab	2.30 a	5.60 a
N	15.33 b	9.17 b	4.17 a
TS	12.93 ab	6.97 b	45.97 a
TSP	11.20 ab	6.77 b	4.97 a
TP	9.67 a	7.20 b	4.77 a
Carbon			
Control 1	280.57 a	282.13 a	359.93 a
N	271.73 a	321.97 a	232.17 a
TS	283.50 a	286.93 a	323.67 a
TSP	356.40 a	238.70 a	392.77 a
TP	387.67 a	276.97 a	329.47 a

3.3.5 Carbon cost of mycorrhiza symbiosis

There was no significant difference between construction cost of plant tissues (C_w), maintenance respiration (R_m) and growth respiration (R_g) of plants growing in different treatments (Table 3.6). However, C_w , R_m , and R_g was high on plants growing on inoculum from natural sites (N) and lowest on plants growing in control 1 treatment at 42 and 56 days after transplanting (Table 3.6). The table also shows the content of phosphorus (P) in plant tissue analysed on each treatment at three different harvesting stages. Despite minor inconsistencies the results in phosphorus content shows no significant differences in phosphorus content between treatments. At 21 days after transplanting, C_w , R_m and R_g was high in plants growing on inoculum from soils rehabilitated with tailings and topsoil and still low in the control 1 treatment. This trend was however not statistically different from other treatments. Plants growing in the inoculum from natural areas also had the highest growth rate while those growing in control 1 had the lowest (results not shown). A correlation regression between plant growth rate and soil nutrient (phosphorus and organic carbon) showed a weak relationship ($R = 0.36$) between the two variables.

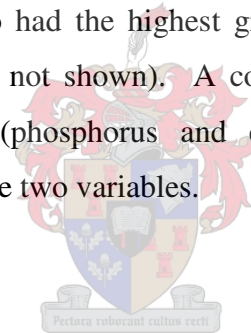
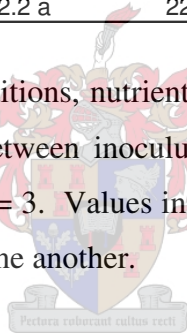


Table 3.6 Carbon cost, maintenance respiration and growth respiration of wheat plant bioassays planted in different soil treatments

DAT	Soil Treatment	Mycorrhiza % infection	Cw (mmolC gDW ⁻¹)	R _m (μmol CO ₂ day ⁻¹)	R _g (mol CO ₂ g DW ⁻¹)	P mol gDW ⁻¹
21 days	Control 1	0 a	3.0 a	6.9 a	0.7 a	4.90 a
	N	7.6 a	5.9 a	27.1 a	2.6 ab	2.23 a
	TS	49.5 b	4.4 a	14.4 a	1.2 ab	2.07 a
	TSP	4.4 a	11.9 a	119.0 a	3.9 ab	2 a
	TP	9.9 a	12.3 a	122.6 a	4.7 b	1.67 a
42 days	Control 1	0 a	14.7 a	191.4 a	4.1 a	0.54 a
	N	2.2 a	58.4 b	3278.9 a	17.1 a	1.20 a
	TS	19.1 b	40.8 ab	1515.0 a	12.2 a	0.85 ab
	TSP	15.4 b	33.6 ab	947.4 a	8.0 a	0.94 ab
	TP	0 a	23.2 ab	692.1 a	7.6 a	1.03 b
56 days	Control 1	1.1 a	29.3 a	790.6 a	9.6 a	1.17 a
	N	4.5 a	87.2 a	9407.3 a	33.1 a	0.53 a
	TS	0 a	29.3 a	774.3 a	9.6 a	0.93 bc
	TSP	44 b	60.9 a	3688.3 a	24.2 a	0.80 b
	TP	0 a	42.2 a	2225.9 a	12.1 a	0.77 b

Plants grown under glasshouse conditions, nutrient analysis carried out at 21, 42 and 56 days after planting. Differences between inoculum tested with a Student Keuls Test, significant differences ($P < 0.05$), $n = 3$. Values in the same column with the same letter are not significantly different from one another.



3.4 Discussion

3.4.1 Mycorrhiza infection on rehabilitated sites

Results shows an overall higher percent infection on plants growing on sites rehabilitated with tailings, topsoil and translocated plants (TSP) as well as on sites rehabilitated with tailings and topsoil (TS) (Figure 3.3 a, b and c). Backfilling mined sites with topsoil with or without translocated plants enhanced mycorrhiza infection better than backfilling with tailings only. It is also clearly shown in the results (Figure 3.3 a, b and c) that topsoil is more important in bringing back AM into the soil because there was very little encounter of AM on soils rehabilitated with tailings and translocated plants (TP) only. The use of topsoil in rehabilitation programme is essential because of its physical and chemical characteristics that are a suitable media for re-establishment of microbial activities and plant growth. Although the relationship between mycorrhizal colonisation and soil properties varies based on tree species and AM strain, topsoil has a great potential of returning microbial activities back to a disturbed site. This is because topsoil provides an environment and soil properties similar to the native soil where mycorrhiza was initially growing. Different mycorrhiza strains get adapted to their native soil (topsoil) in such a way that if topsoil is not used as part of the growing media, mycorrhiza colonization will be minimised (Saxerud and Funke, 1991; Mitchell *et. al.*, 1986, Mitchel, Adelman, Morton, 1986). Topsoil also serve as a reservoir for plant seeds, AM spores and infective propagules that facilitate plant establishment. Topsoils are usually rich in organic matter and nutrient content which enhance water holding capacity and microbial activities in the soil. The benefits of topsoil application in bringing back microbial population in disturbed soil have also been discussed by Claasen and Zasoski, (1993). In their study, they demonstrated that application of topsoil in conjunction with moderate fertilisation increased microbial activities in the soil better than soils where only fertiliser but no topsoil was applied. In other greenhouse experiments conducted, where topsoil and sterilised sand is used as a growing medium, AM infectivity was reduced when less

proportion of topsoil is included in the growing medium (Mitchel, Adelman, Morton, 1986).

Similar proportion of sterilised sandy soil and rehabilitation materials (inoculum) were used as growing media in the nursery, but test plants growing on inoculum from plots rehabilitated with tailings and translocated plants resulted in less mycorrhiza infectivity. This is because tailings have limited ability to facilitate re-establishment of microbial activities in the soil as their normal physical and chemical properties have been altered through mineral extraction process of mining. A variation in mycorrhiza infectivity of rehabilitated soils in this study can therefore be associated with the type of rehabilitation material applied to the post-mined rehabilitated site. In table 3.4 this is shown by low levels of phosphorus, organic carbon and some exchangeable cations (potassium, calcium and magnesium) in these soils. Mahood (2003) also demonstrated the effect of mineral extraction on nutrient content of tailings at Brand se Baai mining areas. This study showed that, except from sodium which increased after mineral extraction all chemical components of the soil were reduced in tailings. Sodium increased because it is a major mineral component of sea water which was used in mineral extraction process. Soil pH was increased from acid soils in natural environments to basic soils in tailings. These changes in chemical and physical composition of tailings are also attributed to low mycorrhiza infection in this treatment. Various strains of mycorrhiza evolutionary establish themselves in soils where they are adapted based on the nutrient content, pH and climate (Mitchell *et. al.*, 1986). For that reason it is also advisable in greenhouse experiments that sterilised sand diluted with inoculum should be from a common area with inoculum so that a limiting factor of environmental adaptation is eliminated.

Although the performance of sites rehabilitated with tailings + topsoil + translocated and those by tailings + topsoil were not following a consistent trend in results of this study, at this stage replacement of topsoil seems advisable because plants on topsoil growth media (TSP and TS) showed a significantly higher AM colonisation than plants on (TP) that lacked topsoil (Table 3.3). There are however lots of inconsistencies in results leading to

erratic results which are difficult to explain. These may be related to inoculum handling and disruption of hyphal network occurred during soil preparation for planting.

The length of the period in which topsoil remains stockpiled is also an important aspect for consideration because it plays a major role in mycorrhiza infection. Stockpiling topsoil for a longer period can have a negative effect on mycorrhiza infection (Rives *et al.* 1980; Gould and Liberta, 1981). Storing topsoil for three years in coal mining site in North America has substantially reduced the level of viable inocula relative to adjacent natural areas. Stockpiling may also favour some and eliminate other strains of mycorrhiza (Visser *et al.*, 1984) that might be more effective in facilitating plant establishment and growth. The maximum period of three months of soil remaining on a stockpile at Namakwa Sands mine is relatively shorter. Determination of critical periods should however be established for the purpose of topsoil management in rehabilitation programme. This could be achieved by testing infectivity of stockpiled soils at spatially and temporally different scales.

The correlation index value for the relationship between plant dry weight production and mycorrhiza infection also shows that plant growth rate is not associated with mycorrhiza infection but rather by other factors like nutrient concentration.

3.4.2 Mycorrhiza infection on natural sites

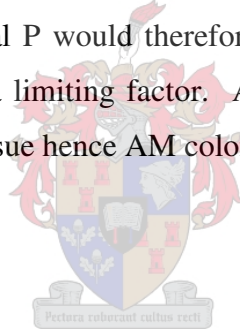
Soil disturbance is one of the known factor which can reduce mycorrhizal infection and microbial activities of the soil (Claasen and Zasoski, 1993; Abott and Robson, 1989). However, in this study mycorrhiza infection was high in plants growing on inoculum from disturbed rehabilitated soil than plants growing on inoculum from natural sites. Apart from soil disturbance, there are other factors associated with mycorrhiza infection on plants which might have affected mycorrhiza infection on plants inoculated with inoculum from natural sites.

Inoculum from natural sites was collected from an undisturbed vegetation of Tall shrub strandveld and short shrub strandveld. These plant communities were dominated mainly by perennial vegetation. According to other studies, ecosystems dominated by perennial vegetations were found to have a low level of spores from which an infection is expected to develop (Molina and Trappe, 1978; Rose, 1980). The low quantity of spores in perennial vegetation occurs because AM production of external spores are reduced during dormant season as the fungi persist within the roots of a perennial host. Other possibilities could be related to the ecology of fungal strain, where in some strains of fungi, sporulation occurs only in wet seasons after sufficient rain is received for germination (Rose, 1980). Assessments of soil infectivity during dry seasons would therefore encounter little infective AM propagules. However, a low level of mycorrhizal propagules in these soils might not necessarily imply low infection in roots of plants composing this vegetation. There are however other mycorrhizal propagules like root fragments and hyphal strand in the soil that could as well initiate mycorrhizal infection, therefore low infection in the N treatment in this study could be attributed to other factors like soil nutrient status.

Analysis of chemical properties and nutrient status of the soil treatments showed a significantly high level of available phosphorus on natural sites (Table 3.4). This high level of phosphorus could also reduce mycorrhiza infection because phosphorus has an inverse effect on mycorrhiza infection (Abbott and Robson, 1982; Jensen and Jakobsen, 1980; Morita and Konishi, 1989). Mycorrhiza infection is high when phosphorus level is low in the soil and vice versa. Schubert and Hayman, 1986, Amijee *et. al.* 1989 demonstrated that at 50 mg kg⁻¹ (50 ppm) or less phosphorus level, mycorrhiza infection was the highest after which it increases at a decreased rate. When the phosphorus level exceeds 100 mg kg⁻¹ mycorrhiza infection start to decline. The P Bray II analysis carried out in this study showed that the amount of P available to the plants was significantly high in N soil treatment (Table 3.4). The high level of phosphorus in these soils perhaps reduced mycorrhiza infection propagules in the N treatment soil and eventually leading to low mycorrhiza infection on bioassays planted in this soil treatment.

Plants only benefits from AM associations when nutrients (mainly phosphorus) are insufficient in the soil. In an environments where phosphorus is readily available in the soil, plants inhibit extensive formation of AM because it would induce a parasitic association whereby less nutritional benefits are acquired by the plant in return of high carbon released to the fungi. The regulatory mechanism behind this is not well understood, but it is based on the sink effect of the fungi as well as the effect of the carbohydrate content of the host plant's roots. The carbohydrate based theory suggests that exudation of soluble carbon compounds (fungal substrate) into the rhizosphere is reduced under high phosphorus nutrition in the soil (Schwab *et. al.*, 1991; Marschner, 1986). This effect would eventually reduce mycorrhiza infections as their sources of food (soluble carbon compounds) are reduced. The fungal sink effect on the other hand, suggests that at optimal level of phosphorus for mycorrhiza colonisation to occur, mycorrhizal plants demand more carbon due to increased root respiration below ground (Snellgrove *et. al.* 1982). Optimal P would therefore encourage existence of the fungi given that carbohydrates are not a limiting factor. A further increase in P is toxic and decrease carbohydrates in plant tissue hence AM colonisation would cease.

3.4.3 Biomass production



At 5 days all seedlings were similar because they were growing in a similar growth medium (vermiculite) before they were transplanted into different treatments. At this stage seedlings can not be significantly different in dry weight production also because of the masking effect of seed nutrient content, as plants derives most of its nutrients from the endosperm of their seeds (Weier *et. al.*, 1982).

High biomass production of plants growing in soil from natural sites at 42 and 56 days after planting can not be related to mycorrhiza infection because in this treatment the least mycorrhiza colonisation was observed. The high biomass production in this treatment could therefore be related to the chemical components of the soil. In Table 3.4 the results show that the level of macro-nutrients obtained on soils from natural sites were relatively high compared to all other soil treatments, hence the higher biomass production was achieved. Soils rehabilitated with tailings, topsoil and plants had the highest biomass

production of all the other rehabilitated soils because this treatment consists of both topsoil and translocated plants a better materials in facilitating soil nutrient content, water holding capacity, and eventually plant growth. Translocated plants also play a major role in creating a micro habitat for seed development in arid regions with minimal annual rain and wind disturbances (Burke, 2001)

For this study, the statistical analysis shows no relation between mycorrhiza and biomass production. This is not surprising especially that mycorrhiza infection observed on wheat bioassays comprised of relatively more undefined hyphae and less proportion of arbuscule structures that are considered to be the sites of nutrient exchange in a given symbiosis (Brundrett, 1991; Smith and Read, 1997). Only 5.5 and 1.1% of arbuscule encounter was observed in TS and N respectively (table 3.3). An association without arbuscules is considered non-functional and can not be definitely defined as AM until further studies are carried out to differentiate between hyphae and vesicles of parasitic fungi and those of AM fungi (Brundrett, 1991). In the presence of infection mainly from hyphae and vesicles, an association could turns out to be parasitic. There was however a strong negative correlation (results not shown) between plant nutrient contents and mycorrhiza infection. This is in line with the earlier arguments by several authors that in soils with sufficient nutrients mycorrhizal associations are limited.

3.4.4 Nutrient content of plant dry matter

The decline in nutrient concentration that occurred as the wheat plants aged is a general trend in plants caused mainly by an increase in proportion of plant structural materials and storage compounds. However the content of carbon increases with time because it is the major component of plant dry weight (Marschner, 1986).

Although results were rather inconsistent, they were significant for nitrogen in plants at 21 and 56 days after planting. Accumulation of phosphorus, nitrogen and carbon in plants assessed in this study was not attributed to mycorrhiza infection but rather to soil chemical properties. Treatments with high mycorrhiza infection like TSP and TS did not result in high concentration of nutrients (nitrogen and phosphorus) in plant tissues.

However high concentrations of soil nutrients in control 1 and N treatments led to high levels of phosphorus and nitrogen in whole plant material. The effect of mycorrhiza on phosphorus uptake can easily be determined when shoot and root plant materials are analysed separately. For this study plant bulk analysis was carried out, therefore further analysis on effects of mycorrhiza on nutrient content of bioassay plants was limited.

3.4.5 Carbon cost of mycorrhiza association

The general picture of results (Table 3.6) shows that mycorrhiza infection had no significant effect on C_w , R_m and R_g . Though not significant, growth construction cost, maintenance respiration and growth respiration of plant tissue were high on plants growing on soil inocula from natural sites (N) and low on plants growing in control 1 treatment (Table 3.6) at all growth stages. This pattern was also observed for plant growth rate and dry matter weight (results not shown). This implies that plants growing in soils with high nutrient grew faster than plants in rehabilitated soils with low nutrient content. For this study high growth rate is associated with high carbon cost and not to mycorrhiza as was hypothesized. Mycorrhiza had no significant effect on carbon cost of these plants. This contradicts findings by other papers where in absence of the mycorrhizal effect, fast growing species were found to spend relatively less of their daily fixed carbon in respiration therefore retain more carbon for plant growth (Poorter, 2002; Poorter *et. al.* 1991).

Although high growth rate in N treatments is associated with high level of nutrients, the relationship between growth rate and carbon cost is not well explained by the data of this study because high carbon cost was observed on treatments with high growth rate. This is due to the growth models (equations) used that assumes a direct relationship between high growth rate (resulting from mycorrhiza infection) and carbon cost. In this case fast growth rate was not due to mycorrhiza infection but rather due to soil factors therefore fast growing plants in N (undisturbed) treatments had high carbon cost as well.

3.5 Conclusions

The use of topsoil with or without translocated plants in backfilling mine sites is beneficial for mycorrhizal re-establishment. Sites rehabilitated with these materials demonstrated a relatively higher mycorrhizal infection in this study. Mycorrhizal infection was strongly related to soil chemical properties of the growing medium used. Natural soils with high concentrations of nutrient inhibited mycorrhizal colonisation of the bioassay plants. Biomass production of the bioassay plants were not correlated to mycorrhizal infection but rather to nutrient content of the soil. In natural soils where nutrient content was high, a high biomass dry weight was achieved while plants growing in control and soils rehabilitated with tailings and plants only had less dry matter weight. Mycorrhizal infection had no effect on carbon cost of the highly infected plants. There was a weak relationship between plant growth rate and soil nutrient content. Plants with high growth rate used more carbon for growth and maintenance respiration.



4 MYCORRHIZA STATUS OF INDIGENOUS PLANTS GROWING ON NATURAL AREAS

4.1 Introduction

About 80% of all vascular plants form associations with mycorrhizal fungi (Smith and Read, 1996; Mauser, 1995; Marschner, 1986). Arbuscule mycorrhiza (AM) fungi are widely distributed in most terrestrial ecosystem because they are less specific to certain plant species (Smith and Gianinazzi-Pearson, 1988). The AM fungi act as decomposers, decomposing complex organic compounds into forms that can be taken up by plants. Several studies confirmed that mycorrhizal association benefit plants by means of increased nutrient uptake, increased tolerance of drought and pathogens as well as enhanced soil stabilisation (Sylvia *et. al.* 1998; Smith and Read, 1997).

Succulent Karoo vegetation along the coast of South Africa is rich in heavy minerals like ilmenite, rutile and zircon that are utilised in paint, ceramic and steel industries (Anglo American Corporation, 2002). This highly diverse vegetation is therefore suffering from the mining activities aimed at the extraction of these minerals. Mining companies are however compelled by law to rehabilitate areas affected by their mining activities. Rehabilitation of these disturbed areas to a functional state of the ecosystem requires knowledge on both macro and micro components of these ecosystems. Arbuscule mycorrhiza fungi appears to be one of the most essential micro component of the ecosystem therefore need to be studied and exploited to the benefit of vegetation re-establishment of post-mined areas. As part of integrated studies assessing the progress of a rehabilitation programme in Namakwa Sand mining areas, this study specifically assessed mycorrhizal infection on a number of selected common plant species occurring in unmined (but grazed) Strandveld Succulent Karoo vegetation of Brand se Baai.

4.2 Methods and materials

4.2.1 Study site

Species were collected from two selected natural sites composing of Tall shrub strandveld and Dwarf shrub strandveld plant community (Figure 2.2). Three permanent plots were established in the two different plant communities, in the vicinity of S31°16' 063" E 17°56'129" and S31°15' 898"E 17°58'391". Plant community classification category follows that of De Villiers *et. al.*, (1999) (Figure 2.2).

Dwarf shrub strandveld community covers the largest part of the West mine area. It is composed of dark-red soils rich in heavy minerals and the most common shrubs within this community belongs to *Ruschia* and *Asparagus* species. Of the other six identified plant communities, this community has the highest number of succulent species belonging to Aizoaceae family. Plots were established outside the proposed mining boundaries but within the same community type.

Tall shrub strandveld on the other hand is associated with deep yellowish sand and a taller vegetation community than other vegetation because of deep sand in which they occur. Species in this community include *Salvia lanceolata*, *Eriocephalus racemosus* and *Helichrysum* species.

4.2.2 Root collection

Roots were collected two times in April 2005 and late June 2005. The second root collection was aimed at collecting roots during rainy season since it rains in winter at the study site. However, rain onset was late and even during the second root collection the area was still dry. During the two periods, similar plants were sampled and roots were collected in the same vicinity of each vegetation community at random. The results from the two analyses were averaged to represent the mean percent infection shown in table 4.1. Other species that were not present on the demarcated plots were also collected outside the plots within each plant community. The total number of 28 different

indigenous plant species were assessed. Roots were recovered from the base of indigenous species at the depth of 30 cm soil profile for mycorrhiza infection analysis. These species were selected based on their predominance on the study site and they included species from Aizoaceae, Asparagaceae, Asteraceae, Chenopodiaceae, Fabaceae, Lamiaceae, Mesembryanthemaceae and Restionaceae families.

Separation of roots of different species in the field was subjective and based on the colour, size and structure of roots. Roots were kept in the solution of 50% ethanol for preservation later to be examined under a compound microscope for mycorrhizal structures.

For each plant, of which its roots were collected, plant specimens were also collected for species and or genus identification. Species identification was carried out with assistance from Prof Sue Milton and Dr Charlie Boucher (University of Stellenbosch). Species identified were confirmed with Compton Herbarium at Kirstenbosch Botanical Garden in Cape Town, South Africa.

4.2.3 Root clearing and staining

Clearing and staining of the roots were conducted according to the method by Phillips and Hayman (1970). Roots that were stored in 50% ethanol were washed with distilled water and cleared (delignified) in 10% potassium hydroxyl (KOH) in an autoclave for 30 minutes at 80°C. Cleared roots were acidified with 1% hydrogen chloride (HCl) to enhance root staining. Roots were then stained with aniline blue stain in the autoclave at 80°C for 5 minutes. After autoclaving, excess stain remaining on roots was removed with a distaining solution of 95% lactic acid.

4.2.4 Slide method for mycorrhiza infection analysis

Mycorrhizal infection in the roots of indigenous plants were analysed by means of slide method. The percentage infection of mycorrhizae along the root length was quantified by mounting 20 segments of 1cm long root on microscopic slides (Caldwell and Virginia,

1989; Giovannetti and Mosse, 1980). The root sections on slides were then examined with a light microscope at 40 X magnification for the presence of mycorrhizal structures. During microscope examination the hairline ruler visible under the microscope was used as the line of intersection. Mycorrhiza observations were recorded only at the intersection between the hairline and the root segments. At each intersection observations for the presence or absence of hyphae, vesicles, arbuscules, were done.

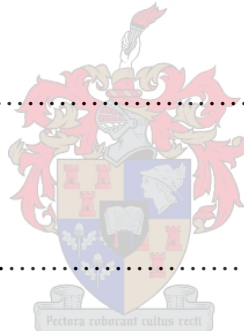
Total Percentage of mycorrhiza infection was therefore calculated by the following equations:

Arbuscular Colonisation (AC)
 $= 100 [(a + (a+v)) / G]$ 4.1

Vesicle Colonisation (VC)
 $= 100 [(v + (a+v)) / G]$4.2

Hyphal Colonisation (HC)
 $= 100 [(G-non) / G]$4.3

Total % infection = $[(a + v + (a+v) + mh + h) / G] * 100$4.5



Whereby:

G = total number of intersections including intersections where mycorrhiza was not observed

non = no fungal structure observed

a = arbuscules

v = mycorrhiza vesicles

a + v = arbuscules and mycorrhiza vesicles

mh = mycorrhiza hyphae (attached to vesicles or arbuscules anywhere in the field of view)

h = hyphae (not seen attached to any mycorrhizal feature)

4.3 Results

Mycorrhiza infection was observed on 85% of the species collected with infection ranging from 8% in *Atriplex lindleyi* and *Drosanthemum hispidum* to 98% in *Salvia lanceolata*. There was no mycorrhizae found in 15% of the assessed species which included *Atriplex semibacata*, one *Hermannia* species, *Tripteris oppositifolium* and one *Zygophyllum* species (Table 4.1).

High total mycorrhizal infection recorded was mainly attributed to hyphae and vesicle colonisation. Arbuscule structures were only observed on *Salvia lanceolata*, *Didelta spinosa* and *Ruschia* species with arbuscule percent colonisation of 12%, 5% and 2% respectively. With exception of the three species, all other assessed species recorded 0% arbuscule colonisation (Table 4.1).

Eighty nine percent of the assessed plant species were perennial and 11% species were short-lived (annual or biennial) shrubs or herbs. Short-lived plant species included *Atriplex lindleyi*, *Atriplex semibacata* and *Salsola kali*. All three are non-indigenous, having been introduced from Australia (*Atriplex* spp.) and Asia.

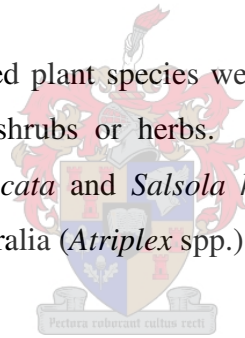


Table 4.1 Percentage mycorrhizal infection on roots of indigenous species in Brand se Baai un-mined vegetation areas.

Species name	Family name	² %AC	%VC	%HC	Total % infection
<i>Salvia lanceolata</i>	Lamiaceae	12	29	98	98
<i>Ericephalus racemosus</i> var. <i>affinis</i>	Asteraceae	0	18	93	92
<i>Mesembryanthemum</i> <i>gaucherianum</i>	Mesembryanthemaceae	0	23	77	78
<i>Ruschia brevibracteata</i>	Mesembryanthemaceae	0	17	56	57
<i>Berkheya spinosissima</i>	Asteraceae	0	16	57	56
<i>Othona fruticosa</i>	Asteraceae	0	18	54	54
<i>Chrysocoma ciliata</i>	Asteraceae	0	16	52	52
<i>Didelta spinosa</i>	Asteraceae	5	5	52	52
<i>Amellus tenuifolius</i>	Asteraceae	0	7	43	43
<i>Asparagus capensis</i> var. <i>litoralis</i>	Asparagaceae	0	8	39	41
<i>Galenia africana</i>	Aizoaceae	0	24	40	40
<i>Ruschia species</i>	Mesembryanthemaceae	2	14	40	40
<i>Lebeckia sericea</i>	Fabaceae	0	0	36	36
<i>Restionaceae species</i>	Restionaceae	0	6	32	33
<i>Chrysanthemoides</i> <i>incana</i>	Asteraceae	0	12	28	29
<i>Zygophyllum</i> <i>morgsanum</i>	Zygophyllaceae	0	3	22	22
<i>Salsola kali</i>	Chenopodiaceae	0	3	19	20
<i>Helichrysum dregeanum</i>	Asteraceae	0	6	18	18
<i>Asparagus capensis</i> var. <i>capensis</i>	Asparagaceae	0	0	17	17
<i>Asparathus spinosa</i>	Asparagaceae	0	4	13	14
<i>Senecio species</i>	Asteraceae	0	3	10	10
<i>Atriplex lindleyi</i>	Chenopodiaceae	0	0	8	8
<i>Drosanthemum</i> <i>hispidum.</i>	Mesembryanthemaceae	0	0	8	8
<i>Atriplex semibaccata</i>	Chenopodiaceae	0	0	0	0
<i>Hermannia species</i>	Sterculiaceae	0	0	0	0
<i>Tripteris oppositifolia</i>	Asteraceae	0	0	0	0
<i>Zygophyllum species</i>	Zygophyllaceae	0	0	0	0

²AC = arbuscule colonisation, VC = vesicle colonisation, HC = hyphal colonisation

4.4 Discussion

4.4.1 Mycorrhizal plants

Mycorrhiza infection of 85% observed in this study indicates the significance of mycorrhiza associations to plant physiology. It is documented in various reviews that mycorrhiza associations are important organs of nutrient uptake in plants and they occur in a wide range of host plants. If a given species naturally forms mycorrhiza and if the response to this association is positive to the plant then mycorrhiza infection may be essential for the establishment and survival of that plant.

The high mycorrhizal colonisation observed in this study was mainly attributed to hyphal and vesicle infection. Very few arbuscules were recorded, and these were only found on *Salvia lanceolata*, *Didelta spinosa* and *Ruschia* species. Mycorrhizal symbioses are usually active during wet season when plant roots are actively growing. Plants also have different requirements of AM based on their phenological stage of growth. A plant in its reproductive state requires more nutrients therefore may develop mycorrhizal association during this stage to accumulate more nutrients required for flowering.

This assessment was carried out during the dry season, therefore this could be the factor associated with low percentage of arbuscular colonisation in plant species sampled. According to Cox and Sanders (1974) during dry season when plants are not actively growing the roots and arbuscules disintegrate gradually into a clump which can not easily be identified. This would represent a non-functional or inactive association (Brundrett, 1991; Rose, 1981) because structures for nutrient transfer are missing. Arbuscules are also ephemeral structures in plants and as the plant ages they die off because their life cycle is generally short. For the fungi colonising rapid growing crops like wheat, the life cycle of their arbuscules is about seven days (Brundrett *et. al.* 1985; Alexander *et. al.* 1988), in slow growing species like perennials shrubs the life cycle is slightly longer. In perennial plants arbuscules occur only on new roots that are formed during periods of active growth. The onset of this period in the Strandveld Succulent Karoo is winter when it is raining. This assessment was carried out in winter but the onset of rain had not yet

started to initiate active root growth in shrubs hence few arbuscules were observed in the species sampled.

A low number of arbuscules could also be an indication of an inter plant hyphal network i.e. hyphae observed on an assessed plant is from a neighbouring host plant (Rose, 1980) where the fungi is actively functional. This situation is quite common in the field where plant roots intermingle in the soil. Many species belonging to family Chenopodiaceae has been found to demonstrate this phenomenon (Rose, 1980).

The mycorrhizal status of the Cape Floristic Regions (Strandveld vegetation included) was studied by Allsopp and Stock (1993) and the results showed that 63% of the species assessed recorded mycorrhizal association with arbuscules and was defined as a functional mycorrhiza association. In their study, the observed fungi with no arbuscules were regarded as functionally non-mycorrhizal.

For a fungal infection to be defined as mycorrhizal, arbuscule structures must also be observed as these structures are only formed in arbuscular mycorrhizae. This therefore implies that there was no functional mycorrhizal association on most of the plants assessed because no arbuscules were observed in most root segments. It is also difficult to accurately determine which hyphal infections are of mycorrhiza infection and which one is from other fungal infections. Infection by other non-mycorrhizal fungi like *Olpidium* species could be mistaken for AM, however they forms no arbuscules but rather cysts and zoosporangia which could be mistaken for AM vesicles. These possibilities are also supported because similar structures (vesicles and hyphae) were observed on Restionaceae and Zygophyllaceae families as well as on the genus *Asparathus* that are generally known to be non-mycorrhizal (Allsopp and Stock, 1993).

An assessment of mycorrhizae in different seasons of the year at a wider spatial variability is therefore required before a definite conclusion can be made about the mycorrhizal status of the vegetation at the site. Even with a full year assessment on mycorrhiza status of plants, a second confirmation is always required because very few

families are consistently with or without mycorrhizae when several consecutive assessments are made (Newman and Reddell, 1987; Brundrett and Kendrick 1988).

Plants have different requirements in terms of mycorrhiza infection based on their growth stage. During periods of active growth, plants require more nutrients hence high mycorrhizal infection, than during dormancy period. *Salvia lanceolata* with the highest arbuscule colonisation and also with highest total infection was flowering at time of the mycorrhiza assessment. There were however many other plant species with flowers during the assessment period, but their infection was not significantly higher. Further investigation regarding mycorrhizal infection levels and plant growth stage is necessary.

4.4.2 Non-mycorrhizal plants

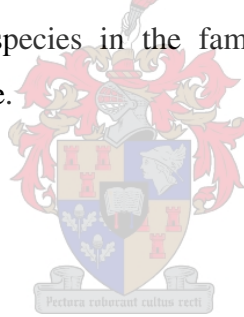
Species recorded as non-mycorrhizal, in this assessment belong to families of Asteraceae, Chenopodiaceae, Sterculiaceae, and Zygophyllaceae. Species belonging to the Chenopodiaceae and Zygophyllaceae have previously been recorded to be non-mycorrhizal in South Africa and North America (Allsopp and Stock, 1993; Allen, 1983; Rose 1980). Species belonging to family Chenopodiaceae have been recorded as either non-mycorrhizal or mycorrhizal depending on type of plants they co-exist with (Rose, 1980). A mycorrhizal association in this family was observed only when species are planted with obligate mycorrhizal plants. In this case the hyphal network of the host plant grows into the non-mycorrhizal species (Allen, 1983; Rose, 1980) where it may not grow actively. Studies on species of these families suggests the status of mycorrhizae in these families and other families growing in less fertile soils should be investigated with respect to soil fertility because it has been found that mycorrhizal associations are absent in members of these families growing in less fertile soils of Cape Floristic Regions and Australia (Allsopp and Stock, 1993; Pendleton and Smith, 1983).

Both the Chenopodiaceae recorded as being non-mycorrhizal in Strandveld are invasive alien *Atriplex* species that were introduced to South Africa from Australia in the late 1800s to cover soils denuded and salinised by overgrazing (Hutchinson, 1946). The

concept of host adaptation to AM associations may also be a limiting factor to mycorrhizal association establishment in these families. A root mapping observation by Allen *et. al.* (1989) suggests that there is an incompatible reaction between the roots of *Salsola kali* species and vesicular arbuscular mycorrhizal fungi, whereby the plant actively rejects AM infection.

4.4.3 Conclusions

Most species in the Strandveld Succulent Karoo grow in association with mycorrhiza. Mycorrhizal colonization observed was mainly attributed to hyphae and vesicle colonization, arbuscules were recorded only in few species and on one of the flowering species. Few arbuscules were recorded in these species because they were not actively growing at the time of assessment. Active growth periods result in development of new roots in perennial vegetation where arbuscules could be established. Mycorrhizal symbiosis was absent in some species in the families Asteraceae, Chenopodiaceae, Sterculiaceae, and Zygophyllaceae.

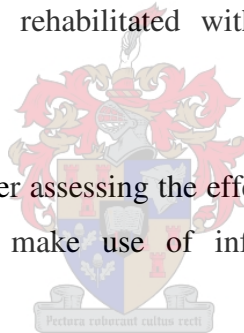


5 GENERAL RECOMMENDATIONS AND CONCLUSIONS

5.1 Recommendations

In order to balance the need for conservation and economic development, rehabilitation of mined sites should be carried out by mining companies to facilitate ecosystem re-establishment. The use of appropriate methods of rehabilitation is very important in ensuring a successful rehabilitation which is in conformity with a prevailing legislation of a given country. In order to increase microbial activities in mined sites, this study shows that it is extremely important to replace topsoil removed during mining activities in order to replace organic matter and microbial propagules that were initially removed during mining. Replacement of topsoil does not immediately bring back microbial activities of the soil but it makes the process of microbial development shorter as it was observed that mycorrhiza colonisation of soils rehabilitated with topsoil were higher than soils rehabilitated with tailings only.

Mycorrhizal studies should consider assessing the effectiveness of different AM fungi so that rehabilitation efforts could make use of infective and effective mycorrhizal propagules.



The best methods to test for soil infectivity are planting bioassay plants in whole soil from the field without diluting the soil with sterilized sand, or planting bioassays in whole soil diluted with sterilized sand at different levels of dilutions. The dilution level at which soil infectivity is not affected by the dilution with sterilized sand can then be determined. This pre-determined level is then confidently used for the experiment testing soil infectivity. Neither one of the above methods was used because it would have required that more heavy sand is carried from the field, and six months was not sufficient to test for the best dilution. Nevertheless, although the best dilution level was not used, the dilution level which was used for this study (as long as it was of similar quantity to all samples) can be expected to show a trend relatively similar to the methods above.

Therefore results can still be compared with one another in determining which soil had the highest soil mycorrhiza infection.

It is also recommended that the sterilised sand portion that is used as planting medium could be collected from a native environment where the inoculum was collected in order to maintain similar soil chemical properties that also facilitate the establishment of AM.

If carbon cost has to be measured accurately analysis for roots and shoots carbon cost should be carried separately so that the extra carbon cost on mycorrhizal roots could be accurately determined.

In order to acquire sufficient data on mycorrhizal infectivity of indigenous species, assessment at different times of the year is required to observe seasonal fluctuation in colonisation levels. These observations should also be conducted during the wet season when plants are actively growing and are in active association with fungi. In order to confirm mycorrhizal status of plants growing at the study site at least three root samples from the same species occurring in different areas at the study site should be collected. This will prevent the bias effect of environmental variations on the results. Spatial scaling is an important consideration in conservation biology; therefore information obtained from homogenous greenhouse environments should also be tested in complex heterogeneous natural ecosystems before definite conclusions are made (Vogt et al, 1991).

5.2 Conclusions

Processing of soil to remove heavy minerals depleted soil carbon and phosphorous significantly relative to concentrations in undisturbed vegetation. Addition of fresh topsoil to mined sites backfilled with tailings, with or without translocated plants, indicated an increased soil infectivity of mined sites. However, this infection had no significant effect on plant growth, plant nutrient content or carbon cost of mycorrhizal plants. Instead soil properties had a pronounced effect on these variables. The study also

showed that most (85%) of the indigenous plants growing on undisturbed sites of the Strandveld Succulent Karoo vegetation of Brand se Baai grow in association with mycorrhiza. Mycorrhiza is therefore probably an essential component of plant community development in this area.

This study has provided new information on the mycorrhizal status of Strandveld plants, and has indicated topsoil has greater mycorrhizal infectivity than tailings. Together, these findings support the notion that saving and replacement of topsoil onto tailings is likely to hasten vegetation recovery of mined landscapes. The short duration of the study and the use of “foreign” sand soil and plants for bioassays were weaknesses that could be overcome in subsequent research. Disturbance to the inoculum soil core could also be minimized to avoid erratic results of different treatments.



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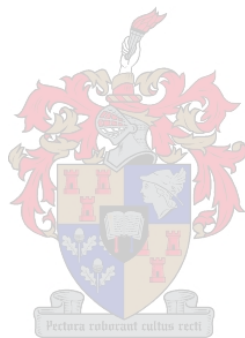
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Nutrient concentration (mmolgDW-1)

Nutrient concentration (mmolgDW⁻¹)

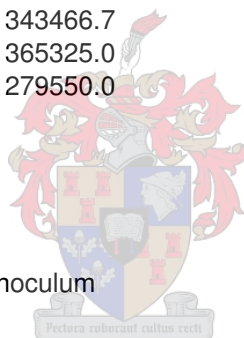
Trt ID	N	P	K	C
C11	40071.4	10354.8	20820.5	67508.3
C11	40071.4	10354.8	20820.5	67508.3
C11	40071.4	10354.8	20820.5	67508.3
C21	40071.4	10354.8	20820.5	67508.3
C21	40071.4	10354.8	20820.5	67508.3
C21	40071.4	10354.8	20820.5	67508.3
N1	40071.4	10354.8	20820.5	67508.3
N1	40071.4	10354.8	20820.5	67508.3
N1	40071.4	10354.8	20820.5	67508.3
TS1	40071.4	10354.8	20820.5	67508.3
TS1	40071.4	10354.8	20820.5	67508.3
TS1	40071.4	10354.8	20820.5	67508.3
TSP1	40071.4	10354.8	20820.5	67508.3
TSP1	40071.4	10354.8	20820.5	67508.3
TSP1	40071.4	10354.8	20820.5	67508.3
TP1	40071.4	10354.8	20820.5	67508.3
TP1	40071.4	10354.8	20820.5	67508.3
TP1	40071.4	10354.8	20820.5	67508.3
C12	24142.9	5516.1	11615.4	189016.7
C12	22857.1	4419.4	11435.9	231450.0
C12	19928.6	4774.2	10820.5	421233.3
C22	32000.0	2419.4	13717.9	393458.3
C22	34500.0	2677.4	15435.9	243016.7
C22	33928.6	3709.7	13512.8	526541.7
N2	36285.7	2225.8	15410.3	295433.3
N2	31928.6	2064.5	15717.9	486041.7
N2	36285.7	2419.4	14948.7	338750.0
TS2	26357.1	2806.5	14820.5	275425.0
TS2	30642.9	1838.7	11410.3	283525.0
TS2	31000.0	1612.9	12641.0	291625.0
TSP2	28714.3	2645.2	11897.4	340225.0
TSP2	35214.3	1935.5	13487.2	324025.0
TSP2	28642.9	1483.9	8153.8	405033.3
TP2	30928.6	1096.8	6769.2	302425.0
TP2	27500.0	2129.0	12461.5	411266.7
TP2	28500.0	1774.2	9666.7	257316.7
C13	8214.3	677.4	2410.3	310525.0
C13	8714.3	580.6	2410.3	324025.0
C13	8500.0	354.8	2102.6	211866.7
C23	15071.4	1322.6	8820.5	243016.7
C23	12642.9	1096.8	8410.3	249250.0
C23	14428.6	1096.8	7923.1	226816.7
N3	21214.3	1483.9	10666.7	346708.3
N3	10785.7	774.2	7179.5	345833.3
N3	14071.4	1354.8	9641.0	273400.0
TS3	9785.7	774.2	5769.2	359666.7
TS3	15857.1	871.0	7487.2	252741.7
TS3	11785.7	903.2	7615.4	248416.7

TSP3	14500.0	1096.8	7205.1	230058.3
TSP3	13571.4	1000.0	8025.6	259216.7
TSP3	7071.4	709.7	5051.3	226816.7
TP3	13857.1	1064.5	7820.5	286641.7
TP3	10928.6	1096.8	7641.0	276375.0
TP3	9142.9	935.5	6205.1	267941.7
C14	11928.6	1129.0	5461.5	368500.0
C14	13428.6	1225.8	5820.5	410433.3
C14	12285.7	1193.5	5461.5	300883.3
C24	10500.0	967.7	5435.9	286641.7
C24	14000.0	1096.8	7025.6	212641.7
C24	14285.7	903.2	6487.2	308450.0
N4	6214.3	580.6	4051.3	405033.3
N4	6071.4	516.1	4256.4	352066.7
N4	5357.1	548.4	4051.3	339908.3
TS4	12142.9	967.7	6000.0	386341.7
TS4	6857.1	774.2	4102.6	289750.0
TS4	11357.1	1000.0	7769.2	294866.7
TSP4	6000.0	806.5	4589.7	421233.3
TSP4	7928.6	871.0	5435.9	373875.0
TSP4	7500.0	709.7	4923.1	383225.0
TP4	9428.6	806.5	5307.7	343466.7
TP4	6142.9	838.7	4897.4	365325.0
TP4	5571.4	677.4	4102.6	279550.0

Legends

N	inocula from natural sites
C1	control with sterilised mixture of inoculum
C2	control with sterilised sand only
TSP	inocula from sites rehabilitated with tailings, topsoil and translocated plants
TS	inocula from sites rehabilitated with tailings and topsoil
TP	inocula from sites rehabilitated with tailings and translocated plants

- 1 Harvesting at 5 days
- 2 Harvesting at 21 days
- 3 Harvesting at 42 days
- 4 Harvesting at 56 days



Chemical properties of rehabilitated soils at Srandveld Succulent Karoo
vegetation at Brand se Baai: final data

Soil ID	pH (KCl)	Resist. (Ohm)	H (cmol/kg)	P Bray II	K
				mg/kg	
N	7.1	547.5	0.0975	101	276.5
N	7.05	635	0	54	228
N	6.85	612.5	0	45	206
TSP	7.85	280	0	7.5	179
TSP	7.65	240	0	7.5	179
TSP	8.4	240	0	11.5	207
TS	8.55	330	0	9.5	161.5
TS	8.3	285	0	8	150
TS	8.15	395	0	8	137
TP	7.15	970	0	5	140.5
TP	7.45	560	0	5.5	184.5
TP	7.7	530	0	5	142
Control 1	7.68	468.75	0.0081	22.29	182.58
Control 1	7.68	468.75	0.0081	22.29	182.58
Control 1	7.68	468.75	0.0081	22.29	182.58

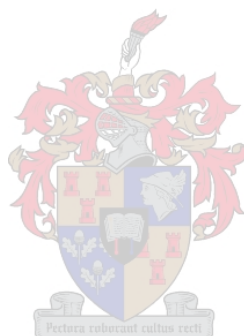
Chemical properties of rehabilitated soils at Srandveld Succulent Karoo
vegetation at Brand se Baai: final data

Na	K	Ca	Mg	%	Soil ID
0.815	0.705	7.62	1.955	1.685	N
0.645	0.585	3.24	2.065	0.69	N
0.4	0.525	2.77	1.395	0.87	N
1.255	0.46	1.33	0.88	0.315	TSP
1.51	0.46	1.34	0.97	0.255	TSP
1.55	0.53	1.83	1.04	0.47	TSP
0.955	0.415	0.71	0.745	0.155	TS
1.015	0.385	1.195	0.63	0.22	TS
0.92	0.355	0.525	0.61	0.24	TS
0.33	0.36	0.435	0.625	0.16	TP
0.67	0.475	0.635	0.775	0.18	TP
0.685	0.365	0.6	0.7	0.14	TP
0.90	0.47	1.85	1.03	0.45	Control 1
0.90	0.47	1.85	1.03	0.45	Control 1
0.90	0.47	1.85	1.03	0.45	Control 1

Mycorrhiza infection on wheat

Root ID	non	%AC	%VC	%HC	Total infectic	rate
control11	30	0.000	0.000	0.000	0.000	0.000
control11	30	0.000	0.000	0.000	0.000	0.000
control11	30	0.000	0.000	0.000	0.000	0.000
control21	30	0.000	0.000	0.000	0.000	0.000
control21	30	0.000	0.000	0.000	0.000	0.000
control21	30	0.000	0.000	0.000	0.000	0.000
N1	30	0.000	0.000	0.000	0.000	0.000
N1	30	0.000	0.000	0.000	0.000	0.000
N1	25	0.000	6.250	21.875	21.875	1.042
TS1	24	0.000	0.000	20.000	20.000	0.952
TS1	17	0.000	28.947	55.263	55.263	2.632
TS1	12	16.216	24.324	67.568	67.568	3.218
TSP1	26	0.000	0.000	13.333	13.333	0.635
TSP1	30	0.000	0.000	0.000	0.000	0.000
TSP1	30	0.000	0.000	0.000	0.000	0.000
TP1	25	0.000	16.667	16.667	16.667	0.794
TP1	29	0.000	3.333	3.333	3.333	0.159
TP1	28	0.000	6.452	9.677	9.677	0.461
control12	30	0.000	0.000	0.000	0.000	0.000
control12	30	0.000	0.000	0.000	0.000	0.000
control12	30	0.000	0.000	0.000	0.000	0.000
control22	30	0.000	0.000	0.000	0.000	0.000
control22	30	0.000	0.000	0.000	0.000	0.000
control22	30	0.000	0.000	0.000	0.000	0.000
N2	29	0.000	0.000	3.333	3.333	0.159
N2	29	3.333	0.000	3.333	3.333	0.159
N2	30	0.000	0.000	0.000	0.000	0.000
TS2	27	0.000	0.000	12.903	12.903	0.614
TS2	24	0.000	15.152	27.273	27.273	1.299
TS2	25	0.000	0.000	16.667	16.667	0.794
TSP2	28	0.000	3.226	9.677	9.677	0.461
TSP2	27	0.000	10.000	10.000	10.000	0.476
TSP2	22	0.000	26.667	26.667	26.667	1.270
TP2	30	0.000	0.000	0.000	0.000	0.000
TP2	30	0.000	0.000	0.000	0.000	0.000
TP2	30	0.000	0.000	0.000	0.000	0.000
control13	30	0.000	0.000	0.000	0.000	0.000
control13	30	0.000	0.000	3.226	3.226	0.154
control13	30	0.000	0.000	0.000	0.000	0.000
control23	30	0.000	0.000	0.000	0.000	0.000

control23	30	0.000	0.000	0.000	0.000	0.000
control23	30	0.000	0.000	0.000	0.000	0.000
N3	28	0.000	6.667	6.667	6.667	0.317
N3	30	0.000	0.000	0.000	0.000	0.000
N3	27	0.000	6.897	6.897	6.897	0.328
TS3	29	0.000	0.000	0.000	0.000	0.000
TS3	30	0.000	0.000	0.000	0.000	0.000
TS3	30	0.000	0.000	0.000	0.000	0.000
TSP3	18	0.000	36.667	40.000	40.000	1.905
TSP3	26	0.000	12.903	16.129	16.129	0.768
TSP3	7	0.000	76.667	76.667	76.667	3.651
TP3	30	0.000	0.000	0.000	0.000	0.000
TP3	30	0.000	0.000	0.000	0.000	0.000
TP3	30	0.000	0.000	0.000	0.000	0.000

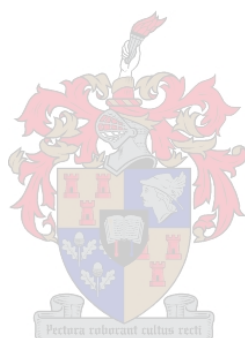


Plant Dry weight

Trtment	Plant DW (g)
Control 5	0.015
Control 5	0.015
Control 5	0.015
N 5	0.015
N 5	0.015
N 5	0.015
TS 5	0.015
TS 5	0.015
TS 5	0.015
TSP 5	0.015
TSP 5	0.015
TSP 5	0.015
TP 5	0.015
TP 5	0.015
TP 5	0.015
Control 21	0.144
Control 21	0.173
Control 21	0.246
N 21	0.191
N 21	0.179
N 21	0.138
TS 21	0.143
TS 21	0.205
TS 21	0.149
TSP 21	0.142
TSP 21	1.000
TSP 21	0.192
TP 21	0.261
TP 21	0.405
TP 21	0.228
Control 42	1.331
Control 42	1.726
Control 42	1.235
N 42	3.663
N 42	4.276
N 42	1.811
TS 42	2.767
TS 42	1.916
TS 42	2.249
TSP 42	2.515
TSP 42	2.556
TSP 42	3.045
TP 42	1.577
TP 42	2.328
TP 42	2.502
Control 56	2.654
Control 56	2.293
Control 56	2.251
N 56	8.088

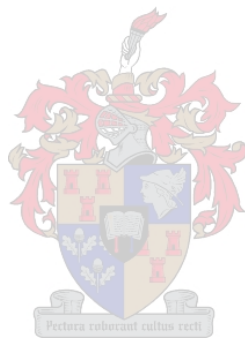


N 56	8.764
N 56	3.832
TS 56	3.744
TS 56	1.833
TS 56	3.400
TSP 56	3.534
TSP 56	5.008
TSP 56	5.013
TP 56	2.410
TP 56	4.252
TP 56	5.157

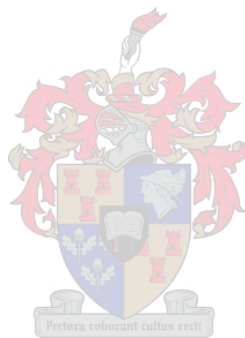


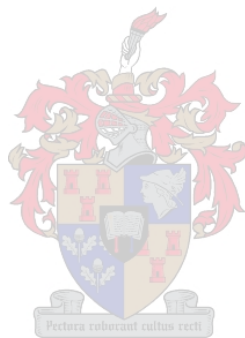
Species name	non	a	v	a+v	m h	h	%G	%AC	%VC	%HC	total infecti
<i>Lebeckia Sericea</i>	7	0	0	0	0	4	11	0	0	36	36
<i>Tripteris oppositifolium</i>	11	0	0	0	0	0	11	0	0	0	0
<i>Zygophyllum morgsanum</i>	12	0	1	0	1	4	18	0	6	33	33
<i>Amellus tenuifolius</i>	8	0	1	0	0	5	14	0	7	43	43
<i>Rhuscia brevibracteata</i>	8	0	4	0	4	7	23	0	17	65	65
<i>Galenia africana</i>	9	0	2	0	1	2	14	0	14	36	36
<i>Asparagus capensis var. cap</i>	10	0	0	0	0	2	12	0	0	17	17
<i>Ruschia spp.</i>	8	0	3	1	3	4	19	5	21	58	58
<i>Galinea africana</i>	11	0	0	0	0	1	12	0	0	8	8
<i>Drosanthemum hispidum.</i>	11	0	0	0	0	1	12	0	0	8	8
<i>Atriplex lindleyi</i>	11	0	0	0	0	1	12	0	0	8	8
<i>Atriplex semibaccata</i>	14	0	0	0	0	0	14	0	0	0	0
<i>Zygophyllum sp.</i>	13	0	0	0	0	0	13	0	0	0	0
<i>Hermannia sp.</i>	11	0	0	0	0	0	11	0	0	0	0
<i>Didelta spinosa</i>	10	1	1	0	2	7	21	5	5	52	52
<i>Helichrysum dregianum</i>	14	0	1	0	0	2	17	0	6	18	18
<i>Salsola kali</i>	5	0	0	0	0	0	5	0	0	0	0
<i>Galenia africana</i>	3	0	7	0	1	1	12	0	58	75	75
<i>Rhuscia brevibracteata</i>	6	0	2	0	1	3	12	0	16	49	49
<i>Chrysocoma ciliata</i>	2	0	3	0	1	8	13	0	21	87	87
<i>Salvia lanceolata</i>	0	1	4	1	2	9	17	12	29	98	98
<i>Asparathus spinosa</i>	10	0	1	0	1	1	11	0	5	14	14
<i>Erioccephalus racemosus var.</i>	1	0	2	0	2	7	12	0	19	92	92
<i>Berkheya spinosissima</i>	5	0	2	0	1	3	11	0	18	56	56
<i>Rhucia spp</i>	5	0	2	0	1	4	12	0	20	60	60
<i>Lampranthus sp</i>	6	0	1	0	0	4	11	0	6	41	41
<i>Mesembryanthemum gauche</i>	3	0	3	0	1	7	14	0	24	78	78
<i>Chrysocoma ciliata</i>	7	0	1	0	0	1	9	0	12	18	18
<i>Othona Fruticosa</i>	6	0	2	0	2	2	12	0	19	54	54
<i>Resitiod sp</i>	7	0	1	0	0	3	10	0	7	33	33
<i>Senecio sp</i>	9	0	0	0	0	1	10	0	3	10	10
<i>Asparagus capensis var. litor</i>	7	0	1	0	0	3	11	0	9	41	41
<i>Chrysanthemoides incana</i>	7	0	1	0	0	2	10	0	13	29	29
<i>Zygophyllum morgsanum</i>	8	0	0	0	0	1	9	0	0	11	11
<i>Rhuscia sp</i>	10	0	0	0	0	0	10	0	3	3	3

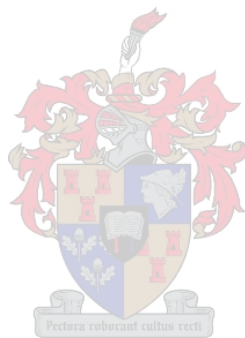












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