

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Soil organisms play a crucial role in ecosystem functioning and potential benefits may be derived from an understanding of the biodiversity (Lee 1994). Knowledge of soil biodiversity will create opportunity to establish their functional significance. Only then can it be determined how to manipulate this biodiversity to increase and sustain production. Fungi are a major component of the soil biomass with considerable importance in regulating ecosystem processes (Hawksworth 1991; Fogel 1980), yet these fungi have received little attention (Hawksworth 1991). The mycorrhizal fungi are proposed as keystone organisms (Power & Mills 1995).

The AMF species associate with a wide range of hosts in diverse soil types and climatic regions of the world and are more widespread than any other symbiotic organisms (Newman & Reddell 1987; Chong-Kyun Kim *et al.* 1989; Allsopp & Stock 1993; Ragupathy & Mahadevan 1993). Members of the *Glomeromycota* (AMF) are obligate symbionts (Harley & Smith 1983) due to their necessity for an autotrophic symbiotic partner to supply their carbon needs, and function as absorptive organs for nutrient acquisition, particularly phosphorus. They are reported to have the ability to transfer nutrients from one host plant to another (Francis *et al.* 1986 and Haystead *et al.* 1988) in a source to sink relationship. The hyphae play an important role in the formation of soil aggregates greater than 240 μm diameter (Hamel *et al.* 1997). AMF may also influence plant biodiversity (van der Heijden *et al.* 1998), help to control pests (e.g nematodes) and

fungal pathogens (Azcon-Aguilar & Barea 1996) and affect fitness of plants in polluted environments (Hildebrandt *et al.* 1999). Information on taxonomic groups of AMF species in Africa is still scarce.

2.2 TAXONOMY

The essential foundation for utilisation of AMF depends on the determination of species, which is not adequately done in most studies undertaken in this group of fungi. The most prevalent problem with this is demonstrated in the lack of ability to replicate experiments. As a result, Morton (1988) and Walker (1992) both emphasized the importance of taxonomy in any experimentation using AMF as a controlled biological variable.

Classification of the *Glomeromycota* started as early as 1809 with the description of the genus *Endogone* Link:Fr. by Link (Gerdemann & Trappe 1974b). Tulasne and Tulasne (1845) first described the genus *Glomus* Tulasne & Tulasne and later transferred it to *Endogone*, when they failed to notice that the zygospores originated in a way distinctly different from two species originally placed in *Endogone*. Nicolson and Gerdemann (1968) included the endomycorrhizal forming fungi as non-sporocarpic members of the genus *Endogone*. In an endomycorrhiza symposium at the University of Leeds, Gerdemann and Trappe (1974a) acknowledged the difficulty in AMF taxonomy and recognized the likelihood of having to change the generic classification as more information became available. This was followed by an era of description of new taxa and the re-organization of classification of the known species. The genus *Endogone* was noted to be too large and species difficult to distinguish. A revision based on a formal Linnean classification was

made by Gerdemann and Trappe (1974b) with sporocarps and mycorrhizal infection used to classify the group into seven genera. They segregated sporocarpic chlamydospore-forming *Endogone* species into *Glomus*. The first identification key was compiled. They revised the genus *Endogone* by describing two new genera, *Acaulospora* Gerdemann & Trappe emend. Berch and *Gigaspora* Gerdemann & Trappe emend. and separated *Glomus* Tulasne & Tulasne (Walker & Sanders 1986).

Based on modes of spore formation, Ames and Schneider (1979) described a new genus *Entrophospora* Ames & Schneider emend. wu, separating it from the genus *Acaulospora*. Wall characteristics (Walker 1983) and the mode of spore germination and auxiliary cells were used (Walker and Sanders 1986) to describe a new genus *Scutellospora* Walker & Sanders separating it from *Gigaspora*. Until Pirozynsky and Dalpe (1989) noted that some genera formed vesicles and arbuscules, all the genera were placed in the family Endogonaceae, order Endogonales. Pirozynsky and Dalpe (1989) proceeded to separate six mycorrhizae forming genera into the family Glomaceae and a non-mycorrhizae forming family the Endogonaceae. Schenck and Perez (1990) produced a manual with species descriptions. Morton and Benny (1990) classified AMF to a new order *Glomales* (correctly referred to as *Glomerales*) based on patterns of morphological characteristics of somatic (hyphae, arbuscules and vesicles) and reproductive stages (spores), patterns of common descent, ontogeny and spore germination. They raised the taxonomic level on the basis of mycorrhizae formation to the order *Glomales* and created two new suborders *Glomineae* and *Gigasporineae*, two new families *Acaulosporaceae* and *Gigasporaceae* and they amended the family *Glomaceae*. A recent addition into the *Glomales* is the fossil genus

Glomites Taylor, Remy, Hass et Kerp. that was first described by Taylor *et al.* (1995). Morton and Benny's (1990) classification was followed by a description of a new class *Glomomycetes* with a brief latin diagnosis (Cavalier-Smith 1998), later corrected to *Glomeromycetes*, within the *Zygomycota*, containing *Glomerales* and *Endogonales* (Shüßler *et al.* 2001). The most recently revised classification is based on molecular techniques with a comprehensive SSU rRNA analysis. The group has been classified in a new monophyletic phylum, the *Glomeromycota* C. Walker & Schuessler, phylum nov. (Shüßler *et al.* 2001) with order *Glomales*, more correctly referred to as *Glomerales* and three new orders *Archaeosporales*, *Paraglomerales* and *Diversisporales* (Figure 2.1).

Phylum

Glomeromycota

Order: *Archaeosporales*

1. Family

(i) *Archaeosporaceae*

Genera

Archaeospora

Diversisporales

(i) *Gigasporaceae*

Gigaspora

Scutellospora

Glomerales

(i) *Glomeraceae*

Glomus group Aa

Glomus group Ab

Glomus group B

Paraglomerales

(i) *Paraglomeraceae*

Paraglomus

2. Family

(ii) *Geosiphonaceae*

Genera

Geosiphon

(ii) *Acaulosporaceae*

Acaullospora

Entrophospora

3. Family

(iii) *Diversisporaceae* fam. ined.

Figure 2.1. Classification of glomeromycotan fungi (Shüßler *et al.* 2001).

Comparative information concerning distribution and diversity of AMF species in different continents is still being assembled. Many species are being re-described and some species merged or assigned to new taxa. Description of new species continues. The International culture collection of Vesicular-arbuscular and Arbuscular Mycorrhizal fungi (INVAM) in the United States and the International Bank for the *Glomeromycota* at Dijon, France, are progressively working on collections from all over the world to bridge this knowledge gap.

There is still need to collect from the different parts of the world. Africa is under-explored and the species diversity of AMF communities in most parts of Africa is not known. With the application of intensive farming practices in Africa, there is danger of losing species before they are recorded.

2.3 ECOLOGY

The existence of soil organisms depends on their relationship with other living organisms (biotic) and the non-living (abiotic) environment (Atlas & Bartha 1986). Kennedy and Smith (1995) suggested that each soil type has an inherent community of soil organisms that has long existed in a state of equilibrium. Disturbance by human activities can greatly influence species diversity and functions of soil organisms. With the rapid increase in population, changes in landscape in Africa are drastic and may have irreversible effects on the soil biodiversity, hence it is crucial to establish the effects of particularly different farming systems on AMF species diversity and how the individual species respond to these changes. Distinct seasonal variations that control plant activities and soil conditions also play a major role in AMF species activities. Distinct seasonal patterns in Africa may therefore greatly affect AMF species.

The most useful method of evaluating the factors influencing a group of organisms in a habitat is to study their diversity. Kennedy and Smith (1995) stated the following as vital reasons for studying biodiversity: “to increase knowledge of the diversity of genetic resources and understand the distribution of diversity on earth, and to increase knowledge of the functional role of diversity and identify changes in diversity associated with

disturbance and management practices”. However, diversity has many meanings to soil biologists. Morton *et al.* (1994) interpreted diversity in terms of species diversity, ecological diversity and the diversity of functional forms. This is a concept to be adapted for this study.

2.3.1 Species diversity

Species diversity is defined (Gaston 1996; Kennedy & Smith 1995; Morton & Bentivenga 1995) as the variety of species. The simplest measure of species diversity is a count of the number of different species termed “species richness” (Gaston 1996; Stiling 1996). The species concept in AMF is interpreted differently from most other organisms. Morton and Bentivenga (1995) conceptualized AMF species as “the smallest assemblage of reproductively isolated individuals or populations diagnosed by epigenetic, morphological or organizational properties of fungal spores that specify a unique genealogical origin based on the criterion of monophyly”. This concept includes terms from evolutionary biology with limited practical importance. Li and Graur (1991) described taxonomic species concept as “a group of organisms resembling each other more than they resemble any other group”. Rosendahl *et al.* (1994) considered Li and Graur (1991) concept more practical and rephrased it as “repeatedly recognizable fungus that can be defined by the morphological characteristics of its spores (allowing differences of gene expressions), and which is internally consistent if found in widely separated geographic locations”. This concept is applied in this study and the spore is thus used as the morphological structure of reference.

The dispersal patterns and biogeographical ranges of most species are not fully established and fungal communities of most habitats particularly in Africa are not known. With the persistent destruction of habitats and expansion of intensive farming systems, there is need to evaluate changes in the diversity of fungal communities as affected by changes in environmental factors and human activities. Among the AMF communities are species with the potential to improve agriculture, forestry and restore degrading habitats. These may be lost.

2.3.2 Ecological diversity

Ecological diversity is observed as the partitioning of a niche in space and time for different species (Morton 1993). Diversity is observed in the distribution and occurrence of the AMF symbiosis in relation to soil nutrients, plant composition and season. Various investigations indicate that abiotic factors such as changes in seasons and soil conditions, biotic factors such as plants and man-made factors could greatly affect the mycorrhizal symbiosis. Depending on the changes in environmental conditions, the symbiosis might shift.

2.3.2.1 Seasonal effects

Distinct seasons determine plant activity and thus influencing the activity of mycorrhizal symbiosis. Plant phenology was observed (Giovannetti 1985; Brundrett 1991) to relate to seasonal changes that influenced the seasonal patterns of spore production. Recent studies by Guadarrama and Sanchez (1999) confirm season to be an important factor in determining the physiological changes of the host and the influence in species diversity and

spore numbers. The highest number of species and spores occurred during the dry season with a marked decrease in the rainy season. These changes were linked to poor root growth in summer and dry season and active growth in spring and wet season in the temperate and tropical regions respectively. Ilag *et al.* (1987) also observed dry season rice to have higher populations of infective propagules than wet season rice. The dry season marks the end of optimum plant growth and it coincides with very slow root growth, absence of root growth or senescence of the host roots. Baylis (1969) suggested that intermittent root growth and drying might stimulate sporulation while adequate soil moisture favours actively growing roots rendering sporulation unnecessary.

Change in soil conditions, particularly moisture and nutrients, also follow a seasonal pattern and they are also used to explain changes in the symbiosis. Studies at the agroforestry site in Malawi noted seasonal fluctuations in levels of ammonium and nitrate in a *Gliricidia sepium* and maize intercrop (Ikerra *et al.* 1999). Rabatin (1979) recorded the highest infection levels in spring when phosphorus was deficient and moisture was low. Seasonal changes of phosphorus are not widely reported, however, seasonal changes in nitrogen are known to affect the balance between nitrogen and phosphorus. Root colonisation was reported to increase as nitrogen content increased if phosphorus levels were moderate and at higher levels of phosphorus, nitrogen application was inhibitory (Bevege 1972). The importance of changes in moisture was more evident under desert conditions as was noted by Jakobsen (1997) in her studies of spore abundance and fungal diversity of the Namibian desert.

AMF species differ in tolerance to changes in seasonal conditions. Sylvia (1986) observed differences in spore numbers of AMF species with spore counts of *Glomus globiferum* increasing by more than 500 % from May to August in summer, while *Glomus aggregatum* Schenck & Smith emend. Koske spore numbers increased by less than 30 %. The majority of spores of *Gigaspora gigantea* (Nicol & Gerd.) Gerdemann & Trappe occurred in late summer and autumn (Gemma & Koske 1988). In a more recent study by Lee Pau-Ju and Koske (1994), healthy spores of *G. gigantea* were most abundant in winter and least in summer. Gemma *et al.* (1989) later reported spore abundance of *Acaulospora scrobiculata* Trappe and *G. gigantea* to be highest in October, spore abundance of *Scutellospora calospora* (Nicol & Gerd) Walker & Sanders was highest in August and *Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders and *Scutellospora persica* (Koske & Walker) Walker & Sanders were highest in May and October. These differences were linked to competition amongst individual fungi for limited nutrients during different stages of plant development (Sylvia 1986).

Studies on the effects of season on AMF in Africa are scarce, yet the continent experiences distinct severe droughts and sometimes floods. Prolonged drought may have devastating effects on the AMF symbiosis. Thompson (1987) noted a total decline in infective propagules in prolonged bare fallow with subsequent decline in crop productivity. The tolerance of AMF species to drought conditions in Africa is not known. Indiscriminate cutting of trees that could protect drought sensitive mycorrhizal species may lead to total loss of these species. Prolonged bare land under dry conditions would probably reduce the diversity of AMF species if not eliminate them completely. The use of perennial plants,

particularly trees and shrubs as fallow in farming systems has proved to be effective fallows for the drought prone southern Africa region of Malawi and Zambia (Kwesiga & Beniest 1998). Trees and shrubs in farming systems compared with bare fallow may alleviate negative effects of extreme conditions on the fungal symbiosis. Based on the many farming interventions used in the African continent to improve crop production, it is important to evaluate their effects on mycorrhizal symbiosis. Improved tree fallow with *Sesbania sesban* and *S. macrantha* are effective fallows in the drought prone regions of eastern Zambia (Kwesiga *et al.* 1997). The effect of improved fallow on AMF symbiosis will be evaluated in this study.

2.3.2.2 Host effects

The host determines the boundary conditions within which the fungus is able to grow and reproduce (Morton 1993). Plant distribution in natural habitats is a reflection of the different demands for water and mineral nutrients from the soil. Each plant species has evolved mechanisms that facilitate uptake of water and nutrients within its habitat. These mechanisms range from physical modification of the root system to chemical exudates from the root system. The root structure and root exudates modify the root rhizosphere and affect the composition and functions of organisms at the rhizoplane and the rhizospheres of the plant (Atlas & Bartha 1986; Paul & Clark 1989). Plant roots are known to provide suitable habitat for the growth of microorganisms (Atlas & Bartha 1986).

Variation in response of host plants to mycorrhizal fungi is widely reported. Baylis (1970) linked differences in plant response to mycorrhizal symbiosis to the root structure. Fitter

(1985) and Douds and Schenck (1990) linked variation in hosts to demands for water, nutrients and allocation of carbohydrates while Morinda *et al.* (1992) and Chabot *et al.* (1992) noted specific plant metabolites exuded by roots, the flavinoids, to also explain the variation in mycorrhizal symbiosis. Azcon and Ocampo (1981) showed wheat cultivars inoculated with *Glomus mosseae* (Nicol & Gerd) Gerdemann & Trappe to differ in mycorrhizal dependence and associated absence of infection with lack of exudation of sugar by roots.

McGonigle and Fitter (1990) observed the species *Holcus lanatus* L. to be predominantly colonized by *Glomus tenue* (Greenhall) Hall and the roots of three other herbaceous species to be predominantly colonised by other mycorrhizal endophytes. Gaur *et al.* (1998) also noted the distribution of mycorrhizal fungal species to differ in two tree species with *Glomus* dominant in *Terminalia arjuna* (Roxb. Ex DC.) Wight & Arn. plots while *Gigaspora* was abundant in a *Populus euphratica* Oliv. plot. Plant species *Agropyron smithii* Rydb. and *Bouteloua gracilis* (HBK) Lag. Ex Steud. affected the fungal community of *G. mosseae*, *Glomus fasciculatum sensu lato* (Thaxter) Gerd. & Trappe, *Glomus macrocarpum* Tulasne & Tulasne, *Entrophospora infrequens* (Hall) Ames & Schneider, and an unidentified species (Stahl & Christensen 1982).

The effect of host plants on the mycorrhizal symbiosis has been reported to vary widely with the type of crops. Kormanik *et al.* (1980) noted corn, millet and sorghum as effective cover crops at increasing inoculum density of *Glomus* species with sorghum the most effective and corn the least. The differences at increasing inoculum density of *Glomus*

species was attributed to the differences in finer root system of sorghum and the coarser root system of corn. Dodd *et al.* (1990) also observed the increase in inoculum density to be dependent on the hosts that were previously planted. Studies in soybeans and bahia grass by Schenck and Kinlock (1980) showed the former to associate with *Scutellospora gregaria* (Schenck & Nicol.) Walker & Sanders, cited as *Gigaspora gregaria* Schenck & Nicol., and two *Gigaspora* species (*G. margarita* and *G. gigantea*), compared to the latter plant associating with numerous spores of *Glomus fasciculatum* and *Glomus clarum* Nicolson & Schenck. Khalil *et al.* (1992) also noted four *Glomus* species as most abundant around the rhizospheres of soybean, *Gigaspora* species as second most abundant and *Acaulospora* species the least. The different tree species used in agroforestry may also affect AMF species symbiosis differently. This study aims to evaluate the effects of agroforestry trees on AMF species.

Depending on the sites, the cropping history largely influenced the type of AMF species. Johnson *et al.* (1991) noted the occurrence of *G. aggregatum*, *Glomus leptotichum* Schenck & Smith now the glomoid morph of *Archaeospora leptoticha* (Schenck & Smith) Morton & Redecker and *Glomus occultum* Walker spores to be high with corn than soybean cropping history and *G. macrocarpum* to be high with soybean history at one site. At another site *G. aggregatum* was not affected by cropping history while three species *G. occultum*, *Glomus albidum* Walker & Rhodes and *G. mosseae* were more abundant in plots with corn history than soybean history. In a pot culture experiment with soils from a field site, Sanders and Fitter (1992) observed six plant species to cause differences in sporulation within a community of AMF species. The *Gliricidia*/maize intercrop and maize monocrop

and the *Sesbania* spp./maize intercrop and maize monocrop experimental sites have different cropping history.

In agroforestry practices, trees and shrubs vary in their ability to improve soil fertility and to coexist with crops. Differences were noted in the decomposition, nitrogen and mineral contents of foliage in *Gliricidia sepium* and *Sesbania sesban* (Mwiinga *et al.* 1994). Variation in growth rates, capacity to fix nitrogen, litter quality, root decomposition and resistance to drought exist in *S. sesban*, *Sesbania macrantha* and *G. sepium* (Kwesiga & Beniast 1998). They also noted that *S. sesban* was more susceptible to insect pests and was highly infected by nematodes compared with *S. macrantha*. The effects of the two *Sesbania* species on AMF symbiosis will also be evaluated.

The differences in survival of agroforestry trees to water stress in Malawi was reported by Maghembe & Prinns (1994) and suggested the mycorrhizal symbiosis as a prerequisite for survival of some tree species. There is evidence in survival of trees inoculated with AMF in water stressed semi-arid lands of Kenya (Wilson *et al.* 1992a), hence mycorrhizal associations have the potential to alleviate water stress in agroforestry species. Variation in root functions in *G. sepium* and *Grevillea robusta* A. Cunn. were attributed to root structure (Odhiambo 1999) while there is a possibility of exudation of organic acids speculated to reduce fixation of phosphorus by some shrubs such as *Tithonia diversifolia* (Hemsl.) Gray (Palm and Rowland 1997). There are no studies undertaken to determine mycorrhizal symbiosis in different agroforestry practices and yet most factors known to vary in trees would influence the quality and the function of the mycorrhizal symbiosis in

the system. *Sesbania sesban* and *S. macrantha* associate differently with the root-knot nematodes and differ in their effect on maize yield. Their effect on AMF symbiosis has not been determined.

2.3.2.3 Soil conditions

Soil is the part of the earth's crust that is a seat of biological activity (Russell 1969). Parks and Cousins (1995) conceived soil as a dynamic system and the core to life on earth maintaining primary producers. Kennedy & Smith (1995) described soil as a key to maintenance of plants and Sanchez (1995) linked insecurity and wars in Africa as a consequence of poverty resulting from a bad soil.

Most agricultural soils in Africa are deficient in nutrients and can hardly support optimum crop production. Nitrogen and phosphorus are the most limiting nutrients to crop production in the tropics (Sanchez 1976). In the study area, both nitrogen and phosphorus are deficient with nitrogen (0.09%) noted as the most limiting nutrient to plant growth (Ikerra *et al.* 1999). Optimum crop production in the region is therefore realized with inorganic fertilizers. The Government's removal of subsidies on fertilizer in Malawi has rendered it unaffordable to the majority of farmers. As a result low-input farming systems were encouraged (Kumwenda *et al.* 1995). Agroforestry farming system was recommended as a low-input practice with great potential for the region. Palm *et al.* (1997) noted the inadequacy of trees alone as a supplement in soil replenishment and recommended a complement with minimal inorganic fertilizers. Hence, agroforestry practices in the region

are complemented with minimal inorganic input. The effect of the low-input agroforestry system on AMF symbiosis has not been established.

Brundrett (1991) regarded the soil environment to establish the initial conditions for mycorrhiza establishment, with the soil acting directly on the hyphal thallus during pre-mycorrhiza formation and on extraradical hypha during later establishment and growth phases. The efficiency of mycorrhizal symbiosis was observed as the function of a particular set of soil conditions (Lambert *et al.* 1980). Haas and Menge (1990) noted edaphic conditions to explain for the differences in fungal species composition in avocado orchards of two sites with similar Mediterranean climates.

Soil

There are contradicting reports on the effects of soil conditions on mycorrhizal symbiosis. Most studies report the AMF symbiosis to function effectively at low fertility. Douds *et al.* (1993) noted biological based farming systems to have more abundant AMF spores than conventional farming systems. They also noted *G. gigantea* to be more numerous in low-input systems than conventional systems. There are also reports on enhanced mycorrhizal symbiosis under high soil fertility. Crush (1975) noted an increase in colonisation levels of sown legumes in fertilized soils and Land & Schonbeck (1991), in studies of arable fertile land, observed a high spore number of *Glomus* species and concluded that it was more adapted to fertile soils. No fertilizer effect on the mycorrhizal symbiosis has been documented by Cooke *et al.* (1992) who noted fertilization of forest soil to have no effects on the incidence of fungi. The variation in response by different fungal species to soil conditions led Sieverding (1991) to classify AMF according to their adaptations to soil

conditions. Classification of species according to adaptations has, however, not been confirmed.

Phosphorus is widely documented as the most important soil nutrient that explains the AMF symbiosis. The effects of phosphorus on the mycorrhizal symbiosis are variable, though generally it is associated with a reduction of the symbiosis. Nielsen *et al.* (1981) reported low numbers of mycorrhizal spores in all soils with high levels of phosphorus and Schwab *et al.* (1983) noted an increase in mycorrhizal formation in phosphorus deficient soils. Studies by Shukla and Vanjare (1990) confirm the declining trend of fungal population in oilseed crops with increase in phosphorus. In natural habitats, Allsopp and Stock (1994) noted a reduction in mycorrhizal colonisation with increase in soil phosphorus levels.

Response of AMF to phosphorus fertilization depends on the inherent phosphorus levels in the soil. Nielsen *et al.* (1981) observed the inherent soil phosphorus levels as a determining factor to mycorrhizal response to phosphorus fertilization. They observed no mycorrhizal response in soils with high phosphorus levels to phosphorus application. They also observed AMF species occurring in soils with low phosphorus status to decline to a greater extent with increasing phosphorus than endophytes from soils with high phosphorus status. This was confirmed by Katiyar *et al.* (1995) who noted a decrease in spore numbers with addition of low doses of phosphorus to P deficient soils.

Jasper *et al.* (1979) noted variation in sensitivity of AMF species to different phosphorus levels. Abbott and Robson (1984) noted shoot growth to increase with inoculation with *G. fasciculatum* at the lowest rates of phosphorus. Schubert and Hayman (1986) noted differences among endophytes in stimulating plant growth at different phosphorus levels with some endophytes showing potential value at low, medium and/or high phosphorus levels. The study site in Malawi has inherently a low phosphorous level although the selected sites had different histories of phosphorus fertilization.

Nitrogen is the most limiting nutrient in the tropics and it was the most limiting at the study site. Contrary to phosphorus, the role of the mycorrhizal symbiosis in nitrogen nutrition has been unnoticed. Sundaresan *et al.* (1988) observed variations in the ability of twelve mycorrhizal species in reducing nitrate to nitrite. There are contrasting reports on the effects of nitrogen on the AMF symbiosis. Early studies by Hayman (1970) reported a decline in colonisation and spore number with addition of nitrogen fertilization while Baltruschat & Dehne (1989) noted general a decline in mycorrhizae formation. In most recent studies Shukla and Vanjare (1990) confirmed nitrogen fertilizer to have adverse effects with increase in nitrogen gradually decreasing fungal populations to zero. Contrasting reports by Furlan and Bernier-Cardou (1989) indicate that nitrogen stimulates root colonisation and spore production. Recent studies by Sreenivasa and Bagyaraj (1990) also observed an increase in the number of spores with nitrogen increase in the form of nitrate and ammonium.

Balanced soil nutrition is acknowledged as a requirement for plant growth (Buresh & Smithson 1997). Sanchez *et al.* (1997) recommended replenishment of nitrogen to follow phosphorus replenishment in agroforestry systems. There are few studies on the effects of balanced soil nutrients on the mycorrhizal symbiosis. Hepper (1983) noted a dense colonisation at high levels of nitrogen compared to low nitrogen and suggested the ratio of nitrate to phosphate as the only explanation for the increase. Hall *et al.* (1984) also noted an increase in AMF symbiosis when phosphorus was combined with nitrogen and also attributed this to an increase in nitrogen:phosphorus ratio with application of nitrogen and suggested the possibility of nitrogen lowering plant phosphorus status. In most farming systems, application of both nitrogen and phosphorus fertilizer is recommended. In agroforestry systems, the two types of fertilizer are applied singly or/and in combination. The effects of combinations of the two fertilizer types on AMF symbiosis will be evaluated.

The advent of sustainable agriculture emphasizes use of organic supplements as soil amendments. The main thrust in agroforestry farming systems is the incorporation of leaf biomass into soil to enhance fertility. Organic inputs contain essential nutrients plus carbon, a source of energy for soil biota (Atlas & Bartha 1986) and thus supporting life in the soil and mediating all processes of nutrient replenishment. There is a decline in organic matter when natural vegetation is converted to cropland. In the miombo region of Zimbabwe, a higher soil macrofauna and microbial biomass was found in soils from the closed miombo than from a maize system (Campbell *et al.* 1998). Tree prunings are an important source of nitrogen for maize in the miombo ecozone (Mafongoya & Dzwowela

1998). Tree prunings are used in agroforestry systems to improve fertility. The effects of tree prunings on AMF symbiosis will be determined in this study.

Hayman (1982) noted better mycorrhizal development with addition of organic matter and St John and Coleman (1983) and Harinkumar and Bagyaraj (1988) observed addition of organic matter to increase the abundance of certain AMF species. These studies were recently confirmed by Verma and Arya (1998) who showed increase in mycorrhizal spore production, root colonisation, shoot phosphorus and height in tissue cultured-raised *Dendrocalamus asper* (Schult. & Schult. f.) Becker ex K. Heyne (bamboo) plantlets with organic manure amendments compared to a sand/soil medium. Negative effects on mycorrhizal symbiosis were reported by Nemec (1978) who noted spore numbers to negatively correlate with soil organic matter, and supported by Baltruschat and Dehne (1989) who noted green manure to have negative effects on inoculum potential in continuous monocropping barley. Whether the positive effects of the recommended agroforestry practice are also reflected on the mycorrhizal symbiosis, needs to be verified.

2.3.3 Functional diversity

Functional diversity is a recent concept and it has many meanings to biologists. Steele (1991) defined functional diversity as a variety of different responses to environmental factors while Zak *et al.* (1994) defined microbial functional diversity in terms of utilization of substrates.

The management and use of AMF species will not only depend on their adaptations to environmental conditions but also in their ability to utilize nutrients and improve plant growth. The mycorrhizal fungi alter plant processes. They promote plant growth by increasing absorption and nutrient transfer (Francis *et al.* 1986; Fogel 1980), improve soil aggregation (Sutton & Sheppard 1976; Forster & Nicolson 1981) and prolong root life and protect roots against soil borne pathogens (Harley & Smith 1983).

Generalization about mycorrhizal functions masks considerable functional diversity in fungal organisms (Kothari *et al.* 1991; Graham *et al.* 1982; Sanders *et al.* 1977) and yet considerable variation exists. AMF species enhance growth in a number of field crops (Ross & Hepper 1970; Khan 1972) and benefits of the mycorrhizal symbiosis are reported in tree species, some of which are commonly used agroforestry species (Michelsen 1993; Sharma *et al.* 1996 and Atayese *et al.* 1993). Plant growth responses showed highly specific responses to different species of mycorrhizal fungi (Allen *et al.* 1995).

Commercial production and utilization of AMF will depend largely on knowledge of the nature of the symbiotic associations. It will also depend on knowledge of the symbiotic efficiency and compatibility with a wide range of host plants. This knowledge is lacking for most AMF species, particularly for species from a similar habitat. Most AMF species tested for efficiency are from different geographical regions.