Influences of agricultural management practices on Arbuscular Mycorrhizal Fungal symbioses in Kenyan agro-ecosystems

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DEDICATION

My husband Samuel Muchai for inspiration and encouragement even when the journey seemed bleak

ABSTRACT

Conservation agriculture (CA) and integrated soil fertility management (ISFM) practices are receiving increased attention as pathways to sustainable high-production agriculture in sub-Saharan Africa. However, little is known about the effects of these practices on arbuscular mycorrhizal fungi (AMF). The study aimed at understanding the long-term effects of (i) ISFM and CA on AMF communities and functioning, and on glomalin concentrations. The study also aimed at understanding the (ii) role of AMF in soil aggregation, plant nutrition and crop yield under field conditions and (iii) combined effect of AMF and earthworms on soil aggregation, plant nutrition and crop yield under greenhouse conditions. The study was conducted in two long-term field trials. The ISFM trial was in Kabete (central Kenya) and compared fertilization (nitrogen and phosphorus) and organic amendments (farmyard manure, crop residue) for 32 years, while the CA trial was in Nyabeda (western Kenya) and compared effect of tillage (conventional versus no-tillage), residue application, cropping system (monocropping versus rotation) and N-fertilization for 5 years. Long-term use of mineral fertilizer and organic amendments, as well as tillage and N fertilization altered AMF species composition, but the changes were relatively minor. Organic amendments alone or in combination with NP fertilization increased AMF incidence, whereas no-tillage in the presence of residue increased spore abundance and root colonization. N fertilization increased root colonization but had a negative effect on spore abundance and species richness. Crop rotation had no effect on AMF. Glomalin was also sensitive to management, but the response was site-specific. Glomalin responded more to CA in Nyabeda than ISFM in Kabete. N fertilization and residue increased glomalin, especially under conventional tillage. Path analysis indicated that AMF symbiosis and glomalin enhanced soil aggregation and crop nutrition and yield in both sites. The positive role of AMF on crop nutrition was stronger in Kabete than Nyabeda. However, yield and nutrient use efficiency were (very) low in Kabete. There was no interaction between AMF and earthworms on soil aggregation, but AMF enhanced soil aggregation. AMF interacted positively with the epigeic earthworm to enhance nutrient uptake and biomass production, but the endogeic earthworm negatively affected AMF symbiosis and function. The study highlights the potential of ISFM and CA practices in enhancing AMF diversity and activity, and indicates factors limiting AMF functioning under ISFM and CA systems. While AMF are important for agro-ecosystem functioning, remedying the non-responsive character of soils, especially Kabete, through judicious management of nitrogen and organic amendments remains a first priority.

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CHAPTER 1

General Introduction

Soil fertility and agriculture in sub-Saharan Africa

Soil degradation through loss of soil organic matter (SOM) and soil fertility decline are considered major limiting factors for achieving household food sufficiency in the majority of tropical and sub-tropical agricultural systems (Scherr, 1999). In particular, loss of SOM, nitrogen (N) and phosphorus (P) deficiencies, and soil acidification are considered as major factors limiting plant growth and crop production in sub-Saharan Africa (SSA) (Okalebo et al., 2007). Despite numerous investigations that demonstrate positive yield responses to mineral nitrogen and phosphorus fertilizer, lack of access to these fertilizers due to their prohibitive costs limits their use in smallholder cropping systems (Odendo et al., 2006). Over the past decades, decline in fertilizer use has been observed for many small-scale farmers in SSA resulting in decline in soil fertility (Sanchez et al., 1997). Whereas use of fertilizer in agriculture has caused considerable yield increases, long-term use of high amounts of fertilizer has in some cases also contributed to decline of soil biodiversity and environmental degradation (Giller et al., 1997; Munyaziza et al., 1997; Plenchette et al., 2005). Conservation agriculture (CA) and integrated soil fertility management (ISFM) practices are currently receiving increased attention as pathways to sustainable and productive agriculture and reduction of off-site problems in SSA (Vanlauwe et al., 2010; Hobbs et al., 2008).

Conservation agriculture (CA) is proposed to restore degraded soils through three CA principles namely; (i) minimal soil disturbance, (ii) permanent soil cover through mulch and (iii) crop rotation (Hobbs, 2007; Hobbs et al., 2008). Reduced erosion, increased infiltration, soil moisture conservation, improved soil fertility, diverse microbial and macro-fauna communities, improved soil physical properties, higher water use efficiency, increased crop production and a variety of environmental services (reduced CO_2 emissions, carbon sequestration) are among the benefits associated with CA practices (Benites and Ashburner, 2003; Hobbs, 2007). However, CA is controversial in the context of African farming systems (Giller et al., 2009). Lack of adequate crop residues, disappearance of crop residues (mulch) through processes such as comminution by termites, and socio-economic factors (labour availability) are major problems limiting adoption of CA practices in SSA (Bationo et al., 2007).

Integrated soil fertility management (ISFM) comprises a set of practices that include combined use of mineral fertilizer and organic inputs alongside improved germplasm to maximize agronomic use efficiency of the applied nutrients (Vanlauwe et al., 2010). Improved soil organic matter contents through organic amendments under ISFM practices enhance nutrient availability, support diverse soil biota, improve soil aggregation and crop production (Vanlauwe

et al., 2011; Ayuke et al., 2011; Kamaa et al., 2011). However, SOM management to maintain SOM contents and soil quality under the current agricultural practices remains a major challenge.

The importance of soil micro-organisms in nutrient cycling and maintenance of soil physical properties is well appreciated (Bremer et al., 2007). In particular, soil micro-organisms are responsible for key ecosystem functions such as decomposition of organic matter and mineralization and cycling of nutrients, humus synthesis, aggregate stabilization, nitrogen fixation and the biological control of soil-borne pests and diseases (Altieri, 1999). Better exploitation of soil-plant-microbe interactions for plant nutrition, and maintenance of soil organic matter is therefore an opportunity that should be explored to enhance agricultural sustainability and productivity in the context of CA and ISFM technologies. Management of Arbuscular Mycorrhizal Fungi (AMF), belonging to phylum Glomeromycota (Schüßler et al., 2001), is considered highly relevant for low-input agricultural systems (Jeffries and Barea, 2001; Jeffries et al., 2003; Gianinazzi et al., 2010). AMF form mutualistic associations with plant roots. They account for a substantial amount of microbial biomass. The fungi benefit from a supply of sugars and the plants benefit from the increased absorbing capacity of mycorrhizal root systems (Smith et al., 2001). The AMF symbiosis has a wide occurrence, and AMF are known to associate with the roots of most angiosperms, gymnosperms, pteridophytes, and even bryophytes (Smith and Read, 2007; Smith et al., 2001).

AMF in agro-ecosystems

AMF determine physical, chemical and biological processes in soil. The AMF symbiosis plays a critical role in plant nutrition. The AMF external mycelium develops around the host plant roots and efficiently exploits a larger volume of soil than roots thereby enhancing mineral acquisition by the plant (Smith and Read, 2007). AMF are particularly important in phosphorus uptake (Koide, 1991, Ortas et al., 1996; Liu et al., 2000; Ortas et al., 2002). The external hyphae of AMF can extend >10 cm away from the root surface in the soil beyond the P depletion zone and access a greater volume of un-depleted soil than the root alone (Jakobsen et al., 1992). The small diameter of hyphae (2–5µm) of the fungal network allows the fungus to access soil pores that cannot be exploited by roots, enabling the AM plant to explore a greater volume of soil than non-mycorrhizal roots. AMF can also enhance P supply of the soil in acidic soils where phosphorus is mainly bound with Fe or Al through excretion of glomalin (Cardoso and Kuyper, 2006).

Unlike P, nitrogen (N), especially in its anionic form nitrate (NO_3^-) is mobile in the soil solution and therefore subject to leaching. Because of nitrate mobility the mycorrhizal symbiosis may not be important for the uptake of mineral N by the host plant. However, under N-deficient

conditions, growth of fungal hyphae in organic patches may be an effective way of supplying N to both the fungus and the host plant (Hodge et al., 2001; Leigh et al., 2009; Hodge and Fitter, 2010). AMF hyphae likely penetrate into the organic material and compete for mineralized NO_3^- or NH_4^+ with other microbes, resulting in increased N acquisition by the plant (Hodge et al., 2001). Extraradical mycelia of AMF convert acquired inorganic N (NO_3^- or NH_4^+) to arginine before transporting it to intraradical fungal structures where the amino acid is broken down and transported to the plant and assimilated into plant proteins (Govindarajulu et al., 2005; Jin et al., 2005). The AMF symbiosis is also associated with increased uptake of other macro- and micro-nutrients and enhanced water uptake (Liu et al., 2000; Augé, 2004; Birhane et al., 2012).

AMF are among the most important biological factors influencing soil structure (Smith and Read, 2007; Jastrow et al., 1998; Rillig et al., 2002; Rillig and Mummey, 2006). Extraradical hyphae of AMF create a skeletal structure that holds soil particles together initiating formation of macro-aggregates, and create conducive conditions for formation of micro-aggregates within macro-aggregates (Tisdall and Oates, 1982; Six et al., 2002). Due to its long residence time in soil and low palatability to fungivorous soil fauna, the AMF network is a major component of soil microbial biomass allowing for a more permanent contribution to soil aggregate stabilization than hyphae of saprobic fungi (Rillig et al., 2002; Purin and Rillig, 2007). AMF also produce glomalin, a glycoprotein currently described as a putative heat shock protein (Wright et al., 1996; Gadkar and Rillig, 2006). The exact nature of glomalin and related soil proteins is not yet resolved (Rillig, 2004). However, recent evidence shows that glomalin may contain other proteins of soil, plant and microbial origin (Rosie et al., 2006; Gillespie et al., 2011). These proposals prompted change of names from glomalin to glomalin-related soil protein (GRSP), and later to Bradford-reactive soil protein (BRSP), indicating the assay used in assessment (Rillig, 2004). In this thesis, I maintain the term glomalin. However, I do not wish to imply that glomalin can be equated solely with this putative heat shock protein produced by AMF; by the term glomalin I refer to the glomalin-related soil protein as quantified in the Bradford assay (Janos et al., 2008).

Glomalin abundance in soils ranges between 2 to > 60 mg g^{-1} (Treseder and Turner, 2007). It is recalcitrant to decomposition in soil and is suggested to have a residence time of 6 to 42 yr in tropical soils (Rillig et al., 2001). Glomalin is closely related to stable soil aggregate formation (Wright and Upadhyaya, 1998), except in soils where soil aggregate stability depends on other stabilizing agents (carbonates and clay) rather than on soil organic material (Rillig et al., 2001; Rillig, 2004: Borie et al., 2008). Due to its recalcitrance, hydrophobicity, and adhesiveness, it is thought to play an important role as cementing material of soil particles, and

at the same time acting as a highly stable form of organic C that represents an important fraction of total soil organic matter (Haddad and Sarkar, 2003; Rillig and Mummey, 2006). The role of glomalin in soil aggregation has remained correlative and the exact mechanisms involved in soil aggregation are unclear (Rillig, 2004). Glomalin contents are often, but not always, related to SOM contents (Treseder and Turner, 2007; Rillig et al., 2001). Glomalin appears an important source of organic matter in soil. Mycelial networks from AMF are supposed to have a high turnover rate (turnover time 6-7 days), but the glomalin they produce seems to by-pass the microbial processing imposed on fresh organic matter, thus contributing directly to the stable soil organic matter pool (Hamel and Strullu, 2006). AMF hyphal networks therefore possess the ability to modify the physical quality of the plant habitat through their contribution to soil organic matter build-up and stabilization of soil aggregates (Rillig and Steinberg, 2002). However, glomalin is sensitive to land use practices and its concentration can decline or increase through agricultural management (Wright et al., 2007; Fokom et al., 2012).

AMF are also important in influencing biological activity in the soil. Studies showing interactions between AMF and other soil biota, in particular earthworms, are increasing and their interactive role in crop nutrition and soil aggregation are being appreciated (Yu et al., 2005). Earthworms influence soil structure and aggregate stability through burrowing, casting and mixing of litter and soil (Six et al., 2004; Curry and Schmidt, 2007). They also change the spatio-temporal availability of C, P, and N through impacting on decomposition and nutrient mineralization resulting in improved plant growth (Scheu, 2003; Li et al., 2012b). AMF and earthworm interactions on plant performance can be antagonistic or synergistic and vary from increased plant nutrient uptake and productivity (Li et al., 2012a; Li et al., 2012b; Ma et al., 2006; Yu et al., 2005) to no interactive effects (Milleret et al., 2009a). AMF are also affected by soil fauna through various mechanisms. Earthworms disrupt and consume mycelial networks thereby diminishing hyphal effectiveness (Pattinson et al., 1997). Earthworms may directly enhance nutrient availability in soil through increased nutrient mineralization in casts and burrow-linings which indirectly influences AMF growth and function (Li et al., 2012b). This effect, however, depends on the accessibility of these available nutrients for AMF and plants through their ability to find earthworm-formed patches (casts, faeces, and burrow-linings). Earthworms also consume spores of AMF; because these spores survive gut passage, earthworms contribute to dispersal of AMF inoculum (Reddell and Spain, 1991). Casts contain viable spores, numbers being usually higher than those in the surrounding soil (Reddell and Spain, 1991; Gange, 1993; Lee et al., 1996). One possible explanation for the occurrence of spores in earthworm casts is selective foraging by earthworms in the rhizosphere of senescent roots;

consumption of spores (without digestion) would then likely entail consumption of the external mycelium (also without complete digestion). A consequence could then be that the structural stability of macro- and micro-aggregates in earthworm casts is (co-)determined by the presence of mycelial fragments and glomalin from these AMF. However, only few studies exist on the interactive role of AMF and earthworms on soil aggregation (Milleret et al., 2009a).

Effect of agricultural management practices on AMF

Because AMF propagate only in the presence of plants, their diversity and function respond rapidly to changes induced by soil disturbance, nutrient management and crop diversification (Douds and Millner, 1999; Jansa et al., 2002; Gosling et al., 2005; Plenchette et al., 2005; Mathimaran et al., 2007). Soil disturbance through tillage, nutrient management and cropping systems are major drivers of AMF change in agro-ecosystems and different AMF species respond differently to management changes in agro-ecosystems (Verbruggen and Kiers, 2010). It is well appreciated that differential function of AMF depends on fungal species and plant species and genotype. The overall effect of AMF symbiosis varies from positive, neutral or negative depending on the identity of fungus, plant host and the environmental context such as nutrient availability and agricultural practices (Bever, 2002; Klironomos, 2003; Reynolds et al., 2005; Reynolds et al., 2006; Hoeksema et al., 2010). Interactions between fungal identity, plant identity and the environmental context make such overall effects sometimes difficult to predict. The functional differences between AMF species belonging to the *Glomeraceae* or *Gigasporaceae* have been highlighted by Hart and Reader (2002a, b). Members of the *Gigasporaceae* are slower root colonizers but produce more extra-radical mycelium and therefore are better soil colonizers. This latter factor could imply that members of the *Gigasporaceae* are more important in soil structure formation and maintenance. However, they seem to be less efficient in transferring P to the host plant. Members of the *Glomeraceae* colonize roots faster and contribute more to nutrient uptake (Dodd et al., 2000; Hart and Reader, 2002a, b). This implies that a change in AMF communities could result in a shift in functional benefits conferred to the crops by these AMF, and an overall effect on plant productivity. In order to ensure AMF sustainability for maximum benefit in low-input agricultural systems, understanding of the ecology of AMF species is therefore imperative. Hereafter, I address the most important management practices affecting AMF and possible consequences for agro-ecosystem functioning.

Nutrient management (Mineral Fertilizer versus Organic Amendments)

Nutrient management is essential for maintenance of soil fertility and crop production. Soluble

fertilizers are often used for maintenance of soil nutrient levels. However, recent evidence has shown that long-term use of soluble fertilizer can lead to soil rigidity through rapid loss of soil organic matter and decline in microbial diversity (Plenchette et al., 2005). Organic resources can increase soil organic matter and enhance soil microbial communities, but they often provide insufficient nutrients to build up longer-term soil fertility and sustain crop yields (Palm et al., 2001). Combined use of organic amendments and soluble fertilizers (ISFM) is therefore proposed for long-term build-up and maintenance of soil fertility and soil organic matter and diverse microbial communities (Vanlauwe et al., 2010). ISFM practices restore and maintain soil fertility, but little is known about their effects on soil biota, in particular AMF.

Generally, formation and function of AMF is affected negatively by higher soil fertility (Grant et al., 2005). Continuous use of soluble fertilizers negatively impacts on total populations of AMF and may stimulate some species while reducing others (Howeler et al., 1987). Agriculture with application of high levels of inorganic fertilizers resulted in a shift in AMF community structure and reduced AMF diversity (Mäder et al., 2000; Kahiluoto et al., 2001; Oehl et al., 2004). This decline is due to readily available soil P (and N), resulting in high plant tissue P due to increased uptake. Schwab et al. (1991), Akiyama et al. (2005), López-Ráez et al. (2008) and Garcia-Garrido et al. (2009) suggested that at higher plant tissue P concentration, plants tend to reduce root exudation such as of strigolactones (a group of apocarotenoids) that act as signal molecules for spore germination and / or hyphal branching of AMF. Reduced exudation results then in low AMF colonization and spore production. Plants also allocate relatively more photosynthate to shoots and leaves and less to roots and AMF when they become enriched with mineral nutrients (Marschner et al., 1997; Johnson, 2010). Members of *Glomeraceae*, which have low requirements for carbohydrates, dominate fertilized agro-ecosystems while members of *Gigasporaceae* decline due to their higher C requirement (Egerton-Warburton and Allen, 2000; Treseder and Allen, 2002; Johnson et al., 2003). In nutrient-deficient soils, increased soil P status following P fertilization may not be sufficient to induce decreases in strigolactones in roots and AMF diversity and colonization may be stimulated (Mathimaran et al., 2007; Muchane et al., 2010).

Alternatively, low-input systems such as organic farming have been shown to be more favourable to AMF diversity and mycorrhizal root colonization (Gosling et al., 2005). Positive effects of organic amendments on AMF are attributed to low P contents in organic inputs that are released slowly over time (Ryan et al., 1994). Organic inputs are also associated with increased levels of soil organic matter, improved soil structure, water retention capacity and microbial activity that stimulate AMF growth (Ryan et al., 1994). AMF root colonization, external AMF

hyphal length and spore abundance are higher in organically-managed systems compared to conventional systems (Ryan et al., 2004; Bending et al., 2004; Oehl et al., 2003, 2004). Members of *Acaulospora* and *Scutellospora* are often more abundant in organic systems than conventional systems (Oehl et al., 2004; Lekberg et al., 2008). However, organic amendments with high available P (animal manure) may reduce AMF diversity and colonization (Jordan et al., 2000; Wang et al., 2011).

Effects of available P on AMF may also vary depending on the availability of other nutrients in the soil. Low P in combination with high concentrations of other nutrients (N) are associated with increased AMF colonization and external hyphal growth while high P concentration accompanied with high concentrations of the other nutrients (N) depresses AMF colonization and hyphal growth (Liu et al., 2000; Valentine et al., 2001). Johnson (2010) provided a trade balance model showing that the interaction between soil N and P availability on the one hand and C supply from the plant and demand by the fungus on the other hand affects AMF activity and function. In her model, four scenarios were predicted. Soils deficient in both N and P support beneficial AM symbioses, but C limitation reduces C-for-P trade resulting in C-limited mutualism. At high N and low P availability strong mutualistic benefits are predicted because a luxury supply rate of N increases the photosynthetic capacity of the host plant. At high P supply and N-deficient conditions benefits of C-for-P trade are eliminated and competition for N and C between plants and fungi are predicted that keeps the fungal C sink in check because N limitation prevents proliferation of the AM fungus. By contrast, when neither N nor P is limiting, fungal growth is only limited by C so the fungal C demand can increase to the point where it may depress plant growth and generate a parasitic (antagonistic) interaction. Soil N: P ratio (and plant N: P ratio) is thus crucial in sustaining AMF activity and functioning in agricultural systems.

Effects of nutrient availability on glomalin concentrations in soils are, however, inconsistent and vary from positive to no effects (Lovelock et al., 2004; Wuest et al., 2005; Antibus et al., 2006). Soil degradation under long-term use of fertilizer may result in no or negative effects on glomalin contents whereas in nutrient-deficient soil, fertilization may lead to improved plant and fungal growth resulting in increased glomalin concentrations (Treseder and Turner, 2007). Use of organic inputs is associated with build-up of soil organic matter and improved AMF communities and soil carbon, resulting in higher glomalin concentration in soils (Valarini et al., 2009; Nie et al., 2007). It is, however, not yet clear whether combined use of organic and inorganic fertilizer would result in enhanced glomalin production.

Soil disturbance (Tillage versus No-tillage)

Conventional tillage (CT) is an integral part of modern intensive agriculture that can modify the physical, chemical and biological properties of a soil (Kabir, 2005). CT disrupts soil aggregates inducing rapid decomposition of soil organic matter and potential loss of C and N (Six et al., 2004). These changes in soil structure can affect soil water, temperature, aeration, increase soil erosion and negatively affect microbial communities. Conservation tillage or no-till systems (NT) under CA practices tend to concentrate crop residues near the soil surface, usually associated with increased soil organic matter contents, improved soil physical properties, increased protection from soil erosion and a more diverse microbial community (Hobbs, 2007). NT systems are associated with a higher AMF diversity and enhanced functioning, while CT systems, used in the management for maximum crop production, have been shown to negatively impact AMF diversity and functioning (Jansa et al., 2003; Castillo et al., 2006). Disruption of the AMF hyphal network is a proposed mechanism by which soil tillage reduces root colonization and hence nutrient absorption (Kabir, 2005; Borie et al., 2008). In undisturbed soil, AMF hyphal length can be substantial (> 20 m g^{-1}), but this can decrease to even less than 0.5 m g^{-1} in cultivated soils (Borie et al., 2006). External hyphae and colonized root fragments are also transported to the upper soil layer during tillage, decreasing and diluting their activity as viable propagules for succeeding crops (Boddington and Dodd, 2000; Kabir, 2005; Borie et al., 2008). Hyphal growth from the extraradical network is considered the main source of inoculum when the host is present and soil is not disturbed, and as a result, disruption of AMF hyphae is connected to low AMF colonization in tilled soil (Evans and Miller, 1990; Kabir et al., 1998; Kabir, 2005). Moreover, AMF propagules such as spores and mycorrhizal root fragments are intimately associated with a more efficient AM hyphal network that is damaged through tillage (Kabir et al., 1998). Consequently, AMF species that proliferate mainly through spores like members of the *Gigasporaceae* decline in tilled soil whereas members of the *Glomeraceae* with their ability to proliferate from spores and other AMF propagule sources such as hyphal fragments and colonized root fragments dominate (Boddington and Dodd, 2000; Jansa et al., 2003; Castillo et al., 2006). Tillage also selects for fast-growing AMF species that are often less beneficial to crop nutrition (Johnson et al., 1992). Moreover, CT systems are often associated with low glomalin concentrations due to high turnover rates of macro-aggregates to micro-aggregates leading to glomalin decomposition and loss (Wright et al., 2007). However, less intensive tillage may not always result in reduced AMF diversity, colonization, glomalin content, nutrient uptake or yield due to a wide range of benefits associated with tillage such as increased N mineralization, increased soil temperature, reduced weed numbers and improved soil physical

properties all of which may impact on the AMF association (Gavito and Miller, 1998; Galvez et al., 2001). More often than not, tillage practices in SSA are not very intensive. Due to low productivity, and hence low levels of residues (mulch), CA also has a relatively small impact (Kihara et al., 2012a). Understanding how AMF community composition and structure respond to tillage practices is therefore important to maximize their functioning for improved soil quality and crop production.

Cropping systems

Crop rotation constitutes a major pillar of CA practices essential for achieving sustainable agricultural production. Diverse cropping systems that include legumes in rotation can change the soil habitat by affecting nutrient status and nutrient use efficiency, crop production, soil aggregation and the microbial habitats, and can stimulate soil microbial diversity and activity (Gan and Goddard, 2008). In particular, crop rotation can affect the diversity and functioning of AMF. Cropping systems that include highly mycorrhiza-dependent or responsive plants can increase AMF populations and consequently improve colonization and AMF functioning for subsequent crops (Thompson, 1987; Plenchette et al., 2005). AMF species generally have a broad host range and a low host specificity or selectivity. Individual species of AMF are known to associate with diverse plant groups from agricultural crops, herbs to long-lived woody perennials (Molina et al., 1992). However, preferential associations between some AMF species or strains and plant species or even genotypes have been reported (Bever et al., 1996, 2001; Vogelsang et al., 2006). Relative growth rates of AMF have been shown to depend greatly on the identity of the plant with which they are associated (Eom et al., 2000; Bever, 2002). These differential responses of AMF to host plant species may regulate AMF species composition and diversity. Mycorrhizal development in the field is dependent on cropping systems and cropping sequence of plants that exhibit a range of mycorrhizal dependencies (Plenchette et al., 2005). Introduction of crops that are strongly mycorrhizal enhances the population of native AMF (Howeler et al., 1987). Cultivation of non-host crops declines AMF propagules in the soil resulting in low AMF colonization of succeeding crops, associated with lower yields (Karasawa et al., 2002). Decline in AMF colonization and consequently yield of crops following non-mycorrhizal crops or low-mycorrhizal crops result in low AMF propagule numbers and delayed AMF colonization of the subsequent crop (Karasawa et al., 2001; Karasawa et al., 2002). Continuous mono-cropping can decrease populations of AMF spores and shift the AMF species composition of the community toward species that may not be beneficial to the crop (Johnson et al., 1992). It is suggested that crop diversification through rotation in low-input systems plays a

decisive role in increasing AMF colonization potential in the soil (Mäder et al., 2000). Recent studies have shown a shift in AMF composition following maize-crotalaria fallow rotation in comparison to maize mono-cropping (Mathimaran et al., 2007). According to Mathimaran et al. (2007), *Gigasporaceae* species were found to be more dominant in comparison to *Glomeraceae* species following maize-crotalaria fallow rotation. There is also evidence that composition of the plant community can influence soil glomalin stocks (Rillig et al., 2002). Plants with extensive root system may increase glomalin content compared to those with less extensive root systems (Bird et al., 2002). Presence of non-mycorrhizal crops in a rotation may decrease glomalin content in soil (Wright and Anderson, 2000). Management of cropping systems for the benefit of mycorrhizal associations through manipulation of cropping sequence and diversification of crops in rotation as proposed under CA practices may be a more direct route towards benefiting from mycorrhizal associations, especially in the tropics where agricultural systems rely more on plant-(beneficial) soil biota interactions (Cardoso and Kuyper, 2006).

Scope of the study

Agriculture in SSA is dominated by small-scale farmers who account for 75% of total agricultural production. Soil degradation is common in small-scale farms due to continuous cropping with little or no use of organic amendments and/or mineral fertilizers. The low-input ISFM and CA practices offer an opportunity to enhance agricultural sustainability through improved soil fertility and enhanced soil organic matter contents. Biological resources such as AMF can contribute to enhanced agricultural sustainability through enhanced soil aggregation and crop nutrition in the context of ISFM and CA practices. However, these practices are often developed and implemented without taking into consideration soil biota (AMF) despite extensive evidence that they can make a major contribution to physical, chemical and biological soil quality (Cardoso and Kuyper, 2006). Understanding the relationship between agricultural practices and management, AMF diversity and agro-ecological functioning is therefore crucial to optimally manage these beneficial fungi and maximize their functioning for improved soil quality and crop production.

This thesis was therefore undertaken to understand the impacts of ISFM and CA practices on AMF communities and their functioning in Kenyan agro-ecosystems. The study also endeavoured to understand how AMF communities and activity changes relate to soil physical conditions, crop nutrition and productivity with the aim of providing an opportunity for gaining insights into the specific effects that land use change, management practices and agricultural intensification have on the abundance, species composition, and functioning of AMF. Such

information will be helpful in management of biodiversity, productivity and in choosing management practices that are compatible with sustainable crop production.

Objectives

The aim of the thesis was to assess (i) AMF communities and glomalin contents in proposed sustainable practices (organic inputs, no-till systems and crop rotations) in comparison to more intensively managed systems (inorganic fertilizer use, conventional tillage and continuous cropping). The study also aimed at (ii) assessing the role of AMF and glomalin on soil aggregation, crop nutrition (P, N) and crop productivity as affected by different management practices. Lastly, the study (iii) assessed the individual and combined effects of earthworms and AMF on some soil physical (soil aggregation) and chemical (uptake of N and P) properties. In this thesis the following major questions are addressed:

- What are the effects of nutrient management (inorganic fertilizer and organic amendments) and agricultural practices (tillage and cropping systems) on AMF communities and their activity?
- How do AMF communities (diversity, hyphal length, root colonization, glomalin concentrations) relate to soil structure (macro- and micro-aggregates), crop nutrition and production?
- Is there an interaction between AMF species and earthworm species in creating and maintaining soil structure (water-stable micro- and macro-aggregates)?

Study site

The study was conducted in two long-term trials in Kenya. The trial in Kabete was established in 1976 in central Kenya and was under continuous cropping for 32 years. The trial in Nyabeda was established in 2005 in a sub-humid site in western Kenya and was under continuous cropping for 5 years (Figure 1.1). The two sites are characterized by two rainy seasons: the long rains from mid-March to June and the short rains from mid-October to December. Maize (*Zea* mays) is the main staple crop and is normally grown either as a mono-crop or in association with legumes, mainly common bean (*Phaseolus vulgaris*) and groundnut (*Arachis hypogaea*). Soybean (*Glycine max*) is being adopted as a cash crop, especially in western Kenya. Both zones have predominantly smallholder settlements, with land sizes ranging from 0.3 to 3 ha per household. Table 1.1 shows the general climatical and soil characterization of the two sites.

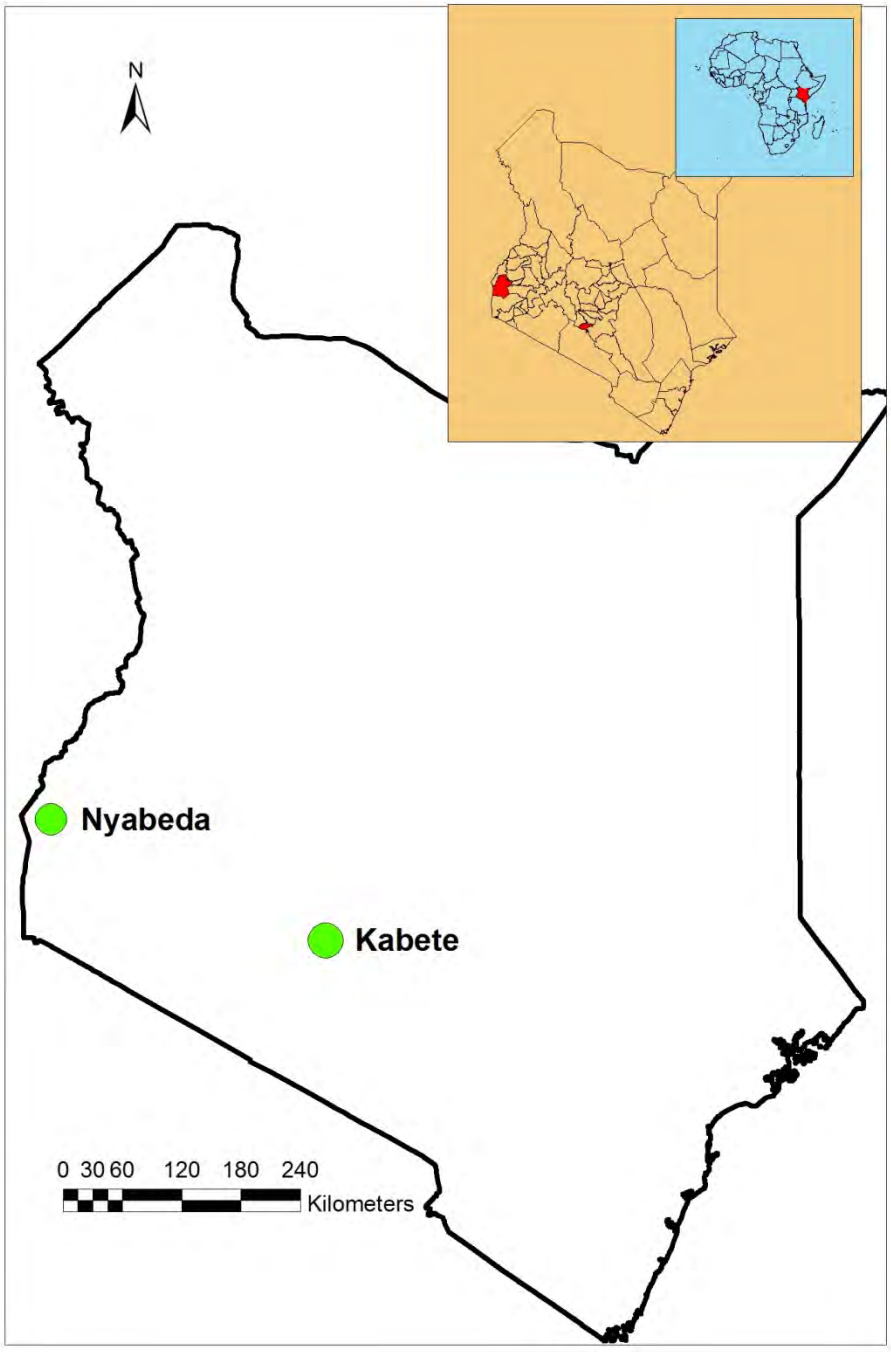

Figure 1.1 Location of study sites.

Table 1.1 Location, climatic and soil characteristics of the study sites. Climatic characteristics were taken from Kibunja et al. (2012) for Kabete and Kihara et al. (2012a) for Nyabeda. Soil characteristics are mean values in the 0-30 cm horizon described during the study period (2008).

Parameter	Kabete	Nyabeda
Agro-climatic zone	Sub-humid (II)	Humid (I)
Latitude	1^0 15' S	0^0 07' N
Longitude	36^0 41'	34^0 24' E
Altitude (m, a.s.l)	1740	1420
Rainfall (mm)	800-1400	1200-1600
Daily Temperature (^{0}C)		
Mean	18	23.2
Minimum	12.6	14
Maximum	23.8	31
Soil type (FAO, 1990)	Humic Nitisol	Ferrasol
Sand: silt: clay ratio	11:22:67	15:21:64
pH (in water)	5.4	5.3
Extractable K (mg kg^{-1})	578.2	84.3
Available P (Olsen, mg kg^{-1})	24.3	10.6
Ca (mg kg^{-1})	1238.9	1036.1
Mg (mg kg^{-1})	231.3	215.9
Total SOC (g kg^{-1})	18.6	18.5
Total nitrogen (g kg^{-1})	1.4	1.5

Outline of the thesis

Chapter 1 provides background on agriculture, describes the importance of AMF for agriculture and the impact of agricultural practices on AMF communities and functioning, and introduces the two study sites in Kenya. Chapters 2 and 3 examine how soil and cropping systems management practices affect AMF diversity in agro-ecosystems in Kabete and Nyabeda. Soil and cropping systems management practices included use of inorganic fertilizer (applications of N and P) and organic amendments (farmyard manure and crop residues), tillage intensity (till versus no-till) and cropping systems (continuous maize cropping versus maize-soybean rotations). Chapter 4 examines glomalin contents across different agricultural practices. Chapter 4 examines the relations between AMF and ecosystem functioning across different management

practices with emphasis on the interactive effects of soil fertility management and AMF diversity on soil aggregation, crop nutrition and production. Chapter 5 explores how agricultural practices and nutrient management affect glomalin content in the whole soil and in soil aggregates of different size classes in both sites. Chapter 6 examines the interaction between AMF and earthworms on soil aggregation as well as on P and N nutrition under greenhouse conditions. In Chapter 7 I synthesise the results of the various chapters and evaluate the results against the background of existing theories, and make suggestions for further research.

CHAPTER TWO

Long-term effects of mineral fertilizer and organic amendments on Arbuscular Mycorrhizal Fungal communities

This chapter will be submitted as: M. N. Muchane, J. Jefwa, B. Vanlauwe and T.W. Kuyper. Long-term effects of mineral fertilizer and organic amendments on Arbuscular Mycorrhizal Fungal communities. *Soil Use and Management*

Abstract

A study was carried out to characterize long-term impacts of mineral fertilizer (nitrogen and phosphorus) and organic amendments (farmyard manure and crop residue) on the diversity and activity of Arbuscular Mycorrhizal Fungi in a long-term field trial in central Kenya. The trial, which was established in 1976, was a three-factor experiment with mineral fertilizer (three rates - zero, 60 kg N and 26.4 kg P ha^{-1}; and 120 kg N and 52.8 kg P ha^{-1}), farmyard manure (three rates - zero; 5 t ha^{-1} and 10 t ha^{-1}) and crop residue (absent or present). We calculated species richness, Shannon diversity, and spore abundance in order to identify patterns of AMF community composition in each treatment. AMF activity was measured by assessing mycorrhizal inoculum potential, hyphal length and colonization in the field. A total of 16 AMF species belonging to five genera (*Acaulospora*, *Entrophospora*, *Glomus*, *Gigaspora* and *Scutellospora*) were recorded. *Acaulospora* species accounted for more than 50% of the total spore abundance. Both long-term use of mineral fertilizer and manure caused a shift in AMF species composition but the effect was small. Manure reduced abundance of two species of *Acaulospora* and one of *Entrophospora*, while NP fertilizer reduced abundance of three species of *Acaulospora*, two of *Entrophospora* and two of *Glomus*. Residue addition had no effect. Species diversity of AMF in the field was affected neither by NP fertilization nor by organic inputs. However, NP fertilizer and manure reduced spore abundance and species richness by 25% in trap cultures. Combined use of inorganic fertilizer and organic amendments increased AMF hyphal length, root colonization and inoculum potential. The implications of long-term use of mineral fertilizer, manure and residue application on AMF communities are discussed.

KEYWORDS: Arbuscular Mycorrhizal Fungi, diversity, mineral fertilizer, nitrogen, phosphorus, farmyard manure, residue

1. Introduction

Low inherent soil fertility in sub-Saharan Africa largely accounts for low and unsustainable crop yields (Okalebo et al., 2006). Integrated soil fertility management (ISFM) combining soluble fertilizer and organic inputs (farmyard manure and plant residue) has been proposed for restoration and maintenance of soil fertility. ISFM is receiving increased attention as a pathway to sustainable high-productive agriculture (Vanlauwe et al., 2010). In addition to restoring soil fertility, ISFM has potential in maintaining diverse microbial and macro-fauna communities (Vanlauwe et al., 2011; Ayuke et al., 2011; Kamaa et al., 2011). However, little is known how ISFM contributes to restoration of communities of arbuscular mycorrhizal fungi (AMF).

AMF are ubiquitous root-symbiotic fungi of phylum Glomeromycota (Schüßler et al., 2001). These fungi form a substantial part of microbial biomass in the soil. AMF contribute to biological, chemical and physical soil quality (Cardoso and Kuyper, 2006). In particular, AMF play a crucial role in plant nutrition (Smith and Read, 2007), water uptake (Augé, 2004), and are an important factor influencing soil structure (Rillig and Mummey, 2006). Since AMF form symbiotic associations with plant roots, and only propagate in the presence of living plants, AMF diversity and activity respond rapidly to changes induced by land management such as cropping systems, soil disturbance, and fertilizer application (Verbruggen and Kiers 2010). AMF may therefore be an early and sensitive indicator of environmental change and health (Oehl et al., 2010; Verbruggen and Kiers, 2010).

Intensive agriculture with high levels of mineral fertilizer may have a negative influence on AMF communities compared to low-input agriculture (Gosling et al., 2006). Application of soluble fertilizers (N and P) is associated with increased availability of soil nutrients that not only affect the total population of AMF, but also stimulate some species while reducing others (Verbruggen and Kiers, 2010). In the presence of readily available nutrients (P and N), plants tend to decrease C allocation to their fungal partners, resulting in decreased AMF activity and spore production (Treseder and Allen, 2002). Low C allocation to roots may in turn lead plants to selection of AMF species with lower carbon requirement, while those with higher C requirement decline (Johnson et al., 2003). Generally, high levels of P and N in soil are associated with increases of *Glomeraceae* species and decline of members of *Gigasporaceae* (Egerton-Warburton and Allen, 2000; Treseder and Allen, 2002; Johnson et al., 2003). However, the effect of fertilization on AMF may also vary depending on the availability of other nutrients and nutrient supply imbalances especially mismatches in levels of N and P (Valentine et al., 2001). Low P availability in conjunction with medium-high supply of N has been associated with increased AMF colonization and extra-radical hyphal development, while high P availability

with high supply of other nutrients has been found to depress AMF development (Liu et al., 2000; Valentine et al., 2001; Treseder and Allen, 2002).

Organic sources of nutrients, such as farmyard manure, compost and crop residues have been shown to stimulate AMF communities (Gosling et al., 2006). Effects of organic amendments on AMF have often been attributed to increases in organic matter, which improves soil structure, water retention capacity and microbial activity (Ryan et al., 1994). Organic inputs are also associated with low phosphorus (P) contents that are gradually released over time, resulting in increases in plant demand for P and consequently increases in C allocation to AMF leading to improved AMF colonization and AMF diversity (Bending et al., 2004). High C allocation to AMF also stimulates proliferation of *Gigasporaceae* and *Acaulosporaceae* species (Oehl et al., 2004). However, the precise effects of organic amendments have been unpredictable when applied on any given soil. The effects of organic amendments on AMF community composition (Wang et al., 2011; Franke-Snyder et al., 2001; Galván et al., 2009, Vestberg et al., 2011) are poorly known. Only very little is known about the impact of long-term mineral fertilization and organic inputs on AMF communities in Kenya (Mathimaran et al., 2007; Muchane et al., 2010). Further studies to enhance our understanding of effects of organic amendments are thus desirable.

In this chapter, we propose a comparative approach to our core hypotheses: NP fertilization and organic inputs (manure and crop residue) will enhance species richness of the indigenous AMF community; AMF colonization and AMF diversity will be higher in management systems using organic amendments compared to systems with mineral fertilizers. Specifically, we hypothesized that:

- Inorganic (NP) fertilizer reduces AMF communities (diversity, species richness, spore abundance, hyphal length and colonization)
- Inorganic fertilizer (NP) causes a shift in AMF species composition (resulting in higher abundance of *Glomeraceae* species and lower abundance of *Gigasporaceae* species).

Our aim was to gain an understanding on how the long-term use of mineral fertilizer (N and P) and organic amendments (farmyard manure and crop residue) affects AMF diversity, species composition and activity in Kenyan agro-ecosystems to ensure an opportunity for their utilization and management.

2. Site and Methods

2.1 Study site

The study was conducted at Kabete long-term field trial established in 1976 at the National

Agricultural Research Laboratories (NARL) of the Kenya Agricultural Research Institute (KARI) situated at Kabete, 36°41′E and 1°15′S, 1740 m above sea level. The site is located 7 km North West of Nairobi. The soil is well drained and very deep, consisting of a dark reddish brown to dark red, friable clay. It is classified as a Humic Nitisol (FAO) and is locally referred to as the Kikuyu Red Clay. The soil before the onset of the experiment had 67% clay, 11% sand and 22% silt (Siderius and Muchena, 1977). Precipitation is bimodal with a long rainy season between mid-March to June and the short rains from mid-October to December. The mean temperature ranges between 13° C and 18° C. The area falls under ecological zone III (dry sub humid) with a precipitation to evaporation ratio of 56% (Siderius and Muchena, 1977).

2.2 Experimental design

The experiment was set to compare the effects of different combinations of mineral fertilizer (nitrogen and phosphorus fertilization; NP) and organic inputs (manure; residues) on the performance of maize (*Zea mays*) and bean (*Phaseolus vulgaris*). The experiment was a factorial experiment with three factors, resulting in 18 treatments (3 x 3 x 2) in a complete randomized block design with three replicates: (1) mineral fertilizer (three levels – no fertilizer; 60 kg N and 26.4 kg P ha^{-1}; 120 kg N and 52.8 kg P ha^{-1}); (2) farmyard manure (FYM: three levels – no manure; 5 t ha^{-1}, equivalent to 10.3% SOC ha^{-1} yr^{-1} and 10 t ha^{-1} equivalent to 20.5% SOC ha^{-1} yr^{-1}); (3) residue (retained versus removed at the end of the long rainy season). Treatment codes are summarized in Table 1. Fertilizer and FYM were applied once per year during the long rainy season, while maize stover was retained annually at end of the long rainy season. Bean residues were completely removed from all treatments after harvest. Nitrogen was applied as calcium ammonium nitrate while P was supplied as triple superphosphate. Plot sizes were 7.0 × 4.5 m. The experiment consisted of maize-bean rotation where maize was grown in the long rainy season, while bean was grown in the short rainy season. Both crops were planted at a spacing of 75 cm x 25 cm, giving a plant population of 50, 000 plants ha^{-1}.

2.3 Soil sampling

Soil for chemical properties and AMF spore assessment was sampled once at the end of the short rainy season (early March, 2008) at two sampling depths (0-15 and 15-30 cm). Soil sampling was done after harvesting of common bean, at the end of the dry season when sporulation is expected to be highest, and when spores are in better condition for identification (Douds and Millner, 1999). AMF extraradical hyphal length was assessed twice (8 weeks after sowing [WAS] in May & 1 week after harvest in September, 2008) during the long rainy season with

maize growth and three times during the short rainy season (4 WAS in November, 8 WAS - December, 2008 and 12 WAS - January, 2009). Roots for AMF colonization were collected once during long-rains season (8 WAS - May, 2008), and twice in short-rains season (4 WAS – November and 8 WAS - December, 2008). Ten random samples were taken from different depths (0-15 and 15-30 cm) in each experimental plot and mixed thoroughly for each depth to obtain a composite sample.

2.4 Soil analysis

Soil analysis was performed at soil analysis laboratory of the World Agroforestry Centre (ICRAF), using near-infrared spectroscopy (NIRS: Shepherd et al., 2003). Soils were first air-dried and passed through a 2-mm sieve. Using the spectral library approach (Shepherd et al., 2003) a subsample of one-third (35 samples) of the total 108 samples was selected for wet chemistry analysis based on their spectral diversity. This was done by conducting a principal component analysis of the first derivative spectra and computing the Euclidean distance based on the scores of the significant principal components. Random samples were then selected from each quartile of the ranked Euclidean distances to make up the 35 samples for analysis by wet chemistry. The 35 selected soil samples were analysed following standard methods for tropical soils (Anderson and Ingram, 1993). Soil pH was determined in water using a 1:2.5 soil : solution ratio. Samples were extracted with 1M KCl using a 1:10 soil : solution ratio, by atomic absorption spectrometry for exchangeable Ca and Mg. Phosphorus and K were extracted with 0.5 M $NaHCO_3$ + 0.01 M EDTA (pH 8.5, modified Olsen) using a 1:10 soil : solution ratio. Exchangeable K was analysed by flame photometer and available P was analysed colorimetrically (molybdenum blue). Organic C (SOC) was determined colorimetrically after H_2SO_4 - dichromate oxidation at 150° C for 30 min. Total N was determined by Kjeldahl digestion with sulphuric acid and selenium as a catalyst. Effective cation exchange capacity (ECEC) was calculated as the sum of exchangeable bases. The results of the 35 selected soil samples were used in prediction of soil properties using the near-infrared spectroscopy by partial least-squares regression (PLSR, Shepherd et al., 2003). For each soil property, a calibration model using PLSR was developed and used to predict the soil properties for the entire set (n = 108). Full hold-out-one cross-validation was done to prevent over-fitting of the model. All calibrations were developed on natural logarithm transformed soil variables.

2.5. Trap cultures

Soil sampled for assessment of AMF spores was used to establish trap cultures, to trap species that may not have sporulated at the time of sampling. Trap cultures were initiated according to the recommendation of Morton et al. (1993). Briefly, a pot culture (each pot representing a single plot) was set up at the National Museums of Kenya, Nairobi using two host plants; sorghum (*Sorghum bicolor*) and cowpea (*Vigna unguiculata*). Sorghum was used because more than 1000 AMF isolates of 98 species from various AMF genera have been able to grow and sporulate with Sudan grass (*Sorghum sudanse*) (Morton et al., 1993). Cowpea has also been shown to be a suitable trap plant for mycorrhizal fungi from tropical soils (Bagyaray and Stürmer, 2008). A sub-sample of 150 g from each soil inoculum was diluted with 150 g of autoclaved medium-sized sand and mixed before being poured into 0.5 l pots. Sorghum was sown at a density of 25 plants while cowpea was sown at a density of 6 plants per pot. Each pot was covered with autoclaved sand to prevent unintentional dispersal of AMF. After seedling emergence pots were watered daily with tap water. Pot cultures were maintained 4 months. During the last week, moisture was successively lowered to stop plant growth and enhance sporulation of AMF.

2.6. Mycorrhizal inoculum potential

For estimation of AM inoculum potential (MIP), three intact soil cores were taken by driving an 8 cm diameter × 10 cm deep steel core into the soil to a depth of 15cm at the end of the short-rains season, February, 2009. Soil cores were transferred to 0.5 l pots and immediately transported for the subsequent greenhouse bioassay. Greenhouse bioassays were executed with three fast-growing crops: sorghum, leek (*Allium ampeloprasum* var. *porrum*) and cowpea. Seeds of these crops were surface-sterilized in 70% alcohol for 1 min and rinsed three times with sterile water. Pretreated seeds were germinated in sterilized water, after which they were planted in each pot. The pots were arranged on greenhouse benches in a randomized complete block design. The plants were grown without nutrient addition under natural light. Tap water was added as required. Plants were allowed to grow in the soil cores for 4 weeks, after which the crops were harvested by carefully washing their roots from the intact core. Roots were separated from organic debris by hand and stored in 70% ethanol before staining.

2.7. Spore extraction and taxonomic analysis

Spores of AMF from both the soil and trap cultures were isolated from 50 g (field soil) and 25 g (trap culture soil) via wet sieving and centrifugation. A very fine sieve (45 μm) was used to collect spores, and coarse material remaining on the top sieve (750 μm) was checked for sporocarps and very large spores. Spores were separated into groups according to general

morphological similarities under a dissecting microscope. Permanent slides of all spores were prepared by placing them in polyvinyl alcohol-lactic acid-glycerin (PVLG) mixed with Melzer's reagent. Spores were cracked open under the cover slip to allow observation of spore wall characteristics and were identified to species according to classical morphological analysis under a compound microscope (Morton, 1988). INVAM isolates and voucher specimens were used as taxonomic references. Spores were usually identified to species, but where this proved impossible, to unnamed morphospecies. Voucher specimens are kept at the NMK collection, Nairobi.

2.8. Assessment of AMF hyphal length

Hyphae were extracted from a 10 g soil subsample by the membrane filter technique (Jakobsen et al., 1992). Soil samples were mixed and suspended in 100 ml of deionized water, to which 12 ml of a sodium hexametaphosphate solution was added. The soil suspension was shaken for 30 s (end-over-end), left on the bench for around 30 min, and then decanted through a 45 µm sieve to retain hyphae, roots and particulate organic matter. The material on the sieve was sprayed gently with deionized water to remove clay particles, and then transferred into a 250 ml flask with 200 ml of deionized water. The flask was shaken vigorously by hand for 5 s, left on the bench for 1 min, and then a 2 ml aliquot was taken and pipetted onto 25 mm Millipore filters. The material on the filter was stained with 0.05% Trypan Blue in glycerol-water (1:1, v:v) and transferred to microscope slides. Hyphal length was measured with a grid-line intersect method at 200-400x magnification. Only non-septate hyphae were assessed.

2.9. Assessment of fractional mycorrhizal colonization

Roots obtained from the soil cores were washed free of soil by first soaking in a bucket of water, followed by wet-sieving (one mm mesh size) with tap water. Roots were separated from organic debris by hand. A sub-sample of field-collected roots was cut into one-cm segments, and stained using the modified procedure of Mason and Ingleby (1998). Roots were cleared in 2.5% KOH in an autoclave for 15 min at 121° C and bleached in a mixture of 30% H_2O_2 and 30% ammonium solution (1:1 v:v) for 30 min to remove phenolics. Roots were then acidified for 2 h with 1% HCl and stained with 0.05% acidified Trypan Blue dissolved in glycerol – water (1:1 v:v) by autoclaving the roots in this solution for 3 min at 121° C. Estimation of AMF colonization was done according to Trouvelot et al. (1986). Thirty root fragments were mounted on two slides each containing 15 root fragments. The fragments were observed under the microscope (magnification 160 – 400 ×) for the presence of hyphae, arbuscules and vesicles. Mycocalc

(www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html) was used to calculate fractional root colonization.

2.10. Data analysis

AMF spore densities in each sample were calculated by summing abundances of all species recorded in the sample. Species richness was calculated as species number in each sample. We calculated Shannon–Wiener diversity index (H′) and species richness for each field sample or trap pot using Ecological Methodology Program (2nd edition, Krebs, 1999). Four-way and three-way analysis of variance (ANOVA) were performed to assess the effects of sampling depth, mineral fertilization, manure, and residue on AMF spore abundance, species richness, species diversity, root colonization and AMF hyphal length. Differences between treatment means were analyzed by multiple range comparison based on least significant difference (LSD) at $P < 0.05$. The effects of mineral fertilization, manure and residue were assessed on AMF community composition (relative abundances of spores of each AMF species) by a multivariate redundancy analysis (RDA) in CANOCO (Version 4.55). All data were tested for normality, and where necessary percentage AMF colonization values were arcsine square root transformed and spore counts were logarithm (log+1) transformed to ensure conformity of the data with ANOVA assumptions.

3. Results

3.1. Soil chemical properties

Table 2 shows how various soil parameters (Ca, Mg, K, ECEC, available P, N, C and pH) varied across treatments. Sampling depth affected all measured properties (Ca, Mg, K, ECEC, available P, N, C and pH) significantly ($P < 0.001$). The upper soil layer (0-15 cm) had higher concentrations of all nutrients than the lower layer (15-30 cm). ECEC was also higher in the lower layer (Table 2). FYM application significantly affected levels of exchangeable cations (Ca, Mg and K), ECEC and available P ($P < 0.05$ in all cases). Both rates of FYM application significantly increased ECEC, Ca, Mg, and available P above control, with more increases in plots with the higher FYM application. C and N contents also significantly increased in plots with FYM, but only in the upper layer. NP fertilization significantly affected contents of N but only in the upper soil layer. NP fertilization did not impact on concentrations of available P. Highest contents of total N were observed in NP2 plots, but N was not different between plots with low or high fertilizer rates. Crop residue and interactions of the three factors (NP x FYM x

residue) were not a significant source of variation for soil properties in the two sampling depths ($P > 0.05$ in all cases).

3.2. Identification of AMF species

Identification based on spore morphology indicated 16 AMF species from five genera. Seven AMF fungi were identified to species level (*Acaulospora scrobiculata, A. denticulata, A. mellea, Glomus aggregatum, Scutellospora pellucida, S. persica,* and *Gigaspora gigantea)* while nine AMF species (*Entrophospora sp.1-2, Acaulospora sp.1, Scutellospora sp.1* and *Glomus sp.1-5)* could not be identified to species level but were morphologically different from the other species in the genera and were thus maintained as separate morphotypes. All 16 AMF species were observed in both field soil and in the sorghum trap culture. *Glomus sp.5* was not detected in trap culture with cowpea. The AMF communities both in the field soil and in trap cultures were dominated by *Glomus* (6 species) and *Acaulospora* (4 species) (Table 3). *Acaulospora* species constituted 60% of total spores, and *Scutellospora* species 20% of total spores (Figure 1). Spore abundance from the other genera declined in the order *Glomus>Entrophospora>Gigaspora* (Figure 1). AMF community composition in the field soil was significantly affected by sampling depth (RDA, F = 11.47, $P < 0.001$) and both NP fertilization (RDA, F = 3.23, $P = 0.002$) and FYM application (RDA, F = 2.24; $P = 0.04$), which explained respectively 10%, 2 % and 1% of the variability in the dataset (Figure 1). FYM application reduced abundance of *Entrophospora sp.1*, *Acaulospora scrobiculata* and *A. sp.2* by 25%, with larger decline observed in the highest rate of manure applications (Figure 2). Similarly low abundance of *A. scrobiculata, A. mellea* and *A. sp.1*, *Glomus sp.1* and *sp.2*, and *Entrophospora sp.1* and *sp.2* were observed with both rates of NP fertilization, with larger decline (decline ranged between 12 -30%) with higher levels of NP applications (Figure 2).

3.3. Spore density in the field and traps

AMF spore abundance was significantly affected by sampling depth (F = 6.99, $P = 0.009$) in the field soil and identity of the host plant in the trap cultures (F = 27.81, $P < 0.001$). The upper 15 cm soil layer recorded lower spore abundance than the lower layer in field-collected soil (Table 4). In the trap cultures spore abundance was higher with sorghum (63 spores per 25 g soil) than with cowpea (41 spores per 25 g soil). Spore abundance in the field soil was unaffected by NP fertilization, FYM, residue and the interaction of the three factors ($P > 0.05$, Table 4). Spore abundance in the trap cultures was significantly affected by FYM, NP fertilizer and FYM x residue interaction, but was unaffected by residue and the other interactions of the three factors

(Table 4). NP fertilization reduced spore abundance by 24% in trap cultures (Table 4). Spore abundance was higher in plots with residue compared to plots with FYM plus residue combined (Table 4).

3.4. AMF species richness and diversity

AMF species richness and diversity were not significantly affected by NP fertilization, FYM, crop residue, and the interactions of the three factors in the field soil ($P > 0.05$; Table 4). In trap cultures the type of plant (sorghum vs. cowpea) significantly affected AMF species richness ($F = 16.43$, $P < 0.001$) and species diversity ($F = 15.35$, $P < 0.001$), but sampling depth was not a significant source of variation ($P > 0.05$). Both AMF species richness and diversity were higher in trap cultures planted with sorghum (8.2 species, H = 1.76) than those planted with cowpea (6.6 species, H = 1.53). Species richness was significantly affected by NP fertilization, FYM and FYM x crop residue ($P < 0.01$) but unaffected by residue and the interaction of the three factors ($P > 0.05$; Table 4). Both NP fertilization and FYM reduced species richness in the trap cultures. Species richness declined from 8.0 species to 7.4 species in NP1 and 7.3 species NP2, whereas in plots with FYM, species richness declined from 8.0 species to 7.6 species in FYM1 and 7.2 species in FYM2. Species diversity was unaffected by NP fertilization, FYM, residue and the interaction of the three factors ($P > 0.05$ in all cases).

3.5. AMF colonization in the field

Root colonization in maize was unaffected by NP fertilization, FYM, residue and by the interaction of the three factors ($P > 0.05$ in all cases). Root colonization in bean was significantly affected by NP fertilization, FYM, residue and the interaction of the three factors in both November and December sampling ($P < 0.05$; Table 5). NP fertilization in combination with organic amendments (FYM and residue) increased root colonization in beans in both November and December sampling.

3.6. Extraradical AMF hyphal length (MEH)

MEH in all sampling dates was significantly affected by sampling depth ($P < 0.05$; Table 5). It was generally higher in the upper 15 cm soil layer than in the lower layer. In May and December MEH was significantly affected by FYM, residue, fertilizer x FYM interaction and fertilizer x FYM x residue interaction ($P < 0.05$; Table 5). FYM alone, and in combination with NP fertilization increased MEH in November and December. NP fertilization in combination with FYM increased MEH above control whereas the plots with NP fertilizer in combination with

FYM plus residue had no significant effect on MEH in May. In December MEH increased significantly in all plots with NP fertilization and organic amendments (FYM, residue) combined (Table 5). In August, November and January MEH was only significantly affected by FYM with exception of MEH in November which was also affected by fertilizer x FYM interaction ($P < 0.05$). Manure applications increased MEH in August and January above control whereas NP fertilization declined MEH.

3.7. AMF colonization in the bioassays

Mycorrhizal colonization in the bioassay was considered a proxy for MIP. AMF colonization varied with plant species identity ($F = 19.15$, $P < 0.001$). Fractional colonization was highest in sorghum (22%), intermediate in leek (16%) and lowest in cowpea (14%). In all three species, fractional colonization was also affected by NP fertilization, FYM, fertilizer x FYM interaction, FYM x residue, and fertilizer x FYM x residue interactions ($P < 0.001$; Table 5). In fertilizer x FYM x residue interactions, all plots tended to increase the MIP above the control, but the increases were more pronounced in plots with FYM alone or FYM plus residue (Table 5).

3.8. Correlations between AMF and soil properties

There were no significant correlations between AMF spore abundance, species richness and diversity (Shannon H index) with the measured soil properties at 0-15 cm soil layer. AMF root colonization correlated positively with ECEC ($r = 0.33$) and Mg ($r = 0.37$) and negatively with soil pH ($r = -0.35$). There was also a positive correlation between AMF hyphal length and N ($r = 0.37$), C ($r = 0.29$) and Mg ($r = 0.35$) at 0-15 cm layer. There were no important correlations in 15-30 cm soil layer.

4. Discussion

The long-term (32 years) effects of NP fertilization and organic amendments were studied to determine their influence on AMF communities and activity. Our results showed that NP fertilization as well as FYM significantly altered AMF species composition, but both factors explained less than 5% of the total variation in the data set. NP fertilization and organic amendments had no effect on AMF diversity under field conditions. Both manure and NP fertilization reduced spore abundance and species richness in trap cultures. Such results were unexpected, considering the well-established negative effects of mineral nutrients (P, but also N) on mycorrhizal functioning (Verbruggen and Kiers, 2010).

Surprisingly, soil analysis indicated that NP fertilization was not a significant source of

variation for any of the chemical parameters assessed (Table 1). We cannot explain why a history of more than thirty years with different rates of P application did not result in different levels of P availability. One possible explanation would be substantial erosion in the field, as a consequence of which treatment effects were eliminated. All plots had high to very high concentrations of available P, usually between 20 and 30 mg P kg^{-1}. Kibunja et al. (2010), who worked in the same sites, also did not observe a significant effect of NP fertilizer on P availability (measured as P-Mehlich) and reported values ranging between 13 and 16 mg P kg^{-1}. High available P concentrations (P-Olsen) were also noted in other Nitisols in Kenya with a history of P fertilization. Kimetu et al. (2006) in another site in Kabete, close to our field site, reported extractable P concentrations of 27 mg P kg^{-1}, while Murage et al. (2000) reported P concentrations ranging from 17 mg P kg^{-1} (non-productive sites) to 55 mg P kg^{-1} (productive sites). In a Ferralsol in Nyabeda (Chapter 3), after P fertilization P concentrations between 10 and 15(-20) mg P kg^{-1} were reported. Application of fertilizer also did not result in changes in SOC contents (Table 1). Our values (around 18.6 g C kg^{-1}) are higher than those reported by Kibunja et al. (2010, 2012), who mentioned SOC contents between 9 and 14 g C kg^{-1}, but comparable to those reported by Ayuke et al. (2011) who also observed no effect of mineral fertilizer on SOC contents. Such SOC contents come close to the critical level of 16 g C kg^{-1}, mentioned by Janssen (2011). Vanlauwe et al. (2010) reported low responses of crops to fertilization under degraded soils (low SOC), and similar condition likely apply to AMF.

While conventional knowledge has suggested large negative effects of P-fertilizer on AMF communities, lack of effects, like in our study, were also reported by Franke-Snyder et al. (2001), Wang et al. (2011), and Vestberg et al. (2011), who did not find any differences in spore numbers and species richness in long-term trials (>15 years) under conventional and low-input farming systems. Galván et al. (2009) did not observe any effect of reduced P-levels on AMF species richness in conventional and organic onion fields. Mathimaran et al. (2007) found no effect of P fertilization on spore abundance and diversity in a Kenyan Ferrasols, but they attributed this effect to low levels of available P in the soil even after P fertilization (resp. 1.7 and 6.6 mg P kg^{-1} resin-extractable P in unfertilized and fertilized plots). Our data, however, did show high P concentrations, but no fertilizer effect.

Whereas mineral fertilizer did not impact on soil properties, FYM increased levels of ECEC, available P, total N, soil organic C and exchangeable cations significantly (Table 1). Increased soil fertility following manure application could be attributed to slow release of nutrients from organic sources, which contributes to the pool of soil organic N and P (Parmer and Sharma, 2002; Shafi et al., 2012). Organic sources may also reduce N leaching and P fixation in

soils, thus increasing contents of N and P in the soil. Organic materials also provide exchangeable bases such as Ca, Mg and K, contrary to NP fertilization. However, the changes due to FYM were relatively small (< 10% increases). Under the current conditions, changes in nutrient availability may not have had pronounced effects on AMF under contrasting management in this system.

Our result show strong effects of organic amendments on AMF activity (root colonization, hyphal length and inoculum potential) especially in combination with mineral fertilizer. Positive effects of organic inputs on AMF colonization have been shown before (Oehl et al., 2003, 2004; Galván et al., 2009; Gosling et al., 2010) and they are associated with gradual release of N and P, resulting in low plant internal P status at early stages of growth which does then not negatively feedback to AMF colonization. Organic sources have also been shown to be an important source of N for AMF (Hodge and Fitter, 2010). Integrated use of organic and inorganic sources of nutrients is associated with C availability and balanced nutrient supply which enhances efficient metabolism of soil microorganisms and improves crop yield (Zheng et al., 2009; Ayeni and Adetunji, 2010). Improved nutrient availability facilitates growth of root and AMF mycelia, resulting in high AMF root colonization (Karunasinghe et al., 2009; Linderman and Davis, 2004). Treseder and Allen (2002) have shown that when plants are provided with ample nutrients, they tend to limit C allocation to AMF.

The hypothesis that NP fertilization favours species belonging to the *Glomeraceae* while organic-managed systems would favour members of the *Gigasporaceae* was not supported in this study. Members of the *Acaulosporaceae* were dominant in this trial and accounted for more than 50% of spore abundance. High abundance of members of the genus *Acaulospora* was reported in other acidic tropical soils (Sieverding, 1991; Castillo et al., 2006). A similar observation was also made in an acidic Ferrasol in Kenya (Mathimaran et al., 2007). *Glomus* is generally the genus of AMF with the greatest number of species in intensively managed agriculture (Sieverding, 1990; Boddington and Dodd, 2000; Jefwa et al., 2009). In this study we found six species of *Glomus*, although their abundance accounted for only 10% of total spore abundance in the field. Possibly low spore abundance is related to low N availability, as several studies have shown decline in spore numbers of *Glomus* in N-limited soils (Treseder and Allen, 2002). N-limitation for AMF is likely due to their substantial N requirement (Hodge and Fitter, 2010).

Both FYM and NP fertilization had negative effects on some AMF species from *Acaulospora, Entrophospora* and *Glomus* (Figure 3). Although NP and FYM had weak effects on the AMF communities, changes in soil conditions may have played a role in the selection of

some AMF species (Johnson, 1993). The lack of a strong effect of both FYM and NP fertilization in our site may also be related to local adaptation of some species. Under long-term field trials, Antunes et al. (2012) have shown that AMF species may become adapted to continuous manipulations of soil fertility. Johnson et al. (2010) have also shown local adaptations of the AMF symbiosis in different P-deficient natural grassland sites.

Low spore density (1-2 spores g^{-1} soil), particularly in the upper 15cm soil layer could be attributed to mechanical disruption of AMF hyphae by regular soil disturbance through tillage. Soil disturbance through tillage is considered a major driver of change in AMF communities in agro-ecosystems (Verbruggen and Kiers, 2010; see also Chapter 3) and is associated with negative effects on hyphal length, fractional colonization, mycorrhizal fungal biomass and ultimately sporulation. Frequent soil disturbances have been shown to negatively affect AMF spore density and species richness (Jansa et al., 2003). We have also shown weak negative effects of tillage on species spore abundance and species composition (Chapter 3). Similar results were observed by Mathimaran et al. (2007) in a maize field in Kenya under regular tillage and by Vestberg et al. (2011) in a long-term field study in the temperate region. These authors attributed such effects to regular soil disturbance. Possibly, regular soil disturbance may also have exerted more pressure on AMF than the effects of NP fertilization and organic amendments.

We recorded 16 AMF species in our study site which is comparable with AMF species richness reported from other agro-ecosystems in Kenya (Jefwa et al., 2009; Mathimaran et al., 2007). Our trap cultures revealed only two species not detected in the field survey, indicating that the observed community assemblage was an accurate reflection of the richness of these communities and a good representation of the AMF community. Although measures of diversity estimated in the pots may not represents the field situation they could be used for comparison of different treatments of the trap pots. In this study, negative effects of manure and NP fertilization on spore abundance and species richness were observed in the traps.

AMF hyphal length (MEH) ranged between 8 and 33 m g^{-1} soil. Although some saprotrophic fungi (members of the Mucoromycotina) may also produce aseptate hyphae, hyphae from these groups have been shown to contribute less than 20% of total hyphal biomass in the soil (Bingham and Biondini, 2009). It is therefore likely that most of hyphal length is due to AMF. Our values are within the range of what has been previously observed in various ecosystems (Purin et al., 2006). Decline of MEH as well as MIP observed in plots with crop residue could be associated with phytotoxicity (allelopathy) by uncomposted crop residue (Kahiluoto et al., 2009, 2012).

Our results raise the question on the long-term sustainability of benefits accrued from

organic amendments on AMF communities in this site. Management of SOC for diverse AMF communities under organic-based management systems also appears to be a prerequisite under these soil conditions. Further studies are desirable to determine how significant these changes in AMF are for the functioning of this agro-ecosystem.

5. Conclusion

This study has shown only weak effects of organic amendments (FYM and crop residue) and mineral fertilizer (N and P) after 32 years on AMF communities. Organic inputs had no effect on AMF spore abundance and species richness contrary to the standard view that organic inputs stimulate AMF (Oehl et al., 2004; but see Franke-Snyder et al., 2001 and Vestberg et al., 2010). Nutrient imbalances, erosion, regular soil disturbance through tillage and local adaptation of AMF may have played a significant role in regulating AMF diversity and species composition. Careful nutrient budgeting and SOC management is imperative in this agro-ecosystem and more general under ISFM practices to sustain AMF diversity. Organic inputs alone or in combination with mineral fertilizer increased AMF activity supporting the integrated use of organic and inorganic sources of nutrients in maintaining AMF activity.

Table 1: Overview of treatments (NP fertilization, manure application and crop residues) in a Humic Nitisol in Kabete, central Kenya

	Treatment	Inorganic	Organic	
			FYM	Crop residue
1	Control	none	none	None
2	R1	none	none	Retained
3	FYM1	none	5 t ha^{-1}	None
4	FYM1R1	none	5 t ha^{-1}	Retained
5	FYM2	none	10 t ha^{-1}	None
6	FYM2R1	none	10 t ha^{-1}	Retained
7	NP1	60 kg N + 26.2 kg P ha^{-1}	none	None
8	NP1R1	60 kg N + 26.2 kg P ha^{-1}	none	Retained
9	NP1FYM1	60 kg N + 26.2 kg P ha^{-1}	5 t ha^{-1}	None
10	NP1FYM1R1	60 kg N + 26.2 kg P ha^{-1}	5 t ha^{-1}	Retained
11	NP1FYM2	60 kg N + 26.2 kg P ha^{-1}	10 t ha^{-1}	None
12	NP1FYM2R1	60 kg N + 26.2 kg P ha^{-1}	10 t ha^{-1}	Retained
13	NP2	120 kg N + 52.4 kg P ha^{-1}	none	None
14	NP2R1	120 kg N + 52.4 kg P ha^{-1}	none	Retained
15	NP2FYM1	120 kg N + 52.4 kg P ha^{-1}	5 t ha^{-1}	None
16	NP2FYM1R1	120 kg N + 52.4 kg P ha^{-1}	5 t ha^{-1}	Retained
17	NP2FYM2	120 kg N + 52.4 kg P ha^{-1}	10 t ha^{-1}	None
18	NP2FYM2R1	120 kg N + 52.4 kg P ha^{-1}	10 t ha^{-1}	Retained

Table 2: Soil properties under nitrogen-phosphorus (NP) fertilization and organic (manure and crop residues) inputs in humic nitisols soil in Kabete, central Kenya. For abbreviations see Table 1. Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Values in bold are significant ($P < 0.05$).

				CEC	Ca	K	Mg	P(O)	pH	C	N
Depth	NP	FYM	CR	cmol/kg	mg/kg	mg/kg	mg/kg	mg/kg		g/kg	g/kg
0-15	None	None	-	15.9(0.2)	1227.5(11.9)	590.2(10.3)	224.8(1.8)	27.0(1.0)	5.1(0.1)	18.6(0.4)	1.4(0.1)
			+	16.1(0.1)	1240.4(6.5)	591.3(11.2)	227.4(0.2)	26.6(1.6)	5.3(0.1)	19.4(0.6)	1.5(0.1)
		FYM1	-	16.0(0.1)	1243.8(0.9)	601.7(8.1)	226.1(1.0)	27.7(1.1)	5.5(0.1)	19.7(0.4)	1.5(0.1)
			+	16.0(0.4)	1227.3(24.3)	574.3(62.9)	228.9(0.4)	24.8(5.0)	5.5(0.1)	18.0(0.2)	1.4(0.1)
		FYM2	-	16.4(0.1)	1250.7(9.6)	623.4(17.7)	227.9(0.6)	28.8(2.3)	5.4(0.1)	19.9(0.6)	1.5(0.1)
			+	16.3(0.1)	1250.0(4.0)	620.4(13.9)	228.0(0.7)	29.9(1.1)	5.4(0.1)	19.7(0.3)	1.5(0.1)
	NP1	None	-	16.2(0.1)	1249.7(7.5)	595.0(10.4)	228.1(1.0)	25.7(2.1)	5.4(0.1)	18.7(0.4)	1.4(0.1)
			+	16.1(0.1)	1246.9(1.5)	608.7(8.7)	226.8(0.7)	27.4(1.0)	5.5(0.1)	19.4(0.3)	1.5(0.1)
		FYM1	-	16.2(0.1)	1241.8(2.9)	583.8(9.7)	228.4(0.7)	25.1(0.8)	5.4(0.1)	19.0(0.3)	1.5(0.1)
			+	16.3(0.1)	1248.3(3.0)	604.3(9.3)	228.5(0.8)	26.4(1.0)	5.4(0.1)	19.7(0.2)	1.5(0.1)
		FYM2	-	16.3(0.2)	1242.5(16.6)	591.8(39.0)	229.6(0.8)	26.5(3.2)	5.4(0.1)	19.6(0.9)	1.5(0.1)
			+	16.4(0.1)	1261.9(5.2)	656.1(15.3)	226.2(0.3)	34.8(2.3)	5.4(0.1)	21.3(0.8)	1.6(0.1)
	NP2	None	-	15.9(0.2)	1231.1(11.5)	562.5(15.9)	226.3(0.8)	25.8(0.9)	5.5(0.1)	19.3(0.3)	1.5(0.1)
			+	16.0(0.1)	1230.4(10.8)	583.6(26.0)	226.3(0.4)	27.4(1.8)	5.5(0.1)	19.0(0.4)	1.5(0.1)
		FYM1	-	16.2(0.1)	1241.4(9.5)	589.1(15.5)	228.0(1.0)	27.2(0.6)	5.5(0.1)	19.3(0.1)	1.5(0.1)
			+	16.6(0.3)	1273.2(22.0)	624.7(16.3)	229.9(1.7)	30.3(2.3)	5.4(0.1)	20.3(0.6)	1.5(0.1)
		FYM2	-	16.4(0.1)	1260.4(2.5)	643.1(18.8)	226.8(0.5)	33.5(2.9)	5.4(0.1)	21.1(0.7)	1.6(0.1)
			+	16.6(0.1)	1273.1(12.3)	637.7(10.9)	229.6(0.2)	32.8(2.2)	5.4(0.1)	20.9(0.4)	1.6(0.1)
15-30	None	None	-	16.3(0.1)	1235.2(2.8)	581.8(22.8)	229.8(2.8)	22.6(1.6)	5.2(0.1)	18.1(0.3)	1.3(0.1)
			+	16.5(0.3)	1238.0(10.7)	569.6(4.1)	233.1(3.3)	20.4(1.7)	5.2(0.1)	17.6(0.2)	1.3(0.1)
		FYM1	-	16.8(0.5)	1217.8(6.7)	537.6(30.6)	237.0(6.6)	21.3(3.5)	5.3(0.1)	17.1(0.6)	1.3(0.1)
			+	16.6(0.3)	1221.2(7.3)	528.0(7.0)	235.9(3.7)	19.7(0.6)	5.3(0.1)	17.1(0.3)	1.3(0.1)
		FYM2	-	16.8(0.5)	1224.2(8.8)	552.4(19.4)	236.1(5.9)	20.7(2.5)	5.3(0.1)	17.4(0.4)	1.3(0.1)
			+	16.4(0.2)	1215.2(17.9)	533.4(34.0)	232.9(2.2)	20.5(2.2)	5.4(0.1)	17.5(0.7)	1.3(0.1)
	NP1	None	-	16.5(0.3)	1207.4(31.1)	504.5(32.4)	235.3(1.4)	16.7(1.9)	5.3(0.1)	16.0(1.2)	1.2(0.1)
			+	16.7(0.3)	1219.4(18.7)	546.3(31.1)	235.9(3.7)	20.2(2.4)	5.3(0.1)	16.6(0.9)	1.2(0.1)
		FYM1	-	16.5(0.2)	1234.0(17.4)	552.5(23.9)	232.9(2.3)	20.7(1.8)	5.4(0.1)	17.7(0.3)	1.3(0.1)
			+	16.8(0.4)	1226.8(2.3)	543.4(19.5)	237.9(5.6)	19.0(2.9)	5.3(0.1)	16.7(0.5)	1.2(0.1)
		FYM2	-	16.7(0.3)	1239.0(6.4)	553.1(16.5)	235.3(4.4)	19.4(3.2)	5.4(0.1)	18.3(0.9)	1.4(0.1)
			+	16.9(0.3)	1254.7(1.3)	590.4(17.6)	235.7(3.9)	21.1(3.2)	5.4(0.1)	18.1(0.6)	1.3(0.1)
	NP2	None	-	16.6(0.2)	1225.1(22.1)	522.8(31.8)	235.8(3.2)	18.5(2.7)	5.3(0.1)	17.3(1.2)	1.3(0.1)
			+	16.6(0.4)	1211.7(13.1)	523.3(36.3)	234.6(4.6)	18.4(3.2)	5.4(0.1)	17.0(0.6)	1.3(0.1)
		FYM1	-	16.3(0.4)	1220.9(10.0)	534.3(5.2)	231.9(4.6)	19.6(2.2)	5.4(0.1)	17.4(0.3)	1.3(0.1)
			+	17.2(0.4)	1275.7(33.6)	612.4(30.7)	236.7(3.9)	22.6(1.5)	5.3(0.1)	18.7(0.7)	1.4(0.1)
		FYM2	-	16.8(0.1)	1237.0(13.0)	566.6(35.6)	235.9(2.5)	20.9(3.7)	5.4(0.1)	17.7(0.8)	1.3(0.1)
			+	16.9(0.2)	1258.0(25.0)	581.6(39.0)	236.0(3.9)	23.6(6.1)	5.3(0.1)	18.7(1.6)	1.4(0.1)
ANOVA											
0-15	Fertilizer (A)			1.7(0.19)	1.8(0.18)	0.2(0.83)	1.4(0.27)	1.5(0.24)	2.4(0.11)	1.8(0.18)	2.3(0.11)
	Manure (B)			**4.2(0.02)**	**7.0(0.00)**	**4.9(0.01)**	**6.5(0.00)**	**6.1(0.01)**	**8.1(0.00)**	**5.1(0.01)**	**9.4(0.00)**
	Residues (C)			1.7(0.20)	1.5(0.22)	1.3(0.26)	2.2(0.14)	1.3(0.26)	1.2(0.28)	0.4(0.53)	0.7(0.41)
	A x C			0.8(0.47)	0.8(0.44)	1.5(0.24)	**7.0(0.00)**	1.8(0.18)	2.1(0.14)	1.5(0.24)	0.8(0.44)
15-30	Fertilizer (A)			0.9(0.42)	0.4(0.70)	0.1(0.89)	0.2(0.82)	0.5(0.64)	0.1(0.91)	0.8(0.47)	1.0(0.40)
	Manure (B)			1.4(0.26)	0.8(0.46)	1.0(0.37)	0.2(0.83)	0.4(0.69)	0.2(0.80)	1.9(0.17)	3.1(0.06)
	Residues (C)			1.3(0.26)	0.8(0.36)	1.1(0.30)	0.3(0.61)	0.1(0.71)	0.2(0.66)	0.1(0.82)	0.0(0.90)

Table 3. Spore numbers of Arbuscular Mycorrhizal Fungal species (per 25 g soil) as affected by NP fertilization and organic inputs (FYM and crop residue) in Humic Nitisol, Kabete, central Kenya. For treatment abbreviations see Table 1.

	NP	None						NP1						NP2					
	FYM	None		FYM1		FYM2		None		FYM1		FYM2		None		FYM1		FYM2	
AMF Species	CR	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Entrophospora sp.1		2.1	3.2	1.8	2.3	2.0	1.9	1.7	1.9	1.3	2.2	1.4	1.1	1.8	2.1	2.1	0.8	1.5	1.4
Entrophospora sp.2		7.6	6.4	5.1	3.1	2.5	2.8	4.6	2.6	2.9	3.6	4.0	2.2	2.1	2.3	2.6	2.1	2.4	1.4
Acaulospora denticulata		3.8	9.9	5.8	3.6	5.7	6.9	5.6	7.1	6.2	7.1	3.9	5.6	5.9	7.8	4.2	8.1	7.6	5.1
A. mellea		8.4	11.7	10.1	6.4	9.1	11.6	11.8	10.3	11.0	5.1	7.6	7.6	6.8	5.7	9.7	5.7	8.2	5.7
A. sp.1		20.2	23.5	7.8	5.7	3.2	5.0	6.4	5.1	5.8	3.1	4.2	10.0	6.1	4.4	7.6	4.2	3.7	5.2
A. *scrobiculata*		6.8	22.9	21.5	4.6	6.3	7.6	10.3	10.8	10.5	3.7	1.4	6.8	7.0	6.1	7.3	3.7	5.6	2.7
Glomus aggregatum		0.0	0.2	0.1	0.2	0.2	0.2	0.0	0.0	0.6	0.1	0.0	0.1	0.3	0.0	0.1	0.1	1.1	0.0
G. sp.1		1.5	2.9	2.4	1.5	7.4	1.4	2.9	1.3	0.8	3.0	1.1	1.2	1.3	2.7	1.4	2.6	2.8	1.1
G. sp.2		0.3	0.6	0.6	0.2	0.5	0.0	0.0	0.4	0.1	0.2	0.1	0.0	0.2	0.3	0.0	0.1	0.9	0.3
G. sp.3		1.2	2.7	1.9	0.9	1.8	1.7	1.0	4.0	1.7	0.6	2.1	3.6	0.6	0.2	0.7	0.5	0.6	0.8
G. sp.4		0.2	0.1	0.1	0.2	0.1	0.4	0.1	0.0	0.2	0.0	0.0	0.3	0.2	0.1	0.1	0.2	0.1	0.2
G. sp.5		0.1	0.0	0.0	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.1	0.0	0.0	0.7
Scutellospora pellucida		5.6	7.7	5.2	11.6	4.9	9.4	7.4	5.1	8.0	6.4	4.5	4.6	5.2	7.1	8.3	6.1	6.7	6.1
S. persica		1.8	2.7	2.3	3.2	3.1	2.1	1.4	3.0	2.3	4.1	4.3	4.2	2.8	2.3	2.5	1.3	1.8	2.4
S. sp.1		0.3	0.5	0.3	0.3	0.2	0.5	0.4	0.2	0.2	0.4	0.4	0.2	0.3	0.1	0.2	0.4	0.3	0.6
Gigaspora gigantea		0.0	0.3	0.1	1.7	0.1	0.1	0.1	0.2	0.1	0.0	0.7	0.1	0.0	0.0	0.4	0.1	0.0	0.1

Table 4: Diversity of AMF (spore abundance, species richness and Shannon (H) index) under NP fertilization and organic inputs (FYM and crop residue) in Humic Nitisol, Kabete, central Kenya. For treatments see Table 1. Richness = species richness, diversity = Shannon H diversity index. Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Values in bold are significant ($P < 0.05$).

				----------Field soil----------			----------Trap cultures----------		
				Abundance	Richness	Diversity	Abundance	Richness	Diversity
	NP	FYM	CR	(spore 25 g soil)		H	(spore 25 g soil)		H
0-15	None	None	-	24.3(4.2)	6.3(0.3)	1.4(0.1)	91.5(22.0)	8.0(0.6)	1.4(0.1)
			+	40.3(2.2)	8.3(0.3)	1.8(0.1)	96.8(13.3)	9.7(0.3)	1.5(0.2)
		FYM1	-	43.0(6.1)	7.3(0.3)	1.8(0.1)	61.2(18.8)	8.0(1.1)	1.7(0.1)
			+	24.0(8.3)	6.0(1.0)	1.6(0.2)	35.3(5.2)	7.3(0.3)	1.7(0.1)
		FYM2	-	34.3(2.3)	8.3(0.9)	1.9(0.1)	47.5(10.4)	6.0(1.0)	1.5(0.2)
			+	32.0(4.6)	7.0(1.5)	1.5(0.2)	43.7(6.3)	7.3(0.8)	1.5(0.2)
	NP1	None	-	36.0(9.5)	7.7(0.7)	1.8(0.1)	61.0(19.2)	8.0(0.7)	1.7(0.1)
			+	28.7(5.5)	7.3(1.2)	1.6(0.3)	42.0(9.5)	7.2(0.3)	1.7(0.1)
		FYM1	-	26.3(9.8)	6.3(1.8)	1.4(0.4)	33.7(3.0)	7.0(0.6)	1.6(0.2)
			+	33.0(6.5)	8.7(1.8)	1.9(0.1)	44.2(7.7)	5.7(0.6)	1.4(0.2)
		FYM2	-	50.7(14.2)	6.7(0.3)	1.6(0.1)	27.3(5.2)	5.8(0.7)	1.5(0.1)
			+	45.0(11.6)	7.7(0.3)	1.8(0.1)	38.5(4.2)	7.8(1.1)	1.7(0.2)
	NP2	None	-	39.7(12.4)	8.7(0.9)	1.8(0.1)	40.3(7.4)	7.31.1)	1.7(0.1)
			+	33.7(4.7)	7.3(0.9)	1.6(0.1)	48.8(10.0)	6.7(0.4)	1.6(0.1)
		FYM1	-	28.0(6.1)	7.3(1.5)	1.7(0.2)	49.2(11.1)	8.2(0.6)	1.7(0.1)
			+	29.0(8.4)	5.7(0.7)	1.4(0.1)	29.5(4.6)	6.3(0.6)	1.5(0.1)
		FYM2	-	29.0(11.0)	8.3(1.3)	1.8(0.1)	52.2(12.0)	9.0(0.4)	1.9(0.1)
			+	33.3(13.4)	5.3(1.8)	1.2(0.3)	26.2(4.0)	6.3(0.7)	1.5(0.2)
15-30	None	None	-	68.3(13.7)	10.3(0.7)	1.9(0.1)	41.2(7.3)	7.3(0.8)	1.7(0.1)
			+	96.0(10.4)	10.3(0.9)	2.1(0.1)	120.8(29.6)	8.8(0.5)	1.6(0.2)
		FYM1	-	53.7(12.5)	8.7(0.7)	1.9(0.2)	85.0(25.2)	8.2(0.7)	1.7(0.1)
			+	77.3(24.2)	9.3(0.9)	1.9(0.1)	50.8(13.3)	8.5(0.4)	1.8(0.1)
		FYM2	-	57.7(18.2)	9.3(0.7)	1.9(0.1)	47.3(12.9)	7.7(0.7)	1.7(0.1)
			+	48.0(11.5)	9.0(0.6)	1.9(0.1)	72.0(14.7)	7.7(0.8)	1.5(0.2)
	NP1	None	-	43.7(15.0)	9.3(0.7)	2.0(0.1)	60.3(16.0)	7.8(0.3)	1.7(0.1)
			+	42.3(17.3)	7.7(1.9)	1.7(0.3)	78.2(19.5)	8.0(0.8)	1.7(0.1)
		FYM1	-	51.3(5.8)	8.7(1.8)	1.9(0.2)	82.0(22.4)	8.2(0.7)	1.6(0.1)
			+	61.0(8.5)	10.7(0.9)	2.1(0.1)	27.2(7.8)	6.8(0.9)	1.6(0.1)
		FYM2	-	40.7(12.2)	8.3(1.2)	1.8(0.2)	33.8(13.0)	7.2(1.2)	1.6(0.1)
			+	28.7(11.9)	6.0(2.3)	1.4(0.4)	67.8(23.2)	6.5(1.6)	1.1(0.2)
	NP2	None	-	39.7(11.2)	8.7(0.9)	1.9(0.1)	42.5(11.3)	7.3(0.6)	1.6(0.1)
			+	46.3(16.2)	9.7(0.9)	2.0(0.1)	34.3(7.4)	6.5(0.4)	1.5(0.1)
		FYM1	-	51.7(9.4)	8.7(0.7)	1.8(0.2)	52.8(8.8)	8.5(0.7)	1.7(0.1)
			+	39.7(9.2)	7.0(1.0)	1.6(0.2)	43.7(9.7)	6.8(0.7)	1.6(0.1)
		FYM2	-	81.3(20.3)	9.0(0.6)	2.0(0.1)	22.8(6.5)	5.2(0.9)	1.2(0.3)
			+	36.3(8.8)	7.7(0.9)	1.8(0.2)	40.2(13.7)	7.3(1.0)	1.6(0.1)
ANOVA									
0-15	Plant			n/a	n/a	n/a	0.2(0.69)	**16.6(0.00)**	**6.5(0.01)**
	Fertilizer(A)			0.1(0.86)	0.2(0.85)	0.5(0.64)	**7.2(0.00)**	1.7(0.20)	0.8(0.44)
	Manure (B)			0.7(0.49)	0.8(0.45)	0.3(0.75)	**7.4(0.00)**	2.7(0.07)	0.1(0.93)
	Residues(C)			0.2(0.64)	0.8(0.37)	0.7(0.40)	0.9(0.34)	0.5(0.47)	1.2(0.27)
	A x B			0.9(0.50)	0.3(0.85)	0.5(0.76)	2.4(0.06)	**2.5(0.05)**	2.0(0.10)
	A x C			0.1(0.89)	2.6(0.09)	**3.1(0.06)**	2.1(0.13)	5.3(0.01)	1.6(0.20)
15-30	Plant			n/a	n/a	n/a	**55.8(0.00)**	**49.5(0.00)**	**5.5(0.02)**
	Fertilizer			**3.7(0.03)**	1.9(0.16)	0.9(0.42)	**10.2(0.00)**	**5.6(0.01)**	1.2(0.32)
	Manure			0.8(0.45)	1.4(0.27)	0.6(0.54)	**6.0(0.00)**	**6.7(0.00)**	5.3(0.01)
	Residues			0.5(0.49)	1.2(0.29)	0.8(0.39)	1.8(0.19)	0.0(0.84)	1.0(0.31)
	B x C			1.7(0.19)	1.0(0.39)	0.8(0.44)	**13.8(0.00)**	2.0(0.14)	0.1(0.90)
	A x B x C			0.2(0.96)	1.2(0.34)	0.6(0.64)	**2.7(0.04)**	**3.1(0.02)**	**3.1(0.02)**

Table 5. Hyphal length, arbuscular mycorrhizal root colonization (in %), hyphal length and inoculum potential (MIP; as fractional colonization, in %) in Humic Nitisol, Kabete, central Kenya. See Table 1 for abbreviations of treatments. LR and SR = long- and short-rains season, March = 8 weeks after sowing (WAS) maize, September = 1 week after maize harvest, October = 4 WAS, December = 8 WAS, January = 12 WAS, MIP = AMF inoculum potential, %M = fractional root colonization. Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Values in bold are significant ($P < 0.05$).

				Hyphal length (m/g soil)					Colonization (%M)			MIP
				LR		SR			Maize	Beans		
Depth	NP	FYM	CR	May	Aug	Nov	Dec	Jan	May	Nov	Dec	%M
0-15	None	None	-	18.2(4.0)	11.4(1.6)	11.0(1.6)	26.3(2.7)	29.7(4.5)	23.9(5.1)	35.3(2.0)	34.4(8.8)	10.5(3.1)
			+	13.2(2.4)	17.3(2.4)	9.1(0.4)	29.4(2.9)	31.7(3.6)	22.3(3.2)	11.1(3.8)	28.1(6.2)	11.2(0.7)
		FYM1	-	16.0(1.2)	14.8(1.3)	8.9(4.5)	28.3(1.6)	39.7(3.7)	21.9(5.6)	46.6(5.9)	19.8(4.3)	29.9(3.2)
			+	18.7(3.4)	16.7(4.0)	12.8(4.2)	33.6(4.2)	40.(1.2)	25.8(4.4)	57.6(4.5)	25.7(3.6)	21.3(0.5)
		FYM2	-	21.2(5.3)	16.8(0.2)	15.8(1.8)	28.7(2.8)	33.5(2.1)	13.6(5.9)	30.9(4.0)	32.7(2.0)	31.3(1.1)
			+	20.8(3.8)	24.0(4.4)	18.2(3.2)	28.0(4.0)	28.5(4.5)	27.6(5.2)	19.4(2.9)	36.1(7.6)	45.7(3.7)
	NP1	None	-	14.7(2.7)	16.9(2.7)	7.6(1.8)	19.8(4.4)	25.3(1.0)	19.2(2.0)	34.4(3.7)	45.6(3.9)	21.2(1.6)
			+	23.3(3.5)	16.2(2.8)	13.1(2.5)	23.6(2.5)	38.2(2.5)	30.0(8.1)	26.9(1.0)	45.8(2.8)	20.6(0.3)
		FYM1	-	21.0(3.2)	14.6(0.9)	10.9(3.0)	28.7(4.2)	29.9(4.2)	28.7(6.4)	32.1(5.8)	59.7(9.9)	19.9(0.6)
			+	22.2(1.9)	11.4(1.4)	14.6(2.0)	28.8(2.8)	27.1(3.3)	28.0(1.5)	50.9(3.6)	54.7(1.8)	27.4(1.1)
		FYM2	-	20.3(3.6)	14.5(2.4)	11.1(1.9)	26.8(4.8)	35.0(3.6)	31.9(1.0)	52.3(6.4)	63.5(6.9)	16.3(2.8)
			+	21.5(2.2)	16.3(1.9)	11.3(1.7)	21.2(1.6)	32.7(5.8)	26.9(3.5)	43.8(6.2)	47.6(6.5)	19.6(2.5)
	NP2	None	-	13.9(2.2)	13.1(0.8)	7.6(0.7)	18.3(3.8)	22.0(1.4)	13.7(5.0)	48.6(3.2)	19.5(4.7)	18.1(0.6)
			+	16.1(3.1)	16.3(1.5)	12.8(1.4)	14.9(2.4)	25.5(1.5)	17.8(6.3)	46.5(7.9)	23.1(5.3)	19.0(2.5)
		FYM1	-	28.6(7.5)	18.3(0.8)	11.9(1.1)	26.4(4.7)	39.8(2.3)	29.0(8.2)	58.9(5.6)	25.5(7.2)	21.7(5.0)
			+	16.6(1.9)	14.4(3.6)	12.4(2.3)	33.4(1.0)	36.6(4.9)	18.5(4.4)	32.1(4.9)	50.4(5.4)	25.2(1.0)
		FYM2	-	20.0(5.9)	18.4(4.3)	9.5(0.7)	28.5(3.6)	39.7(4.1)	25.1(4.4)	36.5(6.0)	21.3(3.8)	24.3(0.5)
			+	14.8(1.5)	19.3(1.6)	8.3(8.3)	32.2(4.7)	39.3(5.3)	21.6(4.4)	25.4(4.4)	20.6(2.4)	16.5(2.4)
15-30	None	None	-	7.6(1.0)	14.0(2.1)	4.1(0.8)	20.1(3.8)	22.9(5.2)	n/a	n/a	n/a	n/a
			+	5.8(0.6)	14.1(5.2)	6.4(1.5)	20.8(1.3)	25.9(2.1)	n/a	n/a	n/a	n/a
		FYM1	-	12.3(2.5)	11.7(2.9)	7.8(1.5)	23.1(2.8)	24.0(4.3)	n/a	n/a	n/a	n/a
			+	12.8(2.1)	16.0(4.0)	7.8(1.8)	22.6(2.3)	22.2(5.3)	n/a	n/a	n/a	n/a
		FYM2	-	20.4(1.5)	19.7(1.9)	7.5(1.3)	24.3(4.7)	22.6(4.5)	n/a	n/a	n/a	n/a
			+	14.0(1.0)	20.8(2.9)	9.9(1.4)	29.4(1.9)	34.8(2.9)	n/a	n/a	n/a	n/a
	NP1	None	-	8.2(0.6)	20.5(3.3)	6.9(0.4)	24.9(1.0)	17.0(4.6)	n/a	n/a	n/a	n/a
			+	13.4(1.5)	14.9(2.4)	7.2(1.6)	24.3(2.1)	22.2(4.4)	n/a	n/a	n/a	n/a
		FYM1	-	14.0(2.0)	9.6(1.9)	11.4(1.9)	24.7(3.8)	25.0(7.3)	n/a	n/a	n/a	n/a
			+	12.8(1.5)	9.9(1.5)	12.4(3.5)	23.1(3.2)	17.5(3.2)	n/a	n/a	n/a	n/a
		FYM2	-	11.7(2.1)	20.4(4.1)	5.6(0.9)	18.4(4.4)	21.9(2.2)	n/a	n/a	n/a	n/a
			+	7.6(1.2)	17.3(5.3)	11.2(1.5)	15.4(1.5)	33.3(3.9)	n/a	n/a	n/a	n/a
	NP2	None	-	8.2(0.6)	14.8(3.6)	8.3(1.6)	22.6(5.0)	16.8(2.8)	n/a	n/a	n/a	n/a
			+	8.8(2.0)	20.7(4.2)	6.9(2.0)	11.1(2.8)	25.2(6.5	n/a	n/a	n/a	n/a
		FYM1	-	17.5(1.8)	15.4(2.9)	11.6(1.2)	25.7(6.4)	21.9(5.9)	n/a	n/a	n/a	n/a
			+	8.8(1.0)	15.9(2.0)	9.5(2.6)	13.9(2.2)	25.1(1.5)	n/a	n/a	n/a	n/a
		FYM2	-	11.1(1.5)	21.1(6.6)	8.8(2.6)	22.6(6.9)	24.3(6.4)	n/a	n/a	n/a	n/a
			+	8.2(0.6)	14.9(2.3)	7.8(1.3)	19.8(1.4)	27.2(2.3)	n/a	n/a	n/a	n/a
ANOVA	Plant			n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	**19.2(0.00)**
0-15	Fertilizer (A)			1.3(0.29)	0.7(0.49)	0.5(0.64)	1.1(0.35)	2.2(0.13)	2.9(0.07)	**5.4(0.01)**	**4.8(0.01)**	**22.6(0.00)**
	Manure (B)			2.3(0.12)	**3.7(0.03)**	0.0(0.96)	2.9(0.07)	1.8(0.17)	1.2(0.31)	**12.6(0.00)**	**8.3(0.00)**	**17.7(0.00)**
	Residues (C)			0.0(0.89)	0.9(0.35)	1.0(0.33)	0.5(0.48)	0.0(0.92)	0.4(0.52)	**10.6(0.00)**	**4.0(0.05)**	0.2(0.62)
	A x B			0.7(0.62)	1.2(0.32)	**3.4(0.02)**	**2.6(0.05)**	**4.1(0.01)**	0.8(0.53)	**11.6(0.00)**	**12.8(0.00)**	**31.3(0.00)**
	A x C			1.7(0.20)	1.7(0.20)	0.1(0.88)	1.4(0.25)	2.2(0.12)	1.1(0.34)	**3.4(0.04)**	2.58(0.09)	1.6(0.21)
	C x B			0.4(0.67)	0.9(0.43)	1.6(0.21)	**3.8(0.03)**	**4.3(0.02)**	0.6(0.56)	**3.4(0.04)**	**4.5(0.02)**	**4.0(0.02)**
	A x B x C			1.2(0.33)	0.4(0.80)	1.3(0.31)	**3.7(0.01)**	**5.4(0.00)**	1.4(0.26)	**6.2(0.00)**	**5.1(0.00)**	**14.5(0.00)**
15-30	Fertilizer (A)			0.8(0.44)	0.5(0.62)	2.1(0.13)	**3.3(0.05)**	0.6(0.57)	n/a	n/a	n/a	n/a
	Manure (B)			**4.9(0.01)**	**3.8(0.03)**	**6.4(0.00)**	0.4(0.70)	2.6(0.09)	n/a	n/a	n/a	n/a
	Residues (C)			**5.3(0.03)**	0.0(0.89)	1.1(0.31)	2.8(0.10)	0.9(0.34)	n/a	n/a	n/a	n/a
	A x B			**7.0(0.00)**	1.4(0.26)	1.0(0.40)	**2.6(0.05)**	0.5(0.77)	n/a	n/a	n/a	n/a
	A x C			1.6(0.21)	0.5(0.59)	2.1(0.14)	**3.5(0.04)**	0.4(0.69)	n/a	n/a	n/a	n/a
	C x B			1.9(0.16)	0.6(0.58)	1.4(0.27)	1.0(0.39)	2.8(0.07)	n/a	n/a	n/a	n/a
	A x B x C			**8.0(0.00)**	0.5(0.74)	0.6(0.65)	0.7(0.60)	0.2(0.95)	n/a	n/a	n/a	n/a

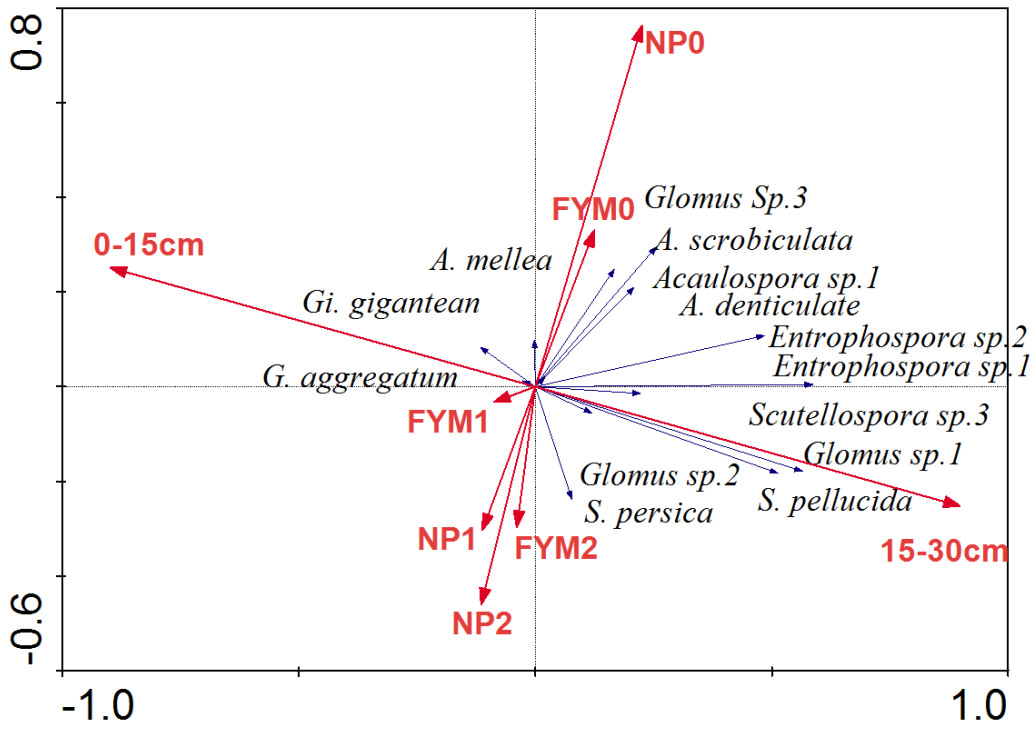

Figure 1. Arbuscular mycorrhizal fungal species composition under NP fertilization and organic inputs (FYM) in a Humic Nitisol, Kabete, central Kenya. Results of redundancy analysis are shown using the spore abundances of the different AMF species in two sampling depths (0-15 cm, 15-30 cm) in the field collected soil. Vectors representing different sampling depth and treatments are shown in red. Size and orientation of the vectors represent correlation among them and with the axes. The smaller the angle between the vectors (or a vector) and the longer the vectors, the more correlated are the variables represented by the vectors. FYM0 = 0 t ha^{-1} manure, FYM1 = 5 t ha^{-1} manure, FYM0 = 10 t ha^{-1} manure, NP0 = 0kg P; 0 kg N ha^{-1}, NP1 = 60 kg N; 26.4 kg P ha^{-1}, NP2 = 120 kg N; 52.8 kg P ha^{-1}.

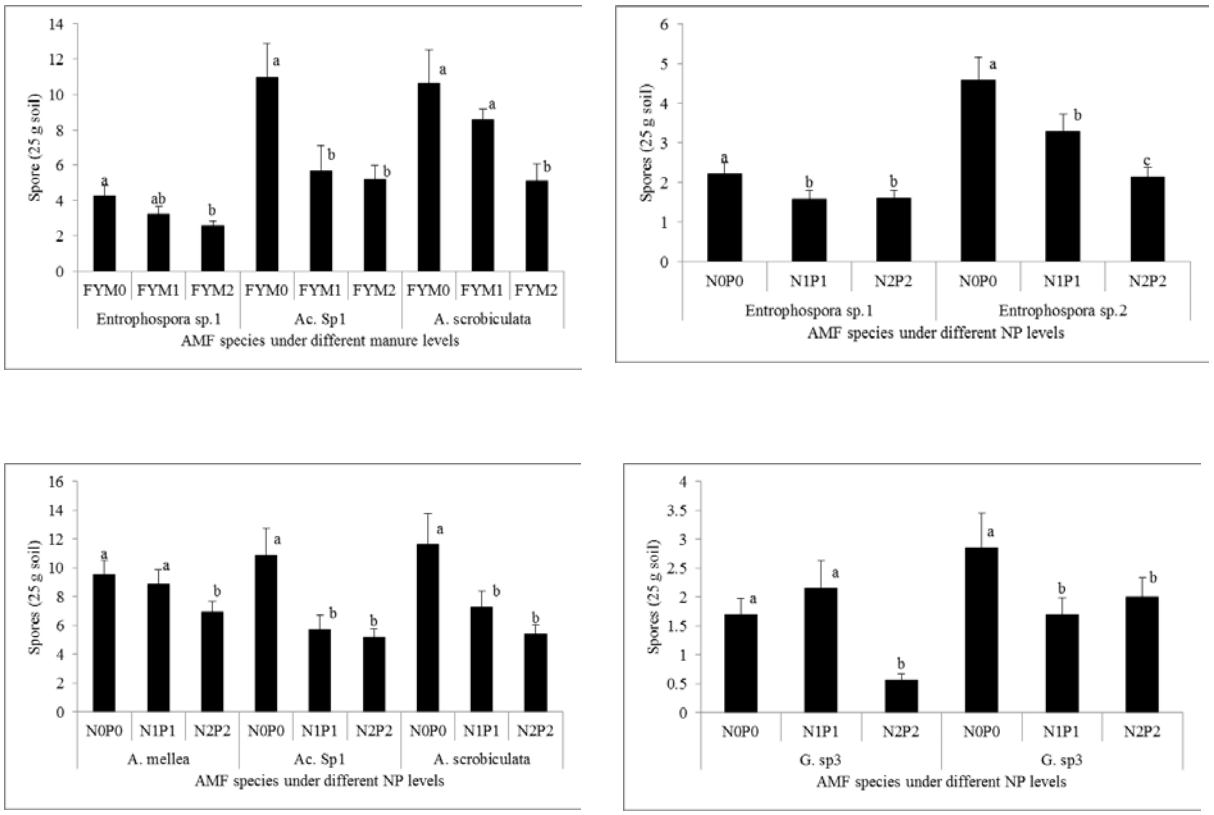

Figure 2. Abundances of spores of AMF species (per 25 gram soil dry weight) in a Humic Nitisol, Kabete, central Kenya. For treatment abbreviations see Table 1. Error bars represent the standard errors of means. Different letters indicate significant differences between the means as determined by LSD multiple range comparison ($P < 0.05$). A. = Acaulospora, G. = Glomus.

CHAPTER THREE

Effects of Conservation Agriculture practices and Nitrogen fertilizer on Arbuscular Mycorrhizal Fungal communities

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Abstract

Conservation agriculture (CA) has been proposed as a remedy against soil degradation in sub-Saharan Africa. However little is known how CA practices influence Arbuscular Mycorrhizal Fungal (AMF) communities in Kenyan agro-ecosystems. This study was carried out during the long-rains season and short-rains season to assess the effect of tillage, cropping practices, residue and nitrogen (N) fertilization on AMF communities. The trial consisted of two tillage practices (Conventional tillage, CT and No-till, NT), two cropping practices (continuous maize and maize-soybean rotation), two residue rates (retained and removed) and two N fertilization rates (0 and 60 kg N ha^{-1}) in a randomized complete block design with three replicates. Eighteen AMF species were isolated from the field site. The site was dominated by members of the genera *Acaulospora*, *Glomus* and *Scutellospora*. Tillage and N fertilization altered AMF species composition, but effects were minor. Spore abundance of *Glomus aggregatum*, *Scutellospora verrucosa* and *Acaulospora* sp.1 was higher in NT than in CT systems whereas abundance of *S. verrucosa*, *S. persica, S.* sp.1 and *G. aggregatum* was lower in fertilized than in unfertilized plots. Species diversity and fractional root colonization by AMF was affected by tillage x residue and tillage x N fertilization interactions. Crop residue addition resulted in highest spore abundance and root colonization in NT systems. N fertilization increased AMF colonization and hyphal length but negatively affected spore abundance and species richness. Species diversity, species composition and AMF activity were unaffected by crop rotation. It is concluded that a lower level of disturbance through NT and crop residue addition can have a positive influence on the AMF symbiosis.

KEY WORDS: Tillage, cropping systems, arbuscular mycorrhiza, crop residue, nitrogen fertilization

1. Introduction

Soil degradation is common in sub-Saharan Africa and is often associated with agricultural practices that decrease soil organic matter and nutrient levels (Drechsel et al., 2001). Conservation agriculture (CA) has been proposed as a remedy to agricultural problems in smallholder farming systems in the tropics (Hobbs et al., 2008). However, CA remains controversial (Giller et al., 2009). Zero-tillage, together with crop residue management (mulch) and crop rotation form the three pillars of CA. It is claimed that CA enhances biological processes above- and below-ground (Benites and Ashburner, 2003). The use of crop residues and reduced tillage may improve soil properties at the macroscopic level, which in turn affects chemical and biological properties of the soil, including Arbuscular Mycorrhizal Fungi (AMF) (Kabir, 2005). These fungi contribute to biological, chemical and physical soil quality (Cardoso and Kuyper, 2006). AMF are considered to be particularly important and useful in low-input agricultural systems for enhanced sustainability (Altieri, 1999; Jeffries and Barea, 2001; Jeffries et al., 2003). AMF constitute the interface between plant roots and soil, and their diversity, abundance, and function respond rapidly to changes induced by disturbance (Boddington and Dodd, 2000; Jansa et al., 2002). Recently Verbruggen and Kiers (2010) reviewed the selection pressures that modern agricultural practices exert on AMF communities. They concluded that tillage, fertilization with mineral nutrients and monocropping all exert strong negative selection pressure, and select for species-poor communities of aggressive colonizers that likely have limited benefit for the plant. They also suggested that alternative agricultural practices could revert this selection, leading to more beneficial (from the farmers' perspective) AMF communities.

Higher abundance of spores of AMF was reported in no-till systems compared to tilled systems (Jansa et al., 2002). A shift in AMF species composition was observed in Conventional Tillage (CT) systems compared to NT systems. Under CT species of *Glomus* dominated, whereas in NT species of *Scutellospora* dominated (Jansa et al., 2003; Boddington and Dodd, 2000). Increased AMF hyphal length (Kabir, 2005) and fractional root colonization (Castillo et al., 2006) were also reported in NT systems. Low AMF colonization, resulting in lower nutrient uptake and reduced yield, have also been shown in CT systems (Gavito and Miller, 1998; Galvez et al., 2001). Tillage does not only disrupt the mycorrhizal network but affects mycorrhizal functioning (Kabir, 2005). Increased N mineralization, increased soil temperature, reduced weed numbers and improved soil physical properties may also dominate over the direct disruptive effects (Gosling et al., 2006).

Crop rotations are associated with reduced pest infestations, improved water use efficiency, increased soil organic carbon (SOC) levels, greater soil aggregation, increased nutrient availability and greater soil biological activity (Hernanz et al., 2002; Wilhelm and Wortmann, 2004; Agyare et al., 2006; Kureh et al., 2006). Crop rotations that include highly mycotrophic plants, can increase AMF functioning (Plenchette et al., 2005), while rotations that include non-mycorrhizal crops (members of the Brassicaceae) can reduce AM functioning (McGonigle et al., 2011). Continuous mono-cropping can decrease mycorrhizal abundance and diversity and shift the AMF species composition toward species that are less beneficial to the crop (Johnson et al., 1992; Verbruggen and Kiers, 2010). Management of cropping systems and tillage practices for the benefit of mycorrhizal associations may be a more direct route towards benefiting from mycorrhizal associations, especially in the tropics where agriculture relies more on plant-soil biota interactions (Cardoso and Kuyper, 2006).

Little is known how CA affects AMF functioning in sub-Saharan Africa. CA is still in its early stage of adoption in Kenya, and nothing is known how the three principles of CA (no-tillage, crop rotation and crop residue management) alone or in combination impact on mycorrhizal functioning in Kenyan agro-ecosystems. Using a long-term trial initiated by Tropical Soil Biology and Fertility institute of CIAT (TSBF/CIAT) in 2005 at Nyabeda, we compared the effect of tillage, cropping systems, crop residue management and nitrogen fertilization on AMF communities. The aim of the study was to assess the effects of (i) tillage (till vs. no-till), (ii) maize-soybean rotation vs. maize mono-cropping, (iii) crop residues (present or absent) and (iv) nitrogen (N) fertilization (0 and 60 kg N ha^{-1}) on AMF communities. We hypothesized, following Verbruggen and Kiers (2010) that the CA practices will support higher AMF diversity and abundance than conventional agricultural practices.

2. Material and methods

2.1. Study site

The study was conducted in Nyabeda (western Kenya; latitude: 0° 06'N; longitude 34° 36'E) at an altitude of 1420 m.a.s.l. The area falls within the humid agro-climatic zone with a mean annual rainfall of 1800 mm, bimodally distributed (mid-march – June; mid-October – December) and a mean monthly temperature of 23° C (Jaetzold et al., 1982). The soil has been classified as a Ferralsol (FAO, 1990) with an average particle size distribution of 64% clay, 21% silt and 15% sand, a pH range of 4.7 to 5.3, and bulk density of 1.16 g cm^{-3} in the plough layer (0-20 cm; Kihara et al., 2012a). Maize (*Zea mays*) is the main staple crop and it

is normally grown either as a mono-crop or in association with legumes, mainly common bean (*Phaseolus vulgaris*) and groundnut (*Arachis hypogaea*). Adoption of the cash crop soybean (*Glycine max*) is currently taking place. Smallholder settlements dominate the area, with land sizes ranging from 0.3 to 3 ha per household.

2.2. Experimental Design

The experiment was set up in 2003 as a split-split-split plot design and involved a factorial combination of tillage system (no-tillage vs. conventional tillage), cropping system (soybean-maize rotation vs. maize monocropping), crop residue (maize stover and soybean residue at 2 t ha^{-1}; added or removed) and nitrogen (N) application (0 or 60 kg N ha^{-1}, as urea). The treatments were laid out in a full factorial, randomized complete block design (RCBD) with 3 replicates (Table 1). Plots were 4.5 m wide and 7 m long. All treatments received 60 kg ha^{-1} phosphorus (P) in the form of triple super phosphate (TSP) and 60 kg ha^{-1} potassium (K) in the form of KCl annually. Maize was planted at an intra-row spacing of 0.25 m and an inter-row spacing of 0.75 m with two seeds per planting hole, later thinned to one plant per hill (53,000 hills ha^{-1}). Soybean was planted at an intra-row spacing of 0.05 m and an inter row spacing of 0.75 m (266,000 seeds ha^{-1}). At the onset of the experiment, land was prepared uniformly across all plots by hand-ploughing to 15 cm depth. In tilled plots (CT) subsequent tilling operations involved hand-hoeing up to 10 cm. In no-till plots (NT) weeding was done by hand-pulling and in some cases by surface-scratching by hand-hoe to 3 cm depth. Prior to establishment of the experiment, the plot had been under native vegetation of grasses and shrubs.

2.3. Soil sampling and analysis

Soil for assessment of chemical properties and AMF spores was sampled once (late February – early March, 2008) at two sampling depths (0-15 and 15-30 cm). Sampling was done after maize harvesting, coinciding with the end of short rains season, when sporulation is high and spores are in a better condition for identification (Douds and Millner, 1999). For assessment of AMF extraradical hyphal length and for fractional root colonization, we sampled thrice during the long rainy season (6, 10 and 14 weeks after planting, April – August, 2008) and thrice during the short rainy season (4, 8 and 12 week after planting maize, October 2008 to February, 2009). Ten random samples were collected from each depth and mixed thoroughly to obtain a composite sample for each plot.

Soil analysis was performed at World Agroforestry Centre (ICRAF), using near infra-red spectroscopy (NIRS) as described by Shepherd et al. (2003). The method uses a Field Spec FR spectro-radiometer (Analytical Spectral Devices Inc., Boulder, Colorado) at wavelengths from 0.35 to 2.5 μm with a spectral sampling interval of 1 μm. In brief, the soils were first air-dried and passed through a 2-mm sieve. Using the spectral library approach, a sub-sample of 25 samples of the total 96 samples were selected for wet-chemistry analysis based on their spectral diversity. This was done by conducting a principal component analysis of the first derivative spectra and computing the Euclidean distance based on the scores of the significant principal components. Random samples were then selected from each quartile of the ranked Euclidean distances to make up the 25 samples for analysis by wet chemistry. These 25 soil samples were analyzed following standard methods for tropical soils (Anderson and Ingram, 1993). Soil pH was determined in water using a 1:2.5 soil : solution ratio. Samples were extracted with 1M KCl using a 1:10 soil: solution ratio, by atomic absorption spectrometry for exchangeable Ca and Mg. P and K were extracted with 0.5 M $NaHCO_3$ + 0.01 M EDTA (pH 8.5, modified Olsen) using a 1:10 soil : solution ratio. Exchangeable K was analyzed by flame photometer and available P colorimetrically by molybdenum blue. Soil Organic Carbon (SOC) was determined colorimetrically after H_2SO_4 - dichromate oxidation at 150° C for 30 min. Total N was determined by Kjeldahl digestion with sulphuric acid and selenium as a catalyst. Effective cation exchange capacity (ECEC) was calculated as the sum of exchangeable bases. The results of the 25 samples were used for prediction of soil properties using NIRS by partial least-squares regression (PLSR). Full hold-out-one cross-validation was done to prevent over-fitting of the model. All calibrations were developed on ln-transformed variables. The validation of the spectral models for exchangeable K, effective cation exchange capacity (ECEC), C and total N indicated moderate accuracy with r^2 values of 0.80, 0.82, 0.93 and 0.83 respectively, that for available P a lower accuracy (0.61), while the spectral models for Ca (0.42), Mg (0.30) and pH (0.30) had r^2 values below 0.50.

2.4. Trap cultures

Soil sampled for assessment of AMF spores was used to establish trap cultures, to also trap species that may not have sporulated at the time of sampling. Trap cultures were initiated according to the recommendation of Morton et al., (1993). Briefly a pot culture (each pot representing a single plot) was set up at the National Museums of Kenya, Nairobi using two host plants; sorghum (*Sorghum bicolor*) and cowpea (*Vigna unguiculata*). Sorghum was used

because 1000 AMF isolates of 98 species in various AMF genera have been able to grow and sporulate with Sudan grass, *Sorghum sudanense* (Morton et al., 1993). Cowpea has also been shown to be a suitable trap plant for tropical soils (Bagyaray and Stürmer, 2008). A sub-sample of 150 g from each soil inoculum was diluted with 150 g of autoclaved medium-sized sand and mixed before being poured into 0.5 l pot. Sorghum was sown at a density of 25 plants while cowpea was sown at a density of 6 plants per pot. Each pot was covered with autoclaved sand to prevent unintentional dispersal of AMF. After seedling emergence pots were watered daily with tap water. Pot cultures were maintained 4 months. During the last week, the moisture was successively lowered to stop plant growth and enhance sporulation of AMF.

2.5. Mycorrhizal inoculum potential

For estimation of AMF inoculum potential (MIP), three intact soil cores were taken by driving an 8 cm diameter × 10 cm deep steel core into the soil to a depth of 15 cm at end of the short-rains season, February, 2009. Soil cores were transferred to 0.5 l pots and immediately transported for the subsequent greenhouse bioassay establishment. Greenhouse bioassays were executed with three crops: sorghum, leek (*Allium porrum*) and cowpea. Seeds of these crops were surface-sterilized in 70% alcohol for 1 min and rinsed three times with sterile water. Pretreated seeds were germinated in sterilized hot water, after which they were planted in each pot. The pots were arranged on greenhouse benches in a randomized complete block design. The plants were grown without nutrient addition under natural light. Tap water was added as required. Plants were allowed to grow in the soil cores for 4 weeks, after which the crops were harvested by carefully washing their roots from the intact core. Roots were separated from organic debris by hand and stored in 70% alcohol before staining.

2.6. Spore extraction and taxonomic analysis

Spores of AMF from both the soil and trap cultures were isolated from 50 g (field soil) and 25 g (trap culture soil) via wet sieving and centrifugation. A very fine sieve (45 μm) was used to collect spores, and coarse material remaining on the top sieve (750 μm) was checked for sporocarps and very large spores. Spores were separated into groups according to general morphological similarities under a dissecting microscope. Permanent slides of spores were prepared by placing them in polyvinyl alcohol-lactic acid-glycerin (PVLG) mixed with Melzer's reagent. Spores were cracked open under the cover slip to allow observation of spore

wall characteristics and were identified to species according to classical morphological analysis under a compound microscope (Morton, 1988). INVAM isolates and voucher specimens were used as taxonomic references. Spores were identified to species when possible, but where this proved too difficult, to unnamed morphospecies. Voucher specimens are being kept at the NMK, Nairobi.

2.7. Assessment of AMF hyphal length

Hyphae were extracted from a 10 g soil subsample by the membrane filter technique (Jakobsen et al., 1992). Soil samples were mixed and suspended in 100 ml of deionized water, to which 12 ml of a sodium hexametaphosphate solution was added. The soil suspension was shaken for 30 s (end-over-end), left on the bench for around 30 min, and then decanted through a 45 μm sieve to retain hyphae, roots and particulate organic matter. The material on the sieve was sprayed gently with deionized water to remove clay particles, and then transferred into a 250 ml flask with 200 ml of deionized water. The flask was shaken vigorously by hand for 5 s, left on the bench for 1 min, and then a 2 ml aliquot was taken and pipetted onto 25 mm Millipore filters. The material on the filter was stained with 0.05% Trypan Blue in glycerol-water (1:1, v:v) and transferred to microscope slides. Hyphal length was measured with a grid-line intersect method at 200-400x magnification. Only non-septate hyphae were assessed.

2.8. Assessment of fractional mycorrhizal colonization

Roots obtained from the soil cores were washed free of soil by first soaking them in a bucket of water, followed by wet-sieving (one-mm mesh size) with tap water. Roots were separated from organic debris by hand. A subsample of field-collected roots was cut into one-cm segments, and stained using the modified procedure of Mason and Ingleby (1998). Roots were cleared in 2.5% KOH in an autoclave for 15 min at 121° C and bleached in a mixture of 30% H_2O_2 and 30% ammonium solution (1:1, v:v) for 30 min to remove phenolics. Roots were then acidified for 2 h with 1% HCl and stained with 0.05% acidified Trypan Blue dissolved in glycerol – water (1:1, v:v) by autoclaving the roots in this solution for 3 min at 121° C. Estimation of AMF colonization was done according to Trouvelot et al. (1986). Thirty root fragments were mounted on two slides, each containing 15 root fragments. The fragments were observed under the microscope (magnification 160 – 400 ×) for the presence of hyphae,

arbuscules and vesicles. Mycocalc (www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html) was used to calculate fractional root colonization.

2.9. Data analysis

AMF spore densities in each sample were calculated by summing abundances of all species in the sample. Species richness was calculated as species number in each sample. Shannon–Wiener diversity index (H′) was calculated for each field sample or trap pot. Analysis of variance (ANOVA) was performed to assess the effects of tillage, cropping system, residue, and N fertilization on AMF spore abundance, species richness, species diversity, root colonization and AMF hyphal length. Differences between treatment means were analyzed by multiple range comparison based on least significant difference (LSD) at $P < 0.05$. The effects of tillage, cropping system, residue, and N fertilization were assessed on AMF community composition (relative spore abundance of each AMF species) by a multivariate redundancy analysis (RDA) in CANOCO (Version 4.55). All data were tested for normality, and where necessary percent values were arcsine square root transformed; spore counts were logarithm (log+1) transformed to ensure conformity of the data with ANOVA assumptions.

3. Results

3.1. Soil chemical properties

Sampling depth significantly affected all soil parameters (Table 2). Several soil properties were significantly affected by tillage, residue, and N fertilization, whereas cropping system was not a significant source of variation. Several interactions, depth × tillage, depth × N fertilization, tillage × residue and tillage × N fertilizer were also significant. Available P, SOC, total N, pH, and ECEC were significantly higher in the 0-15 cm layer than in the 15-30 cm layer. CT increased levels of Ca, ECEC (at lower depth), K, available P, SOC and total N (at both depths) compared to NT. Soil pH was lower under NT than under CT in the 0-15 cm layer, but was unaffected by tillage practices in the 15-30 cm layer. Higher levels of P and Mg were observed in plots with residue than those without residue. Nitrogen application reduced levels of Ca, K, ECEC, SOC (only at 15-30 cm) and available P (at both depths). Soil pH declined after N fertilization at 0-15 cm but was unaffected in 15-30 cm. Soil pH was higher in NT+CR plots than NT-CR plots, but was unaffected by residue under CT.

3.2. AMF species composition and abundance of spores

In total 18 species from six genera of AMF were observed (Table 3). Eight taxa were identified to species level (*Acaulospora scrobiculata, A. mellea, Glomus aggregatum, G. multicaule, Scutellospora verrucosa, S. pellucida, S. persica,* and *Gigaspora gigantea)* while ten further species (*Entrophospora sp1. Paraglomus sp1., Acaulospora sp.1-3, Scutellospora sp.1-2* and *Glomus spp.1-3*) could not be identified to species level but were morphologically clearly distinct species. All 18 species were observed in sorghum trap cultures. In field soil *Acaulospora sp.2* and *sp.3* were not observed, while in the cowpea trap cultures *Entrophospora sp.1* and *Gigaspora gigantea* were not found. Species of *Glomus* and *Acaulospora* were most abundant, each constituting about 40% of total spores. Within *Glomus*, *G. aggregatum* was dominant, especially with sorghum as trap crop. *Acaulospora* species tended to be more abundant under CT than under NT, and *Glomus* species more abundant in N fertilized plots. The AMF community composition in the field-collected soil was significantly affected by both N fertilization (RDA, F = 5.52; P = 0.002) and tillage (RDA, F = 2.66, P = 0.008), and these factors explained 5% and 2% respectively of the variability in the dataset (Figure 1). In the trap cultures, tillage, cropping system, residue and N fertilization had no significant effect on AMF species composition. In the field soil, tillage negatively affected *G. aggregatum* (F = 5.82, P = 0.02), *S. verrucosa* (F = 4.89, P = 0.03) and *Acaulospora sp.1* (F = 10.15, P = 0.002), while N fertilization negatively affected *G. aggregatum* (F = 15.34, $P < 0.001$), *S. verrucosa* (F = 7.56, P = 0.007), *S. persica* (F = 7.49, P = 0.008) and *Scutellospora sp.1* (F = 3.59, P = 0.05) (Figure 2).

Mean spore abundance in field-collected soil was 107 ± 7 spores per 25 g soil, while in trap cultures spore abundance was much higher, 566 ± 41 per 25 g soil. Spore abundance was significantly affected by sampling depth in both field soil and trap cultures as well as by identity of host plant in trap cultures. Spore abundance was higher in 0-15 cm than in 15-30 cm in field-collected soil, but in trap cultures the opposite was observed. Spore abundance with sorghum as trap was twice that of cowpea as trap (Table 4). Tillage and the tillage × residue interaction were significant sources of variation in field soil only. Generally, NT systems had higher spore abundance than CT. NT systems with crop residue supported higher spore numbers than NT systems without residue (160 vs. 106 spores in 25 g soil). Addition of crop residue had no effect on spore numbers under CT systems. Presence of crop residues also increased spore number by almost 50% in the trap cultures. N fertilization affected spore abundance significantly in field-collected soil and in the trap cultures ($P < 0.05$ in both cases; Table 4). N fertilization reduced spore numbers by 37% in the field soil and 29% in the trap cultures (Table 4). The depth x tillage x N fertilization interaction was also observed in trap

cultures (F = 4.16, $P = 0.04$). NT systems with N fertilization recorded lower spore numbers (444 spores in 25 g soil) in 15-30 cm depth compared to NT system without N fertilization (1027 spores in 25 g soil), CT systems with (865 spores per 25 g soil), and plots without (947 spores in 25 g soil) N fertilization.

3.3. AMF species richness and diversity

AMF species richness and diversity was higher in field-collected soil than in trap cultures. On average 8.8 species were found in field soil and 7.6 species in trap cultures. Species diversity (Shannon-Wiener) was on average 1.52 in field soil while in trap cultures it was 1.15. Sampling depth affected both richness and diversity in trap cultures, but only richness was affected in field-collected soil (Table 4). Host plant identity affected species diversity in trap cultures. Higher richness occurred in 0-15 cm in field soil than in 15-30 cm, but in trap cultures the opposite pattern was observed (Table 4). Diversity was always higher in 0-15 cm than in 15-30 cm in trap cultures with cowpea supporting higher AMF diversity than sorghum. In field soil, species richness was affected by N fertilization ($P = 0.01$) and the interaction tillage × N fertilization ($P = 0.02$) while diversity was affected only by tillage ($P = 0.01$). Species richness was lower in fertilized than in unfertilized CT systems (8.1 versus 9.6 species). Species diversity was higher in CT than in NT systems (Table 4). In trap cultures, both species richness ($P = 0.002$) and diversity ($P < 0.001$) were affected by host identity × depth interaction, but only species richness was affected by residue ($P = 0.03$) and host identity × residue interaction ($P = 0.008$). Residue application significantly increased species richness in 0-15 cm (6.5 vs. 7.7 species), but not in 15-30 cm.

3.4. AMF colonization in the bioassay

Fractional root colonization in the bioassay was used as a measure of Mycorrhiza Inoculum Potential (MIP). MIP varied with plant species (F = 160.25, $P < 0.001$). MIP was highest in leek (39%), intermediate in cowpea (17%) and lowest in sorghum (13%). MIP in all three plants was significantly affected by N application and residue, but not by tillage and cropping system. N application increased MIP, while residue decreased MIP (Table 5). MIP was also affected by tillage × cropping system interaction, cropping system × residue interaction, cropping system × nitrogen interaction, and tillage × residue × nitrogen interaction (Table 5). NT systems with maize-soybean rotation had lower MIP compared to NT systems with continuous maize systems. Presence of residue in maize-soybean rotation reduced MIP

compared to continuous maize with residue, but in absence of residue maize-soybean rotation had higher MIP than continuous maize (Table 5). An increase in MIP was observed after N-fertilizer application in maize-soybean rotation compared to continuous maize. In tillage × residue × N application interaction, N fertilized NT systems plus residues addition had low MIP compared to N fertilized NT systems minus residue additions. Similar effect was also observed in CT systems.

3.5 AMF colonization in field crops (maize and soybean)

Mycorrhizal root colonization was affected by tillage, cropping system, residue and nitrogen application (Table 5). It was also affected by residue × tillage interaction, nitrogen × residue interaction, and nitrogen × cropping systems interaction. Table 5 shows how AMF colonization varied across different treatments during various sampling times. Briefly, root colonization was higher in NT than in CT at most sampling dates, with consistently higher colonization in NT than CT in the presence of residue. Application of N fertilizer increased root colonization, especially in continuous maize. Effect of residue varied with sampling time, with low root colonization in early sampling during long rainy season and high colonization in early sampling during short rainy season. Presence of residue improved root colonization in N-fertilized plots in November, but this effect disappeared in December.

3.6. Extraradical hyphal length of AMF

Extraradical hyphal length (EH) ranged between 7.1 and 32.8 m g^{-1} soil. The EH varied across season. It was low at early growth period, increased at mid-period of growth and declined just before harvest. EH was higher in 0-15 than at 15-30 cm at most sampling dates. EH was significantly affected by nitrogen application during May sampling (13.8 in fertilized versus 8.9 m g^{-1} in unfertilized soil), and unaffected by tillage, cropping system, residue and their interactions.

3.7. Relationship between AMF and soil properties

There were no significant correlations between measured soil parameters and AMF spore abundance, species richness and species diversity in the 0-15 cm depth. However there was a significant positive correlation between species richness and levels of Ca (r = 0.31), and ECEC (0.30) at 15-30 cm depth ($P < 0.05$ in both cases). AMF extraradical hyphal length was significantly positively correlated with levels of Mg (r = 0.36), Ca (r = 0.28), ECEC (r =

0.32), N (r = 0.30) and SOC (r = 0.31) and negatively correlated with soil pH (r = -0.32). AMF colonization of field crops correlated positively with levels of Ca (r = 0.22), K (r = 0.35), and negatively with P (r = -0.31), N (r =- 0.26) and SOC (r = -0.27).

4. Discussion

Our study showed that NT systems plus crop residue supported high spore abundance and AMF colonization. This result is in line with studies showing soil disturbance through tillage as one of the important factors negatively affecting AMF communities (Kabir et al., 1998; Galvez et al., 2001; Jansa et al., 2002; Castillo et al., 2006; Celik et al., 2011). Crop residues addition under NT systems is associated with improvement of the micro-climate (reduced soil evaporation) and improved water infiltration, which are responsible for maintaining soil biota (Erenstein, 2002; Bationo et al., 2007). Our result supports the importance of crop residue in sustaining AMF diversity and their activity under NT systems. However undecomposed crop residue might also have negative effects on AMF inoculum potential (Table 5), probably due to phytotoxicity to AMF hyphae due to undecomposed crop residues (Kahiluoto et al., 2012).

Negative effects of CT on spore abundance and alteration of AMF community composition are attributed to mechanical disruption of the mycorrhizal network, dilution of AMF inoculum, changes in nutrient availability, changes in microbial activity, and changes in density of weeds that serve as alternative hosts for AMF (Jansa et al., 2003). The undisturbed hyphal network in NT systems likely explains the survival of *Scutellospora* species in our site. Although extraradical AMF hyphal length (MEH) was not affected by tillage, it is possible that tillage affected hyphal production of some species like members of the genus *Scutellospora*. High abundance of weedy AMF species (*G. aggregatum*) might have compensated for the disrupted hyphal network, thus masking the general effect of disturbance. The MEH reported here (between 7 and 33 m g^{-1} soil) is in the range of what has been reported earlier in annual cropping systems (Purin et al., 2006). Although some saprotrophic fungi (especially members of the Mucoromycotina such as *Morteriella*) also form non-septate hyphae, they commonly contribute less than 20% of total hyphal length (Bingham and Biondini, 2009). Hence hyphal length data are considered to be of AMF origin. We noted a lack of tillage effects on AMF hyphal length. The same effect was reported by Castillo et al. (2006). Our results contradict other studies that showed that disturbance reduced mycorrhizal activity in particular hyphal networks (Goss and De Varennes, 2002; Miller, 2000; Miller et

al., 1995). Studies on effect of tillage on active AMF mycelia may be desirable since tillage affects the viability of hyphae more than total length (Borie et al., 2006).

Tillage improved soil fertility (Ca, ECEC, K, P, N and SOC) in this study. However, levels of available P were still low. This result was surprising and contradicts claims on the beneficial effects of CA (reduced or no-tillage) on soil organic matter and nutrient availability (Yoo et al., 2006; Gal et al., 2007). Inadequate amounts of residue in NT systems and the intensity of tillage might have influenced our results. The amount of residue applied (2 t ha^{-1} yr^{-1}) was low compared to much higher amount of residue (8-16 t ha^{-1} yr^{-1}) used elsewhere (Blanco-Canqui and Lal, 2007). Soil tillage in CT was also quite shallow (0-10 cm) and rather comparable to reduced tillage in other studies (Borie et al., 2010). Similar results were reported by Follett et al. (2005) and Blanco-Canqui and Lal (2008) indicating that under certain condition NT systems may not always increase soil fertility and SOC levels.

Shifts in AMF composition, reduced spore abundance and species richness following N fertilization has been reported regularly (Egerton-Warburton and Allen, 2000; Treseder and Allen, 2002; Johnson et al., 2003). N addition (and that of other nutrients) results in lower C allocation to AMF (Treseder 2004). Especially species from *Gigasporaceae* have high C requirements and these species are thus more sensitive to nitrogen fertilization (Egerton-Warburton and Allen, 2000; Treseder and Allen, 2002; Egerton-Warburton et al., 2007). Decline in spore abundance of *G. aggregatum* at higher N levels is therefore difficult to understand, as this species has weedy attributes (Hart and Reader, 2002a, b). Increased levels of AMF colonization, extraradical hyphal length and mycorrhizal inoculum potential following N fertilization could be due to improved agronomic performance (increased root length) of crops in fertilized soils (Kihara et al., 2012a). Although N fertilization had negative effects on AMF diversity, our result show that N addition is still vital for improved AMF activity, especially under NT systems.

Crop plant and type of rotation can affect diversity and function of AMF (Karasawa et al., 2002; Verbruggen and Kiers, 2010). Our results, however, did not show a significant effect of crop rotation on AMF diversity and activity. Soybean was chosen as second crop in this study due to its high biomass production and its ability to fix atmospheric nitrogen (Kihara et al., 2012b). However, its influences on AMF in this site are not fully understood. Mathimaran et al. (2007) found no effect of crop rotation on AMF diversity, though they recorded a shift in species composition in a rotation of maize with Crotalaria (*Crotalaria grahamiana*). A similar study found high AMF colonization in maize-Tithonia (*Tithonia diversifolia*) rotation but not in maize-Crotalaria rotation (Muchane et al., 2010). This

suggests that the effect of crop rotation on AMF depends on the choice of the host species. It is also possible that long-living weeds under NT systems acted as alternative host for AMF, masking the effect of crop rotation. Further studies are desirable to understand the influences of soybean on AMF.

We recorded 18 AMF species which is within the range of AMF species numbers reported from agro-ecosystems in Kenya (Jefwa et al., 2009; Mathimaran et al., 2007). We recorded higher spore numbers compared to earlier studies in this region (Mathimaran et al., 2007). Five years before the onset of the trial the site was under secondary fallow which may have supported high spore numbers as shown by other fallows in Africa (Onguene and Kuyper, 2005). Our study showed dominance of *G. aggregatum* which dominated the AMF community and which was preferentially enhanced by the sorghum trap culture. This observation supports earlier reports that trap cultures act as a filter allowing sporulation of part of the indigenous AMF species that are fast colonizers that sporulate in a fast-growing host in a short time span (Leal, 2009). Hart and Reader (2002a) had earlier shown that *G. aggregatum* is a fast root colonizer, colonizing roots one week after inoculation. Nevertheless, trap cultures revealed two species not found in field-collected soil, justifying the use of trap cultures for more complete AMF surveys than direct isolation of spores from field soils (Mathimaran et al., 2007).

5. Conclusion

Agricultural practices such as tillage, fertilization and mono-cropping are hypothesized to have negative impacts on the functioning of AMF communities over ecological and evolutionary time scales (Verbruggen and Kiers, 2010). We therefore expected that CA practices have a beneficial impact on AMF. However, we found limited support for this hypothesis. We observed a positive effect of NT plus crop residue addition on spore numbers and root colonization, and a shift in AMF species composition following soil disturbance through tillage and N fertilization. However, effects were weak. One major reason has been suggested by Kihara et al. (2012a), who noted that the various treatments unlikely exerted strong selective pressure: conventional tillage was shallow, and little fertilizer was added. Despite that, our result suggests that no-till systems have the potential in sustaining AMF communities in Kenyan agro-ecosystems. However, success of NT (and of CA in general) is dictated by accumulation of adequate amounts of organic matter to prevent sealing, crusting and soil compaction in these Ferralsols. Further research is needed to translate such changes

into functional consequences for agro-ecosystem functioning, the more so as conservation agriculture is still subject to heated debate about socio-economic benefits (Giller et al., 2009).

Table 1: Overview of tillage, cropping systems, crop residue application, and N fertilization in Nyabeda field trial, Western Kenya.

	Tillage	Cropping systems	Crop residue	N fertilization	Treatment Code
1	No till	Continuous maize	Removed	0 kg N ha^{-1}	NT-CM-R0-0N
2	No till	Continuous maize	Removed	60 kg N ha^{-1}	NT-CM-R0-60N
3	No till	Continuous maize	Retained	0 kg N ha^{-1}	NT-CM-R1-0N
4	No till	Continuous maize	Retained	60 kg N ha^{-1}	NT-CM-R1-60N
5	No till	Maize-soybean rotation	Removed	0 kg N ha^{-1}	NT-SM-R0-0N
6	No till	Maize-soybean rotation	Removed	60 kg N ha^{-1}	NT-SM-R0-60N
7	No till	Maize-soybean rotation	Retained	0 kg N ha^{-1}	NT-SM-R1-0N
8	No till	Maize-soybean rotation	Retained	60 kg N ha^{-1}	NT-SM-R1-60N
9	Tillage	Continuous maize	Removed	0 kg N ha^{-1}	CT-CM-R0-0N
10	Tillage	Continuous maize	Removed	60 kg N ha^{-1}	CT-CM-R0-60N
11	Tillage	Continuous maize	Retained	0 kg N ha^{-1}	CT-CM-R1-0N
12	Tillage	Continuous maize	Retained	60 kg N ha^{-1}	CT-CM-R1-60N
13	Tillage	Maize-soybean rotation	Removed	0 kg N ha^{-1}	CT-SM-R0-0N
14	Tillage	Maize-soybean rotation	Removed	60 kg N ha^{-1}	CT-SM-R0-60N
15	Tillage	Maize-soybean rotation	Retained	0 kg N ha^{-1}	CT-SM-R1-0N
16	Tillage	Maize-soybean rotation	Retained	60 kg N ha^{-1}	CT-SM-R1-60N

Table 2: Effect of tillage, cropping system, crop residue, and nitrogen fertilization on average (± SE) soil properties: effective cation exchange capacity (ECEC), exchangeable cations, calcium (Ca), potassium (K), and magnesium (Mg), available phosphorus (P-Olsen), total nitrogen (N), soil organic carbon (C) and soil pH in the Nyabeda field trial, Kenya. For treatment abbreviations see Table 1. Interaction tested factors, but only significant interactions are presented. ANOVA table shows F-value and p-value in parentheses. Values in bold are significant ($P < 0.05$).

				CEC	Ca	K	Mg	P	pH	C	N
Depth	Treatment	N	CR	(meq/100g)		ppm				g/kg	
0-15	NTCM	-	-	13.5(0.2)	1095.1(7.6)	84.5(5.1)	224.8(3.8)	9.4(0.7)	5.2(0.1)	19.4(0.5)	1.4(0.1)
		-	+	13.1(0.1)	1065.5(11.2)	99.5(9.4)	216.0(1.6)	13.6(1.4)	5.3(0.1)	20.0(0.5)	1.5(0.1)
		60N	-	13.4(0.3)	1079.5(31.8)	83.5(13.5)	223.6(2.8)	9.4(1.8)	5.2(0.1)	19.3(1.2)	1.5(0.1)
		60N	+	12.9(0.1)	1053.4(4.2)	90.1(7.5)	216.1(2.7)	11.5(1.2)	5.3(0.1)	19.6(0.3)	1.5(0.1)
	NTSM	-	-	13.2(0.1)	1073.2(4.2)	94.9(6.7)	218.7(1.6)	11.5(1.2)	5.3(0.1)	19.3(0.6)	1.5(0.1)
		-	+	13.3(0.1)	1078.7(4.6)	99.3(5.9)	219.0(2.3)	13.2(1.1)	5.3(0.1)	20.1(0.2)	1.5(0.1)
		60N	-	13.8(0.3)	1082.9(19.7)	72.9(5.1)	230.4(2.2)	9.2(0.7)	5.2(0.1)	20.6(0.6)	1.5(0.1)
		60N	+	13.2(0.2)	1072.9(11.0)	100.4(9.5)	217.4(1.9)	13.3(1.3)	5.3(0.1)	19.6(0.3)	1.4(0.1)
	CTCM	-	-	13.2(0.4)	1083.4(30.4)	117.8(21.8)	215.3(3.8)	15.7(3.1)	5.4(0.1)	20.4(1.2)	1.5(0.1)
		-	+	13.3(0.1)	1091.0(1.0)	126.5(9.0)	214.3(1.5)	16.4(0.6)	5.4(0.1)	19.7(0.6)	1.5(0.1)
		60N	-	13.1(0.1)	1058.1(5.9)	89.2(5.2)	217.7(2.6)	11.9(1.0)	5.3(0.1)	19.9(0.1)	1.5(0.1)
		60N	+	13.6(0.1)	1092.2(3.8)	113.5(4.4)	218.6(0.3)	16.1(0.7)	5.3(0.1)	21.4(0.6)	1.6(0.1)
	CTSM	-	-	13.1(0.1)	1078.4(9.3)	112.8(4.0)	215.3(2.6)	15.6(0.5)	5.4(0.1)	20.1(0.3)	1.5(0.1)
		-	+	13.4(0.1)	1099.4(11.8)	136.0(17.3)	213.2(2.0)	18.4(1.8)	5.4(0.1)	21.8(0.1)	1.6(0.1)
		60N	-	13.4(0.2)	1088.1(5.2)	103.6(13.4)	220.4(4.6)	13.6(1.9)	5.3(0.1)	20.1(0.4)	1.5(0.1)
		60N	+	13.6(0.1)	1087.4(10.5)	109.2(19.0)	220.4(3.4)	14.9(2.7)	5.3(0.1)	20.6(0.1)	1.5(0.1)
15-30	NTCM	-	-	12.0(0.1)	1006.1(16.3)	59.1(5.8)	215.9(2.3)	5.6(1.0)	5.4(0.1)	15.6(0.7)	1.3(0.1)
		-	+	12.2(0.2)	1016.1(16.6)	73.5(3.9)	212.6(2.2)	8.7(0.4)	5.4(0.1)	17.9(0.4)	1.5(0.1)
		60N	-	11.4(0.6)	958.3(52.2)	48.9(15.7)	212.2(5.3)	4.8(1.8)	5.4(0.1)	15.7(2.1)	1.4(0.1)
		60N	+	11.1(0.3)	922.2(20.8)	41.4(7.3)	208.1(0.5)	4.2(0.7)	5.4(0.1)	15.4(0.3)	1.4(0.1)
	NTSM	-	-	12.0(0.2)	991.5(19.9)	58.9(9.5)	215.1(0.6)	6.9(2.0)	5.4(0.1)	16.6(1.5)	1.4(0.1)
		-	+	12.4(0.3)	1020.6(14.8)	69.9(1.4)	215.6(3.1)	9.0(0.5)	5.4(0.1)	18.0(0.3)	1.5(0.1)
		60N	-	11.0(0.6)	923.2(46.5)	40.8(8.5)	208.4(4.5)	3.4(0.8)	5.4(0.1)	14.7(1.1)	1.4(0.1)
		60N	+	10.6(1.0)	894.9(72.5)	42.2(13.4)	203.7(6.3)	4.0(1.6)	5.5(0.1)	13.5(1.9)	1.3(0.1)
	CTCM	-	-	12.2(0.6)	1020.3(42.7)	76.3(18.6)	213.8(4.5)	9.3(2.9)	5.4(0.1)	18.2(1.5)	1.5(0.1)
		-	+	12.8(0.1)	1058.1(5.3)	90.1(5.1)	216.6(1.9)	11.6(1.3)	5.4(0.1)	19.0(0.7)	1.5(0.1)
		60N	-	12.7(1.0)	1039.0(85.5)	107.9(8.8)	216.7(1.3)	14.4(6.5)	5.3(0.1)	19.3(3.0)	1.5(0.1)
		60N	+	12.2(0.4)	996.6(39.5)	60.0(11.4)	217.1(3.1)	7.0(1.9)	5.3(0.1)	17.0(1.4)	1.4(0.1)
	CTSM	-	-	12.6(0.2)	1047.6(11.1)	90.3(4.1)	214.3(2.1)	12.2(0.8)	5.4(0.1)	19.3(0.3)	1.5(0.1)
		-	+	12.7(0.1)	1057.8(4.8)	99.9(3.3)	213.5(0.7)	13.0(0.7)	5.4(0.1)	18.9(0.3)	1.5(0.1)
		60N	-	11.6(0.2)	967.8(15.8)	54.3(1.9)	211.6(1.8)	5.8(0.1)	5.4(0.1)	16.6(0.2)	1.4(0.1)
		60N	+	11.8(0.2)	959.4(16.2)	51.7(6.6)	211.7(0.7)	5.2(1.0)	5.3(0.1)	15.9(0.8)	1.4(0.1)
ANOVA											
0-15	Tillage (A)			0.1(0.81)	2.1(0.16)	**16.8(0.00)**	**8.2(0.01)**	**26.1(0.00)**	**7.4(0.01)**	**7.0(0.01)**	3.9(0.06)
	Cropping systems (B)			1.4(0.24)	0.7(0.41)	0.3(0.59)	0.6(0.44)	0.9(0.35)	0.0(0.83)	1.4(0.25)	0.1(0.72)
	Crop residues (C)			0.1(0.80)	0.0(0.92)	**6.6(0.02)**	**8.4(0.01)**	**11.8(0.00)**	2.8(0.11)	2.7(0.11)	1.4(0.24)
	Nitrogen (D)			1.3(0.27)	0.7(0.41)	**5.9(0.02)**	**6.8(0.01)**	**5.2(0.03)**	**7.8(0.01)**	0.0(0.87)	0.0(0.87)
	AxC			**11.7(0.00)**	**4.5(0.04)**	0.0(0.85)	**6.2(0.02)**	0.2(0.64)	**6.5(0.02)**	1.0(0.33)	0.0(0.97)
15-30	Tillage (A)			**8.8(0.01)**	**7.4(0.01)**	**28.6(0.00)**	3.7(0.06)	**14.5(0.00)**	3.5(0.07)	**10.4(0.00)**	**5.9(0.02)**
	Cropping systems			0.8(0.38)	1.0(0.32)	1.8(0.19)	2.4(0.13)	0.5(0.47)	0.2(0.69)	0.7(0.40)	0.3(0.62)
	Crop residues			0.0(0.86)	0.0(0.86)	0.0(0.83)	0.6(0.46)	0.0(0.97)	0.0(0.90)	0.0(0.98)	0.0(0.84)
	Nitrogen			**10.6(0.00)**	**13.4(0.00)**	**21.7(0.00)**	**5.0(0.03)**	**10.8(0.00)**	0.1(0.81)	**8.5(0.01)**	2.1(0.16)
	AxD			1.5(0.23)	0.6(0.46)	0.0(0.88)	**4.4(0.04)**	0.0(0.98)	**7.7(0.01)**	0.2(0.65)	0.0(0.86)
	BxD			2.0(0.17)	1.5(0.23)	**5.9(0.02)**	2.2(0.15)	**4.5(0.04)**	1.0(0.34)	2.8(0.10)	2.4(0.13)
	CxD			1.4(0.25)	1.8(0.19)	**8.3(0.01)**	0.4(0.55)	3.7(0.06)	0.3(0.61)	2.9(0.10)	1.7(0.20)
	AxBXD			0.1(0.77)	0.3(0.59)	**5.0(0.03)**	0.0(0.85)	1.9(0.17)	1.7(0.20)	0.0(0.88)	0.3(0.61)

Table 3: Arbuscular mycorrhizal fungal species spore abundance (25 g soil) in the field collected soil in 0–30 cm soil depth under different treatments (see Table 1 for abbreviations) at Nyabeda field trial, Kenya.

Tillage	----------NT----------								----------CT----------							
Cropping systems	----------CM----------				----------MS----------				----------CM----------				----------SM----------			
Crop residues	-------R0-------		-------R1-------		-------R0-------		-------R1--------		--------R0-------		--------R1-------		---------R0------		---------R1------	
N fertilization	0N	60N	0N	60N	0N	60N	0N	60N	0N	60N	0N	60N	0N	60N	0N	60N
Glomus aggregatum	28.7	14.8	72.3	35.3	44.0	21.6	76.9	24.3	23.9	18.3	41.4	13.3	40.8	12.6	34.4	14.3
G.multicaule	0.1	0	0.3	0.3	0.5	0.1	0.3	0.4	0	0.8	1.4	0.3	0.9	0.3	2.0	0.3
G. sp.1	4.8	4.6	3.5	5.4	5.2	2.3	3.5	7.7	3.4	6.1	5.2	1.7	3.8	4.4	2.8	3.8
G. sp.2	7.8	7.3	2.0	4.1	3.7	9.2	3.0	4.4	1.6	7.0	3.2	0.7	1.3	1.8	2.8	3.3
G. sp.3	0	0	0	0	0	0	0	0	0.2	0	0	0	0	0	0	0
Acaulospora mellea	0.3	0.1	0.9	0.5	2.2	1.6	0.8	0.6	0.3	0.8	0.5	0.2	2.5	0.4	0.5	0.3
A. scrobiculata	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0.1	0	0
A .sp.1	20.5	10.8	51.8	31.7	41.2	30.0	43.3	88.4	19.1	17.0	16.8	13.1	43.0	18.3	24.2	18.2
Scutellospora pellucida	5.2	3.0	2.1	1.6	2.2	4.4	3.6	3.3	4.8	2.0	1.8	0.7	1.2	1.8	2.3	0.3
S. verrucosa	42.8	17.1	59.9	17.8	24.8	15.8	33.7	36.1	32.5	18.5	22.9	10.4	18.2	17.3	15.7	10.1
S. persica	0.8	0.6	1.6	0.8	1.6	0.8	1.3	0.5	1.0	1.2	0.8	0.1	1.3	0.3	0.6	0.3
S. sp.1	3.2	1.6	2.4	1.9	2.3	0.9	2.8	2.0	2.6	2.3	0.9	0.5	2.3	1.5	1.0	0.8
S. sp.2	3.6	0.1	0	0	0.8	1.0	0	0	1.1	0.2	0.3	0.2	0.3	0	0	0.1
Entrophospora sp.1	2.5	0	1.0	1.8	2.6	2.2	0.3	3.7	2.0	1.2	0.7	0.4	7.9	0.2	0.9	1.2
Gigaspora gigantea	0.5	0	0.4	0.4	0.2	1.4	0	0	0.5	0	0.2	0	0	0	0.2	0.1
Paraglomus sp.1	0.3	3.7	0.9	0.3	18.0	0.9	0.1	1.9	1.8	0.5	1.5	0.2	0.7	0.6	2.8	1.1

Table 4: Diversity of AMF populations (spore abundance per 25 g soil; species richness; and Shannon (H) diversity index) under different treatments (see Table 1 for treatment abbreviations). Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Interaction tested for all factors, but only significant interactions are presented. Values in bold indicate significant effects ($P < 0.05$).

				----------Field soil----------			----------Trap cultures----------		
				Abundance	Richness	Shannon	Abundance	Richness	Shannon
Depth	Treatment	N	CR	(25 g soil)		(H)	(25 g soil)		H
0-15	NTCM	-	-	115.5(32.3)	8.3(0.3)	1.5(0.1)	77.89(26.6)	7.0(0.7)	1.4 (0.2)
		-	+	223.7(59.7)	10.3(0.3)	1.4 (0.1)	227.5(92.4)	7.8(0.7)	1.5(0.2)
		60N	-	69.2(13.7)	8.8(0.3)	1.6 (0.1)	157.3(45.7)	5.8(0.5)	1.1(0.1)
		60N	+	144.7(33.2)	10.7(0.3)	1.6 (0.1)	403.7(211.4)	8.5(0.9)	1.4(0.1)
	NTSM	-	-	179.8(24.1)	10.0(0.6)	1.6 (0.1)	374.0(230.5)	6.8(0.8)	1.2(0.2)
		-	+	209.0(74.5)	8.3(0.7)	1.3 (0.3)	418.8(202.1)	8.2(0.7)	1.3(0.2)
		60N	-	89.8(34.7)	9.7(1.5)	1.3 (0.3)	485.3(261.6)	6.7(0.3)	1.0(0.2)
		60N	+	226.3(96.7)	9.0(0.6)	1.3 (0.1)	222.5(81.1)	6.3(0.9)	1.3(0.2)
	CTCM	-	-	86.7(22.7)	9.3(0.3)	1.5 (0.2)	137.8(25.5)	6.5(0.9)	1.3(0.2)
		-	+	149.2(21.0)	10.7(0.9)	1.5 (0.1)	634.0(482.1)	7.3(0.8)	1.2(0.3)
		60N	-	98.3(13.4)	10.0(0.6)	1.8 (0.1)	327.7(85.5)	6.5(0.4)	1.4(0.2)
		60N	+	55.3(14.5)	7.0(1.0)	1.5 (0.2)	247.7(130.4)	6.7(0.5)	1.3(0.2)
	CTSM	-	-	148.8(32.4)	10.3(0.9)	1.6 (0.1)	393.3(272.2)	6.2(0.5)	1.2(0.2)
		-	+	122.7(25.7)	9.7(0.3)	1.5 (0.1)	332.3(174.7)	8.5(0.7)	1.4(0.1)
		60N	-	66.3(15.2)	8.7(1.3)	1.6 (0.1)	98.2(51.2)	6.3(0.3)	1.5(0.2)
		60N	+	60.8 (19.6)	8.0(0.1)	1.6 (0.2)	347.3(275.2)	8.2(0.6)	1.5(0.3)
15-30	NTCM	-	-	126.2(5.7)	8.7(0.9)	1.5(0.2)	704.8(258.2)	8.5(0.6)	1.0(0.2)
		-	+	174.7(12.2)	7.3(1.2)	1.2(0.2)	1080.2(266.1)	8.0(0.4)	1.0(0.2)
		60N	-	57.7(12.2)	6.7(0.9)	1.3(0.1)	310.0(127.0)	8.2(0.5)	1.4(0.1)
		60N	+	59.2(16.3)	7.3(0.3)	1.5(0.1)	673.2(203.6)	8.0(0.7)	0.9(0.2)
	NTSM	-	-	118.2(7.1)	8.7(0.9)	1.5(0.1)	729.3(323.1)	8.8(0.3)	1.2(0.2)
		-	+	130.2(62.9)	8.0(0.6)	1.4(0.1)	1594.3(502.7)	8.8(0.6)	0.9(0.3)
		60N	-	94.7(25.5)	9.3(0.9)	1.7(0.1)	321.2(87.4)	6.7(0.9)	0.8(0.2)
		60N	+	120.0(75.4)	8.0(1.5)	1.6(0.1)	472.7(139.8)	8.2(0.8)	1.1(0.2)
	CTCM	-	-	102.5(54.1)	10.0(1.2)	1.7(0.1)	520.8(125.9)	7.5(1.1)	1.1(0.2)
		-	+	45.7(4.9)	9.3(1.2)	1.7(0.1)	1083.2(379.5)	8.5(0.9)	0.9(0.1)
		60N	-	53.2(17.4)	7.7(1.2)	1.5(0.2)	695.8(162.9)	8.8(0.3)	1.0(0.2)
		60N	+	27.7(1.7)	7.7(0.7)	1.6(0.1)	1327.5(275.2)	7.8(0.9)	0.9(0.1)
	CTSM	-	-	99.2(26.3)	9.7(0.9)	1.6(0.1)	1250.5(249.2)	8.0(0.5)	0.7(0.1)
		-	+	57.3(18.4)	8.0(0.6)	1.7(0.1)	935.0(200.1)	6.7(0.8)	0.9(0.2)
		60N	-	52.7(11.9)	7.3(1.3)	1.3(0.3)	795.7(88.8)	8.2(0.6)	0.9(0.1)
		60N	+	47.2(9.8)	8.7(1.2)	1.7(0.1)	640.7(252.7)	7.8(0.7)	1.2(0.2)
ANOVA									
0-15 cm	Plant (P)			n/a	n/a	n/a	**19.1(0.00)**	2.2(0.14)	**18.8(0.00)**
	Tillage (A)			**8.3(0.01)**	0.3(0.60)	3.8(0.06)	0.2(0.62)	0.1(0.78)	0.6(0.46)
	Cropping systems (B)			0.8(0.38)	0.2(0.67)	1.2(0.28)	0.9(0.35)	0.1(0.71)	0.2(0.67)
	Crop residues (C)			3.7(0.06)	0.2(0.63)	1.9(0.17)	3.0(0.09)	**11.2(0.00)**	1.3(0.25)
	Nitrogen (D)			**10.5(0.00)**	3.6(0.07)	0.5(0.48)	0.4(0.54)	1.3(0.26)	0.1(0.82)
	P*A*B			n/a	n/a	n/a	**4.2(0.04)**	0.8(0.39)	0.0(0.90)
	AxC			**5.0(0.03)**	2.6(0.12)	0.0(0.95)	1.4(0.24)	1.2(0.28)	1.4(0.24)
	AxD			0.1(0.74)	**6.4(0.02)**	0.3(0.58)	2.3(0.14)	1.2(0.29)	0.6(0.46)
	AxBxC			0.0(0.95)	**5.4(0.03)**	0.7(0.40)	0.2(0.64)	0.4(0.52)	**4.0(0.05)**
15-30 cm	Plant (P)			n/a	n/a	n/a	**13.4(0.00)**	**9.0(0.00)**	1.0(0.33)
	Tillage (A)			**7.8(0.01)**	1.1(0.30)	3.2(0.08)	2.8(0.10)	0.6(0.45)	1.2(0.28)
	Cropping systems (B)			0.2(0.66)	0.6(0.45)	0.3(0.61)	0.2(0.67)	0.7(0.42)	0.2(0.67)
	Crop residues (C)			0.5(0.49)	0.7(0.40)	0.1(0.78)	**4.4(0.04)**	0.1(0.74)	0.1(0.72)
	Nitrogen (D)			**7.4(0.01)**	3.1(0.09)	0.0(0.97)	**7.4(0.01)**	0.2(0.66)	0.4(0.51)

Table 5. Treatment effects (see Table 1 for treatment abbreviations) on arbuscular mycorrhizal inoculum potential expressed as fractional root colonization (%M). Numbers in parentheses are standard errors (SE). Interactions tested for all factors but presented only for significant ones. ANOVA table shows F-value and p-value in parenthesis. Values in bold indicate significant effects ($P < 0.05$).

			AMF Colonization				
			Long rains		Short rains		MIP
Treatment	N	CR	May	July	November	December	%M
NTCM	-	-	30.4(2.3)	33.1(8.2)	26.9(7.3)	18.7(4.2)	27.0(7.2)
	-	+	25.6(3.1)	61.8(6.3)	23.2(6.0)	41.6(8.7)	29.1(7.9)
	60N	-	44.7(4.0)	50.8(6.6)	32.7(8.3)	24.8(6.4)	25.9(7.1)
	60N	+	23.8(3.0)	64.6(6.5)	48.4(6.3)	39.0(9.8)	18.3(3.4)
NTSM	-	-	34.5(5.7)	17.7(1.1)	36.6(17.2)	26.7(4.5)	19.1(3.5)
	-	+	28.1(2.8)	23.7(2.9)	42.0(10.4)	45.0(4.7)	13.6(1.8)
	60N	-	37.4(7.1)	26.7(9.8)	32.3(10.2)	29.7(9.6)	29.6(9.7)
	60N	+	26.6(6.6)	30.3(7.1)	53.0(10.9)	11.4(2.8)	15.6(2.8)
TCM	-	-	22.8(4.0)	46.0(6.8)	15.9(6.13)	31.3(2.8)	18.9(4.7)
	-	+	23.6(3.2)	36.7(1.6)	28.9(3.0)	15.3(6.3)	27.4(5.4)
	60N	-	27.4(4.0)	56.3(10.4)	51.9(11.9)	37.7(3.4)	21.7(1.9)
	60N	+	43.4(4.6)	39.4(4.0)	43.3(5.3)	25.8(3.4)	26.2(7.8)
TSM	-	-	26.2(6.1)	29.3(2.7)	17.8(5.5)	21.9(5.9)	23.8(5.7)
	-	+	14.0(0.9)	26.4(7.3)	39.5(7.2)	39.0(11.7)	15.4(2.1)
	60N	-	27.1(4.3)	21.6(3.5)	27.1(3.0)	27.4(9.2)	29.2(7.5)
	60N	+	17.7(3.5)	27.5(6.5)	32.4(5.0)	16.2(2.0)	28.4(7.1)
ANOVA	Plant (P)		n/a	n/a	n/a	n/a	**136.0(0.00)**
	Tillage (A)		**5.1(0.03)**	0.7(0.40)	0.9(0.36)	0.6(0.44)	1.8(0.18)
	Cropping systems (B)		2.4(0.13)	**53.6(0.00)**	0.0(0.86)	0.5(0.47)	2.7(0.10)
	Crop residues (C)		**5.7(0.02)**	1.4(0.24)	**4.4(0.05)**	0.1(0.75)	3.6(0.06)
	Nitrogen (D)		3.4(0.08)	2.6(0.12)	**7.1(0.01)**	1.0(0.32)	**3.8(0.05)**
	A x B		2.4(0.13)	2.2(0.15)	1.9(0.18)	0.0(0.84)	**3.9(0.05)**
	A x C		2.8(0.10)	**9.0(0.01)**	0.0(0.88)	**4.6(0.04)**	**5.1(0.03)**
	B x C		2.4(0.13)	0.0(0.93)	1.1(0.30)	0.0(0.88)	**7.7(0.01)**
	B x D		2.2(0.15)	0.9(0.34)	3.5(0.07)	**7.3(0.01)**	**11.2(0.00)**
	C x D		0.0(0.83)	0.3(0.59)	0.1(0.75)	**6.3(0.02)**	1.8(0.19)
	A x B x C		**4.4(0.04)**	**5.0(0.03)**	0.0(0.92)	**7.6(0.01)**	0.2(0.65)
	A x C x D		0.0(0.90)	1.4(0.25)	0.0(0.97)	**5.4(0.03)**	**4.3(0.04)**

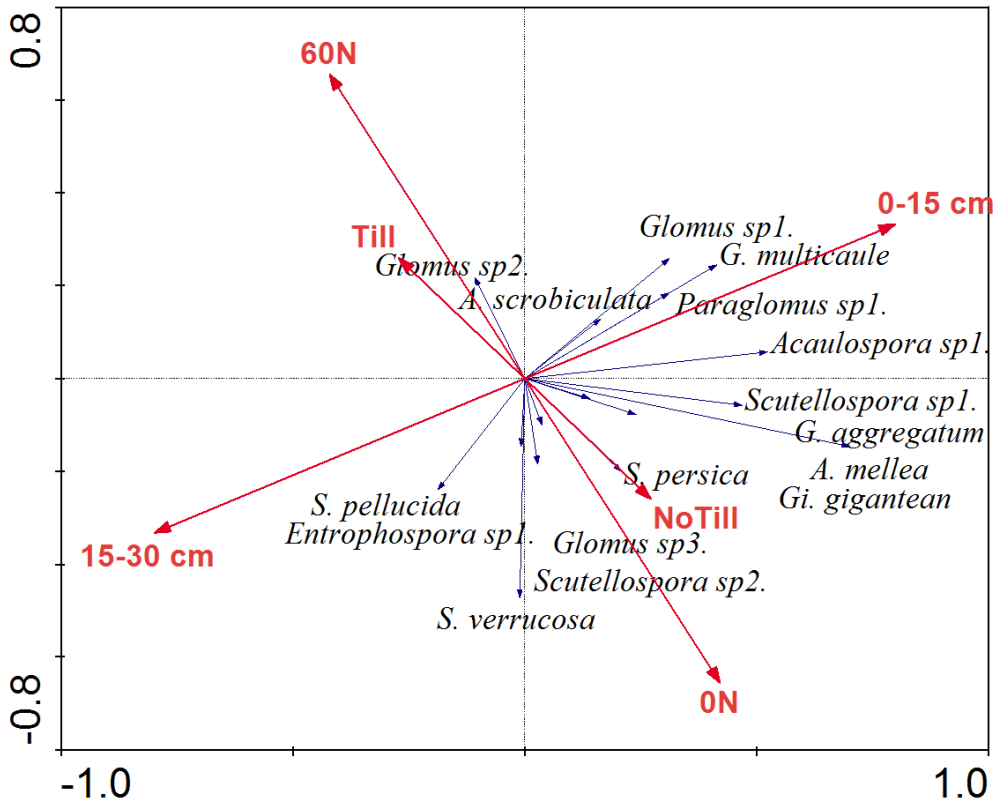

Figure 1. Effects of tillage and N fertilization on the community composition (relative species abundances) of AMF in the field soil. Results of redundancy analysis are shown using the spore abundance of the different AMF species. Vectors representing different sampling depth and treatments are shown in red. Size and orientation of the vectors represent correlation among them and with the axes. The smaller the angle between the vectors (or a vector) and the longer the vectors, the more correlated are the variables represented by the vectors. 0N and 60N represents plots with 0 and 60 kg N ha^{-1}. Till and No till represents two tillage practices.

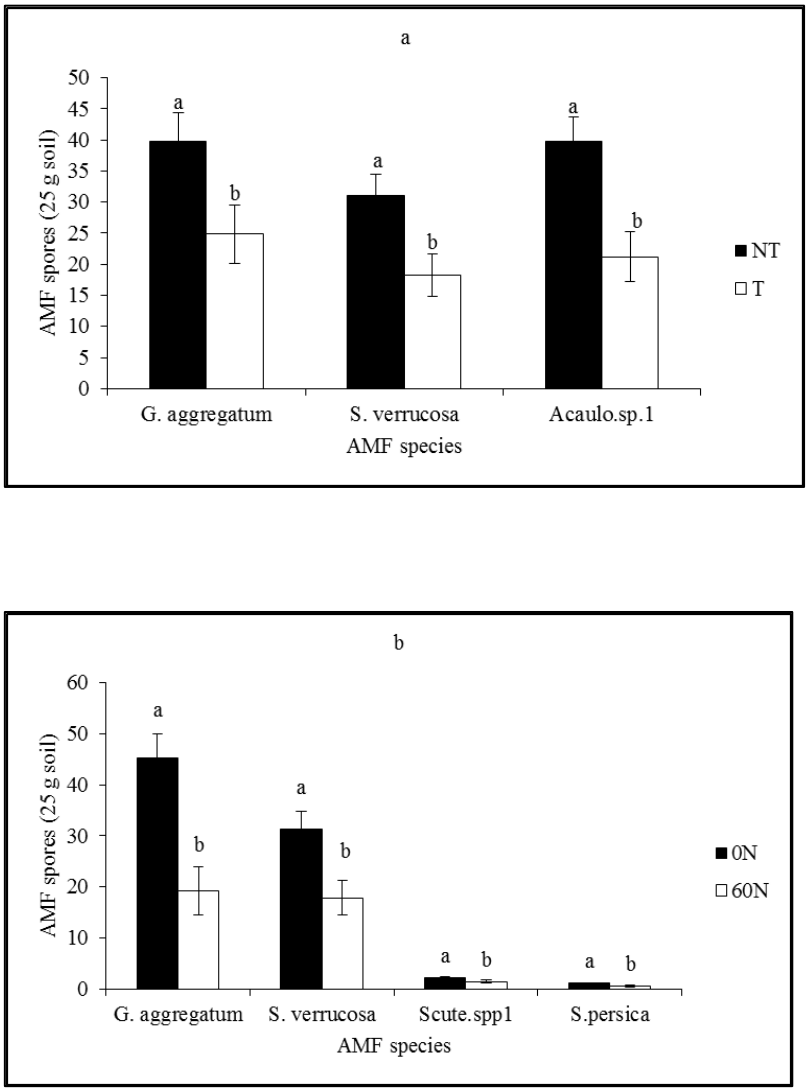

Figure 2. Abundance of spores of AMF species (per 25 g soil dry weight) as affected by tillage (a) and N fertilization (b). Error bars represent standard errors of means. NT = no till, T = tillage, 0N = 0 kg N ha^{-1} , 60N = 60 kg N ha^{-1}. Different letters between treatments indicate significant differences between the means as determined by LSD multiple range comparison ($P < 0.05$).

CHAPTER FOUR

The role of arbuscular mycorrhizal fungi on soil aggregation and crop nutrition in agro-ecosystems

This chapter will be submitted as: M. N. Muchane, B. Vanlauwe, J. Jefwa, C. Kibunja and T.W. Kuyper. The role of arbuscular mycorrhizal fungi on soil aggregation and crop nutrition in agro-ecosystems. *Pedobiologia.*

Abstract

Arbuscular mycorrhizal fungi (AMF) are essential for increasing the sustainability of agro-ecosystems. However, the importance of AMF in soil aggregation, crop nutrition and productivity especially under agricultural systems proposed to restore degraded soils is often not quantified. We investigated the relative importance of AMF and roots in influencing soil aggregation, and crop nutrition and production in two field trials in Kenyan agro-ecosystems. The Kabete field trial on a Humic Nitisol tested organic (farmyard manure, residue) and inorganic (NP fertilizer) management practices for 32 years while the Nyabeda field trial on a Ferralsol tested conservation agriculture practices (no-till and conventional tillage system with or without crop residues) and nitrogen fertilization for 5 years. We measured aggregate size distribution, crop yield, nitrogen and phosphorus uptake as well as AMF spore abundance, species richness, hyphal length, glomalin levels (both total glomalin and easily extractable glomalin), fractional root colonization and inoculum potential. Structural equation modeling (path analysis) indicated that AMF played a role in soil aggregation, N and P uptake, and crop production in the two sites, but their role was dependent on soil conditions, management and agricultural practices. Glomalin was important in explaining soil macro-aggregates in Nyabeda, and both macro-aggregates and micro-aggregates in Kabete. Root length and hyphal length were also important in explaining soil aggregates in both sites. In addition, AMF positively correlated with N and P uptake and subsequent crop yield in Kabete, while differences in AMF did not seem to explain crop nutrition and production in Nyabeda, except in CT systems. AMF spore abundance and species richness had either no or a negative relation with soil aggregation, crop nutrition and production. The result of this study highlights the importance of AMF in soil aggregation, and crop nutrition and production in Kenyan agro-ecosystems.

Key words: Glomalin, soil aggregation, nitrogen, phosphorus, uptake, yield, arbuscular mycorrhizal fungi

1. Introduction

Arbuscular mycorrhizal fungi (AMF) from phylum Glomeromycota (Schüßler et al., 2001) are ubiquitous soil microbes forming a substantial part of soil microbial biomass. AMF are currently receiving wide attention as part of an active and diverse soil biological community essential for increasing the sustainability of agricultural systems, in particular in organic and conservation agriculture practices (Galvez et al., 2001; Cardoso and Kuyper, 2006; Galván et al., 2009; Gianinazzi et al., 2010). AMF play an important role in soil aggregation, and are among the most important biological factors influencing soil structure (Smith and Read, 2007; Jastrow et al., 1998; Rillig et al., 2002; Rillig and Mummey, 2006). Extra-radical hyphae of AMF enmesh micro-aggregates to form macro-aggregates (Six et al., 2004). AMF has also been shown to produce glomalin, a microbial glue (Wright and Upadhyaya, 1996) proposed to be involved in soil aggregation, because of the correlation between glomalin and soil water aggregate stability (Rillig et al., 2002). AMF also impact on the composition of soil microbial communities, hence affecting microbial by-products (polysaccharides) that are important in soil aggregate stabilization (Gupta and Germida, 1988). However, despite the role played by AMF in soil aggregation, very few studies have been undertaken to understand the role of AMF in soil aggregation in African agro-ecosystems (Preger et al., 2007; Fokom et al., 2012).

In addition to soil aggregation, the AMF symbiosis also plays a critical role in plant nutrition, in particular of phosphorus (P), based on the ability of the external mycelium developing around the host plant roots to efficiently exploit a larger volume of soil, thereby enhancing mineral acquisition by the plant (Smith and Read, 2007). AMF is also important in uptake of ammonium (NH_4^+), which is less mobile than nitrate (NO_3^-) (Cardoso and Kuyper, 2006), and in exploitation of soil organic N (Hodge et al., 2001; Feng et al., 2002, Leigh et al., 2009). AMF can acquire substantial quantities of N from organic sources, but utilize significant amounts N for their own growth and metabolism and transfer only part of it to the plant (Hodge and Fitter, 2010). In organic (or organically amended) soils AMF therefore have the potential to improve N uptake, but might at the same time compete with host plants, especially under conditions of N-limitation (Hodge and Fitter, 2010).

Conservation agriculture (CA) practices utilizing zero-tillage, crop residue addition, and crop rotations support higher AMF species diversity and greater AM colonization than conventional practices with high rates of inorganic fertilizers and intensive tillage (Jansa et al., 2003; Castillo et al., 2006; Borie et al., 2010; Verbruggen and Kiers, 2010). Organically

managed agro-ecosystems may also sustain more diverse AMF communities (Ryan et al., 2000, Oehl et al., 2004, Borie et al., 2010). A diverse and abundant community of AMF can compensate, to some degree, for the reduced use of soluble P fertilizers, resulting in improved crop production (Gosling et al., 2006).

However, the importance of AMF in enhancing soil aggregation, crop nutrition and productivity in various agro-ecosystems, in particular under proposed agricultural practices for soil fertility and soil biodiversity restoration, is uncertain. Improved AMF symbiosis was associated with improved crop nutrition and production under impoverished soil (Muchane et al., 2010). However, under intensively managed agricultural systems with high levels of soluble fertilizer, AMF either had no effect or was negatively associated with crop nutrition and production (Ryan et al., 2000). Ryan and Graham (2002) in their review argued that AMF may have no crucial role in nutrition or growth of plants in production agricultural systems. This calls for further studies especially in sub-Saharan Africa where soil is highly depleted of soil nutrients particularly phosphorus (P) and nitrogen (N). In such cases, AMF may play a substantial role in enhancing crop nutrition and production.

The objective of the study was to assess the roles of AMF in (i) soil aggregation, (ii) N and P uptake and (iii) crop production in two study sites in Kenya (chapters 2 and 3). Previous work in the Kabete site examining long-term effects of organic and inorganic amendments on AMF diversity and activity found improved AMF activity (colonization, inoculum potential and hyphal length) but no changes in AMF community composition and diversity due to organic amendments (Chapter 2). No-till systems in Nyabeda supported higher spore abundance, species richness and root colonization than conventional tillage systems. Higher AMF performance could enhance crop nutrition (N and P) and subsequent crop yield (Smith and Read, 2007). In order to explore hypotheses on the role of AMF in (i), (ii), and (iii), we developed a conceptual model of the interrelationships between root length, mycorrhizal fungal characteristics, and aggregate size distribution and tested the adequacy of the model using path analysis (Miller and Jastrow, 1990; Rillig et al., 2002).

2. Materials and Methods

2.1. Study sites

The study was conducted using soils from two long-term trials with different management practices. The Kabete site is described in detail by Kibunja et al. (2012a) and the Nyabeda site by Kihara et al. (2012). The Kabete long-term field trial, located in central Kenya at 1°15’S and 36°41′E, was established in 1976. The Nyabeda trial, located in western Kenya at

0°06'N and 34° 36'E, was established in 2003. The soil in Kabete is a Humic Nitisol and in Nyabeda a Ferralsol (FAO 1990). The experiment in Kabete consisted of maize-bean rotation in a randomized complete block design with three replicates. Maize was grown during the long-rains season and common bean during the short-rains seasons. Plot sizes were 7.0 × 4.5 m. Agricultural inputs consisted of organic inputs (farmyard manure and maize stover) and inorganic fertilizer (calcium ammonium nitrate (N) and triple superphosphate (P). The treatments comprised three levels of farmyard manure (0, 5 or 10 t ha^{-1} manure), three levels of nitrogen-phosphorus (NP) fertilizer (0kg N P ha^{-1}, 60 kg N, 26.4 kg P ha^{-1} and 120 kg N, 52.8 kg P ha^{-1}) and two levels of crop residues (0 or 2 t ha^{-1} residues) resulting in 18 treatments. The treatments in Nyabeda consisted of two tillage systems (Conventional tillage, CT and No-till, NT), two cropping systems (continuous maize and maize-soybean rotation), two crop residue management practices (0 or 2 t residue ha^{-1} yr^{-1}) and two N fertilization levels (0 and 60 kg N ha^{-1}; P and K were added to all plots), resulting in 16 treatments in a randomized complete block design with three replicates. In the two study sites precipitation is bimodal with the long-rains season from Mid-march to June and the short-rains season from mid-October to December. Maize is the main staple crop and it is normally grown either as a monocrop or in association with legumes, mainly common bean, soybean or groundnut. Adoption of soybean, which is treated as a cash crop, is taking place in the region. Smallholder settlements dominate this area, with land sizes ranging from 0.3 to 3 ha per household.

2.2. Soil sampling

Soil for glomalin and aggregate analysis was sampled once during the end of the short-rains season in February 2008 at three depths (0-15, 15-30 and 30-45 cm). Ten random samples were collected from different depths in each plot and mixed thoroughly for each depth to obtain a composite sample. Soil for assessment of AMF extraradical hyphal length and root length was sampled when the crops were in their productive phase, when root growth is expected to be maximum. Soil was sampled randomly in ten different points per plots. The samples from the ten points were pooled to make composite samples for analysis.

2.3. Assessment of external AMF hyphal length and plant root length

Hyphae were extracted from a 10 g subsample by an aqueous extraction and membrane filter technique by Jakobsen et al. (1992). Soil samples were mixed and suspended in 100 ml of

deionized water, to which 12 ml of a sodium hexametaphosphate solution was added. The soil suspension was shaken for 30 s (end-over-end), left on the bench for around 30 min, and then decanted quantitatively through a 45 µm sieve to retain hyphae, roots and organic matter. The material on the sieve was sprayed gently with deionized water to remove clay particles, and then transferred into a 250 ml flask with 200 ml of deionized water. The flask was shaken vigorously by hand for 5 s, left on the bench for 1 min, and then a 2 ml aliquot was taken and pipetted onto 25 mm Millipore filters. The material on the filter was stained with 0.05% Trypan Blue and transferred to microscope slides. Hyphal length was measured with a grid-line intersect method at 200-400 x magnification, separating septate from non-septate hyphae. Only non-septate hyphae were measured. Roots were extracted from 10 g soil samples by flotation and wet-sieving. Soils were suspended in 1 l of water in a beaker and stirred vigorously. The floating roots were decanted onto a 0.50 mm sieve, rinsed, and collected with a forceps. This process was repeated until no roots were retained on the sieve. Roots were dried overnight at 60° C, and root lengths were measured with an image analysis system (Win-Rhizo, Regent Instruments INC, Québec, Canada).

2.4. Separation of Water-stable aggregates (WSA)

The separation of aggregates into separate size classes of water-stable-aggregates (WSA) was carried through the wet-sieving method described by Elliott (1986). A subsample of 80 g was spread evenly on a 2000 µm sieve, immersed in distilled water, and left for 5 minutes before starting the sieving process. Then aggregates were separated by moving the 2000 µm sieve up and down by about 3 cm with 50 repetitions in 2 minutes. The aggregates >2000 µm were collected as large macro-aggregates (LM) and the same sieving procedure was repeated for the 2000-250 µm fraction (SM) with the 250 µm sieve. Then the fraction 250-53 µm was obtained by sieving with a 53 µm sieve as free micro-aggregates (Mi). The aggregates remaining on top of each sieve were backwashed into labeled and pre-weighed containers and oven-dried at 60° C overnight before the final weight was determined. Soil material that passed through 53 µm was determined by taking a 300 ml subsample from the supernatant water of the whole volume after thoroughly shaking the suspension and was dried in the same way as done for the rest of the fractions. Weights were then corrected for the size of the sub-sample as compared to the whole volume and the fractions were recorded as free silt and clay.

2.5. Isolation of Micro-aggregates

Subsamples from LM and SM were bulked to isolate micro-aggregates held within macro-aggregates (mM) following the method described by Six et al. (2000). First, 10 g of macro-aggregates was taken from oven-dried large M and small M proportional to their initial weight, and mixed thoroughly. Then, 5 g of the mixture was used for micro-aggregate isolation. A device (Micro-aggregate Isolator) was used to completely break up macro-aggregates while minimizing the breakdown of the released micro-aggregates. The macro-aggregates were immersed in distilled water on top of a 250 µm mesh screen and gently shaken with 50 metal beads (diameter = 4mm). A continuous and steady water flow through the device ensured that micro-aggregates were immediately flushed onto a 53 µm sieve and not further disrupted by the metal beads. After all the macro-aggregates were broken up, the sand and coarse POM remaining on the 250 µm sieve were washed off and collected. The material collected on the 53 µm sieve was sieved according to Elliott (1986) to ensure that the isolated micro-aggregates were water-stable. Silt and clay fractions (<53 µm) were obtained by subsampling the supernatant water after measuring the total volume and gently shaking the suspension (wet-sieving). All fractions; sand and coarse POM (>250 µm), micro-aggregates within macro-aggregates (250-53 µm), and silt and clay (<53 µm) from the isolation step, were dried at 60° C overnight in an oven before final weight was determined.

2.6. Extraction of glomalin from soil samples aggregates

Glomalin extraction from whole-soil sub-samples, stable macro-aggregates (both LM and SM), stable micro-aggregates (Mi) and micro-aggregates within macro-aggregates (mM), was carried out as described by Wright and Updahyaya (1998). Glomalin levels were determined by the Bradford assay (see Chapters 5 and 6).

2.7. Plant sample and plant nutrient (P and N) analysis

Plant harvesting and dry matter determination was done as described by Kihara et al. (2012a) and Kibunja et al. (2012). Briefly, maize and bean were harvested above-ground from the 3 middle rows in each plot leaving two border plants (0.25 m spacing) on both ends of the row and one row (0.75 m spacing) from the other end to eliminate edge effects. The harvested plants from each plot were divided into stover (stalk and leaves), cob, and grains. The stover and cobs were chopped into small pieces, weighed and subsampled for dry matter determination. Grain was weighed, moisture content measured using a moisture meter

(Agromatic Mark II, Farmer Tronic, Denmark), and then subsampled for dry matter determination. Plant subsamples for dry matter determination were oven-dried at 60° C for 48 h in a ventilated oven to constant weight. The weights of oven-dry subsamples were recorded and used to calculate total above-ground dry matter yields. Plant subsamples were then finely ground for subsequent digestion and nutrient analysis. N and P were analyzed by Kjeldahl digestion with concentrated sulphuric acid and determined colorimetrically (Anderson and Ingram, 1993; ICRAF, 1995).

2.8. Statistical analysis

Three and four-way analysis of variance was applied to test for significant sources of variation in root length, aggregate fractions, crop yield, N and P uptake in the various agricultural practices. Results were considered significant at $P < 0.05$. Significant *P*-values were separated by Fisher's Least Significant Difference (LSD) test. Further, Pearson *r* correlation analysis was carried out to observe the degree of association between AMF parameters (fractional root colonization, inoculum potential, abundance, species richness, and diversity) and nutrient uptake (N and P) and crop yield, as well as degree of association between AMF parameters (glomalin, hyphal length, root length, AMF abundance, species richness and diversity) and soil aggregation. Path analysis was used to design the model and calculate path coefficients, squared multiple correlations, and model fit to test the influence of these factors in explaining levels of soil aggregation, crop nutrition and crop yield. Path analysis is a type of multiple regression analysis for modelling the correlation structure among variables, allowing testing of complex patterns of interrelationships. Path analysis has been used before to show interactions between biological factors on aggregate stability (Miller and Jastrow, 1990, Jastrow et al., 1998, Rillig et al., 2002). We used path analysis (structural equation modelling) to test causal relationships among interacting AMF factors (glomalin, spore abundance and hyphal length) in addition to fine root length with aggregate stability. Initially, we included species richness in the model but this did not improve model fit. We also explored links between root length and glomalin, but this was later dropped since there were no correlations between roots and glomalin in both sites (Nyabeda, 0.07 and Kabete, -0.17; $P > 0.05$). The variance inflation factors (VIF) in the multiple regression model were <5 (all data) and condition index based on Eigen values was <50 indicating multicollinearity was not a problem. All analyses were done using SPSS (PASW statistics 19) software except for path analysis where we used AMOS 18 program (Arbuckle, 2009).

3. Results

3.1. AMF parameters

AMF spore abundance, species richness and AMF activity (root colonization, hyphal length and inoculum potential) in both sites were affected by agricultural management (Chapter 2 and 3; Table 1). Spore abundance was two times higher in Nyabeda than in Kabete, while levels of glomalin were two times higher in Kabete than in Nyabeda. In Kabete, spore abundance, species richness, extraradical hyphal length and total glomalin were unaffected by NP fertilization, manure, residue, and the interaction of the three factors. Root colonization and mycorrhizal inoculum potential (MIP) were significantly affected by NP fertilization and manure ($P < 0.05$). Manure application increased MIP, while NP fertilization reduced MIP. There were also significant effects of interactions between manure x NP fertilization, NP fertilization x Manure x Residue on MIP and root colonization (Table 1). In Nyabeda, spore abundance and total glomalin were significantly affected by tillage practices and N fertilization. Conventional Tillage (CT) plots as well as N-fertilized plots had lower spore numbers and species richness compared to No-Till (NT) plots and unfertilized plots. CT plots had higher levels of total glomalin than NT, whereas N-fertilized plots had lower total glomalin compared to unfertilized plots. Cropping system and residue had no significant effect on spore abundance, species richness and levels of total glomalin ($P > 0.05$). MIP was significantly affected by cropping system, residue, and N fertilization. N fertilization increased MIP and root colonization, while crop residue reduced MIP, especially in NT systems.

3.2. Root length

Root length in both sites was affected by agricultural management (Tables 2 and 3). In Kabete, root length was significantly affected by manure in both long rain and short rains season (F =17.4 and 30.0, $P < 0.001$) but not by NP fertilizer and crop residue (Table 2). Manure application increased root length with highest root length observed at the highest manure application rate. In Nyabeda, root length was significantly affected by crop residue (F = 6.54, $P = 0.02$) and N fertilization (F = 11.86, $P = 0.002$). Both crop residue and N fertilization increased root length.

3.3. Water-stable aggregates

In Kabete, long-term application of NP significantly influenced large macro-aggregates (>2000 µm, LMa) only in the upper 15 cm soil layer, but had no effect on small macro-aggregates (250-2000 µm, SMa), free micro-aggregates (53-250 µm, Mi) and micro-aggregates within macro-aggregates (53-250 µm, mM) (Table 2). LMa were higher in plots with high NP application rates, intermediate in plots with low NP application rates and lowest in plots without fertilizer. Organic amendments also significantly influenced water-stable LMa, SMa, Mi and silt and clay (<53, SC) in the upper 15 cm layer, but had no effect on mM. Levels of water-stable Ma were highest at high rates of manure application (FYM2), intermediate at low rates of manure (FYM1) applications and lowest in plots without manure. Levels of Mi and SC were higher in control plots, intermediate in FYM1 plots and lower in FYM2 plots. Organic amendments significantly affected mM in both 15-30 and 30-45 cm layers, but NP fertilization affected mM only in 15-30 cm layer (Table 2, data for 30-45cm depth not shown). There was no interaction of NP and organic amendment observed in both layers.

In Nyabeda, there was a significant effect of tillage on mM at 0-15 cm depth, on LMa at 15-30 cm depth and on SMa, Mi and SC at 30-45 cm depth ($P < 0.05$ in all cases, Table 3, data presented only for the two upper depths). NT systems increased mM in 0-15 cm, LMa in 15-30 cm, Mi and SC in 30-45 cm, but reduced SMa in 30-45 cm compared to CT. Crop residue also significantly affected SMa and Mi in 0-15 cm, LMa in 15-30 cm and SMa, Mi and SC in 30-45 cm ($P < 0.05$ in all cases). Crop residue increased levels of SMa and reduced levels of Mi and SC in two sampling depths (0-15 and 30-45 cm). There was also a general decline of LMa in plots with crop residue at all sampling depths with a significant decline observed in 15-30 cm. N fertilization had no effect on water-stable aggregates in 0-15 cm, but significantly affected Mi and SC in 15-30 cm, and SMa and Mi in 30-45 cm. N-fertilized plots had higher levels of Mi, but lower levels of SC in 15-30 cm and SMa in 30-45 cm compared to unfertilized plots. Cropping system had no significant effect on aggregate fractions in all sampling depths but there was a significant tillage x cropping system interaction for Mi in 0-15 cm, SMa, Mi and SC in 15-30 cm. A significant tillage x crop residue interaction was also observed in 30-45 cm for SMa.

3.4. Glomalin levels in various aggregate fractions

In Chapter 5 we have provided detailed data on glomalin in various aggregate size fractions as well as the total glomalin : soil organic matter (TG : SOM) ratio in each fraction. Briefly,

glomalin was high in SC (11.4 mg g^{-1}), intermediate in Mi (5.9 mg g^{-1}) and lowest in Ma (4.5 mg g^{-1}) in Kabete. In Nyabeda, glomalin was high in Mi (5.5 mg g^{-1}), intermediate in SC (4.6 mg g^{-1}) and lowest in Ma (4.4 mg g^{-1}). In the two sites (Kabete vs. Nyabeda) the glomalin: SOM ratio for SC was highest (0.29 vs. 0.12), followed by Mi (0.15 vs. 0.14) and Ma (0.11 in both sites) had the lowest.

3.5. Crop yield and nutrient (P and N) uptake

Crop yield and N and P uptake were three times higher in Nyabeda than in Kabete (Table 4 and 5). In Kabete, N and P uptake was significantly affected by NP fertilization and manure application but unaffected by residue and the interactions of the three factors ($P < 0.05$; Table 4). This pattern was observed for both maize (grain and stover) and bean (haulm) yield with exception of bean grain yield that was not affected by NP fertilization (Table 4). Generally NP-fertilized plots had higher nutrient uptake and crop yield than control, with a larger increase with higher rate of NP fertilization. Similarly manure application resulted in higher nutrient uptake and crop yield than in the control, with a larger increase in plots with higher rates of manure applications.

In Nyabeda, N uptake was significantly affected by tillage, cropping system and N-fertilization ($P < 0.05$). There was also a significant tillage x residue interaction, tillage x cropping system x N fertilization interaction, and tillage x residue x N fertilization interaction on N uptake. P uptake was also affected by N fertilization, tillage x residue interaction, cropping system x N fertilization interaction, and residue x N-fertilization interactions ($P < 0.05$, Table 5). NT systems showed lower N uptake than CT systems. N uptake was higher in fertilized than in unfertilized plots, in both NT and CT systems. Soybean-maize rotation showed higher N uptake than continuous maize, especially in fertilized systems (Table 5). Crop residue increased N uptake in CT systems but reduced it in NT systems. P uptake was high in N-fertilized plots, in particular in plots under CT systems with residue. However, NT systems with residue had low P uptake irrespective of N fertilization. High P uptake was also observed in soybean-maize rotation under NT systems, but not under CT systems. Similar effects were observed for maize yield where tillage, crop rotation and N fertilization affected grain, stover and total yield significantly during both seasons ($P < 0.05$, table 5). Maize yield was generally higher in plots under CT systems than under NT systems. Fertilized (N) plots also recorded higher maize yield than unfertilized plots. Maize in the soybean-maize rotation also had higher yield than maize under continuous maize. Plots with residue had higher maize

yield than plots without residue under CT, but under NT plots with residue had lower maize yield than those without residue. Soybean yield during the long-rains season was only affected by crop residues ($P < 0.05$). Plots with residue showed higher yield than those without residue.

3.6. Path models

Table 6 and Figure 2 show the contributions of root length, hyphal length, glomalin content, and spore abundance to water-stable aggregates in both sites. Almost 25% of the variability in water-stable aggregates was explained by the model variables of the path diagram (Table 6). Root length and MEH had about comparable direct paths to SMa, with 0.22, and 0.25 in Kabete ($P < 0.05$). Glomalin had a strong negative direct path to LMa (-0.42) and a positive direct path to Mi and SC (0.23 in both cases).

In Nyabeda only root length and glomalin had a strong positive direct path to SMa (0.32), and a negative path to Mi and SC (-0.31 and -0.22 respectively). The direct path from extraradical hyphal length to Ma and the direct paths from spore abundance to various aggregate size fractions were all negligible in both sites ($P > 0.05$ in all cases). Root length had a stronger total effect to various aggregate fractions than hyphal length in both sites (Kabete: SMa 0.37 vs. 0.25, Nyabeda: SMa 0.29 vs. -0.03). There was a strong path from spore abundance to glomalin (0.33) in Kabete. Using maximum likelihood estimate, we obtained a $\chi 2$ of <5 for the two models used in the two study sites (df = 3; $P > 0.05$), showing model fit was good (Table 6). The comparative fit index (CFI, ranging from 0 to 1) was between 0.80-0.94 and RSMEA was <1 in all models, further supporting that an acceptable fit of the model to the data was achieved. The variance inflation factors (VIF) were < 2.5 (all data) and condition index based on eigen values was < 50, indicating lack of multicollinearity in the data set. In both sites, there were strong correlations between total glomalin and levels of LMa (Kabete; r = 0.52, Nyabeda; r = 0.29), SMa (Nyabeda; r = 0.49), Mi (Nyabeda; r = -0.55) and mM (Kabete, r =- 0.41) even at 30-45 cm depth.

3.7. Correlations between AMF and crop yield

The AMF parameters used in the multivariate regression were important in explaining P and N uptake (r^2=0.23) as well as crop production r^2=0.35) in Kabete (Table 7). Root colonization and MIP were important factors explaining P and N nutrition and crop production positively (r between 0.27-0.39) while AMF diversity indices were negatively correlated with yield and

nutrition (r between -0.30 to -0.36). In Nyabeda, AMF parameters were not important in explaining crop nutrition or production. However, when the two tillage system (NT and CT) were separated, there were significant positive correlations between root colonization and P uptake (r = 0.57) and significant negative correlations between AMF diversity indices and N uptake (r = -0.46) as well as grain yield (r = -0.41) in CT systems.

4. Discussion

4.1. Arbuscular mycorrhiza and soil aggregation

The aim of this study was to determine the degree of association between various AMF parameters and soil aggregation using path analysis. Path analysis does not allow testing causality between variables. A priori knowledge is used to construct a conceptual model (path diagram) of the causal relationships among the measured variables (Jastrow et al., 1998). However, model comparisons are possible, and we constructed path models with and without a direct link between roots and glomalin levels. However, that alternative model did not provide support for a role of plant material in contributing to the glomalin pool, contrary to Chapter 6. Previous studies also found a better model fit with root length (Jastrow et al., 1998, Rillig et al., 2002), and we included root length (fine root <2 mm diameter) in our model. Root length was linked with MEH and with aggregates, while MEH was casually linked to glomalin and directly to aggregates (Miller and Jastrow, 1990, Rillig et al., 2002). We also constructed a direct path from AMF spore abundance to hyphal length, to total glomalin and to SMa (Wright and Upadhyaya, 1998).

Our results indicated that AMF contributed to soil aggregation in the two sites, but that their role was dependent on site, soil management and agricultural practice. Glomalin, MEH and root length were the most important parameters explaining soil aggregation. Glomalin and root length were the most important explanatory variables of levels of SMa in Nyabeda. In Kabete, root length and hyphal length were important in explaining levels of SMa while glomalin was important in explaining levels of Mi. There were positive correlations between glomalin and LMa and SMa, even in deeper soil layers (30-45 cm) in both sites.

The roots and MEH contributed positively to levels of stable macro-aggregate in line with various studies showing the roles of MEH and roots in soil aggregation (Wilson et al., 2009; Piotrowski et al., 2004). Plant roots and MEH create a skeletal structure that holds soil particles together, initiating formation of macro-aggregates, and creating conducive

conditions for formation of micro-aggregates within macro-aggregates (Six et al., 2002, 2004). MEH also releases glomalin that acts as a microbial glue to hold particles together (Rillig and Mummey, 2006). We observed increased MEH and root length following organic amendments, and higher root length in NP-fertilized plots. Organic amendments also increased levels of Ma and reduced levels of Mi and SC in Kabete in line with a study by Ayuke et al. (2011). NP fertilization increased levels of LMa but had no effect on SMa, Mi and SC. The total effect of roots was generally higher than that of hyphal length, supporting various studies showing a larger contribution of roots in soil aggregation than that of hyphal length (Jastrow et al., 1998). Consistent with the larger role of roots, NP fertilization increased root length (in Kabete) but had no effect on hyphal length.

There was apparently no role of MEH on levels of soil aggregates in Nyabeda. We also observed minimal effects of agricultural practices on MEH. Whereas tillage and N fertilization affected AMF abundance and species richness, they explained only 7% of variation in AMF community structure, suggesting that >90% of total variation was due to other factors. Agricultural practices in this site may have had a stronger influence on soil aggregation than AMF. Tillage is known to affect soil aggregation by disrupting soil aggregates, especially macro-aggregates, resulting in increased levels of micro-aggregates (Bronick and Lal et al., 2005). In our study NT only increased levels of LMa, while CT increased levels of SMa and reduced levels of LMa. Similarly mixing of crop residues under CT may have influenced incorporation of SOC in SMa more than under NT, thus explaining the positive influence of residue on SMa levels, especially under CT. Crop rotation only increased levels of Mi under NT, but had no effect on levels of water-stable Ma. It is possible that the macro-aggregates formed during the soybean phase break up during the cereal phase (Kihara, 2009).

Although glomalin is considered a specific glycoprotein that is exclusively produced by AMF (Rillig, 2004), it is likely that several glycoproteins and humic materials are co-extracted during glomalin assessment (Gillespie et al., 2011). Nevertheless, glomalin has been shown to be the most important factor in explaining soil aggregation (Rillig et al., 2002). Our result, however, showed variable influences of glomalin on soil aggregation in both sites. Glomalin was important in explaining increasing levels of Ma in Nyabeda and increasing levels of Mi and declining levels of Ma in Kabete. The Nyabeda results are in accordance with various studies showing a positive correlation between glomalin and soil aggregation (Rillig et al., 2002; Wright et al., 2007; Fokom et al., 2012), while Kabete results support other studies that showed no relationship between glomalin and soil aggregates

(Rillig et al., 2003b; Borie et al., 2002, 2008). It is hypothesized that glomalin acts as glue with hydrophobic properties (Wright and Upadhyaya, 1998), but the direct biochemical mechanisms involved are still unclear, and the role of glomalin has remained correlative (Rillig and Mummey, 2006). Positive correlations between glomalin and aggregates are considered as support for its role in soil aggregation. However, such positive correlations between glomalin and soil aggregate stability apparently apply only to hierarchically structured soils, in which organic material is the main binding agent (Rillig et al., 2003a). Rillig et al. (2003b) found no relationship between glomalin and aggregates in soils where carbonates were the main binding agent. Borie et al. (2008) have also shown no relationship between glomalin and aggregates in Andisols (containing large amounts of non-crystalline clay that contribute to soil structure) and Mollisols (with fossilised humic content).

A positive correlation between glomalin and Mi could indicate both a role of Mi in stabilizing glomalin and a role of glomalin in Mi formation. The positive correlation between SC and glomalin levels suggests that clay plays an important role in stabilizing glomalin. In fact, the lack of a positive relationship between glomalin and Ma may reflect the degree of glomalin stabilization in well-aggregated soils. In path models it is not possible to test feedbacks loops and reciprocal effects. However, we also observed that the glomalin : SOM ratio in SC and Mi was larger than 0.15 in Kabete, whereas that ratio in Ma was < 0.10. This suggests that in well-aggregated soil, clay particles protect SOM including glomalin. Schmidt et al. (2011) recently argued that physical disconnection between decomposers and organic matter determines persistence of SOM (and, by implication, glomalin), rather than its supposed chemical recalcitrance. The positive correlations found in the deeper layer (30-45 cm) between glomalin and soil aggregation suggests a role for glomalin in soil aggregation even deeper in the soil profile. Kramer and Gleixner (2008) have shown that microbial products contribute more to SOM in subsoil horizons than plant compounds. In both sites we indeed noted increasing levels of stable aggregates with soil depth, suggesting the role of these microbial products in stabilizing soil aggregates. Glomalin may thus be a major microbial product deeper in the soil profile that causes the increasing aggregate stability.

Spore abundance and species richness of AMF were not important in explaining soil aggregation. Although several studies have reported a decline in AMF diversity in agro-ecosystems (Jansa et al., 2003; Castillo et al., 2006), it is not yet clear to what degree diversity decline impacts on their symbiotic functioning. It has been hypothesized that members of the Gigasporaceae may contribute more to soil structure stability than members of the Glomeraceae, since Gigasporaceae have hyphal clusters and a larger biomass in the soil

(Hart and Reader, 2002a, b). However, in both sites changes in AMF community composition due to the treatments were very small (Chapters 2 and 3). We have also shown that *Scutellospora verrucosa* (Gigasporaceae) was not better than *Glomus etunicatum* (Glomeraceae) in enhancing soil aggregation under greenhouse conditions (Chapter 6). Our results therefore suggest that AMF activity (MEH) is more important in soil aggregation than AMF abundance and diversity.

4.2. Arbuscular mycorrhiza and crop nutrition

AMF correlated with nutrient uptake and crop production, but their role was dependent on site, soil management and agricultural practices. In Kabete, root colonization and MIP explained crop nutrition and production. In Nyabeda, AMF colonization and MIP were not important in explaining nutrient uptake and crop production, but P uptake and root colonization under CT were positively correlated. AMF spore abundance, species richness and diversity had either a negative or no influence on crop nutrition and production.

Our results corroborate other studies showing relationships between AMF colonisation, nutrient uptake, and crop yield (Bi et al., 2003; Emmanuel et al., 2012). AMF have the ability to explore a larger area of the soil, the ability to absorb P at lower rhizosphere concentration than roots and have some solubility effect on fixed or adsorbed P (Smith et al., 2001; Cardoso and Kuyper, 2006). Similarly AMF can increase organic N availability through enhanced mineralization and transport organic N directly to the plant (Hodge and Fitter, 2010). Improved AMF activity (colonization and MIP) in plots with manure alone or in combination with NP fertilizer suggests a role in crop nutrition and subsequent crop yield, and a positive feedback between plant performance and mycorrhizal fungal performance. This may explain N and P uptake in Kabete in organically-amended plots compared to that in fertilized plots. N and P uptake and yield were increased by both manure and NP fertilization compared to the control, but there were no significant differences among these treatments. Our data, and those of Chapter 2 and 5 suggest that low AMF activity in plots with high NP, next to declining SOC levels cause low crop yield. Low SOC has been associated with low responses of crops to fertilization in degraded soils (Vanlauwe et al., 2010; Janssen, 2011). The result of our study suggests that management of AMF under such conditions may be more beneficial than increasing inputs of mineral nutrients.

We did not observe a role for AMF in crop nutrition and yield in Nyabeda, except for CT systems. Although NT systems showed high AMF abundance and colonization levels,

crop nutrition and yield was lower than in CT systems. The positive correlation between root colonization and P uptake in CT systems suggests that AMF is still important in this site in enhancing P uptake, but that other factors are more important in explaining crop yield. For example, crop residues retained in NT systems (2 t ha^{-1} yr^{-1}) may not have been adequate. Relatively higher bulk density under NT than under CT (Terano, 2010), suggests soil degradation in the absence of adequate amounts of residues (Govaerts et al., 2005, 2007; Lichter et al., 2008). In addition, large amounts of maize stovers with a high C:N ratio were left on the surface in NT systems and these may have resulted in net N immobilization, as indicated by low N and P uptake in plots with residues. Presence of perennial weeds particularly in NT systems may also have influenced our result through competition for nutrients (Barberi and Cascio, 2001).

Improved N nutrition and crop yield in soybean-maize rotations could not also be explained by AMF community since we found very little differences. This could be attributed to biological nitrogen fixation by soybean resulting in improved N availability (Kihara et al. 2012b), and crop yield. Kahiluoto et al. (2012) noted that differences in AMF communities were not of major importance in explaining benefits of AMF under contrasting management practices. This suggests that AMF activity is more important in crop nutrition than changes in AMF abundance and species richness in agro-ecosystems.

5. Conclusion

The results of this study highlight the importance of AMF in enhancing soil structure, crop nutrition (N and P uptake) and production. The role of AMF was, however, dependent on site, soil properties and agricultural practices. Root length and MEH were important in explaining levels of Ma, while in Nyabeda glomalin was important in explaining macro-aggregates. In addition, AMF enhanced N and P uptake and subsequent crop yield in Kabete, but not in Nyabeda. N immobilization following retention of residues may have had a strong influence on crop nutrition and production, masking effects of AMF. The study further showed that enhancing AMF activity was more important in explaining soil aggregation, crop nutrition and production than changes in AMF spore abundance and diversity. Finally, our study suggests that management of AMF under low-input technologies may be more beneficial than increasing mineral nutrient inputs.

Table 1. Spore abundance, species richness, hyphal length, root colonization, inoculum potential and total glomalin of Arbuscular Mycorrhizal Fungi (AMF) in Nyabeda and Kabete field trials, Kenya. For treatment abbreviations in Kabete, see Chapter 2, table 1; for treatment abbreviations in Nyabeda, see Chapter 3, table 1. Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Interactions tested for all factors, but only significant interactions shown. Values in bold are significant at $P < 0.05$.

				Abundance	Richness	MEH	Glomalin	Colonization		MIP
Site	NP	FYM	CR	(25 g soil)		m g^{-1} soil	(mg g^{-1})	4 WAS	8 WAS	%M
Kabete	None	None	-	25.3(4.2)	6.3(0.3)	15.2(4.0)	8.0(0.1)	n/a	34.4(1.1)	10.5(3.1)
			+	40.3(2.2)	8.3(0.3)	13.2(2.4)	8.9(0.3)	n/a	18.9(3.5)	11.2(0.7)
		FYM1	-	43.0(6.1)	7.3(0.3)	16.0(1.2)	8.8(0.4)	n/a	43.0(3.4)	29.9(3.2)
			+	24.0(8.3)	6.0(1.0)	18.7(3.4)	7.4(0.2)	n/a	49.4(2.7)	21.3(0.5)
		FYM2	-	34.3(2.3)	8.3(0.9)	21.2(5.3)	7.8(0.5)	n/a	33.7(2.4)	31.3(1.1)
			+	32.0(14.6)	7.0(1.5)	20.8(3.8)	7.5(0.6)	n/a	26.0(2.1)	45.7(3.7)
	NP1	None	-	36.0(9.5)	7.7(0.7)	14.7(2.7)	7.8(0.3)	n/a	35.8(2.2)	21.2(1.6)
			+	28.7(5.5)	7.3(1.2)	23.3(3.5)	8.7(0.2)	n/a	31.2(0.6)	20.6(0.3)
		FYM1	-	26.3(9.8)	6.3(1.8)	21.0(3.2)	7.9(0.3)	n/a	34.3(3.7)	19.9(0.6)
			+	33.0(16.5)	8.7(1.8)	22.2(1.9)	8.7(0.7)	n/a	45.5(2.1)	27.4(1.1)
		FYM2	-	50.7(14.2)	6.7(0.3)	20.3(3.6)	8.4(0.4)	n/a	46.3(3.8)	16.3(2.8)
			+	45.0(11.6)	7.7(0.3)	21.5(2.2)	7.5(0.7)	n/a	41.3(3.6)	19.6(2.5)
	NP2	None	-	39.7(12.4)	8.7(0.9)	13.0(1.8)	8.7(0.3)	n/a	44.2(1.3)	18.1(0.6)
			+	33.7(4.7)	7.3(0.9)	20.3(1.9)	8.6(0.3)	n/a	45.7(5.8)	19.0(2.5)
		FYM1	-	28.0(6.1)	7.3(1.5)	31.3(12.1)	8.4(0.4)	n/a	48.9(5.2)	21.7(5.0)
			+	29.0(8.4)	5.7(0.7)	16.6(1.9)	7.7(0.8)	n/a	34.3(3.0)	25.2(1.0)
		FYM2	-	29.0(11.0)	8.3(1.3)	20.0(5.9)	8.0(0.2)	n/a	37.0(3.6)	24.3(0.5)
			+	33.3(13.4)	5.3(1.8)	14.8(1.5)	8.3(0.2)	n/a	30.0(3.0)	16.5(2.4)
	ANOVA	Fertilizer (A)		0.1(0.91)	0.3(0.71)	0.8(0.44)	0.2(0.84)	n/a	**5.4(0.01)**	**18.9(0.00)**
		Manure (B)		0.6(0.53)	0.7(0.50)	2.0(0.16)	2.9(0.12)	n/a	**12.6(0.00)**	**14.8(0.00)**
		Residues (C)		0.6(0.72)	0.5(0.50)	0.2(0.67)	0.2(0.64)	n/a	**10.6(0.00)**	0.2(0.65)
		A x B		0.4(0.78)	0.8(0.53)	0.8(0.51)	1.1(0.36)	n/a	**11.6(0.00)**	**26.2(0.00)**
		A x C		2.8(0.07)	0.0(0.98)	2.1(0.14)	1.5(0.24)	n/a	**3.4(0.04)**	1.3(0.28)
		B x C		0.5(0.61)	0.1(0.93)	0.7(0.52)	1.2(0.30)	n/a	**3.4(0.04)**	**3.4(0.05)**
		A x B x C		1.0(0.40)	0.9(0.46)	1.3(0.31)	1.6(0.19)	n/a	**6.2(0.00)**	**12.2(0.00)**
	Treatment	N	CR							
Nyabeda	NTCM	-	-	115.5(32.3)	8.3(0.3)	23.9(5.6)	3.5(0.2)	26.9(7.3)	18.7(4.2)	27.0(7.2)
		-	+	223.7(59.7)	10.3(0.3)	19.3(4.4)	3.7(0.2)	23.2(6.0)	41.6(8.7)	29.1(7.9)
		60N	-	69.2(13.7)	8.8(0.3)	31.5(12.7)	2.8(0.2)	32.7(8.3)	24.8(6.4)	25.9(7.1)
		60N	+	144.7(33.2)	10.7(0.3)	18.7(4.7)	3.2(0.3)	48.4(6.3)	39.0(9.8)	18.3(3.4)
	NTSM	-	-	179.8(24.1)	10.0(0.6)	30.3(4.8)	3.6(0.4)	36.6(17.2)	26.7(4.5)	19.1(3.5)
		-	+	209.0(74.5)	8.3(0.7)	24.5(2.7)	3.8(0.3)	42.0(10.4)	45.0(4.7)	13.6(1.8)
		60N	-	89.8(34.7)	9.7(1.5)	31.5(4.0)	2.8(0.1)	32.3(10.2)	29.7(9.6)	29.6(9.7)
		60N	+	226.3(96.7)	9.0(0.6)	30.9(3.2)	3.3(0.3)	53.0(10.9)	11.4(2.8)	15.6(2.8)
	CTCM	-	-	86.7(22.7)	9.3(0.3)	19.3(1.0)	3.8(0.1)	15.9(6.13)	31.3(2.8)	18.9(4.7)
		-	+	149.2(21.0)	10.7(0.9)	29.8(11.5)	3.9(0.1)	28.9(3.0)	15.3(6.3)	27.4(5.4)
		60N	-	98.3(13.4)	10.0(0.6)	34.4(6.7)	3.5(0.3)	51.9(11.9)	37.7(3.4)	21.7(1.9)
		60N	+	55.3(14.5)	7.0(1.0)	22.2(5.1)	3.7(0.2)	43.3(5.3)	25.8(3.4)	26.2(7.8)
	CTSM	-	-	148.8(32.4)	10.3(0.9)	18.7(3.1)	4.0(0.2)	17.8(5.5)	21.9(5.9)	23.8(5.7)
		-	+	122.7(25.7)	9.7(0.3)	23.3(5.1)	4.3(0.1)	39.5(7.2)	39.0(11.7)	15.4(2.1)
		60N	-	66.3(15.2)	8.7(1.3)	36.2(15.5)	3.5(0.1)	27.1(3.0)	27.4(9.2)	29.2(7.5)
		60N	+	60.8 (19.6)	8.0(0.1)	24.5(5.1)	3.0(0.3)	32.4(5.0)	16.2(2.0)	28.4(7.1)
ANOVA	Tillage (A)			**7.4(0.01)**	0.1(0.72)	0.1(0.78)	**10.8(0.00)**	0.7(0.42)	1.0(0.33)	2.2(0.15)
	Cropping systems (B)			0.5(0.50)	0.0(0.80)	0.2(0.68)	0.6(0.43)	0.2(0.69)	0.9(0.34)	**5.0(0.03)**
	Residues (C)			3.2(0.09)	0.4(0.51)	0.4(0.53)	2.2(0.15)	4.0(0.06)	0.3(0.60)	**5.5(0.03)**
	N-fertilization (D)			**10.5(0.00)**	**4.4(0.04)**	1.1(0.29)	**28.5(0.00)**	**6.6(0.02)**	0.7(0.42)	**4.2(0.05)**
	A x B			0.8(0.37)	0.3(0.60)	2.8(0.11)	0.1(0.75)	1.3(0.26)	0.0(0.98)	**6.3(0.02)**
	A x C			**5.2(0.03)**	3.3(0.08)	2.0(0.17)	1.7(0.20)	0.1(0.81)	3.9(0.06)	**8.6(0.01)**
	A x D			0.2(0.68)	**7.5(0.01)**	0.4(0.51)	0.0(0.91)	0.1(0.78)	1.8(0.19)	3.7(0.06)
	B x C			0.2(0.64)	**4.4(0.04)**	3.5(0.07)	0.3(0.61)	0.7(0.41)	0.0(0.94)	**14.1(0.00)**
	B x D			0.0(0.86)	0.2(0.65)	1.2(0.28)	3.1(0.09)	**4.4(0.04)**	**6.3(0.02)**	**20.3(0.00)**
	C x D			0.0(0.88)	0.9(0.35)	**6.9(0.01)**	0.0(0.92)	0.0(0.83)	**7.5(0.01)**	1.9(0.18)
	A x B x C			0.1(0.82)	**4.6(0.04)**	**7.3(0.01)**	0.7(0.42)	0.0(0.90)	**9.2(0.01)**	0.5(0.47)
	A x B x D			0.2(0.66)	0.0(0.85)	0.7(0.40)	2.0(0.17)	0.9(0.36)	0.1(0.79)	0.4(0.51)
	A x C x D			1.5(0.23)	2.9(0.10)	0.2(0.70)	1.6(0.22)	3.4(0.07)	0.6(0.44)	**5.9(0.02)**
	B x C x D			2.2(0.15)	**4.7(0.04)**	**5.1(0.03)**	0.5(0.49)	0.0(0.86)	**6.7(0.01)**	1.6(0.21)

Table 2: Aggregate fractions as affected by agricultural practices in the Kabete trial. For treatment abbreviations, see Chapter 2, table 1. LM = large macro-aggregates, SM = small macro-aggregates, Mi = micro-aggregates, SC = silt and clay, mM = micro-aggregates within macro-aggregates, POM = coarse particulate organic matter, SCm = silt and clay within macro-aggregates. LR and SR = long- and short-rains season. Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Interaction tested for all factors, but only significant ones shown. Values in bold indicate significant effects ($P < 0.05$).

				--------Aggregate fractions (g) in 100 g soil----------				Fractions (g) in 5g TM (>250µm)			Root length (cm g^{-1} soil)	
				LM	SM	Mi	SC	POM	mM	SCm		
Depth	NP	FYM	CR	>2000µm	250-2000µm	53-250µm	<53µm	>250µm	53-250µm	<53µm	LR	SR
0-15	None	None	-	0.7(0.2)	35.0(1.7)	50.6(1.8)	13.6(1.6)	0.8(0.1)	3.5(0.2)	0.7(0.1)	1.7(0.2)	1.2(0.3)
			+	0.7(0.1)	36.5(1.9)	50.0(1.0)	12.8(0.9)	0.9(0.1)	3.5(0.1)	0.6(0.1)	0.9(0.4)	0.7(0.1)
		FYM1	-	1.0(0.1)	38.0(2.6)	48.3(1.5)	12.6(1.2)	0.7(0.1)	3.5(0.1)	0.7(0.1)	3.8(0.6)	1.8(0.4)
			+	2.2(0.9)	40.2(2.1)	47.1(2.9)	10.6(0.1)	0.7(0.1)	3.6(0.1)	0.7(0.1)	3.8(0.4)	1.8(0.5)
		FYM2	-	1.9(0.5)	40.5(1.2)	46.7(1.2)	10.9(0.5)	0.7(0.1)	3.3(0.1)	1.0(0.1)	4.3(0.3)	2.0(0.3)
			+	2.9(0.1)	46.3(2.7)	40.5(2.4)	10.4(0.4)	0.6(0.1)	3.4(0.1)	0.9(0.1)	4.0(0.1)	2.0(0.2)
	NP1	FYM0	-	1.9(1.4)	40.4(2.7)	45.4(1.9)	12.3(1.7)	1.2(0.3)	3.2(0.3)	0.6(0.1)	2.1(0.3)	1.3(0.6)
			+	1.1(0.5)	39.0(1.7)	47.8(1.2)	12.1(0.7)	0.7(0.2)	3.7(0.2)	0.6(0.1)	2.2(0.2)	1.2(0.4)
		FYM1	-	1.9(0.5)	37.1(0.2)	49.8(0.9)	11.2(1.0)	0.9(0.2)	3.5(0.1)	0.7(0.1)	3.9(0.6)	2.2(0.3)
			+	1.9(0.3)	40.8(1.1)	46.8(0.8)	10.5(0.1)	0.7(0.1)	3.5(0.1)	0.8(0.1)	3.7(0.6)	2.0(0.2)
		FYM2	-	2.8(0.8)	42.8(4.5)	44.7(4.8)	9.7(0.5)	1.0(0.1)	3.2(0.1)	0.8(0.1)	4.6(0.9)	2.4(0.3)
			+	3.5(1.2)	44.4(1.8)	41.7(2.1)	10.4(0.8)	0.8(0.2)	3.4(0.1)	0.8(0.1)	5.0(0.9)	2.5(0.4)
	NP2	FYM0	-	3.2(0.9)	38.5(0.8)	46.5(0.7)	11.7(0.9)	1.8(0.4)	2.7(0.3)	0.5(0.1)	3.0(0.8)	1.6(0.2)
			+	1.0(0.3)	35.1(2.5)	51.6(3.0)	12.3(0.9)	0.8(0.1)	3.5(0.2)	0.7(0.1)	2.9(0.5)	2.0(0.2)
		FYM1	-	1.5(0.1)	41.4(3.5)	47.3(3.1)	9.9(1.6)	1.0(0.1)	3.4(0.1)	0.6(0.1)	3.9(0.2)	2.0(0.2)
			+	5.7(2.0)	42.2(2.1)	42.5(2.8)	9.5(0.9)	1.0(0.3)	3.1(0.2)	0.9(0.1)	3.7(1.0)	2.3(0.5)
		FYM2	-	3.0(1.2)	40.5(3.9)	45.5(4.6)	11.1(0.5)	0.7(0.1)	3.5(0.1)	0.8(0.1)	4.8(0.3)	2.5(0.5)
			+	4.2(1.0)	45.3(0.6)	40.9(1.1)	9.6(0.4)	0.9(0.1)	3.2(0.1)	0.9(0.1)	4.8(0.6)	2.5(0.3)
15-30	None	None	-	0.6(0.3)	41.2(2.6)	45.5(2.2)	12.7(1.6)	0.5(0.1)	3.9(0.1)	0.6(0.1)	0.8(0.4)	0.9(0.3)
			+	0.6(0.1)	43.9(0.8)	44.2(2.0)	11.3(1.3)	0.3(0.1)	3.9(0.1)	0.7(0.1)	0.4(0.1)	0.6(0.1)
		FYM1	-	1.2(0.4)	59.8(4.1)	31.4(3.4)	7.6(1.1)	0.4(0.1)	3.5(0.1)	1.1(0.1)	1.6(0.1)	1.1(0.8)
			+	0.6(0.1)	44.9(5.3)	42.9(2.3)	11.6(3.3)	0.5(0.1)	3.9(0.1)	0.6(0.1)	1.5(0.1)	1.1(0.7)
		FYM2	-	1.0(0.3)	48.2(0.4)	40.1(1.7)	10.8(1.0)	0.4(0.1)	3.7(0.1)	0.9(0.1)	3.4(0.8)	2.4(0.8)
			+	1.8(0.7)	50.0(7.5)	38.9(6.6)	9.3(2.3)	0.2(0.1)	3.7(0.1)	1.1(0.1)	2.5(0.6)	1.1(0.4)
	NP1	FYM0	-	0.3(0.1)	50.2(10.5)	38.7(8.5)	10.8(2.2)	0.3(0.1)	4.0(0.1)	0.7(0.1)	0.6(0.2)	0.7(0.3)
			+	0.6(0.1)	48.8(6.6)	40.3(5.5)	10.3(1.3)	0.4(0.1)	3.7(0.1)	0.9(0.1)	0.8(0.3)	0.7(0.2)
		FYM1	-	1.0(0.4)	47.1(8.5)	41.1(6.2)	10.8(2.6)	0.6(0.1)	3.9(0.1)	0.5(0.1)	1.4(0.1)	1.6(0.6)
			+	0.5(0.2)	45.5(6.4)	44.4(4.8)	9.6(1.4)	0.3(0.1)	3.8(0.1)	0.9(0.1)	1.1(0.3)	1.6(0.3)
		FYM2	-	1.0(0.2)	47.3(5.8)	41.2(3.8)	10.4(2.4)	0.5(0.1)	3.6(0.1)	0.9(0.1)	2.4(0.4)	1.8(0.4)
			+	0.9(0.1)	49.0(1.5)	39.5(2.3)	10.5(2.3)	0.5(0.1)	3.5(0.1)	1.0(0.2)	2.8(0.1)	1.7(0.3)
	NP2	FYM0	-	1.2(0.8)	49.3(2.5)	39.7(1.3)	9.9(1.4)	0.4(0.2)	4.1(0.3)	0.5(0.1)	1.1(0.6)	0.5(0.1)
			+	0.9(0.3)	41.7(3.1)	46.3(1.3)	11.0(1.9)	0.3(0.1)	3.6(0.1)	1.1(0.1)	1.4(0.6)	0.8(0.3)
		FYM1	-	0.8(0.3)	42.1(1.4)	45.2(2.3)	11.8(1.3)	0.5(0.1)	3.7(0.1)	0.7(0.1)	2.4(0.1)	1.9(0.8)
			+	2.1(1.5)	46.8(3.7)	40.8(2.3)	10.4(1.0)	0.7(0.3)	3.4(0.3)	0.9(0.1)	2.4(0.1)	1.1(0.4)
		FYM2	-	1.2(0.8)	50.9(3.7)	37.6(4.2)	10.3(1.2)	0.4(0.1)	3.6(0.1)	1.0(0.1)	2.3(0.6)	2.1(0.7)
			+	2.2(0.9)	49.5(7.7)	35.5(4.7)	12.7(4.8)	0.8(0.1)	3.4(0.2)	0.8(0.1)	2.8(0.2)	1.5(0.7)
ANOVA												
0-15	Fertilizer (A)			**4.9(0.01)**	0.5(0.59)	0.6(0.55)	2.3(0.11)	**5.1(0.01)**	**4.9(0.01)**	**3.3(0.05)**	2.8(0.07)	**3.4(0.05)**
	Manure (B)			**5.3(0.01)**	**9.4(0.00)**	**7.6(0.00)**	**8.7(0.00)**	**3.6(0.04)**	0.8(0.46)	**25.5(0.00)**	**30.0(0.00)**	**11.7(0.00)**
	Residue (C)			2.1(0.16)	2.5(0.13)	2.4(0.13)	1.5(0.23)	**6.5(0.02)**	2.9(0.10)	**9.4(0.00)**	0.2(0.65)	0.0(0.88)
	A x C			0.6(0.54)	0.4(0.65)	0.2(0.84)	0.5(0.62)	1.4(0.27)	0.8(0.46)	**6.0(0.01)**	1.1(0.36)	0.3(0.85)
	B x C			**4.2(0.02)**	1.9(0.17)	**3.3(0.05)**	0.4(0.70)	2.8(0.07)	**4.0(0.03)**	1.3(0.29)	0.1(0.90)	0.1(0.94)
	A x B x C			1.8(0.15)	0.4(0.82)	0.5(0.73)	0.5(0.77)	2.3(0.08)	**2.8(0.04)**	0.3(0.87)	0.2(0.95)	0.3(0.90)
15-30	Fertilizer (A)			2.1(0.13)	0.1(0.91)	0.0(0.97)	0.3(0.75)	2.3(0.12)	1.4(0.27)	0.1(0.92)	2.9(0.07)	0.2(0.84)
	Manure (B)			1.7(0.19)	0.5(0.63)	0.8(0.48)	0.2(0.81)	2.5(0.10)	5.2(0.01)	**4.2(0.02)**	**32.9(0.00)**	**6.8(0.00)**
	Residue (C)			0.8(0.39)	0.5(0.50)	0.6(0.46)	0.0(0.98)	0.0(0.89)	3.0(0.10)	**5.0(0.03)**	0.1(0.82)	1.6(0.22)
	A x C			0.8(0.48)	0.1(0.91)	0.1(0.86)	0.3(0.71)	1.9(0.16)	3.8(0.03)	3.0(0.07)	1.5(0.24)	0.3(0.74)
	B x C			0.5(0.64)	0.2(0.78)	0.4(0.64)	0.0(0.96)	0.7(0.49)	0.9(0.41)	**3.2(0.05)**	0.1(0.91)	0.7(0.50)
	A x B x C			0.8(0.52)	1.0(0.42)	1.1(0.39)	0.7(0.60)	**2.6(0.05)**	0.6(0.69)	**6.2(0.00)**	0.6(0.67)	0.4(0.83)

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Table 3: Aggregate fractions as affected by agricultural practices in the Nyabeda field trial, Kenya. For treatment abbreviations, see Chapter 3, table 1. LM = large macro-aggregates, SM = small macro-aggregates, Mi = micro-aggregates, SC = silt and clay, mM = micro-aggregates within macro-aggregates, POM = coarse particulate organic matter, SCm = silt and clay within macro-aggregates. LR and SR = long- and short-rains season. Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Interaction tested for all factors, but only significant interactions shown. Values in bold are significant ($P < 0.05$).

				----------Aggregate fractions (g) in 100 g soil----------				Fractions (g) in 5g TM (>250µm)			Root length (cm g^{-1} soil)	
				LM	SM	MI	SC	POM	mM	SCm		
Depth	Treatments	N	CR	>2000µm	250-2000µm	53-250µm	<53µm	>250µm	53-250µm	<53µm	LR	SR
0-15	NTCM	-	-	1.9(0.5)	43.6(2.4)	43.8(0.6)	10.8(1.5)	0.5(0.1)	3.3(0.1)	1.2(0.1)	1.3(0.2)	1.7(0.3)
		-	+	1.7(0.4)	54.5(5.2)	35.8(4.2)	8.0(1.3)	0.5(0.1)	3.3(0.1)	1.2(0.1)	2.9(0.5)	3.8(0.3)
		60N	-	4.3(1.5)	47.1(1.2)	41.4(1.7)	9.5(0.7)	0.3(0.1)	3.3(0.1)	1.4(0.1)	2.0(0.5)	3.4(0.3)
		60N	+	2.0(0.4)	49.9(1.4)	39.3(0.9)	8.7(0.9)	0.4(0.1)	3.3(0.1)	1.3(0.1)	3.3(0.4)	4.0(0.8)
	NTSM	-	-	2.0(0.6)	54.4(5.8)	35.5(5.0)	8.2(1.1)	0.4(0.1)	3.3(0.1)	1.2(0.1)	1.1(0.2)	1.5(0.6)
		-	+	1.9(0.2)	55.6(2.2)	34.0(1.4)	8.4(1.2)	0.5(0.1)	3.2(0.1)	1.4(0.1)	1.8(0.4)	3.6(0.5)
		60N	-	3.8(1.2)	48.6(2.9)	38.9(1.4)	8.6(1.2)	0.4(0.1)	3.2(0.1)	1.4(0.1)	1.2(0.1)	4.3(2.1)
		60N	+	2.4(0.2)	55.2(2.3)	34.2(2.0)	8.2(0.7)	0.5(0.1)	3.1(0.1)	1.3(0.1)	1.8(0.4)	4.6(0.1)
	CTCM	-	-	4.0(2.4)	54.6(4.1)	32.7(4.5)	8.6(1.1)	0.4(0.1)	3.0(0.1)	1.6(0.1)	2.3(0.4)	3.7(0.5)
		-	+	2.6(0.9)	59.4(4.0)	29.3(3.8)	8.7(0.9)	0.5(0.1)	3.2(0.1)	1.2(0.1)	3.6(0.3)	3.9(0.6)
		60N	-	2.2(0.6)	53.7(3.5)	35.3(3.8)	8.7(0.5)	0.5(0.1)	3.2(0.1)	1.3(0.1)	3.2(0.5)	4.6(1.6)
		60N	+	1.9(0.7)	51.1(3.8)	37.9(3.8)	9.1(0.6)	0.5(0.1)	3.0(0.2)	1.5(0.1)	3.7(0.4)	5.3(0.3)
	CTSM	-	-	1.2(0.2)	52.8(7.6)	36.5(6.2)	9.6(1.6)	0.5(0.1)	3.3(0.1)	1.2(0.1)	1.6(0.4)	2.7(0.2)
		-	+	3.3(1.6)	57.8(4.5)	31.3(5.0)	7.6(1.0)	0.5(0.1)	3.2(0.2)	1.3(0.1)	1.7(0.2)	4.2(0.8)
		60N	-	1.9(0.3)	46.8(6.6)	42.3(6.9)	8.9(0.5)	0.5(0.1)	3.2(0.1)	1.3(0.1)	2.4(0.6)	3.8(0.5)
		60N	+	2.5(0.7)	54.1(3.2)	35.2(3.3)	8.2(0.7)	0.5(0.1)	3.0(0.1)	1.6(0.1)	2.7(0.1)	5.7(0.7)
15-30	NTCM	-	-	4.2(1.9)	54.2(1.8)	34.8(2.4)	6.8(0.7)	0.4(0.1)	3.3(0.2)	1.3(0.2)	1.1(0.3)	1.7(0.3)
		-	+	2.4(0.5)	58.6(1.6)	33.2(2.0)	5.8(0.1)	0.4(0.1)	3.4(0.2)	1.2(0.1)	1.4(0.1)	2.7(0.3)
		60N	-	4.8(0.7)	55.1(1.5)	33.6(1.1)	6.5(0.3)	0.3(0.1)	2.3(1.0)	1.3(0.1)	1.5(0.3)	2.4(0.2)
		60N	+	2.5(0.6)	58.8(2.6)	33.1(2.5)	5.6(0.3)	0.4(0.1)	3.4(0.2)	1.2(0.2)	0.9(0.3)	3.0(0.4)
	NTSM	-	-	1.9(0.2)	60.6(2.2)	31.8(1.8)	5.7(0.6)	0.3(0.1)	3.7(0.2)	1.1(0.1)	1.4(0.1)	1.3(0.1)
		-	+	1.5(0.2)	60.3(2.8)	31.6(2.5)	6.5(0.6)	0.4(0.1)	3.4(0.2)	1.2(0.2)	0.9(0.2)	2.6(0.8)
		60N	-	8.0(3.8)	56.5(1.4)	29.6(2.9)	5.9(0.4)	0.4(0.1)	3.1(0.2)	1.6(0.2)	1.1(0.1)	2.4(0.8)
		60N	+	1.7(0.7)	59.7(4.9)	32.9(5.1)	5.7(0.7)	0.3(0.1)	3.6(0.3)	1.1(0.2)	0.3(0.1)	3.0(0.7)
	CTCM	-	-	3.8(1.2)	62.2(1.0)	27.5(0.8)	6.5(0.7)	0.3(0.1)	3.5(0.2)	1.2(0.2)	1.2(0.1)	1.1(0.6)
		-	+	1.9(0.2)	62.1(1.7)	29.7(1.5)	6.3(0.4)	0.5(0.1)	3.5(0.1)	1.1(0.1)	1.3(0.1)	2.0(0.4)
		60N	-	2.4(0.2)	58.6(0.6)	32.8(0.6)	6.1(0.3)	0.4(0.1)	3.3(0.2)	1.3(0.1)	1.1(0.1)	1.8(0.2)
		60N	+	2.1(0.2)	60.7(1.2)	31.4(1.0)	5.8(0.2)	0.4(0.1)	3.5(0.2)	1.1(0.1)	1.2(0.2)	2.8(0.7)
	CTSM	-	-	1.3(0.1)	60.1(3.9)	32.0(3.2)	6.6(0.8)	0.5(0.1)	3.4(0.1)	1.1(0.1)	0.8(0.2)	1.4(0.1)
		-	+	1.1(0.1)	56.9(0.6)	33.4(0.5)	8.6(0.9)	0.5(0.1)	3.4(0.1)	1.1(0.1)	1.1(0.1)	2.1(0.5)
		60N	-	2.7(0.3)	60.8(2.0)	30.4(1.9)	6.1(0.6)	1.4(0.1)	2.4(0.9)	1.2(0.1)	0.8(0.1)	2.2(0.3)
		60N	+	2.0(0.3)	56.8(2.1)	34.8(1.9)	6.4(0.4)	0.4(0.1)	3.4(0.3)	1.1(0.2)	0.9(0.1)	2.9(0.3)
ANOVA												
0-15	Tillage (A)			0.1 (0.79)	1.3 (0.26)	1.7 (0.20)	0.1 (0.90)	2.8 (0.10)	**4.0 (0.05)**	0.9 (0.36)	**13.7(0.00)**	4.3(0.05)
	Cropping systems (B)			0.1 (0.82)	0.2 (0.70)	0.1 (0.80)	0.9 (0.36)	0.2 (0.67)	0.1 (0.80)	0.0 (0.98)	**22.8(0.00)**	0.0(0.87)
	Residue (C)			0.3 (0.59)	**5.4 (0.03)**	**4.4 (0.05)**	2.5 (0.12)	1.8 (0.20)	2.3 (0.14)	0.4 (0.51)	**17.8(0.00)**	**8.3(0.01)**
	Fertilization (D)			0.1 (0.71)	2.1 (0.16)	2.4 (0.14)	0.0 (0.91)	1.2 (0.28)	2.1 (0.16)	4.0 (0.06)	**6.7(0.01)**	**10.1(0.00)**
	A x B			0.1 (0.77)	3.5 (0.07)	**4.2 (0.05)**	07 (0.42)	0.3 (0.62)	1.5 (0.24)	1.9 (0.18)	0.0(0.86)	0.2(0.63)
15-30	Tillage (A)			**4.7 (0.04)**	2.0 (0.17)	0.5 (0.50)	3.5 (0.07)	2.8 (0.11)	0.0 (0.96)	1.8 (0.18)	0.0(0.88)	2.2(0.15)
	Cropping systems (B)			0.5 (0.56)	0.2 (0.67)	0.2 (0.66)	2.0 (0.17)	1.4 (0.25)	0.0 (0.93)	0.0 (0.98)	**6.9(0.01)**	0.0(0.83)
	Residue (C)			**7.6 (0.01)**	0. 9 (0.35)	0.4 (0.56)	0.0 (0.95)	0.7 (0.42)	3.2 (0.08)	3.0 (0.09)	1.5(0.24)	**12.5(0.00)**
	Fertilization (D)			3. 5 (0.07)	0. 5 (0.51)	0.1 (0.83)	**5.4 (0.03)**	0.4 (0.55)	2.3 (0.14)	1.9 (0.18)	2.5(0.13)	**8.5(0.01)**
	A x B			0.1 (0.72)	**8.2 (0.01)**	6.1 (0.02)	**5.2 (0.03)**	2.8 (0.11)	2.9 (0.10)	0.0 (0.92)	0.0(1.00)	0.5(0.47)

Table 4. Phosphorus (P) and nitrogen (N) uptake and crop (maize and soybean) yield as affected by agricultural practices in Kabete field trial, Kenya. For treatment abbreviations, see Chapter 2, table 1. LR is the long-rains season, SR is short-rains season, total yield is sum of grain, stover and cob yield for maize and grains and haulms/stalks for beans. N and P uptake was assessed for maize grains in the long-rains season. Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Values in bold are significant ($P< 0.05$).

			----------LR (Maize)----------			----------SR (Bean)----------			Nutrient uptake LR (grains)	
			Grain	Stover	Total	Grains	Haulms	Total	N	P
NP	FYM	CR	----------t ha^{-1}----------						----------kg ha^{-1}----------	
None	None	-	0.4(0.1)	2.2(0.3)	2.6(0.4)	0.3(0.1)	0.3(0.2)	0.7(0.3)	5.0(2.0)	0.7(0.3)
		+	0.2(0.1)	3.2(0.2)	3.5(0.2)	0.1(0.1)	0.3(0.1)	0.4(0.1)	4.6(1.0)	0.5(0.1)
	FYM1	-	1.7(0.1)	6.4(1.0)	8.1(0.9)	0.3(0.1)	0.5(0.2)	0.8(0.3)	27.1(3.8)	2.9(0.4)
		+	2.0(0.1)	8.1(1.9)	10.1(2.1)	0.5(0.1)	0.8(0.1)	1.3(0.2)	32.0(2.2)	4.0(0.3)
	FYM2	-	1.9(0.4)	5.2(1.5)	7.0(1.8)	0.5(0.1)	1.1(0.2)	1.6(0.3)	32.9(7.9)	4.7(1.5)
		+	2.2(0.2)	9.4(0.9)	11.6(0.8)	0.4(0.1)	1.0(0.2)	1.4(0.2)	45.0(1.7)	4.2(0.1)
NP1	None	-	1.0(0.2)	3.5(0.4)	4.5(0.6)	0.3(0.1)	0.3(0.1)	0.6(0.2)	15.0(3.6)	1.7(0.5)
		+	0.8(0.1)	4.6(1.1)	5.4(1.0)	0.2(0.1)	0.4(0.1)	0.5(0.1)	14.6(1.9)	1.5(0.10
	FYM1	-	2.7(0.2)	9.1(1.3)	11.8(1.5)	0.3(0.1)	0.9(0.2)	1.2(0.3)	46.9(5.8)	6.0(1.6)
		+	2.0(0.1)	7.9(0.8)	10.0(0.7)	0.4(0.1)	0.8(0.1)	1.2(0.1)	34.1(4.9)	4.2(0.2)
	FYM2	-	2.4(0.1)	10.3(1.0)	12.8(1.0)	0.4(0.1)	1.1(0.3)	1.5(0.4)	45.7(1.7)	5.5(0.1)
		+	2.5(0.3)	9.4(1.1)	11.9(1.4)	0.4(0.2)	1.3(0.3)	1.7(0.5)	45.8(4.1)	5.8(0.8)
NP2	None	-	0.9(0.1)	5.9(1.1)	6.7(1.1)	0.3(0.1)	0.9(0.3)	1.2(0.3)	14.8(2.2)	1.9(0.3)
		+	1.1(0.1)	4.0(0.8)	5.1(0.8)	0.4(0.1)	0.8(0.1)	1.2(0.2)	19.8(1.9)	2.2(0.2)
	FYM1	-	1.9(0.5)	5.2(1.5)	7.1(1.9)	0.3(0.1)	0.9(0.2)	1.2(0.2)	37.0(10.3)	4.4(1.4)
		+	2.7(0.3)	7.8(0.7)	10.5(0.4)	0.5(0.1)	1.2(0.2)	1.7(0.2)	45.9(2.5)	6.3(0.5)
	FYM2	-	3.2(0.2)	6.8(0.6)	10.0(0.6)	0.3(0.1)	1.2(0.2)	1.5(0.2)	55.7(4.5)	6.6(0.2)
		+	2.2(1.1)	8.5(1.0)	10.7(2.1)	0.5(0.1)	1.3(0.2)	1.7(0.2)	60.6(10.5)	7.0(1.7)
ANOVA	Fertilizer (A)		**5.2(0.01)**	**4.3(0.02)**	**5.1(0.01)**	0.6(0.57)	**5.9(0.01)**	**4.3(0.02)**	**13.4(0.00)**	**8.8(0.00)**
	Manure (B)		**41.8(0.00)**	**29.8(0.00)**	**41.8(0.00)**	**5.4(0.01)**	**16.4(0.00)**	**15.6(0.00)**	**81.4(0.00)**	**46.6(0.00)**
	Residues (C)		0.0(0.98)	3.5(0.07)	2.5(0.12)	0.3(0.56)	0.5(0.49)	0.6(0.45)	1.1(0.29)	0.2(0.70)
	A x B		0.1(0.98)	1.8(0.15)	1.4(0.26)	0.4(0.82)	0.8(0.51)	0.8(0.54)	0.8(0.52)	0.3(0.88)
	A x C		0.6(0.58)	2.4(0.10)	2.4(0.11)	1.3(0.30)	0.1(0.95)	0.3(0.73)	2.2(0.13)	1.2(0.32)
	B x C		0.4(0.67)	0.8(0.44)	0.5(0.60)	1.9(0.17)	0.7(0.52)	1.2(0.30)	0.5(0.61)	0.1(0.90)
	A x B x C		2.1(0.10)	1.9(0.13)	1.7(0.17)	0.9(0.50)	0.3(0.85)	0.4(0.81)	0.8(0.56)	1.0(0.41)

Table 5: Phosphorus (P) and nitrogen (N) uptake and crop (maize and soybean) yield as affected by agricultural practices in Nyabeda field trial, Kenya. For treatment abbreviations, see Chapter 3, table 1. LR is the long-rains season, SR is short-rains season, total yield is sum of grain, stover and cob yield for maize and grains and haulms/stalks for soybean. N and P uptake was assessed for maize grains in short-rains season. Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Values in bold are significant ($P < 0.05$).

			----------LR (Total)----------		----------SR (Maize)----------			Uptake in SR (grain)	
			Maize	Soybean	Grain	Stover	Total	N	P
Treatment	N	CR	----------t ha^{-1}----------					----------kg ha----------	
NTCM	-	-	4.2(0.8)	n/a	2.2(0.1)	1.7(0.3)	4.3(0.3)	46.8(13.2)	10.2(1.6)
	-	+	5.7(1.1)	n/a	2.6(0.6)	3.5(0.8)	6.6(1.4)	68.6(12.0)	9.8(1.4)
	60N	-	7.9(0.6)	n/a	3.9(0.6)	4.9(0.1)	9.6(0.7)	122.0(19.7)	19.7(0.5)
	60N	+	9.6(1.6)	n/a	3.7(0.6)	4.4(1.0)	9.0(1.6)	79.0(8.2)	11.3(1.5)
NTSM	-	-	n/a	1.6(0.1)	4.5(0.7)	3.8(0.9)	9.0(1.6)	78.0(14.9)	13.0(2.8)
	-	+	n/a	2.7(0.6)	3.9(0.1)	3.5(0.4)	8.1(0.4)	94.2(20.0)	16.0(0.3)
	60N	-	n/a	1.7(0.6)	5.5(0.4)	4.6(0.7)	11.1(1.0)	150.6(13.5)	18.9(3.4)
	60N	+	n/a	2.8(0.2)	4.5(0.4)	5.7(1.3)	11.2(1.5)	118.1(7.8)	10.3(1.4)
CTCM	-	-	6.9(0.9)	n/a	2.3(0.4)	3.6(0.2)	6.4(0.7)	49.5(10.0)	11.5(1.2)
	-	+	6.1(1.2)	n/a	2.6(0.5)	3.9(0.6)	7.0(0.6)	47.0(9.0)	12.5(1.2)
	60N	-	10.4(0.8)	n/a	3.7(0.2)	4.9(0.7)	9.4(0.5)	84.6(2.3)	18.1(1.3)
	60N	+	11.1(1.4)	n/a	5.1(0.1)	4.9(0.4)	11.1(0.4)	155.4(8.6)	21.7(3.2)
CTSM	-	-	n/a	1.8(0.1)	4.2(0.1)	6.2(0.4)	11.1(0.5)	118.6(5.5)	10.6(1.1)
	-	+	n/a	2.4(0.2)	4.8(0.5)	5.2(0.5)	11.0(0.0)	133.4(20.0)	16.7(3.8)
	60N	-	n/a	2.3(0.2)	4.8(0.5)	5.3(0.7)	11.1(1.2)	121.5(5.9)	17.7(1.3)
	60N	+	n/a	2.9(0.3)	6.0(0.2)	5.6(0.4)	12.9(0.5)	163.0(15.2)	16.3(2.1)
ANOVA									
Tillage (A)			**5.1 (0.04)**	0.6 (0.45)	2.7 (0.11)	**8.1 (0.01)**	**8.5 (0.01)**	**5.1 (0.03)**	3.8 (0.06)
Cropping systems (B)			n/a	n/a	**53.6 (0.00)**	**9.5 (0.00)**	**35.5 (0.00)**	**40.6 (0.00)**	0.6 (0.56)
Residues (C)			0.9 (0.35)	**11.46 (0.00)**	1.6(0.21)	0.5(0.48)	1.6(0.21)	2.9(0.10)	0.4(0.55)
Nitrogen (D)			**26.1 (0.00)**	1.3 (0.28)	**37.1(0.00)**	**11.3(0.00)**	**33.2(0.00)**	**49.4(0.00)**	**17.5(0.00)**
A x C			1.1 (0.31)	0.9 (0.36)	**8.5(0.01)**	0.9(0.36)	0.7(0.41)	**10.1(0.00)**	**8.7(0.01)**
B x D			n/a	n/a	3.9(0.06)	2.1(0.15)	4.1(0.05)	3.9(0.06)	**6.2(0.02)**
C x D			0.3 (0.62)	0.0(0.99)	0.2(0.64)	0.0(0.92)	0.01(0.77)	0.1(0.79)	**9.3(0.01)**
A x B x D			n/a	n/a	0.3(0.57)	0.4(0.54)	0.5(0.48)	**5.7(0.02)**	0.1(0.83)
A x C x D			0.2 (0.69)	0.0(0.95)	2.3(0.14)	0.5(0.48)	1.6(0.22)	**17.6(0.00)**	3.3(0.08)

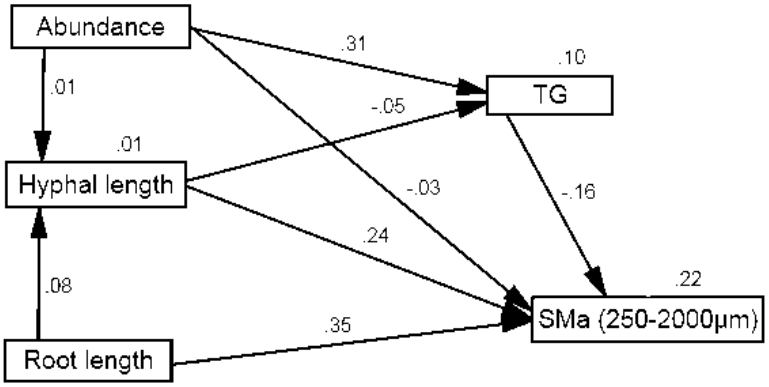
Table 6. Pearson correlation matrix and multiple regression direct estimates (r^2) matrix between selected AMF parameters with soil size fractions (aggregates) in the two field trials (Kabete and Nyabeda), Kenya. TG = total glomalin in whole soil, Abu=AMF spore abundance, Div = AMF Shannon H diversity index, Rich = species richness, MEH= AMF hyphal length, REH=root length, LMa and SMa = large & small macro-aggregates, Mi = micro-aggregates, SC = silt and clay, mM = micro-aggregate within macro-aggregate, n/a = not applicable. Values in bold are significant at $P < 0.05$.

			Multiple regression direct estimates (r^2) matrix					
			Parameters used in model				Multiple regression	
			TG	ABU	MEH	REH	r^2	Model fit
Kabete	0-15	LMa	**-0.42**	0.05	0.11	0.15	0.21	$\chi2$=2.18, p=0.34)
		SMa	-0.16	-0.03	**0.24**	**0.35**	0.22	
		Mi	**0.23**	0.01	**-0.27**	**-0.27**	0.21	
		SC	**0.23**	0.01	-0.01	**-0.35**	0.18	
		mM	0.18	-0.21	-0.11	0.03	0.08	
	15-30	LMa	0.21	0.13	-0.04	**0.38**	0.19	$\chi2$=4.33, p=0.12)
		SMa	-0.03	0.04	-0.01	0.07	0.01	
		Mi	-0.15	-0.02	0.03	-0.2	0.05	
		SC	**0.34**	0.02	0.04	0.06	0.12	
		mM	-0.09	0.1	-0.04	**-0.43**	0.25	
Nyabeda	0-15	LMa	-0.15	-0.2	0.1	-0.05	0.08	$\chi2$=1.43, p=0.49)
		SMa	**0.31**	0.19	0.02	**0.29**	0.23	
		MI	**-0.30**	-0.14	0.01	**-0.26**	0.18	
		SC	-0.09	-0.15	-0.14	-0.22	0.10	
		mM	-0.21	**0.25**	-0.2	**-0.31**	0.22	
	15-30	LMa	**-0.30**	0.02	0.10	-0.01	0.11	$\chi2$=4.93, p=0.09)
		SMa	0.11	0.02	-0.11	0.07	0.03	
		MI	0.01	0.01	0.05	-0.04	0.01	
		SC	**0.28**	-0.05	0.00	-0.21	0.12	
		mM	0.10	0.15	**0.26**	-0.10	0.12	

Table 7. Pearson correlation matrix of selected AMF parameters with nitrogen and phosphorus nutrition and crop yield (grain, stover and total), in the two field trials (Kabete and Nyabeda), Kenya. Abu=AMF spore abundance, Div = AMF Shannon H diversity index, Rich = species richness, WAS= weeks after sowing, %M= percentage root colonization, CT= conventional tillage, NT= no till systems. n/a = not applicable. Values in bold are significant at $P < 0.05$. In column with multiple regression, ANOVA results are shown with F-value and p-value in the parentheses.

			--AMF Diversity indices----			--%Colonization---		MIP	Multiple
Site	Agric. System	Uptake/yield	Abu	Rich	Div.	4 WAS	8 WAS	%M	Regression
Kabete	Organic + Inorganic	N uptake	**-0.30**	-0.006	**-0.33**	n/a	0.14	**0.30**	**2.4(0.05)*, r^2=0.23**
	(n=54)	P uptake	**-0.34**	-0.06	**-0.36**	n/a	0.21	0.23	**2.4(0.04)*, r^2=0.23**
		Grain yield	-0.22	-0.13	-0.222	n/a	**0.27**	**0.28**	1.9(0.10), **r^2=0.19**
		Stover yield	**-0.34**	0.049	**-0.32**	n/a	**0.33**	0.12	**4.3(0.002)*, r^2=0.36**
		Total yield	**-0.33**	0.003	**-0.31**	n/a	**0.32**	0.17	**4.2(0.002)*, r^2=0.35**
Nyabeda	CT systems	N uptake	**-0.41**	**-0.46**	0.05	0.27	-0.02	-0.04	1.4(0.26), r^2=0.34
	(n=24)	P uptake	-0.36	-0.30	0.08	**0.57**	0.14	0.23	2.0(0.11), r^2=0.35
		Grain yield	**-0.41**	-0.40	0.02	0.18	-0.13	0.29	1.19(0.36), r^2=0.29
		Stover yield	-0.04	-0.19	0.03	0.17	0.02	0.10	0.3(0.92), r^2=0.10
		Yield	-0.30	-0.36	0.04	0.21	-0.08	-0.20	0.8(0.53), r^2=0.24
	NT systems	N uptake	-0.30	-0.06	-0.08	0.11	0.08	0.02	1.0(0.46), r^2=0.26
	(n=24)	P uptake	-0.23	-0.25	-0.17	0.06	0.06	0.24	0.3(0.93), r^2=0.31
		Grain yield	0.02	0.02	-0.19	0.24	-0.03	-0.15	0.7(0.67), r^2=0.10
		Stover yield	-0.01	0.14	0.13	0.30	-0.08	-0.01	0.6(0.73), r^2=0.19
		Yield	-0.05	0.09	-0.02	0.31	-0.07	-0.10	0.62(0.71), r^2=0.18
	NT + CT systems	N uptake	-0.23	-0.14	0.14	0.26	-0.08	0.20	1.3(0.27), r^2=0.16
	(n=48)	P uptake	-0.26	-0.19	0.07	0.19	-0.01	0.18	1.2(0.30), r^2=0.15
		Grain yield	-0.16	-0.22	-0.05	0.20	-0.09	0.06	0.7(0.62), r^2=0.10
		Stover yield	-0.14	-0.03	0.18	0.21	-0.08	0.07	0.7(0.67), r^2=0.09
		Yield	-0.17	-0.15	0.08	0.22	-0.10	0.10	0.8(0.56), r^2=0.10

A



B

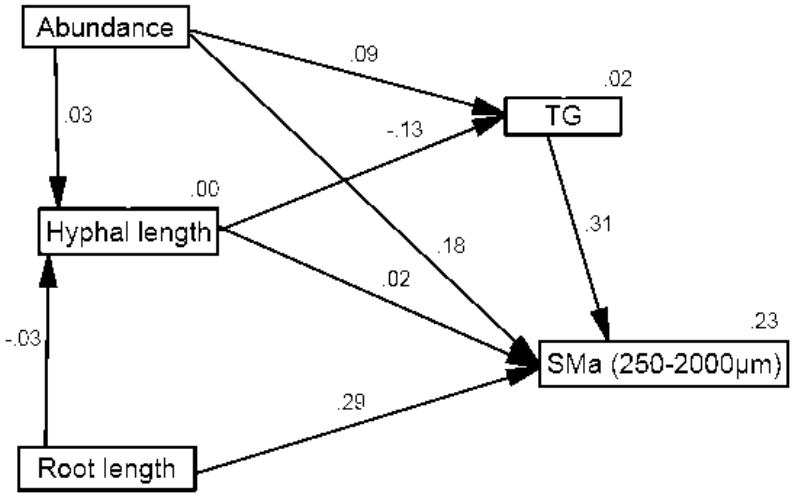


Figure 1: Path model depicting the hypothesized causal relationship of soil aggregates and AMF parameters in (a) Nyabeda and (b) Kabete. Numbers on arrows are standardized path coefficients and are estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable. Each arrow signifies a hypothesized direct causal relationship in the direction of the arrow. Indirect causal effects occur if one variable is linked to another via other, intermediate variables. The model fit is significant (a: χ^2=2.18, df=2 p=0.34 vs. b: χ^2=1.43, df=2, p=0.49).

CHAPTER FIVE

Effect of agricultural practices on glomalin – comparing soil with macro- and micro-aggregates

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Abstract

We assessed the effects of nutrient management and conservation agriculture on glomalin in two Kenyan agro-ecosystems. The Kabete field trial studied effects of various amendments, comparing in a factorial trial the effects of inorganic fertilizer (NP, 3 different rates), farmyard manure (3 rates) and crop residue (present or absent), for 32 years. The Nyabeda field trial studied conservation agriculture practices, comparing in a factorial experiment the effects of tillage (no-till, NT versus conventional tillage, CT), cropping system (either continuous maize cropping (CM) or soybean-maize rotation (SM)), residue (with or without crop residue), and nitrogen fertilization (0 or 60 kg N ha^{-1}), for 5 years. We assessed concentrations of total glomalin (TG) and easily extractable glomalin (EEG) in whole soil and in aggregates of various size classes. Total glomalin pools in both whole soil and different aggregate fractions were sensitive to agricultural management practices, but magnitude of the responses was variable and site-specific. Use of organic and inorganic amendments in Kabete had no significant effects on glomalin pools. N fertilization alone or in combination with crop residue increased glomalin pools by 20% in aggregate fractions in Nyabeda trial. Five-year-old NT systems had lower glomalin concentrations than CT systems. N fertilization increased glomalin concentrations in both NT and CT systems with larger increases in CT systems. Crop rotation (SM) increased glomalin concentrations in micro-aggregates under NT compared to CT, suggesting a potential of rotation in enhancing glomalin pools. Glomalin concentrations were higher in micro-aggregates than macro-aggregates, suggesting a role of glomalin in formation or stabilization of micro-aggregates, and a role of micro-aggregates in physical protection of glomalin against decomposition. Soil organic carbon contents were positively correlated to total glomalin contents, but the ratio of TG: soil organic carbon was different between both trials. Also the ratio of EEG : TG differed between both trials. The results of this study highlight the importance of glomalin in soil quality change due to agricultural management practices.

Keywords: *Glomalin, organic, inorganic, tillage, cropping systems*

1. Introduction

Conventional agricultural practices including frequent and intensive tillage and the extensive use of fertilizers can result in a loss of soil organic matter (SOM), leading to degradation and a decline in soil quality (Peigné et al., 2007). SOM plays a fundamental role in soil processes that maintain productivity, replenish nutrients removed by crops, and enhance soil physical condition and biological activity (Watson et al., 2002). Maintenance of SOM in agro-ecosystems is thus an important component for enhanced physical soil quality and chemical soil fertility. However, little is known about the effects on SOM of agricultural practices such as organic amendments and conservation agriculture (CA), proposed to mitigate land degradation and restore SOM in Sub-Saharan Africa.

Glomalin, a glycoprotein produced on the hyphal walls of arbuscular mycorrhizal fungi (AMF) (Driver et al., 2005) and quantified from soil as immuno-reactive glomalin or as Bradford-reactive Soil Protein (BRSP) is a recently described SOM fraction linked to the stable soil carbon and N pool (Haddad and Sarkar, 2003, Nichols and Wright, 2005; Lovelock et al., 2004a). Glomalin is characterized as a putative homolog of heat shock protein (HSP 60; Gadkar and Rillig, 2006). It has received much attention over the past few years because of its contribution to SOM and to aggregate stability. Strong relationships between glomalin and soil aggregate stability has been reported, resulting in enhanced ecosystem productivity through improved soil aeration, drainage, and microbial activity (Wright and Upadhyaya, 1998; Wilson et al., 2009, Fokom et al., 2012). Glomalin constitutes an important pool of soil organic carbon and nitrogen accounting for upto 37 to 89% of the SOC (Lovelock et al., 2004a; Cornejo et al. 2008). Because of its low turn-over rate, is linked to long-term C sequestration (Rillig et al., 2001). Glomalin has been proposed as a sensitive indicator of soil C changes produced by land-use practices that impact on C-sequestration (Rillig et al., 1999). Understanding glomalin pools in soils could be an important criterion to consider when building up agricultural management strategies in degraded soils.

Standing stocks of glomalin in soil are determined by its production and decomposition, and land use practices influence these two processes differently (Treseder and Turner, 2007). Agricultural practices that degrade soil properties and decrease SOM may also reduce levels of glomalin (Rillig, 2004). Long-term use of inorganic inputs are associated with soil rigidity and with a decline in microbial communities including AMF communities (Gryndler et al., 2006), which in turn reduces glomalin levels. Use of organic inputs on the other hand is associated with build-up of SOM and enhanced microbial (including AMF) activity, which in turn increases glomalin production (Valarini et al., 2009). Whereas organic inputs have been shown to result in

increased glomalin levels, inorganic inputs such as N and P fertilizers have shown contradictory results, with some studies showing higher glomalin levels (Wuest et al., 2005) while others have shown no effect (Lovelock et al., 2004, Antibus et al., 2006). With increasing interest in the build-up and maintenance of SOM, further understanding of the long-term effects of NP fertilization and organic amendments on organic matter fractions such as glomalin is vital.

Soil disturbance through tillage increases carbon turnover rates by disrupting aggregates, and affecting formation of micro-aggregates within macro-aggregates (Six et al., 2004). Tillage also results in the physical disruption of the more transient AMF hyphal network (Kabir, 2005), which negatively affects AMF communities (Jansa et al., 2003) and possibly rates of glomalin production (Wright et al., 2007). Reduced-tillage and no-tillage (NT) systems generally accumulate SOM (at least in upper layers; Lal, 2009; Luo et al., 2010), and result in larger soil microbial communities, higher aggregate stability, abundance of root channels, and water-holding capacity (Liebig et al., 2004). Such practices have a direct role in reducing SOM turnover rates. Minimal soil disturbance and crop residue addition in NT systems enhance diversity and activity of various groups of soil micro-organisms such as AMF that promote plant growth (Oehl et al., 2004; Gosling et al., 2006). Management of cropping systems may also influence glomalin production and decomposition. Composition of the plant community influences soil glomalin stocks (Rillig et al., 2002), and presence of non-mycorrhizal crops in a rotation may reduce levels of glomalin (Wright and Anderson, 2000). Plants with extensive root system may increase glomalin levels compared to those with less extensive root systems (Bird et al., 2002). Although crop rotation is a major practice in low-input agricultural systems, only few studies exist on the effect of crop rotation on glomalin levels.

Very few studies have examined how glomalin is distributed in different aggregate size fractions such as macro- and micro-aggregates (Wright et al., 2007). Macro- and micro-aggregates play important role in stabilization of carbon (Six et al., 2002). Since glomalin constitutes a major part of soil carbon, different aggregate fractions will also play an important role in glomalin stabilization. Macro-aggregates were reported to have higher glomalin levels in less disturbed soils under NT, whereas in highly disturbed soil higher glomalin levels are shown in micro-aggregates (Wright et al., 2007). In order to examine the changes in glomalin levels due to organic amendments (manure and crop residues) and inorganic fertilizer (N and P), and conservation agricultural practices (tillage and cropping system), we hypothesized that:

(1) Organic amendments will increase glomalin levels more than inorganic amendments since organic amendments support increased AMF activity (extraradical hyphal length), which will have positive consequences for glomalin production and stabilization;

(2) Conversion from conventional tillage to no-till practices will increase glomalin levels since tillage will physically disrupt AMF hyphal networks and reduce AMF activity. Tillage also increases organic matter turnover rates, which may increase glomalin decomposition rates and reduce stocks;

(3) Crop rotation (maize plus soybean) will increase glomalin production compared to continuous monocropping with maize due to increases in AMF and other soil organisms such as rhizobia;

(4) Macro-aggregates will have higher glomalin levels than micro-aggregates since AMF hyphae play an important role in enmeshing micro-aggregates to form macro-aggregates. Macro-aggregates are also sensitive to land use practices (Six et al., 2002), their turnover rate may be high in tilled systems and inorganic systems with only transiently bonding systems such as roots and hyphae, and this may have negative consequences for glomalin pools.

In order to test these hypotheses, two long-term field trials with different contrasting management practices were used. The Kabete field trial (Chapter 2), consisting of the long-term use of inorganic fertilizer and organic amendments in a maize-common bean cropping system, was used to test the effects of organic amendments on glomalin levels in different aggregate size fractions. The Nyabeda field trial (Chapter 3), consisting of different conservation agriculture practices (till vs. no-till, continuous maize cropping vs. maize-soybean rotation, with or without N fertilization, with or without addition of crop residue) was used to test these effects on glomalin levels.

2. Materials and Methods

2.1 Study Site

The study was conducted using soils from two long-term trials with different management practices. The Kabete trial in described in detail by Kibunja et al. (2012) and the Nyabeda trial by Kihara et al. (2012a). The Kabete long-term trial was established in 1976 and is located in central Kenya (36°41′E and 1°15′S). The Nyabeda trial was established in 2003 and is located in western Kenya (0° 06N and 34° 36E). The soil in Kabete is a Humic Nitisol and in Nyabeda a Ferralsol (FAO 1990). The trial in Kabete consists of maize-common bean rotation in a randomized complete block design with three replicates. Maize was grown during the long rainy season and common bean during the short rainy season. Plot size was 7.0×4.5 m. Agricultural inputs consisted of organic inputs (farmyard manure and maize stover residue) and inorganic fertilizer supplied as calcium ammonium nitrate (N) and triple superphosphate (P). The treatments comprised three levels of farmyard manure (0, 5 or 10 t ha^{-1} manure), three levels of nitrogen-phosphorus (NP) fertilizer (0kg N and P ha^{-1}, 60 kg N plus 26.4 kg P ha^{-1} and 120 kg N plus 52.8 kg P ha^{-1}), and two levels of crop residue (0 or 2 t residue ha^{-1}), resulting in 18 treatments. The

treatments in Nyabeda consisted of two tillage systems (Conventional tillage, CT and No-till, NT), two cropping systems (continuous maize and maize-soybean rotation), two crop residue management practices (0 or 2 t residue ha^{-1} yr^{-1}), and two N fertilization levels (0 and 60 kg N ha^{-1}) resulting in 16 treatments in a complete randomized block design with three replicates.

In the two study sites precipitation is bimodal with the long rainy season occurring between mid-march to June and the short rainy season between mid-October to December. Maize is the main staple crop in both regions and it is normally grown either as mono-crop or in association with legumes, mainly common bean or groundnut. Adoption of soybean, which is treated as a cash crop, is also taking place in the region. Smallholder settlements predominate the areas, with land sizes ranging from 0.3 to 3 ha per household.

2.2 Soil sampling for glomalin and aggregate analysis

Soil was sampled once during the end of the short rainy season in February 2008 at three depths, 0-15, 15-30 and 30-45 cm. Ten random samples were collected from different depths in each plot, mixed thoroughly, for each depth to obtain a composite sample.

2.3 Separation of water-stable aggregates (WSA)

The separation of aggregates into separate size classes of water-stable aggregates (WSA) was carried out through the wet-sieving method described by Elliott (1986). A subsample of 80g was spread evenly on a 2000 µm sieve, immersed in distilled water, and left for 5 minutes before starting the sieving process. Then, aggregates were separated by moving the 2000 µm sieve up and down by about 3 cm with 50 repetitions in 2 minutes. The aggregates >2000 µm were collected as large macro-aggregates (large Ma) and the same sieving procedure was repeated for the 2000-250 µm fractions (small Ma) with the 250 µm sieve. Then, the fraction 250-53 µm was obtained by sieving with the 53 µm sieve as free micro-aggregates (Mi). The aggregates remaining on top of each sieve were backwashed into labeled and pre-weighed containers and oven-dried at 60° C overnight before the final weight was determined. Soil materials that passed through 53 µm were determined by taking 300 ml sub-sample from the supernatant water of the whole volume after thoroughly shaking the suspension and were dried in the same way as done for the rest of the fractions. The weights were then corrected for the size of the sub-sample as compared to the whole volume and the fractions were recorded as free silt and clay (SC).

2.4 Isolation of micro-aggregates (53-250 µm) within macro-aggregates

Sub-samples from small Ma and large Ma were bulked, and micro-aggregates held within macro-aggregates were isolated following the method described by Six et al. (2000). Briefly, 10 g of macro-aggregates was taken from oven-dried large Ma and small Ma proportional to their initial weight, and mixed thoroughly. Then, 5 g of the mixture was and used for micro-aggregate isolation. A device (Micro-aggregate Isolator) was used to completely break up macro-aggregates while minimizing the breakdown of the released micro-aggregates. The macro-aggregates were immersed in distilled water on top of a 250 µm mesh screen and gently shaken with 50 metal beads (diameter 4 mm). Continuous and steady water flow through the device ensured that micro-aggregates were immediately flushed onto a 53 µm sieve and not further disrupted by the metal beads. After all the macro-aggregates were broken up, the sand and coarse POM (particulate organic matter) remaining on the 250 µm sieve were washed off and collected. The material collected on the 53 µm sieve was sieved according to Elliott (1986) to ensure that the isolated micro-aggregates were water-stable. Silt and clay fractions (<53 µm) were obtained by sub-sampling the supernatant water after measuring the total volume and gently shaking the suspension (wet-sieving). All the fractions; sand and coarse POM (>250 µm), micro-aggregates within macro-aggregates (250-53 µm), and silt and clay (<53 µm) from the isolation step, were dried at 60° C overnight in the oven before the final weight was determined.

2.5 Extraction of glomalin from different aggregate fractions

Glomalin extraction from whole-soil sub-samples, stable macro-aggregates (both LMa and SMa), stable micro-aggregates (Mi), micro-aggregates within macro-aggregates (mM) and silt and clay (SC) was carried out as described by Wright and Updahyaya (1998). Easily-extractable glomalin (EEG) was extracted with 20 mM citrate, pH 7.0 at 121^{o} C for 30 min while total glomalin (TG) was extracted with 50 mM citrate, pH 8.0 at 121^{o} C in rounds of 60 min each. For the sequential extractions, the supernatant was removed by centrifugation at 5000x*g* for 20 min. Extraction of a sample continued until the supernatant showed none of the red-brown color typical of glomalin. Protein was determined by the Bradford assay (Wright and Upadhyaya, 1998). The glomalin extraction for SC was only done for TG but not for EEG since the amount of the soil fraction was in most cases less than 1.5g.

2.6 Soil analysis

Soil analysis was performed at the soil analysis laboratory of the World Agroforestry Centre (ICRAF), Kenya, using near infrared spectroscopy (NIRS). The method is described in detail in chapters 2 and 3. Briefly soils were first air-dried and passed through a 2-mm sieve. Based on

their spectral diversity a sub-sample of one-third of the total samples were selected for wet chemistry. The selected soil samples were analyzed following standard methods for tropical soils (Anderson and Ingram, 1993). Soil pH was determined in water using a 1:2.5 soil/ solution ratio. Samples were extracted with 1M KCl using a 1:10 soil/solution ratio, by atomic absorption spectrometry for exchangeable Ca and Mg. Phosphorus and K were extracted with 0.5 M $NaHCO_3$ + 0.01 M EDTA (pH 8.5, modified Olsen) using a 1:10 soil/solution ratio. Exchangeable K was analyzed by flame photometer and available P was analyzed colorimetrically (molybdenum blue). Organic C (SOC) was determined colorimetrically after H_2SO_4 - dichromate oxidation at 150° C for 30 min. Total N was determined by Kjeldahl digestion with sulphuric acid and selenium as a catalyst. Effective cation-exchange capacity (ECEC) was calculated as the sum of exchangeable bases. The results of the selected soil samples were used in prediction of soil properties using the near infrared spectroscopy by partial least-squares regression (PLSR). All calibrations were developed on natural logarithm transformed variables.

2.7 Statistical analysis

Analysis of variance (ANOVA) was used to test for significant sources of variation in EEG and TG pools in various agricultural practices. Results were considered significant at the $P < 0.05$ level. Significant p-values were separated by Fisher's Least Significant Difference (LSD) test. Further, a Pearson *r* correlation analysis was carried out to observe the degree of association between various soil properties and AMF parameters with glomalin. Treatments effects were statistically analysed using SPSS (PASW statistics 19) software.

3. Results

3.1 Whole-soil glomalin

Easily extractable glomalin (EEG) and total glomalin (TG) were present at every soil depth in both sites. TG and EEG were affected by depth (Table 1 and 2). TG and EEG were high in upper 15 cm, intermediate in 15-30 cm and lowest in 30-45 cm depth (Figure 1). EEG was relatively similar in the two upper layers and represented 39% and 30% of the TG in Kabete, while at 30-45 cm, EEG presented 43% of TG. In Nyabeda, EEG was relatively higher, presenting 56-62% of TG in the three layers (Figure 1).

3.2 Glomalin in aggregates of various size

TG and EEG were present in aggregates of different sizes, total macro-aggregates (TM), free micro-aggregate (Mi), micro-aggregate within macro-aggregate (mM) and silt and clay (SC). In both sites TG and EEG in the various aggregate size classes were significantly affected by depth ($P < 0.001$ in all cases). In Kabete TG in TM- and SC-fractions was highest in 0-15 cm depth, intermediate in 15-30 cm depth and lowest at 30-45 cm depth. TG in Mi- and mM-fractions was highest in 15-30 cm depth, intermediate in 0-15 cm depth and lowest in 30-45 cm depth (Figure 1). EEG in various aggregate size classes was highest in 15-30 cm depth, intermediate in 0-15 cm depth and lowest at 30-45 cm depth in all the four classes. In Nyabeda, TG and EEG in the different aggregate size classes declined significantly with depth (Figure 1). Levels of TG and EEG in the different aggregate classes differed significantly in both trials ($P < 0.001$, Figure 1). Levels of TG were high in SC- and Mi-fractions, while levels of EEG were high in TM- and mM-fractions in the upper two layers (Figure 1).

3.3 Effect of inorganic fertilizer and organic amendments on whole-soil glomalin

TG was only affected by manure in the upper soil layer ($P = 0.02$), but was unaffected by NP fertilization in all depths ($P > 0.05$ in all cases). TG was higher in plots without manure than in manure-amended plots. Crop residue only affected EEG in the two deeper layers (Table 1). Residue-amended plots had higher EEG than unamended plots. There were no interactions between organic and inorganic inputs for TG and EEG.

3.4 Effect of inorganic fertilizer and organic amendments on glomalin in aggregates of various sizes

The mM-fraction EEG in 0-15 cm layer was affected by manure but unaffected by NP, residue and the interaction of the three factors (Table 1). The mM-fraction EEG in manure-amended plots was higher than in plots without manure. Retention of crop residue also affected Mi-fraction EEG ($P < 0.05$). The Mi-fraction TG was higher in residue-added plots (0.98 mg g^{-1}) than in unamended plots (0.83 mg g^{-1}). There was a significant residue x NP fertilizer interaction on TM-fraction TG in 15-30 cm depth (Table 1). TM-fraction TG in fertilized plus residue-addition plots was higher than in unfertilized plots without residue (Table 1). At 30-45 cm layers, Mi-fraction TG was affected by NP fertilizer, manure and the interaction between the three factors ($P < 0.05$ in all cases, data not presented). The SC-fraction TG was also affected by manure and NP fertilizer x manure interaction and manure x residue interaction ($P < 0.05$). Fertilization combined

with organic amendments (manure and residue) had lower Mi-fraction TG than either fertilized or unfertilized treatments (Table 1).

3.5 Effect of CA practices on whole-soil glomalin

TG was affected by tillage and N fertilization in all depths as well as by cropping system x N-fertilization interaction at 15-30 cm layer (Table 2). EEG was also affected by tillage x cropping systems interaction in the upper 15 cm layer and tillage x N-fertilization interaction in the two deeper layers (Table 2). TG was higher in CT than NT in all depths. TG in CT systems was 10%, 20% and 31% higher than TG in NT systems in the three soil depths. TG was also higher in unfertilized than in N-fertilized systems all the three depths (Table 2).

3.6 Effect of CA practices on glomalin in aggregates of various sizes

Tillage and N-fertilization affected levels of TG in various aggregate size classes in all depths (Table 2). Fertilized plots had generally higher TG and EEG concentrations than unfertilized plots for most aggregate size classes (TM, Mi, mM and SC) in two upper layers, but the opposite was observed in 30-45 cm. CT plots also had higher TG and EEG levels than NT plots in the different aggregate size classes (Table 3). The Mi-fraction TG was also affected by cropping system, residue, tillage x cropping system, residue x N-fertilization, cropping system x residue, and cropping system x N-fertilizer interactions in the upper 15 cm (Table 2). The Mi-fraction TG was higher in soybean-maize rotation (SM) than in continuous maize (CM) systems, with more increases in either fertilized or residue-amended plots. The Mi-fraction TG in both SM and CM was higher in NT than in CT plots (Table 2).

3.7 Glomalin as a fraction of SOM

The ratio TG : OM was three times higher than the ratio EEG : OM in Kabete (5.5 vs. 19.6%) and two times higher in Nyabeda (4.5 vs. 8.1%). In various aggregate size classes, the TG : OM ratio was high in SC (20.7%), followed by Mi (16.7) and mM (13.3%), and lowest in TM (10.7%) in Kabete. In Nyabeda, the TG : OM ratio was highest in Mi (14.3), followed by SC (11.9%) and TM (10.9%) and lowest in mM (7.6%). The TG : OM and EEG : OM ratios in the whole soil and various aggregate size classes were affected by soil depth in Kabete (Table 3). In Nyabeda, soil depth affected whole-soil TG : OM ratio, and Mi- and mM-fractions as well as whole-soil EEG : OM ratio and mM-fraction (Table 4). The whole-soil TG : OM ratio, and the ratio in TM- and SC was higher in the upper layer than in the lower layers in Kabete (Table 3). However, whole-soil EEG : OM ratio and that in various aggregate size classes, as well as the TG : OM ratio in Mi-

and mM was higher in the layers 15-30cm than in the upper layer. In Nyabeda, whole-soil TG : M and EEG : OM ratio and that in mM was generally higher in the upper soil layer than in deeper layers (Table 4).

The whole soil TG : OM ratio and that in Mi-fraction was affected by manure but unaffected by fertilizer, residue and interactions of these factors with depth (Table 3). The ratio was lower in manure-amended plots than in plots without manure (Table 3). Similar observations were made for the EEG : OM ratio in TM. Whole-soil EEG : OM ratio was also affected by residue in the 15-30 cm layer ($P < 0.05$). The EEG : M ratio in TM was higher in plots with residue than in plots without residue. Plots with organic inputs alone or in combination with NP-fertilization had a higher TG : OM ratio in Mi than either sole NP-fertilized plots or plots without inputs (Table 3). Whole-soil TG : OM ratio and the ratio in TM and Mi was higher in CT than in NT plots. N-fertilized plots recorded higher whole-soil TG : OM ratio and that in TM and Mi than unfertilized plots (Table 4). Fertilized plots under soybean-maize rotation had a TG : OM ratio in Mi than under continuous maize (Table 4).

3.8 Correlations between glomalin soil properties and AMF

The correlation matrix showing relationships between TG and EEG with soil properties in both trials is shown in Table 5. Correlations with whole-soil TG were generally better in the 15-30 cm layer than in the 0-15 cm layer. These were no significant correlations between soil properties and whole-soil EEG. For whole-soil TG in 0-15 cm correlations were positive for C N, P and pH and negative for Mg, while in 15-30 cm correlations with cations were negative in Kabete but positive in Nyabeda. For the various aggregate size classes most correlations were not significant, with remarkable differences between both glomalin pools, soil depths and sites.

Correlations between TG and EEG in the 0-15 cm layer and mycorrhizal parameters are shown in Table 6. AMF spore abundance, species richness and diversity correlated positively with whole-soil TG and EEG in Kabete or Nyabeda. Whole-soil TG and EEG generally were not significantly correlated with extraradical hyphal length. For the various aggregate size classes most correlations were again not significant, with notable differences between TG and EEG, and between sites. In general correlations in Nyabeda were more often significant than in Kabete.

4. Discussion

4.1 Glomalin in agro-ecosystems

Concentrations of whole-soil glomalin in both agro-ecosystems were comparable to those reported from various tropical agro-ecosystems (Fokom et al., 2012; Lovelock et al., 2004) and temperate regions (Curaqueo et al., 2010). Fokom et al. (2012) reported a glomalin concentration of 8.5 mg g^{-1} in an agricultural field in Cameroon. Glomalin levels ranged between 0.8-12.5 mg g^{-1} in tropical soils of Costa Rica (Lovelock et al., 2004). High glomalin levels could be due to the method of extraction (Bradford rather than immune-assays) and mostly likely high clayey soils (67% clay) resulting to more stabilized glomalin.

Glomalin levels generally declined with depth. Our result is in accordance with studies by Rillig et al. (2003a) and Tang et al. (2009) who also found decreasing glomalin concentrations with depth. This pattern is due to high root and AMF densities and high SOC levels in the upper soil layer. Decreasing glomalin levels were noted in all aggregate size fractions. TG levels declined especially strongly with depth in the SC-fraction in Kabete, but less so in Nyabeda. Tillage reduced levels of whole-soil TG and EEG and TG and EEG in the various aggregate size classes in Nyabeda. Regular tillage is associated with increased turnover of mM, and minimal stabilization and protection of organic matter from decomposition (Six et al., 2002; Denef et al., 2007).

Glomalin is usually associated with stabilization of macro-aggregates and very little attention has been devoted to its role in stabilization of micro-aggregates (Rillig, 2004). Our result show higher levels of TG in Mi and SC than in TM. The TG : OM ratio was also higher in Mi and SC than in TM. Our results imply that glomalin plays a role in stabilization of both macro-aggregates and micro-aggregates, and the effect may be even stronger in micro-aggregates. Glomalin, the product produced by AMF mycelia, has a primary role in the living fungus and a secondary role in soil aggregation (Gadkar and Rillig, 2006; Purin and Rillig, 2007). This implies that as the AMF hyphae inside the macro-aggregate decompose, glomalin is released, which together with other soil organic matter fractions interacts with clay to form macro-aggregates. As the binding agents in macro-aggregates degrade over time, resulting in loss of macro-aggregate stability, stable micro-aggregates formed within macro-aggregates are released and become the building blocks of the next cycle of macro-aggregates (Six et al., 2000). In such micro-aggregates glomalin may be physically protected against further degradation. High glomalin levels in SC also suggest that clay contributes to stabilization of glomalin. Higher whole soil glomalin than in some of the aggregates in Kabete could reflect aggregates disruption which resulted to increased glomalin extraction efficiency as suggested by Janos et al (2008).

High levels of EEG in TM and mM suggest that glomalin in these fractions is less stabilized compared to that in free Mi. The EEG pool was initially considered as the younger (less

transformed) glomalin, but we now consider that the EEG pool reflects material that is less strongly bound to clay (or to the iron and aluminium coating around clay) and therefore may be liable to extraction in a weaker medium. This suggests that stable micro-aggregates physically protect occluded glomalin against decomposition. This is in line with current knowledge that free micro-aggregates plays a major role in stabilization and protection of organic material unlike macro-aggregates that are sensitive to land use practices and exhibit a much lower degree of physical protection (Six et al., 2002; Pulleman et al., 2005; Schmidt et al., 2011). Our data suggest that the degree of stable micro-aggregation might play a more important and direct role in the relation between glomalin stabilization and agricultural management.

Glomalin pools were sensitive to agricultural management, however the magnitude of responses was variable and site specific. We noted large differences in TG and in the TG : OM ratio between Kabete and Nyabeda. For EEG the differences between both sites were much smaller. Kabete, which was under continuous cropping for 32 years, recorded two times higher whole-soil TG levels than Nyabeda, which was under cultivation for 5 years. Glomalin stabilizes aggregates, which in turn protects it from degradation (Wright and Upadhyaya, 1998). The degree of glomalin stabilization may depend on management practices, and duration of the management. Glomalin stabilization of micro-aggregates could consequently reduce micro-aggregate turnover, leading to lower carbon turn-over and nitrogen cycling.

4.2 Effects of NP-fertilization and organic amendments on glomalin

Our data did not show pronounced effects of NP fertilization and organic inputs on glomalin pools in Kabete. This was in contrast to various studies showing positive effects of organic amendments to glomalin levels (Wuest et al., 2005, Valirini et al., 2009). Our results are in agreement with others showing lack of pronounced effect of N and P fertilization on glomalin (Antibus et al., 2006; Nie et al., 2007; Garcia et al., 2008). Organic inputs might have increased glomalin levels through increased AMF activity (Chapter 2). However, other organic (humified) materials can also be Bradford-reactive (Rosier et al., 2006), and such materials may have shown a faster turn-over due to the inputs of organic and inorganic materials. The positive correlation between glomalin with SOC and N in the lower layer (15-30 cm) further suggests that in more stabilized C pools the levels of glomalin also higher and even relatively increased.

Kibunja et al. (2010) and Kamaa et al. (2011) reported dominance of bacterial communities in this soil. Bacteria-dominated systems may lead to faster mineralization and release of nutrients compared to fungi-dominated systems that may immobilize nutrients. Fungi also have higher carbon utilization efficiency than bacteria (Holland and Coleman, 1987). Under

such conditions, decompositions rates are high resulting in lack of significant effects on levels of glomalin levels. High temperature (in tropics) accompanied by regular soil disturbance of soil through tillage may have further raised glomalin decomposition rate hence homogenizing glomalin levels under contrasting management practices. Finally, the weak treatment effects, as reported in Chapters 2 and 3, will likely have resulted in small changes in the glomalin and SOC pools.

Indeed, the TM : SOM ratio in Mi was lower after farmyard manure addition, suggesting that the degree of glomalin stabilization under various management forms affected the amount of glomalin extracted. There was also a trend of lower levels of TG and EEG in plots with organic amendments than in those without them. Long-term application of chemical fertilizer in agricultural soils is associated with increases of soil rigidity, which results in lower levels of SOC and soil biota (AMF) leading to less stable soil macro-aggregates. We reported high levels of Mi and SC in soils without inputs as well as soils with sole NP fertilization (Chapter 4) which suggest reduced C and cycling and hence degradation of soil quality. Soil with lower levels of stable macro-aggregates has less physical protection of glomalin (as reflected by high levels of EEG) resulting to high glomalin content whereas glomalin was more stabilized (protected) in systems under continued application of organic inputs.

Stable soil aggregates, especially micro-aggregates, physically protect occluded soil organic matter against decomposition (Pulleman et al., 2005; Schmidt et al., 2011), and may consequently affect the amount of glomalin extracted, especially in soils with high clay content (Nichols and Wright, 2005). Nichols and Wright (2005) and Wright et al. (2006) showed interaction of glomalin and soil properties. Clay, and Fe- and Al-(hydr-)oxides negatively affect the extraction efficiency of glomalin. In soils with high clay content, Fe- and Al-(hydr-)oxide bridges between organic matter and clay minerals form organo-mineral complexes (Degens, 1997) that are likely responsible for the formation of humin, and probably the recalcitrant glomalin pool (Rice, 2001; Nichols and Wright, 2005). Denef et al. (2004) have also shown stabilization of soil organic matter within aggregates in soils with 1:1 kaolinite clay. Under such conditions treating soil with citric acid before glomalin extraction and the use of high temperature during citrate extraction may be desirable as suggested by Nichols and Wright (2005). Future studies are desirable to test the responses of immuno-reactive TG and EEG fractions following NP-fertilization and manure amendments, since this immune-reactive fraction may be a more specific pool of pure (that is AMF) glomalin than the Bradford-reactive pool (Wuest et al., 2005; Treseder et al., 2007).

We observed a negative correlation between whole-soil TG and cations (K, Ca, Mg, and

ECEC) in the upper soil layer in Kabete and partly also in the lower layer. In lower layer, however, positive correlations between whole-soil TG and C, N, available P, and pH were noted. While these positive correlations indicate glomalin stabilization, especially of the more stabilized part, it is not clear whether the negative correlations in the upper layer reflect a negative role of cations on glomalin production or on stabilization

4.3 Effect of CA practices on glomalin

We hypothesized that soil under NT would contain more glomalin than soils under CT. Contrary to our hypothesis, soils under CT had higher whole-soil glomalin and glomalin in various aggregate size fractions than soils under NT, forcing us to reject the hypothesis. This result was in contrast to the view that CT has a negative effect on glomalin (Wright et al., 2007; Curaqueo et al., 2010). We attribute our observations to intensity of tillage and amount of crop residues under NT. Tillage in Nyabeda was carried out by hand and hoe and only disturbed the upper 10 cm soil layer. Reduced tillage, disturbing only the upper 15 cm layer, was shown to have similar beneficial effects as NT systems on glomalin (Borie et al., 2006) and SOC levels (Alvarez, 2005).

Crop residue under NT is responsible for creating a favourable micro-climate and for reducing soil erosion (Hobbs et al., 2008). However, plant-available nutrient levels are altered considerably under NT depending on the quantity and type of organic mulch (Schomberg et al., 1994). In Nyabeda, NT plots had lower plant-available nutrients as well as lower SOC (Chapter 3), suggesting that crop residues were insufficient to alter carbon and nutrient levels. Because glomalin and SOC are subject to similar decomposition dynamics (Rillig et al., 2001; Bedini et al., 2007), our results corroborate the results from various studies (Treseder and Turner, 2007). We also observed a positive correlation between glomalin levels and AMF hyphal length, suggesting that improved AMF activity also contributed to glomalin production. Positive correlations were also observed between whole-soil TG levels and some other soil properties. Interestingly, in Nyabeda at 15-30 cm we observed positive correlations between basic cations and whole-soil TG, in contrast to the negative correlation between these properties in the upper layer in Kabete. We cannot explain this differential relationship.

Poor crop growth under NT might have had a negative impact on root growth, AMF hyphal quality and root exudates, as well as soil properties that in return affected glomalin production. Although NT systems are known to affect glomalin levels in upper 5 cm soil depth due to concentration of litter on the soil surface, previous studies in this site did not find improved soil properties in upper 5 cm layers (Terano 2010), which may also suggest also minimal change to glomalin levels.

Nitrogen fertilization increased glomalin pools in various aggregate size classes, with more increases under CT than under NT. Studies looking at effects of N fertilization on glomalin have shown inconsistent results. Wilson et al. (2009) and Wuest et al. (2005) showed positive effects of N fertilization on glomalin and attributed the effects to increased plant biomass production, improved AMF symbiosis and high C levels. Increased plant biomass contributes directly to higher SOC levels and probably also glomalin levels through a more dense rooting system and higher amount or changed quality of root exudates (Russel et al., 2009). Unfertilized soil has also been suggested to have rapid decomposition of glomalin due to utilization of glomalin-N by N-limited microbes (Treseder and Turner, 2007).

Soybean-maize (SM) rotation under NT increased glomalin pools in micro-aggregates compared to CT. A positive effect of crop rotation on glomalin levels has been shown before (Wright and Anderson, 2000). This effect may be due to changes in microbial communities (AMF), in nutrients, especially N, and in rooting systems. Although we observed minimal changes in AMF activity (hyphal length and root colonization) in Nyabeda due to rotation, it may have affected other members of the soil microbial community (bacteria) resulting in changes in glomalin production (Kihara et al., 2012b). It is also likely that differences in rooting systems influenced glomalin production (Bird et al., 2000). This implies that SM rotations are important for sustained glomalin production under CA.

5. Conclusion

Glomalin pools in both whole soil and in aggregate size fractions are sensitive to agricultural management, but the magnitude of responses is highly, and soil and management-specific. Long-term use of organic and inorganic amendments had no pronounced effect on glomalin pools in Kabete, while N-fertilization alone and in combination with crop residue increased glomalin pools in Nyabeda. Poor plant growth in all treatments in Kabete may have caused lack of clear effects on glomalin. Contrary to the received view that NT increases glomalin levels (Wight et al., 2007), this study shows negative effect of a five-year-old NT system on glomalin pools in Nyabeda. Improved plant growth (higher amount of plant roots) and improved AMF symbiosis may have played a major role in enhancing glomalin pools under CT, especially in combination with N-fertilization. Use of a maize-legume rotation (maize-soybean) has potential in enhancing glomalin pools under NT. The study provides support for the view that glomalin (either TG or EEG, and either as whole-soil glomalin or glomalin in various aggregate size classes) can be used as an indicator of soil quality. However, our study also suggests that higher glomalin levels (and

especially higher TG : SOM levels) are not necessarily indicators of better physical soil quality or chemical soil fertility, as shown by high values in the unproductive site of Kabete.

Table 1. Glomalin levels (TG, EEG; mg g^{-1}) as affected by different agricultural practices in Kabete. TG and EEG = total and easily extractable glomalin. NP = Nitrogen-Phosphorus fertilization, NP1 = 26.6 kg P-60 kg N ha^{-1}, NP2 = 52.2 kg P-120 kg N ha^{-1}; FYM = farmyard manure, FYM1 and FYM2 =5 and 10 t ha^{-1}; CR = crop residues, -/+ = absence / presence of crop residue. TM = macro-aggregates, Mi = micro-aggregates, SC = silt and clay, mM = micro-aggregates within macro-aggregates, Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Interaction tested for all factors but presented only for the significant ones.

				Total glomalin (mg/g)					Easily extractable glomalin (mg/g)			
				Soil	TM	Mi	mM	SC	Soil	TM	Mi	mM
Depth	NP	FYM	CR	(W)	(≥250 µm)	(53-250 µm)	(53-250 µm)	(≤250 µm)	(W)	(≥250 µm)	(53-250 µm)	(53-250 µm)
0-15 cm	None	None	-	8.02(0.1)	4.48(0.2)	6.62(0.4)	4.11(0.2)	11.37(1.1)	2.23(0.1)	1.55(0.4)	0.69(0.1)	1.11(0.1)
			+	8.93(0.3)	4.69(0.1)	6.64(0.4)	4.19(0.8)	10.87(0.7)	2.15(0.1)	1.05(0.1)	0.76(0.2)	1.06(0.1)
		FYM1	-	8.81(0.4)	4.41(0.6)	6.20(0.8)	4.38(0.7)	12.57(0.7)	1.93(0.1)	1.15(0.1)	0.78(0.1)	1.14(0.1)
			+	7.44(0.2)	4.97(0.2)	6.12(0.3)	4.17(0.3)	11.29(0.8)	1.84(0.1)	0.91(0.1)	0.68(0.1)	1.20(0.3)
		FYM2	-	7.75(0.5)	4.19(0.3)	5.59(0.2)	4.45(0.5)	11.56(0.6)	1.99(0.1)	0.94(0.1)	0.77(0.2)	1.26(0.2)
			+	7.47(0.6)	5.38(0.6)	5.50(0.2)	4.14(0.1)	11.37(1.5)	2.19(0.3)	0.97(0.1)	0.57(0.1)	1.23(0.1)
	NP1	None	-	7.77(0.3)	3.71(0.4)	5.90(0.3)	3.82(0.3)	10.58(0.3)	1.79(0.1)	0.99(0.1)	0.48(0.1)	0.91(0.1)
			+	8.65(0.2)	4.36(0.2)	5.94(0.3)	5.16(0.2)	11.73(0.3)	2.06(0.1)	1.07(0.1)	0.70(0.1)	1.26(0.2)
		FYM1	-	7.89(0.3)	4.87(0.6)	5.33(0.1)	4.23(0.5)	10.98(1.1)	2.02(0.1)	0.85(0.1)	0.62(0.1)	1.12(0.1)
			+	8.73(0.7)	4.53(0.5)	6.00(0.6)	3.97(0.5)	11.79(0.5)	2.05(0.2)	1.11(0.1)	0.62(0.1)	1.26(0.1)
		FYM2	-	8.37(0.4)	3.92(0.6)	6.66(0.9)	4.38(0.4)	11.81(1.0)	2.18(0.1)	0.93(0.1)	0.64(0.1)	1.28(0.1)
			+	7.67(0.7)	4.31(0.6)	5.64(0.2)	4.02(0.3)	12.04(1.1)	2.02(0.1)	0.94(0.1)	0.45(0.1)	1.37(0.1)
	NP2	None	-	8.76(0.5)	4.13(0.1)	6.14(0.3)	4.47(0.7)	11.77(0.1)	2.08(0.1)	0.92(0.1)	0.66(0.1)	1.22(0.1)
			+	8.79(0.2)	4.76(0.4)	5.88(0.4)	5.10(0.1)	12.38(0.8)	2.09(0.1)	1.05(0.1)	0.64(0.3)	1.14(0.1)
		FYM1	-	8.39(0.2)	4.91(0.9)	6.81(0.3)	4.07(0.4)	11.08(0.3)	2.44(0.4)	0.98(0.1)	0.65(0.1)	1.35(0.1)
			+	7.72(0.8)	4.23(0.7)	4.73(0.7)	3.86(0.5)	10.81(0.5)	2.07(0.2)	1.11(0.1)	0.81(0.1)	1.16(0.1)
		FYM2	-	7.98(0.2)	4.21(0.2)	5.34(0.5)	4.21(0.3)	13.09(1.0)	1.98(0.1)	1.08(0.1)	0.53(0.2)	1.39(0.1)
			+	8.27(0.2)	4.53(0.6)	5.67(0.1)	4.21(0.4)	8.92(1.8)	2.03(0.2)	0.74(0.3)	0.59(0.1)	1.34(0.1)
15-30 cm	None	None	-	6.68(0.4)	3.60(0.1)	7.34(0.4)	5.63(0.9)	4.03(0.7)	2.18(0.1)	1.48(0.1)	0.96(0.1)	3.25(0.1)
			+	6.90(0.4)	3.33(0.5)	7.10(0.6)	5.58(0.4)	4.16(0.6)	2.67(0.6)	1.44(0.1)	0.90(0.1)	3.29(0.1)
		FYM1	-	6.49(0.7)	3.34(0.6)	6.19(0.5)	5.36(0.5)	4.04(1.0)	1.82(0.1)	1.52(0.1)	0.85(0.1)	3.35(0.2)
			+	6.15(0.3)	4.00(0.8)	5.51(0.5)	5.06(0.4)	4.37(0.8)	2.14(0.1)	1.43(0.1)	0.87(0.1)	3.29(0.1)
		FYM2	-	5.91(0.5)	3.20(0.1)	7.08(0.2)	5.83(0.6)	4.25(0.7)	2.07(0.1)	1.45(0.1)	0.80(0.1)	3.36(0.1)
			+	6.01(0.3)	2.59(0.1)	5.94(0.4)	4.80(0.7)	3.98(0.6)	1.94(0.1)	1.39(0.1)	0.88(0.2)	3.05(0.1)
	NP1	None	-	6.34(0.9)	3.00(0.3)	6.26(0.9)	4.87(0.5)	4.20(0.7)	1.65(0.1)	1.35(0.1)	0.79(0.1)	3.24(0.2)
			+	6.87(0.2)	4.00(0.3)	6.31(0.4)	5.47(0.3)	4.48(0.3)	2.11(0.2)	1.48(0.1)	1.21(0.3)	3.31(0.1)
		FYM1	-	6.61(0.3)	3.19(0.1)	6.03(0.5)	5.55(0.2)	5.14(0.6)	2.00(0.1)	1.40(0.1)	0.83(0.2)	3.46(0.1)
			+	6.11(0.3)	4.53(0.2)	6.79(0.5)	5.59(0.3)	4.64(0.9)	2.16(0.1)	1.52(0.1)	1.11(0.1)	3.51(0.1)
		FYM2	-	6.08(0.4)	3.72(0.1)	5.53(0.2)	5.30(0.6)	4.04(0.7)	2.03(0.2)	1.45(0.1)	0.77(0.1)	3.35(0.2)
			+	5.69(1.2)	3.07(0.1)	6.77(0.3)	5.55(0.5)	4.24(0.6)	2.51(0.1)	1.43(0.1)	1.04(0.1)	3.47(0.1)
	NP2	None	-	6.39(0.8)	4.00(0.2)	6.82(0.3)	6.02(0.2)	4.26(1.3)	1.93(0.2)	1.41(0.1)	0.79(0.1)	3.14(0.1)
			+	6.60(0.2)	2.83(0.3)	6.69(0.5)	5.26(0.6)	4.34(0.3)	2.03(0.2)	1.40(0.1)	0.95(0.2)	3.38(0.2)
		FYM1	-	6.26(0.3)	3.69(0.5)	6.48(0.7)	5.20(0.6)	4.67(1.3)	1.74(0.1)	1.40(0.1)	0.65(0.1)	3.06(0.1)
			+	6.28(0.5)	3.12(0.4)	6.30(0.5)	6.00(0.3)	4.28(0.7)	2.31(0.4)	1.52(0.1)	0.97(0.1)	3.47(0.2)
		FYM2	-	6.29(0.6)	3.42(0.1)	5.65(0.5)	5.30(0.4)	3.62(1.0)	1.99(0.1)	1.53(0.1)	1.04(0.1)	3.54(0.3)
			+	6.23(0.4)	3.01(0.6)	5.90(0.3)	5.58(0.8)	4.14(0.6)	1.82(0.3)	1.47(0.1)	0.87(0.1)	3.54(0.2)
ANOVA	0-15cm	Fertilizer (A)		0.2(0.80)	1.5(0.23)	0.9(0.42)	0.1(0.95)	0.1(0.92)	1.7(0.20)	1.3(0.29)	1.5(0.24)	1.1(0.33)
		Manure (B)		**4.3(0.02)**	0.8(0.44)	1.5(0.23)	1.0(0.39)	0.1(0.95)	0.2(0.83)	2.1(0.14)	1.0(0.37)	**3.5(0.04)**
		Residues (C)		0.3(0.58)	1.5(0.23)	1.5(0.22)	0.1(0.74)	1.6(0.21)	0.1(0.72)	0.5(0.47)	0.0(0.92)	0.2(0.68)
	15-30cm	Fertilizer (A)		0.0(0.97)	0.6(0.56)	0.5(0.59)	0.9(0.40)	0.3(0.76)	2.2(0.12)	0.4(0.66)	1.2(0.30)	0.9(0.40)
		Manure (B)		1.8(0.18)	1.2(0.30)	1.9(0.17)	0.1(0.93)	0.6(0.56)	1.8(0.18)	1.1(0.34)	0.0(0.97)	0.9(0.41)
		Residues (C)		0.0(0.92)	0.4(0.53)	0.1(0.78)	0.2(0.69)	0.0(0.90)	**14.7(0.00)**	1.0(0.32)	2.4(0.13)	0.7(0.42)
		A x B		0.2(0.93)	**2.7(0.05)**	1.5(0.23)	0.4(0.78)	0.1(0.97)	**5.0(0.00)**	0.6(0.64)	0.5(0.75)	1.2(0.33)
		A x C		0.0(0.96)	**3.7(0.03)**	**3.2(0.05)**	1.4(0.26)	0.0(1.00)	0.8(0.47)	0.9(0.40)	0.0(0.99)	1.6(0.21)

Table 2. Glomalin levels (TG, EEG; mg g^{-1}) as affected by different agricultural practices in Nyabeda. TG and EEG = total and easily extractable glomalin. NT = No-till, CT = Conventional tillage; CM = continuous maize, SM = soybean-maize rotation. 60N = Nitrogen fertilization (60 kg N ha^{-1}); CR = crop residue, -/+ absence / presence of crop residue. TM = macro-aggregates, Mi = micro-aggregates, SC = silt and clay, mM = micro-aggregates within macro-aggregates, Values in parentheses are standard errors. ANOVA table shows F-value and p-value in parentheses. Interaction tested for all factors but presented only for the significant ones.

				----------Total glomalin (mg/g)----------					----------Easily extractable glomalin (mg/g) ---------			
				Soil	TM	Mi	mM	SC	Soil	TM	Mi	mM
Depth	TRT	N	CR	W	(≥250 µm)	(53-250 µm)	(53-250 µm)	(≤53 µm)	W	(≥250 µm)	(53-250 µm)	(53-250 µm)
0-15cm	NTCM	-	-	3.48(0.2)	2.60(0.4)	5.11(0.4)	3.48(0.3)	3.95(0.5)	2.42(0.1)	0.83(0.1)	1.82(0.2)	2.08(0.1)
		-	+	3.66(0.2)	2.96(0.6)	5.11(0.3)	2.82(0.1)	3.02(0.6)	2.46(0.2)	0.99(0.2)	2.37(0.5)	2.37(0.1)
		60N	-	2.78(0.2)	5.24(0.3)	4.00(0.9)	2.56(0.1)	5.70(0.2)	1.80(0.1)	1.32(0.2)	0.61(0.1)	1.39(0.2)
		60N	+	3.18(0.3)	5.57(0.2)	6.28(0.3)	3.14(0.1)	5.32(1.3)	1.63(0.1)	1.49(0.1)	0.61(0.1)	1.52(0.1)
	NTSM	-	-	3.64(0.4)	3.03(0.5)	5.35(0.3)	3.12(0.2)	3.03(0.8)	2.18(0.2)	1.30(0.1)	1.69(0.1)	2.10(0.1)
		-	+	3.84(0.3)	2.91(0.2)	4.95(0.4)	3.42(0.3)	3.79(0.7)	2.10(0.3)	1.44(0.4)	1.90(0.3)	2.29(0.1)
		60N	-	2.81(0.1)	5.13(0.3)	6.93(0.3)	3.12(0.1)	4.18(0.2)	1.58(0.2)	1.41(0.3)	0.61(0.1)	1.73(0.1)
		60N	+	3.33(0.3)	5.48(0.2)	6.81(0.4)	3.25(0.2)	5.63(0.5)	1.57(0.1)	1.48(0.1)	0.74(0.1)	1.78(0.1)
	CTCM	-	-	3.75(0.1)	3.35(0.4)	4.55(0.2)	3.02(0.2)	3.85(0.6)	2.15(0.3)	1.02(0.2)	1.66(0.1)	2.13(0.1)
		-	+	3.88(0.1)	3.82(0.5)	5.24(0.2)	3.05(0.1)	4.20(0.5)	2.49(0.1)	1.53(0.1)	2.06(0.4)	2.19(0.3)
		60N	-	3.47(0.3)	5.81(0.6)	5.43(0.4)	3.81(0.6)	5.97(0.2)	1.50(0.1)	1.55(0.4)	0.78(0.1)	1.75(0.1)
		60N	+	3.67(0.2)	5.56(0.5)	6.40(0.1)	3.49(0.3)	6.23(0.5)	1.42(0.1)	1.50(0.1)	0.72(0.1)	1.72(0.2)
	CTSM	-	-	3.98(0.2)	3.36(0.4)	4.71(0.1)	3.23(0.1)	4.22(1.1)	2.41(0.1)	1.05(0.1)	1.93(0.1)	2.10(0.1)
		-	+	4.33(0.1)	3.95(0.3)	4.68(0.4)	3.52(0.2)	3.59(0.5)	2.50(0.1)	1.43(0.4)	2.27(0.3)	2.22(0.1)
		60N	-	3.50(0.1)	6.24(0.6)	5.55(0.6)	3.21(0.3)	5.89(0.5)	1.45(0.1)	1.46(0.1)	0.80(0.1)	1.77(0.1)
		60N	+	3.02(0.3)	5.61(0.4)	6.83(0.1)	3.35(0.3)	5.60(0.4)	1.63(0.1)	1.47(0.2)	0.79(0.2)	1.81(0.2)
15-30cm	NTCM	-	-	2.83(0.2)	1.93(0.4)	2.74(0.4)	2.79(0.3)	3.50(0.4)	2.09(0.3)	1.21(0.2)	1.48(0.3)	1.42(0.2)
		-	+	3.08(0.5)	2.10(0.2)	2.57(0.3)	3.52(0.1)	3.53(0.4)	2.00(0.1)	1.15(0.3)	1.43(0.1)	1.42(0.1)
		60N	-	1.86(0.1)	4.11(0.3)	6.40(0.7)	1.49(0.5)	3.07(0.6)	0.74(0.2)	0.76(0.2)	0.52(0.2)	0.58(0.1)
		60N	+	1.81(0.4)	4.61(0.4)	6.78(0.4)	2.15(0.4)	4.25(0.3)	1.29(0.1)	1.08(0.1)	0.68(0.1)	0.94(0.3)
	NTSM	-	-	2.75(0.4)	2.76(0.3)	3.38(0.2)	3.32(0.3)	2.70(0.8)	2.02(0.3)	1.29(0.2)	1.51(0.5)	1.75(0.3)
		-	+	3.38(0.2)	2.44(0.6)	2.66(0.5)	3.13(0.1)	3.84(0.3)	2.16(0.1)	1.13(0.1)	1.42(0.2)	1.26(0.1)
		60N	-	0.89(0.3)	3.85(0.3)	5.72(0.5)	1.22(0.5)	2.44(0.3)	0.73(0.2)	1.05(0.1)	0.40(0.1)	0.65(0.1)
		60N	+	1.87(0.2)	4.66(1.0)	6.51(0.9)	1.85(0.4)	3.32(0.5)	1.04(0.1)	0.94(0.2)	0.53(0.1)	0.75(0.2)
	CTCM	-	-	3.21(0.4)	3.06(0.4)	3.91(0.2)	2.59(0.5)	3.77(1.4)	1.58(0.5)	1.82(0.1)	1.26(0.2)	1.29(0.6)
		-	+	3.64(0.1)	3.11(0.3)	3.93(0.2)	2.66(0.3)	7.10(1.1)	1.59(0.2)	1.59(0.2)	1.79(0.2)	1.76(0.2)
		60N	-	2.15(0.7)	4.60(0.9)	6.60(0.8)	1.45(0.1)	3.89(1.0)	1.12(0.3)	0.84(0.2)	0.59(0.3)	0.71(0.2)
		60N	+	1.45(0.6)	3.49(0.7)	5.40(0.3)	1.60(0.4)	4.33(0.4)	0.87(0.3)	0.89(0.2)	0.35(0.1)	0.54(0.1)
	CTSM	-	-	3.86(0.1)	3.59(0.3)	4.32(0.2)	3.35(0.5)	5.04(0.9)	2.02(0.2)	1.44(0.3)	1.65(0.1)	1.53(0.2)
		-	+	3.90(0.2)	3.52(0.5)	4.10(0.5)	3.87(0.4)	3.64(0.2)	1.65(0.4)	1.33(0.1)	1.95(0.1)	1.60(0.1)
		60N	-	1.71(0.1)	4.57(0.4)	6.38(0.3)	1.91(0.4)	7.68(3.3)	1.07(0.1)	0.97(0.1)	0.85(0.2)	0.88(0.2)
		60N	+	2.18(0.1)	5.01(0.5)	6.93(0.5)	2.31(0.1)	4.04(0.5)	1.24(0.1)	1.13(0.2)	0.57(0.1)	0.63(0.1)
ANOVA	0-15cm	Tillage (A)		**9.8(0.00)**	**8.6(0.01)**	0.5(0.47)	3.0(0.09)	3.8(0.06)	0.1(0.74)	0.7(0.40)	0.6(0.45)	0.5(0.47)
		Cropping systems (B)		0.4(0.54)	0.2(0.62)	5.5(0.03)	0.7(0.41)	0.8(0.37)	0.6(0.43)	0.8(0.37)	0.0(0.91)	1.3(0.27)
		Residues (C)		2.6(0.11)	0.5(0.50)	**8.9(0.005)**	0.2(0.64)	0.1(0.82)	0.3(0.59)	2.4(0.13)	3.2(0.08)	2.1(0.16)
		N-fertilization (D)		**26.9(0.00)**	**132.1(0.00)**	**29.5(0.00)**	0.1(0.80)	**34.4(0.00)**	**118.7(0.00)**	**5.5(0.03)**	**133.1(0.00)**	**47.8(0.00)**
		A x B		0.2(0.62)	0.1(0.79)	**4.6(0.04)**	0.9(0.35)	0.0(0.87)	**5.4(0.03)**	1.8(0.19)	1.4(0.24)	0.5(0.47)
		B x C		0.1(0.73)	0.2(0.67)	**4.2(0.05)**	1.5(0.23)	0.6(0.44)	0.0(0.91)	0.0(0.83)	0.1(0.81)	0.0(0.92)
		B x D		2.5(0.13)	0.0(0.89)	**7.6(0.01)**	1.0(0.32)	0.4(0.55)	0.1(0.71)	1.0(0.33)	0.2(0.69)	1.8(0.19)
		C x D		0.1(0.81)	0.9(0.36)	**7.0(0.01)**	0.3(0.57)	0.4(0.56)	0.7(0.41)	1.3(0.26)	2.8(0.11)	0.7(0.43)
	15-30cm	Tillage (A)		**10.3(0.00)**	**8.9(0.01)**	**13.9(0.00)**	0.3(0.60)	**6.5(0.02)**	0.4(0.55)	4.5(0.04)	2.7(0.11)	0.4(0.52)
		Cropping systems (B)		0.4(0.51)	0.0(0.88)	0.5(0.47)	0.8(0.38)	0.0(0.97)	0.0(0.94)	0.6(0.45)	0.2(0.69)	0.1(0.82)
		Residues (C)		2.2(0.15)	0.0(0.84)	0.1(0.77)	**4.2(0.05)**	0.2(0.65)	0.2(0.64)	0.0(0.86)	0.3(0.60)	0.0(0.93)
		N-fertilization (D)		**83.6(0.00)**	**29.1(0.00)**	**150.6(0.00)**	**59.1(0.00)**	0.0(0.98)	**52.7(0.00)**	**22.0(0.00)**	**89.2(0.00)**	**51.5(0.00)**
		A x D		0.2(0.63)	0.3(0.58)	2.2(0.15)	0.9(0.36)	0.1(0.79)	5.6(0.02)	3.4(0.08)	0.1(0.78)	0.0(0.93)
		B x D		**5.3(0.03)**	2.8(0.10)	**4.6(0.04)**	4.0(0.06)	1.5(0.23)	1.7(0.20)	0.8(0.38)	0.9(0.34)	0.1(0.75)
		A x B x C		0.8(0.38)	0.2(0.69)	0.2(0.67)	0.3(0.61)	**6.8(0.01)**	0.1(0.71)	0.3(0.59)	0.1(0.75)	0.3(0.57)

Table 3: Total glomalin : SOM and easily extractable glomalin : SOM ratios in Kabete. For abbreviations see Table 1.

				----------Total glomalin : SOM ----------					-------Easily extractable glomalin : SOM ---------			
				W	Tma	MI	Mm	SC	W	Tma	MI	Mm
	NP	FYM	CR	soil	>250µm	53-250µm	53-250µm	<53µm	soil	>250µm	53-250µm	53-250µm
0-15cm	None	None	-	23.68(0.5)	11.99(0.3)	17.73(0.9)	11.05(0.6)	30.52(3.0)	5.99(0.2)	4.16(1.1)	1.85(0.4)	2.97(0.2)
			+	23.08(0.4)	12.16(0.6)	17.13(0.6)	10.83(2.0)	28.12(0.80	5.58(0.3)	2.72(0.1)	1.94(0.4)	2.72(0.1)
		FYM1	-	22.37(0.6)	11.29(1.7)	15.7091.7)	11.10(1.6)	32.04(2.50	4.89(0.3)	2.93(0.3)	1.97(0.2)	2.89(0.1)
			+	21.29(2.5)	14.18(1.6)	17.69(2.8)	12.14(2.30	31.85(1.90	5.21(0.4)	2.63(0.4)	1.91(0.3)	3.24(0.4)
		FYM2	-	19.50(1.2)	10.51(0.3)	14.09(0.7)	11.16(1.0)	29.05(0.7)	5.00(0.1)	2.38(0.1)	1.91(0.4)	3.14(0.4)
			+	19.01(1.7)	13.67(1.5)	13.96(0.5)	10.50(0.3)	28.83(3.8)	5.57(0.9)	2.46(0.2)	1.45(0.2)	3.12(0.1)
	NP1	None	-	20.77(1.0)	9.96(1.3)	15.77(0.7)	10.26(1.8)	28.31(1.3)	4.79(0.4)	2.65(0.1)	1.27(0.2)	2.42(0.1)
			+	22.26(0.8)	11.19(0.4)	15.28(0.8)	13.27(0.50	30.17(0.9)	5.30(0.3)	2.75(0.1)	1.80(0.2)	3.26(0.6)
		FYM1	-	20.72(0.6)	12.84(1.6)	13.99(0.1)	11.0891.1)	28.82(2.7)	5.30(0.3)	2.25(0.3)	1.62(0.3)	2.94(0.2)
			+	22.16(1.6)	11.55(1.4)	15.21(1.4)	10.06(1.20	29.99(1.5)	5.22(0.5)	2.83(0.1)	1.57(0.2)	3.19(0.1)
		FYM2	-	21.51(1.8)	10.01(1.3)	16.87(1.5)	11.24(1.1)	30.07(1.6)	5.59(0.4)	2.41(0.4)	1.64(0.2)	3.25(0.2)
			+	18.04(1.6)	10.09(1.2)	13.28(0.6)	9.47(0.7)	28.25(1.8)	4.77(0.2)	2.21(0.2)	1.05(0.1)	3.21(0.1)
	NP2	None	-	22.69(1.4)	10.70(0.2)	15.88(0.7)	11.60(1.8)	30.46(0.5)	5.37(0.2)	2.39(0.3)	1.72(0.1)	3.15(0.1)
			+	23.12(1.0)	12.50(1.0)	15.43(1.0)	13.39(0.5)	32.47(1.5)	5.49(0.4)	2.76(0.1)	1.64(0.6)	3.00(0.1)
		FYM1	-	21.72(0.7)	12.74(2.4)	17.63(0.7)	10.53(1.0)	28.69(0.7)	6.31(1.0)	2.55(0.2)	1.69(0.3)	3.49(0.4)
			+	19.18(2.5)	10.49(1.7)	11.80(2.1)	9.59(1.40	26.74(1.9)	5.15(0.7)	2.76(0.3)	2.01(0.2)	2.88(0.4)
		FYM2	-	19.01(1.1)	10.03(0.7)	12.62(0.7)	9.97(0.3)	31.09(2.5)	4.70(0.1)	2.58(0.2)	1.23(0.4)	3.29(0.1)
			+	19.82(1.0)	10.84(1.4)	13.55(0.1)	10.07(0.8)	21.33(4.2)	4.87(0.5)	1.80(0.8)	1.43(0.4)	3.20(0.1)
15-30cm	None	None	-	18.49(0.5)	9.94(0.3)	20.24(0.8)	15.53(2.4)	11.16(2.2)	6.04(0.4)	4.08(0.1)	2.67(0.2)	8.98(0.4)
			+	19.54(0.9)	9.41(1.4)	20.10(1.4)	15.83(1.2)	11.77(1.5)	7.56(1.7)	4.08(0.1)	2.56(0.2)	9.35(0.2)
		FYM1	-	18.92(1.5)	9.86(1.9)	18.11(1.3)	15.73(1.7)	11.67(2.5)	5.35(0.4)	4.45(0.1)	2.49(0.2)	9.81(0.4)
			+	17.97(0.6)	11.68(2.2)	16.17(1.9)	14.86(1.5)	12.78(2.4)	6.27(0.3)	4.20(0.2)	2.54(0.3)	9.6(0.5)
		FYM2	-	16.97(1.4)	9.22(0.6)	20.38(0.7)	16.70(1.5)	12.13(1.8)	5.95(0.3)	4.16(0.3)	2.28(0.2)	9.67(0.1)
			+	17.22(0.5)	7.45(0.4)	17.04(1.0)	13.73(1.9)	11.51(2.0)	5.55(0.4)	4.01(0.3)	2.51(0.6)	8.74(0.3)
	NP1	None	-	19.85(3.0)	9.45(1.1)	19.39(1.8)	15.27(1.6)	12.99(1.4)	5.19(0.4)	4.24(0.2)	2.50(0.4)	10.20(1.0)
			+	20.79(1.2)	12.06(0.7)	18.96(0.5)	16.48(0.5)	13.46(0.4)	6.34(0.5)	4.46(0.10	3.61(0.8)	10.03(0.8)
		FYM1	-	18.64(0.5)	9.01(0.5)	17.03(1.4)	15.68(0.6)	14.56(1.9)	5.64(0.2)	3.94(0.1)	2.35(0.5)	9.77(0.5)
			+	18.31(0.7)	13.61(0.9)	20.36(0.4)	16.82(1.2)	13.83(2.3)	6.50(0.6)	4.55(0.1)	3.31(0.3)	10.54(0.5)
		FYM2	-	16.58(0.3)	10.22(0.8)	15.15(0.8)	14.47(1.4)	10.89(1.5)	5.55(0.5)	3.97(0.2)	2.10(0.4)	9.18(0.5)
			+	15.50(2.7)	8.47(0.3)	18.77(1.5)	15.31(1.1)	11.61(1.4)	6.94(0.2)	3.95(0.3)	2.86(0.1)	9.58(0.1)
	NP2	None	-	18.36(1.3)	11.74(1.4)	19.74(0.5)	17.52(1.4)	11.99(2.9)	5.69(1.0)	4.11(0.4)	2.28(0.2)	9.12(0.6)
			+	19.51(1.1)	8.34(0.8)	19.66(0.8)	15.45(1.4)	12.77(0.7)	5.97(0.4)	4.14(0.2)	2.76(0.6)	9.94(0.3)
		FYM1	-	17.97(0.6)	10.62(1.3)	18.65(2.1)	14.97(1.7)	13.43(2.8)	5.00(0.2)	4.04(0.4)	1.88(0.2)	8.80(0.4)
			+	16.81(1.2)	8.37(0.9)	17.00(1.9)	16.16(1.3)	11.36(1.7)	6.18(1.0)	4.08(0.2)	2.62(0.2)	9.32(0.6)
		FYM2	-	17.65(1.0)	9.69(0.5)	15.88(0.9)	14.89(0.7)	9.97(2.5)	5.61(0.1)	4.30(0.2)	2.90(0.2)	9.96(0.7)
			+	16.87(1.5)	8.13(1.5)	16.03(1.5)	15.32(2.8)	11.23(1.8)	5.02(1.0)	4.03(0.6)	2.35(0.3)	9.54(0.4)
ANOVA	0-15cm	Fertilizer (A)		0.4(0.71)	1.8(0.17)	2.6(0.09)	0.1(0.92)	0.8(0.44)	0.3(0.71)	0.8(0.46)	1.9(0.17)	0.6(0.54)
		Manure (B)		**7.1(0.00)**	1.6(0.22)	**4.8(0.02)**	1.8(0.18)	1.3(0.27)	0.9(0.41)	3.3(0.05)	1.9(0.17)	1.9(0.16)
		Residue (C)		0.5(0.49)	1.4(0.24)	1.8(0.18)	0.1(0.80)	1.5(0.23)	0.2(0.69)	0.0(0.89)	0.0(0.94)	0.1(0.81)
		A x B x C		1.3(0.28)	1.0(0.43)	**3.5(0.02)**	0.8(0.56)	1.3(0.28)	1.4(0.26)	1.2(0.35)	0.6(0.64)	1.3(0.28)
	15-30cm	Fertilizer (A)		0.2(0.86)	1.4(0.25)	0.7(0.53)	0.1(0.93)	0.6(0.56)	1.2(0.33)	0.1(0.89)	1.3(0.28)	1.8(0.18)
		Manure (B)		**5.4(0.01)**	**3.7(0.03)**	**5.9(0.01)**	0.6(0.57)	1.2(0.32)	0.5(0.60)	0.5(0.62)	0.6(0.53)	0.3(0.77)
		Residue (C)		0.0(0.88)	0.2(0.64)	0.0(0.93)	0.0(0.90)	0.0(0.86)	**5.1(0.03)**	0.0(0.85)	**5.3(0.03)**	0.3(0.58)
		A x C		0.0(0.97)	**5.5(0.01)**	**3.8(0.03)**	0.8(0.46)	0.0(0.99)	0.6(0.55)	1.0(0.36)	2.4(0.11)	0.6(0.55)
	0-30cm	Depth (D)		**43.2(0.00)**	**16.5(0.00)**	**54.1(0.00)**	**96.6(0.00)**	**616.2(0.00)**	**10.9(0.00)**	**189.6(0.00)**	**65.5(0.00)**	**2353.3(0.00)**
		Fertilizer (A)		0.3(0.73)	0.7(0.48)	2.8(0.07)	0.0(1.00)	0.7(0.48)	0.8(0.44)	1.5(0.22)	0.5(0.63)	1.4(0.25)
		Manure (B)		**13.2(0.00)**	**4.7(0.01)**	**10.4(0.00)**	2.0(0.15)	2.49(0.10)	1.1(0.33)	**3.6(0.03)**	1.6(0.21)	0.2(0.78)
		Residues (C)		0.4(0.55)	0.4(0.55)	1.0(0.32)	0.0(0.95)	0.6(0.43)	2.6(0.11)	0.4(0.56)	2.9(0.09)	0.4(0.55)

Table 4: Ratio TG : SOM and EEG : SOM in Nyabeda. For abbreviations see Table 2.

				----------Total glomalin : SOM ----------					-----Easily extractable glomalin : SOM------			
				W	TM	MI	Mm	SC	W	TM	MI	Mm
Depth	Treatment	N	CR	soil	>250µm	53-250µm	53-250µm	<53µm	soil	>250µm	53-250µm	53-250µm
0-15cm	NTCM	-	-	8.95(0.4)	6.68(0.8)	13.16(0.8)	9.04(1.0)	10.27(1.5)	6.25(0.1)	2.16(0.5)	4.69(0.4)	5.36(0.1)
		-	+	9.17(0.7)	7.45(1.6)	12.77(0.5)	7.05(0.3)	7.64(1.6)	6.15(0.3)	2.47(0.4)	5.99(1.4)	5.91(0.1)
		60N	-	7.20(0.2)	13.59(0.2)	10.67(2.8)	6.65(0.1)	14.81(0.5)	4.72(0.4)	3.42(0.2)	1.56(0.2)	3.59(0.2)
		60N	+	8.08(0.7)	14.20(0.6)	16.01(0.8)	8.01(0.3)	13.56(3.4)	4.15(0.3)	3.80(0.3)	1.55(0.2)	3.88(0.3)
	NTMS	-	-	9.40(0.8)	7.79(1.0)	13.88(0.9)	8.08(0.4)	7.81(2.0)	5.64(0.2)	3.32(0.9)	4.39(0.4)	5.45(0.3)
		-	+	9.54(0.8)	7.23(0.6)	12.31(1.2)	8.52(0.7)	9.43(1.8)	5.23(0.9)	3.56(0.7)	4.70(0.8)	5.68(0.4)
		60N	-	6.83(0.4)	12.50(1.0)	16.83(0.9)	7.57(0.3)	10.12(0.2)	3.85(0.5)	3.44(0.4)	1.48(0.1)	4.20(0.1)
		60N	+	8.50(0.8)	14.00(0.6)	17.36(0.6)	8.27(0.4)	14.34(1.1)	4.02(0.2)	3.77(0.5)	1.88(0.2)	4.53(0.2)
	CTCM	-	-	9.28(0.5)	8.32(1.3)	11.27(0.9)	7.42(0.3)	9.38(1.0)	5.39(0.9)	2.56(0.4)	4.12(0.5)	5.28(0.4)
		-	+	9.86(0.4)	9.67(1.2)	13.30(0.3)	7.74(0.2)	10.70(1.4)	6.33(0.4)	3.93(1.0)	5.28(1.1)	5.58(0.4)
		60N	-	8.71(0.7)	14.58(1.5)	13.64(1.0)	9.57(1.5)	14.99(0.4)	3.77(0.1)	3.90(0.1)	1.95(0.2)	4.39(0.9)
		60N	+	8.58(0.3)	12.97(0.9)	14.99(0.3)	8.14(0.5)	14.59(1.2)	3.34(0.2)	3.51(0.3)	1.68(0.2)	4.01(0.2)
	CTMS	-	-	9.88(0.3)	8.32(0.8)	11.72(0.1)	8.05(0.3)	10.42(2.6)	5.99(0.1)	2.61(0.3)	4.78(0.2)	5.22(0.6)
		-	+	9.92(0.2)	9.06(0.6)	10.73(0.9)	8.05(0.4)	8.24(1.1)	5.73(0.1)	3.28(0.9)	5.21(0.7)	5.08(0.3)
		60N	-	8.70(0.2)	15.46(1.2)	13.85(1.7)	7.97(0.6)	14.61(0.9)	3.61(0.2)	3.65(0.3)	1.99(0.1)	4.41(0.4)
		60N	+	7.32(0.8)	13.60(0.9)	16.57(0.3)	8.11(0.7)	13.56(0.9)	3.95(0.1)	3.56(0.5)	1.91(0.4)	4.39(0.4)
15-30cm	NTCM	-	-	9.01(1.3)	6.18(0.5)	8.82(1.1)	9.05(1.3)	11.26(1.0)	6.80(1.2)	3.96(0.9)	4.76(1.1)	4.58(0.6)
		-	+	8.61(0.5)	5.81(0.8)	7.21(1.1)	9.86(0.6)	9.92(1.3)	5.59(0.3)	3.20(0.7)	3.99(0.3)	3.99(0.5)
		60N	-	6.21(1.2)	13.20(0.6)	20.69(1.6)	4.56(1.2)	9.59(0.9)	2.35(0.4)	2.33(0.3)	1.57(0.4)	1.80(0.3)
		60N	+	5.84(1.2)	15.00(1.0)	22.03(0.8)	6.94(1.3)	13.84(1.1)	4.19(0.2)	3.50(0.4)	2.22(0.3)	3.02(0.8)
	NTMS	-	-	8.19(0.6)	8.23(1.6)	10.29(0.6)	10.13(1.1)	7.82(1.9)	6.06(0.5)	3.92(0.5)	4.49(1.4)	5.42(1.1)
		-	+	9.39(0.6)	6.74(2.8)	7.41(1.4)	8.70(0.2)	10.66(0.8)	6.00(0.4)	3.14(0.2)	3.92(0.5)	3.48(0.1)
		60N	-	3.17(1.0)	13.40(1.9)	19.95(3.0)	4.38(1.8)	8.44(1.3)	2.59(0.8)	3.61(0.3)	1.41(0.4)	2.29(0.5)
		60N	+	5.37(0.2)	13.72(4.5)	20.75(4.3)	6.21(1.7)	17.10(4.2)	3.43(0.8)	3.41(0.6)	1.42(0.6)	2.08(0.8)
	CTCM	-	-	8.79(0.5)	8.40(0.3)	10.88(0.9)	7.46(2.0)	9.92(2.8)	4.49(1.7)	5.06(0.3)	3.61(0.8)	3.83(1.7)
		-	+	9.58(0.3)	8.17(0.8)	10.33(0.3)	7.08(1.1)	18.89(3.7)	4.17(0.5)	4.19(0.5)	4.70(0.4)	4.67(0.7)
		60N	-	5.68(1.7)	12.19(2.1)	17.57(2.0)	3.91(0.5)	9.74(1.4)	3.15(1.0)	2.37(0.7)	1.44(0.6)	1.85(0.4)
		60N	+	6.50(1.9)	14.89(2.3)	20.75(2.2)	6.81(1.3)	12.20(1.8)	3.62(0.8)	3.38(0.6)	1.68(0.2)	1.81(0.3)
	CTMS	-	-	10.04(0.2)	9.33(0.7)	11.25(0.7)	8.74(1.5)	13.13(2.3)	5.25(0.3)	3.73(0.8)	4.29(0.1)	3.97(0.5)
		-	+	10.34(0.7)	9.32(1.3)	10.89(1.6)	10.24(1.0)	9.62(0.6)	4.38(1.0)	3.50(0.2)	5.15(0.3)	4.24(0.2)
		60N	-	5.14(0.5)	13.75(1.3)	19.16(0.8)	5.73(1.1)	22.94(9.7)	3.23(0.2)	2.91(0.2)	2.56(0.6)	2.65(0.5)
		60N	+	5.92(0.6)	14.81(2.1)	20.72(2.6)	5.90(0.7)	10.64(2.1)	3.31(0.3)	2.98(0.7)	1.70(0.5)	2.39(0.4)
ANOVA	0-15	Tillage (A)		**4.3(0.05)**	**4.8(0.04)**	2.5(0.12)	0.6(0.45)	1.8(0.18)	1.4(0.24)	0.2(0.64)	0.1(0.77)	0.0(0.86)
		Cropping systems (B)		0.0(0.91)	0.0(0.90)	2.9(0.10)	0.2(0.69)	1.4(0.25)	1.7(0.20)	0.4(0.52)	0.0(0.84)	0.4(0.51)
		Residue (C)		0.8(0.36)	0.1(0.81)	**4.3(0.05)**	0.0(0.85)	0.0(0.95)	0.0(0.85)	1.6(0.21)	2.0(0.17)	0.6(0.43)
		Fertilizer (D)		**30.0(0.00)**	**140.5(0.00)**	**22.7(0.00)**	0.0(0.89)	**34.2(0.00)**	**92.5(0.00)**	**5.5(0.03)**	**118.0(0.00)**	**48.0(0.00)**
		B x D		1.5(0.23)	0.0(0.99)	**6.5(0.02)**	0.6(0.45)	0.3(0.61)	0.4(0.54)	0.7(0.40)	0.4(0.51)	2.6(0.12)
		C x D		0.0(0.98)	0.9(0.36)	**6.2(0.02)**	0.7(0.42)	0.3(0.59)	0.2(0.68)	1.2(0.29)	1.9(0.18)	0.2(0.64)
		A x B x C		1.3(0.27)	0.0(0.92)	1.0(0.33)	0.0(0.83)	**4.9(0.03)**	0.3(0.60)	0.0(0.91)	0.0(0.99)	0.0(089)
		A x B x C x D		0.5(0.48)	0.2(0.64)	3.4(0.08)	**4.1(0.05)**	0.1(0.80)	0.3(0.57)	0.2(0.66)	0.0(0.84)	0.1(0.77)
	15-30	Tillage (A)		2.7(0.11)	1.3(0.26)	0.3(0.56)	0.6(0.43)	2.1(0.15)	3.1(0.09)	0.2(0.63)	0.3(0.59)	0.2(0.66)
		Cropping systems (B)		0.5(0.49)	0.5(0.47)	0.1(0.78)	0.8(0.39)	0.2(0.69)	0.0(0.97)	0.1(0.72)	0.1(0.70)	0.1(0.73)
		Residue (C)		2.0(0.17)	0.3(0.61)	0.0(0.84)	2.4(0.13)	0.6(0.43)	0.1(0.80)	0.1(0.79)	0.1(0.79)	0.1(0.81)
		Fertilizer (D)		**63.3(0.00)**	**42.8(0.00)**	**128.5(0.00)**	**29.1(0.00)**	1.1(0.30)	**30.0(0.00)**	**8.1(0.01)**	**70.5(0.00)**	**33.3(0.00)**
		A x D		0.1(0.81)	1.1(0.30)	4.0(0.06)	0.8(0.38)	0.2(0.68)	**5.0(0.03)**	2.5(0.12)	0.0(0.95)	0.0(0.93)
		C x D		0.2(0.69)	1.1(0.29)	2.7(0.11)	1.9(0.18)	0.1(0.76)	3.4(0.07)	**4.6(0.04)**	0.1(0.81)	0.6(0.46)
		A x B x C		1.5(0.22)	0.0(0.88)	0.0(0.96)	0.2(0.70)	**8.0(0.01)**	0.1(0.73)	0.2(0.62)	0.1(0.73)	0.5(0.45)
		A x C x D		0.0(0.90)	0.0(1.00)	0.0(0.90)	0.3(0.57)	**4.5(0.04)**	0.5(0.48)	0.0(0.88)	3.4(0.08)	3.1(0.09)
	0-30cm	Depth (E)		**25.5(0.00)**	0.1(0.79)	**5.2(0.03)**	**5.1(0.03)**	0.6(0.43)	**7.5(0.01)**	0.5(0.47)	1.6(0.22)	**61.3(0.00)**
		Tillage (A)		**6.1(0.02)**	**4.1(0.05)**	0.1(0.77)	0.1(0.70)	3.7(0.06)	**4.4(0.04)**	0.5(0.50)	0.4(0.55)	0.2(0.64)
		Cropping systems (B)		0.3(0.58)	0.5(0.48)	1.2(0.27)	0.9(0.34)	0.0(0.87)	0.4(0.53)	0.0(0.84)	0.0(0.89)	0.4(0.54)
		Residues (C)		2.8(0.10)	0.3(0.58)	1.5(0.23)	1.7(0.19)	0.5(0.50)	0.0(0.89)	0.5(0.47)	1.3(0.26)	0.0(0.88)
		Fertilizer (D)		**92.7(0.00)**	**127.8(0.00)**	**148.6(0.00)**	**22.8(0.00)**	**12.5(0.00)**	**86.1(0.00)**	0.1(0.74)	**183.6(0.00)**	**69.2(0.00)**
		B x D		**4.5(0.04)**	0.3(0.58)	0.9(0.35)	1.3(0.26)	1.9(0.18)	0.0(0.91)	0.3(0.61)	0.1(0.79)	1.0(0.31)

Table 5: Correlation matrix (Pearson) between total glomalin and easily extractable glomalin and chemical soil properties in the layers 0-15 and 15-30 cm. Soil (W) = whole soil, TM = total macro-aggregates, Mi = free micro-aggregates, mM – micro-aggregates within macro-aggregates, SC – silt and clay. ** - correlation is significant at $P < 0.01$; and *- correlation is significant at $0.01 < P$ 0.05.

			----------Total glomalin (mg/g)----------					----------Easily extractable glomalin (mg/g) ---------			
			Soil	TM	Mi	mM	SC	Soil	TM	Mi	mM
Site	Depth		W	(≥250 µm)	(53-250 µm)	(53-250 µm)	(≤53 µm)	W	(≥250 µm)	(53-250 µm)	(53-250 µm)
Kabete	0-15cm	Ca	**-.344***	-0.075	-0.218	-0.143	0.031	-0.115	**-.367****	0.089	**.430****
(n=54)		CEC	**-.351****	0.033	-0.238	-0.208	0.005	-0.044	**-.372****	0.063	**.407****
		K	**-.290***	0.03	-0.098	-0.097	**.284***	-0.005	-0.155	0.147	**.594****
		Mg	**-.281***	0.123	**-.284***	-0.104	**-.372****	-0.052	**-.452****	0.036	-0.052
		P(O)	-0.248	-0.044	-0.104	-0.083	**.290***	-0.039	-0.147	0.09	**.587****
		pH	**.319***	-0.052	0.257	0.26	0.108	0.011	**.375****	0.008	-0.262
		C	0.092	-0.084	-0.054	-0.034	0.215	0.041	0.251	0.185	**.610****
		N	-0.06	0.003	-0.108	-0.029	0.095	0.142	**.281***	0.132	**.614****
	15-30cm	Ca	0.101	-0.034	0.212	0.235	**.269***	0.191	**.275***	0.242	**.327***
		CEC	**-.338***	0.019	0.016	0.141	-0.133	0.111	0.235	0.223	0.153
		K	**.305***	-0.151	.279*	0.164	**.381****	0.247	0.23	**.362****	**.421****
		Mg	**-.428****	0.137	-0.079	0.096	-0.267	0.049	0.179	0.06	0.003
		P(O)	**.489****	-0.176	0.131	0.016	**.415****	0.043	0.033	0.137	**.356****
		pH	**.426****	-0.045	0.121	-0.04	**.289***	-0.001	-0.104	-0.092	0.006
		C	**.351****	-0.089	0.189	0.186	**.383****	0.113	0.153	0.128	**.339***
		N	**.348****	-0.008	0.08	0.197	**.323***	0.134	0.081	0.033	**.274***
Nyabeda	0-15	Ca	0.219	-0.069	-0.12	0.079	0.187	0.135	0.103	0.021	0.117
(n=48)		CEC	-0.111	0.101	0.129	0.01	-0.037	0.151	-0.159	-0.042	-0.104
		K	**.376****	-0.16	-0.19	0.114	**.363***	-0.013	0.197	0.132	**.297***
		Mg	**-.310***	0.185	0.204	-0.088	**-.295***	0.147	-0.217	-0.132	-0.274
		P(O)	**.399****	-0.11	-0.109	0.177	**.371****	0.002	0.121	0.141	0.261
		pH	**.400****	-0.221	-0.252	0.101	**.336***	-0.11	0.27	0.106	**.295***
		C	**.344***	0.172	0.027	**.311***	0.152	0.112	-0.115	0.078	0.014
		N	**.399****	0.215	-0.063	**.307***	0.096	0.132	-0.052	0.158	0.015
	15-30	Ca	**.571****	-0.113	-0.258	**.344***	**.358***	0.253	**.341***	**.313***	**.490****
		CEC	**.535****	-0.082	-0.199	**.323***	**.328***	0.237	**.330***	**.290***	**.439****
		K	**.613****	-0.047	-0.226	0.274	**.290***	0.232	0.276	**.305***	**.458****
		Mg	**.380****	-0.041	-0.131	0.231	0.235	0.143	**.386****	0.241	.329*
		P(O)	**.552****	0.008	-0.16	0.26	0.256	0.258	0.229	0.212	**.433****
		pH	-0.100	-0.091	-0.106	-0.143	-0.058	-0.032	-0.185	-0.061	-0.054
		C	**.562****	0.053	-0.143	**.292***	0.259	0.271	0.274	**.295***	**.450****
		N	**.446****	0.234	-0.016	0.11	0.125	0.186	0.135	0.202	**.328***

Table 6: Correlation matrix (Pearson) between total glomalin and easily extractable glomalin and mycorrhizal parameters. Abundance = spore number g^{-1} soil; LR = long rainy season, SR = short rainy season; H1-H6 = sampling dates, H1 = May 2008, H2 = July, H3 = August, H4 = November, H5 = December, H6 = January 209. For other abbreviations see Table 5

		----------Total glomalin (mg/g)----------					----------Easily extractable glomalin (mg/g) ---------			
		Soil	TM	Mi	mM	SC	Soil	TM	Mi	mM
		W	(≥250 µm)	(53-250 µm)	(53-250 µm)	(≤53 µm)	W	(≥250 µm)	(53-250 µm)	(53-250 µm)
AMF parameters										
Kabete	Abundance	**.282***	0.081	0.202	0.256	**.486****	-0.002	**.273***	**.427****	0.202
	Richness	0.153	0.036	0.156	0.177	0.261	-0.007	0.139	0.21	0.145
	H' index	0.123	0.043	0.203	0.155	**.445****	0.031	0.201	**.337***	0.181
LR	H1	0.244	0.048	0.117	0.011	0.027	0.034	-0.157	0.041	0.208
	H3	0.063	0.144	-0.173	-0.016	-0.219	0.107	-0.223	-0.051	0.139
SR	H4	0.058	0.135	-0.111	0.005	0.16	0.046	0.169	0.112	-0.011
	H5	0.018	0.112	-0.161	-0.089	-0.163	-0.054	-0.147	0.069	0.117
	H6	0.11	0.037	0.055	-0.136	-0.01	-0.004	0.066	-0.066	0.088
Nyabeda	Abundance	**.288****	**-.324****	-0.18	**.269****	-0.157	**.306****	0.014	**.323****	**.395****
	Richness	**.309****	-0.068	-0.13	0.087	-0.016	**.237***	.250*	**.228***	**.298****
	Diversity	-0.05	0.042	-0.068	-0.159	0.072	-0.044	.214*	-0.116	-0.066
LR	H1	-0.002	**.324****	0.183	0.082	**.311****	-0.092	**.368****	**.219***	0.019
	H2	-0.127	0.046	0.152	-0.033	0.112	-0.027	**.211***	-0.03	0.073
	H3	-0.071	-0.076	0.139	-0.138	-0.129	**.208***	-0.085	-0.024	-0.167
SR	H4	0.05	0.073	0.148	0.06	0.065	-0.064	-0.046	-0.058	0.052
	H5	0.108	0.009	0.038	0.074	**.271****	-0.126	-0.072	-0.026	-0.024
	H6	0.171	**.257***	0.09	0.133	0.113	0.157	0.073	0.04	**.236***

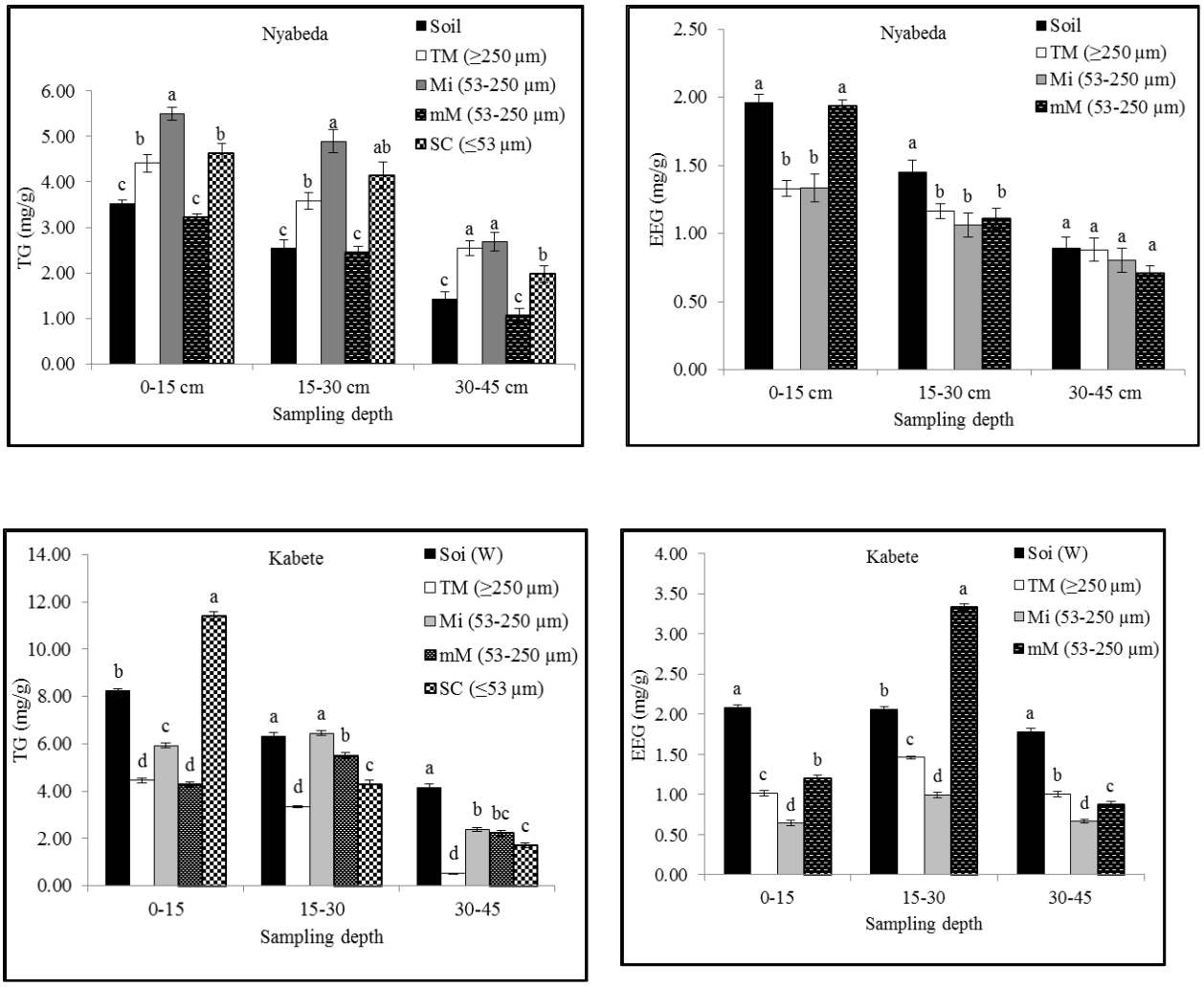

Figure 1: Distribution of total and easily extractable glomalin (TG and EEG) in whole soil and aggregate size fractions across three sampling depths (0-15cm, 15-30cm and 30-45 cm) in Nyabeda and Kabete; Bars within each sampling depth that have different letters are significantly different ($P < 0.05$). Error bars are standard errors. Soil (W) = whole soil, TM = total macro-aggregates, Mi = free micro-aggregates, mM = micro-aggregates within macro-aggregates, SC = silt and clay.

CHAPTER SIX

Impact of Arbuscular Mycorrhizal Fungi and earthworms on aggregate stability

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Abstract

Earthworms and arbuscular mycorrhizal fungi (AMF) play important roles in modifying soil physical and chemical properties. Both may enhance soil aggregation, which in turn may affect nutrient and water use efficiency by crops. However, little is known about their single and interactive effects on water-stable aggregation, crop nutrition and plant growth. A greenhouse experiment was run for 9.5 months to investigate single effects of earthworm (*Pontoscolex corethrurus* (endogeic) and *Dichogaster bolaui* (epigeic) and AMF species (*Glomus etunicatum* and *Scutellospora verrucosa*), and earthworm-AMF interactions on soil aggregate stability and growth and N and P uptake of maize (*Zea mays*) and pigeonpea (*Cajanus cajan*). The study used a humic nitisol, with grass residues placed on top. Crop height was measured weekly. At harvest, water-stable macro-aggregates ($WSA_{>250\mu m}$) and micro-aggregates ($WSA_{<250\mu m}$), AMF colonization, extraradical hyphal length, glomalin levels in various aggregates, and plant biomass, and P and N uptake were assessed. Crop and earthworm, but not AMF, were the most important factors influencing soil aggregation and glomalin pools in various aggregate size fractions. *Dichogaster* improved soil aggregation by increasing levels of large macro-aggregates by 32% and reducing micro-aggregates by 19%. *Pontoscolex* had no effect. *Dichogaster* also increased glomalin pools in stable aggregates and improved biomass and nutrient uptake by 50% in pigeonpea. There was a significant crop x AMF interaction on soil aggregation and glomalin. Interactions between AMF and earthworm were also observed on nutrient (P, N) uptake and biomass, but not on soil aggregation. This study highlights the importance of crops, soil macro-fauna (*Dichogaster)* and AMF on soil aggregation, crop nutrition and crop productivity.

Key Words: *arbuscular mycorrhizal fungi, endogeic, epigeic, phosphorus, nitrogen, water-stable aggregates, glomalin*

1. Introduction

Integrated Soil Fertility Management (ISFM) which entails the combined use of organic amendments and inorganic fertilizers to maintain soil fertility and improve nutrient use efficiency has been proposed as a means to restore soil fertility and soil biodiversity in the tropics and sub-tropics (Vanlauwe et al. 2010). However, the success of ISFM in terms of increased soil fertility and nutrient use efficiency depends on its effectiveness in improving chemical, physical and biological soil quality. Soil biota contribute to the maintenance and productivity of agro-ecosystems by regulating nutrient cycling and improving the soil structure (Giller et al. 1997; Wardle 2002; Kuyper and Giller 2011). In particular, earthworms and AMF are soil biota known to influence soil physical and chemical properties (Milleret et al. 2009a, b).

AMF usually form mutualistic symbioses with the majority of plant species, including most crops and can account for >50% of the soil microbial biomass (Olsson et al. 1999; Plenchette et al. 2005). AMF can enhance the uptake of P, Zn, Cu, N, and K by extending the external hyphae (MEH) from the root surface to the soil beyond the P depletion zone to a greater volume of soil than the root alone (Smith and Read 2007). AMF also influence soil structure by binding and enmeshing soil particles into larger aggregates (Rillig et al. 2002; Rillig and Mummey 2006; Treseder and Turner, 2007). On the other hand, AMF play a role in mediating water uptake and enhancing water use efficiency, especially during drought stress (Augé 2004; Birhane et al. 2012). Differential functioning of AMF depends on the AMF species. Members of the Gigasporaceae are slower root colonizers but better soil colonizers, producing denser extra radical mycelium than members of the Glomeraceae (Hart and Reader 2002a). The latter factor could imply that Gigasporaceae are more important in soil structure formation and maintenance than Glomeraceae. However, they also seem to be less efficient in transferring P to the host plant (Dodd et al. 2000; Hart & Reader 2002b) compared to *Glomaraceae*.

Earthworms play a major role in the build-up and maintenance of soil structure through burrowing and cast formation. Earthworms are known to ingest organic matter together with mineral soil particles passing this mixture through their gut and excreting organo-mineral excrements (casts) that form micro- and macro-aggregates (Brown et al. 2000; Six et al. 2004). The contribution of earthworms to soil structure however varies with their ecological categories. Endogeic earthworms live in the upper layer of the mineral soil and feed on soil enriched with organic matter. They make horizontal burrows and are considered major agents of aggregation and soil organic matter stabilization, compared to epigeic earthworms which live in the organic layer at the soil surface and rarely make burrows (Lavelle and Spain 2001). Earthworms also change the spatio-temporal availability of P, N and C through nutrient mineralization resulting to

improved plant growth (Scheu 2003; Li et al 2012b).

Earthworms may also influence the activity of AMF by selective feeding on spores and hyphae, thus damaging the fungal network and reducing mycorrhizal effectiveness (Pattinson et al. 1997). Nevertheless, earthworms can also foster AMF dispersal through ingesting spores and hyphal fragments without digesting them (Reddell and Spain 1991; Gange 1993; Lee et al. 1996), and concentrating them in their faecal material (Reddell and Spain 1991; Harinikumar and Bagyaraj 1994; Lee et al. 1996). A consequence of earthworm grazing on AMF is hypothesized that macro- and micro-aggregate stability in casts is (co-) determined by the presence of AMF hyphae and glomalin. AMF-earthworm interaction may also influence nutrient uptake and plant performance, but the results this far are contradictory varying from an increased plant nutrient uptake and productivity (Li et al 2012a; Li et al 2012b; Ma et al 2006; Yu et al 2005) to no interactive effects (Milleret et al 2009a). The interaction between AMF and earthworms in enhancing soil structure and crop nutrition may thus depend on AMF × earthworm species combinations.

While studies on the interactions between AMF and earthworms are increasing, very few studies have investigated interactive effects of AMF and earthworms on soil aggregation (Milleret et al. 2009a, b). Information on the combined impact of AMF and earthworms on glomalin pools is equally scanty. The aim of this study was to examine the (single and interactive) effects of two earthworms (*Pontoscolex corethrurus* Müller 1857 – endogeic; *Dichogaster bolaui,* Michaelsen, 1891 - epigeic) and two AMF (Glomaraceae – *Glomus etunicatum* W.N. Becker & Gerd. Gigasporaceae – *Scutellospora verrucosa* C. Walker & F.E. Sanders) species on aggregate stability, glomalin pools and crop performance. We used two major tropical crops (Maize; *Zea mays* L. and pigeon pea; *Cajanus cajan* (L.) Mills p.) that respond positively to AMF. We hypothesized that there is an interaction between earthworms and AMF in soil aggregation, and that the combination of *Pontoscolex* and *Scutellospora* would contribute most to soil aggregation. We predicted that *Scutellospora* produces denser and more extraradical mycelium than *Glomus*, implying that it is more important in soil structure formation and maintenance. The endogeic *Pontoscolex* is expected to contribute more to soil structure than the epigeic *Dichogaster*.

2. Materials and Methods

2.1. Soil and earthworm collection

Soil used in this study was collected at the National Agricultural Research Laboratories (NARL) of the Kenya Agricultural Research Institute (KARI) situated at Kabete (1°15′S; 36°41′E), 7 km NW of Nairobi. The soil, a humic nitisol (FAO 1990), was collected from the upper 30 cm soil

layer. The soil was mixed with sand (ratio 1:1) to improve water drainage and passed through a 0.5 cm sieve to remove large soil and sand particles. The main characteristics of this soil after mixing with sand (ratio 1:1) were 14.4 g kg-1 soil carbon, 1.1 g kg-1 total N; pH 5.09, 36 ppm P, 1.96 cmol kg^{-1} K, 11.8 cmol kg^{-1} Ca, 2.09 cmol kg^{-1} Mg, 41.5% sand, 26.8% clay, and 31.6% silt. AMF inoculum (*Glomus etunicatum* and *Scutellospora verrucosa*) was obtained from the Kenya Forestry Research Institute (KEFRI) at Muguga, Kenya. Earthworms were collected using hand-sorting (Anderson and Ingram 1993). *P. corethrurus* was collected from a maize field near KARI - Embu (0°30'N; 37°27'E) while *D. bolaui* was collected from a maize field in Kabete (near KARI Kabete, 36°41′E; 1°15′S). *Pontoscolex* is an introduced species now dominant in this region, while *Dichogaster* is a common indigenous species (Ayuke et al. 2011). Earthworms of similar size (young adults) placed in containers filled with moistened soil and stored at room temperature before the inoculation.

2.2. Greenhouse experiment

A three-factorial greenhouse experiment was conducted at the National Museums of Kenya Greenhouse facilities in Nairobi from December, 2009 to September, 2010. The temperature in the greenhouse ranged from 25 to 30° C. The experiment contained three factors: (1) earthworm (none, *Pontoscolex*, *Dichogaster*), (2) AMF (none, *Glomus*, *Scutellospora*), and (3) crop (maize, pigeon pea) in a complete randomized design with four replicates (Table 1). A mixture of soil and sand (ratio 1:1) was sterilized in an autoclave for 1 hr at 121° C. Seventy two pots (30 cm diameter, 45 cm depth) were filled with 12 kg sterilized soil each. In the treatments with AMF inoculation, 50g of AMF inoculum was added to the surface of each bucket. Pots that were not inoculated received a similar amount of steam-sterilized inoculum. In addition, all pots received 40 ml of a microbial wash to ensure similar microbiota. This was prepared from 500g fresh soil from the field site. The soil (500g) was dispensed in 1.5 l de-ionized water and filtered through a 25µm mesh for eliminating AMF spores (Schroeder and Janos 2004). The soil was then allowed to stand for 14 days for soil micro-organisms to get re-established. Each of the earthworm treatments received 24 sub-adults earthworms (equivalent to 440 worms m^{-2}). To prevent earthworms escaping, the pots were covered by a cloth net, which was removed after two weeks not to interfere with plant growth. Rhodes grass (*Chloris gayana* Kunth) mulch (150g per pot, equivalent of 20 t ha^{-1} , C:N ratio of 24) was added to all pots to protect earthworms from heat and as source of food. It also prevented the earthworm from crawling out of the pots. Two pre-germinated seeds of maize and pigeon pea were then planted per pot. The pigeon pea and maize were watered as required (300ml per day in all pots). Maize was grown in three consecutive

periods (1st cycle: December 2009 – February 2010; 2nd cycle: March – May 2010 and 3rd cycle: June – August 2010) while pigeonpea was grown in two consecutive periods (1st cycle: December 2009 –February 2010 and 2nd cycle: March-August 2010). The experiment lasted 38 weeks (9.5 months).

2.3. Data collection

At the end of each cycle crop shoots (maize and pigeonpea) were removed and oven-dried at 70° C) and weighed. Roots were only removed at the end of experiment (38.5 weeks). Soil adhering to roots was carefully washed using tap water, and total fresh weight determined. From each pot, a sub-sample of approximately 2 g of fresh roots was removed and cut into 1-cm segments for subsequent AMF assessment. The remaining part of roots was also oven-dried (at 70º C) for dry weight determination. The ratio of fresh to dry weight of roots was determined and total root dry weight calculated. The dried shoots were ground and analyzed for P and N. N and P were determined after wet digestion colometrically using a spectrophotometer. Numbers of earthworm that survived during the study period were also enumerated at the end of the experiment. In total 15±3 individuals of *Pontoscolex* were recovered in pots with maize and 17±4 individuals in pots with pigeon pea out of the 24 earthworms initially added to each pot. For *Dichogaster* 17±5 individuals were found in pots with maize and 15±3 in pots with pigeon pea. This indicates that the majority of the earthworms survived the experimental setup. We also collected juveniles and eggs of *Pontoscolex* suggesting that the experimental conditions were conducive for normal earthworm activity.

2.4. Root staining and assessment for AMF colonization

Root colonization was assessed once at the end of the experiment. A sub-sample of roots was stained using the modified procedure of Mason and Ingleby (1998). Roots were cleared in 2.5% KOH for 15 min at 121° C and later bleached in a mixture of 30% H_2O_2 and 30% ammonium solution (1:1 v:v) for 30 min to remove phenolic compounds. The roots were then acidified for 2 h with 1% HCl and stained with 0.05% acidified Trypan blue dissolved in glycerol – water (1:1 v:v) for 3 min at 121° C. Estimation of AMF colonization was done according to Trouvelot et al. (1986). Thirty root fragments were mounted on two slides each containing 15 root fragments. The fragments were observed under the microscope (magnification 160 - 400×) for the presence of hyphae, arbuscules and vesicles. Mycocalc program (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html) was used to calculate fractional root colonization.

2.5. Extraction of AMF hyphae from soil

AMF hyphal length was assessed once after the end of the experiment. Hyphae were extracted from a 10 g soil sub-sample by an aqueous extraction and membrane filter technique following Jakobsen et al. (1992). Soil samples were mixed and suspended in 100 ml of deionized water, to which 12 ml of a sodium hexametaphosphate solution was added. The soil suspension was shaken for 30 s (end-over-end), left on the bench for around 30 min, and then decanted through a 45 µm sieve to retain hyphae, roots and organic matter. Material on the sieve was sprayed gently with deionized water to remove clay particles, and then transferred into a 250 ml flask with 200 ml of deionized water. The flask was shaken vigorously by hand for 5 s, left on the bench for 1 min, and then a 2 µl aliquot was taken and pipetted onto a 25 mm diameter Millipore filter (25 µm pore size). The material on the filter was stained with 0.05% Trypan Blue and transferred to microscope slides. Hyphal length was measured with a grid-line intersect method at 100x magnification.

2.6. Assessment of water-stable micro- and macro-aggregates

The separation of aggregates into separate size classes of water-stable-aggregates (WSA) was carried out using the wet-sieving method described by Elliott (1986). A subsample of 80g was spread evenly onto a 2000 µm sieve, immersed in distilled water, and left for 5 minutes before starting the sieving process. Then, aggregates were separated by moving the 2000 µm sieve up and down by about 3 cm with 50 repetitions in 2 minutes. The aggregates >2000 µm were collected as large macro-aggregates (LMa) and the same sieving procedure was repeated for the 2000-250 µm fraction with the 250 µm sieve to give small macro-aggregates (SMa). Then, the fraction 53-250µm was obtained by sieving with 53 µm sieves as free micro-aggregates (Mi). The aggregates remaining on top of each sieve were backwashed into labeled and pre-weighed containers and oven-dried at 60° C overnight before final weight was measured. Soil material that passed through 53 µm was determined by taking a 300 ml sub-sample from the supernatant water of the whole volume after thoroughly shaking the suspension, and dried in the same way as the other fractions. The weights were then corrected for the size of the sub-sample as compared to the whole volume and the fractions were recorded as free silt and clay (SC). The different soil fractions except silt and clay were corrected for sand. The amount of sand in various aggregates was isolated using sodium hexametaphosphate, and later wet-sieved as above. The proportion of sand (250-2000µm) in various aggregates was 0.03 in large macro-aggregates, 0.40 in small macro-aggregates and 0.07 in micro-aggregates.

2.7. Assessment of glomalin

Glomalin extraction from water-stable macro-aggregates (>250µm) and micro-aggregates (53-250 µm) was carried out as described by Wright and Upadhyaya (1998). Briefly, easily-extractable glomalin (EEG) was extracted with 20 mm citrate, pH 7.0 at 121^{o} C for 30 min. Total glomalin (TG) was extracted with 50 mm citrate, pH 8.0 at 121^{o} C in rounds of 60 min each. For the sequential extractions, the supernatant was removed by centrifugation at 5000xg for 20 min. Extraction of a sample was done till the supernatant showed none of the red-brown color typical of glomalin, and glomalin was determined by Bradford assay (Wright and Upadhyaya 1996, 1998). To account for differences in amount of sand (with which there is no glomalin associated), the following correction factor was applied:

$$\text{Sand free Glomalin} = \frac{\text{Glomalin in soil size fraction}}{\text{1-(Sand proportion , 250-2000µm) fractions}}$$

2.8. Data analysis

A three-way analysis of variance (ANOVA) was used to test for significant sources of variation in aggregate size distribution, glomalin content in aggregate fractions and AMF hyphal length. AMF hyphal length was analyzed only for those treatments inoculated with AMF. Two-way ANOVA was applied for pigeon pea and maize separately for shoot and root biomass, and for N and P uptake. Post-hoc analysis was performed whenever a significant *p*-value (at $P < 0.05$) was observed using Fisher's LSD (Least Significant Difference). The relationship between mycorrhizal parameters, soil structure parameters (water-stable micro- and macro-aggregates) and glomalin pools were tested by Pearson correlation analyses. All statistical analyses were performed using GENSTAT 14TH Edition software (VSN international) except for Pearson correlation analyses which was carried out by SPSS (PASW Statistics 19).

3. Results

3.1. Extraradical hyphal length and root colonization of AMF

There were no mycorrhizal structures observed in roots and no aseptate hyphae were observed in pots without mycorrhizal inoculum. Extraradical hyphal length (MEH) at the end of the experiment was significantly affected by AMF and earthworm, but not by crop and the interaction of the three factors ($p>0.05$, Fig. 1). Hyphal length was significantly higher in pots with *Scutellospora* (4.98 mg^{-1} soil) than in treatments with *Glomus* (4.08 mg^{-1} soil). *Pontoscolex* declined hyphal length by 35% as compared to controls whereas *Dichogaster* had no significant

effect (Fig. 1). Maize and pigeonpea root colonization was also affected by earthworm ($p<0.001$), but was unaffected by AMF and their interaction. In the presence of *Pontoscolex* declined root colonization by 56.5% in maize and 72.1% in pigeon pea as compared to GE and SV treatments (Fig. 1).

3.2. Water-stable aggregates

The recovery rate of water-stable aggregates ranged between 98-100% indicating minimal losses of aggregates during wet sieving. Water stable macro-aggregates (TM, >250µm), micro-aggregates (Mi, 53-250µm) and silt and clay (SC, <53µm) were affected by crop, earthworm and crop x AMF interactions ($p<0.05$) but were unaffected by AMF (Table 2). Only SC was affected by crop x earthworm interactions ($p<0.05$). *Dichogaster* increased levels of TM by 9% and declined levels of Mi by 19% when compared to control (Fig. 2a, b, c). The levels of TM were also slightly higher in *Pontoscolex* but not significantly different from control (Fig. 2a). *Dichogaster* also had lower levels of SC in pigeonpea than in maize. The EPSV and EPGE treatments also declined levels of Mi by 22% when compared to controls (Fig. 2c). On the other hand, *Scutellospora* had higher levels of TM in pigeonpea (47%) than in maize (43%) and lower levels of Mi and SC in pigeonpea (20% vs. 3%) than in maize (20% vs. 4%, Fig. 2a, b, c).

3.3. Total glomalin (TG) in water stable aggregates

The Mi-fraction TG levels was 3-4 times higher than the TM-fraction TG (Fig. 3a, b). The Mi-fraction TG was significantly affected by crop, earthworm and AMF × earthworm interaction but was unaffected by AMF whereas the TM-fraction TG was affected by crop × earthworm interactions, AMF species × earthworm interaction and plant × earthworm × AMF interaction, but was unaffected by crop, AMF and earthworm (Table 2). Pigeonpea recorded higher levels of Mi-fraction and TM fraction TG than maize (Fig. 3b). The Mi-fraction TG was also higher in EPSV and EPGE treatments than in ENSV ($p<0.05$; Figure 3b). The TM-fraction TG in pigeonpea was higher in EPSV than in EPGE treatments. In maize the TM-fraction TG was higher in ENGE treatments when compared to treatments with EPGE and EPSV (Fig. 3a).

3.4. Easily extractable glomalin (EEG) in aggregates

The Mi-fraction EEG levels was also 2 times higher than TM-fraction EEG (Fig. 3c, d). The Mi-fraction EEG was affected by crop, earthworm and the interaction of the three factors whereas in TM-faction EEG was significantly affected by earthworm and interaction of the three factors

($p<0.05$, Table 2). The TM-fraction EEG were only increased by EPSV above control in maize whereas in pigeonpea the TM-faction EEG was higher in *Scutellospora* treatments compared to control (Fig. 3c). All other treatments had no significant effect on TG in TMa in both maize and pigeonpea (Fig. 3c). All treatments increased the Mi-fraction EEG above control in pigeonpea. The ENSV treatments had the highest EEG levels (Fig. 3d). In maize, the Mi-fraction EEG was only increased by *Dichogaster* above control was not significantly different among the treatments (Fig. 3d).

3.5. Crop performance (biomass, growth)

Earthworm and AMF x earthworm interactions affected pigeonpea root and shoot biomass during the two harvests (Table 3). *Dichogaster* increased biomass, which was further enhanced by *Scutellospora* (EPSV) treatments ($p<0.05$, Table 3). *Pontoscolex* as well as ENSV and ENGE treatments had no effect on biomass compared to control treatments (Table 3). AMF only affected shoot biomass in pigeonpea. *Scutellospora* increased shoot biomass above control, but *Glomus* had no significant effect (Table 3). In maize, AMF and AMF x earthworm interactions affected shoot biomass in all the three harvest (February, June and August, Table 3). *Scutellospora* and *Glomus* as well as EPSV and ENSV treatments increased shoot biomass above control in February, but declined both biomass in the subsequent harvestings. Only *Pontoscolex* and EPGE treatment increased shoot biomass in August. Growth (height) followed similar trends as biomass and strongly correlated with biomass hence not presented.

3.6 Phosphorus and nitrogen content

a) Pigeonpea: Total P concentration was significantly affected by AMF x earthworm interaction ($p<0.05$) during the two harvest. This was expressed as a lower P concentration in *Dichogaster* treatments as well as ENSV, ENGE, EPSV and EPGE treatments compared to control in the initial harvest in February (Table 3b). In subsequent harvesting in August, treatments with *Glomus* and EPSV increased P concentrations by 24% above control while ENSV treatments declined P concentrations by 20% (Table 3b). Other treatments during the two harvestings had no significant effects. Total N concentration was also affected by AMF x earthworm interaction in February ($p<0.05$, Table 3b). N concentration was 8% higher in *Dichogaster*, EPSV, EPGE and ENGE than in control but was 30% lower in *Scutellospora*. *Glomus* and *Pontoscolex* had no effect on N concentrations. The P:N ratio was in average 7.3 and was affected by AMF x Earthworm interaction in February ($p<0.05$, Table 3b). This was expressed as higher P:N ratio in treatments with *Dichogaster* (10.3), ENSV (9.3), ENGE (11.3), EPSV (9.4) and EPGE (9.5) than in control

(6.8). Treatments with *Scutellospora*, *Glomus* and *Pontoscolex* had no effect on P:N ratio (Table 3b).

b) Maize: Total P concentration was affected by AMF, earthworm and AMF x Earthworm interaction in final harvest in August, but was only affected by AMF in the initial harvest in February ($p<0.05$, Table 3b). AMF (*Scutellospora*, *Glomus*) and earthworm (*Pontoscolex*, *Dichogaster*) treatments increased P concentration by 14% above the control treatments (Table 3b). EPGE and EPSV also increased P concentration above the control by 63% and 11% respectively but ENSV and ENGE treatments had no effect. Total N concentration was only affected by AMF in final harvest in August ($p=0.03$, Table 3b). The two AMF species declined N concentration from 15.6 g kg^{-1} biomass to 13.2 g kg^{-1} in *Scutellospora* treatments and 10 g kg^{-1} in *Glomus* treatments (Table 3b). P:N ratio in maize was in average 5.3 and was also affected by AMF x Earthworm interaction during final harvest in August. This was expressed as a lower P:N ratio in *Scutellospora* and *Glomus* treatments compared to control. ENSV, ENGE, EPSV and EPGE treatments also had a lower P:N ratio with EPSV (3.4) and EPGE (2.9) treatments recording the lowest. Dichogaster had no effect on P:N ratio. No significant effect was observed in N:P ratio, N and P concentrations in June harvest.

3.7. Correlations between parameters

There were significant correlations at the end of the experiment between aggregates, glomalin and external hyphal length (Table 4). Total glomalin (TG) in micro-aggregates correlated negatively with the fraction micro-aggregates ($r = -0.25$). Extraradical hyphal length was positively correlated with TG in macro-aggregates ($r = 0.38$) and negatively with EEG in micro-aggregates ($r = -0.37$). Pigeonpea biomass correlated positively with TG and EEG both for macro-aggregates and micro-aggregates, whereas no significant correlations were noted for maize at final harvest. Pigeonpea biomass (shoots and roots) correlated positively with extraradical hyphal length ($r = 0.72$ and 0.43 respectively), whereas the relationship between maize biomass at final harvest and extraradical hyphal length was not significant. Pigeonpea biomass also correlated significantly with mycorrhizal colonization ($r = 0.53$ and 0.52 respectively), and again there was no significant correlation between maize biomass and mycorrhizal colonization. Nitrogen and phosphorus content of pigeonpea followed pigeonpea biomass in being significantly correlated with some glomalin factions, and with extraradical hyphal length and fractional root colonization. For maize nutrient content was not significantly correlated with any of these parameters.

4. Discussion

Our results showed AMF-earthworm interaction on crop performance (growth and biomass) and nutrition (N, P uptake). Presence of *Dichogaster* and AMF improved both maize (first phase) and pigeonpea performance but presence of both *Pontoscolex* and AMF had no effect on pigeon pea and maize (2^{nd} and 3^{rd} phase) performance. Positive effects of *Dichogaster* and AMF on crop performance are in accordance to recent studies by Li et al. (2012b) who reported positive effects of the epigeic earthworm (*Eisenia fetida*) on maize shoot and root biomass. He attributed such effects to improved activities of urease, acid phosphatase, and cellulase and increased availability of N, P, and K contents. High tissue N concentrations in treatments *Dichogaster* suggest that *Dichogaster* improved availability of these nutrients in soils which resulted to improved crop performance. Earthworms are known to change the spatio-temporal availability of P, N and C through nutrient mineralization resulting to improved plant growth (Scheu 2003). *Dichogaster* thus affected nutrient availability through ingestion and decomposition of grass mulch placed on the surface. In addition, *Dichogaster* had no negative effects on AMF hyphal length and root colonisations (Fig. 1) since it forages within organic matter and forms little burrows (5-10 cm) within the soil (Sahu et al., 1988; Kale and Karmegam 2010). AMF in return forms prominent symbioses with roots which increases plant potential to mobilize mineralized nutrients in particular P (Smith and Read, 2007). AMF increased shoot P concentrations (Table 3) suggesting potential in mobilizing P uptake in our systems. Together, *Dichogaster* and AMF thus increased plant nutrient supply via very different but congruent mechanisms, and additively improved nutrient uptake and crop performance.

Our results further indicate positive effects of *Dichogaster* on formation of stable macro-aggregation. The changes though relatively small (on average 43% stable macro-aggregate in *Dichogaster* treatment in comparison to 47% in pots without earthworm), indicates the potential for strong effects under circumstances in which aggregate stability is low (e.g. highly disturbed soil). Such effects could be attributed to ingestion and incorporation of partly decomposed residues into their casts. Earthworms might have incorporated grass mulch placed on the soil surface into the aggregates resulting in enhanced aggregate stability. Sahu et al. (1988) reported *Dichogaster bolaui* as an epigeic earthworm foraging within organic residues with a vertical distribution of <10cm within the soil. It is therefore likely that *Dichogaster* ingested both crop residues placed on surface and soil within upper 10 cm soil layer resulting to improved soil aggregation. A decline in the levels of micro-aggregates and silt and clay content (Fig. 1c) indicates that some of the original micro-aggregates were bound together to form macro-aggregates.

In deed *Dichogaster* increased total glomalin and easily extractable glomalin in two soil size (macro- and micro-aggregate) fractions (Fig. 3c, d), suggesting that *Dichogaster* stimulated incorporation of residue-derived organic matter into the mineral soil reflected by increasing levels of occluded glomalin in stable aggregates. Although glomalin was considered a specific glycoprotein, exclusively produced by AMF (Treseder and Turner, 2007), it being recognized that several glycoproteins and humic materials are also co-extracted (Gillespie et al. 2011). Under such conditions, ingested and partly decomposed residues into casts could have intimately mixed with mineral soil in the process of biogenic aggregate formation contributing to the glomalin pool. Nie et al. (2007) measured substantial increases in glomalin levels after addition of rice straw, further supporting the claim that humified plant materials may end up in this operationally defined pool. Taken in this perspective, our results are thus in agreement with those by Bossuyt et al. (2006) and Giannopoulos et al. (2011), showing improved soil aggregation and stimulated incorporation of residue-derived organic matter in the aggregates where epigeic earthworms were added and residues placed on the surface. Our differences were however smaller compared to former study by Bossuyt et al. (2006) who reported larger proportion of fresh residue in both macro-aggregates and micro-aggregates within macro-aggregates in treatments with epigeic earthworms and residues on the surface. This could be due to the fact in our experiment we mixed soil and sand to improve soil drainage and did not crush original macro-aggregates. Nevertheless our results demonstrates potential of Epigeic earthworms in improving soil aggregation especially when crop residues are placed on the surface and the effect can be greater in soil with low aggregate stability such as disturbed soils.

Lack of positive effects of *Pontoscolex* (endogeic) and AMF on crop performance coincides with results from studies by Tuffen et al. (2002), Eisenhauer et al., (2009) and Milleret et al. (2009b). Our results were however in contrast to other studies showing positive effects of Endogeic earthworm-AMF interactions on crop performance and nutrition (Ma et al. 2006; Li et al. 2012a). A number of factors could explain these contradictory results. *Pontoscolex* negatively affected AMF functioning by declining root colonization and soil hyphal length through regular mechanical disruption of mycorrhizal network. Negative effects of Endogeic earthworms on AMF hyphal length is common (Pattinson et al. 1997; Tuffen et al., 2002; Ortiz-Ceballos et al. 2007) and are often associated with negative effects on AMF functioning (Tuffen et al., 2002). Tuffen et al. (2002) found that mechanical disruption of MEH by earthworm eliminated the effect of AMF on ^{32}P transfer between mycorrhizal plants. Though *Pontoscolex* tended to increase tissue N concentration, it negatively affected P concentrations in both maize and pigeonpea (Table 3) which suggests impaired AMF functioning. In addition, *Pontoscolex* produces a very compact

impermeable superficial layer in absence of food substrates (crop residues) which affects structural pore volume and infiltration (Blanchart et al. 2004). In our system, we observed (visual observation) excess water on the surface of the mesocosms with *Pontoscolex*. Increased water logging, poor aeration, increased bulk density and reduced AMF activity thus led to lack of positive effects of *Pontoscolex*+AMF on crop performance.

Changes in aggregate size distribution due to *Pontoscolex* were also very limited in our study system. Although *Pontoscolex* contributed to the glomalin pools, it only increased glomalin significantly in micro-aggregate. It's possible that *Pontoscolex* in our study systems had no access to the residues when left on the surface, and mainly fed on the soil organic matter available in the soil, thereby disrupting existing aggregates and probably making others. Under such conditions (absence crop residues and low organic matter 1.4%) *Pontoscolex* may not have contributed to the formation of stable macro-aggregates. Similar results were shown by Milleret et al. (2009a), who reported no significant effect of earthworms on stable aggregate size distribution in a greenhouse mesocosms using *Allolobophora chlorotica* and leek (*Allium porrum*). Milleret et al. (2009b) pointed out that in addition to burrowing habit, the effect of earthworms on soil compaction (compacting versus de-compacting species – see Blanchart et al. (2004) is important. Compacting species (like *Pontoscolex* and *A. chlorotica*) therefore negatively affected soil aggregation by decreasing structural pore volumes and disappearance of structural pore radii. Taken together, our results and those of other scholars (e.g. see Milleret et al. 2009b) are surprising, as it has previously been repeatedly shown that Endogeic earthworms increase water-stable aggregates (see review by Six et al. 2004). Our experiment was carried out in a greenhouse under controlled conditions where only one earthworm species was added in each pot, and no crop residues incorporated in the soil. Giannopoulos et al. (2010) found effect of the endogeic species *Aporrectodea caliginosa* on soil aggregation when residues were incorporated in the soil but not when placed on top. Our system was also one-species ecosystem. However, under natural (field) conditions, earthworm species richness is larger than one and species mixtures of various functional groups co-occur. Our result implies that positive effects of Endogeic earthworm (e.g. *Pontoscolex*) on soil aggregation and crop nutrition will thus depend on presence of food substrate.

Presence of AMF did not result in consistent differences among the various aggregate fractions, however our result show that AMF–host species combinations differentially controlled the percentage of water-stable soil aggregates. Our results are in line with other studies showing interaction between AMF with respect to soil aggregation (Piotrowski et al., 2004; Hallett et al., 2009; Rillig et al., 2002; Milleret et al., 2009b). Such differences could be associated with

differences in root exudates, root structure and distribution (rooting depth), plant cover, quality and quantity of carbon inputs, glomalin secretion from AMF, microbial communities and their activities and the enmeshing role of both roots and fungi (Rillig et al., 2002; Milleret et al., 2009b). Positive correlation between macro-aggregates and glomalin as well as MEH suggests their positive contribution to soil aggregation. Consequently, differences in glomalin levels between two plants can partly explain the observed differences on soil aggregation as affected by the two plants. Although crop species had no significant effect on MEH length, it is possible that root exudates, root and MEH branching differed among the two plant species (Piotrowski et al. 2004). Further studies are however desirable to understand AMF-plant interaction on soil aggregation.

Our hypothesis regarding the interactive role of AMF and earthworm in increasing water stable aggregates was rejected under our study. We attribute this result partly to negative effect of earthworms, particularly *Pontoscolex* on AMF activity, evidenced by reduced AMF external mycelium and root colonization of both maize and pigeon pea in pots with *Pontoscolex* (Fig. 1). Although *Dichogaster* had no negative effect on AMF hyphal length and fractional root colonization, lack of a main mycorrhizal effect on soil aggregation and indirect effects of plants through roots may have influenced this observation. Lack of *Pontoscolex* effect on soil aggregation contrary to our expectation also influenced the observed result. Similar to AMF, there was significant plant × earthworm interaction (Table 2) on levels of SC suggesting that earthworm effect on soil aggregation may also be important with certain plants. *Dichogaster* tended to decline levels of SC fraction in pigeon pea but not in maize. *Scutellospora* recorded longer hyphal length than pots with *Glomus*, confirming earlier reports that *Scutellospora* is a better soil colonizer than *Glomus* (Hart and Reader 2002a). However, *Scutellospora* was not better in soil aggregation than *Glomus*. Plant species in addition to crop residue placement may have played an important role in influencing our results. Similar results were reported by Milleret et al (2009 a, b) using *Glomus intraradices* and *Allolobophora chlorotica* (endogeic). However, in order to conclusively reject possible interaction between AMF and earthworm on soil aggregation, we propose further studies taking into consideration residue placement and plant species effect to clearly demonstrate lack of such interactions.

Crop, AMF and earthworm were important factors explaining variation in the glomalin pool in the two aggregate fractions. *Dichogaster* contributed more to glomalin in AMF inoculated pigeon pea while *Pontoscolex* contributed more to AMF inoculated maize. Earthworm may influence glomalin pools in various ways. It can increase glomalin production and physical protection from decomposers through incorporation of plant residues in to aggregates and

improved plant growth and AMF functioning as well as increased aggregate stability. *Dichogaster* may have influenced both glomalin production and stabilization by increasing stable aggregates and improving AMF functioning. *Pontoscolex* influenced glomalin by disrupting existing aggregates and impairing plant growth and AMF functioning. Crop species may influence glomalin through differences in root systems, root exudates, and AMF dependency (Bird et al., 2000; Wright et al., 2000; Rillig et al. 2003). Our result thus suggests a plant-AMF-earthworm interaction, which can be tailored for enhanced soil organic matter stabilization.

Higher glomalin levels in micro-aggregate than macro-aggregate coincides with findings by Wright et al (2007) who found higher glomalin levels in micro-aggregates in disturbed soil. The soil used for experiment was collected in regular tilled soil and was further disturbed during potting and sterilization. Since macro-aggregates change with management (Six et al. 2000), this suggest that micro-aggregate are more stable in storing glomalin either in the slow or recalcitrant soil C fraction than macro-aggregates. In fact, micro-aggregate have been found to have high carbon levels (on weight mass) than macro-aggregate (Gulde et al. 2008, Green et al. 2005), indicating its role in glomalin stabilization.

Efficiency of AMF in enhancing crop performance declined with subsequent maize harvesting cropping relative to control (Table 3). This could be related to N deficiency in our experimental systems as evidenced by N: P ratios of 6-8 for pigeon pea and 4-6 for maize. Crop N: P ratios have been regularly used to assess the question whether N or P is the limiting factor. Güsewell (2004) reviewed the existing literature and concluded that at N: P ratios below 10 there is N-limitation and at ratios above 20 there is P-limitation. Because the mycorrhizal role in nutrient uptake is more important for poorly available nutrients such as P (where diffusion is the main mechanism for uptake) than for more readily available N (where mass flow is the main mechanism) it is not surprising that the mycorrhizal benefits were relatively low and declined with subsequent harvesting in maize.

Our experiment was allowed to run for 38 weeks to maximize the chance of recording significant changes in water-stable aggregates fractions and glomalin levels. Consequently, three harvests of maize and two harvest for pigeon pea were made in entire period of the experiment. While the above-ground materials were weighed and nutrients removed, the roots of the crops remained in the mesocosms. It is therefore likely that other factors such presence of dead maize roots affected nutrient dynamics for the following cycle of crops. Low quality organic residues (e.g. maize roots with C:N ratio of 64) is associated to N immobilization (Sakala et al., 2000). Johnson (2010) has shown impaired AMF functioning in N limited soil due to competition for C and N between plant and AMF. Li et al. (2012a) have shown impaired AMF functioning when soil

is N deficient. Hodge and Fitter (2010) has also shown competition for organic N between AMF and plant. In our experiment, AMF inoculated pots had low N concentrations which indicate competition between AMF and plant for N. Although we reported total soil N of 1.1 g kg^{-1} which may be seem adequate for crop nutrition, total soil N is often a weak indicator of soil N availability and in soils with high clay content (like our case), clay can strongly protect soil organic matter lowering N mineralization potential and availability (Vanlauwe et al. 2002). Similar observations were found in a humic nitisols soil in Kabete where N deficiencies were associated with declining crop production due to N-P imbalance despite high N fertilization (Janssen 2011; Muchane et al. unpublished). Since pigeonpea may partially increase N availability through biological N fixation and high N mineralization (C:N 12 ratio) of dead roots (Sakala et al., 2000), the N deficiency was therefore more in maize. In deed pigeonpea recorded slightly higher N: P ratio than in maize indicating improved N uptake under pigeon pea. Lack of significant correlations between levels of colonization of maize and biomass and uptake of N and P further indicate that AMF role was minimal under maize systems.

Experimental results obtained under controlled conditions though cannot be representative of field conditions, the microcosm approach may help us better understand cause–effect relationships between mycorrhizal symbiosis, earthworm and soil quality. Our finding highlights the importance of epigeic earthworm on soil aggregation, regulation of glomalin and crop performance. Earthworm densities in our mesocosms were 440 worms individuals m^{-2}. Ayuke et al. (2011) reported on a density of 75 - 395 worms in a humic nitisols soils under organic amendments which improved soil aggregation by 34% when compared to systems with no input. The result of our experiment may also reflect relatively similar processes occurring under field conditions. This may have greater implication in organic based systems as well as the no till systems where huge amounts of low quality organic residues (maize stover) are applied. Further, our study highlights the importance of organic amendments in enhancing earthworm activities. Moreover, this study suggests a plant-AMF-earthworm interaction on glomalin pools which can be tailored to enhance soil quality. This can be important under the context of Integrated Soil Fertility Management (ISFM) for enhancing nutrient availability and soil aggregation which in return results to improved crop performance. Future studies focusing on such interaction under field conditions would be interesting.

5. Conclusion

Our study highlights the role of crops, earthworms and AMF, and their interactions on soil aggregation. Plants and earthworm species appeared to have larger positive impacts than AMF on

the fraction of large macro-aggregates and levels of glomalin. Our initial hypothesis regarding the interactive role of an endogeic earthworm (*Pontoscolex*) and *Gigasporaceae* AMF species (*Scutellospora*) which form extensive soil network was rejected. In the presence of the earthworm mycorrhizal colonization and external hyphal length was reduced, which resulted in lower crop performance. Also, the absence of sufficient organic material in the soil (rather than at the soil surface) resulted in soil compaction and excess water, and this result may also have negatively impacted crop performance and hence carbon inputs to the soil. The epigeic earthworm (*Dichogaster*) contributed more to glomalin pools than the endogeic species, especially because of the superficial addition of grass mulch. Under such conditions the role of earthworms in increasing N uptake may have been more important than the role of AMF in enhancing P uptake. Further long-term experimental studies are required to underscore the role of AMF and earthworm communities on performance of a variety of different crops.

Table 1 Treatment structure of the greenhouse experiment. All treatments were applied to maize and pigeon pea, resulting in a total of 18 treatments.

	Earthworm	AMF	Treatment code
1	none	None	Control
2	none	*S. verrucosa*	SV
3	none	*G. etunicatum*	GE
4	*P. corethrurus* (Endogeic)	None	EN
5	*P. corethrurus* (Endogeic)	*S. verrucosa*	ENSV
6	*P. corethrurus* (Endogeic)	*G. etunicatum*	ENGE
7	*D. bolaui* (Epigeic)	None	EP
8	*D. bolaui* (Epigeic)	*S. verrucosa*	EPSV
9	*D. bolaui* (Epigeic)	*G. etunicatum*	EPGE

Table 2 Results of the 3-way ANOVA for the factors plant, arbuscular mycorrhiza fungi (AMF) and earthworm on water stable aggregates and total and easily extractable Bradford related soil protein (BRSP) in macro-aggregates and micro-aggregates. Water stable aggregate fractions comprised of total macro-aggregates (TM), micro-aggregates (MI) and silt and clay (SC). The table presents F-values, followed by p-values (between parentheses). Values in bold have a p-value below 0.05. The effect of block was not significant, hence not presented in the table.

	---------Soil aggregates in 100 g soil----------			----Bradford related soil protein (BRSP) in aggregates---			
	TM	MI	SC	--------Total Glomalin-----		Easily extractable glomalin	
Sources	(>250µm)	(53-250 µm)	(<53 µm)	>250 µm	(53-250 µm)	>250 µm	(53-250 µm)
Crop (A)	**10.59(0.00)**	**5.76(0.02)**	**27.97(0.00)**	2.84(0.10)	**198.75(0.00)**	2.17(0.15)	**84.10(0.00)**
AMF (B)	1.18(0.32)	1.36(0.27)	0.29(0.75)	1.24(0.30)	0.58(0.57)	3.16(0.06)	0.53(0.59)
Earthworm (C)	**12.21(0.00)**	**8.51(0.00)**	**11.20(0.00)**	2.54(0.09)	**27.65(0.00)**	**44.80(0.00)**	**11.47(0.00)**
A x B	**4.50(0.02)**	**3.87(0.03)**	**3.66(0.03)**	2.38(0.10)	2.29(0.11)	**9.40(0.00)**	**7.84(0.00)**
A x C	1.53(0.23)	0.50(0.61)	**5.65(0.01)**	**14.35(0.00)**	1.29(0.28)	**21.57(0.00)**	**6.83(0.00)**
B x C	3.00(0.06)	2.94(0.07)	2.07(0.10)	**8.15(0.00)**	**4.74(0.00)**	**8.92(0.00)**	**5.45(0.00)**
A x B x C	2.03(0.10)	1.93(0.12)	1.30(0.28)	**6.47(0.00)**	0.85(0.50)	**3.55(0.02)**	**9.22(0.00)**

Table 3 The effects of the factors arbuscular mycorrhiza fungi (AMF) and earthworm on dry shoot and root biomass in maize and pigeonpea grown in greenhouse conditions from December 2009 to August 2010. Treatment codes are presented in Table 1.

	Shoot biomass (g)					Root biomass	
	Maize			Pigeonpea			
	Feb	June	Aug	Feb	Aug	Maize	Pigeonpea
Control	4.2(0.2)cd	14.0(2.3)	25.0(5.1)b	3.8(0.3)	7.9(1.0)d	10.7(1.0)	6.1(1.2)cd
SV	9.5(1.2)a	6.8(0.4)	12.0(3.5)bc	11.0(1.1)	27.5(2.9)b	13.3(3.6)	7.1(1.3)c
GE	7.0(1.3)ab	5.2(0.9)	8.9(1.2)d	8.3(1.7)	27.6(2.6)b	8.3(1.4)	6.0(0.9)cd
EN	9.4(0.5)a	8.5(0.7)	35.8(1.4)a	2.0(0.1)	16.2(2.0)c	11.8(0.3)	3.1(0.2)e
ENSV	9.9(1.4)a	5.0(0.6)	6.9(1.8)d	3.3(0.9)	5.6(0.3)d	6.7(0.4)	4.9(1.00)de
ENGE	3.2(0.4)d	5.2(1.8)	17.8(3.6)bc	3.3(0.5)	6.6(1.3)d	11.1(2.9)	6.1(1.1)cd
EP	6.3(1.1)bc	10.1(1.8)	15.9(2.2)c	6.0(1.2)	39.9(7.2)a	10.2(2.3)	16.1(0.9)a
EPSV	8.0(1.2)ab	4.4(1.1)	9.6(1.9)dc	12.0(1.4)	38.7(3.2)a	7.1(1.2)	13.2(0.3)b
EPGE	5.7(0.6)cd	9.0(1.5)	29.2(3.0)ab	8.3(3.0)	29.8(4.3)b	10.0(0.7)	10.9(1.1)c
ANOVA							
AMF (A)	**11.6(0.00)**	**13.2(0.00)**	**23.3(0.00)**	**9.3(0.00)**	0.6(0.57)	0.7(0.50)	0.6(0.53)
Earthworm (B)	0.6(0.56)	2.3(0.12)	2.2(0.13)	**15. 8(0.00)**	**46.9(0.00)**	0.6(0.56)	**69.4(0.00)**
A x B	**5.8(0.00)**	2.6(0.06)	**11.5(0.00)**	1.5(0.24)	**8.1(0.00)**	2.1(0.12)	**4.8(0.01)**

Maize was grown in three consecutive phases; December-February (Feb), March to May (May) and June to August (Aug). Fresh: dry weight ratio was in average 4 in pigeon pea and 6 in maize. Different letters in each column, indicate significant differences between treatments at P≤0.05. SED is standard error of the difference.).

Table 4 The effects of the factors arbuscular mycorrhiza fungi (AMF) and earthworm on N and P concentrations (and N:P ratio) in maize and pigeonpea grown in greenhouse conditions from December 2009 to August 2010. Treatment codes are presented in Table 1.

	Maize (g kg^{-1} plant biomass)									Pigeon pea (g kg^{-1} plant biomass)					
	N			P			N:P			N		P		N:P	
Treatments	Feb	June	Aug	Feb	June	Aug	Feb	June	Aug	Feb	Aug	Feb	Aug	Feb	Aug
None	17.5(2.9)	15.8(2.3)	14.9(0.9)	3.0(0.5)	3.2(0.6)	2.7(0.2)c	6.2(1.2)	5.2(0.7)	5.6(0.4)a	22.9(0.7)b	21.0(1.4)	3.4(0.1)b	3.1(0.5)b	6.8(0.3)b	7.4(1.4)
SV	15.8(2.3)	15.4(1.8)	14.0(0.1)	4.0(0.3)	2.4(0.5)	3.1(0.1)b	3.9(0.5)	7.4(1.3)	4.5(0.3)c	19.3(2.3)b	20.1(2.2)	3.5(0.3)b	3.0(0.4)b	5.7(1.0)b	7.4(1.5)
GE	14.9(2.6)	12.3(2.3)	12.1(1.0)	3.4(0.6)	2.3(0.5)	3.1(0.1)b	4.8(1.2)	6.9(2.5)	4.0(0.4)c	16.0(0.9)c	23.6(2.6)	3.4(0.4)b	3.9(0.6)a	5.0(0.7)b	6.5(1.2)
EN	14.3(1.5)	18.4(4.4)	15.3(0.8)	3.0(0.3)	3.2(0.3)	3.2(0.1)b	4.9(0.8)	5.6(0.8)	4.8(0.4)b	21.0(1.4)b	21.0(5.2)	4.1(0.3)ab	3.6(0.3)ab	5.2(0.5)b	6.0(1.4)
ENSV	12.3(1.0)	21.0(4.0)	14.0(1.4)	3.5(0.2)	2.6(0.3)	2.8(0.1)c	3.5(0.1)	9.4(3.0)	5.0(0.6)b	22.8(1.0)b	16.6(1.7)	2.2(0.1)d	2.5(0.2)c	9.3(1.1)a	6.7(0.6)
ENGE	14.0(2.0)	18.4(2.2)	14.9(1.7)	3.1(0.4)	2.0(0.3)	2.9(0.1)c	4.6(0.2)	9.5(1.1)	5.1(0.4)b	25.0(1.5)a	21.4(1.5)	2.5(0.3)c	3.0(0.2)b	11.3(0.6)a	7.4(0.7)
EP	13.1(0.9)	14.0(2.5)	16.6(1.7)	3.1(0.1)	2.9(0.3)	3.1(0.1)b	4.3(0.3)	4.9(0.7)	5.4(0.5)a	24.1(1.5)a	17.5(2.9)	2.4(0.2)c	2.7(0.3)bc	10.3(1.2)a	6.5(0.4)
EPSV	13.5(1.5)	15.8(1.0)	10.5(2.0)	3.2(0.1)	2.9(0.2)	3.0(0.2)b	4.3(0.5)	5.5(0.6)	3.4(0.5)d	24.5(1.4)a	21.9(0.9)	2.7(0.2)c	3.8(0.3)a	9.4(0.4)a	5.8(0.4)
EPGE	12.4(0.8)	14.9(1.7)	12.3(1.0)	3.1(0.2)	2.8(0.3)	4.4(0.3)a	5.8(0.6)	5.5(0.8)	2.9(0.5)d	25.4(1.7)a	16.6(2.2)	2.6(0.1)c	3.0(0.3)b	9.5(1.0)a	5.8(1.0)
ANOVA															
AMF (B)	0.4(0.67)	0.6(0.56)	**4.0(0.03)**	**3.2(0.05)**	2.8(0.08)	**8.2(0.002)**	2.9(0.07)	2.0(0.15)	**7.0(0.003)**	0.1(0.88)	0.1(0.89)	**4.0(0.03)**	0.2(0.82)	1.5(0.23)	0.0(1.0)
Earthworm (B)	2.3(0.12)	2.9(0.07)	(1.1(0.34)	2.9(0.07)	0.4(0.65)	**10.1(0.001)**	0.6(0.54)	2.7(0.09)	**5.1(0.01)**	**10.4(0.00)**	1.0(0.380	**10.1(0.001)**	0.5(0.60)	**19.9(0.00)**	0.8(0.47)
A x B	0.28(0.90)	0.2(0.93)	1.5(0.21)	1.1(0.37)	0.6(0.63)	**8.8(0.00)**	1.3(0.29)	0.4(0.80)	**3.1(0.03)**	**3.8(0.01)**	1.3(0.29)	**7.3(0.00)**	**3.1(0.03)**	**7.5(0.00)**	0.4(0.83)

Table 5 Correlation matrix (Pearson) between selected variables measured during a greenhouse experiment with two crops (pigeonpea and Maize), two AMF (S. *verrucosa* and *G. etunicatum*) and two earthworm (Endogeic and Epigeic) between December, 2009 and August 2010. n=72 for the correlation between soil size fractions (aggregates) and glomalin pools and hyphal length while n=24 for relationship between AMF parameters (colonization hyphal length and glomalin pools) with dry biomass, soil size fractions and N and P nutrition. *: significant at $0.01 < P < 0.05$; **: significant at $P < 0.01$.

							AMF
		TG		EEG			Colonization
		TM (>250 µm)	Mi (53-250 µm)	TM (>250 µm)	Mi (53-250 µm)	EH	%M
Aggregates	SM (>250µm)	0.07	.27*	.35**	0.08	0.19	n/a
	Mi (53-250 µm)	-0.15	-.25*	-.37**	-0.02	-0.14	n/a
	SC (<53 µm)	-0.04	-.38**	-.45**	-0.18	-0.24	n/a
Biomass							
Pigeonpea	Shoot	0.59**	0.17	0.34*	0.08	0.72**	0.53**
	Root	0.30	0.20	0.43*	0.17	0.43*	0.52**
Maize	Shoot	-0.08	0.11	-0.14	-0.19	0.09	-0.07
	Root	0.02	0.05	-0.13	-0.36	0.02	-0.02
Nutrient uptake							
Pigeonpea	N	0.55**	0.10	0.32	0.28	0.67**	0.60**
	P	0.62**	0.12	0.27	0.23	0.77**	0.58**
Maize	N	-0.01	0.07	-0.16	-0.15	0.03	-0.06
	P	-0.11	0.22	-0.06	-0.17	0.03	-0.02
Hyphal	EH	0.38**	0.054	0.23	-0.370**	1	n/a

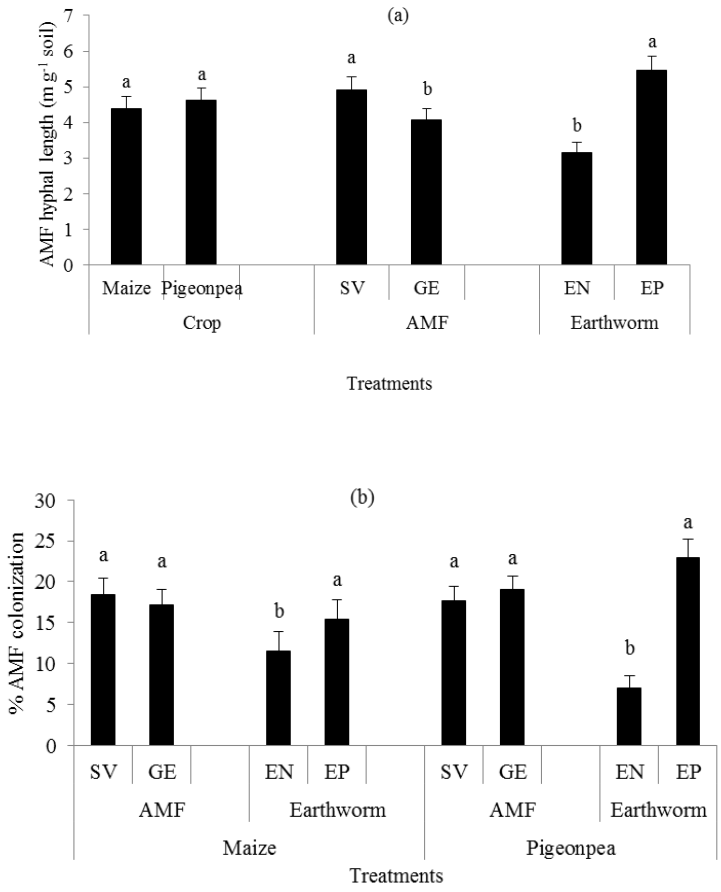


Fig. 1 Effect of crop, arbuscular mycorrhiza fungi (AMF) and earthworm species on AMF hyphal length (m/g soil. a) and percentage AMF root colonization (b) under greenhouse conditions during December, 2009 to August, 2010. Hyphal length was affected by AMF ($F=4.69$, $p=0.04$) and Earthworm ($F=12.92$, $p<0.001$) while root colonization was affected by earthworm ($p<0.001$) was significant. Treatment codes are presented in Table 1. Errors bars are standard error (SE). Bars with same letters per given factor are not significantly different at $p\leq0.05$.

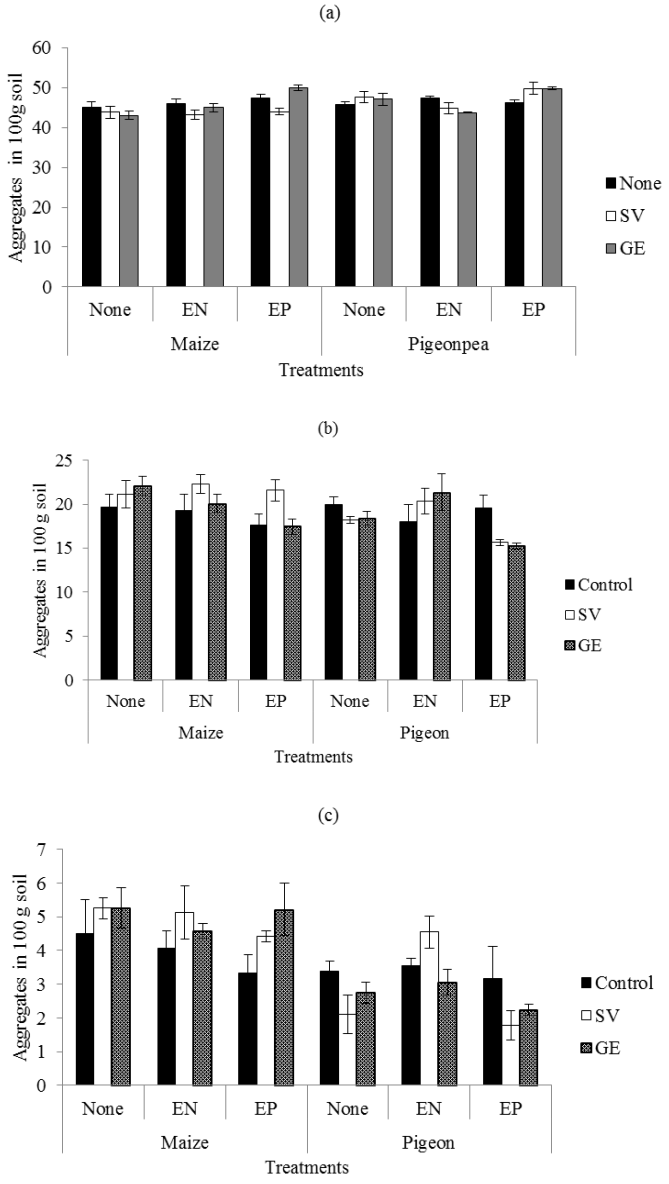


Fig. 2 Effect of arbuscular mycorrhiza fungi (AMF) and earthworm species on water stable aggregates. Water stable aggregates comprised of water stable macro-aggregates (>250µm, a), micro-aggregate (53-250µm, b) and silt and clay (<53µm, c) under maize and pigeonpea grown in greenhouse conditions during December, 2009 to August, 2010. Aggregates were affected by crop, earthworm, crop x AMF interactions and crop x earthworm interactions ($P<0.05$ in all cases, Table 2). Errors bars are standard error (SE). Bars with same letters are not significantly different at $P \leq 0.05$. Treatment codes are presented in Table 1.

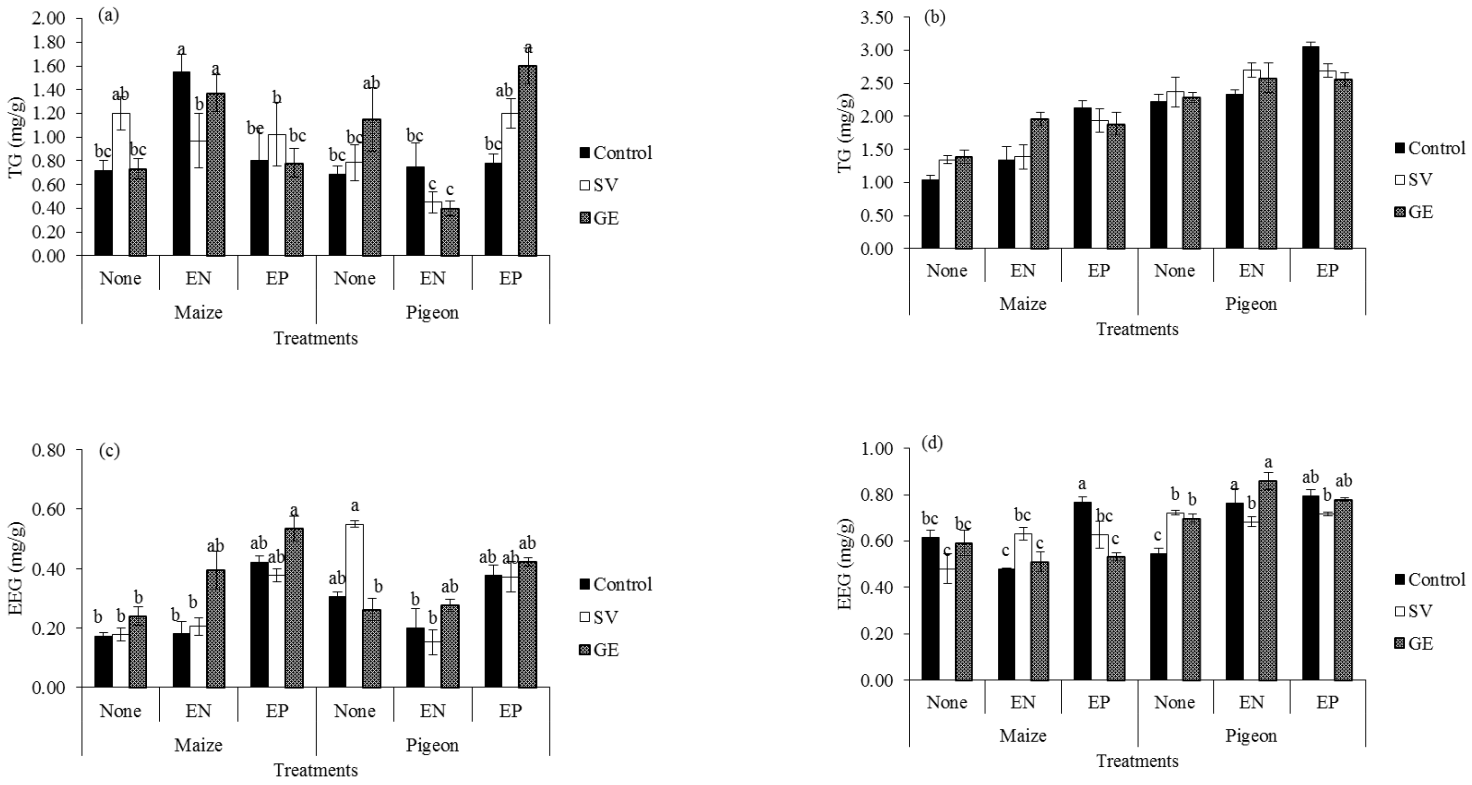


Fig. 3 Effect of arbuscular mycorrhiza fungi (AMF) and earthworm species on total and easily extractable glomalin (TG and EEG) in macro-aggregates (a & c) and micro-aggregates (b & d) under maize and pigeonpea grown in greenhouse conditions during December, 2009 to August, 2010. TG and EEG were affected by crop, earthworm, crop x AMF x earthworm interactions ($p<0.05$ in all cases, Table 2). Errors bars are standard error (SE). Bars with same letters are not significantly different at $p\leq0.05$. Treatment codes are presented in Table 1.

CHAPTER SEVEN

General Discussion and Synthesis

1. Introduction

Soil degradation is common in Sub-Saharan Africa and is often associated with declining soil fertility and low crop productivity. Integrated soil fertility management (ISFM) and conservation agriculture (CA) have been proposed for restoration of degraded soils in smallholder farming systems in the tropics (Hobbs et al., 2008; Vanlauwe et al., 2010). Apart from improving soil fertility, the proposed technologies also aim at restoring soil biodiversity and biological activity. Increased biological activity can to some extent counter the challenges of food production problems and contribute to enhanced agricultural sustainability through increased nutrient acquisition and use efficiency, soil aggregate stability and improved organic matter formation and stabilization. Arbuscular mycorrhizal fungi (AMF) are among the most important soil microbiota influencing physical, chemical and biological soil properties, and can enhance sustainability and productivity of agricultural systems (Cardoso and Kuyper, 2006). Since AMF form mutualistic associations with plants roots, and only propagate in the presence of plants, they are sensitive to soil disturbance, land management and cropping practices, and they may therefore be an early and sensitive indicator of environmental change and health (Oehl et al., 2010). Understanding how AMF communities respond to agricultural practices ensures an opportunity for their utilization and management. AMF also produce glomalin, a glycoprotein quantified as glomalin-related soil protein (GRSP) or Bradford-reactive soil protein (BRSP) and linked to long-term C and N storage (Wright and Upadhyaya, 1998; Haddad and Sarkar, 2003; Nichols and Wright, 2006). Although the exact nature of glomalin using the Bradford assay is unknown and it may likely contain a mixture of several glycoproteins of (mycorrhizal) fungal and plant origin (Gillespie et al., 2011; see also Chapter 6), glomalin content has been proposed as an important criterion for agricultural management strategies in degraded soils (Wright et al., 2007; Fokom et al., 2012). However, very little is known how current agricultural practices (ISFM and CA), proposed to mitigate land degradation, restore soil AMF communities and their products (glomalin) in sub-Saharan Africa. This study was thus undertaken to investigate the effects of agricultural management practices (tillage, cropping systems, organic vs. inorganic inputs) on AMF communities and on the possible roles of AMF on soil aggregation, crop nutrition and production. More precisely, my objectives were to: (i) assess the diversity and activity of AMF under contrasting agricultural management practices; (ii) to determine the impact of these practices on glomalin; (iii) to explore possible roles of AMF diversity and

activity, and glomalin levels in influencing soil structure, crop nutrition and productivity and (iv) to investigate interactions between AMF and other soil biota, in particular earthworms.

2. Effect of agricultural management on AMF communities

Soil disturbance through tillage, soil nutrient management and crop diversification either through rotation or intercropping are the strongest drivers of change in AMF communities in agro-ecosystems (Verbruggen and Kiers, 2010). In Chapter 2 and 3, I took a close look at the long-term effect of agricultural management on AMF communities and their activities. In Chapter 2, I assessed the long-term (32 years) effect of organic (farmyard manure and maize stover) and inorganic amendments (N pus P fertilization), whereas in Chapter 3, I assessed the effects of tillage, cropping system, crop residue and N fertilization in a 5-year-old trial. In these chapters I showed that nutrient management (NP fertilization and organic inputs) and soil disturbance through tillage altered AMF species composition. However, their effects were relatively weak and ordinations revealed that these factors explained less than 10% of the variation in the dataset. This finding was rather unexpected because studies from the temperate zone (both Western and Central Europe, and the USA) have shown strong effects of tillage, mineral fertilizers (especially P, but also N), organic amendments, and cropping systems on AMF communities (Verbruggen and Kiers, 2010). These studies were generally performed in soils that were not severely nutrient (N and P) deficient, had adequate contents of SOC, and were subject to more intensive tillage practices (affecting the upper 30 cm of the soil), whereas our soils were P- fixing, N-deficient (with N being even more limiting plant production than P), had low and sometimes inadequate levels of SOC, and were subject to less intensive tillage practices. However, my result coincides with others showing no significant effects of inorganic and organic fertilization (Franke- Snyder et al., 2001; Wang et al., 2011; Vestberg et al., 2011) and less intensive tillage practices (Borie et al., 2006) on AMF communities under long-term field trials.

It is commonly known that organic amendments increase AMF species diversity, based on temporal differences in P availability between inorganic and organic sources. Organic inputs such as manure are associated with a gradual release of P, providing better opportunities for synchronization with plant demand. Furthermore, organic amendments improve soil physical conditions that favour plant growth and AMF diversity and functioning (Gosling et al., 2006). However in my study system the effects of organic amendments on AMF communities were

limited. Several factors may explain such results. First the soil under the study was humic nitisols, reported to have high available P levels (Olsen) ranging between 17 mg kg^{-1} in unproductive soil to 55 mg kg^{-1} in productive soil (Murage et al. 2000). Such P concentrations when accompanied by low N availability may result in unbalanced N-P proportions. Unbalanced N-P proportion in return results to N deficiencies. The effect may be further aggravated by rapid losses of organic matter (OM) through regular soil disturbance in presence of humidity and high temperature throughout the years and SOC stabilization by clay. In soils with high clay content (like our case), clay can strongly protect soil organic matter lowering N mineralization potential and availability (Vanlauwe et al. 2002). In Kabete none of the inputs (organic or inorganic) were able to sustain SOC levels relative to initial levels before the onset of the trial. Only plots with organic amendments (manure and residue) in combination with NP fertilizer kept SOC levels close to initial levels (Kapkiyai et al., 1999; Ayuke et al., 2011; Chapter 2). Janssen (2011) noted that organic matters in Kabete came close to the critical concentration of 16 g kg^{-1} soil, below which soils become unresponsive to fertilizer (Vanlauwe et al., 2010). Although soil organic matter is not a requirement for plant growth, it influences availability of nutrients, particularly N. Low N availability likely affected the AMF community in Kabete. Hodge and Fitter (2010) and Treseder and Allen (2002) have shown that AMF require substantial amounts of N for their own growth, and under N-deficient conditions the N-demand of the fungi may lead to reduced N transfer to the plant, ultimately leading to no or even negative mycorrhizal responsiveness.

Tillage in Sub-Saharan African countries is done by hand and hoes (affecting only the 10 cm upper soil layer) and is usually much less intensive than in temperate countries (Erenstein, 2003) where tillage is by mechanical means. Low-intensive tillage, such as practiced here, may be beneficial in improving soil aeration and reducing crop competition with weeds. I showed in Chapter 3 improved soil fertility (SOC, N, available P, and exchangeable bases) in conventional tillage (CT) systems compared to no-till (NT) systems, especially in the upper 30 cm profile. However this finding was contrary to my initial hypothesis on benefits of NT on AMF functioning. Alvarez (2005) reported no differences between reduced tillage (affecting 0-15 cm depth, chisel plough) and zero tillage on SOC levels. However, it is also likely that lack of sufficient crop residue under NT influenced my results. Although crop residue plays a significant role in CA, the amount of crop residue and extent of soil cover required are not accurately defined. According to Allmaras and Dowdy (1985), 30% of soil should be under cover under NT

systems to reduce soil erosion by 80%. In several studies in temperate regions, the amount of crop residue ranged between 8-20 t ha^{-1} (Blanco-Canqui and Lal, 2007). This strongly suggests that the amount applied in our system in Nyabeda (2 t ha^{-1}) was inadequate. Nevertheless, the modest application of crop residue increased AMF spore abundance and soil P levels and exchangeable bases. This result suggests that surface addition of crop residue was important in both NT and CT systems.

My results showed a decline of several species of *Glomus*, *Entrophospora, Acaulospora* and *Scutellospora* due to tillage and fertilization. I associate the decline in tilled soils to mechanical disruption of the hyphal network and dilution of AMF propagules, while in fertilized plots I associate the differences to the often observed negative effects of mineral P on AMF. Although *Glomus* species are often reported to dominate intensively managed agro-ecosystems (e.g., high fertilizer applications), my result showed a decline in spore abundance. Possibly, this decline is due to other factors, such as the still low N availability that may have selected against these ruderal *Glomus* species, given that several studies have reported their decline in N-deficient conditions (Treseder and Allen, 2002).

Based on the result of those two chapters, it is clear that the proposed technologies (ISFM and CA) for restoration of soil fertility and soil biodiversity have only limited potential in sustaining AMF spore abundance and AMF activity (root colonization, hyphal length and inoculum potential) in Kenyan agro-ecosystems. Organic inputs alone or in combination with soluble fertilizers are essential to enhance AMF activity and functioning in these agro-ecosystems. NT systems in the presence of residue supported higher AMF activity. Nitrogen fertilization appeared to be a prerequisite in both NT and CT systems for improved AMF activity, even though it negatively affected AMF spore abundance and species richness. Surprisingly, I did not observe any effect of soybean-maize rotation on AMF communities and activity, possibly due to the presence of long-living weeds in NT systems that acted as an alternative host for AMF species in the monocropped maize fields, thus masking the effects of rotation.

3. Role of AMF in soil structure and crop production

One question that is often asked is whether high AMF diversity and activity observed in organically managed systems and under CA practices would result in improved crop nutrition

and higher sustainability of agro-ecosystems. Improved AMF functioning has been proposed as a mechanism to reduce nutrient inputs in agriculture, thereby reducing costs and increasing environmental sustainability (Gosling et al., 2006). The formation and functioning of the AMF symbiosis is thus expected to play an important role in sustainable agriculture, especially under the newly advocated CA practices and under ISFM practices that combine soluble fertilizer and organic inputs. In Chapter 4, I employed structural equation modelling using path analysis to further explore the roles of AMF on soil aggregation, crop nutrition (N and P), and production.

In this chapter, I highlight the importance of AMF on soil aggregation, crop nutrition and production. The degree of its influences, however, depends on agricultural management. Organic-based system alone or in combination with mineral fertilizer resulted in higher AMF activity (hyphal length, root colonization) leading to enhanced soil aggregation and crop nutrition. Root and hyphal length were particularly important in explaining levels of stable macro-aggregates in both sites. Glomalin was also important in influencing levels of stable macro- and micro-aggregates in these trials. However, it is worth noting that the role of glomalin in soil aggregation has remained correlative and very little is known about the mechanisms involved. Hence, correlations between glomalin and levels of aggregates should be interpreted with caution, especially in soils where organic matter is not the only binding agent. Understanding the mechanism involved should therefore be given greater priority in future studies. Similar results were reported by Rillig et al., (2003b) who found a negative or no correlation between macro-aggregates and glomalin in soils where carbonates were the main binding agents. Borie et al. (2008) also showed no correlation between glomalin and macro-aggregates in Ultisols and Mollisols.

AMF was important in explaining crop nutrition and production in Kabete but had limited effects in Nyabeda. The differences between the two sites are highlighted in Table 7.1. Kabete trial had high soil P content, low AMF spore numbers (<2 spore per g soil) especially in upper soil profile, extremely low crop yield, low N and P uptake despite high applications of inorganic and organic inputs compared to Nyabeda. One major further difference, related to the differences in productivity, was apparent nutrient recovery efficiency (ANR, kg nutrient taken up per kg nutrient applied). ANR is defined as (Kimetu et al., 2011);

$$(1)\ \%\ ANR = \frac{(\text{Nutrient uptake F, kg - Nutrient uptake C, Kg})}{(\text{Quantity of nutrient applied, kg})} \times 100$$

Where F represents nutrient uptake in fertilized plots and C represents uptake in control plots (with no organic or inorganic inputs). ANR in Kabete was extremely low. Out of the NP fertilizer added only 16% vs. 8% of applied N and 3.8 % vs. 2.3% of applied P (low vs. high fertilized plots) was removed in grains. While some N and P may have ended up in vegetative biomass that is also removed, it is clear that most of the nutrients added were effectively lost, through fixing and / or leaching. Consequently, the plots in Kabete belong to the category of non-responsive soils (Vanlauwe et al., 2010). Organic amendments, particularly farmyard manure, in fact had a larger impact on crop nutrition than NP fertilization. Kimetu et al. (2006) carried out a study to understand partial balances of N inputs in form of inorganic fertilization (urea) and organic amendments (*Tithonia diversifolia* residues) in a humic nitisol soil adjacent to the Kabete long term trial. From this study N losses through leaching were more in N fertilized soil (9% in N fertilized soil compared to 0.7% in soil with *Tithonia*) while N immobilization was high in organic amended soil (38.7% N immobilized in soil with *Tithonia* soil compared to 8% in N fertilized soil). This indicates that most of applied N in this soil may either be lost or immobilized making it un-available to plants. This suggests that N management is vital in restoring crop productions. Studies understanding N losses and role of soil biota especially AMF in acquisition of organic N may be vital in enhancing N nutrition.

For Nyabeda, I could only calculate ANR for N (because P was applied in all plots). ANR ranged between 120% in NT systems minus residues and <50% in NT systems plus residues additions. This indicates that ANR in Nyabeda was high and nearly all fertilizer applied was utilized by the crops in soil without crop residue. Though I reported high AMF spore abundance and activity in NT systems, there was minimal effect of AMF on crop nutrition. However the AMF colonization increased even in fertilized plots in CT systems, indicating that the plants were still responsive to AMF. Strong correlations between root colonization and P uptake in CT systems indicated that AMF were still important for enhancing P uptake. N immobilization and lack of sufficient amounts of high-quality crop residues therefore masked the effect of AMF on crop nutrition in NT systems. Kihara et al. (2012a) also attributed low crop yield in NT systems to lack of adequate crop residues of high quality and N immobilization in this site. Under such

conditions, the high N demand of AMF could even further aggravate N-limitation to the crops (see above).

A further way to demonstrate the importance of a positive interaction between inorganic fertilizers and organic amendments is to predict N and P uptake, under the assumption that the combined application has only additive effects (Gentile et al., 2011):

$$(2)\ \text{Unexplained uptake} = (\text{Uptake}_{F+M} - \text{Uptake}_C) - (\text{Uptake}_F - \text{Uptake}_C) - (\text{Uptake}_M - \text{Uptake}_C)$$

Where F represents nutrient uptake in sole fertilized plots, M represents nutrient uptake in plots with sole organic (manure or residues) inputs, F+M represents nutrient uptake in plots with combined NP fertilization and organic inputs (manure or residues) and C represents uptake in control plots (with no organics or inorganic inputs). Values larger than zero then indicate a positive interaction between both amendments, indicating that the organic amendment contributes to enhanced ANR after application of mineral fertilizer. In fact, when the effect of sole NP fertilization and sole organic inputs was removed, assuming that NP fertilization and organic inputs had similar effect to P and N uptake when combined, NP fertilization explained less than 20% of N and P uptake while manure explained more than 50% of the N and P uptake. Though organic amendments increased availability of P and N, which could partly explain the high effect of manure, the increases were relatively low (<10%). This meant that changes in microbial activities which include mycorrhizal activity thus influenced crop nutrition in this site.

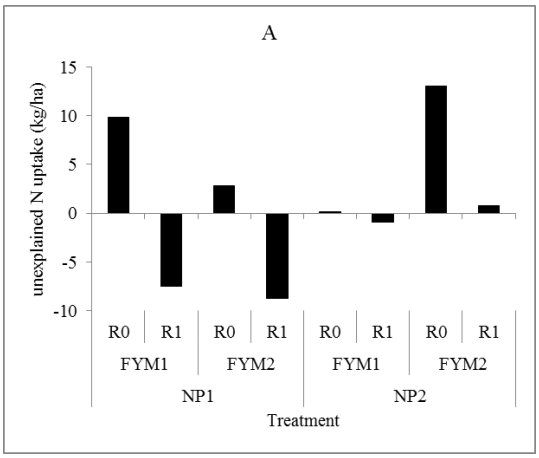
High AMF activities (root colonization, inoculum potential and hyphal length) after organic amendments, positive correlations between AMF colonization and N and P uptake, and a significant contribution of AMF to crop nutrition and production, indicate a mycorrhizal fungal role in N and P uptake. Kahiluoto et al. (2012) have shown that AMF provide better access to pools of sparingly soluble residual P from cumulative fertilisation under organic-based systems resulting in enhanced P uptake. However, it remains controversial whether AMF directly contribute to increased uptake of organic P or whether glomalin production by AMF increased the pool of soil solution P by interacting with iron (hydr-)oxides (Cardoso et al., 2006). Under the two fertilization regimes, other factors which may include AMF appeared to play a significant role in low NP and manure inputs (NP1FYM1) treatments. Approximately 40% of N and P uptake in plots with NP1FYM1 could not be attributed to either organic (manure) or NP fertilization compared to approximately 10% N uptake in plots with high NP and low manure

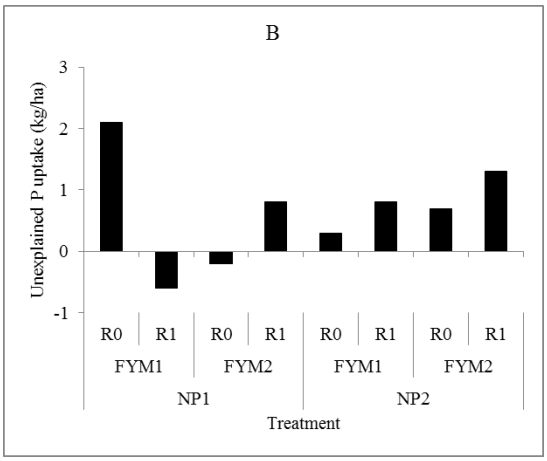
inputs (NP2FYM1, Figure 7.1). In plots with high rates of manure (FYM2), 25% of N uptake in both NP1 and NP2 plots and 13% of P uptake in NP2 plots was unexplained (Figure 7.1). This indicates that management of AMF under low-input technologies may be more beneficial than increasing nutrient inputs in the absence of adequate levels of soil organic matter. AMF communities in organic-based systems were shown to be superior in enhancing crop nutrition and production (Kahiluoto et al., 2012; Antune et al., 2012).

The contribution of AMF diversity to soil aggregation and crop nutrition was minimal in both trials. AMF species differ in their ability to enhance plant growth and P uptake often because of temporal differences in colonization or variation in the length of external hyphae (Graham et al., 1982; Kough and Linderman, 1986). Koide (2000) suggested that a more diverse AMF community would span a broader range of functions, resulting in more benefits conferred to the crops. However, Kahiluoto et al. (2012) and Antune et al. (2012) have shown that AMF diversity changes play a minimal role in determining AMF functioning and in nutrient-deficient soils diverse AMF communities can even be unbeneficial.

Table 7.1 Soil characteristics, AMF parameters, nutrient (N and P) uptake and crop yield of the two study sites. Soil characteristics and AMF parameters are mean values (± SE) in 0-30 cm soil profile, averaged over all treatments. Data from Chapters 2, 3, 4 and 5.

	Parameters	Kabete	Nyabeda
Soil nutrients	Soil type	Humic nitisols	Ferrasols
	Sand: silt: clay ratio	11:22:67	15:21:64
	pH	5.4±0.01	5.3±0.01
	Extractable K (mg kg^{-1})	231.3±4.7	84.3±2.7
	P (mg P kg^{-1})	24.3±0.6	10.6±0.5
	Ca (mg kg^{-1})	1238.9±2.3	1036.1±7.2
	Mg (mg kg^{-1})	578.2±0.6	215.9±0.7
	Total SOC (g kg^{-1})	18.6±0.1	18.5±0.3
	Total nitrogen (g kg^{-1})	1.4±0.01	1.5±0.01
	CEC (cmol kg^{-1})	16.4±0.04	12.6±0.1
AMF parameter	Spore abundance (25 g^{-1} soil)	43.8±2.4	108.8±6.7
	Species richness	8.0±0.2	8.8±0.2
	Diversity	1.8±0.03	1.5±0.02
	Hyphal length (m g^{-1} soil)	18.8±0.4	21.7±0.5
	Total glomalin (TG, mg g^{-1})	7.0±0.1	3.0±0.1
	Easily extractable glomalin (EEG, mg g^{-1})	2.1±0.03	1.7±0.05
	TG/EEG ratio	3.6±0.1	1.9±0.07
	TG/SOC ratio	0.4±0.01	0.2±0.01
Nutrient uptake	N (kg ha^{-1})	32.3±1.2	101.8±3.2
	P (kg ha^{-1})	3.9±0.2	14.6±0.5
	Tissue N:P ratio	8.6±0.2	7.2±0.3
Crop yield	Maize (kg ha^{-1})	1.8±0.1	4.0±0.1
	Bean (Kabete), Soybean (Nyabeda) (kg ha^{-1})	0.4±0.1	2.3±0.1





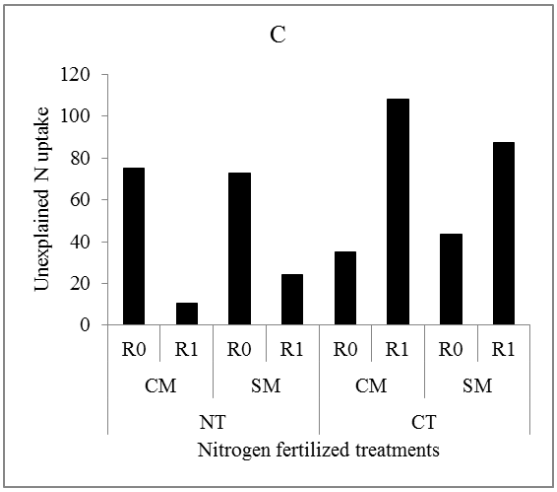


Figure 7.1 Synergism in N (A) and P (B) uptake in Kabete in treatments with combined application of NP fertilizer and manure and N uptake (C) in Nyabeda. FYM1 & FYM2 = 5 & 10 t/ha manure, NP1 & NP2 = 26.2 P, 60 N kg/ha & 52.4 P, 120 N kg/ha, R0/R1 = absence/retained crop residues. NT = no till systems, CT = tillage systems, CM = continuous maize, SM = soybean-maize rotation.

4. Glomalin in managed agricultural systems

Maintenance of optimum SOC levels in agro-ecosystems is an important component for physical, chemical and biological soil quality (Watson et al., 2002). In chapter 4, I assessed the effects of ISFM and CA on glomalin. I demonstrated that glomalin is sensitive to agricultural practices and is an important component of SOC. However, interpretation of changes in glomalin levels need to be undertaken with care, especially taking into account the physical protection and the degree to which glomalin is bound to soil particles. It seems therefore useful to consider both absolute glomalin levels (expressed per g soil) and relative glomalin levels (expressed per g organic carbon or organic matter).

It is generally accepted that agricultural practices that degrade soil properties and decrease soil organic matter, also reduce glomalin pools in soils (Rillig et al., 2004). However, this scenario may apply in well-structured soil where organic matter is the main or only binding agent, but in soil with other strong binding agents (clays) the reverse can be observed. In Table 1 I show that in Kabete glomalin levels were more than twice those of Nyabeda, despite similar SOC levels. The glomalin pool in Kabete represented 40% of SOC whereas in Nyabeda it represented less than 20% of SOC. Under a simplistic interpretation that considers higher glomalin levels as more desirable from the perspective of soil quality and soil fertility, absolute glomalin levels would indicate that soils in Kabete are less degraded than those in Nyabeda. However, in reality the reverse is true, and this is reflected by higher TG / SOC ratios in Kabete than in Nyabeda.

Soils in both sites have high clay content (Table 7.1), contributing to soil aggregation and stabilization of SOC. The soil in Kabete is a Humic Nitisol and that in Nyabeda a Ferrasol; both have likely a high Fe-content as well. Even though inherent soil fertility in Kabete is higher than in Nyabeda (to judge from higher ECEC and levels of basic cations, and also of levels of available P; Table 7.1), the site is characterized by severe N deficiency due to low organic levels (close to the critical level as defined by Janssen, 2011) and consequently very low rates of N-cycling, which constrain availability and result in very severe N-limitation. The SOC in Kabete is likely recalcitrant and / or physically protected against decomposition. High glomalin levels (both absolute and relative) are consistent with physical protection of glomalin (and other humic compounds) and unlikely due to higher rates of glomalin production, as mycorrhizal activity (extraradical hyphal length) was not higher in Kabete than in Nyabeda.

Regular tillage and continuous cultivation for 32 years in Kabete increased the turnover rate of macro-aggregates resulting in more glomalin more tightly bound in micro-aggregates, compared to Nyabeda that was under continuous cropping for only five years. However, it could also be possible that glomalin extraction methods present further complications, with site-specific extraction efficiencies both for TG and EEG, depending on clay and iron content. Nichols and Wright (2005) observed diminishing extraction efficiency of glomalin in soil with higher soil organic matter, higher clay content and in soil with other cations (especially Fe) that bind glomalin. Extraction of glomalin was also shown to be easier in sandy soils than clay soil (Nichols and Wright, 2005; Zhu et al., 2010). This suggests that extraction procedure should be re-examined to be more suitable for a wider range of soils with large differences in texture, levels of OM, and iron and aluminum (hydr-)oxides.

My results are consistent with a role for glomalin in stabilization of micro-aggregates and stabilization of glomalin in these micro-aggregates. This was concluded from higher glomalin levels in micro-aggregates than macro-aggregates (Chapter 5). Currently, it had been assumed that low turn-over rates of glomalin (half-life of 6-42 years according to Rillig et al., 2001) are due to its chemical nature; however, my result suggests that low turn-over may be due to physical protection, especially in micro-aggregates. My results would fit with a general shift in organic matter science, where chemical recalcitrance is currently de-emphasized and physical protection is gaining more prominence (Schmidt et al., 2011). Hence the stability of aggregates determined the amount of glomalin extracted under contrasting management.

Though organic amendments (manure and crop residue) may have increased levels of glomalin through enhanced AMF activity and also through humic materials from the crop residues, this effect was likely masked by high levels of micro-aggregates in plots without organic amendments. Soil physical degradation in fertilized plots and plots without inputs therefore resulted in high turn-over rate of macro-aggregates to micro-aggregates (with some glomalin associated to the free silt and clay fraction), resulting in increased glomalin levels. This explanation fits with observations made in Nyabeda, where turn-over rates of large macro-aggregates to small macro-aggregates due to tillage increased glomalin levels in CT systems, resulting in higher levels in CT than in NT systems (chapter 5). My explanation, however, is contrary to the received view that tillage has negative effects on glomalin levels in soils (Wright et al., 2007). It is worth noting that most of previous studies were done in soils with high sand

content (>40%) and low clay content, where physical protection of glomalin was less important. Tillage practices were also less intensive in Nyabeda compared to the studies reported from temperate countries. Borie et al. (2006) found no significant difference between glomalin levels in NT systems and reduced tillage systems. This suggests that intensity of tillage is a major determinant of changes in levels of glomalin.

Higher SOC levels have repeatedly been correlated with higher glomalin levels (Nichols and Wright, 2005; Fokom et al., 2012), but not always (Rillig et al., 2001; Lovelock et al., 2004). However a causal mechanism to link both OM fractions has been lacking. In my study levels of SOC correlated positively with levels of SOC with the exception of glomalin concentrations in some aggregates and in the upper soil profile in Kabete. However, the relation between both fractions does not always follow a 1:1 line. Consequently, such relations obtained within sites where organic amendments were added, but not between sites where similar SOC levels in Kabete and Nyabeda went together with very different glomalin levels (Table 7.1). Positive correlations may also be caused by reduced decomposition of organic material (including glomalin) via the formation of aggregates.

There have been inconsistent effects of inorganic resources such as N, P, and exchangeable cations on soil glomalin (Treseder and Turner, 2007), and the mechanisms involved in such relations are not yet clear. Lovelock et al. (2004) found declining levels of glomalin with increasing levels of soil fertility (P, K, N) in tropical soils. Rillig et al. (2003b) found weak positive correlations between divalent cations (Mg and Ca) and glomalin concentrations but they found no relationship with availability of P and K. My studies showed positive correlations between glomalin with availability of inorganic resources such as P, N (Kabete; r=0.58, 0.611, Nyabeda; r=0.65, 0.54) and exchangeable bases (Kabete; r=0.41, -0.66, 0.18, Nyabeda; r=0.66, 0.34, 0.67 for K, Mg and Ca), especially in the 0-30cm soil profile. However, SOC levels often correlate with levels of N, P and basic cations (this underlies the relative effectiveness of NIRS for soil analysis, see Chapters 2 and 3). I therefore employed multiple regression to understand roles of these elements on glomalin levels. My results showed that the soil elements (P, K, Mg, Ca, N and SOC) were important in explaining glomalin concentrations in the two field trial (Kabete, F= 3.3, p=0.007, Nyabeda; F=5.0, p=0.001). However only levels of total C significantly explained increasing levels of glomalin content (t=2.6, p=0.01) while levels of P explained decreasing levels of glomalin content (t=2.5, p=0.02)

at Kabete field trial. This suggested that under contrasting management practices it is likely that differences in rates of production, the extent of incorporation within soil aggregates, and/or rates of decomposition of glomalin exist at various rates affecting the sensitivity of the glomalin fractions to the concentration of soil elements. Further studies are required to test and elucidate the mechanism involved.

Two glomalin fractions can be operationalized based on extraction conditions. The easily extractable glomalin (EEG) is extracted after autoclaving soil (1g) mixed with 20 mM citrate at pH 7.0 for 30-60 minutes while total glomalin (TG) is determined by an exhaustive extraction with 50 mM citrate solution at pH 8.0 for 60 minutes in autoclave. Initially it was thought that the EEG pool consists of the more recently produced glomalin, but it was later shown that TG also contains recently modified glomalin (Steinberg and Rillig, 2003). In this thesis, I showed higher levels of EEG in macro-aggregates (both size classes) and micro-aggregates within macro-aggregates than in micro-aggregates, but higher levels of TG in micro-aggregates suggesting some form of glomalin stabilization within the aggregates during ageing of glomalin. Under such conditions of strong aggregation, TG may give therefore a more accurate account of glomalin than EEG.

In conclusion, glomalin appeared to be sensitive to agricultural management. However, interpretation of different levels of glomalin under contrasting management should take into consideration the physical protection of glomalin and not only differential rates of production as a function of differences in mycorrhizal activity. Assessment of glomalin in different aggregate size fractions as well as assessment of the amount of SOC in each fraction can indicate the degree of physical protection of glomalin in soil.

5. Interaction between AMF and earthworm on soil aggregation and crop nutrition

Earthworms and AMF are considered as major soil ecosystem engineers influencing biological, chemical and physical soil properties (Six et al., 2002, Cardoso and Kuyper, 2006). In chapter 6, I assessed the interactive role between AMF (*Glomus*-better root colonizer and *Scutellospora*-better soil colonizer) and earthworms (the endogeic-burrowing *Pontoscolex* and epigeic-surface-dweller *Dichogaster*) on soil aggregation, crop nutrition (N and P) and biomass production. My results highlighted the important role played by plant species, earthworm, AMF and their

interaction on soil aggregation, crop nutrition and production. Their influence was, however, dependent on residue placement and nutrient availability. The epigeic earthworm was important in improving soil aggregation and glomalin allocation in different aggregate fractions. This result was unexpected given that epigeic earthworms have been shown to have a minor effect on soil aggregation (Six et al., 2004). However, similar results were reported by Bossuyt et al. (2006) and Giannopoulos et al. (2011), especially when crop residues are placed on the surface. I therefore attribute my results to ingestion of residue placed on the surface and incorporation of partly decomposed residues into casts. Since endogeic earthworms burrow within the soil, it had no access to residue placed on the surface, resulting in small effects on soil aggregation and glomalin enrichment. The stability of the casts is shown to depend on the quality of the ingested organic matter (Shipitalo and Protz, 1988; Coq et al., 2007). When food supply is limited, earthworms ingest more soil in an effort to obtain sufficient food, consequently casts tend to be less stable (Martin, 1982). My result further showed that AMF–host species differentially controlled the fraction of water-stable aggregates as previously reported (Piotrowski, 2004; Milleret et al., 2009b, Hallett et al., 2009). AMF were particularly important for soil aggregation in presence of the pigeonpea (more than with maize) in my study.

No interactive role between AMF and earthworms on soil aggregation was observed in this study, however, my study did not conclusively reject this hypothesis. Studies on interactions between AMF and earthworm on soil aggregation are few. One such study with the endogeic earthworm *Allolobophora chlorotica* and the AMF species *Glomus intraradices* also observed no interaction between AMF and earthworms (Milleret et al., 2009a). Many unexpected factors, which my experiment did not take into account, may have influenced my observations. Briefly, the compacting nature of endogeic earthworm in absence of crop residue, its destructive effect on AMF activity (reduced hyphal length), and plant species may have played a role influencing my results. Other factors such as root exudates and the enmeshing role of both roots and fungi also influenced soil stability.

Plant growth depends on limiting nutrients (N and P), and nutrient availability influenced crop nutrition. According to Güsewell (2004), plants are limited by N when the tissue N:P ratio is <10, and limited by P when N:P ratio if >20. My results showed that N was severely limiting in the experiment. The N:P ratio was less than 8 in both maize (5.0) and pigeonpea (6.7). Under such conditions, earthworms particularly epigeic earthworms, enhance N uptake through

increased N mineralization resulting in higher N availability. This effect was more evident in mycorrhizal inoculated pigeonpea than maize. Nutrient immobilization (N) by roots and grass mulch in pots with maize after two consecutive harvests also influenced the results in mesocoms with maize. Under these conditions I showed a minimal effect of the endogeic earthworm on crop nutrition and negative effects in mycorrhizal maize and pigeonpea. I attributed these effects to severe N limitation, especially in maize mesocoms, and to constant mechanical disruption of the mycorrhizal network by the endogeic earthworm. AMF have a high demand for N for their own growth and under N-limiting conditions the mycorrhizal symbiosis can result in negative plant responses (Hodge and Fitter, 2010). Mechanical disruption of hyphae also negatively affects AMF functioning (Tuffen et al., 2002).

The outcome of my study shows that interactions among earthworms, AMF and plants are apparently not obvious, but complex with many physical, chemical and biological processes responsible for controlling soil structure and crop nutrition. Factors such as soil type, nutrient availability, plant species, root exudates, microbial communities, residue placement, etc. need to be taken into consideration to fully understand and appreciate their role in agro-ecosystems. I propose future studies taking into consideration plant-AMF-earthworm species interactions in addition to those other factors under greenhouse and field conditions to fully understand the interaction between AMF and earthworms on soil aggregation and crop nutrition.

6. Conclusions, recommendations and way forward

In my thesis I have shown that ISFM and CA have potential in restoring AMF activity in Kenyan agro-ecosystems. Improved AMF activity in return has the potential to enhance soil aggregation and increase crop nutrition resulting in higher crop productivity. Profound differences were, however, observed between the two study sites with regard to levels of carbon and nutrients (P levels, cations), AMF parameters (including absolute and relative glomalin levels), crop nutrition and productivity (Table 7.1). Kabete soils had higher available P and cation levels, lower AMF spore numbers (<2 spore per g soil), especially in the upper soil profile, higher absolute and relative glomalin levels compared to Nyabeda, and extremely low N and P uptake and crop yield despite high doses of inorganic fertilizers. Furthermore, plant tissue N:P ratio was <10, indicating that plants were N limited. N deficiency may have affected AMF functioning. Interestingly, under intensive agricultural practices AMF species may be found in deeper soil

profiles that are unaffected by management. Oehl et al. (2005) found AMF species not found in upper soil profile in deeper soil horizons (50–70cm) that were unaffected by agricultural practices. In my thesis I also found higher spore numbers in the 15-30 cm soil layer indicating that AMF genotypes that are not adapted to current practices may be found at greater depths and provide a bank of mycorrhizal species diversity available after management has been improved (Hamel and Strullu, 2006). Future studies should focus on management of nutrient imbalances and maintenance of soil organic matter under ISFM and CA to stimulate AMF functioning and to improve crop production.

Another striking result from my thesis was the low fertilizer use efficiency (<15% N and <5% P was utilized by crops in Kabete) under continuous NP fertilization, with only a small increase after organic amendments. Organic inputs instead contributed more than 50% of N and P uptake in fertilized plots while >30% was unexplained (rather implying an effect of organic matter-induced increased nutrient use deficiency in non-responsive soils). Under such conditions, soil biota (including AMF) are important for crop nutrition. This implies that management of AMF next to that of other beneficial soil biota (including saprobic micro-organisms and soil fauna) may be more important in systems with organic amendments than increasing levels of fertilizer without organic amendments. Understanding the functional diversity of AMF may merit further studies. I noted declining abundance of *Glomus* species in the two trials. *Glomus* species prevail under intensively managed agricultural systems. However, studies in Kenyan agro-ecosystems have so far shown prevalence of AMF species that belong to other genera than *Glomus* (Mathimaran et al., 2007). It is likely that the acidic nature of our soils selects in favour of *Acaulospora* species. There is also a possibility that low N availability selects against *Glomus* species. Several studies have shown decline of *Glomus* species in N-deficient conditions (Treseder and Allen, 2002). Hence, understanding the factors selecting AMF species, and characterization of their functional attributes in agro-ecosystem may thus help in their management and utilization for sustainability of agro-ecosystems.

Despite the potential of CA in sustaining AMF spore abundances and activity, lack of adequate crop residue and nutrient immobilization in residues appeared to limit AMF functioning. Maintenance of crop residue as mulch is among the three pillars of CA, although the recommended (minimum) level for residue addition in NT systems is not been specified. The amount of crop residues (2 t ha^{-1}) used in my experiment was based on what was easily available

and affordable by small-scale farmers in these regions. The amount, however, seemed inadequate and the type of crop residue (maize stover) used as mulch was accompanied by nutrient immobilization, irrespective of N fertilization, thereby negatively impacting on the benefits accrued from the systems. Future studies should therefore endeavour to determine the amount of mulch required for creation of favourable micro-climate in NT systems and explore other options (alternative crop residues) and ways to manage N immobilization. Other organic sources of N (manure) may be desirable due to negative effects of N fertilization on AMF spore abundance.

Conservation agriculture, though increasingly promoted in Sub-Saharan Africa as an alternative for coping with the need to increase food production on the basis of more sustainable farming practices, still has a very low adoption rate in the region (Gowing and Palmer, 2008). Giller et al. (2009) have highlighted the main factors that may lead to lack of adoption of CA by small-scale farmers in SSA. Some of these biophysical factors include weed management problems due to high costs of herbicides, lack of adequate crop residues, and N immobilization following incorporation of low-quality crop residue such as maize stover leading to poor crop yield. Lack of markets for leguminous grains was also cited as a major constraint hindering adoption of cereal-legume rotations. In this thesis, I showed that soil fertility levels and crop yield were lower in NT compared to CT systems even after five years of CA. I cited N immobilization as major factor contributing to low crop yield as evidenced by low N and P uptake and low N:P ratio of crops. Since small-scale agriculture is need-driven, successful adoption of promising environmentally friendly agricultural technologies will depend on their immediate and tangible benefits rather than intangible long-term ecosystem services. Hence lack of immediate returns in terms of crop yield may thus constraint adoption of CA. Ways of how to improve crop production under CA may thus be vital.

To date, the composition of the extracted soil glomalin is not yet known and its link with AMF is still not clear (Gillespie et al., 2011). Questions that often arise are whether extracted soil glomalin is of fungal origin or also includes humic substances derived from plants. My study cannot answer this question since I did no test the properties of extracted glomalin. The recent study by Gillespie et al. (2011) has shown that quantified soil glomalin yields a mixture of compounds containing proteins from other organisms, where the AMF contribution was minimal. Rosier et al. (2006) also showed that soil organic matter influenced the Bradford assay glomalin by co-extraction of plant-derived humic materials. To date, only few studies have

shown positive correlations between glomalin and AMF parameters such as hyphal length and spore abundance (Bedini et al., 2007). Such findings are often attributed to the gap between production and turn-over rate of hyphae and that of glomalin (Rillig et al., 2003a; Steinberg and Rillig, 2003). Glomalin residence time was estimated at 6-42 years while that of AMF hyphae may range between days to months (Rillig et al., 2001; Steinberg and Rillig, 2003). However, there is evidence that AMF contributed to glomalin. Glomalin concentrations declined when AMF was eliminated (Rillig et al., 2003a; Steinberg and Rillig, 2003). Several studies have also extracted glomalin from sterile in-vitro conditions using transformed carrot root (Rillig and Steinberg, 2002; Gadkar and Rillig, 2006, Hammer and Rillig, 2012). Significant effects of earthworm on glomalin suggest that earthworms either reduced glomalin turnover rate through increased aggregate stabilization or through incorporation of decomposed residues into aggregates that were co-extracted. Positive correlations between glomalin and soil organic carbon suggest that both pools (glomalin and SOC) are subject to similar production and decomposition dynamics or that glomalin contains substantial amount of humic substances. Future research should therefore endeavour to establish the amount of glomalin that is of AMF origin. Assessment of relative glomalin amounts (the ratio TG / SOC or the ratio EEG / TG; Table 7.1) may be useful in this regard.

In conclusion, the results of my thesis show that the proposed agricultural technologies (organic amendments and CA) have some potential in maintaining and restoring AMF community and activity. Enhanced AMF activity is important in enhancing soil aggregation, crop nutrition, and crop production. However, under long-term applications of mineral fertilizer in the absence of organic amendments, the benefits of AMF communities may not be accrued due to soil unresponsiveness and / or nutrient imbalances and (too) low levels of soil organic matter under continuous cultivation. Nutrient management, especially of N, accompanied by organic matter management is necessary to maintain AMF communities. NT systems plus crop residue addition were the most important practices in enhancing AMF activity. Further work is necessary to determine the amount of crop residue required to maintain a favourable micro-climate, and ways to manage N immobilization following crop residue addition. N fertilization (in combination with organic amendments to increase SOC levels and thereby nutrient use efficiency) is a prerequisite in enhancing AMF activity in Kenyan agro-ecosystems.

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THESIS SUMMARY

Current practices in soil management, in particular soil disturbance through tillage, application of mineral fertilisers and monocropping (rather than intercropping or crop rotation), are considered as measures that have a strong negative impact on the occurrence and functioning of communities of arbuscular mycorrhizal fungi (AMF) in agro-ecosystems. AMF are important for physical, chemical and biological soil quality. Mycorrhizal functioning is therefore of great importance for sustainable production in agro-ecosystems. However, our current knowledge is still limited on the effects of agricultural management practices that have been proposed to regenerate soil fertility and to enhance soil life, and especially those of AMF in Africa, in particular Kenya. The purpose of my study was to increase knowledge on the effects of agricultural management practices on AMF, with special attention for their roles in formation or aggregates, and their roles in plant nutrition and crop productivity. More specifically my research was aimed at: (i) understanding long-term effects of mineral fertilisers and organic amendments on AMF; (ii) understanding long-term effects of conservation agriculture on AMF; (iii) quantifying glomaline, a glycoprotein with humus-like properties that is produced by AMF and that is assessed in the Bradford assay as Bradford-reactive soil protein; (iv) assessing relationships between AMF, soil aggregation (soil structure), plant nutrition and crop productivity; (v) investigating interactions between earthworms and AMF regarding soil structure, plant nutrition and crop productivity.

Both field and greenhouse research was done. Field observations were done in two long-term trials in agro-ecosystems in Kenya. One experiment took place in central Kenya (Kabete), where since 1976 the effects of mineral fertiliser (a combination of nitrogen and phosphorus) and organic amendments (farmyard manure, crop residue) on crop production have been investigated. The other experiment took place in western Kenya (Nyabeda), where in 2003 an experiment was set up to study the effects of conservation agriculture (minimum tillage, crop rotation, crop residue addition) and nitrogen fertilisation on crop productivity. During my research these experiments ran for 32, resp. 5 years. In both localities I collected soil material and plant roots. I used two trap crops, cowpea and sorghum, to assess diversity of AMF. I isolated spores from field soil and from soil from trap cultures, assessed abundance and identified spores to species level. Next to species richness and abundance I calculated Shannon diversity. I measured length

of the extraradical mycorrhizal fungal network in soil and also fractional root colonisation. Colonisation was assessed in field soil and in soil with trap plants (cowpea, sorghum, leek). Finally I assessed aggregate size distribution (large and small macro-aggregates, free micro-aggregates, micro-aggregates within macro-aggregates, silt plus clay), levels of glomalin and organic matter (including the glomaline : soil organic matter ratio) in these various aggregate size classes, and nitrogen and phosphorus mass fractions in the crops, which together with plant biomass indicates nutrient uptake. In order to derive hypotheses on the roles of mycorrhizal associations in soil structure and plant nutrition, I applied path analysis, a multivariate technique that allows assessment of direct and indirect effects of important factors. For the study of the interactions between earthworms and AMF regarding soil structure, nutrient uptake (nitrogen, phosphorus) en plant performance (maize, pigeonpea). I executed a greenhouse experiment that lasted 9.5 months. I studied two species of earthworms (the endogeic *Pontoscolex corethrurus* and the epigeic *Dichogaster bolaui*) and two species of AMF (*Glomus etunicatum*, a fast root coloniser but a poor soil coloniser and *Scutellospora verrucosa*, a good soil colonisers but a poor root coloniser) in a factorial experiment (with controls without worms and / or AMF).

The results of my study showed that various agricultural management practices (use of mineral fertiliser, application of organic amendments such as farmyard manure and crop residues, tillage) have an effect on species composition, diversity and abundance of AMF. However, these effects were relatively minor and less than 10% of the variation in an ordination diagram was explained by these factors in both experiments. My results therefore contradict those of earlier studies in other parts of the world where it was shown that application of mineral fertiliser and tillage have strong negative effects on AMF. A possible explanation could be that my treatments were relatively mild compared to those in agricultural management in temperate areas: mineral fertiliser did not lead to nitrogen or phosphorus saturation and tillage was shallow. In agreement with earlier studies I observed a positive effect of organic amendments (with and without simultaneous addition of mineral fertiliser) on extraradical hyphal length and on fractional root colonisation. The combination of minimum tillage and residue addition increased spore abundance and also increased fractional root colonisation. There was no effect of crop rotation (compared to cereal monocropping) on mycorrhiza. From the results of both experiments I conclude that practices, aimed at restoration of soil life and soil fertility, have some positive effect. However, the main factor that constrains crop productivity is nitrogen

limitation (rather than phosphorus limitation), and nitrogen should therefore be applied, together with organic amendments.

Path analysis did show positive correlations between mycorrhiza, soil structure, plant nutrition and crop yield. Application of organic amendments in Kabete resulted in a relatise of the fraction macro-aggregates, while mineral fertiliser had no effect. The relative fraction of micro-aggregates showed the opposite pattern. Both mineral fertiliser (nitrogen and phosphorus) and organic amendments increase nutrient uptake and resulted in higher crop yield. Minimum tillage in Nyabeda resulted in an increase of the fraction macro-aggregates. However, nutrient uptake and crop yield in Nyabeda were higher in plots with conventional tillage than in plots with minimum tillage. Crop response to nitrogen fertiliser was much larger in Nyabeda than in Kabete. In Kabte I observed positive correlations between the fraction macro-aggregates, fine root length and length of the extraradical network, whereas in Nyabeda there was only a positive correlation between fraction macro-aggregates and fine root length but not with extraradical hyphal length. In Kabete mycorrhizal incidence partly explained crop productivity, but this was not the case for Nyabeda. On the basis of these data I conclude that an increased activity of AMF in agro-ecosystems, where organic amendments are applied, can have a positive impact on soil structure, plant nutrition and crop yield.

In Chapter 5 I report levels of glomalin (total glomalin and easily extractable glomalin). In Kabete there was a positive correlation between glomalin levels and fraction micro-aggregates (and hence a negative correlation with fraction macro-aggregates), whereas in Nyabeda the results were exactly the opposite. Total glomalin content, and the ratio glomalin : soil organic matter were much higher in Kabetre and Nyabeda. Glomalin levels were unaffected by fertiliser application, and organic amendments increased glomalin levels. In Nyabeda conventional tillage, in combination with organic amendments, resulted in higher glomaline levels than minimum tillage in combination with organic amendments. A comparison between glomalin levels in the different aggregate size classes and whole-soil glomalin levels showed that sample pretreatment (when aggregates are destroyed) had a great impact on measured total levels.

A major question is to what extent the results of both experimental sites can be compared. In both localities soils were clayey and acidic and had relatively low soil organic matter levels. Glomalin levels (and the ratio glomaline : soil organic matter) was substantially higher in Kabete than in Nyabeda. Crop yield in Kabete was much lower, and nutrient use efficiency after

fertiliser application was also much lower than in Nyabeda. A possible explanation could be that the site in Kabete (after 32 years of experimentation) represents a so-called non-responsive soil, these are soils with a critically low levels of soil organic matter that results in lack of response to fertiliser. In such non-responsive soils a major part of organic matter in present in stable micro-aggregates. Recalcitrant organic matter (humic compounds, also glomalin) is protected in these micro-aggregates against further degradation. Consequently, carbon and nutrient cycling proceed slowly and this slow cycling negatively affects crop productivity. For my research these results imply that high glomalin levels cannot always be considered as indicators for high soil quality. High glomalin levels in combination with (and protected by) a large fraction of micro-aggregates rather indicate organic matter stability and lack of active organic matter, and hence indicate a soil where organic matter dynamics are too low to sustain crop yield. Restoration of soil organic matter levels in these soils is a prerequisite, also in order to improve nitrogen use efficiency after mineral fertilisers have been applied.

In the greenhouse experiment the epigeic worm had a larger effect on macro-aggregates than the endogeic worm. This effect was contrary to current literature reports where it has been commonly reported that epigeic worms have a much smaller effect on soil structure than endogeic worms. Presumably my results were caused by addition of mulch to the surface of the pots (to prevent desiccation of the pots and to provide sufficient food for earthworms). Epigeic worms had access to this food source and mixed it with mineral soil particles, resulting in improved soil structure. For the endogeic worm these residues were unavailable. The endogeic worm used (*Pontoscolex*) is also known as a compacting worm. Both species of AMF increased fraction macro-aggregates in pots with pigeonpea but not in pots with maize. There was no significant interaction between worms and AMF; their combined effect on soil structure is the sum of the separate effect of both groups of soil biota. For crop performance an earthworm x AMF interaction was observed. The combination of epigeic worm and AMF increased nutrient uptake and plant growth, whereas the combination endogeic worm plus AMF reduced these parameters, possibly because the endogeic earthworm fed on and disrupted the mycelial network in soil thereby reducing mycorrhizal effectiveness. From my experiment it became clear that interactions between earthworms (with different feeding strategies), AMF (with different strategies regarding root and soil colonisation) and crop species (with maize as a N-limited and pigeonpea as P-limited species) are very complex, and that simple generalisation are not (yet)

possible.

In my final chapter I conclude that Integrated Soil Fertility Management (ISFM, a combination of mineral fertiliser and organic amendments) and Conservation Agriculture (CA, a combination of minimum tillage, crop rotation and residue amendment through mulching) can have a positive effect on occurrence and functioning of communities of AMF. This positive effect could result in improved soil structure and plant nutrition, ultimately resulting in higher crop productivity. However, the effects should be evaluated in the framework of other agricultural management practices. Application of nitrogen fertiliser and an increase in nitrogen use efficiency after fertiliser application are of essential importance for the resource-poor African farmer.

THESIS SAMENVATTING

Hedendaagse praktijken in bodembeheer, in het bijzonder bodemverstoring via ploegen, toepassen van kunstmest en het continu telen van één gewas (in plaats van mengteelt of wisselteelt) worden beschouwd als maatregelen die een sterke negatieve invloed uitoefenen op het voorkomen en functioneren van gemeenschappen van arbusculaire-mycorrhizaschimmels in landbouwsystemen. Deze mycorrhizaschimmels zijn van belang voor de fysische, chemische en biologische bodemkwaliteit. Het functioneren van deze mycorrhizasymbiose is daarom van groot belang voor de duurzaamheid en productiviteit van landbouwsystemen. We weten echter nog te weinig van de effecten van de huidige landbouwbeheersvormen, zoals die zijn voorgesteld om de bodemvruchtbaarheid te herstellen en het bodemleven te bevorderen, op het voorkomen en functioneren van deze mycorrhizaschimmels in Afrika, in het bijzonder Kenya. Deze studie had als doel inzicht te verwerven in de effecten van verschillende vormen van beheer op deze mycorrhizaschimmels, en in het bijzonder op hun rol bij de vorming van bodemaggregaten, plantenvoeding en de productiviteit van het gewas. Meer specifiek waren de doelstellingen van dit onderzoek (i) begrijpen van lange-termijneffecten van minerale meststoffen (kunstmest) en toevoegingen van organisch materiaal op mycorrhizaschimmels; (ii) begrijpen van lange-termijneffecten van conserveringslandbouw op mycorrhizaschimmels; (iii) kwantificeren van glomaline, een eiwit-koolhydraatcomplex met humusachtige eigenschappen dat door deze schimmels wordt geproduceerd en dat door middel van de zogenaamde Bradford-reactie wordt bepaald; (iv) vaststellen van verbanden tussen mycorrhiza, bodemaggregaten (bodemstructuur), plantenvoeding en gewasproductiviteit; (v) onderzoeken van de interactie tussen mycorrhizaschimmels en regenwormen bij bodemstructuur, plantenvoeding en gewasproductie.

Onderzoek werd zowel in het veld als in de kas uitgevoerd. Veldwaarnemingen werden gedaan in twee lange-termijn veldproeven in agro-ecosystemen in Kenya. Het ene experiment werd uitgevoerd in centraal Kenya (Kabete), waar sinds 1976 een proef loopt naar het effect van kunstmest (stikstof en fosfaat) en van toevoegingen van organisch materiaal (dierlijke mest, gewasresten) op gewasproductie. Het andere experiment werd uitgevoerd in westelijk Kenya (Nyabeda), waar in 2003 een proef werd gestart naar de effecten van conserveringslandbouw (minimale grondbewerking, wisselteelt, toevoegen van gewasresten) en stikstofbemesting op de gewasproductie. Ten tijde van het onderzoek waren deze proeven dus 32 resp. 5 jaar oud. In

beide proefvelden verzamelde ik bodemmateriaal en wortels voor analyse. Daarnaast gebruikte ik twee vanggewassen, ogenboon en gierst, om de mycorrhizadiversiteit te bepalen. Ik isoleerde sporen vanuit de veldbodem en vanuit de grond van de potten met de vanggewassen, bepaalde hun talrijkheid en determineerde ze tot op soortniveau. Naast soortenrijkdom en –talrijkheid bepaalde ik de diversiteit volgens Shannon. Ook bepaalde ik de lengte van het mycorrhizanetwerk in de bodem en bestudeerde ik welk deel van de wortels door deze schimmels gekoloniseerd was. Kolonisatie werd zowel bestudeerd aan wortelmonsters die direct in het veld verzameld werden alsook aan wortelmonsters in potten met vanggewassen (ogenboon, gierst, prei). Tot slot bepaalde ik de verdeling van aggregaten over klassen van verschillende grootte, onderzocht het gehalte aan glomaline en organische stof in elk van deze klassen van aggregaten, en bepaalde het gehalte aan stikstof en fosfaat in het gewas, dat samen met gegevens over het plantgewicht inzicht gaf in de opname van beide voedingsstoffen. Om uit deze reeks gegevens hypothesen te kunnen afleiden over de rol van mycorrhiza bij bodemstructuur en plantenvoeding gebruikte ik padanalyse, een multivariate techniek om directe en indirecte effecten van belangrijke factoren te kunnen onderscheiden. Voor de studie naar de interactie tussen regenwormen en mycorrhizaschimmels en hun effect op bodemstructuur, nutriëntopname (van stikstof en fosfaat) en gewasgroei (van mais en duivenerwt) deed ik een kasproef gedurende 9,5 maand, waarbij ik twee soorten regenwormen (de grondeter *Pontoscolex corethrurus* en de strooiseleter *Dichogaster bolaui*) en twee soorten mycorrhizaschimmels (*Glomus etunicatum*, die wortels snel koloniseert maar slechts een beperkt netwerk in de bodem maakt, en *Scutellospora verrucosa*, die een groter schimmelnetwerk in de bodem maakt maar de wortels minder uitgebreid koloniseert) in een factoriële proef (met controles zonder wormen en / of schimmels) onderzocht.

De resultaten van mijn studie lieten zien dat verschillende landbouwpraktijken (gebruik van kunstmest, toepassen van organische materialen zoals dierlijke mest of gewasresten, grondbewerking) effecten hebben op de soortensamenstelling, diversiteit en talrijkheid van arbusculaire-mycorrhizaschimmels. De effecten waren echter beperkt, doordat minder dan 10% van de variatie in de beide proeven door deze factoren werden verklaard. Mijn resultaten waren daarmee in tegenspraak tot die van andere studies in andere delen van de wereld, waar vooral toediening van kunstmest en grondbewerking een sterk negatief effect hadden. Een mogelijke verklaring hiervoor is dat de behandelingen minder ingrijpend waren dan in landbouwsystemen

in de gematigde streken: de kunstmestgiften leidden niet tot stikstof- en fosfaatverzadiging van de grond, en de grondbewerking was relatief ondiep. In overeenstemming met wat in de literatuur bekend was, vond ik een positief effect van toediening van organisch materiaal (al dan niet in combinatie met kunstmest) op de lengte van het schimmelnetwerk in de bodem en op de kolonisatiegraad van wortels. De combinatie van minimale grondbewerking met toevoegen van gewasresten leidde tot een groter aantal sporen van mycorrhizaschimmels en verbeterde eveneens de kolonisatiegraad van wortels. Er bleek geen effect te zijn van wisselteelt (in vergelijking met continuteelt van hetzelfde gewas) op mycorrhiza. Uit beide proeven trek ik de conclusie dat maatregelen, die gericht zijn op herstel van bodemleven en van bodemvruchtbaarheid, enig positief effect hebben. Echter, de belangrijkste factor voor de lage gewasproductiviteit is het gebrek aan stikstof (eerder dan aan fosfaat); en deze moet via kunstmest, in combinatie met organische stof, worden toegevoegd.

De padanalyse liet zien dat er directe positieve verbanden waren tussen mycorrhiza, bodemstructuur, plantenvoeding en gewasproductie. Door toepassen van organische-meststoffen in Kabete nam het relatieve aandeel van macro-aggregaten toe, terwijl door toepassen van kunstmest dat aandeel niet veranderde. Het relatieve aandeel van micro-aggregaten toonde een omgekeerd patroon. Zowel kunstmest (stikstof en fosfaat) en organische toevoegingen vergrootten de opname van plantenvoedende stoffen, hetgeen leidde tot een grotere gewasproductie. In Nyabeda leidde minimale grondbewerking tot een vergroting van het relatieve aandeel van macro-aggregaten. Echter in deze proef bleek de nutriëntopname en de gewasproductie hoger te zijn bij conventionele grondbewerking dan bij minimale grond-bewerking. De reactie van het gewas op stikstoftoediening was in Nyabeda veel sterker dan in Kabete. In Kabete waren er positieve correlaties tussen het aandeel macro-aggregaten en de lengte van fijne wortels en het schimmelnetwerk, terwijl in Nyabeda er alleen een positief ver-band was tussen aandeel macro-aggregaten en wortellengte. In Kabete kon het relatieve voor-komen van mycorrhiza de opbrengst gedeeltelijk verklaren, terwijl dat in Nyabeda niet het geval was. Op grond van deze proeven concludeer ik dat een toegenomen activiteit van mycorrhizaschimmels in landbouwsystemen waar regelmatige organische bemesting wordt toegepast een positief effect kan hebben op bodemstructuur, plantenvoeding en gewasgroei.

In hoofdstuk 5 bestudeerde ik gehalte aan totaal glomaline en gemakkelijk extraheerbaar glomaline. In Kabete was er een positief verband tussen gehaltes aan glomaline en het relatief

aandeel aan micro-aggregaten (en dus een negatief verband met het relatieve aandeel macro-aggregaten), terwijl in Nyabeda de uitkomst precies omgekeerd was. De absolute niveaus aan totaal glomaline en de verhouding tussen glomaline en totale organische stof waren veel hoger in Kabete dan in Nyabeda. Gehaltes aan glomaline werden niet beïnvloed door de toepassing van kunstmest, terwijl toepassing van gewasresten leidde tot toename van het glomalinegehalte. In Nyabeda leidde conventionele grondbewerking, in aanwezigheid van gewasresten, tot hogere gehaltes aan glomaline dan minimale grondbewerking. Een vergelijking tussen het glomalinegehalte in de verschillende klassen van aggregaten en dat van de grond in zijn geheel liet zien dat de voorbehandeling (waarbij de aggregaten vermalen worden) grote effecten heeft op het totale gehalte.

Een belangrijke vraag is in hoeverre de resultaten van beide proefvelden vergeleken kunnen worden. In beide locaties was de bodem zuur en kleiig en was het organische-stofgehalte relatief laag. Het gehalte aan glomaline (en de verhouding glomaline en organische stof) was aanzienlijk hoger in Kabete dan in Nyabeda. De gewasproductie in Kabete was veel lager, en ook was de efficiëntie waarmee kunstmest benut kan worden daar veel lager. Een mogelijke verklaring voor dat verschil is dat de locatie in Kabete (na 32 jaar) behoort tot de zogenaamde niet-responsieve bodems, bodems waarbij het organische-stofgehalte zo laag is dat stikstofkunstmest niet meer effectief benut kan worden. In zulke gronden is het grootste deel van de organische stof aanwezig in stabiele micro-aggregaten. Vooral de slecht afbreekbare delen daarvan (humusachtige verbindingen maar ook glomaline) zijn in die micro-aggregaten beschermd tegen verdere afbraak. Het gevolg is dat de koolstof- en nutriëntenkringloop erg langzaam verlopen, waardoor de gewasgroei beperkt is. Voor mijn onderzoek betekent dit dat hoge gehaltes aan glomaline niet zonder meer kunnen gelden als indicator voor goede bodemkwaliteit. Hoge gehaltes aan glomaline in associatie met (en beschermd door) micro-aggregaten zijn eerder een aanwijzing voor stabiliteit van de organische stof en het ontbreken van actieve organische stof, en dus voor een bodem waar de organische-stofdynamiek te laag is. Herstel van bodemorganische stof in zulke gronden is een eerste vereiste, ook ter verbetering van de efficiëntie waarmee stikstof en andere kunstmest kan worden benut.

In de kas bleek de strooiseletende worm een groter effect te hebben op macro-aggregaten dan de grondeter. Dit effect was tegengesteld aan wat in de literatuur meestal gerapporteerd werd, namelijk dat strooiseletende wormen een veel kleiner effect op bodemstructuur hebben dan

grondetende wormen. Vermoedelijk wordt mijn resultaat veroorzaakt doordat ik gewasresten aan de oppervlakte in de potten toevoegde (om uitdroging te voorkomen en om de wormen van voldoende voedsel te voorzien). De strooiseletende wormen hadden daar toegang tot en vermengden dit plantaardige materiaal met bodemdeeltjes, wat gunstig uitwerkte voor de bodemstructuur. Voor de grondetende wormen waren deze strooiselresten niet beschikbaar. Bovendien behoorde de door mij gekozen grondetende worm tot de bodemverdichters. Beide mycorrhizaschimmels vergrootten het aandeel macro-aggregaten in potten met duivenerwt maar niet in potten met mais. Er bleek geen sprake van een significante interactie tussen wormen en mycorrhizaschimmels; hun gecombineerde effect op bodemstructuur kan worden beschreven als de som van beide afzonderlijke effecten. Voor de gewasgroei was er wel sprake van een interactie. De combinatie van de strooiseletende worm en mycorrhiza vergrootte nutriëntenopname en groei van duivenerwt. De combinatie van grondetende worm en mycorrhiza had een negatief effect, mogelijk doordat de worm zich voedde met het schimmelnetwerk en daardoor de effectiviteit van mycorrhiza reduceerde. Uit de proef werd duidelijk dat de interactie tussen regenwormen (met verschillende voedselstrategieën), mycorrhizaschimmels (met verschillend strategieën) en plantensoorten (waarbij mais meestal door stikstof en duivenerwt meestal door fosfaat beperkt wordt) buitengewoon complex is, en dat simpele generalisaties vooralsnog niet mogelijk zijn.

In hoofdstuk 7 concludeer ik dat geïntegreerd bodemvruchtbaarheidsbeheer (ISFM, een combinatie van toepassen van organisch materiaal met kunstmest) en conserveringslandbouw (CA, een combinatie van minimale grondbewerking, wisselteelt en toepassen van gewasresten) een positief effect kunnen hebben op het voorkomen en het functioneren van gemeenschappen van arbusculaire-mycorrhizaschimmels. Dit positieve effect kan op zijn beurt leiden tot een betere bodemstructuur en een betere plantenvoeding, uiteindelijk resulterend in een hogere gewasproductie. Echter, de effecten daarvan moeten beschouwd worden in het kader van het totaal aan landbouwkundige maatregelen. Toepassen van stikstofbemesting, en vergroting van de efficiëntie waarmee deze stikstof door het gewas kan worden benut, zijn voor de Afrikaanse boer van essentieel belang.

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CURRICULUM VITAE

Mary Nyawira was born in Kenya on December 20th, 1974. She attended Mutuma primary school and later joined Kabare Girls School for her secondary education. In September 1994, she joined Moi University in Eldoret, and graduated with a Bachelor's Degree in Forestry in December, 1998. She did her B.Sc. dissertation on 'Effects of ectomycorrhiza inoculation on incidence and severity of fusarium root rot in *Pinus radiata*'. She then worked as secondary-school teacher for 16 months before joining Maseno Regional Research Centre, Kisumu in May, 2000. She worked there as project staff in a collaborative research project between Kenyan Forestry Research Institute (KEFRI), Kenya Agricultural Research Institute (KARI) and International Centre for Research in Agroforestry (ICRAF). Her main work was based at KEFRI Biotechnology Laboratory, and comprised of extraction, culture and assessment of arbuscular mycorrhizal fungi, rhizobia and nematodes associated with improved fallow systems. In November 2001, Mary began her M.Sc studies at Soil Science Department of Moi University. She studied the 'Influences of improved fallow systems on arbuscular mycorrhizal symbiosis in maize' in a collaborative project between ICRAF and ETH-Zürich (Switzerland). In June 2004, she joined the National Museums of Kenya (NMK), Mycology section of Botany Department as a volunteer and was offered a job as assistant research scientist in March 2005. Her main work was to research, collect, document and preserve Kenyan fungi with main emphasis on arbuscular mycorrhizal fungi. In August 2007 she joined the Department of Soil Quality of Wageningen University as a sandwich PhD student. In this period she studied the influence of agricultural practices and management on arbuscular mycorrhizal fungal communities in Kenyan agro-ecosystems. During this time she continued to work on various activities at mycology section, NMK. Mary is married to Samuel Muchai and they have two children.

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PE&RC PhD Training Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Impact of Arbuscular mycorrhiza on agricultural management practices and their effects on soil structure, nutrient use efficiency and interactions with soil macro-fauna (2007)

Writing of project proposal (4.5 ECTS)

- Impact of Arbuscular mycorrhiza on agricultural management practices and their effects on soil structure, nutrient use efficiency and interactions with soil macro-fauna (2007)

Post-graduate courses (3.9 ECTS)

- Soil ecology; PE&RC (2010)
- Linear models; PE&RC (2010)
- Root ecology; PE&RC (2012)

Laboratory training and working visits (2.1 ECTS)

- Glomalin analysis; NIOO, Heteren (2007)
- In vitro mycorrhiza; UCL, Louvain, Belgium (2012)

Invited review of (unpublished) journal manuscript (2 ECTS)

- Applied Soil Ecology: managing AMF diversity in tropical agro-ecosystems (2012)
- Ecosystems: spatial variability of microbial biomass assesses by stable isotopes of PLFA and NLFA (2012)

Deficiency, refresh, brush-up courses (3 ECTS)

- Advanced statistics (2007)

Competence strengthening / skills courses (3.3 ECTS)

- PhD Competence assessment; WGS / PE&RC (2007)
- Project and time management; WGS / PE&RC (2007)
- Scientific publishing; WGS / PE&RC (2007)
- Information literacy and introduction Endnote; WGS / PE&RC (2008)
- Fundraising and scientific writing workshop; Kenya (2010)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC Day: is our civilisation able to stand the test of time? (2007)
- PE&RC Weekend (2012)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- Statistics, mathematics and modelling in production ecology and resource conservation (2007)
- Stable isotope in ecological research (2007)
- National Museums of Kenya; monthly seminars (2008-2011)
- Conservation and sustainable management of below-ground biodiversity (2010)
- Integrating agro-biodiversity with conservation to improve livelihoods (2010-2011)
- Biochar: the soil is the limit (2012)

International symposia, workshops and conferences (6.6 ECTS)

- Mycorrhizal symbioses – a biological tool for sustainable development in Africa; Dakar (2010)
- Agro-environment conference; Wageningen (2012)
- Integrated soil fertility management practices-from microbes to markets; Nairobi (2012)