IMPORTANCE OF ARBUSCULAR MYCORRHIZA FUNGI IN THE RESTORATION OF A DISTURBED SITE IN KAKAMEGA FOREST

BY

SIMON NGURU WANDETO

B.Sc. (ENVIRONMENTAL STUDIES); KENYATTA UNIVERSITY

A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE (BIOLOGY OF CONSERVATION); UNIVERSITY OF NAIROBI

JULY 2014

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Simon Nguru Wandeto
School of Biological Sciences
University of Nairobi

Sign:.....

Date: 30/06/2014

This thesis has been submitted for examination with our approval as the University supervisors.

Prof. Jenesio Kinyamario
School of Biological Sciences
University of Nairobi

Sign:.....

Date: 30/06/2014

Dr. Winfred Musila
Formerly of National Museums of Kenya

Sign: .....

Date: 30.06.2014

Dr. Joyce Jefwa
Formerly of International Center for Tropical Agriculture (CIAT)

Sign:.....

Date: 26. June 2014

DEDICATION

I would like to dedicate this thesis to my wife Waitherero for her unwavering support and encouragement through the work.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following individuals and institutions for providing me with the opportunity and inspiration toembark and complete this work. Firstly, my appreciation goes to my supervisors – Prof. Jenesio Kinyamario, Dr. Winfred Musila formerly of the National Museums of Kenya and Dr Joyce Jefwa formally of the International Center for Tropical Agriculture (CIAT) for their guidance and oversight in the research. Their kind and meticulous input gave me the motivation and courage to forge ahead and complete this task. I especialy thank Dr. Jefwa for her encouragement and valuable advise in the mycorrhizal studies which formed the bulk of the work. Secondly, my gratitude goes to BIOTA East Project for granting me a scholarship and the funding to carry out this research. I would not have been able to enroll for the Master of Science programme or carry out the research withour their support. I am grateful to Dr. Musila – the local BIOTA East Project Coordinator for her confidence and patience with me even when, due to complexities of the task and other extreneous factors, the work seemed to have been abandoned.

I also wish to thank my colleagues and friends both from the National Museums of Kenya and the University of Nairobi; John Nyaga, Susan Kanyari, Victor Otieno, Morris Obunga, Christopher Chesire, Salome Thuo and Rebecca Kariuki who in many ways contributed to the success of this work. My deepest gratitude goes to God for His faithfulness and goodness towards me.Nothing would have would have been possible without Him. He gave me the strength, health, wisdom and a sound mind to be able to pursue this endeavor.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

ACRONYMS AND ABBREVIATIONS

AMF	Arbuscular Mycorrhiza Fungi
ANOVA	Analysis of Variance
ASTER	Advanced Space-borne Thermal Emission Reflection Radiometer
BIOTA	Biodiversity Monitoring Transect Analysis
EC	Electrical Conductivity
EDTA	Ethylenediaminetetraacetic Acid
GPS	Global Positioning System
HCL	Hydrochloric Acid
INR	Near Infra-Red
KIFCON	Kenya Indigenous Forest Conservation Network
KOH	Potassium Hydroxide
RMD	Relative Mycorrhizal Dependency
SOM	Soil Organic Matter

ABSTRACT

Kakamega forest, a major reservoir of biodiversity in Kenya has experienced degradation over a long period of time due to human disturbance. This disturbance has resulted in a heterogeneous mixture of vegetation including disturbed primary forest, secondary forest, clearings and glades, and timber plantations. Restoration of the forest ecosystem has included the re-establishment of native tree species in order to accelerate recovery of the degraded areas and connect formally joined forest fragments. The degraded habitats, besides losing the above ground vegetation, have also lost the below-ground biological organisms. Often, restoration efforts have disregarded these below-ground organisms, some of which are crucial in maintaining plant community structure. It was hypothesised that the mycorrhizal abundance and diversity in soils of these degraded areas would affect plant growth and establishment rate in the restoration efforts. The study developed a vegetation map of a disturbed site within the Forest and assessed the mycorrhizal abundance and diversity in soils of the identified vegetation stands. Arbuscular mycorrhiza fungi abundance and diversity was found to be low at the disturbed site and also varied according to vegetation type and soil properties. The study also sought to determine the mycorrhizal dependancy of *Erythrina abyssinica*, *Markhamia lutea*, and *Senna spectabilis* seedlings which were some of the tree species identified for planting in the restoration efforts. The seedlings were found to be facultatively mycorrhizal but contamination of the experiment obscured the clear benefit of arbuscular mycorrhiza fungi colonization on seedling establishment.

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The intense exploitation of tropical forests has led to degradation of once stable ecosystems. FAO (2005a) found that degraded forestlands and secondary forests cover significant areas throughout the tropics and that in most countries, the degraded forestlands now exceed areas covered by primary forests. The cycle of tropical deforestation typically begins with excessive logging that reduces the original forest to a non-commercial resource leading to changes in biotic and abiotic soil properties which hamper the re-establishment of proper vegetation cover (Shono *et al.*, 2007; Miller, 1987). Research has shown that tropical soils are dominated by soils low in available nutrients and moisture (Khanam, *et al*., 2006; Schlesinger, 1997; Kauffman *et al.,* 1995). In addition to the low levels of available nutrients, the soils of disturbed sites also lack the nitrogen-fixing bacteria and mycorrhizal fungi usually associated with root rhizospheres (Quilambo, 2003).

Arbuscular mycorrhizae (AM) are symbiotic associations between plants and soil fungi which ranges, on a continuous scale, from parasitic to mutualistic (Harrison, 2005; Harley and Smith, 1983). Most plant species form mycorrhizae and the effect of the mycorrhizal fungi on plant performance has far reaching ecological consequences from increasing productivity of ecosystems to enhancing plant diversity (Lovelock *et al.,* 2003). Kungu (2004) asserts that deforested and degraded areas no longer regenerate into woody perennials partly due to lack of Mycorrhiza propagules for re-colonization but rather into the so-called derived savannas. Mukhongo *et al.* (2011)

further opine that depletion of the soil seed bank had also contributed to the slow regeneration of the natural forest in some of these degraded areas. Disturbance disrupts the soil fungi by removing the plants on which these obligate mutualists depend for their carbon and by breaking the hyphal mycelia and thereby reducing the inoculum density for newly establishing seedlings (Allen *et al.*, 1998). Mycorrhizal fungi are depleted in soils that have been damaged by tillage, erosion, mining or other mechanical damage and may be relatively slow to re-colonise (Allen *et al.*, 1997).

The need to restore degraded forest ecosystems to improve their productive capacity, environmental functions, and biodiversity value is widely recognized (Parotta, 2000). However, the restoration is in many cases erratic disregarding successional stages and adaptation to degraded soil conditions that are not necessarily suitable for establishment of the selected tree species. Degraded habitats do not only encompass loss of the above ground trees, but also the associated belowground biological organisms such as fungi, some of which are crucial in maintaining plant community structures. The importance of biological components of terrestrial ecosystems has been overlooked during the development of most habitat restoration strategies. Commonly used practices place a premium on above-ground production or diversity at the expense of below-ground function (Miller and Jastrow, 1992).

Amongst organisms that play key roles in maintaining plant communities are the mycorrhizae which form mycorrhizal symbiosis, a mutually beneficial relationship between plants and fungi. Association with plants ranges from obligate to facultative with association more of a rule rather than an exception. The mycorrhizal association is key to well-functioning plant communities due to the role of mycorrhiza in nutrient cycling, creation of nutrient reserves by their contribution to development of the soil

structure, and involvement of mycorrhiza in whole plant processes such as nutrient uptake, water regulation, growth and reproduction, prevention of disease and maintenance of plant diversity (Miller and Jastrow, 1992). Understanding the dynamics of mycorrhizal symbiosis along the disturbance gradient of proposed restoration ecosystems is crucial in devising restoration strategies that will guarantee a sustainable and productive forest ecosystem.

1.2 Study background

Kakamega Forest is one of the most important reservoirs of biodiversity in Kenya and one of the last remaining indigenous forests of Kenya (Lung and Schaab, 2004; Tsingalia, 1988, Wass, 1995). It is the largest moist lowland forest ecosystem in Kenya with important biological resources similar in characteristics to the Central Africa equatorial forest (Kokwaro, 1988). The forest harbours world famous and rare birdlife and primates and a high plant species diversity, including high value timber species. The forest is enriched by its contact with the montane forests of the rift valley escarpment and the Mt Elgon forests (KIFCON, 1994). The main forest block consists of a heterogeneous mixture of vegetation in different successional stages including disturbed primary forest, secondary forest, clearings and glades as well as tea and timber plantations (Bennun and Njoroge, 1999). Due to its situation in an agricultural area with a high human population density, Kakamega forest continues to face conflicts between forest conservation and land use needs of an increasing population. The results have been severe forest degradation through extensive commercial and local exploitation of timber. Several studies by KIFCON (1994), Mutangah (1996) and Bleher *et al*., (2006) have attempted to quantify the extent of this degradation.

Fragmentation and disturbance is high in the forest such that the forest now comprises of a main forest block surrounded by small forest fragments which may have been connected to the main forest in the past (Kokwaro, 1988; Lung and Schaab, 2004; Wass, 1995). Kakamega forest, once measuring up to 13,888.3 ha of natural forest is now smaller, down to only 8,245ha (Mutangah, 1996). The rapidly increasing human population in areas adjacent to the forest has immensely contributed to land fragmentation and consequently degradation of habitat (Bleher *et al*., 2006).

Initiatives to restore the forest ecosystem have been undertaken with establishments of both indigenous (732.8ha) and exotic plantations (858.9ha) (Mutangah, 1996).A restoration programme aimed at planting native tree species in order to accelerate recovery of the degraded areas and connect formerly joined forest fragments has also been initiated by BIOTA East Project (Musila, 2006). The project is currently in the process of planting tree islands or clumps of selected tree species to stimulate regeneration by serving as perches for seed dispersers such as birds and monkeys. Three corridors have also been identified totalling 40 ha which will be planted with indigenous trees to link remnant forests and rejoin the isolated populations of plants. Mukhongo *et al.* (2011) established that one of the factors that could be hindering regeneration of the natural forest is a depletion of the soil seed bank in the degraded areas. The soil seed banks were found to be dominated by graminoid seeds and those of shrubs. Viability of the seeds of tree species was also low ranging between 1.3 and 33.8%.

1.3 Literature Review

1.3.1 The Role of Arbuscular Mycorrhiza Fungi (AMF)

The AMF form specialized tree-like structures inside the root cells called arbuscules, and other structures such as the intra and extra-radical spores, intra and extra-radical hyphae, and fungal storage structures called the vesicles.

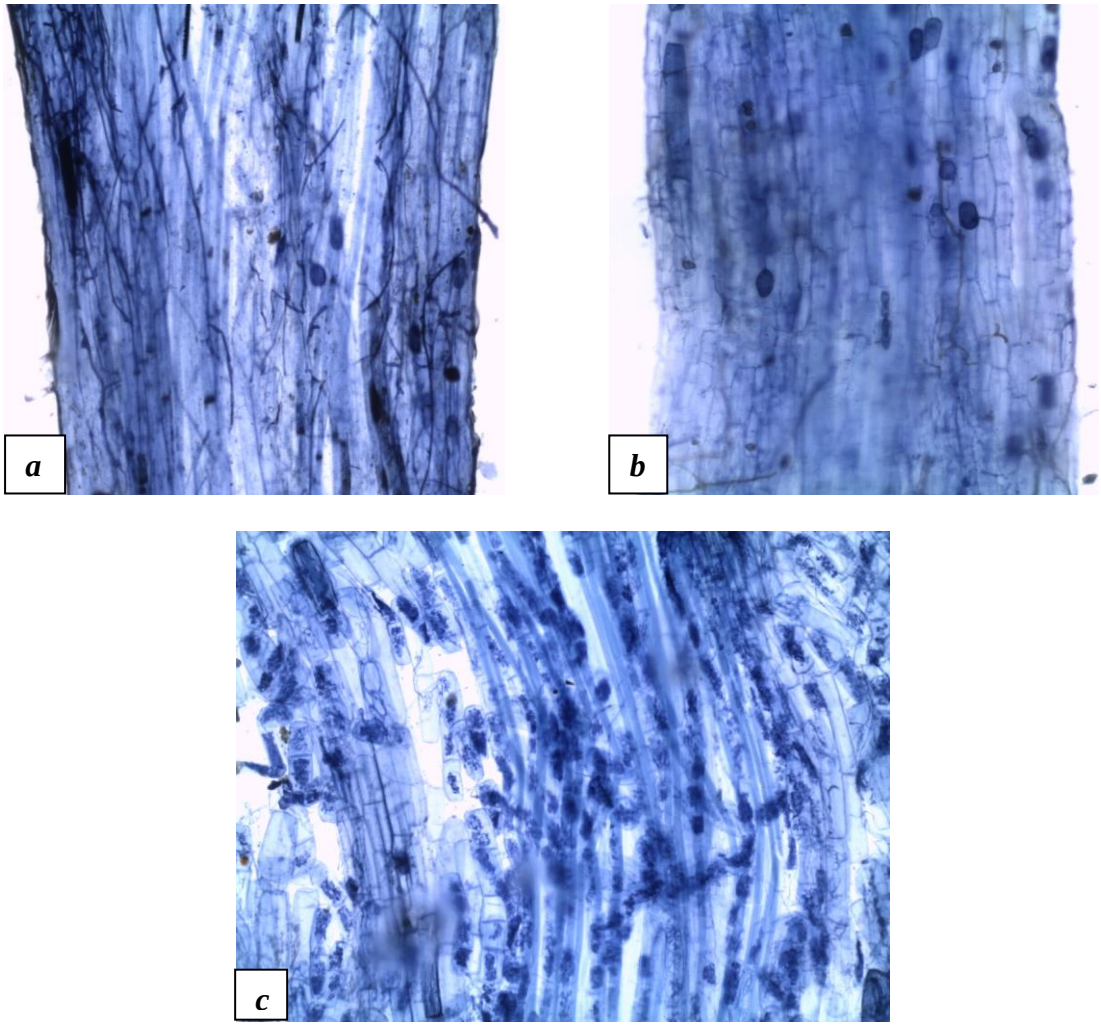


Plate 1.1: mycorrhizal structures in trypan blue - stained roots showing; a) hyphae, b) vesicles and c) arbuscules

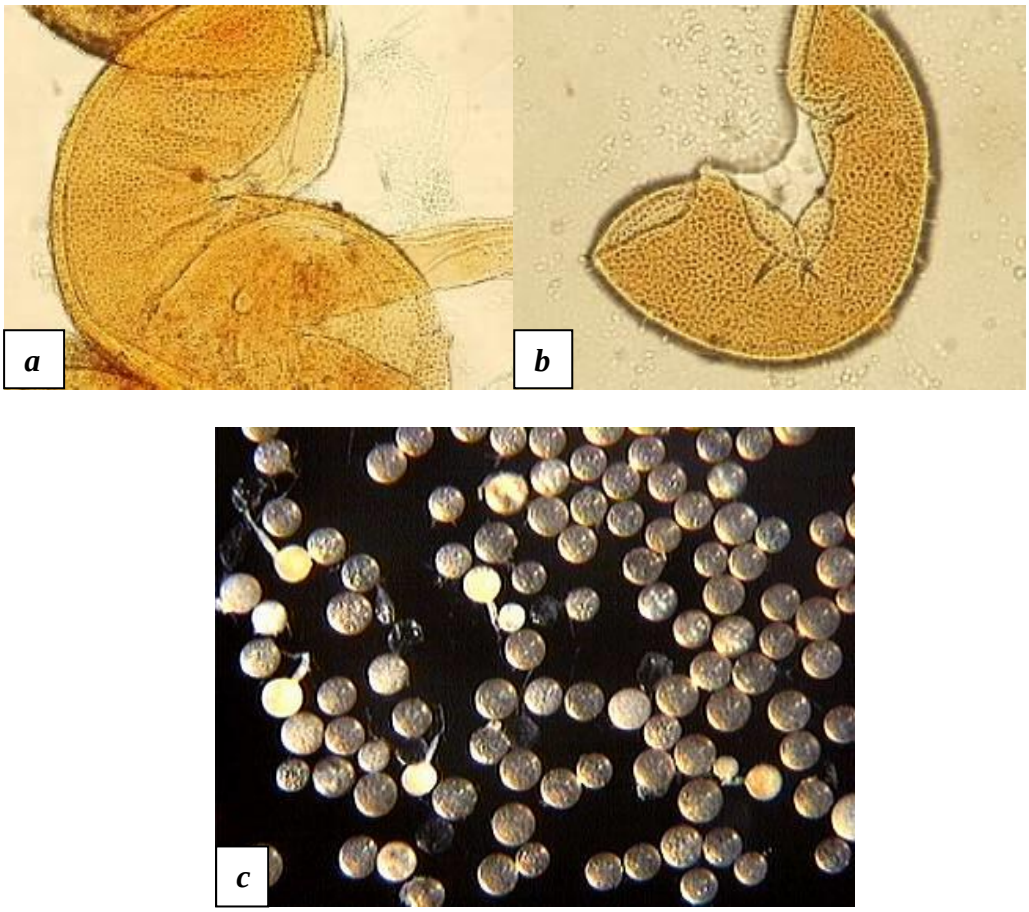


Plate 1.2: Spores of *Acaulospora scrobiculata*: *a* & *b)* in Melzer's reagent at x40 magnification, and *c*) whole spores at x10 magnification

The fundamental role of the hyphae is to bridge the annular space, producing a physical connection between the root-surface and surrounding soil (Miller and Jastrow, 1992). The extra-radical hyphae spreads around the root system and performs important ecosystem functions such as increasing the plant's ability to explore the soil for nutrients, especially phosphorus and water, and increasing the plant's ability to resist diseases. The fungus also helps in improvement of soil texture. In turn, the fungus obtains photosynthates from the host plant (Dalpe and Monreal, 2004; Barea *et al*. 1996; Selvaraj and Chellappan, 2006).

In forests, seedling recruitment is a vital stage in the determination of forest structure and diversity. Seeds are dispersed without their mycorrhizal symbionts and

acquire a symbiont at germination. Spatial and temporal variation in the Arbuscular Mycorrhiza fungal community and the effectiveness of those fungi may therefore give rise to microsites that are more or less favourable for seedling establishment and growth (Lovelock *et al*., 2003). However, not all plants and fungi form mycorrhizae. A plant is independent of mycorrhizae if it can survive as an individual, compete in a community and reproduce without them. Plants or fungi can be facultative if they are capable of forming mycorrhizae but are also capable of completing their lifecycle without forming mycorrhizae. Thus, plants may be regarded as facultative mycotrophs if they function without mycorrhizae in some natural situations and with mycorrhizae in others. Obligate mycotrophs cannot complete their lifecycles without mycorrhizae. Data indicates that about 12% of the angiosperms are facultative, 18% are non-mycorrhizal, and 70% are obligate mycotrophs (Molina *et al.*, 1992).

1.3.2 Effects of AMF Diversity on Plant Community Structure

AMF diversity is the major factor in the maintenance of plant biodiversity and ecosystem stability and function (Quilambo, 2003). Diversity of the AMF determines plant community structure through the response of individual plant species to this diversity. The AMF influence the plant's ability to establish and cope with stress situations such as nutrient deficiency, drought, trace element imbalance and soil disturbance. The AMF also determines successional processes by altering the relative success of plants both at the seedling stage and during later development (Barea *et al*., 1996; Aziz *et al*., 1995).

Conversely, plant community structure can affect the composition of AMF community. A change in host-plant composition is likely to alter the activity and

species composition of AMF (Aziz *et al*., 1995). Living roots release a wide variety of soluble, insoluble and volatile exudates, some of which act as chemical messengers that assist in coordinating the formation of mycorrhizae. Events mediated by these compounds include spore germination, directional growth of germ tubes and hyphae, hyphal growth rate and branching, and the positioning of hyphae near potential entry sites on the root surface (Koske and Gemma, 1992). Arbuscular mycorrhizae fungi exhibit host specific chemotaxis whereby hyphal growth is higher in fungi found in exudates of plants starved of phosphorus (Aziz *et al*., 1995).

Arbuscular mycorrhizae communities may also differ because tree species differ in growth rates and phenology. Root morphologies are also distinctive and trees deploy their roots in different regions of the soil profile. Some trees have abundant roots found very close to or on the soil surface while others are deployed deeper in the soil profile (Lovelock *et al*., 2003). Trees can also alter differentially fertility and other physical and chemical characteristics of soils which can in turn affect arbuscular mycorrhizae community structure and abundance (Lovelock *et al*., 2003; Aziz *et al*., 1995).

1.3.3 Host Preference of AMF and Mycorrhizal Dependency of Plant Species

Although arbuscular mycorrhizal fungi are not host specific, they may have preferences for certain plants (Luis *et al.,* 2009.) and are also influenced by soil properties such as soil pH, temperature, soil carbon and nitrogen. The level of benefit to plant growth thus varies depending on the fungal community-host combination, prevailing environmental conditions and genotype of the fungal species (Lovelock *et al*., 2003). Vaidya *et al.* (2007) thus opines that managing soils could be a potential

way to optimize the proliferation of indigenous arbuscular mycorrhiza fungi. Plant growth responses are highly specific to different mycorrhizal species (Allen *et al.*, 1992) and some plant species are more dependent on mycorrhizal association. This has been attributed to the ability of plant species to absorb Phosphorus from soils low in available Phosphorus (Mosse *et al.*, 1973), morphological root properties i.e. root geometry, rate of growth, density and length of root hairs (Plenchette, 1991) and possibility of the size of seeds.

Koide and Mosse (2004) however point out that arbuscular mycorrhizal fungi do not always cause plant growth increases. There are notable cases of growth depression apparently caused by arbuscular mycorrhizal fungi in "non-host" species or in host species when phosphate availability is high.

1.3.4 Restoration of Degraded Forest Ecosystems

Degraded ecosystems can be returned to their original conditions by applying appropriate management technologies to soil-plant systems based on ecological principles (Barea *et al.*, 1996). Since mycorrhizae is important to the growth of tropical trees, their dynamics in gaps and other kinds of disturbance may be related to vegetation recovery (Allen *et al.*, 1998). The rate of recovery and of succession in degraded ecosystems can be hastened by inoculation or manipulation of the mycorrhizal fungus population under conditions where the fungal propagules densities are very low (Barea *et al.*, 1996; Miller and Jastrow, 1992). The mycorrhizal fungi do not facilitate skipping of successional stages, rather, they prevent stagnation of community development (Miller and Jastrow, 1992). It is also important to select a mycorrhizal fungus that is symbiotically efficient and physiologically compatible with the plant (Barea *et al.*, 1996). Selection of the most specific and appropriate plant-

fungus association for each specific environmental and ecological situation is one of the main challenges in current research on arbuscular mycorrhiza (Khanam *et al*., 2006).

Inoculation is often recommended as a measure to improve restoration but is costly and often not practical due to the inability to culture most species of mycorrhizal fungi. Because viable populations of mycorrhizal fungi must be maintained, most restoration projects rely on indigenous populations of the arbuscular mycorrhiza fungi, their natural dispersal mechanisms from the surrounding undisturbed vegetation, and management practices that increase reproduction and dispersal (Allen *et al*., 1997; Miller and Jastrow, 1992). In severely disturbed ecosystems, the phenomena of disturbance and facultativeness influence successional pattern. Non-mycorrhizal and facultatively mycorrhizal plants are well known early colonizers of disturbed ecosystems (Molina *et al.,* 1992) and thus can also be used in initial restoration efforts.

1.4 Justification

Tree species used in restoration have variable survival strategies upon which there are subsequent effects on their growth under different soil conditions. Some plant species establish easily on highly degraded soils while others require modifications to soil conditions for them to survive. The establishment of pine trees in Kenya for example has failed due to absence of their mycorrhizal symbionts. The survival strategies of tree seedlings are determined by their ability to explore for nutrients and water and resist disease and shock during transplanting, functions performed by the root systems. Plants with fibrous roots are able to explore nutrients better in nutrient limiting environments than plants with thick roots. The mycorrhizal symbiosis enhances root

functions, and is necessary in the establishment of plant species with thick root systems and also trees in soils with limited soil nutrients.

Disturbance of the vegetation, soil and associated micro-flora could lead to the decline in soil nutrients and abundance and diversity of mycorrhizal fungi in the affected areas. Establishment of trees in these areas and subsequent recovery of these areas may depend upon the re-establishment of mycorrhizal fungi during restoration efforts where the fungus is completely absent. Selection of the most appropriate plant-fungus association for each vegetation type identified in the disturbed areas is also an important consideration (Johnson *et al*., 1991). This is because the low density and diversity of AMF may limit the degree of mycorrhizae colonization in transplanted seedlings and consequently hamper the seedling establishment and growth in those areas (Quilambo, 2003). This study was designed to establish the distribution of plant species at the sites, mycorrhizal dependency of the trees used in restoration and the mycorrhizal fungi abundance and diversity along disturbance gradients. This will provide useful information on choice of plant species for introduction in these areas and whether inoculation with mycorrhizae is necessary prior to field establishment.

1.5 Study Objectives

1.5.1 General objective

The main objective of the study was to assess mycorrhizal abundance and diversity in soils from different vegetation types of a disturbed site in Kakamega forest and their effects on growth and establishment of selected restoration trees.

1.5.2 Specific objectives

The specific objectives of the study were:

1. To determine plant species distribution and soil nutrient conditions along the disturbance gradient;
2. To analyse the mycorrhizae abundance and diversity in each of the identified vegetation types along the restoration corridor; and
3. To assess the mycorrhizal dependency of selected restoration tree species.

1.6 Hypothesis

The hypotheses of the study were:

1. Plant species composition and soil properties vary along the disturbance gradient on a disturbed site;
2. Mycorrhizal abundance and diversity in soils of degraded areas is a vital determinant of plant growth and establishment in restoration efforts;
3. *Markhamia lutea*, *Erythrina abyssinica* and *Senna spectabilis* are obligately mycorrhizal requiring inoculation with arbuscular mycorrhiza fungi in restoration of a disturbed site.

CHAPTER TWO: MATERIALS AND METHODS

2.1 Study Area

2.1.1 Location

Kakamega forest is located in Shinyalu division of Kakamega District in the Western Province of Kenya, approximately 40 kilometres North West of Lake Victoria. It lies between latitudes 00°08’30.5” N and 00°22’12.5” N, and longitudes 34°46’08.0”E and 34°57’26.5” E. The forest is bordered by the Nandi Escarpment to the East and South and lies at an altitude between 1500 – 1700 m above sea level (Musila, 2007). Figure 1 below shows an image of Kakamega main forest and its fragments in western Kenya, with official forest boundaries as gazetted in 1933.

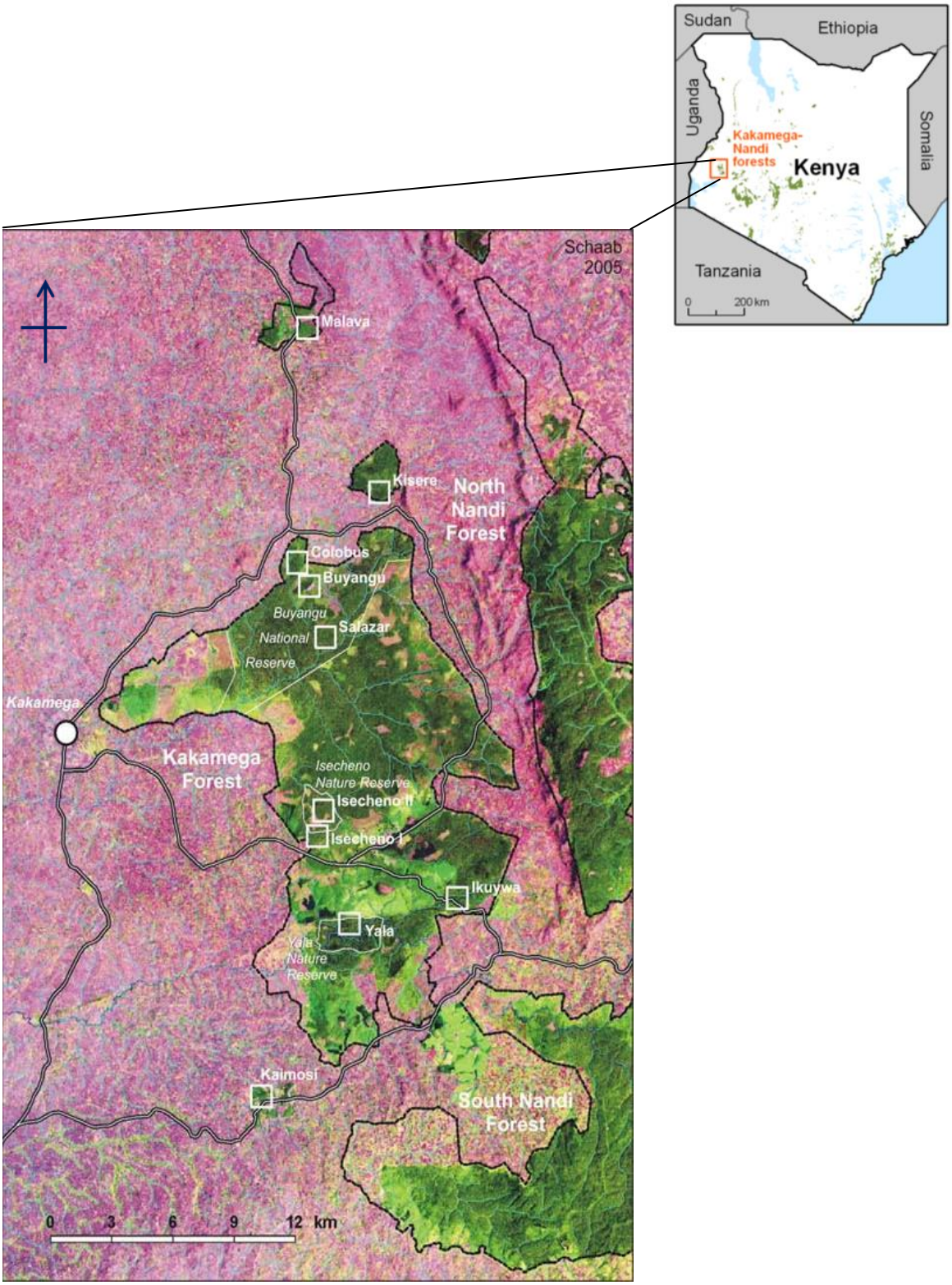


Figure 2.1*:* Landsat ETM+ (7) satellite image (05 Feb 2001, spectral bands 5/4/3, contrast enhanced) of Kakamega Forest, its peripheral fragments and the Nandi Forests (source: BIOTA-E02, G. Schaab, F.H Karlsruhe)

2.1.2 Climate

The Forest experiences heavy precipitation due to its proximity to Lake Victoria and position near the Equator (Musila, 2007). Annual rainfall in Kakamega forest according to Muriuki and Tsingalia (1990) is approximately 2007mm as averaged from the Forest Department records at Isecheno Forest Station from 1982-2001. The rainfall is highly seasonal with a rainy season from April to November and a short dry season from December to March (Muriuki and Tsingalia, 1990; Mitchell and Schaab, 2008). The average monthly maximum temperature ranges from 18°C to 29° C while the average monthly minimum ranges from 4°C to 21° C.

2.1.3 Geology and Soils

Kakamega forest is characterized by the Nyanzian and Kavirondian rock formations, the oldest rocks in Kenya formed in the lower Pre-Cambrian time. The underlying rocks of the forest area consist of undifferentiated mudstone and ancient gneisses which are associated with gold bearing quartz veins (Ojany and Ogendo, 1987). The northern and eastern edge of the forest consists of small strips of granitic rock types (Jatzold and Schmidt, 1982). Kakamega soils have been classified as Acric Ferralsols (FAO, 1990b).

2.1.4 Vegetation

Due to past anthropogenic disturbance, most of Kakamega forest is a middle aged forest with some parts having young successional vegetation (Althof, 2005; Musila, 2007). The vegetation consists of a mixture of grasslands (glades), shrub lands and degraded forest sites. There are two types of grasslands: Natural glades which have been in existence for a long period of time and whose origin is not certain, and the other type being recent man-made glades resulting from abandoned farmlands. Genetic

and species losses in Kakamega forest have been attributed to various factors such as physical disturbance, forest excisions for agricultural development and exploitation for food such as game hunting, fruits and vegetables (Onyango *et al.,* 2004).

Succession has begun in some of the man-made glades which are now colonized by pioneer species namely *Psidium guajava*, *Acanthus pubescens*, *Harungana madagascarienses* and *Bersama abyssinica*. The forested sites are characterized by plant species such as *Funtumia latifolia*, *Prunus africana, Olea capensis* and *Celtis africana.*

2.2 Determination of plant species distribution and soil properties along a disturbance gradient

2.2.1 Vegetation mapping

A vegetation map of the study area was developed using remote sensing techniques following the guidelines of Brocklehurst *et al.* (2007). An August 2008 ASTER (Advanced Space-borne Thermal Emission Reflection Radiometer) image of 15 x 15 meter spatial resolution, and in raw image bands was acquired and projected to Arc 1960 datum and Clarke 1880 spheroid. Bands 1, 2 and 3n of the image were composited in IDRISI software to give a false color image for clarity of interpretation and differentiation of the different vegetation types.

The area of study boundary was generated based on the ongoing activities by BIOTA East project. The composited image was clipped based on this boundary using ArcView software image analysis extension. Interpretation of the image was then done in GEOVIS software using on-screen digitization method where homogeneous parts were captured as polygons. Nine vegetation types were identified in this process and

included the Natural forest, *Psidium guajava* and *Vernonia sp* shrub-lands, *Eucalyptus saligna, Pinus patula* and *Cupressus lusitanica* plantations, *Hyparrhenia* sp. and *Brachiaria brizantha* grasslands. Strips of *Acanthus pubescens* vegetation were also identified. GPS coordinates were also taken around the various vegetation types on the study site and these also aided in the digitization of vegetation polygon types. An attribute table was then created and the different vegetation type names inserted and linked to the digitized polygons. Digitization of road and river shape files was also done on the satellite image as arcs. The polygons and arcs captured were exported to ArcView shape files as vector layers, after which the vector layers were taken to ArcMap software for map layout preparation. Other map components like the grid, scale bar, north arrow and neat-line were cartographically added.

2.2.2 Soil Sampling

A total of 39 sampling points were selected on a stratified random basis covering the seven identified vegetation types. The number of soil samples from each vegetation type was determined according to the size of the vegetation stand. The sampling points were distributed as follows:

(i) 7 samples in *Psidium guajava* dominated shrubland;

(ii) 7 samples in *Vernonia* sp. dominated shrubland;

(iii) 4 samples in *Eucalyptus saligna* plantation;

(iv) 4 samples in *Cupressus lusitanica* plantation;

(v) 6 samples *in Hyparrhenia* sp. dominated grassland (natural);

(vi) 4 samples in *Brachiaria brizantha* dominated grassland(artificial); and

(vii) 7 samples in Natural forest vegetation.

Soil was sampled from each randomly identified point in each stratum using a soil auger at four depths of 0 – 10 cm, 10 – 20 cm, 20 – 30 cm, and 30 - 40 cm. The AMF spore abundance and diversity, and soil chemical characteristics i.e. pH, texture, electrical conductivity (EC) and Phosphorus were determined at each depth for the soils of the seven vegetation types sampled.

2.2.3 Soil Analysis

a) Soil pH determination

Soil pH was determined using a pH meter and an electrode immersed in water using the method described in Jones and Reynolds, 1996. A composite soil sample of each depth was obtained for each vegetation type out of which 10g was taken for the test. One part of soil by volume was mixed with two parts distilled water (pH 7) and an electrode immersed in the solution. A reading was taken after 10 minutes.

b) Soil extractable phosphorus determination

Soil Phosphorus in oven dried soil (400° C for 48hrs) was determined by elemental analysis as described by Okalebo *et al.* (2002). Olsen P was extracted using 0.5 M sodium bicarbonate + 0.01M ethylenediaminetraacetic acid (EDTA) at pH 8.5, and was estimated colorimetrically using a spectrophotometer.

c) Particle size analysis

This procedure was done to determine the particle size distribution of the soil samples using the hydrometer method as described by Gee and Bauder (1986). The method employed sieving and sedimentation of a soil/water/dispersant suspension to separate the particles. The sedimentation technique was based on an application of Stokes' law to a soil/water suspension and periodic measurement of the density of the suspension.

Sub-samples measuring 100g were oven dried at 100˚C then broken up to particles fine enough to pass through 4.75 mm and 2 mm sieves. Weighing was done for particles less than 2mm. 200 ml of deionised water and 20 ml of 25% sodium hexametaphosphate were added to the samples in a shaking bottle and shaken for 16 hours at 15rpm. The prepared and dispersed samples were then transferred to 1 litre measuring cylinders and filled to the 1litre mark with deionised water. Stirring was done to ensure that all material at the bottom was suspended. A blank solution (without the soil sample) was also prepared and poured into a l litre measuring cylinder. A hydrometer was immersed to a depth slightly below its floating position and allowed to float freely. Readings were taken after 40 seconds. The temperature of the suspension and the blank solution was also taken. A similar procedure was followed taking hydrometer readings from the blank solution. Other readings were taken after 8 hours.

Calculations:

$$sand + silt\ (\%) = \frac{(First\ reading - blank)}{weight}\ x\ 100\ (A) \quad \text{.....Equation 1}$$

$$Clay\ (\%) = \frac{(Second\ reading - blank)}{weight} x100\ (B) \text{ .....Equation 2}$$

$$Silt\ (\%) = A - B \text{.....Equation 3}$$

$$Sand\ (\%) = 100 - A - B \text{.....Equation 4}$$

2.3 Mycorrhizal abundance and diversity

2.3.1 Spore extraction

Spores were extracted from 50 g sub-samples for each of the 156 (39 x 4) soil samples. Extraction was through wet sieving and decanting (Gerdmann and Nicolson, 1963) followed by sucrose centrifugation (Jenkins, 1964). The sieving was done through 710 and 45µm mesh sieves, while 60w/v of sucrose was used in sucrose centrifugation.

Two isolations were performed for each sample. The spores were distinguished into morphotypes under reflected light on a stereomicroscope with features such as: colour of spore, spore size, attachments on spore, and surface appearance of spore, used for diagnosis. The number of spores was counted for each morphotype. The Edinburgh Botanic Gardens colour chart for fungi was used for determination of spore colour. Voucher specimens were prepared for each AMF morphotype and further described under a compound microscope with spore germination characteristics, spore wall characteristics, i.e. spore wall size and number of layers, and reaction to Melzer's reagent used as diagnostic characteristics. The Shannon-Weiner function was used to establish AMF species diversity in the soils of the seven vegetation stands. Where H' is the index of species diversity, S is the number of species, and p_i the proportion of total sample belonging to the *i*th species (Krebs, 1985).

$$H' = -\sum_{i=1}^{S}(p_i \ln p_i)$$ .....Equation 5

2.4 Assessment of mycorrhizae dependency of tree species

2.4.1 Experimental design

Three tree species were selected as the study plants and included *Senna spectabilis*, *Erythrina abyssinica,* and *Markhamia lutea.* A greenhouse experiment was set up using a factorial design comprising of 7 vegetation types, 4 treatments and 3 tree species. Each treatment combination consisted of 4 replicated units. The four treatments were applied on the study plants as follows: (i)**Treatment 1**-Establishment of the study plants on un-treated soil from each of the 7 vegetation types under investigation, (Non-sterile field soil) (ii) **Treatment 2** - Establishment of the study plants on autoclaved soil from each of the 7 vegetation types under investigation

(sterilised field soil), (iii) **Treatment 3** - Establishment of the study plants on inoculated soil from each of the 7 vegetation types under investigation, (Non-sterile and inoculated field soil), and (iv) **Treatment 4** - Establishment of the study plants on autoclaved and inoculated soil (Sterilized and inoculated field soil). Four replicates of each plant species were established on soil from each of the seven vegetation types and allowed to grow for a period of 18 weeks. Table 2.1 below summarises the experimental set-up of the study.

Table 2.1: Experimental treatment structure

Treatment	Treatment description	vegetation types	Species per vegetation type	Replicates	No. of Pot Plants
Treatment 1	Non-sterile soil	7	3	4	84
Treatment 2	Sterile soil	7	3	4	84
Treatment 3	Non-sterile soil + mixed inoculum	7	3	4	84
Treatment 4	Sterile soil + mixed inoculum	7	3	4	84
Total					336

2.4.2 Mixed Inoculum preparation (for dependency experiment)

A composite soil sample from the seven vegetation types was used to produce inoculum for assessment of mycorrhizae dependency of the selected tree species in the green house experiment. Equal portions of soil from the various vegetation types were thoroughly mixed together after which the composite sample was mixed with sterile river sand in the ratio of 3: 1(soil : sand). Leek *(Allum sepa*) - a highly mycorrhizal plant was sown in basins of the mixture and allowed to grow for a period of 2 months under greenhouse conditions. 50 grams of the inoculum was added to the soil in pots of Treatment 3 and Treatment 4 of the experimental set-up.

Plate 2.1: preparation of mixed inoculum showing Leek (*Allum sepa*) growing in basins

2.4.3 Plant growth parameters

Growth parameters such as the number of leaves, basal diameter, and shoot height were taken weekly to determine the plants' growth rate over 18 weeks. Harvesting of the plants was done after the 18 weeks growth period and their wet weights determined. A sub-sample (30%) of the roots from each plant was taken and processed to determine root colonization by AMF. The rest of the plant material, i.e. shoot and roots, were loosely packed in paper bags and stored in a well-ventilated room in which air drying took place for a period of two weeks. Dry weights of the shoots and roots were then determined using a weighing scale.

2.4.4 Assessment of Mycorrhizal colonization

a) Root processing

Root processing was done following the method proposed by Koske and Gemma (1989). The 30% portion of harvested roots were washed and rinsed in several changes of tap water.10% KOH was added and the roots autoclaved at 120^{o}C for 15 minutes. The roots were then bleached using Hydrogen Peroxide and Ammonia solution. Acidification was then done overnight by adding 1% HCL to the roots. The HCL was then decanted and 0.05% trypan blue in lacto-glycerol added and to the roots and

autoclaved at 120^{o}C for 5 minutes. The stain was then decanted and root pieces stored in glycerol acidified with a few drops of HCL until examination for the presence or absence of mycorrhizae. In assessment of colonization, the roots were cut into approximately 1 cm segments and mounted on slides. A minimum of thirty - 1 cm piece root segments were mounted on a single slide per root sample for examination under a compound microscope (Biermann and Linderman, 1980).

b) Frequency and intensity of root colonization

The frequency and Intensity of AMF colonization, i.e. the occurrence of arbuscules, coils, vesicles, internal and external hyphae was recorded for each slide. Care was taken to distinguish artifacts from AMF structures. The frequency of AMF was recorded as the number of root fragments infected with AMF and was expressed in percentage. The intensity of AMF colonization was recorded as the percentage cover of AMF colonization in each root fragment as described in McGonigle *et al*. (1990). The percentages obtained from the quantification of Intensity were categorized into classes (1-20, 21-40, 41-60, 61-80 and 81-100). The number of roots in each category was multiplied by the median number in each class (10v + 30w + 50x + 70y + 90z) where v, w, x, y, z are the number of roots in each class. The AMF intensity was expressed as follows:

$$AMF\ Intensity = \frac{(10v+30w+50x+70y+90z)}{(v+w+x+y+z)} \quad \text{.....Equation 6}$$

c) Relative mycorrhizal dependency

The relative mycorrhizal dependency refers to the degree of plant responsiveness to mycorrhizal colonization by producing maximum growth or yield at a given level of soil fertility (Gerdemann, 1975). Relative mycorrhizal dependency is related to

morphological and physiological properties of root systems (Mosse *et al*., 1973; Gianinazzi-Pearson, 1984). The relative mycorrhizal dependency (RMD) was determined by expressing the difference between the dry weight of the shoot of the mycorrhized plant and the average dry weight of the shoot of the non-mycorrhized plant as a percentage of the dry weight of the mycorrhized plant shoot (Plenchette *et al.*, 1983).

$$RMD = \frac{(MDW\ of\ plants\ with\ AM - MDW\ of\ plants\ without\ AM)}{MDW\ of\ plants\ with\ AM}\ x\ 100$$ .....Equation 7

Where, (MDW = Mean Dry Weight)

2.5 Statistical analysis

Analysis of variance (ANOVA) with GENSTAT version 8 analytical package and MS-Excel Analysis Toolpak was carried out on all data to test the treatment effects on various measured parameters. Means were separated by Fisher's othorgonal comparisons of treatment means ($p \leq 0.05$).

CHAPTER THREE: RESULTS

3.1 Plant species distribution

3.1.1 Vegetation Mapping

Various vegetation types were delineated in the vegetation map. These included the following:

3.1.1.1 Natural forest

This was the dominant vegetation stand delineated in the map. The forest stand comprised of various tree species such as *Olea capensis*, *Prunus africana*, *Ficus sur*, *Funtumia lantifolia*, *Trichilia emetica*, *Zanthoxylum gilletii*, and *Celtis africana*. The forest had a dense shrub layer consisting mainly of *Dracaena fragrans*. Other plant species identified within the forest stand were *Pollia condensata*, and *Dorstenia brownii*. *Desmodium repandum* was also identified in open gaps within the forest. No distinction was however made between the primary forest and secondary forest although Lung (2004) in his Landsat imagery classification identified near natural/old secondary forest (Forest of lowest disturbance level, dense canopy, older than 50 years as well as old secondary forest of between 30 to 50 years), and secondary forest (this being mid-aged secondary forest of 20-30 years as well as aged *Maesopsis eminii* plantations mixed with indigenous species).

3.1.1.2 *Cupressus lusitanica* and *Pinus patula* plantations

These were closed canopy monoculture plantation stands with little undergrowth mostly comprised of herbs/shrublets and climbers such as *Justicia flava*, *Dorstenia brownii*, *Afromomum* sp. and *Desmodium repadum.*

3.1.1.3 *Eucalyptus saligna* plantation

This was an open canopy *Eucalyptus saligna* tree stand interspersed with grasses mostly *Brachiaria brizantha* and herbs such as *Justicia flava.*

3.1.1.4 *Psidium guajava* and *Vernonia* sp. shrub-land

This was a transitional zone linking the main forest block with the natural forest fragment nearest the access road.

3.1.1.5 *Acanthus pubescens* shrub-land

This shrub populated the main forest block edge forming a belt between the forest and the *Hyparrhenia* sp. grassland. The shrub was also delineated along the edge of the *Cupressus lusitanica* plantation.

3.1.1.6 *Hyparrhenia* sp. and *Brachiaria brizantha* grasslands

These were partially due to origin and partially from clearings. The *Hyparrhenia* sp. grassland was distinct and in patches some of which were located in un-disturbed forest areas. These patches had little if any, tree or shrub cover. The *Brachiaria brizantha* grassland on the other hand was interspersed with shrubs and *Eucalyptus saligna, Cupressus lucitanica,* and *Pinus patula* trees, an indicator that that the grassland was in a phase of succession after recent disturbance. The vegetation map is shown in Figure 3.1 below.

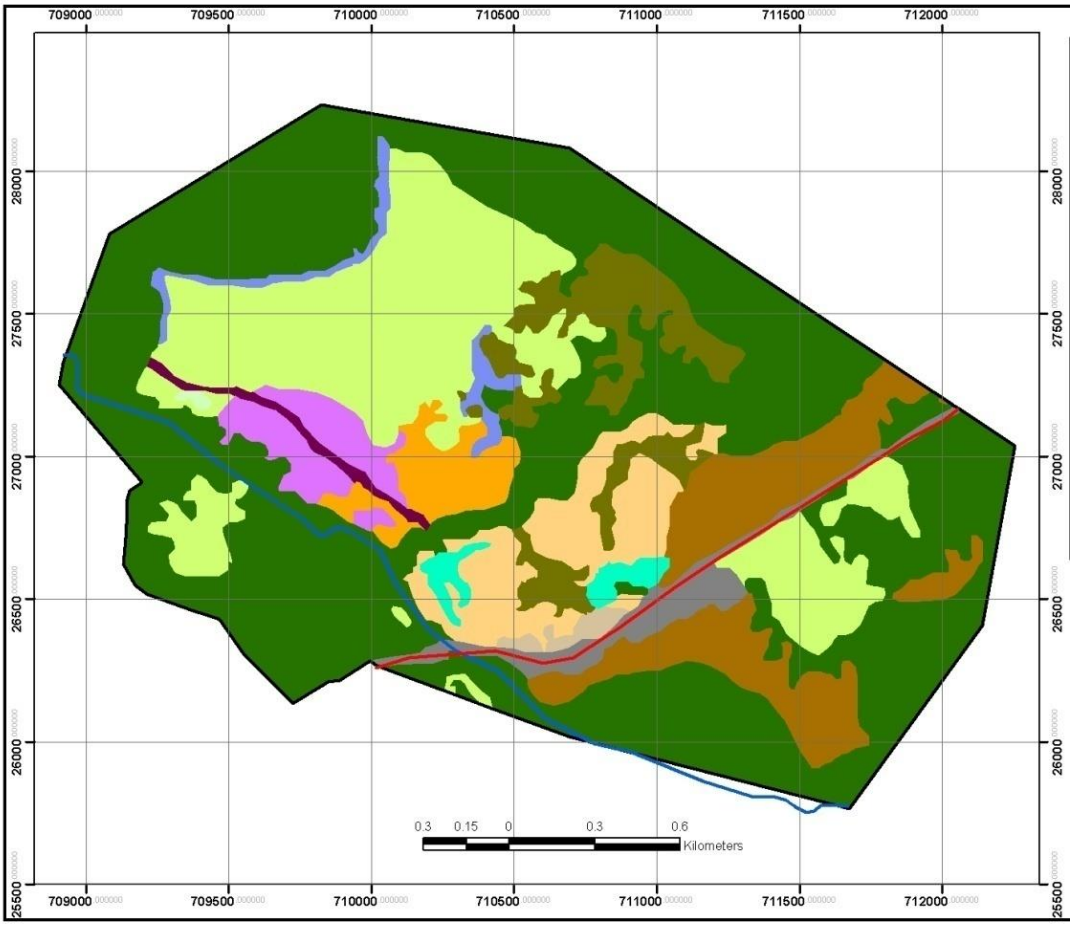


Figure 3.1: A vegetation map of the study site

Legend

	Road
	River
	Natural forest
	Pinus patula plantation
	Acanthus pubescens cover
	Eucalyptus saligna plantation
	Cuppressus lusitanica plantation
	Psidium guajava cover
	Vernonia sp. cover
	Hyparrhenia sp. cover
	Brachiaria brizantha cover
	Bare Ground
	Fire Break

3.2 Soil physical and chemical properties

3.2.1 Electrical Conductivity

a) Electrical conductivity variance with soil depth

The table below indicates electrical conductivity variance with soil depth for the seven vegetation stands

Table 3.1: Comparison of electrical conductivity means for soils from each vegetation type at varying soil depth

Vegetation type	Depth (cm)				SED
	0 -10	10 -20	20-30	30-40	
Psidium guajava	199**a**	218**a**	127**a**	153**a**	**49.2**
Eucalyptus saligna	264**a**	124**a**	123**a**	99**a**	**67.4**
Vernonia sp.	290**a**	166**b**	92**b**	93**b**	**49.8**
Cuppressus lisitanica	376**a**	143**b**	168**b**	210**a**	**74.2**
Hyparrhenia sp. grassland	103**a**	70**a**	50.3**a**	66.7**a**	**16.78**
Brachiaria brizantha sp. grassland	205**a**	148**a**	218**a**	185**a**	**85.2**
Natural Forest	358**a**	228**ab**	157**b**	126**b**	**65.8**

Significant differences at $p<0.05$ were observed in the electrical conductivity of soils from the *Vernonia* sp. vegetation stand ($p=0.013$). At the 0-10 cm depth, electrical conductivity was significantly higher (290 µs) than in subsequent depths (10-20cm, 20-30 cm and 30-40cm). The electrical conductivity of soils from *Cupressus lusitanica* plantation was marginally significant across the four depths ($p=0.052$). At the 0-10 cm depth, electrical conductivity was significantly higher than that in the 10-20 cm, and 20-30 cm depths. However, electrical conductivity of the soils in 0-10cm and 30-40cm depths did not vary significantly.

Electrical conductivity of soils from the natural forest stand varied significantly with depth ($p=0.032$). The top two depths (0-10 cm and 10-20cm) were similar but significantly higher than that of the bottom two depths (20-30 cm, and 30-40 cm).

There were no significant differences in electrical conductivity of the soils from *Hyparrhenia* sp. grassland (p=0.071), *Brachiaria brizantha* grassland (p=0.854), *Psidium guajava* (p=0.307), and *Eucalyptus saligna* plantation (p=0.134). In general, electrical conductivity was found to be highest within the 0-10 cm depth of soils from a majority of the seven vegetation types and decreased with an increase in depth.

b) Electrical conductivity Variance with vegetation type

Electrical conductivity varied significantly at 0-10cm depth across the seven vegetation stands (p=0.046). Soils from the Natural forest, *Eucalyptus saligna*, *Cupressus lusitanica* and *Vernonia* sp.. stands were higher in electrical conductivity and different from those from *Brachiaria brizantha*, *Psidium guajava*, and *Hyparrhenia* sp. vegetation stands. Comparatively, soils from the *Hyparrhenia* sp. grassland had the lowest electrical conductivity of all the seven types.

Table 3.2: Comparison of Electrical Conductivity means for soils from the different vegetation types at each soil depth

	Vegetation Type							
Soil Depth	*Psidium guajava*	*Eucalyptus saligna*	*Vernonia* sp.	*Cuppressus lisitanica*	*Hyparrhenia* sp. grassland	*Brachiaria brizantha* sp. grassland	Natural Forest	SED
0 -10 cm	199**a**	264**ab**	290**ab**	376**b**	103**a**	205**a**	358**ab**	**79.3**
10 - 20 cm	218**a**	124**ab**	166**a**	143**ab**	70**b**	148**ab**	228**a**	**43.8**
20 – 30 cm	127**a**	123**a**	92**a**	168**a**	50**a**	218**a**	157**a**	**66.3**
30 – 40 cm	153**a**	99**a**	93**a**	210**a**	67**a**	185**a**	126**a**	**52.3**

Similar patterns were observed at the 10-20cm depth where electrical conductivity varied significantly (P=0.039). Soils from the Natural forest had the highest electrical conductivity while those from the *Hyparrhenia* sp. grassland had the lowest. There were however no significant differences in electrical conductivity at depths 20-30 cm (p=0.305), and 30-40cm (p=0.135) across the seven vegetation types.

3.2.2 pH variance with depth and vegetation type

a) pH variance with depth

There was no vertical stratification in pH levels from a depth of 0-40cm for soils from six of the seven vegetation stands. Table 3.3 below shows the mean pH values and the p-values for ANOVA across the four depths for each vegetation stand.

Table 3.3: Comparison of pH means of soil from each vegetation type at varying with soil depth

Vegetation Type	Soil Depth (cm)				SED	p-value
	0-10	10-20	20-30	30-40		
Psidium guajava	5.59**a**	5.53**a**	5.72**a**	5.63**a**	0.316	0.939
Eucalyptus saligna	5.39**a**	5.56**a**	5.55**a**	5.43**a**	0.313	0.926
Cupressus lusitanica	6.24**a**	6.26**a**	5.87**a**	5.64**a**	0.309	0.213
Hyparrhenia sp.	4.87**a**	4.89**a**	4.93**a**	4.83**a**	0.103	0.826
Brachiaria brizantha	5.76**a**	5.89**a**	5.76**a**	5.66**a**	0.401	0.949
Natural Forest	6.32**a**	5.82**a**	5.73**a**	5.62**a**	0.349	0.273
Vernonia sp.	5.64**a**	5.64**a**	5.47**a**	5.14**b**	0.099	0.003

However, there was a significant difference in pH at the various depths for soil from *Vernonia* sp. Vegetation (p=0.003). The pH of soil at a depth of 30-40 cm was significantly lower than that of soil at the top three depths.

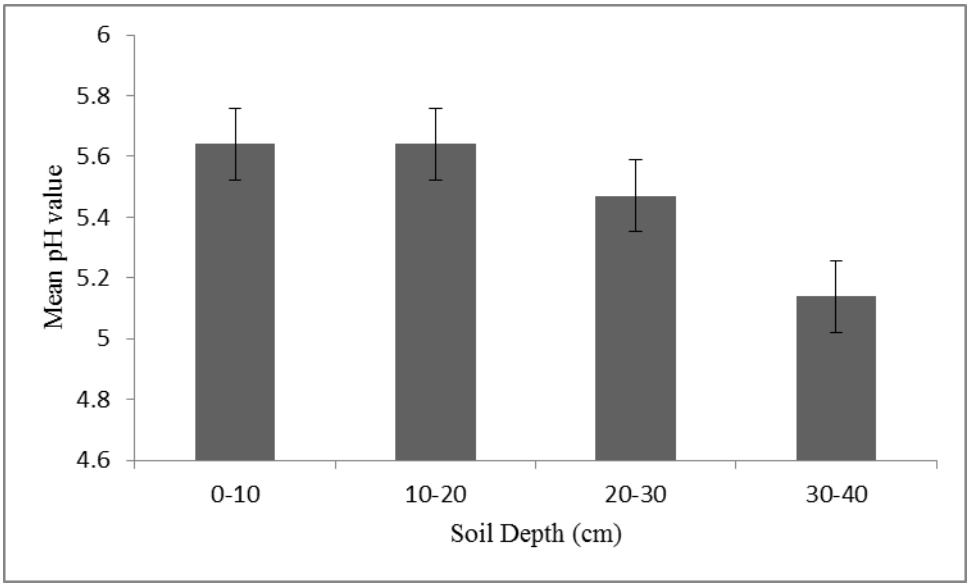


Figure 3.2: pH variance with soil depth in the *Vernonia* sp. vegetation stand

b) pH variance with vegetation type

At 0-10cm depth, the pH of soils varied significantly across the seven vegetation types ($p<0.001$). The pH of soil from the Natural forest stand and *Cupressus lusitanica* plantation was similar but significantly higher than that of soils from all other vegetation types.

Table 3.4: Comparison of pH means for soils from the different vegetation types at each soil depth

	Vegetation Type								
Soil Depth	*Psidium guajava*	*Eucalyptus saligna*	*Vernonia* sp.	*Cuppressus lisitanica*	*Hyparrhenia* sp. grassland	*Brachiaria brizantha* sp. grassland	Natural Forest	*p*	SED
0-10 cm	5.59**a**	5.39**a**	5.64**a**	6.24**b**	4.89**c**	5.76**a**	6.32**b**	<0.001	0.1914
10-20 cm	5.53**a**	5.56**a**	5.64**ab**	6.26**b**	4.89**a**	5.89**ab**	5.82**ab**	0.021	0.3083
20–30 cm	5.72**a**	5.55**a**	5.47**ab**	5.87**a**	4.93**b**	5.76**a**	5.73**a**	0.056	0.2686
30–40 cm	5.63**a**	5.43**ab**	5.14**ab**	5.64**a**	4.83**b**	5.66**a**	5.62**a**	0.251	0.3706

Similar patterns were also observed at the 10-20 cm depth where pH varied significantly (p=0.021). Soils from the *Cupressus lusitanica, Brachiaria brizantha* sp. grassland, and natural forest stands had the highest pH while those from *Hyparrhenia* sp. grassland and *Psidium guajava* shrub-land had the lowest. There were however no significant differences in the pH at depths of 20-30 cm (p=0.056) and 30-40 cm (p=0.251) for soils from the seven vegetation stands. Generally, the *Hyparrhenia* sp. grassland had soils with the lowest pH while the *Cuppressus lisitanica* plantation stand had soils with the highest pH.

3.2.3 Soil Phosphorus

ANOVA results indicate that there was no significant vertical stratification in Olsen P from 0-40 cm depths for soils from the seven vegetation stands as shown in Table 3.5 below.

Table 3.5: Phosphorus variance with soil depth

Vegetation Type	Depth (cm)				SED	p-value
	0-10	10-20	20-30	30-40		
Psidium guajava	2.59	1.68	1.79	1.67	0.574	0.377
Vernonia sp.	1.31	4.48	1.81	2.10	2.229	0.530
Eucalyptus saligna plantation	1.48	1.25	2.42	1.76	0.970	0.668
Cupressus lusitanica plantation	0.23	1.81	0.85	1.04	0.816	0.351
Hyparrhenia sp. grassland	1.74	0.76	1.43	2.46	0.870	0.336
Brachiaria brizantha grassland	0.97	1.63	2.85	1.17	0.885	0.221
Natural Forest	2.19	1.75	2.22	2.39	1.156	0.952

There were also no significant differences in Olsen P at similar depths across the seven vegetation types as shown in Table 3.6 below.

Table 3.6: Phosphorus variance with vegetation type

Soil Depth	*Psidium guajava*	*Eucalyptus saligna*	*Vernonia* sp.	*Cuppressus lisitanica*	*Hyparrhenia* sp. grassland	*Brachiaria brizantha* grassland	Natural Forest	SED	*p*-value
0-10	2.59	1.48	1.31	0.23	1.74	0.97	2.19	1.062	0.421
10-20	1.68	1.25	4.48	1.81	0.76	1.63	1.75	1.742	0.500
20-30	1.79	2.42	1.81	0.85	1.43	2.85	2.22	0.612	0.093
30-40	1.67	1.76	2.10	1.04	2.46	1.17	2.39	1.026	0.729

3.3 AMF spore abundance and diversity

3.3.1 AMF spore abundance by depth

Spore abundance varied significantly according to depth (p =0.03) and decreased with increase in depth in soils from all the vegetation stands as shown in Figure 3.3 below.

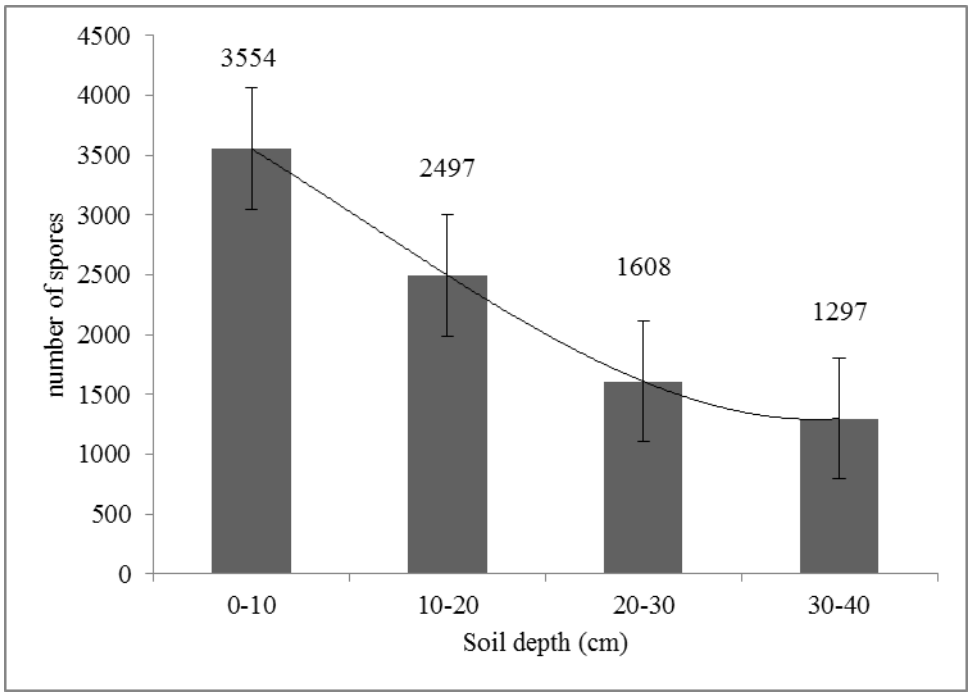


Figure 3.3: Spore abundance with soil depth

3.3.2 AMF spore abundance by vegetation type

Spore counts in soils from the *Eucalyptus saligna* plantation and *Brachiaria brizantha* grassland were up to 1.7 times higher (45-49 spores)per 50g of soil than those from *Vernonia* sp., *Hyparrhenia* sp., *Psidium guajava,* and Natural Forest vegetation stands (24 -29 spores) per 50g of soil. Soils from the *Cupressus lusitanica* vegetation stand had the lowest spore counts at 13 spores per 50g of soil.

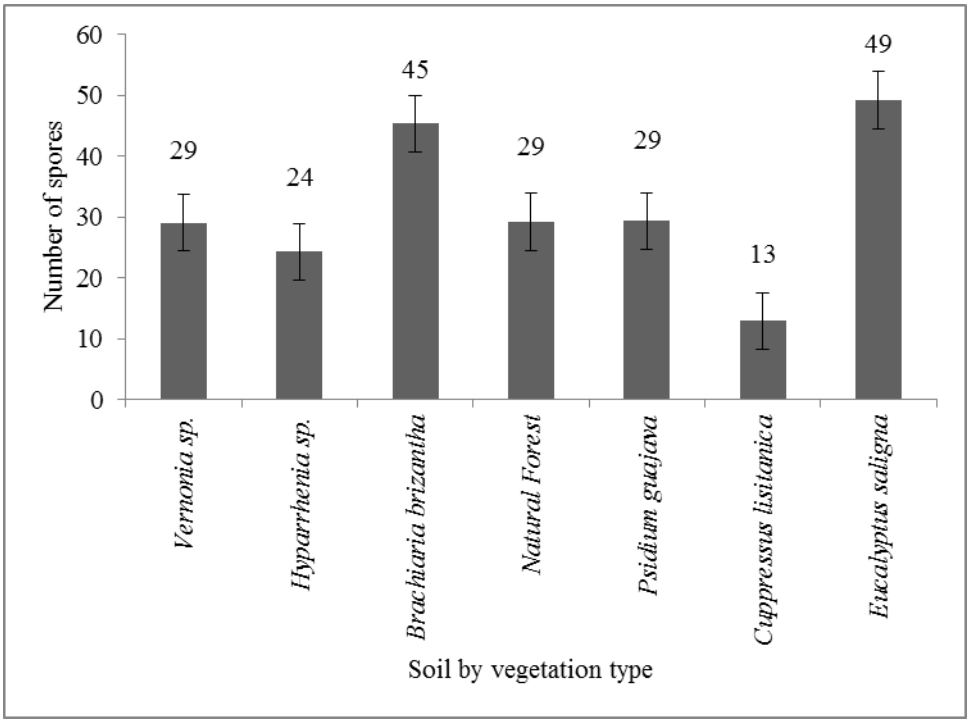


Figure 3.4: Comparison of spore counts per 50g of soil in soils from different vegetation stands

3.3.3 AMF species richness and diversity

The highest number of AMF species was recorded in soils from the Natural forest stand at 20 species, while the lowest numbers were recorded in soils from the *Eucalyptus saligna* and *Cupressus lusitanica* vegetation stands at 14 and 15 species respectively.

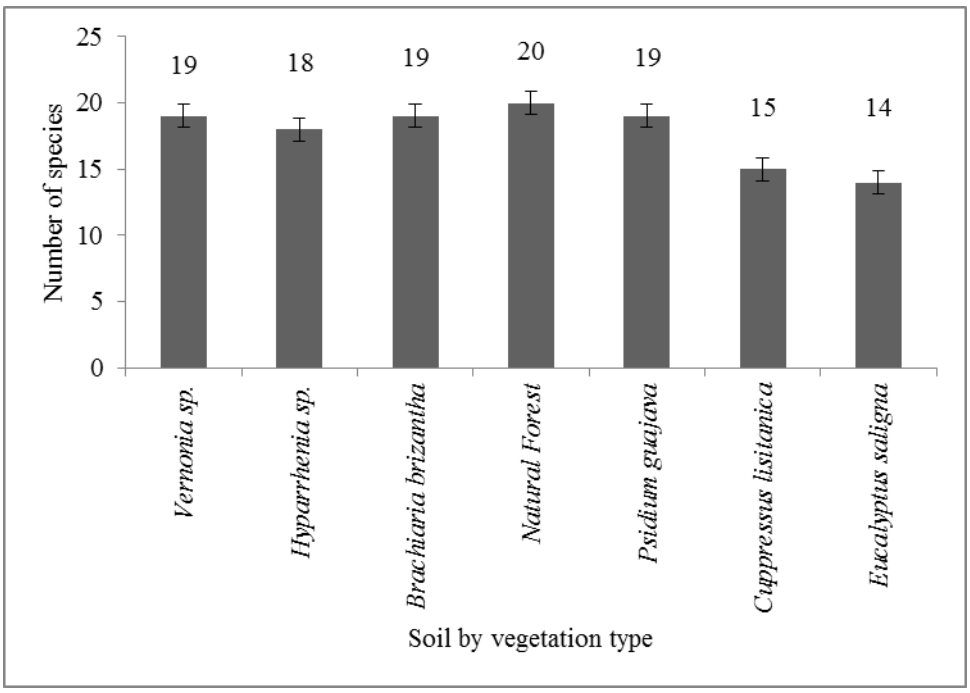


Figure 3.5: Number of AMF species in each vegetation stand

Soils from the *Brachiaria brizantha* grassland had the highest AMF species diversity index while those from the *Eucalyptus* sp. plantation had the lowest AMF species diversity index. Table 3.7 below shows the Shannon-Weiner index of AMF species diversity in the seven vegetation stands.

Table 3.7: Shannon-Weiner's Index of diversity

Vegetation Stand	Diversity Index (H')
Vernonia sp.	2.26
Hyparrhenia sp.	1.82
Brachiaria brizantha	2.49
Natural Forest	2.47
Psidium guajava	2.06
Cupressus lusitanica	2.04
Eucalyptus saligna	1.4

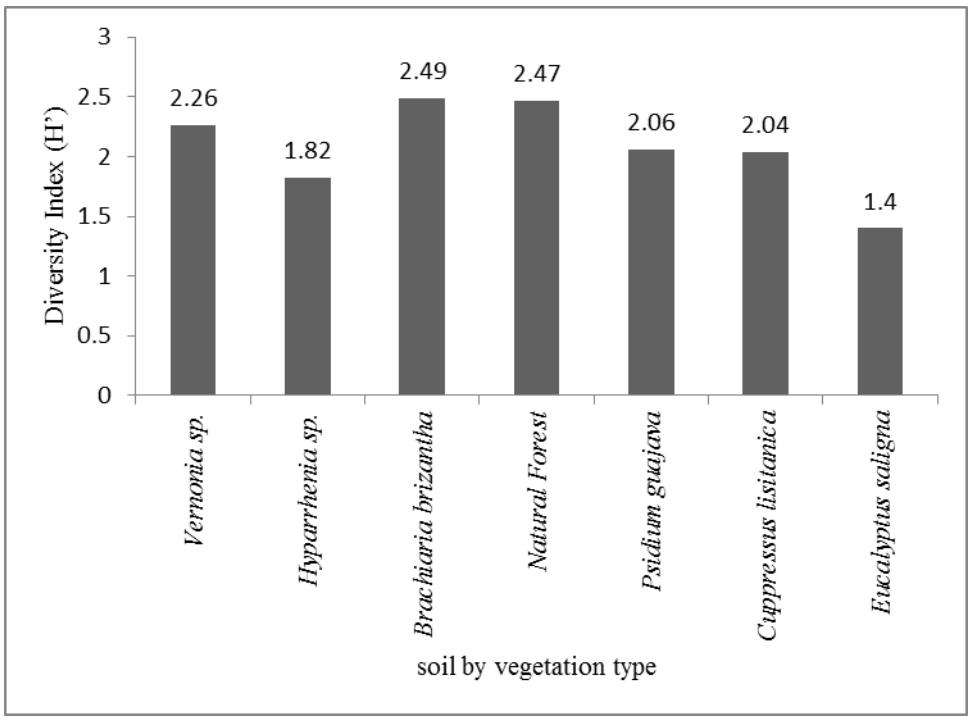


Figure 3.6: Shannon-Weiner's Index of diversity of spores from the soils of different vegetation stands

3.4 Effect of sterilization and inoculation on shoot Dry Weight

3.4.1 Effects on Erythrina abyssinica seedlings

There were no significant differences in the shoot dry weights of *Erythrina abyssinica* seedlings grown on soils from the different vegetation stands except for seedlings grown in soil from the *Hyparrhenia* sp. grassland ($p<0.001$) and *Vernonia* sp. shrub land ($p=0.002$). Seedlings grown in sterile soil (T2) from the *Hyparrhenia* sp. grassland, and non-sterile soil (T1) from the *Vernonia* sp. shrubland had significantly higher shoot weights than those grown in soils treated otherwise. Table 3.8 below shows a summary of the mean dry shoot weights in grams of seedlings on soil under different treatments. Treatment 3 consistently produced seedlings with the lowest shoot weights while Treatment 2 in most cases produced seedlings with the highest shoot weights.

Table 3.8: Shoot dry weights of *Erythrina abyssinica* seedlings under different treatments

Source of soil	Treatment				p-value	SED	LSD
	T1	T2	T3	T4			
Brachiaria brizantha	1.63	2.69	1.14	1.83	0.07	52.3	114
Cupressus lusitanica	1.70	1.75	1.28	3.05	0.256	87.7	191
Eucalyptus saligna	1.71	2.08	0.87	1.94	0.099	46.2	102.9
Natural forest	0.82	1.73	0.86	1.77	0.129	48.5	106.6
Hyparrhenia sp.	1.11	2.93	0.70	0.90	<0.001	13.43	29.27
Psidium guajava	2.27	2.80	1.18	1.62	0.325	89.2	194.3
Vernonia sp. shrub land	2.73	1.52	0.89	1.42	0.002	37.8	85.4

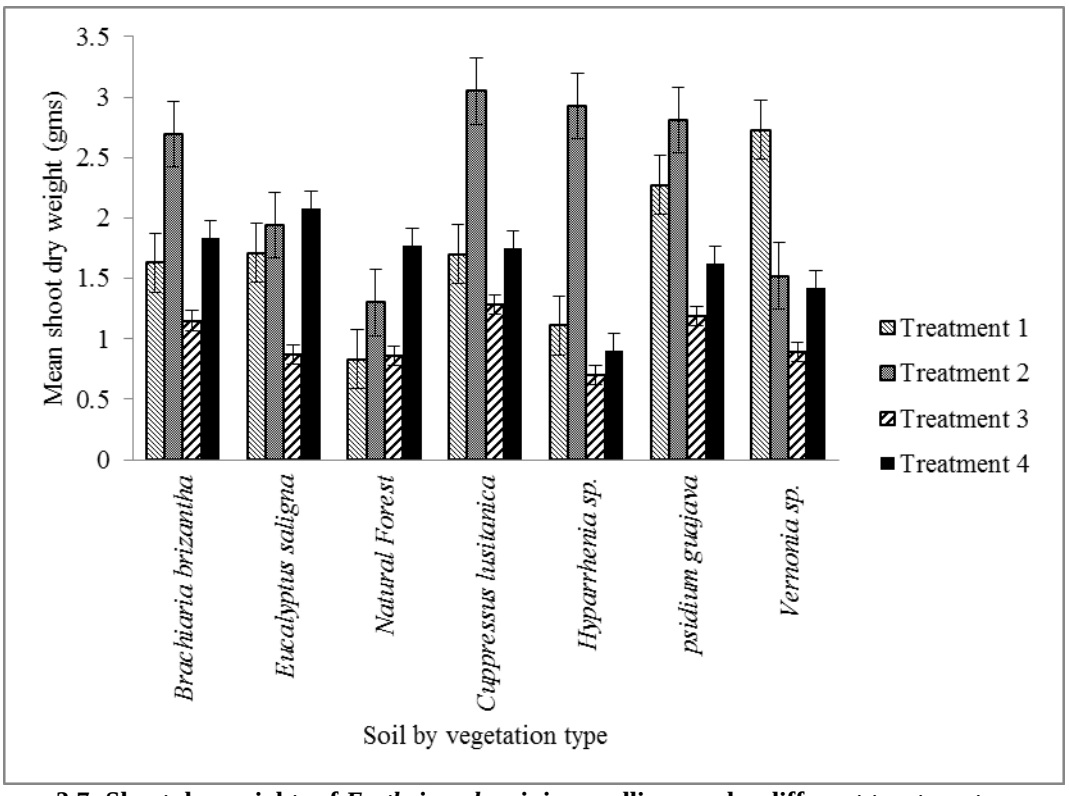


Figure 3.7: Shoot dry weights of *Erythrina abyssinica* seedlings under different treatments

3.4.2 Effect on Markhamia lutea tree seedlings

Significant differences were observed in the shoot dry weights of *Markhamia lutea* seedlings grown in soils subjected to different treatments. These are summarized in Table 3.9 below.

Table 3.9: Shoot dry weights of *Markhamia lutea* seedlings under different treatments

Source of soil	Treatment				p-value	SED	LSD
	T1	T2	T3	T4			
Brachiaria brizantha	1.23	1.68	0.28	0.76	0.018	38.3	83.5
Cupressus lusitanica	1.41	2.09	0.17	1.63	<0.001	24.63	53.66
Eucalyptus saligna	1.53	1.47	0.18	1.58	<0.001	26.2	57.08
Natural forest	2.23	2.10	0.18	0.57	<0.001	18.64	40.61
Hyparrhenia sp.	0.09	0.34	0.19	1.00	0.006	22.37	48.75
Psidium guajava	0.61	1.60	0.10	0.98	<0.001	24.82	54.08
Vernonia sp.	1.30	0.91	0.14	1.54	0.006	32.9	71.6

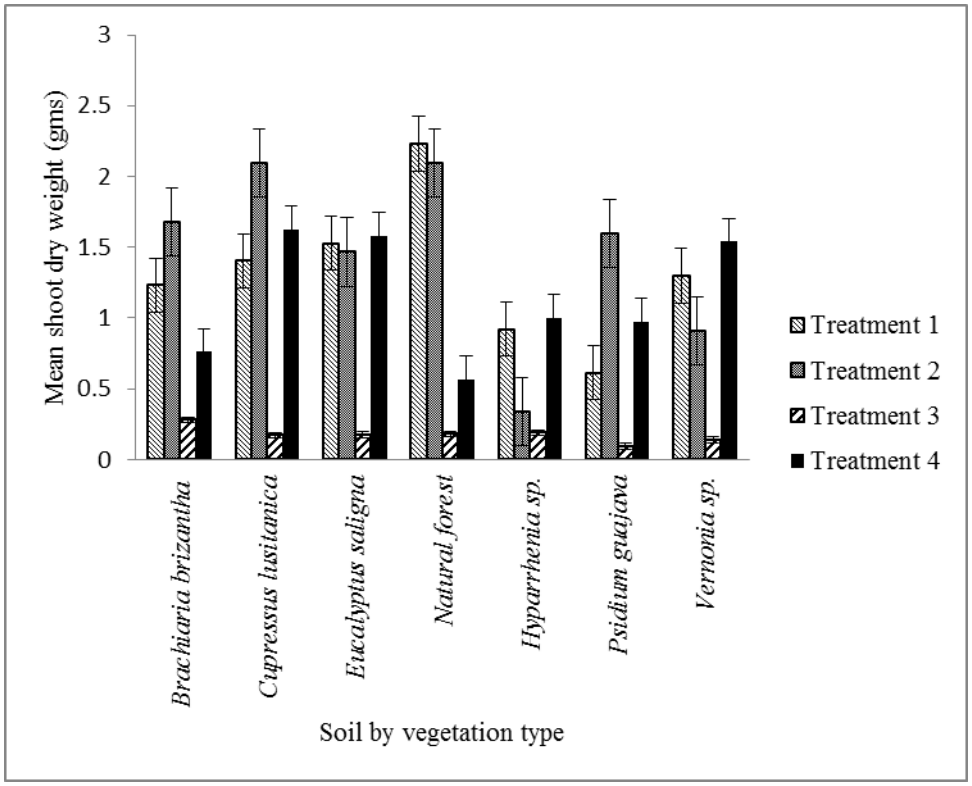


Figure 3.8: Shoot dry weights of *Markhamia lutea* seedlings under different treatments

The seedlings performed better on soils from the Natural Forest stand and *Brachiaria brizantha* grassland which were subjected to T1 (unsterilized field soil) than those grown on soils subjected to T3 (unsterilized soil with inoculum). Sterilization of the soils did not have a significant effect on the shoot weights of

Markhamia lutea seedlings. In addition, a combination of sterilization and inoculation of the soil did not have a significant effect on the shoot weights of the seedlings grown on soils from these two vegetation stands as T3 and T4 did not produce significantly different results.

Seedlings grown in soils from *Cupressus lusitanica* and *Vernonia* sp. vegetation stands and which were subjected to T1 had higher shoot weights than those grown in soils subjected to T3. Sterilization also produced higher shoot weights in the seedlings as T4 was significantly different from T3 for soils from the two vegetation stands. For seedlings grown on soil from the *Eucalyptus saligna* vegetation stand and which were subjected to either T1 or T2, there was no significant difference in shoot weight. However, those subjected to T4 were significantly higher in shoot weight than those subjected to T3.

Seedlings grown on soils from *Hyparrhenia* sp. grassland and which were subjected to either T1 or T2 were not significantly different in shoot weight. Introduction of inoculum also did not produce any significant difference as weights of seedlings subjected to either T1 or T3 did not vary. However, seedlings subjected to T4 were higher in shoot weights than those subjected to T3 indicating that a combination of sterilization and inoculation produced better results than inoculation alone.

Seedlings grown on soil from *Psidium guajava* vegetation stand and subjected to T2 had higher shoot weights than those subjected to T1 indicating that in this case, sterilization of soil produced better results. Inoculation alone did not have any significant effect on shoot weight as seedlings subjected to T1 were not significantly different in weight than those subjected to T3. However, a combination of sterilization

and inoculation had a significant effect on shoot weight as seedlings subjected to T4 were heavier than those subjected to T3.

3.4.3 Effects on Senna spectabilis

Significant differences were observed in the shoot weights of *Senna spectabilis* seedlings grown on soils from six vegetation types and subjected to different treatments as shown in Table 3.10 below. However, seedlings grown on soils from *Psidium guajava* shrub land and subjected to different treatments did not vary in weight.

Table 3.10: Shoot dry weights of *Senna spectabilis* seedlings under different treatments

Source of soil	Treatment				p-value	SED	LSD
	T1	T2	T3	T4			
Brachiaria brizantha	1.23	1.07	0.12	0.79	0.001	21.27	46.82
Cupressus lusitanica	1.41	1.81	0.17	0.78	<0.001	27.6	60.13
Eucalyptus saligna	1.15	1.93	0.12	1.26	<0.001	27.33	59.55
Natural forest	1.83	1.40	0.26	1.75	<0.001	23.28	50.72
Hyparrhenia sp.	0.18	0.70	0.07	0.63	<0.001	13.78	30.02
Psidium guajava	1.22	0.89	0.80	1.42	0.532	46.4	101.2
Vernonia sp.	1.14	1.17	0.15	1.13	<0.001	16.62	36.58

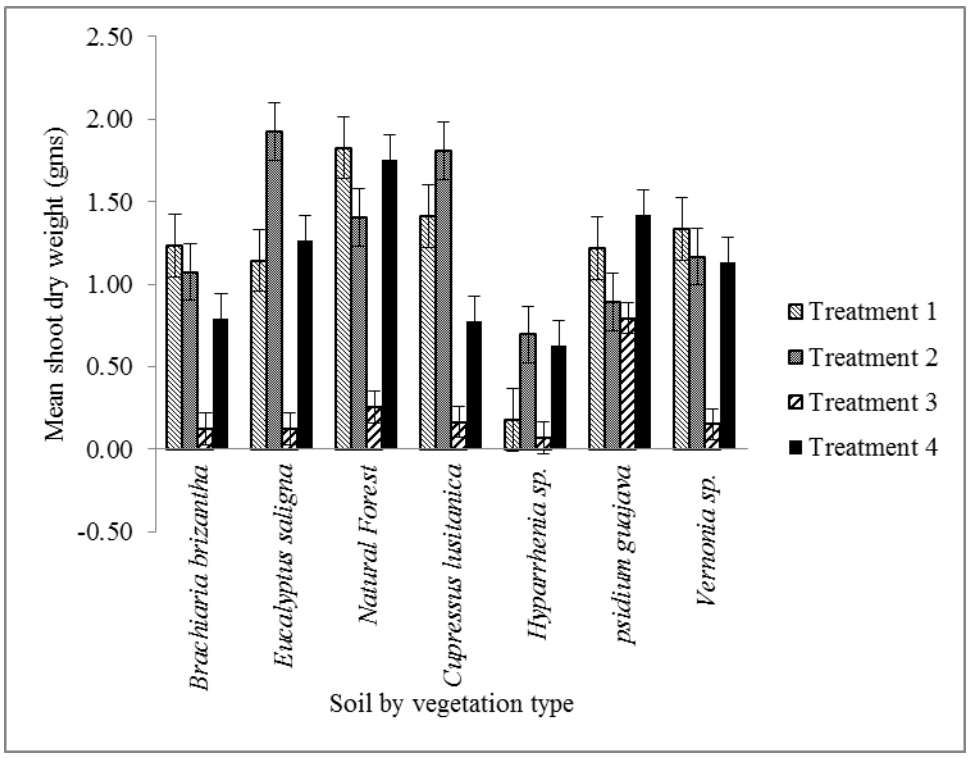


Figure 3.9: Shoot dry weights of *Senna spectabilis* seedlings under different treatments

Senna spectabilis seedlings grown on soils from *Brachiaria brizantha*, *Cupressus lusitanica*, Natural Forest, and *Vernonia* sp. vegetation stands, and subjected to either T1 or T2 were not significantly different in weight, i.e. sterilization of soil had no significant effect on shoot weight. For seedlings grown on soils from *Eucalyptus saligna* and *Hyparrhenia* sp. grassland however, soil sterilization resulted in higher shoot weights.

Inoculation adversely affected the shoot weights of *Senna spectabilis* seedlings grown in non-sterile soil from all the vegetation types except the *Hyparrhenia* sp. grassland where T1 and T3 did not result in significantly different shoot weights. A combination of sterilization and inoculation resulted in higher shoot weights than inoculation alone.

3.5 Root colonization

3.5.1 Percentage and intensity of root colonization in field samples

Analysis of colonization in root fragments obtained from field soil during sampling indicate that percentage colonization was generally low with majority falling below 50%. The intensity of colonization was also low for the root fragments. No significant differences were observed in percentage colonization of root fragments in soils from the seven vegetation types across the four depths of 0-10, 10-20, 20-30 and 30-40 cm as shown in Table 3.11 and Figure 3.10 below.

Table 3.11: Percentage colonization of root fragments in field soils

	Percentage colonization				
	0-10	**10-20**	**20-30**	**30-40**	**P-Value**
Natural forest	39.71	26.41	39.88	24.76	0.70
Vernonia sp.	47.52	64.01	51.75	46.09	0.82
Psidium guajava	55.79	18.00	35.33	38.92	0.48
Cupressus lusitanica	55.83	76.24	31.93	72.50	0.29
Eucalyptus saligna	44.17	30.11	31.32	49.40	0.61
Brachiaria brizantha	63.20	34.17	49.17	39.13	0.56
Hyparrhenia sp.	58.19	36.34	37.64	44.04	0.88

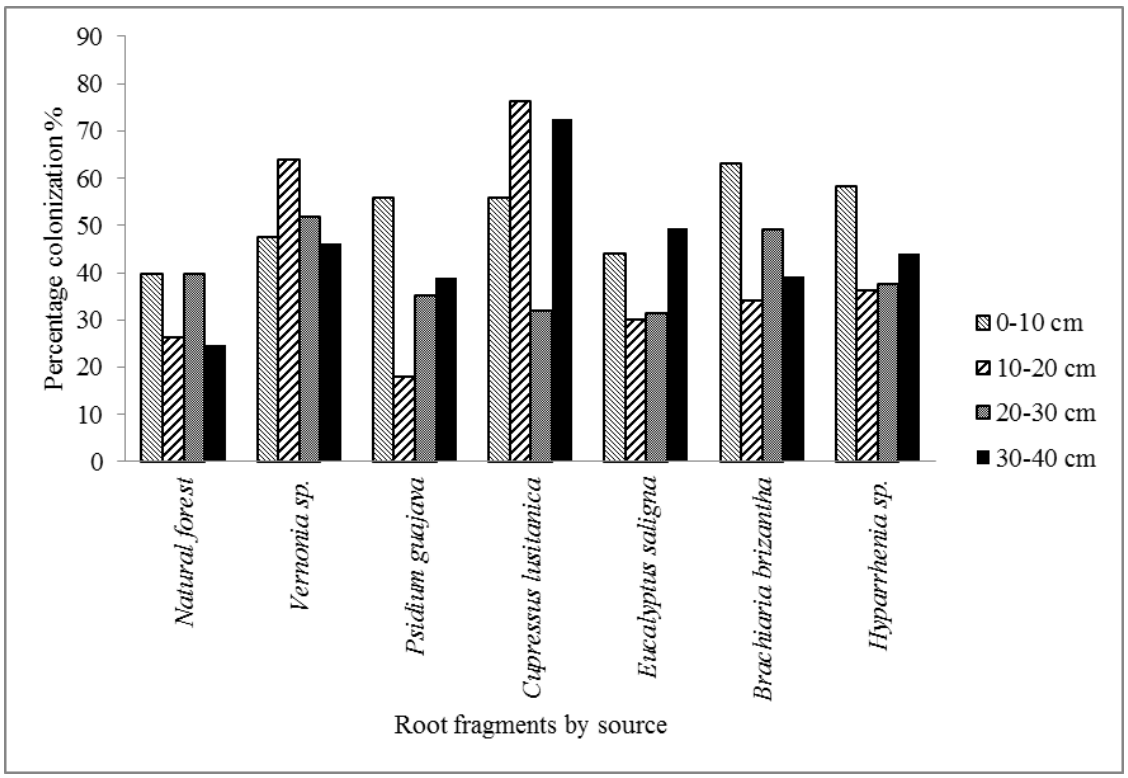


Figure 3.10: Percentage colonization of root fragments in field soils

Table 3.12: Intensity of colonization or root fragments in field soils

	Intensity				
	0-10	**10-20**	**20-30**	**30-40**	**P-Value**
Natural forest	27.51	20.30	24.85	17.87	0.80
Vernonia sp.	36.79	37.98	23.28	24.70	0.56
Psidium guajava	35.87	6.62	19.06	11.99	0.16
Cupressus lusitanica	22.70	52.07	17.06	30.91	0.23
Eucalyptus saligna	28.76	20.31	15.39	15.95	0.53
Brachiaria brizantha	30.19	20.51	23.21	14.47	0.56
Hyparrhenia sp.	27.37	20.36	20.30	22.76	0.88

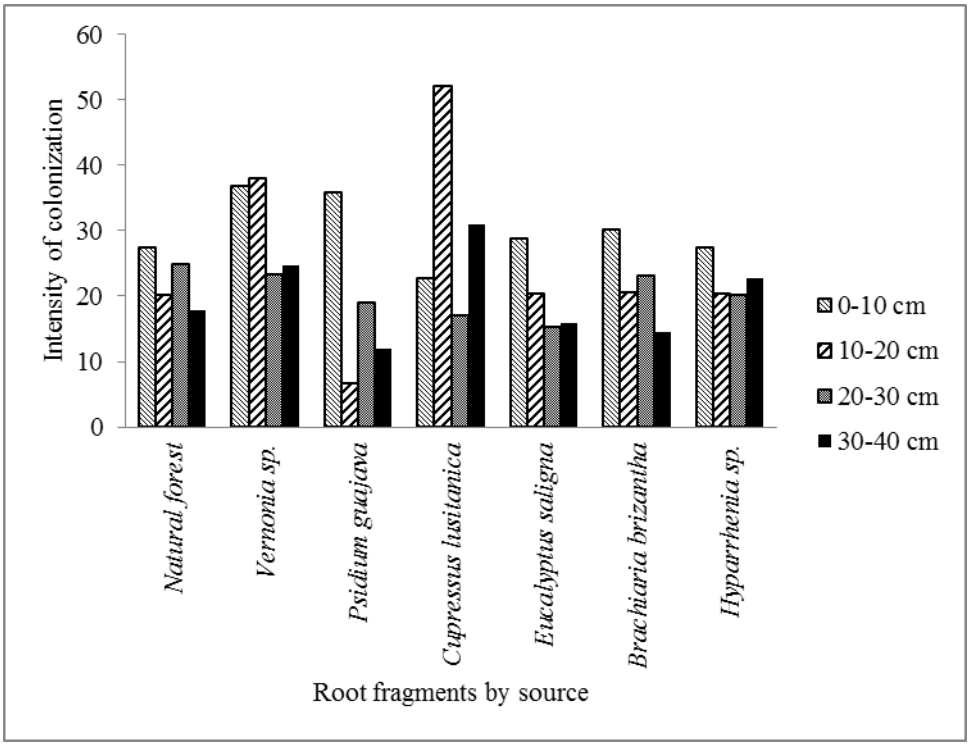


Figure 3.11: Intensity of colonization of root fragments in field soils

3.5.2 Percentage and intensity of colonization in roots of Erythrina abyssinica, Senna spectabilis, and Markhamia lutea seedlings

ANOVA results indicate that there was no significant difference at $p<0.05$ in percentage colonization among the four treatments T1, T2, T3 and T4. Percentage colonization significantly differed only in roots of *Erythrina abyssinica* seedlings grown in soils from the natural forest stand where colonization of roots under T2 was much higher (81.5%) in comparison with other treatments (T1 = 25%, T3 = 18.3%, and T4 = 28.9%)

Table 3.13: p-values of ANOVA in percentage colonization of roots under T1, T2, T3 and T4

	Natural Forest	*Hyparrhenia* sp.	*Vernonia* sp.	*Cupressus lusitanica*	*Brachiaria brizantha*	*Eucalyptus saligna*	*Psidium Guajava*

Erythrina abyssinica	0.009*	0.235	0.677	0.723	0.087	0.099	0.192
Markhamia lutea	0.072	0.437	0.889	0.476	0.057	0.237	0.967
Senna spectabilis	0.089	0.06	0.195	0.785	0.127	0.092	0.898

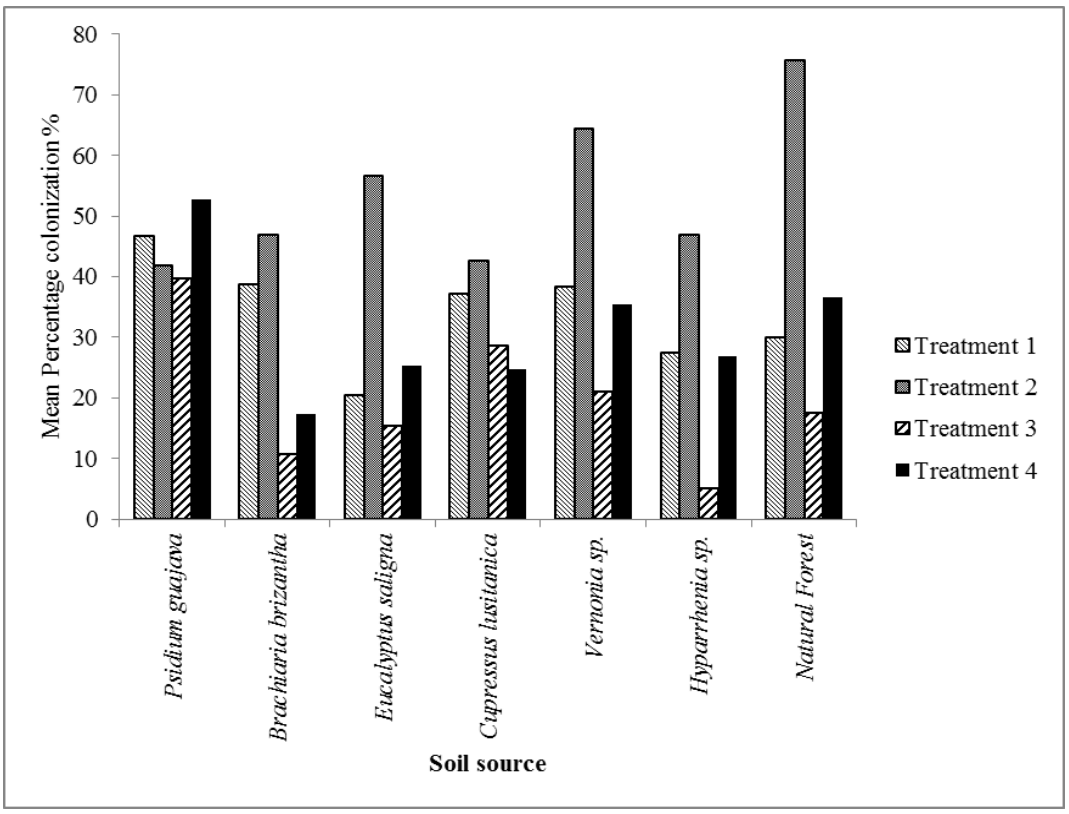


Figure 3.12: Percentage colonization in roots of *Senna spectabilis*

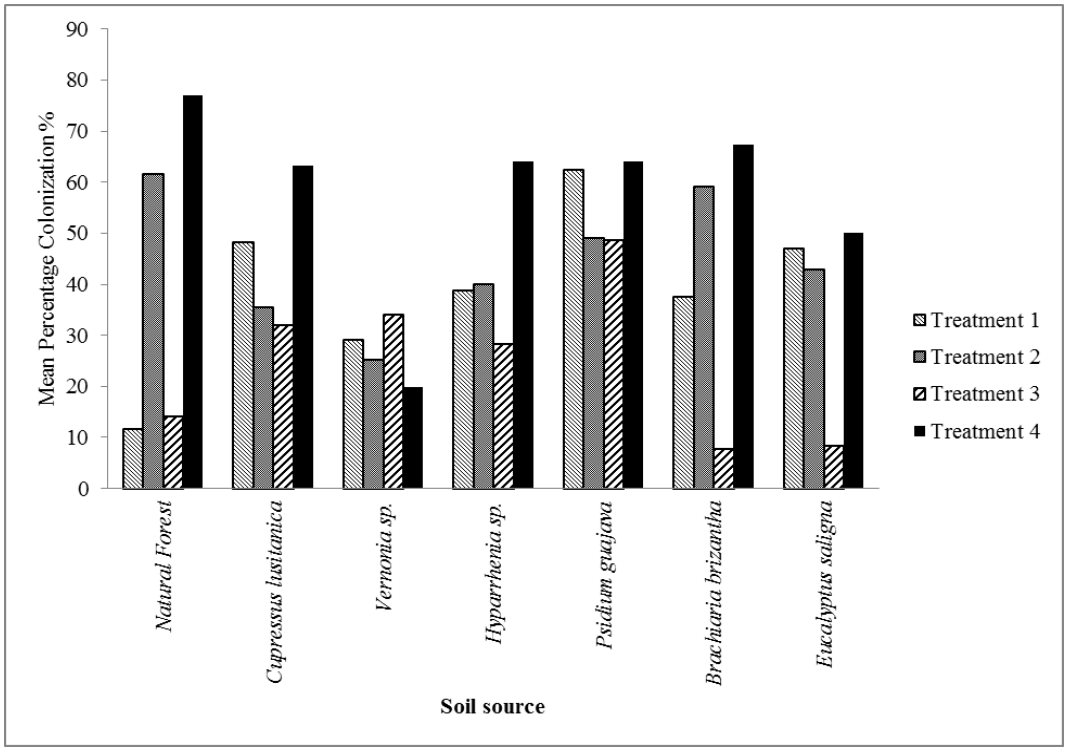


Figure 3.13: Mean percentage colonization of roots of *Markhamia lutea* seedlings

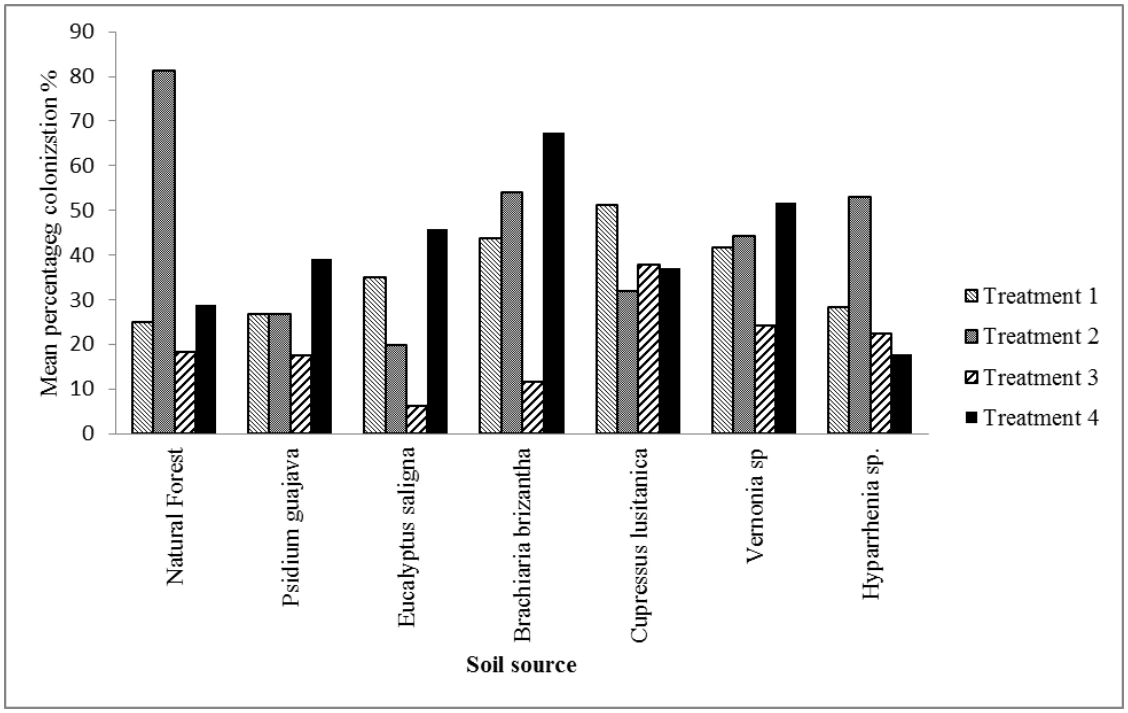


Figure 3.14: Mean percentage colonization of the roots of *Erythrina abyssinica* seedlings

Intensity of colonization only differed in the roots of *Erythrina abyssinica* seedlings grown in soils from the Natural forest, *Hyparrhenia* sp., and *Brachiaria brizantha* grasslands, *Vernonia* sp. shrubland, and *Eucalyptus* sp. plantation as shown in Table 3.14 below. For soils from these vegetation stands, intensity of colonization was higher under T2 than in other treatments except in soils from the Eucalyptus sp. plantation where intensity of colonization was significantly higher under T1 and T4 than under T2 and T3.

Table 3.14: p-values of ANOVA in intensity of colonization of roots under T1, T2, T3, and T4

	Natural Forest	*Hyparrhenia* sp.	*Vernonia* sp.	*Cupressus lusitanica*	*Brachiaria brizantha*	*Eucalyptus saligna*	*Psidium Guajava*
Erythrina abyssinica	0.039*	0.003*	0.039*	0.07	0.044*	<0.001*	0.076
Markhamia lutea	0.087	0.842	0.62	0.249	0.073	0.135	0.567
Senna spectabilis	0.078	0.085	0.373	0.311	0.079	0.576	0.981

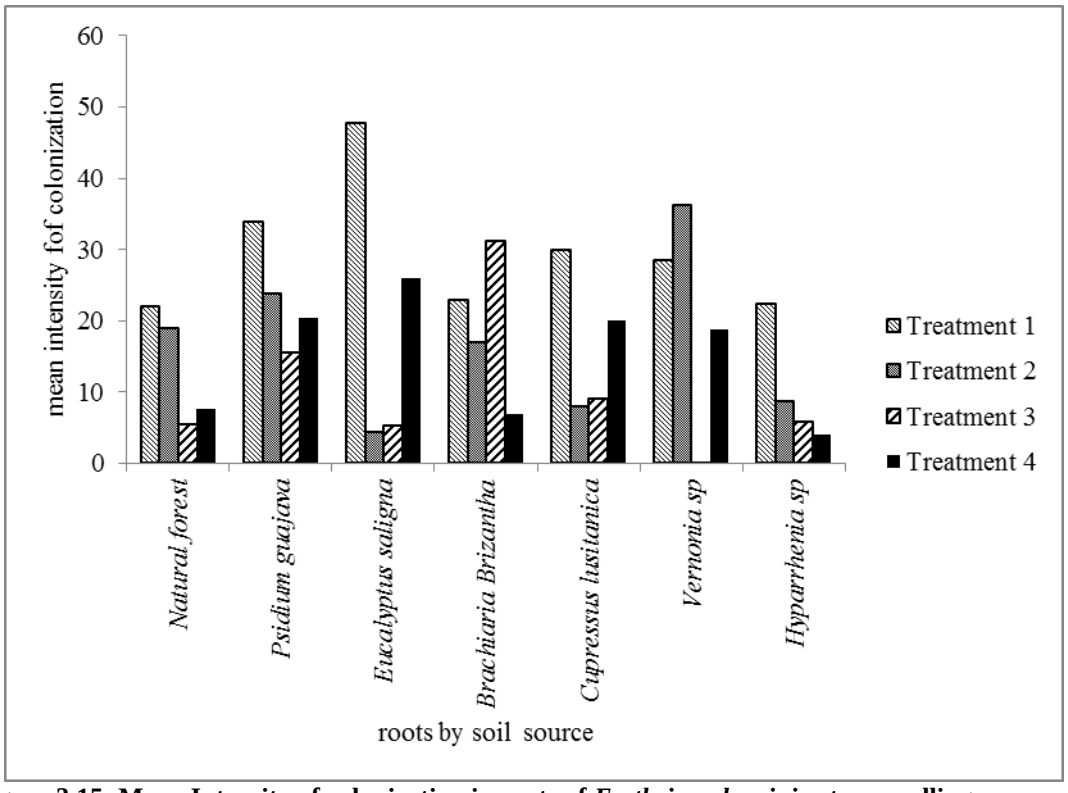


Figure 3.15: Mean Intensity of colonization in roots of *Erythrina abyssinica* tree seedlings

a) Relative mycorrhizal dependency

It was observed that most tree seedlings were mainly negatively mycorrhizal. However, there were few instances where these were positively mycorrhizal. When grown in soils from the *Vernonia* sp. vegetation stand, all three tree species responded

positively to inoculation as opposed to other soil types where inoculation adversely affected plant growth.

Table 3.15: Relative mycorrhizal dependency of the tree species

Vegetation type	*Markhamia lutea*	*Erythrina abyssinica*	*Senna spectabilis*
Brachiaria sp.	-402	-91	-149
Hyparrhenia sp.	58	-57	-49
Eucalyptus saligna	-41	-4	-83
Vernonia sp.	36	24	5
Cupressus lusitanica	-27	-131	-55
Psidium guajava	-146	-77	32
Natural forest	-490	21	27

CHAPTER FOUR: DISCUSSION

4.1 Vegetation mapping

The application of visible Near Infrared (NIR) reflectance spectroscopy produced a vegetation map with a high level of accuracy due to the relative homogeneity in species composition of the different vegetation stands and the relatively large sizes of the stands to enable representation by pure pixels (Lung and Scaab, 2004). Discrimination was possible due to the unique spectral features that are related to the biochemical composition (chlorophyll), leaf –water absorption features, and structure of the leaves, which depend on factors such as the plant species, the development or microclimate position of the leaf on the plant (Jacquemoud and Ustin, 2001, Kokaly *et al.*, 2007). The different spectral features produced varying image colors, texture and tone which were interpreted as different vegetation groups. This coupled with ground truthing confirmed the expanse and composition of the vegetation stands. The mapping however failed to identify a seasonal river that flows under the densely vegetated strip of natural forest between the two types of grasslands to join Isiukhu River.

It was established that the *Eucalyptus saligna*, *Cupressus lusitanica,* and *Pinus patula* plantations delineated were the product of reforestation initiatives by the Kenya Forest Service and other community based organizations in the area to re-vegetate disturbed sites. Althof (2005) confirms that enumeration of the forest stand started simultaneously as reforestation and silvicultural improvements which included eviction of the local people out of the forest took place under the watch of the Forest Department. In addition, the *Brachiaria brizantha* grass had grown to cover previously cultivated sites, while the *Psidium guajava* and *Vernonia* sp. covered areas proximal to water courses.

Psidium guajava invades disturbed, and to a lesser degree undisturbed, sites and forms dense thickets (ISSG, 2010). The shrub is also considered an invasive due to its easy spread of seeds and regrowth (Berens *et al*., 2008) as well as an important pioneer species in the process of forest recovery on abandoned pasture (Aide *et al*., 2000). The shrub propagates easily in areas with moderate to heavy rainfall and although it tolerates many soil conditions, it produces better in rich soils high in organic matter. Loam and alluvial types of soils which are well-drained and have a pH range of 5 to 7 are most ideal. The pH of the soils at the disturbed sites lay between this range. At the study site, the seeds of guava fruits are consumed by the numerous birds and monkeys found around the forest edges, which disperse guava seeds in their droppings and cause spontaneous clumps of guava trees growing near the water courses and forest edges. Various studies (Anitha and Gandhi, 2012; Chapla and Campos, 2010; Gutierrez *et al.*, 2008; Monteiro and Vieira, 2002; Brown *et al*., 1983; and Bovey and Diaz-Colon, 1968) have identified chemical products belonging to the groups with allelopathic properties in *Psidium guajava* leaves, a factor that could explain dominance of the shrub and low plant diversity wherever it is found on the disturbed site.

Vernonia sp. shrubs also invade disturbed areas but their propagation may be slower in comparison to *Psidium guajava* due to the sensitive nature of the *Vernonia* sp. seeds and the environmental factors necessary to break dormancy. (Nyamongo *et al*, 2009; Binggeli, 1997; and Wienke *et al.*, 1995). The location of the *Vernonia* sp. shrub land further away from the main road could indicate a chronology in disturbance whereby the *Psidium guajava* shrub land may have been more recently disturbed than the former.

Acanthus pubescens is an aggressive pioneering sub-woody shrub species that populates recent clearings in primary and secondary forests, and forest margins (Vollesen

2007; Paul *et al*., 2004). The shrub is widely distributed in Eastern Africa in such areas as margins of forest/grassland mosaics and tall fire – swept grasslands. The shrub often forms a belt between tall *Hyparrhenia* sp. grassland and forest or woodland and acts as a firebreak preventing the often fierce grass-fires from entering the forest. The dominance of this shrub along the study site's forest edges is an indicator of the previous natural forest extent before disturbance. Paul *et al* (2004) established that regeneration in *Acanthus pubescens* dominated sites is suppressed as compared to regeneration in an adjacent forest largely due to the periodic collapse of *Acanthus pubescens*. Collapses are enhanced by the arching, vine-like growth form of the shrub, which results in large networks of stems collapsing, often during the rainy season, and sometimes over an area of several square meters, smothering seedlings. It is probable that the natural forest extended up to these areas leaving a natural expanse of *Hyparrhenia* sp. grassland inside the forest. This would thus define the limits of future restoration exercises. In any case, the soil conditions of the *Hyparrhenia* sp. grassland were found to be the poorest and would be most limiting to seedling establishment.

4.2 Soil Properties

Top soils in the *Vernonia* sp., *Cupressus lusitanica,* and Natural Forest vegetation stands were mainly clay loams as compared to those in *Hyparrhenia* sp. and *Brachiaria brizantha* grasslands, *Psidium guajava* shrub land, and *Eucalyptus* sp. plantation, which were mainly clays. The former soils being clay loams tend to be more fertile, contain more organic matter, have higher cation exchange and buffer capacities, are better able to retain moisture and nutrients, and permit less rapid movement of air. The latter soils however, being clays would most likely exhibit properties which are somewhat difficult to manage or overcome.

A directional increase in clay content was observed along a disturbance gradient from the natural forest to the more disturbed sites moving from clay loams to clays. Similar results were obtained by Bahrami *et al* (2010) and perhaps this increase was as a result of intensified chemical soil degradation due to cultivation practices earlier on involving the input of nitrates as fertilizer. The electrical conductivity of these soils also varied and was most likely influenced by the amount of moisture held, particle size and texture, and Cation Exchange Capacity (Grisso *et al*, 2009). A higher pH in the less disturbed soils could also be attributed to higher soil organic matter content than in the more disturbed soils. The low pH values of the soils in the more disturbed sites could possibly be due to the intensive application of nitrogen fertilizers during cultivation. This could also be attributed to the addition of litter and plant residuals to the soils in the more disturbed sites. In addition, the oxidation of nitrogen and sulphur could have resulted in an intensified decomposition of soil organic matter in the disturbed sites leading to a reduction in the soil pH (Bahrami *et al* 2010).

In spite of the apparent homogeneity of soil origin, variations in the physical, chemical and biological properties are largely influenced by the nature of the vegetation cover (Phil-Eze, 2010). The soil in turn affects various vegetation characteristics, including productivity, structure, and floristic composition (Eni *et al*, 2011).The soil properties most affected by variations in vegetation cover are sand content of the soil, soil moisture content, soil microorganisms, soil organic matter content and soil cation exchange capacity (CEC). It is probable that over time, and as the natural vegetation cover was lost through forest clearance to pave way for cultivation, there was a decline in nutrient cycling through weak CEC. This followed a reduction in soil microbial activities that generated humus, a binding/aggregating agent in the soil.

4.3 Spore abundance and diversity

Spore densities were higher in soils from the *Eucalyptus* sp. plantation, *Brachiaria brizantha* grassland, and *Psidium guajava* shrubland than in soils from *Cupressus lusitanica* plantation, *Vernonia* sp. shrubland and natural forest vegetation stands. It would seem that sporulation of fungi was higher in the former soils than in the latter. However, since percentage colonization of root fragments collected from all the vegetation stands did not differ significantly, it is possible that lower rates of spore decomposition could be the cause of higher spore densities in soils from *Eucalyptus* sp. plantation, *Brachiaria brizantha* grassland, and *Psidium guajava* shrubland.

Soil fertility showed no significant effect on the composition of AM fungal communities, and soil textural differences rather than fertility may be attributed to differences in community structure of the sporulating AM fungi in disturbed sites. Of more importance in species diversity however, is the level of disturbance of a site. Various studies (Shi *et al*. 2007; Oehl *et al.,* 2003; Picone 2000) have shown that AMF species composition is higher in un-disturbed to less disturbed sites than in the more disturbed sites. This could possibly explain why more AMF species were found in the Natural forest stand than in other areas.

4.4 Soil sterilization, inoculation and mycorrhizal dependency of experimental plants

In most instances, inoculation adversely affected the growth of the experimental plants. On the other hand, sterilization had a positive effect on the growth of the plants. It is possible that soil pathogens in the inoculum caused seedling mortality or stunted growth. Autoclaving of the soil killed most soil biota-mainly pathogens alongside the mycorrhizal fungi resulting in a nutrient flush which probably caused the increased survival of the

seedlings in sterilized soil. Troelstra *et al.* (2001), and He and Cui (2009) agree that besides the occurrence of a direct nutrient flush, the temporary elimination of the microbial biomass, and thus the temporary absence of any immobilization, may also cause a temporary increase in the availability of nutrients. Various studies (Al-Khaliel, 2010; Troelstra *et al*. 2001; Eissa, 1971) have shown that soil steaming kills pathogens by heating the soil to levels that cause protein coagulation or enzyme inactivation leading to a better starting position, quicker growth and strengthened resistance against plant disease and pests. Soil steaming can also cure soil fatigue through the release of nutritive substances blocked within the soil.

Screening of roots extracted from T2 pot plants revealed a level of colonization similar to that of T1, T3 and T4 indicating that contamination of the treatments may have occurred in the greenhouse resulting in the re-introduction of fungi and other soil biota into the otherwise sterile soil thus compromising the experiment. Contamination could have occurred whereby the soil-borne pathogens in infected soil inside or surrounding the greenhouse were spread by wind or irrigation/wash water. Some soil-borne pathogens, such as *Sclerotinia sclerotiorum* or *Rhizoctonia solani* are known to produce aerial sexual spores that are ejected into the air and spread by wind (Raaijmakers *et al*, 2008). The oomycetes, which produce motile swimming zoospores, are especially adapted for movement in water. Other possible explanations for contamination could be inadequate spacing between the experimental plants, or inadequate sterilization of the soil used in the experiment.

From the results obtained, it is evident that the experimental plants did not benefit from AMF colonization. It would appear that the plants are neither obligatorily mycorrhizal (due to the lack of benefit from colonization) nor non-mycorrhizal since the

roots were colonized to a considerable extent. Non-mycorrhizal plants have roots that are highly resistant to colonisation by mycorrhizal fungi and do not form functional associations (Brundrett, 2002). Facultative mycorrhizas are balanced associations, where plant benefits are conditional on soil fertility. The facultatively mycorrhizal plants benefit from VAM only when soil phosphorus levels are relatively low and these plants typically have relatively long, narrow and highly branched roots with long root hairs in comparison with obligately mycorrhizal species. The practical designation of plants as facultatively mycorrhizal is often not based on physiological data (Brundrett, 2003). Field surveys have shown that plant species generally have (i) consistently high levels of mycorrhizas, (ii) inconsistent, low levels of mycorrhizas or (iii) are not mycorrhizal; those in the second category have traditionally been designated as facultatively mycorrhizal. Where such plants are grown in relatively fertile soils, the benefit provided by mycorrhizas can actually decrease as the degree of mycorrhizal colonisation of roots increases (Clapperton and Read, 1992; Gange, 1999).

4.5 Conclusions

In most cases, successful recruitment of seedlings into permanent vegetation such as forests or grasslands is limited by low levels of light or nutrients. The integration of seedlings into mycorrhizal mycelial systems may be a pre-requisite for successful establishment under such circumstances (Read & Birch 1988). Vegetation mapping identified distinct vegetation types along a disturbance gradient on the study site. Subsequent investigations were carried out with the premise that soils from the different vegetation types of the disturbed site had varying physical and chemical properties such as texture, pH, and phosphorus which could determine seedling recruitment success.

Further, an experiment was set up on the premise that seedling recruitment would be more successful when mixed inoculum from the field soil was added.

The studies established that the soils of the site varied from clays to clay loams which could be a factor in determining the type of vegetation growing on the disturbed grounds in the early stages of succession. Under normal circumstances, these soils possess different properties such as organic matter, cation exchange capacities, moisture and nutrient levels. The studies also established that while physical properties differed between these soils, phosphorus levels were similar. All these factors could alter AMF community structure but not colonization. This is supported by the fact that the level of colonization in root fragments from all soil types was similar. It can therefore be concluded that the soil physical properties of the disturbed site are of more significance in seedling establishment than their AMF composition. The inoculum potential of the soils from the study site was also low as evidenced by low spore densities and percentage colonization of root propagules in these soils. This was probably due to the high disturbance levels and high moisture content of the soil at the site.

From the experiment, it can be concluded that *Erythrina*, *Markhamia lutea* and *Senna spectabilis* are facultatively mycorrhizal, only benefiting from association in extremely harsh soil conditions, of which the disturbed site had not yet attained. Their seedlings could therefore be established in restoration efforts without inoculation. Contamination within the experimental plants however obscured the effect of AMF inoculum addition on seedling growth.

4.6 Recommendations

The following recommendations are made in the restoration of the disturbed site:

- Presently, the soil conditions of the disturbed site are still favourable for the establishment of the seedlings without inoculation. Planting of *Erythrina abyssinica*, *Markhamia lutea* and *Senna spectabilis* seedlings will not require inoculation. However, while *Erythrina abyssinica* and *Markhamia lutea* are native to East Africa, *Senna spectabilis* is native to Central and South America. It is thus recommended that restoration of Kakamega Forest only focuses on indigenous tree species;
- Restoration efforts should exclude planting of seedlings in the *Hyparrhenia* sp. grassland as the vegetation stand appears to be in its original form;
- Clearing of the *Acanthus pubescens* vegetation along the forest edges should be done and sustained to enable tree seedling recruitment along these edges;
- In the event that other tree species are proposed for use in the restoration efforts, investigations about their mycorrhizal association should be done using the most common AMF species isolates identified in the spore abundance and diversity assessments as inoculum;
- Determination of the mycorrhizal dependency of other tree species through a greenhouse experiment should be done in a highly controlled environment taking caution against potential contamination from exposure to wind, water or inadequate sterilization of soil.

CHAPTER FIVE: REFERENCES

Aide T. M., Zimmerman J. K., Pascarella J. B., Rivera L., and Marcano-Vega H. 2000. Forest regeneration in a chronosequence of tropical abandoned pastures: implications for restoration ecology. Restoration Ecology 8:328–338.

Allen M.F. 1991. The Ecology of Mycorrhizae. Cambridge University Press, New York.

Allen E. B, Espejel I., and Siguenza C. 1997. Role of Mycorrhizae in Restoration of Marginal and Derelict Land and Ecosystem Stability. *In:* Palm M. and Chapela I. A. (Eds.) Mycology in Sustainable Development: Expanding Concepts, Vanishing Borders. Parkway Publishers, North Carolina.

Allen E. B., Rincon E., Allen M. F., Jimenez A. P., and Huante P. 1998. Disturbance and Seasonal Dynamics of Mycorrhizae in a Tropical Deciduous Forest in Mexico. *Biotropica*,. 30 (2): 261-274.

Al-Khaliel A. S. 2010. Effects of Arbuscular Mycorrhization in Sterile and Non-sterile Soils. Tropical Life Sciences Research, 21(1), 55–70.

Althof A. 2005. Human Impact on Flora and vegetation of Kakamega Forest, Kenya: Structure, Distribution and Disturbance of Plant communities in an EastAfricanRain Forest. Ph.D dissertation, University of Koblenz-Landau, Germany.

Althof A., Killmann D., Solga A., and Fischer E. 2004. Influence of Natural and Anthropogenic Fragmentation on the Vegetation in Montane Rainforests of Kakamega and Mt. Kenya.

An Z.Q., Hendrix J. W., Hershman D. E., andHenson G. T. 1990. Evaluation of the "Most Probable Number" (MPN) and Wet-Sieving Methods for Determining Soil-

Borne Populations of Endogonaceous Mycorrhizal Fungi: *Mycologia*, Vol. 82, No. 5 pp. 576-581.

Anitha S. and Gandhi P. K. 2012. Allelopathic Effect of *Spinacia oleracea* L. and *Psidium guajava* L. on *Vigna radiata* L. Var. KM-2 and Vamban-2. IOSR Journal of Pharmacy and Biological Sciences. ISSN : 2278-3008 Volume 1, Issue 5 (July-August 2012), PP. 21-23.

Aziz T., Sylvia D. M., and Doren R. F. 1995. Activity and Species Composition of Arbuscular Mycorrhizal Fungi Following Soil Removal. *Ecological Applications*, Vol. 5, No. 3, pp. 776-784.

Bahrami A., Emadodin I., Atashi M. R., and Bork H. R., 2010. Land-use change and soil degradation: A case study, North of Iran. Agriculture and Biology Journal Of North America.

Barea J.M., Requena N., and Jimenez I. 1996. A Revegetation Strategy Based on The Management of Arbuscular Mycorrhizae, Rhizobium and Rhizobacterias for The Reclamation of Desertified Mediterranean Shrubland Ecosystems. CIHEAM - Options Mediterraneennes.

Bennun L. and Njoroge P. 1999. Important Bird Areas in Kenya. The East Africa Natural History Society, Nairobi, Kenya.

Berens, D.G., Farwig, N., Schaab, G. and Böhning-Gaese, K. 2008. Exotic guavas are foci of forest regeneration in Kenyan farmland. *Biotropica*, 40: 104-112.

Biermann B. and. Linderman R. G. 1980. Quantifying Vesicular-Arbuscular Mycorrhizae: A Proposed Method Towards Standardization. *New phytol*. 87, 63-67.

Binggeli P., 1997. An Overview of Invasive Woody Plants in the Tropics

Bleher B., Uster D., and Bergsdorf T. 2006. Assessment of Threat Status and Management Effectiveness in Kakamega Forest, Kenya. *Biodiversity and Conservation* 15:1159–1177.

Bovey, R. W. and Diaz-Colon, J. D. 1968. Occurrence of plant growth inhibitors in tropical e subtropical vegetation. *Phys. Plant*., 22, 253-259.

Brocklehurst P., Lewis D., Napier D., and Lynch D. 2007.Northern Territory Guidelines and Field Methodology for Vegetation Survey and Mapping. Technical Report No. 02/2007D. Department of Natural Resources, Environment and the Arts, Palmerston, Northern Territory.

Brown R. B., 2003. Soil Texture. Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Fact Sheet SL-29.

Brown, R.L., Tang, C. S. and Nishimoto, R. K., 1983. Growth-inhibition from guava roots exudates. *Hortscience,* 18, 316-318.

Brundrett M. 2003. Diversity and classification of mycorrhizal associations. *Biol. Rev*. (2004), 79, pp. 473–495.

Brundrett, M. C. 2002. Coevolution of roots and mycorrhizas of land plants. New Phytologist, 154: 275–304.

Chapla T. E. and Campos J. B. 2010. Allelopathic Evidence in Exotic Guava (*Psidium guajava* L.) *Braz. Arch. Biol. Technol*. v.53 n. 6: pp. 1359-1362.

Clapperton M. J. and Read D. J. 1992. A relationship between plant growth and increasing VAM mycorrhizal inoculum density. *New Phytologist* 120, 227–234.

Dalpé Y, and Monreal M. 2004. Arbuscular Mycorrhiza Inoculum to Support Sustainable Cropping Systems. Plant Management Network.

Egerton-Warburton L. M, Allen E. B.,2000. Shifts in Arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecol Appl* 10:484-496.

Eissa M. F. M.1971. The Effect of Partial Soil Sterilization on Plant Parasitic Nematodes and Plant Growth. H. Veenman & Zonen N.V. – Wageningen.

Eni D. D., Iwara A. I. and Offiong R. A., 2011. Analysis of Soil-Vegetation Interrelationships in a South-Southern Secondary Forest of Nigeria. International Journal of Forestry Research Volume 2012.

FAO (Food and Agriculture Organization of the United States). 2005a. State of the world's forests. FAO, Rome, Italy.

FAO, 1990b. Soil Map of the World: Revised Legend. World Soil Resources Report 60. FAO, Rome, Italy.

Gange A. C. 1999. On the relation between arbuscular mycorrhizal colonization and plant 'benefit'. *Oikos* 87, 615–621.

Gee, G.W. and J.W. Bauder, 1986. Particle-size Analysis. Pages 383-411 in Methods of Soil Analysis Part 1. A. Klute (edi.), Soil Science Society of America Book Series 5, Madison, Wisconsin, USA.

Gerdmann J.W.,and Nicolson T.H. 1963. Spores of mycorrhizal endogone species extracted by wet sieving and decanting. *T Brit Mycol Soc* 46: 235-244.

Gerdmann J.W. 1975. Vesicular-arbuscular mycorrhizae. In: The development and Function of Roots. Torrey JG, Clarkson DT (eds). Academic Press, New York and London, pp 575-591.

Gianinazzi-Pearson V. 1984. Host-fungus specificity in mycorrhizae. *In*: Verma D.P.S, and Hohn T.H. (eds). Genes involved in plant-microbe interactions. Springer, Vienna, pp, 225-253.

Grisso R., Alley M, Wysor W.G. Holshouser D, and Thomason W.,2009. Precision Farming Tools: Soil Electrical Conductivity. College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University. Virginia Cooperative Extention, Publication 442-508.

Gutierrez, R.M., Mitchell, S and Solis, R.V. (2008). *Psidium guajava* L. A review of its traditional uses, Phytochemistry and Pharmacology. J. *Ethnopharmacol*., 117, 1-27.

Harley, J. L. and Smith, S. E. (1983). Mycorrhizal Symbiosis. Academic Press, Toronto.

Harrison M. J. 2005. Signaling In The Arbuscular Mycorrhizal Symbiosis. Annual Review of Microbiology Vol. 59: 19-42.

He W. M. and Cui Q. G. 2009. Manipulation of soil biota in ecological research. *Web Ecol*. 9: 68–71.

Invasive Species Specialist Group (ISSG), 2010: Global Invasive Species Database. IUCN Species Survival Commission.

Jacquemoud S. and Ustin S. L., 2001. Leaf Optical Properties: A State of the Art; Proceedings of the 8th International Symposium on Physical Measurements and Signatures in Remote Sensing; Aussois, France. pp. 223–232.

Jatzold R. and Schmidt H. 1982. Natural Conditions and farm management information; A: West Kenya (Nyanza and Western Provinces).

Jenkins W.R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. Plant Disease Report 73: 288-300.

Johnson N. C, Tilman D, Wedin D., 1992. Plant and soil controls on mycorrhizal fungal communities. Ecology 73:2034-2042.

Johnson N. C., Zak D. R., Tilman D., and Pfleger F. L. 1991. Dynamics of Vesicular-Arbuscular Mycorrhizae during Old Field Succession. *Oecologia*, Vol. 86, No. 3, pp. 349-358.

Jones J. C. and Reynolds J. D. 1996**.** Environmental Variables. In: Sutherland W. J (Ed) Ecological Census Technique; A hand Book. Cambridge University Press, New York, USA.

Kauffman S., Sombroek W. and Mantel S. 1995. Soils of Rainforest, Characterisation and major constraints of dominant forest soils in the humid tropics. International Soil Reference and Information Centre.

Khanam D., Mridha M.A.U., and Solaiman A.R.M. 2006. Comparative Study of Arbuscular Mycorrhizal Association with Different Agricultural Crops among Four AEZS of Bangladesh. *J. Agric. Res*., 44(2).

KIFCON 1994. Kakamega Forest, the Official Guide.

Koide R T. and Mosse B. (2004) A History of Research on Arbuscular Mycorrhiza. Mycorrhiza (2004) 14:145–163.

Kokaly R. F., Despain D. G., Clark R. N., and Livo K.E. 2007. Integrated Geoscience Studies in the Greater Yellowstone Area— Volcanic, Tectonic, and Hydrothermal Processes in the Yellowstone Geoecosystem. U. S Geological Survey.

Koske R.E, and Gemma J.N., 1989. A modified procedure for staining roots to detect Vesicular Arbuscular Mycorrhizas. *Mycol Res* 92:486–488.

Koske, R.E., and Gemma, J.N. 1992. Fungal reactions to plants prior to mycorrhizal formation. In Mycorrhizal functioning: an integrative plant fungal process. (ed. Allen M.F.). Chapman & Hall, New York. pp. 3–27.

Kokwaro J.O., 1988. Conservation Status of the Kakamega Forest in Kenya: The Easternmost Relict of the Equatorial Rain Forests of Africa. Monogr. Syst. Bot. Missouri Bot. Garden 25: 471–489.

Krebs C. J. 1985. Ecology: The Experimental Analysis of Distribution and Abundance. Third Edition. Harper and Row, New York.

Kungu J.B (2004) Effect of Vesicular Arbuscular Mycorrhiza (VAM) inoculation on growth performance of *Senna spectabilis*. In: Bationo A (ed) Managing nutrient cycles to sustain soil fertility in sub Saharan Africa. Academy Science Publishers, Nairobi, pp 433–446.

Lovelock C. E., Andersen K., and Morton J. B., 2003. Arbuscular Mycorrhizal Communities in Tropical Forests Are Affected by Host Tree Species and Environment. *Oecologia*, Vol. 135, No. 2, pp. 268-279.

Luis I., Eduardo S., Humberto L. A. and Pekka N. 2009. Characterization and Host Preference of Arbuscular Mycorrhizae associated to a Theobroma Cacao – Inga Edulis Agroforestry System In the Humid Tropics of Costa Rica.

Lung T. and Schaab G. 2006. Assessing Fragmentation and Disturbance of West Kenyan Rainforests by means of Remotely Sensed Time Series Data and Landscape Metrics. *Afr. J. Ecol*. 44, 1–16.

Lung T. and Schaab G., 2004. Change-Detection in Western Kenya – The Documentation of Fragmentation and Disturbance for Kakamega Forest and Associated Forest Areas by means of Remotely-Sensed Imagery.

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L. and Swan, J. A., 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol*. 115:495-501.

Miller, R.M. (1987) The ecology of vesicular-arbuscular mycorrhizae in grass and shrublands. In: G.R. Safir (editor), Ecophysiology of VA Mycorrhizal Plants. CRC. Press, Boca T Raton, pp. 135-170.

Miller R. M. and Jastrow J.D. 1992. Application of Vesicular Arbuscular Mycorrhizae to Ecosystem Restoration and Reclamation. In: Allen M.F. (Ed). Mycorrhizal Functioning: An Integrative Plant Fungal Process. Chapman & Hall, London

Mitchell N. and Schaab G. 2008. Developing a Disturbance Index for Five East African Forests Using GIS to Analyze Historical Forest Use as an Important Driver of Current Land Use⁄Cover. Blackwell Publishing Ltd, *Afr. J. Ecol*.

Molina R.**,** Massicotte H.**,** and Trappe J.M. 1992. Specificity Phenomena in Mycorrhizal Symbiosis: Community –Ecological Consequences and Practical Implications In: Allen M.F. (Ed). Mycorrhizal Functioning: An integrative Plant Fungal Process. Chapman & Hall, London.

Monteiro, C. de A. and Vieira, E. L. (2002), Substâncias alelopáticas. In: Castro, P. R. de C. e, Sena, J. O. A. de and Kluge, R. A. Introdução à fisiologia do desenvolvimento vegetal. Maringá-PR: Eduem.

Morton J.B, Bentivenga S.P, and Bever J.D. 1995. Discovery Measurement And Interpretation Of Diversity In Arbuscular Endomycorrhizal Fungi (Glomales, Zygomycetes). *Can J Bot* 73:25–32.

Mosse B, Hayman D.S., and Arnold D.J. 1973. Plant growth response to vesicular-arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labeled with 32P. *New Phytol*. 72:809-815.

Mukhongo J. N., Kinyamario J. I., Chira R. M. and Musila W. (2011). Assessment of soil seed bank from six different vegetation types in Kakamega forest, Western Kenya. African Journal of Biotechnology Vol. 10(65), pp. 14384-14391.

Muriuki J.W. and Tsingalia M.H. 1990. A New Population of De Brazza's Monkey in Kenya. *Oryx* 24: 157–162.

Musila W. M. 2006. Experimental Restoration and rehabilitation of degraded forest sites and threatened tree species in Kakamega forest. In: BIOTA EAST Project proposal for Phase III, Vol. 1.

Musila W. M. 2007. Multi-scale Analysis of Spatial Heterogeneity of Kakamega Tropical Forest Soils; Role of Disturbance, Succession, Soil Depth, Trees and Near Infra Red Spectroscopy. Ph.D dissertation, University of Hohenheim, Institute of Botany, Stuttgart, Germany.

Mutangah J. G. 1996. An investigation of vegetation status and process in relation to human disturbance in Kakamega forest, Western Kenya. Doctoral Dissertation, University of Wales, Aberystwyth, UK.

Nyamongo, D. O., Nyabundi, J. and Daws, M. I. (2009), Germination and dormancy breaking requirements for Vernonia galamensis (Asteraceae). *Seed Sci. & Technol*., 37, 1-9.

Oehl, F., Sieverding, E., Ineichen, L.,Mader, P., Boller, T. and Wienmken, A. 2003. Impact of Land Use Intensity on the Species Diversity of Arbuscular Myocrrhial Fungi in Agroecosystems of Central Europe. *Appl. Env. Microbiol*. 69: 2816-2824

Ojany F. F. and Ogendo R. B. 1987. Kenya: A study in physical and human geography. Longman, Kenya.

Okalebo J. R., Gathua K. W. and Woomer P. L. (2002). Laboratory Methods of Soil and Plant Analysis: A Working Manual 2nd Edition Tropical Soil Biology and Fertility, Soil Science Society of East Africa, pp 128.

Onyango J. C., Nyunja R. A. O. and Bussmann R. W. 2004. Conservation of Biodiversity in the East African tropical Forest. *Iyonia*, Vol 7(2).

Parrotta, J. A. 2000. Catalysing natural forest restoration on degraded tropical landscapes. Pages 45–54 in S. Elliot, J. Kerby, D. Blakesley, K. Hardwick, K. Woods, and V. Anusarnsunthorn, editors. Forest restoration for wildlife conservation. International Tropical Timber Organization and the Forest Restoration Research Unit, Chiang Mai University, Chiang Mai, Thailand.

Paul J. R., Randle A. M., Chapman C. A. and Chapman L. J. 2004. Arrested succession in logging gaps: is tree seedling growth and survival limiting? *Afr. J. Ecol.*, 42, 245–251.

Phil-Eze P. O. 2010. Variability of Soil Properties Related to Vegetation Cover in a Tropical Rainforest Landscape. Journal of Geography and Regional Planning Vol. 3(7), pp.177-184.

Picone C. M. 2000. Diversity and Abundance of Arbuscular Mycorrhizal Fungus Spores in Tropical Forest and Pasture. *Biotropica*, 32: 734-750.

Plenchette C. (1991). Utilisation des mycorhizes en agriculture et horticulture. In : Strullu D. G, Garbaye J, Perrin P, Plenchette C. (eds) Les Mycorhizes des Arbres et Plantes Cultivées. Technique et Documentation, Lavoisier, pp. 131-196.

Plenchette C., Fortin J.A., Furlan, V. (1983). Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. *Plant and Soil*, v.70, p.199-209.

Quilambo O. A. 2003. The Vesicular-Arbuscular Mycorrhizal Symbiosis. *African Journal of Biotechnology* Vol. 2 (12), pp. 539-546.

Raaijmakers J. M, Paulitz T. C, Steinberg C., Alabouvette C., and Moënne-Loccoz Y. (2008). The Rhizosphere: A Playground and Battlefield for Soilborne Pathogens and Beneficial Microorganisms. *Plant Soil* (2009) 321:341–361.

Read, D. J. and Birch, C. P. D. (1988). The effects and implications of disturbance of mycorrhizal mycelial systems. *Proc. R. Soc. Edin. B* 94**,** 13-24.

Schenck N. C., and Perez Y. (eds.) 1990. Manual for the identification of VA Mycorrhizal fungi. Synergistic Publications, Gainesville.

Schlesinger, W.H. 1997. Biogeochemistry: An Analysis of Global Change. 2nd edition. Academic Press, San Diego.

Selvaraj T. and Chellappan P. 2006. Arbuscular Mycorrhizae: A Diverse Personality. *Journal of Central European Agriculture*. Volume 7, No. 2 pp. 349-358.

Shi, Z., Y., Wang, F. and WeI Y. L. 2007.Natural Forest and Forest Plantation Affect Diversity of Arbuscular Mycorrhizal Fungi in the Rhizosphere of Diptorocarpaceae. American-Eurasian J. Agric. Environ. Sci.

Shono K. Cadaweng E. A. and Durst P. B. 2007. Application of Assisted Natural Regeneration to Restore Degraded Tropical Forestlands. *Restoration Ecology* Vol. 15, No. 4, pp. 620–626.

Troelstra S. R., Wagenaar R., Smant W. and Peters B. A. M. 2001. Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. – *New Phytol*. 150: 697–706.

Tsingalia M.H. 1988. Animals and the Regeneration of an African Rainforest Tree. PhD dissertation, University of Nairobi, Nairobi.

Vaidya G. S., Shrestha K., Khadge B. R., Johnson N. C., and Wallander H. 2007. Study of Biodiversity of Arbuscular Mycorrhizal Fungi in Addition with Different Organic Matter in Different Seasons of Kavre District (Central Nepal). *Scientific World*, Vol. 5, No. 5.

Vollesen K. 2007. Synopsis of the species of Acanthus (Acanthaceae) in tropical East and Northeast Africa and in tropical Arabia. *Kew Bulletin* 62 (2): 233–249.

Wass P. 1995. Kenya's Indigenous Forests – Status, Management and Conservation. IUCN, Gland, Switzerland.

Wienke T. M. S., Filho L., Antonio O., and Elio K., 1995. Germination and vigor of guava seeds (*Psidium guajava* I.) submitted to different methods to supress dormancy. *Rural Science*. Vol.25, n.1, pp. 11-15.

Zar J. H. 1984. Biostatistical Analysis. Prentice – Hall Inc. U.S.A.

APPENDICES

Appendix 1: Soil analysis results (Soil phosphorus, Electrical Conductivity and pH) of soils from the seven vegetation types

Soils by vegetation type	Depth (cm)	Replicate	MgP/kg	pH	EC (µs)
Psidium guajava	0-10	1	2.59	5.34	200
Psidium guajava	0-10	2	2.38	5.66	172
Psidium guajava	0-10	3	2.80	5.78	226
Psidium guajava	10-20	1	1.68	5.04	315
Psidium guajava	10-20	2	1.59	5.67	239
Psidium guajava	10-20	3	1.77	5.88	99
Psidium guajava	20-30	1	1.79	5.30	168
Psidium guajava	20-30	2	1.49	5.97	125
Psidium guajava	20-30	3	2.08	5.90	89
Psidium guajava	30-40	1	0.20	5.15	171
Psidium guajava	30-40	2	2.87	6.09	141
Psidium guajava	30-40	3	1.96	5.64	147

Soils by vegetation type	Depth (cm)	Replicate	MgP/kg	pH	EC (µs)
Vernonia sp..	0-10	1	0.30	5.66	200
Vernonia sp..	0-10	2	2.45	5.49	350
Vernonia sp..	0-10	3	1.18	5.78	320
Vernonia sp..	10-20	1	1.15	5.58	95
Vernonia sp..	10-20	2	10.38	5.63	193
Vernonia sp..	10-20	3	1.90	5.71	210
Vernonia sp..	20-30	1	0.49	5.34	53
Vernonia sp..	20-30	2	3.00	5.43	117
Vernonia sp..	20-30	3	1.96	5.65	106
Vernonia sp..	30-40	1	2.66	5.12	50
Vernonia sp..	30-40	2	2.59	5.06	68
Vernonia sp..	30-40	3	1.05	5.23	161

Continuation of Appendix 1

Soils by vegetation type	Depth (cm)	Replicate	MgP/kg	pH	EC (µs)
Eucalyptus saligna	0-10	1	2.26	5.77	385
Eucalyptus saligna	0-10	2	0.39	5.15	107
Eucalyptus saligna	0-10	3	1.80	5.26	300
Eucalyptus saligna	10-20	1	3.11	5.98	158
Eucalyptus saligna	10-20	2	0.37	5.08	93
Eucalyptus saligna	10-20	3	0.26	5.63	122
Eucalyptus saligna	20-30	1	2.41	5.82	171
Eucalyptus saligna	20-30	2	2.84	5.18	48
Eucalyptus saligna	20-30	3	2.00	5.66	149
Eucalyptus saligna	30-40	1	3.01	5.72	95
Eucalyptus saligna	30-40	2	1.99	4.97	61
Eucalyptus saligna	30-40	3	0.28	5.60	140

Soils by vegetation type	Depth (cm)	Replicate	MgP/kg	pH	EC (µs)
Cupressus lusitanica	0-10	1	-0.38	6.02	267
Cupressus lusitanica	0-10	2	0.98	6.34	503
Cupressus lusitanica	0-10	3	0.10	6.36	358
Cupressus lusitanica	10-20	1	3.15	6.22	119
Cupressus lusitanica	10-20	2	0.78	6.21	168
Cupressus lusitanica	10-20	3	1.49	6.35	143
Cupressus lusitanica	20-30	1	0.18	5.96	186
Cupressus lusitanica	20-30	2	0.45	5.43	187
Cupressus lusitanica	20-30	3	1.93	6.23	131
Cupressus lusitanica	30-40	1	-0.18	6.31	138
Cupressus lusitanica	30-40	2	1.85	5.13	131
Cupressus lusitanica	30-40	3	1.44	5.49	362

Soils by vegetation type	Depth (cm)	Replicate	MgP/kg	pH	EC (µs)
Hyparrhenia sp. Grassland	0-10	1	2.38	4.87	127
Hyparrhenia sp. Grassland	0-10	2	1.17	4.76	101
Hyparrhenia sp. Grassland	0-10	3	1.67	5.03	81
Hyparrhenia sp. Grassland	10-20	1	0.73	4.85	84
Hyparrhenia sp. Grassland	10-20	2	-0.27	4.78	67
Hyparrhenia sp. Grassland	10-20	3	1.83	5.05	59
Hyparrhenia sp. Grassland	20-30	1	0.62	4.95	50
Hyparrhenia sp. Grassland	20-30	2	2.35	4.92	40
Hyparrhenia sp. Grassland	20-30	3	1.33	4.92	61
Hyparrhenia sp. Grassland	30-40	1	1.36	4.67	101
Hyparrhenia sp. Grassland	30-40	2	4.20	4.84	50
Hyparrhenia sp. Grassland	30-40	3	1.82	4.99	49

Continuation of Appendix 1

Soils by vegetation type	Depth (cm)	Replicate	MgP/kg	pH	EC (µs)
Brachiaria brizantha	0-10	1	-0.20	5.88	161
Brachiaria brizantha	0-10	2	2.34	5.86	225
Brachiaria brizantha	0-10	3	0.75	5.53	229
Brachiaria brizantha	10-20	1	2.08	6.17	128
Brachiaria brizantha	10-20	2	1.65	5.99	198
Brachiaria brizantha	10-20	3	1.15	5.51	117
Brachiaria brizantha	20-30	1	2.26	6.11	145
Brachiaria brizantha	20-30	2	2.73	5.82	436
Brachiaria brizantha	20-30	3	3.54	5.34	72
Brachiaria brizantha	30-40	1	0.46	6.16	160
Brachiaria brizantha	30-40	2	0.09	6.09	249
Brachiaria brizantha	30-40	3	2.95	4.72	146

Soils by vegetation type	Depth (cm)	Replicate	MgP/kg	pH	EC (µs)
Natural Forest	0-10	1	0.63	6.65	453
Natural Forest	0-10	2	5.28	5.99	185
Natural Forest	0-10	3	0.65	6.32	436
Natural Forest	10-20	1	2.00	6.45	249
Natural Forest	10-20	2	1.23	5.11	202
Natural Forest	10-20	3	2.03	5.91	234
Natural Forest	20-30	1	2.36	6.14	182
Natural Forest	20-30	2	2.04	5.32	164
Natural Forest	20-30	3	2.25	5.72	125
Natural Forest	30-40	1	2.80	5.63	170
Natural Forest	30-40	2	2.88	5.65	80
Natural Forest	30-40	3	1.50	5.59	128

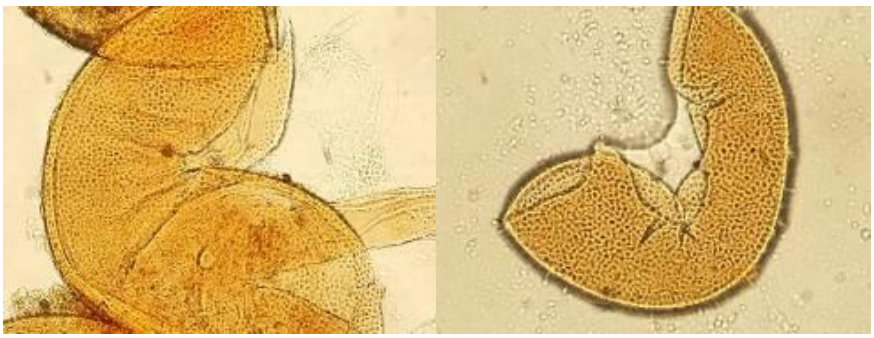
Appendix 2: Soil types, Clay, silt and sand percentages of soils from the seven vegetation stands

Sample ID	Vegetation Type	Depth (cm)	PSSLWT	% CLAY	% Sand	% Silt	Textural Class
SPGV4	*Psidium guajava*	0---10	50.02	27.1	45.9	27.1	sandy clay loam
SPGV4	*Psidium guajava*	10---20	50.02	29.4	48.6	22.0	sandy clay loam
SPGV4	*Psidium guajava*	20---30	50.07	65.3	14.7	20.0	clay
SPGV4	*Psidium guajava*	30---40	50.08	63.3	16.7	20.0	clay
SPGV5	*Psidium guajava*	0---10	50.05	36.1	37.9	26.0	clay loam
SPGV5	*Psidium guajava*	10---20	50.01	51.4	14.6	34.0	clay
SPGV5	*Psidium guajava*	20---30	50.09	51.3	14.7	33.9	clay
SPGV5	*Psidium guajava*	30---40	50.03	44.1	20.6	35.3	clay
SPGV6	*Psidium guajava*	0---10	50.06	23.4	40.6	36.0	loam
SPGV6	*Psidium guajava*	10---20	50.05	34.1	29.9	36.0	clay loam
SPGV6	*Psidium guajava*	20---30	50.05	38.1	27.9	34.0	clay loam
SPGV6	*Psidium guajava*	30---40	50.01	41.4	16.6	42.0	silty clay
SPVR1	*Vernonia sp.*	0---10	50.02	37.4	42.6	20.0	clay loam
SPVR1	*Vernonia* sp..	10---20	50.06	51.4	28.6	20.0	clay
SPVR1	*Vernonia* sp..	20---30	50.04	57.4	20.6	22.0	clay
SPVR1	*Vernonia* sp..	30---40	50.09	65.3	16.7	18.0	clay
SPVR2	*Vernonia* sp..	0---10	50.02	41.4	34.6	24.0	clay
SPVR2	*Vernonia* sp..	10---20	50.01	33.4	40.6	26.0	clay loam
SPVR2	*Vernonia* sp..	20---30	50.07	44.1	23.9	32.0	clay
SPVR2	*Vernonia* sp..	30---40	50.01	72.1	11.9	16.0	clay
SPVR3	*Vernonia* sp..	0---10	50.05	30.1	45.9	24.0	sandy clay loam
SPVR3	*Vernonia* sp..	10---20	50.02	32.1	43.9	24.0	clay loam
SPVR3	*Vernonia* sp..	20---30	50.01	55.4	22.6	22.0	clay
SPVR3	*Vernonia* sp..	30---40	50.07	50.1	25.9	24.0	clay
SPPE1	*Eucalyptus saligna*	0---10	50.06	37.4	40.6	22.0	clay loam
SPPE1	*Eucalyptus saligna*	10---20	50.07	51.4	24.7	24.0	clay
SPPE1	*Eucalyptus saligna*	20---30	50.07	61.4	16.7	22.0	clay
SPPE1	*Eucalyptus saligna*	30---40	50.00	63.4	16.6	20.0	clay
SPPE2	*Eucalyptus saligna*	0---10	50.01	59.4	20.6	20.0	clay
SPPE2	*Eucalyptus saligna*	10---20	50.07	51.4	28.7	20.0	clay
SPPE2	*Eucalyptus saligna*	20---30	50.06	60.1	18.7	21.3	clay
SPPE2	*Eucalyptus saligna*	30---40	50.06	68.1	17.9	14.0	clay
SPPE4	*Eucalyptus saligna*	0---10	50.05	16.1	61.9	22.0	sandy loam
SPPE4	*Eucalyptus saligna*	10---20	50.04	40.1	31.9	28.0	clay
SPPE4	*Eucalyptus saligna*	20---30	50.03	44.1	25.9	30.0	clay
SPPE4	*Eucalyptus saligna*	30---40	50.02	43.4	30.6	26.0	clay
SPPC1	*Cupressus lusitanica*	0---10	50.03	25.4	50.6	24.0	sandy clay loam
SPPC1	*Cupressus lusitanica*	10---20	50.03	57.4	16.6	26.0	clay
SPPC1	*Cupressus lusitanica*	20---30	50.03	44.1	27.9	28.0	clay

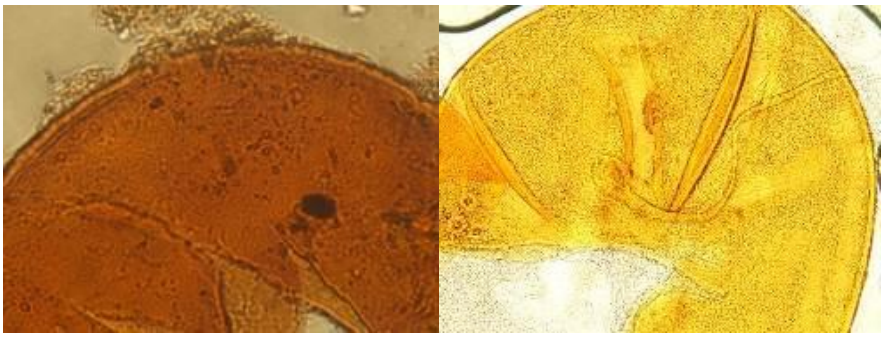
Sample ID	Vegetation Type	Depth (cm)	PSSLWT	% CLAY	% Sand	% Silt	Textural Class
SPPC1	*Cupressus lusitanica*	30---40	50.08	54.1	18.0	28.0	clay
SPPC3	*Cupressus lusitanica*	0---10	50.08	29.4	46.6	24.0	sandy clay loam
SPPC3	*Cupressus lusitanica*	10---20	50.07	47.4	28.7	24.0	clay
SPPC3	*Cupressus lusitanica*	20---30	50.01	46.2	25.9	28.0	clay
SPPC3	*Cupressus lusitanica*	30---40	50.07	51.4	28.7	20.0	clay
SPPC4	*Cupressus lusitanica*	0---10	50.01	31.4	38.6	30.0	clay loam
SPPC4	*Cupressus lusitanica*	10---20	50.03	31.4	42.6	26.0	clay loam
SPPC4	*Cupressus lusitanica*	20---30	50.02	35.4	30.6	34.0	clay loam
SPPC4	*Cupressus lusitanica*	30---40	50.07	46.1	23.9	30.0	clay
SPGLN1	*Hyparrhenia* sp.	0---10	50.09	46.1	39.9	14.0	clay
SPGLN1	*Hyparrhenia* sp.	10---20	50.03	36.1	49.9	14.0	sandy clay
SPGLN1	*Hyparrhenia* sp.	20---30	50.06	50.1	31.9	18.0	clay
SPGLN1	*Hyparrhenia* sp.	30---40	50.06	28.1	55.9	16.0	sandy clay loam
SPGLN4	*Hyparrhenia* sp.	0---10	50.04	40.1	29.9	30.0	clay
SPGLN4	*Hyparrhenia* sp.	10---20	50.07	50.1	21.9	28.0	clay
SPGLN4	*Hyparrhenia* sp.	20---30	50.02	52.1	17.9	30.0	clay
SPGLN4	*Hyparrhenia* sp.	30---40	50.08	44.1	30.0	26.0	clay
SPGLN6	*Hyparrhenia* sp.	0---10	50.08	50.1	22.0	28.0	clay
SPGLN6	*Hyparrhenia* sp.	10---20	50.06	46.1	30.6	23.3	clay
SPGLN6	*Hyparrhenia* sp.	20---30	50.04	42.1	36.6	21.3	clay
SPGLN6	*Hyparrhenia* sp.	30---40	50.07	56.1	16.7	27.2	clay
SPGLA2	*Brachiaria brizantha*	0---10	50.06	42.1	32.6	25.2	clay
SPGLA2	*Brachiaria brizantha*	10---20	50.09	62.0	12.7	25.2	clay
SPGLA2	*Brachiaria brizantha*	20---30	50.03	62.1	14.6	23.3	clay
SPGLA2	*Brachiaria brizantha*	30---40	50.02	60.1	18.6	21.3	clay
SPGLA3	*Brachiaria brizantha*	0---10	50.00	48.2	28.6	23.3	clay
SPGLA3	*Brachiaria brizantha*	10---20	50.03	50.1	26.6	23.3	clay
SPGLA3	*Brachiaria brizantha*	20---30	50.09	58.1	20.7	21.2	clay
SPGLA3	*Brachiaria brizantha*	30---40	50.01	68.1	10.6	21.3	clay
SPGLA4	*Brachiaria brizantha*	0---10	50.06	44.1	32.6	23.3	clay
SPGLA4	*Brachiaria brizantha*	10---20	50.06	60.1	18.7	21.3	clay
SPGLA4	*Brachiaria brizantha*	20---30	50.09	66.0	14.7	19.2	clay
SPGLA4	*Brachiaria brizantha*	30---40	50.03	72.1	12.6	15.3	clay
SPNF1	Natural Forest	0---10	50.04	58.1	28.6	13.3	clay
SPNF1	Natural Forest	10---20	50.06	44.1	20.7	35.2	clay
SPNF1	Natural Forest	20---30	50.05	50.1	18.6	31.2	clay
SPNF1	Natural Forest	30---40	50.05	60.1	16.6	23.3	clay
SPNF2	Natural Forest	0---10	50.03	24.1	32.6	43.3	loam
SPNF2	Natural Forest	10---20	50.02	30.1	28.6	41.3	clay loam
SPNF2	Natural Forest	20---30	50.09	32.1	22.7	45.2	clay loam

Sample ID	Vegetation Type	Depth (cm)	PSSLWT	% CLAY	% Sand	% Silt	Textural Class
SPNF2	Natural Forest	30---40	50.06	34.1	24.7	41.2	clay loam
SPNF7	Natural Forest	0---10	50.01	40.2	34.6	25.3	clay
SPNF7	Natural Forest	10---20	50.05	52.1	28.6	19.3	clay
SPNF7	Natural Forest	20---30	50.05	66.1	18.6	15.3	clay
SPNF7	Natural Forest	30---40	50.05	44.1	22.6	33.2	clay

Appendix 3: Photos of identified spores

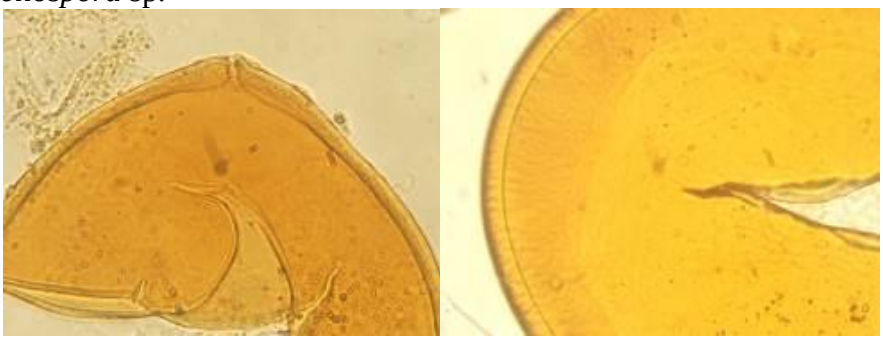


Acaulospora scrobiculata



Gigaspora sp.

Scutellospora sp.



Glomus aff. etunicatum