SOIL MICROBIAL PROPERTIES AND APPLE TREE PERFORMANCE UNDER CONVENTIONAL AND ORGANIC MANAGEMENT

By

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DECLARATION

By submitting this thesis, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

Conventional (CON) soil management that permits the use of agrochemicals is currently the most common form of management in Western Cape deciduous fruit orchards. There is increasing pressure to minimise or eliminate synthetic agrochemical usage due to its potentially harmful effect on the environment, particularly to non-target soil microorganisms, and to the functions and processes they perform or mediate. In apple orchards, organic (ORG) practices exclude the use of synthetic pesticides and herbicides making use instead of organic fertilisers and naturally derived products as defined by organic certification programs. ORG practices aim to improve nutrient availability, yield, and long-term orchard sustainability relative to CON orchard management practices. If ORG and CON orchard floor management practices affect orchard ecosystems differently, such differences should be measurable in terms of differences in microbiological parameters.

In this thesis it is hypothesised that ORG practices would induce positive soil microbiological responses in Western Cape apple orchards relative to CON practices, and by inference general soil health and apple tree performance. To test this hypothesis a polyphasic approach was adopted. This involved measurement of soil microbial activities and functional diversities, by enzyme activity (using colorimetric assays) and carbon-substrate utilisation (using the BIOLOG™ system), respectively. With reference to the enzyme analyses, the performance of a literature-validated, enzyme-based soil health index was also tested. The analyses were supported by coarse-level comparisons of the magnitude of bacteria, fungi, actinobacteria and total heterotroph populations using traditional culturing techniques (dilution plating on growth media). The extent to which the microbial status differed between the applied ORG and CON treatments was thought likely to reflect such treatment-induced variables as soil nutrient status, apple tree nutritional response, tree growth and yield, all of which were determined. Because the root systems of deciduous fruit trees commonly extend to depths >60 cm in well-prepared soils, microbial enzyme activities in the soil depth intervals corresponding to the lower rootzone, were also investigated. This research was carried out in a randomized field trial. Finally, to gain a broader understanding of the effects of contrasting soil management systems on soil microbiology under a greater variety of environmental conditions, arbuscular mycorrhizal (AM) fungal dynamics were explored in a survey of commercial apple orchards. These orchards were selected to span the range of environmental conditions that occur in the apple production areas of the Western Cape.

Orchard soils under ORG management promoted richer microbial ecosystems, and appeared to be better able to sustain community metabolic diversity and, by inference, the functions mediated by soil microbial communities, than those under CON management. This

implies that ORG approaches possibly afford a better option to sustain critical ecosystem functions than CON management. This possibly explains why use of straw mulches and compost in accordance with ORG practices, compared with CON practices, promoted *β*-glucosidase, acid phosphatase and urease activities rather than affecting the abundance of the micro-organisms that produce these enzymes. Enzyme activities in the 0–30 cm soil intervals were also more effectively promoted by ORG than CON practices, although no differences were observed at lower depth intervals. ORG practices promoted functional AM associations more effectively than CON practices, but the abundance of glomalin, a beneficial by-product of AM fungi, was unaffected. The greater enzyme activities and higher root colonisation levels in the ORG treatments probably contributed to improved nutritional effects that caused greater vegetative growth, but lower yields, in the ORG treatments. Yield suppression was conceivably due to excessive vegetative growth induced by oversupply of compost and the mineral nutrients contained therein. The survey of Western Cape apple orchards suggested that neither glomalin nor root colonisation bore any specific relationship to production area, cultivation practice, scion x rootstock combination, or, in the case of root colonisation, with any chemical parameters. However, the effect of season on glomalin was conclusively shown, being higher in summer than in spring, as was the lack of any effect of year on glomalin and root colonisation.

Collectively, these results showed that ORG soil management promote soil microbiology, soil nutrient status, and apple tree performance compared to CON management.

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DEDICATION

This thesis is dedicated to my late father, Dennis Jacobus Meyer, for his loving support and sacrifices over all the years.

PREFACE

This thesis presents a compilation of articles, herein presented as experimental chapters, published in different peer-reviewed scientific journals. Some repetition between chapters, therefore, has been unavoidable. One style has been adopted.

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Literature review

1.1. Introduction

Deciduous fruit production is a major agricultural industry in South Africa with a turnover of ZAR 7.2 billion in 2014 (Rabé 2014). Much of the industry is located in the winter rainfall region of the Western Cape Province where about 23 000 ha of land in the cooler, higher-lying intermontane valleys of the Cape Fold Mountain belt are utilised for apple production, mainly Golden Delicious and Granny Smith. About 43% of the total apple crop (c. 27 million 12.5 kg cartons in 2014) is exported (Rabé 2014). The apple industry supports around 26 800 workers and 107 000 dependents (Rabé 2014), and is thus a major contributor to the economy, and to social security in the Western Cape, which is a mostly agricultural region where employment opportunities are few.

That the apple sector of the deciduous fruit industry has flourished since the early 1900's is evidence that, the Mediterranean climate of the area is suitable for apple production, notably in terms of adequate winter chill, under the present climatic conditions. Scheduled irrigation is nevertheless a prerequisite (Taylor and Gush 2009). In view of the acid, nutrient-depleted nature of the soils of the area, it is nevertheless unlikely that economic apple production levels could have been achieved, or sustained, if lime (finely ground calcium (Ca) and magnesium (Mg) limestones) and fertilisers (macro: nitrogen (N), phosphorus (P) and potassium (K)) and micro nutrient elements (copper (Cu), zink (Zn), manganese (Mn), and boron (B), which are routinely applied as foliar sprays) had not been regularly supplied. Determining how much lime and fertiliser to apply, and when, was the subject of extensive orchard and vineyard research in the mid to late 20th century. This work culminated in the compilation of analysis-based nutritional guidelines for tree and bush crops (Kotzé 2001) and also for vines (Conradie 1996). In response to global warming, vines are increasingly being grown in the traditional apple growing areas which are cooler than the lowlands. Where nutritional supplementation was required, the necessary mineral nutrient elements were, and for the most part continue to be supplied in the form of synthetic fertilisers.

During the late 20th century the desirability of using synthetic chemicals in all branches of agriculture began to be questioned by environmental and consumer groups. Pressure for greener, organic production methods presented an opportunity for retailers to market produce as "organic", indicating that production took place in accordance with rules specified and enforced by independent certifying organisations. Organic (ORG) agricultural production is a systematic approach to management which focuses on the food web and on nutrient

cycling in the absence of synthetic agro chemicals (El-Hage Scialabba and Hattam 2002), as used in conventional (CON) orchard management practices. A related perspective is that deciduous fruit producers need to reduce their carbon (C) footprints in line with the climate change/ global warming imperative. Soil C sequestration is seen as both beneficial and measurable in ways that can be used to benefit the conservation orientated image of the industry and promote the marketing of its produce. Switching from CON to ORG practices was thought likely to promote the required soil C storage. However, recent work has shown that the ability of the soil to function as a stable repository for C, a factor in climate change modeling has been overestimated. According to Van Groenigen et al. (2014), increased atmospheric CO_2 stimulates plant growth but also provides more C for microbial metabolic activity with the net result that the return of CO_2 to the atmosphere from both new and old-established soil C reserves remains appreciable. Increasing atmospheric CO_2 therefore increasingly limits potential soil C storage. According to Six et al. (2002), the ability to sequester C in soil is in any case limited to a maximum value dictated by the inherent physicochemical parameters of the individual soil.

An early response to consumer pressure to eliminate synthetic agrochemicals by the South African deciduous fruit industry was to implement a system intended to minimize environmental impacts due to undesirable practices, amongst which synthetic agrochemical usage was considered the most detrimental. This system, which is sometimes known as integrated production (IP) (Cross and Dickler 1994) permits the use of agrochemicals, but imposes market penalties for each usage, is currently the most common form of management in Western Cape deciduous fruit orchards, and will be referred to in this thesis as conventional (CON). Apple production practices that conform to the requirements of organic certification organisations, all of which specifically prohibit synthetic agrochemical usage, have also been developed in the Western Cape. Henceforward, these will be referred to as organic (ORG). Control of insect pests apart, the main differences between CON and ORG practices concern the orchard floor.

The importance of the orchard floor is that it is not only the bearing surface for all forms of transport but the interface through which water and fertilisers must traverse before entering the body of the soil. It is also the boundary layer through which gas exchange takes place, and the zone that is inhabited by green cover plants and weeds which root in the soil and photosynthesize in the overlying atmosphere. The characteristics of this surface are affected by orchard floor management practices (Fourie 2007).

The effects of soil surface management practices have been extensively researched in vineyards, notably by Fourie (2007) and Fourie and Raath (2010), but to a lesser extent in orchards (Fourie et al. 2011; Wooldridge et al. 2013a, 2013b). CON orchard floor management entails the supply of mineral nutrients as synthetic fertilisers and the use of

synthetic herbicides to control weed growth in the tree rows (Wooldridge et al. 2013a). Conversely, in ORG orchards, mineral nutrients are supplied in organic form (compost, or liquid compost derivatives), and organic mulches (commonly cereal straw) are used to suppress weeds in the tree rows (Wooldridge et al. 2013a, 2013b). Mulching of the tree rows, for all or part of the year, is usually carried out in combination with green cover crops in the access lanes (work rows or alleys) (Fourie et al. 2011).

Published research concerning the effects of CON and ORG treatments in South African apple orchards mainly stems from an orchard trial at the Elgin Research Farm of the Agricultural Research Council in the Overberg deciduous fruit growing area. In this trial a variety of CON and ORG treatments were applied to 'Cripp's Pink'/M7 apple trees over seven consecutive seasons. The principle findings were that, relative to CON treatments, the application of commercial compost to the tree rows (ORG) led to increased soil P, K and pH levels (Wooldridge et al. 2013a). The ORG treatments were also associated with excessive vegetative growth and poor bearing. The effects of the treatments on soil N and C levels were nevertheless inconsistent (Wooldridge et al. 2013b). The overall conclusion was that the implementation in apple orchards of practices that make use of commercial composts are likely to lead to reduced yields and, potentially, to poor fruit quality. These undesirable performance parameters were ascribed to the fact that the compost used in the ORG treatments supplied N, P and K in amounts and proportions that were not conducive to the production of high yields of good quality apple fruit (Wooldridge et al. 2013a, 2013b). By inference, unless approved organic nutrient carriers are manufactured which deliver N, P and K in appropriate amounts at for each of the orchards' phenological stages, CON practices are likely to be preferred by apple producers for the excellent reason that nutrient supply can be more effectively tailored to the changing demands of the tree than by the use of compost, which delivers all elements at much the same, but diminishing rates, over a protracted period of time (Wooldridge et al. 2013b). A matter that is of concern is that many commercial compost products derive their N from municipal sewerage sludge, which invariably contains heavy metals, or from chicken or kraal manure, both of which may carry antibiotics (Chen and Jiang 2014). The potential effects of these materials on soil microbiology as well as on consumers of foodstuffs grown on compost-supplemented soil are unclear, as are the potential effects of microbes which survive the composting process.

Linked with the increasing consumer preference for organic produce in a progressively more health and environmentally conscious world is the widespread advertising and popular publication-driven belief that ORG production techniques promote healthier (*sensu lato*) orchard ecosystems, of which soil microbiology is a major component (as outlined in the Harvard Soil Health Manual, Gugino et al. 2009) and, by inference, healthier food, than CON techniques. Evidence for and against this belief is sparse, of doubtful provenance and

usually ambiguous. The issue of organic versus synthetic sources of mineral nutrient elements is nevertheless a socially emotive one (El-Hage Scialabba and Hattam 2002), which is potentially able to affect the marketing and consumer acceptability of “organic” produce (as variously defined by retailers).

From the viewpoint of the apple producers, who must optimise production techniques to meet medium to long-term consumer preferences, clarification of the real (if any), as opposed to popularly perceived benefits of ORG over CON management, is urgently required. The basic decisions that producers have to make concern how to balance their enterprise structures in view of the fact that although certified organic produce realises a higher price than that from conventional orchards, yields of marketable organic fruit tend to be appreciably lower due to nutrition-induced imbalance in the trees between vegetative and reproductive (buds, flowers, fruit) growth, and damage to the fruit caused by poorly controlled orchard pests. Cases may potentially arise where, in order to fulfil quotas, unprofitable organic production is subsidised by conventional production (Wynen 2002).

In environmental terms, a valid case may be made for promoting organic production of agricultural crops through the conversion of existing organic wastes into nutrients. This process generates very little greenhouse gas, and utilises less energy than synthetic fertiliser manufacture (Reginald et al. 2001). The case for organic production would probably be strengthened if evidence were found that organic production leads to improvements in soil microbiology that outweigh any associated loss in production.

If ORG and CON orchard floor management practices do indeed affect orchard ecosystems differently, such differences should be measurable in terms of differences in microbiological parameters between similar orchard soils that have been simultaneously subjected to CON and ORG managements. If differences between these treatments can be established by experimentation, then the null hypothesis – that ORG and CON treatments do not affect soil microbiology – will have been disproven (under the prevailing experimental conditions).

In order to be acceptable to the deciduous fruit industry, the research described in this thesis was carried out under the conditions that prevail in commercial apple orchards of the winter rainfall region of the Western Cape, South Africa. Specifically, the research presented here was, with the exception of the survey described in Chapter 8, carried out in the same trial orchard, and over much the same time period, as the trials of Fourie et al. (2011) and Wooldridge et al. (2013a, 2013b). Information concerning the effects of a number of CON and ORG treatments on soil, cover crops and tree performance parameters, was therefore available to complement the soil microbiological data that was obtained during the research that formed the body of this thesis.

1.2 Soil perspective

1.2.1 Physical setting, soils

The soils of South Africa, the southern extremity of the sub-Saharan African subcontinent, form a diverse assemblage (Fey 2010). The landscapes and soils are products of the erosional downcutting and sub aerial weathering that prededed, and intensified following the fragmentation of the Gondwana supercontinent in late Mesozoic times. Erosion still continues, with little rejuvenation from uplift in the Western Cape, and no evidence of glacial rejuvenation. Most apple varieties require chilling during winter. For this reason most apple production takes place in the cool, upland valleys between the sandstone ramparts of the mountains of the Cape Fold Mountain Belt. This belt parallels the south western Cape coastline where it is exposed to the cooling effect of the south easterly trade winds in summer. Rainfall mainly occurs in winter when the south western Cape falls under the influnce of storm-front bearing winds from the south west. The climate is Mediterranean, with cool wet winters and warm, dry summers (Schulze 1972). This thesis mainly concerns the Elgin apple producing area, which is an intermontane, faulted, basin-like depression in the hiatus zone at the intersection of the limbs of the Cape Fold Mountain Belt (Gresse 1988; Wooldridge and Beukes 2003). The basin floor has an undulating topography and is floored by Bokkeveld shale with overlying drift deposits from the surrounding Table Mountain Sandstone highlands (Wooldridge and Beukes 2003; Oberholzer and Schloms 2013). The soil at the trial site is fairly typical of the area, grading from sandy loam, enriched at some depth intervals with relict plinthitic gravel, into clay loam or silty clay loam derived by *in situ* weathering of Bokkeveld shale, which tends to be kaolinitic. Signs of wetness (mottling) usually increase with depth. Bottomland soils are generally plinthic, and include such forms as Dundee (stratified alluvium), Westleigh, Fernwood, Cartref, Pinedene, Longlands and Kroonstad. Bainsvley, Pinedene and Avalon may occur in orchards on the mid slopes (Soil Classification Working Group 1991; Fey 2010).

1.2.2 Soil properties and orchard soil preparation

In their virgin, veld state the soils of the Elgin area are vegetated with fynbos, locally planted with pine, and are depleted in base cations (and therefore acidic), P, and in trace elements, particularly Zn, Cu, B and Mn. Levels of soil organic C are generally below 1% (Wooldridge 2009). Prior to planting, virgin and existing orchard soils are cleared, topdressed with calcitic/dolomitic lime and P (based on prior chemical analysis), and cultivated by cross ripping or ploughing with a delve (deep) plough to 60 cm or (rarely) 90 cm depth. This soil depth is usually adequate for apple rootstocks which tend to be bushy because tap roots are severed during lifting from the nursery bed. Few apple roots were observed below the

cultivation depth by Wooldridge et al. (2013b). The final cultivation is carried out in a direction that is diagonally down slope, to facilitate drainage. During soil preparation the aim is to neutralise exchangeable acidity, bring the soil pH (KCl) to about 5.5 and achieve a Bray II extractable soil P level of 25-30 mg/kg over the full cultivated soil depth (Conradie 1996, vineyards; Kotzé 2001, tree and bush orchards). Exchangeable Ca, Mg and K are usually maintained at levels of, respectively, 70-80%, 10-12% and 4% of the sum of exchangeable cations (S-value) throughout the economic life of the orchard. Because of its solubility, K is usually supplied as a topdressing. Nitrogen application rates are calculated on the basis of tree vigour and anticipated yield (Kotzé 2001). Increasingly, nutrients are being applied at specific phenological stages (Kangueehi et al. 2011).

Unlike soil chemical properties, which can be adjusted with lime and fertilisers, soil mineralogy and texture are permanent. Bulk mineral / textural composition can nevertheless be rendered more homogenous by the mixing action of deep cultivation which disrupts texturally disparate layering, whether of depositional origin or created *in situ* by pedogenic processes (Soil Classification Working Group 1991; Fey 2010). In South Africa and elsewhere, research concerning physical / mechanical soil preparation has been more extensive in vineyards than in orchards (White, 2015). Vineyard soil preparation procedures developed by Van Huyssteen (1977, 1989) were adapted to Western Cape orchards. In the absence of cultivation, stratification would probably inhibit uniform root penetration, water and air diffusion, and the movement of slowly mobile elements, notably P, into the subsoil. The soil preparation that was carried out at the Elgin trial site (Wooldridge et al. 2013a) was designed to overcome these problems. The effects of this preparation and of subsequent manipulations on soil chemistry over the full trial period have been described by Wooldridge et al. 2013a). The effects of this preparation and of certain management practices applied to the prepared soil surface, on the performance of ‘Cripps Pink’/M7 apple trees were described by Wooldridge et al. (2013b).

Irrigation is a necessity in commercial Western Cape apple orchards during the warm, dry summer. Volschenk (2013) showed that gross farm income from apple in the Cold/Koue Bokkeveld increased with increasing seasonal rainfall plus irrigation. Irrigation is critical in ‘Golden Delicious’ apple during the vegetative growth/cell division and fruit ripening stages (Volschenk and Gindaba 2014). In ‘Forelle’ pear, deficit irrigation during fruit ripening led to wrinkling and reduced fruit mass (Volschenk 2014). To what extent irrigation affects soil microbiology in apple orchards is unclear but, in view of the operational necessity of irrigation, the matter is not of great practical significance.

Soils (mineral materials which pass through a 2 mm sieve) are classified in terms of the percentage (weight basis) of particles that fall into recognised size ranges (sand, silt, clay). Class names reflect the proportions in which materials in these three size ranges are

represented in the < 2.0 mm fraction. Soils also contain organic matter in all stages of decomposition, air and water. They are thus complex, multicomponent systems. Soil organic matter (SOM), which ranges from < 0.5% in arid region soils to > 40% in peat soils, may be generated within the soil, mainly by root growth, exudate production and microbial synthesis of carbohydrates and proteins, or it may be carried into the mineral soil by faunal activity (Brady 1974). The kneading action of root growth, combined with organic breakdown products, creates aggregates of mineral particles. These aggregates may remain stable for protracted periods, contribute to a porous, low density soil structure through which gases and water permeate readily, and present extensive surface areas on which cation exchange may take place. Humus, the end stage of organic decomposition, has cation exchange capacities that are two to three times higher than any of the minerals in the clay fraction (Brady 1974). Microbial (enzymatic) decomposition of SOM progresses through stages in which carbohydrate breakdown releases metabolisable energy, and minerals are released to the soil solution, pending root or microbial uptake and tissue synthesis, uptake on exchange sites or loss through leaching or volatilization (McGill and Cole 1981; Gerke et al. 1999; Davet 2004; Wolf and Wagner 2005).

SOM levels vary with climate and topography (catena, or slope position). SOM contents of Bokkeveld shale soils from the B21 horizon of a catena in the upland, Elgin deciduous fruit growing area varied from 1.52% (upper slope/crown) to 2.78% (footslope) (Wooldridge 1988). Smuts (2001) found that of a population of 78 Western Cape soils, 54% contained <1.0 % C, 27% contained 1.0-2.0% C, and 19% contained >2.0% C. Of 38 vineyard soils from the warm coastal lowlands, 20 contained <0.5% C, 8 contained 0.5-1.0 %C and 10 (all topsoils) contained >1.0% C (Van Schoor 2001). Thus SOM, which acts as a metabolisable substrate for microbes and is the most powerful exchange medium (and chelating agent) in soils, is present in very low concentrations in the orchard and vineyard soils of the Western Cape. By inference, management practices that increase SOM levels should bring about increased microbial activity and nutrient buffering/retention, although organic acids generated during SOM breakdown are likely to lead to acidification and base depletion, coupled with a concomitent increase in the solubility of aluminium, which may reach phytotoxic levels at pHs below c. 4.7 (1 M KCl) (Smuts 2001).

Mobility of air and water in soils is dependent on pore volume and on the interconnectedness of the soil pores. These parameters are influenced by the distribution of mineral particle sizes, and also on the volume of free space between soil agregates. Aggregates are usually formed by the kneading action of roots, and depend for their stability thereafter on the adhesive-like effects of such organic decomposition products as glomalin, a bi-product of arbuscular mycorrhizal fungal activity (Carrizo et al. 2015). Soils also contain a wide variety of living organisms, mostly microscopic. The combined effects of the

interactions between the different soil components determine the physical, chemical and biological properties of the soil. According to Gugino et al. (2009) soil properties can be seperately scored against established norms. The completed score sheet serves as an indicator of overall soil quality *sensu lato* (“health”, Gugino et al. 2009), and indicates areas where remediation is necessary. Soil properties (indicators) used in the scoring process are broadly categorised as physical, chemical and biological. By considering biological factors, the Cornell score sheet (Gugino et al. 2009) goes beyond the scope of the assessment methods currently used for Western Cape soils which emphasise physical (Van Huyssteen 1977, 1989; White 2015) and chemical (Conradie 1996; Kotzé 2001) parameters.

1.3. Microbiological perspective

1.3.1 Soil microflora

Microorganisms, which constitute only 1% to 3% of the total organic C in soil, inhabit less than 5% of the overall available space in the soil (Ingham et al. 1985; Voroney, 2007), in so-called microhabitats (Stotzky 1997; Voroney 2007). Yet, the biomass supported by these micro-zones is enormous. Populations of soil microorganisms in these zones are greatest seeing that access to nutrients is usually easiest (Ingham 2000; Schütz et al. 2009). Therefore, soil organisms are concentrated around roots, in litter, on humus, on the surface of soil aggregates and in spaces between aggregates. Coexistence of microorganisms appears to be mostly due to better resource partitioning (Giller 1996; Ettema and Wardle 2002). Different groups of organisms can be distinguished in the soil (Brussaard and Juma 1996). Microflora, i.e. bacteria, fungi and actinobacteria (previously known as actinomycetes), together with the fauna, are key in the decomposition chain (Neher 1999).

Having strong links with aboveground ecosystems, microflora and fauna are absolutely central to natural, as well as agricultural systems, particularly with regards to crop production and soil fertility (Setälä 1995; Laakso and Setälä 1999; Davet 2004; Gobat et al. 2004). An active, thriving microbial population is generally a good indication of soil fertility. Changes due to dissimilar management practices are likely to be reflected by the changes in substrate availability and hence, in the prevalence of different microbial groups (Cardoso et al. 1992). In accordance with rough calculation, bacteria make up the most abundant group of microorganisms in the soil, followed by actinobacteria, fungi, yeast, algae and protozoa (Pelczar et al. (2010). As per biomass, fungi are nevertheless the most superior due to the larger diameter and extensive network of finely-branched filaments. The amount of hyphae in the soil is measured in hundreds or thousands of meters of length per gram of soil. It is estimated that the total length of hyphae in a gram of soil can, at any given time, contain up to a kilometre of fungal hyphal tissue (Voroney 2007). The next few paragraphs provide a

brief summation of some important organisms in terms of their key biological and ecological attributes and relevance to agriculture, mostly from a beneficial point of view.

Microorganisms have a wide variety of nutritional requirements, depending on the characteristics of individual species. Depending on the source of C, microorganisms are classified into autotrophs and heterotrophs (Alexander 1977; Pelczar et al. 2010). Autotrophs use inorganic CO_2 as their C-source for growth; their N comes from inorganic compounds such as NH_3, NO_3^-, or N_2. Heterotrophs require organic C-sources (sugars, proteins, fats or amino acids) that are directly supplied as a substrate from an exogenous source. Microorganisms also require energy (ATP) which in most cases is obtained through an electron transfer process via metabolic pathways (Alexander 1977; Pelczar et al. 2010). There are three main sources from which microorganisms gain energy. These are light, and inorganic and organic compounds. Through these different methods of electron transfer, microorganisms can be further classified into even smaller subgroups, photo-autotrophs and chemo-autotrophs (Alexander 1977; Pelczar et al. 2010). Photo-autotrophs use light, and the process of photosynthesis to produce energy. Chemo-autotrophs use organic and inorganic chemicals to produce energy.

Most bacteria, actinobacteria and fungi are aerobic heterotrophs (Davet 2004). Some bacteria are autotrophs, some photo-autotrophs, and others chemo-autotrophs. All autotrophic bacteria possess essentially the same organic cellular constituents found in heterotrophic bacteria. However, from a nutritional viewpoint, the autotrophic mode of metabolism is unique, occurring only in bacteria. Bacteria are important in agricultural soils because they contribute to the C-cycle by fixation (photosynthesis) and decomposition. Chemo-autotrophic bacteria, like nitrifying bacteria and certain free-living bacteria, like the nitrobacters species, are capable of fixing atmospheric N (Postgate 1998), which is the conversion of atmospheric N into N-containing compounds (such as ammonia) that can be used by plants. This recycling process, by which organic and inorganic N compounds are used metabolically and recycled among bacteria, plants, and animals, involves a series of processes including ammonification, mineralisation, nitrification, denitrification, and N fixation (Sylvia et al. 2005). Biological N fixation is highly beneficial in agriculture (Mehboob et al. 2009). Other groups of chemo-autotrophic N-fixing bacteria, collectively called rhizobia, form a symbiosis with the roots of higher plants (e.g. legumes) inside root nodules and fix N from the air into ammonium N that can be used by plants (Postgate 1998). The amount of autotrophic bacteria is small compared to heterotrophic bacteria, but are nevertheless very important because most plants and other organisms requires N in some way, and would have no way of obtaining it if not for N-fixing bacteria.

Actinobacteria resembles fungi, but are actually a type of filamentous bacteria, lacking nuclei, like other bacteria (Erickson 1949; Pelczar et al. (2010). Actinobacteria share some

characteristics with fungi, including shape and branching properties, spore formation and secondary metabolite production. They are responsible for the distinct ‘’earthy’’ smell of the soil from the production of their metabolic by-product, geosmin, that is usually noticeable during rain falls after a dry spell of weather (Gerber and Lechevalier 1965). As decomposers, actinobacteria specialize in chemically degrading or digesting (via enzymatic action) tough (structurally complex) molecules such as cellulose and lignin, the component of cell walls of plants or woody material, and chitin found in the hard exoskeletons of insects, as well proteins. The breakdown of these materials, particularly pertinent to composting (McCarthy and Williams 1992), makes nutrients once more available to plants.

Fungi, like bacteria, are important for immobilizing or retaining nutrients in the soil. Because most fungi consume organic matter for nutrition, the quality and quantity of organic matter in the soil has a direct correlation to their growth. They are typical heterotrophs. Variation in organic substrate availability, depending on the soil management strategy, can thus affect nutrient levels and hence can cause a shift in dominance of decomposers from bacterial to fungal. They are essential for decomposing the C-ring structures in some pollutants (Peng et al. 2008). Fungi, like the actinobacteria, are one of the few kinds of microorganisms that secrete enzymes that are able to break down cellulose into glucose (Enari and Markkanen 1977), enabling other microorganisms to continue the decomposition process when most of the cellulose has been exhausted. Like actinobacteria, fungi also decompose lignin, the fungi being the only known microorganisms that degrade lignin completely, whereas the metabolism of lignin in bacterial systems is less oxidatively powerful (Brown and Chang 2014). Mycorrhizas are a unique group of beneficial fungi that form a dense network of thin filaments that reach far into the soil, acting as extensions of the plant roots that they are in symbiosis with. These fungi facilitate the uptake of water and a wide range of nutrients and offer other numerous benefits to the plant and soil (see section 1.4).

Soil microorganisms respond rapidly to changes, hence they adapt to environmental conditions (Nielsen and Winding 2002), and the microorganisms that are best adapted, dominate. The numbers, kinds and activities of microorganisms present in soil, may therefore depend on such factors as soil moisture, soil temperature, degree of aeration, soil pH, soil depth, etc. (Prescott et al. 1999). Microbial functioning is most effective under conditions optimal for metabolizing their substrates. The decomposition rate is directly proportional to the numbers of microbes present. Thus, soil environmental conditions that favour the growth of microorganism will most likely favour fast decomposition rates. Both quantity and quality of mineralizable C-substrates have been shown to decrease as the depth of the soil column increase (Blume et al. 2002; Bausenwein et al. 2008; Gelsomino and Azzellino 2011), with soil organic matter becoming less degradable with depth (Richter and Markewitz 1995; Ajwa et al. 1998; Trumbore 2000), which is reflected in the changes in

the assemblages of microorganisms down through the soil profile (Zelles et al. 1995; Bossio and Scow 1998; Griffiths et al. 1999; Schütz et al. 2009).

One of the most influential factors affecting the microbial community in soil is pH. Soil pH may control the biomass composition of fungi and bacteria (Fierer et al. 2006), in agricultural soils (Arao 1999, Bardgett et al 2001). The majority of soil microbes, especially most bacteria, grow best in an environment with a narrow pH range near neutrality (Rousk et al. 2009) between pH 6.5 and 7.5 due to the high availability of most nutrients in this pH range. Plant nutrient availability is greatly influenced by soil pH (Kemmitt et al. 2005; Kemmitt et al. 2006; Pietri et al. 2008). There are, however, microbes, for example fungi that are adapted to a wide range of pH between 1 and 13 (Sylvia et al. 2005), and they thrive in acidic environments (Rousk et al. 2009), dominating the conditions that most bacteria and actinobacteria do not normally tolerate. Those bacteria that grow at pH extremes are classed as acidophiles (acid-loving) or alkalinophiles (base-loving). Some of these include the *Halomonas* and Archea *Archaeoglobus* (LaPaglia et al. 1997). Alterations in pH can render essential microbial enzymes inactive and/or denature proteins within the cells and prevent microbial activity from occurring (Sylvia et al. 2005). Changes in pH can also affect microbes in their access to metals and organics that react differently under varied pH régimes (Flis et al. 1993; Sylvia et al. 2005). In contrast, in high pH, humic soils, actinobacteria usually dominate over fungi (Goodfellow and Williams 1983). Changes due to altered soil management caused by practices such as liming are likely to be reflected in shifts from fungal to bacterial (Cardoso et al. 1992).

Depending upon oxygen requirements, soil microorganisms are grouped into categories either as aerobic (require oxygen for life processes), anaerobic (do not require oxygen) and microaerophilic (requiring low levels of oxygen (Prescott et al. 1996; Hogg 2005). Microorganisms proliferate best in the moisture range of 20% to 60%. Under excess moisture or water logged conditions, anaerobic microflora predominates whereas the oxygen-reliant aerobes are inhibited due to a lack of soil oxygen. Fungi, and also actinobacteria, grow well in dry, soil conditions as they are very much aerobic, whereas bacteria are much less tolerant of these conditions (Griffin 1981). This may explain why fungi typically reside just beneath the soil surface layer. By comparison, bacteria may thrive under high moisture and low oxygen conditions, and are responsible for most of the biological and chemical changes that occur under anaerobic conditions (Sommers et al. 1981).

Next to moisture, temperature is probably the most important environmental factor influencing microorganisms, their population dynamics, as well the functions and processes they mediate and catalysed. Depending on the temperature range at which microorganisms can grow and function, they are divided into three groups, i.e. psychrophiles (growing at low temperature below 10°C), mesophiles (growing well in the temp range of 20°C to 45°C) and

thermopiles (tolerating temperature above 45°C). Most soil microorganisms are mesophilic and optimum temperature for most mesophiles is 37°C. True psychrophiles are almost absent in soil. True thermophiles are more abundant in decaying manure and compost heaps where high temperature prevails (Lechevalier 1988; Phae et al. 1990; Hatsu et al. 2002). Seasonal changes in soil temperature affect microbial population and activities especially in temperate regions. In winter, when temperature is lowest, the number and activities of microorganisms drop, and as the soils warms up in spring, there numbers and activities start to pick up again, reaching a maximum in summer (Cartes et al. 2009).

1.3.2 Soil microfauna

The soil fauna are a diverse group of soil animals divided into various categories according to size, which include macrofauna (>2 mm, e.g. earthworms), mesofauna (0.1-0.2 mm, e.g. mites and nematodes), and microfauna (<0.1 mm, e.g. protozoa). The meso- and macrofauna are not small enough to be considered microscopic. Nevertheless, soil fauna are important from an organic matter breakdown and nutrient recycling point of view and are therefore also briefly discussed. Of these, protozoa, nematodes and earthworms have arguably been studied the most for their role in soil fertility (Alexander 1977; Yeates 1979; Forge et al. 2003; Gobat et al. 2004).

Microfauna feed heterotrophically. Protozoa, which is the simplest form of animal life, is probably the best known, having abilities to either adsorb solubilized organic and inorganic substances, or directly feed upon microbial cells or other particulate matter by what is termed, phagotrophic nutrition (Alexander 1977). They typically prey upon algae, microfungi and bacteria. The metabolites produced by protozoa seem to be particularly stimulating to bacteria (Clarholm 1985) and combined with certain feeding preferences, are instrumental in controlling bacteria populations and biomass (Seastedt 1984). Because of their relentless grazing abilities the contribution of protozoa to mineralisation of N in agricultural systems are thought to be very prominent (Gugino et al. 2009). Protozoa themselves fall prey to certain larger organisms. In the absence of sufficient moisture, the activities of protozoa are very much limited.

Mesofauna (0.1 - 2 mm in diameter) includes mainly microarthropods, such as nematodes, springtails, mites, and the worm-like enchytraeids. Mesofauna have limited burrowing ability and generally live within soil pores, feeding on organic materials, microflora, microfauna and other invertebrates. Soil-inhabiting nematodes are arguably the best-known due to their feeding habits and economic damage to cultivated plants by root feeding nematodes, the so-called herbivores. Most known nematodes are actually free living and are generally beneficial to plants (Gobat et al. 2004). Bacterial-feeding nematodes (bacterivores) and fungal-feeding nematodes (fungivores) contribute much to N mineralisation (Ekschmitt

et al. 1999) and, depending on their food preference, may alter the fungal-bacterial balance, and cause changes in species composition (Ferris et al. 2001). Predators feed indiscriminately on other soil nematodes, both plant parasitic and free-living, and other animals of comparable size. Predatory nematodes are nevertheless less common, but some are found in most soils.

The macrofauna are large macroscopic organisms, which are visible to the naked eye (generally >2 mm in diameter), e.g. earthworms, insects, and millipeds. They all play an important role for improving aggregation, soil drainage and aeration due to their burrowing nature). They require well-aerated environments, adequate moisture and warm temperatures (Gobat et al. 2004). In both natural and agricultural systems, soil macrofauna are important regulators of decomposition, nutrient cycling, soil organic matter dynamics, and pathways of water movement as a consequence of their feeding and burrowing activities (Gugino et al. 2009).

1.3.3 Soil organic matter

The soil organic fraction encompasses all organic matter in the entire soil body, both living and dead, that makes up the smallest part of the total soil volume, yet is fundamental in crop production and soil fertility (Davet 2004; Gobat et al. 2004). Soil organic matter can be conveniently divided into three major pools (Brady and Weil 1999), including (1) living biomass of soil organisms of various sizes (microflora and fauna), along with microscopically small remains of plants (including roots) or animals that are freshly returned to the soil (serving as parent material for SOM), (2) decomposing (active, or easily decomposed) organic residues at various stage of decomposition (detritus), and (3) a relatively stable, dark coloured organic matter fraction that had undergone extensive modification, to become the final product of decomposition, humus. (Tisdall and Oades 1982; Parton et al. 1987; Stevenson 1994; Juma 1998). As a general rule, surface litter is not part of SOM.

Evidently, although SOM can be partitioned conveniently into different forms or various fractions (Tate 1987; Theng 1987), these do not merely represent static end products, but instead reflect a dynamic equilibrium that sustain critical life processes.

1.3.3.1 Decomposition and nutrient cycling

Decomposition is largely a biological process that occurs naturally and includes the physical breakdown and biochemical transformation of complex organic molecules into simpler organic and inorganic molecules (Juma 1998). Depending on the chemical structure, the process of decomposition can be rapid (active) (sugars, starches and proteins), slow (cellulose, fats, waxes and resins) and very slow (passive) (lignin) (Brady and Weil 1999; Brown and Chang 2014). This active fraction is influenced strongly by weather, soil moisture

status, growth stage of the vegetation, entering of organic matter residues, and certain cultural practices. The processing and recovery of key nutrients from SOM is really a specialized function bestowed mainly to soil microflora and fauna that rely on organic matter for a source of food and energy (Alexander 1977; Larson and Pierce 1991; Tisdall 1996; Davet 2004).

The decomposition process of organic matter involves a series of progressive stages. Micro-organisms are the first to arrive and to colonise the organic material, but the process really sets off initially as being mainly mechanical, the organic matter being torn apart then eaten by soil fauna, mostly macrofauna, mainly by bioturbation by soil- and litter-feeding invertebrates such as insects and nematodes, snails, by earthworms via digestive enzymes (Bouché 1977; Lavelle 1981; Yeates and Coleman 1982; Ponge 1991). The disintegrated organic material produced by these animals is termed detritus. Earthworms are examples of detritivores, or organisms that consume detritus for energy, performing crucial functions as mixing residues into the soil and increasing the surface area to be more susceptible to microbial attack (Seastedt 1984; Paul and Clark 1996). The formation of particulate organic matter (POM), consisting of fine particulate detritus (Cambardella and Elliot 1992; Brady and Weil 1999) is at a transitional stage of decomposition, yet relatively stable, and serving as an important long-term supply of nutrients (Wander et al. 1994). Once mixed into the soil, decomposition rapidly becomes more biochemically-driven as progressively more microorganisms colonise the detritus to further the decomposition process. The actions involved are mainly enzymatic, mainly by bacterial and fungal enzymatic catalysis, requiring the production (secretion) of a suite of extracellular enzymes to process complex organic compounds into assimilable monomeric subunits (sugars, amino acids, etc.) (Dick 1994; Nannipieri and Badalucco 2003).

This way, bacteria (including actinobacteria) and fungi, being saprophytic, feed upon the organic matter by breaking down C structures and rebuilding completely new ones (Foth and Turk 1972). Initially, the activities of fungi appear to be stimulated, at the same time bacteria are maintained in a constant population so long as nutrients are available, whilst actinobacteria become more prominent at a later stage of decay. Bacteria have difficulty breaking down the more complex molecules like cellulose and lignin, whereas the metabolism of lignin in fungal systems is probably the most advanced. Fungi enable bacteria to continue the decomposition process when most of the cellulose has been exhausted. The nutrients remaining are used by other microorganisms (Wolf and Wagner 2005), or is released in plant-available form (Jenkinson and Ladd 1981; McGill and Cole 1981) via the process of mineralisation. The saprophytes themselves are fed upon by microfauna (e.g. Protozoa), and the microfauna by mesofauna (such as microarthropods) and macrofauna (such as earthworms).

The waste products produced by micro-organisms also count as SOM, although it is less decomposable than the original detritus, but it can be used by a large number of organisms. As one organism feeds on the other a whole series of conversions of energy and nutrients takes place, from primary producers to consumers. The total complement of soil organisms involved in this type of trophic interactions, forms, what is termed, the ‘’soil food web’’ (Gugino et al. 2009). Evidently, cycling of nutrients involves the movement of nutrients from the physical environment into living organisms, and subsequently, recycling (back) to the physical environment. The nutrient cycle must be balanced and stable if the organisms inhabiting the environment are to flourish and to be maintained in a constant population.

In contrast to the preceding active and slow soil organic matter stages, the organic matter, not completely decomposed, moves into a passive stage (pool) of organic matter. This portion of organic material is the resultant final (highly stable) product left after extensive chemical and biological breakdown that does not decompose readily because of its intimate interactions with soil mineral phases and is chemically too complex to be used further by most soil organisms (Prasad and Power 1997). This portion of organic matter that does not get mineralised is generally termed, humus, and the process, humification (Hernando 1975) (Tisdall and Oades 1982; Parton et al. 1987; Stevenson 1994).

The complex array of substances contained in humus, largely of vegetal origin, includes modified lignins, oils, fats and waxes and newly synthesized compounds, like polysaccharides and polyuronids. Humic substances, due its complexity, are often divided into three components based on solubility and to stability (Pettit 2004). Fulvic acids, which are produced in the earlier stages of humus formation, contain light yellow to yellow-brown organic materials that have the lowest molecular weight, are soluble in water under all pH conditions, and are susceptible to microbial attack. Humic acids contain intermediate dark brown to black organic materials, with medium molecular weight, are soluble in water, except for conditions more acid than pH 2, and exhibit intermediate resistance to microbial attack. Humin is the portion of materials, darkest in colour, with highest molecular weight, are not soluble in water at any pH, unable to be extracted with a strong base as sodium hydroxide (NaOH), and are most resistant to microbial attack. Due to its resilience caused by the complex structure of humic substances, humus remains in the soil for relatively protracted periods (Pettit 2004).

1.3.3.2 Mineralisation, immobilisation, and the C:N ratio

The microbiologically-driven process in soil by which organic compounds in organic matter are chemically broken down by enzymatic oxidation and are converted to other organic compounds, simpler organic compounds or inorganic compounds, is referred to as mineralisation (Benbi and Richter 2002;Sylvia et al. 2005), the final end product being

nutrients in mineral form. When the enzymes have disrupted the compounds, bacteria and fungi use some of the parts released in this process as nutrients as a food source for energy. They store the C into their own biomass (e.g. their cell wall structures and cellular contents) for growth and reproduction. In addition to meeting the C and energy needs of microorganisms, other nutrients also become available, for example, when microorganisms mineralise protein, made up of C, N, P and sulphur (S), these additional nutrients are released. During mineralisation, protein may undergo various transformations into simpler organic molecules before the C is converted to CO_2, the N to ammonium, the P to phosphate and the S to sulphate (Jenkinson and Ladd 1981; McGill and Cole 1981), all of which being inorganic forms of nutrients, available to both microorganisms and plants for direct uptake.

The amount of C relative to the amount of N present in organic matter (C:N ratio) (Sylvia et al. 2005) may nevertheless determine the rate of decomposition and the rate of N supply to decomposing microorganisms and the release of N for plant uptake. If the organic material has a narrow C:N ratio, i.e. having a reasonably similar amount of C and N content, or the amount available for microorganisms is exceeded, decomposition releases N sufficiently into the surrounding soil solution for plant uptake. However, when the content of N (and often of nutrients as S and P) is low in organic residues proportional to the C composition of microorganisms (high C:N ratio residues greater than 30:1), decomposition is slow as the organic residues lack sufficient N to meet microbial needs. Subsequently, N, if present in soil solution, is absorbed directly and incorporated into organic molecules within microbial cells (i.e. into the microbial biomass), by a process commonly known as immobilisation, the opposite of mineralisation. Immobilisation is thus critical since it repositions mineral nutrients into pools with relatively quick turnover rates (Clarholm 1985; Freckman 1988; Ekschmitt et al. 1999), preventing possible loss by leaching. Immobilisation could however, temporarily deplete the soil's supply of soluble N, in which case plants will probably become N deficient, as plants, which take up nutrients in mineral form only, are out-performed by microorganisms competing for N in soil solution (Jackson et al. 1989).

Thus, incorporating organic matter of high C:N ratio, like straw mulch, is likely to cause some N deficiency in the crops, at least in the short-term. Nitrogen addition in the form of mineral salts would normally alleviate the problem to meet plant N needs and lessen competition between plants and microorganisms (Hogue and Neilsen 1987; Lipecki and Berbec 1997). Thus, the balance between mineralised and immobile forms of nutrients, by supply of nutrients via mineralisation and desorption, and binding of nutrients via immobilisation and adsorption, respectively (Carter and Stewart 1996; Reeves 1997), is critical to ensure a nevertheless steady supply of nutrients available to both microorganisms and plants (Mckenzie et al. 2001; Davet 2004; Ball 2006).

1.3.3.3 Functional attributes

Organic matter is an essential component of soil, as it serves multiple functions that help sustain physical, chemical and biological properties of soil. Humus contributes to the buffering ability of soil, improving properties such as soil pH (Stevenson 1986; Pieri 1992), with plant nutrient availability being greatly reliant on soil pH. Due to its chemical make-up, humus has the distinct ability to increase the soils' capacity to retain and supply nutrients on exchange sites, called the cation exchange capacity (CEC), thus acting as a major depository of plant nutrients, especially N, P, and sulfur (S), along with other equally important elements present in even smaller quantities, the micronutrients (Schnitzer 1986); all of these get slowly released upon mineralisation. The ability to withhold nutrients (and water), is very important from a soil fertility point of view as it gives plants the capacity for growth. Particulate organic matter (Brady and Weil 1999) serves as an important long-term supply of nutrients (Wander et al. 1994). Besides benefitting plant growth directly through physiological and nutritional effects, some organic substances perform numerous other functions in relation to plant growth, for example, as natural plant hormones (auxines and gibberillins), with some sugars capable of improving seed germination and root initiation (Schnitzer 1986)

A most striking characteristic of humic substances is that they supply organic compounds (chelates) to the soil solution that are able to react with (binds) metals (i.e. trace elements) such as Cu, iron (Fe), Mn, and Zn, protecting them from precipitating and hence from becoming insoluble and unavailable to plants (Stevenson 1994; Havlin et al. 1999). Iron, for instance, becomes nearly insoluble above neutral soil pH and chelation can greatly increase their availability (Havlin et al. 1999). Chelation can be increased through the use of commercial chelating agents, synthetic organic compounds, etc. Additionally, organic chemicals have been shown to inhibit precipitation of calcium phosphate minerals, possibly keeping fertiliser P in soluble form for longer periods (Grossl and Inskeep 1991). Organic complexes can also contribute to a reduction of toxicity, e.g. of aluminium (Al) in acid soils (Tan and Binger 1986), or to entrapped pollutants, i.e. herbicides such as Atrazine or pesticides such as Tefluthrin, in the cavities of the humic substances (Vermeer 1996).

Furthermore, the active and some of the resistant soil organic components, together with microorganisms (especially fungi, particularly AM fungi via the production of glomalin), are involved in cementing (binding) soil particles into larger aggregates. Aggregation is important for good soil structure, aeration, water infiltration, resistance to erosion, crusting and to improve decay of nutrients by certain microfauna and mesophauna (Stevenson 1994). Soil organic matter serves as a major source and sink of C in soil, the greater part of soil organic matter being made up of C (Périé and Ouimet 2008). Aggregation has been

linked with levels of total C (Matson et al. 1997), organic C (Dalal and Mayer 1986a, 1986b) and more recently, with the labile C fractions, in agricultural systems (Blair and Crocker 2000). Hence it is not surprising that the two concepts 'soil organic C' and ‘soil organic matter’ are often used synonymously without a clear distinction between them. SOM relative to the CO_2 pool in the atmosphere and the C pool in terrestrial plants, represent the largest sink for C and is recognized for its central role in the global C-cycle (Delgado and Follett 2002). Sequestering C in soil can be accomplished through soil conservation practices that not only reduce soil erosion, but also increase the SOM content, contributing to general soil health and possibly lowering overall input costs (Diacono and Montemurro 2010).

1.3.4 Soil enzymes

1.3.4.1 Origin of soil enzymes

All living systems, ranging from bacteria to the animal kingdom, from algae and fungi to the higher plants, contain a vast number of enzymes catalyzing both simple and complex networks of chemical reactions (Tabatabai and Dick 2002). Enzymes are found in ponds, lakes, rivers, water treatment plants, animal manures, and soils.

Soil contains intracellular enzymes contained within microbial cells as part of the living biomass, immobilized extracellular enzymes released outside the cell (separated from their origins) and stabilised by a compact three-dimensional arrangement of macromolecules, and free enzymes (Tabatabai and Dick 2002). Soils are thus, enzymatically active (McLaren et al. 1975), containing a whole complement of enzymes that are either of plant, animal or microbial derivation. Some may include amylase, arylsulphatases, *β*-glucosidase, cellulose, chitinase, dehydrogenase, phosphatase, protease, and urease, that are released from plants (Tabatabai and Dick 2002), animals, microorganisms (Tabatabai and Dick 2002), or from both plants and microorganisms (Nannipieri et al. 2011).

Nevertheless, microbially-secreted enzymes are thought to account for most of the enzyme activity in soil environments (Tabatabai 1994;), being secreted mainly by bacteria and fungi (Tabatabai and Dick 2002), and probably because of their large biomass, high metabolic activity, and short lifetimes, allowing them to produce and release relatively large amounts of extracellular enzymes in comparison to plants and animals.

1.3.4.2 State of enzymes in soils

Upon entering the soil environment, considerable amounts of enzymes from microorganisms or plant roots are inhibited by soil constituents, rapidly degraded by soil protease, or both (Tabatabai 1994; Dick 1997). Nevertheless, a diminutive, yet significant, fraction of measured active enzyme protein in soil that is of microbial derivation, sorbed to clays or

humic colloids to become stabilised, persists in the soil over protracted periods (Hayano and Tubaki 1985; Boyd and Mortland 1990; Busto and Perez-Mateos 2000).

Upon physicochemical adsorption or immobilisation, enzymes are exposed to a different microenvironment, the characteristics of which are determined by the chemical nature of the support material (Goldstein 1976). Mineral colloids have active surfaces that may affect the stability of enzymes either positively by enhancing their activity (Naidja and Huang 1996) or negatively by inhibiting their activity (Carrasco et al. 1995). Some aspects of interactions of proteins, including enzymes, with clays have been discussed in detail by Theng (1979). The protective influence of soil on extracellular enzymes by association of enzymes with humus through various bonding mechanisms (Ladd and Butler JHA 1975), to form humus–enzyme complexes, has also been sufficiently demonstrated (Boyd and Mortland 1990). Numerous studies have supported this conclusion by showing that enzyme activities in soils are significantly correlated with organic matter content (Tabatabai 2994). For example, studies have shown that extracellular urease associated with soil organo-mineral complexes is more stable than urease in the soil solution (Burns 1986) and the humus-urease complexes extracted from soil are highly resistant to denaturing agents such as extreme temperatures and proteolytic attack (Nannipieri et al. 1978). Ladd and Butler (1975) suggested that enzymes bind to soil humus by hydrogen, ionic, or covalent bonding. Butler and Ladd (1969) proposed that enzyme–organic matter complexes are formed through the formation of amino–carboxyl salt linkages. That enzymes are bound to humus and, humus, by itself, binds to clay minerals, suggests combined influence of clay–humus matter complexes on enzyme activity and stabilization.

Collectively, the organic and mineral fractions in both bulk and rhizosphere soils have a marked influence on the total enzymatic activity of the specific soil concerned. These may hold serious implications for soil fertility. Boyd and Mortland (1990) in their overview summarized pertinent investigations on enzyme interactions with clays and clay-organic complexes.

1.3.4.3 Fundamentals in enzyme catalysis

Enzymes, which are proteins with catalytic properties, are composed of linear chains of amino acids that fold to produce a three-dimensional structure. The sequence of the amino acids specifies the structure which in turn determines the catalytic activity of the enzyme (Anfinsen 1973). Catalysts are substances that speeds up or accelerates a chemical reaction, by lowering its activation energy without itself undergoing conformational changes. The molecules at the beginning of the process are called substrates and the enzyme converts these into different molecules, called products. Enzymes must bind their substrates before they can catalyse any chemical reaction and are very specific as to

what substrates they bind. Specificity is achieved by binding pockets with complementary shape, charge and hydrophilic/hydrophobic characteristics to the substrates (Jaeger and Eggert 2004). Some enzymes require non-protein molecules called cofactors to be bound for full activity (de Bolster 1997). Cofactors can be either inorganic (e.g., metal ions and iron-sulfur clusters) or organic compounds (e.g., flavin and heme), like the coenzymes, which are small organic molecules loosely or tightly bound to enzymes, which transport chemical groups from one enzyme to another. Examples include NADH, NADPHand adenosine triphosphate (ATP). Enzyme activity can be affected by inhibitors, molecules that decrease enzyme activity, and activators, molecules that increase activity (Wagner 1975).

1.3.4.4 Enzyme catalysis key to nutrient attainment

Breakdown and mineralisation of organic matter is a specialized role bestowed to microorganisms (Bardgett et al. 2005). In soil, microorganisms thrive primarily because they have relatively easy access to organic matter, the main substrate from which they acquire C or limiting nutrients serving as a food source for energy, essential for growth and cell maintenance (Alexander 1977; Larson and Pierce 1991; Tisdall 1996; Davet 2004). However, microorganisms are unable to directly translocate these macromolecules into their cytoplasm. Instead, they depend on the mediation and catalytic actions of a range of enzymes that they generate and secrete into the surrounding soil environment. Being the driving forces of fundamental metabolic processes in soil, these extracellular enzymes help break down and process complex organic compounds into assimilable subunits (sugars, amino acids, NH_4^+, PO_4) (Sinsabaugh et al. 1991;Dick, 1997; Paul and Clark 1996; Wolf and Wagner 2005). In essence, microbially-secreted enzymes depolymerize organic compounds and generate soluble oligomers and monomers, which are subsequently recognized by cell wall receptors, and transported across the outer membrane into the microbial cell. The levels and species of enzymes in soils may nevertheless differ as each soil type may vary in terms of the quantity and quality of the mineralizable C-source (Gil-Sotres et al. 2005; Trasar-Cepeda et al. 2008). Therefore, substrate presence induces respective enzyme synthesis (Suto and Tomita 2001). Whereas hydrolytic enzymes (e.g. *β*-glucosidase, N-acetyl-*β*-D-glucosaminidase, xylosidase) are responsible for the decay of organic substrates with faster turnover times like carbohydrates or chitin, oxidative enzymes (e.g. phenol and peroxidase) have an important function in the degradation of SOM components with slower turnover times (e.g. lignin) (Horwath 2007). For this reason activities of enzymes have frequently been found to relate to the chemical composition of SOM and its C and N content (Sinsabaugh et al. 2008; DeForest et al., 2012).

1.3.4.5 Functional attributes pertaining to soil health

Management-induced changes that may affect the health of the soil used to be almost entirely measured in terms of the chemical changes that soils underwent (Pagliai and Vittori Antisari 1993). Chemical changes may nevertheless be slow and several years may pass before anything significant is recorded (Sparling 1992). However, indications are that changes in soil caused by natural or anthropogenic factors can be both reflected fairly accurately in the changes in the activities of soil enzymes (Gupta et al. 1988, Dick 1997; Bandick and Dick 1999) and that subtle improvement or decline in soil quality can be anticipated long before they are detected by chemical measurements (Ndiaye et al. 2000). Being able to detect subtle changes in soil chemical and physical properties, soil enzyme activities may therefore, be useful as early signs of soil improvements or early warnings of soil degradation (Dick 1997; Bergstrom et al. 1998; Margesin et al. 2000; Trasar-Cepeda et al. 2000; Bastida et al. 2008) and thus, are well-suited to measure the impact of pollution on the quality of soil.

Heavy metals are considered to be one of the major contributors to soil pollution (Huang and Shindo 2000). The presence of heavy metals in the soil, especially of Cu, Ni, Cd, Zn, Cr, and Pb has been reported by many authors to have caused long-term hazardous effects on soil ecosystems and soil biological processes (Kuperman and Carreiro 1997; Speir et al. 1999; Huang and Shindo 2000; Kunito et al. 2001; Lorenz et al. 2006; Malley et al. 2006). The sources may vary, for instance, the negative effects of Cu on microbial enzyme activity in agricultural soils are commonly observed where Cu-based fungicides have been used over protracted periods (Merry et al. 1983). It has been proposed that the negative effects on soil biological processes are mainly a consequence of inhibition of both microoganisms and their enzyme activities, as claimed by many researchers (Speir et al. 1999; Kunito et al. 2001; Shen et al. 2005; Malley et al. 2006; Oliviera and Pampulha 2006; Khan et al. 2007; Kahkonen et al. 2008; Kizilkaya 2008). Yang et al. (2007a, 2007b) has drawn particular attention to the negative effects of metal contaminants on soil fertility. The mechanisms by which heavy metals may inhibit enzyme activities have been postulated to be either via masking of catalytically active groups, or having denaturing effects on the conformation of proteins, or competing with the metal ions involved in the formation of enzyme–substrate complexes (Gianfreda and Bollag 1996). Inhibition of enzyme activities is nevertheless reliant upon certain soil characteristics as the clay, silt and organic matter contents and the pH conditions (Doelman and Haanstra 1986; Geiger et al. 1998). Thus, it is possible to use enzymatic activities as early sensors of soil stress in reaction to management practices to timely forewarn that soil degradation is about to occur (Bergstrom et al. 1998, Margesin et al. 2000).

Other attributes include sensitivity to management-induced changes in the soil due to tillage (Gupta and Germida 1988; Kandeler et al. 1999; Acosta-Martínez and Tabatabai, 2001; Alvear et al. 2005), municipal refuse amendments (Perucci 1992; Ros et al. 2003), cropping systems (Bandick and Dick 1999; Ndiaye et al. 2000; Ekenler and Tabatabai 2002), land use (Gewin et al. 1999), organic versus conventional farming (Benitez et al. 2006; Melero et al. 2006; mulching (Yang et al. 2003), pH (Tabatabai 1994; Perucci et al. 2000; Quiquampoix 2000; Makoi and Ndakidemi 2008; Das and Varma 2011), temperature (Zogg et al. 1997), moisture (Kieft et al. 1993; Lundquist et al. 1999; Schimel et al. 1999), herbicide additions (Seghers et al. 2003), and fertiliser use (Zakarauskaitė at al. 2008; Balezentiene and Klimas 2009).

There may be further merits favouring the use of enzyme activities as soil health indicators. For example, they meet the requirements of being relatively straightforward and inexpensive, compared to most other soil analyses (Gianfreda and Bollag 1996; Ndiaye et al. 2000; Nannipieri et al. 2002), the results can be used in a comparative manner and the information can be correlated to or integrated with other physicochemical soil properties (Moore et al., 2000; Ndiaye et al., 2000; Trasar-Cepeda et al., 2000), and enzyme activities are closely linked to the cycling of nutrients in ecosystems (Taylor et al. 1989, Johansson et al. 2000). Moreover, they are the direct expression of the soil microbial community to metabolic requirements and nutrient availability (Dick et al. 1996; Badiane et al. 2001; Moore-Kucera and Dick 2008) and thus, indicative of the soil's potential to sustain overall microbiological activity (Paul and Clark 1989; Masciandaro et al. 1997) while also producing relevant information on the capacity of a soil to perform certain functions that help maintain overall soil fertility and productivity (Skujins 1967, Burns 1978, Frankenberger 1983; Dick 1994; Dick 1997; Garcia et al. 1997; Pascual et al. 2001; Ros et al. 2003; Caldwell 2005; Bastida et al. 2008).

The majority of reports are thus, in favour of using enzyme activity as valid indicators of soil health (Dick 1994, Dick et al. 1996, Marx et al. 2001; Paz-Ferreiro et al. 2009). However, some studies on individual enzyme activities suggest strong temporal and spatial variability, dependent on the function of the enzyme and the type of land use under consideration thus, often leading to conflicting results (Aon et al. 2001; Trasar-Cepeda et al. 2008; Garcı´a-Ruiz et al. 2009). It is this complexity of the behaviour of the soil enzymes that raises doubts, among some researchers, about the suitability of enzyme activities as a measure of general soil health. However, the activities of individual enzymes are rather difficult to interpret and a number of reports proposed using simultaneous estimation of multiple enzyme activities, equations or single numerical values (indices), as indicators of soil health or quality (Pankhurst et al. 1997; Trasar-Cepeda et al. 1998; Saviozzi et al. 2001; Killham and Staddon 2002). One such index, alteration index three (AI3), quantifies the balance between the three

most commonly studied microbially-secreted enzymes, *β*-glucosidase, phosphatase and urease, each participating in the biogeochemical cycling of, respectively, C, P and N. These enzymes are sensitive to alterations in soil characteristics caused by management practices (Puglisi et al. 2006). Internationally, notably in Italy, testing has shown that the AI3 index could be used successfully in soils that ranged from mine spoil to forest (Puglisi et al. 2006; Bastida et al. 2008). Alteration of the soil, whether by over-utilisation or other detrimental practices, results in AI3 values that are higher than those of control soils (Puglisi et al. 2006, and references therein). AI3 is potentially useful for determining soil quality in temperate grasslands (Paz-Ferreiro et al. 2009), and as an index of soil degradation due to agricultural practices (Bastida et al. 2008).

Evidently, a wealth of information on the functional attributes of enzymes in soils has been gathered, and collectively these strengthen the prospect of enzyme activities being used as suitable indicators of general soil health.

1.4 Root symbiotic relationships

1.4.1 The root-soil interface

Soil in close proximity to the roots of higher plants is chemically, physically and biologically different from soil found some distance away (bulk soil) (Jenny & Grossenbacher 1963; Hawes & Pueppke 1986; Young 1995). The root–soil interface embodies soil, clung to and/or immediately adjacent to the root surface, marginally extending into the soil matrix. Operationally defined as the rhizosphere, this zone under the direct influenced of the roots of higher plants, is characterised by accelerated biological activity (Tate 2000), the densities of microorganisms being 10 to 1000 times more than bulk soil (Smalla et al. 2006). This zone, through which water is also drawn, represents a major depository of compounds in massive amounts, is loaded with plant constituents derived from photosynthesis and other plant processes, and is home to (or populated by) an extraordinarily diverse assemblage of fungi, bacteria and other microorganisms. Plant roots exude a variety of molecules into the rhizosphere, including acids, sugars, polysaccharides, as well as proteins as ectoenzymes. As much as 40% of the plant's primary C is believed to be lost through rhizodeposition (Berendsen et al. 2012). Several factors which include the plant species, cultivar, age, stage of development, presence of other microorganisms and environmental conditions, including soil properties, particularly levels of physical, chemical and biological stress, all have an influence on the extent to which rhizodeposition occurs (Bowen and Rovira 1999; Yao et al. 2005; Houlden et al. 2008; Berg and Smalla 2009; Dennis et al. 2010; Carvalhais et al 2011; Bulgarelli et al. 2012). Exudates either directly affect plants, e.g., through increasing nutrient solubility (Uren and Reisenauer 1988; Grayston et al. 1996)

or indirectly through influencing the activity of soil organisms (Werner 1998; Walker et al. 2003; Badri and Vivanco 2009; Berendsen et al. 2012; Lundberg et al. 2012). For instance, many kinds of microflora, like bacteria, feed on sloughed-off plant cells, which in turn are grazed upon by protozoa and nematodes. Thus, much of the nutrient cycling and disease suppressing duties by plants are confined to the immediate zone bordering the roots. Microbial activity in the rhizosphere furthermore affects rooting patterns, as well as mineralisation and immobilisation processes, and in turn, alters both the quantity and quality of the root exudates (Bowen and Rovira 1999). This interrelatedness between roots and microorganisms, alongside conditions around the rhizosphere, play an important role in plant productivity and soil functioning (Sturz and Christie 2003).

1.4.2 Root symbiotic relationships and signalling

Secondary metabolites in root exudates are believed to act as chemical triggers in the rhizosphere (Cook and Baker, 1983), setting off an array of root-root and root-microbe interactions (Bais et al. 2004; Perry et al. 2007). Differences in triggering due to differences in quantity and quality of the root exudates, largely determine which microorganisms predominate in the rhizosphere, and which not. The root-microbe interactions between symbiotic N-fixing bacteria and leguminous plants, and between obligate root-symbionts, arbuscular mycorrhizal (AM) fungi and higher plants, are arguably some of the best described.

Symbiotic N-fixing bacteria, such as *Rhizobium* species, detect compounds like flavonoids (McNaught et al. 1997) by the roots of leguminous plants. In reaction, so-called nodulation (nod) factors are produced by the bacteria serving as a trigger to the plant to form what is called, root nodules (Zuanazzi et al. 1998). In these nodules, bacteria are sustained by nutrients from the plant, and in return they convert N gas readily available for plant uptake (Postgate 1998). The legumes produce leghemoglobin to carry away any oxygen that would inhibit nitrogenase activity. Non-symbiotic (or "free-living") N-fixing bacteria may also reside in the rhizosphere of certain plants (including many grasses), and "fix" N gas in a similar fashion, despite being loosely associated with plants. In this way, N-fixing bacteria in the rhizosphere of the rice plant are able to fully meet plant N demand (Sims et al. 1984).

Strigolactones is the name given to certain root secreted plant hormones that stimulate the branching and growth of AM fungi, increasing the probability of contact and establishment of a symbiotic association between the plant and AM fungus (Akiyama et al. 2005; López-Ráez et al. 2009). This highly developed mutualistic relationship is arguably the most prevalent plant symbiosis known to date (Simon et al. 1993). Tremendous advances in

research on mycorrhizal physiology and ecology over the past few decades have led to an in depth understanding of the multiple roles of AM fungi in ecosystems, some of which are summarized in the next section.

1.4.3 Arbuscular mycorrhizae

1.4.3.1 Origin, definition, and types of mycorrhizae

"Mycorrhiza", which describes fungus-root associations (Harley 1969), is a term derived from a combination of two words namely, "mykes" (Greek) meaning fungus, and "rhiza" (Latin) meaning root. Currently, "mycorrhiza" refers to highly evolved, close, prolonged, mutualistic associations between soil fungi and the roots of about 80% of the known vascular plant species (Harley and Smith, 1983; Brundrett 1991). The fungal partner essentially extend plant reach to water and nutrients via a multi network of underground hyphal ''bridges'' between root cortical cells and the soil (Augé 2001; Smith and Read 2008). The fungus sources its energy (catabolized photosynthetically derived C compounds) from a continuous plant root supply (Bago et al. 2003). Being unable to grow and reproduce separately from a plant host, these fungi are considered obligate biotrophs. Harley and Smith (1983) proposed a classification system based on the type of host plant as well as the type of fungus involved.

AM fungi belong to the fungal phylum, Glomeromycota (Schübler et al., 2001). Currently, mycorrhizas are grouped into at least seven types, including arbuscular; ecto; arbutoid; ericoid; monotropoid, orchid and ectendo (Brundrett 1991; Smith and Read 1997). Of these, the ectomycorrhizas and arbuscular mycorrhizas are the most widespread and therefore the best described. Vesicular arbuscular mycorrhizas were originally applied to symbiotic associations formed by all fungi in the order Glomales (Morton and Benny 1990). However, because a major suborder in this order lacks the ability to form vesicles in roots, the term arbuscular mycorrhiza is now commonly accepted. As opposed to ectomycorrhizae, which are more pertinent in forests, AM are important in agriculture.

1.4.3.2 Root colonisation characteristics and developmental stages

1.4.3.2.1 Presymbiotic (external) soil phase

According to Brundrette et al. (1996), mycorrhizal symbiosis is a step-wise process that occurs in two main phases, an external and internal phase. The external phase applies to the development of AM fungi prior to root contact and penetration. During the presymbiosis stage AM fungi embark on three main stages: spore germination, hyphal growth, and host recognition and appressorium formation.

1.4.3.2.1.1 Spore germination

In the presymbiosis stage, AM fungi propagate through resting-phase propagules, spores, which are thick-walled multi-nucleate structures, and usually composed of several wall layers (Sylvia and Jarstfer 1992; Brudrette et al. 1996; Wright 2005). These structures form swellings on one or more AM subtending hyphae in the soil or in roots. Germination of spores is accelerated in the presence of host root exudates (Douds and Nagahashi 2000). Spore germination may also depend on suitable conditions of the soil matrix, temperature, CO_2 concentration, pH, and P concentration (Wright 2005).

1.4.3.2.1.2 Hyphal growth

Soil hyphae, also known as extraradical hyphae or external hyphae, are filamentous AM fungal structures that ramify through the soil (Brundrett et al. 1996). These hyphae are believed to be responsible for nutrient acquisition, propagation of the association and spore formation. Soil hyphae may either originate from germinating spores or from fragments of roots, but in many cases a pre-existing network of hyphae is already present in the soil resulting from previous root activity. Soil hyphae resulting from spore germination have a limited capacity to grow and may die if they do not encounter a susceptible root (Kabir et al. 1997). The growth of AM hyphae in soil is controlled by host root exudates, strigolactones, and also is reliant on the soil P concentration (Akiyama et al. 2005). Low-P concentrations in the soil increase hyphal growth and branching as well as induce plant exudation of compounds that control hyphal branching intensity (Nagahashi et al. 1996; Douds et al. 2000).

1.4.3.2.1.3 Host recognition

Mycorrhizal associations are initiated by means of a chemotaxic response, i.e. directing their growth towards the roots of the host plant in response to exudates acting as chemical stimulus. Chemotaxis has been claimed to increase the efficacy of root colonisation under low-P soil conditions (Nagahashi et al. 1996; Douds and Nagahashi 2000). Molecular techniques have confirmed this host-specific chemotaxis (Tamasloukht et al. 2003).

1.4.3.2.1.4 Appressorium formation and penetration

Upon establishing contact with host plant roots, appressoria or 'infection structures' form between adjacent epidermal cells (Brundrett et al. 1996). An appressorium basically provides an entryway for hyphae to penetrate the root cortical cells (Gianinazzi-Pearson 1996). Signaling, however, between symbionts may not necessarily be important for appressorium formation, but rather, is required for further growth once appressoria have

formed (Douds, and Nagahashi 2000). After appressorium formation, aseptate hyphae may cross the hypodermis through passage cells if these are present in the exodermis (Brundrett et al. 1996). These hyphae start to branch in the outer cortex to form colonies (infection units). The extrametrical mycelium may give rise to a number of entry points in the root, depending on the season and age of the root (Mosse 1959; Nicolson 1959).

1.4.3.2.2. Symbiotic (internal) root phase

1.4.3.2.2.1 Hyphal proliferation and modes of spreading

The different ways in which hyphae may spread through the root appears to be linked to the anatomy of the plant root and fungus involved (Brundrett et al. 1996). Spreading depends on the wall thickening pattern of the outer exodermis cells, since suberin in the walls of these cells was implicated as a factor influencing the pathway of hyphal root penetration. There are two morphologically distinctive modes of spreading within the root cortex, the *Paris* and *Arum* type of spread. The *Paris* type is characterized by the intracellular growth of hyphae through cell-to-cell passage mainly due to the lack of continuous longitudinal air spaces between root cortical cells (Brundrett et al. 1996). Resulting colonies generally have a coiled appearance. The *Arum* type is characterized by the growth of hyphae in the intermediate layers of the cortical parenchyma where they dilate the intercellular air channels (spaces) between the walls of the root cells, sometimes in bundles of three or four (Brundrett et al. 1985; Brundrett et al. 1996). These bundles sometimes give them a wavy and linear appearance, up to several millimeters long (Brundrett et al. 1985; Brundrett and Kendrick 1988). They often form intermittent projections and are at times swollen (Abbott and Robson 1979). In the absence of these spaces, the slower *Paris* type AM association may develop (Cavagnaro et al. 2001). The choice between *Paris* type and *Arum* type is primarily determined by the host plant family, although some families or species are capable of either type (Yamato and Masahide 2005).

1.4.3.2.2.2 Formation of arbuscules

As root colonisation progresses, various fungal structures are formed. Formation of arbuscules is initiated shortly after root penetration (Brundrett et al. 1996; Gianinazzi-Pearson 1996). These are highly branched structures representing the sites of exchange for P, C, water, and other nutrients (Wright 2005). Arbuscules are known to be formed only by AM fungi. Thus, from a functional viewpoint, arbuscules are most significant in the AM complex (Cox et al. 1975; Scannerini and Bonfante-Fasolo 1983). Major modifications are required in the plant host cell to accommodate arbuscules. There is a decondensation of the plant's chromatin, which indicates increased transcription of the plant's DNA in arbuscule-

containing cells (Gianinazzi-Pearson 1996). The vacuoles shrink and other cellular organelles proliferate. The plant cell cytoskeleton is reorganized around the arbuscules. This deterioration is characterised by the collapse of the arbuscular branches until only the trunk remains (Cox and Tinker 1976; Brundrett et al. 1996). Arbuscules are short-lived and may start to collapse only four days after formation. Arbuscule degeneration has been shown to be tightly coupled with plant P and N status. Both inorganic P (Pi) and N transport occurs at the periarbuscular membrane, a plant-derived membrane that encloses the arbuscule. Using *Medicago pt4* mutant plants, it was demonstrated that Pi import was crucial for wild-type arbuscule dynamics, and that the Pi ion itself could act as local, cell-autonomous signal that triggers accommodation and maintenance of the arbuscule by the host cell (Javot et al. 2007). Furthermore, that arbuscule lifespan could be restored when *Medicago pt4* mutants were grown at low N concentrations (Javot et al. 2011), suggests that, in addition to Pi-, N-delivery is equally important in the regulation of arbuscule lifespan in a cell-autonomous fashion. Arbuscules may appear and disappear numerous times in roots over long periods of time, as long as the fungus is growing, which in turn depends mostly on the formation of new roots by the host (Brundrett et al. 1996). Roots collected from the field often show large arbuscular clumps, originating from smaller arbuscules, filling the host cell (Bonfante-Fasolo 1978). In nature and particularly in older plant roots, senescent arbuscules are more frequently encountered than active arbuscules (Bonfante-Fasolo 1984). Active arbuscules are usually associated with young mycorrhizal roots.

1.4.3.2.2.3 Formation of vesicles and vesicle-like structures

Vesicles start to form soon after the first arbuscules, but continue to develop when the arbuscules senesce (Brundrett et al. 1996). Vesicles are thin-walled intercalary or terminal hyphal swellings formed on internal hyphae, which may be found both in the inner and outer root cortex, within or between cells, and develop to accumulate storage products (e.g. lipids) (Brundrett et al. 1996). The cytological organization of the vesicles and the fact that vesicle numbers increase in old or dead roots, in which they develop thick walls, suggest that these structures are mainly resting organs (Bonfante-Fasolo 1984). With age vesicles become vacuolated (Mosse 1959; Nicolson 1959). By the time root colonisation has reached a matured stage, starch granules are found near arbuscular clumps (Bonfante-Fasolo 1984; Brundrett et al. 1996). External vesicles, also known as accessory bodies or auxiliary cells, are clustered swellings on external soil hyphae (Brundrett et al. 1996). These structures are often ornamented with spines or knobs. Only *Scutellospora* and *Gigaspora* species form external vesicles. External vesicles do not function as propagules and may form to allow partitioning of nutrients (e.g. P/C) and nuclei prior to spore formation (Boddington and Dodd 1999).

1.4.3.2.2.4 Hyphal growth from the root into the soil

Once colonisation has occurred, short-lived runner hyphae grow from the plant root into the soil. These are the hyphae that take up P and micronutrients, which are conferred to the plant (see following section). There are also hyphae that grow from the roots and colonize other host plant roots. Although they fulfil different functions, the different types of hyphae are morphologically distinct (Wright 2005).

1.4.3.3 AM facilitated nutrient uptake and exchange

As obligate symbionts, having limited saprobic ability, AM fungi depend on their host to meet their C requirements (Harley and Smith 1983). Carbon transfer from plant to fungi typically occurs through the arbuscules, or intraradical hyphae (Pfeffer et al., 1999). Inside the intraradical mycelium, hexose is converted to the C storage forms, trehalose and glycogen. Balance between synthesis and degradation of these constituents may buffer the intracellular sugar concentrations (Pfeffer et al. 1999). The intraradical hexose may enter the oxidative pentose phosphate pathway, which produces pentose for nucleic acid production. Lipids deriving from intraradical mycelium may be transported to extraradical mycelium, either to be stored, or further processed into hexoses (Pfeffer et al. 1999). Of the approximate 20% of the C in plants that is thought to be translocated to the AM fungi (Pfeffer et al. 1999), up to a quarter is stored in the extraradical hyphae (Hamel 2004). Evidently, host plants contribute much to the below-ground organic C pool by investing a significant amount of C in mycorrhizal networks.

In exchange for a constant provision of catabolized photosynthetically derived C compounds, in the form of hexoses, the fungus help capture plant available nutrients such as P, S, N and micronutrients from the soil (Harley and Smith 1983), being referred to as a bi-directional transfer of nutrients (Smith et al. 1994). Up to 80% of plant P, 25% of plant N, 10% of plant K, 25% of plant Zn and 60% of plant Cu may be acquired (Marschner and Dell 1994). For this reason, AM-colonised plants generally show greater nutrient-uptake ability compared to AM-free plants. AM-facilitated nutrient uptake is nevertheless restricted to nutrients, like P, with low diffusion rates, and those present at low concentrations in the soil (Bolan et al. 1987).

1.4.3.4 AM fungi's unique P-uptake ability

The main benefit of mycorrhizas to plants has been attributed to increased uptake of nutrients, especially P. Soil P gets transported to the root via diffusion and is absorbed as phosphate in the form of $H_2PO_4^-$-ions, which are present at low concentrations in the soil solution (Mengel 1997). During P absorption by roots, the zone surrounding the roots has to

be simultaneously filled by mass flow of the soil solution, but research showed that diffusion of P to the roots is often slower than the rate of P-uptake (Koide 1991). This zone is referred to as the P-depleted zone and can be up to 2 mm from the root surface (Koide 1991). Subsequently, an insufficient P supply is available to the roots and explain why plants often suffer from P deficiencies even under conditions of adequate P concentrations in the soil. AM roots however, can overcome this shortage by exploring larger soil volumes beyond the P-depleted zone, reducing the the distance required for diffusion, and reaching soil volumes unavailable to plant roots. AM fungi effectively increase the root surface area through a vast network of finely branched hyphae (Sanders and Tinker 1973; Li et al. 1991) that have higher surface-to-volume ratios than plant roots (Augé 2001; Tuomi et al. 200). The much finer hyphae penetrate soil pores that are inaccessible to root hairs (Bolan 1991), which is evident from their 2-4 µm diameter compared to the generally larger than 10 µm diameter of root hairs (Barley 1970). In some cases, the role of P-uptake can be completely taken over by the mycorrhizal network, and all of the plant's P may be of hyphal origin (Smith et al. 2003).

Improve P-uptake can also be explained from a plant physiological point of view. Since transpiration rates of AM plants are higher than normal, water uptake per unit root length is also expected to be higher. Subsequently, the mass flow of soil solution to the root surface is higher than normal, and the rate of P absorption is likely to be influenced accordingly. This explains why AM-plants exhibit higher P concentrations compared to AM-free plants (Possingham and Obbink 1971; Karagiannidis et al. 1995). It has been estimated that the influx rate of P to AM fungal roots may be up to six times higher that of root hairs (Sanders and Tinker 1973; Bolan 1991; Jakobsen et al. 1992)

It further appears that a decreased in soil pH increases the solubility of P precipitates (Raven et al. 1978; Bolan 1991; Mengel 1997). AM fungi however, may produce organic acids with chelating properties which can chemically modify bound P (Bolan et al. 1987). AM fungi lower the root zone pH by selective uptake of NH_4^+ (ammonium-ions) and by releasing H^+ions. The hyphal NH_4^+ uptake also increases the N flow to the plant as the soil's inner surfaces absorb ammonium and distribute it by diffusion (Hamel 2004).

Nevertheless, species of AM fungi differ in their abilities to supply the plant with P (Smith et al. 2003). In some cases, AM fungi are poor symbionts, providing little P while taking relatively high amounts of C (Smith et al. 2003). As shown by Wooldridge (1999), the effects of AM fungi on growth parameters on juvenile apple rootstocks grown in a common growth medium may differ between combinations of AM fungal species and rootstock cultivar. This variability might be attributable to differences in AM fungi x rootstock specificity, but could

also indicate that AM fungal species differ in their abilities to utilize the elements and compounds that are present in the soil.

1.4.3.5 AM facilitated plant growth, disease resistance and water use efficiencies

AM fungi also help improve host plant growth (Makarian et al. 2013), mainly because of improved nutrient uptake. AM-plants appear to be healthier than AM-free plants and are generally better able to resist disease (Whipps 2004). AM fungi have been shown to improve plant tolerance to abiotic environmental factors such as salinity and benefit plant growth and productivity (Porcel et al. 2012). They are better able to sustain drought stress than AM-free plants (Safir and Nelsen 1985; Subramanian et al. 1997; Cruz et al. 2000), displaying higher water use efficiencies (Al-Karaki 1998). Reduction in drought stress may be as a result of increased rates in transpiration and photosynthesis after periods of water stress. On the contrary, at higher soil nutrient and moisture concentrations, AM fungi become less prevalent and diverse (Augé 2001), seemingly because plants allocate less C to AM fungi and AM fungi reallocate their resources to intraradical hyphae in these conditions (Johnson et al. 2003). Over the long term, AM fungi may become tolerant to these environmental conditions (Johnson et al. 2010). For example, slightly above 7 mg/kg P was found to inhibit AM root colonisation when tested at lab-scale (Brundrett et al. 1996), whereas under field conditions, AM root colonisation appeared to be unaffected under much higher soil P conditions (Plenchette et al. 1983; Schubert et al. 1990); Menge et al.,1978b; Meyer et al. 2003).

1.4.3.6 Glomalin: a by-product of AM fungi

AM fungi can also benefit the physical characteristics of the soil because their hyphae form a mesh to help stabilise soil aggregates. This is accomplished by glomalin, a water-stable product with biochemical binding properties, which is released upon decomposition of AM fungal hyphal and spore walls (Driver et al. 2005; Purin and Rillig 2007; Fokom et al. 2012). Glomalin is exclusively produced AM fungi (Wright et al. 1996).

Glomalin, being composed of c. 37% C and 3-5% N, has been investigated for its C and N storing properties; it contributes 3% to the storage of soil C and 5% to N (Purin and Rillig 2007; Treseder and Turner 2007; Fokom et al. 2012). Glomalin is a significant component of SOM and act to bind mineral particles together, improving soil aggregate water stability and decreasing soil erosion, and thus improving soil quality (Comis 2002); Rillig 2004). A strong correlation has been found between glomalin and soil aggregate water stability in a wide variety of soils where organic material is the main binding agent, although the mechanism is not known (Rillig 2004).

Due to its slow decomposition rate, total glomalin remains relatively persistent and stable over protracted periods (several decades) (Steinberg and Rillig 2003), and may accumulate to concentrations of several mg g^{-1} of soil (Wright and Upadhyaya 1996; Rillig et al. 2001). Rate of decline of glomalin is slower than for SOM following land-use change (Preger et al. 2007). Glomalin may thus sequester relatively large amounts of C in soil (Treseder and Alen 2000; Quiquampoix and Burns 2007). That similar trends have been found for organic C in clay-size fractions, suggests that glomalin is protected by association with clay minerals (Lobe et al. 2001). Correlations of various fractions of glomalin with C under different soil conditions (Wright and Upadhyaya 1998; Nichols and Wright 2005; Peng et al. 2015) support this supposition.

1.5 Methodological considerations for measuring the microbial component of soil

Broad understanding of soil ecosystem functioning is somewhat limited by the difficulty of quantitative and representative recovery of microorganisms from soil samples. The microbial component in soil can nevertheless be measured in different ways, in terms of microbial numbers, microbial biomass, microbial diversity, functional activity (such as respiration and N-minerlization), enzyme activities, probing of specific genotypes, etc. Molecular approaches, in particular, allowed new insights during the last two and a half decades or so, showing an unexpected greater diversity of genomes in soil (Thies 2006). The aforementioned measurements are either direct (by counting) or by inference (from chemical and physical measurements) and include, among others, approaches as microscopic counts, plate counts, biochemical analyses, physiological assays, and molecular approaches through the analysis of soil extract DNA, such as DNA-fingerprinting (Torsvik et al. 1996). Some may be culture-dependent; others culture-independent (Hill et al. 2000). Some may be used to assess the total microbial community, others the specific members of the community, and others both. Not all these methods are necessarily suited to produce generally accepted results, but they give relative information, among others, about soil fertility, productivity and general soil health (Skujins 1967; Burns 1978; Frankenberger and Dick 1983) and can be combined. Soil microflora are most frequently assessed in terms of their abundance, activity and function, and diversity or community composition; the following sections are a summation of the methods involved.

1.5.1 Microbial abundance

Customary measurements of microbial abundance have relied on phenotypic characteristics using direct (microscopic) and indirect (plating on a variety of artificial culture media) counting methods, designed to maximize the recovery of different microbial species, as well

as methods involving extraction of specific cell components or molecules through measuring their concentration (Pankhurst et al. 1997; Thies 2006).

Despite the attempts to devise suites of culture media formulations to maximize the recovery of diverse microbial groups from soils (Balestra and Misaghi 1997; Mitsui et al. 1997), it has been well established that plate counts account for only a small percentage of the total microflora (1-10%), as in the case of typical agricultural soils (Torsvik et al. 1990a; Atlas and Bartha 1998). The type of growth medium thus greatly affects colony formation (Sørheim et al. 1989; Johnsen and Nielsen 1999). The discrepancy in results is mainly due to the interrelatedness between different organisms in natural ecosystems, in the inability to match (in pure culture) the environmental conditions microorganisms are exposed to, and the knowledge that some microbial species are culturable only under certain physiological conditions (Bakken 1997; Muyzer and Smalla, 1998; Heuer et al. 2001). Evidently, methods of extraction and enumeration are both subject to bias. These enumeration methods therefore, underestimate population size and diversity and fail to account for the functioning of soils (Amann et al. 1995). As for the remaining 99% or more, of soil microorganisms not accounted for, soil microbial physiologic and taxonomic information is effectively lost. In addition, the procedures are laborious. Despite these shortcomings, enumeration methods may still be relevant to obtain quantitative data on soil microbial communities and is especially useful for comparative purposes (Mazzola 2004) or in cases when specific microorganisms are examined.

Direct cell enumeration using advanced fluorescence microscopy, with the aid of several stains specific to proteins or nucleic acids, can reflect a 100-1000 times more the numbers obtained by plate counting (Johnsen et al. 2001). This culture-independent procedure however, does not allow counting specific microbial species, and some of the stains used, do not discriminate between living and dead microbial cells.

Chloroform fumigation extraction (CFE) is often used to estimate the total microbial biomass C and/or N of soils (Brookes et al. 1985; Needelman et al. 2001). Soil microbial biomass as an important source and sink of nutrients, are particularly recognized in agriculture. Another approach of assessing the composition of soil microflora, without the need to cultivate them, is the employment of structural components like phospholipid fatty acid (PLFA) or fatty acid methyl esters (FAME) analysis (Tunlid and White, 1992; Bossio and Scow 1998; Frostegard and Baath 1996; Zelles 1999; Pankhurst et al. 2001). PLFA is based on the extraction, fractionation, methylation and chromatography of the phospholipid component of soil lipids. The presence and abundance of these signature fatty acids in soil reveals the presence and abundance of particular organisms or groups of organisms in which those signatures can be found. Results can be interpreted by reference to a database of pure cultures and known biosynthetic pathways (Zelles 1999).

Other methods include detection of specific molecules (e.g ATP, ergosterol, glomalin,) associated with the soil (Jenkinson and Ladd 1981; Newell et al. 1988). ATP is found in and around living cells, and quantified by measuring the light produced through its reaction with the naturally occurring enzyme, luciferase, using a luminometer. The amount of light produced is directly proportional to the amount of ATP present in the sample. Ergosterol is a fungal-index molecule to measure fungal biomass. Glomlain is a breakdown-resistant glycoproteinaceous substance (Curaqueo et al. 2010) that is produced by AM fungi, and released into the soil upon decomposition of AM fungal hyphal and spore walls. Traditionally, soil glomalin content has been indirectly analysed by the Bradford protein assay (Wright and Upadhyaya 1998) and has been renamed as glomalin-related soil protein (GRSP) (Rillig 2004) as it is suspected to include other proteins, and not only glomalin, in its composition. Nevertheless, glomalin and GRSP, are used as synonymous terms. The Bradford protein assay utilises extraction protocols which enable two main fractions of soil glomalin to be quantified, though in relative terms. Easily extractable glomalin (EEG), i.e. easily extractable-GRSP (EE-GRSP) represents glomalin that has been freshly released into the soil as opposed to total glomalin (TG), i.e. total-GRSP (T-GRSP) in the recalcitrant, slowly decomposable state. These fractions have not yet been fully described, and are operationally defined on the basis of their ease of extractability. The recent discovery by Gillespie et al. (2011) that glomalin may also have a non-AM origin, could potentially change the way how glomalin has been interpreted in soil research.

On the molecular front, quantitative polymerase chain reaction (Q-PCR) is a quick, relatively cheap, accurate and highly sensitive and easy to implement method for sequence quantification that can also be used to quantify microbial groups (Smith and Osborn 2009). The limitation is that it can only be used for targeting of known sequences and DNA impurities and artifacts may create false-positives or inhibit amplification.

The preceding approaches are never stand-alone methods. To characterize the soil, or to understand differences among soils, or obtain answers on functionality, additional and/or complimentary approaches are needed.

1.5.2 Microbial activity and function

Insofar as monitoring microorganisms are concerned, it is not possible to evaluate the ecological significance of microorganisms simply by determining their numbers. In this regard, process-level studies can be used, whereby microorganisms themselves are not isolated or identified, but rather their activities are measured (Dick 1994; Pinkart et al. 2001; Kirk et al. 2004). This view was strongly emphasised by Alexander (1977) and by Naseby and Lynch (1997) who considered information on the activity of microorganisms to be of greater importance than microbial measures, since they can be made with higher precision.

Microbial activity indicates the vast range of activities carried out by microorganisms in soil. Measurements of microbial activity in soils are based on the presence of intact and active microbial cells; they reflect the physiological state of microbial cells (Alef and Nannipieri 1995). The study of microbial activity lends itself to wide application through measuring the rate of certain soil biochemical processes as basal respiration, substrate induced respiration, N mineralisation, nitrification rate, potential denitrification activity, dehydrogenase activity, ATP content and enzyme activity (Alef and Nannipieri 1995; Hill et al. 2000; Nannipieri et al. 2003). The activities of enzymes have arguably been measured more frequently and are of direct, practical importance to agriculture. Enzyme activity measurements have a long history of use as an indirect assessment or indicator of the overall activity of specific groups of microorganisms in the soil (Hofrichter 2002; Baldrian 2008), whilst generating helpful functional data on the ability of a soil to perform specific functions critical in sustaining soil fertility (Dick 1994; Dick 1997; Garcia et al. 1997; Pascual et al. 2001; Ros et al. 2003; Caldwell 2005; Bastida et al. 2008).

Enzyme techniques often differ in the use of a variety of reaction substrates, the reaction conditions (temperature, use of buffers, time of reaction), and/or in the mode of detection (spectrophotometry, fluorescence, radiolabelling) (Tabatabai 1994, Alef and Nannipieri 1995, Gianfreda and Bollag 1996). Some of these detection methods have been in use for decades already, for example, fluorescein diacetate (FDA), has been used as a measure of total microbial activity since the early 1980's (Swisher and Carroll, 1980). The widespread use of artificial colorimetric (Tabatabai 1994) and fluorometric substrates (Marx et al., 2001) along with multi-well plate reader technology (Marx et al. 2001) allows for the rapid and inexpensive development of large data sets compared to other biochemical analysis (Ndiaye et al. 2000), and the results are correlated to other soil properties (Moore et al. 2000; Ndiaye et al. 2000; Trásar-Cepeda et al. 2000). Numerous enzymes have been investigated; the choice of method being largely depended on the geochemical cycle under investigation. For example, *β*-glucosidase, urease and phosphatase, are active in the cycling of, respectively, C, N and P cycles, as has been extensively reviewed, among others, by Makoi and Ndakidemi (2008). Enzyme analyses in recent studies demonstrated a high level of spatial variability of soil enzyme activity both in space and depth (Trásar Cepeda et al. 2000; Andersson et al. 2004; Wittmann et al. 2004). Furthermore, enzyme activity in soils is regulated by seasonally-dependent variables such as temperature, moisture and the input of fresh litter (Wittmann et al. 2004). In accordance with Baldrian (2009), seasonality must be taken into account during the interpretation of enzyme activity data, and more than one sampling time during a season are usually needed to cover the annual variability of soil enzymatic processes. Moreover, to exclude random climatic effects, seasonality studies should be repeated during the following years.

Since only a few decades ago, molecular approaches have been used to investigate soil microbial communities. Several methods are used today to study soil DNA or RNA (Rincon-Florez et al. 2013). The approaches have added much to our understanding of the composition and functions of microorganisms in soil (Torvisk and Øvreås 2002). Methods such as quantitative polymerase chain reaction (Q-PCR) can be used to quantify target genes that mirror the capacity of microorganisms to perform certain critical soil functions, for example, nitrite reductase, to quantify denitrifying soil bacteria in a given sample (Henry et al. 2004). Genomic and transcriptomic approaches have helped to understand the response of microbial communities to changes in their environment in relation to soil microbial structure, activity and functions (Hirsch et al. 2010; Sofo et al. 2010; Carvalhais et al. 2012). Nicolaisen et al., (2008) has demonstrated that direct analysis of functional gene expression dynamics by quantification of mRNA was possible in natural soil. Most of the discrepancies in the genetic potential and apparent functions associated with enzyme production appear to be mendable, although many improvements are still to be made (Nannipieri 2006; Cañizares et al. 2011).

1.5.3 Microbial diversity and community composition

Microbial diversity is a general term used to include genetic diversity, i.e. the amount and distribution of genetic information, within microbial species; diversity of bacterial and fungal species in microbial communities; and ecological diversity, i.e. variation in community structure, complexity of interactions, number of trophic levels, and number of associations. Microbial diversity is measured by various traditional techniques as well as newer molecular-based procedures (Rincon-Florez et al. 2013).

Soil microbial communities exhibit certain C-substrate utilisation patterns that may be determined using BIOLOG Eco Plates™ (BIOLOG™, Hayward, CA, USA), which is derived from the original method proposed by Garland and Mills (1991). A particularly useful aspect of this method is that it gives insight into the functional (or metabolic) diversity or composition of microbial communities (Garland and Mills 1991; Sigler,2004). Reportedly (Mayr et al. 1999; Yan et al. 2000), such metabolic fingerprints (community-level physiological profiles; CLPP) are sensitive and reliable in terms of their ability to identify changes in the soil environment and microbial communities that stem from changes in soil management. The technique is rapid and simple, but not without any drawback as it is culture dependent, and reproducible results can be obtained only if replicates contain identical community profiles and are of similar inoculation density (Insam 1997), the samples are required to be incubated before any changes in the microbial community occur (Smalla et al. 1998), and it excludes the contribution of fungi due to their general slow growth (Haack et al. 1995).

Community profiling based on PLFA and FAME analysis can estimate gross changes in the abundance of specific organisms or groups of organisms in which unique PLFA and FAME signatures can be found (Zelles 1999; Rincon-Florez et al. 2013). Detection at the species level is not possible with PLFA, but FAME has been used as an accepted taxonomic discriminator for species identification. Gross changes have been reflected by soil disturbances such as cropping practices (Zelles et al. 1995), pollution (Frostegard et al. 1993), and changes in soil quality (Bossio et al. 1998; Petersen et al. 1998). Equations that calculate species richness and evenness and diversity indices, which combine both richness and evenness, e.g. the Shannon-Weaver index (Pankhurst et al. 1997), have also become very useful (Kennedy and Smith 1998).

Further insight into the diversity of soil microbial communities can be gained by taking advantage of the variation in their genetic make-up (Torsvik et al. 1990). The DNA extracted from soil is purified and can be used either with DNA-DNA hybridization to detect specific genes in the soil (Holben et al. 1988; Torsvik et al. 1990; Hill et al. 2000). Alternatively, the unique feature of 16S ribosomal RNA (rRNA) genes (i.e. encoded by rDNA) in prokaryotes and 5S or 18S rRNA genes in eukaryotes (Ward et al. 1992) can be easily amplified using polymerase chain reaction (PCR). These small subunit (SSU) rDNA molecules are found universally in all three forms of life: the domains Bacteria, Archaea, and Eucarya (Woese et al. 1990); they are composed both of highly conserved regions and also of regions with considerable sequence variation (Woese 1987). The conserved regions deviate only among taxonomically distant groups, while the variable regions may show differences even among different strains of a single species. Primers determine the portions of the DNA that is amplified. The amplification products are typically analysed by gel electrophoresis to produce unique patterns (or profiles) of separated DNA fragments that can be used as genetic fingerprints (Thies 2006). Some methods match individual rDNA sequences to a database of previously encountered sequences in order to assess diversity (Olsen and Woese 1993). Of the diversity methods commonly used include terminal restriction fragment length polymorphisms (T-RFLP) (Liu et al. 1997), denaturing or temperature gradient gel electrophoresis (DGGE/TGGE) (Muyzer and Smalla 1998) and automated ribosomal intergenic spacer analysis (ARISA) (Ranjard et al. 2001). Several more molecular methods have been reviewed by Rincon-Florez et al. (2013). These include the new exciting high-throughput sequencing approaches (also referred to as next generation sequencing; NGS). NGS is increasingly being used for estimates of microbial diversity in complex environments (including as soils) in culture-independent manners and include both metagenomic (DNA-based) and metatranscriptomic (RNA-based) approaches. Undoubtedly, NGS technologies have revolutionized research on environmental microbiology and have a great potential to shed further light on relevant questions in agriculture and soil biology. However, the large

amounts of generated data pose serious challenges with respect to analyses, processing and interpretation of the data.

Evidently, the preference of one technique over another is subjective to the researcher's hypothesis, availability of samples, and resources including equipment and funding to carry out the experiments. A combination of traditional and newer molecular techniques would possibly be ideal.

1.6 Soil management effects on selected soil microbial and chemical properties relating to crop performance

1.6.1. Effects of conventional management practices

1.6.1.1 Effect of inorganic fertiliser

Fertilisation has become an indispensable practice in modern agricultural production. In simple terms, any amendment to the soil with the purpose of enhancing soil fertility can be seen as a form of fertilisation (Martens et al. 1992; Graham et al. 2005). Organic and inorganic fertilisation (inorganic fertilisation, being the more radical method of the two) are methods focussed on soil nutrient balance improvement, both with the aim of augmenting the level of or supplying certain nutrients essential for plant growth, crop maintenance and yield increase.

Soil nutrient balance improvement is tied to soil nutrient dynamics and balance, soil carrying capacity, and soil health and system sustainability. Given their direct involvement in soil processes such as organic matter decomposition and nutrient cycling (Bohme et al. 2005), changes in soil microbial properties, as opposed to chemical and physical properties, can more accurately and sensitively reflect changes relating to soil fertility and general soil health (Anderson 2003; Bending et al. 2004) and thus, the productive capacity of soils. The effect of inorganic fertilisers, in particular, on soil microbial properties has been the subject of various studies, which include effects with respect to parameters as microbial biomass (Belay et al. 2002; Zhong and Cai 2007; Juan et al, 2008; Wei-Dong et al. 2008), microbial numbers and functional groups, including bacteria, fungi, oligonitrophilic bacteria, *Azotobacter*, actibobacteria and heterotrophs (Belay, et al., 2002; Mandic et al., 2011; Chen et al. (2011); Ai et al. 2013; Okore et al. 2014; Tianaa et al. 2015), microbial activity, including enzyme activity (Lee et al. 2013; Okore et al. 2014; Zhang et al. 2015a) and microbial community structure and diversity (Zhong and Cai 2007; Juan et al. 2008 Wei-Dong et al. 2008; Zhang et al. 2012; Geisseler and Kate 2014; Tianaa et al. 2015).

However, the effects of inorganic fertilisers on the microbiology of the soil that are reported in literature are often conflicting. Though there is clear evidence that certain chemical products can induce negative effects (Barabasz et al. 2002; Ayoola and Adeniyan

2006; Zakarauskaitė at al. 2008; Zhang et al. 2015a), some effects may be positive (Fauci and Dick 1994), whereas in other instances, no noticeable changes were observed, often in cases when simple fertiliser treatments were singularly applied (Belay et al. 2002; Okore et al. 2014; Tianaa et al. 2015). Other contributing factors explaining some of the discrepancies in results associated with mineral fertiliser usage could be due to the differences that stem from the use of different formulations, e.g. NPK (Lee et al. 2013; Zhang et al. 2015a) as opposed to LAN (limestone ammonium nitrate) (Wooldridge et al., 2013a), simple (N, P or K) (Belay, et al. 2002) as opposed to balanced (NPK) fertilisers (Belay et al. 2002; Juan et al. 2008; Lee et al. 2013; Okore et al. 2014; Zhang et al. 2015a), and combined use of inorganic and organic fertilisers instead of using inorganic fertilisers alone (Lee et al. 2013; Zhang et al. 2015a). Recently, Zhang et al (2015a) demonstrated that by applying inorganic fertilisers alone, especially in high amounts, in greenhouse fields, the effects on soil quality parameters were more detrimental than when inorganic and organic fertilisers were applied jointly. Time of sampling in relation to rain events, temperature peaks, crop physiological stages and fertiliser application may also be critical as soil environmental conditions impinge greatly on general microbial activity (Mandal et al 2007).

Furthermore, the effects of inorganic fertilisers on soil microbial properties may be direct or indirect (Wei et al. 2008; Zhong and Cai 2007); direct effects constitute injury to microorganisms that come in contact with the chemical, especially ammonium-based fertilisers in high concentrations, whereas indirect effects are the consequence of changes caused by the chemical to the environment and/or food source of the organism. Direct effects of inorganic fertilisers can be short-term, usually visible in the first season after application of the fertiliser (Balezentiene and Klimas 2009; Zhang et al. 2015a) and often when used in combination with organic fertilisers, or can be long-term if repeated additions are required (Marschner et al. 2003; Dolfing et al. 2004). Direct non-target effects on microorganisms are generally observed only in the band of application and can further depend on the rate of fertilisers applied (Stark et al. 2007; Mandic et al. 2011; Okore et al. 2014; Uz and Tavali. 2014); very high levels of the chemical are required to cause damage to microorganisms (Monokrousos et al. 2006). Indirect effects are usually long-term as they take more than one season to develop.

Irrespective of the uncertainties that remain, some serious concerns about the long-term, indirect effects have been expressed, i.e. soils might suffer cumulative detrimental effects, leading to the deterioration of soil physical, chemical and biological properties (Belay et al. 2002; Zhong and Cai 2007; Balezentiene and Klimas 2009; Zhang et al. 2015a). These, in the long run, could impact on the general health and productive capacity of soils (Barabasz et al. 2002; Ayoola and Adeniyan 2006; Zhang et al. 2015a). Indirect, long-term effects on soil microbial properties are evident from the changes in soil pH, electrical

conductivity (EC), CEC, and in the availability of C and nutrients (Zhong and Cai,2007; Zhang et al. 2015a).

Soil pH is widely accepted to be a major factor shaping microbial communities and their activities (Lauber et al. 2009; Rousk et al. 2010; Shen et al. 2012). They regulate a range of soil biological processes (Pietri and Brookes 2008) that have direct impact on the availability of nutrients and subsequent plant growth and crop performance. As catalysers of all biochemical reactions in soil, enzymes respond to mineral fertiliser induced changes long before any soil chemical and physical quality indicator changes are detectable (Bandick and Dick, 1999). Enzyme activities decrease under long-term mineral fertilisation applications (Shen et al. 2012; Zhang et al. 2015a), which are mainly, in part, due to lowering in soil pH. That enzyme activities are generally sensitive to soil pH has been well-documented (Makoi and Ddakidemi 2008; Das and Varma 2011). Acosta-Martinez and Tabatabai (2000) found that 13 out of 14 enzymes were significantly and positively correlated with soil pH.

Lower soil pH's are conceivably attributed to acidification (Bünemann et al. 2006). Frankenberger and Johanson (1982) found that the weakening of enzymatic activity that accompanies soil acidification is a function of decreasing bond strength in the active sites of the enzymes. Most N-containing inorganic fertilisers (e.g. LAN), unless specially treated, tend to acidify soil. This is mainly due to the fact that most fertilisers supply NH_4^+ or result in its production. Upon oxidation, NH_4^+ can release H^+-ions which are potential sources of soil acidity (Magdoff et al. 1997). Assimilation of urea by soil microorganisms lead to the production of acidic metabolites as organic acids (He and Suzuki 2004). The extent to which soil pH decrease under mineral fertilisation may nevertheless vary (Belay et al. 2002; Jorquera et al. 2014; Zhang et al. 2015a) and could be attributed to different duration of fertilisation time, different fertiliser application amounts and different plant types involved. It was found that other abiotic soil factors (not only related to soil acidity) such as Al, Ca, Mg, Mn and B, could also drive microbial population dynamics (Navarrete et al. 2012), a finding supported by Zhang et al. (2015b) who suggested that soil nutrients, rather than soil pH, may be more critical in shaping microbial communities. That no significant correlation was detected between bacterial communities and soil pH under a typical clay loamy anthrosol (Zhao et al. 2014), perhaps support this supposition. Nevertheless, this theory is very much debatable and remains unsubstantiated.

Conflicting results concerning the effects of pH on AM fungi have been reported (Carrenho et al. 2007). AM root colonisation may increase at low pH (Ingrid et al. 2002) or, in other cases, at high pH (Porteret al. 1987). Giovannetti et al. (2010) showed that AM species, and even isolates of the same species, responded differently to pH. Where differences in AM parameters were induced by acidity or heavy metals, it was found that germination and germ tube formation were the most severely affected (Giovannetti et al.

2010). Carrenho et al. (2007) speculated that susceptibility to colonisation of roots was higher under acid soil conditions because the need for mycorrhization was greater due to the generally lower mineral nutrient availability and more restricted root function in acid than in neutral soils. This finding is supported by the work by Van Aarle et al. (2002) who found that increasing soil pH suppressed colonisation of *Plantago lanceolata* roots by AM fungi. Inorganic P fertilisation has been consistently reported to decrease AM fungi, but the extent to which this occurs is reliant on the species of the fungus involved and the level of plant available P. Under conditions of high soil P concentrations, plants absorb soil P directly and not through mycorrhizae, resulting in increasing P concentrations in the root tissue that inhibits AM root colonisation (Menge et al. 1978b; Jasper et al. 1979). A concentration above 7 mg/kg P was found to inhibit AM root colonisation under controlled lab conditions (Brundrett et al. 1996). Conflicting results with respect to P fertiliser application is often evident from field observations (Douds et al. 1993; Meyer et al. 2003). Douds et al. (1993) observed low infection potentials and low incidences of AM fungi in high-input agricultural soils (Douds et al. 1993)), whereas Meyer et al. (2003) showed that relatively high AM root colonisation levels and high incidences of AM species in a high-input vineyard soil in which the soil P concentrations were markedly higher than the recommended phosphate fertilisation level for that particular vineyard. Plenchette et al. (1983) and Schubert et al. (1990) made similar observations in this regard. Thus, the work by Douds et al. (1993) accords with Carrenho et al. (2007) that colonisation increases with plant demand, whereas the work by Meyer et al. (2003), Plenchette et al. (1983) and Schubert et al. (1990) conflicts with the viewpoint of Carrenho et al. (2007). It was nevertheless suggested that root P rather than the soil P concentration is to be considered the determining factor in AM root colonisation (Graham et al. 1981).

Long-term N fertilisation also seems to lead to an increase in EC, indicating that it causes soil salinization (Pernes-Debuyser and Tessier 2004; Shi et al. 2009; Shen et al 2010). Salts tend to modify the ionic conformation of the active centre of enzymes; specific ion toxicities can also result, causing nutritional imbalances for microbial growth and subsequent enzyme synthesis (Frankenberger and Bingham 1982). In addition, Pankhurst et al. (2001) found that agriculture-induced salinity caused a shift towards a less active, less diverse, bacteria-dominated community). High fertiliser N inputs also decreased soil CEC, thus decreasing the soil's nutrient supplying capacity. Research showed that α- and *β*-glucosidase tend to be adsorbed on kaolinite and goethite (Lammirato et al. 2010), with adsorption of the protein increasing as pH falls below neutrality, leading to a concurrent decrease in enzyme activities (Quiquampoix et al. 1989).

Soil C is considered an important indicator of soil quality tied to major soil functions, such as aggregate stability, nutrient retention and availability, and nutrient cycling. Reduction

in soil organic C contents is obvious from the reduced input of organic matter when excessive inorganic fertiliser is used (Bünemann et al. 2006; Wooldridge et al. 2013a; Zhang et al. 2015a). It was shown that soil organic C in the vast majority of soil samples taken from many sites decreased after long-term N fertilisation (Miao et al. 2011; Zhang et al. 2015a). High inorganic N inputs induces a positive priming effect resulting in a net loss of soil C (Kuzyakov et al. 2000; Russell et al. 2005; Hamer et al. 2009), especially when crop residue is not returned to the soil and underground biomass is small (Kuzyakov et al. 2000). It appears that N fertilisers can enhance the activities of heterotrophic soil microorganisms that use C derived from crop residues or soil organic matter and thus stimulate decomposition (Mack et al. 2004; Khan et al. 2007). A decreased C:N ratio can cause a shift from fungal to bacterial-dominated microbial communities that could result in faster decomposition of soil organic C (Kuzyakov et al. 2000; Miao et al. 2011). That soil microbial enzyme activities significantly decrease due to a reduction in soil organic C content was reported by several studies (Stark et al. 2008; Zhang et al. 2015a).

1.6.1.2 Effect of insecticides

The use of insecticides is a necessary practice in modern agricultural production and is meant to protect plants against different groups of pests to ensure quality production of crops. Insecticides like the organophosphorus types (e.g., chlorpyrifos, phorate, phosalone, and pirimiphose methyl) have been extensively used for more than 40 years are used throughout the world to control a wide range of sucking and chewing insects and mites on a range of crops, including deciduous fruit trees, citrus fruit, bananas, vegetables, potatoes, beet, sugar cane, coffee, cocoa, tea, tobacco, cotton, and rice. It is also used to control agricultural soil-dwelling insects (Tomlin 2003). Due to the frequent use of insecticides, there is a significant amount that reaches the soil where it accumulates and persists (Gupta et al. 2008), especially in the top layers where the greater part of microorganisms reside (Alexander 1978).

Several groups of microorganisms are capable of decomposing a great variety of insecticides to derive energy and other nutrients for their metabolism, the information of which has been widely reported on (Hayatsu et al. 2000 Ghassempour et al. 2002; Karpouzas et al. 2005; Yasouri 2006; Lakshmi et al. 2008). As a result, their biomass may increase which may favourably influence nutrient transformations in soil (Agnihotri et al. 1981; Das and Mukherjee 1994). It has been revealed that high organic matter content led to a reduction in organophosphorus insecticides bioavailability and their degradation rate (Karpouzas and Walker 2000), whereas other studies, like that of Singh et al. (2006), showed no significant effect on fenamiphos and chlorpyrifos dissipation.

Nevertheless, there is great concern about the environmental stability, bioaccumulation, and toxicity of a variety of insecticides to non-target microorganisms and their associated transformations in soil (Martinez-Toledo et al. 1992; Pandey and Singh 2004). The insecticidal effects on soil microorganisms and their functions they perform or mediate, such as N-fixation, nitrification, ammonification, organic matter decomposition, sulfur oxidation and P solubilization, are very specific since the response by certain members within a group may vary with the level of toxicity (Simon- Sylvestre and Fournier 1979). The effects are with respect to different types of insecticides (Das and Mukherjee 1998), insecticide doses (Das and Mukherjee, 1998), soil depths (Digrak and Ozcelik 1998; Tawfic et al. 1998), number of microorganisms, alterations in biochemical activity (including enzyme activities), quantitative and qualitative decrease of the microbial community (Vig et al., 2008; Filimon et al. 2015), growth and activities of microorganisms (Das and Mukherjee 1998), transformations of nutrients (Das and Mukherjee 1998); soil fungi (Slavikova and Vadkertiova 2003; Abd El-Mongy and Abd El-Ghany 2009) ecophysiological groups of bacteria (Filimon et al. 2015).

Nonetheless, the effects of insecticides on microbial communties in soil remain difficult to determine because many other variables should be considered like habitat, soil structure, organic and inorganic composition, texture, pH and temperature (Monkiedje et al. 2002, Beulke et al. 2004).

1.6.1.3 Effect of nematicides

Plant parasitic nematodes are important pests of the greater part of cultivated crops. Root-knot nematodes (*Meloidogyne* spp.), which have been recognized as one of the major nematode pest of a wide range of crops, particularly of vegetables, are found in abundance, especially in sandy soils, and causes dramatic yield losses in agriculture (Sikora and Fernandez 2005). In the same way as for crop damage caused by other pests and pathogens, the size of crop losses caused by nematodes is of great concern to farmes in general.

There are a number of nematicides to effectively control nematode pests of annual crops (van Berkum and Hoestra 1979; Mohan 2011). In cases where effective control of plant-parasitic nematodes in roots and soil by systemic nematicides were achieved, their population numbers usually reduced substantially (Hague and Gowen 1987; Mohan 2011). However, without repeated applications of nematicides the prospect of effectively controlling many of the pests of perennial crops would have been very little. It is precisely why, with repeated application of nematicides, that they are believed to cause non-target detrimental effects to organisms in soil (Bollen 1993). They are thought to radically alter soil organisms, including bacteria and fungi, due to their broad-spectrum activities (Ibekwe 2004). Fumigant usage resulted in the absence of nematode competitors, predators, and parasites in soils

(Bell et al. 2006). The elimination of mycorrhizas by methyl bromide resulted in poorer plant growth (Klein et al. 1996). Long-term aldicarb treatment of potato fields decreased the number of bacterial genera and species, decreased the population levels of plant growth promoting rhizobacteria, but also increased total bacterial biomass, compared to untreated soils (Sturz and Kimpinski 1999). Other reports, like that of Suneja et al. (2008), suggest little to no effect on microbial populations and activities in soil. Nematicide usage is nonetheles problemetic with respect to the environmental as they cause soil and groundwater contamination (Sukul et al. 2001).

As evident from the above, future control of nematodes will arguably increasingly rely on site-specific, sustainable management practices, as well as on integrated pest management involving sensible use of nematicides. Nonchemical strategies available to growers include crop rotation, altered planting time, resistant germplasm, solarization, fallow and nematode suppressive soil amendments (Akhtar and Malik 2000; Randhawa et al. 2001; Stirling et al. 2005; Mohan 2011). Many of these strategies are less expensive, but sometimes could also be less effective than traditional chemical control.

1.6.1.4 Effect of herbicides

In modern agricultural production, herbicide application is a regular practice. As farmers continue to realize the usefulness of herbicides, larger quantities are applied to the soil (Liu et al. 1997). After application, herbicides may be washed away through surface run-off, leached (in which case groundwater is contaminated), inactivated by plants, or incorporated in soil directly during plant treatment (Ayansina et al. 2003). There is an increasing concern however, that herbicides use in conventional crop management not only affect the target organisms (weeds), but that these chemicals may also exert certain effects on non-target organisms, including soil microorganisms (Wardle and Parkinson 1990a, 1990b; Sebiomo et al. 2011; Druille et al. 2013), and the critical functions they perform or mediate, such as organic matter degradation and nutrient cycling (e.g. the N-cycle) (Hutsch 2001; Sebiomo et al. 2011). It appears that even only minor alterations in the diversity of microbial communities could affect soil health and ecosystem function (Dunfield and Germida 2004).

Several studies have reported on the negative non-target effects of herbicides. For example, it has been observed that alachlor and paraquat are toxic to bacteria (Sahid et al. 1992). Paraquat has also been shown to temporarily stress and inhibit bacterial populations (Kopytko et al. 2002). Glyphosate also has been observed to cause a decrease in pseudomonad populations (Kremer and Means 2009). In a study by Sebiomo et al. (2011), bacterial, fungal and actinobacteria populations decreased, percentage organic matter decreased and dehydrogenase activity decreased upon treatment with herbicides (atrazine, primeextra, paraquat and glyphosate). Dehydrogenase activities were also found to be

inhibited by glyphosate in sandy loam soil by Dzantor and Felsot (1991) and under laboratory conditions by Nada and Mitar (2002). Glyphosate apparently inhibits protein synthesis via the shikimic acid pathway in bacteria and fungi (Bentley 1990; Franz et al. 1997). Reduced enzymatic activities were also found by Dzantor and Felsot (1991) in a study on the interference of atrazine with phosphatase, dehydrogenase and esterase activity of soil. Application of imazethapyr to a silty loam and a loamy soil lead to a shift in the soil community structure as seen in a reduction from soil microbial biomass C (Zhang et al. 2010).

Most herbicides used at normal field rates are generally considered to have no major or long-term effect on gross soil microbial activities (Subhani et al. 2000; Zabaloy et al. 2008) and that positive effects of herbicides on non-target organisms are also possible. Herbicide application to soil may lead to the proliferation of general or specific organisms that can utilise a particular chemical in the herbicide for nutrition (Paulin et al. 2011). Increases in the populations of actinobacteria and fungi (Araujo et al. 2003) and soil microbial biomass (Hanley et al. 2002), and stimulation in microbial respiration (Busse et al. 2001), enzyme activity (Perucci et al. 2000) and nutrient cycling processes (Carlisle and Trevors 1986), after treatment with glyphosate, were observed. Both glyphosate and paraquat have been reported to cause activation in soil urease and invertase soil enzymes (Sannino and Gianfreda 2001), while diquat and paraquat increased fungal populations (Mewatankarn and Sivasithamparam 1987). Moreno et al. (2007) reported that an increase in metabolic activity with atrazine concentration and with incubation time can be deduced from their work. Similar results were presented by Rossel et al. (1997). The stimulation of bacterial populations in soil by atrazine (Ros et al. 2006) as well as the stimulation of aerobic heterotrophic bacterial populations by glyphosate, 2,4-D-dichlorophenoxyacetic acid (2,4-D) and metsulfuron (Zabaloy et al. 2008) has also been documented.

It nevertheless appears that the effects of herbicides on non-target organisms rely heavily on the dose at which they are applied. For example, high doses of atrazine and alachlor (3 and 4 l ha^{-1}, respectively) caused decreases in the total number of bacteria, ammonifiers and azotobacters and they reduced dehydrogenase activity (Konstantinoviã et al. 1999). A tenfold dose of glyphosate affected negatively, the activity of this oxide-reducing enzyme by 5% (11 weeks after herbicide application) (Schuster and Schröder 1990). From the above, it is evident that an increase in herbicide dose tends to amplify its negative effect on microorganisms.

Still, other studies, like that of Busse et al. (2001), reported no long-term change in microbial populations in response to herbicide (glyphosate) application at the recommended field rate. Also, no response in soil dehydrogenase activity to herbicide application was detected by Lethbridge et al. (1981) and Nakamura et al. (1990). These accords with the

conclusions of Bünemann et al. (2006) and Banks et al. (2014) that herbicides had minimal or no effect on soil microbes.

The effects of herbicides on root colonisation by AM fungi have also been researched and reportedly range from positive to negative (Ronco et al. 2008). Negative effects of glyphosate have been recently reported by Druille et al. (2013) and Zaller et al. (2014). Causes of negative effects may be direct, i.e., damage to the external hyphae and/or AM spores, or indirect, whereby the production, amounts or quality of root exudates, the chemical trigger for root colonisation by AM fungi, is reduced by the herbicide-induced death of targeted host plants (Yao et al. 2005; Druille et al. 2013). Root colonisation by AM fungi appears greater at high root densities (under mulching) due to an increased probability of root-to-root colonisation than at low root densities (under herbicide strips) due to a decreased probability of root-to-root colonisation (Atkinson and White 1980; Atkinson 1983; Yao et al. 2009). Thus, herbicide weed or covercrop control in intercropping systems, those typical used in vineyards and orchards systems, could potentially affect the link (root-to-root AM colonisation) between work row cover plant roots and roots of vines or trees in planting rows, and subsequently the performance of the crop.

Fate of herbicides in the soils has also become increasingly important. Herbicides, once applied to soil, apart from leaching, may become subject to chemical or microbiological degradation. Rate of herbicide decomposition in soil is influenced by the application rate, the physical and chemical soil properties, soil moisture and temperature, plant cover, soil cultivation method and kinds of microorganisms present (Schuster and Schröder 1990; Radosevich et al. 1995). Experiments have shown that microorganisms may use atrazine as a source of C (Radosevich et al. 1995) or N (Cook and Hutter 1981), or glyphosate as a source of C, N and P (Partoazar et al. 2011; Duke et al. 2012). Plants too have an important role in herbicide decomposition. They incorporate herbicides via roots, stems and leaves. Herbicide dose and mode of application determine how it is incorporated by the aboveground plant parts or the roots. Experiments of Barriuso and Houot (1996) showed that atrazine's triazine rings were mineralised faster in soils under corn treated with this herbicide each year than in the untreated soils under wheat or grasses. Increased moisture and temperature accelerate the degradation of atrazine and 2,4-D (Willems et al. 1996). The rate of atrazine mineralisation is slow (<2%), further decreasing in deeper soil layers, at low temperature and at low moisture content.

It is evident that the documented evidence of the effects of herbicides on soil micro-organisms is inconsistent and even contradictory. It is therefore difficult to discern the general trend of herbicide effects on soil microorganisms since the information extends to a wide range of treatment combinations with other herbicides, application rates, target crops, non-target microorganisms and soil types, under different experimental conditions. In a

recent review, Wolmarans and Swart (2014) stressed that controversy persists regarding the positive and negative effects of glyphosate, and of herbicides generally, on non-target biota and conclude by stressing the need to utilise management practices likely to optimise crop production efficiency and promote sustainable weed control.

1.6.1.5 Effect of fungicides

The success of modern agriculture is, by and large, accredited to the use of different agrochemicals, including fungicides, to control fungal plant diseases and maintain production of quality crops. Unfortunately, a serious of ecotoxicological risks arises from the accumulation of fungicides in soil when it is indiscriminately used. Although intended to protect crops from plant pathogens, fungicide control often goes beyond this intended role and the frequent use of these chemicals in fields and orchards may exert a harmful effect on non-target soil organisms whose functions are vital to soil fertility, general soil health and crop performance (Wainwright 1978; Wang et al. 2009).

As demonstrated by several authors, fungicides are much more damaging to certain microbial groups than herbicides (Kruglov 1991; Bünemann et al. 2006). Fungicides are usually applied at high rates (Ojo et al. 2006). The negative effects of high fungicide doses on soil-residing microorganisms were confirmed by a study in which the fungicides Unix 75 WG and Swing Top 183 SG, applied in quantities larger than the recommended dose, greatly inhibited copiotrophic bacteria and actinobacteria (Jastrzębska 2006). Many other reports have shown that use of fungicides in the long-run can lead to a disturbance of soil microbial communities by reducing the biomass of non-target microorganisms (Pal et al. 2008; Tejada et al. 2011) or by changing their biochemical activity (Bending et al. 2007; Wang et al. 2009; Milenkovski et al. 2010; Muñoz-Leoz et al. 2011). The application of fungicides may also change the structural (Wang et al. 2009) and functional diversity of bacterial communities (Muñoz-Leoz et al, 2011; Wang et al. 2012). Negative effects on microbial enzyme activity where Cu-based fungicides sprays have been used in agricultural soils over protracted periods, are commonly observed (Merry et al. 1983).

If applied at the commercial field dose, most fungicides are likely to effectively combat diseases, but in cases of indiscriminate use, fungicides may also have side effects with detrimental consequences on AM fungi (Kjøller and Rosendahl 2000; Thingstrup et al. 2000). However, from studies elucidating the efficacy of several fungicides on AM fungi, it is clear that the effects of different fungicides are not necessarily of the same magnitude (Kjøller and Rosendahl 2000). Benomyl, an agricultural fungicide, is particularly known for its deleterious effects on AM fungi. However, it has been shown that this fungicide may sometimes have a fungistatic rather than a fungicidal effect on spore germination (Venedikian et al. 1999). Nevertheless, the environment and detrimental side effects on AM

fungi necessitates an environmental friendly alternative to fungicides, as was suggested for methyl bromide fumigation (Thomas 1996; Sances and Ingham 1997).

Soil microbial communities, particularly those residing in the superficial layers of soils of vineyards and orchard floors, are especially susceptible to the non-target harmful effects of fungicides that are repeatedly applied during the growing season. Cu-based fungicides sprays in conventional production systems, in both vineyard (Fernández-Calviño et al. 2010) and orchard (Wang et al. 2009) systems have been shown to inhibit microbial enzyme activities. Fungicides common to orchard practices usually belong to the azole group, the most important being imidazoles and triazoles (Verweij et al. 2009). Azole fungicides, which are demethylation inhibitors (DMIs), affect the biosynthesis of fungal ergosterol through the inhibition of cytochrome P45014-α steroldemethylase (Amer et al. 2007). Sułowicz and Piotrowska-Seget (2015) have shown that microbial activity in an apple orchard soil had dreased after having been treated with tetraconazole. The application of tetraconazole also significantly changed the structure of the microbial communities in the same orchard soil.

Resistance of microorganisms to fungicides is also of concern. Intensive and consecutive use of anazole fungicides can create fungal populations with a reduced sensitivity to DMIs (Holb and Schnabel 2007). The spread of this resistance to applied triazoles forces the application of new compounds which showed activity against pathogens (Holb and Schnabel 2007; Verweij et al. 2009). No significant impact on the CFU number of bacteria was found in soils treated with the fungicides imazalil (Thirup et al. 2003) and carbendazim (Tortella et al. 2013). The lack of changes in the number of bacteria in the orchard soil may be a result of the development of resistance by the bacterial community in response to successive fungicide stress. Moreover, many bacteria in soils with a long history of fungicide application are able to use these chemicals as sources of C (Arbeli and Fuentes 2007), and their proliferation may compensate for the loss of pesticide-sensitive microorganisms. No significant changes in the biomass of bacterial fatty acids in soil that had been treated with captan and the bactericide oxytetracycline, were reported by Piotrowska-Seget et al. (2008). Similarly, White et al. (2010) found a negligible effect of triazole usage on the soil fungal biomass.

However, the application of captan caused a significant reduction in the biomass of fungal fatty acids. The study by Zhang et al. (2014) revealed significant decrease in the total and bacterial PLFAs in a silty loam soil treated with different dosages of tetraconazole. The negative impact of tetraconazole on the substrate utilisation was also observed (Zhang et al. 2014). Sułowicz and Piotrowska-Seget (2015) using Biolog profiles revealed a decrease in the catabolic activity of the microbial communities in a grassland soil that had been treated with tetraconazole over time.

It is clear that evaluating the effects of fungicides on soil microorganisms appears to be quite challenging because of their huge diversity, the influence of soil properties, the limited knowledge of their interactions (Jacobsen and Hjelmsø 2014), and that there is no universal method that adequately assesses the risks associated with application of these chemicals (Ramsey et al. 2006; Montecchia et al. 2011). Puglisi (2012) nevertheless indicated that PLFA and Biolog methods are the most sensitive and useful tools for determining the effects of fungicides on microbial communities.

1.6.2 Effects of organic management practices

1.6.2.1 Effect of compost and manure applications

There is increasing concern that soils might suffer long-term degradation due to damage to soil structure, reductions in soil organic matter levels and loss of soil fertility under intensive agricultural systems (Anon 1970; Allen-Stevens 1999). As an alternative, the application of organic soil amendments (including compost, sewage sludge and farmyard manure) has gained enormously importance worldwide since it offers a whole range of benefits, both agronomic and environmental.

Organic matter is a key determinant of soil fertility and quality (Stockdale et al. 2001; Perez-Piqueres et al. 2006; Garland et al. 2010) with positive effects on the physical, chemical and biological soil properties (Ciećko et al. 2001, Talgre et al. 2012; Wyszkowska et al. 2013). Organic matter is a source of C to microorganisms and of different nutrients, like N and P, and micronutrients, to plants, (Scherer and Sharma 2002). Reganold (1988) found greater organic C and N, in organic than in conventionally managed soils. Long-term application of sewage sludge, compost and farmyard manure resulted in an increase in the total C and N content of the soil (Werner et al. 1988). Soil organic C is the key determinant of shifts in microbial communities (Drenovsky et al. 2004) and although Tianaa et al. (2015) is of the opinion that compost application is not always beneficial to soil quality, and might decrease soil diversity, numerous studies in the past have frequently reported that microbial diversity is affected by fertilisation regimes and that higher size and diversity of microbial communities were found in organically than in inorganically fertilized systems (Esperschütz et al. 2007; Zhang et al. 2012). Besides that compost usually increases the content of soil organic C (Achiba et al. 2010), it also increased soil microbial activity and gene copies of bacteria, archaea, and ammonia-oxidizing bacteria (Tianaa et al 2015). Interestingly, microbial activity was found to have increased thirteen years after a single sewage sludge application in comparison with mineral fertiliser application (Barbarick et al. (2004). In another study, large amounts of different composts had no effect on the relative abundance of actinobacteria, with actinobacteria having been the predominant species across the

different composts applied (Tianaa et al. 2015). Apparently, this is not uncommon as actinobacteria appear to be relatively ineffective competitors under high nutrient conditions versus other microorganisms, as was shown by Ryckeboer et al. (2003). With regards to the effects on AM fungi, Purin et al. (2006) demonstrated that when mineral fertilisers were substituted by organic fertilisers, AM activity generally improved.

Low fertility of soils is the primary constraint to increased crop yields (Ogbodo 2009ab 2010). Good soil fertility management, as in organic systems, to ensure adequate nutrient availability to plants and yield increase, is reliant on various catalyzing reactions necessary for the decomposition of organic matter and nutrient cycling in soil; these are vital functions that are controlled by microbes and soil enzymes (Monokrousos et al. 2006, Karaca et al. 2011). Three microbial enzymes, i.e. ß-glucosidase, urease and phosphatase, which are active in the cycling of principle nutrients C, N and P, respectively, are particularly sensitive to management-induced changes in the soil environment due to their strong relationship with soil organic matter content and quality (Masciandaro and Ceccanti 1999; Caravaca et al. 2002). Many studies support the generalised view that enzyme activity increase with increase organic input to the soil (Marcinkeeviciene and Pupaliene 2009). Organic manure incorporation increased enzyme activities to different degrees and gave a significant and positive relationship with organic C and total N (Nayak et al. 2007). Enzymatic activities are all more sensitive to the changes in soil quality than soil organic matter (Allison et al. 2008), which makes them candidate ‘sensors’ of soil stress for management practices to timely forewarn soil degradation (Bergstrom et al. 1998; Margesin et al. 2000). When compost, produced from animal manures, was intensely and repeatedly applied, it resulted in accumulation of heavy metals and inhibition of growth of microorganisms in soils (Baldantoni et al. 2010; Sun et al. 2014). Given that enzymes function as a measurement of the metabolic state of soil microorganisms (Watts et al. 2010), high concentrations of heavy metals may inhibit soil microbial and enzyme activities (Wyszkowska and Wyszkowski 2003), a finding that Scherer et al. (2011) could nevertheless not confirmed. The toxicity of heavy metals may nevertheless depend on their bioavailability and not necessarily on the total concentration (Davis and Carlton-Smith 1981; Scherer et al. 2011). On the other hand, even if organic amendments exert positive effects on soil microbial properties, plant growth may still be inhibited by toxic levels of heavy metals in soil (Werner et al. 1987; Scherer et al. 2011). In deficiency situations, supplying such elements may nevertheless promote soil microbial and enzyme activity (Ashman and Puri 2002). Herencia et al. (2008a, 2008b) mainly attribute the higher concentrations of some soil micronutrients that are generally found in organically managed soils, compared to those that are non-organically managed, to increased solubility. In the case of Mn, increased solubility is due to microbial decomposition of organic compounds under reducing conditions (Herencia et al. 2008b). It is clear that the

information available on the effects of heavy metals in soil amendments on microbial properties as enzyme activity is contradictory and both beneficial and detrimental responses are possible.

Organic materials are generally believed to stabilise or even increase soil pH (Reganold 1988; Deng et al. 2006; Zhong et al. 2010; Gopinath and Mina 2011; Gopinath et al. 2011; Wooldridge et al. 2013a; Uz and Tavali 2014). This may be due to the fact that certain organic materials have alkaline reaction (Wooldridge et al. 2013a), which is why supplemental liming to counteract the resulting acidifying effect of certain inorganic fertilisers is often necessary. Soil enzyme activities are sensitive to soil pH (Makoi and Ndakidemi 2008; Das and Varma 2011). The effect of pH on enzyme activities is influenced by the buffering effects of organic C compounds (Acosta-Martínez and Tabatabai 2000). Frankenberger and Johanson (1982) found that pH alters the ionisation state of the enzyme's active centre. Applications of organic fertilisers are also known to increase soil EC. However, the increase in EC by vermicompost and farmyard manure, as shown by Uz and Tavali (2014), did not cause any salinity problem. Several researchers obtained similar results and concluded that, in general, organic fertilisers do not cause salinity problems when applied at moderate levels (Gerke et al. 1999; Kirchmann and Bergström 2001). Regular additions of organic matter, such as manure compost, have been shown to increase CEC (Pernes-Debuyser and Tessier 2004; Zhang et al. 2015b). Reganold (1988) found increases in CEC in organic compared to conventional management systems, thus increasing the soil's nutrient supplying capacity under organic management.

Regular additions of organic matter, such as manure compost, have been shown to improve water holding capacity, cohesion and soil stability (Pernes-Debuyser and Tessier 2004). Improved soil stability has also been tied to glomalin, a glycoproteineous, water-stable substance with binding properties in soil, released during decomposition of AM fungal hyphal and spore walls (Driver et al. 2005; Purin and Rillig 2007; Fokom et al. 2012). This may explain why, in the work of Rillig et al. (2003), both soil C and N, being the main components of soil organic matter, were strongly correlated with glomalin across different soils and within different land-use types. Correlations of various fractions of glomalin with C under different soil conditions were also shown by others (Wright and Upadhyaya 1998; Nichols and Wright 2005; Peng et al. 2015). Rate of decline of glomalin is slow (Preger et al. 2007) and therefore, glomalin may sequester relatively large amounts of C in soil (Treseder and Alen 2000; Quiquampoix and Burns 2007), as does soil organic matter (De Neve et al. 2003; Nendel and Reuter 2007). That sequestration of soil C in organic matter has been tied to clay-size fractions, suggests that glomalin is protected by association with soil clay minerals (Lobe et al. 2001). Enzyme activities are similarly tied to soil organic matter via its enzyme stabilising effect (Tabatabai 1994). Immobilisation of soil extracellular enzymes stem

from the three-dimensional network of clay-humus complexes (Tabatabai 1994; Naidja et al. 2000). Increases in the levels of enzyme activity in organic-amended soil may therefore, reflect a greater amount of protective sites within the soil as a result of enhanced humus content.

1.6.2.2 Effect of mulches

Weed control, one of the major management challenges of modern day agriculture, is usually carried out through herbicide application in conventional systems. However, the constant use of these chemicals can be harmful to the environment, including the microorganisms that reside in the soil (Schutte 2002). To avoid or reduce these negative effects, some orchards are managed utilizing conservationist methods of soil management. Covering soils with different types of mulches to suppress the growth of weeds, such as bark, peat, sawdust and straw, or even synthetic mulches, has become common practice in agriculture. Unlike synthetic mulches, which need to be removed after a period of time, organic mulches decompose and they need to be re-applied regularly.

Appropriate mulching is a key tool in the management of most woody perennial crops, which, apart from controlling weeds (Verdú and Mas 2007; Sinkevičienė et al. 2009, (Wooldridge et al. 2013a, 2013b) also impropves soil organic matter content, soil physical health (including texture) ro protects soil from erosion (Jafari et al. 2012), and maintains soil structures conducive to rapid gas exchange (Tisdall et al. 1984; Hogue and Neilsen 1987), improves crop productivity and quality (Hogue and Neilsen 1987; Pervaiz et al. 2009; Siczek and Frąc 2012; Zhu et al., 2010; Gong et al. 2013), provides greater relative stability by buffering drastic changes (diurnal/seasonal fluctuations) in soil temperature (Sinkevičienė et al. 2009; Jafari et al. 2012), increases water use efficiency (conserving soil moisture) by reducing evaporation (Sinkevičienė et al. 2009; Siczek and Frąc 2012), and suppresses plant diseases (Robinson 1988; Hoitink and Boehm 1999; Grundy and Bond 2007) Organic mulches also improve soil conditions that supports microbial biomass and microbial activities, such as soil respiration (Hogue and Neilsen 1987; Grundy and Bond 2007; de Oliveira Almeida et al. 2011) and enzyme activities (Yang et al. 2003; Siczek and Frąc 2012) which, in turn, drives the decomposition processes in soil and releases nutrients (Haynes 1980; Sinkevičienė et al. 2009). Interestingly, straw mulch applied to heavy metal-contaminated soil, releases allelochemicals that regulate hyperaccumulator growth and heavy metal accumulation (Lin et al. 2014). Organic acids and other organic matter such as humus derived from the straw through decay and decomposition change the rhizosphere pH, redox potential, and nutrient availability, and thereby affect the bioavailability of heavy metals in the rhizosphere soil (Blanco-Canqui and Lal 2007; Ge et al. 2014).

Some favorable soil conditions as described above, such as increases in soil organic matter, greater cation exchange capacity, higher rates of soil microbial respiration, and the presence of disease-suppressive soil microflora, promote tree root growth under mulch (Yao et al. 2005; Yao et al. 2009; Kotze 2012). For example, in a study of the effects of conventional and organic practices on apple performance, root numbers in the 0-30 cm soil depth interval where the soil was kept bare under herbicide treatment, averaged 245 in conventional treatments compared to 516 under mulching in organic treatments (Wooldridge et al. 2013b). In return, higher root densities could increase the frequency of colonisation of apple roots by AM fungi compared to lower root densities under unmulched treatments (Kotze 2012), supposedly because higher root densities under mulch increases the probability of root-to-root colonisation by AM fungi (Atkinson 1983).

Mulching exert other positive effects on the soil and crops. For example, straw mulch (Sønsteby et al. 2004) and grass mulch (Cadavid et al. 1998) significantly increased the available P and K in the soil. Sønsteby et al. (2004) established increased amounts of P and K levels in crop leaves in plots mulched with wood chips. Mulching also improves plant growth, yield and yield quality (Sharma and Sharma 2003). An increase in grain yield by mulching was attributed primarily to decrease in soil temperature and improved soil moisture regimes (Lal 1974). Nevertheless, some researchers have clamied no effect of mulching with wood chips on crop yield (Gruber et al. 2008) and no improvement on potato yields with mulching (Johnson et al. 2004; Döring et al. 2005).

Conversely, some reports claim that certain mulches (straw, peat, sawdust) may negatively affect agricultural crops (Johnson et al. 2004; Sønsteby et al. 2004). Applying straw mulch (high C:N ratio) to the soil surface may dry up soil N due to a wide C:N ratio (Johnson et al. 2004; Sønsteby et al. 2004; Tolk et al. 1999) This is because the growth of microorganisms, utilising these high C:N sources, become N-limited in which case microorganisms revert to using the soil's remaining N supplies to meet their N requirements, which eventually lead to temporary N deficiency in soil (Tolk et al. 1999). Instead, nutrient addition via fertilisation could stimulate their growth (Aber 1992; Wardle 1992; Vanotti et al. 1995). Conversely, decomposition of organic matter with a C:N ratio less than 30:1 may result in C limitation of microbial growth (Aber 1992; Wardle 1992; Kaye and Hart 1997) in which case external C inputs are needed.

1.6.2.3 Effect of cover crops as an intercrop

Grasses, legumes, and forbs that are planted to offer seasonal cover for arable soil, are referred to as cover crops. Depending on the season for soil cover, cover crops are further divided into summer and winter types. A cover crop that is interplanted and grown with an annual row crop is also known as living mulch. Sowing of cover crops is becoming

increasingly popular in perennial agroecosystems like vineyards and deciduous fruit orchards as a way to provide several benefits to the cropping habitat.

The primary function of cover crops is for soil and water conservation. Often in combination with other conservation agriculture techniques (minimum tillage or no-till), cover crops provide many benefits to the soil and main crop, including controlling weeds through competition (Lanini et al. 1989; Liebl et al. 1992; Den Hollander et al. 2007), reducing erosion (Lanini et al. 1989; Liebl et al. 1992; Novara et al. 2011), enhancing fertility (Lanini et al. 1989), improving soil quality (Lanini et al. 1989), enhancing soil organic matter (Steenwerth and Belina, 2008), enhancing soil C dynamics (Jackson et al. 2004; Smith et al. 2008; Steenwerth and Belina 2008; Celette et al. 2009; Peregrina et al. 2010, 2012) enhancing soil N dynamics, N availability, microbiological functions, as N mineralisation, nitrification and denitrification (Steenwerth and Belina 2008), enhacing microbial enzyme activity (Virto et al. 2012; Peregrina et al. 2014), enhancing soil C sequestration (Grandy and Roberston, 2007), improving productivity of degraded soils (Bruce et al. 1995; Sainju et al. 2002), and facilitating vast underground networks of AM fungal hyphae and spores which interconnect interplanted cover crop and row crop roots (Meyer et al. 2003). Evidently, cover crops are both of environmental and ecological significance to the production of agricultural crops.

In contrast, when using cultivation, the soil's capacity to deliver on the abovementioned benefits, are diminished (Steenwerth and Belina 2008). For example, soil tillage buries residues, disrupts macroaggregates, and rushes SOM decomposition that results in C loss from soil to the atmosphere as CO_2 (Reeves 1997); whereas cover cropping together with no-till, slows down SOM turnover by reducing disturbance. Cover crops also avoid the non-target negative effecs stemming from the use of herbicides, on soil microorganisms (Wardle and Parkinson 1990a, 1990b; Sebiomo et al. 2011; Druille et al. 2013), on the enzymes they produce (Dzantor and Felsot 1991; Sebiomo et al. 2011) and the functions they perform (Hutsch 2001; Sebiomo et al. 2011) as well as on the direct and indirect harmful effects on AM fungi (Druille et al. 2013). Use of cover crops also avoids disturbance effects of tillage on general microbial enzyme activities (Acosta-Martínez and Tabatabai 2001) and to the delicate underground AM fungal hyphal networks; cultivation has particularly negative effects on glomalin levels in field soils (Avio et al. 2013).

Compared to individual species, cover crop mixtures can improve soil productivity by building active organic matter pools and increasing N mineralisation due to their increased biomass production (Mutch and Martin 1998). A field study showed significantly higher soil C concentration under three winter cover crop treatments (i.e. rye, hairy vetch, and crimson clover) than no cover crop control (Sainju et al. 2002); the effects were dependent on cover crop species and tillage practices (Steenwerth and Belina 2008). In addition to the total

amount of organic C, the quality of soil organic matter (chemical composition of crop biomass) is also reported to vary with different cover crops (Ding et al. 2006). High lignin content generally retards decomposition (Trofimov 1997). Indeed, cover crops can increase N supply and reduce the amount of N fertiliser required for succeeding summer crops (McCracken et al. 1994; Kuo et al. 1997). They fix N_2 through rhizobium symbiosis, by which rhizobia provide N for host plants through N_2 reduction to ammonia (Atkins 1984). In this way, legumes are utilised as an efficient strategy to increase soil N supply, especially in N deficient soils, whereas less than 40% of legume plant N was recovered by non-legume crops due to asynchrony between N mineralisation and plant N uptake, N loss via leaching, microbial N assimilation, and low plant N use efficiency on account of non-legume crops (Hesterman et al. 1987; Harris et al. 1994).

Despite being regarded by many as a conservationist method of soil management, use of cover crops often go along with termination methods by which cover crops are chemically killed and returned back to the soil. A killing method using synthetic herbicides after rolling was reported to reach a termination rate up to 90%, which was 10% higher than using physical means alone (Kornecki et al. 2012). Cover crop residues can be cut into small pieces or remain intact. After termination, cover crops can be placed either on the surface or incorporated into the soil. Surface spreading is good for maintaining soil moisture and reducing temperature fluctuation (Ramakrishna et al. 2006; Sarkar and Singh 2007; Sarkar et al. 2007; Murungu et al. 2011). However, the non-target negative effects of herbicies on soil microorganisms (Wardle and Parkinson, 1990a, 1990b; Sebiomo et al. 2011; Druille et al. 2013) need to be seriously considered.

1.7. Research objectives

1.7.1 Overall objective

In this thesis it is hypothesised that If ORG and CON orchard floor management practices affect orchard ecosystems differently, such differences would be measurable in terms of differences in microbiological parameters, and that ORG practices would induce positive soil microbiological responses in Western Cape apple orchards relative to CON practices, and by inference general soil health and apple tree performance. Any differences revealed would be evidence in favour of rejecting the null hypothesis, i.e. that CON and ORG treatments do not affect soil microbiological parameters.

Therefore, the main objective of the study was to investigate the effects of conventional (utilising synthetic fertiliser and herbicide) and organic (nutrients supplied in compost, weeds controlled with straw mulch) orchard floor management practices on selected soil microbial properties and to establish if these translate into differences in soil nutrient status and apple

performance under the conditions that prevail in commercial apple orchards of the winter rainfall region of the Western Cape, South Africa.

1.7.2 Specific objectives

1) To measure the effects of CON and ORG practices in a 'Cripp's Pink'/M7 apple orchard on the activities of *β*-glucosidase, acid phosphatase and urease, and on the relative abundance of actinobacteria, bacteria, fungi and heterotrophs. Certain of these parameters were correlated with soil and tree factors to investigate possible links between soil microbial populations and enzyme activities, soil chemical characteristics and orchard performance.
2) To investigate the depth-wise variation in urease and *β*-glucosidase activities in a 'Cripp's Pink'/M7 apple orchard after seven years under CON and ORG orchard floor management, for the reason that the root systems of deciduous fruit trees commonly extend to depths >60 cm in well-prepared soils, which may affect the microbial enzyme activities in lower rootzone soil layers.
3) To test the ability of AI3, an enzyme-based soil alteration index, to distinguish between apple orchard soils under CON and ORG production protocols, and to reflect soil nutrient status, growth and yield performance in maturing 'Cripps Pink'/M7 apple trees.
4) To deteremine the effect of ORG and CON practices on C-substrate utilisation by the soil microbial community in a 'Cripps Pink'/M7 apple orchard to establish which of the two practices, ORG or CON, afford a better option to sustain critical apple orchard ecosystem functions.
5) To determine the effects of CON and ORG orchard floor management practices on AM fungi, and on soil glomalin contents, in the rootzone of a Cripps Pink'/M7 apple orchard.
6) To compare production areas, cultivation practices (conventional (CON) vs organic (ORG)), rootstocks, scions and rootstock x scion combinations, with regard to their effects on EEG stocks and AM root colonizaton levels, and to determine whether environmental variation within and between apple growing seasons affect soil EEG and AM fungal root colonisation levels.

1.8. References

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CHAPTER 2

Effect of conventional and organic orchard floor management practices on enzyme activities and microbial counts in a 'Cripp's Pink'/M7 apple orchard

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Abstract

Organic (ORG) production practices are increasingly being used in South African apple orchards. Whether ORG orchard floor management practices differ in their effects on soil enzyme activities and microbial populations from conventional (CON) practices have not been adequately investigated, particularly with regard to soil chemical characteristics and orchard performance. To seek clarification a randomized field trial was carried out in the winter rainfall region of the Western Cape. In this trial 'Cripps Pink'/M7 apple trees received straw mulch with compost (ORG) or synthetic fertiliser and herbicide (CON) in the tree rows. Soil microbial enzyme activities and microbial counts were determined by colorimetric assays and dilution plating, respectively. Activities of *β*-glucosidase, acid phosphatase and urease, and actinobacteria counts, tended to be greater in the ORG than the CON treatments. Activities correlated positively with soil zinc and manganese concentrations and with leaf zinc, but negatively with soil copper. *β*-glucosidase and acid phosphatase activities also correlated negatively with ammonium and nitrate nitrogen in the soil, and with leaf nitrogen concentration. Yields decreased with increasing *β*-glucosidase and acid phosphatase activities. Therefore, although ORG practices increased soil microbiological activity relative to CON management, they did not improve yield.

Keywords: acid phosphatase, actinobacteria, *β*-glucosidase, heterotrophs, urease

2.1. Introduction

Western Cape apple producers are increasingly using organic (ORG) orchard floor management practices. ORG production entails the use of compost and mulches to supply nutrients and control weeds, respectively. These functions are performed in conventionally managed (CON) orchards by synthetic fertilisers and herbicides, the latter commonly containing glyphosate. ORG practices tend to oversupply phosphorus (P), potassium (K) and organic carbon (C), to increase soil pH (Wooldridge et al. (2013a), and to increase vegetative growth, but suppress yield (Wooldridge et al. 2013b) in apple, compared to CON practices. The compost and mulch used in ORG management supply appreciable amounts

of variably decomposed organic material to the soil surfaces of the tree rows, compared to CON practices. It is therefore likely that soil microbiological populations and enzyme activities will differ between ORG and CON treatments due to differences in the availability of metabolisable organic matter and nutrients (Van Schoor 2009).

Bacteria are the most abundant soil microorganisms, followed by actinobacteria, fungi, yeast, algae and protozoa (Pelczar et al. 2010). These microorganisms facilitate metabolic processes in soils mainly through enzyme synthesis (Dick 1994). The activities of soil enzymes may be affected by agricultural management practices (Dick 1994). *β*-glucosidase, urease and phosphatase, which are active in the cycling of, respectively, C, nitrogen (N) and P, have been shown to be sensitive to management-induced changes in the soil environment by Caravaca et al. (2002). Typically, enzyme activities correlate with soil OM content, soil OM being the site of enzyme synthesis and enzyme stabilization (Tabatabai 1994). Activities of these enzymes are indicative of short (Bandick and Dick 1999) and medium to long-term (Jin et al. 2009) changes in their respective pools of organic nutrients. Potentially, enzyme activity may be a useful indicator of the effects of different orchard floor management practices on soil conditions, which may, in turn, affect tree performance.

The research reported in this article concerns the effects of CON and ORG practices in an apple orchard on the activities of *β*-glucosidase, acid phosphatase and urease, and on the relative abundance of actinobacteria, bacteria, fungi and heterotrophs. Certain of these parameters were correlated with soil and tree factors to investigate possible links between soil microbial populations and enzyme activities, soil chemical characteristics and orchard performance.

2.2. Materials and methods

This research formed part of a wider field trial carried out on gravelly clay loam soil at ARC Overberg Research Farm, Elgin (34.1363°S; 19.0216°E; altitude 327 m) (Wooldridge et al. 2013a, 2013b). Before planting, the soils received sufficient calcitic lime and single superphosphate to increase the pH to 5.6 (1 M KCl), neutralize exchangeable acidity, adjust the exchangeable sodium (Na), K, calcium (Ca) and magnesium (Mg) to, respectively, <1%, 3.5-4%, 70% and 10-12% of the sum of base cations (S-value) and the Bray II extractable soil P concentration to 25-30 mg kg^{-1}. These values are close to ideal for apple trees in the winter rainfall region of the Western Cape (Terblanche et al. 1980).

The trees were planted at a spacing of 1 m x by 4.0 m (2 500 trees ha^{-1}) in winter 2000. Each experimental unit (plot) consisted of nine ‘Cripp’s Pink’/M7 trees separated from adjacent in-row plots by one or more pollinator trees (‘Hillieri’/Seedling). Experimental rows were separated by side rows. Irrigation was carried out at c. 7-day intervals, at c. 60% plant available water depletion. Two CON (T1 and T2) and three ORG (T3, T4 and T5) soil

surface management treatments were compared (Table 2.1). These were applied in spring 2003 and annually thereafter until spring 2010. Each treatment was replicated in three blocks. The treatments and blocks were laid out in a fully randomized complete block design. Trace element sprays, pruning, thinning and other routine orchard procedures were carried out in accordance with normal industry practice.

Table 2.1: Conventional (CON) and organic (ORG) orchard floor management treatments applied in a 'Cripp's Pink'/M7 apple trial orchard (Adapted from Wooldridge et al. 2013a)

Treatment	Work row		Tree row
	Tree dormant	Tree active	(Drip line to drip line)
T1 (CON)	Weed cover	Cover mown, natural die-back in summer	Chemical weed control[a] + inorganic N[b]
T2 (CON)	Soil tillage + cover crop[c]	Cover crop[c], chemical weed control in spring[a]	Chemical weed control[a] + inorganic N[b]
T3 (ORG)	Straw mulch[d]. Hand weeded	Straw mulch[d]. Hand weeded	(Compost + mulch)[e]. Hand weeded
T4 (ORG)	Soil tillage + cover crop[c]	Cover crop[c], mown, natural die-back in summer	(Compost + mulch)[e] + compost tea[f]. Hand weeded
T5 (ORG)	Weed cover	Cover mown, natural die-back in summer	(Compost + mulch)[e]. Hand weeded

[a]Roundup 360SC® applied at 5 L ha^{-1} (3%) in September and January

[b]Nitrogen as limestone ammonium nitrate (LAN) applied at full bloom, six weeks after full bloom and after harvest at rates of 9.2 g N, 5.2 g N and 18.0 g N $tree^{-1}$, respectively, in seasons 2003/04 to 2007/08. Applied after harvest only, at 18.0 g N $tree^{-1}$, in 2008/09 and 2009/10. Applied to the tree row in a continuous strip 1 m wide

[c]Rye (*Secale cereale* L. cv. Henog) and 'Pallinup' oats (*Avena sativa* L. cv. Pallinup) planted in tilled soil (0-10 cm) after leaf senescence in alternate seasons. Nitrogen (as LAN) applied to the cover crops and weed covers at 14 kg N ha^{-1} in June

[d]Wheat or oat straw (mulch) applied as a 5 cm layer in a continuous strip, c. 100 cm wide, along the tree row. Mulch applied at 3.2 kg straw $tree^{-1}$. Work rows covered with a 5 cm layer of straw mulch. Mulches replenished annually in October

[e]Compost applied at 20 kg per $tree^{-1}$ (128 g N, 3.7 g P, 6.3 g K $tree^{-1}$) in spring and autumn 2003/04, 10 kg $tree^{-1}$ in spring and autumn 2004/05 to 2006/07 and 10 kg $tree^{-1}$ in spring 2008 and 2009. Spread in a continuous strip, c. 1 m wide, along the tree row. Covered with a

5 cm layer (c. 3.2 kg per tree^{-1}) of wheat or oat straw (mulch). Mulch reapplied annually, in October.

[f]Compost tea (aerated, aqueous, microbial digest) surface-applied around each tree at one L tree^{-1} per 4-week interval from full bloom to leaf senescence. Principal components (mg ml^{-1}): P, 7.5; K, 190; Ca, 51; Mg, 34.6; Na, 163. Nitrogen: 0.06%.

Soil sampling for microbiological analysis took place over seasons 2006/07 to 2010/11 in spring (September/October) and summer (January/February). On each occasion, composite samples (four subsamples per plot) were obtained from the 0-15 cm soil depth interval using a 10 cm-wide spade rinsed with 70% ethyl alcohol between samples. This depth interval was variably populated with mainly fine apple feeding roots. The samples were taken from each plot on alternate sides of the tree row, adjacent to the trunks of every second tree, after removing organic material from the mineral soil surface. The samples were sealed in polythene bags, placed on ice in cooler boxes, transported to the laboratory, sieved (<2 mm) and stored at 4 °C. Analysis occurred within 48 h of sample collection.

Soil sampling for chemical analysis was carried out in early spring (September) from 2006 to 2010. On each occasion, composite samples (three subsamples per plot) were removed by auger from incremental depth intervals (Wooldridge et al. 2013a). Parameters determined in the air-dry, <2.0 mm soil fractions included pH (1 M potassium chloride), Bray II (0.03 M ammonium fluoride in 0.01 M hydrochloric acid) extractable P and K, and 0.2 M ammonium acetate exchangeable acidity, Na, K, Ca and Mg. The trace elements copper (Cu), zinc (Zn) and manganese (Mn) were extracted using 0.02 M Na-EDTA. These analyses were carried out using standard methods prescribed by the Non-Affiliated Soil Analysis Work Committee (1990), except that an inductively-coupled plasma atomic-emission spectrometer (ICPAES) (Vista MPX) was used to quantify the mineral elements. Ammonium (NH_4^+) and nitrate (NO_3^-) N were measured in a potassium chloride extract by Auto Analyser (Technicon Auto analyser III). Organic C contents were determined by the dichromate oxidation method of Walkley-Black using a correction factor for incomplete oxidation of 1.33 (Walkley and Black 1934). Data from these analyses have been reported by Wooldridge et al. (2013a).

Fruit yields were determined each summer, and stem circumferences 40 cm above ground level were measured in winter. Leaf sampling and analysis was performed as described by Wooldridge et al. (2013b).

β-glucosidase activity (EC 3.2.1.21) was determined in field-moist soil in a reaction mixture containing 1.0 g soil, 0.25 ml toluene, 1.0 ml 25 mM *p*-nitrophenyl-*β*-D-glucopyranoside as substrate, and 4.0 ml modified universal buffer (MUB) at pH 6.0 (Eivazi and Tabatabai 1988). The reaction mixture was incubated at 37 °C for 60 min after which the

reaction was terminated by adding 1.0 ml of 0.5 M calcium chloride and 4.0 ml of 0.1 M, pH 12, tris (hydroxymethy1) aminomethane buffer. The amount of *p*-nitrophenol liberated during enzymatic hydrolysis was determined at 410 nm with a digital UV-Vis spectrophotometer by reference to a calibration curve corresponding to a *p*-nitrophenol standard (Sigma-Aldrich) incubated with each soil sample under the same conditions as for the soil samples, after subtracting the absorbance values of the controls. In the controls, the substrate was not added until after the reaction was stopped, immediately before filtration of the resulting soil suspension through Whatman no. 2V filter paper.

Acid phosphatase (EC 3.1.3.2) activity was determined as described for *β*-glucosidase activity except that the reaction mixture consisted of 1.0 ml 25 mM *p*-nitrophenol phosphate as substrate, 4.0 ml MUB, 0.25 ml toluene, and that the released *p*-nitrophenol was extracted with 4 ml of 0.5 M NaOH at pH 6.5 (Tabatabai and Bremner 1969). This method quantifies acid phosphatase of both plant and microbial origin under low pH soil conditions (Tarafdar et al. 2001; Nannipieri et al. 2011, and references therein), as in the present orchard trial. Activities of *β*-glucosidase and of acid phosphatase were expressed as micrograms *p*-nitrophenol per gram per hour.

Urease activities (EC 3.5.1.5) were determined by the method of Kandeler and Gerber (1988). Each 5 g field-moist soil sample received 2.5 ml of non-buffered urea solution (80 mM) and was incubated for 2 h at 37 °C. Each soil was accompanied by a reference sample (control) in which the soil was incubated with deionised water instead of urea solution. The NH_4^+ released by enzymatic action was extracted with 50 ml of a potassium chloride solution (1 M potassium chloride and 0.01 M hydrochloric acid). The solutions were shaken for 30 min on an orbital shaker. Determinations were based on the reaction of sodium salicylate with NH_4^+ in the presence of sodium dichloroisocyanurate. Extinction was measured at 690 nm with a digital UV–Vis spectrophotometer against the reagent blank. The NH_4^+ content was calculated by reference to a calibration curve spanning the range from zero to 2.5 mg NH_4^+ ml^{-1}. Sodium nitroprusside was used as a catalyst. Activity was expressed as micrograms NH_4^+ per gram per 2 h. Two replicates from each soil were analysed for the *β*-glucosidase and acid phosphatase assays, and three replicates for the urease determinations. Enzyme activities were expressed on a moisture-free basis. Soil moisture content was determined gravimetrically from the loss in weight after drying at 105 °C for 24 h (Hillel 1980).

Nutrient agar (Sigma-Aldrich), malt extract agar (Sigma-Aldrich), plate count agar (Sigma-Aldrich) and actinomycete isolation agar (Sigma-Aldrich) were used to isolate culturable bacteria, fungi, total fast-growing heterotrophic bacteria and actinobacteria, respectively, using the standard spread-plate count method. For the enumeration of bacteria, 20 μg ml^{-1} nystatin (to inhibit fungal growth) was aseptically added to the nutrient agar after

sterilization in an autoclave at 121 °C for 15 min before pouring the plates. For the enumeration of fungi on malt extract agar, filter sterilised (filter size: 0.2 μm) streptomycin sulphate (0.5 g l^{-1}) was added to the medium (to inhibit bacterial growth) immediately before pouring the plates. The plate count agar and actinomycete isolation agar growth media were prepared and utilised in accordance with the manufacturer's instructions.

Microbial enumerations, performed on air-dried samples, were diluted 10-fold in sterile 0.15 M sodium chloride (w/v) diluent. Aliquots (0.1 ml) at dilutions spanning the range 10^{-4} and 10^{-5} were plated in triplicate. The inoculated plates were incubated in an inverted position at 25 °C for 48 h for bacteria, 28 °C in the dark for 72 h for fungi, 26 °C for 120 h for total heterotrophs and 30 °C for 72 h for actinobacteria. Counts were expressed as $\log_{10}$ colony-forming units (CFU) per gram dry soil.

2.2.1. Statistical analysis

The enzyme activity data were tested for normality (Shapiro and Wilk 1965), observed to be acceptably normally distributed and subjected, untransformed, to an analysis of variance (ANOVA) using the General Linear Model procedure of SAS 9.1.3 (SAS Institute Inc. 2008). Factors were treatment and sampling date. Student's *t* least significant difference (LSD) values were calculated at the 5% probability (*p*) level to facilitate comparison between treatment means (Snedecor and Cochran 1980). Pearson correlation coefficients (*r*) were calculated for relationships between soil and plant parameters using the CORR procedure of SAS 9.1.3. The enumeration data were transformed ($\log_{10}$) prior to ANOVA. Means that differed at $p < 0.05$ were considered significantly different.

2.3. Results

Over the period 2007/08 to 2010/11, *β*-glucosidase activity decreased in the sequence T4, T5 > T1, T2 in spring and T4 > T1, T2 in summer (Table 2.2). *β*-glucosidase activity in the full-surface straw mulch treatment (T3) was intermediate between the CON and ORG treatments in spring. Relative to T5, the additional supply of compost tea (T4) had no significant effect on *β*-glucosidase activity in either season. Averaged over all treatments, season did not significantly affect *β*-glucosidase activity.

Acid phosphatase activity was not significantly affected by the treatments in either spring or summer, but averaged over all treatments, was higher in spring than in summer (Table 2.3). Urease activity in spring was greater in T3, T4 and T5 than in T1 and T2 (Table 2.4). In summer, T2 suppressed urease activity relative to T3 only. Over all five treatments, urease activity was significantly higher in summer than in spring.

Table 2.2: Effect of orchard floor management practices on *β*-glucosidase activity[1] in a 'Cripp's Pink'/M7 apple orchard. Combined data over seasons 2007/08-2010/11. Values in the same column and data set, followed by the same letter, do not differ at $p < 0.05$

Treatment	Spring	Summer
T1	106[b]	111[b]
T2	119[b]	125[b]
T3	174[ab]	175[ab]
T4	221[a]	225[a]
T5	241[a]	173[ab]
Mean	172[a]	162[a]

[1] Expressed as *μ*g *p*-nitrophenol g^{-1} h^{-1}

Table 2.3: Effect of orchard floor management practices on acid phosphatase activity[1] in a 'Cripp's Pink'/M7 apple orchard. Combined data over seasons 2007/08 to 2010/11. Values in the same column and data set, followed by the same letter, do not differ at $p < 0.05$

Treatment	Spring	Summer
T1	435[a]	792[a]
T2	424[a]	760[a]
T3	597[a]	852[a]
T4	766[a]	936[a]
T5	786[a]	835[a]
Mean	604[a]	835[b]

[1] Expressed as *μ*g *p*-nitrophenol g^{-1} h^{-1}

Table 2.4: Effect of orchard floor management practice on urease activity[1] in a 'Cripp's Pink'/M7 apple orchard. Combined data over seasons 2007/08 to 2010/11. Values in the same column and data set, followed by the same letter, do not differ at $p < 0.05$

Treatment	Spring	Summer
T1	11.2[b]	26.5[ab]
T2	11.9[b]	19.1[b]
T3	29.9[a]	38.8[a]
T4	23.2[a]	33.7[ab]
T5	26.8[a]	29.0[ab]
Mean	20.9[b]	29.4[a]

[1] Expressed as *μ*g NH_4^+ g^{-1} 2 h^{-1}

Activities of acid phosphatase, *β*-glucosidase and urease correlated positively and reasonably strongly with soil pH, Zn and Mn, but negatively with Cu. Acid phosphatase and *β*-glucosidase activities were suppressed by NO_3^-, but not significantly by NH_4^+ (Table 2.5). Acid phosphatase correlated most strongly with leaf Zn, Na, Ca and Mg, whereas *β*-glucosidase and urease correlated with leaf Zn (Table 2.6). Leaf N and yield correlated negatively with acid phosphatase and *β*-glucosidase activities.

Table 2.5: Correlations between soil parameters and rootzone (0-15 cm) enzyme activity in a 'Cripp's Pink'/M7 apple orchard soil. Combined data from five orchard floor management practices over four seasons

Soil parameter	Acid phosphatase[1]		*β*-glucosidase[1]		Urease[2]	
	r[3]	*p*	*r*	*p*	*r*	*p*
pH (KCl)	0.5075	0.0008	0.4877	0.0014	0.6019	<0.0001
P (mg kg^{-1})	0.3697	0.0189	0.3500	0.0268	0.5703	0.0001
K (mg kg^{-1})	0.1718	0.2893	0.2161	0.1805	0.5658	0.0001
Cu (mg kg^{-1})	-0.8023	<0.0001	-0.6969	0.0006	-0.6371	0.0025
Zn (mg kg^{-1})	0.7704	<0.0001	0.6476	0.0020	0.5505	0.0119
Mn (mg kg^{-1})	0.8030	<0.0001	0.6908	0.0007	0.5642	0.0096
C (%)	0.4034	0.0004	0.3732	0.0011	0.0343	0.7717
NO_3^- (mg kg^{-1})	-0.5126	<0.0001	-0.4895	0.0002	0.1987	0.1458
NH_4^+ (mg kg^{-1})	-0.0711	0.6093	-0.1967	0.1541	0.4180	0.0015

[1] Expressed as *μ*g *p*-nitrophenol g^{-1} h^{-1}

[2] Expressed as *μ*g NH_4^+ g^{-1} 2 h^{-1}

[3] Pearson correlation coefficient

Table 2.6: Correlations between tree parameters and root-zone (0-15 cm) enzyme activity in a 'Cripp's Pink'/M7 apple orchard. Combined data from five orchard floor management practices over four seasons

Tree parameter	Acid phosphatase[1]		*β*-glucosidase[1]		Urease[2]	
	r[3]	*p*	*r*	*p*	*r*	*p*
Leaf N (%)	-0.4595	0.0029	-0.4709	0.0022	-0.2695	0.0466
Leaf P (%)	0.3656	0.0221	0.4540	0.0037	0.3809	0.0035
Leaf K (%)	0.2489	0.1215	0.3478	0.0279	0.3755	0.0047
Leaf Ca (%)	0.7844	<0.0001	0.5110	0.0008	0.2544	0.0609
Leaf Mg (%)	0.7334	<0.0001	0.5548	0.0002	0.3081	0.0221
Leaf Na (mg kg^{-1})	0.8060	<0.0001	0.5678	0.0001	0.4561	0.0031
Leaf Zn (mg kg^{-1})	0.9897	<0.0001	0.8198	<0.0001	0.7369	<0.0001
Circ. Increase (cm)	0.1062	0.5143	0.4066	0.0092	0.3480	0.0092
Yield (t ha^{-1})	-0.7934	<0.0001	-0.4985	0.0011	0.0132	0.9248

[1] Expressed as *μ*g *p*-nitrophenol g^{-1} h^{-1}

[2] Expressed as *μ*g NH_4^+ g^{-1} 2 h^{-1}

[3] Pearson correlation coefficient

Counts of bacterial and fungal populations did not differ significantly between treatments (Table 2.7), but actinobacteria counts were greater in T4 than in T1 and T2. Heterotroph counts in both spring and summer were higher in T4 and T5 than in T1 (Table 2.8). Over all treatments the spring and summer counts did not differ. For the combined spring and summer heterotroph data, counts in T1 were lower than in treatments T2 to T5.

Table 2.7: Effect of orchard floor management practices on populations of actinobacteria, bacteria and fungi in spring (average of years 2006 to 2008) in a 'Cripp's Pink'/M7 apple orchard. Values within a column, followed by the same letter, do not differ at $p < 0.05$. CFU = colony forming unit

Treatments	Actinobacteria		Bacteria		Fungi	
	CFU (g^{-1} soil)	Log_{10}CFU (g^{-1} soil)	CFU (g^{-1} soil)	Log_{10}CFU (g^{-1} soil)	CFU (g^{-1} soil)	Log_{10}CFU (g^{-1} soil)
T1	2.33×10^6	6.3301^{b}	1.43×10^7	6.9549^{a}	1.55×10^5	5.0381^{a}
T2	2.29×10^6	6.1522^{b}	3.40×10^6	6.8229^{a}	1.33×10^5	4.9938^{a}
T3	2.66×10^6	6.5887^{ab}	1.26×10^7	6.8530^{a}	1.49×10^5	5.2899^{a}
T4	6.31×10^6	6.7866^{a}	9.72×10^6	6.7586^{a}	1.78×10^5	5.2684^{a}
T5	3.84×10^6	6.5643^{ab}	8.92×10^6	6.7578^{a}	1.43×10^5	5.2164^{a}

Table 2.8: Effect of orchard floor management practices on average heterotroph counts in spring and summer (average of years 2009 to 2011) in a 'Cripp's Pink'/M7 apple orchard. Values within a column or data set, followed by the same letter, do not differ at $p < 0.05$. CFU = colony forming unit

Treatments	Heterotrophs					
	Spring		Summer		Spring and summer	
	CFU (g^{-1} soil)	Log_{10}CFU (g^{-1} soil)	CFU (g^{-1} soil)	Log_{10}CFU (g^{-1} soil)	CFU (g^{-1} soil)	Log_{10}CFU (g^{-1} soil)
T1	1.73×10^7	6.9284^{b}	5.79×10^6	6.6020^{b}	1.15×10^7	6.7652^{b}
T2	1.55×10^7	7.0068^{ab}	3.14×10^7	7.3232^{a}	2.35×10^7	7.1650^{a}
T3	2.08×10^7	7.2083^{a}	1.51×10^7	6.9991^{ab}	1.77×10^7	7.1877^{a}
T4	2.00×10^7	7.2119^{a}	1.47×10^7	7.1440^{a}	1.71×10^7	7.1757^{a}
T5	3.02×10^7	7.4140^{a}	1.64×10^7	7.1587^{a}	2.33×10^7	7.2863^{a}
Mean	-	7.1548^{a}	-	7.0383^{a}	-	-

2.4. Discussion

2.4.1. Effects of treatments on enzyme activities

Enzyme activities in the CON and ORG treatments did not differ consistently, but averaged 75%, 32% and 76% higher in the ORG treatments for *β*-glucosidase, acid phosphatase and urease, respectively. This tendency for enzyme activities in the ORG treatments to be higher than in the CON treatments was probably due to differences in soil pH (Bünemann et al. 2006). Enzyme activities, notably acid and alkaline phosphatase activities, have different pH optima (Herbien and Neal 1990). Acid phosphatases generally prevail in acidic soils (Nannipieri et al. 2011, and references therein), hence its inclusion in the present trial. Acid phosphatases are produced by both plants and microorganisms (Tarafdar et al. 2001; Nannipieri et al. 2011, and references therein), but no distinction was made between acid phosphatases of plant and microbial derivation in the present trial. However, microbial acid phosphatases release P from organic matter more efficiently than plant-derived acid phosphatase (Tarafdar et al. 2001). Microbially-derived acid phosphatases probably account for the largest proportion of the total measured acid phosphatase activity in the rhizosphere

(Nannipieri et al. 2011, and references therein). Thus, acid phosphatase of microbial origin probably also contributed most to the results observed in the present study.

The lower tree row soil pH levels in CON compared to the ORG treatments (Wooldridge et al. 2013a) was attributed to the acidifying effect of synthetic nitrogenous fertilisers in T1, T2 (Bünemann et al. 2006), contrasting with the liming effect of the compost applied to T3, T4 and T5 (Wooldridge et al. (2013a). The 76% difference in urease activity between the CON and ORG treatments supports Balezentiene and Kilimas (2009) who found that urease activity decreased under mineral fertilisation. The effect of pH on enzyme activities is influenced by the buffering effects of organic C compounds (Acosta-Martinez and Tabatabai 2000). The C contents of the CON soils were lower than in the ORG soils due to the absence of compost and mulch in the tree rows, and the herbicide-induced lack of a rooted vegetation cover during the growing season in the CON treatments (Wooldridge et al. 2013a).

The systemic herbicide glyphosate (active ingredient in Roundup®) may also have affected the soil microorganisms. At high concentrations, glyphosate may stimulate microbial respiration (Busse et al. 2001), enzyme activity (Perucci et al. 2000), and nutrient cycling processes (Carlisli and Trevors 1986). Some microorganisms utilise glyphosate as a source of C, N and P (Partoazar et al. 2011). Conversely, glyphosate inhibits protein synthesis via the shikimic acid pathway in bacteria and fungi (Bentley, 1990; Franz et al. 1997). In a recent review, Wolmarans and Swart (2014) indicated that controversy persists regarding the positive and negative effects of glyphosate, and of herbicides generally, on non-target biota and conclude by stressing the need to utilise management practices likely to optimise crop production efficiency and promote sustainable weed control. Nonetheless, the generally lower activities of *β*-glucosidase (Table 2.2) and urease (Table 2.4), and a similar trend in acid phosphatase (Table 3), in the CON compared with the ORG treatments, supports research by Bentley (1990) and Franz et al. (1997), which collectively point to a negative effect of glyphosate on soil microbial activity.

Whether the observed differences in enzyme activities between the CON and ORG treatments (Tables 2.2–2.4) were due to suppression by synthetic agrochemicals, or to positive effects stemming from the addition and decomposition of compost and mulch, was unclear. Enzyme activity usually increases with increasing organic input to the soil, and Yang et al. (2003) have shown that enzyme activity is normally higher in mulched than unmulched plots. In the present trial, the compost applied to T3, T4 and T5 promoted higher Ca and Mg saturations (of T-value) and reduced soil acidity (Wooldridge et al. 2013a), factors which may have promoted microbial enzyme activity (Acosta-Martinez and Tabatabai 2000).

The rate at which N is released or immobilized by organic matter in the soil depends primarily on the C:N ratio (Janssen 1996). Applying straw mulch (high C:N ratio) to the soil

surface may therefore have induced a temporary deficiency of N due to the utilisation of this element by decomposers (Tolk et al. 1999). In this trial, however, any N deficiency that might have arisen from decomposition of the straw mulch would probably have been offset by the supply of N from the compost. To what extent the lower *β*-glucosidase and acid phosphatase activities in T3 (straw-mulched work row) relative to T4 and T5 (green work rows) could be ascribed to the absence of a green cover in the work row of T3, or to inhibition by allelopathic exudates leached into the soil from the straw mulch of T3 by winter rains (Das Neves and Gaspar 1990), could not be ascertained.

2.4.2. Effects of time of sampling on enzyme activities

The effects of time of sampling were inconsistent. Over all treatments, *β*-glucosidase activity did not differ between spring and summer (Table 2.3), acid phosphatase activity was greater in summer than spring (Table 2.4), and summer urease activity exceeded that in spring (Table 2.5). Higher urease activities in summer, compared to spring, are consistent with the findings of Cartes et al. (2009) that urease activity increases with increasing temperature. In general, seasonal variation in soil enzyme activity is affected by both soil microclimate and soil chemical factors, notably metabolisable substrate availability (Sinsabaugh et al. 1992).

Thus, microbial activity is likely to be favoured by green covers and mulches relative to exposure of the herbicided, weed-free soil surface to sunlight. Green covers and mulches also promote greater relative stability in terms of soil temperature, moisture content and organic substrate availability, and the maintenance of soil structures conducive to rapid gas exchange (Tisdall et al. 1984; Hogue and Neilsen 1987). Mulching favours microbial decomposition processes in the soil (Haynes 1980), and also improves soil and crop quality, relative to herbicide usage (Hogue and Neilsen 1987).

2.4.3. Relationships between enzyme activities and soil parameters

A prominent result was the negative relationship between Cu and the activities of acid phosphatase, *β*-glucosidase and urease (Table 2.5). Negative effects of Cu on microbial enzyme activity in agricultural soils are commonly observed where Cu-based fungicides have been used over protracted periods (Merry et al. 1983). High concentrations of heavy metals may inhibit soil microbial and enzyme activities (Wyszkowska and Wyszkowski 2003). In deficiency situations, however, supplying such elements may promote soil microbial and enzyme activity (Ashman and Puri 2002).

The positive relationships between enzyme activities and soil Zn and Mn levels (Table 2.5) may have been due to the amelioration of previously existing deficiencies of these elements by the compost. Herencia et al. (2008a, 2008b) mainly attribute the higher concentrations of some soil micronutrients that are generally found in organically managed

soils, compared to those that are non-organically managed, to increased solubility. In the case of Mn, increased solubility is due to microbial decomposition of organic compounds under reducing conditions (Herencia et al. 2008b).

The positive relationship between *β*-glucosidase and urease activities, and soil pH (Table 2.5), supports Acosta-Martinez and Tabatabai (2000) who found that the activities of 13 out of 14 enzymes correlated positively with pH. Acid phosphatase activity correlated positively with pH in the present trial, but negatively in the work of Acosta-Martinez and Tabatabai (2000). This contradiction supports the need for caution when interpreting enzyme activity levels, notably phosphatases, where xenobiotic compounds, such as herbicides are applied (Perucci et al. 2000), P being a key component of glyphosate.

Positive relationships between microbial enzyme activities and soil C were reported by Schaller (2009). The correlations between acid phosphatase and *β*-glucosidase activities, and C (Table 2.5), support these findings. Enzyme activities tend to correlate with soil organic matter content due to the link between soil microbial biomass and enzyme synthesis, combined with the enzyme stabilizing effect of soil OM (Tabatabai, 1994). Frankenberger and Johanson (1982) found that the weakening of enzymatic activity that accompanies soil acidification is a function of decreasing bond strength in the active sites of the enzymes.

In the case of urease, where activity depends on the availability of metabolisable substrate, a link between urease activity and C appears likely (Sinsabaugh et al. 1992). However, urease activity and soil C were not significantly correlated in the present trial (Table 2.5). Why the activities of *β*-glucosidase and acid phosphatase correlated negatively with NO_3^-, but bore no significant relationship to NH_4^+, was unclear. Implicit in the mineralisation process is the relationship between urease activity and the abundance of NH_4^+ (a product of the enzymatic hydrolysis of amino-N). The observed (Table 2.5) positive relationship between urease activity and NH_4^+ is explicable in terms of the positive effects of base cations on mineralisation (Brady 1974). Urease activity correlated positively with both soil P and K (Table 2.5). As shown by Wooldridge et al. (2013a), soil P and K levels (Tables 2.2 and 2.3) were generally higher in the mulched and composted ORG than in the CON treatments.

2.4.4. Relationships between enzyme activities and plant parameters

Acid phosphatase, *β*-glucosidase and urease activities correlated positively with leaf P, Mg, Na, and, most strongly, with Zn (Table 2.6). Conceivably, an enzyme production or function limiting undersupply of Zn in the soil was rectified by the supply of Zn from the compost. The relationships between the three enzymes and leaf N was, however, negative; as was the relationship between acid phosphatase and *β*-glucosidase activities and soil NO_3^- content.

Soil N, notably in the form of NO_3^-, therefore suppressed the activities of acid phosphatase and *β*-glucosidase.

Acid phosphatase and *β*-glucosidase activities decreased as yields increased, whereas *β*-glucosidase and urease activities increased with increasing stem circumference. This pattern reflected the inverse relationship between vegetative growth and yield observed in the trial orchard by Wooldridge et al. (2013b). A direct causative relationship between enzyme activity and yield could, however, not be demonstrated.

2.4.5. Population sizes of actinobacteria, bacteria, fungi and total heterotrophs

Counts of bacteria and fungi did not differ between treatments (Table 2.7), perhaps because the bacterial and fungal populations were less sensitive to differences between the CON and ORG treatments than the actinobacteria. Since the all-treatments heterotroph counts did not differ between spring and summer, despite the generally higher summer soil temperatures, microbial populations would appear not to vary greatly in number within seasons.

Averaged over all treatments, counts tended to decrease in the sequence: bacteria > actinobacteria > fungi, in the ratio 64:23:1, which agrees with Atlas and Bartha (1998) and Pelczar et al. (2010). The finding that counts of bacteria and fungi (Table 2.7) did not differ between treatments nevertheless contrasts with Varga et al. (2004) who found that, in apple orchards, numbers of total bacteria, cellulose decomposing bacteria and fungi under straw and pine bark mulch exceeded those in unmulched soils. The greater abundance of actinobacteria in T4 (ORG) than in T1 and T2 (CON) may have been a response to the application of compost tea, in addition to mulch and compost, to T4.

In the combined spring and summer count data (Table 2.8), heterotroph counts in T1 were lower than in all other treatments. Differences in heterotroph count between T1 and T2 may have been a carry-over effect from herbicide used in the work rows of T2 into the tree rows. Lower counts in T1, compared to ORG treatments T3, T4 and T5, could have been due to the higher soil C levels in the ORG relative to the CON treatments (Wooldridge et al. 2013a). Since soil microorganisms are often C-limited (Wardle et al. 2001), differences in plant biomass inputs across soil treatments could have affected the soil biota.

2.5. Conclusions

Compared with CON orchard floor management practices, ORG practices tended to promote *β*-glucosidase, acid phosphatase and urease activities. They also led to increased actinobacteria counts, but had no effect on numbers of bacteria and fungi, suggesting that the ORG treatments facilitated enzyme activity, rather than affecting the abundance of the micro-organisms that produce these enzymes. The greater enzyme activities in the ORG treatments probably contributed to the complex of factors that caused the greater vegetative

growth, but lower yields, in the ORG treatments. Notable among these factors were soil pH, P, K, Ca and Mg saturations, and NO_3^-; all of which were higher in the ORG treatments. However, the relative contributions to tree performance made by the biological and soil chemical parameters could not be separately quantified. It is nevertheless evident that the activities of acid phosphatase and *β*-glucosidase, and vegetative growth, were promoted by ORG practices, though to the detriment of yield. This yield suppression probably represented an imbalance between vegetative growth and yield that was induced by oversupply of compost and the nutrients therein.

2.6. References

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CHAPTER 3

Variation in urease and *β*-glucosidase activities with soil depth and root density in a 'Cripp's Pink'/M7 apple orchard under conventional and organic management

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Abstract

The effects of conventional (CON: utilising synthetic fertiliser and herbicide), and organic (ORG: nutrients supplied in compost, weeds controlled with straw mulch) orchard floor management practices on depth-wise variation in urease and *β*-glucosidase activities in tree-row soils were compared in a Western Cape 'Cripp's Pink'/M7 apple orchard. Urease and *ß*-glucosidase activities were determined spectrophotometrically in soils from five depth intervals from the walls of trenches excavated across the tree rows after seven years of treatment application. Soil pH, organic carbon (C), nitrate (NO_3^-) and ammonium (NH_4^+) nitrogen were also determined, as was root density. Enzyme activities were higher in the ORG than the CON topsoils but did not differ significantly ($p < 0.05$) at depths >30 cm. The positive effects of the ORG treatments were attributed to the liming effect and C and nitrogen contributions of the compost. Urease and *β*-glucosidase activities correlated strongly. Activities of both enzymes correlated significantly and positively with C, NO_3^- and pH; urease more strongly than *β*-glucosidase. Only urease correlated with root density. Organic orchard floor management practices may be more effective than CON practices in promoting microbial enzyme activities in the 0-30 cm soil depth intervals of Western Cape apple orchard soils.

Keywords: compost, fertiliser, herbicide, root density, straw mulch

3.1. Introduction

Nutrient supply and weed control are critical aspects of orchard floor management in Western Cape apple orchards. Whereas synthetic fertilisers and herbicides are used for these respective purposes in conventional (CON) orchards, nutrients are supplied in compost and mulch is used to control weeds in organic (ORG) orchards (Wooldridge et al. 2013a). Compared to topsoils, which have greater microbial loads and are the sites of higher rates of organic decomposition and mineralisation than subsoils (Bardgett et al. 2005), microbial communities in the deeper soil material are poorly researched (Rumpel and Kögel-Knabner, 2011; Kramer et al. 2013). Because the root systems of deciduous fruit trees

commonly extend to depths >60 cm in well prepared soils (Wooldridge et al. 2013b), information concerning microbial activity in the lower rootzone is also required. Both the quantity and quality of metabolisable organic carbon (C) substrate decrease with increasing depth (Blume et al. 2002; Bausenwein et al. 2008; Gelsomino and Azzellino 2011). Irrespective of depth, populations of soil microorganisms are greatest in zones where access to nutrients is easiest (Schütz et al. 2009). Densities of microorganisms in soil profiles therefore reflect gradients in mineralisable substrates (Bossio and Scow 1998; Griffiths et al. 1999; Schütz et al. 2009). Soil factors that affect microbial populations also affect the availability and activity of enzymes produced by those microorganisms (Paul and Clark 1996; Sinsabaugh et al. 1993; Kramer et al. 2013). *β*-glucosidase and urease facilitate cycling of C and nitrogen (N), respectively. Activities of these enzymes are sensitive to management-induced changes in the soil (Masciandaro and Ceccanti 1999; Caravaca et al. 2002). Notable variables are soil pH (Perucci et al. 2000; Makoi and Ndakidemi 2008; Das and Varma 2011), temperature (Zogg et al. 1997) moisture (Kieft et al. 1993; Lundquist et al. 1999; Schimel et al. 1999), and rhizo-deposition of root exudates reflecting vertical gradients in root distribution (Smalla et al. 2001; Yao et al. 2005).

Topsoils experience greater and more rapid variation in temperature and moisture content than subsoils (Brady and Weil 2002). Microbial communities in topsoils under orchard tree rows that are kept weed-free with herbicides and exposed to sunlight should therefore differ from those in mulch-covered topsoils (Hogue and Neilsen 1987; Tisdall et al. 1984). Variability in soil moisture and temperature and rate of degradation of organic material decreases with increasing depth. The effects of contrasting soil management practices on microorganisms and enzyme activities are therefore also likely to decrease with increasing depth. Potentially, soil management-induced changes in the dynamics of soil organic matter, and in root distribution and exudate production, may be reflected by the activity levels of soil enzymes (Badiane et al. 2001, Moore-Kucera and Dick 2008). Management systems that facilitate decomposition, nutrient cycling and nutrient retention should promote plant nutrient acquisition (Harrison et al. 2011) and sustain long-term soil fertility and crop production (Wardle et al. 2001). Trial data concerning the effects of ORG and CON practices on enzyme activities in Western Cape apple orchards is nevertheless limited.

This paper describes an investigation of depth-wise variation in urease and *β*-glucosidase activities in a ‘Cripp’s Pink’/M7 apple orchard after seven years under CON and ORG orchard floor management practices.

3.2. Materials and methods

3.2.1. Location, treatments and design

This research, which formed part of a wider field trial, was carried out on the Overberg Research Farm, Elgin (34.1363°S; 19.0216°E; altitude 327 m) (Wooldridge et al. (2013a, 2013b). Before planting, the gravelly clay loam soils received sufficient calcitic lime and single superphosphate to increase the pH to 5.6 (1 M KCl), neutralize exchangeable acidity, adjust the exchangeable sodium, potassium, calcium and magnesium to, respectively, <1%, 3.5-4%, 70% and 10-12% of the sum of base cations and the Bray II extractable soil phosphorus concentration to 25-30 mg kg^{-1}. These values are close to ideal for apple trees in the winter rainfall region of the Western Cape (Terblanche et al. 1980). The ameliorants were incorporated by cross-ripping at 120° to a depth of 60 cm. The trees were planted at spacings of 1 m x by 4.0 m (2 500 trees ha^{-1}) in winter 2000. Each experimental unit (plot) consisted of nine 'Cripp's Pink'/M7 trees separated from adjacent in-row plots by one or more pollinator trees ('Hillieri'/Seedling). Experimental rows were separated by side rows. Plots extended across the experimental tree row and work rows to the adjacent side rows. Irrigation was carried out at ca 7-day intervals, at ca 60% plant available water depletion. Five orchard floor management treatments were compared (Table 3.1). These consisted of combinations of practices applied to the tree and adjacent work rows, and commenced in spring 2003. Each treatment was replicated in four blocks in a fully randomized design. Trace element sprays, pruning, thinning and other routine orchard procedures were carried out in accordance with normal industry practice.

3.2.2. *Soil sampling and analysis*

Soil sampling for the determination of orchard soil chemical parameters was carried out in early spring (September) 2010. Composite soil samples (three subsamples) were obtained by auger from the 0-7.5, 7.5-15, 15-30 and 30-60 cm depth intervals in each plot. Samples were analysed by an independent laboratory using standard methods prescribed by the Non-Affiliated Soil Analysis Work Committee (1990). pH (1 M KCl) was determined in the air-dry soil (<2.0 mm). Ammonium (NH_4^+) and nitrate (NO_3^-) nitrogen (N) were measured in a KCl extract by Auto Analyser (Technicon Auto analyser III). Organic C contents were assessed by the dichromate oxidation method (Walkley and Black 1934) using a correction factor for incomplete oxidation of 1.33.

Table 3.1: Conventional (CON) and organic (ORG) orchard floor management treatments applied in a 'Cripp's Pink'/M7 apple trial orchard (Adapted from Wooldridge et al. 2013a)

Treatment	Work row		Tree row
	Tree dormant	Tree active	(Drip line to drip line)
T1 (CON)	Weed cover	Cover mown, natural die-back in summer	Chemical weed control[a] + inorganic N[b]
T2 (CON)	Soil tillage + cover crop[c]	Cover crop[c], chemical weed control in spring[a]	Chemical weed control[a] + inorganic N[b]
T3 (ORG)	Straw mulch[d]. Hand weeded	Straw mulch[d]. Hand weeded	(Compost + mulch)[e]. Hand weeded
T4 (ORG)	Soil tillage + cover crop[c]	Cover crop[c], mown, natural die-back in summer	(Compost + mulch)[e] + compost tea[f]. Hand weeded
T5 (ORG)	Weed cover	Cover mown, natural die-back in summer	(Compost + mulch)[e]. Hand weeded

[a]Roundup 360SC® applied at 5 L ha^{-1} (3%) in September and January

[b]Nitrogen as limestone ammonium nitrate (LAN) applied at full bloom, six weeks after full bloom and after harvest at rates of 9.2 g N, 5.2 g N and 18.0 g N $tree^{-1}$, respectively, in seasons 2003/04 to 2007/08. Applied after harvest only, at 18.0 g N $tree^{-1}$, in 2008/09 and 2009/10. Applied to the tree row in a continuous strip 1 m wide

[c]Rye (*Secale cereale* L. cv. Henog) and 'Pallinup' oats (*Avena sativa* L. cv. Pallinup) planted in tilled soil (0-10 cm) after leaf senescence in alternate seasons. Nitrogen (as LAN) applied to the cover crops and weed covers at 14 kg N ha^{-1} in June

[d]Wheat or oat straw (mulch) applied as a 5 cm layer in a continuous strip, c. 100 cm wide, along the tree row. Mulch applied at 3.2 kg straw $tree^{-1}$. Work rows covered with a 5 cm layer of straw mulch. Mulches replenished annually in October

[e]Compost applied at 20 kg per $tree^{-1}$ (128 g N, 3.7 g P, 6.3 g K $tree^{-1}$) in spring and autumn 2003/04, 10 kg $tree^{-1}$ in spring and autumn 2004/05 to 2006/07 and 10 kg $tree^{-1}$ in spring 2008 and 2009. Spread in a continuous strip, c. 1 m wide, along the tree row. Covered with a 5 cm layer (c. 3.2 kg per $tree^{-1}$) of wheat or oat straw (mulch). Mulch reapplied annually, in October.

[f]Compost tea (aerated, aqueous, microbial digest) surface-applied around each tree at one L $tree^{-1}$ per 4-week interval from full bloom to leaf senescence. Principal components (mg ml^{-1}): P, 7.5; K, 190; Ca, 51; Mg, 34.6; Na, 163. Nitrogen: 0.06%.

Soil samples for enzyme analysis were obtained in July 2011. Each sample consisted of multiple subsamples taken from the 0-7.5, 7.5-15, 15-30, 30-60 and 60-90 cm depth intervals of trenches excavated perpendicularly across the tree rows to a depth of 1.5 m, from drip line to drip line, in each treatment. Subsamples from each plot and depth interval were collected using a 10-centimetre-wide spade rinsed with 70% alcohol between samples. Subsamples were combined in a common polythene bag, transported to the laboratory, sieved (<2 mm), homogenised, stored at 4°C and analysed within 48 hours of collection.

β-glucosidase activity (EC 3.2.1.21) was determined in field-moist soil in a reaction mixture containing 1.0 g soil, 0.25 ml toluene, 1.0 ml 25 mM *p*-nitrophenyl-*β*-D-glucopyranoside as substrate, and 4.0 ml modified universal buffer (MUB) at pH 6.0 (Eivazi and Tabatabai 1988). The reaction mixture was incubated at 37°C for 60 min after which the reaction was terminated by adding 1.0 ml of 0.5 M $CaCl_2$ and 4.0 ml of 0.1 M tris (hydroxymethyl) aminomethane buffer (pH 12). Amount of *p*-nitrophenol liberated during enzymatic hydrolysis was determined at 410 nm with a digital UV–Vis spectrophotometer by reference to a calibration curve corresponding to a *p*-nitrophenol standard (Sigma-Aldrich) incubated with each soil under the same conditions as the samples and after subtracting the absorbance values of the control. In the reference samples (controls), the substrate was not added until after the reaction was stopped, immediately before filtration of the resulting soil suspension through Whatman no. 2V filter paper. *β*-glucosidase activity was expressed as micrograms *p*-nitrophenol per gram per hour.

Urease activity (EC 3.5.1.5) was determined by the non-buffered method of Kandeler and Gerber (1988) in which unbuffered urea solution (80 mM) was added to field-moist soil samples. These were incubated for 2 h at 37°C. Controls received deionized water. NH_4^+ released by the action of urease on its substrate was extracted with KCl solution. The solutions were shaken for 30 min on an orbital shaker. Determinations were based on the reaction of sodium salicylate with NH_4^+ in the presence of sodium dichloroisocyanurate. Extinction was measured at 690 nm with a digital UV–Vis spectrophotometer against the reagent blank. The NH_4^+ content was calculated by reference to a calibration curve compiled using standards containing 0, 1.0, 1.5, 2.0 and 2.5 mg NH_4^+ ml^{-1}. Sodium nitroprusside was used as a catalyst. Activity was expressed as micrograms NH_4^+ per gram per 2 h. Two replicates and one control from each soil were analysed for the *β*-glucosidase, and three replicates and one control for the urease determinations. Soil moisture contents were determined from the weight loss after drying at 105°C for 24 h. All enzyme activities were expressed on a moisture-free basis.

Root distributions were assessed by the profile trench method (Böhm 1979) in July 2011 after seven years of treatment application at the same time as the microbiology samples were taken. In two randomly-selected plots per treatment, a 1.5 metre deep trench was dug

from the base of the central tree perpendicularly across the adjacent work row. A 2.0 m x 1.2 m square-mesh (10 cm) reference grid was pegged to the trench wall with its long upper edge level with the soil surface and one side beneath the stem. Numbers of roots protruding from the loosened trench wall within each grid square were recorded (Wooldridge et al. 2013b).

3.2.3. Statistical analysis

The enzyme activity data were tested for normality (Shapiro and Wilk 1965), found to be acceptably normally distributed and subjected to an analysis of variance using the General Linear Model procedure of SAS (Statistical Analysis System) 9.1.3 (SAS Institute 2008). Factors were treatment and sampling date. Least significant difference (LSD) values were calculated at the 5% probability (*p*) level (Student's *t*-test) to facilitate comparison between treatment means (Snedecor and Cochran 1980). Means that differed at $p < 0.05$ were considered significantly different. Pearson correlation coefficients (*r*) were calculated for relationships between enzyme activities, soil parameters and root density using the CORR procedure of SAS 9.1.3. Means designated *, **, *** and **** differed at $p < 0.05$, 0.01, 0.001 and 0.0001, respectively. The data were also subjected to discriminate analysis (DA).

3.3. Results

3.3.1. Enzyme activity

Urease and *β*-glucosidase activities tended to decrease with increasing depth (Figure 3.1). Rates of decrease were greatest in the 0-30 cm depth interval. Averaged over all treatments, activities in the 0-7.5, 7.5-15, 15-30 and 60-90 cm depth intervals differed significantly (Table 3.2). Activities in the 30-60 cm interval were intermediate between, and did not differ from those in the 15-30 and 60-90 cm intervals. Averaged over all depth intervals, urease activities in the ORG treatments (T3, T4 and T5) exceeded those in the CON treatments (T1 and T2). Average *β*-glucosidase activity was greater in T5 than in T1 and T4. At soil depths below 15 cm, urease and *β*-glucosidase activities did not differ between treatments. Urease activities in the ORG treatments exceeded those in the CON treatments in the 0-15 cm soil material. *β*-glucosidase activity increased in the sequence in T2, T5 > T1 at 0-7.5 cm and T5 > T3 > T1 at 7.5-15 cm.

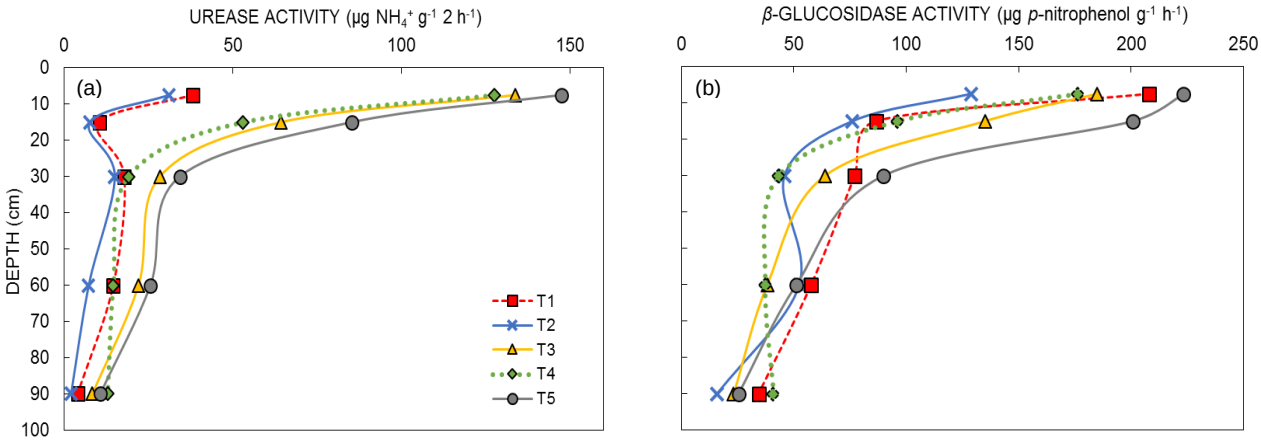

Figure 3.1: Effect of conventional (T1 and T2) and organic (T3, T4 and T5) orchard floor management practices on depth-wise change in urease (a) and *β*-glucosidase (b) activities in a 'Cripp's Pink'/M7 apple orchard soil after seven years of treatment application

Table 3.2: Effect of conventional (CON) and organic (ORG) orchard floor management treatments, and of soil depth, on urease and *β*-glucosidase activities. Values in the same data set, that are followed by the same letter, do not differ at $p < 0.05$

Enzyme	Treatment	Soil depth interval (cm)					Mean
		0-7.5	7.5-15	15-30	30-60	60-90	
Urease (μg NH_4^+ g^{-1} 2 h^{-1})	T1 (CON)	38.3^{cde}	$10.5f^{gh}$	17.9^{efgh}	14.6^{efgh}	4.0^{h}	18.0^{b}
	T2 (CON)	31.2^{defg}	$7.8f^{gh}$	15.1^{efgh}	7.3^{gh}	2.1^{h}	13.4^{b}
	T3 (ORG)	133.9^{a}	64.4^{bc}	28.4^{defgh}	22.1^{efgh}	$8.4f^{gh}$	54.5^{a}
	T4 (ORG)	127.7^{a}	53.1^{cd}	19.1^{efgh}	14.6^{efgh}	12.9^{efgh}	47.8^{a}
	T5 (ORG)	147.5^{a}	85.4^{b}	34.4^{def}	25.6^{efgh}	10.7^{fgh}	64.3^{a}
	Mean	95.7^{a}	44.2^{b}	23.0^{c}	16.9^{cd}	7.6^{d}	-
β-glucosidase (μg *p*-nitrophenol g^{-1} h^{-1})	T1 (CON)	128.7^{bcd}	76.0^{defg}	45.8^{efgh}	52.4^{efgh}	15.9^{h}	70.2^{b}
	T2 (CON)	208.1^{a}	86.7^{cdef}	77.1^{defg}	57.8^{efgh}	34.3^{fgh}	97.0^{ab}
	T3 (ORG)	185.0^{ab}	135.0^{bc}	63.9^{efgh}	$38.2f^{gh}$	23.2^{gh}	93.8^{ab}
	T4 (ORG)	176.1^{ab}	95.9^{cde}	43.1^{efgh}	37.2^{fgh}	40.9^{efgh}	81.3^{b}
	T5 (ORG)	223.4^{a}	200.7^{a}	89.9^{cdef}	51.1^{efgh}	25.4^{gh}	124.7^{a}
	Mean	184.3^{a}	118.9^{b}	65.3^{c}	$47.0c^{d}$	27.9^{d}	-
Urease : *ß*-glucosidase[1]		1:1.92	1:2.69	1:2.84	1:2.78	1:3.67	-

[1] Ratios over all treatments

3.3.2. Soil parameters

Over all sampled depth intervals within the 0-60 cm cultivated zone, the pH, C and NO_3^- levels were higher in the ORG than the CON treatments (Table 3.3, Figure 3.2), by c. 33%, 56% and 476%, respectively. Levels of NH_4^+, however, decreased in the order T1 > T3, T5 > T4, T1 exceeding T4 by 187%. Over all treatments, the average pH and C levels were higher in the 0-15 than the 15-60 cm soil material. Levels of NO_3^- were appreciably greater, and NH_4^+ lower, in the 0-30 cm than in the 30-60 cm soil material. Higher pH's were observed in the ORG than the CON treatments at all five depth intervals. Similar patterns were shown by

C and NO_3^- within the 0-30 cm interval. Below 30 cm differences between CON and ORG treatments were inconsistent. Ammonium levels did not differ significantly between treatments in the 0-7.5 cm interval, but decreased in the sequence T1, T2, T3 > T5 > T4 at 7.5-15 cm, T1, T2, T3, T5 > T4 at 15-30 cm and T1 > T3, T5 > T4 at 60-90 cm. Ratios of NO_3^- to NH_4^+ were lower in the CON (1:3.52) than the ORG treatments (1:0.36).

Table 3.3: Effect of conventional (CON) and organic (ORG) orchard floor management treatments, and of soil depth, on soil pH and on carbon (C), nitrate (NO_3^-) and ammonium (NH_4^+) contents. Values in the same data set, that are followed by the same letter, do not differ at $p < 0.05$

Parameter	Treatment	Soil depth interval (cm)				Mean
		0-7.5	7.5-15	15-30	30-60	
pH	T1 (CON)	4.43^{b}	4.53^{b}	4.38^{b}	4.45^{b}	4.82^{b}
	T2 (CON)	4.88^{b}	4.56^{b}	4.58^{b}	4.53^{b}	4.63^{b}
	T3 (ORG)	6.08^{a}	6.20^{a}	5.95^{a}	6.08^{a}	6.08^{a}
	T4 (ORG)	6.43^{a}	6.50^{a}	6.28^{a}	6.10^{a}	6.33^{a}
	T5 (ORG)	6.45^{a}	6.38^{a}	6.28^{a}	6.25^{a}	6.34^{a}
	Mean	5.65^{a}	5.61^{a}	5.49^{b}	5.48^{b}	-
C (%)	T1 (CON)	1.82^{b}	1.82^{b}	1.55^{b}	1.67^{b}	1.70^{b}
	T2 (CON)	2.00^{b}	1.86^{b}	1.60^{b}	1.72^{b}	1.79^{b}
	T3 (ORG)	3.56^{a}	2.81^{a}	2.61^{a}	2.33^{a}	2.83^{a}
	T4 (ORG)	3.10^{a}	3.14^{a}	2.50^{a}	1.01^{ab}	2.69^{a}
	T5 (ORG)	3.13^{a}	2.85^{a}	2.51^{a}	2.19^{a}	2.67^{a}
	Mean	2.72^{a}	2.50^{a}	2.15^{b}	1.98^{b}	-
NO_3^- (mg kg^{-1})	T1 (CON)	16.5^{b}	13.6^{b}	11.4^{b}	6.8^{ab}	12.0^{b}
	T2 (CON)	11.0^{b}	10.1^{b}	9.4^{b}	5.4^{b}	8.8^{b}
	T3 (ORG)	70.2^{a}	75.9^{a}	73.2^{a}	16.0^{ab}	58.9^{a}
	T4 (ORG)	73.3^{a}	74.1^{a}	70.3^{a}	11.8^{ab}	57.4^{a}
	T5 (ORG)	74.8^{a}	80.2^{a}	69.2^{a}	39.6^{a}	66.0^{a}
	Mean	49.2^{a}	50.8^{a}	46.7^{a}	15.9^{b}	-
NH_4^+ (mg kg^{-1})	T1 (CON)	24.4^{ab}	25.9^{a}	29.5^{a}	71.9^{a}	37.9^{a}
	T2 (CON)	21.4^{ab}	27.5^{a}	28.0^{a}	64.6^{ab}	35.4^{ab}
	T3 (ORG)	28.4^{a}	28.3^{a}	22.8^{a}	24.3^{cd}	25.9^{b}
	T4 (ORG)	14.4^{b}	12.0^{c}	10.6^{b}	15.8^{d}	13.2^{c}
	T5 (ORG)	18.4^{ab}	19.4^{b}	25.4^{a}	41.7^{bc}	26.2^{b}
	Mean	21.4^{b}	22.6^{b}	23.3^{b}	43.6^{a}	-

3.3.3. Correlations

Urease and *β*-glucosidase activities were well correlated (Table 3.4). Activities of both enzymes correlated significantly with soil C, NO_3^- and pH. Correlations between *β*-glucosidase and C, NO_3^- and pH were weaker than for urease, especially with regard to pH. Urease, but not *β*-glucosidase activity, correlated with number of tree roots per soil depth interval. Soil C correlated positively with NO_3^- and pH, but negatively with NH_4^+. Nitrate also correlated negatively with NH_4^+. pH correlated positively with C and NO_3^- but, like C and NO_3^-, correlated negatively with NH_4^+. Tree root density correlated with none of the above soil chemical parameters.

3.3.4. Discriminant analysis

Within the space defined by the F1 and F2 axes (Figure 3.3), centroids marking the XY-ordination of ORG treatments T3, T4 and T5, plotted at significantly ($p < 0.05$) higher values along the F1 axis than those of CON treatments T1 and T2. The F1 axis represented 96.0% of the total cumulative variability of 99.0%. Centroids for the CON and ORG treatments did not differ greatly along the F2 axis which accounted for only 3.1% of the total variability.

3.4. Discussion

The observed separation between centroids representing the CON and ORG treatments in the discriminate analysis (Figure 3.3) implies that the parameters *β*-glucosidase activity, urease activity, C, pH, NO_3^- and NH_4^+ differed between the CON and ORG treatments. That urease activity, pH, C and NO_3^- differed consistently between the CON and ORG treatments in the 0-7.5 and 7.5-15 cm intervals, and in some cases deeper (Tables 3.2 and 3.3), suggests a causative linkage between urease activity and the parameters pH, C and NO_3^-. Correlations between urease activity and soil pH ($r = 0.552$****), C ($r = 0.731$****) and NO_3^- ($r = 0.610$****) support this supposition. Soil enzyme activities are sensitive to pH (Makoi and Ndakidemi 2008; Das and Varma 2011) which usually alters the ionization state of the enzyme's active center (Frankenberger and Johanson 1982). Since dichromate-oxidisable organic matter is the raw substrate on which soil microorganisms and their enzymes act, correlations between enzyme activity and soil C may be anticipated. Enzyme activity increases with increasing organic matter input (Marcinkevičienė and Pupalienė 2009) and is normally higher in mulched than unmulched plots (Yang et al. 2003).

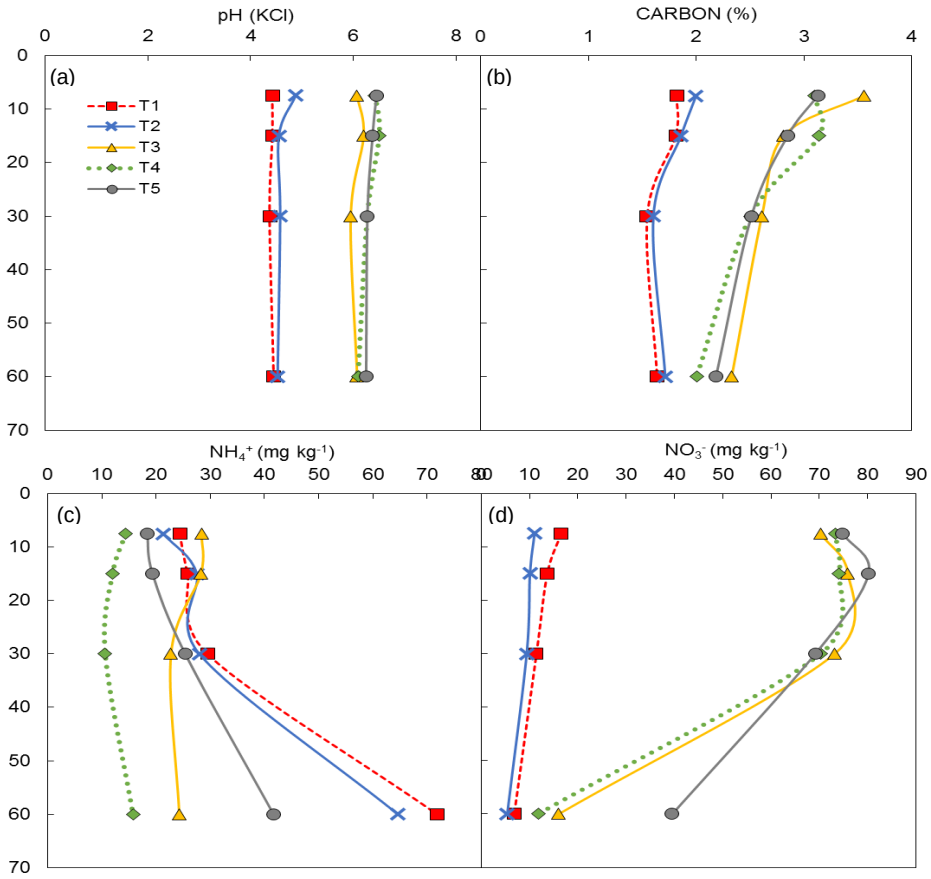

Figure 3.2: Effect of conventional (T1 and T2) and organic (T3, T4 and T5) orchard floor management practices on depth-wise change in pH (a), organic C (b), NH_4^+ (c) and NO_3^- (d) in a 'Cripp's Pink'/M7 apple orchard soil after seven years of treatment application

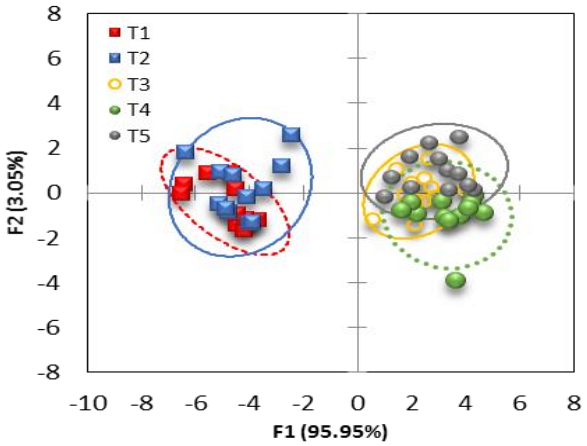

Figure 3.3: XY-ordination plot of conventional (T1 and T2) and organic (T3, T4 and T5) orchard floor practices in the space defined by the first (F1) and second (F2) factors (axes) of the discriminate analysis of *β*-glucosidase and urease activities and of C, NO_3^-, NH_4^+ and pH. Symbols represent the mean of four replicates. Values in the same ellipsoid do not differ at $p < 0.05$. Values in brackets indicate the percentage of total variation attributable to each discriminate analysis axis

Table 3.4: Pearson correlation coefficients between microbial enzyme activities, soil chemical parameters and root density in a 'Cripp's pink'/M7 apple orchard soil under different orchard floor management practices (n = 68)

Sources of variation	Urease (μg NH_4^+ g^{-1} 2 h^{-1})	β-glucosidase μg *p*-nitrophenol g^{-1} h^{-1}	Organic C (%)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	pH (KCl)
β-glucosidase	0.80382****					
Organic C (%)	0.73091****	0.49766****				
NO_3^--N (mg kg^{-1})	0.60984****	0.34560**	0.72783****			
NH_4^+-N (mg kg^{-1})	-0.24362^{ns}	-0.22141 ^{ns}	-0.37057***	-0.29600**		
pH (KCl)	0.55220****	0.28794*	0.71891****	0.74776****	-0.42881****	
Root density	0.47243*	0.36110 ^{ns}	0.37884 ^{ns}	0.32134 ^{ns}	-0.27780 ^{ns}	0.37322 ^{ns}

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. ^{ns} = non-significant.

For urease activity to correlate with soil N a source of urea is required. Urea, a mammalian excretory product, was probably introduced into the ORG treatments as a component of compost. Lack of correlation between urease activity and NH_4^+ may have been due, in part, to inhibition in accordance with mass action principles of enzymatic hydrolysis of amino-bearing compounds by NH_4^+ from the limestone ammonium nitrate (LAN) applied to the CON treatments (Masciandaro et al. 2004). That *β*-glucosidase activity correlated reasonably well with C (r = 0.498****), which is supported by Ma et al. (2010), but more weakly correlated with NO_3^- (r = 0.346**) and pH (r = 0.388*), suggests that *β*-glucosidase activity was less sensitive to NO_3^- and pH than urease, despite the observed reasonably strong correlation between urease and *β*-glucosidase activities (r = 0.804****). This strong correlation implies that, overall, the production and activity of both enzymes are facilitated by a similar set of environmental parameters. The weak correlation between *β*-glucosidase activity and NO_3^- was ascribed to the fact that, since *β*-glucosidase and its derivatives reduce *β*-D-glucosides to glucose in soils (Lehninger et al. 2000) they are unlikely to be greatly influenced by soil N unless the amounts present place limits on microbial activity. The weaker correlation between *β*-glucosidase activity and pH (r = 0.288*), compared with urease and pH (r = 0.552****), may reflect pH-related differences in the entrapment of these separate enzymes on the soil colloids. Collectively, these findings were consistent with the known involvement of *β*-glucosidase and urease in the biogeochemical cycling of C and N, respectively (Makoi and Ndakidemi 2008). The observed correlations between pH and the activities of urease and *β*-glucosidase (Table 3.4) corroborate work by Acosta-Martinez and Tabatabai (2000).

Significant differences in urease activity between the CON and ORG treatments only occurred in the 0-7.5 and 7.5-15 cm intervals, although the trend was similar in the 15-30 and 30-60 cm intervals. All-treatment average urease activities decreased in the sequence: 0-7.5 cm > 7.5-15 cm > 15-30 cm. Thresholds for pH, C and NO_3^-, above which increases in these parameters through the supply of compost are likely to lead to increased urease activities, may therefore lie between the average values in the ORG treatments in the 7.5-15 cm and 15-30 cm depth intervals, i.e. pH 6.4-6.2; C 3.9-2.5% and NO_3^- 76.7-70.9 mg kg^{-1}). Balance between these soil parameters is likely to be critical, but could not be investigated in this trial. Possibly important was the increase in the ratio of NO_3^- to NH_4^+ with depth in the ORG treatments, from 1:2.8 at 7.5-15 cm to 1:3.6 at 15-30 cm. That the average NH_4^+ levels were higher in the 30-60 cm interval than in the overlying soil, notable in T1 and T2 (Figure 3.3), may have been due to the supply of more NH_4^+ by the LAN used in these treatments than could be converted to NO_3^-, utilised by root uptake or retained by cation exchange sites in the 0-30 cm soil depth interval. Since pH and C in T1 and T2 did not vary significantly with depth (Table 3.3, Figure 3.2) it is unlikely that differences in the abundance of pH-dependent

cation exchange sites on organic colloids contributed to the high exchangeable NH_4^+ levels in the 30-60 cm intervals of these treatments. The substantially higher NO_3^- levels in the ORG, compared to the CON treatments in the 0-30 cm soil material were attributed to enzymatic oxidation of NH_3^- and NH_4^+ formed from amino compounds during the composting process, hence the (albeit weak) negative relationship between NH_4^+ and NO_3^-. That NO_3^- levels in the ORG soils were lower in the 30-60 cm than the overlying soil material was counter to expectation, based on the assumption that anions leach faster than cations. Since the relationship between NO_3^- and root density was not significant, it is unlikely that downward leaching of NO_3^- was unduly limited by root uptake in the 0-30 cm interval.

Unlike urease, *β*-glucosidase activities overlapped between CON and ORG treatments such that T1 was exceeded by T2 which did not differ from T3, T4 and T5 at 0-7.5 cm, and that T1, T2 and T4 did not differ at 7.5-15 cm. *β*-glucosidase activity was therefore less sensitive to differences in the soil environment between the CON and ORG orchard floor management practices than urease. In relative terms and over all depths, urease:*β*-glucosidase activity ratios in T1 (1:3.9) and T2 (1:7.4) (CON) tended to be lower than in ORG treatments T3 (1:1.7), T4 (1:1.7) and T5 (1:1.9). *β*-glucosidase and urease activity levels therefore differed less in the ORG than the CON treatments. Ratios of urease activity to *β*-glucosidase activity were higher in the 0-7.5 cm interval than in the 7.5-60 cm interval. Between 60 and 90 cm, beneath the zone of pre-plant soil preparation, the ratio of urease to *β*-glucosidase activity was low. Reactions involving urease and *β*-glucosidase activities therefore proceed at rates that are dependent on depth, and on those soil parameters that change with depth. Soil preparation therefore reduced differences in activity between urease and *β*-glucosidase relative to the underlying material. In the prepared soil material (0-60 cm), the urease and *β*-glucosidase activities were closer in the 0-7.5 cm interval (i.e. the material closest to the surface on which the treatments were applied) than in the underlying soil material, indicating that the effects of the CON and ORG treatments on urease and *β*-glucosidase activities were largely confined to the 0-7.5 cm soil interval. Since the ratio of urease to *β*-glucosidase activity in T3 (straw mulched work rows) did not differ appreciably from those in T4 and T5, the presence of a permanent straw mulch in the work row had little or no carry-over effect on the activities of these enzymes under the canopy, even though increasing soil C levels have been reported to increase microbiological immobilisation of NO_3^- (Recous et al. 1990).

Urease and *β*-glucosidase activities declined more rapidly with increasing depth than pH, C, NO_3^- and NH_4^+. Thus, urease and *β*-glucosidase activities in the 15-30 cm soil interval in the ORG treatments only averaged c. 20% and 34%, respectively, of their values in the 0-7.5 cm soil interval, whereas pH, C, NO_3^- and NH_4^+ values in the 15-30 cm intervals were, respectively, 98%, 79%, 97% and 96% of their values at 0-7.5 cm. Likewise, in the CON

treatments, urease and *β*-glucosidase activities, pH, C, and NO_3^- in the 15-30 cm intervals were, respectively, 47%, 37%, 96%, 82% and 97% of their values in the 0-7.5 cm intervals. Conversely, NH_4^+ concentrations in the 15-30 cm intervals of the CON treatments averaged 25.5% higher than in the 0-7.5 cm horizons. That NH_4^+ tended to be more abundant in the 15-30 cm than in the 0-7.5 cm soil depth interval was probably due to downward leaching of NH_4^+ derived from the surface-applied LAN.

According to Wooldridge et al. (2013b), root density did not differ between the 0-30 cm and 30-60 cm soil depth intervals. Neither, in the present trial (Table 3.4), were significant correlations observed between root density and the soil parameters C, NO_3^-, NH_4^+ or pH, possibly because the levels of these parameters were adequate for root activity at all depth intervals above 60 cm, which was the depth to which soil preparation was carried out and in which 83% of the total roots to a depth of 90 cm were located (Wooldridge et al. 2013b). Urease activity correlated significantly with root density, but *β*-glucosidase activities did not. In view of the abundance of organic material in the soil, particularly in the ORG treatments, the likelihood that a causative relationship exists between root density/exudate abundance and urease activity is probably low.

Although ORG practices promoted urease activity in the superficial soil material, compared to CON practices, they did not improve orchard performance, as evidenced by the finding of Wooldridge et al. (2013b) that yield efficiencies (yield per unit stem cross sectional area) were lower in the ORG than the CON treatments. Lower yield efficiencies in the ORG treatments were attributed to the promotion of excessively vigorous vegetative tree growth by the higher levels of mineral nutrients supplied by compost to the ORG, compared with the CON treatments. Orchard floor management practices that improve the activities of microbially generated enzymes are therefore not necessarily associated with higher yields of apples per unit stem cross sectional area.

3.5. Conclusions

Compared to CON orchard floor management practices using synthetic fertilisers and herbicides, ORG practices utilising compost and straw mulch promote urease activity within the 0-30 cm soil depth interval, but do not affect *β*-glucosidase activity consistently. The activities of both enzymes correlate with soil C content and decrease with increasing depth at rates that exceed those of pH and C.

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CHAPTER 4

Relationship between soil alteration index three (AI3), soil organic matter and tree performance in a 'Cripp's Pink'/M7 apple orchard

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Abstract

Alteration index three (AI3), which calculates the balances between three microbially-secreted enzymes, potentially enables differences between soils due to contrasting management practices to be quantified in relative terms. The ability of AI3 to distinguish between apple orchard soils under conventional and organic production protocols, and to reflect tree performance, were tested in a maturing 'Cripp's Pink'/M7 apple orchard. Activities of *β*-glucosidase, phosphatase and urease were determined colourimetrically in extracts of tree-row top-soils (0–15 cm) taken during September and January over five consecutive seasons. Soil organic matter content was determined by dichromate oxidation. Stem circumference and yield were measured manually. AI3 correlated significantly ($p < 0.05$) with soil organic matter, yield and yield efficiency. AI3 may thus be a useful indicator of relative apple tree performance under organic and conventional soil surface management practices.

Keywords: AI3, compost, enzyme activity, organic, straw mulch

4.1. Introduction, Results and discussion, Materials and methods, and Conclusions

Organic (ORG) orchard floor management protocols, using compost as a nutrient source and mulches to control weeds, differ in their effects on soil parameters and apple tree performance from conventional (CON) practices using synthetic fertilisers and herbicides (Wooldridge et al. 2013a, 2013b). Whether ORG and CON practices differ in their effects on the activities of soil enzymes has not been tested in Western Cape apple orchards. Neither has it been established whether enzyme activity ratios bear any relationship to tree performance.

Alteration index three (AI3) quantifies the balance between three microbially-secreted soil enzymes and is sensitive to alterations in soil characteristics caused by management practices (Puglisi et al. 2006). Alteration of the soil, whether by over-utilisation or other detrimental practices, results in AI3 values that are higher than those of control soils (Puglisi et al. 2006 and references therein). AI3 is potentially useful for determining soil quality in

temperate grasslands (Paz-Ferreiro et al. 2009), and as an index of soil degradation due to agricultural practices (Bastida et al. 2008).

To clarify the effects of ORG and CON treatments on soil enzyme activity ratios in orchard soils, AI3 was determined in extracts obtained from two CON and three ORG treatments (Table 4.1) in the latter stages of an ongoing field trial in a 'Cripp's Pink'/M7 apple orchard on a sandy loam soil in the Elgin area (Wooldridge et al. 2013a). Treatments were applied to nine-tree plots replicated in four randomised blocks from September 2003 (beginning of the third growth season) to January 2011.

Composite soil samples were taken from the zone of highest white feeding root concentration (c. 0–15 cm depth) on both sides of the tree row, beneath the canopy drip zone, from September 2006, when the trees were approaching maturity to January 2011. The soil organic matter (SOM) contents and chemical characteristics of these samples were determined by Wooldridge et al. (2013a). Activities of *β*-glucosidase, urease and phosphatase were assessed colourimetrically in extracts from the 0–15 cm drip-zone soil samples (Tabatabai and Bremner 1969; Eivazi and Tabatabai 1988; Kandeler and Gerber 1988). AI3 values were calculated with the equation:

AI3 = (7.87 x *β*-glucosidase) – (8.22 x phosphatase) – (0.49 x urease)

where enzyme activities were expressed in micromoles of, respectively, *p*-nitrophenyl-*β*-D-glucoside and *p*-nitrophenylphosphate per gram of soil per hour, and micrograms of urea per gram of soil per hour.

Stem circumferences were measured 40 cm above ground level using a flexible tape at the end of seasons 2003/04 to 2009/10 (Wooldridge et al. 2013b). Yields were determined annually, and yield efficiencies (yield in kg cm^{-2} stem cross-sectional area) were calculated for seasons 2006/07 to 2010/11 (Wooldridge et al. 2013b). Data were subjected to analysis of variance using SAS 9.1.3 (SAS Institute 2008). Least significant difference values were calculated at $p < 0.05$ (Student's *t*-test) to facilitate comparison between treatment means. Pearson correlation coefficients (*r*), correlating AI3 with the SOM, stem circumference, yield and yield efficiency data of Wooldridge et al. (2013a, 2013b) were derived using the CORR procedure of SAS 9.1.3.

Over all of the seasons in which treatments were applied (2003–2010), SOM contents in ORG treatments T3, T4 and T5 were greater than in CON treatments T1 and T2 (Table 4.2). Average increases in stem circumference over this period were also greater in T3, T4 and T5 than in T1 and T2 due to higher levels of phosphorus and potassium, and lower acidity, in the ORG than the CON soils (Wooldridge et al. 2013b).

Table 4.1: Conventional (CON) and organic (ORG) orchard floor management treatments applied in a 'Cripp's Pink'/M7 apple trial orchard (Adapted from Wooldridge et al. 2013a)

Treatment	Work row		Tree row
	Tree dormant	Tree active	(Drip line to drip line)
T1 (CON)	Weed cover	Cover mown, natural die-back in summer	Chemical weed control[a] + inorganic N[b]
T2 (CON)	Soil tillage + cover crop[c]	Cover crop[c], chemical weed control in spring[a]	Chemical weed control[a] + inorganic N[b]
T3 (ORG)	Straw mulch[d]. Hand weeded	Straw mulch[d]. Hand weeded	(Compost + mulch)[e]. Hand weeded
T4 (ORG)	Soil tillage + cover crop[c]	Cover crop[c], mown, natural die-back in summer	(Compost + mulch)[e] + compost tea[f]. Hand weeded
T5 (ORG)	Weed cover	Cover mown, natural die-back in summer	(Compost + mulch)[e]. Hand weeded

[a]Roundup 360SC® applied at 5 L ha^{-1} (3%) in September and January

[b]Nitrogen as limestone ammonium nitrate (LAN) applied at full bloom, six weeks after full bloom and after harvest at rates of 9.2 g N, 5.2 g N and 18.0 g N $tree^{-1}$, respectively, in seasons 2003/04 to 2007/08. Applied after harvest only, at 18.0 g N $tree^{-1}$, in 2008/09 and 2009/10. Applied to the tree row in a continuous strip 1 m wide

[c]Rye (*Secale cereale* L. cv. Henog) and 'Pallinup' oats (*Avena sativa* L. cv. Pallinup) planted in tilled soil (0-10 cm) after leaf senescence in alternate seasons. Nitrogen (as LAN) applied to the cover crops and weed covers at 14 kg N ha^{-1} in June

[d]Wheat or oat straw (mulch) applied as a 5 cm layer in a continuous strip, c. 100 cm wide, along the tree row. Mulch applied at 3.2 kg straw $tree^{-1}$. Work rows covered with a 5 cm layer of straw mulch. Mulches replenished annually in October

[e]Compost applied at 20 kg per $tree^{-1}$ (128 g N, 3.7 g P, 6.3 g K $tree^{-1}$) in spring and autumn 2003/04, 10 kg $tree^{-1}$ in spring and autumn 2004/05 to 2006/07 and 10 kg $tree^{-1}$ in spring 2008 and 2009. Spread in a continuous strip, c. 1 m wide, along the tree row. Covered with a 5 cm layer (c. 3.2 kg per $tree^{-1}$) of wheat or oat straw (mulch). Mulch reapplied annually, in October.

[f]Compost tea (aerated, aqueous, microbial digest) surface-applied around each tree at one L $tree^{-1}$ per 4-week interval from full bloom to leaf senescence. Principal components (mg ml^{-1}): P, 7.5; K, 190; Ca, 51; Mg, 34.6; Na, 163. Nitrogen: 0.06%.

Over the period 2006 to 2010, however, when the trees were mature, increases in stem circumferences did not differ significantly between treatments. Percentage increase in stem

circumference in T3 and T4 were nevertheless greater than in T1. Average yields between 2006 and 2010 were greater in T1 than in T3, T4 and T5. Over the same period, average yield efficiencies in T1 and T2 exceeded those in T3, T4 and T5. September AI3 values in T4 and T5 were lower than in T1 and T2. January AI3 values were lower in T3, T4 and T5 than in T2. That the AI3 value in T2 tended to be lower than in T1 may have been due to carry-over of the effects of work-row tillage in T2 into the drip zone from which the samples were obtained. According to Bergstrom et al. (1998), tillage may affect enzyme activities. Similarly, Puglisi et al. (2006) found that control soils were characterised by lower AI3 values than soils that had been altered by detrimental management practices. The altered soils were inferred to be less able to support plant growth than the control soils.

Yields and yield efficiencies were higher in the treatments in which stem circumferences over the whole period of treatment applications (2003–2010) were lowest, which support the principle that vegetative growth in deciduous fruit trees is inversely related to fruitfulness (Jerie et al. 1989). Low yields and yield efficiencies in the present trial were attributed to high mineral nutrient levels, notably in the ORG treatments, which led to excessive vegetative growth in the young trees, necessitating girdling (Wooldridge et al. 2013b).

The observation that the September and January AI3 values were less well correlated than the September and January SOM contents (Table 4.3) implies that AI3, which reflects microbiological activity, is more variable within seasons than SOM, probably because SOM indicates the abundance of carbon in living organisms and in their no-longer living residues. AI3 therefore appears to be more sensitive to environmental conditions than SOM. Likewise, Jin et al. (2009) found that enzyme activities in soils vary within seasons. AI3 was more highly correlated with SOM in September than in January. AI3 correlated significantly with neither stem circumference nor percentage increase in stem circumference over the period 2006–2010, either in September or January. Yield and yield efficiency correlated significantly with AI3 in September, but not January, indicating that sampling for enzyme assays in apple orchards should be done in September. The September and January SOM values correlated with both yield and, more strongly, with yield efficiency. In the combined September and January data, yield and yield efficiency were well correlated.

AI3 values determined in drip-line top-soils in September were generally higher, and yields and yield efficiencies were also higher, in maturing ‘Cripp’s Pink’/ M7 orchards under CON than under ORG management protocols. AI3 correlates with SOM. Both AI3 and SOM can therefore be used as indicators of yield and yield efficiency, although AI3 was more sensitive to intra-seasonal changes than SOM. The AI3 value range that corresponds to optimum soil conditions for apple production, as opposed to vegetative growth, must still be determined. Testing of AI3 in commercial apple orchards will commence in September 2014.

Table 4.2: Effect of conventional (CON) and organic (ORG) management on soil alteration index three (AI3), and on soil organic matter (SOM) content, stem circumference, yield, yield efficiency (kg cm^2 stem cross-sectional area) in a 'Cripp's pink'/M7 apple orchard. Values in the same column, that are followed by the same letter, do not differ at $p < 0.05$

Treatment	SOM 2003-2010 (%)[1]	Average increase in stem circumference 2003-2010 (cm)[2]	Average increase in stem circumference 2006-2010 (cm)[2]	Increase in stem circumference 2006-2010 (%)[2]	Average yield 2006-2010 (t ha^{-1})[2]	Average yield efficiency 2006-2010 (kg cm^2)[2]	AI3 September 2007-2010	AI3 January 2008-2011
T1 (CON)	1.06^{d}	12.60^{b}	6.3^{a}	11.5^{b}	38.71^{a}	0.386^{a}	-23^{ab}	-47^{ab}
T2 (CON)	2.03^{cd}	12.58^{b}	6.5^{a}	12.6^{ab}	32.59^{ab}	0.363^{a}	-20^{a}	-43^{a}
T3 (ORG)	3.22^{ab}	17.15^{a}	8.0^{a}	15.0^{a}	29.30^{bc}	0.218^{b}	-33^{bc}	-51^{b}
T4 (ORG)	3.54^{a}	16.05^{a}	7.6^{a}	14.8^{a}	22.51^{c}	0.186^{b}	-36^{c}	-51^{b}
T5 (ORG)	3.45^{a}	15.90^{a}	6.8^{a}	13.9^{ab}	21.74^{c}	0.168^{b}	-36^{c}	-51^{b}

[1] Tree row including drip zone. Data of Wooldridge et al. (2013a).

[2] Data of Wooldridge et al. (2013b).

Table 4.3: Pearson correlation coefficients (*r*) between soil alteration index three (AI3), soil organic matter (SOM) content and plant performance parameters in a 'Cripp's pink'/M7 apple orchard in September 2006 to 2010 and January 2007 to 2011 (n = 20)

Parameters correlated	*r*	*p*
September AI3 x January AI3	0.6069	0.0045
September SOM x January SOM	0.9847	<0.0001
September AI3 x September SOM	-0.7761	<0.0001
January AI3 x January SOM	-0.4613	0.0406
September AI3 x stem circ. increase	-0.1760	0.4580
January AI3 x stem circ. Increase	-0.1027	0.6667
September AI3 x percentage increase in stem circumference	-0.0480	0.5733
January AI3 x percentage increase in stem circumference	-0.0515	0.5458
September AI3 x yield	0.5110	0.0213
January AI3 x yield	0.2416	0.3049
September AI3 x yield efficiency	0.6565	0.0017
January AI3 x yield efficiency	0.4160	0.0681
September SOM x yield	-0.5885	0.0063
January SOM x yield	-0.6151	0.0039
September SOM x yield efficiency	-0.8296	<0.0001
January SOM x yield efficiency	-0.8516	<0.0001
Yield x yield efficiency	0.8175	<0.0001

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CHAPTER 5

Effect of organic and conventional practices on carbon-substrate utilisation by the soil microbial community in a 'Cripp's Pink'/M7 apple orchard

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Abstract

Changes in microbial community metabolic profiles can potentially be used to identify differences in soil characteristics caused by dissimilar orchard floor management practices. The impacts of orchard floor management practices on the microbial community metabolic diversity (CMD) were examined in apple orchard soils subjected to two conventional (CON) and three organic (ORG) orchard floor management practices. The CMD, as indicated by carbon (C) substrate utilisation (BIOLOG™ system), was determined in composite soil samples (0-15 cm) from the tree rows. CON treatments reduced, and ORG treatments increased, C-source utilisation and, hence, CMD. Within CON treatments C-source utilisation in summer was favoured by winter cover crops in work rows, controlled with herbicide in spring, compared to uncontrolled weed-covered work rows. Orchard soils under ORG regimes more closely resembled soils of natural ecosystems in that they were better able to sustain CMD, and possibly afford a better option to sustain critical ecosystem functions than under CON management which relies heavily on synthetic agrochemical usage.

Keywords: BIOLOG™, community metabolic diversity, compost, fertiliser, herbicides

5.1. Introduction, Results and discussion, Materials and methods, and Conclusions

Changes in soil microbial communities due to altered orchard floor management practices are likely to be reflected by their abilities to perform such ecosystem-level processes as decomposition of organic matter and nutrient cycling (Neher 1999). Soil microbial communities differ in terms of the types of carbonaceous substrate that they are able to utilise. Carbon (C) substrate utilisation patterns may be determined using BIOLOG Eco Plates™ (BIOLOG™, Haward CA). This approach quantifies the complexities of microbial communities in terms of their capacities to utilise 31 different carbonaceous substrates on a single microtitre plate. These substrates have high relevance to soil bacterial communities, or are known to be contained within plant root exudates (Preston-Mafham et al. 2002). Reportedly (Rashedul et al. 2010), such metabolic fingerprints (community level

physiological profiles, or CLPP) are sensitive and reliable in terms of their ability to identify changes in the soil environment and microbial communities that stem from changes in soil management. A particularly useful aspect of CLPP is that it describes microbial communities in terms of their metabolic diversities, i.e. the community metabolic diversity (CMD) (Sigler 2004).

This research concerns the effects of conventional (CON) and organic (ORG) orchard floor management practices on CMD in a 'Cripp's Pink'/M7 apple orchard on a sandy loam soil in the Elgin area, South Africa. This trial has been described by Wooldridge et al. (2013). Briefly, five tree row x work row treatments (Table 5.1) were applied to nine-tree plots in spring (September) 2003 when the trees were entering their third season. Applications continued until January 2011. Each treatment was replicated in four randomised blocks. CMD was calculated by summing the number of C substrates utilised by the microbial community (positive responses represented by purple-coloured wells) in each BIOLOG™ microtitre plate (Sigler 2004). Positive responses were defined as any absorbance > 0.25 (590 nm) after 48-hours (curve inflection point) incubation at c. 23°C. The CMD's were determined in composite soil samples from the 0-15 cm depth interval on both sides of the tree row, beneath the canopy drip line, in spring (September) 2008 and 2009, and in summer (January) 2010. The BIOLOG™ data (% of 31 C sources) were subjected to analysis of variance using SAS 9.1.3 (SAS 2008). Student's *t* least significant difference (LSD) values were calculated at the 5% probability (*p*) level to facilitate comparison between treatment means. The spring 2008 and summer 2010 data were also subjected to principal component analysis (PCA) (Hirst and Jackson 2007).

Carbon substrate utilisation in CON treatments T1 and T2 were low compared to those in ORG treatments T4 and T5 in 2008, and to ORG treatments T3, T4 and T5 in 2009 (Table 5.2). Utilisation in T1 was exceeded by that in T2, T3, T4 and T5 in 2010. Averaged over all sampling dates, C substrate utilisation in T1 and T2 were lower than in T4, and utilisation in T1 was lower than in T3, T4 and T5. Over all treatments, utilisation increased in the sequence: spring 2008 < spring 2009 < summer 2010.

PCA analysis of the 2008 data (Figure 5.1a) showed that, within the space defined by the PC1 and PC2 axes, centroids marking the XY-ordination of the ORG treatments plotted at higher, more positive, values along the PC1 axis than those of the CON treatments. The PC1 axis represented 36.6% of the total cumulative variability of 63.65%. Centroids for T1 and T2 were separated more widely along the PC2 axis, which accounted for 27.05% of the variability, than were those of the ORG treatments. PCA plots for summer 2010 (Figure 5.1b) resembled those for spring 2008 in that centroids for T3, T4 and T5, plotted at higher values on the PC1 axis, which in 2010 accounted for a much higher percentage (76.54%) of the total variability (90.16%) than in 2008.

Table 5.1: Conventional (CON) and organic (ORG) orchard floor management treatments applied in a 'Cripp's Pink'/M7 apple trial orchard (Adapted from Wooldridge et al. 2013a)

Treatment	Work row		Tree row
	Tree dormant	Tree active	(Drip line to drip line)
T1 (CON)	Weed cover	Cover mown, natural die-back in summer	Chemical weed control[a] + inorganic N[b]
T2 (CON)	Soil tillage + cover crop[c]	Cover crop[c], chemical weed control in spring[a]	Chemical weed control[a] + inorganic N[b]
T3 (ORG)	Straw mulch[d]. Hand weeded	Straw mulch[d]. Hand weeded	(Compost + mulch)[e]. Hand weeded
T4 (ORG)	Soil tillage + cover crop[c]	Cover crop[c], mown, natural die-back in summer	(Compost + mulch)[e] + compost tea[f]. Hand weeded
T5 (ORG)	Weed cover	Cover mown, natural die-back in summer	(Compost + mulch)[e]. Hand weeded

[a]Roundup 360SC® applied at 5 L ha^{-1} (3%) in September and January

[b]Nitrogen as limestone ammonium nitrate (LAN) applied at full bloom, six weeks after full bloom and after harvest at rates of 9.2 g N, 5.2 g N and 18.0 g N $tree^{-1}$, respectively, in seasons 2003/04 to 2007/08. Applied after harvest only, at 18.0 g N $tree^{-1}$, in 2008/09 and 2009/10. Applied to the tree row in a continuous strip 1 m wide

[c]Rye (*Secale cereale* L. cv. Henog) and 'Pallinup' oats (*Avena sativa* L. cv. Pallinup) planted in tilled soil (0-10 cm) after leaf senescence in alternate seasons. Nitrogen (as LAN) applied to the cover crops and weed covers at 14 kg N ha^{-1} in June

[d]Wheat or oat straw (mulch) applied as a 5 cm layer in a continuous strip, c. 100 cm wide, along the tree row. Mulch applied at 3.2 kg straw $tree^{-1}$. Work rows covered with a 5 cm layer of straw mulch. Mulches replenished annually in October

[e]Compost applied at 20 kg per $tree^{-1}$ (128 g N, 3.7 g P, 6.3 g K $tree^{-1}$) in spring and autumn 2003/04, 10 kg $tree^{-1}$ in spring and autumn 2004/05 to 2006/07 and 10 kg $tree^{-1}$ in spring 2008 and 2009. Spread in a continuous strip, c. 1 m wide, along the tree row. Covered with a 5 cm layer (c. 3.2 kg per $tree^{-1}$) of wheat or oat straw (mulch). Mulch reapplied annually, in October.

[f]Compost tea (aerated, aqueous, microbial digest) surface-applied around each tree at one L $tree^{-1}$ per 4-week interval from full bloom to leaf senescence. Principal components (mg ml^{-1}): P, 7.5; K, 190; Ca, 51; Mg, 34.6; Na, 163. Nitrogen: 0.06%.

Utilisation by T2 was appreciably greater along the PC1 axis in 2010 than in 2008. As in 2008, utilisation by T1 and T2 differed along the PC2 axis, which in 2010 accounted for a lower percentage (13.62%) of the variability than in 2008. As indicated by their trajectories, utilisation data for 2008 and 2010 (Figure 5.1c) differed appreciably along the PC1 axis (32.16% of variability) in T2, T3, T4 and T5, but not T1.

The observed higher CMD in the ORG compared to the CON treatments may have been due to, respectively, higher and lower, soil organic matter (SOM) in these systems (Grayston et al. 1998), the CMD profiles reflecting variation in substrate diversity and availability (Grayston et al. 2004). Likewise, differences in CMD between spring 2008 and summer 2010 may have been due to higher SOM turnover rates in summer compared to spring (Haynes 1980). Over the trial period SOM levels were higher in ORG than CON treatments (Wooldridge et al. 2013).

The higher summer CMD in T2 (work rows were seeded with cover crops each winter), compared with T1 (weed-covered work rows) may have been due to carry-over from the work rows into the tree rows, across the drip line from which the samples were taken. If so, the winter cover crop, which was chemically controlled in spring, may have stimulated CMD by releasing a greater variety of C compounds into the soil through root exudation and decomposition, than did the uncontrolled weeds, which persisted into summer (Grayston et al. 1998; Yao et al. 2005).

Relative to CON orchard floor management practices (herbicides and inorganic fertilisers applied), ORG practices (mulching, no agrochemicals) promote richer microbial ecosystems and appear to be better able to sustain CMD and, by inference, the functions mediated by soil microbial communities. The approach used in this research potentially lends itself to wider application, both as a measure of microbial metabolic diversity and as an indicator of C availability in agricultural soils.

Table 5.2: Effect of conventional (CON) and organic (ORG) management on community metabolic diversity (CMD), indicated by positive wells (carbon sources utilised) on BIOLOG Eco Plates™ (> 0.25 absorbance unit), in a 'Cripp's pink'/M7 apple orchard. Values within the same column followed by the same letter do not differ significantly at $p < 0.05$

Treatment	Community metabolic diversity (%)[1]			Mean
	2008 spring	2009 spring	2010 summer	
T1 (CON)	70.97^{e}	71.77^{de}	70.16^{e}	70.97^{c}
T2 (CON)	70.97^{e}	75.00^{de}	90.32^{ab}	78.76^{bc}
T3 (ORG)	75.81^{cde}	91.94^{a}	95.16^{a}	87.63^{ab}
T4 (ORG)	79.03^{cd}	91.94^{a}	96.77^{a}	89.25^{a}
T5 (ORG)	83.07^{bc}	90.32^{ab}	93.55^{a}	88.98^{ab}
Mean	75.97^{c}	84.19^{b}	89.19^{a}	

[1] Percentage utilisation of total carbon sources (n = 31)

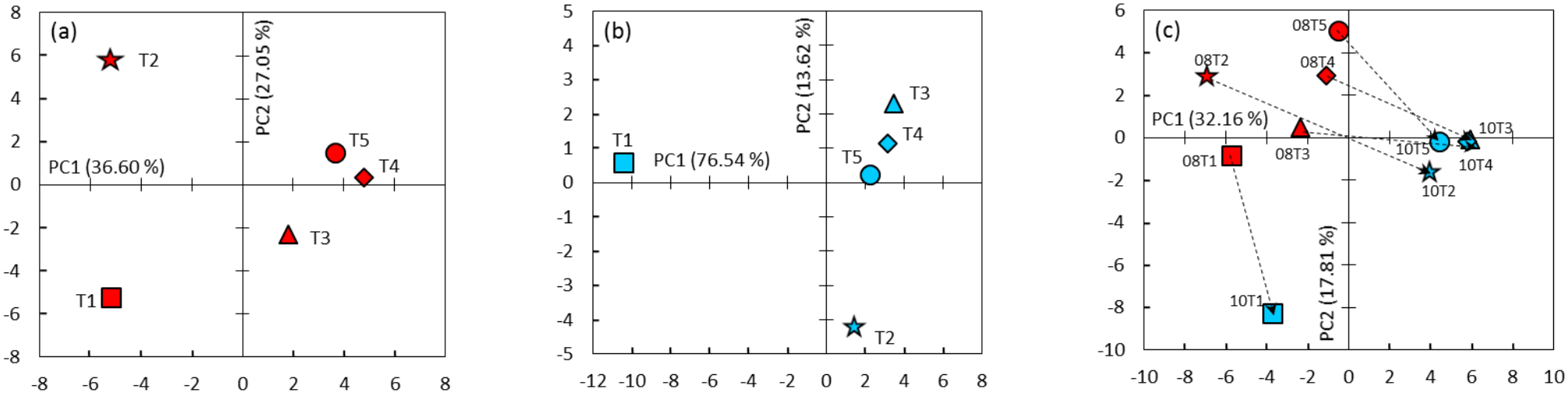

Figure 5.1. XY-ordination plot of conventional (T1 and T2) and organic (T3, T4 and T5) treatments in the space defined by the PC1 and PC2 axes of the principal component analysis (PCA) of carbon-substrate utilisation (BIOLOG™) data. (**a**) spring 2008, (**b**) summer 2010, (**c**) change in ordination (arrowed dashed lines) between spring 2008 and summer 2010. Symbols represent the mean of four replicates. Values in brackets indicate the percentage of total variation attributable to each principal component axis

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CHAPTER 6

Effect of conventional and organic orchard floor management practices on arbuscular mycorrhizal fungi in a 'Cripp's Pink'/M7 apple orchard soil

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Abstract

Arbuscular mycorrhizal (AM) fungi are key components of agricultural soil-plant systems which may be affected by agricultural practices. In organically managed (ORG) orchards, nutrients are supplied in the form of compost, and weeds are suppressed with mulches, whereas synthetic fertilisers and herbicides are used for these respective purposes in conventional (CON) orchards. The effects of ORG and CON orchard floor management practices on native AM fungi in apple orchards were investigated in a randomized field trial. AM root colonisation, spore abundance, infectivity potentials and soil glomalin contents were determined in the 0-30 cm soil depth interval, in tree rows, over consecutive seasons. Root colonisation was higher in the ORG than the CON treatments, but intermediate where straw mulch was substituted for green work-row covers. Glomalin levels were not affected by the treatments. Root colonisation by AM fungi increased with increasing soil pH, P, C, K, Zn, and Mn concentrations, but was suppressed by Cu. Colonisation correlated positively with leaf P, Ca and Mg, and with stem circumference, but negatively with leaf N and yield. ORG orchard floor management practices therefore promoted functional AM associations more effectively than CON practices.

Keywords: compost, glomalin, root colonisation, spore count, straw mulch

6.1. Introduction

Arbuscular mycorrhizal (AM) fungi are abundant in agricultural soils, and AM/plant functional symbioses are critical components of agro-ecosystems, notably those that are focussed on sustainable crop production (Schreiner and Bethlenfalvay 1995). Deciduous fruit trees are also considered to benefit from associations between their roots and AM fungi (Hoepfner et al. 1983). Nevertheless, as shown by Wooldridge (1999), the effects of AM fungi on growth parameters on juvenile apple rootstocks grown in a common growth medium may differ between combinations of AM fungal species and rootstock cultivar. This variability might be attributable to differences in AM fungi x rootstock specificity, but could also indicate that AM

fungal species differ in their abilities to utilize the elements and compounds that are present in the soil.

AM fungi propagate through resting-phase propagules (Sylvia and Jarstfer 1992), or by infection from colonised roots (Augé 2001). AM hyphae form bridges between root cortical cells and the soil, where they ramify through and exploit a greater volume of soil than non-mycorrhizal roots (Augé 2001).

AM/root relationships are generally mutualistic. The AM fungi supply the root with water (Augé 2001) and mineral nutrients, notably those (like P) that have narrow diffusion zones (Smith and Read 2008). They also improve host plant growth (Makarian et al. 2013) and disease resistance (Whipps 2004). In exchange, AM fungi obtain photosynthetically fixed carbon from the roots (Bago et al. 2003). High levels of root colonisation signify that the association is well adapted. In such cases both plant and AM fungi probably benefit from the relationship.

AM fungi contribute to soil aggregate stability (Schreiner and Bethlenfalvay 1995), mainly through the particle-binding effects of their underground hyphae which are facilitated by the large amounts of glomalin (a breakdown-resistant glycoproteinaceous substance) (Curaqueo et al. 2010) that are produced in the hyphae and spores, and which may accumulate in soils to concentrations of several mg g^{-1} of soil (Wright and Upadhyaya 1998). Despite its stability, glomalin is sensitive to differences between agricultural management practices (Wright and Anderson, 2000). Cultivation has particularly negative effects on glomalin levels in field soils (Avio et al. 2013).

In apple orchards, nutrient supply and weed control are critical aspects of orchard floor management. In organic (ORG) orchards, nutrients are supplied in the form of compost, and weeds are controlled using organic mulches, whereas synthetic fertilisers and herbicides are used for these respective purposes in conventional (CON) orchards. ORG practices reputedly increase the frequency of AM fungal sporulation (Vaidya et al. 2008) and of AM fungal colonisation of apple roots (Kotze 2012). Mulching, for instance, promotes root development (Yao et al. 2005; Yao et al. 2009; Kotze 2012), which may, in turn, increase the probability of root-to-root colonisation by AM fungi (Atkinson 1983), compared with CON practices.

According to Fontaine et al. (2003), energy availability in soil to microorganisms is often limited by the poor quality (degraded state) of the available organic carbon sources. In such cases supplying additional energy in the form of fresh compost or of a mulch consisting of even minimally decomposed organic material, as in the ORG treatments in the present trial, may stimulate microbiological activity through a priming effect. Germinating AM spores and extending AM haustoria are as likely to benefit, albeit indirectly, from this increased energy supply, and from the concomitant increase in nitrogen availability, as are decomposers. As

shown by Sheik et al. (2013), the effects of ORG treatments on AM spore abundance may nevertheless be inconsistent, which accords with the observation by Wooldridge (1999) that, in a common growth medium, the effects of AM fungi on apple rootstock performance differ between combinations of AM fungal species and rootstock cultivars.

Compared to ORG orchard floor management practices, CON practices, as shown by Gosling et al. (2006), may result in AM communities becoming impoverished and less infective (Douds et al. 1993), resulting in declining colonisation levels and increasing impairment of the symbiotic relationship (Helgason et al. 1998). For instance, under herbicided strips, where the tree rows are kept mainly weed-free and apple root density is usually low (Atkinson and White 1980), the probability of AM colonisation of apple roots through root-to-root colonisation (Atkinson and White 1980), is also low. An herbicide, like glyphosate, may also affect AM root colonisation directly (Druille et al. (2013). The effects of herbicides nevertheless range from positive to negative (Malty et al. 2006; Ronco et al. 2008). Apart from the practices applied in the tree rows, activities in the work rows, such as the sowing of cover crops, especially legumes, which are good hosts of AM fungi, could facilitate AM colonisation of apple roots through root-to-root colonisation (Atkinson and White 1980) between cover crop and apple tree roots at the interface between work and tree rows.

This article reports on the effects of CON and ORG orchard floor management practices on AM fungi, and on soil glomalin contents, in the rootzone of an apple orchard in the winter rainfall region of the Western Cape Province, South Africa.

6.2. Materials and methods

6.2.1. Trial parameters

This research was carried out on a gravelly clay loam soil at the ARC Overberg Research Farm, Elgin (34.1363°S; 19.0216°E; altitude 327 m) and formed part of a larger trial, aspects of which have been described by Wooldridge et al. (2013a, 2013b). Before planting, the soils received sufficient calcitic lime and single superphosphate (10.5% P) to increase the pH to 5.6 (1 M KCl), neutralize exchangeable acidity, adjust the exchangeable Na, K, Ca and Mg to, respectively, <1%, 3.5-4%, 70% and 10-12% of the sum of base cations (S-value) and the Bray II extractable soil P concentration to 25-30 mg kg^{-1}. These values were close to ideal for apple trees in the winter rainfall region of the Western Cape (Terblanche et al. 1980).

The trees were planted at intervals of 1 m (in-row) x by 4.0 m (between rows) in winter 2000. Each treatment was represented by an experimental unit (plot) consisting of nine free-standing ‘Cripps Pink’/M7 trees, and their adjacent work rows. Each plot was represented in four blocks. The plots and blocks were fully randomized. Buffer rows separated the

experimental tree rows. Plots were separated in the tree rows by one or more pollinator trees ('Hillieri'/Seedling). Irrigation (micro-sprinkler) was carried out at c. 7-day intervals, at c. 60% plant available water depletion.

Five orchard floor management treatments were compared. These were fully described by Wooldridge et al. (2013a), and are summarized in Table 6.1. Treatment applications commenced in spring 2003. Each treatment was replicated in three blocks, distributed in a randomized block design. Foliar sprays, pruning, thinning and other routine orchard procedures were carried out in accordance with normal industry practice.

6.2.2. Sampling and analysis

6.2.2.1. Mycorrhizal parameters

Composite soil samples were obtained from the 0-30 cm soil depth interval using a 10 cm wide spade. This depth interval was variably populated with mainly fine apple feeding roots and, in the work row, with roots from the cover crop. Sampling was carried out in spring (September/October) and summer (January/February) from 2006/07 to 2010/11. On each occasion, four subsamples were taken from alternate sides of the tree row, adjacent to every second tree, from the outer margin of the canopy drip zone between the tree row and work row. Superficial organic material was removed from the mineral soil surface before sampling. The excavated soil and roots were separated, and the soil subsamples were transferred to a single plastic bag. The root subsamples were placed in separate capped vials containing 50% ethanol. All tools were rinsed with 70% ethanol between samples. The soils were air-dried, passed through a 2-mm sieve to remove stone, and archived.

Easily extractable glomalin (EEG) was extracted from the air-dried soil samples by the method of Wright and Upadhyaya (1998), which involves a 30-min extraction with 20 mM citrate buffer at pH 7.0 (121°C), followed by centrifuging at 10 000 x *g*. EEG was determined in the supernatant by the Bradford dye-binding assay, using bovine serum albumin as standard. Each EEG assay was triplicated.

Determination of AM root colonisation was performed on 5.5 g root samples that were washed in tap water and cut into 10 mm long pieces. These root segments were cleared in 2.5 % (w/v) KOH solution in an autoclave (120 kPa, 121°C) for 6 minutes, and afterwards washed in deionized water several times before it was acidified in a 1% (v/v) HCl solution for 16 h. The root bits were then stained in a 0.05% solution of Aniline Blue [0.5 g Aniline Blue, 50 ml 1% (v/v) HCl, 700 ml glycerol and 250 ml deionized water] for 2-3 days at room temperature before they were destained in destain solution [50 ml 1 % (v/v) HCl, 700 ml

Table 6.1: Conventional (CON) and organic (ORG) orchard floor management treatments applied in a 'Cripp's Pink'/M7 apple trial orchard (Adapted from Wooldridge et al. 2013a)

Treatment	Work row		Tree row
	Tree dormant	Tree active	(Drip line to drip line)
T1 (CON)	Weed cover	Cover mown, natural die-back in summer	Chemical weed control[a] + inorganic N[b]
T2 (CON)	Soil tillage + cover crop[c]	Cover crop[c], chemical weed control in spring[a]	Chemical weed control[a] + inorganic N[b]
T3 (ORG)	Straw mulch[d]. Hand weeded	Straw mulch[d]. Hand weeded	(Compost + mulch)[e]. Hand weeded
T4 (ORG)	Soil tillage + cover crop[c]	Cover crop[c], mown, natural die-back in summer	(Compost + mulch)[e] + compost tea[f]. Hand weeded
T5 (ORG)	Weed cover	Cover mown, natural die-back in summer	(Compost + mulch)[e]. Hand weeded

[a]Roundup 360SC® applied at 5 L ha^{-1} (3%) in September and January

[b]Nitrogen as limestone ammonium nitrate (LAN) applied at full bloom, six weeks after full bloom and after harvest at rates of 9.2 g N, 5.2 g N and 18.0 g N $tree^{-1}$, respectively, in seasons 2003/04 to 2007/08. Applied after harvest only, at 18.0 g N $tree^{-1}$, in 2008/09 and 2009/10. Applied to the tree row in a continuous strip 1 m wide

[c]Rye (*Secale cereale* L. cv. Henog) and 'Pallinup' oats (*Avena sativa* L. cv. Pallinup) planted in tilled soil (0-10 cm) after leaf senescence in alternate seasons. Nitrogen (as LAN) applied to the cover crops and weed covers at 14 kg N ha^{-1} in June

[d]Wheat or oat straw (mulch) applied as a 5 cm layer in a continuous strip, c. 100 cm wide, along the tree row. Mulch applied at 3.2 kg straw $tree^{-1}$. Work rows covered with a 5 cm layer of straw mulch. Mulches replenished annually in October

[e]Compost applied at 20 kg per $tree^{-1}$ (128 g N, 3.7 g P, 6.3 g K $tree^{-1}$) in spring and autumn 2003/04, 10 kg $tree^{-1}$ in spring and autumn 2004/05 to 2006/07 and 10 kg $tree^{-1}$ in spring 2008 and 2009. Spread in a continuous strip, c. 1 m wide, along the tree row. Covered with a 5 cm layer (c. 3.2 kg per $tree^{-1}$) of wheat or oat straw (mulch). Mulch reapplied annually, in October

[f]Compost tea (aerated, aqueous, microbial digest) surface-applied around each tree at one L $tree^{-1}$ per 4-week interval from full bloom to leaf senescence. Principal components (mg ml^{-1}): P, 7.5; K, 190; Ca, 51; Mg, 34.6; Na, 163. Nitrogen: 0.06%

glycerol and 250 ml deionized water] overnight. Stained root segments (25, 10 mm long, 0.3-0.5 mm diameter) were mounted in Polyvinyl-Lacto-Glycerol (PVLG) on each of four microscope slides (76 x 26 mm) per sample.Mounted segments were covered with glass cover slips (50 x 24 mm), flattened by applying slight downward pressure, and examined under a compound microscope at 200x magnification (VANOX Olympus Research Microscope Model AHBS). A root segment was considered mycorrhizal (positively colonised) if the vertical eye-piece cross-hair, when moved to randomly selected positions, either cut a hypha, arbuscule or vesicle, or any combination of these characteristic structures of AM fungi, within a single field of view of the microscope. The extent of colonisation by AM fungi was calculated and expressed in percent (Brundrett *et al.*, 1994).

AM spores were separated from each soil sample by wet sieving and decantation, followed by sucrose gradient centrifugation (Brundrett et al. 1994). Spores were quantified microscopically. Infectivity (inoculum) potential was determined by the most probable number (MPN) method of Smith and Dickson (1997).

6.2.2.2. *Soil chemical parameters*

Routine orchard soil sampling was carried out in spring (September) from 2006 to 2010. Composite samples (three subsamples) were removed from each plot, by auger, from incremental depth intervals. The composite samples were analysed by an independent laboratory. Parameters determined in the air-dried soil (< 2.0 mm) fractions included pH (1.0 M potassium chloride), Bray II (0.03 M ammonium fluoride in 0.01 M hydrochloric acid) extractable P and K, and 0.2 M ammonium acetate exchangeable acidity, Na, K, Ca and Mg. Copper, Zn and Mn were extracted using 0.02 M Na-EDTA. The methods used were as prescribed by the Non-Affiliated Soil Analysis Work Committee (1990), except that an inductively-coupled plasma atomic-emission spectrometer (ICPAES) (Vista MPX) was used to quantify the mineral elements. Ammonium and nitrate N were determined in a KCl extract by Auto Analyser (Technicon Auto analyser III). Organic C contents were determined by dichromate oxidation using a correction factor for incomplete oxidation of 1.33 (Walkley and Black 1934).

6.2.2.3. Tree parameters

Apple fruit yields were determined each summer. Stem circumferences were measured 40 cm above ground level at the end of each growing season. Leaf sampling and analysis were performed as described by Wooldridge et al. (2013b).

6.2.3. Statistical analysis

The data were tested for normality (Shapiro and Wilk 1965) and were found to be acceptably normally distributed. They were then subjected to an analysis of variance (ANOVA) using the General Linear Model procedure of SAS 9.1.3 (SAS Institute Inc. 2008). Factors were treatment and sampling date. Student's *t* least significant difference (LSD) values were calculated to facilitate comparison between treatment means (Snedecor and Cochran 1980). Means that differed at the 5% probability level were considered to be significantly different. Pearson correlation coefficients (*r*) were calculated for relationships between soil parameters and the variables root colonisation (%) and EEG content. Correlation coefficients were also calculated for the relationships between plant parameters, root colonisation and EEG. In both cases *r* was calculated using the CORR procedure of SAS 9.1.3.

6.3. Results and discussion

6.3.1. Effects of year, season and treatment on AM parameters

Averaged over all treatments, spore counts in spring were lower in 2008 than in the 2006 and 2007 seasons (Table 6.2). In 2008, counts were higher in T1 than in T2, T4 and T5, whereas in 2006 and 2007 there were no differences in AM spore counts. Yearly variation in AM spore count in fruit plantations were also observed by De Oliveira and De Oliveira (2005) and by Khanam (2007). Although AM spore count in the present study was carried out only in spring, the findings by De Oliveira and De Oliveira (2005) suggest that AM spore count could also be influenced by differences between spring and summer.

Over the period spring 2006 to summer 2010 all-treatment average root colonisation by AM fungi was greater in summer than spring (Table 6.3). AM activity in steppe plant species varies with metabolic rate and nutrient demand, peaking during the active growth phase, early in the growing season, and again during the seed production stage (Su et al. 2011). If AM activity in irrigated apple orchards is also linked with metabolic rate and nutrient demand, then the higher apple root colonisation rates observed in summer, compared with spring (Table 6.3), was probably ascribable to the high mid to late summer energy demand associated with the maturation of the apple crop. Root colonisation in spring was greater in treatments T4 and T5, both of which received compost overlain by straw mulch in the tree rows, than in the uncomposted and unmulched CON treatments T1 and T2 (where the tree rows were kept mainly weed-free with herbicide), and in treatment T3 (in which straw mulch was substituted for green work-row covers).

Table 6.2: Effect of orchard floor management treatments on arbuscular mycorrhiza fungal spore counts (100 g^{-1} soil) in spring in rootzones of apple orchards, over three successive years. Values in the same column and data set, followed by the same letter, do not differ at $p < 0.05$

Treatment	2006	2007	2008
T1	60[a]	89[a]	59[a]
T2	120[a]	140[a]	21[b]
T3	89[a]	170[a]	31[ab]
T4	74[a]	94[a]	17[b]
T5	89[a]	71[a]	19[b]
Mean[a]	82[a]	113[a]	29[b]

[a] Overall mean

Table 6.3: Effect of orchard floor management treatments on percentage (%) arbuscular mycorrhizal root colonisation of apple trees. Combined data over four seasons. Values in the same column and data set, followed by the same letter, do not differ at $p < 0.05$

Treatment	Spring	Summer
T1	41.7[b]	54.7[bc]
T2	34.6[b]	49.9[c]
T3	42.9[b]	68.1[ab]
T4	66.6[a]	80.9[a]
T5	69.6[a]	83.5[a]
Mean[a]	50.8[b]	67.1[a]

[a] Overall mean

Root colonisation levels in summer followed a similar pattern to those in spring, except that in spring the root colonisation rate in T1, T2 and T3 did not differ, whereas in summer the root colonisation rate in T3 was significantly greater than in T2 and tended to be greater than in T1. Given that T3, T4 and T5 were all organic treatments, but that the root colonisation rate in T3 was significantly lower than in T4 and T5 in spring, and tended to be lower than T4 and T5 in summer, it is possible that the straw mulch in the work rows of T3 had an inhibiting effect on AM root colonisation, particularly in spring. Since straw has a high C:N ratio and the work rows in T3 received no compost, most of the N required for microbial decomposition of the straw must have been drawn from the soil, thereby reducing the availability of N to the tree roots, which may, in turn, have suppressed AM root colonisation, relative to T4 and T5, which did not have straw-mulched work rows. Allelopathic responses to products leaching from the straw mulch (Das Neves and Gaspar 1990) could also have contributed to the lower root colonisation in T3 compared to T4 and T5.

The basis for the overall differences in root colonisation levels between CON treatments T1 and T2, and ORG treatments T4 and T5, is likely to be twofold. The lower and higher AM

root colonisation levels in CON and ORG treatments, respectively, could be due to a negative reaction to synthetic fertiliser and/or herbicide usage in the CON treatments, or alternatively to contrasting positive responses to the mulch and/or compost applied to the ORG treatments.

The effects of herbicide glyphosate (active ingredient in Roundup®) on root colonisation by AM fungi reportedly range from positive to negative (Malty et al. 2006; Ronco et al. 2008). Negative effects of glyphosate, supportive of the findings in the present study, have been recently reported by Druille et al. (2013) and Zaller et al. (2014). Causes of negative effects may be direct, i.e. damage to the external hyphae and/or AM spores, or indirect, as where root exudate production is reduced by the herbicide-induced death of targeted host plants, as in T1 and T2 at Elgin, where the tree rows were kept mainly weed-free with glyphosate.

Under herbicided strips, apple root density is also usually low (Atkinson and White 1980), whereas roots appear to be more dense under mulch in the topsoil when compared to herbicide strips (Yao et al. 2009). Favorable soil conditions such as increased soil organic matter, greater cation exchange capacity, higher rates of soil microbial respiration, and disease-suppressive soil microflora apparently promote tree root growth under mulch (Yao et al. 2005). At Elgin, apple root numbers in the 0-30 cm soil depth interval averaged 245 in T1 and T2 (CON), compared to 516 in T4 (ORG) (Wooldridge et al. 2013b). That root colonisation by AM fungi appears greater at high than at low root densities due to an increased probability of root-to-root colonisation (Atkinson 1983) thus explains, in part, why spring and summer AM root colonisation levels were lower in the CON than in ORG treatments (Table 6.3). Derkowska et al. (2013) also found that organic mulches promoted mycorrhizal associations.

Since the tree rows of T1 and T2 were treated the same, the observed difference in 2008 AM spore count (Table 6.2) and, to a lesser extent, in percentage root colonisation levels (Table 6.3) between T1 and T2, were probably due to differences in the work row treatments. Work rows in T1 retained a living weed cover throughout summer whereas T2 retained a cover crop which was suppressed with herbicide in spring. According to Druille et al. (2013), host plant characteristics indirectly affect root colonisation. Thus, differences in genetic make-up could have influenced the mycorrhizal dependency of the different plants by affecting the amounts and quality of their root exudates (Yao et al. 2005), which are the chemical trigger for root colonisation by AM fungi (Druille et al. (2013). In turn, differences in triggering could have influenced root-to-root AM colonisation (Atkinson 1983), affecting the link between work row cover plant roots and apple tree roots (Atkinson and White 1980). Besides the differences in triggering, killing of cover crops with herbicide in T2 conceivably reduced cover crop root density and, by implication, the probability of root-to-root colonisation between the work row cover crop roots and apple tree roots in the tree row,

which may possibly have contributed to the lower levels of AM root colonisation in T2 compared to T1.

MPN was higher in T2 and T5 than in T1, T3 and T4 (Table 6.4). No reason was apparent for the wide differences in MPN between treatments. That MPN was lowest in T3 (straw-mulched work rows) perhaps reflected a response to allelopathic products from the decomposing straw that was applied in both the tree and work rows (Das Neves and Gaspar 1990). That MPN in T2 was significantly higher than in T1 is counter to the trend in AM spore count and root colonisation levels (Tables 6.2 and 6.3), both these being MPN variable factors. Reports on the relationship between AM spore count and root colonisation are nevertheless inconsistent (Moreira et al. 2006; Khakpour and Khara 2012). For instance, AM spore number and root colonisation correlated positively in the work of Khakpour and Khara (2012), were inversely related in the work of Moreira et al. (2006), whereas in the present study, AM spore count and root colonisation were not related (data not shown).

Table 6.4: Effect of orchard floor management treatments on infectivity potential (MPN) (most probable number of propagules 200 g^{-1} soil) in summer 2010. Values followed by the same letter do not differ at $p < 0.05$

Treatment	MPN
T1	135[b]
T2	390[a]
T3	10[b]
T4	45[b]
T5	395[a]

6.3.2. Effects of year, season and treatment on EEG

EEG levels did not differ between treatments (Table 6.5). That EEG levels in T1 and T5, both of which were untilled, did not exceed those in T2 and T4 (tilled) contrasted with the findings of Curaqueo et al. (2010) who found that glomalin levels were lower in tilled than untilled soils. Averaged over all treatments, the EEG counts were lower in summer, when root colonisation was high, than in spring (Table 6.5). The lower summer EEG levels in the orchard soils differed from the situation under grassland (Burrows 2014) where glomalin levels were higher in late summer than in spring. A likely reason for these different results is that whereas perennial grasslands remain active hosts for AM fungi throughout the year, thereby facilitating continuous hyphal growth and EEG production, the growth of weeds or cover crops during summer in non-irrigated work rows in Western Cape apple orchards is suppressed by herbicide and by drought. In the present trial, soil EEG content was not significantly correlated with spore count or colonisation (Table 6.6). Neither, in a survey of 22 ecosystems, was glomalin found to correlate with AM abundance, although the

relationship between glomalin and primary productivity was positive (Treseder and Turner 2007). That EEG and spore count were unrelated in the present trial supports the supposition that glomalin resists decomposition and accumulates as successive generations of AM fungi proliferate and decompose. Under grassland, however, glomalin levels correlated positively with AM spore density (Burrows 2014). By inference, tree roots that extend from herbicided or composted/mulched tree row soils into work rows soils under green covers will pass through soils in which the relationships between AM fungi, root colonisation and glomalin differ markedly. In apple orchards, tree row soils are more likely to affect overall tree performance than work row soils, due to the greater availability of nutrients and water, and lack of competition from weeds, in the former.

Table 6.5: Effect of orchard floor management treatments on easily extractable glomalin concentrations (mg EEG g^{-1} soil) in rootzones of apple orchard soils. Combined data over four seasons. Values in the same column and data set, followed by the same letter, do not differ at $p < 0.05$

Treatment	Spring	Summer
T1	1.709[a]	1.446[a]
T2	1.885[a]	1.489[a]
T3	1.819[a]	1.740[a]
T4	1.568[a]	1.414[a]
T5	1.683[a]	1.532[a]
Mean[a]	1.733[a]	1.524[b]

[a] Overall mean

6.3.3. Relationships between soil, EEG and AM parameters

Colonisation of the apple roots by AM fungi correlated positively with soil pH, P, Zn, Mn and C, but negatively with Cu (Table 6.6). These positive correlations conflict with the observation by Douds et al. (1993) that low infection potentials, and low incidences of AM fungi, often occur in high-input agricultural soils, although a number of interacting factors may be involved (Gosling et al. 2010). The negative relationship between Cu and root colonisation supports wider experience concerning the fingicidal properties of Cu, and its use in commercial fungicides (Morgan and Johnston 1991). Root colonisation was not significantly correlated with soil N levels in either the nitrate or ammonium form (Table 6.6). This finding was unexpected in view of the observation by Avio et al. (2013) that N affects AM fungal communities in Mediterranean soils.

Conflicting results concerning the effects of pH on AM fungi have been reported (Carrenho et al. 2007). AM root colonisation may increase at low pH (Ingrid et al. 2002) or, in

other cases, at high pH (Porter et al. 1987). Giovannetti et al. (2010) showed that AM species, and even isolates of the same species, may respond differently to pH. Where

Table 6.6: Relationships between soil parameters and percentage arbuscular mycorrhizal root colonisation of apple trees, and easily extractable glomalin (EEG) content of rootzone soils in spring. Combined data from five orchard floor management treatments over five years

Parameter	Root colonisation[a]		EEG[b]	
	r[c]	*p*	*r*	*p*
pH (KCl)	0.5139	0.0019	-0.0680	0.6766
P Bray II (mg kg^{-1})	0.4154	0.0146	-0.1845	0.2545
K Bray II (mg kg^{-1})	0.3201	0.0650	-0.2650	0.0940
Cu (mg kg^{-1})	-0.6013	0.0065	0.2494	0.2889
Zn (mg kg^{-1})	0.5308	0.0194	-0.2846	0.2240
Mn (mg kg^{-1})	0.5854	0.0085	-0.2842	0.2247
C (%)	0.5154	0.0002	0.0346	0.8321
NO_3^- (mg kg^{-1})	-0.0130	0.9453	-0.0346	0.8849
NH_4^+ (mg kg^{-1})	-0.1945	0.3030	0.3310	0.1541

[a] expressed as percentage (%) of root colonised

[b] expressed as mg EEG g^{-1} soil

[c] Pearson correlation coefficient

differences in AM parameters are induced by acidity or heavy metals, germination and germ tube formation are the most severely affected (Giovannetti et al. 2010, and references therein). In contrast to the positive correlation between pH and AM root colonisation shown in Table 6.6, Van Aarle et al. (2002) found that increasing soil pH suppressed colonisation of *Plantago lanceolata* roots by AM fungi. Carrenho et al. (2007) speculate that susceptibility to colonisation of roots is higher under acid soil conditions because the need for mycorrhization is greater due to the generally lower mineral nutrient availability and more restricted root function in acid than in neutral soils. According to Marschner (1995), mineral mobility, and, hence, uptake by AM fungi and infectivity, may be sensitive to pH. That root colonisation in the present study positively correlated with soil pH (Table 6.6) was probably a positive response by the AM fungi to the addition of compost, which had a pH (1 M KCl) of 7.5 and in which Ca was the dominant cation (0.84%) (Wooldridge et al. 2013a). Greater soil acidity in the CON, compared with the ORG treatments (Wooldridge et al. 2013a) may have been due, in part, to oxidation of the inorganic nitrogenous fertilisers (limestone ammonium nitrate) used in the CON treatments (Bünemann et al. 2006). Purin et al. (2006) also demonstrated that when mineral fertilisers were substituted by organic fertilisers, AM activity improved.

According to Carrenho et al. (2007), P and C have little effect on mycorrhizal development when considered as individual factors. However, when C is applied in

combination with lime and P, AM root colonisation was suppressed, apparently because microbial activity and nutrient availability increased. By inference, supplying P in the form of compost, which also supplies C, should have suppressed root colonisation at Elgin, whereas increased colonisation was actually observed (Table 6.3). The observed positive relationship between AM root colonisation and soil P, the levels of which ranged from adequate (average 24 mg Bray P kg^{-1}) in the CON to high (144 mg Bray P kg^{-1}) in the ORG treatments thus, conflicts with the supposition of Carrenho et al. (2007) that colonisation increases with plant demand.

The observation that mulched and composted treatments (T3, T4 and T5) promoted percentage AM root colonisation of apple trees (Table 6.3) accords with the positive correlation between soil C content and percentage AM root colonisation (Table 6.6). Similarly, Kotze (2012) reported that mulching increased the frequency of colonisation of apple roots by AM fungi compared to unmulched treatments.

Contrary to the relationship between AM fungi and certain soil parameters, EEG was not significantly correlated with any of the soil parameters tested in spring (Table 6.6), perhaps because soil EEG content in spring was weakly and negatively correlated with overall (spring and summer) AM root colonisation (r = -0.3910, p = 0.0167, n = 37). According to Treseder and Turner (2007), AM parameters such as hyphal length and root colonisation correlate poorly with glomalin, mainly because glomalin persists longer in soils than hyphae, leading to accumulation of the glomalin stock. EEG also did not correlate with soil C, even though C is a major component of glomalin (Rillig et al. 2003). The contribution made by glomalin to the total soil C content, as quantified by the dichromate oxidation method, therefore appears to be small.

6.3.4. Relationships between plant, EEG and AM parameters

AM root colonisation correlated positively with leaf P, Ca and Mg, and with increase in stem circumference, in summer (Table 6.7). Conversely, root colonisation correlated negatively with leaf N and yield. This negative correlation probably arose because, in apple, leaf N status and tree vigour act independently (Marsh et al. 1996), potentially leading to situations where leaf N levels may be abnormally low in high N soils due to the diluting effect on leaf N levels of excessively vigorous vegetative growth. Vigorous vegetative growth in the Elgin trial was also found to be associated with low yields (Wooldridge et al. 2013b).

Although soil EEG content was not significantly correlated with the soil parameters listed in Table 6.6, EEG concentrations increased with leaf N, and with fruit yield; but decreased with increasing leaf Ca, Mg and Na (Table 6.7). Under the prevailing trial conditions, soil EEG content therefore appears to have reflected tree rather than soil parameters.

Table 6.7: Relationships between tree parameters and percentage arbuscular mycorrhizal root colonisation of apple trees, and of easily extractable glomalin (EEG) content of rootzone soils in summer. Combined data from five orchard floor management treatments over five years

Parameter	Root colonisation[a]		EEG[b]	
	r[c]	*p*	*r**	*p*
Leaf N (%)	-0.6494	<0.0001	0.6348	<0.0001
Leaf P (%)	0.6076	0.0002	0.2198	0.2116
Leaf K (%)	0.3429	0.0508	0.2778	0.1061
Leaf Ca (%)	0.3702	0.0340	-0.7257	<0.0001
Leaf Mg (%)	0.4395	0.0105	-0.7738	<0.0001
Leaf Na (mg kg^{-1})	-0.1182	0.6406	-0.6095	0.0043
Leaf Zn (mg kg^{-1})	0.1104	0.6627	0.3133	0.1787
Circ. Increase (cm)	0.5534	0.0008	0.0624	0.7217
Yield (t ha^{-1})	-0.6051	0.0002	0.6912	<0.0001

[a] expressed as percentage (%) of root colonised

[b] expressed as mg EEG g^{-1} soil

[c] Pearson correlation coefficient

6.4. Conclusions

Compared to CON practices, the use of straw mulches and compost in accordance with ORG management principles promoted increased colonisation of apple roots with native AM fungi under the prevailing trial conditions. This increased colonisation was positively linked with soil pH, and with the availability of P, Zn, Mn and C. Although colonisation correlated positively with leaf P, Ca and Mg, and with increased in stem circumference, the extent to which these increases in leaf element levels, and in stem circumference were due to the mycorrhizal associations, rather than to normal root uptake, could not be determined. Further investigation is needed, preferably under more tightly controlled conditions than can be achieved in open-orchard field trials. Root colonisation by AM fungi seemed to have been suppressed where leaf N levels were high, but was unaffected by soil N. Soil EEG levels did not vary between ORG and CON treatments, and did not correlate with soil C or N levels. Copper, a component of commercial fungicides, suppressed root colonisation by AM fungi.

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CHAPTER 7

Factors affecting glomalin and arbuscular mycorrhizal (AM) fungi in apple orchards in the Western Cape of South Africa

Article in preparation for submission to Applied Soil Eclogy: André H. Meyer, John Wooldridge, Joanna F. Dames: Factors affecting glomalin and arbuscular mycorrhizhal (AM) fungi in apple orchards in the Western Cape of South Africa.

Abstract

Arbuscular mycorrhizal (AM) fungi are root-symbionts that facilitate uptake of mineral nutrients by their plant hosts. AM fungi produce glomalin, a glycoprotein, which is considered to improve soil structural stability. Though common in agroecosystems, the dynamics of AM fungi in South African apple orchard soils have not been well studied. This work reports on a 2-year survey of native AM fungal root colonisation and soil glomalin content in 30 apple orchards. These orchards contained trees that were combinations of two scion cultivars ('Golden Delicious', 'Cripps Pink') and two rootstocks (M7, M793). The survey extended across nine apple production areas and included organic (ORG) and conventionally (CON) managed orchards. Correlation of AM root colonisation and of the easily extractable component of soil glomalin (EEG) in summer, with the soil parameters: P, K, NH_4^+-N and (total) N, were not significant ($p < 0.05$). Spring EEG correlated with soil organic carbon (C) content over two years. Spore count correlated moderately strongle with soil NH_4^+-N content. AM colonisation was greater under CON management in 2009 than under ORG management in 2010. Averaged over two years, soil EEG content was higher in soils under 'Golden Delicious'/M7, than under 'Golden Delicious'/M793 trees. EEG levels were higher in summer than in spring, and were higher in Somerset West than in the Vyeboom area. AM colonisation in the Ceres Warm Bokkeveld area exceeded those in the Grabouw, Misgund and Villiersdorp areas.

Keywords: apple orchard, arbuscular mycorrhiza, easily extractable glomalin, root colonisation, spore count

7.1. Introduction

Colonisation of the land by green plants was facilitated by the establishment of symbiotic relationships with fungi (Delaux el al. 2015). Fossil evidence of fungal structures in early land plants was presented by Humphreys et al. (2010). Trees and decomposers are connected via mycorrhizal fungi, implying a role in the prediction of carbon (C) cycling in the context of climate change related shifts in plant communities (Averill et al. 2014). Also according to Averill et al. (2014), plant communities containing both ecto- and endo-mycorrhizal

communities (EEM) contain about 70% more C per unit nitrogen (N) than arbuscular mycorrhiza (AM)-dominated communities because EEM fungi produce enzymes that provide access to forms of organic N that at not available to AM fungi.

AM fungi are obligate root symbionts in over 80% of terrestrial plants (Smith and Read 1997; Averill et al. 2014). AM fungi propagate through resting-phase propagules (spores) (Sylvia and Jarstfer 1992), or by infection from colonised roots (Augé 2001). In petunia, the root hormone strigolactone, in association with the transporter protein PhPDR1 has been shown to attract AM fungal hyphae, encouraging mycorrhization under arid or waterlogged conditions where mycorrhization is typically delayed (Kretzschmar et al. 2012). According to Plett et al. (2014) colonisation follows injection by the fungus of effectors, notably MiSSP7, which immobilize the plant's immune response system. Conversely, Jayaraman et al. (2014), suggest that fungi and bacteria mechanically stimulate the host cell into a receptive state before penetrating and establishing the symbiotic relationship. AM hyphal networks form links between root cortical cells and the soil through which they ramify, facilitating exploitation of greater soil volumes than are accessible to non-mycorrhizal roots (Augé 2001). AM fungi are important in sustainable agriculture because of their ability to facilitate the transfer of mineral nutrients from the bulk soil material to plant roots. They also transfer C from plant roots to the soil (Miller and Jastrow 2000; Zhu and Miller 2004; Smith and Read 2008; Averill et al. 2014). This C occurs in forms that stabilise soil structures, thereby contributing to the sustainability of the soil ecosystem (Write and Upadhyaya 1998; Miller and Jastrow 2000; Carrizo et al. 2015). Soil aggregate stability is improved by the glycoproteineous substance glomalin. This water-stable product, which has biochemical binding properties, is released by decomposing AM fungal hyphae and spore walls (Driver et al. 2005; Purin and Rillig 2007; Fokom et al. 2012). Glomalin, which is not produced by non-AM soil fungi (Wright et al. 1996), is composed of c. 37% C and 3-5% N. Glomalin contributes 3% to the storage of soil C and 5% to the store of N, and represents a potential source of both C and N (Purin and Rillig 2007; Fokom et al. 2012). By inference, glomalin could potentially be used as an indicator of relative soil quality or health (Gugino et al. 2009)

Traditionally, soil glomalin content has been indirectly analysed by the Bradford protein assay (Wright and Upadhyaya 1998) and has been renamed as glomalin-related soil protein (GRSP) (Rillig 2004) as it is suspected to have other proteins, and not only glomalin, in its composition. Nevertheless, glomalin and GRSP, are often used as synonymous terms. For the purposes of the present article glomalin is used as a proxy for GRSP. The Bradford protein assay utilises extraction protocols which enable two fractions of soil glomalin to be quantified, though in relative terms. Easily extractable glomalin (EEG) represents glomalin that has been freshly released into the soil as opposed to glomalin in the recalcitrant, slowly decomposable state. These fractions have not yet been fully described, and are

operationally defined on the basis of their ease of extractability. The recent discovery by Gillespie et al. (2011) that GRSP may also have a non-AM origin, could potentially change the way how GRSP has been interpreted in soil research. Association with clay-fraction minerals stabilises both glomalin and C (Lobe et al. 2001). Glomalin fractions in soil aggregates nevertheless appear to be better protected from breakdown by chemicals and soil organisms, allowing them to remain in soils for decades (Rillig et al. 2001; Steinberg and Rillig 2003; Zhu and Miller 2004), and to accumulate to concentrations of several mg g^{-1} of soil (Wright and Upadhyaya 1996; Rillig et al. 2001). By contrast, C in bulk soil is more susceptible to microbial breakdown. As a result, glomalin (*sensu lato*) concentrations decline at lower rates than other forms of soil organic matter following changes in land use (Preger et al. 2007). Glomalin may thus sequester relatively large amounts of C in soil (Treseder and Alen 2000; Quiquampoix and Burns 2007). Glomalin fractions correlate with C under a variety of soil conditions (Wright and Upadhyaya 1998; Nichols and Wright 2005; Peng et al. 2015).

Soil type, cultivation practices, water (irrigation) (Wright and Upadhyaya 1998; Wright and Anderson 2000) altitude (Peng et al. 2015), land use change (Rillig et al. 2003), tillage practices (Wright et al. 1999), and crop rotation systems (Wright and Anderson 2000), may affect soil glomalin content. Seasonal changes in glomalin have also been demonstrated (Lutgen et al. 2003; Sumathi at el. 2008; Emran et al. 2012; Wu et al. 2013; Burrows 2014). According to Emran et al. (2012), seasonal effects on glomalin are greatest in the fraction that is of most recent origin, i.e. EEG, appearing to be more abundant during seasons of driest and warmest soil conditions.

Evidently, glomalin and AM fungi are potentially important with regard to the sustainability of agricultural soils, and merit further investigation; notably with regard to apple trees, the roots of which are known to form associations with AM fungi (Reich and Barnard 1984; Miller et al. 1985). Apple orchards from the Western Cape in South Africa, given the wide diversity of soils and variety of cultivars grown, could potentially benefit form such an investigation. However, information about the spatial distribution of glomalin and AM fungi in apple orchards from the Western Cape, the production areas of which varying in local edaphic and climatic conditions, are relatively unknown. Growth responses to AM fungi by juvenile apple trees (pot-grown) were nevertheless found to be either positive or negative, depending on the rootstock (Wooldridge 1999a, 1999b). Furthermore, conventional (CON) soil management that permits the use of agrochemicals is currently the most common form of management in Western Cape apple orchards; few farms are managed in accordance with organic (ORG) protocols. Therefore, the case for ORG production would probably be strengthened if evidence were found that organic production leads to improvements in soil microbiology.

The present study aimed to compare cultivation practices (CON vs ORG), production areas, rootstocks, scions and rootstock x scion combinations, with regard to their effects on EEG stocks, AM root colonisaton levels and spore density, and to determine whether environmental variation within and between apple growing seasons affect these respective parameters.

7.2. Materials and methods

7.2.1.Trial parameters

The survey, which was carried out over two southern hemisphere apple production seasons (2009/10 and 2010/11), included orchards containing mature (nine to 18 years in 2009) trees representing four scion x rootstock combinations: 'Cripp's Pink'/M7 (n = 6), 'Cripp's Pink'/M793 (n = 9), Golden Delicious/M7 (n = 6) and Golden Delicious/M793 (n = 9) (Table 7.1). Of the total of 30 qualifying orchards, 26 were subjected to CON (use of synthetic herbicides and fertilisers permitted), and four ORG (nutrients supplied as compost and weeds in tree rows suppressed with compost and straw mulch) management practices (Meyer et al. 2015). The CON orchards were selected based on similar orchard floor management practices applied, as was the ORG orchards. The smaller number of ORG, compared with CON orchards, was indicative of the skew in the industry toward conventional production. Typically, CON orchards underwent soil preparation (cross ripping to c. 60 cm to incorporate surface-applied lime, P and K) before planting in keeping with Bray II extractable soil P and K levels in mature apple orchards of around 30 and 120 mg kg^{-1}, respectively (Kotzé, 2001). These apple orchards lie in the valleys of the Cape Fold Mountain Belt where the soil materials are mainly derived from sandstones and shales, and are variably mixed during colluvial and alluvial transport and by depositional processes. The orchards were distributed across nine of the apple growing areas of the south western Cape, extending from the Koue Bokkeveld in the north west of the Western Cape Province to Joubertina in the Eastern Cape Province.

7.2.2. Sampling procedures

Sampling was carried out in summer (January/February) 2009, spring (September/October) 2009, summer 2010 and spring 2010. On each occasion, organic material was removed from the mineral soil surface before sampling. For each of the CON orchards, 20 soil samples were taken randomly from the 0-30 cm soil depth interval in the tree rows using a small (10 cm wide) spade, rinsed with 70% ethyl alcohol between samples, and treated with Sporekill™. Randomly selected pairs of these soil samples were combined and mixed to

Table 7.1: Apple orchards included in a survey of native arbuscular mycorrhizal (AM) fungi

Production area	Farm/orchard	Cultivation practice	Scion x rootstock	Plant year
Ceres Koue Bokkeveld				
(32°15' S; 19°18' E, alt. 850-1050 m)	Lindeshof[a]	CON	'Cripp's Pink'/M793	1997
	Lindeshof[b]	CON	'Cripp's Pink'/M793	1997
	Lindeshof[c]	CON	Golden Delicious/M793	1998
	Lindeshof[d]	CON	Golden Delicious/M793	1997
	Wakkerstroom	CON	Golden Delicious/M7	1999
Ceres Warm Bokkeveld				
(33°21' S; 19°24' E, alt. 500-600 m)	Laastedrif Boerdery[a]	CON	'Cripp's Pink'/M7	1998
	Laastedrif Boerdery[b]	CON	Golden Delicious/M7	2000
	Laastedrif Boerdery[c]	CON	Golden Delicious/M7	2000
	Laastedrif Boerdery[d]	CON	Golden Delicious/M7	1998
Grabouw				
(34°08' S; 19°02' E', alt. 300-400 m)	Elgin Experiment Farm	CON	'Cripp's Pink'/M7	2000
	Lorraine Farm Trust[a]	ORG	Golden Delicious/M793	1993
	Lorraine Farm Trust[b]	ORG	Golden Delicious/M793	1991
	Monteith[a]	CON	Golden Delicious/M7	1998
	Monteith[b]	CON	Golden Delicious/M7	1998
	Monteith[c]	CON	'Cripp's Pink'/M793	1998
	Monteith[d]	CON	'Cripp's Pink'/M793	1998
	Monteith[e]	CON	Golden Delicious/M793	1998
	Monteith[f]	CON	Golden Delicious/M793	1998
Joubertina				
(33°49' S; 23°52' E, alt. 500-550 m)	South Cape Produce[a]	CON	'Cripp's Pink'/M7	1999
	South Cape Produce[b]	CON	'Cripp's Pink'/M7	2000
Misgund				
(33°46' S; 23°31' E, alt. 750-800 m)	Fairview Landgoed[a]	ORG	'Cripp's Pink'/M793	*
	Fairview Landgoed[b]	ORG	Golden Delicious/M793	*
Piketberg				
(32°47' S; 18°14' E, alt. 500-750 m)	Stawelklip Estates[a]	CON	'Cripp's Pink'/M793	2000
	Stawelklip Estates[b]	CON	'Cripp's Pink'/M793	2000
	Stawelklip Estates[c]	CON	Golden Delicious/M793	2000
Somerset West				
(43°04' S; 18°54' E, alt. 80-130 m)	Lourensford	CON	'Cripp's Pink'/M7	2000
Villiersdorp				
(33°59' S; 19°17' E, alt. 300-400 m)	Riverside Farm[a]	CON	'Cripp's Pink'/M7	1999
	Riverside Farm[b]	CON	'Cripp's Pink'/M793	2000
Vyeboom	Carica Estates	CON	'Cripp's Pink'/M793	2000
(34°04' S; 19°07' E, alt. 300-400 m)	Graymead farm	CON	Golden Delicious/M793	*

CON = Conventional, ORG = Organic, [(a, b, c, d, e, f)] Different letters signify different orchards on the same farm
*Plant year unconfirmed

create 10 composite samples from each CON orchard. Since the diversity of green cover plants in the tree rows of the ORG orchards was considerable, the number of soil samples taken from the ORG orchards was increased to 40, of which four randomly selected soil samples were pooled to create 10 composite samples per ORG orchard. The composite soil samples were conveyed to an independent laboratory, air dried, passed through a 2 mm sieve to remove stone and archived. Apple root samples (young roots, <1 mm in diameter) were randomly collected from each of the trees from around which the soil samples had been taken. The root samples were obtained from the 0-30 cm soil depth interval, within 30 cm of the tree trunk, in the tree row midline. In total, 20 root samples, one from each tree, were obtained from the CON, and 40 from each ORG orchard. Root samples from the same tree were placed in a capped vial containing preservative (50% ethyl alcohol) pending AM root colonisation analysis.

7.2.3. Determination of soil physical and chemical parameters

Sub samples were obtained from each of the archived soils using a sample splitter. Particle size distribution was determined by the hydrometer method (Day 1956; Van der watt 1966). Chemical parameters determined included pH (1.0 M potassium chloride), Bray II (0.03 M ammonium fluoride in 0.01 M hydrochloric acid) extractable phosphorus (P) and potassium (K). Standard methods prescribed by the Non-Affiliated Soil Analysis Work Committee (1990) were used throughout, except that an inductively coupled plasma atomic-emission spectrometer (Vista MPX) was used to quantify the mineral elements. Ammonium (NH_4^+) and nitrate (NO_3^-) N were measured in a potassium chloride extract by auto analyser (Technicon Auto analyser III). Total N was determined by combustion in a Total Nitrogen Analyser and expressed as % N. Organic C content (expressed a % C) was determined by the dichromate oxidation method of Walkley-Black using a correction factor for incomplete oxidation of 1.33 (Walkley and Black 1934).

7.2.4. Mycorrhizal parameters

7.2.4.1. EEG analysis

EEG was quantified by the method of Wright and Upadhyaya (1998) in triplicated samples of each soil. In each case 1.00 g of the dry-sieved <2 mm soil fractions were autoclaved with 8 ml of sodium citrate buffer extractant (20 mM; pH 7.0), at 121 ºC for 30 min. After autoclaving, samples were centrifuged (10 000 x *g* for 5 min) to remove soil particles, and the quantity of the protein (glomalin) in the supernatant was determined by the Bradford dye-binding assay using bovine serum albumin (BSA) as standard (Wright et al. 1996). Extracts from each replicate were pooled and then analysed.

7.2.4.2. AM root colonisation analysis

Determination of AM root colonisation was performed on 5.5 g samples of the young, thin (<1 mm in diameter) roots of each tree. The roots were washed in tap water and cut into 10 mm long segments. These segments were cleared in 2.5% (w/v) KOH solution in an autoclave (120 kPa, 121 ºC) for 6 min, washed in deionized water several times then acidified in a 1% (v/v) HCl solution for 16 h. The root segments were stained in a 0.05% solution of Aniline Blue [0.5 g Aniline Blue, 50 ml 1% (v/v) HCl, 700 ml glycerol and 250 ml deionized water] for 2–3 days at room temperature before being destained in destain solution [50 ml 1% (v/v) HCl, 700 ml glycerol and 250 ml deionized water] overnight. Stained root segments (25 mm to 10 mm long, 0.3–0.5 mm diameter) were mounted in Polyvinyl-lacto-glycerol (PVLC) on each of four microscope slides (76 x 26 mm), accommodating 100 root segments from each tree sample. Mounted segments were covered with glass cover slips (50 x 24 mm), flattened by applying slight downward pressure, and examined under a compound microscope at 200 x magnification (VANOX Olympus Research Microscope Model AHBS). A root segment was considered mycorrhizal (positively colonised) if the eye-piece cross-hair, when moved to randomly selected positions, either cut a hypha, arbuscule or vesicle, or any combination of these characteristic structures of AM fungi, within a single field of view. The extent of colonisation by AM fungi was calculated and expressed in percent (Brundrett et al. 1994).

7.2.4.3. Spore extraction and enumeration

Spores of AM fungi were extracted from the composite soil samples by wet sieving and decanting and by sucrose centrifugation as described by Brundrett et al. (1994). One hundred grams of soil was suspended in 1000 ml tap water. The mixture was vigorously shaken for 30 seconds to free the spores from the soil and roots, and the coarse particles were allowed to settle for 1-2 minutes. The soil water suspension was decanted through superposed analytical sieves arranged in descending order of mesh pore size (500, 250, 100 and 38 µm). The decanting and sieving procedure was repeated twice with the settled soil to allow the majority of spores to be extracted. The content of the top sieve was examined for sporocarps >500 µm in diameter. The sievings (containing the spores and minimal amount of organic particles) retained on the rest of the sieves, were transferred to 100 ml centrifuge tubes with a fine stream of water from a wash bottle, followed by centrifugation at 2000 rpm for 5 min. Floating organic debris was discarded with the supernatant; debris adhered to the side of the tube was carefully removed, avoiding disturbance of the pellet.

The pellet in each tube was re-suspended in chilled 50% (w/v) sucrose solution by vigorously shaking the tightly stoppered tubes, and centrifuged at 2000 rpm to separate

spores (and any remaining organic debris) from denser soil components, and applying the brake to stop the centrifuge after 1 min. Immediately after centrifugation, the sucrose supernatant was carefully poured onto the finest mesh sieve (38 µm) and carefully rinsed with tap water until the sucrose was completely removed. The material caught on the sieve was washed into a specimen jar with deionized water, made up to 50 ml volume, thoroughly mixed by hand, and the spore suspension was vacuum filtered through a pre-wetted 0.45 µm gridded Millipore® membrane filter using a Millipore® glass funnel filter system. Spores were examined and counted under a stereo-microscope at 40x magnification (Nikon SMZ1500 stereoscopic zoom microscope). Spore density was expressed as the number of spores per 100 g of dry soil.

7.2.5. Statistical analysis

The data were tested for normality (Shapiro and Wilk 1965), found to be acceptably normally distributed and subjected, untransformed, to an analysis of variance (ANOVA) using the General Linear Model procedure of SAS (Statistical Analysis System) 9.1.3 (SAS Institute Inc. 2008). Factors were cultivation practice, scion, rootstock, scion x rootstock combination, production area, season (spring, summer) and year of sampling. Student's *t* least significant difference (LSD) values were calculated at the 5% probability (*p*) level to facilitate comparison between treatment means (Snedecor and Cochran 1980). Pearson correlation coefficients (*r*) were calculated for relationships between soil and plant parameters using the CORR procedure of SAS 9.1.3. Means that differed at $p < 0.05$ were considered significantly different.

7.3. Results

7.3.1. Soil physical parameters

Soil textures differed widely both within and between areas (Table 7.2). This variability is typical of the apple growing areas of the Western Cape.

7.3.2. Soil chemical parameters

Few new orchard soils were particularly acid, relative to a norm of pH 5.5 (1 M KCl) (Kotzé, 2001) (Table 7.3). Variation in P and K levels, as in Table 7.3, is a reflection of how recently maintenance topdressing was carried out. Levels of N and C in the soils were consistently above those encountered in unprepared veld soils. These values were consistent with soil conditions in commercial apple orchards of the Western Cape.

Table 7.2: Soil physical characteristics of apple orchards sampled in a survey of native arbuscular mycorrhizal (AM) fungi

Farm/orchard	Clay	Silt	Fine sand	Medim sand	Coarse sand
		%			
Carica Estates	13.7	25.7	40.4	2.6	17.6
Elgin Experiment Farm	5.4	16.0	50.2	15.4	13.0
Fairview Landgoed[a]	1.0	6.4	49.2	35.2	8.2
Fairview Landgoed[b]	3.0	9.0	52.3	25.2	10.5
Graymead farm	5.4	18.0	64.6	6.5	5.5
Laastedrif Boerdery[a]	5.2	10.2	67.4	10.6	6.6
Laastedrif Boerdery[b]	3.0	1.0	88.0	4.0	4.0
Laastedrif Boerdery[c]	2.0	5.0	51.1	28.8	13.1
Laastedrif Boerdery[d]	6.0	8.0	75.3	7.7	3.0
Lindeshof[a]	3.0	2.0	31.4	20.4	43.2
Lindeshof[b]	3.4	11.0	69.0	8.3	8.0
Lindeshof[c]	3.2	2.2	32.2	23.0	39.4
Lindeshof[d]	3.4	13.0	68.7	6.9	8.0
Lorraine Farm Trust[a]	2.0	3.0	77.2	2.6	15.2
Lorraine Farm Trust[b]	2.4	19.0	45.8	8.0	24.8
Lourensford	3.0	2.0	81.3	11.1	2.6
Monteith[a]	3.0	2.0	47.0	27.4	20.6
Monteith[b]	15.4	27.0	37.9	3.7	16.0
Monteith[c]	3.0	1.0	48.4	23.2	24.4
Monteith[d]	13.4	26.0	39.0	4.2	17.4
Monteith[e]	3.0	3.0	40.7	32.4	20.8
Monteith[f]	10.4	29.0	45.8	6.0	8.8
Riverside Farm[a]	3.0	5.0	58.1	24.9	9.0
Riverside Farm[b]	3.0	6.0	59.6	24.0	7.4
South Cape Produce[a]	3.0	8.0	81.0	6.0	2.0
South Cape Produce[b]	3.2	1.8	87.1	4.3	3.6
Stawelklip Estates[a]	3.0	1.0	39.5	42.5	14.0
Stawelklip Estates[b]	3.0	2.0	35.8	45.2	14.0
Stawelklip Estates[c]	2.6	1.6	61.6	24.5	9.7
Wakkerstroom	3.7	14.7	67.3	3.4	10.9

[(a, b, c, d, e, f)] Different letters signify different orchards on the same farm

7.3.3. EEG

Over all treatments, average EEG levels did not differ in either 2009 or 2010 (Table 7.4), but were appreciably (207%) higher in summer than spring. Spring EEG levels did not differ significantly between production areas but, in summer, more EEG was present in the soils from Piketberg and Somerset West than from Vyeboom. Averaged over both spring and summer, only Somerset West and Vyeboom differed. Average EEG levels were slightly (10.3%) higher under CON than ORG management. This difference was also non-significant. Scion cultivar did not affect EEG. Summer EEG levels were significantly higher (21.5%) under trees on M7 than on M793 rootstocks. Averaged over year and season, soil

EEG levels were higher (49.2%) under 'Golden Delicious'/M7 than under 'Golden Delicious'/M793 trees. This difference was apparent in summer, but not spring.

Table 7.3: Soil chemical characteristics of apple orchards sampled in a survey of native arbuscular mycorrhizal (AM) fungi

Farm/orchard	pH	P Bray II	K	NO_3^--N	NH_4^+-N	N	C
	(KCl)	mg kg^{-1}				%	%
Carica Estates	4.7	21	236	31.28	12.48	0.115	2.66
Elgin Experiment Farm	4.3	37	85	13.54	11.88	0.152	2.26
Fairview Landgoed[a]	4.5	75	98	78.02	11.88	0.345	1.69
Fairview Landgoed[b]	5.1	127	106	7.74	9.79	0.248	1.29
Graymead farm	4.9	31	107	19.18	11.68	0.173	1.92
Laastedrif Boerdery[a]	6.2	56	87	92.16	12.02	0.198	1.96
Laastedrif Boerdery[b]	5.3	70	76	55.30	10.28	0.078	0.69
Laastedrif Boerdery[c]	*	*	*	*	*	*	*
Laastedrif Boerdery[d]	6.2	126	177	6.72	12.25	0.256	2.53
Lindeshof[a]	5.7	61	96	28.68	12.93	0.127	1.71
Lindeshof[b]	5.9	49	138	19.28	18.54	0.111	1.72
Lindeshof[c]	5.8	66	145	103.35	11.76	0.115	1.43
Lindeshof[d]	5.4	117	259	13.58	10.84	0.176	2.38
Lorraine Farm Trust[a]	6.1	77	140	34.86	11.70	0.093	1.80
Lorraine Farm Trust[b]	6.0	302	377	33.79	18.12	0.303	4.62
Lourensford	4.6	59	79	15.52	9.86	0.090	1.61
Monteith[a]	5.8	295	99	36.19	9.14	0.374	1.40
Monteith[b]	6.4	26	142	19.01	8.39	0.397	2.25
Monteith[c]	6.2	63	70	12.84	11.64	0.210	0.94
Monteith[d]	5.6	20	142	39.25	12.78	0.376	2.47
Monteith[e]	5.5	167	60	16.98	8.05	0.111	0.95
Monteith[f]	6.3	61	225	31.94	8.89	0.446	2.54
Riverside Farm[a]	5.2	97	72	8.18	7.92	0.325	1.03
Riverside Farm[b]	5.4	52	109	7.92	9.58	0.225	1.00
South Cape Produce[a]	4.7	191	264	129.31	9.84	0.342	2.13
South Cape Produce[b]	4.6	140	373	167.87	13.66	0.512	2.65
Stawelklip Estates[a]	5.1	166	73	26.97	10.68	0.105	1.28
Stawelklip Estates[b]	4.7	134	67	29.10	10.12	0.122	1.40
Stawelklip Estates[c]	4.2	69	75	15.64	9.56	0.135	0.51
Wakkerstroom	5.2	155	114	43.64	10.39	0.085	2.59

[(a, b, c, d, e, f)] Different letters signify different orchards. *Orchard removed

7.3.4. AM root colonisation

Neither rootstock, scion, nor rootstock x scion combination had significant effects on AM root colonisation (Table 7.5). Colonisation was significantly higher (21.3%) in CON orchards in 2009 than in ORG orchards in 2010. Averaged over both years colonisation levels were 12.0% higher in the CON than the ORG orchards. This latter difference was not significant. Colonisation did not differ between production areas in 2009, but in 2010 was greater in the Ceres Warm Bokkeveld than the Grabouw, Joubertina, Misgund, Villiersdorp and Vyeboom areas. Across both years and seasons, colonisation levels at Ceres Warm Bokkeveld

exceeded those at Grabouw, Misgund and Villiersdorp. Over all treatments, the AM colonisation levels did not differ between 2009 and 2010.

7.3.5. Relationships between EEG, soil C and N, and silt+clay and coarse sand fractions

No significant correlations were established between EEG and the soil textural parameters, soil C or soil N (Table 7.6). However, the silt+clay fraction correlated positively and reasonably strongly with both C and N, whereas soil N correlated with soil C.

7.3.6. Relationship between AM parameters and selected soil factors

No significant correlations were observed between root AM colonisation in spring or summer (2009, 2010) and the soil factors pH, P, K, NO_3^--N, NH_4^+-N, N or C (Table 7.7). Soil EEG levels correlated positively with soil K, N and C, but negatively with NH_4^+-N in spring 2009. In 2010, spring EEG content correlated only with soil P and C. The only correlation that was consistently significant during both spring 2009 and spring 2010 was that between soil EEG and soil C. These correlations were only moderately strong. A single soil factor (NH_4^+-N) correlated with AM spore count in spring 2009.

7.4. Discussion

The observation that average summer EEG exceeded that in spring (Table 7.4) agreed with work by Lutgen et al. (2003), Sumathi at al. (2008), Emran et al. (2012), Wu et al. (2013) and Burrows (2014). This difference may have been due, in part, to the longer period available for root activity and exudate production between the onset of root growth in early spring and the summer sampling date, compared with the period between root growth onset (usually early September) and the spring sampling date. Warmer soil temperatures in early summer, compared to those in spring, may also have contributed by accelerating the rate of biological, including AM fungal activity, prior to and around the summer sampling date. To this effect Heinemeyer and Fitter (2004) showed that percentage root colonisation by AM fungi, and length of extra-radical mycelium in *Plantago lanceolata* correlated positively with temperature. In apple orchards high soil EEG levels in summer may confer benefits to the trees, as well as to the broad soil microbial population, by conserving water and slowing the onset of desiccation during the later, drying, stages of each irrigation cycle (Emran et al. 2012) as in the hydrophobicity trials of Feeney et al. (2004). That different AM species could have been present during the change in seasons (summer vs spring) and contributed to the difference in measured EEG, is also possible. To this effect, Su et al. (2011) showed that season had a notable effect on AM species richness and diversity in steppe plant species.

Thus, perhaps more insight on AM fungal dynamics would have been gained had fall and winter also been included in the present study.

In view of the wide geographical spread of the sample sites (Table 7.1), and of the wide diversity of soil textures (Table 7.2) and chemical characteristics encountered (Table 7.3), it is surprising that EEG levels varied only minimally between areas.

Table 7.4: EEG[1] contents of apple orchards included in a survey of native arbuscular mycorrhizal (AM) fungi

Variable	Year		Season		Mean[4]
	2009[2]	2010[2]	Summer[3]	Spring[3]	
Production area					
Ceres Koue bokkeveld	2.504 [a5]	1.510 [a]	3.352 [ab]	0.993 [de]	2.173 [ab]
Ceres Warm bokkeveld	2.259 [a]	1.993 [a]	3.248 [ab]	0.873 [e]	2.180 [ab]
Grabouw	2.092 [a]	1.729 [a]	2.968 [abc]	1.016 [de]	1.968 [ab]
Joubertina	1.581 [a]	1.655 [a]	2.152 [abcd]	1.060 [de]	1.606 [ab]
Misgund	2.305 [a]	1.848 [a]	3.337 [ab]	0.968 [de]	2.153 [ab]
Piketberg	2.515 [a]	2.068 [a]	3.911 [a]	0.821 [e]	2.366 [ab]
Somerset West	3.193 [a]	1.790 [a]	4.290 [a]	1.160 [de]	2.725 [a]
Villiersdorp	1.874 [a]	1.753 [a]	2.642 [abcd]	1.025 [de]	1.833 [ab]
Vyeboom	2.504 [a]	1.735 [a]	1.420 [cde]	1.078 [de]	1.249 [b]
Cultivation practice					
Conventional	2.214 [a]	1.771 [a]	3.134 [a]	0.999 [b]	2.067 [a]
Organic	1.954 [a]	1.713 [a]	2.780 [a]	0.967 [b]	1.874 [a]
Scion x rootstock					
'Cripp's Pink'/M7	2.171 [ab]	1.727 [ab]	3.064 [ab]	1.081 [c]	2.014 [ab]
Golden Delicious/M7	2.890 [a]	1.909 [ab]	3.955 [a]	1.029 [c]	2.583 [a]
'Cripp's Pink'/M793	2.265 [ab]	1.837 [ab]	3.273 [ab]	0.972 [c]	2.122 [ab]
Golden Delicious/M793	1.766 [ab]	1.662 [b]	2.512 [b]	0.951 [c]	1.731 [b]
Rootstock					
M7	2.530 [a]	1.810 [a]	3.523 [a]	1.057 [c]	2.290 [a]
M793	1.961 [a]	1.730 [a]	2.809 [b]	0.959 [c]	1.884 [a]
Scion					
Golden Delicious	2.083 [a]	1.727 [a]	2.928 [a]	0.972 [b]	1.966 [a]
'Cripp's Pink'	2.229 [a]	1.793 [a]	3.195 [a]	1.015 [b]	2.080 [a]
Mean[6]	2.145 [a]	1.756 [a]	3.040 [a]	0.991 [b]	

[1] EEG expressed as mg g^{-1} soil

[2] Combined data over summer and spring

[3] Combined data over 2009 and 2010

[4] Marginal mean per variable of all years and seasons combined

[5] Values in the same data set, followed by the same letter, do not differ at $p < 0.05$

[6] Marginal mean (inclusive of all variables) of respectively 2009, 2010, summer and spring

Table 7.5: Percentage arbuscular mycorrhizal (AM) root colonisation of apple orchards included in a survey of native AM fungi

Variable	Year		Mean[2]
	2009[1]	2010[1]	
Production Area			
Ceres Koue bokkeveld	71.05 [ab3]	72.20 [ab]	71.63 [ab]
Ceres Warm bokkeveld	67.58 [ab]	80.36 [a]	73.70 [a]
Grabouw	60.19 [b]	56.47 [b]	58.33 [bc]
Joubertina	69.88 [ab]	55.50 [b]	62.69 [abc]
Misgund	59.50 [b]	56.00 [b]	57.75 [c]
Piketberg	69.08 [ab]	61.58 [ab]	65.33 [abc]
Somerset West	69.00 [ab]	69.25 [ab]	69.13 [abc]
Villiersdorp	61.75 [ab]	54.00 [b]	57.88 [c]
Vyeboom	64.13 [ab]	60.13 [b]	62.13 [abc]
Cultivation practice			
Conventional	65.63 [a]	63.80 [ab]	64.72 [a]
Organic	61.44 [ab]	54.13 [b]	57.78 [a]
Scion x rootstock			
Cripp's Pink/M7	64.08 [a]	67.38 [a]	65.73 [a]
Golden Delicious/M7	65.90 [a]	66.05 [a]	65.97 [a]
Cripp's Pink/M793	64.58 [a]	57.42 [a]	63.44 [a]
Golden Delicious/M793	65.69 [a]	62.31 [a]	61.56 [a]
Rootstock			
M7	64.91 [a]	66.79 [a]	65.84 [a]
M793	65.14 [a]	59.86 [a]	62.50 [a]
Scion			
Golden Delicious	65.77 [a]	60.40 [a]	63.11 [a]
Cripp's Pink	64.38 [a]	64.33 [a]	64.36 [a]
Mean[4]	65.05 [a]	62.45 [a]	

[1] Combined data over summer and spring

[2] Marginal mean per variable of all years and seasons combined

[3] Values in the same data sets, followed by the same letter, do not differ at $p < 0.05$

[4] Marginal mean (inclusive of all variables) of respectively 2009, 2010, summer and spring

The only significant difference between areas was between Somerset West (Lourensford), and Vyeboom. Like many Western Cape sandstone-derived soils, those at Lourensford are dominated by medium sand, have low clay and silt contents and diverse clay mineralogies, and have disproportionately large cation exchange capacities (CECs) per unit clay content, particularly in the limed state (Wooldridge 1989). Strong CEC responses to liming in low-clay sandy soils are consistent with the presence of both interstratified 2:1 minerals and organic C, perhaps in the form of EEG, as implied by Table 7.4. Conversely, the low EEGs at Vyeboom, an intermontane valley (synform), are typical of situations where the clay and silt

fractions are dominated by kaolinite, which has a low CEC, and where insufficient soil C is present to offset the low CEC of the kaolinite (Wooldridge, 1989, 1990).

Table 7.6: Relationship (*r, P*) between EEG, C, N, silt + clay fractions, and coarse sand fractions in a survey of native arbuscular mycorrhizal (AM) fungi. Combined data over years 2009 and 2010

AM / soil factor variable	Coarse sand	EEG	[1]Silt + Clay	C
	(%)	(mg g^{-1})	(%)	(%)
EEG (mg g^{-1})	0.1067^{ns}			
[1]Silt + Clay (%)	-0.0502^{ns}	0.2313^{ns}		
C (%)	-0.2913^{ns}	0.2360^{ns}	**0.5195****	
N (%)	-0.0036^{ns}	0.2746^{ns}	**0.7790*****	**0.7251*****

, *, respectively, $p <$ 0.01 and 0.001. ^{ns} = not statistically significant

[1]Silt and clay fractions combined

Average soil EEG levels did not differ significantly between rootstocks or scions (Table 7.4). EEG nevertheless tended to be higher under trees on M7 than M793. Soil EEG levels were significantly higher in soils associated with 'Golden Delicious'/M7 than 'Golden Delicious'/M793. Since M7 confers 20%-30% less growth vigour than M793 (M. North, personal communication) this finding was counterintuitive. Possibly glomalin production is promoted in situations where the rootstock is not well suited to the prevailing soil conditions and is under stress to maintain the scion and the crop. Under such conditions, stress-related root exudate production could stimulate both AM activity and glomalin production in accordance with Kretzschmar et al. (2012). Increased soil glomalin levels could contribute to improved soil conditions for both the AM fungi and the tree root system, with greater opportunities for successful AM root colonisation. According to Druille et al. (2013), host plant characteristics (genetics) indirectly affect AM root colonisation; so may host preference of AM fungi also be affected (Su et al. 2011). Further, as shown by Wooldridge (1999), growth responses to the same indigenous AM fungi differ between common apple rootstock varieties. Whilst rootstock genetics influence the quantity and quality of the root exudates, the genome of the scion affects photosynthate production and scion/root hormone cycling (Yao et al. 2005). Root exudates potentially act as chemical signaling agents which stimulate AM spore germination and root colonisation (Druille et al. 2013). However, the observed levels of root colonisation (Table 7.5) differed by only 5.3% between rootstocks, which was not significant. Neither did root colonisation differ between scion x rootstock combinations, despite the above-mentioned higher soil EEG contents associated with the 'Golden Delicious'/M7 compared with the 'Golden Delicious'/M793 trees. Root colonisation in spring

was not related to any of the variables listed in Table 7.7. Since EEG is a product of the decomposition of AM spores and hyphae, some link between AM activity and EEG might have been expected. However, glomalin is refractory in soils, and may accumulate over time, reaching a long term equilibrium that is only minimally affected by short term variation in AM activity (Steinberg and Rillig 2003).

Unlike average soil EEG content, which was higher at Somerset West than at Vyeboom, AM root colonisation did not differ significantly between these two areas. Instead, colonisation in Ceres Warm Bokkeveld was higher than in Grabouw, Misgund and Villiersdorp. No soil textural or chemical explanation for this difference was apparent. Like EEG, AM root colonisation bore no apparent clear relationship to soil texture, which is supported by the finding that EEG did not correlate significantly with the coarse sand fraction (Table 7.6). Neither was EEG correlated with the fine (silt + clay) component. Both soil C and N were nevertheless positively correlated with the silt + clay fraction, which corroborates the work of Razafimbelo et al. (2013) that soil C and the clay + silt fraction are linked. Linkage between soil C and soil texture has been attributed to a chemical stabilization of soil C by physicochemical adsorption of soil C on the sufaces of the mineral grains (Feller and Beare 1997). The soil C and N fractions also correlated reasonably strongly with silt + clay in the present study (Table 7.6). There was thus a link between the abundance of the main chemical components (C and N) of the soil, which included EEG, and the finer soil materials consisting of the combined silt + clay fractions, even though there was no relationship between EEG itself, as determined by the Bradford protein assay, and the silt+clay fractions. The percentage clay content, or alternatively clay + silt content, thus indicates the likely presence of C in the soil (Nichols et al. 1984), but not that of EEG itself.

Since EEG represents a source of soil C and N, it was counterintuitive to observe that EEG correlated with soil C in spring (2009 and 2010), but not in summer when EEG levels were higher and that EEG correlated with N only in spring 2009 (Table 7.7). In the work of Rillig et al. (2003), soil C and N were strongly correlated with glomalin across different soils and within different land-use types. The fact that the percentage AM root colonisation levels did not differ between 2009 and 2010, across all categories (Table 7.5), may suggest that variation between growing seasons does not have an effect on AM root colonisation of apple. This supposition, although also supported by the overall mean values for glomalin, nevertheless requires further substantiation. Orchard management practice practice did not significantly affect either EEG (Table 7.4) or root colonisation (Table 7.5), although both of these parameters tended to be higher in the conventional than the organic orchards. However, more conventional than organic orchards were included in this survey (a function of availability). This result requires verification.

Table 7.7: Relationship (*r, P*) between root colonisation, EEG, spore count and selected soil factors in a survey of native arbuscular mycorrhizal (AM) fungi

AM parameter / soil factor variable	pH (KCl)	P Bray II (mg kg^{-1})	K (mg kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	N (%)	C (%)
[1]Root colonisation (spring 2009)	0.079^{ns}	0.085^{ns}	0.233^{ns}	0.048^{ns}	-0.138^{ns}	0.277^{ns}	0.312^{ns}
[1]Root colonisation (spring 2010)	0.082^{ns}	-0.128^{ns}	-0.095^{ns}	-0.237^{ns}	0.244^{ns}	-0.196^{ns}	-0.233^{ns}
[2]EEG (summer 2009)	0.048^{ns}	0.065^{ns}	-0.119^{ns}	nd	nd	nd	0.128^{ns}
[2]EEG (summer 2010)	0.012^{ns}	-0.116^{ns}	-0.154^{ns}	-0.139^{ns}	0.106^{ns}	-0.215^{ns}	-0.094^{ns}
[2]EEG (spring 2009)	-0.139^{ns}	-0.027^{ns}	**0.388***	-0.014^{ns}	**-0.476****	**0.541****	**0.479****
[2]EEG (spring 2010)	-0.030^{ns}	**0.450***	0.129^{ns}	0.276^{ns}	-0.073^{ns}	-0.056^{ns}	**0.372***
[3]Spore count (spring 2009)	0.059^{ns}	0.039^{ns}	-0.152^{ns}	0.028^{ns}	**0.415***	-0.211^{ns}	-0.146^{ns}

*, **, respectively, $p < 0.05, 0.01$. ^{ns} = not statistically significant. nd = no data

[1]expressed as percentage (%) roots colonised

[2]expressed as mg EEG g^{-1} soil

[3]expressed as counts 100 g^{-1} soil

7.5. Conclusions

The finding that the EEG levels in summer generally exceeded those in spring prompts speculation that EEG is maximally generated in response to root stress induced by the higher temperatures and greater soil moisture tensions that are experienced in mid summer. Support for this supposition arises from the observation that higher soil EEG levels were associated with M7, which was the less resilient, and probably more highly stressed of the two rootstocks tested. Nevertheless, the lack of significant diffrerences in EEG levels among production areas suggest that the effect of climatic and environmental factors on EEG production appears to be less pronounced in so far as these factors vary from area to area across the Western Cape. That more production areas differed in AM root colonisation levels than in EEG levels implies that root colonisation by AM fungi is more variable within seasons and thus, more sensitive to site-specific orchard conditions, than EEG. Correlation between soil N and soil C, and the combined clay + silt fractions, implies that the availability of these elements is texture dependent in so far as metabolisable reserves of these elements are likely to be sparser in sandy than in heavier textured soils. The observed correlation between soil N and soil C may mean that both N and C are derived from the same type of organic parent material. Soil C and EEG in spring appears to be subjected to similar deposition and decomposition dynamics, which is why studies of agricultural practices intending to increase carbon in the soil, should also include the effects on the production of glomalin. Further studies are needed to demonstrate the generality of these patterns.

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CHAPTER 8

General discussion and conclusion

8.1. Discussion

The background against which the research described in this thesis was carried out was that, compared to CON, ORG practices increased soil fertility levels, notably with regard to pH, P and K. They also promoted the vegetative growth vigour of the trees, but suppressed yield and yield efficiency (ratio of yield to stem cross section area) (Wooldridge et al. 2014a, b).

The negative relationship between vigour and yield was ascribed to oversupply of mineral nutrients in the ORG treatments, compounded by imbalance between mineral elements, and by supply rates that did not match the requirements of the trees at the various phenological stages (Kotzé 2001; Kangueehi et al. 2011; Wooldridge et al. 2013a 2013b). Since the nutrient minerals contained in the compost had been applied in excess of orchard requirements in the CON treatments, evidenced by the imbalance between vigour and yield, it is reasonable to assume that the soil conditions in the CON and ORG treatments were sufficiently different to induce detectable differences in soil microbiology. Assuming, of course that supplying organic material in composted form does indeed affect soil microbiology. Any differences revealed by the research in this thesis would be evidence in favour of rejecting the null hypothesis, i.e. that CON and ORG treatments do not affect soil microbiological parameters.

Where differences were observed in the ANOVAs between the CON (T1, T2) and ORG (T3, T4 and T5) treatments, the values obtained were rarely clear cut from the viewpoints of consistency and statistical significance. In some cases (e.g. urease activity, Table 2.4) differences were less clear in summer than in spring. In other cases (e.g. *β*-glucosidase activity in spring) the values observed in T3 were intermediate between T1, T2 and T4, T5. Similarly, Wooldridge et al. 2013b observed low root numbers in T4 (T3 in the present work). They ascribed these low root numbers to possible carry-over of negative allelomorphic effects associated with the permanent work-row straw mulch into the tree row. If T3 is regarded as anomalous in some respects because of this suspected allelomorphism, then differences between CON and ORG should perhaps be assessed only in terms of differences between T1, T2 (CON) and T4, T5 (ORG), i.e., excluding T3. Instances where reasonably convincing numerical differences were observed between CON and ORG treatments (as defined above) include *β*-glucosidase activity (spring) (Table 2.2), acid phosphatase (Table 2.3) and urease (Table 2.4) activities in spring, actinobacteria count (Table 2.7), community metabolic diversity (Table 5.2), root colonisation by AM fungi in spring and summer (Table 6.3) and possibly heterotroph counts (which reverse order

between spring and summer (Table 2.8). Further, as shown in Table 4.2, soil organic matter (C) in T3 and T4 exceeded, and AI3 tended to exceed the levels in T1 and T2, whereas the converse tended to be true for yield efficiency. Outside the confines of the Elgin trial, only four out of 30 (13.3%) commercial apple orchards in the Western Cape that fulfilled the survey criteria (combinations of two rootstocks and two scion cultivars, sampled over two successive years) were organic (Chapter 7). This finding reflects a common sentiment in the Western Cape apple industry that organic apple production is unrewarding. Data from the survey showed that AM root colonisation was 12.0% (Table 7.5, and easily extractable glomalin 10.3% (Table 7.4) higher in the CON than the ORG orchard. These differences were not significant ($p < 0.05$), and were probably of doubtful integrity in view of the numerical imbalance between ORG and CON orchards in the survey. If further testing of a more balanced orchard population showed the above differences to be repeatable, then consideration would need to be given to the possibility that ORG management does not promote AM colonisation of apple roots. A possible reason why this should be the case is that ORG management (in the soil populations tested in this thesis) increased soil P levels to the point, where AM colonisation may be inhibited.

Whether the abovementioned differences constitute sufficient evidence to justify rejection of the null hypothesis is dabateable. However, evidence pertinent to the null hypothesis concerns the relationships between microbiological and soil chemical parameters as revealed by correlation analysis, bearing in mind that, as shown by Wooldridge et al. (2013a), the values of most such parameters were appreciably higher in the ORG than the CON treatments. To this effect significant ($p < 0.05$) positive relationships were observed between acid phosphatase, *β*-glucosidase and urease activities and soil pH and P, urease and K, and acid phosphatase and *β*-phosphatase and Zn, Mn and C (Table 2.5). All three enzymes reacted negatively to Cu, and acid phosphatase and *β*-glucosidase activities were suppressed by NO_3^- (Table 2.5). Likewise, as shown in Table 3.4, urease correlated positively with soil C, NO_3^- and pH, and *β*-glucosidase correlated with C, NO_3^- and pH. Root colonisation by AM fungi correlated positively with soil pH, P, Zn and Mn (Table 6.6). As was the case for the activities of acid phosphatase, *β*-glucosidase and urease, AM root colonisation was suppresed by soil Cu. Use of Cu containing fungicides has been linked with reduced earthworm activity in South African vineyards (Eijsackers et al., 2005). Soil alteration index three (AI3) correlated positively with yield efficiency (bearing in mind that AI3 had negative values), whereas soil organic matter (C) correlated negatively with both yield and yield afficiency (Table 4.3). These relationships suggest that increasing soil pH, mineral nutrient and C levels (the primary effect of the high compost application rates used in this research and that of Wooldridge et al. 2013a, 2013b), was to promote enzyme activity (Table 2.5) (in the 0-30 cm soil depth interval, Figure 3.1, Table 3.2) and AM root colonisation (Table 6.6). Further, AI3 correlated with C (Table 4.3), which was

higher in the ORG than the CON treatments (Table 4.2), and with both yield and yield efficiency (Table 4.3).

With the notable exception of Cu (a common component of fungicides used in CON orchards, which tends to accumulate in orchard topsoils over time), the affects on ORG, relative to CON treatments, on AM and soil enzyme activities were therefore generally positive and significant ($p < 0.05$), although correlation coefficients ranged from weak to strong. From the survey of apple orchards described in Chapter 7, easily extractable glomalin (a by-product of AM fungi), where assessed in spring over two years, was found to correlate with soil C content (Table 7.7), which in turn correlated with total soil N (Table 7.6). Both soil C and soil N correlated with the fine (silt+clay) contents of the surveyed soils, which may mean that nutrients (N in this case) that are applied in organic (as oposed to synthetic) form are more likely to be retained by fine than by coarse textured soils.

Collectively, these correlations provide further, albeit indirect support, for the contention that ORG orchard floor management practices affect at least some soil microbiological parameters differently to CON practices.

The combined ANOVA and correlation data are considered to provide sufficient grounds for rejection of the null hypothesis in favour of the assumption that ORG and CON orchard floor management techniques have different effects on at least some important aspects of apple orchard soil microbiology.

8.2. Implications for the deciduous fruit industry

Acceptance of the fact that the effects of ORG orchard floor management practices differ from those of CON management practices has implications for South African apple producers and, by inference, for the broader deciduous fruit industry. These implications stem from the findings that although the organic treatments used in this research and that of Wooldridge et al. (2013a, 2013b) had several beneficial effects on soil microbiology (this thesis), they also had negative effects on orchard performance (Wooldridge et al. 2013b). The management objective must therefore be to engineer a situation where both soil microbiology (“soil health”, in the broad sense used by Gugino et al. 2009) and orchard performance are balanced to maximise both soil health and orchard profitability.

Poor tree performance in the compost-based ORG treatments used in the Elgin trial orchard was attributed to three factors (after Wooldridge et al. 2013b).

1. The availability of P, K and probably N (N availability to roots could not be reliably determined) exceeded the requirements of the trees, resulting in luxury uptake and imbalance between vegetative growth and production (yield). Seasonal nutrient application rates adjusted for soil and leaf analysis, vigour and anticipated yield, have been firmly established (Kotzé, 2001).

2. The major nutrient elements supplied by the compost were present in relative proportions that did not match the requirements of the tree. That is, the nutrients were not balanced (Kotzé 2001; Kangueehi et. al. 2011).
3. The supply rates of the different elements, most notably of N, could not be adjusted to match the phenological stage-dependent requirements of the trees. (Release of nutrient mineral elements from compost is a continuous, slow-release process which slows with time, but which is subject to fluctuation with varying temperature.)

These three factors represent challenges which must be addressed, singly and jointly, if the twin objectives of maximised soil health (*sensu lato*), and optimal economic orchard performance are to be achieved.

Devising workable solutions to these challenges lies outside the scope of this thesis. It is nevertheless self evident that the availabilities of the separate mineral nutrients, and the balance between them, can only be controlled by adjusting the components used in the manufacture of the compost. If the mineral nutrient element contents of an organic product are known, and the ratios between the elements are also known, the product can be applied at calculated rates in the same way as conventional synthetic fertilisers. At that point the difference between organic and synthetic products will lie in the nature of the material carrying the nutrients, i.e., whether it is of organic derivation or synthetic. The nature of the carrier may also be expected to contribute to the rates at which the nutrient elements are released. Whether such organic-based carriers/products would have the same effects on soil microbiology as the compost used in the trials described in this thesis, is unclear. Clarification will need to be obtained through field trials once testable products have been produced and have become available. Regarding the design of such trials it may be pertinent to remember that, in the orchard survey described in Chapter 7, easily extractable glomalin (an AM fungal breakdown product) levels differed between two rootstocks (M7 and M793) grafted onto the same scion (Table 7.4). This finding suggests that rootstock variety, preferably grafted to the same scion cultivar, should be a factor in the testing of all organic products.

8.3. Conclusion

The research described in this thesis sought to show that ORG and CON orchard floor management practices induced different soil microbiological responses in Western Cape apple orchard soils. A number of differences were observed. Microbiological parameters for which ORG treatments induced positive responses, compared with CON treatments, included:

1. Actinobacteria counts.
2. Urease, phosphatase and *β*-glucosidase activities in the topsoils.
3. Soil alteration index three (AI3).

4. Carbon source utilisation (microbial community metabolic diversity).
5. AM fungal root colonisation.

The null hypothesis, i.e. that CON and ORG practices do not affect soil microbiology, is therefore rejected.

8.4. References

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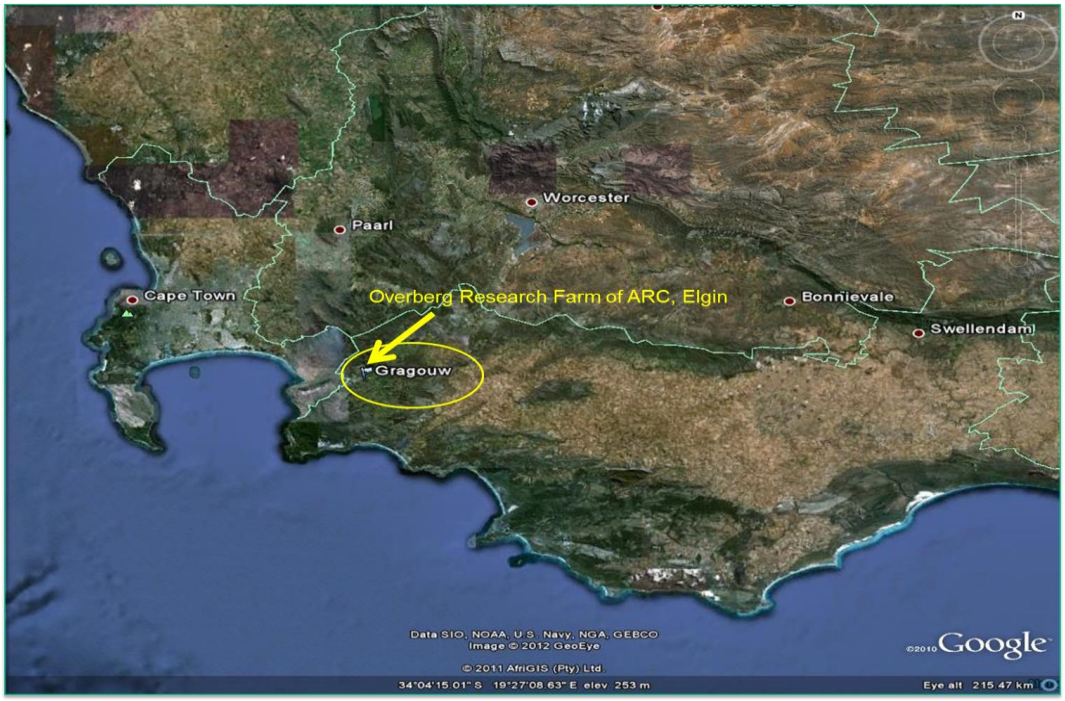
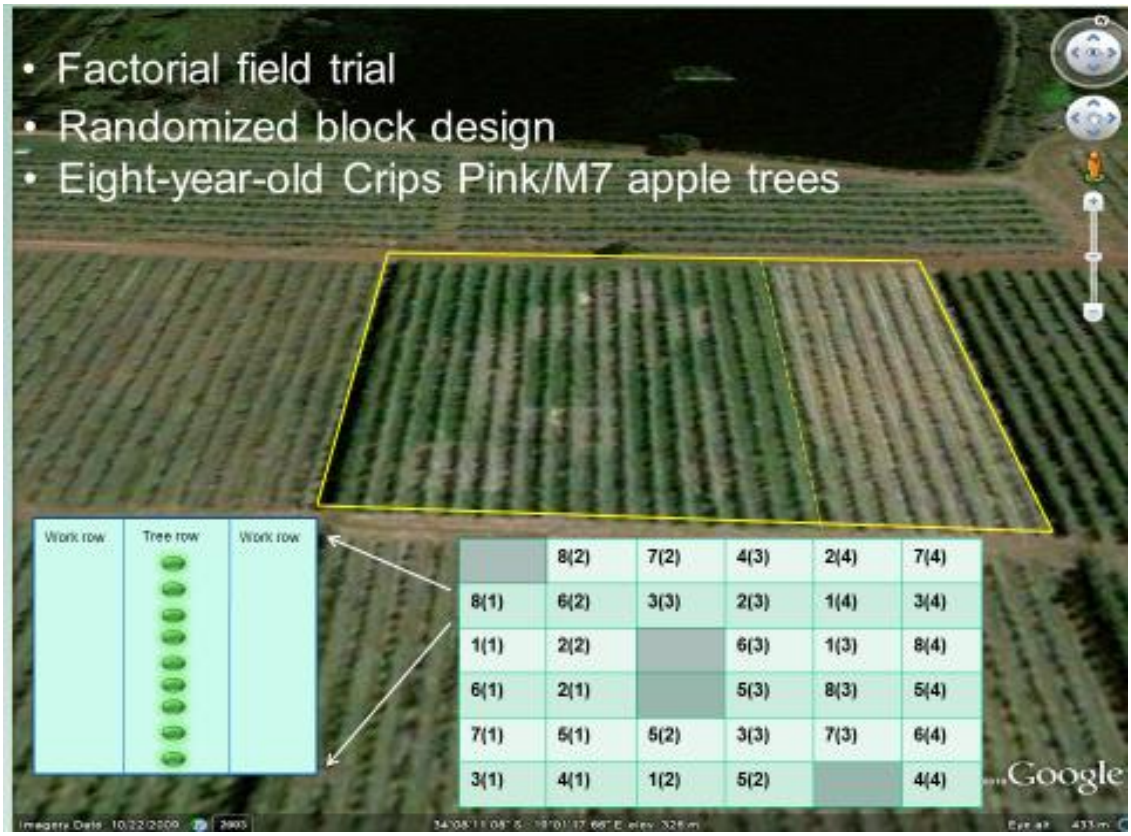
APPENDIX A1: Location of Overberg Research farm of ARC



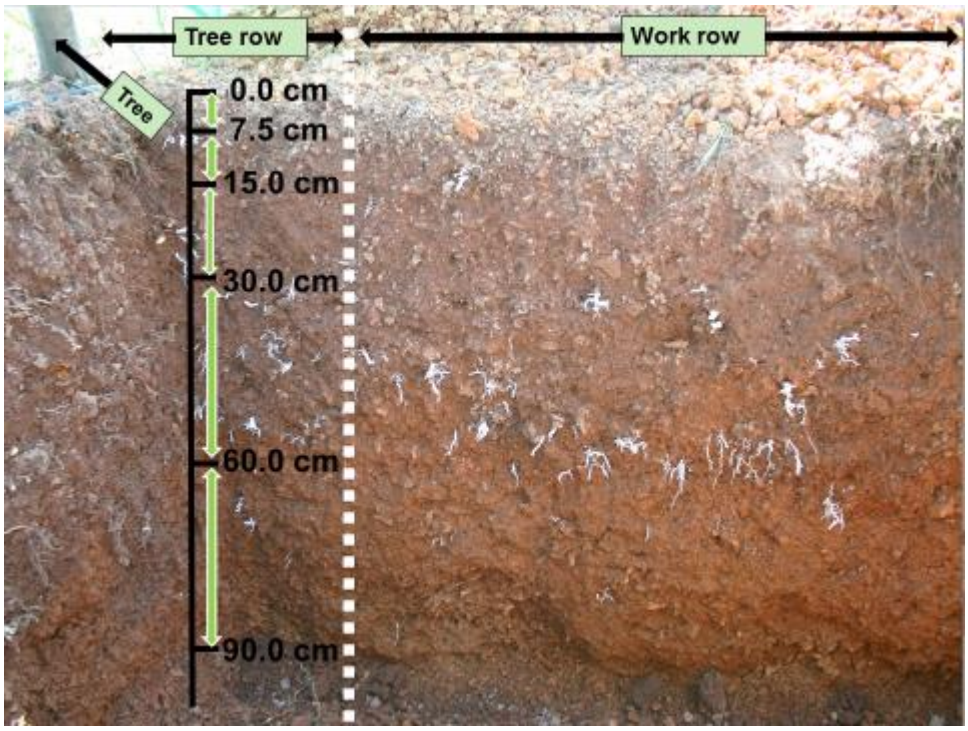
APPENDIX A2: Location of orchard trial site at Overberg Research farm of ARC in Elgin

APPENDIX A3: Experiment layout of the Elgin trial at Overberg Research farm in Elgin

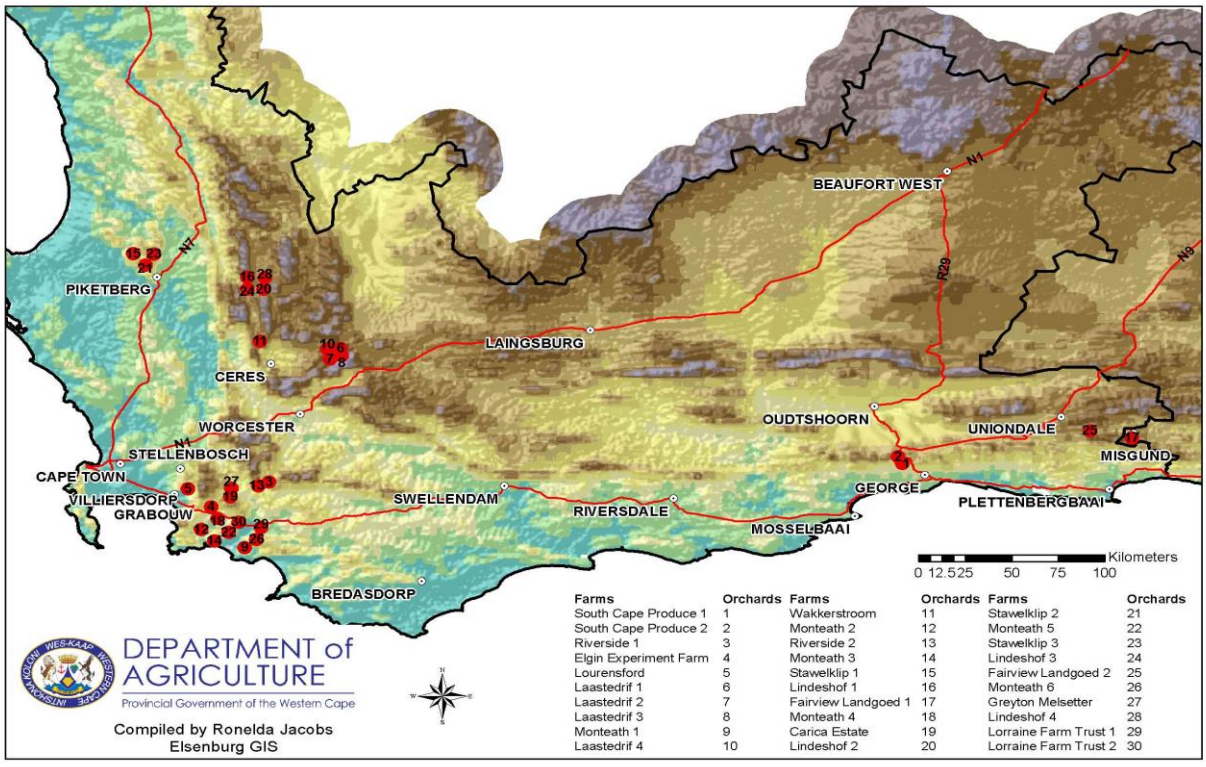


APPENDIX A4: The apple orchard at Overberg Research farm in Elgin during spring 2007. Soil surface treatments were applied in the tree and work rows. Mulch clearly visible at the forefront

APPENDIX A5: The trench dug at the base of the central apple tree perpendicularly across the adjacent work row. Numbers of roots protruding from the loosened trench wall within each grid square were recorded



APPENDIX B: Map of the farms participating in the survey on naturally occurring arbuscular mycorrhizal (AM) fungi associated with various apple orchards of the Western and Eastern Cape apple production areas



Farms	Orchards	Farms	Orchards	Farms	Orchards
South Cape Produce 1	1	Wakkerstroom	11	Stawelklip 2	21
South Cape Produce 2	2	Monteath 2	12	Monteath 5	22
Riverside 1	3	Riverside 2	13	Stawelklip 3	23
Elgin Experiment Farm	4	Monteath 3	14	Lindeshof 3	24
Lourensford	5	Stawelklip 1	15	Fairview Landgoed 2	25
Laastedrif 1	6	Lindeshof 1	16	Monteath 6	26
Laastedrif 2	7	Fairview Landgoed 1	17	Greyton Melsetter	27
Laastedrif 3	8	Monteath 4	18	Lindeshof 4	28
Monteath 1	9	Carica Estate	19	Lorraine Farm Trust 1	29
Laastedrif 4	10	Lindeshof 2	20	Lorraine Farm Trust 2	30