EFFECT OF BIOLOGICAL AMENDMENTS ON SOIL MICROBIAL PROPERTIES AND PERFORMANCE OF POME FRUIT TREES

By

Louise van Schoor

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Promoter: Dr. P.J.C. Stassen
Department of Horticultural Science
Stellenbosch University
South Africa

Co-promoter: Prof. A. Botha
Department of Microbiology
Stellenbosch University
South Africa

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DECLARATION

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SUMMARY

The global movement in agriculture is towards more environmentally friendly, sustainable production practices, since the role of soil microbial functions in ensuring crop production and soil fertility has become more evident in agricultural systems. Furthermore, with the impeding phase-out of methyl bromide, apple replant disease (ARD) is becoming an increasingly important problem and biological management practises are needed. Since microbial activity is generally carbon-limited in agricultural soil, it is widely accepted that management practices providing a range of organic compounds on a regular basis will tend to maintain an active and diverse microbial population. It was hypothesised that the application of various biological amendments can affect soil microbial numbers and function, thereby having a positive effect on fruit tree growth and yield. The effect of continued applications of organic material, various microbial inoculants and biostimulants on tree performance were evaluated in conventional management systems. Field trials were established in a conventional pear orchard, potential apple replant disease sites, as well as an optimally managed, high density apple orchard under controlled fertigation. The use of compost, compost extracts, a *Bacillus* inoculant and humates were investigated intensively. Furthermore, to improve our understanding of soil biological systems a combination of simple, practical methods were used to evaluate the effect of biological amendments on soil microbial properties and effects were related to tree performance.

Regular application of compost extract in combination with compost showed the most significant effect in improving tree performance in commercial pome fruit orchards under various conditions. In the pear orchard, cumulative yield over the first two seasons was improved by more than 50% compared to controls, while in the fertigated orchard yield was improved by 22%. Biological amendments also showed improved growth in orchards suffering from stunted growth symptoms typical of ARD. However, in severe ARD cases methyl bromide fumigation showed the most consistent effects. Other biological amendments which showed positive effects on yield were application of *Bacillus* inoculants (Biostart®) in combination with a labile C source and a low dosage humate product, as well as a combination of compost and humates. It was clear that a combination of labile organic matter and a diverse group of microorganisms showed most promise. Although for some specific treatments increased microbial numbers and activity may have resulted in improved tree performance, in general, changes in culture-based plate counts, soil enzyme activity and carbon utilisation profiles could not be used as an indicator of yield. It was suggested that improved synchronisation of nutrient release and plant uptake, as well as microbial phytohormone production, may play an important role in improving tree performance with application of biological amendments. More research is needed on the exact mechanisms through which compost extracts improve yield and studies on root growth proliferation, as well as effects in the rhizosphere are recommended.

UITWERKING VAN BIOLOGIESE TOEVOEGINGS OP GROND MIKROBIOLOGIESE ASPEKTE EN PRESTASIE VAN KERNVRUGBOME

OPSOMMING

Binne lanbouverband is daar tans wêreldwyd die neiging om die uitwerking van produksie-praktykte op die omgewing in ag te neem en sodoende meer verantwoordelik op te tree. Omdat die belangrike rol wat grondmikro-organisme funksionering in volhoubare verbouingspraktyke speel nou deeglik besef word, word meer volhoubare bestuurspraktyke bepleit. Hiermee saam, noodsaak aspekte soos die uitfasering van metielbromied vir die beheer van appelhervestigingsiekte, dat biologiese bestuurspraktyke meer aandag geniet. Daar word geredelik aanvaar dat gereelde toediening en aanvulling van organiese materiaal ‘n aktiewe, diverse mikrobe populasie in die grond tot gevolg sal hê. Die hipotese is gestel dat die toediening van ‘n verskeidenheid biologiese produkte grondmikrobe getalle en werking gunstig kan beïnvloed. Dit kan moontlik weer aanleiding gee tot positiewe reaskies wat die groei en drag van vrugtebome betref. In hierdie studie is die uitwerking van voortgesette toedienings van organiese materiaal, mikrobiese inokulante, asook biostimulante, op die prestasievermoë van vrugtebome ondersoek. Veldproewe is uitgelê in ‘n konvensionele peerboord, verskeie boorde met moontlike appelhervestigingsiekte probleme, asook ‘n hoë-digtheidsaanplanting appelboord onder optimale bestuur. ‘n Deeglike ondersoek is gedoen met betrekking tot die gebruik van kompos, komposekstrak, *Bacillus*-inokulante en humate. Eenvoudige, praktiese metodes is aangewend om vas te stel hoe biologiese toevoegings grondmikrobe eienskappe beïnvloed en of dit verband hou met veranderinge in boomprestasie.

Die studie het aangetoon dat die gereelde toediening van komposekstrak saammet kompos, betekenisvolle verbetering in boomprestasie van kernvrugboorde teweeg bring onder verskeie omstandighede. Die kumulatiewe opbrengs van ‘n peerboord is oor twee seisoene met meer as 50% verhoog teenoor die kontrole. In ‘n optimaal bestuurde appelboord onder sproeibemesting, is opbrengs met 22% verhoog in vergelyking met die kontrole. Biologiese toevoegings het ook groei verbeter in boorde waar appelhervestigingsiekte bome se groei vertraag het. In die geval van ernstige appelhervestigingsimptome het metielbromied egter steeds die mees konstante positiewe uitwerking gehad. Ander biologiese toevoegings wat ‘n gunstige uitwerking op opbrengs getoon het, was ‘n kombinasie van *Bacillus* inokulante, ‘n lae dosis humaat en ‘n aktiewe koolstofbron, asook kompos in kombinasie met humate. Dit is duidelik dat ‘n kombinasie van ‘n maklik afbreekbare koolstofbron (soos kompos) tesame met ‘n diverse groep mikroorganismes mees belowend is vir gebruik in biologiese verbouingssisteme. Resultate toon dat veranderings in aantal organismes gemeet deur plaattellings, die aktiwiteit van grondensieme, en verbruikspotensiaal van verskillende koolstofbronne, nie as ‘n aanduiding van boomprestasie gebruik kan word nie. Daar is voorgestel dat verbeterde sinkronisasie van voedingselementvrystelling en plantopname, sowel as produksie van plantgroeihormone deur mikrobe, moontlik ‘n rol speel by boomreaksies op

biologiese toevoegings. Meer navorsing wat verband hou met die meganisme waardeur komposekstrak opbrengs verbeter, is nodig. Verder word studies op fynwortelontwikkeling sowel as aspekte van die wortelrisosfeer aanbeveel.

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LITERATURE REVIEW

1.1 INTRODUCTION

The global movement in agriculture is towards more environmentally friendly, sustainable production practices. Motivations for shifting from chemically intensive management strategies to more biologically based practices, include concern for protecting animal and human health from potential hazards of pesticides, protecting non-renewable resources, as well as the need to lower escalating production costs (Fraser et al., 1988; Matson et al., 1997; Tillman, 1999).

In natural ecosystems, cycling of mineral nutrients and carbon as well as development of soil structure are regulated by the interactions of a highly diverse and complex web of soil flora and fauna that is sustained by the influx of organic matter into the soil (Alexander, 1977; Larson and Pierce, 1991; Tisdall, 1996; Davet, 2004). Conventional agriculture attempts to maximize yield by controlling biological functions normally executed by the soil microbial community. Initially these practices increased production levels, but over time they have affected the functioning of agroecosystems. Impacts are now being realized in increased compaction and erosion, reduced levels or shifts in species composition of soil flora and fauna, increased crop susceptibility to biotic and abiotic stress, as well as a reduction in soil organic matter (SOM) (Grayston et al., 1996; Loveland and Webb, 2003; Phelan, 2004).

The negative impact of conventional management practices on soil productivity has renewed interest in the integration of biological soil amendments into standard management systems in order to improve soil productivity by affecting soil microbial activity. The beneficial effect of organic matter on soil physical and chemical properties is well established (Hudson, 1994; Stevenson, 1994; Carter and Stewart, 1996; Swift, 2001). The connection between SOM application and increased biological functioning in agroecosystems becomes clear when comparing chemically intensive and organic or biologically integrated farming practices (Bolton et al., 1985; Doran et al., 1987; Reganold et al., 1993; Wander et al., 1994; Drinkwater et al., 1995; Katayama et al., 1998; Mäder et al., 2002; Flieβbach et al., 2007). Application of soil inoculants has shown benefits especially in improving plant health and improved uptake of nutrients (Glick, 1995; Zahir et al., 2004). The addition of biostimulants, such as seaweed extracts and humic substances (HS) is also widely advocated (Russo and Berlyn, 1990; Chen and Aviad, 1990). Furthermore, the use of microbial inoculant mixtures containing a diversity of unspecified soil microorganisms such as compost teas and effective microorganisms (EM) are being promoted (Higa, 1994; Ingham, 1999a) with little scientific literature to back up the claims made.

The aim of this review was to study the main biological amendments used to improve soil microbial activity in agriculture and to evaluate their effect on plant performance. However, it is essential to first

understand the functioning of the soil-plant system in order to know how to manage agricultural systems productively and in a sustainable manner. There has been a dramatic increase in the number of publications studying the effect of various biological management practices on soil microbial activity in the past few years. From these studies it is clear that our knowledge of soil ecosystem functioning is limited in part by the complexity of measuring soil microorganisms. Furthermore, the direct relationship between plant performance and microbial activity is not well studied. In order to apply biological amendments successfully in orchard management systems, a better understanding is needed of mechanisms involved in the relationship between soil biological activity and plant performance. Therefore, an overview of various mechanisms involved in improving plant performance will be provided. Lastly, from current knowledge, the potential application of biological amendments in deciduous fruit production will be evaluated in terms of improving plant performance, reducing chemical inputs, and addressing specific industry problems.

1.2 THE SOIL-PLANT SYSTEM

The soil-plant system is the direct environment in which roots grow, absorb water and nutrients and release some inorganic ions and organic material into the soil as exudates, which in turn serve as nutrients and energy for the growth and development of microorganisms. Soil is an essential natural resource that provides important ecological functions in promoting plant growth and production and therefore represents the basis for food production (Larson and Pierce, 1991; Karlen et al., 1997; Loveland and Webb, 2003; Magdoff and Weil, 2004, Komatsuzaki and Ohta, 2007). It is essential to understand the functioning of the soil-plant system in order to manage agricultural systems productively. The basic principles of soil functioning are well established. Soil is composed of both a mineral and an organic fraction, with the remaining soil volume composed of pore space filled with air or water. The porosity of soils is affected by the state of aggregation. A well aggregated soil structure ensures appropriate soil tilth, soil-plant water relations, water infiltration rates, soil aeration, and root penetrability, all contributing to soil productivity (Miller and Jastrow, 2000) which is linked to plant productivity (Abbott and Murphy, 2003). Through root exudations plants actively participate in soil processes and continually adjust their interactions with the soil environment, particularly within the rhizosphere (Tate, 2000). Research during the past 50 years has placed much emphasis on the importance of mineral nutrients for crop productivity, with notably less research on the importance of the soil organic fraction and biological processes performed by soil organisms.

1.2.1 The soil organic fraction

Soil organic matter consists of a variety of components in varying proportions and intermediate stages. These include fresh organic residues, plant roots and living soil biota, as well as an active organic fraction consisting of decomposing material with a relatively short turnover time. The input of carbon through plant roots also affects the microbial community and plant performance (Rovira, 1959). Furthermore, there is also a pool of carbon that is physically protected or in chemical forms with a more intermediate turnover time, as well as more stabilised organic matter that is difficult to decompose (Tisdall and Oades, 1982; Parton et al., 1987). This stabilised organic matter is generally termed humus (Stevenson, 1994).

1.2.1.1 Soil microflora and fauna

Although the living portion of the soil body makes up the smallest part of the total soil volume, it is central to crop production and soil fertility (Davet, 2004; Gobat et al., 2004). Alexander (1977) described the major groups of soil microorganisms and some essential information in understanding their functioning in soil are summarised in this section. The most important soil microflora consist of bacteria, actinomycetes and fungi.

Although bacteria are by far the most abundant microorganisms in soil, fungi make up a more significant part of the biomass due to the large diameter and extensive network of their filaments. Fungi, as well as actinomycetes, show relative uniformity in terms of metabolism, and are aerobic heterotrophs, requiring preformed organic nutrients to serve as a source of energy and carbon (Davet, 2004). Although most bacteria are heterotrophs, some are autotrophs and capable of using CO_2 to satisfy their carbon need. These organisms obtain their energy from either sunlight (photo-autotrophs) or by oxidation of inorganic compounds (chemo-autotrophs). The nitrifying bacteria, as well as bacteria which fix nitrogen (N) from the atmosphere are chemo-autotrophs and play an essential role in the N cycle.

Fungi are adapted to a wide pH range, therefore microbial communities in areas of low pH are dominated by fungi due to low numbers of bacteria and actinomycetes. Liming can greatly increase the abundance of bacteria and actinomycetes. Fungi and actinomycetes are more tolerant of drier conditions. In contrast, bacterial respiration declines rapidly under these conditions (Griffin, 1981). However, when moisture levels are excessive, fungi are among the first to suffer and are therefore mostly concentrated in the few inches of soil below the surface. In anaerobic environments, bacteria account for almost all biological and chemical changes (Sommers et al., 1981). Actinomycetes form the dominant fraction of the microflora in relatively dry, humic soils with a high pH (Goodfellow and Williams, 1983). Most soil microflora are mesophilic, with optimum growth temperatures between 25 ºC and 35 ºC. However, the microbial biomass is only directly sensitive to large shifts in temperature (Wardle, 1992). There are also numerous bacteria (such as the *Bacillus* spp.) that grow at temperatures of 45 ºC to 65 ºC (thermophiles). These thermophiles, as well as actinomycetes, can regulate transformations at high temperatures, and are particularly abundant in compost heaps, and manures (Lechevalier, 1988; Phae et al., 1990; Hatsu et al., 2002).

The soil fauna are a diverse group divided into various categories according to size and of which the protozoa, nematodes and earthworms have been extensively studied for their role in soil fertility (Alexander, 1977; Yeates, 1979; Forge et al., 2003; Gobat et al., 2004). Release of nutrients from the microbial biomass, is partly regulated through grazing by the soil fauna, playing an important role in nutrient cycling. The most important soil microfauna are the protozoa, the simplest form of animal life. These organisms feed heterotrophically, obtaining nutrients from soluble organic and inorganic substances, or by phagotrophic nutrition characterized by direct feeding upon microbial cells or other particulate matter (Alexander, 1977). As predators, they prey upon algae, bacteria and microfungi and food source

preferences are very specific (Alexander, 1977). The metabolites produced by protozoa stimulate bacterial populations that provide their sustenance (Clarholm, 1985) and together with selective feeding play a vital role in controlling bacteria populations and biomass (Seastedt, 1984). Protozoa themselves are also an important food source for larger creatures and the basis of many food chains. Adequate soil moisture is essential for their physiological activity and lateral and vertical movement.

The soil mesofauna include the nematodes, microarthropods, mites, and springtails. Nematodes are the best-known because of the detrimental effect of parasites that feed on plant roots. However, most nematodes are free living microbial feeders, omnivores or carnivores and generally beneficial to plants (Gobat et al., 2004). Bacterial- and fungal-feeding nematodes contribute significantly to nitrogen mineralization (Ekschmitt et al., 1999). Nematodes can also through their feeding preference (fungi or bacteria), significantly alter the fungal-bacterial balance, and cause changes in species composition (Ferris et al., 2001).

The macrofauna which include earthworms, insects, arthropods and enchytraeids play an important role in building soil structure. They require well-aerated environments, adequate moisture and warm temperatures (Gobat et al., 2004).

1.2.1.2 Decomposition and nutrient cycling

The active organic matter and the living soil biota are central to nutrient cycling. Soil organisms perform a key role in plant nutrition as both a source and sink for mineral nutrients and can conduct a multitude of biochemical transformations (Jenkinson and Ladd, 1981).

In the decomposition process, carbon is recycled as carbon dioxide, nitrogen is converted to ammonium, and other associated elements are released in plant available form (Jenkinson and Ladd, 1981; McGill and Cole, 1981). Bacteria and fungi, possessing a greater suite of enzymes for chemical breakdown of organic material, are the major decomposers and are also considered as the labile pool of carbon (C), nitrogen (N), phosphate (P), and sulphur (S), called the soil microbial biomass. The soil fauna is crucial for initial decomposition steps, such as mixing of residues into the soil, and increasing surface area in preparation of further microbial attack (Seastedt, 1984; Paul and Clark, 1996). During the initial period of decomposition, stimulation of fungal action seems to be greatest. Bacteria respond promptly to organic amendment and remain numerous as long as nutrients are available, while actinomycetes become more pronounced only at a later stage of decay, when there are more readily available nutrients and less competition. Fungi have evolved a remarkable metabolic versatility and have a critical role in breakdown of the more complex carbon sources. They can utilise lignin that is particularly resistant to bacterial degradation. Actinomycetes, on the other hand, play an important role in decomposition of organic materials such as cellulose and chitin.

The proportion of mineral elements released by microorganisms and immediately available for plants depends on the nature of the substrate. Microbes generally out-compete plants for nutrients in the presence of sufficient carbon sources (Jackson et al., 1989). If the contents of nitrogen, sulphur and phosphorus are low in the residues compared to their carbon composition, these elements will be immobilised in the microbial biomass. The addition of mineral salts would be necessary in this case to preclude competition between plants and the microorganisms (Hogue and Neilsen, 1987; Lipecki and Berbec, 1997). However, immobilisation is only temporary and a portion of these nutrients is continuously mineralised through death of microbes. Grazing by soil fauna on microbial communities and predation on micro- and mesofauna are responsible for a significant portion of the mineralisation of nitrogen in soil (Clarholm, 1985; Freckman, 1988; Ekschmitt et al., 1999). Furthermore, the microbial grazing mesofauna affect growth and metabolic activities of the soil microbes and alter community composition, thus regulating decomposition rates of organic matter (Seastedt, 1984). With a large microbial population turning over rapidly, the cyclic flux between immobile and mineralised forms can provide a gradual, continuous supply of nutrients (Mckenzie et al., 2001; Davet, 2004; Ball, 2006). Therefore the complexity in trophic groups of the soil food web plays an important role in plant performance (Setälä, 1995; Laakso and Setälä, 1999).

1.2.1.3 Soil organic matter functions

During decomposition, new compounds are formed from decomposition products which do not occur in plants and organisms (Foth and Turk, 1972). These include the humic substances (HS), which are large, complex compounds and comprise 65-70% of humus (Hernando, 1975). Soil organic matter contributes to various soil physical, chemical and biological properties.

Chelating and buffering are considered by many to be the most important property of soils, since without this, agriculture would require much more intensive management (Loveland and Webb, 2003). SOM contributes in a large part to this buffering ability, improving properties such as the cation exchange capacity (CEC) (Thompson et al., 1989) and soil pH (Stevenson, 1986; Pieri, 1992). Furthermore, these chelating substances react with trace elements such as iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn), protecting them from precipitating and becoming insoluble and unavailable to plants (Hodges, 1991; Stevenson, 1994). Indirect effects of SOM on plant performance through improved soil physical conditions include increased porosity, soil aggregate formation, reduced bulk density, as well as increased water holding capacity and reduced erosion (Hudson 1994; Carter and Stewart, 1996; Swift 2001; Loveland and Webb, 2003). The more active SOM provides an important reservoir of nutrients for plants, with the mineralisation of SOM being the primary source of available N, P and S in natural systems (Brady and Weil, 1999). Furthermore, the availability of all major nutrients is influenced by the presence of SOM as it supplies an available nutrient pool via mineralization and desorption and binds nutrients via immobilisation and adsorption reactions (Carter and Stewart, 1996; Reeves, 1997).

The most important single element in the biological realm and the substance that serves as the cornerstone of cell structure is carbon (Alexander, 1977). Since SOM contains the organic carbon and nitrogen needed for microbial development, it is the dominant food reservoir for the microbial biomass and greatly influences the biological processes critical for soil functioning.

1.2.2 Importance of biological processes in agriculture

Soil organisms perform a key role in soil fertility and plant nutrition (Jenkinson and Ladd, 1981; Jeffries et al., 2003). In addition to decomposition, they influence the availability of nutrients via a range of activities such as immobilisation of nutrients, mineralisation, improved nutrient availability and uptake, nutrient retention and nutrient cycling. Various bacteria and fungi also have the ability to solubilise nutrients from insoluble forms as well as enhance nutrient uptake (Glick, 1995; Rodriguez and Fraga, 1999; Zahir et al., 2004). Specific groups of bacteria can also fix N from the atmosphere (Kennedy and Islam, 2001). In addition, the cell material and excretions of soil microorganisms act as cementing agents, affecting soil physical structure through the formation and stabilitation of soil aggregates (Gupta and Germida, 1988; Tisdall 1994; Beare, 1997; Wright and Upadhyaya, 1998; Miller and Jastrow, 2000). Soil microorganisms are also involved in the detoxification of organic and inorganic substances that would impede plant growth (Lynch, 1983; Bollag et al., 1992; Lambais and Cardoso, 1993; Dalal, 1998; Barea et al., 2005). Furthermore, microbial activity in the rhizosphere plays an important role in pest and disease suppression through biological control (Baker and Cook, 1974; Bowen and Rovira, 1999; Whipps, 2001). Other functions that soil organisms perform in the agroecosystem are the production of plant growth promoting compounds which directly effect plant physiology, especially root growth (Glick, 1995; Zahir et al., 2004). Growth stimulating substances present in SOM can also be released by microbes during decomposition (Frankenberger and Arshad, 1995).

In order to perform these key soil functions, the presence of a large and diverse microbial community, with the ability to break down a wide range of chemical bonds is essential (Murphy et al., 2003; Kennedy et al., 2004). Diverse systems have higher agricultural productivity, resilience to stress and provide better protection against pests and diseases (Giller et al., 1997). However, considerable functional redundancy exist at species level (Andren et al., 1995), meaning that individual taxa may have multiple functions, while several taxa appear to have similar functions. There is still a continuing debate whether or not species diversity and ecosystem function are causally related (Huston, 1997; Brussaard et al., 2004). However, this does not mean that there is no need to preserve the biological richness of the soil (Phelan, 2004), since taxa performing the same function are often isolated spatially, temporally or by microhabitat preference (Beare et al., 1995). Therefore, although redundancy of single functions is common, distinct physiological and environmental requirements drive species of the same functional group to play widely different roles in soil ecosystem processes.

1.2.3 Plant –microbial interaction

The rhizosphere is that portion of the soil under the direct influence of the roots of higher plants and a site of maximized biological activity (Tate, 2000). A multitude of compounds are released into the rhizosphere of soil-grown plants, most of which are organic compounds and normal plant constituents derived from photosynthesis and other plant processes. Whipps (1990), estimated that as much as 40% of the plant's primary carbon production may be lost through rhizodeposition. The relative and absolute amounts of root exudates produced vary with 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 (Rovira, 1959; Hale et al., 1978; Hale and Moore, 1979; Bowen and Rovira, 1999).

Root exudates may have a direct effect of immediate benefit to the plant, e.g., an increase in nutrient solubility (Uren and Reisenauer, 1988; Grayston et al., 1996) or have an indirect effect on the plant through controlling the activity of soil organisms (Barber and Lynch, 1977; Xu and Juma, 1993; Werner, 1998; Walker et al., 2003). Microbial activity in the rhizosphere furthermore affects rooting patterns, as well as mineralization and immobilization processes, thereby modifying in turn the quality and quantity of root exudates (Bowen and Rovira, 1999). Secondary metabolites in root exudates have the potential to perform numerous important functions as chemical signals in the rhizosphere, mediating an array of root-root and root-microbe interactions (Bais et al., 2004; Perry et al., 2007).

It is therefore clear that root exudates determine to a great extent which organisms will reside in the rhizoplane (Cook and Baker, 1983). The interaction between microorganisms and plant roots, as well as soil conditions surrounding the rhizosphere, therefore plays an important role in plant productivity and soil functioning (Sturz and Christie, 2003). Farrar et al. (2003) stated that root exudation is a combination of complex multidirectional fluxes operating simultaneously and that a better understanding is needed of its overall importance in plant nutrition, root growth and pathogen response. This will aid in establishing the rhizosphere that is needed for optimum plant performance and development of management practices that can induce this state.

1.2.4 Soil quality and soil health

With the current focus on sustainability, terms such as soil quality and soil health are used to describe the state of the soil as a means of improving recognition of the importance of soil as a resource. Doran and Parkin, (1994) defined soil quality as "the continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries, sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health". Van Bruggen and Semenov (2000) defined a healthy soil as a stable system with resilience to stress, high biological diversity, and high levels of internal nutrient cycling. The soil quality concept, furthermore, addresses the associations among soil

management practices, observable soil characteristics, soil processes, and the performance of soil ecosystem functions (Lewandowski et al., 1999).

Although soil quality is influenced by many properties inherent to a particular soil and environment, soil quality also reflect the condition of soil resulting from alteration in soil properties by human use and management (Larson and Pierce, 1991; Carter et al., 1997). Soil health is more often used to describe aspects of soil quality that reflect the condition of the soil as expressed by management-sensitive properties (Larson and Pierce, 1991; Doran and Parkin, 1994; Islam and Weil, 2000) and is mainly associated with biological diversity and stability. A positive relationship has generally been found between the microbial biomass and soil organic carbon levels (Fraser et al., 1988; Houot and Chaussod, 1995; Burgos et al., 2002; Magdoff and Weil, 2004). Soil organic carbon has therefore become an important indicator of soil quality and agricultural sustainability because of its impact on physical, chemical and biological soil properties (Reeves, 1997).

1.3. INFLUENCE OF SOIL MANAGEMENT PRACTICES ON SOIL BIOLOGICAL PROPERTIES

Management practices that cause a decline in soil functioning reduce soil quality, while proper management systems can be expected to restore ecosystem function (Reeves, 1997). It is clear from the above section that soil microorganisms play an essential role in soil quality and plant productivity through various key processes. Therefore, it is important to know the effect of agricultural management practices on the soil microbial community for a broader understanding of soil health and to establish sustainable management practices (Hill et al., 2000). However, our knowledge of soil ecosystem function is limited in part by the complexity of measuring soil microorganisms.

1.3.1 Measuring soil microbial communities

The main approaches that are used to measure soil microbial communities include microscopy, biochemical methods, physiological assays, and molecular analyses such as DNA-fingerprinting (Torsvik et al., 1996). Traditional techniques commonly rely on phenotypic characteristics and are restricted to organisms that can be isolated or cultured. Since <1%, of soil microorganisms can be cultured, these techniques can underestimate population size and diversity (Amann et al., 1995). These techniques may however be useful in discerning relative differences between soil microbial communities, without determining the abundance or identity of specific microorganisms in the population (Mazzola, 2004). Process-level studies can also be used, where microbes themselves are not isolated or identified but their activities measured (Dick, 1994; Pinkart et al., 2001; Kirk et al., 2004). However, the most promising advances are made in the use of molecular methods (Thies, 2006), with soil-extracted nucleic acids, which do not rely on the capacity to culture organisms.

Soil microflora are most frequently assessed in terms of their abundance, activity or function, and diversity or community composition. The total microbial community or specific members of the community can be assessed. Alternatively, indicators that reflect the capacity of the soil to function can also be measured (Doran and Parkin, 1994; Idowu et al., 2008).

1.3.1.1 Abundance

Traditional methods of measuring abundance include culturing organisms on artificial media, direct microscopy and extraction of specific cell components or molecules through measuring their concentration (Pankhurst et al., 1997; Thies, 2006). The most common biochemical methods used to assess abundance are fumigation-extraction (Vance et al., 1987) to measure microbial biomass carbon and/or nitrogen. Analysis based on phospholipid fatty acids (PLFA) or fatty acid methyl esters (FAME) are useful due to their presence in all living cells. Specific groups of organisms can be distinguished through their unique fatty acids but cannot be characterized to species level (Zelles, 1999). Other methods include detection of specific molecules (e.g ATP, glomalin, ergosterol.) associated with the soil (Jenkinson and Ladd, 1981; Newell et al., 1988).

1.3.1.2 Microbial activity and function

Generally, the rate of a specific biochemical process can be measured, for example the ability to transform one compound to another (carbon or nitrogen mineralisation) (Pankhurst et al., 1997), or the ability to metabolise specific compounds. Studies of microbial activity have been commonly conducted at a broad-scale level, through measuring microbial respiration (Hill et al., 2000). Enzymatic activities have been used as an indicator of the overall microbial activity in soils while also producing useful functional information on the capacity of a soil to carry out specific activities important in maintaining soil fertility (Dick, 1994; Dick, 1997; Garcia et al., 1997; Pascual et al., 2001; Ros et al., 2003; Caldwell, 2005; Bastida et al., 2008). Furthermore, molecular techniques, such as real-time or quantitative polymerase chain reaction (PCR) can be used to quantify target genes that reflect the capacity of microorganisms to perform specific functions, e.g. nitrite reductase, to quantify denitrifying soil bacteria in a given sample (Henry et al., 2004).

1.3.1.3 Diversity and community composition

Community level physiological profiling (CLPP) is a method that has been extensively used to obtain insight into functional diversity or composition of microbial communities (Garland and Mills, 1991). The Biolog® system is commonly used where utilization of various carbon sources are employed. The pattern of substrates oxidized can then be compared among soil samples as an indication of differences in physiological or metabolic function. Community profiling based on PLFA and FAME analysis can also be used to measure diversity or structural composition of the microbial community based on the groupings of fatty acids or presence and abundance of specific fatty acids extracted from soil (Ibekwe and Kennedy, 1998). Another method used is diversity indices, for example the Shannon-Weaver index which includes parameters such as species richness and evenness (Pankhurst et al., 1997).

The heterogeneity of DNA recovered from soil can also be used as a reflection of community diversity (Torsvik et al., 1990). Extracted DNA can be used either with DNA-DNA hybridization to detect specific genes in the soil (Holben et al., 1988; Torsvik et al., 1990), or with primers to amplify portions of the DNA, generating electrophoretic patterns of DNA fragments separated in different ways, resulting in profiles used as genetic fingerprints (Thies, 2006). These methods 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). Some DNA approaches compare individual rDNA sequences to a database of previously encountered sequences in order to assess diversity (Olsen and Woese, 1993). These DNA-based methods in a large part make use of PCR for amplification of the small subunit rRNA gene found universally in all life forms and composed of highly conserved regions as well as regions with considerable sequence variation (Woese et al., 1990; Ward et al., 1992).

1.3.1.4 Soil quality indicators

Soil ecosystem functioning are difficult to measure directly, therefore more easily measurable soil properties are used as indicators. Various indicators have been selected based on soil physical, chemical and biological properties (Doran and Jones, 1996), to aid in the routine analyses of soil quality. These indicators need to be inexpensive, sensitive to changes in management practices, highly reproducible and represent soil processes relevant to soil functions (Doran and Parkin, 1994) or more specifically, crop production (Idowu et al., 2008).

Soil physical indicators can include bulk density, porosity, available water capacity, penetration resistance and aggregate stability (Arshad et al., 1996). Soil chemical parameters are generally assessed on a routine basis and soil properties such as CEC, soil pH, extractable phosphorous and potassium as well as minor element contents, can all give indications of soil functioning (Doran and Jones, 1996; Idowu et al., 2008). Given the important role of SOM in ecosystem functions, SOM-related properties can be important soil quality indicators, especially active SOM fractions which can change in relatively short time periods (Weil et al., 2003; Flieβbach et al., 2007). Biological indicators include counts of soil fauna such as earthworms, free living nematodes, arthropods, and protozoa (Pankhurst et al., 1997). Mycorrhizal infection rates, presence of pathogens or a general root health rating, can also be used. Some groups have identified a minimum data set for monitoring soil quality, including a combination of various physical, chemical and biological indicators (Doran and Parkin, 1994; Idowu et al., 2008).

1.3.1.5 Interpretation of results

Due to the great complexity and variability of soil systems, no single method has been widely accepted for assessing soil quality, since each method has its own limitations. Although the most promising advances have been made with molecular studies, results are still dependent on DNA extraction protocols and have known biases associated with them due to the PCR process (Von Wintzingerode et al., 1997). However,

when the limit of any one technique is recognized and appropriate methods are applied, various methods can provide suitable characterization of the soil microbial community (Mazzola, 2004), especially if a combination of methods is used. Results also need to be interpreted with caution and natural environmental factors taken into account (Pankhurst et al., 1997). Since optimum or standard values are difficult to establish, it is not clear what changes are related to a significant difference in functioning of the ecosystem (Kennedy and Papendick, 1995; Bünemann et al., 2006). However, comparisons between various management systems can be made within a soil type. Also, spatial variation in soil can be reduced by appropriate sampling method (Van Elsas et al., 2002). In many cases, due to a large number of variables and interactions, multivariate statistical analyses can be employed to assist in result interpretation. These include redundancy analysis (Rumberger et al., 2004), principal component analysis (Garland and Mills, 1991), discriminant analyses, hierarchical cluster analysis (Yao et al., 2005), and correlation analysis (Bulluck et al., 2002)

Despite the potential use of the methods above, it must be borne in mind that conclusions drawn on plant productivity from microbial measurements made under controlled conditions, is problematic (Phelan, 2004). It is known that the use of microcosms, hydroponic systems, sterile planting substrates, as well as systems with no plant interaction, make it extremely difficult to predict effects in a field soil environment.

1.3.2 Effect of conventional management practices on microbial communities

Conventional farming practices have largely neglected the importance of the soil biological component in agricultural ecosystem functioning. In intensively managed agroecosystems, the role of soil organisms in nutrition and disease management has partly been replaced by the use of external inputs such as inorganic fertiliser and pesticides. Although agricultural management practices ultimately seek to increase or optimise plant productivity, globally, sustainability is becoming an important factor. In order to identify more sustainable management practices the effect of current conventional practices on soil microbial properties have been investigated over the past two decades.

1.3.2.1 Inorganic fertiliser

The importance of rhizosphere processes in nutrient availability has been largely neglected due to the supply of luxurious quantities of synthetic fertilizers. However, there is little evidence for significant direct negative effects of inorganic fertiliser on soil organisms (Bünemann et al., 2006) and criticism is mainly against its sole use, leading to soils low in organic matter (Flieβbach et al., 2007). Indirect effects are related mainly to increased biological activity with inorganic fertiliser application due to increased plant productivity, crop residues and soil organic matter (SOM) levels (Allison, 1973; Martyniuk and Wagner, 1978; Bünemann et al., 2006). Decreased biological activity is related to a decrease in soil pH (Wardle, 1992). The injudicious use of N fertiliser, especially ammonium fertilisers, is a major contributor to soil acidification (Pierre et al., 1971; Rasmussen and Rohde, 1989) and effects bacterial populations negatively.

Different views exist on the effect of inorganic N on SOM levels. Since microbial activity is often limited by N and the readily available N leads to rapid microbial decomposition of organic matter in soils, excessive N fertiliser applications can result in a decline in organic matter (Jenkinson et al., 1985; Green et al., 1995; Ball, 2006). However, a study by Allison, (1973) suggested that there is only a short term stimulation of organic matter decomposition and that long-term SOM levels are not affected.

A specific group of soil fungi, the arbuscular mycorrhizal fungi (AM fungi) form mutualistic associations with tree roots and benefit the plant through improved nutrition. It has been reported that mycorrhizal inoculation is sensitive to phosphorus enrichment (Abbott et al., 1984) and also that high nitrogen concentrations (Johnson et al., 2003; Blanke et al., 2005) can decrease AM fungal colonization. This increased availability of nutrients to microbes induces plants to allocate less carbon to the roots, affecting root exudates, as well as reducing the amount of carbon available to AM fungi (Douds and Johnson, 2003). Fertilisation can also change species composition and select for less mutualistic AM fungi (Johnson, 1993) increasing the chance of parasitic interaction.

1.3.2.2 Pesticides

Soil organisms and thus soil functions can directly be affected by pesticides, but it is unclear if these changes are long-term (Bünemann et al., 2006). Direct effects of pesticides on soil organisms depend on type, specificity, and rate of application. Furthermore, repeated applications to the same soil can lead to more effective microbial degradation so that the efficacy of the pesticide is reduced dramatically, with a negative effect on the plant (Roeth, 1986). Organophosphate and carbamate insecticides have generally been shown to have a significant negative effect on soil microbial numbers and activity, as well as earthworm populations (Pandey and Singh, 2004; Menon et al., 2005). Insecticides also critically disturb soil protozoa (Foissner, 1997) and are often toxic to non-target insects such as predacious and parasitic arthropods (Koehler, 1992). Insecticides also impact the diversity and abundance of nematode trophic groups (Yeates and Bongers, 1999).

The negative affect of fungicides on the soil fauna and flora seem to be even greater than insecticides (Bünemann, et al., 2006). Pascual et al. (2002) suggested that some fungicides have a non-specific effect acting not only on the pathogen, but also on non target organisms. Especially with copper-based fungicides these negative effects are likely to persist for many years, as copper accumulates in surface soils and is not prone to biodegradation (Filser et al., 1995). Merrington et al. (2002) showed that residues from copper fungicides were responsible for significant reductions in microbial biomass. Negative effects have also been found with benomyl on mycorrhizal associations (Smith et al., 2000).

Some herbicides affect earthworm populations negatively (Pizl, 1993; Amorim et al., 2005). Herbicides used in conventional systems may furthermore be related to reduced disease resistance. Increased soilborne root diseases can be caused by glyphosate, via glyphosate inhibition of systemic resistance to the crop it is

meant to benefit (Liu et al., 1997; Descalzo et al., 1998). In addition, routine use of herbicides, especially pre-emergence herbicides, leads to a reduction in orchard floor organic matter coverage and can reduce total microbial populations as a result of reduced input of organic residues (Wainwright, 1978).

Suppressive effects of non-fumigant nematicides on non-target organisms have been reported, depending on the nematicide used. Long-term negative effects of fenamifos on free-living nematodes and microbial functional diversity was documented by Pen-Mouratov and Steinberger (2005) and Kaffe-Abramovich and Steinberger (2006). However, the organophosphates imicyafos and fosthiazate were less toxic to growth of fungi and bacteria (Wada and Toyota, 2008). Nematicides were also found to temporarily increase microbial activity under certain conditions (Sumner and Bell, 1982), possibly due to rapid degradation of the nematicide used as a carbon source.

However, with increasing specificity and reduced dosages of active ingredients with new formulations, negative effects on soil microorganisms are less frequently recorded (Fraser et al. 1988; Murphy et al., 2003).

1.3.2.3 Fumigation

Apart from its effect on plant pathogens and soilborne pests, fumigation affects microbial activity as well as the structure and functionality of the soil microbial community. Pronounced negative effects have been shown in the first few weeks after fumigation, as well as persistent effects on some microbial parameters up till two years. However, laboratory microcosm experiments have been used in many studies to investigate the effect of fumigants on soil microbial properties and it is not clear to which extent results are applicable to field conditions.

Effects on broad-scale properties such as total culturable bacteria and microbial biomass, were generally found to be less persistent (Ridge, 1976; Sinha et al., 1979; Toyota et al., 1999; Stromberger et al., 2005). However, in the studies of Tanaka et al., (2003) as well as Yamamoto et al. (2008) chloropicrin (CP) had a large and long-term impact on microbial biomass and activity. Sinha et al. (1979) reported that fungi and *Rhizobia* populations were drastically reduced in fumigated soils, while populations of bacteria, actinomycetes and *Azotobacter* populations gradually increased again over a 45 day incubation period after fumigation. Soil fumigation is often followed by a shift of the bacterial community composition towards Gram positive bacteria dominating the community structure (Ibekwe et al, 2001). Gram positive bacteria are seemingly less affected by fumigation (Zelles et al., 1997; Klose et al., 2006), however, gram negative bacteria can recover rapidly (Toyota et al., 1996; Xiao and Duniway 1998). Increases in Gram negative bacteria were observed within three weeks after fumigation and persisted for up to one year (Porter et al., 1999).

Persistent effects of fumigation on microbial diversity have been found, depending on the fumigant used. Various studies indicated an inhibition of nitrification for long periods (Rovira, 1976; Malkomes, 1995; Tanaka et al., 2003; Stromberger et al., 2005). In a greenhouse study by Toyota et al. (1999) it was shown that the size of the ammonium and nitrite oxidizer populations was reduced by four orders of magnitude and started showing slight recovery only after 100 days. Klose and Ajwa (2004) concluded that organic matter turnover and nutrient cycling and therefore long term soil productivity, was unaffected in soils fumigated with the fumigants methyl iodide (Midas), propargyl bromide (PrBr) and chloropicrin (CP), but that the combination of methyl bromide (MeBr) and CP showed a severe effect. Wada et al. (2008) concluded that disturbances of organic matter decomposition and denitrification, associated with dichloropropene (1,3-D) and CP disinfestation may be temporary so that soil microbial function recovered significantly during the cropping season. Ibekwe et al. (2001) in their study found distinct shifts in both species composition and relative abundance of different bacterial groups with fumigation. High diversity was maintained for control soils as well as soils fumigated with methyl isothiocyanate (MITC) and 1,3-D with CP. However, diversity significantly decreased with MeBr fumigation, although it did show some recovery after 12 weeks. In contrast to results by Ibekwe et al. (2001), Toyota et al. (1999) found substantial changes in carbon utilisation profiles even 105 days after fumigation with MITC, showing a drastic reduction in community function**.** Yao et al. (2006) showed that in an apple orchard, soil fumigation with a mixture of 1,3-D and CP, resulted in an altered rhizosphere bacterial community even one year after planting, however differences were less significant after 22 months.

Klose et al. (2006) found that soil enzyme activities were generally reduced by various fumigants over a 90 day period in microcosm studies. Furthermore, it was evident that some fumigants were more toxic to specific enzyme reactions than to the overall microbial community as measured through the FAME profiles. Stromberger et al. (2005) showed in strawberry field plots with various fumigants that the effect on arylsulfatase and acid phosphatase activity were most severe and persisted through the full 37 week trial period.

The negative effect of fumigation on indigenous AM fungi is well established (Menge, 1982; Trappe et al., 1984; Klose et al., 2006). McGraw and Hendrix (1984) linked stunted growth and P, Cu, and Zn deficiency occurring after fumigation to the destruction of AM fungi. Furthermore, soil fumigation can nullify natural soil suppressiveness to plant diseases and thereby have a negative impact on plant growth (Van Os et al., 1999).

1.3.2.4 Tillage

The greater the intensity of energy inputs to the soil through tillage, the greater the rates of organic matter decomposition (Neher, 1999; Watts et al., 2000). Tillage therefore plays an important role in the management of soil nutrients through its influence on SOM dynamics. The action of tillage enhances aeration, resulting in favourable conditions for rapid mineralization of C and other organically bound

nutrients (Parr and Papendick, 1978). In addition, the breaking apart of macro-aggregates by tillage increases the availability of occluded SOM to soil organisms (Six et al., 1999) and thereby increases the potential for soil erosion (Magdoff and van Es, 2000; Johnson et al., 2005). Many of the soil fauna, as well as fungi, are extremely sensitive to tillage practices (Osler, 2003; SP-IPM, 2004), therefore reduced tillage systems have different food webs than intensively tilled systems (Verhoef and Brussaard, 1990).

The effect of tillage has a less significant role in perennial fruit crops, however, weed control through intensive cultivation can also degrade soil structure, disrupt soil faunal communities and accelerate organic matter loss (Hoagland et al., 2008).

1.3.3 Promoting biological activity in soil

Management strategies must provide a favourable environment for soil fauna and microflora because of their dominating role in mineralisation and immobilisation processes. There is increasing evidence of the impact of organic amendments and soil inoculants on soil health and the presence of beneficial microorganisms (Abawi and Widmer, 2000; Albiach et al., 2000; Bulluck et al., 2002; Van Bruggen and Semenov, 2000; Van Bruggen and Termorshuizen, 2003). Biological activity in soil can be promoted by stimulation of the resident soil microbes through addition of available carbon sources, by improving root growth proliferation and plant health or by addition of microbes to the soil. Management practices that can promote biological activity in soil therefore mainly include strategies that increase or retain SOM, the addition of supplemental microbial food sources or biostimulants, as well as direct soil inoculation with specific microorganisms, or mixtures of various microbial groups.

1.3.3.1 Organic matter amendment

Microbial activity is generally carbon-limited in agricultural soil and the addition of an available carbon source tends to increase microbial proliferation (Campbell, 1989; Magarey, 1999; Termorshuizen et al., 2004; Bünemann et al., 2006). Therefore, although amendments vary widely in terms of characteristics of material used and rates applied, application of organic material is one of the most effective ways to increase microbial activity and numbers of beneficial organisms (Albiach et al., 2000; Bailey and Lazarovits, 2003; Pérez-Piqueres et al., 2006). Differences in soil biological properties between conventional and organic systems are not necessarily the direct result of application of inorganic fertilizer and pesticides, per se, but rather results from increased substrate availability and a better soil environment with the use of organic amendments (Fraser et al., 1988). Organic matter can also have an indirect positive effect on soil microbial activity through its beneficial effect on soil physical properties, soil chemical properties and plant and root growth.

Management practices that can be applied to increase SOM mainly include crop residue management, organic mulching, application of raw or composted organic material (urban sludge, biosolids, manure, compost) and the use of cover crops or green manure (Magdoff and Weil, 2004).

1.3.3.1.1 Compost and Manure application

Composting is the controlled biological decomposition of biodegradable material under predominantly aerobic conditions that allow the development of thermophillic temperatures as a result of biologically produced heat, in order to achieve compost that is sanitary, uniform and stable (Epstein, 1997). Interest has also increased progressively in vermicomposting, a process involving the use of earthworms to promote microbial activity in organic waste, breaking it down into materials that can be used in crop production (Edwards and Flectcher, 1988). The most common raw materials used in composting are municipal solid waste (or biosolids), sewage sludge, wastes of the timber and food processing industries and manure (Raviv, 1998). Many factors must be controlled to obtain consistent effects with the use of organic amendments. Compost quality is affected by its input materials, moisture content as well as the maturity of the end product (Hoitink et al., 1997; Lazarovits, 2001; Hoitink and Changa, 2004).

Manure has not undergone aerobic composting and can be defined as animal excrement which may contain large amounts of bedding (Litterick et al., 2004). The type of animal, as well as bedding content (C:N ratio) plays an important role. Magdoff and van Es (2000) noted that dairy and beef manures containing high amounts of lignified substances (bedding and undigested forage) contributed more to SOM maintenance than poultry manure. Manure is furthermore a less stable source of organic matter because it consists primarily of easily decomposable organic mater and its effect on SOM content will therefore be of a more temporary nature (Termorshuizen et al., 2004). Volatilisation is a problem with handling and storage of manure, as well as when surface applied (Kotzé and Joubert, 1992).

Animal manures are mainly used to supply N in systems where use of chemicals is reduced or eliminated (Jenkinson, 1991) and generally has a high content of macro and micronutrients, depending on the type of manure. Fraser et al. (1988) found that soluble P levels were eight fold greater in manure amended surface soils. However, Kotzé and Joubert (1992) concluded that poultry manure holds no particular advantage in deciduous fruit over inorganic fertiliser and that the rate of application as well as N content should be taken into consideration. Nutrients in compost are usually less available than in fresh manure because of stabilisation by microbial assimilation during the composting process (Hadas et al., 1996). In general, compost shows low lability of N and large amounts may be initially needed to supply enough nutrients to achieve optimum yield (Eriksen et al., 1999; Chung et al., 2000). Also, compared to synthetic fertilisers, benefits are not always apparent over the short term (Carpenter-Boggs et al., 2000). However, an increase of SOM in soil continually applied with compost for multiple years can result in improvement of soil quality, facilitating nutrient availability and uptake (Pascual et al., 1997; Roe, 1998; Pinamonti, 1998; Termorshuizen et al., 2004).

Application of either compost or manure can rapidly improve biological aspects of soil quality and positive effects on soil microbial communities were documented for a diversity of agricultural systems, including fruit trees (Renagnold et al., 2001; Mäder et al., 2002), grain crops and vegetables (Fraser et al., 1988; Parr

and Hornick, 1992; Temple et al., 1994; Angers et al., 1995; Drinkwater et al., 1995; Gunapala and Scow, 1998; Carpenter-Boggs et al., 2000; Flieβbach et al., 2007). Greater microbial activity or biomass has been found, as well as higher diversity of bacteria (Bolton et al., 1985; Doran et al., 1987; Reganold et al., 1993; Wander et al., 1994; Drinkwater et al., 1995; Katayama et al., 1998; Mäder et al., 2002). Furthermore, a significant effect on soil microbial community structure was found within a group of organisms, as well as ratios of various organism groups (Marschner et al., 2003; Yao et al., 2006; Lejon et al., 2007). Toyota and Kuninaga (2006) showed from utilisation of different carbon sources that soil microbial communities from manure amended soil had the ability to utilise a greater variety of substrates. Furthermore, actinomycetes seem to dominate in manure amended soil (Lechevalier, 1988). In general, minor impacts have been found with organic amendments on fungal communities compared to prokaryotic communities (Marschner et al., 2003; Rumberger et al., 2004; Lejon et al., 2007; Yao et al., 2006), with the exception of the AM fungi (Oehl et al., 2003). However, increased capacity and complexity of macro-, micro-, and mesofauna have been documented widely (Freckman and Caswell, 1985; Drinkwater et al., 1995; Van Bruggen, 1995; Hartley et al., 1996; Ferris et al., 1998; Bulluck et al., 2002; Mäder et al., 2002; Mulder et al., 2003).

The duration of observed effects on microbial parameters, however, depends on the amount of readily decomposable carbon substrates added and availability of nutrients, especially N (Hartz et al., 2000; Adediran et al., 2003). Therefore, microbial characteristics can often return to baseline within a few years (García-Gil et al., 2004) if there is not continued application such as is the case in organic or biological systems. Furthermore, although direct C addition through organic amendments plays a major role in stimulating soil organisms, the role of C quality is not yet well understood (Bünemann et al., 2006).

Compost can suppress disease through modification of the microbial community structure (Hoitink and Fahey, 1986; De Ceuster and Hoitink, 1999; Hoitink and Boehm, 1999; Litterick et al., 2004; Noble and Coventry, 2005). These studies have demonstrated suppressive effects of composts on soilborne diseases such as damping off, root rots (*Pythium, Phytophthora, Rhizoctonia*) and wilts (*Fusarium*), mainly in container media, and ornamental crops. However, compost has also shown to suppress several diseases in the field (Malajczuk, 1983; Hoitink et al., 1991) although effects are generally smaller and less consistent. The impact of manure on pests and diseases is much less predictable than that of composts (Litterick et al., 2004). However, compost variability is an important factor when using compost for disease control (Hoitink et al., 1997). Few composts are universally effective and specific compost properties have significant effects on disease suppressiveness (Hoitink and Fahey, 1986; Litterick et al., 2004). To maximise natural disease control, composts should be mature (Kuter et al., 1988) and can also be inoculated after peak heating with specific biological control agents or mixes.

The application of organic material also has the potential to show harmful effects on plant and soil. Excessive rates of application can cause overloading of nutrients in soil. Manure based compost treatments showed high P and K surplus after three years, showing that loading levels of these nutrients need

consideration, especially when using compost to satisfy crop N needs (Reider et al., 2000). Nutrient or metal loading in raw or partially composted amendments can negatively impact the soil microbial community, especially naturally occurring antagonists such as nematode trapping fungi and predacious nematodes (Giller et al., 1999; Jaffee, 2004; Forge et al., 2008). Results from Varga et al. (2004) showed lower mycorrhizal colonisation rate with addition of livestock manure, suggesting that high nutrient release from manure can adversely affect apple root colonization by AM fungi.

In practise, these organic amendments are mainly applied as a top dressing, or mixed into the top layer of orchard soil. However, there is no clear indication from literature which method is most beneficial. Application of manure to the soil surface may result in volatilisation (Kotze and Joubert, 1992) and increased organic matter breakdown, although Kayuki and Wortmann (2001) found that mineralization were faster when high-quality organic residues (low in lignin and phenols) were incorporated. Application to the soil surface also reduced labour costs and improved soil cover. Lejon et al. (2007), as well as Marschner et al. (2003), found that the size of carbon biomass was increased whatever the type of organic matter input or agricultural practise (surface application vs. incorporation) and that specific changes in the soil microbial community were more dependent on the type of organic amendment, as well as the type of soil (Pérez-Piqueres et al., 2006; Lejon et al., 2007).

1.3.3.1.2 Organic mulches and crop residue management

Mulching is a protective layer of material that is maintained on the surface of cultivated soil. Various forms of organic material, including crop residue, can be used as mulches. Conservation of soil moisture is considered one of the most significant advantages of mulching, especially in the case of fruit trees (Woodbury et al., 1917; Baxter, 1970; Tisdall et al., 1984; Hogue and Neilsen, 1987; Merwin et al., 1994; Walsh et al., 1996*;* Neilsen et al., 2003b, 2007). It is most likely a consequence of both improved infiltration capacity and reduced evaporation from the soil surface so that maximum field capacity is maintained longer at all depths (Schroch and Shribbs, 1986; Kotzé and Joubert, 1992; Faber et al., 2001). In addition, mulching provides isolation from extreme temperature fluctuations (Gregoriou and Raj Kumar, 1984; Wooldridge and Harris, 1991; Hartley et al., 1996; Pinamonti, 1998). Furthermore, mulches suppress weeds and therefore competition for nutrients (Lanini et al., 1988; Niggli et al., 1990; Autio et al., 1991; Pinamonti et al., 1995; Faber et al., 2001).

Mulching provides a favourable environment for microbial activity and fine feeder root development, especially in surface soil. Mulch treatments have generally been found to increase soil respiration and total microbial biomass as well as increase or at least conserve SOM content (Hogue and Neilsen, 1987; Niggli et al., 1990; Merwin et al., 1994; Marsh et al., 1996; Werner, 1997; Tiquia et al., 2002; Yao et al, 2005). Varga et al. (2004) found in apple orchards that numbers of total bacteria, cellulose decomposing bacteria and fungi were higher under straw and pine bark mulch than in soil where no mulch was applied. Results by Yang et al. (2003) indicated that the effect of organic mulches on soil bacterial communities one year

after application was dependent upon the type of mulch used and effects were mostly exerted in the top few centimetres of the soil (<5 cm). In a study by Tiquia et al. (2002) mulching with compost influenced structure of the microbial rhizosphere community more than plots mulched with woody material.

Studies on orchard floor management practices in apple, have demonstrated that the use of organic materials as mulches can have profound effects on the structure of the soil food web, specifically beneficial nematodes (Forge et al., 2003; Yao et al., 2005). Surface application of organic matter can also greatly stimulate earthworm populations (Syers and Springett, 1984).

However, mulching can in some cases affect soil biological properties negatively. Saw and wool dust used as mulches in apple orchards decreased the total number of earthworms (Hartley et al., 1996), as well as bacterial and fungal biomass. The higher ratio of basal to substrate induced respiration (SIR) also suggested that these mulches caused soil microflora to respond with lower efficiency. Larsson et al. (1997) found significantly higher respiratory activity per unit biomass (qCO_2) associated with wood chip mulch which is similar to effects on qCO_2 found in sites with reduced fertilizer compared to adequate fertliser (Insam et al., 1991). Furthermore, moisture conserving properties associated with mulching may in certain conditions and soil types be undesirable (Hogue and Neilsen, 1987). Wooldridge (1992) found that, with hay mulch, the period over which free water was retained after rain was increased. Merwin et al. (1992) ascribed *Phytophtora* crown and root rot development in 35% of straw mulch plots to prolonged soil saturation beneath mulched apple trees at poorly drained sites. Faber et al. (2001) also stated that irrigation had to be adjusted with mulching to accommodate higher soil moisture contents that could be conducive to root rots.

1.3.3.2 Cover crops

In most organic systems, soil management involves the use of mowed or tilled cover crops (Sánchez et al., 2007). Cover crops supply organic matter, provide soil cover to preserve soil structure, and can immobilise and retain available soil N to prevent leaching (Lewan, 1994; Magdoff and Weil, 2004; Komatsuzaki and Ohta, 2007). Often a legume is chosen to add the benefit of nitrogen fixation (Marsh et al., 1996). Additional benefits include breaking of the pest cycle and weed suppression. Furthermore, soil ecological diversity is enhanced through root exudates and decaying residues from the cover crop, contributing to the labile carbon compounds that stimulate microbial activity (Rovira et al., 1990; Gu and Mazzola, 2003).

In addition to providing material for mulching, cover crop root systems continuously provide organic matter as roots die off and are decomposed (Bolton et al., 1985). Wander et al. (1994) stated that belowground C inputs may play a more significant role in SOM accumulation than mulching. In vineyards Fourie et al. (2007) showed that cover crops had the ability to significantly increase the organic matter content of a sandy soil over a 5 year period. Soil organic matter of the top 0-30 cm soil was improved to a greater extent if the cover crop was controlled chemically, rather than mechanically. Similarly, SOM in the

top soil layer was improved more when cover crop material was left on the soil surface compared to when it was incorporated into the soil. Sanchez et al. (2007) found that disking of cover crops may decrease SOM content and therefore was not recommended since it leads to poor vigour.

Root exudates determine to a great extent which organisms will reside in the rhizoplane (Cook and Baker, 1983). Cover crops can therefore enhance populations of specific resident microbial antagonists to create disease suppressive conditions (Vargas-Ayala et al., 2000; Mazzola and Gu, 2002; Weller et al., 2002). Furthermore, the use of cover crops can significantly effect AM fungal composition (Douds and Johnson, 2003) and has the potential to increase AM fungal spore numbers, thereby enhancing the inoculation of the following crop. Furthermore, biofumigant cover crops can also be used or applied as soil amendment for disease control (Brown and Morra, 1997). These crops are from the Brassicaceae family and produce glucosinolates which upon hydrolysis yield compounds with antimicrobial activity. Some evidence also suggest that these plant species may operate in suppression of fungal pathogens irrespective of their glucosinolate content, by transforming the bacterial community structure (Cohen et al., 2005).

A significant amount of research has been carried out on residues of some species of cover crops that can suppress populations of plant parasitic nematodes or reduce infection levels of roots when applied to soil (D'Adabbo, 1995; Abawi and Widmer, 2000; Akhtar and Malik, 2000). Marigold (*Tagetes* spp.) and sudangrass (*Sorghum sudanense* × *sudanense*) are widely used in agriculture to reduce populations of root-knot nematodes (Siddiqui and Alum, 1987; Ploeg and Maris, 1999). Some *Brassica* species have also been shown to suppress plant parasitic nematodes (Brown and Morra, 1997; Cohen et al., 2005). Vargas-Ayala et al. (2000) observed that cropping soils to velvet bean altered the composition of microbial communities in the soil rhizosphere, possibly through enhanced parasitism of *Meloidogyne incognita* eggs by a diverse group of bacteria and fungi.

1.3.3.3 Microbial soil inoculants

In agricultural systems, the integration of beneficial microorganisms into production systems can to some extent shift the balance of the microbial communities towards a population structure more conducive to increased plant health and productivity (Avis et al., 2008). Plant-beneficial microbial interactions can be divided into microorganisms playing a role in nutrition, those preventing the effects of phytopathogenic organisms in the rhizosphere and those that can directly affect growth, for example through production of phytohormones. (Frankenberger and Arshad, 1995; Glick, 1995; Rodriguez and Fraga, 1999; Kennedy and Islam, 2001; Zahir et al., 2004). Inoculation of soil with beneficial rhizosphere organisms can include specific species or strains, but also mixture of various microorganisms. Inoculants containing broad diversity without exact definition of the active microbes, includes effective microorganisms (EM Technology™) (Higa, 1994) and compost extracts or teas (Ingham, 1999a; Litterick et al., 2004).

1.3.3.3.1 Inoculation with specific rhizosphere microorganisms

Two of the most important and beneficial root interactive microbes are the plant growth promoting rhizobacteria (PGPR) and AM fungi (Kloepper et al., 1980; Azcon-Aguilar and Barea, 1997; Van Loon et al., 1998). These microorganisms cause changes in root architecture and enhanced root function that can influence plant health, growth, yield and product quality (George, 2000; Zahir et al., 2004). To produce the desired effect, inoculated soil organisms must survive in the soil and have the ability to compete with resident microflora to successfully establish in the rhizosphere (Hirsch, 1996). Growth responses to microbial inoculation have also been reported to be subject to strain, crop, as well as site specificity (Martin, 2003; Zahir et al., 2004).

Deciduous fruit trees are widely considered to benefit from the formation of mutualistic associations between their roots and AM fungi, mainly through improved plant nutrition, as well as increased protection against various stress factors, but also possibly by generating a distinct soil microbial community due to changes in root chemistry and exudates (Linderman, 2000). AM fungal communities are studied specifically in agricultural systems, where conventional practices such as the use of pesticides, herbicides and soluble fertilisers impact natural AM fungal infection negatively (Purin et al., 2006). Since AM symbiosis can benefit plant growth and plant health there is increasing interest in ascertaining their effectiveness in agricultural systems and, consequently, in manipulating them so that they can be incorporated into production practices. Specific microbial populations in the rhizosphere can benefit the establishment of mycorrhizal symbioses (Gryndler, 2000). A typical example is that exerted by ‘mycorrhiza-helper-bacteria’ (MHB), known to stimulate mycelial growth of AM fungi as well as ectomycorrhiza (Garbaye, 1994). The bacteria produce compounds that increase the rates of root exudation, which in turn stimulate mycorrhizal mycelia, or facilitate root penetration.

Plant growth promoting rhizobacteria have become a new class of biofertilisers and physiological stimulators in recent years (Zahir et al., 2004) and their use to increase plant productivity has been extensively reviewed (Lazarovits and Nowak, 1997). These inoculants hold great promise as potential agricultural inoculants and if effective, could reduce the use of agrochemicals including fertilisers and pesticides. Plants are treated in several ways such as seed coating, root dips for transplants and watering into the soil.

1.3.3.3.2 Effective microorganisms (EM)

Effective microorganisms (EM), was developed in Japan in the 1970’s and has mainly been used in nature farming systems in Asian countries. It is a mixed microbial culture of naturally occurring beneficial microorganisms, consisting primarily of photosynthetic and lactic acid bacteria, yeasts and actinomycetes that co-exist in liquid culture (Higa, 1994). Microorganisms are blended in a molasses or sugar medium and maintained at low pH. It has been used to improve soil quality and growth and yield of crops, as well as control disease and is usually used on plant seeds, in soils, sprayed on organic matter or on plant leaves.

The adoption and use of EM in nature farming systems has preceded the essential fundamental research on the exact mechanisms of how it affects soil-plant ecosystems. Although exact mechanisms of how it elicits beneficial effects are largely unknown, the principle activity of EM is thought to be through increasing the biodiversity of the microflora. The EM concept may be considered controversial in some quarters due to lack of scientific evidence to support all of its claims. Condor-Golec et al. (2007) reviewed EM literature and principles and concluded that there is a great amount of non-reliable information about EM and that the effects in soil health were minimal. However, many farmers still use EM. Also, some positive results have been reported, although mostly from Asian countries (Higa, 1994; Higa,1998; Parr et al., 1998; Xu, 2000). Higa and Wididana (1991) stated that EM is not a substitution for other management practices but is an additive for optimising al other amendments and practices used for crop production.

1.3.3.3.3 Compost extracts

Application of compost extracts, also commonly referred to as compost teas, has mainly been shown to improve plant health through reduced incidence and severity of foliar disease (Weltzein, 1991; Scheurell and Mahaffee, 2002). However, its use as a soil inoculant to improve beneficial microbial activity has also recently been advocated (Litterick et al., 2004). Unfortunately, very little scientific research has been done to confirm or quantify these benefits. Indications are that the extracts act more as a microbial inoculant that stimulates soil microbial population effectiveness, than as a nutrient source (Carpenter-Boggs, 2005) and it was suggested that these amendments should mainly be used for disease control. Considering the diverse microbial community present in compost extracts, it can be expected that multiple modes of activity can be associated with observed effects.

Compost tea is produced by mixing compost with water and incubating it for a defined period, either actively aerated (aerated compost teas, ACT) or not (non-aerated compost teas, NCT) and with or without additives intended to increase microbial population densities during production (Scheuerell and Mahaffee, 2002). A commonly recommended additive is molasses (Ingham, 1999b), presumably because this is a readily available nutritional source for microbial growth and is relatively inexpensive. The efficacy of compost teas depends greatly on the production process (Bess, 2000; Bess et al., 2002) and the compost used. Factors that influence the efficacy of compost teas include compost material, compost maturity, water ratio, fermentation time, added nutrients or other amendments, temperature and pH (Litterick et al., 2004). Most studies have been conducted with the use NCT. However, the necessity to aerate during compost tea production has been debated in order to prevent re-growth of human pathogens in the starting material. Concern was also raised that additive nutrients such as molasses, are accessible to virulent *Escherichia coli* types present in the compost due to inadequate composting or improper handling (Duffy et al., 2004). However, it seems that if the compost is devoid of *E. coli*, compost teas also did not contain *E. coli* (Kannangara et al., 2006).

Limited work has been published on the efficacy of ACT and there is currently little scientific evidence to demonstrate that they are any more effective than NCT. In a study on soilborne diseases Scheurell and Mahaffee (2004) found that both ACT and NCT could significantly reduce disease on cucumber caused by *Pythium ultimum* in peat based growing media. However, the most consistent formulation to induce suppressiveness was ACT produced with kelp and humic acid additives. Furthermore, ACT produced with a molasses-based additive negated the suppression of damping-off. Heating and dilution with water (1:9) also negated suppression. There was also an indication that for disease suppression the selection of additives was more critical than the source of compost used. This could be important for widespread application, since these additives can be standardised, whereas the properties of compost vary to a great extent.

1.3.3.4 Biostimulants

Biostimulants are being used increasingly in horticulture, however peer reviewed research results are not abundant. Although definitions vary, these are generally non-nutritional products which have a beneficial effect on plant growth, especially root growth, and may reduce fertilizer use, or provide resistance to water and temperature stress (Russo and Berlyn, 1990). Commercial biostimulants mainly include HS, seaweed extracts, and combinations thereof with other plant metabolites /organic substances such as vitamins (Kelting et al., 1998).

1.3.3.4.1 Humic substances

Humic substances (HS) comprise a major part of SOM and are classified into humic acids (HA), fulvic acids (FA) and humin on the basis of their solubility in water as a function of pH (Swift, 1999). Humic acids are not soluble in water under acidic conditions, but soluble at higher pH values, while FA is soluble in water at all pH conditions and humin insoluble in water. These substances are part of a supra-molecular complex containing several heterogeneous compounds with relatively low molecular mass but dynamically associated with hydrophobic interactions and hydrogen bonds (Sutton and Sposito, 2005). Therefore, despite their high molecular mass, these substances can express bioactivity and are involved in many reactions contributing to various soil physical, chemical and biological properties.

Their most important role is the chelation of ions, increasing their availability to organisms, including plants. However, results from Mylonas and Mccants, (1980), showed that HA and FA affected root development and improved growth of tobacco seedlings by means other than a source of nutrients. Humic substances can also directly stimulate plant biomass production, especially root growth (Vaughn and Malcolm, 1985; Visser, 1985a; Chen and Aviad, 1990; Nardi et al., 2002). The typical response curve shows increasing growth with increasing concentrations of HS, followed by a decrease in growth at very high concentrations.

In recent years there have been increasing interest in amending soil with HS to increase fertility of soils with low organic matter content (Mann, 1986) and a wide variety of organic amendments containing HS of different origin are available commercially. These products are mostly derived from brown coal (leonordite or lignite), or peat and applied as humates, which are the salts of humic acids that hold ions, such as K or Na, instead of hydrogen on the ion-exchange sites (Wallace and Terry, 1998). However, it is important to keep in mind that these commercial humates do not have the same biological and chemical properties as soil HS and therefore cannot be expected to have the same effect (Stevenson, 1979). Valdrighi et al. (1995) compared HA potassium salts from leonardite and compost, and found more positive effects on growth with the compost humate, which also showed less negative effects at high rates of application. Soil application has generally been reported to be more effective than foliar application (Lee and Bartlett, 1976; Cooper et al., 1998) but effects are highly dependent on concentration. Chen and Aviad (1990) concluded that the net direct effect of humic materials on growth probably involves interactions of a series of biochemical stimulations and inhibitions, thereby partially explaining the dependence of effects on HS concentration as well as the type and degree of effects observed. It is also well known that the effect of HS varies between plant species (Vaughn and Malcolm, 1985).

Humic substances, especially lower molecular weight fractions, affect soil microbial properties. Visser (1985a) showed that HA could increase the growth of a wide range of soil bacteria, as well as introduce a change in metabolism, allowing organisms to proliferate on substrates which previously they could not utilise. Valdrighi et al. (1996) also showed that ammonium and nitrite oxidisers increased with HS application. However, only slight effects have been reported on actinomycetes and fungi (Vallini et al., 1993; Bünemann et al., 2006). Various studies have also shown increased soil enzyme activities associated with HS application (Visser 1985 a, b; Vallini et al., 1993; Lizarazo et al., 2005). Results from Lizarazo et al. (2005) showed that HS from various origins, including plant residue, lignite and peat, differed in their HA and FA content and behaved differently in stimulating microbial activity. Phosphatase activity of these three humic products was highly correlated to the FA added, which was most prominent in the plant residue. Also, soils treated with humus plant residue showed higher enzyme activities and inorganic N concentrations. Ayuso et al. (1996) found that when looking at germination, HA derived from different forms of organic matter had a less favourable effect on germination than HS. This may be related to a higher content of the lower molecular fraction FA, which are contained in HS but not HA. Lower molecular weight fractions from compost also showed greater microbial stimulation than higher fractions from brown coal (Garcia et al., 1991; Valdrigi et al., 1995). These compounds of smaller molecular weight seem to show the greatest degree of bioactivity (Dell'Amico et al., 1994).

Some positive results in reducing the effect of phytopathogenic fungi have also been found with humic as well as fulvic fractions of compost and soil (Moliszewska and Pisarek, 1996; Pascual et al., 2002). Pascual et al. (2002) underlined the importance of the humic fraction of compost in reducing *Pythium* counts.

1.3.3.4.2 Seaweed extracts

Seaweed extracts derived from marine algae such as *Ascophyllum nodosum* (North Atlantic Ocean) and *Ecklonia maxima* (southern hemisphere), has been widely used in horticulture. (Verkleij, 1992). It is a commonly used organic supplement to increase plant growth and stress resistance (Russo and Berlyn, 1990). Application of seaweed preparations have increased plant growth, and specifically root growth, in various studies (Finnie and van Staden 1985; Metting et al., 1990; Crouch and van Staden, 1991).

Results from some researchers have indicated that the effect of seaweed is most beneficial when the plant is coping with adverse environmental conditions (Mooney and van Staden, 1985; Nus, 1993) such as nutrient stress or pest and diseases (Beckett and van Staden, 1989). Furthermore, the ability of crops to respond depends on soil type, type of crop, growth stage of crop, and quality of the extract (Verkleij, 1992). Liquid seaweed extract is generally applied as a foliage spray, but also as a drench into the soil and should be applied several times during the growing season, because of its cumulative effect (Verkleij, 1992). Van Staden et al. (1995) showed that application of a seaweed concentrate was optimal early in the seedling life of *Eucalyptus* species, but had little benefit in application after transplantation. The most documented active ingredients of seaweed extracts are trace nutrients and plant hormones, while osmoprotectants, such as betaines, have also been identified. Seaweed extracts can also be a source of microelements (Pattison, 1994), although big differences are found in trace elements between products of various manufacturers (Verkleij, 1992). Some products have also been found to contain macronutrients (P, Ca). Nelson and van Staden (1986) suggested that maximum yields achieved at sub-maximal application rates as well as low content of trace elements at applied rates, indicated that seaweed extracts showed a growth stimulant effect rather than a direct nutrient effect. However, seaweed extracts may also affect nutrition through acting as nutrient chelators (Metting et al., 1990).

It is widely believed that the application of seaweed extracts results in enhanced root zone microbial growth, development and activity, resulting in increased organic matter breakdown and nutrient availability (Dixon and Walsh, 1998; Hunter, 2004). Seaweed extracts can also alter the mode of activity of microorganisms, thus directly or indirectly affecting characteristics such as root colonisation and penetration, and biological control mechanisms (Kuwada et al., 1999; Dixon and Walsh, 2004). Results from Kuwada et al. (1999) also suggested that extracts contain growth stimulatory substances to AM fungi.

1.4 MECHANISMS THROUGH WHICH BIOLOGICAL AMENDMENTS AFFECT PLANTS

Mechanisms through which plant performance is affected by biological amendments can not be easily separated and result from a number of direct and indirect effects. These mainly include stimulation of soil microbial activity and improved soil physical conditions, leading to increased root proliferation, improved nutrient availability and uptake, protection against pests and diseases and decontamination of pollutants.

1.4.1 Soil physical properties

One of the most important effects of organic amendment application and the resulting stimulation in microbial activity is the improvement in soil physical properties. Main effects are increased total porosity and reduced bulk density (BD) and penetration resistance, as well as improved soil water conditions through the formation and stabilisation of soil aggregates (Tester, 1990; Roe, 1998; Neilsen et al., 2003a; Magdoff and Weil, 2004). These parameters have been indicated as accurate predictors of root system performance by Thompson et al. (1987). Fraser et al. (1988) found that soil microbial numbers are greatly stimulated by the addition of manure through modification of soil physical characteristics. The favourable effects of mulching on root proliferation is widely documented (Boynton and Oberly, 1966; Baxter,1970; Hogue and Neilsen 1987; Moore-Gordon et al., 1996; Pinamonti, 1998; Yao et al., 2005; Forge et al., 2008). This has been attributed to improved soil moisture conditions under the mulch that are more beneficial for surface root activity. Pinamonti et al. (1995) showed that improved porosity, and water retention, as well as reduced temperature fluctuations in vineyards supplied with compost were more important in improving nutrient uptake than increased availability of nutrients.

1.4.2 Plant nutrition

Biological amendments can affect plant nutrition either directly by supplying bulk nutrients, or indirectly through increasing the availability and uptake of nutrients, increasing cation exchange capacity (CEC), affecting pH, or preventing leaching of nutrients. Furthermore, changes in soil biological activity and diversity can improve nutrient cycling. Microbial activity in the rhizosphere is a major factor that determines the availability of nutrients to plants and has a significant influence on plant health and productivity (Jeffries et al., 2003).

1.4.2.1 Nutrient supply

Organic amendments serve as a direct source of nutrients released through mineralisation. Nearly all N and large amounts of the phosphorous (P) and sulphur (S) found in soils occur as constituents of SOM (Ashworth and Harrison 1983). Soil organic matter serves as both the principal long-term storage medium and as the primary short-term source of these nutrients and others. However, Roe (1998) found that although organic amendments generally improve the chemical and physical soil environment of the crop it does not always result in increased plant nutritional concentration. Effects of organic matter on soil chemical properties depend to a great extent on the mineral content of constituent organic material, and are therefore variable (Gallardo-Lara and Nogales, 1987; Neilsen et al., 2003a; Neilsen et al., 2007). Nitrogen availability is furthermore largely dependent on the maturity and C:N ratio of the organic material used (Haynes, 1980; Gallardo-Lara and Nogales, 1987). In material with a high C:N ratio (30:1), N will temporarily be immobilised in microbial tissue which can create N-deficient conditions. It is therefore advised to use nitrogen fertilizer after introducing natural mulches to compensate for immobilisation (Hogue and Neilsen, 1987; Geiger et al., 1992; Lipecki and Berbec, 1997).

A substantial amount of nutrients, especially N, can be sequestered in the rhizosphere microbial biomass (Jenkinson and Ladd, 1981). Greater soil microbial populations turning over rapidly through organic matter addition can therefore increase the reserves of biological N (Fraser et al., 1988), gradually supplying nutrients and minimising leaching losses (Mckenzie et al., 2001; Davet, 2004; Ball, 2006). A large portion of the N taken up by crops comes from organic pools that cycle through the microbial biomass (Reddy and Reddy, 1993). Conventional management practices in crop rotation systems had more N in mineral pools as indicated by higher nitrate-N, whereas organic systems, making use of manure, had higher N in the microbial biomass, indicating a shift in the N pools between the two systems (Briar et al., 2007).

Predatory organisms in higher trophic levels (such as protozoa and free-living nematodes) can substantially increase microbial turnover rates and mineralisation (Clarholm, 1985; Kuikman and van Veen, 1989; Ferris et al., 1998; Akhtar and Malik, 2000; Bonkowski, 2004) and are important in decreasing immobilisation of nutrients associated with high C:N ratio substrates (Clarholm, 1985; Ferris et al., 1998; Forge et al., 2003). Blue gamma grass withdrew more nitrogen from fertilised soil in the presence of protozoa than in their absence (Zwart et al., 1994). It was also found by Hunt et al., (1987) that 14% of the N extracted by plants is accounted for by predation of bacteria by protozoa.

1.4.2.2 Increased availability and uptake

The spatial availability of nutrients is mainly governed by root growth parameters and therefore increased by conditions that improve root growth and development. The positive effect of organic amendments, biostimulants and various soil rhizosphere organisms are well documented (Boynton and Oberly, 1966; Vaughan and Malcolm, 1985; Chen and Aviad, 1990; Crouch and van Staden, 1991; Kotzé and Joubert, 1992; Moore-Gordon et al. 1996; Pinamonti, 1998; George 2000; Zahir et al., 2004). However, the presence of nutrients in a form available for plant uptake also plays a decisive role in nutrient acquisition and is affected by SOM, plant root exudates and associated microorganisms.

Nutrient availability is influenced by organic molecules (chelators) binding to metal ions such as Fe, Cu, Zn and Mn, and maintaining them in a soluble state (Gobat et al., 2004). These chelating agents are present in SOM and organic amendments such as compost, as well as HS (Chen and Aviad, 1990; Alvarez et al., 1999). Solubilisation of nutrients from their inorganic forms can be a major factor in the promotion of plant growth in soils by HS and has been the focus of many publications (Chen and Stevenson, 1986; Varanini et al., 1993; Stevenson, 1994). De Kock (1955) found that HS not only increased the solubility of Fe in solution but also affected translocation from roots to shoots. The response of the plasma membrane (PM) in root cells plays a primary role in the interactions between roots and soil components, such as HS, present in the rhizosphere (Varanini and Pinton, 2007). The stimulatory effect of HS on plant nutrition and growth might, at least in part, be explained on the basis of both a direct action of low molecular weight (LMW) humic molecules on PM H^+-ATPase activity (Varanini et al., 1993; Canellas et al., 2002) and specific modification of cell membrane permeability (Vaughn et al., 1985).

Chelating agents produced by soil microorganisms, such as PGRB, as well as plant roots, can increase the mobility and availability of micronutrients by the formation of high affinity Fe-chelating siderophores (Zahir et al., 2004; Gupta and Murali, 2008). Numerous plants are capable of using bacterial Fe siderophore complexes as a means of obtaining Fe from soil. Seaweed extracts may also affect nutrition through acting as nutrient chelators (Metting et al., 1990).

Total phosphorous in the soil may be high, but mainly present in unavailable forms. Rhizosphere microorganisms improving P uptake mainly include nutrient solubilising bacteria and mycorrhiza that can also change root morphology and extend root exploration (Glick, 1995; George, 2000). Jones et al. (1998) reported that the efficiency with which mycorrhizal plants take up P, compared with non-mycorrhizal plants is 3.1 to 4.7 times higher. The principal mechanism for solubilisation of inorganic P is the production of organic acids by soil microorganisms (Sundara and Sinha, 1963), while P-hydrolysing enzymes play a major role in the mineralization of organic P in soil (Rodriques and Fraga, 1999). Plant roots, as well as microorganisms produce phosphatases, while only microorganisms produce phytases which is needed to decompose phytin, the major form of stored organic P (Abbott and Murphy, 2003). The extra-radical hyphae of AM fungi also have phosphatase activity associated with their cell walls (Joner et al., 2000; Ezawa et al., 2005). Humic substances can also increase the activity of secreted acid phosphatases (Zancani et al., 2009).

Biological nitrogen fixation (BNF) by soil organisms is considered one of the major mechanisms by which plants benefit from beneficial microorganisms. Significant levels of BNF from sources other than nodulated legumes (symbiotic N_2 fixation) have shown potential to improve plant growth (Kennedy and Islam, 2001). Asymbiotic N_2 fixation (ANF) was defined by Kennedy and Islam (2001) as any N_2 fixation by microbial cells growing independently (free living) in soil as saprophytes, where BNF occur in loose or close association with the plant rhizosphere, endophytically, but not requiring morphologically defined nodules. The highly organised nature of the symbotic systems are in strong contrast to the disorganised nature of the asymbiotic system. *Bacillus* strains showed ability to fix N_2 asymbiotically in apricot (Esitken et al., 2003), raspberry (Orhan et al., 2006) and apple (Aslantas et al., 2007). Karlidag et al. (2007) found that some *Bacillus* strains could be associated with increased plant N content.

1.4.3 Decontamination of polluted soils

Organic material affects the mobility of heavy metals (Magdoff and Weil, 2004) and can precipitate metals, or through microbial activity catalyse the breakdown of organic pollutants, thereby increasing plant growth (Romantschuk et al., 2000). Insoluble organic matter usually forms insoluble organo-metal complexes or absorbs metal ions, making them less available (Sauve et al., 1998). The addition of organic residues to acidic soils can reduce Al toxicity (Haynes and Mokolobate, 2001). During residue decomposition soluble humic molecules as well as low molecular weight aliphatic organic acids are released or synthesised by decomposer microflora (Stevenson, 1994). These substances can form

complexes with phytotoxic monomeric Al in soil solution, detoxifying it. Humic substances can also influence the effect and mobility of non-ionic organic compounds, such as pesticides and pollutants, by removing these from aqueous solution (Chiou, 1990). Pesticides or their degradation intermediates can be polymerised or incorporated into humus by the action of soil microbial enzymes (Bollag et al., 1992).

The use of living organisms for decontamination of soils is termed bioremediation (Kumar et al., 1995). The process can involve immobilisation of heavy metals in soil (Barea et al., 2005; Turnau et al., 2005), or extraction into the plant. Lambais and Cardoso (1993) suggested that AM fungi can induce the synthesis of metal binding proteins in the root as a means of protecting the plant against potential phytotoxic elements, such as Cd, Lb, Al, Mn. Rhizobacteria and AM fungi can also act synergistically. However, a key aspect is the use of heavy metal-adapted microbes.

1.4.4 Crop protection against phytopathogens

Disease suppression through biological amendments typically results from multiple mechanisms (Hoitink and Fahey, 1986; De Ceuster and Hoitink 1999; Hoitink and Boehm, 1999; Litterick et al., 2004; Noble and Coventry, 2005). Conditions for improved root growth and plant nutrition can increase plant vigour, leading to disease escape or masking of symptoms (Campbell, 1989). Huisman (1982) found that rapidly moving root tips are less likely to become infected, as the portion of root of greatest exudation as well as susceptibility moves past the fungal propagule by the time it has germinated. However, suppressiveness is in most cases either directly or indirectly related to changes in microbial activity and diversity (Hoitink and Fahey, 1986). Pathogens can be affected directly through parasitism, competition, the release of antimicrobial compounds and decomposition products detrimental to the pathogen, or indirectly through the production of volatile and soluble compounds that induce plant resistance and result in changes in rhizospere community composition (Lockwood 1988; Windels, 1997; Van Loon et al., 1998; 1988; Bowen and Rovira, 1999; Tenuta and Lazarovits, 2002; Zahir et al., 2004). However, Windels (1997) stated that high populations of pathogens or parasites, or unusually favourable conditions for disease development can negate beneficial aspects of biological amendments.

1.4.4.1 Predation or parasitism against pathogens

The spectrum of parasitism could range from the simple attachment of cells to hyphae, to complete lysis and degradation of pathogen hyphae through production of extracellular cell wall degrading enzymes (Whipps, 1997). The ability of bacteria, especially actinomycetes, to parasitise and degrade spores of fungal plant pathogens is well established (EL-Tarabily et al., 1997). Mycoparasitism is the most documented action of the well known biological control fungus, *Trichoderma* (Harman et al., 2004). *Trichoderma* species often colonise compost spontaneously or are stimulated in soil after compost amendment (Bullock and Ristaino, 2002) and act against various soil-borne pathogens. In studies by EL-Masry et al. (2002) unautoclaved compost in *in vitro* studies reduced hyphal growth of pathogenic fungi,

compared to autoclaved compost, due to the presence of various lysogenic enzymes playing a role in fungal degradation.

1.4.4.2 Antibiosis

Antibiosis is antagonism mediated by specific or non-specific metabolites of microbial origin including antibiotics, or other volatile compounds and toxic substances (Baker and Cook, 1974; Fravel, 1988). Antibiotic production by both fungi and bacteria is a well documented phenomenon (Howell and Stipanovic, 1993). Among prokaryotes a wide range of bacteria such as *Agrobacterium*, *Bacillus*, *Streptomyces*, *Burkholderia* and *Pseudomas* spp. have been shown to be effective antagonists (Yasuda and Katoh, 1987; Fenton et al., 1992). Among the eukaryotes, although a wide variety of fungal species display antagonistic properties, *Trichoderma* species clearly dominate (Harman et al., 2004; Barea et al., 2005). Non-pathogenic species of *Pythium* and *Fusarium* are also receiving increased attention as antibiotic producers. Lugtenberg and Leveau (2007) concluded that it is likely that root colonization is required as the delivery system of the antibiotic.

With the use of compost teas, Cronin et al. (1996) found that sterilised NCT retained suppressive qualities, but if the compost were sterilised before fermentation, suppressiveness was lost. They suggested that antibiosis was partly responsible for the disease suppressive effects. Yohalem et al. (1996) also found that the disease control with compost extract application was probably related to microbial produced metabolites which were extracted from the compost.

Certain high N content organic amendments were shown to provide disease suppression through biological activity acting upon the amendment, resulting in the release of antimicrobial metabolites (Tenuta and Lazarovits, 2002; Lazarovits, 2001). The biocidal nature of biofumigant plants resides in their capacity to produce glucosinolates, of which hydrolysis yields various biologically active compounds with broad spectrum antimicrobial activity, including isothiocyanates (Brown and Morra, 1997).

1.4.4.3 Competition for substrate, space and nutrients

Competition for substrate colonization and space is another well documented mechanism of biological control (Baker and Cook, 1974). Pathogens that grow saprophytically on plant residues can be managed by pre-colonisation with non-pathogens (Cook and Baker, 1983). Colonisation of roots by non pathogenic strains (including mycorrhiza) can also result in competition for root infection sites (Weller, 1988; Azcón-Aguilar and Barea, 1997). When sterilisation of compost extracts results in loss of disease suppressiveness (Scheurell and Mahaffee, 2002), microbial competition is generally indicated as the mechanism of action.

Competition for nutrients can lead to energy stress resulting in repression of microbial spore germination. Siderophore mediated competition for Fe is an important mechanism of biocontrol of *Fusarium* and *Pythium* spp. (Kloepper et al., 1980; Duijff et al., 1994). Siderophores are low molecular weight Fe binding

compounds, and have also been associated with the fungal pathogen suppressive properties of several *Pseudomonas* spp. (Kloepper et al., 1980; Leong, 1986; O'Sullivan and O'Gara, 1992). The Fe sequestering abilities deprive the pathogen of available Fe. Results from Dixon and Walsh (1998) showed that seaweed extracts encouraged the growth of the beneficial bacteria *Pseudomonas putida*, as well as their capacity to form siderophores. Diánez et al. (2006) found microorganisms present in grape marc compost that excreted siderophores, which when extracted into the ACT, prevented the development of various phytopathogens.

1.4.4.4 Induced systemic resistance (ISR)

Induced systemic resistance (ISR) is a physiological state of enhanced defence capacity of the host plant that is activated by specific inducing stimuli (biotic or abiotic), whereby the plant's innate defences are potentiated against subsequent biotic challenges and is effective against a broad range of pathogens and parasites (Kloepper et al., 1992; Van Loon et al., 1998). Generally, induced resistance is systemic, because the defensive capacity is increased not only in the primary infected plant parts, but also in non-infected, spatially separated tissues. A network of signalling pathways regulates ISR against plant pathogens, of which the primary components are plant signal molecules such as salicylic acid (SA). jasmonic acid (JA), ethylene (ET) and nitric oxide (NO) (Choudary et al., 2008). Changes that have been observed in plant roots exhibiting ISR include, strengthening of cell walls, increased levels of enzymes such as chitinase and peroxidase, enhanced phytoalexin production and enhanced expression of stress related genes (Whipps, 2001).

The greatest growth area in biocontrol in the past few years has perhaps been concerned with ISR (Kloepper et al., 1992). The use of specific soil inoculants such as B*acillus* and *Pseudomonas* rhizobacteria has received increased attention as inducers of systemic resistance in plants (Van Loon et al., 1998; Choudary and Johri, 2008), as well as AM fungal associations (Pozo et al., 2002). The protective effect of compost and compost extracts (Zhang et al., 1996, 1998) as well as some seaweed extracts (Dixon and Walsh, 2004) is partly due to the induction of systemic resistance. Some *Bacillus* spp. can produce antibiotics, but are poor colonizers of the root. In this respect, some antibiotics also appear to be able to induce systemic resistance (Lugtenberg and Leveau, 2007).

1.4.4.5 Altering microbial rhizosphere composition

Shifts in microbial community structure and the resulting microbial equilibria is one of the mechanisms through which mycorrhizal fungi exert a biological control effect on soil pathogens (Linderman, 2000; Whipps, 2004). Another possible strategy in biological control is also utilisation of soil amendments with the capability to selectively enhance activity or populations of microbial components that function in disease suppression. Mazzola et al. (2001) observed that changes in the resident soil microbial community contribute to the control of *Rhizoctonia* root rot of apple in response to the application of brassicaceuos seed meal amendments in orchard systems. The observed disease control appeared to function through the

proliferation of resident *Streptomyces* possessing the capacity to produce NO, known to induce plant host defence responses (Cohen et al., 2005).

Plant cultivation is also a viable means to manipulate the composition and function of resident soil microflora. The effect on soil microbial communities generally results from changes in root exudation chemistry (Marschner et al., 2001). Cultivation of various cover crops have been shown to enhance the growth of effective antagonists (Vargas-Ayala et al., 2000; Mazzola and Gu, 2002; Weller et al., 2002). Some studies demonstrated a plant-genotype dependant capacity to select for specific functional microbial elements that contribute to soil suppressiveness and their activity in the rhizosphere (Latour et al., 1996; Marschner et al., 2001; Notz et al., 2001; Gu and Mazzola, 2003).

Results from Mazzola and Gu (2002) indicated that wheat cover cropping during orchard renovation may reduce the incidence of root infection caused by both *Pythium* and *Rhizoctonia* spp. and thereby improve growth of apple in apple replant disease (ARD) soils where these pathogens are involved. Changes in the composition of the fluorescent pseudomonad community contributed to the reduction in disease severity (Mazzola, 1999). Furthermore, bacterial and fungal communities in the rhizosphere of rootstocks susceptible to ARD differed from tolerant rootstocks (Rumberger et al., 2007) and it was therefore suggested that certain rootstock genotypes may promote pre-emptive root colonizing symbionts that suppress root pathogens and provide indirect forms of ARD suppression.

1.4.4.6 Compost and disease suppression

Compost typically suppresses disease through multiple mechanisms (Litterick et al., 2004). Although disease suppression associated with compost is generally through biological mechanisms (De Ceuster and Hoitink 1999; Hoitink and Boehm, 1999; Noble and Coventry, 2005), it is not always clear if the suppression functions through the enhanced activity of the indigenous microbial community or through introduced populations resident in compost. Kowalchuk et al. (2003) found with the use of DNA fingerprinting that the suppression of *Pythium* was functioning through a biological community native to the compost and that different microbial communities can lead to suppression against *Pythium*. This was concluded since restoration of suppressiveness in compost-amended soils was associated with a different microbial community than observed in untreated, suppressive soils. Pérez-Piqueres et al. (2006), in microcosm lab experiments, studied the short term effect of non-amended, amended soil and the amendments itself on soil microbial DNA profiles. They suggested that shifts in microbial community structure and density were mainly due to amendment with new community members originating from the compost, although there also was stimulation of the resident soil microflora. In a study on *Fusarium* wilt suppression it was however shown that the resident soil microflora was more effective than the compost microflora in limiting disease development (Cotxarrera et al., 2002).

Pascual et al. (2002) concluded that control of *Pythium ultimum* with the humic fraction of municipal solid waste compost was associated with increased *Pseudomonas* and *Trichoderma* colony forming units (CFU) in the soil. They attributed the significant increases to the carbon and nutrients provided by the compost, due to similar effects with sterilized and unsterilised compost. Application of the compost also resulted in higher *Pseudomonas* and *Trichoderma* counts than when a water-soluble extract of the compost was applied, indicating the importance of the carbon provided.

Distinction must furthermore be made between general and specific disease suppression. In systems associated with general suppression, suppression typically occurs as a result of an overall increase in microbial activity and biomass (Cook and Baker, 1983). *Pythium* and *Phythophtora* can in most cases be controlled through general suppression (Hoitink et al., 1997). However, this general mechanism of suppression is not universal as control of certain pathogens such as *Rhizoctonia solani* with compost amendments, appears to have greater biological specificity (Hoitink and Boehm, 1999). Only about 20% of compost tested, naturally suppress *Rhizoctonia* (Hoitink and Boehm 1999; Tuitert et al., 1998) and suppression is normally generated through the activities of one or several specific populations of soil organisms (Cook and Baker, 1983). To improve consistency in disease control, compost can be inoculated with specific antagonists, such as *Trichoderma* and *Gliocladium* (Hoitink et al., 1991).

Pérez-Piqueres et al. (2006) in their study indicated that changes in community structure induced by compost, related both to soil and type of organic amendment. Termorshuizen et al. (2006), in bioassays involving 18 composts and 17 pathosystems, found significant disease suppression in 54% of cases and disease suppression was related to the properties of compost-substrate mixes rather than just the pure compost. Both these studies concluded that disease suppression will therefore be better predicted based on evaluation of soil-compost mixes, rather than compost on its own.

One of the biggest impediments in disease suppression with compost is variability. Compost can be expected to have variable effects as organic materials with variable properties are applied (Hoitink and Fahey, 1986; Litterick et al., 2004). Maturity of the composting material is an important factor in disease suppression. Addition of fresh material in early stages of decomposition can lead to temporary increases in populations of pathogens such as *Rhizoctonia* and *Pythium* spp because they can reproduce easily in such material (Rush et al, 1986; Van Bruggen and Termorshuizen, 2003). Metabolic by-products of microbial decomposition of fresh material can also be phytotoxic, predisposing the plant to attack by pathogens (Linderman, 1989). Synthesis of lytic enzymes involved in parasitism are also repressed in fresh organic matter due to high concentrations of available nutrients (De la Cruz et al., 1993) and the same may be true for antibiotic production.

1.4.4.7 Soil inoculants and disease suppression

There are several factors hampering the large-scale inoculation of agricultural soil with beneficial microorganisms. Firstly, to achieve the desired effect in the field, the inoculant organism must not only survive but establish itself, as well as dominate in the soil or rhizosphere (Bünemann et al., 2006). Effective use of soil inoculants also strongly depend on soil physical and chemical factors (e.g. adequate nutrition), environmental conditions, as well as the plant involved (Kennedy et al., 2004). From literature it is clear that the response with different AM fungal species and *in vitro* propagated rootstocks are variable and that there are distinct genetic variations in responsiveness to AM fungi within plant species (Smith et al., 1992; Allen 1996). Inoculant strains can also be restricted to the plant roots of certain species because of special nutritional needs such as high sugar concentration (Kennedy et al., 2004). A high degree of crop, as well as site specificity are exhibited by various microbial isolates towards controlling different pathogens, as well as producing biologically active substances (Martin 2003; Zahir et al., 2004).

The addition of multiple microorganisms to a given system appears attractive, considering the multifaceted beneficial effects of various rhizosphere organisms (Avis et al., 2008). Several reports have shown potential in combining different biocontrol agents with various disease-suppression mechanisms in the field (De Boer et al., 2003). However, in trials on strawberries testing different inoculation patterns on the effect of five diverse rhizosphere microorganisms, Vestberg et al. (2004) found that the biocontrol fungi did not establish well, while *Bacillus* bacteria were the most promising PGPR. Growth promotion effects were not consistent and dual inoculation did not seem to provide a significant additional benefit. Growth promoting effects of some PGPR can also be significantly reduced in the presence of other rhizobactera and possible competitiveness between microorganisms must be taken into account (Barea et al., 2005; Avis et al., 2008). Combinations of rhizobacteria which individually produce beneficial levels of plant hormones may also in combination produce inhibitory amounts (Bent et al., 2001).

Compost and other substrates can be used as carriers to augment survival and function of introduced inoculants (Hoitink and Boehm, 1999). Another strategy which may help contribute to the establishment of introduced organisms is through the use of selected communities of endophytic microorganisms (Sturz and Nowak, 2000). These microorganisms for part of their life cycle invade tissue of living plants and cause unapparent and asymptomatic infections entirely within plant tissue, but cause no disease symptoms. However, much research is still needed regarding their community structures, principal functions and ecological stability.

1.4.5 Control of pests

1.4.5.1 Plant parasitic nematodes

Similar mechanisms are involved in the nematicidal effects of biological amendments than with biocontrol of plant pathogens (Rodriguez-Kabana, 1986; Stirling, 1991; D'Addabbo, 1995; Akhtar and Malik, 2000). Amending soil with organic material has been associated with decreased population densities of plant

parasitic nematodes in various vegetable crops (Abawi and Widmer, 2000). In apple orchards, shredded paper mulch as well as alfalfa straw decreased *Pratylenchus penetrans* (root lesion nematode) population densities in roots (Forge et al., 2008). Also in apple, nematodes were lower in compost treated soil compared to control plots for eight months after application (Leinfelder and Merwin, 2006), which is consistent with results from Forge et al. (2008) and Hoitink and Fahey (1986). Daneel et al. (2000) concluded that the direct effect of HS on parasitic nematode populations was insignificant, but that HS products could render the plant more resistant to nematode attacks, by allowing plants to compensate for root damage. Some studies have also reported nematicidal activity and insect resistance with seaweed extracts. (Featonby-Smith and Van Staden, 1983; Whapman et al., 1993). Jenkins et al. (1998) concluded that betaines present in the seaweed extract played a major role in the reduction of root knot nematodes invading roots of tomato plants and egg recovery from plants was also significantly lower.

Several soil microorganisms are known for their biological control activity against root parasites. These include various *Bacillus* rhizobacteria as well as *Burkholderia cepacia* (Akhtar and Malik, 2000). Another group of nematode antagonists, the endospore-forming bacteria *Pasteuria penetrans*, are obligate parasites of nematodes and currently appear to have the greatest potential as economically practical biological control agents (Chen et al., 1996). Nematicidal fungi include *Trichoderma, Hirsutella* spp., *Paceilomyces lilacinus* as well as various others (Spiegel and Chet, 1998; Akhtar and Malik, 2000; Sharon et al., 2001). Nematicidal properties of antibiotics produced by actinomycetes (*Streptomyces* spp.) have also stimulated interest (Samac and Kinkel, 2001). Furthermore, free-living nematodes promote rhizosphere colonization of beneficial rhizobacteria (Knox et al., 2003). Nahar et al. (2006) found that the incorporation of either raw or composted manure increased the abundance of bacterial and fungal feeding nematodes as well as omnivorous and predatory nematodes, while decreasing plant parasitic nematode populations.

Nematode-suppressive cover crops through phytochemical based strategies have also received much attention in nematode control (Chitwood, 2002). These crops produce natural compounds that are toxic to nematodes and are released from roots of living plants or by incorporation of plants into soil as green manures. Marigold (*Tagetes species*) is one of the most highly studied crops for its ability to suppress nematodes with antagonistic phytochemical exudates, namely the polythienyls. Research also demonstrates that rhizobacteria living in association with Marigold roots are suppressive to root lesion and other nematodes (Siddiqui and Alam, 1987; Sturz and Kimpinski, 2004). Furthermore, Marigolds can act as a trap crop preventing nematodes from maturing or reproducing once they have entered the root. *Brassica* spp. (e.g., rapeseed and mustard) also show a nematode-supressive effect that is attributed to glucosinolate compounds contained in plant residues (Brown and Morra, 1997). Toxicity with these crops is attributed to enzymatically induced breakdown products of glucosinolates, a large class of compounds known as isothiocyanates and nitriles that suppress nematodes by interfering with their reproductive cycle. Mazzola et al. (2001) also observed that soil amendment with *Brassica napus* seed meal reduced the lesion

nematode *P. penetrans*. However, effects were cultivar dependant and some *Brassica* spp. can even support high populations of parasitic nematodes.

1.4.5.2 Insect pests

Lower pest pressure with the use of organic amendments could result from the abundance of beneficial insects, or alternatively the production of healthy plants that are more resistant to insects. Drinkwater et al. (1995) found that the abundance of arthropod herbivores was similar in organic and conventional systems but natural enemy communities were distinct. The use of compost as a mulch reduced herbivores and increased predatory arthropods in apple orchard ecosystems, making them beneficial to insect pest management (Brown and Tworkoski, 2004). Compost application increased biodiversity of arthropods inhabiting the orchard floor and also increased soil detritivores by six fold (Matthews et al., 2002). This abundance in detritivores could aid in predator stabilization, by providing an alternative food resource for predators and subsequently enhance biocontrol of orchard pests that spend part of their life-cycle on the orchard floor. Damavandian (1999) showed that the physical effect of a mulch can affect migration of *Eriosoma lanigerum* (woolly apple aphid). Fewer nymphs were found in the canopy, possibly due to more predators on the orchard floor, although infestation of roots was not affected by compost application.

However, the relationship between SOM and the ability of a crop to resist aboveground losses to insects and diseases are not simple. Reduced insect damage is not always associated with increased predator densities, or changes in herbivore behavioural preferences. Evidence has been found suggesting that plant physiology may possibly mediate differences in pest levels (Phelan et al., 1995) and that plants from organically managed sites differ biochemically. The concept of biological buffering was proposed, which asserts that a sustained influx of SOM provides the resource base needed for the soil community, whose interaction act to lessen the changes in the soil environment. Phelan (1997) asserted that the primary mechanism for biological buffering of plant health is the modulation of mineral availability in the soil solution by the SOM decomposer food web. They proposed a mineral balance hypothesis, similar to the plant stress hypothesis of White (1984), to explain lower pest pressure in organic systems. Plants require an optimal balance of mineral nutrients, therefore not only absolute levels are important but also ratios of nutrients. Mineral nutrition effects most plant chemistry and also the external communication systems. Imbalances block certain paths, potentially resulting in slower growth, too low levels of certain metabolites, or build up of soluble precursors such as amino acids. These changes in plant physiology can increase host quality for insect herbivores (Cockfield, 1988). Phelan (1997) suggested that organically managed soils are better able to approximate the optimal plant nutrient balance because they are closer to the natural soil environment that the plant evolved in. Furthermore, one of the most beneficial aspects of organic matter is its capacity to buffer mineral availability.

1.4.6 Production of phytohormones

The positive effect of PGPR on plant growth is generally correlated to remarkable changes in root morphology and architecture (Arshad and Frankenberger, 1998). It is assumed that this developmental response is triggered by phytohormone or plant growth regulating substance production by rhizosphere organisms. Microbial production of phytohormones is one of the major and most plausible mechanisms in modifying growth and yield of plants (Glick et al., 1998). The stimulatory effects of seaweed extracts on plant growth, especially root growth, were mostly attributed to phytohormones being the main active ingredient in these extracts (Crouch and van Staden, 1993). Furthermore, the hormone-like activity of HS has been investigated intensively (Cacco and Del'agnola, 1984; Vaughan and Malcom, 1985; Piccolo et al., 1992; Nardi et al., 1996; 2002).

The exogenous supply of plant hormones can provide supplemental quantities to the plant's endogenous levels, stimulate endogenous changes in plant phytohormone levels via a chemical signal, affect the sensitivity of the plant to phytohormones, resulting in altered plant growth or affect plant growth indirectly through modifying the rhizosphere environment (Glick, 1995; Arshad and Frankenberger, 1998). It should be emphasized that phytohormones do not act alone but rather interact with one another in a variety of ways. Therefore, plant response is regulated by the net balance of applied phytohormones as well as endogenous ones. Since endogenous levels of phytohormones vary with the different stages of growth, responses would also depend on time of application (Arshad and Frankenberger, 1998). Furthermore, under certain conditions plants may not have the capacity to synthesize sufficient endogenous phytohormones for optimal plant growth and then respond more favourably to exogenous application.

1.4.6.1 Microbial production

Production of all major plant hormones by rhizosphere microflora has been reported. Data indicated that several known pseudomonad strains, shown to increase root growth in the absence of pathogens (Glick, 1995), produced the cytokinin (CK) dihydrozeatin riboside (DHZR) in pure culture (Garcia de Salamone et al., 2001). Results from Patten and Glick (2002), using indole acetic acid (IAA) deficient mutants of the bacterium *Pseudomonas putida*, suggested that bacterial produced IAA (auxin) plays a major role in the development of the host plant root system. It is also clear that auxin production by microorganisms proceeds via more than one pathway (Gaudin et al., 1994). Among natural sources, soil microbiota are the most potent producers of ethylene (Arshad and Frankenberger, 1989). Barea et al., (1976) reported that among 50 bacterial isolates collected from the rhizosphere of various plants, 86, 58 and 90% produced auxin, gibberellins and CK-like substances respectively. Protozoa can also stimulate plant growth by altering concentrations of plant hormones such as auxin (Jentschke et al., 1995). The free-living diazotrophs *Azotobacter* and *Azospirillum*, as well as the micro-symbiont *Rhizobia*, have significant effects on plant growth and development which has been attributed to production of auxins, gibberellins and CK (Arshad and Frankenberger, 1998). The ability of *Azospirillum* species to produce auxin-type phytohormones may even be more important than their N_2 fixing activity (Dobbelaere et al., 1999).

Production of abscisic acid is also a common characteristic of soil and rhizosphere microflora (Neill et al., 1982; Crocoll et al., 1991) although they have not been evaluated extensively. Microbially released phytohormones can be of valuable ecological significance to the agricultural industry in providing a continuous supply that may prove better than one-time applications of synthetic phytohormones (Arshad and Frankenberger, 1998).

The type and amount of phytohormones produced varies between different microorganisms (Zahir et al., 2004). Aslantas et al. (2007) found that several PGPR species were capable of producing IAA, but amounts varied between species and higher amounts of both IAA and CK production appeared to be directly correlated with plant growth and yield. Bent et al. (2001) also observed bacteria species-specific affects in root hormone levels, where auxin levels were elevated in roots inoculated with *Pseudomonas polymyxa* while CK levels were elevated with *P. fluorescens*. Furthermore, not all strains of *Pseudomonas fluorescens* tested by Jeon et al. (2003) produced IAA.

Although the direct benefit of phytohormone production by soil microorganisms is not clear, it is possible that their production may benefit the microorganism through control of its environment (Bent et al., 2001). By affecting plant metabolism, the microbe in turn affects the chemical composition of plant exudates and, hence, its nutritional supply. Beyrle (1995) reviewed the role of phytohormones in the function and biology of mycorrhizae. Auxin production is widespread among many mycorrhizal fungi which may indicate that this hormone plays a role in the symbiosis (Arshad and Frankenberger, 1998). Barker and Tagu (2000) suggested that CK is involved in signalling between AM fungi and the host cell. Their research on AM fungi has shown that CK accumulation throughout the plant is specifically enhanced by the symbiosis and a pathway was proposed linking enhanced CK to development of AM fungal associations.

The involvement of enzymes such as aminocyclopropane carboxylic acid (ACC) deaminase, produced by *Pseudomonas putida* have also been suggested in modifying the root growth of different plants (Glick et al., 1998). This bacterium hydrolyses ACC, a precursor of ethylene in higher plants, thereby lowering endogenous ACC levels with a subsequent increase in root elongation. This effect is due to reduced inhibition caused by ETH, resulting in plant growth promotion (Gravel et al., 2007).

1.4.6.2 Hormone-like activity of humic substances

Humic substances affect plant nutrient uptake, but can also show a specific, direct effect on plant metabolism (Pinton et al., 1992; Varini et al., 1993; Chen et al., 2004) through the active uptake of LMW components of HS (Vaughn et al., 1985; Valdrigi et al., 1995). Humic substances have also been reported to act as electron donors, intervening in the respiratory chain of cells thereby increasing the energy supply to cells (Sanchez-Sanchez et al., 2002). Both the photosynthesis and respiration rate of plants were enhanced by the presence of HS (Vaughan et al., 1985), and Vaughan and Malcolm (1979) also observed interaction of HS in the production of nucleic acids. The effects of HS on plant physiology include

modification of root morphology, proliferation of lateral roots and root hairs and higher differentiation rate of root cells (Concheri et al., 1996; Canellas et al., 2002). Hormone-like activity has been suggested for several humic fractions whose biological action appears to mimic the response induced by various phytohormones, such as gibberellic acid and IAA. The fact that HS exhibit regulatory effects on enzyme activity and plant cell growth and development also indicates possible hormonal activity (Cacco and Del'agnola, 1984; Vaughan and Malcom, 1985; Piccolo et al., 1992; Nardi et al., 2002). Enzyme activity effects have been investigated particularly in IAA metabolism. (Mato et al., 1972 a, b; Chen and Aviad, 1990). Inhibition of the catabolic enzyme IAA oxidase hinders IAA destruction, thereby causing an increase of endogenous hormone concentration in plant tissue. Cacco and Del'agnola (1984) showed quantifiable auxin- and CK-like action of soluble humic complexes, although at low activity. Nardi et al. (1994) concluded that the LMW fraction was the component with hormone-like activity.

The hormone effect of HS has been debated for almost a century, with mainly indirect evidence being provided (Muscola et al., 1998; Canellas et al., 2002, Nardi et al., 2002; Dobbs et al., 2007; Zandonadi et al., 2007). Chen et al. (1994) directly examined the presence of auxin, gibberellins, CK and abscisic acid in HS extracts and failed to provide evidence of their presence. Schmidt et al. (2007) provided evidence that LMW fractions alter root development without significantly affecting the auxin homeostasis of the plant. They demonstrated that water-extractable HS could induce an increase in the root absorptive surface by affecting genes involved in epidermal cell fate specification, but could not find evidence for a role in the expression of auxin-related genes. However, HS acting as plant growth regulating hormones can not be ruled out and further research continues (Chen et al., 2004).

Frankenberger and Arshad (1995) found that the active ingredients in humus were organic substances and biologically-active metabolites of various microbes and proposed that HS could perhaps be considered as a 'memory' of microbial population and plant cover. Nardi et al. (2002) therefore concluded that it was not clear if the hormonal effect of HS is strictly linked to the chemical structure of HS or whether it depends on hormones of microbial origin forming a bioactive part of the HS molecule.

1.4.6.3 Hormones as active ingredients of seaweed extracts

De Villiers et al. (1983) stated that if the observed effects with liquid seaweed extract (LSE) are induced through plant hormone action, it could explain the dependence of effects on crop type, plant growth stage with application, concentration, as well as composition of the extract. Results from various studies indicate that CK are involved in the activity of seaweed extracts (Blunden and Wildgoose, 1977; Featonby-Smith and Van Staden, 1984; Finnie and van Staden, 1985; Chouliaras et al., 1997). There are several reports suggesting that the accumulation level of CK and export by the roots is closely correlated with the nutritional status of the plant (Menary and van Staden, 1976; Sattelmacher and Marschner, 1978, Horgan and Wareing, 1980; Samuelson and Larsson 1993; Wagner and Beck, 1993; Takei et al., 2001).

The wide range of physiological effects obtained after application of seaweed extracts imply that more than one group of plant growth regulators are possibly involved (Crouch and van Staden, 1993). Auxins were also identified from seaweed extracts (Sanderson et al., 1987; Crouch and van Staden, 1992). Results from Stirk and van Staden (1997) indicated that levels of CK- and auxin-like activity were similar for six commercial seaweed extracts tested.

1.5 EFFECT ON HORTICULTURAL PERFORMANCE OF THE CROP

Although the beneficial effects of biological management practices on microbial activity and other soil functions have been extensively documented and a combination of mechanisms studied through which the plant can be affected, it is not always easy to demonstrate influence on crop yield under field conditions. However, improved root growth proliferation and better fruit quality is of equal importance, although economic benefits may not always be immediate.

1.5.1 Root growth proliferation

Changes in root architecture and physiology affect water and nutrient absorption, therefore the activity of the root system plays a central role in adaptation to environmental conditions and ultimately, plant performance. Organic matter application increased root number, root length, growth rate and branching indices in maize seedlings (Sangakkara et al., 2008). The effect of HS on root growth, especially in annual crops, are widely documented (Bryan, 1976; Mylonas and McCants, 1980; Rauthan and Schnitzer, 1981; Chen and Aviad, 1990; Crouch and van Staden, 1991; van de Venter et al., 1991; Adani et al., 1998) and both increases in root length and stimulation of the development of secondary roots have been observed (Vaughan and Malcolm, 1985). Application of seaweed extracts also increased root growth in various studies (Finnie and van Staden 1985; Metting et al., 1990; Crouch and van Staden, 1991). Furthermore, soil inoculants such as PGPR and AM fungi were shown to cause changes in root morphology and architecture (Glick, 1995; Azcón-Anguilar and Barea, 1997; Van Loon et al., 1998; George, 2000; Zahir et al., 2004; Gravel et al., 2007).

Few detailed root studies have been executed on the effect of biological amendments on root growth in fruit crops. Mulching has been shown to increase fine feeder root biomass (Boynton and Oberly, 1966; Yao et al., 2005; Forge et al., 2008) with greater root density beneath the mulch near the soil surface and roots extending into the mulch itself (Baker, 1943; Pinamonti, 1998). Moore-Gordon et al. (1996) found more prolonged and extensive root growth in mulched plots. In vineyards, Reynolds et al. (1995) noted that fresh and dry weight responses of roots to increasing levels of humate application followed a quadratic trend. Webb and Bings (1988) observed favourable effects of humate soil amendments in mature, declining Valencia orange trees that showed enhanced water uptake one year after application. Field observations in fruit trees with compost application also showed increased rooting densities in the top 30cm (Kotze and Joubert, 1992).

1.5.2 Growth and yield

1.5.2.1 Organic matter application

Strickling (1975) found that SOM levels accounted for more than 80% of the variation in corn yield, regardless of fertilisation levels. Improvement of tree vigour and maximisation of fruit yield has generally been reported with mulching of fruit trees, as reviewed by Cockroft and Tisdall (1974) and Hogue and Neilsen (1987). Much less research has been conducted on the effects of incorporated organic matter on fruit tree yield and results in general are more variable, due to great variability in compost quality factors. Mulching has also been combined with other organic matter applications such as compost, biosolids and manure.

The importance of increased vigour to initial yield has been indicated by various researchers. Kotzé and Joubert (1992), in a sandy soil, achieved significant increases in annual trunk circumferences of apricot trees, when applying an organic mulch and concluded that tree responses to mulching could be expected to be higher for young trees, where the surface exposed to radiant energy is higher. The bigger trees had increased cumulative yield by 22% compared to non mulched plots. In the same study, it was also found that tree growth and production were significantly increased by pre-plant compost application, mixed with the soil to a depth of 30 cm. Tree growth improved from the first year after planting, and cumulative yield over four years increased by 55% compared to plots not receiving compost. Shribbs and Schroch (1986a) found in non-irrigated plots that first year growth of apple trees was significantly increased with rye straw mulch and that in the fourth year of application, trunk diameter was still significantly greater compared to control plots, despite similar relative growth rates.

In a high density, fertigated apple orchard, Neilsen et al. (2003a) found that vigour and yield over the first five fruiting seasons was increased by soil management practices of various surface mulches (alfalfa, biosolids, paper) widely differing in nutrient contents. Yield was lowest for the plots receiving the standard commercial practice of maintaining a 2 m-wide weed free strip with multiple glyphosate applications. In a similar study (Neilsen et al., 2007), trunk cross-sectional area was consistently greater for trees with paper mulch each year for the 6-year trial period, but effects on yield was less pronounced compared to the study of Neilsen et al., (2003a). In both the above studies, maintenance of paper mulch affected vigour more than periodic applications of biosolids. However, potential benefits of the high nutrient content of biosolids in terms of plant nutrition may have been masked by a strong fertigation regime used. In another experiment where municipal compost was rotovated into the top 30 cm prior to planting, first year shoot growth of Braeburn apple was improved (Neilsen et al., 2004), however the effect on yield was not established. Niggli et al. (1990), in a field study using various organic mulches, also showed lowest fruit yield for un-mulched, herbicide treated plots. Furthermore, mulching reduced strong annual yield fluctuations in a cultivar where this poses a problem. Marsh et al. (1996) in a study on orchard understorey management in apple, found that in three years mulching induced greater growth than trees with a mown understorey and yield was increased more by mulch treatments than compost application. Van Schoor et al. (2009) found

that compost in combination with a straw mulch significantly increased growth of newly established apple trees.

In a study by Varga et al. (2004) cumulative yield over the first two bearing seasons of apple were significantly greater for trees with pine bark mulch, but not with straw mulch and livestock manure application. Trunk circumference area of trees planted with these various organic mulches differed only slightly from clean cultivation plots after four seasons. Pinamonti et al. (1995) studied the effect of compost mulch applications on growth and yield of apple and vines and found significant effects in the early years of the trial period. Autio et al. (1991) in two studies on newly planted apple trees showed that peat moss amendment in the planting hole significantly increased growth compared to the controls in the establishment years of the orchard. Composted horse manure only had an effect on tree growth in one of the studies.

Although results on growth and yield have been favourable in general, some studies have shown little or no significant effect of mulching on yield, despite increased growth with organic mulches (Merwin et al., 1994; Merwin et al., 1995). Hartley and Rahman (1994) found no significant effect on tree branch extension, trunk growth or yield over a 3-year period with application of various mulches, including compost, to two and 3-year old orchards. In pear, yield was not increased by biosolid compost applied to the soil surface (Korcak, 1986). In a study on grapevine, Pinamonti (1998) found that two different composts, applied twice over 5 years improved growth only during the first year and over four cropping seasons only plastic mulch had a significant effect on yield. Neilsen et al. (2004) observed that effects were also not observed at sites with strong fertigation regimes, fertile soils, or when sites had overriding growth limitations unaffected by the treatment. Compost and other organic amendments have also been reported by some researchers to be ineffective in improving growth of apple trees on sites with apple replant disease (ARD) (Granatstein and Mazzola, 2001; Rumberger et al., 2004; Neilsen et al., 2004; Wilson et al., 2004).

Yield can also be negatively affected when mulching creates conditions favourable for pest and disease development (Merwin et al., 1992), or reduced aeration (Robinson and O'Kennedy, 1978). Furthermore, Marsh et al. (1996) stated that sustained tree vigour with organic mulch application can become problematic in high-density plantings. Faber et al. (2001) also noted that irrigation had to be adjusted with mulching to accommodate higher soil moisture contents that could be conducive to root rots. Neilsen et al. (2004) concluded that an adjustment in irrigation regime may be required to maximise the benefits of mulching, since it is difficult to maintain sufficient N in the root zone under these conditions.

With the application of raw or composted organic materials, rate of application, maturity of the material, heavy metal content and nutrient content are all factors that can influence the effect on yield negatively (Linderman, 1989; Roe, 1998; Van Bruggen and Termorshuizen, 2003). Planting should therefore be delayed after incorporation of fresh organic matter, to reduce possible negative effects on yield. Ros et al. (2003) found that the incorporation of compost seems to be more effective than fresh organic matter due to

its stabilized organic matter content. Gallardo-Lara and Nogales (1987) found that combining mineral fertilizer with compost generally resulted in higher yield than either alone. It is also advised to use N fertilizer for the first few years after introducing organic mulches to prevent N-immobilisation (Hogue and Neilsen, 1987; Geiger et al., 1992).

1.5.2.2 Microbial inoculants

Crop yield and quality are affected by microorganisms associated with the rhizosphere (Glick, 1995; Rodriguez and Fraga, 1999). However, little research has been done on the use of microbial inoculants in promoting growth and yield in fruit tree crops. Their effect in horticulture has mostly been studied in relation to disease suppression, thereby indirectly affecting plant growth.

The AM fungi are one of the most researched groups of soil microorganisms. Since AM symbiosis can benefit plant growth and health there is an increasing interest in their incorporation into plant production practices (Azcón-Aguilar and Barea, 1997). In this regard, fruit crops have received more attention than vegetables and ornamentals. It is accepted that the first evidence of positive influence of AM symbiosis on horticultural field production was provided from a study by Menge et al. (1977) on establishment of citrus plants in nursery beds. Growth stimulation with AM fungi in apple trees has also been observed in different stages of plant development (Plenchett et al., 1981; Morin et al., 1994; Von Bennewitz, 2006). Several reports have shown that AM fungi are more efficient in promoting growth of apple plants than applying fertiliser only (Geddeda et al., 1984; Miller et al., 1985). Plenchette et al. (1981) demonstrated increased growth and mineral uptake of apple trees colonized by mycorrhizal fungi in conventionally managed apple orchards. Apple growth responses to mycorrhiza have also been shown in various pot studies (Covey et al., 1981; Koch et al., 1982; Gnekow and Marschner, 1989).

Generally, results seem to be more favourable when AM inoculum is introduced into a sterile environment where the introduced strains do not have to compete with other soil microbial communities or already established strains (Plenchette et al., 1981; Menge et al., 1983; Catska and Taube-baab, 1994; Forge et al., 2001). Lack of positive results with AM fungi in some studies may be ascribed to inoculum containing low numbers of viable propagules or incompatibility with the host plant (Abbott and Murphy, 2003). Futhermore, effects may be masked in soils with high P (Geddeda et al., 1984; Runjin, 1989; Morin et al., 1994; Marschner and Dell, 1994), as well as by indigenous AM fungi (Dodd et al., 2000; Meyer et al., 2004). However, in other studies AM fungi was shown to be a factor even under high P levels (Wooldridge, 1999; Schubert and Lubraco, 2000; Douds and Reider, 2003; Douds et al., 2007) and their effect should therefore not be ignored in these systems.

Several studies indicate that PGPR may act as natural elicitors for improving the growth and yield of wheat, maize and potato (Xia et al., 1990; Zahir et al., 1996; Zahir and Arshad, 1996; Javed and Arshad, 1997; Javed et al., 1998; Dobbelaere et al., 2001). In various studies it was also found that PGPR could

stimulate growth and increase yield and fruit quality in citrus, apricot, sweet cherry and raspberry (Kloepper, 1994; Esitken et al., 2003; Orhan et al., 2006). In a recent study on young apple trees and the effect of PGPR, Aslantas et al. (2007) found *Pseudomonas* and *Bacillus* spp. effective in promoting shoot growth and yield of different apple cultivars. The various rootstock-cultivar combinations responded differently to inoculation with the various PGPR isolates, indicating very specific plant-microbe interactions. In floriculture, *Bacillus subtilis* increased flower fresh weight as well as total inflorescence production significantly and produced higher numbers of mature flowers than uninoculated plants (Flores et al., 2007).

Few effects on yield of fruit tree crops with EM and compost teas are documented in the scientific literature. However, preliminary results from a study in South Africa showed significant increases in tree growth when compost extract was applied in addition to compost (Van Schoor et al., 2008).

Microbial inoculants can also affect plants negatively. According to Johnson et al. (1992), AM fungal species, and even isolates within species, may vary from mutualistic to neutral to pathogenic. When nutrients and water are in unlimited supply and pathogens absent, the cost of AM symbioses may outweigh their benefits and plant growth can be depressed (Johnson et al., 1997). Furthermore, co-inoculation does not always provide synergistic effects. Flores et al. (2007) found that the increase in inflorescence production was higher when a *B. subtilis* strain was applied alone, than when applied in combination with an AM fungus. In cases where the plant and PGPR strain are incompatible, a decline in yield may be associated with inoculation (Nguyen et al., 2002).

1.5.2.3 Humic substances and seaweed extracts

Positive effects on plant growth with HS can be summarised as general stimulation of plant biomass production (Vaughn and Malcolm, 1985; Chen et al., 2004). Increased yield with the use of commercial HS has been demonstrated for various herbaceous annual crops (Lee and Bartlett, 1976; Brownell et al., 1987; Gonet et al., 1996). Commercial HA applications on tomato also improved growth in hydroponics culture (Adani et al., 1998).

Few scientific studies have focused on the application of HS on fruit tree crops, although some studies have been conducted on grapevines. Brownell et al. (1987) found increased yield with applications of commercial leonordite extracts. The most pronounced effects were obtained using a combination of early soil treatment with a post-emergence foliar spray. They hypothesised that a flowering response was triggered. Reynolds et al. (1995) investigated the effect of granular and liquid humates on growth of Chardonnay vines in pots at various concentrations and found that shoot growth responded to increasing levels of humate application in a predominantly cubic trend, with higher concentrations showing negative effects. Zachariakis et al. (2001) showed that grapevine rootstocks grown in calcareous soil in the presence

of HS had increased growth and total leaf chlorophyll content. Significant increases in shoot and root dry matter, as well as shoot carbohydrate content were also documented.

Webb and Bings (1988) found favourable effects of humate soil amendments on citrus trees under stress as well as in new plantings. After application on mature trees, growth flushes and productivity were enhanced after two years. Mature, declining orange trees, treated with humates, were greener with more vigorous growth flushes than control plots. In newly planted trees, treatment with humate at various concentrations significantly increased stem cross-sectional area one year later. In contrast to this, Nemec (1992) found no significant increase in trunk diameter or yield of Valencia orange trees after the first three growing seasons, when applying humates before planting. In a study by Van Zyl (1996), growth of citrus seedlings in sand was significantly increased when a humate was applied in combination with bark, but not when applied alone. Fallahi et al. (2006) could show no consistent effect on yield with apple trees receiving a granular formulation of HA, and the only significant difference in yield during the three years of application, was after two years of application where the product was applied in combination with a high N rate.

Most published results consistently show that high concentrations and excessive applications of HS can negatively influence plant growth (Vallini et al., 1993; Reynolds et al., 1995; Valdrighi et al., 1995; Atiyeh et al., 2002). Lee and Bartlett (1976) also showed that if HS levels are already high in the soil, addition of more may not necessarily benefit the crop. Chen and Aviad (1990) concluded that commercial humates applied to normally productive agricultural soils at rates recommended by their promoters would not appear to contain sufficient quantities of the necessary ingredients to produce the claimed beneficial effects. The most benefit is typically obtained in high pH, calcareous soils, that are low in available iron and with low organic matter content and extractable HA (Chen and Barak, 1982; Zachariakis et al., 2001). Soils high in Fe and Al (mostly acidic soils in humid areas), may inactivate humates (Rowberry, 1977). Beneficial effects on plants can therefore be expected to be more effective in arid and semi-arid regions than humid regions (Chen et al., 2004).

Improved growth and yield have also been reported with the application of seaweed extracts, as well as earlier production and reduced fruit drop (Finnie and van Staden 1985; Metting et al., 1990; Crouch and van Staden, 1991; Passam et al., 1993; Fornes et al., 1993; Koo and Mayo, 1994; Nabati et al., 1994; Chouliaras et al., 1997; Isaac, 2000). Positive effects have mainly been indicated with seedlings (Nelson and van Staden, 1986; Van Staden et al., 1995; Rijkenberg, 1994) However, the majority of research has been conducted on the effect of seeweed extract on fruit quality.

1.5.3 Fruit quality

In studies comparing different management systems, the majority of results show higher fruit firmness for apples produced in organic systems (DeEll and Prange, 1992; Andrews et al., 2001; Renagold et al., 2001; Peck et al., 2006). Compost applications on their own have shown few significant effects on fruit quality

(Kotzé and Joubert, 1992; Pinamonti et al., 1995; Neilsen et al., 2003a; Neilsen et al., 2007). However, Kotzé and Joubert (1992) found that fruit size was not influenced negatively, despite heavy crops on trees treated with compost. Furthermore, increased N with the addition of compost can reduce fruit firmness (Marsh et al., 1996). Results from Moore-Gordon et al. (1996) showed that a vigorous root system, ameliorated by mulching, can result into more and bigger fruit. Bark mulches also improved fruit quality in a study by Niggli et al. (1990). Specific *Bacillus* strains applied to young apple trees in a study by Aslantas et al., (2007) improved fruit size significantly. In field trials on grapevines with seaweed extract, Norrie et al. (2002) showed a consistent increase in berry size, weight and firmness for several grape varieties and locations.

Increased shelf- life with seaweed extracts have also been found for peaches (Skelton and Senn 1969). Mamaguti et al. (2002) found that foliar sprays of seaweed extracts improved red colour intensity and percentage of fruit skin covered by red colour in Gala, but not Fuji. However, applications had no effect on yield, fruit weight, or vegetative growth. In floriculture, Flores et al. (2007) found improvements in flower quality with enhanced xanthophyll content when an AM fungus was applied. Plants inoculated with a *Bacillus* or *Bacillus* in combination with an AM fungus, showed improved intensity and clarity of colour.

According to de Jager (1994), CK in seaweed extracts can be a major factor when applied to apple in promoting growth of fruiting spurs, and can reduce premature dropping of fruit as well as improve fruit firmness and skin colour. Seaweed extracts have also been applied in combination with Ca sprays required to prevent bitter pit in apples to aid in the uptake of Ca. However, North and Wooldridge (2003) found no beneficial effects that could specifically be attributed to LSE applications where it was applied in addition to calcium nitrate sprays. De Villiers et al. (1983) concluded that seaweed foliar sprays over two seasons in a field trial did not have an effect on yield and fruit quality of apples, peaches and table grapes.

In a study by Fallahi et al. (2006) investigating the effect of HS and N on ‘Early spur Rome’ apple, a significant effect on fruit quality was found. Fruits where soil was treated with HS once a year for three years had higher soluble solid concentrations compared to the control. Some studies also found higher TSS (total soluble solid) levels with organic treatments in apple (DeEll and Prange, 1992; Andrews et al., 2001; Renagold et al., 2001). However, Norrie et al. (2002) found that TSS levels tended to be lower in seaweed extract treated grape berries. Kangueehi (2008) found increases in TSS, malic and citric acid with application of humates and also application of compost in combination with compost extract. Effects of biological amendments on fruit nutrition and polyphenol content are not widely documented. Peck et al. (2006) found that organically grown apples contained a higher percentage total antioxidant activity, while Fauriel et al. (2007) found higher fruit polyphenol content of peaches produced from organically managed orchards, compared to conventionally managed orchards. It is has also been suggested that EM can improve produce quality (Daly and Stewart, 1999; Cao et al, 2000).

1.5.4 Relationship between soil microbial activity and plant performance

Few studies have been conducted relating changes in microbial activity to tree performance. Renagold et al. (2001) compared the sustainability of various apple management systems and found no difference in cumulative yield, despite major differences in soil quality. Carrera et al. (2007) found that tomato yield was not significantly increased by hairy vetch cover cropping, although microbial community structure was significantly affected. Drinkwater et al. (1995), comparing conventional and organic tomato agroecosystems, found no distinct difference in fruit yield, although differences were demonstrated in nitrogen mineralization potential and microbial diversity. Hoagland et al. (2008), in newly established organic apple orchards, found that maintenance of a living cover understory increased soil N concentration and availability as well as soil biological activity; however tree growth was less than with tillage weed control where soil biological properties were not improved. On the other hand, application of wood chip mulch, providing substantial inputs of total C, resulted in exceptional tree growth although soil N as well as tree leaf N was lower and soil biological activity not improved.

In fertigated apple orchards, Neilsen et al. (2003b) found that rankings of treatments based on optimum soil physical parameters and soil fertility (Forge et al., 2003), favoured treatments involving the application of biosolids while herbicide treated control plots, not receiving organic matter, ranked lowest. These control plots also showed lowest yield efficiency, compared to treatments where the orchard floor was covered with organic matter. However, biosolids applied alone did not improve vigour or yield compared to herbicide treated control plots. Furthermore, yield did not differ significantly from treatments receiving paper mulch without biosolid application. Although few of the soil chemical and physical properties measured in their study could be directly related to long term tree performance, soil pH related parameters appeared most important to tree performance and improved with paper mulch treatments, probably due to high Ca content. An important factor in improving yield with shredded paper mulch applications was possibly also the reduction in population densities of *Pratylenchus penetrans* (Forge et al., 2003).

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In a study by Varga et al. (2004) numbers of cellulose decomposing bacteria and microscopic fungi was higher under pine bark mulch and highest with livestock manure application. Tree performance did not confirm the advantage of manure in terms of soil microbial activity and cumulative yield over the first two bearing seasons and yield efficiency were higher only for the pine bark mulch. However, manure applications significantly reduced mycorrhizal colonisation of roots, possibly affecting yield negatively.

Investigating the effect of various orchard ground management systems in apple, Yao et al. (2005) found that bacterivorous and fungivorous nematodes as well as soil respiration rates were consistently higher in mulched plots compared to herbicide treated plots. Soil DNA studies of the soil microbial communities making use of DGGE profiles, also showed that mulched plots harboured a unique fungal community compared to the other treatments. However, trunk circumference growth after 11 years was similar for mulch treatments and control plots treated with post-emergence herbicides. Over nine harvesting seasons,

both these treatments also showed the most consistent, positive effect on yield. Grass treatments were generally least productive, despite similar microbial community profiles, and microbial activity, compared to post-emergence herbicide treatment. However, looking at root growth measured from excavated trees, fine feeder root biomass was lowest for grass treatments. In a bioassay using orchard soil from these various treatments, Laurent et al. (2008) found that widely accepted factors of soil quality indicators, such as soil respiration rate, populations sizes of culturable bacteria and fungi, SOM content and nutrient availability, were not significant predictors of apple seedling biomass in these soils. However, growth of apple seedlings was closely linked with bacterial community composition of orchard soil in each ground cover management system. Furthermore, in the bioassay, in contrast to field trial results, apple seedlings grown in soil from plots treated with pre-emergence residual herbicides performed the best.

In studies by Rumberger et al. (2004) and Yao et al. (2006) on the use of different rootstock genotypes in ARD management, results showed that bacterial communities of susceptible rootstocks differed from tolerant rootstocks (Rumberger et al., 2007) and correlated with improved yield (Leinfelder and Merwin, 2006).

1.6 APPLICATION OF BIOLOGICAL AMENDMENTS IN DECIDUOUS FRUIT PRODUCTION

In deciduous fruit production, interest in organic and microbial amendments in integrated disease management and plant growth stimulation is increasing due to environmental, as well as economic pressures. Application of biological amendments can improve tree efficiency in soil of low or marginal suitability for crop production. Furthermore, the economics of intensive planting systems depend on rapid establishment of orchard canopy and precocious cropping (Foote et al., 2001). In addition to improved tree performance, reduced chemical and fertiliser application, as well as protection of non-renewable resources are all possible prospects with biological management systems.

1.6.1 Improving tree efficiency under unfavourable conditions

Hogue and Neilsen (1987) concluded that mulch application can be especially important in improving fruit tree performance under adverse soil conditions. In shallow soils, Baxter (1970) found greater blossom density per unit of tree circumference, double the yield, as well as larger fruit size with mulch compared to clean cultivation in peach and apple orchards. Autio et al. (1991), when mulching in an ARD site, only found a dramatic effect on growth and bloom when rainfall was below normal. At a site where leaf N was generally low, first year trunk circumference increment were significantly increased by compost mulch treatments (Neilsen et al., 2004). Results from various researchers have also indicated that the effects of organic matter applications and biostimulants are most beneficial when the plant is coping with adverse environmental conditions, or nutrient stress (Mooney and van Staden, 1985; Beckett and van Staden, 1989; Nus, 1993).

1.6.2 Orchard establishment

Although many studies indicate a short term stimulation of tree vigour by organic amendments, it has been suggested that any advantage given to the young tree at establishment is maintained throughout the lifespan of the tree. Propagation, especially micro-propagation is one of the target systems in horticulture where AM fungal inoculation can be most practically applied (Azcon-Aquillar and Barea, 1997). Moderate amounts of AM fungal infection are achieved before transfer to the field, which is an environment much lower in nutrients. Colonisation in micro-propagated apple plants significantly increased plant growth and P concentration of shoots (Cavallazzi et al., 2007). Wooldridge (1999) found that the presence or absence of AM fungi is a factor that may affect early growth of *in vitro*-propagated apple plants even under high P levels, although young fruit trees do not respond uniformly to AM under conditions of adequate nutrition. Schubert and Lubraco (2000) also found that inoculation of transplanted micro-propagated apple rootstocks enhanced growth and increased P uptake, despite high P levels in the substrates. However, rather than being propagated *in vitro*, many apple rootstocks are grown under field conditions where roots become naturally infected with indigenous AM fungi (Wooldridge, 1999). According to Marschner (1995) indigenous AM-infected rootstocks will not necessarily show growth reactions following inoculation, unless indigenous AM are damaged or weakened. Mycorrhizal inoculation has been used to enhance plant growth and production in orchard establishment after fumigation (Menge et al., 1978).

In apple, Neilsen et al. (2004) found that the application of a high P organic amendment in the root zone prior to planting can stimulate first year growth more that supplying mineral P fertiliser in the early stages of root growth. Previous research by Neilsen and Yorston (1991) also indicated that high P is important in accelerating establishment, early growth and fruiting of apple.

1.6.3 Reduction of chemical inputs

1.6.3.1 Alternatives to fumigation in ARD management

Apple replant disease (ARD) is a disorder associated with poor growth of young apple trees planted on previous apple sites. Symptoms include stunted growth and reduction in tree vigour and productivity (Savory, 1966; Hoestra, 1968; Mai and Abawi, 1981). Although the disease is not lethal, it has great economic importance due to its lasting effect on production. The economics of intensive planting systems depend on rapid establishment of orchard canopy and precocious cropping (Foote et al., 2001), therefore any growth-retarding factor is adversely felt. In South Africa in 1998, it was estimated that 40% of apple orchards suffered from ARD (Honeyborne, 1996). It was also identified as one of the biggest problems in establishing an economically viable apple orchard by the deciduous fruit industry. However, no direct economic value has been associated with ARD in South Africa. The etiology of ARD is still not fully understood, but accumulated research results point to a biological origin, involving a shift in microbial community composition in ARD development, towards pathogens dominating the soil microbial profile (Mazzola 1998; 1999; Manici et al., 2003). Apple replant disease is successfully controlled through routine fumigation prior to orchard establishment. However, due to the biological nature of ARD etiology,

integration of various biological and cultural practices can possibly provide alternative control methods to broad spectrum fumigation. In field trials, under marginal production conditions for apple, Van Schoor, et al. (2009) found that compost in combination with a straw mulch consistently increased growth to the same or greater extent as the standard fumigant treatments. These results confirmed results from Engel et al. (2001) who found that compost mixed with replant soil at planting and subsequent mulching with apple wood chips was the most effective treatment in improving vegetative and reproductive growth. In a study by Autio et al. (1991), during the first season, peat moss and composted manure treatments in the planting hole resulted in significantly greater increases in trunk circumference and shoot growth than controls. The effect dissipated and growth was similar to the controls in the third season, but peat treated trees were still significantly larger.

Several studies have shown that inoculation of apple seedlings or rootstocks with beneficial rhizosphere bacteria including *Agrobacterium radiobacter* (Catska and Hudska, 1993), *Bacillus subtilis* (Utkhede and Smith, 1992), *Enterobacter agglomerans* (Utkhede and Smith, 2000) and *Pseudomonas putida* (Biró et al., 1998; Mazzola et al., 2001) has potential for biological management of ARD. The causal complex of ARD include pathogenic fungi from the genera *Cylindrocarpon, Phytophthora, Pythium* and *Rhizoctonia* (Jaffee et al., 1982; Dullahide et al., 1994; Braun, 1995; Mazzola, 1998; Manici et al., 2003; Tewoldemedhin et al., 2007; Van Schoor et al., 2009). *Bacillus subtilis* has the potential to control crown rot caused by *Phytophthora cactorum* (Utkhede et al., 2001) while the frequency of root colonization by *Cylindrocarpon destructans* was effectively reduced by fluorescent pseudomonad spp (Caesar and Burr, 1987), also correlating with increased growth of apple. The humic fraction of municipal solid waste compost was highly effective in controlling *Pythium ultimum* (Pascual et al., 2002), possibly associated with increased *Pseudomonas* and *Trichoderma* colony forming units (CFU) in the soil. Greenhouse trials also showed a significant increase in seedling growth when ARD soil was inoculated with a mycorrhizal strain (Utkhede, 1992). These AM fungi could be inoculated at the time of replanting or possibly used in apple nurseries, to establish infection before replanting.

Changes in the rhizosphere microbial communities through the effect of rootstock exudates as well as root exudates from specific cover crops can also induce ARD suppression (Gu and Mazzola, 2003; Rumberger et al., 2004, 2007). However, effects are cultivar specific and more research is needed.

1.6.3.2 Reduced inorganic fertilizer application

The amount of fertiliser application can be reduced either through an increase in availability of nutrients through biological amendment application or improved nutrient uptake or fertilizer use efficiency. Compost mulches in vineyards and apple orchards, (Pinamonti et al., 1995) substituted for chemical fertilizer with no resulting loss of tree vigour or fruit yield and quality. Drinkwater et al. (1995) concluded that differences between agroecosystems with and without organic matter input suggested that biological processes can compensate for reductions in the use of synthetic fertilisers through enhanced nutrient

cycling. Mulching with organic material can also significantly contribute to nutrient cycling (Akhtar and Malik, 2000; Kayuki and Wortmann, 2001; Forge et al., 2003).

Better fertiliser use efficiency is essential where costs can constrain production as well as for reducing environmental pollution through nutrient leaching. Ma et al. (1999) found that available N in a manured system was better synchronized with plant demand than N from inorganic fertiliser systems, therefore reducing N losses. Increased root proliferation in the top soil with organic amendment application can also reduce leaching as well as result in improved nutrient uptake. An increased leaf K concentration is one of the most frequently recognized consequences of mulching with organic materials (Boynton and Oberly, 1966; Kotzé and Joubert, 1992; Merwin et al., 1994; Marsh et al., 1996; Smith et al., 2000; Neilsen et al., 2003a; Neilsen et al., 2007). Available P has also been shown to increase with the use of organic mulches (Hogue and Neilsen, 1987; Merwin et al., 1994; Pinamonti, 1998; Smith et al., 2000; Yao et al., 2005). In addition, mulches suppress competition for nitrogen by weeds (Lanini et al., 1988; Niggli et al., 1990; Autio et al., 1991; Faber et al., 2001; Neilsen et al., 2003b) and also reduce chemical weed control (Pinamonti et al., 1995). In field trials, Kotzé and Joubert (1992) found improved fertiliser use efficiency with compost application to fruit trees.

Russo and Berlyn (1990) suggested that the use of organic biostimulants shows most potential for improving fertiliser use efficiency, by reducing fertiliser application without negatively affecting plant growth. Results are also favourable for the combined use of HS and mineral fertiliser. The presence of HS substantially increases effective assimilation of mineral nutrient elements (Chen and Aviad, 1990). Pandeya et al. (1998) observed that the efficiency of Fe-FA as a fertiliser is much greater than that of $FeCl_3$. Reynolds et al., (1995) found that humates increased petiole and lamina Fe in Chardonnay vines. Fe nutrition is a big problem in arid and semi-arid regions and HS application can possibly be a remedy for lime induced iron chlorosis in these soils (Chen and Barak, 1982; Zachariakis et al., 2001).

Increased P fertiliser use efficiency has frequently been found in soils amended with organic matter (Violante and Gianfreda, 1993). Delgado et al. (2002) found an increase in recovery of applied P with the application of a HA-FA mixture. They furthermore stated that manures and other organic sources of P were more efficient in increasing available P than inorganic P fertilizers. The use of P solubilising bacteria as inoculants can simultaneously increase P uptake and crop yield (Rodriguez and Fraga, 1999) and strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* show most potential. The most prominent and consistent effect of AM fungi is to improve the uptake of nutrients that are scarce, diffusion-limited and immobile, particularly P, Cu and Zn (Habte, 2006). Karlidag et al. (2007) and Jeon et al. (2003) found specific *Pseudomonas* and *Bacillus* strains capable of dissolving insoluble phosphates.

The use of EM inoculum along with organic or inorganic materials was found to be an effective technique for stimulating release of nutrients from these nutrient sources (Khaliq et al., 2006). Economic analyses

suggested the use of half of the mineral NPK with organic matter and EM, saves the mineral N fertiliser by almost 50% compared to a system with only mineral NPK application.

1.6.4 Natural resource conservation

1.6.4.1 Soil Carbon

Evaluations of the agricultural impact on soil carbon sequestration emphasize the importance of the return of carbon to the soil (Freibauer et al., 2004). Knorr et al. (2005) claim that rising temperatures brought about by climate change will cause microorganisms in soils to decompose organic matter more rapidly, releasing extra CO_2 and over the long term accelerating climate change. Depletion of soil organic C from the root zone will in addition to environmental quality, strongly affect soil productivity. By adapting management practices that accumulate carbon or slow down the decomposition process, CO_2 emissions from agricultural fields can be reduced (Termorshuizen et al., 2004). Recommended management practices for soil C sequestration includes; reduced tillage, surface residue management, mulching of bare soil, manure application, the use of cover crops, as well as use of composted material including a fraction resistant to decomposition (Follett et al., 2005). However, it is important to keep in mind that soil organic C is reactive and an increase may also have negative impacts on the local environments if the soil is not properly managed (Komatsuzaki and Ohta, 2007).

1.6.4.2 Water conservation

Globally, the pressure on water resources is increasing dramatically. Therefore, any management practice that can lead to water saving could play an important role in improving sustainability. One of the most beneficial factors of mulching is soil moisture conservation, due to protection from extreme temperature fluctuations, improved water permeability and storage and reduced evaporative losses. Pinamonti et al. (1995) found in vineyards and apple, as well as Kotzé and Joubert (1992) in grapevines and apricots respectively, that soil moisture was consistently higher with compost applied as a mulch. Lakatos et al. (2000), also in apple, showed that straw and pine bark mulch resulted in better uniformity of distribution of water as opposed to clean cultivation, while Neilsen et al., (2003b) found improved water distribution in the root zone of irrigated crops receiving a mulch treatment. In a study by Wooldridge (1992), mulching with hay more than doubled the irrigation cycle length and reduced the amount of water needed to sustain pear trees through the season by 55%. Neilsen et al. (2003a) found that application of biosolids to apple trees increased water infiltration rate, but of more practical significance was the increase in water retention capacity when applied in combination with a shredded paper mulch. Furthermore, in a study by Fallahi et al. (2006), apple trees receiving a granular formulation of 70% HA showed significantly higher water retention in the root zone.

1.6.5 Economic considerations

Producers' acceptance of the implementation of biological strategies in commercial agriculture will involve practical as well as economic considerations such as transportation and labour costs, availability of

organic material and efficacy of treatments compared to conventional treatments. It is therefore important to determine if benefits from these amendments compensate for additional expenses.

Cost of initiation and maintenance of mulching or compost application may seem high (Shribbs and Schroch 1986a; b), however with modern, high density orchard systems, these inputs can be optimised for maximum production. Furthermore, although crop yield is important, benefits of improved soil and environmental quality achieved through biological inputs are equally valuable without showing immediate reward in the market place (Renagold et al., 2001). Reduction in chemical fertiliser usage and pesticides also need to be taken into account. The amount of material needed for mulching can create severe logistical problems and is both time consuming and labour intensive to apply and maintain (Lipecki and Berbec, 1997). Therefore ways need to be investigated to improve plant biomass production in the orchard to use the residue as mulch. More research is needed on the use of cover crops in orchard systems.

Improvements in soil fertility through biological amendment applications and water conservation under mulches are potential long-term benefits that eventually may compensate for greater establishment and maintenance costs (Merwin et al., 1995). Neilsen et al. (2003b) also speculated that improved soil conditions may have greater impact only for future orchards planted on the same site. Future challenges are the incorporation of the value of ecosystem processes into the traditional market place, as well as financially supporting producers in their attempts to employ both economically and environmentally sustainable production practices.

1.7 CONCLUSION

Soil is the substance in which the majority of biochemical reactions occur which are critical to soil functioning. These processes are mediated by soil microorganisms, which in turn are sustained by SOM. Any cultivation practice executed or amendment applied, either directly or indirectly affect soil biology and thereby plant health and productivity. There is much controversy surrounding the sustainability of conventional practices. Complete loss of soil function due to conventional management practices is the exception rather than the rule (Bünemann et al., 2006). However, loss of specific soil functions carried out by only a few species, as well as effects on soil organisms critical in nutrient cycling, is a big threat to agroecosystem functioning. Therefore more emphasis needs to be placed on the ability of soil to recover from external inputs or environmental factors (Griffiths et al., 2001). The role of biodiversity in securing crop protection and soil fertility seems clear and most agriculturists would agree that implementing strategies to enhance SOM, will improve soil quality, and aid in the movement towards more sustainable production practices.

Research on the use of biological amendments in deciduous fruit crops is limited, and due to various applications methods, and different rates and materials used, it is difficult to draw exact conclusions.

However, there are indications from the literature on amendments used and expected outcomes. It can be summarised that various biological amendments affect soil microbial activity positively, and are certainly of value in improving plant nutrition as well as attaining disease suppression. Furthermore, effects on root growth development and proliferation are pronounced. Although beneficial effects on yield are not always apparent, in terms of fruit quality some benefit has been shown. With the emphasis on getting young trees off to a good start, there is also definite scope for application of biological amendments in orchard establishment, especially in orchards with replant problems or marginal production conditions. Furthermore, increased fertiliser use efficiency with some biological amendments can lead to significant reductions in fertiliser application.

According to Bünemann et al., (2006), much of the potential for microbial inoculants is yet to be realised. More research is also needed on the effects of compost extracts. These inoculants are easy to apply and not very costly. Microbial inoculants often struggle to compete in complex field situations. Therefore, it seems plausible that persistence of disease suppressiveness operating through a biological mechanism will be greater if the organic amendment functions through enhanced activity of the resident soil microbial community (Mazzola, 2004). Research need to be expanded on the effects of cover crops and their root exudates on resident rhizosphere communities.

Baker et al. (1967) previously stated that ‘considering the great amount of inherent variability across various organic amendments, specific microbial isolates, plant susceptibility, soil characteristics and other environmental factors that influence microbial processes, a lack of uniform, concise management recommendations is not surprising’. Consequently, effective use of biological amendments strongly depends on the type of soil as well as the environmental conditions. Furthermore, extrapolation of results from greenhouse or controlled studies to field application can lead to inconsistent effects. Therefore, the evaluation of biological amendments in field conditions is of great importance. A much better understanding of the mechanisms involved in generating positive or negative functioning in field environments is necessary to develop management strategies that can be applied in commercial agricultural systems. In the past few years there has been a dramatic increase in the number of publications studying the effect of various biological management practices on soil microbial activity, however, our knowledge of soil ecosystem functioning is limited in part by the complexity of measuring soil microorganisms. Although various methods are available and literature is abundant, no single method has been widely accepted, since each method has its own limitations. Furthermore, the relationship between plant performance and microbial activity is not well studied in fruit trees.

1.8 RESEARCH OBJECTIVES

1.8.1 Overall objective

The main objective of the study was to investigate the effect of organic material, microbial inoculants and biostimulants, most widely used in the study area, on tree performance and selected soil microbial properties in conventional management systems of commercial pome fruit production.

It was hypothesised that the application of biological amendments can affect soil microbial numbers, activity or function, thereby having a positive effect on tree growth, yield or fruit quality. Literature has shown that the mechanisms through which these amendments affect plant growth is mainly through either direct or indirect effects on root development and soil microbial communities, leading to improved plant nutrition, crop protection against pests and diseases or changes in plant phytohormone balances.

1.8.2 Specific objectives of the study were the following:

1) To establish if biological management practices can improve tree performance in a pear orchard established on BP1 rootstock, that suffers from poor root development in the initial years after planting.
2) To evaluate the potential use of biological soil amendments for use as alternative management practices to soil fumigation in reducing the effects of apple replant disease under South African conditions.
3) To determine whether application of biological amendments in an optimally managed, high density apple orchard can lead to complimentary improvement in tree performance.
4) To establish if changes in the soil microbial properties measured can be related to effects on tree performance.
5) To extract general conclusions on the changes to be expected with biological amendments on selected soil microbial properties.
6) To extract general conclusions on the use of biological amendments in pome fruit orchards in South Africa.

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CHAPTER 2

EFFECT OF ORGANIC MATERIAL AND BIOLOGICAL AMENDMENTS ON PEAR TREE PERFORMANCE, NUTRIENT AVAILABILITY AND SOIL BIOLOGICAL PROPERTIES

ABSTRACT

The long-term effect of continued applications of organic material (straw mulch, compost), various microbial inoculants (compost extract, *Bacillus* inoculants, Effective Microorganisms) and biostimulants (seaweed extracts, humates) on tree performance was investigated in a conventional management system. A field trial was conducted in a commercial 'Early Bon Chretien' (Williams) pear orchard established on BP1 rootstock (generally suffering from poor root development) in the Vyeboom region (34° 08' S; 019° 02' E), Western Cape, South Africa. The experimental layout was a split-plot design consisting of straw mulched and non-mulched plots (main treatments) and nine biological management practices (sub treatments). Organic material and biological amendments were applied from orchard establishment in 2002 and then every season up till the harvest of 2008. The effect of these biological management practices on nutrient availability, uptake and soil microbial properties was investigated in order to aid in the interpretation of tree performance effects. Soil microbial properties were measured by making use of soil enzyme activity assays (urease, acid phosphatase and β-glucosidase), conventional microbial plate counts (*Bacillus* spp. and actinomycetes) and community level physiological profiles (Biolog). Over the six year trial period maintaining a wheat straw mulch in the tree row showed few effects on nutrition and no effect on tree performance, despite significant changes in soil microbial functioning and activity. Annual compost applications improved soil microbial, as well as chemical properties. However, tree performance, in terms of vigour and yield, was only improved when in addition to compost, compost extract was applied monthly. It was suggested that monthly compost extract applications resulted in maximum efficiency of nutrient utilisation through synchronisation of nutrient release with plant demand. Results indicated that no simple relationship was apparent between yield and the parameters measured in this study.

Keywords: *compost, compost extract, Bacillus, seaweed extract, manure, humate, straw mulch*

2.1 INTRODUCTION

Environmental pressures in combination with escalations in production costs have renewed interest in the integration of biological soil amendments into standard agricultural management systems. Conventional agriculture has mainly relied on inorganic nutrient applications with low organic matter input and intensive use of pesticides to maximise crop productivity. Much less attention has been given to the importance of soil biological processes in maintaining plant health and yield. The development of soil structure (Tisdall and Oades, 1982; Gupta and Germida, 1988; Beare, 1997; Wright and Upadhyaya, 1998; Miller and Jastrow, 2000), soil fertility and plant nutrition, (Jenkinson and Ladd, 1981; Jeffries et al., 2003; Glick, 1995; Zahir et al., 2004), as well as disease suppression (Baker and Cook, 1974; Bowen and Rovira, 1999; Whipps, 2001) are regulated by the interactions of a highly diverse and complex web of soil flora and fauna that is sustained by the influx of organic matter into the soil (Alexander, 1977; Larson and Pierce, 1991; Tisdall, 1996; Murphy et al., 2003; Davet, 2004; Magdoff and Weil, 2004). Although complete loss of soil function due to conventional management practices are highly unlikely (Bünemann et al., 2006), loss of specific soil functions carried out by only a limited number of species, as well as shifts in soil fauna and flora communities, can have a significant effect on agroecosystem functioning (Griffiths et al., 2001), affecting plant growth and yield negatively.

Microbial activity is generally carbon-limited in agricultural soil (Campbell, 1989; Magarey, 1999; Bünemann et al., 2006) and it is widely accepted that management practices providing a range of organic compounds on a regular basis, will tend to maintain an active and diverse microbial population (Kennedy and Gewin, 1997; Magdoff and Weil, 2004). The connection between soil organic matter application and biological functioning in agroecosystems is clearly shown when comparing chemically intensive and organic or biologically integrated farming practices (Bolton et al., 1985; Doran et al., 1987; Fraser et al., 1988; Reganold et al., 1993; Wander et al., 1994; Drinkwater et al., 1995; Katayama et al., 1998; Mäder et al., 2002; Flieβbach et al., 2007). Improvement of tree performance in deciduous fruit have been found with organic amendment application either as mulches or incorporated into the top soil (Hogue and Neilsen, 1987; Autio et al., 1991; Wooldridge, 1992; Kotzé and Joubert, 1992b; Reganold et al., 1993; Marsh et al., 1996; Pinamonti, 1998; Neilsen et al., 2003a, 2007; Van Schoor, et al., 2009).

The addition of biostimulants, such as seaweed extracts and humic substances (HS) is also widely advocated in biological management systems (Russo and Berlyn, 1990; Chen and Aviad, 1990; Verkleij, 1992). However, the effects of these amendments in fruit production have received relatively little attention, although some positive effects have been found in citrus (Webb and Bings, 1988) and grapevine (Reynolds et al., 1995; Zachariakis et al., 2001) with the application of commercial HS. Application of soil inoculants has shown benefits especially in improving plant health and improved uptake of nutrients (Glick, 1995; Zahir et al., 2004). Plant growth promoting rhizobacteria (PGPR) have furthermore been shown to increase yield and crop quality (Kloepper, 1994; Zahir and Arshad, 1996; Rodriguez and Fraga,

1999; Dobbelaere et al., 2001; Esitken et al., 2003; Orhan et al., 2006). In a recent study on young apple trees, Aslantas et al. (2007) found that *Pseudomonas* and *Bacillus* spp. could effectively promote shoot growth and yield of different apple cultivars. Furthermore, the use of microbial inoculant mixtures containing a diversity of unspecified soil microorganisms are being promoted with little scientific literature to back up claims made. Effective microorganisms (EM Technology™) and more recently the use of compost extracts or compost teas, has been advocated as microbial inoculants that can stimulate and enhance the soil microflora (Higa, 1994; Ingham, 1999; Litterick et al., 2004). These inoculants are used locally in organic agriculture to a wide extent and since virtually no scientific literature is available on their use in deciduous fruit production it is important to establish their value in terms of improving tree performance.

Although research on deciduous fruit crops is limited, it was proposed that the potential of soil for deciduous fruit production can be increased considerably by biological amendment application. The mechanisms through which these amendments affect plant growth is mainly through either direct or indirect effects on root development (Glick, 1995; Moore-Gordon et al., 1996; Pinamonti, 1998; Yao et al., 2005; Forge et al., 2008) and soil microbial communities, leading to improved plant nutrition (Jenkinson and Ladd, 1981; Glick, 1995; Ferris et al., 1998), crop protection against pests and diseases or changes in plant hormonal balances (Elliott and Prevatte, 1996; Arshad and Frankenberger, 1998). Optimal fruit production and sustained high fruit yield are associated with good root proliferation and high soil fertility consequently rendering the plant more tolerable to stress.

We hypothesised that the application of various biological amendments can affect soil microbial numbers and function, thereby having a positive effect on fruit tree growth and yield. The objective of the study was to investigate the long-term effect on tree performance of continued applications of organic material, various microbial inoculants and biostimulants, in a conventional management system. For this purpose, a field trial was conducted in a pear orchard established on BP1 rootstock that generally suffers from poor root development in the initial years after planting. Furthermore, the effect of these amendments on nutrient availability and uptake and soil microbial properties was investigated in order to aid in the interpretation of tree performance effects.

2.2 MATERIALS AND METHODS

2.2.1 Orchard study site and experimental design

The experiment was conducted in a newly established commercial orchard in the Vyeboom region (34° 08' S; 019° 02' E), one of the main pome fruit production regions in the Western Cape, South Africa. The study site consisted of 'Early Bon Chretien' (Williams) pear (*Pyrus communis*) trees on BP1 rootstock planted in the winter of 2002 at a spacing of 4.5 m x 2.0 m (within row) on a soil not previously planted to apple or pear. The soil was a gravelly, sandy loam soil (7% clay, 30% silt, 63% sand and 45% stone) and

with orchard establishment pH (KCl) values averaged 6.5, total soil carbon 1.5%, and cation exchange capacity (CEC) (pH 7) 4.5. Plots were separated by two guard trees. The experimental layout was a split-plot design consisting of two main treatments and nine sub treatments, blocked three times, with an experimental unit consisting of six trees. Irrigation was supplied through a micro irrigation system (discharge rate 54 $L.h^{-1}$), 2-3 times per week by C-probe scheduling to keep water between field capacity and 50% plant available water. An approximate amount of 6000 $m^3.ha^{-1}$ was applied yearly to bearing trees. All treatments were treated equally in terms of fertiliser and pesticide application, as per the standard orchard practice. Glyphosate ($4L.ha^{-1}$) was applied to all treatments in spring and again in autumn to control weeds. Organic material and biological amendments were applied from orchard establishment in 2002 and then every season up till the harvest of 2008.

2.2.2 Treatment application

Main treatments consisted of a 10 cm thick wheat straw mulch application, maintained throughout the trial period, and a non-mulched treatment. Subplot treatments consisted of organic material application, soil inoculants and biostimulants and included:

1) Untreated control plots, managed to industry norm.

2) Chicken manure applied at planting at ($5\ ton.ha^{-1}$) to a 1 m^2 soil surface area around the tree as a once-off application.

3) Commercial compost, applied at 15 $ton.ha^{-1}$ of which a third was mixed with soil in the planting hole and the rest was applied as a top dressing in spring. Surface application of compost was repeated annually in spring at 15 $ton.ha^{-1}$. Compost consisted of aerobically composted chipped garden waste material (60%), horse manure (15%), grape waste (5%), vegetable waste (5%), mature compost (10%) clay (5%) and a powdered inoculum (produced locally, BioEarth, Stellenbosch) sprayed onto the rows with the first turn of the compost row.

Soil inoculants were applied as a drench with planting and thereafter annually, on a monthly basis throughout the growing season and included:

4) Compost extract (BioEarth, Stellenbosch, SA) applied at 500 $L.ha^{-1}$, diluted 50:1 sprayed onto the soil with each application. The compost extract was prepared by adding 1000 L of water to 50 kg of re-composted compost (same as used previously) and actively aerating the suspension for 48 h, with no additional additives.

5) Effective miroorganisms (EM) were applied as a diluted suspension (1:1000) of stock EM in combination with molasses ($1\ L.ha^{-1}$) for each application. EM primarily consists of photosynthetic and lactic acid bacteria, yeasts, actinomycetes and fermenting fungi (Higa, 1994).

6) Biostart® (Microbial Solutions, Kya Sand, SA) inoculant was applied at $1\ L.ha^{-1}$. Biostart® is a soil applied, liquid three-strain, living Bacillus bacterial formulation consisting of *Bacillus laterosporus, B. chitinosporus* and *B. licheniformis*. The liquid living bacterial formulations were applied in combination with a Microboost® Activator as a carbon source to sustain the activity of the introduced bacteria. From

the third growing season this product was applied in combination with a low dosage humic acid product, a 14% Potassium humate liquid, at 10 $L.ha^{-1}$. Humic substances comprise a major part of soil organic matter (SOM) and are classified into humic acids (HA), fulvic acids (FA) and humin on the basis of their solubility in water as a function of pH (Swift, 1999). Humic acid salts are termed humates.

Biostimulants applied, included two liquid seaweed extracts and a humate product:

7) A potassium humate product was applied anually, at 50 $L.ha^{-1}$ split into a spring and an autumn application (Omnia, SA).

8) Kelpak (Kelp Products, Simonstown, SA) derived from *Ecklonia maxima* and containing natural auxins (11 $mg.L^{-1}$) and cytokinins (0.031 $mg.L^{-1}$) were applied annually, as an initial soil drench in spring at 25 $L.ha^{-1}$, followed by three foliar sprays at a 1:500 dilution.

9) Goëmar (Goëmar Laboratoires, St. Malo, France) a product specifically formulated for soil application and derived from *Ascophyllum nodosum* (auxins, cytokinins, gibberellins ca 200 $\mu g.kg^{-1}$ and traces of amino acids and betaïnes) were applied as four soil drenches at 2 $L.ha^{-1}$ each for the first two growing seasons and replaced with Goëmar FoliphosE (containing P_2O_5 340 $g.L^{-1}$ and K_2O 65 $g.L^{-1}$ in addition to the original product) applied twice at 5 $L.ha^{-1}$ for the last four seasons of the trial period.

Chemical properties of the compost and compost extracts used are shown in Appendix A. The structure of the microbial consortium within the compost and compost tea was not determined. Recently, it was found that the functional diversity among microorganisms within an ecosystem may outweigh the diversity among taxonomic entities (Dinsdale et al., 2008). Thus, it was decided to rather determine the effect of these biological amendments on the soil ecosystem, than to compare their microbial composition to that of the soil. Also, previous experience with compost extracts, of which the microbial composition was monitored using molecular analyses of taxonomic informative gene-sequences, indicated that the microbial consortium within these extracts continually changes. (Prof A. Botha, personal communication).

2.2.3 Tree performance evaluation

Trees were permanently marked 20 cm above the graft union and trunk circumference measured every year during winter. Total growth (shoot growth and leader growth) were measured at planting and at the end of each growing season for the first to fourth season (trees were cut back at planting, and again after the second growing season). Annual yield on a per tree basis was recorded for the 2006/2007 season (the first season trees were allowed to bear) and the 2007/2008 season, after five and six seasons of applications respectively. Cumulative yield efficiency for the past two seasons were calculated by adding 2007 and 2008 yield and dividing by trunk section area as measured with the 2008 harvest. The number of harvested fruit was determined for each tree in order to calculate average fruit mass per plot for each treatment replicate. Fruit quality parameters were measured for the 2007 and 2008 season and included fruit firmness, total soluble solids (TSS) and total titratable acids (TTA). These parameters were evaluated at harvest, after 8 weeks (2007) and 12 weeks (2008) storage at -0.5 ºC under regular atmosphere (RA), and

then after 7 days at room temperature (21-24 ºC) (shelf life period). For each treatment combination 35 fruit were evaluated from each plot for each evaluation.

2.2.4 Leaf nutrient analyses

In order to relate leaf nutrient content to effects on tree performance, leaf nutrient analyses were done for treatments showing contrasts in tree performance. Control plots and biological amendments showing the most potential for improving tree performance according to shoot and trunk growth measurements were selected. Leaf nutrient analyses were done for the last three seasons (2006-2008) to provide insight into tree performance effects after continued application over three seasons. A combined 50 leaf sample of mature leaves in the mid shoot section of the current years growth was collected at the end of January from the six trees in each plot. Leaf samples were washed in 1% v/v HCL solution and then rinsed twice, first with tap water and then with deionised water, after which it was dried at 80 ºC. Samples were analysed for both macro- and micronutrients by a commercial laboratory (Bemlab®, Strand, SA) using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Varian MPX-OEX, Varian Inc. Co., Palo Alto, California, USA) and a nitrogen analyzer (LECO FP528 Nitrogen analyzer, LECO Cooperation, St. Joseph, Michigan, USA).

2.2.5 Soil sampling and analyses

Due to economical constraints, only control treatments and treatments with biological amendments showing the most potential in terms of growth improvement based on shoot growth measurements, were sampled. Soil was sampled within the root zone of the top soil where microbial activity is expected to be greatest, at a depth of 0-25 cm. Samples were taken at a distance of 30-40 cm from the tree base, from two holes beneath four trees in each plot and composite samples prepared for each treatment from the eight sub-samples in each of the three block replicates. Soil samples were taken from selected biological amendment treatments in Oct 2006 (spring), after 5 seasons of annual application of biological amendments, but before commencement of the next seasonal applications. Soils were again sampled in Dec 2006 (summer), Apr 2007 (two weeks after the last application of the season in autumn) and again in Dec 2007.

Sub-samples of all the soil samples taken in Oct 2006, Apr 2007 and Dec 2007 were analysed for chemical soil properties by Bemlab® soil analyses laboratory (Strand, SA), using standard methods (Soil Science Society of South Africa, 1990; Soil and Plant Analysis Council, 1999). Soil pH (in 1M KCl), resistance (ohm), available macro- and micronutrients (ICP-OES), organic carbon (Walkley-Black method) and total N% (LECO Analysis) were measured. The resistance of a saturated soil/water paste was determined in a standard USDA soil cup. Soil P was determined using the Bray II method. K, Mg and Ca were extracted in ammonium acetate, Cu, Fe, Mn, and Zn in 0.1 M HCl and B in hot water. Bulk density (BD) was measured for the Dec 2007 sampling date. Field moist sub-samples were sieved through a 2 mm mesh screen for

microbial analyses. Visible root pieces and un-decomposed organic matter were removed and soil stored at 4ºC for no more than two weeks before analyses.

2.2.6 Soil microbial analyses

2.2.6.1 Plate counts. Conventional dilution spread-plating on laboratory media was performed to assess total *Bacillus* bacteria and actinomycetes only after treatments were applied. Numbers of *Bacillus* bacteria were counted on 1/10 strength tryptone soy agar (TSA, Difco) after pasteurization of soil samples for 10 min at 80 ºC. Sodium caseinate agar (Du Plessis et al., 2005) was used for enumeration of actinomycetes. Plates were inoculated in triplicate and incubated at 25 ºC. Total heterotrophic numbers were counted after 72 h incubation and actinomycetes were counted after 7-10 days depending on colony growth. Since less than 1%, of soil microorganisms can be cultured, these techniques can underestimate population size and diversity (Amann et al., 1995) and therefore additional methods were applied.

2.2.6.2 Soil enzyme activity. Soil enzyme systems are associated with organic residue management, and therefore affect the rate at which nutrients become available to crop and other soil organisms (Tabatabai, 1982; Perruci et al., 1984). Enzymatic activities have therefore been used as an indicator of the overall microbial activity in soils while also producing useful functional information on the capacity of a soil to carry out specific activities important in maintaining soil fertility (Dick, 1994, 1997; Garcia et al., 1997; Pascual et al., 2001; Ros et al., 2003; Caldwell, 2005; Bastida et al., 2008). Acid phosphatase, β-glucosidase and arylsulfatase activity were determined based on the release and spectrophotometric detection of *p*-nitrophenol (Tabatabai and Bremner, 1969; Tabatabai, 1982). Urease hydrolysing activity was determined by the non-buffered method of Kandeler and Gerber (1988). Controls were performed for all enzymes assayed by the addition of the substrate after incubation, but prior to analysis of the reaction product.

2.2.6.3 Substrate utilisation profiles. In addition to the functional formation provided by enzyme activity, substrate utilisation of the soil microbial community can be assessed. Commercially available Biolog® EcoPlates (Biolog® Inc., Hayward, USA) was used to provide community level physiological profiles (CLPPs) as an indication of community function, according to a modified procedure described by Buyer and Drinkwater (1997). The Biolog plates contain 31 ecologically relevant carbon sources replicated three times within each plate to help account for variability in inoculum density. Soil suspensions were prepared by shaking 10 g of field moist soil in 90 mL sterile deionised water for 30 min on a rotary shaker and allowing it to settle for 2 h to remove larger soil particles. Using sterile deionised water the supernatant was subsequently diluted to a final dilution of 1:1000 and each well inoculated with 125 µL. Plates were incubated at 25 ºC and tetrazolium violet reduction measured spectrophotometrically after 24 h, as well as 38 h at 595 nm using a Biorad microtiter plate reader. Incubation for 24 h was used to estimate substrate utilization of soil microbial communities with a fast metabolism and 38 h incubation to also detect

microbial communities with a slower metabolism. Analyses of each sample were performed in triplicate. Optical density (OD) values from the Biolog plates were analysed using the average well colour development (AWCD) technique as described by Garland and Mills (1991). AWCD were calculated as the average OD across all wells per plate. OD values were corrected by subtracting values of the control well (blank). Substrate utilisation ability was calculated as the number of substrates metabolised per 31 substrates as done by Zak et al., (1994). Data were then transformed to eliminate variation in AWCD caused by different cell densities, by calculating AWCD for each plate and then dividing the values of the individual wells by the AWCD value of the plate. The transformed data were then used to analyse microbial community function.

2.2.7 Statistical analysis

A standard split-plot analysis of variance (ANOVA) was performed on tree performance data, microbial plate counts, soil enzyme activity, as well as soil physiochemical characteristics using, SAS Statistical Software (SAS, 2002-2003). Trunk circumference measurements over the trial period were analysed as repeated measurements by comparing the slopes (b values) of linear regressions fitted to the data (R^2 = 0.99) in an ANOVA. Student's t-LSD was calculated at a 5 % significance level to compare the treatment means. Profiles of carbon substrate utilisation were statistically analysed by principal component analysis (PCA) (Garland and Mills, 1991; Palojärvi et al., 1997; Buyer and Drinkwater, 1997; Larkin, 2003), using the correlation matrix (Rencher, 2002). Pearson Product Moment correlation coefficients (r) were calculated (SAS, 2002-2003) for averages of parameters measured over the trial period, as well as for the last sampling date of soil, leaves and yield of 2008. In addition, a Stepwise regression was performed to predict yield (SAS, 2002-2003). Furthermore, stepwise discriminant analysis (SDA) was used to select a sub-set of variables from an initial of 36 variables including leaf nutrient contents, soil chemical and biological parameters, as well as yield parameters. The subset of variables contained those variables which best differentiate or discriminate between the soil amendments and were used for canonical discriminant analysis (CDA). A PCA bi-plot was constructed, illustrating the relationship between the variables and their association to the different soil treatments. Multivariate analyses were performed using XLStat software.

2.3 RESULTS

2.3.1 Growth measurements

There was no significant interaction between mulching and the various biological treatments for trunk circumference or shoot growth and therefore only the main effects are presented (Table 1). Trunk circumference measurements showed that all trees were of a similar size at planting. No significant effect on trunk circumference or shoot growth was measured with maintaining a wheat straw mulch in the tree row over six seasons (Table 1). However, with biological amendment application, plots receiving compost both with and without compost extract, as well as plots treated with Biostart, Goëmar or EM, significantly

increased the rate of trunk circumference increase during the trial period when compared to the control and plots treated with manure. Shoot growth was significantly increased by compost extract application in the first season when compared to all other treatments, as well as in the second year after establishment in comparison to all treatments except Goëmar (Table 1). After the third growing season, although results were not significant, compost extract treated trees still showed the most shoot growth, improving growth by 45% compared to control plots. A positive trend was also noted with Biostart application on shoot growth after three growing seasons when compared to the control.

2.3.2 Yield and fruit quality

There was also no significant interaction between the main and sub treatments for yield and main effects are presented in Table 2. The first yield was recorded in 2007 and second yield in 2008 after five and six seasons of application, respectively. There were no significant differences in yield ($kg.tree^{-1}$) of trees between mulched and non-mulched plots (Table 2). Application of the various biological amendments did also not result in significant differences in yield. However, there was a clear trend with compost extract application showing highest yield and yield efficiency, for both harvest seasons. The addition of compost extract with compost application resulted in a 51% increase in cumulative yield on a per tree basis when compared to control trees. It was observed that most of the biological treatments had a positive effect on cumulative yield and yield efficiency over the two seasons, when compared to the control. Application of manure, as well as EM in combination with molasses resulted in similar cumulative yields than the control trees over the two harvest seasons. There were no significant differences in fruit mass with mulching or any of the biological amendments. However, the greater number of fruit yielded by the compost extract treated plots did not affect fruit size negatively.

Fruit quality parameters for the 2007 harvest season showed no significant interaction. There were also no significant treatment differences with mulching or biological amendment application on fruit quality at harvest or directly following storage (Table 3). Evaluation after the shelf-life period did show significantly firmer fruit for mulched compared to non-mulched plots, but differences were very small and of no commercial value (Table 3). Fruits were stored for 12 weeks during the 2008 season, compared to the 8 weeks of the 2007 season. In the 2008 season there was significant interaction between mulching and the biological amendments for fruit firmness at harvest, as well as TTA following a shelf-life period (Table 4). Although differences in fruit firmness with harvest were small, in the non-mulched system all biological amendments except Goëmar improved firmness significantly compared to controls (Table 4). Compost extract application in the mulched system resulted in significantly lower firmness compared to controls, compost treatment on its own, as well as Biostart treatment. Furthermore, mulched control plots resulted in significantly firmer fruit than the non-mulched control. Biostart treatment resulted in significantly lower fruit TTA than the control when applied in combination with a mulch. However, although not significant, results were opposite in the non-mulched system and Biostart treatment increased TTA. When there was no interaction, main effects showed that between mulched and non-mulched plots the only significant

difference in fruit quality was found for TSS evaluation after 12 weeks storage, where results showed higher TSS levels for fruit from mulched plots (Table 4). Biological amendments showed no significant differences at harvest and after storage, but following a shelf life period, TTA was significantly higher for fruit from control plots compared to most of the biological amendments, with the exception of K-Humate.

2.3.3 Microbial analyses

Due to the laborious nature of these analyses and to reduce storage effects of soil to a minimum, treatments that showed the most improvement in growth when compared to untreated trees were selected for microbial analyses, in order to compare contrasts in tree performance. These treatments included Biostart, Göemar, compost and compost extract applied in combination with compost.

2.3.3.1 Plate counts. There were no significant interaction between mulching and biological amendments and only main effects are presented in Table 5. Actinomycete counts showed no significant sub or main treatment differences over the four sampling dates (Table 5). *Bacillus* counts also showed no significant difference between mulch and non-mulched plots for any of the sampling dates. *Bacillus* counts for Dec 2007 showed significantly higher counts for compost application on its own, as well as in combination with compost extract, in comparison to the control, Goëmar and Biostart treated soil. When taking average counts over all four sampling dates (Table 5), *Bacillus* counts were significantly higher in the two treatments receiving compost, than the control and Biostart treatments. There was no significant interaction with time for both the main and the sub treatments.

2.3.3.2 Enzyme activity. There was significant interaction between mulching and the biological treatments with urease activity for both summer samples in 2006 and 2007 (Table 6). The interaction indicated that in Dec 2006 increases in urease activity with compost extract was significant in a non-mulched system, but generally high for all the treatments if compared in the mulched system (Table 6). Application of mulch to the control plots also resulted in significantly higher urease activity. In Dec 2007, compost extract application again had a significant effect when applied in a non-mulched system, but in the mulched system compost application on its own performed superior to all other treatments and significantly increased urease activity compared to all treatments except Biostart. Furthermore, in Dec 2007 mulched soil showed significantly higher urease activity with all treatments, except compost extract application, when compared to non-mulched soil. There were no significant differences in urease activity between mulched and non-mulched plots for the other sampling dates. There was a general trend over the four sampling dates for urease activity to be higher with treatments where compost was applied. Biostart and Göemar application did not result in significant changes in urease activity levels compared to the control.

Acid phosphatase activity only showed a significant interaction between mulching and the biological treatments in Dec 2006 (Table 6). The interaction indicated that compost application, as well as Goëmar treatment when combined with mulching, resulted in significantly higher phosphatase activity compared to

mulched controls. However, without mulch application phosphatase activity were similar between the various treatments, except for compost extract application resulting in significantly lower phosphatase activity compared to the control. In Dec 2006, mulch application also significantly increased phosphatase activity in the control, as well as the Biostart treatment. Similar to urease activity in Dec 2007, phosphatase activity in Dec 2007 was significantly higher in mulched soil compared to non-mulched soil. Phosphatase activity was similar between mulched and non-mulched plots for the first three sampling dates. Biological amendments applied as sub treatments had no significant effect on phosphatase activity.

β-glucosidase activity showed no significant difference between mulched and non-mulched plots or the various biological amendments and the control for the Oct 2006, Dec 07 and Apr 07 sampling dates. β-glucosidase activity showed significant interaction between main and sub treatments for the Dec 2007 sample (Table 6). This was similar to the interaction found with urease activity in Dec 2006, showing a significant increase in enzyme activity with compost extract application in the non-mulched system compared to the control, while in a mulched system β-glucosidase activity was similar for all treatments, except compost extract showing significantly lower enzyme activity compared to Biostart treatment. Similar to phosphatase activity in Dec 2006, mulch application significantly increased phosphatase activity in the control, as well as the Biostart treatment.

Arylsulfatase activity was determined for the last two sampling dates and was consistently higher for mulched plots, but results were only significant in Dec 2007 (Table 6). No significant treatment differences were found for the sub treatments.

2.3.3.3 Substrate utilisation. Results obtained after 24 h incubation of inoculated Biolog plates, showed that there was a significant interaction between mulching and the biological amendments only in Dec 2007 (Table 7). For this sampling date, in a mulched system, microbial communities from all treatments utilised a similar number of substrates, except Goëmar, which utilised less substrates compared to the control and compost treatment. Mulching in combination with control plots, as well as both compost treatments, resulted in more substrates utilised when compared to non-mulched plots (Table 7). However, in a non-mulched system, significantly more substrates were utilised by soil microbial communities subjected to Biostart treatment when compared to all other treatments. The number of substrates utilised by microbial communities subjected to Biostart without mulch application was also similar to the number of substrates utilised in the mulched system. Mulching as a main effect showed a similar trend for the Oct and Dec 2006 sampling dates although results were not significant (Table 7). After 38 h incubation of plates differences in the number of substrates utilised between soil microbial communities from mulched and non-mulched soil were only significant for the Dec 2006 sample. There were no significant effects for the number of substrates utilised with the various biological amendments.

Average well colour development (AWCD) values of the 31 carbon substrates contained by the Biolog plates were analysed by PCA using the mean AWCD values over the four sampling dates to compare community level physiological profiles (CLPPs) of the selected treatments. Incubating the inoculated Biolog plates for 24, as well as 38 h, revealed that mulching did not result in microbial communities with similar substrate utilisation profiles, when subjected to different biological amendments in addition to the mulch (Figures 1A and 1B). Furthermore, microbial communities from soil treated with the same biological amendment, either with or without the addition of a straw mulch, did not always show similar CLPPs.

After 24 h incubation of Biolog plates (Figure 1A) there were clear differences in CLPPs between compost with and without mulch application, as well as Biostart with and without mulch, while compost extract and Goëmar treatments showed similar profiles irrespective of mulching. The variation in substrate utilisation of microbial communities not subjected to any biological amendment (control treatment without a mulch) was very high but CLPPs still differed significantly from microbial communities subjected to compost treatment, with and without compost extract, Biostart treatment with mulch, as well as Goëmar treatments. Specifically looking at the microbial communities from the four treatments receiving compost, it was observed that after 24 h incubation of inoculated plates, the addition of compost extract resulted in different substrate utilisation in the mulched management system, but not in the non-mulched system. The percentage accounting for total variability in substrate utilisation was only 34%. This may be due to the execution of the PCA on data including block replicates (in order to calculate standard deviations) showing more variability than when analysing average values.

Incubating inoculated Biolog plates for 38 h, revealed that microbial communities from control plots not receiving a mulch showed distinct CLPPs from all treatment combinations, except Goëmar applied with mulch (Figure 1B). For all biological amendments, as well as controls, the addition of a straw mulch changed CLPPs of the soil microbial community. Differences in CLPPs with and without mulching were most distinct for controls and treatments not receiving compost and less distinct with the addition of compost extract. It was noted that for CLPPs from the microbial communities subjected to compost treatments, results were similar than for 24 h incubation, with the addition of compost extract resulting in different CLPPs in the mulched system, but not in the non-mulched system. The percentage accounting for total variability was again only 35%.

2.3.4 Soil chemical analyses

In general, Ca and K were high in soil for all treatments including the control, while Mg was low when compared to industry norms (Kotzé, 2001) (Table 8). Soil extractable P increased from low in Oct 2006 to high in Dec 2007 in control soils. Furthermore, Mn was generally high for all treatments, while Cu, Zn and B were low compared to industry norms. The main effects of treatments on soil chemical parameters measured in Oct 2006, Apr 2007 and Dec 2007 are presented in Table 8. However, there was significant

interaction between the main and sub treatments for soil carbon (%), Mg, Ca, the T value and micronutrients Zn, Mn and B. Sub treatment effects showed that compost application, either with or without the addition of compost extract, was the only biological amendment that had a significant effect on soil chemical properties for some sampling dates, although not for all parameters measured.

There was no significant difference in soil pH or resistance between mulched and non-mulched plots for any of the sampling dates. However, resistance was lower in compost treated soil than for any of the other biological treatments in both the 2007 samples, although results were only significant in Dec 2007. The only significant treatment effects on pH when compared to controls, was with compost extract application in Dec 2007. The interaction for soil carbon was significant in both Apr 2007 and Dec 2007 (0-5 cm sample). Results showed that total soil carbon was similar for all treatments in a mulched system, with the exception of the Biostart treatment in Apr 2007 showing lower soil carbon compared to all other mulched treatments. In a non-mulched system the addition of compost significantly increased soil carbon, as well as Biostart application in Apr 2007 (Table 8). Mulching significantly increased soil carbon for control and Goëmar treatment in both Apr 2007 and Dec 2007 (0-5 cm sample), and also with Biostart application in Dec 2007 (0-5 cm sample). Main effects showed significant increases in carbon % with compost application for all sampling dates and both soil depths in Dec 2007 (Table 8). Furthermore, straw mulch application significantly increased carbon % in Oct 2006. There was no significant difference in total N (%) between mulched and non-mulched plots, except for the 0-5 cm sample. In general, for all sampling dates, total N was amongst the highest for treatments receiving compost. However, results were only significant in Dec 2007 and in Apr 2007 and for Apr 2007 only where compost was applied without compost extract.

Soil extractable P was higher in mulched soil for the first two sampling dates, differing significantly from non-mulched soils, only in Apr 2007. Soil extractable K showed similar trends with mulching as soil P. Levels of soil extractable P showed no significant difference between sub treatments for the first two sampling dates, although soil P was much higher with compost extract application in Apr 2007 compared to all other treatments. This could possibly be due to high variation between samples. There was a dramatic increase in soil P from Oct 2006 to Apr 2007 in all treatments and a further increase in P with compost application for Dec 2007. In Dec 2007 soil extractable P was significantly higher for both treatments receiving compost and P levels were very high compared to the industry norm (Kotzé, 2001). Soil K was significantly increased by compost application for the last two sampling dates. Furthermore, soil extractable Ca was higher with compost application and results were significant for the Dec 2007 sample. There was a significant interaction between the main and sub treatments for Ca in Apr 2007. In the non-mulched system compost application without the addition of compost extract significantly increased soil Ca compared to all other treatments. However, in the mulched system treatment application did not affect soil Ca significantly. Mulching resulted in significantly lower soil Ca when applied in combination with compost and significantly higher soil Ca when applied with Goëmar. There were no significant differences

in Ca and Mg content of soils when comparing mulched and non-mulched plots, except for interaction between mulching and biological amendment application for both Mg and Ca in Apr 07. The interaction showed significantly higher soil extractable Mg from compost treated soil compared to al other treatments in both mulched and non-mulched systems. However, in the non-mulched system compost without the addition of compost extract also significantly increased soil Mg when compared to compost with compost extract treatment. Soil extractable Mg was significantly increased by compost treatment compared to the control in both Oct 2006 and Dec 2007. The T value was significantly higher with compost application and results were significant in Dec 2007. There was also a significant interaction between main and sub treatments in Apr 2007. The interaction data showed that effects of compost application on the T value was similar in the non-mulched system as found for Mg, while there were no significant treatment differences in T values in the mulched system, except for Biostart which showed a significantly lower T value compared to compost treatment.

There were no consistent differences in soil micronutrients with mulching, except possibly for soil extractable Cu which was generally lower in mulched soil and the effect was significant for the Oct 2006 sample (Table 8). Soil micronutrients Zn and Cu were significantly higher in compost treated plots for all three sampling dates when compared to controls. There was a significant interaction between mulching and the biological treatments for Zn in Apr 2007. Mulching resulted in significantly lower soil Zn when applied with compost. Furthermore, in both the mulched and non-mulched system compost application significantly increased soil Zn. Soil extractable Mn and B showed significant interactions between main and sub treatments. In Oct 2006 soil Mn levels were only significantly higher with compost application when applied in combination with a mulch. Although Mn was generally higher in compost treated soil, sub treatment effects were only significant compared to the control in Dec 2007 when compost extract was applied in combination with compost. Soil B showed significant interaction in both Oct 2006 and Apr 2007. In Oct 2006 compost applied on its own was the only treatment to significantly increase soil extractable B in the mulched system and results were significant compared to all other treatments. In Apr 2007 compost in combination with compost extract was the only treatment to significantly increase soil B in the non-mulched system compared to the control. For this sample date soil B levels of plots receiving biological amendments in addition to a mulch did not differ significantly from mulched controls.

Bulk density (BD) was measured for the last sampling date (data not shown). Differences between mulched and non-mulched treatments were small (1.23 vs 1.30 $kg.L^{-1}$) but statistically significant ($P = 0.0263$), while no differences were found between the various biological amendments.

2.3.5 Leaf nutrient analyses

Values of macronutrients were mainly within the acceptable range for pear production (Kotzé, 2001), except for leaf K and Mg levels that were low for all treatments in the 2007 and 2008 season (Table 9). A significant interaction between main and sub treatments were only found for leaf P content in 2007. The

interaction showed that leaf P levels differed significantly with biological treatment application only in a mulched system and was significantly higher with Biostart application and significantly lower with compost and humate application compared to controls. Mulching also significantly increased leaf P concentrations when applied to the control, compost extract treatments, as well as Biostart. There was also a general decrease in leaf macronutrient content from 2006 to 2008, especially for P. However, no significant differences in leaf P were found in 2006 and 2008. In general, the majority of significant treatment differences were found in 2008. No significant differences with main effects were found between leaf nutrient content of mulched and non-mulched plots.

Sub treatment effects showed that leaf N was significantly lower in compost extract and humate treated trees in 2008 compared to the control. Results were similar for 2007, although only leaf N from humate treated plots differed significantly from the control. Leaf K content did not differ significantly between treatments, while leaf Ca content was significantly higher in 2006 with both treatments where compost was applied, as well as Göemar treatments compared to the control. In 2008, for leaf Mg content and most of the macronutrients leaf nutrient contents were lower for the compost extract and humate treatments, compared to the control, although effects were generally not significant and nutrient levels within the acceptable range. Furthermore, compared to the 2007 analyses, this was only consistent for N and Mg. In 2008 for leaf Mg, nutrient content of compost extract and humate treated trees was also significantly lower than with compost applied on its own.

Leaf micronutrient concentrations of Mn, Fe and Zn were consistently very high compared to industry norms, for all treatments across the three sampling dates (Table 9). There was a general decrease in Cu and B levels over the three seasons and in 2008 levels were in the lower range for both elements despite foliar applications. Biological soil amendments did not have a consistent effect on leaf micronutrients and results were again mainly significant for the 2008 season. During this season leaf B was significantly lower for compost extract and humate treated trees compared to control, as well as compost treated plots. Leaf Cu was lower for all treatments compared to the control in 2008, and the effect was significant with the majority of treatments, except compost. Leaf Mn levels in 2008 showed similar results to leaf Cu.

2.3.6 Correlations and multivariate analyses

Pearson's correlation coefficients were calculated for the averages of the different sample dates over which parameters were measured during the trial period. The most significant correlation was between *Bacillus* numbers in the soil and soil Zn content ($r = 0.640$; $p = 0.0001$). A significant negative correlation was found between arylsulfatase and BD ($r = -0726$; $p < 0.0001$). Furthermore, correlation coefficients were calculated for data recorded for the Dec 2007 soil sample, yield measured in Jan 2008, as well as leave nutrient analyses in Jan 2008 after harvest. Correlations of *Bacillus* numbers were again significant with soil Zn content ($r = 0.727$; $p < 0.0001$), but also soil Mg ($r = 0.704$; $p = 0.0001$), soil K ($r = 0.603$; $p = 0.0004$), and soil P ($r = 0.750$; $p < 0.0001$), and soil Ca ($r = 0.589$; $p = 0.0006$). In addition, Cu showed a

significant negative correlation with *Bacillus* numbers (r = -0.617; p = 0.0003). There were significant correlations between total soil C% and urease (r = 0.474; p = 0.0094), β-glucosidase (r = 0.474; p = 0.0093), phosphatase (r = 0.568; p = 0.0013) and *Bacillus* numbers (r = 0.467; p = 0.0093). Yield efficiency was negatively correlated to leaf N content (r = -0.600; p = 0.0020), as well as leaf K content (r = -0.669; p = 0.0001).

Principal component analysis (PCA), as well as a SDA were also performed on the averages of the different sample dates over which parameters were measured during the trial period. The PCA bi-plot illustrating the relationship between the various parameters measured and their association to the different soil biological amendments are presented in Figure 2. The percentage accounting for total variability in the data was 65%. All mulched treatments were found to group in the top two quadrants of the ordination axes, and non-mulched treatments in the bottom quadrants. Furthermore, both treatments receiving compost showed distinct differences from other treatments. Soil receiving compost and mulch associated more closely with the biological parameters measured, except for *Bacillus* counts, which was more closely related to compost applications without mulching and the association was even closer when compost extract was added. Yield parameters were most closely associated with leaf Ca and Zn content, as well as soil pH and were grouped in the same quadrant as the two treatments receiving compost without mulching. Furthermore, soil N content and leaf N showed a negative relation to yield and were associated with treatments that were mulched but did not receive compost. When evaluating the variables as predictors of yield with Stepwise regression, only leaf N content remained in the model (Cumulative Yield = 814.57 – 290.43 x Leaf N) and explained 62% of the variation.

Eight discriminant elements (variables) which had the most discriminatory powers for subsequent analysis were identified by SDA. These included leaf P content, soil pH, N%, Ca%, actinomycete and *Bacillus* counts, as well as urease and β-glucosidase enzyme activities. The selected variables were subjected to CDA analysis to establish whether discrimination between the various biological soil amendments could be achieved. Canonical variants 1 and 2 explained 94% of the total dispersion (Canonical variant 1 explained 75% of the variation, while canonical variant 2 explained the remaining 19% of the variation) (Figure 3). Standardised canonical discriminant function coefficients for canonical variant 1 was highest for soil pH, Ca%, N% and urease, while for canonical variant 2 coefficients were highest for β-glucosidase, urease and soil pH. Compost applications without mulch could be separated on the first canonical variable from all other treatments not mulched. Variables associated with soil pH and N was most likely responsible for this discrimination. Furthermore, all treatments receiving compost could be separated from other treatment combinations on canonical variable 2. Urease, β-glucosidase and soil pH was responsible for this separation. Control plots and soil treated with Biostart or Göemar without mulch could not be clearly separated from each other.

2.4 DISCUSSION

2.4.1 Effect of biological management practices on tree performance

Over the six year trial period, regular application of compost extract in addition to annual compost applications significantly improved shoot growth, as well as trunk circumference growth over the trial period. After the third growing season the addition of compost extract resulted in 46% more total growth compared to the control. Additional growth possibly resulted in an increase in bearing positions, resulting in the increase in yield and fruit number per tree with compost extract application in the 2007 and 2008 season. Application of Biostart (applied in combination with a low dosage humate), Goëmer and compost on its own, also resulted in a significant increase in trunk growth tempo and Biostart treated trees also showed increased shoot growth after three growing seasons. Furthermore, all the biological amendments showed a positive effect on cumulative yield over the two seasons, in comparison to the control, although effects were only significant for compos extract. Manure was only applied in the first two seasons, and may have resulted in significant positive effects if applied seasonally over the whole trial period, or at higher concentrations. No significant effects were observed on tree performance with maintaining a wheat straw mulch in the tree row.

Although no consistent significant effects on fruit quality were found over the two harvest seasons, there was significant interaction between mulching and Biostart, as well as compost extract, for some parameters measured. Further investigation is needed to confirm these effects. High N levels in fruit were shown to reduce fruit firmness (Marsh et al., 1996), however no negative effects on fruit quality were found with increased soil N resulting from the addition of compost. In literature, compost application on its own has shown few significant effects on fruit quality (Kotzé and Joubert, 1992b; Pinamonti et al., 1995; Neilsen et al., 2003a; Neilsen et al., 2007). In studies comparing different management systems, the majority of results show higher fruit firmness for apples produced in organic systems, higher TSS and lower acidity (DeEll and Prange, 1992; Andrews et al., 2001; Renagold et al., 2001; Peck et al., 2006). This corresponds with our results only for the 2008 season, where higher TSS values were found in mulched plots after 12 weeks storage.

2.4.2 Effect of mulching and biological amendments on nutrition

Straw mulching had little influence on soil chemical parameters or leaf nutrient content after six years of maintaining a wheat straw mulch, when compared to soil that was not mulched. In contrast to this, compost application in the planting whole with establishment, followed by a top soil dressing every season (total amount applied = 90 ton.ha^{-1}), significantly improved total soil N, as well as extractable P, K, Ca, and Mg. Soil P levels increased dramatically throughout the trial period. This was ascribed to the cumulative effects of repeated compost applications, containg P, as well as a possible increase in P-solubilising microorganisms resulting in higher extractable P levels. High soil extractable P can negatively affect uptake of K (Kotzé, 2001). Although leaf nutrient levels did not show K deficiency, it was within the lower

range for the 2008 season and high soil P content may therefore pose problems in future, in trees receiving regular compost applications, unless supplemental K is applied. In literature, increases in soil N (Neilsen et al., 2003b), P (Pinamonti, 1998; Marsh et al., 1996; Neilsen et al., 2003b), K (Kotzé and Joubert, 1992a; Marsh et al., 1996; Pinamonti, 1998), and Ca (Marsh et al., 1996) have been reported with compost application. However, effects of organic matter on soil chemical properties depend to a great extent on the mineral content of constituent organic material, and are therefore variable (Gallardo-Lara and Nogales, 1987; Neilsen et al., 2003b).

Micronutrients were less affected by the various biological management practices, although soil extractable Zn and B was increased with compost application. Soil Zn was generally low, but in 2008 increased with compost application to levels within the acceptable range for pear production (10-15 $mg.kg^{-1}$). However, leaf Zn concentrations were not deficient. Soil B content was very low in general and corresponded with low leaf B content in the 2008 season, in spite of foliar B applications. In compost extract treatments in 2008, low leaf B content indicated possible B deficiency, although soil B was highest in compost extract treated soil. Results from Yermiyahu et al. (2001) indicated that organic matter plays an important role in controlling B concentration in the soil solution, and that it has a prominent effect on reducing B uptake by plants. However, the form in which B occurs in soil, and plant B uptake, are highly pH dependent. The reduced B uptake observed in the extract treatment, relative to the compost, may therefore have been brought about by a pH shift induced by the extract in the rhizosphere.

Changes in soil extractable nutrients did not always correspond with changes in leaf nutrient content. Furthermore, no consistent effects on leaf nutrient content were observed over the trial period. Our results are in agreement with various other studies on organic matter application in fruit trees that found increased soil extractable nutrients, with no clear effect on leaf nutrient content (Kotzé en Joubert, 1992a; Gallardo-Lara and Nogales, 1987; Pinamonti, 1998; Roe 1998; Marsh et al., 1996; Andrews et al., 2001; Neilsen et al., 2003b). However, these increased levels of soil extractable nutrients are available to the plant and can therefore potentially have a positive effect on plant performance. Additionally, increased soil extractable nutrients can lead to reduced fertiliser application, reducing input costs, thereby increasing the economic benefits of these biological amendments.

Humate, Biostart, EM, seaweed extracts and chicken manure application had no significant effects on nutrition compared to untreated controls. Chicken manure was only applied at planting and it is possible that more regular applications or higher application rates would have resulted in more significant changes in nutrition.

2.4.3 Effect of mulching and biological amendments on soil microbiology

Total soil C% showed most significant increases with the application of organic material (straw mulch and compost). Organic material application was also the treatments that significantly affected soil microbial

activity. Mulching conditions provide a favourable environment for microbial activity and fine feeder root development, especially in surface soil (Boynton and Oberly, 1966; Kotze and Joubert, 1992a; Pinamonti, 1998). Our results were in agreement with other studies which generally found increased soil respiration and total microbial biomass, as well as soil organic matter content with mulch application (Hogue and Neilsen, 1987; Merwin et al., 1994; Marsh et al., 1996; Niggli et al., 1990; Werner, 1997; Tiquia et al., 2002; Yao et al, 2005). In our study, although numbers of actinomycetes, as well as *Bacillus* bacteria remained similar, mulching resulted in a general increase in soil enzyme activity. There was a significant interaction between mulching and some of the biological amendments and increased urease activity were mainly significant in a non-mulched system. This may be due to an overriding effect of the mulch on enzyme activity. In addition, BD with mulch application was negatively correlated to arylsulfatase activity. Arylsulfatase activity can be used as an indicator of fungal activity (Tabatabai, 1982), therefore these results suggest that increased fungal activity resulted in reduced BD, which could lead to improved root performance and nutrient uptake. Furthermore, increases in phosphatase enzyme activity with mulch application could also be related to higher soil P content with mulching. Although increases in leaf P content were not significant, these P-hydrolysing enzymes can play a major role in the mineralisation of organic P in soil (Rodriques and Fraga, 1999). High soil P values did not seem to have a negative effect on soil phosphatase activity. Similar results have been observed in other trials in the Western Cape (Wooldridge, 2009, personal communication). Furthermore, in various studies AM fungi, which also produce phosphatase enzymes, were shown to be a factor even under high P levels (Wooldridge, 1999; Schubert and Lubraco, 2000; Douds and Reider, 2003; Douds et al., 2007).

Compost application consistently increased *Bacillus* numbers in soil, as well as urease actvity. It was shown in numerous studies that application of composted material can rapidly improve biological aspects of soil quality and positively affect soil microbial communities as documented for a diversity of agricultural systems, including fruit trees, grain crops and vegetables (Fraser et al., 1988; Parr and Hornick, 1992; Temple et al., 1994; Angers et al., 1995; Drinkwater et al., 1995; Gunapala and Scow, 1998; Carpenter-Boggs et al., 2000; Reganold et al., 2001; Mäder et al., 2002; Flieβbach et al., 2007). In general, an increase in various soil enzyme activities have been reported with application of organic amendments in long-term field experiments (Martens et al., 1992; Masciandaro et al., 1997; Albiach et al., 2000; Garcia-Gill et al., 2000; Ros et al., 2003; Bastida et al., 2008). Urease plays an important role in the N cycle by hydrolysing urea and producing CO_2 and NH_3 (Tabatabai, 1982). Increased urease activity could therefore result in increased availability of soil N, which corresponds with our soil chemical analyses. *Bacillus* species, as thermophillic bacteria, form an integral part of composted material (Phae et al., 1990; Hatsu et al., 2002) and the increased numbers of these bacteria in soil treated with compost therefore suggest that microbial communities from the compost could have established in the orchard soil.

Results from the PCA bi-plot in our study also showed that soil microbial properties related mainly to soil applications including both compost and mulch. The exception was *Bacillus* numbers, which were only

associated with treatments where compost was applied without a mulch and the association was even closer when compost extract was added. Compost extracts was shown by EL-Masry et al. (2002) to contain various *Bacillus* spp. Lejon et al. (2007), Pérez-Piqueres (2006), as well as Marschner et al. (2003), found that the size of carbon biomass was increased whatever the type of organic matter input or agricultural practice (surface application vs. incorporation), but that specific changes in the soil microbial community were more dependent on the type of organic amendment. Direct inoculation with *Bacillus* species (Biostart) showed no consistent effect on numbers of *Bacillus* colony forming units (CFU) in soil. However, the interaction between these various *Bacillus* spp. in these various amendments, as well as resident *Bacillus* spp. is complex and changes in *Bacillus* counts do not reflect changes in species composition.

There was an indication of decreased availability of Cu in soil with mulch, as well as compost application, although soil extractable Cu were not very high overall. Soil organic matter affects the mobility of heavy metals by forming insoluble organometal complexes or through absorbing metal ions and thereby decreasing its availability (Sauve et al., 1998; Magdoff and Weil, 2004). This may have had a positive effect on soil microbial activity, since soil microorganisms and microbial processes are generally sensitive to soil Cu (Giller et al., 1998; Vulkan et al., 2000; Du Plessis et al., 2005). In our study a significant negative correlation was found between soil Cu content and *Bacillus* numbers in soil.

Significant effects on soil microbial community structure have been found with organic amendments in various studies (Bolton et al., 1985; Doran et al., 1987; Reganold et al., 1993; Wander et al., 1994; Drinkwater et al., 1995; Katayama et al., 1998; Mäder et al., 2002; Marschner et al., 2003; Yao et al., 2006; Lejon et al., 2007). In our study, changes in community function, as indicated by CLPPs and enzyme activity, were more pronounced between mulched and non-mulched soils, than changes in microbial numbers. The addition of a straw mulch changed substrate utilisation of the soil microbial community after 38 h incubation of Biolog plates, when added to the various biological amendments, including compost. Biostart application induced the most drastic differences in CLPPs between mulched and non-mulched soils and compost extract, the most similar CLPPs. Although Biostart had no effect on microbial activity or numbers, it is possible that the introduced *Bacillus* spp. could not compete with the resident microbes, but that the soil microbial community function was changed by the carbon source with which the Biostart was applied, or the addition of the low dosage HS. Visser (1985) showed that HA could increase the growth of a wide range of soil bacteria, as well as introduce a change in metabolism, allowing organisms to proliferate on substrates which previously they could not utilise.

Incubation of the inoculated Biolog plates for 38 h, showed that microbial communities from control treatments where no mulch and no biological amendments were applied, showed different metabolic function from microbial communities originating from all other treatment combinations, except Göemar applied with a mulch. Addition of compost extract to compost on its own changed the metabolic

functioning of the soil microbial community in the mulched system, but not the non-mulched system. This was in contrast to results with soil enzyme activities, where increase in activity was only significant when mulch was not applied. It is however important to keep in mind that due to the functional redundancy of soil microorganisms (Marschner et al., 2003), one function in soil can be performed by a range of different microorganisms and therefore microbial communities showing similar CLPPs may show very different species composition and enzyme activity.

Furthermore, it has to be kept in mind that differences in soil microbial properties between mulched and non-mulched systems may have partly resulted from herbicide application. Although glyphosate, a systemic herbicide, was applied conservatively and is broken down fairly rapidly in the soil, its effect on the soil microbial community can not be excluded. Glyphosate was applied to all treatments, and the mulch layer, also providing some degree of weed control, may have reduced possible negative effects on the soil biology. Differences in root exudates associated with weeds compared to where soils were mulched could also lead to significant differences in soil rhizosphere communities and activity.

2.4.4 Relation of variables measured to tree performance

Over the six year trial period maintaining a wheat straw mulch in the tree row showed few effects on nutrition parameters measured, as expected, and no effect on tree performance, despite significant changes in soil microbial properties and lower BD. Annual compost applications improved soil microbial, as well as chemical properties, but when compost extract was added no additional effects on soil microbial parameters was observed. Nevertheless, vigour and yield was only significantly improved when in addition to compost, compost extract was applied on a monthly basis. Biostart application also showed positive results on tree performance. However, this treatment did not result in significant improvement in nutrition or soil microbial properties measured, although differences were shown in substrate utilisation profiles. In RDA and subsequent CDA analysis, separation of the various management practices was mainly caused by soil pH and urease activity, which showed high canonical discriminant coefficients for both canonical variables. Soil pH, but not urease, was also positively associated with yield in the PCA bi-plot. Separation of the various treatments in the CDA could not be related to yield differences.

Other studies using broad level measurement, as well as studies using DNA extraction methods, have also recognised the difficulty of relating the performance of deciduous fruit crops to specific soil microbial properties (Renagold et al. 2001; Forge et al., 2003; Neilsen et al. 2003b; Varga et al., 2004; Yao et al., 2005; Hoagland et al., 2008). It is therefore clear that factors determining yield are complex and that the effect of biological amendments on general microbial activity or functioning cannot consistently predict tree performance across a wide range of soil conditions and environments, even in the same crop. These results from literature also show the importance of site-specific effects and that although certain soil microbial properties are improved by soil management strategies, associated negative effects on nutrition or pest and disease development are more critical in affecting plant performance.

It is also possible that, although differences in soil community functioning did not always result in improved yield in the current study, effects on productivity may be more pronounced under conditions of stress. Although considerable functional redundancy exist at species level (Andren et al., 1995; Marschner et al., 2003), diverse systems have higher resilience to stress and provide better protection against pests and diseases, since distinct physiological and environmental requirements drive species of the same functional group to play widely different roles in soil ecosystem processes (Beare et al., 1995; Giller et al., 1997). In our study, root systems were healthy and there were no indications of nematode or disease problems. Furthermore, soil nutrient levels were high in general. Also, irrigation scheduling was based on non-mulched plots which could have masked possible benefits on plant productivity due to conservation of soil moisture with mulching (Hogue and Neilsen, 1987; Wooldridge, 1992; Merwin et al., 1994; Walsh et al., 1996*;* Neilsen et al., 2003b).

2.4.5 Possible mechanisms of improved yield and shoot growth

The application of compost extract showed a consistent positive effect on tree performance, although there were no differences in nutrition, soil microbial numbers or enzyme activity when compost extract was applied in combination with compost. Little research has been conducted on the mechanisms through which compost extracts can improve plant performance. The majority of research has focused on the use of compost extracts in disease suppression (Litterick et al., 2004), through mechanisms of biological control. However, in our study no difference in disease incidence was observed between the various treatments. Improved tree performance with compost extract application could also not be explained by improved plant nutrition parameters as indicated in leaf nutrient contents of the various treatments. However, depending on soil physical properties (Pinamonti et al., 1995), fertiliser regimes (Neilsen et al., 2003a,b) and competition from either weed or fruit load (Andrews et al., 2001), leaf nutrient analyses may not always reflect differences in soil nutrient content in perennial fruit crop systems. Due to the higher fruit load with compost extract treatments, improvement in plant nutrition may not have manifested in improved leaf nutrient contents. It is also possible that improved synchronisation of nutrient release and plant uptake may have resulted in better tree performance. One of the biggest problems in soil biotic management is the relative unpredictability of system performance (Hendrix et al., 1990). Nutrient availability that is synchronised with the plants phenologic development, plays a key role (McGill and Myers, 1987; Myers et al., 1994). Furthermore, the presence of nutrients in a form available for plant uptake plays a decisive role in nutrient acquisition. It therefore makes sense that synchronisation should be easier with regular application of solubilised nutrients available for plant uptake, as contained in the monthly compost extract applications. Additionally, compost significantly increased soil nutrients available to the trees that can affect tree performance positively if nutrient uptake is improved. Bacterial secretion of phytohormones can impact root architecture, increasing nutrient and water uptake, thus contributing to growth (Persello-Cartieaux et al., 2003). Actively growing root tips are an important source of CK production and translocation (Salisburry and Ross, 1992) and the production of CK by plant-associated bacteria has been well documented (Nieto and Frankenberger, 1990; Garcia de Salamone et al., 2001). Specific effects on

plant growth promotion and yield, related to CK production, has recently been shown with *Ballicus* inoculation (Arkiphova et al., 2005; Aslantas et al., 2007; Flores et al., 2007; Ortiz-Castro et al., 2008). Several studies have indicated a positive effect of the *Bacillus* spp on growth and yield of fruit trees (Kloepper, 1994; Esitken et al., 2003; Arkhipova et al., 2005; Orhan et al., 2006; Aslantas et al. 2007). Furthermore, specific effects on plant growth promotion and yield, related to CK production, has recently been shown with *Ballicus* inoculation (Arkiphova et al., 2005; Aslantas et al., 2007; Flores et al., 2007; Ortiz-Castro et al., 2008). In our study, although correlations between soil biological properties measured and yield were not significant, the PCA bi-plot showed a relationship between *Bacillus* numbers and yield parameters. These bacteria are also among the most powerful phosphate solubilisers (Rodrigues and Fraga, 1999). Our results showed increased soil extractrable P in Dec 2007 where compost and compost extract was applied. This could indicate a positive effect on yield through root associations with *Bacillus* bacteria from compost and compost extracts, resulting in increased nutrient availability and affecting plant growth hormone production and translocation.

2.5 CONCLUSION

The application of a compost extract in addition to compost treatment improved growth and yield in a conventionally managed pear orchard compared to untreated controls and results were consistent for the 2007 and 2008 season. Long-term maintenance of a straw mulch, as well as annual compost applications from orchard establishment, indicated that the application of organic material increased soil enzyme activity. Furthermore, organic material and the application of biological amendments affected substrate utilisation of the soil microbial community. Application of compost also significantly increased numbers of *Bacillus* bacteria in soil. Although compost extract application did not result in additional changes in *Bacillus* counts and urease activity when compared to compost applied on its own, other effects on the soil microbial community can not be excluded. Compost extracts are dynamic products, consisting of a diverse microbial community. Application of compost extract to compost treated plots in a mulched system resulted in changes in substrate utilisation and therefore possibly community function. It was suggested that monthly compost extract applications resulted in maximum efficiency of nutrient utilisation through synchronisation of nutrient release with plant demand.

Crop productivity represents the outcome of complex interactions among plant, soil and management practices. In our study, performance of trees in sites where organic material and biological amendments were applied, did not always differ significantly from untreated controls and no simple relationship was apparent between yield and microbial properties. Soil was sampled from soil in the root zone, but not specifically the rhizosphere. Since release of carbon from roots has always been considered to be a major factor controlling microbial growth in soil, more research needs to be focused on effects in the rhizosphere. This includes investigation of the role of microbially produced phytohormones in affecting tree performance in these biological systems. Maximum efficiency of nutrient utilisation occurs when nutrient

release from organic residues are synchronised with plant demand. Therefore, knowledge on the breakdown rate and the rate of nutrient release from residues will enable management systems to utilise biological amendments more effectively.

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Table 1. Effect of various biological management practices on trunk circumference over the five year trial period and total shoot growth for the first three growing seasons of 'Early Bon Chretien' pear trees planted on BP1 rootstock. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

	Trunk circumference (cm)		Shoot growth (cm)		
Treatment Main[x] Sub[z]	At planting (2002) (cm)	Rate of increase over the trial period[y]	Total growth 2003	Total growth 2004	Total growth 2005
No Mulch	4.18	3.75	293.9	837.4	1706
Mulch	4.40	3.80	312.7	921.8	1777
Control	4.25	3.52 bc	269.3 b	784.5 b	1451
Manure	4.45	3.45 c	303.0 b	821.6 b	1421
Compost	4.13	3.90 a	263.2 b	814.8 b	1714
Compost + extract	4.36	3.98 a	440.2 a	1199.5 a	2112
Kelpak	4.35	3.79 ab	262.8 b	804.7 b	1737
Goëmar	4.43	3.84 a	309.7 b	985.3 ab	1847
Biostart	4.44	3.92 a	326.5 b	890.1 b	1960
K-humate	4.32	3.77 ab	301.7 b	806.7 b	1702
EM	4.33	3.78 a	253.5 b	808.4 b	1683
P values					
Main Treatment	0.3276	0.6641	0.4743	0.1049	0.6626
Sub Treatment	0.3304	0.0185	0.0386	0.0165	0.1142
Main x Sub	0.4510	0.6425	0.1968	0.0907	0.2886

[x] Values for the main treatments are means from the nine biological amendments in each of three block replicates, with measurements from four trees in each treatment plot for each replicate.
[z] Values for the sub treatments are means from two main treatments (mulched and non mulched) in each of three block replicates, with measurements from four trees in each main treatment plot for each replicate.
[y] Slope (b value) of linear regressions ($R^2 = 0.99$) fit to trunk circumference measured from 2002 to 2008, indicating growth tempo.

Table 2. Yield parameters, fruit number and fruit mass for the 2007 and 2008 harvest seasons of 'Early Bon Chretien' pear trees planted in 2002 on BP1 rootstock under various biological management practices. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means followed by no letters are not significantly different.

Treatment Main / Sub	Fruit number per tree		Average Fruit Mass (g/fruit)		Yield kg.tree^{-1}		Yield Efficiency (kg.cm^{-2})		Cumulative Yield (kg.tree^{-1})	Cumulative Yield Efficiency (g.cm^{-2})
	2007	2008	2007	2008	2007	2008	2007	2008		
No Mulch	69	119	156	158	10.46	18.66	1.138	695	28.90	1.082
Mulch	67	109	159	159	11.12	17.22	1.128	634	28.29	1.039
Control	54	103	159	154	8.32	15.10	0.958	551	23.42	0.886
Manure	62	108	163	159	10.10	17.27	1.107	672	26.91	1.043
Compost	64	114	158	157	10.47	17.66	1.089	646	28.28	1.038
Compost + extract	88	136	155	156	13.50	21.83	1.350	771	35.33	1.249
Kelpak	69	111	155	159	10.89	17.46	1.125	652	28.11	1.051
Goëmar	70	113	157	158	11.01	18.12	1.145	673	28.67	1.065
Biostart	71	114	159	164	11.52	18.79	1.211	679	29.93	1.088
K-humate	71	118	155	157	10.83	18.33	1.170	684	29.51	1.095
EM	63	105	156	157	9.93	16.36	1.030	621	25.93	0.982
P value										
Main Treatment	0.6715	0.2382	0.2603	0.4695	0.5042	0.2356	0.9013	0.2412	0.8016	0.6377
Sub Treatment	0.1358	0.5124	0.5309	0.2676	0.0870	0.2853	0.1951	0.4005	0.1330	0.2680
Main x Sub	0.6906	0.1945	0.4962	0.6193	0.6716	0.2839	0.7370	0.0966	0.5670	0.3615

Table 3. Effect of biological management practices on fruit quality parameters for 'Early Bon Chretien' pear planted on BP1 rootstock as determined during the 2007 harvest season, at harvest, after cold storage (at -0.5 ºC for 8 weeks), as well as cold storage following a shelf life period of 7 days at room temperature (21-24 ºC) (shelf life period). Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different. TSS = Total soluble solids, and TTA = Total titratable acids.

Treatment	Evaluation at harvest[z]			Evaluation after storage			Evaluation after shelf life		
Main / Sub	TSS (%)	TTA (%)	Firmness ($kg.cm^{-2}$)	TSS (%)	TTA (%)	Firmness ($kg.cm^{-2}$)	TSS (%)	TTA (%)	Firmness ($kg.cm^{-2}$)
No Mulch	12.97	0.54	9.69	13.49	0.46	9.62	14.02	0.35	1.03 b
Mulch	12.64	0.51	9.62	13.47	0.47	9.60	13.68	0.33	1.05 a
Control	12.77	0.57	9.75	13.42	0.46	9.63	13.80	0.35	1.00
Compost	12.63	0.60	9.44	13.35	0.47	9.65	13.70	0.32	1.09
Compost + extract	12.92	0.55	9.67	13.58	0.46	9.29	14.52	0.35	1.08
Goëmar	12.87	0.49	9.62	13.43	0.46	9.72	13.63	0.34	1.03
Biostart	12.92	0.47	9.85	13.45	0.42	9.64	13.60	0.34	1.05
K-humate	12.73	0.49	9.63	13.65	0.48	9.69	13.83	0.34	1.02
P value									
Main treatment	0.1242	0.4318	0.5067	0.7128	0.1912	0.8702	0.3975	0.5008	0.0399
Sub treatment	0.6629	0.2572	0.2362	0.7780	0.9739	0.4131	0.2961	0.9810	0.3235
Main x Sub	0.0507	0.4238	0.3246	0.7841	0.9838	0.8925	0.7431	0.2669	0.4171

[z] For each main x sub treatment combination 35 fruit from each block were analysed per evaluation.

Table 4. Effect of biological management practices on fruit quality parameters for 'Early Bon Chretien' pear planted on BP1 rootstock as determined during the 2008 harvest season, at harvest, after cold storage (at -0.5 ºC for 8 weeks), as well as cold storage following a shelf-life period of 7 days at room temperature (21-24 ºC). Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment		Evaluation at harvest[z]			Evaluation after storage			Evaluation after storage and shelf-life		
Main / Sub		TSS (%)	TTA (%)	Firmness ($kg.cm^{-2}$)	TSS (%)	TTA (%)	Firmness ($kg.cm^{-2}$)	TSS (%)	TTA (%)	Firmness ($kg.cm^{-2}$)
No Mulch		11.55	0.60	-	12.96 b	0.55	8.41	13.60	0.30	1.04
Mulch		11.53	0.61	-	13.21 a	0.55	8.45	13.55	0.29	1.06
Control	No Mulch[*]	11.68	0.58	8.89 e	13.13	0.55	8.50	13.83	0.33 ab	1.03
	Mulch			9.49 abc						
Compost	No Mulch	11.50	0.60	9.30 abcd	13.20	0.55	8.49	13.22	0.27 c	1.03
	Mulch			9.65 a						
Cextract	No Mulch	11.25	0.59	9.30 abcd	12.88	0.56	8.47	13.63	0.28 c	1.02
	Mulch			9.13 de						
Goëmar	No Mulch	11.65	0.61	9.20 bcde	13.23	0.54	8.31	13.87	0.28 c	1.08
	Mulch			9.34 abcd						
Biostart	No Mulch	11.53	0.60	9.27 bcd	13.25	0.56	8.49	13.40	0.29 bc	1.05
	Mulch			9.55 ab						
K-humate	No Mulch	11.63	0.61	9.41 abcd	12.93	0.55	8.40	13.23	0.34 a	1.10
	Mulch			9.26 bcd						
Main treatment		0.6167	0.2156	0.0998	0.0247	0.7680	0.6848	0.7880	0.2660	0.2148
Sub treatment		0.2857	0.5390	0.3835	0.6006	0.9773	0.6599	0.1266	0.0067	0.4476
Main x Sub		0.4252	0.6795	0.0476	0.9602	0.9974	0.0827	0.7767	0.0251	0.5919

[z] For each main x sub treatment combination 35 fruit from each block were analysed per evaluation.
[*]Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments.

Table 5. Effect of biological management practices on actinomycete and *Bacillus* numbers of colony forming units (CFUs) in soil, measured at four sampling dates, Oct 2006, Dec 2006, Apr 2007 and Dec 2007. The average numbers over the four sample dates are also shown. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment Main / Sub	Actinomycetes x10^6 CFU/g soil					*Bacillus* x10^5 CFU/g soil				
	Oct 2006	Dec 2006	Apr 2007	Dec 2007	Average	Oct 2006	Dec 2006	Apr 2007	Dec 2007	Average
No Mulch	67.53	53.47	60.80	80.0	65.5	35.07	25.86	33.00	30.8	32.5
Mulch	71.91	70.18	57.07	80.3	69.7	34.75	25.50	27.87	35.7	29.7
Control	69.00	61.17	58.50	81.3	67.5	32.00	22.83	23.33	26.8 b	26.3 c
Compost	67.50	52.67	59.50	89.3	67.3	44.17	33.17	33.83	41.5 a	38.2 a
Compost +extract	64.00	66.33	57.67	84.2	68.0	32.67	26.00	32.33	44.0 a	33.8 ab
Goëmar	65.33	61.67	62.83	66.7	64.3	34.00	23.00	33.17	27.8 b	30.2 bc
Biostart	81.00	61.60	56.17	79.3	69.4	31.33	21.67	29.50	26.2 b	27.2 c
P value										
Main Treatment	0.6887	0.1028	0.8005	0.8437	0.5877	0.9114	0.8511	0.4490	0.0699	0.0607
Sub Treatment	0.6302	0.6199	0.9887	0.2730	0.9916	0.2447	0.0742	0.5217	<0.0001	0.0019
Main x Sub	0.1825	0.2705	0.5059	0.6520	0.9073	0.6233	0.4542	0.4780	0.3567	0.2879

Values are means from three plate replicates performed for each sub treatment and main treatment combination in each block.

Table 6. Effect of biological management practices on urease, phosphatase, β-glucosidase and arylsulfatase soil enzyme activities at four sampling dates, Oct 2006, Dec 2006, Apr 2007 and Dec 2007. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment		Urease[z]				Acid phosphatase[x]				β-Glucosidase				Arylsulfatase	
Main		(µg N/g soil/2h)				(mg PNP/kg soil/h)				(mg PNP/kg soil/h)				(mg PNP/kg soil/h)	
Sub		Oct06	Dec06	Apr07	Dec07	Oct06	Dec06	Apr07	Dec07	Oct06	Dec06	Apr07	Dec07	Apr07	Dec07
No Mulch		39.83	-	50.04	-	315.8	-	382.3	305.5 b	249.2	281.8	239.2	-	97.5	80.47 b
Mulch		42.75	-	52.29	-	325.9	-	401.2	361.9 a	264.2	276.4	235 .0	-	125.1	122.29 a
Control	NMulch[*]	35.56	30.43 c	48.46	33.31 e	318.1	322.4 c	384.5	300.0	232.3	258.1	226.5	175.0 c	108.2	97.52
	Mulch		40.52 ab		42.36 bcd		428.2 a						216.8 ab		
Compost	NMulch	43.69	39.90 ab	55.47	39.14 cde	323.4	397.8 ab	390.9	365.0	252.1	285.6	240.5	182.9 bc	111.0	109.37
	Mulch		40.52 ab		56.93 a		392.2 abc						208.8 abc		
CExtract	NMulch	44.29	44.25 a	49.27	44.44 bc	322.7	432.8 a	383.6	342.6	263.7	291.2	221.8	217.2 ab	118.2	107.58
	Mulch		37.88 abc		44.74 bc		347.9 bc						179.6 bc		
Goemar	NMulch	45.35	39.90 ab	55.47	35.13 de	322.4	412.2 ab	408.5	318.9	263.7	283.4	257.4	179.7 bc	120.9	90.85
	Mulch		39.85 ab		47.39 bc		400.1 ab						210.8 abc		
Biostart	NMulch	38.87	33.38 bc	47.17	31.04 e	315.9	321.9 c	391.2	331.8	271.3	280.7	239.5	183.3 bc	98.1	101.58
	Mulch		41.92 a		50.57 ab		398.1 ab						239.4 a		
P value															
Main treatment		0.3378	0.2977	0.6165	0.0064	0.1983	0.7125	0.6102	0.0078	0.1478	0.5729	0.6292	0.0727	0.0890	0.0151
Sub treatment		0.0788	0.1775	0.3168	0.0379	0.9896	0.3158	0.9457	0.2857	0.2261	0.6933	0.5492	0.6118	0.2029	0.3633
Main x Sub		0.6609	0.0179	0.6405	0.0316	0.5749	0.0060	0.2595	0.1435	0.4853	0.0960	0.6666	0.0298	0.6802	0.5414

[z] Urease hydrolysing activity was determined by the non-buffered method of Kandeler and Gerber (1988).

[x] Acid phosphatase, β-glucosidase and arylsulfatase activity were determined based on the release and spectrophotometric detection of *p*-nitrophenol (Tabatabai and Bremner, 1969; Tabatabai, 1982).

[*] Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments

Table 7. Number of substrates utilised after 24h and 38h incubation of Biolog® Ecoplates inoculated with soil microbial communities subjected to various biological management practices. Biolog Ecoplates contain 31 different carbon sources, replicated three times on a plate and substrates utilised were assayed Oct 2006, Dec 2006, Apr 2007 and Dec 2007. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment		Number of substrates utilised after 24 h				Number of substrates utilised after 38 h			
Main									
Sub		Oct 2006	Dec 2006	Apr 2007	Dec 2007	Oct 2006	Dec 2006	Apr 2007	Dec 2007
No Mulch		8.2	2.6	9.3	-	21.2	17.2 b	19.6	18.9
Mulch		12.2	7.0	9.2	-	21.9	19.4 a	19.7	20.0
Control	No Mulch*	9.5	3.4 b	9.0	0.8 d	20.8	17.8	21.1	20.3
	Mulch				4.0 a				
Compost	No Mulch	11.2	4.2 b	9.2	1.5 cd	21.3	18.6	18.9	19.8
	Mulch				4.0 a				
CExtract	No Mulch	9.5	3.8 b	9.3	1.2 d	22.0	18.2	19.5	18.3
	Mulch				3.5 ab				
Goëmar	No Mulch	11.7	3.5 b	9.8	1.2 d	23.0	17.2	18.8	19.1
	Mulch				2.0 bcd				
Biostart	No Mulch	10.8	7.4 a	9.1	3.2 abc	21.5	18.8	19.8	19.9
	Mulch				2.5 abcd				
P value									
Main treatment		0.1197	0.0511	0.9628	0.0268	0.1660	0.0103	0.9024	0.1150
Sub treatment		0.8142	0.0197	09846	0.3101	0.5863	0.7499	07327	0.3109
Main x Sub		0.0930	0.1214	0.5060	0.0426	0.0743	0.3622	0.9033	0.5995

*Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments.

Table 8. Effect of various biological management practices on soil chemical properties of the top 0-25 cm of a gravelly, sandy loam soil measured at three times throughout the trial period, Oct 2006, Apr 2007 and Dec 2007. Total C% was also measured in the top 0-5 cm for the last sampling date. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment		pH (KCl)			Resistance (ohm)			Total C (%)				Total N (%)			
Main Sub		Oct06	Apr07	Dec07	Oct06	Apr07	Dec07	Oct06	Apr07	Dec07 0-25 cm	Dec07 0-5 cm	Oct06	Apr07	Dec07 0-25 cm	Dec07 0-5 cm
No Mulch		7.00	6.78	6.79	551	875	570	1.88 b	-	1.79	-	0.24	0.250	0.247	0.296 b
Mulch		6.94	6.71	6.69	496	619	479	2.17 a	-	1.94	-	0.23	0.239	0.278	0.324 a
Control	Nmulch*	7.03	6.73 ab	6.73 bc	550	832	672 a	1.70 b	1.48 d	1.56 b	1.35 c	0.215	0.225 b	0.226 b	0.269 b
	Mulch								1.81 bc		2.16 ab				
Compost	NMulch	7.02	6.88 a	6.85 ab	513	613	394 b	2.31 a	2.24 a	2.15 a	2.52 a	0.255	0.280 a	0.324 a	0.342 a
	Mulch								2.00 ab		2.41 ab				
CExtract	NMulch	6.95	6.88 a	6.93 a	492	635	357 b	2.22 a	2.06 ab	2.17 a	2.21 ab	0.245	0.242 b	0.292 a	0.361 a
	Mulch								1.87 bc		2.50 a				
Goëmar	NMulch	6.97	6.63 b	6.62 c	543	848	656 a	1.87 b	1.59 cd	1.62 b	1.64 c	0.220	0.243 b	0.231 b	0.278 b
	Mulch								1.98 ab		2.09 b				
Biostart	NMulch	6.90	6.60 b	6.57 c	543	808	578 a	1.89 b	1.92 b	1.85 ab	1.65 c	0.233	0.231 b	0.240 b	0.294 b
	Mulch								1.47 d		2.41 ab				
Main Treatment		0.4474	0.4444	0.2136	0.4680	0.0780	0.3894	0.0122	0.7030	0.3485	0.0930	0.4775	0.4016	0.2688	0.0201
Sub Treatment		0.3456	0.0055	0.0016	0.6795	0.0907	0.0023	0.0023	0.0011	0.0044	0.0002	0.2647	0.0256	0.0008	<0.0001
Main x Sub		0.6726	0.3052	0.4854	0.5510	0.1127	0.2441	0.8776	0.0019	0.0845	0.0156	0.3068	0.0633	0.5317	0.0522

*Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments

Table 8. (Continue)

Treatment		P BrayII (mg.kg^{-1})			K (mg.kg^{-1})			Ca (cmol.kg^{-1})			Mg (cmol.kg^{-1})			T-Value (cmol.kg^{-1})		
Main Sub		Oct06	Apr07	Dec07	Oct06	Apr07	Dec07	Oct06	Apr07	Dec07	Oct06	Apr07	Dec07	Oct06	Apr07	Dec07
No Mulch		19.93	51.93 b	98.5	328.0	319 b	350.0	10.73	-	10.38	1.10	-	1.05	12.80	-	12.52
Mulch		30.40	81.00 a	106.1	379.8	364 a	339.5	11.00	-	10.52	1.28	-	1.14	13.37	-	12.71
Control	Nmulch*	9.83	56.50	55.8 b	333.5	287.5 b	225.8 b	10.53	8.84 d	9.62 b	0.85 c	0.84 e	0.75 b	12.34	10.4 e	11.06 b
	Mulch								10.22 bcd			1.03 de			12.24 b-e	
Compost	NMulch	26.6	53.33	161.5 a	383.3	449.7 a	504.5 a	11.41	12.24 a	11.64 a	1.54 a	1.70 a	1.44 a	14.10	15.3 a	14.61 a
	Mulch								10.53 bc			1.31 b			13.19 b	
CExtract	NMulch	29.0	93.00	175.2 a	359.5	421.5 a	535.5 a	11.30	10.34 bcd	12.04 a	1.37 ab	1.25 bcd	1.47 a	13.72	12.78 bc	15.22 a
	Mulch								9.67 bcd			1.29 bc			12.29 bcd	
Goëmar	NMulch	27.7	74.83	61.3 b	334.7	272.2 b	238.3 b	10.75	8.93 cd	9.47 b	1.01 bc	0.92 e	0.87 b	12.74	10.64 de	11.07 b
	Mulch								10.85 ab			1.18 bcd			12.89 bc	
Biostart	NMulch	30.5	54.67	57.8 b	335.8	278.2 b	219.8 b	10.21	9.04 cd	9.47 b	1.04 bc	0.87 e	0.95 b	12.22	10.67 de	11.11 b
	Mulch								9.34 bcd			1.04 de			11.3 cde	
Main Treatment		0.1826	0.0148	0.6016	0.0756	0.0251	0.8355	0.4258	0.7263	0.6483	0.2215	0.2551	0.2086	0.1562	0.5678	0.6483
Sub Treatment		0.1511	0.2163	<0.0001	0.4091	<0.0001	<0.0001	0.2788	0.0109	0.0010	0.0055	<0.0001	<0.0001	0.0864	0.0006	<0.0001
Main x Sub		0.1693	0.9656	0.4778	0.2902	0.4839	0.0761	0.8678	0.0267	0.5238	0.7872	0.0102	0.3837	0.8863	0.0167	0.3580

*Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments

Table 8. (continue)

Treatment		Cu (cmol.kg^{-1})			Zn (cmol.kg^{-1})			Mn (cmol.kg^{-1})			B (cmol.kg^{-1})		
Main Sub		Oct06	Apr07	Dec07	Oct06	Apr07	Dec07	Oct06	Apr07	Dec07	Oct06	Apr07	Dec07
No Mulch		2.62 a	1.429	1.83	5.947	-	6.79	38.63 b	24.34	36.2	-	-	0.89
Mulch		2.17 b	1.226	1.66	7.092	-	7.22	40.12 a	23.42	34.7	-	-	0.84
Control	Nmulch*	2.86 a	1.57 a	2.46 a	4.70 b	4.47 d	4.65 b	40.87 ab	23.17 ab	34.45 bc	0.737 b	1.17 bc	0.77
	Mulch					4.87 cd		37.27 b			0.657 b	1.43 abc	
Compost	NMulch	1.69 b	0.86 c	0.93 b	8.37 a	9.30 a	10.32 a	39.27 b	25.45 a	36.72 ab	0.730 b	1.40 abc	0.99
	Mulch					6.93 b		41.27 ab			1.303 a	1.41 abc	
CExtract	NMulch	2.08 b	1.12 bc	1.01 b	7.65 a	7.03 b	10.47 a	37.30 b	25.47 a	38.35 a	1.220 a	1.65 a	1.14
	Mulch					6.73 b		45.20 a			0.680 b	1.14 c	
Goëmar	NMulch	2.96 a	1.48 ab	2.05 a	5.67 b	4.90 cd	4.73 b	38.80 b	23.87 a	33.14 c	1.177 a	1.18 bc	0.66
	Mulch					6.10 bc		37.85 b			1.223 a	1.53 ab	
Biostart	NMulch	2.77 a	1.61 a	2.30 a	5.50 b	4.23 d	4.87 b	36.90 b	21.45 b	34.53 bc	0.700 b	1.49 abc	0.76
	Mulch					4.67 d		36.77 b			0.587 b	1.20 bc	
Main Treatment		0.0206	0.0680	0.0630	0.2626	0.8429	0.6451	0.0243	0.4139	0.6447	0.0589	0.7288	0.8172
Sub Treatment		0.0014	0.0017	<0.0001	0.0030	<0.0001	<0.0001	0.2102	0.0134	0.0430	0.0057	0.9292	0.1193
Main x Sub		0.7658	0.0747	0.2066	0.4988	0.0086	0.4391	0.0409	0.4529	0.7898	0.0018	0.0184	0.7018

*Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments

Table 9. Leaf nutrient analyses as affected by the various biological management practices for the 2006-2008 seasons. Results are expressed as percentage for macronutrients and mg.kg^{-1} DW for micronutrients. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

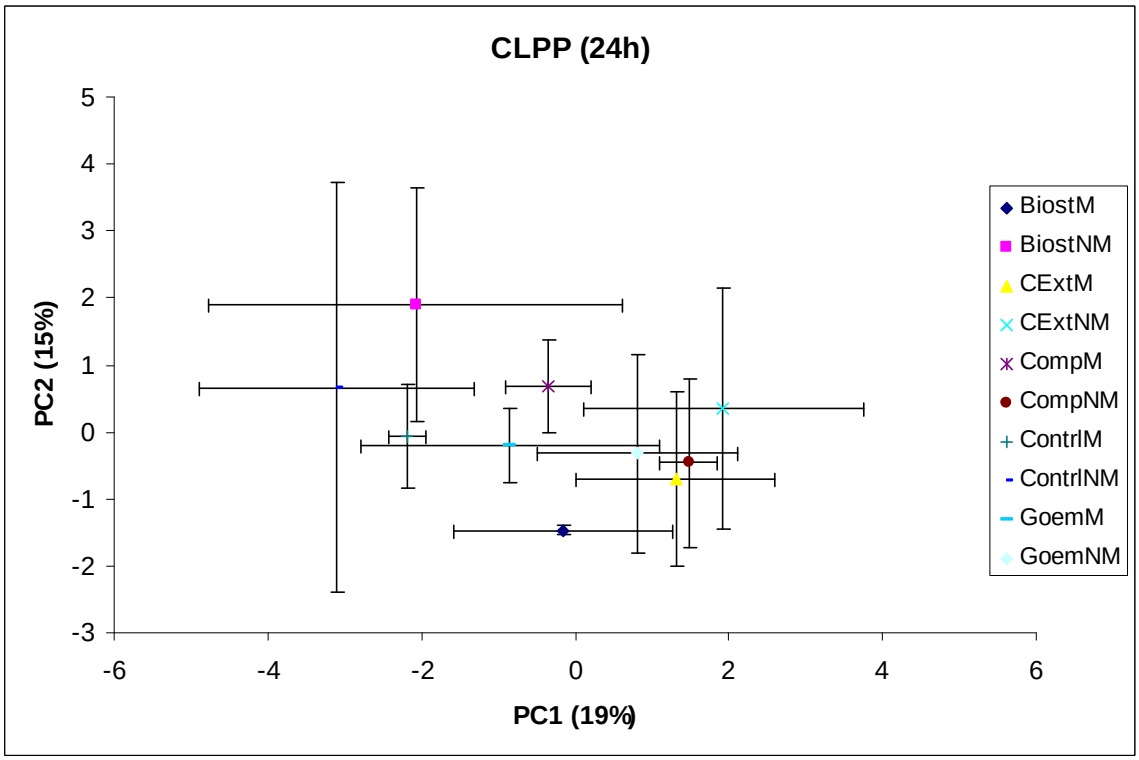
Treatment		Leaf-N (%)			Leaf-P (%)			Leaf-K (%)			Leaf-Ca (%)			Leaf-Mg (%)		
Main	Sub	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
No Mulch		2.78	2.522 b	2.618	0.208	-	0.151	1.25	1.172	1.124	1.721	1.488	1.348	0.394	0.273	0.293 a
Mulch		2.83	2.565 a	2.579	0.221	-	0.163	1.31	1.192	1.152	1.646	1.421	1.309	0.362	0.276	0.266 b
Control	Nmulch*	2.805	2.547 ab	2.680 a	0.200	0.157 d	0.152	1.25	1.19	1.21	1.488 b	1.455	1.378	0.380	0.283	0.313 a
	Mulch					0.180 bc										
Compost	NMulch	2.750	2.560 a	2.633 ab	0.217	0.175 bcd	0.164	1.24	1.15	1.19	1.788 a	1.488	1.487	0.387	0.297	0.300 a
	Mulch					0.157 d										
CExtract	NMulch	2.902	2.478 bc	2.530 bc	0.225	0.163 cd	0.137	1.37	1.20	1.06	1.733 a	1.445	1.232	0.357	0.258	0.245 c
	Mulch					0.180 bc										
Goemar	NMulch	2.783	2.622 a	2.678 a	0.207	0.167 bcd	0.193	1.29	1.19	1.17	1.752 a	1.415	1.343	0.380	0.270	0.292 ab
	Mulch					0.185 ab										
Biostart	NMulch	2.783	2.593 a	2.563 abc	0.223	0.167 bcd	0.162	1.25	1.19	1.09	1.657 ab	1.448	1.245	0.387	0.255	0.272 abc
	Mulch					0.200 a										
Humate	NMulch	-	2.460 c	2.505 c	-	0.167 bcd	0.134	-	1.17	1.11	-	1.475	1.287	-	0.282	0.253 bc
	Mulch					0.157 d										
Main treatment		0.6637	0.0315	0.4193	0.6808	0.1764	0.3418	0.5948	0.8204	0.4351	0.1631	0.3276	0.2782	0.4596	0.6525	0.0480
Sub treatment		0.3256	0.0019	0.0275	0.8327	0.0180	0.1549	0.5814	0.9533	0.6013	0.0208	0.9584	0.1040	0.9307	0.5805	0.0258
Main x Sub		0.6450	0.3095	0.2564	0.3221	0.0084	0.6727	0.7159	0.0744	0.7729	0.5643	0.1712	0.4697	0.9316	0.0938	0.2816

Footnotes: Kotzé (2001) norms: N (2.1-2.6%), P (0.14-0.19%), K (1.2-1.4%), Ca (1.45-1.60%), Mg (0.30-0.40%), Na (500 mg.kg^{-1}), Mn (20-90 mg.kg^{-1}), Fe (80-150 mg.kg^{-1}), Cu (5-10 mg.kg^{-1}), Zn (30-50 mg.kg^{-1}), B (30-35 mg.kg^{-1}). Each leaf sample consisted of 50 leaves. Samples were analysed by a commercial laboratory (Bemlab®, Strand, SA) using an inductively coupled plasma-optical emission spectrometer and a nitrogen analyzer.

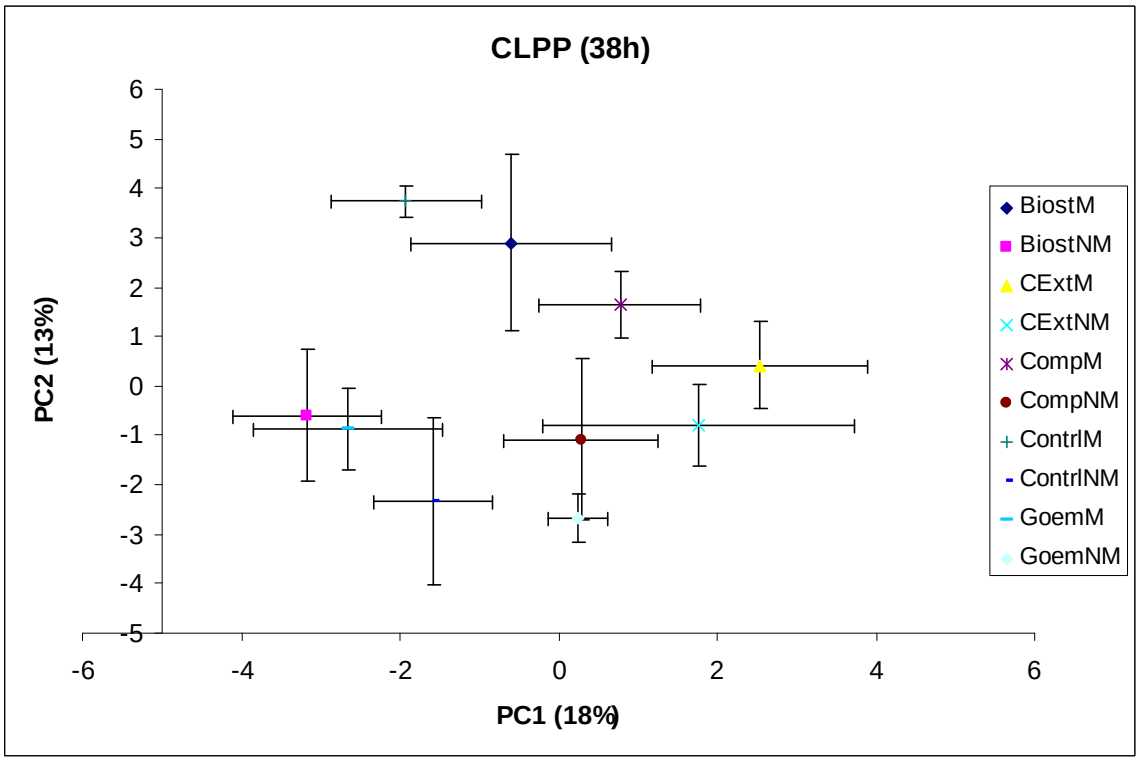
*Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments

Table 9. (Continue)

Treatment Main Sub	Leaf-Mn (mg.kg^{-1})			Leaf-Cu (mg.kg^{-1})			Leaf-Zn (mg.kg^{-1})			Leaf-B (mg.kg^{-1})		
	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
No Mulch	139	318	229 b	10.4	9.83	7.61	48.6	52.7	44.2	42.4	35.6	29.0
Mulch	126	310	254 a	10.7	9.94	7.50	40.7	47.5	41.4	41.9	33.9	28.2
Control	125	308	274 a	11.2	10.2	8.67 a	41.7	50.2	42.7	41.1	35.5	31.0 a
Compost	143	325	246 ab	10.0	9.8	7.83 ab	46.3	51.8	46.8	42.2	34.0	30.5 ab
Compost +Extract	145	312	243 b	10.5	9.7	7.00 b	46.5	50.2	41.5	42.2	34.3	26.2 c
Goemar	127	312	226 b	10.3	10.2	7.50 b	43.2	49.5	43.3	43.3	34.0	28.3 bc
Biostart	124	302	231 b	10.8	10.2	7.33 b	45.7	48.3	40.0	42.0	35.3	28.5 abc
Humate	-	325	231 b	-	9.3	7.00 b	-	50.7	42.3	-	33.7	27.2 c
Main treatment	0.2444	0.5509	0.0126	0.5598	0.7735	0.6349	0.0731	0.1210	0.1912	0.4825	0.1562	0.5286
Sub treatment	0.2177	0.7487	0.0495	0.5121	0.5461	0.0048	0.2741	0.9400	0.3988	0.9265	0.6135	0.0073
Main x Sub	0.5520	0.5201	0.8982	0.5662	0.7051	0.6796	0.7552	0.9672	0.2304	0.9639	0.2269	0.4565



A



B

Figures 1A-B. Ordination plots of principal components (PCs) 1 and 2 community level physiological profiles (CLPP) of soil where organic material and biological amendments were applied. Principal component analysis was conducted on 24h incubation data from Biolog EcoPlates for the average substrate utilisation of samples taken in spring 2006, summer 2006, autumn 2007 and summer 2007. Error bars represent ±1 standard error of the mean. Values in brackets indicate the percent of total variation accounted for by each principal component axis.

BiostM: Biostart with mulch, BiostNM: Biostart without mulch, CExtM: compost with compost extract and mulch, CExtNM: compost with compost extract wtihout mulch, CompM: compost with mulch, CompNM: compost without mulch, ContrlM: control with mulch, ContrlNM: no organic or biological amendments (control without mulch), GoemM: Goemar with Mulch, GoemMN: Goemar without mulch.

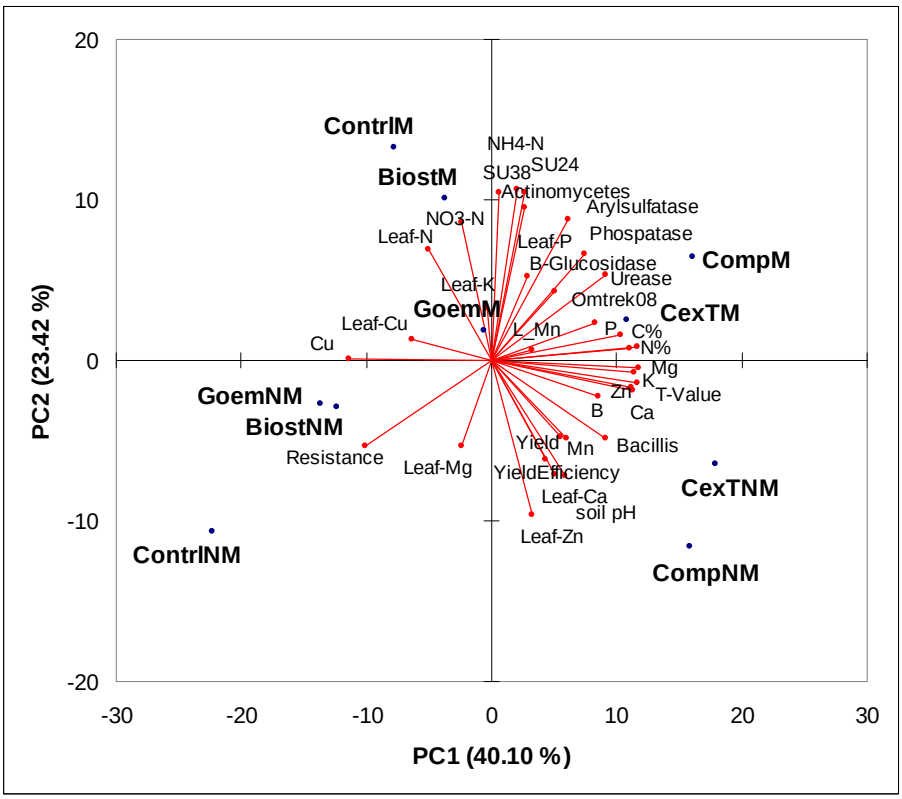


Figure 2. Principal component analysis (PCA) Bi-plot of the different variables (chemical and biological soil properties, leaf nutrient content and yield) in relation to the various soil treatments. Values in brackets indicate the percent of total variation accounted for by each principal component axis.
Mulch (M); Without mulch (NM)

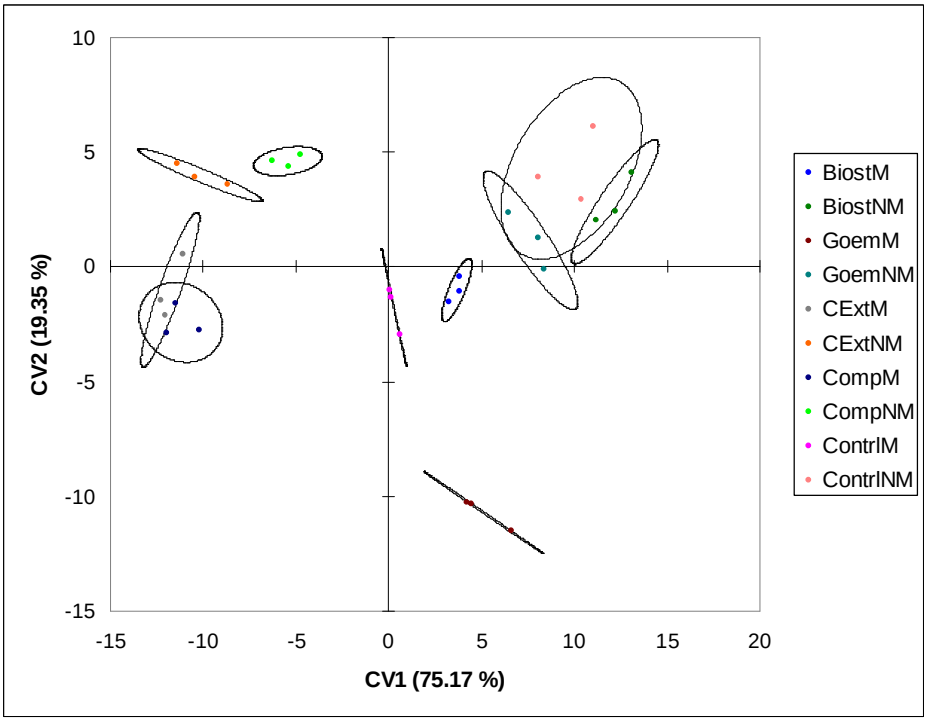


Figure 3. Plots of the first two canonical variables (CVs) from a canonical discriminant analysis (CDA), showing separation of the various soil treatments. Values in brackets indicate the percent of total dispersion explained by each CV.
Biostart with mulch, BiostNM: Biostart without mulch, CExtM: compost with compost extract and mulch, CExtNM: compost with compost extract wtihout mulch, CompM: compost with mulch, CompNM: compost without mulch, ContrlM: control with mulch, ContrlNM: no organic or biological amendments (control without mulch), GoemM: Goemar with Mulch, GoemMN: Goemar without mulch.

CHAPTER 3

BIOLOGICAL SOIL AMENDMENTS AND THEIR EFFECTS ON TREE PERFORMANCE AND SOIL MICROBIAL PROPERTIES IN MANAGING APPLE REPLANT DISEASE

ABSTRACT

The effects of biological soil amendments were investigated for use as alternative management practices to reduce the effects of apple replant disease (ARD) under marginal South African apple production conditions. The study site consisted of 'Fuji' and 'Ruby Gala' apple trees on M793, in a commercial orchard with a history of ARD problems in Vyeboom (34° 08' S; 019° 02' E), Western Cape, South Africa. The trial was conducted as a randomised complete block design with nine biological soil management treatments. Organic material and biological amendments were applied from orchard establishment in 2003, for every season up till the 2008 harvest season. Tree performance was measured in terms of growth, yield and fruit quality. Furthermore, changes in selected soil chemical properties, as well as leaf nutrient content were measured after four years of treatment application. Additionally, the effect of treatments on soil microbial properties was measured by making use of soil enzyme activity assays (urease, acid phosphatase and β-glucosidase), conventional microbial plate counts and community level physiological profiles (Biolog). Methyl bromide fumigation showed the most significant and consistent response in terms of early growth improvement and yield. However, regular application of *Bacillus* soil inoculant (Biostart®) in combination with a labile C-source and low dosage of humate, as well as compost extract applied with compost, showed improved yield over the four year trial period when compared to untreated plots. Changes in soil microbial properties were most significant with compost extract treatment in increasing *Bacillus* and actinomycete numbers in soil, as well as soil phosphatase activity. After 38h incubation of inoculated Biolog plates, microbial communities from compost extract treated soil showed the most distinct community level physiological profile, when compared to the other soil treatments. Biostart® application also resulted in significant increases in soil enzyme activity. Pre-plant fumigation had no effect on broad-scale soil microbial properties three years after methyl bromide application, but persistent effects on substrate utilisation of the microbial community was indicated.

Keywords: *Actinomycetes, Bacillus, Biostart®, Biolog®, compost, compost extract, humate, methyl bromide, mustard gas, soil enzyme activity*

3.1 INTRODUCTION

There is a general movement in agriculture in the direction of more environmentally friendly, sustainable production practices. This is mainly due to the adverse effect on soil biological activity and diversity through conventional management practices. Monoculture practices, intensive use of chemicals and low organic matter input (Grace et al., 1994) has over the long term resulted in shifts in soil fauna and flora communities, affecting agroecosystem functioning (Griffiths et al., 2001) and thereby plant growth and yield, negatively (Grayston et al., 1996; Loveland and Webb, 2003; Phelan, 2004). Soil microbial rehabilitation is of prime importance in orchards suffering from apple replant disease (ARD). This disorder is associated with poor growth of young apple trees planted on previous apple sites. Symptoms include stunted growth and reduction in tree vigour and productivity and root systems are typically small with few functional root hairs (Savory, 1966; Hoestra, 1968; Mai and Abawi, 1981). Although the disease is not lethal, it has great economic importance due to its lasting effect on production (Mazzola, 1998). The economics of intensive planting systems depend on rapid establishment of orchard canopy and precocious cropping (Foote et al., 2001), therefore any growth-retarding factor is adversely felt.

The etiology of ARD is still not fully understood, but accumulated research results point to a biological origin involving a complex of soil fungi, bacteria, as well as nematodes that can vary across geographic regions or even between orchards in the same region (Savory, 1966; Hoestra, 1968; Covey et al., 1979; Mai and Abawi, 1981; Sewell, 1981; Jaffee et al., 1982; Slykhuis and Li, 1985; Utkhede et al., 1992; Dullahide et al., 1994; Braun, 1995; Mazzola, 1998; Manici et al., 2003). Studies by Mazzola (1998; 1999) indicated a shift in microbial community composition in ARD development, towards pathogens dominating the soil microbial profile. Due to its complex etiology, ARD has been controlled successfully in most cases by the application of a broad spectrum fumigant of which methyl bromide is the most effective and extensively used. However, due to environmental and economic pressures biological alternatives are needed. The biological nature of ARD etiology, has initiated research into induction of soil suppressiveness as a more sustainable approach in ARD management (Gu and Mazzola, 2003). Furthermore, due to the adverse effects of ARD on root proliferation and development, stimulation of root growth can play an important role in improving plant performance and managing ARD symptoms.

The disease-suppressive effects of compost have received growing attention (Hoitink et al., 1997; Ristaino and Thomas, 1997; De Ceuster and Hoitink, 1999; Pascual et al., 2002; Noble and Coventry, 2005). Furthermore, general biological activity of soil is stimulated by addition of labile organic material (Campbell, 1989; Magarey, 1999) and soils with a diversity of beneficial microorganisms are more likely to be suppressive to disease development (Lazarovits, 2001; Van Elsas et al., 2002). Positive effects have been found with the use of compost in ARD management (Autio et al., 1991; Engel et al., 2001; Moran and Schupp, 2001; Van Schoor, et al., 2009). Humic substances (HS) comprise a major part of soil organic matter (SOM) and in recent years there have been increasing interest in amending soil with commercial HS

to increase soil fertility (Mann, 1986). Positive effects of HS on root growth (Bryan, 1976; Mylonas and McCants, 1980; Rauthan and Schnitzer 1981; Vaughn and Malcolm, 1985; Chen and Aviad, 1990; Crouch and van Staden, 1991; Adani et al., 1998) and soil microbial activities (Visser 1985 a, b; Vallini et al., 1993; Lizarazo et al., 2005) have been shown.

Application of soil inoculants has shown benefits in improving plant health and yield through changes in root development (Van Loon et al., 1998; George, 2000; Gravel et al., 2007), improved uptake of nutrients (Glick, 1995; Rodriguez and Fraga, 1999; Zahir et al., 2004), as well as phytohormone production (Arshad and Frankenberger, 1998; Aslantas et al., 2007). Under greenhouse conditions, several studies have shown that inoculation of apple seedlings or rootstocks with plant growth promoting rhizobacteria (PGPR) of *Pseudomonas* and *Bacillus* spp. (Utkhede and Smith, 1992; Catska and Hudska, 1993; Biro et al., 1998; Utkhede and Smith, 2000; Mazzola et al., 2001), as well as arbuscular mycorrhizal (AM) fungi (Utkhede, 1992) has potential for biological management of ARD. Furthermore, the use of compost extracts, also referred to as compost teas, has been advocated as an inoculant to stimulate and enhance the soil microflora (Ingham, 1999; Litterick et al., 2004). However, very little scientific research has been done to confirm or quantify these benefits. In pot trials, the application of sterilised and unsterilised compost extracts, significantly increased growth of apple seedlings in ARD soils in addition to nutritional effects (Van Schoor et al., 2009).

The objective of the study was firstly, to evaluate the potential use of various biological soil amendments for use as alternative management practices to reduce the effects of ARD under marginal South African apple production conditions. Secondly, in an attempt to establish if reduced ARD effects can be associated with changes in soil microbial properties, the effect of treatments on soil microbial community activity was measured by making use of soil enzyme activity assays, conventional microbial plate counts and community level physiological profiles (CLPPs).

3.2 MATERIALS AND METHODS

3.2.1 Orchard study sites and treatment application

The experiment was conducted in a newly established commercial orchard with a history of ARD problems, in Vyeboom (34° 08' S; 019° 02' E), one of the main pome fruit production regions in the Western Cape, South Africa. Annual rainfall in the area is 600 mm and Utah chill units have averaged 700 for winter months the past 10 years. The average maximum temperature in summer is 28ºC, with an average minimum 14ºC. The study site consisted of ‘Fuji’ and ‘Ruby Gala’ apple (*Malus domestica*) trees on M793 planted in 2003 at a spacing of 4.0 m x 1.5 m (within row) on a site with ARD. The site was selected based on a bioassay using apple seedlings and showing more than 100% growth increase with fumigation. The soil is a loamy sand (3% clay, 11% silt and 86% sand). With orchard establishment, the top 30 cm pH (KCl) values averaged 6.6, total soil carbon 2.0%, and stone 9%. The experimental layout

was a randomised complete block design with nine treatments applied to plots consisting of six trees, replicated in three blocks for each of the cultivars. Plots were separated by two guard trees. Irrigation was supplied through a micro sprinkler system (discharge rate 37 $L.h^{-1}$), 2-3 times per week. Irrigation scheduling was done using a wetting front detector that was calibrated to keep soil moisture above 50% plant available water. The total amount of water applied was between 6000 $m^3.ha^{-1}$ and 7000 $m^3.ha^{-1}$. All treatments were treated equally in terms of fertiliser and pesticide application, as per the standard orchard practice. Glyphosate ($3L.ha^{-1}$) was applied to all treatments twice a year to control weeds.

3.2.2 Treatment application

Treatments consisted of organic material application, soil inoculants and biostimulants and included:

1) Untreated control plots, managed to industry norm.

2) Methyl bromide treated plots fumigated (300 g per running m) the autumn before planting. Fumigated plots were covered with plastic as a standard practice with fumigation and trees planted into the plastic at orchard establishment. Plastic was left intact until the autumn of 2006.

3) and 4) A product derived from mustard seed (TA, Nematrol Inc.) applied as a biofumigant, releasing alyll isothiocyanate with the addition of water. The standard recommended dosage (TA1) and double the standard dosage (TA2) was applied as a post plant treatment for the first two seasons.

5) Commercial compost A (CompostA), applied at 15 $ton.ha^{-1}$ of which a third was mixed with soil in the planting hole and the rest was applied as a top dressing in spring. Surface application of compost was repeated annually in spring at 20 $ton.ha^{-1}$. CompostA was applied on its own for the first growing season and from the second growing season, because of positive results in the study site from Chapter 2, compost extract was applied seven times during the growing season, on a monthly basis, in combination with the annual compost application. Turned aerobic windrow composting was used and CompostA consisted of aerobically composted peat (15%), straw (15%), wood shavings (5%), chicken manure (30%) and 35% of pre-composted, inoculated green garden waste material, used as a starter. A commercial compost extract (BioEarth, Stellenbosch, SA) was applied at 500 $L.ha^{-1}$, diluted 50:1 and sprayed onto the soil with each application. The compost extract was prepared every month by adding 1000 L of water to 50 kg of re-composted compost and actively aerating the suspension for 48 h, with no additional additives. Field applications were made within 6 hours after preparation.

6) CompostA in combination with EM Bokasi (250 $g.m^{-2}$). EM Bokashi is produced through EM Technology (see Chapter 2), and contains anaerobically fermented organic material with a high nutrient content (Higa, 1994).

7) Commercial compost B (CompostB) applied on its own, at the same dosage as CompostA. Forced aerated static pile composting was used to produce CompostB and it consisted of chipped green garden waste material (60%), grape rests (10%) and a mixture of cow manure and straw (20%). CompostB was also coarser than CompostA.

8) A potassium humate product was applied annually at an initial rate of 100 $L.ha^{-1}$ in both spring and autumn. However, due to adverse effects on growth, application rates were reduced to 50 $L.ha^{-1}$ in the

second year. Humic substances are classified into humic acids (HA), fulvic acids (FA) and humin on the basis of their solubility in water as a function of pH (Swift, 1999). Humic acid salts are termed humates.

9) Biostart® (Microbial Solutions, Kya Sand, SA) *Bacillus* inoculant was applied at 1 $L.ha^{-1}$, in combination with a Microboost® Activator (Microbial Solutions) as a carbon source to sustain the activity of the introduced bacteria. In addition to the inoculant, a low dosage humic acid product, a 14% potassium humate liquid, was applied at 10 $L.ha^{-1}$, in October, December and March of each season.

Organic material and biological amendments were applied from orchard establishment in 2003 (except for compost extract, which were only applied from 2004), in every season until the 2008 harvest season. Soil inoculants (Biostart, EM and compost extract) were applied as a drench with planting and thereafter monthly throughout the growing season. Chemical properties of the compost and compost extracts used are shown in Appendix A. Biological properties were not measured (see Chapter 2).

3.2.3 Tree performance evaluation

Trees were permanently marked 20 cm above the graft union and trunk circumference measured at planting and every year during winter. Total shoot growth was measured at the end of the first and second growing season. Annual yield was recorded as the average yield (kg/tree) of the six trees from each plot for the first commercial yield in 2006/2007 and again in 2007/2008 season, after four and five seasons of applications respectively. The number of harvested fruit was determined for each tree in order to calculate average fruit mass per plot for each treatment replicate. Yield efficiency was calculated by dividing yield by trunk cross section area at harvest. Fruit quality was evaluated for the 2007/2008 season and parameters measured included fruit firmness, total soluble solids (TSS) and total titratable acids (TTA). Evaluations were done at harvest, after 12 weeks storage at -0.5 ºC under regular atmosphere (RA), and then following 7 days at room temperature (21-24 ºC) (shelf life period). For each evaluation 35 fruit from each treatment and block combination was analysed.

3.2.4 Leaf nutrient analyses

Leaf nutrient analyses were done for the last three seasons (2006-2008) to provide insight into tree performance effects with continued application after three seasons. Leaf nutrient analyses were done for the control plots, fumigated plots and the majority of the biological amendments, except TA2. TA1 was selected because it resulted in a more positive effect on shoot growth. A combined 50 leaf sample of mature leaves in the mid shoot section of the current years' growth was collected at the end of January from the six trees in each plot for the 2006-2008 seasons. Leaf samples were prepared and analysed as described in Chapter 2.

3.2.5 Soil sampling and analyses

Only five selected treatments from a total of five replicates were sampled within the two cultivars due to the labour intensive nature of the microbial analyses and in order to limit the effect of storage on soil

microbial properties. Soil was sampled from experimental plots where treatments showed the biggest contrasts in growth improvement after two seasons, control plots, as well as from plots where treatments performed similar to controls. Furthermore, treatments were selected that showed similar tree performance in both cultivars. The treatments selected were fumigation, CompostA+Extract, CompostA+Bokashi and Biostart. Soil was sampled within the root zone of the top soil where microbial activity is expected to be greatest, at a depth of 0-25 cm. Samples were taken at a distance of 30-40 cm from the tree base, from two holes beneath four trees in each plot and composite samples prepared for each treatment from the eight sub-samples in each of the five block replicates. Soil samples were taken in autumn 2006 (May06), after 3 seasons of annual application of biological amendments, in spring 2006 (Oct06), before commencement of the seasonal applications, in summer 2006 (Dec06) and again in autumn 2007 (May07), two weeks after the last application of the season.

Sub-samples of soil sampled in summer 2006 were analysed for selected soil chemical properties using the methods described in Chapter 2. Field moist sub-samples of all four sampling dates were sieved through a 2 mm mesh screen for microbial analyses. Visible root pieces and un-decomposed organic matter were removed and soil stored at 4 ºC for no more than two weeks before analyses.

3.2.6 Soil microbial analyses

3.2.6.1 Plate counts. Conventional dilution spread-plating was performed to assess total heterotrophic counts, bacilli and actinomycetes. Medium R2A (Difco) was used for total heterotrophic counts, and *Bacillus* numbers were counted on 1/10 strength tryptone soy agar (TSA, Difco) after pasteurization of soil samples for 10 min at 80 ºC. Sodium caseinate agar (Du Plessis et al., 2005) was used for enumeration of actinomycetes. Plates were inoculated in triplicate and incubated at 25 ºC. Total heterotrophic numbers were counted after 72 h incubation and actinomycetes were counted after 7-10 days depending on colony growth.

3.2.6.2 Soil enzyme activity. Acid phosphatase and β-glucosidase activity were determined based on the release and spectrophotometric detection of *p*-nitrophenol (Tabatabai and Bremner, 1969; Tabatabai, 1982). Urease hydrolysing activity was determined by the non-buffered method of Kandeler and Gerber (1988). Controls were performed for all enzymes assayed by the addition of the substrate after incubation, but prior to analysis of the reaction product. Urease activity was only measured for three of the four sampling dates.

3.2.6.3 Substrate utilization profiles. Soil microbial community function within each of the soil samples was determined using substrate utilization profiles from commercially available Biolog® EcoPlates (Biolog® Inc., Hayward, USA) according to a modified procedure of Buyer and Drinkwater (1997), described in Chapter 2.

3.2.7 Nematode analyses

Nematode community structure was assessed and classified into trophic levels according to the scheme of Yeates et al. (1993). Data for the separate taxa were summarised into the abundance of bacterivore, fungivore-root hair feeder, omnivore–predator and plant-parasitic trophic groups. Abundance of enrichment opportunist nematodes was calculated as the sum of *Rhabditidae, Diplogasteridae* and *Panagrolaimidae* (Forge et al., 2003).

3.2.8 Statistical analysis

A standard analysis of variance (ANOVA) was performed on tree performance data, using the general linear means (GLM) procedure of SAS Statistical Software (SAS, 2002-2003). Trunk circumference measurements over the trial period were analysed as repeated measurements by comparing the slopes (b values) of linear regressions fitted to the data (R^2 between 0.97 and 0.99) in an ANOVA. Results were analysed separately for the two cultivars. Data from the two cultivars were pooled for soil chemical and microbial parameters, as well as leaf nutrient concentration after homogeneity of the cultivar variances was established by Levene's homogeneity test. A combined ANOVA was then performed on the data. Student's t-LSD was calculated at a 5 % significance level to compare the treatment means. Profiles of substrate utilisation were statistically analysed by principal component analysis (PCA) (Garland and Mills, 1991; Buyer and Drinkwater, 1997; Palojärvi et al., 1997; Larkin, 2003), using the correlation matrix (Rencher, 2002). Pearson Product Moment correlation coefficients (r) were calculated (SAS, 2002-2003) for averages of parameters measured over the trial period, as well as for the last sampling date of soil, leaves and yield of 2008. Furthermore, stepwise discriminant analysis (SDA) was used to select a sub-set of variables from an initial of 36 variables including leaf nutrient contents, soil chemical and biological parameters, as well as yield parameters. The subset of variables contained those variables which best differentiate or discriminate between the soil amendments and were used for canonical discriminant analysis (CDA). A PCA bi-plot was constructed, illustrating the relationship between the variables and their association to the different soil treatments. Multivariate analyses were performed using XLStat software.

3.3 RESULTS

3.3.1 Tree performance

3.3.1.2 Growth

Fuji. Trunk circumference measurements showed that all trees were of a similar size at planting (Table 1A). Increase in trunk circumference from planting showed significant increases with MeBr fumigation for the first season (Table 1A). Biostart application also significantly increased trunk circumference after the first growing season compared to the control, as well as compost extracted treatment, TA2 application and humate treatment. However, there were no significant treatment differences in trunk circumference growth tempo over the trial period. Total growth from MeBr treated plots were significantly more after only one growing season (2004), and growth was still significantly more (48% growth improvement) after two

growing seasons compared to all other treatments. None of the biological treatments had a significant effect on growth after one season of application relative to controls. However, after two growing seasons, Biostart application significantly improved growth and a positive effect was also observed with compost extract application, as well as CompostB although results were not significant. CompostA applied with Bokashi at planting, as well as two seasons of mustard gas (TA) at both a higher and lower dosage, resulted in similar shoot growth as control trees. Humate application appeared to have a negative effect on first year growth, and dosages were lowered in the second growing season.

Ruby Gala. Trunk circumference measurements showed that all trees were of a similar size at planting. Fumigation with MeBr significantly improved trunk circumference after the first growing season compared to all other treatments (Table1B). However, similar to results from 'Fuji', there were no significant treatment differences in trunk circumference growth tempo over the trial period. Total growth from MeBr treated plots were significantly more after the first, as well as the second growing season when compared to the control. None of the biological treatments had a significant effect on shoot or trunk growth compared to control plots after two seasons of application. However, compost in combination with compost extract application produced similar shoot growth than trees from fumigated plots after the second growing season. CompostB, CompostA applied with Bokashi, humate application, as well as mustard gas applications had no significant effect on growth compared to the control.

3.3.1.3 Yield and fruit quality

Fuji. Methyl bromide fumigation had a significant effect on yield parameters. Fumigation significantly increased the number of fruit per tree in both seasons compared to all other treatments and more than doubled yield in 2008 compared to control plots (Table 2A). None of the biological treatments improved yield or yield efficiency significantly in the 2007 or 2008 seasons compared to control trees. However, biological treatments showing the most potential for improving yield were Biostart and CompostB, as well as TA1. None of the treatments showed a negative effect on yield. Average fruit mass calculated at harvest did not show significant differences between the treatments. However, in the fruit quality analysis (Table 3) both CompostA treatments, TA1, MeBr, as well as Biostart significantly increased fruit size compared to fruit from control plots.

Ruby Gala. Yield for 2007 varied between trees in the same plot and none of the treatments improved yield compared to trees from the control plots (Table 2B). However, in 2008 yield was significantly higher for trees from fumigated plots, as well as Biostart treated plots compared to the control, with yields for fumigation being significantly higher than yields with Biostart. Although compost with compost extract application did not significantly improve yield in any one of the seasons, cumulative yield was significantly higher than from control plots. Cumulative yield was again highest for trees planted in fumigated soil. Biostart did not show significant improvement in cumulative yield, because of very low yield in 2007. However, it was not clear what caused this. Although some treatments showed a negative effect on yield in

2007, it was not consistent for 2008. Except for MeBr, Biostart and compost extract application, none of the other soil treatments showed a positive effect on yield. Average fruit mass calculated at harvest in both 2007 and 2008, showed no significant differences between the treatments. Results were similar for fruit size evaluations with fruit quality analysis in 2008 (Table 3).

Fruit quality parameters measured showed no significant effect for 'Ruby Gala' apples and few significant effects for 'Fuji' (Table 3). Furthermore, few consistent trends could be observed between the two cultivars over the three evaluation periods. Fruit firmness at harvest was lowest with compost extract for both cultivars, but results were only significant for 'Fuji'. Results were significant compared to all treatments, except MeBr and Biostart. After storage differences in fruit firmness were not significant in either of the cultivars. TSS showed no significant differences with any of the evaluations for 'Fuji'. For 'Ruby Gala' lower TSS was observed in MeBr treatment after storage compared to all other treatments, but results were not significant. There were no significant differences in TTA, background and red colour, or starch conversion for 'Fuji' or 'Ruby Gala'. However, for 'Ruby Gala' there was a trend for fruit from compost extract treated plots to ripen earlier than control fruit when comparing higher TSS, lower TTA and fruit firmness and higher starch conversion at harvest. This was in contrast to greenest skin colour (background colour) at harvest with compost extract application.

3.3.1.4 Soil chemical properties

Soil extractable P and K were generally very high for all treatments and levels were already high when the trial was established (P 150 $mg.kg^{-1}$, and K 183 $mg.kg^{-1}$). Although differences in pH were minor, MeBr fumigation resulted in soil with significantly higher pH compared to the control and soil treated with CompostA with Bokashi, as well as Biostart (Table 4). Soil resistance was significantly lower in soil sampled from plots where compost was applied when compared to all other treatments and there was no significant difference between application of CompostA with Bokashi and CompostA in combination with compost extract. Total soil C and N, as well as ammonium- and nitrate-N showed no significant differences between the treatments. There was great variation in soil extractable P between samples from the same treatment, possibly resulting in non-significant statistical difference between the various treatments. However, after four seasons of biological amendment application, soil extractable P from MeBr fumigated plots was the lowest. Soil extractable K was significantly higher in soil treated with compost compared to all other treatments and application of CompostA in combination with compost extract resulted in significantly higher soil extractable K compared to CompostA with Bokashi. Results were similar for Na. There were no significant differences between the treatment means for soil extractable Ca and Mg. Treatments where compost was applied, showed lower Ca% due to high Na and K%. Soil micronutrients showed no significant differences among the soil treatments. Soil extractable Zn and Cu were low compared to industry norms for all treatments, possibly as a result of high soil extractable P. There were no significant differences in bulk density (BD) and water holding capacity. However, Biostart treatment resulted in both highest water holding capacity and lowest BD.

3.3.1.5 Leaf nutrient analyses

Analyses over the 2006-2008 seasons showed consistent trends for most of the macronutrients, although results were not always significant for all seasons. Leaf N content was high in general and there were no significant differences between treatments for all three seasons (Table 5). Leaf P content was within industry norms for most of the treatments and although results were not significant there was a consistent trend for leaf P content to be highest with application of compost extract in addition to CompostA. This was significant compared to the controls only for the 2006 season. Leaf K content showed a similar trend than P and was significantly higher in 2006 with compost extract application compared to all treatments where compost was not added. Leaf Ca content was low for all treatments, but not under the critical minimum level, and results varied among seasons. In both 2006 and 2008, leaf Ca content was highest in trees from fumigated plots and results were significant for the 2008 season compared to all treatments except compost extract and Biostart application. In the 2008 season, leaf Ca content was also significantly higher in trees from Biostart treated plots when compared to untreated control trees. There were no significant differences in 2007 in leaf Ca content between the treatments. Leaf Mg content showed no significant differences in among the treatments and Mg levels decreased in all treatments from 2006 to 2008.

Leaf micronutrient concentration showed less consistent trends over the three seasons than macronutrients (Table 5). No significant differences in leaf Mn were found. Leaf Fe concentrations showed inconsistent results over the three seasons. In 2006 leaf Fe was significantly higher compared to control plots in both treatments where CompostA was applied, as well as where humate was applied. There were no significant differences in 2007 in leaf Fe between the treatments. However, in 2008, with the exception of compost extract application, leaf Fe was significantly lower in most treatments compared to the control. Leaf Cu concentration showed a general decline from 2006 to 2008, but was still within the industry norms for apple and treatments did not result in significant effects for 2006 or 2007. In 2008 leaf Cu was significantly higher for CompostA with Bokashi and TA1 treatment, compared to controls and the other compost treatments. Leaf Zn concentration showed no significant treatment differences in any of the seasons, but a trend was noted with fumigated plots, as well as Biostart treated plots showing highest leaf Zn in 2006 and 2008. In the 2008 season leaf Zn concentration of all the treatments were very high compared to industry norms. Leaf B concentration also showed no significant differences between the treatments.

3.3.1.6 Nematode analyses

Although *Xiphinema* counts were not at a high enough level to cause concern, counts from Biostart treated soil were significantly lower compared to all other treatments (Table 6). No other significant differences were observed in nematode counts among the various treatments. This may be attributed to the characteristic variation found in counts from field sites. However, several trends were noted. For plant parasitic nematodes, *Pratylenchus* counts from roots were generally high for all treatments, including MeBr fumigation (Table 6). It was observed that fungivorous nematodes were also more abundant in soil treated

with Biostart. Bacterivorous nematodes were highest in compost treated soil, followed by Biostart and compost extract treated soil, respectively. Omnivorous and predacious nematodes were generally low, but were lowest in control soil and highest in fumigated soil and compost extract treated soil. Abundance of enrichment opportunist nematodes was used by Forge et al. (2003) as indicator of enhanced nutrient fluxes. Results showed that counts from compost amended soil were highest for enrichment opportunist nematodes, followed by compost extract and Biostart treated soil, respectively.

3.3.1.7 Soil microbial analyses

3.3.1.7.1 Plate counts. Plate counts of colony forming units (CFUs) for actinomycetes showed few significant differences among treatments, except for the last sampling date (autumn 2007), where counts were significantly increased with compost extract application (Figure 1). This was also a general trend over the four sampling dates. *Bacillus* numbers was consistently higher with compost extract application compared to control plots and Biostart treated plots and the effect was significant in spring 2006 and autumn 2007 (Figure 2). When calculating the averages of the *Bacillus* numbers over the four seasons, compost extract significantly increased *Bacillus* numbers compared to all other treatments (Figure 2). Fumigation, addition of CompostA with Bokashi and Biostart application had no significant effect on microbial numbers measured throughout the sampling period.

3.3.1.7.2 Enzyme activity. Urease and β-glucosidase activity showed similar trends for the various treatments across the sampling period and results were only significant for the autumn 2006 sample, with Biostart treatments showing higher urease activity compared to all treatments except compost extract application (Figures 3A and B). It was observed that for the average urease activity over the three sampling dates, enzyme activity was significantly increased by Biostart application, as well as the addition of compost extract in combination with compost compared to the control and fumigated plots. Results for the first sampling date showed that fumigation in combination with plastic had a negative effect on soil urease activity. Over the four sampling dates, effects on enzyme activity were most consistent for phosphatase activity, with compost extract significantly increasing activity in autumn 2006 and autumn 2007. Similar trends where noted in the other two sampling dates. Average phosphatase activity over the sampling period was significantly higher with compost extract, as well as Biostart treatment, when compared to the control (Figure 3C). Average phosphatase activity was also significantly higher for CompostA with extract compared to CompostA with Bokashi, as well as MeBr fumigation. There was no significant negative effect of fumigation and plastic cover on phosphatase activity in autumn 2006, or any of the sampling dates.

3.3.1.7.3 Substrate utilisation. Results obtained after 24 h incubation of inoculated Biolog plates, showed that soil microbial populations with higher metabolic activity, originating from soil treated with compost extract, were capable of utilising the most substrates in three of the sampling dates (Table 7). The number of substrates used was significantly more compared to substrates used by microbial communities from

control soils in autumn and spring 2006. In the autumn 2006 sample, microbial communities from Biostart and fumigated soil utilised a similar number of substrates as those from compost extract treated soil. In spring 2006, microbial communities from soil treated with CompostA either with Bokashi or compost extract, showed significantly more substrates utilised compared to all other treatments. However, this was not consistent for the other three sampling dates. In autumn 2007, Biostart showed a lower number of substrates utilised compared to fumigated, compost extract treated and control plots (Table 7). There were no significant differences in summer 2006. Results obtained after 38 h incubation of inoculated Biolog plates were similar to results after 24 h incubation, but effects were not significant in spring 2006.

Average well colour development (AWCD) values of the 31 carbon substrates on the Biolog plates were analysed by PCA using the mean AWCD values over the four sampling dates to compare community level physiological profiles (CLPPs) of the selected treatments. Incubating the plates for 24 h, revealed that CLPPs of the selected treatments differed from profiles of the microbial communities of untreated control plots (Figure 4A). Microbial communities from soil treated with Biostart and those treated with compost extract in addition to CompostA, showed similar CLPPs, while profiles from microbial communities treated with CompostA and Bokashi were more similar to CLPPs of microbial communities from fumigated soil. In comparison to profiles from control soils, CompostA with Bokashi showed most similar CLPPs. Incubating the plates for 38 h, resulted in different CLPPs than that after 24 h incubation, with microbial communities from compost extract treated soil showing the most distinct profile (Figure 4B). Fumigated soil still showed CLPPs most similar to CompostA with Bokashi treatment, but both were distinct from the control. Microbial communities from control and Biostart treated soil showed similar CLPPs when inoculated plates were incubated for 38 h.

3.3.1.8 Correlations and regressions

Pearson's correlation coefficients were calculated for the averages of parameters measured over the trial period, and showed most significant correlations between microbial properties measured. There was a significant positive correlation between *Bacillus* numbers in soil and urease activity ($r = 0.5495$; $p = 0.0020$), as well as urease activity and enrichment opportunist nematodes ($r = 0.5809$; $p = 0.0037$). Furthermore, phosphatase activity was significantly correlated with actinomycete ($r = 0.5238$; $p = 0.0042$), as well as *Bacillus* numbers ($r = 0.5156$; $p = 0.0050$) in soil. Yield only showed significant correlations with leaf nutrient content. Cumulative yield was positively correlated to leaf Na ($r = 0.7061$; $p = <0.0001$) and leaf Ca content ($r = 0.4639$; $p = 0.0148$). When calculating correlations for each cultivar separately, the correlation with leaf Ca was most significant for the 2008 yield ('Ruby Gala': $r = 0.7225$; $p = 0.0035$; 'Fuji': $r = 0.6653$; $p = 0.0010$).

Principal component analysis (PCA) was performed on the averages of yield, nutrition and microbial parameters measured over the trial period. The PCA bi-plot illustrating the relationship between the various parameters measured and their association to the different soil treatments are presented in Figure 5. The

percentage accounting for total variability in the data was 62%. Soil treatments on the two PC components showed distinct differences between controls, compost extract treatment, Biostart application and fumigation with MeBr. Treatment with CompostA and Bokashi could also be distinguished from compost extract treatment, as well as fumigation, but could not clearly be separated from Biostart application and controls. Yield parameters were more closely associated with fumigation, as well as leaf Na content, soil pH and tree size. Biological amendments were closely associated with soil microbial properties measured. From the various microbial parameters measured, compost extract treatment was most closely associated with *Bacillus* and actinomycete counts, phosphatase enzyme activity, as well as the amount of substrates utilized on inoculated Biolog plates by microbial communities from this soil treatment. Furthermore, soil extractable K and ammonium N showed association to compost extract treatment. Biostart treatment was most closely associated with β-glucosidase activity and fungivorous nematodes, while CompostA with Bokashi was associated with bacteriovorous nematodes. Leaf Fe content, as well as soil extractable P, also showed a strong association to Biostart treatment.

Ten discriminant elements (variables) which had the most discriminatory powers for subsequent analysis were identified by SDA. These included cumulative yield, phosphatase activity, actinomycete counts, amount of substrates utilized by soil microbial communities after 38 h incubation of biolog plates, leaf N, leaf P and leaf Cu and soil extractable P, K and Mg. The selected variables were subjected to CDA analysis to establish whether discrimination between the various biological soil amendments could be achieved. Canonical variants 1 and 2 explained 99% of the total dispersion (canonical variant 1 explained 93% of the variation, while canonical variant 2 explained the remaining 6% of the variation) (Figure 6). Standardized canonical discriminant function coefficients for canonical variant 1 was highest for cumulative yield, soil extractable P, actinomycete counts and soil extractable K while for canonical variant 2 coefficients were highest for Leaf N content, soil extractable K and Mg. Soil fumigation treatment could be separated from all biological treatments, as well as the controls. Furthermore, treatments receiving compost extract in addition to CompostA could be separated from the other biological treatments, as well as the control. Control plots, and Biostart treatments were most closely associated.

3.4 DISCUSSION

3.4.1 Effect of fumigation on soil microbial properties

Pre-plant soil fumigation with MeBr showed no reduction in actinomycete and *Bacillus* numbers, beneficial nematode levels, or enzyme activities, measured three years after fumigation. Plastic sheeting applied as part of the fumigation process, seemed to have a more negative effect on enzyme activity, since β-glucosidase and urease activity returned to levels of the control plots after removal of the plastic in autumn 2006. This may have been attributed to high temperatures reached in the top soil with the black plastic sheeting affecting microbial activity negatively. Results with fumigation are in agreement with literature, showing few persistent effects on broad-scale properties such as total culturable bacteria, microbial

biomass and soil respiration (Ridge, 1976; Sinha et al., 1979; Toyota et al., 1999; Stromberger et al., 2005; Yao et al., 2006). Some negative effects on enzyme activity were shown in a trial period of 37 weeks (Stromberger et al., 2005), but results varied with different fumigants and enzymes and recovery of activity after 37 weeks was not recorded. Wada et al. (2008) suggested that fumigation effects may be temporary and that soil microbial function recovered significantly during cropping. However, in our study fumigation still showed different CLPPs for microbial communities after three years when compared to the control. This is in agreement with various studies that found significant effects of soil fumigation on soil microbial community composition and diversity (Toyota et al., 1996; Zelles et al., 1997; Xiao and Duniway, 1998; Ibekwe et al., 2001; Porter et al., 2005; Klose et al., 2006; Yao et al., 2006). However, few studies have shown persistent effects with fumigation in the field after more than two years.

3.4.2 Effect of biological soil treatments on soil microbial properties

Biological activity in soil can be promoted by stimulation of the resident soil microbes through addition of an available carbon source (Campbell, 1989; Magarey, 1999; Termorshuizen et al., 2004), by improving root growth proliferation and plant health, thereby affecting rhizosphere organisms and root exudates (Cook and Baker, 1983; Bowen and Rovira, 1999; Sturz and Christie, 2003), or by direct addition of microbes to the soil.

In our study PCA bi-plots showed that soil microbial properties were closely associated with biological treatments, in contrast to control and fumigation treatments. Furthermore, the majority of the biological parameters where more closely associated with compost extract application. Results also showed that the same compost applied annually for four seasons, did not have a similar effect on soil microbial properties when combined with different amendments under ARD conditions. Bokashi (a high nutrient source) was applied as a once-off application with planting and its effect on microbial properties was probably negligible after 3 seasons, when soil was sampled. Soil microbial properties measured were therefore mainly the resulting effect from the compost application, which did not lead to significant changes in soil microbial numbers or activity. However, where compost extract was combined with the same compost and a continued source of soil microbes applied monthly, significant changes were found in soil microbial properties. In contrast to these results, Drenovsky, et al. (2005) found that application of compost, as a source of labile organic matter, was the main factor that influenced microbial populations when applied in combination with microbial soil inoculants. This is in agreement with results found in Chapter 2. Lejon et al. (2007), as well as Marschner et al. (2003) found that the size of carbon biomass was increased by various sources of organic matter input, while specific changes in the soil microbial community were more dependent on the type of biological amendment applied. Few composts are therefore universally effective and specific compost properties, as well as inherent soil microbial properties can have effects on disease suppressiveness and affect tree performance (Hoitink and Fahey, 1986; Litterick et al., 2004).

Bacillus and actinomycete counts, as well as phosphatase and urease activity were significantly increased by compost extract application when added to compost. Furthermore, significant correlations were found between these microbial parameters. Furthermore, incubation of Biolog plates inoculated with soil communities obtained from the various treatments, revealed higher utilisation ability of substrates by soil microbial populations from plots treated with compost extract. However, in contrast to our findings with compost in Chapter 2, soil analyses from the current study did not show significant changes in soil carbon% and could therefore not be related with increased microbial activity. However, Wander et al. (1994) concluded that qualitative rather than quantitative changes in soil organic carbon are important during the first decade of organic management.

Compost applications, as well as Biostart treatments increased numbers of bacteria-feeding nematodes, including enrichment opportunists which make an important contribution to nutrient cycling (Forge et al., 2003.) Bacterial numbers were not directly affected by *Bacillus* application with the Biostart treatment, but increased urease and phosphatase activity were found. Effective use of soil inoculants strongly depend on their survival and establishment (Kennedy et al., 2004) in the soil or rhizosphere. Furthermore, a high degree of specificity are exhibited by various microbial isolates towards controlling different pathogens, as well as producing biologically active substances (Martin, 2003) and growth responses to microbial inoculation have been reported to involve strain to crop, as well as site specificity (Zahir et al., 2004). It therefore seems likely that the increase in enzyme activity observed in our study with Biostart application was an indirect effect on the resident soil microbial population due to the regular addition of carbon sources applied in combination with the Biostart (HS and a labile carbon source). Various studies have shown increased soil enzyme activities associated with HS application (Visser, 1985 a,b, Vallini et al., 1993; Lizarazo et al., 2005).

Herbicides were applied to all treatments and possible effects on broad-scale microbial parameters can therefore not be excluded. This may be if more significance in plots where biological amendments were not applied regularly, and may partially explain differences between effects of compost on its own and in combination with compost extract.

Significant effects on soil microbial community composition were found with organic amendments in various studies (Bolton et al., 1985; Doran et al., 1987; Reganold et al., 1993; Wander et al., 1994; Drinkwater et al., 1995; Katayama et al., 1998; Mäder et al., 2002; Marschner et al., 2003; Yao et al., 2006; Lejon et al., 2007). Results from our study revealed that the use of substrates represented by CLPPs of microbial communities differed for the various treatments. Results also differed when incubating the inoculated plates for either 24 h or 38 h, indicating differences in reaction of soil microbial populations with higher metabolic activity (already active after 24 h) and slower metabolic activity (only utilising substrate after 38 h incubation). Community level physiological profiles after 24 h incubation showed a distinct profile for soil microbial populations from control soils from both fumigation, as well as biological

treatments. After 38 h incubation of inoculated plates, microbial communities from compost extract treated soil showed the most distinct substrate utilisation profile of the 31 carbon substrates, when compared to the other soil treatments. Microbial communities from soil treated with Biostart showed similar profiles to control soils. However, CLPPs only indicate functional aspects of the cultivable fraction of the soil community that grows on the C sources used in the plates (Widmer et al., 2001) and results need to be interpreted in combination with other microbial parameters measured.

3.4.3 Relation to tree performance and mechanisms involved

3.4.3.1 Fumigation

In our study, MeBr fumigation before orchard establishment (with the addition of plastic cover) had an immediate positive effect on plant growth in both 'Fuji' and 'Ruby Gala' from the first growing season. Trees from fumigated plots also produced the highest cumulative yields after the first two harvest seasons, for both cultivars. These positive effects of fumigation on tree growth and yield in replanted apple orchards are well documented (Mai and Abawi, 1981; Traquair, 1984). Results from the PCA-biplot showed that MeBr fumigation was the only treatment that could be associated with yield parameters. Furthermore, separation of the various soil treatments in the CVA was based partially on yield effects, significantly separating fumigation treatments from biological treatments and controls.

Fumigation in our study showed few direct effects on soil available nutrients, three years after establishment. Results by Porter et al. (2005), showed large increases in ammonium N following fumigation. However, in South Africa, orchards are fumigated 3-4 months before apple trees are planted. In this period it is likely that soil microorganisms will utilise these nutrients before orchard establishment and the effect at planting is therefore unclear. The only significant effect on leaf nutrient concentrations with fumigation was higher leaf Ca. Leaf Ca was low in general when compared to critical norms, possibly due to high soil extractable K. This indicates a potential advantage in Ca uptake with MeBr application, which may be ascribed to improved root proliferation and an increase in the number of root tips, improving Ca uptake. Leaf Ca was also significantly correlated to yield for both cultivars.

Improved yield with MeBr is usually ascribed to its broad spectrum biocidal activity, resulting in improved root growth and plant health. Pathogen status associated with the various soil treatments in our study where not quantified, but since increased pathogen effects are characteristic of ARD development the control of soilborne pathogens probably resulted in increased growth with fumigation. However, apart from its direct effect on plant pathogens and soilborne pests, fumigation affects microbial activity, as well as the structure and functionality of the soil microbial community (Zelles et al., 1997; Ibekwe et al., 2001; Klose et al., 2006). From literature it is clear that the effect of fumigation on soil microbial properties is most significant in the initial months after fumigation. Porter et al. (2005) showed reduced fungal and bacterial colonization on roots for at least 17 weeks after fumigation, and found a significant correlation with increased root growth. Although in our study no changes in microbial numbers and activity, or nematodes were found

three years after fumigation, effects were probably significant with orchard establishment, which represents a critical period for initial root proliferation. Microbes generally out-compete plants for nutrients in the presence of sufficient carbon sources (Jackson et al., 1989). Therefore, improvement in growth with fumigation may be ascribed to reduction in competition for resources from microbes in the early stage of root development. In our study substrate utilisation profiles also still showed differences with fumigation after three years, suggesting more long-term effects, even in cropped systems.

3.4.3.2 Biological treatments

Since ARD mainly develops through changes in soil microbial populations, there is merit in the integration of biological soil amendments into production systems in order to shift the balance of the microbial communities towards a population structure more conducive to increased plant health and productivity (Gu and Mazzola, 2003; Avis et al., 2008). In our study, significant effects on trunk and shoot growth were mainly found with fumigation. However, Biostart treatment also significantly increased shoot growth in 'Fuji' after three growing seasons. Positive effects on shoot growth were also observed in both cultivars with compost extract, in 2005 after it was first applied in 2004. Furthermore, trees from all treatments showed a similar rate of trunk circumference increase over the trial period. Cumulative yield of 'Ruby Gala', but not 'Fuji' trees were significantly increased with compost extract application compared to the control. Biostart application showed a positive effect on yield for both cultivars, but only after the second harvest. Although both cultivars were planted on the same rootstock, cultivar differences can be expected due to differences in trees size and growth habit of different cultivars, affecting nutrient as well as plant growth hormone translocation in the plant. Differences in cultivar susceptibility to replant problems have also been indicated for various deciduous fruit crops (Yadava and Doud, 1980).

Improved growth with organic amendments in ARD orchards was indicated in another field study in South Africa (Van Schoor et al., 2009). In this study compost applied as a soil dressing in combination with a straw mulch significantly increased shoot growth, but not trunk circumference, over three seasons, and results compared favourably to fumigation. Pot trials forming part of this same study, also showed that the application of compost, as well as sterilised and unsterilised compost extracts, significantly increased growth of apple seedlings in six ARD soils. In contrast to positive effects with Biostart and compost extract application in our study, application of CompostA with Bokashi at planting, as well as CompostB did not result in significant changes in tree performance in either of the cultivars. This is in agreement with other studies reporting compost to be mainly ineffective in controlling ARD (Granatstein and Mazzola, 2001; Neilsen et al., 2004). Furthermore, Leinfelder and Merwin (2006) found that pre-plant treatments with compost had no significant effect on tree growth or yield of trees planted on an old orchard site. However, in their study soil fumigation also did not result in growth improvement, but effects of ARD tolerant rootstocks were significant and results explained by changes in the rhizosphere soil microbial communities associated with the various rootstocks.

Application methods can also influence results with organic amendments. Wilson et al. (2004) found no positive effect with incorporation of organic matter into the top 20 cm of orchard soil but replacing soil from the planting hole with soil not previously planted to an apple orchard, improved growth significantly. In our study, compost was initially mixed with soil in the planting whole. Direct protection of roots from pathogens or favourable conditions for root establishment may explain these positive effects. Differences in results with compost in various studies can also be explained by compositional variability of the organic material applied, as well as site specific effects. Furthermore, ARD effects can be aggravated by site specific abiotic factors. Engel et al. (2001) found that compost mixed with replant soil at planting and subsequent mulching with apple wood chips was effective in improving vegetative and reproductive growth mainly due to abiotic factors putting the plant under stress. In our study irrigation was scheduled on plant available water percentage and trees were therefore not subjected to water stress. To reduce effects of compositional variability on disease suppression due to the site specific etiology of ARD, inoculation of composts was suggested (Kuter et al., 1988; Hoitink et al., 1997). Our results also suggest that application of soil inoculants and biostimulants in combination with organic material, showed more promise in managing ARD than applying compost on its own.

Although Biostart application showed little effect on soil chemical properties and leaf nutrient concentration, urease and phosphatase activity were increased in some seasons. Increased enzyme activity can lead to increased availability of nutrients. It was noted in the previous section that bacterial numbers were not directly affected by *Bacillus* application and that improved microbial properties were possibly related to the effect of the labile carbon sources and HS applied in combination with the inoculant. The effect of HS on root growth has been widely documented (Vaughan and Malcolm, 1985; Chen and Aviad, 1990; Crouch and van Staden, 1991; Van De Venter et al., 1991; Reynolds et al., 1995). Effects on soil structure, such as increased total porosity, reduced BD, as well as improved soil water conditions (Tester, 1990; Roe, 1998; Neilsen et al., 2003; Magdoff and Weil, 2004) have been indicated as accurate predictors of root system performance (Thompson et al., 1987). In our study, soil structure seemed to be more favourable with Biostart application, possibly having a positive effect on root development. Furthermore, fungi-feeding nematodes were highest in soil where Biostart treatment was applied. Increased capacity and complexity of the mesofauna with organic amendments are well documented (Freckman and Caswell, 1985; van Bruggen, 1995; Drinkwater et al., 1995; Ferris et al., 1998; Bulluck et al., 2002; Mäder et al., 2002; Mulder et al., 2003) and free living nematodes that feed on microbes contribute significantly to nutrient mineralisation (Ekschmitt et al., 1999; Nahar et al., 2006).

Application of a humate product on its own did not show a positive effect on yield and even retarded early growth to some extent. This could possibly be ascribed to a too high dosage applied initially. The typical response curve with HS applications shows increased growth with increasing concentrations of HS, followed by a decrease in growth at high concentrations (Chen and Aviad, 1990). The net direct effect of humic materials on growth probably involves interactions of a series of biochemical stimulations and

inhibitions (Chen and Aviad, 1990), thereby partially explaining the dependence of effects on HS concentration. High concentrations of humates may also reduce the availability of chelated nutrients (Ruthann. and Schnitzer, 1981), thereby having a negative influence on plant growth.

Compost extracts contain a diverse group of microbes, as well as easily available nutrients and in our study its addition to compost was associated with higher *Bacillus* and actinomycete numbers, as well as phosphatase and urease enzyme activity. Compost water extracts were found to contain *Bacillus* spp. and various actinomycetes in a study by EL-Masry et al. (2002). In our study extractable soil macronutrients were also highest with compost extract application. Furthermore, increased leaf K, P and to a lesser extent Ca content, was found with this treatment. CLPPs of microbial communities from compost extract treated soil also indicated an effect on microbial community function, especially the microbes with slower metabolic activity. Soil microorganisms play an important role in changing root morphology and architecture (Azcón-Anguilar and Barea, 1997; Glick et al., 1998; Van Loon et al., 1998; George, 2000; Zahir et al., 2004; Gravel et al., 2007), as well as directly improving nutrient solubilisation and supply (Rodriguez and Fraga, 1999; Habte, 2006). The use of P solubilising bacteria as inoculants, specifically *Bacillus* spp., can increase P uptake and crop yield (Rodriguez an Fraga, 1999; Jeon et al., 2003; Karlidag et al., 2007). Phosphatase enzymes play a major role in the mineralisation of organic P in soil (Rodriques and Fraga, 1999; Joner et al., 2000; Ezawa et al., 2005). In our study, improved uptake of P was possibly caused by increased phosphatase activity with compost extract application, which in turn may have been linked to increased *Bacillus* numbers. Furthermore, actinomycetes (Carpenter-Boggs et al., 1995), as well as *Bacillus* spp. (Xavier and Germida, 2003) can facilitate mycorrhizal root colonization through associations with AM fungi and are therefore termed mycorrhiza helper bacteria (MHB) (Garbaye, 1994).

Although effects on pathogens were not measured in this study, both actinomycetes and *Bacillus* spp. are capable of inducing biological control of fungal pathogens (EL-Tarabily et al., 1997; Van Loon et al., 1998; Utkhede et al., 2000; Cohen et al., 2005; EL-Tarabily, 2006), as well as parasitic nematodes (Akhtar and Malik, 2000; Samac and Kinkel, 2001) implicated in ARD development. In our study levels of lesion nematodes were approaching damaging levels and it is possible that plants were protected from nematode attack by changes in the soil microbial populations with biological amendment. Biostart has specifically been formulated to control plant parasites and soil pathogens. With Biostart application increases in fungal feeding nematodes were found. *Xiphenema* counts were also significantly lower in these soils. Although counts were not at damaging levels in 2006, numbers may have increased in the following years, giving Biostart treated trees an advantage in terms of yield. Daneel et al. (2000) concluded that the direct effect of HS on parasitic nematode populations was insignificant, but that HS products could render the plant more resistant to nematode attacks, by allowing plants to compensate for root damage.

Changes in phytohormone levels mainly control growth and developmental processes in plants (Davies, 1995; Weyers and Paterson, 2001). Various *Bacillus* strains have been found to produce plant growth

promoting hormones (Arshad and Frankenberger, 1998; Gutierez-Mañero et al., 2001; Arkhipova et al., 2005; Aslantas et al., 2007). In a recent study by Aslantas et al. (2007) it was found that PGPR strains, including *Bacillus* spp., were effective in promoting growth and yield of different apple cultivars and that the plant growth promoting effect appeared to be related partly to the production of IAA and CK. Hormone-like activity has also been suggested for several humic fractions (Cacco and Del'agnola, 1984; Vaughan and Malcom, 1985; Piccolo et al., 1992; Nardi et al., 1996, 2002). In deciduous fruit, CK plays a crucial role in regulating lateral bud burst and development (Pillay and Railton, 1983; Faust et al., 1997; Cook et al., 2001; Zhang et al., 2003), the quality of fruiting spurs (de Jager, 1994) and fruit set (Stevens and Westwood, 1984; Zhang et al., 2008). Furthermore, there are several reports suggesting that the accumulation level of CK and export by the roots is closely correlated with the nutritional status of the plant (Menary and van Staden, 1976; Sattelmacher and Marschner, 1978; Horgan and Wareing, 1980; Wagner and Beck, 1993; Samuelson and Larsson, 1993; Takei et al., 2001). Although increased shoot growth was not always significant with biological amendment application, it is possible that there were changes in bud break or spur development which could have a significant effect on future yield.

Phosphatase activity was the microbial parameter showing the most discriminative power in separating the various treatments from each other in the CVA analysis. However, phosphatase activity could not be related to yield. In our study soil were sampled within the root zone, but not from the rhizosphere directly. In recent studies by Yao et al. (2006) on the use of different rootstock genotypes in ARD management, results showed that bacterial communities in the rhizosphere of susceptible rootstocks differed from tolerant rootstocks (Rumberger et al., 2007) and correlated with improved yield (Leinfelder and Merwin, 2006).

3.5 CONCLUSION

Fumigation was the treatment that showed the most significant and consistent response in terms of tree performance in this ARD site. However, regular application of Biostart® soil inoculant in combination with a labile C-source and low dosage of humate, as well as compost extract applied with compost, showed significant improvement in tree performance compared to untreated plots.

Changes in broad-scale soil microbial properties were significant with biological amendments that was applied monthly every season, but not with fumigation three years after MeBr application. Fumigated sites also did not show differences in parasitic nematode counts when compared to untreated soil. Effects with fumigation therefore seem to be dramatic and immediate, allowing trees to establish in an environment with little microbial competition, and in the absence of soil pathogens. However, in a study by Mazzola (1999) it was shown that cultivation of apples can induce microbial communities capable of inciting ARD within two to three years, having negative implications for the next orchard to be established. Effects with biological amendments are more gradual and long term. Early application is therefore important, in order

for trees to be colonized by beneficial organisms protecting them from pathogen attack. Furthermore, young trees still trying to establish an efficient root system can benefit from improved nutrient solubilisation and microbial growth hormone production in the rhizosphere. With repeated application, changes induced in soil microbial communities should prevail, making it more difficult for soilborne pathogens to dominate. This can possibly have more positive implications for the next orchard.

Biological approaches to ARD control are knowledge and management intensive and ultimately should form part of an integrated biological management system to maximise benefits. More research is needed on the effects of compost extracts. These inoculants are easy to apply and not very costly. The use of ARD tolerant rootstocks in combination with these biological amendments may also result in more favourable effects compared to fumigation. However, many issues remain to be addressed before there can be sufficient confidence in the reliable use of these biological amendments. Furthermore, a trade-off between short and long term advantages and/or disadvantages must be considered for sustainable management.

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Table 1A. Effect of biological soil management practices in comparison with methyl bromide fumigation on trunk circumference growth from orchard establishment in 2003 to 2008 and effect on total shoot growth for the first two growing seasons of 'Fuji' apple trees planted on M793 rootstock in a replant site (loamy sand soil). Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	Trunk circumference (cm)			Shoot growth (cm)	
	At planting 2003	Growth 03-04	Growth tempo over trial period[**]	Total growth 2004	Total growth 2005
Control	5.47	2.96 cd	3.86	423.9 bc	1571.3 cd
Methyl bromide[x]	5.77	5.20 a	3.98	970.9 a	2484.1 a
CompostA+Extract[y]	5.34	3.04 cd	4.05	466.5 bc	1972.2 bc
CompostA+Bokashi[*]	5.37	3.48 bc	3.97	502.1 b	1582.9 cd
CompostB	5.50	3.14 bcd	4.26	445.8 bc	1848.4 bc
Biostart	4.89	3.81 b	3.75	485.1 b	2037.7 b
TA1[z]	5.28	3.26 bcd	3.88	376.9 bc	1600.1 cd
TA2	5.43	2.97 cd	3.54	364.2 bc	1310.2 d
Humate	5.13	2.72 d	3.49	250.6 c	1268.1 d
Treatment (P value)	0.6619	<0.0001	0.1173	0.0011	0.0007

Treatment means are the average of three block replicates, with measurements from six trees in each treatment plot for each replicate.

[x] Plastic cover applied with fumigation was left intact with planting and removed in the autumn of 2006.
[y] Biological treatments were applied from establishment, except for compost extract which was only applied from the second growing season.
[z] A product derived from mustard seed (TA, Nematrol Inc.) applied as a biofumigant. TA1 = standard dosage, TA2 = 2 x standard dosage
[*] EM Bokashi is produced through EM Technology (Higa, 1994), and contains anaerobically fermented organic material with a high nutrient content.
[**] Slope (b value) of linear regressions (R^2 between 0.97 and 0.99) fit to trunk circumference measured from 2003 to 2008, indicating growth tempo.

Table 1B. Effect of biological soil management practices in comparison with methyl bromide fumigation on trunk circumference growth from orchard establishment in 2003 to 2008 and effect on total shoot growth for the first two growing seasons of 'Ruby Gala' apple trees planted on M793 rootstock in a replant site (loamy sand soil). Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	Trunk circumference (cm)			Shoot growth (cm)	
	At planting 2003	Growth 03-04	Growth tempo over trial period [x]	Total growth 2004	Total growth 2005
Control	4.46	3.43 bc	4.61	506.6 bc	1615.9 bcd
Methyl bromide	4.93	5.30 a	4.79	1138.1 a	2316.4 a
CompostA+Extract	5.17	3.70 bc	4.22	589.9 bc	2002.6 ab
CompostA+Bokashi	5.37	3.51 bc	3.96	567.7 bc	1547.1 cd
CompostB	5.13	3.40 bc	3.81	433.0 c	1323.6 d
Biostart	4.67	4.18 b	4.03	469.0 bc	1694.7 bcd
TA1	5.12	3.41 bc	4.12	652.0 b	1837.1 bc
TA2	5.16	3.15 c	4.14	424.8 c	1704.7 bcd
Humate	4.64	3.39 bc	4.38	443.7 bc	1494.2 cd
Treatment (P value)	0.1991	0.0044	0.1677	<0.0001	0.0025

[x] Slope (b value) of linear regressions (R^2 between 0.97 and 0.99) fit to trunk circumference measured from 2003 to 2008, indicating growth tempo.

Table 2A. Effect of methyl bromide fumigation and various biological treatments on yield and fruit size of 'Fuji' apple trees planted in 2003 on M793 rootstock in a replant site (loamy sand soil). Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	Fruit size (g)		Fruit number per tree		Yield (kg.tree^{-1})		Yield efficiency (kg.cm^{-2})		Cumulative Yield (kg.tree^{-1})
	2007	2008	2007	2008	2007	2008	2007	2008	
Control	161	144	109 b	84 b	20.3	12.5	1.147	0.482	32.8 bc
Methyl bromide	164	149	219 a	216 a	36.4	30.6	1.766	1.119	66.9 a
CompostA+Extract	177	159	104 b	110 b	18.7	17.3	1.052	0.728	36.1 bc
CompostA+Bokashi	163	142	109 b	108 b	19.9	15.2	1.087	0.583	35.1 bc
CompostB	159	156	146 b	115 b	26.1	16.9	1.404	0.726	43.0 bc
Biostart	168	153	121 b	134 b	23.9	20.3	1.282	0.893	44.2 b
TA1	185	151	118 b	119 b	23.2	17.9	1.306	0.769	41.1 bc
TA2	152	153	102 b	98 b	17.1	14.4	1.020	0.656	31.5 bc
Humate	164	151	96 b	78 b	18.3	11.8	0.985	0.544	30.0 c
Treatment (P value)	0.2772	0.2556	0.0334	0.0458	0.1733	0.0502	0.3442	0.1407	0.0008

Table 2B. Effect of methyl bromide fumigation and various biological treatments on yield and fruit size of 'Ruby Gala' apple trees planted in 2003 on M793 rootstock in a replant site (loamy sand soil). Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	Fruit size (g)		Fruit number per tree		Yield (kg.tree^{-1})		Yield efficiency (kg.cm^{-2})		Cumulative Yield (kg.tree^{-1})
	2007	2008	2007	2008	2007	2008	2007	2008	
Control	129	126	173 abc	114 c	24.3 ab	14.33 c	1.312 ab	0.531 c	38.6 c
Methyl bromide	139	124	165 abc	388 a	22.6 abc	48.14 a	1.094 bcd	1.648 a	70.7 a
CompostA+Extract	137	127	205 a	184 bc	27.7 a	23.35 bc	1.466 a	0.892 bc	51.1 b
CompostA+Bokashi	128	122	188 ab	124 bc	23.0 ab	15.21 bc	1.279 abc	0.591 c	38.3 c
CompostB	123	125	96 d	80 c	11.8 e	16.58 bc	0.719 e	0.627 bc	27.5 d
Biostart	124	124	134 bcd	107 bc	16.5 cde	27.59 b	0.947 de	1.046 b	44.1 bc
TA1	126	125	189 ab	133 bc	23.2 ab	16.74 bc	1.279 abc	0.670 bc	40.9 bc
TA2	129	127	148 bcd	151 bc	20.4 bcd	19.32 bc	1.150 abcd	0.573 c	39.7 c
Humate	132	127	119 cd	114 bc	15.7 de	20.66 bc	0.921 de	0.756 bc	36.4 cd
Treatment (P value)	0.6588	0.9374	0.0196	0.0019	0.0037	0.0116	0.0183	0.0016	<0.0001

Table 3. Effect of fumigation and biological treatments on fruit quality parameters for 'Fuji' and 'Ruby Gala' planted in 2003 on M793 as determined during the 2008 harvest season, after cold storage (at -0.5 ºC for 8 weeks), as well as cold storage following a shelf life period of 7 days at room temperature (21-24 ºC). Probability values shown at the bottom of the tables are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	Fruit size	Evaluation at harvest						After storage			After shelf life		
	(mm)	Firmness (kg m^{-2})	TSS (%)	TTA (%)	Skin Colour	Red Colour	Starch*	Firmness (kg m^{-2})	TSS (%)	TTA (%)	Firmness (kg m^{-2})	TSS (%)	TTA (%)
Fuji													
Control	71.41 d	8.13 ab	15.13	0.79	5.83	6.83	40.4	7.5	15.4	0.61	7.77	16.2	0.51
Methyl bromide	73.38 a	7.83 abc	15.90	0.75	5.67	4.33	47.7	7.4	15.9	0.59	7.67	15.9	0.51
CompostA+Extract	72.84 ab	7.47 c	14.93	0.76	4.00	5.67	50.5	7.6	15.4	0.58	7.47	15.5	0.49
CompostA+Bokashi	73.39 a	8.03 ab	15.23	0.75	7.00	3.00	53.0	7.7	15.7	0.53	7.73	15.7	0.53
CompostB	71.63 cd	7.93 ab	15.47	0.75	3.83	1.33	50.7	7.8	15.6	0.53	7.70	15.7	0.51
Biostart	73.70 a	7.73 bc	15.13	0.77	1.83	5.00	47.8	7.5	15.7	0.57	7.63	15.5	0.46
Humate	70.82 d	8.23 a	14.75	0.70	3.00	4.00	49.7	7.8	16.0	0.54	7.60	15.7	0.51
TA1	72.35 bc	8.17 ab	15.20	0.74	4.83	5.83	47.4	7.6	15.7	0.57	7.80	15.9	0.47
Treatment (P value)	<0.0001	0.0313	0.2460	0.6637	0.1043	0.1087	0.2244	0.1380	0.5544	0.9038	0.3549	0.2261	0.5912
Ruby Gala													
Control	66.47	8.9	12.9	0.92	5.2	5.3	27.3	8.4	14.0	0.73	7.4	14.1	0.54
Methyl bromide	65.96	8.7	12.6	0.84	5.0	4.2	39.3	8.0	13.3	0.66	6.9	13.8	0.60
CompostA+Extract	66.91	8.4	13.1	0.78	6.2	4.2	34.8	8.3	13.7	0.69	7.1	14.1	0.61
CompostA+Bokashi	66.17	8.8	12.8	0.80	4.0	5.8	28.9	8.2	13.9	0.68	7.3	14.0	0.60
CompostB	65.68	8.8	12.8	0.88	3.3	4.0	29.9	8.2	13.6	0.70	7.5	14.0	0.63
Biostart	66.77	8.8	12.9	0.80	3.8	3.5	31.0	8.0	13.9	0.71	7.2	14.1	0.57
Humate	65.35	8.6	12.5	0.78	3.5	2.5	26.8	8.0	13.9	0.72	7.2	13.9	0.64
TA1	66.04	8.7	12.7	0.88	2.3	3.7	31.2	8.1	13.6	0.72	7.3	13.9	0.63
Treatment (P value)	0.4023	0.3042	0.4070	0.0947	0.4831	0.7920	0.8152	0.0738	0.0546	0.9323	0.4821	0.7847	0.4749

*Percentage starch converted to sugar

For each cultivar 35 fruit from each treatment and block combination were analysed per evaluation.

Table 4. Soil chemical properties from fumigated soil, as well as soil amended with biological applications (loamy sand soil). Soil samples were taken in December 2006 in the top 0-25 cm soil layer from both cultivars. Data from the two cultivars were pooled after homogeneity of the cultivar variances was established. A combined analyses of variance indicated no significant cultivar and treatment interaction and only main effects are presented. Probability values are shown at the bottom of the table. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	pH (KCl)	Resist. (ohm)	C %	$NO3^-$-N ($mg.kg^{-1}$)	$NH4^+$-N ($mg.kg^{-1}$)	N %	P Bray II ($mg.kg^{-1}$)	K ($mg.kg^{-1}$)	Na ($cmol.kg^{-1}$)	Ca ($cmol.kg^{-1}$)	Mg ($cmol.kg^{-1}$)	T-value ($cmol.kg^{-1}$)
Control	6.88 b	767 a	1.82	28.07	9.65	0.176	205.8	266.3 c	0.065 c	11.5	1.51	13.70
Methyl bromide	6.98 a	738 a	2.12	29.96	10.10	0.189	156.8	283.3 c	0.065 c	12.4	1.61	14.81
CompA+Extract	6.93 ab	417 b	2.06	25.95	11.44	0.190	200.4	532.0 a	0.187 a	12.6	1.74	15.89
CompA+Bokashi	6.88 b	433 b	1.79	31.30	10.31	0.185	219.6	402.8 b	0.145 b	11.6	1.66	14.42
Biostart	6.85 b	703 a	1.71	33.18	9.71	0.188	214.6	285.0 c	0.073 c	11.8	1.49	14.04
P Value	0.0404	<0.0001	0.3211	0.2368	0.6192	0.8203	0.3152	<0.0001	0.0010	0.7522	0.6217	0.2243

Treatment	Cu ($mg.kg^{-1}$)	Zn ($mg.kg^{-1}$)	Mn ($mg.kg^{-1}$)	B ($mg.kg^{-1}$)	Na %	K %	Ca %	Mg %	BD $kg.L^{-1}$	Water holding capacity	
										10 kPa %	$mm.m^{-1}$
Control	1.18	6.00	20.68	0.99	0.48 b	4.95 b	83.7 a	10.90	1.268	9.8	146
Methyl bromide	1.02	6.63	20.78	0.91	0.46 b	5.02 b	83.7 a	10.86	1.270	7.2	151
CompA+Extract	0.78	5.75	19.32	1.06	1.21 a	8.76 a	79.1 b	10.94	1.255	9.2	148
CompA+Bokashi	1.02	6.75	19.53	0.91	1.01 a	7.15 a	80.5 b	11.39	1.268	6.6	154
Biostart	0.94	5.38	19.07	0.97	0.52 b	5.20 b	83.6 a	10.64	1.248	11.2	143
P Value	0.8418	0.5255	0.7507	0.5612	<0.0001	0.0002	0.0032	0.8419	0.6536	0.3422	0.1774

Table 5. Comparison of leaf nutrient analyses of macro and micro elements for three consecutive seasons from fumigated soil, as well as soil amended with biological applications. Data from the two cultivars were pooled after homogeneity of the cultivar variances was established. A combined analyses of variance indicated no significant cultivar and treatment interaction and only main effects are presented. Probability values are shown at the bottom of the table. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	Leaf N (%)			Leaf P (%)			Leaf K (%)			Leaf Ca (%)			Leaf Mg (%)		
	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
Control	2.96	2.42	2.43	0.178 b	0.150	0.173	1.76 bc	1.57	1.52	0.926	1.238	1.023 c	0.344	0.294	0.213
Methyl bromide	3.05	2.41	2.47	0.186 b	0.158	0.136	1.60 c	1.55	1.50	1.094	1.285	1.182 a	0.336	0.293	0.222
CompostA+Bokashi	3.08	2.41	2.39	0.194 b	0.148	0.186	1.79 ab	1.52	1.65	0.914	1.182	1.046 c	0.344	0.308	0.232
CompostA+Extract	2.96	2.42	2.51	0.230 a	0.206	0.218	1.93 a	1.71	1.66	1.012	1.352	1.093 abc	0.370	0.278	0.225
CompostB	3.10	2.44	2.55	0.188 b	0.195	0.218	1.82 ab	1.64	1.61	0.934	1.208	1.020 c	0.334	0.293	0.223
Biostart	3.10	2.37	2.66	0.200 ab	0.158	0.222	1.77 b	1.46	1.49	0.940	1.180	1.150 ab	0.373	0.305	0.238
Humate	3.00	2.45	2.50	0.180 b	0.158	0.154	1.73 bc	1.47	1.60	0.864	1.175	1.044 c	0.306	0.295	0.230
TA1	3.03	-	2.56	0.204 ab	-	0.188	1.76 bc	-	1.57	0.950	-	1.078 bc	0.326	-	0.235
P value	0.3774	0.8521	0.1013	0.0331	0.1490	0.2342	0.0295	0.2807	0.5721	0.0889	0.1992	0.0241	0.6297	0.7287	0.8062

Treatment	Leaf Mn ($mg.kg^{-1}$)			Leaf Fe ($mg.kg^{-1}$)			Leaf Cu ($mg.kg^{-1}$)			Leaf Zn ($mg.kg^{-1}$)			Leaf B ($mg.kg^{-1}$)		
	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
Control	82	186	317	191 c	168	151 a	6.8	5.8	4.3 c	67	39.6	97.2	39	32.6	30.7
Methyl bromide	87	190	361	195 bc	167	97 c	7.0	5.3	5.2 abc	79	41.3	115.0	38	33.3	31.6
CompostA+Bokashi	84	188	322	231 a	178	100 c	6.0	5.2	6.0 ab	65	41.6	97.4	41	32.8	32.0
CompostA+Extract	83	200	324	220 ab	162	144 ab	6.6	5.4	4.7 c	59	41.2	97.8	39	34.2	32.5
CompostB	73	178	303	191 c	170	96 c	6.4	5.5	4.8 c	58	40.6	87.3	40	36.0	33.0
Biostart	82	183	349	203 bc	169	120 bc	6.2	5.5	5.0 bc	73	40.5	105.7	43	33.3	34.0
Humate	75	181	327	241 a	171	102 c	7.0	5.3	5.4 abc	65	42.3	97.4	39	34.3	31.2
TA1	74	-	307	198 bc	-	103 c	6.6	-	6.3 a	62	-	89.0	42	-	31.0
P value	0.9288	0.9200	0.2274	0.0010	0.7784	0.0042	0.1318	0.3586	0.0118	0.7665	0.9967	0.0692	0.3612	0.6548	0.0976

Footnotes: Kotzé (2001) norms: N (2.1-2.6%), P (0.14-0.19%), K (1.2-1.4%), Ca (1.45-1.60%), Mg (0.30-0.40%), Na (500 $mg.kg^{-1}$), Mn (20-90 $mg.kg^{-1}$), Fe (80-150 $mg.kg^{-1}$), Cu (5-10 $mg.kg^{-1}$), Zn (30-50 $mg.kg^{-1}$), B (30-35 $mg.kg^{-1}$). Each leaf sample consisted of 50 leaves. Samples were analysed by a commercial laboratory (Bemlab®, Strand, SA) using an inductively coupled plasma-optical emission spectrometer and a nitrogen analyzer.

Table 6. Effect of fumigation and biological treatments on the abundance of parasitic nematodes, as well as fungivore, omnivore–predator, bacterivore nematode trophic groups and enrichment opportunists in an apple replant disease site (loamy sand soil). Data from the two cultivars were pooled after homogeneity of the cultivar variances was established. A combined analyses of variance indicated no significant cultivar and treatment interaction and only main effects are presented. Probability values are shown at the bottom of the table. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	*Xiphinema*	*Pratylenchus*	Fungivores	Omnivore-predators	Bacterivores	Enrichment opportunists
Control	52 a	512	22	6	532	339
Methyl bromide	36 a	688	36	47	510	276
CompostA+ Extract	48 a	784	43	40	749	536
CompostA+ Bokashi	43 a	852	54	17	1268	1075
Biostart	6 b	734	77	10	797	486
P value	0.0171	0.9234	0.5592	0.2271	0.7347	0.6934

Nematode counts per 250 cm^3 soil, except *Pratylenchus* which is per 5g of roots

Table 7. Number of substrates utilised after 24 h and 38 h incubation of Biolog Ecoplates inoculated with soil microbial communities subjected to various biological management practices, as well as fumigation. Biolog Ecoplates contain 31 different carbon sources, replicated three times on a plate and substrates utilised were assayed in May 2006, Oct 2006, Dec 2006 and Apr 2007. Data from the two cultivars were pooled after homogeneity of the cultivar variances was established. A combined analyses of variance indicated no significant cultivar and treatment interaction and only main effects are presented. Probability values are shown at the bottom of the table. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	Number of substrates utilised after 24h				Number of substrates utilised after 38h			
	Autumn 2006	Spring 2006	Summer 2006	Autumn 2007	Autumn 2006	Spring 2006	Summer 2006	Autumn 2007
Control	9.9 bc	3.4 b	4.3	11.9 a	20.7 c	19.2	18.7	22.9 ab
Methyl bromide	11.1 abc	4.2 b	8.1	11.1 a	23.9 ab	18.4	19.9	20.8 bc
CompostA+ Extract	13.4 a	10.0 a	5.4	14.1 a	25.7 a	22.6	21.1	23.8 a
CompostA+ Bokasshi	8.7 c	9.3 a	3.4	10.7 ab	22.6 bc	22.0	18.0	20.1 c
Biostart	12.5 ab	3.4 b	5.8	7.4 b	24.7 ab	20.4	19.0	19.7 c
P value	0.0087	0.0121	0.0815	0.0189	0.0139	0.1736	0.0921	0.0202

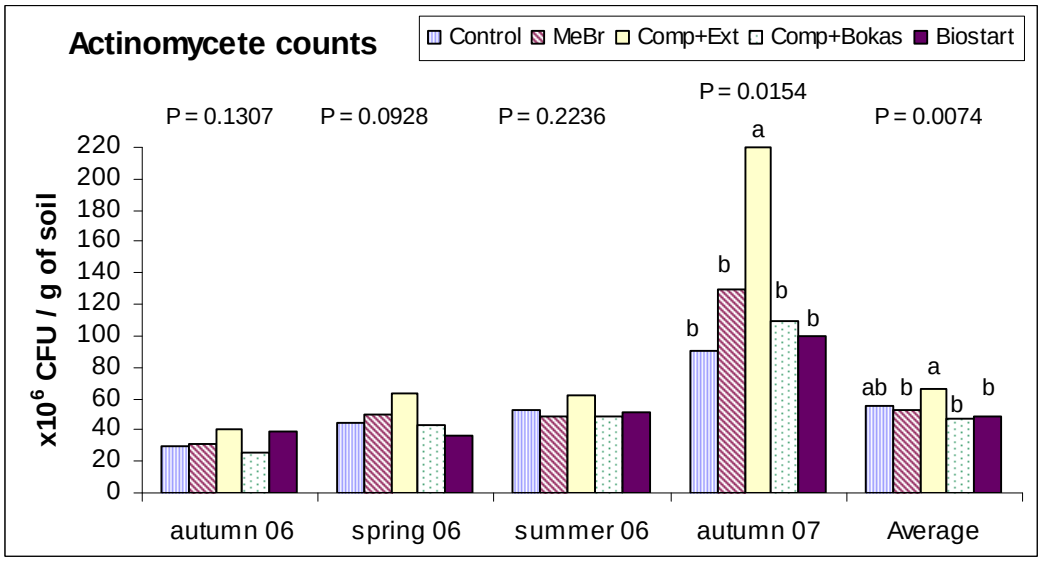


Figure 1. Effect of biological amendments and fumigation on number of colony forming units (CFUs) of actinomycete bacteria isolated from soil at four sampling dates. The average over the four sampling dates is also shown. Data from the two cultivars were pooled after homogeneity of the cultivar variances was established. A combined analyses of variance indicated no significant cultivar and treatment interaction and only main effects are presented. Probability values are shown for each sampling date. Bars within sampling dates topped by the same or no letter are not significantly different according to Student's t-LSD at a 5 % significance level.

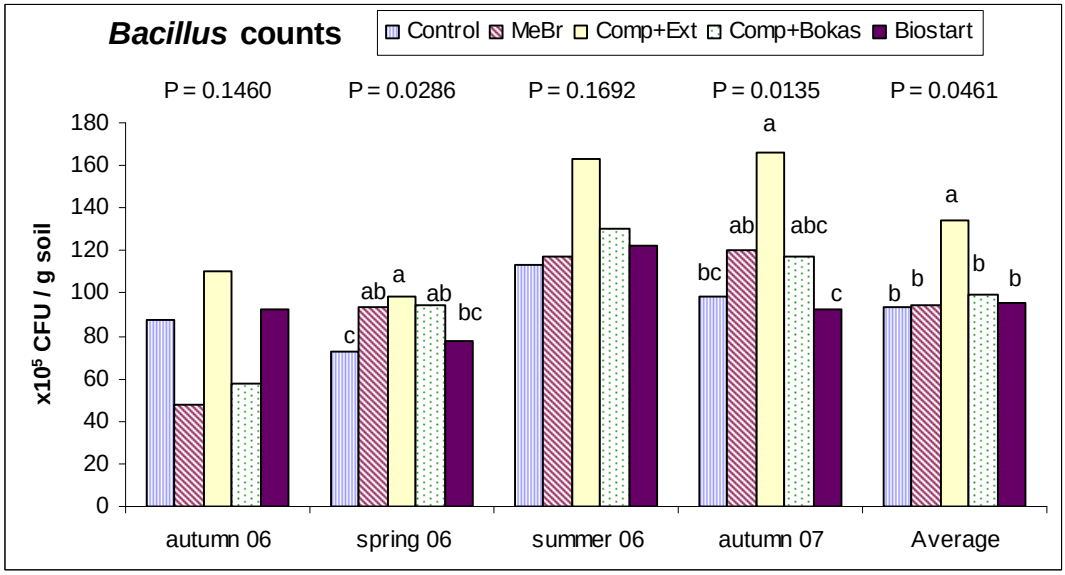
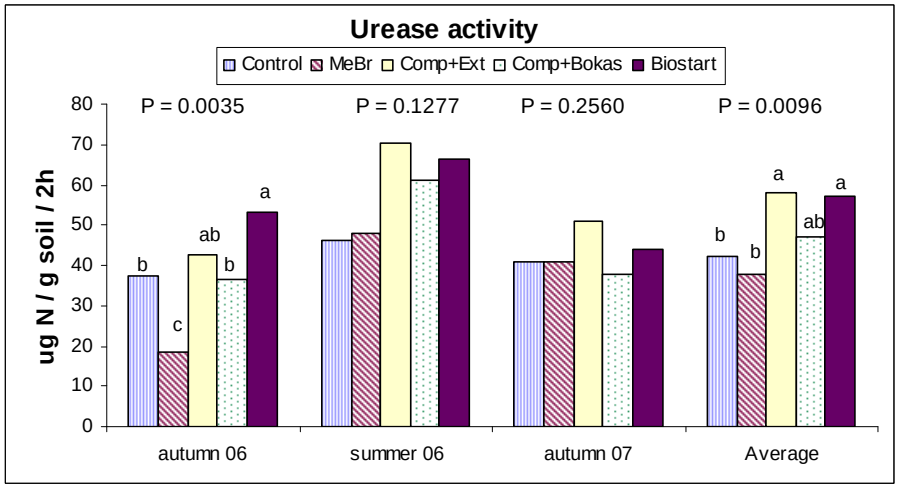
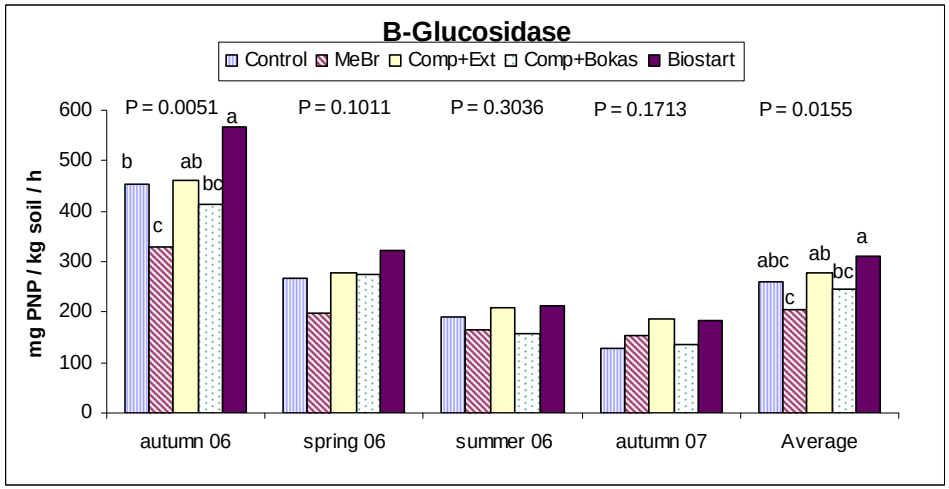


Figure 2. Effect of biological amendments and fumigation on number of colony forming units (CFUs) of total *Bacillus* bacteria isolated from soil at four sampling dates. The average over the four sampling dates is also shown. Data from the two cultivars were pooled after homogeneity of the cultivar variances was established. A combined analyses of variance indicated no significant cultivar and treatment interaction and only main effects are presented. Probability values are shown for each sampling date. Bars within sampling dates topped by the same or no letter are not significantly different according to Student's t-LSD at a 5 % significance level.



A



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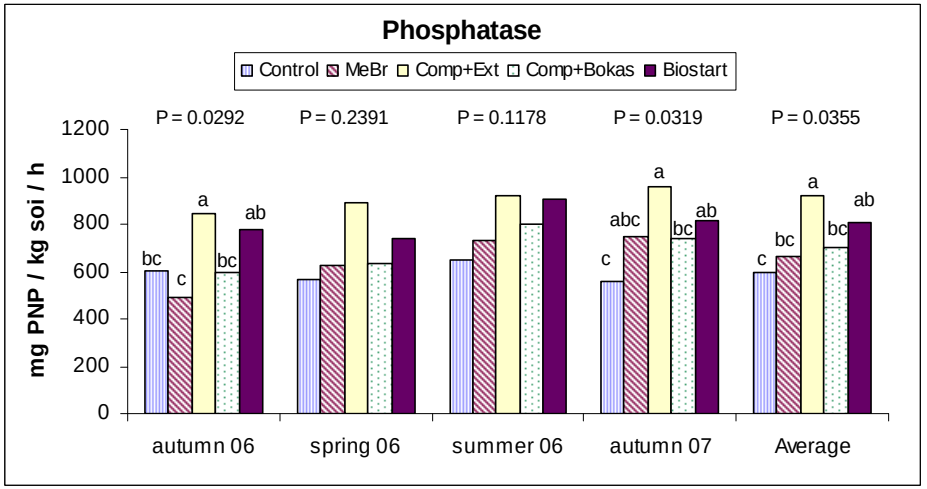
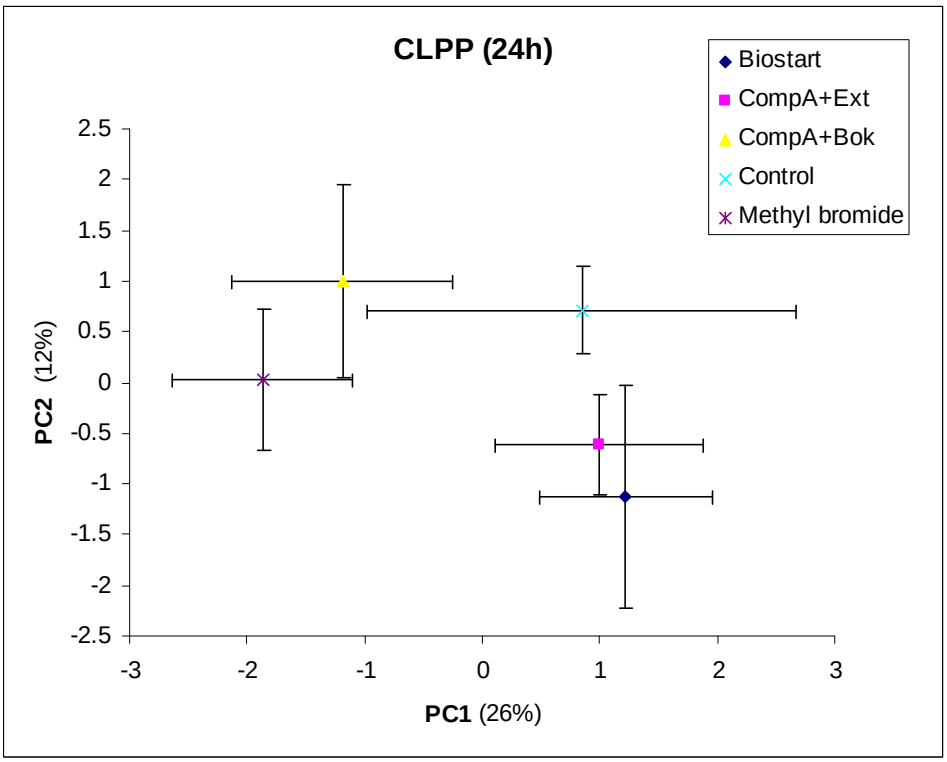
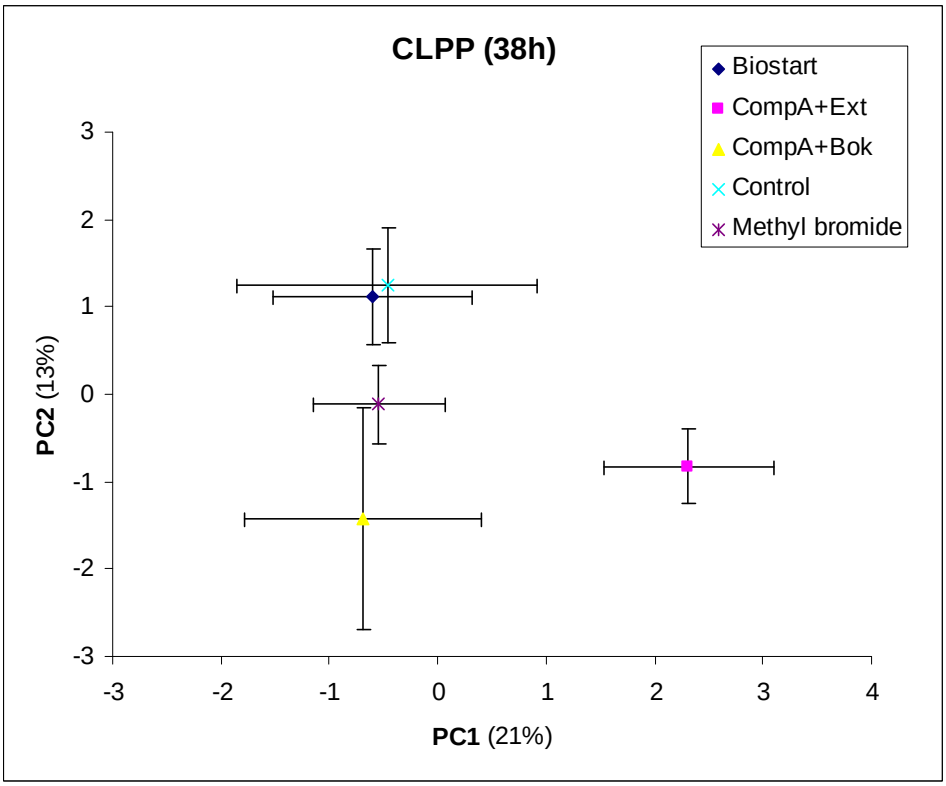


C

Figures 3A-C. Effect of biological soil amendments and methyl bromide fumigation on soil enzyme activity; A)Urease activity, B) β-Glucosidase activity, C) Phosphatase activity. Soil from the top 0-25 cm soil was sampled in May 2006, Oct 2006, Dec 2006 and Apr 2007. The average soil enzyme activity of the four sampling dates is also shown. Data from the two cultivars were pooled after homogeneity of the cultivar variances was established. A combined analyses of variance indicated no significant cultivar and treatment interaction and only main effects are presented. Probability values are shown for each sampling date. Bars within sampling dates topped by the same or no letter are not significantly different according to Student's t-LSD at a 5 % significance level.



A



B

Figures 4A-B. Ordination plots of principal components (PCs) 1 and 2 from community level physiological profiles (CLPP) of Biolog Ecoplates inoculated with soil from the various treatments. Principal component analysis was conducted on the average values of four sample dates for A) 24h and B) 38h incubation. Error bars represent ±1 standard error of the mean. Values in brackets indicate the percent of total variation accounted for by each principal component axis.

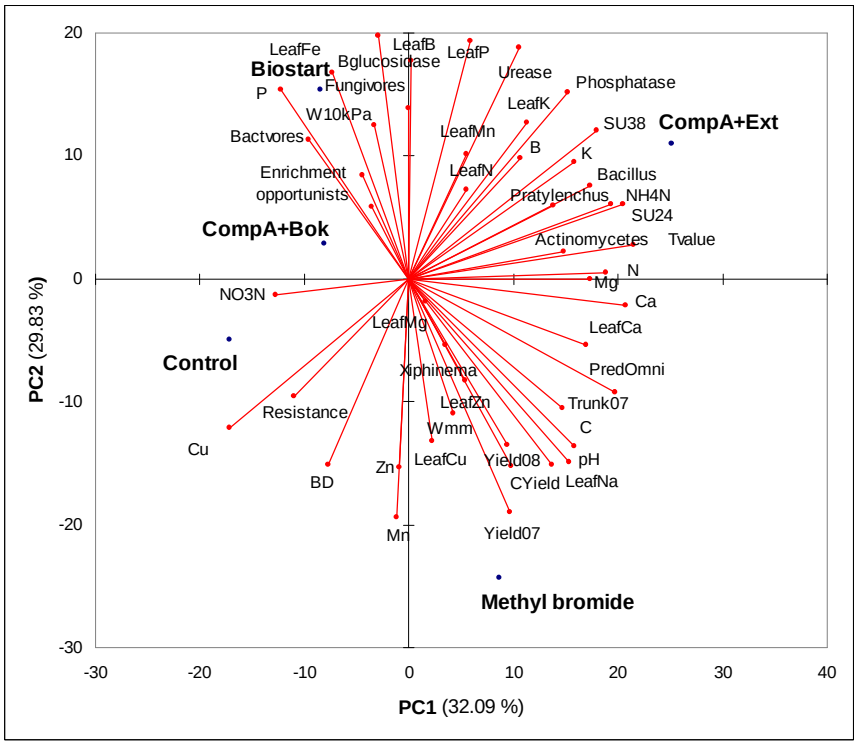


Figure 5. Principal component analysis (PCA) Bi-plot of the different variables (chemical and biological soil properties, leaf nutrient content and yield) in relation to the various soil treatments. Values in brackets indicate the percent of total variation accounted for by each principal component axis. CompostA with bokashi (CompA+Bok); CompostA with compost extract (CompA+Ext).

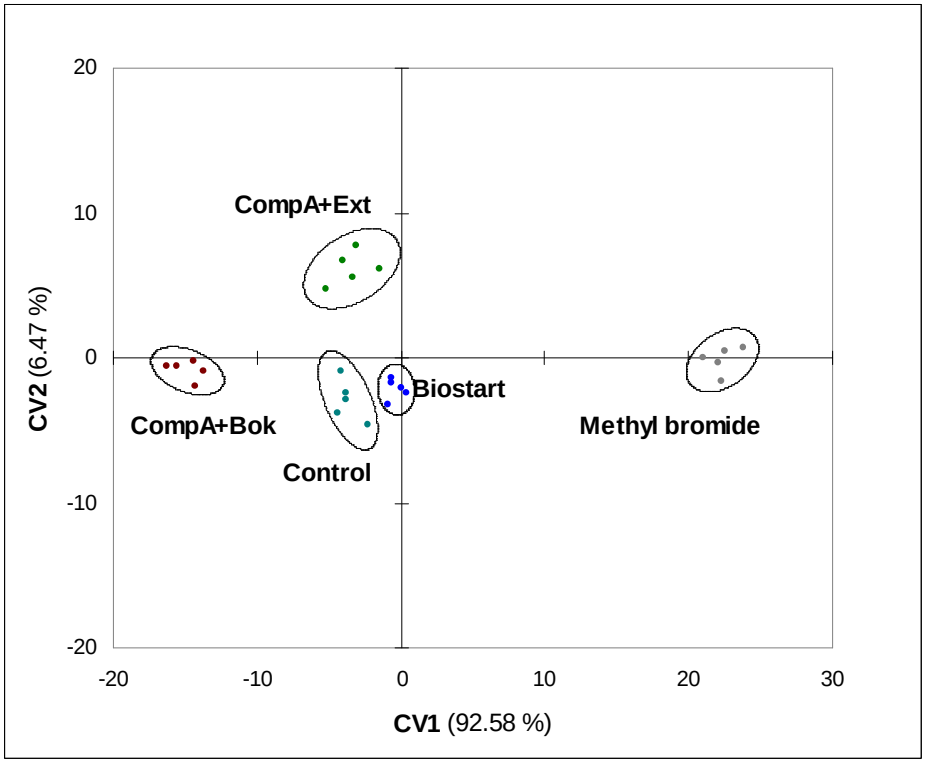


Figure 6. Plots of the first two canonical variables (CVs) from a canonical discriminant analysis (CDA), showing separation of the various soil treatments. Values in brackets indicate the percent of total dispersion explained by each CV. CompostA with bokashi (CompA+Bok); CompostA with compost extract (CompA+Ext).

CHAPTER 4

POTENTIAL USE OF COMPOST EXTRACT AND *BACILLUS* INOCULANTS IN COMBINATION WITH COMPOST IN MANAGING APPLE REPLANT DISEASE

ABSTRACT

Dual application of compost with compost extract or *Bacillus* inoculants were evaluated for its potential use to reduce the negative effects of apple replant disease (ARD) on early growth of apple. Differences in effects on plant growth of compost extract produced with additives (ExtractB) and without (ExtractA) were also investigated. Effects on tree performance with biological amendments were compared to methyl bromide fumigation treatment effects. Three trials were established in commercial orchards with a history of ARD problems in the Vyeboom (34° 08' S; 019° 02' E) and Elgin region (34° 10' S; 018° 85' E), Western Cape, South Africa. The first two study sites were sandy soils planted with 'Fuji' apple trees on M793 (Graymead) and M7 (Eikenhof). The third site (Monteith), with higher clay content, consisted of 'Royal Gala' apple trees on M793 rootstock. In the Graymead site where ARD symptoms were most severe and fumigation showed the most significant response, biological amendments resulted in little improvement in yield when compared to untreated plots. In the other two sites ARD was less severe and biological amendment application improved tree growth to the same extent as fumigation over three growing seasons. Results showed no clear indication of which amendment in addition to compost resulted in the best tree performance, except possibly the treatment where compost application was combined with compost extract treatment, as well as *Bacillus* inoculants and humic substances. This treatment was also the only biological amendment treatment that showed increased enzyme activity. Compost, and not the inoculant added, seemed to be the dominant factor affecting CLPPs, as well as soil extractable nutrients at these sites. Results suggest that in replant disease orchards improvement in microbial activity or changes in microbial community function can not be used to predict effects on tree performance.

Keywords: *Biological management, soil enzyme activity, Biostart®, Biolog®*

4.1 INTRODUCTION

The development of apple replant disease (ARD) and its control through fumigation is possibly one of the most extreme examples of the negative effect of monoculture combined with low organic matter inputs on plant performance. The ARD disorder is associated with poor growth of young apple trees planted on previously cultivated apple sites (Hoestra, 1968). Symptoms include stunted growth and reduction in tree vigour, resulting in delayed productivity. Root systems are typically small with few functional root hairs and a marked reduction in lateral root development (Savory, 1966; Hoestra, 1968; Mai and Abawi, 1981). Although the disease is not lethal, it is of great economic importance because of its lasting effect on production. Furthermore, the problem is intensified as suitable land, not previously planted to apple becomes limited. In South Africa serious ARD symptoms occur in approximately 40% of replantings (Honeyborne, 1996). Although ARD is one of the major impediments to the establishment of an economically viable apple orchard on sites previously planted to apple, its economic effect has not been quantified in South Africa.

In spite of extensive research on ARD, the etiology remains to be fully elucidated. The problem is rarely caused by a single agent, but rather a complex of factors that vary across geographic regions or even between orchards in the same region. Numerous soilborne organisms including plant parasitic nematodes, pathogenic fungi, actinomycetes and bacteria have been implicated as being potential causal factors (Savory, 1966; Hoestra, 1968; Covey et al., 1979; Mai and Abawi, 1981; Sewell, 1981; Jaffee et al., 1982; Slykhuis and Li, 1985; Utkhede et al., 1992; Dullahide et al., 1994; Braun, 1995; Mazzola, 1998; Manici et al., 2003). Studies by Mazzola (1998; 1999) indicated a shift in microbial community composition in ARD development, towards pathogens dominating the soil microbial profile. A complex of pathogenic fungi including the genera *Cylindrocarpon, Phytophthora, Pythium* and *Rhizoctonia* was implicated (Dullahide et al., 1994; Braun, 1995; Mazzola, 1998; Manici et al., 2003). A biological origin of ARD in South Africa was also suggested by Tewoldemedhin et al. (2007), as well as Van Schoor et al. (2009), since isolates of *Pythium* and *Cylindrocarpon* spp. were consistently isolated from replant soils indicating that these fungi may play a role in ARD etiology in South Africa.

Due to the uncertain and complex etiology of ARD, control has traditionally been achieved through the use of biologically broad-spectrum soil fumigants (Mai and Abawi, 1981), and in particular the application of methyl bromide. However, the high cost of chemical control and its potential hazard to human health and the environment, necessitates the development of more sustainable means of ARD control. There is strong evidence that shifts in microbial community composition can influence the growth and health of plants (Barea et al., 2005) and that this could possibly be established through the implementation of suitable soil management practices (Reeves, 1997; Cohen et al., 2005; Yao et al., 2006). The disease-suppressive effects of compost have received growing attention (Hoitink et al., 1997; Ristaino and Thomas, 1997; De Ceuster and Hoitink, 1999; Pascual et al., 2002; Noble and Coventry, 2005). Results reported in literature on the

use of compost in ARD management vary from positive (Autio et al., 1991; Engel et al., 2001; Moran and Schupp, 2001; Van Schoor, et al., 2009), to no effect (Granatstein and Mazzola, 2001; Neilsen et al., 2004; Rumberger et al., 2004; Wilson et al., 2004). However, the site-specific etiology of ARD means that elements implicated in disease development in other countries may have only a limited role locally, and vice versa.

The use of compost extracts or compost teas have been advocated as an inoculant to stimulate and enhance the soil microflora (Ingham, 1999a; Litterick et al., 2004). Various methods have been proposed in the production of compost extracts (Bess, 2000; Scheurell and Mahaffee, 2002), however it is unclear to which extent the efficacy of the extract is affected by different production processes. The use of additives to compost, intended to increase microbial population densities during compost extract production has been recommended (Ingham, 1999b). Molasses, kelp and humic-based additives in compost extract production were shown to positively affect disease suppression (Scheurell and Mahaffee, 2004). This could be important for widespread application, since additives can be standardised, whereas the properties of compost vary to a great extent. In previous studies in South Afica the application of sterilised and unsterilised compost extracts, significantly increased growth of apple seedlings in pot trials with six ARD soils in addition to augmenting nutrition (Van Schoor et al., 2009), however results were not verified under field conditions. Positive effects of compost in combination with compost extract, as well as the use of a *Bacillus* inoculant on tree performance were also shown in previous studies in newly established pear orchards (Chapter 2), as well as an ARD site (Chapter 3). Furthermore, positive effects on growth and yield of apple with *Bacillus* inoculants have also been indicated by other studies (Utkhede and Smith, 1992; Utkhede and Smith, 2000; Aslantas et al., 2007).

The objective of this study was to establish if dual application of compost with compost extract or *Bacillus* inoculants could be used to reduce the negative effects of ARD if used with orchard establishment under South African conditions. Differences in effects on plant growth of compost extract produced with additives and without were also investigated. Furthermore, the effect of treatments on soil microbial communities was measured by making use of soil enzyme activity assays, and community level physiological profiles (CLPPs).

4.2 MATERIALS AND METHODS

4.2.1 Orchard study sites and treatment application

Three trials were established in commercial orchards with a history of ARD problems. Two of the sites were conducted in the Vyeboom (34° 08' S; 019° 02' E) region, and one in the Elgin/Grabouw region (34° 10' S; 018° 85' E), both in the Western Cape, South Africa. The first two study sites consisted of ‘Fuji’ apple (*Malus domestica*) trees planted in 2006 at a spacing of 4.0 m x 1.5 m (within row) on M793 rootstock in the first site (Graymead) and M7, in the second site (Eikenhof). Graymead had a sandy soil

(2% clay, 10% silt and 88% sand) and at orchard establishment, in the top 30 cm, pH (KCl) values averaged 6.5, total soil carbon 1.6% and stone 43%. Eikenhof also had a sandy soil (2.5% clay, 9.5% silt and 88% sand) and at orchard establishment, in the top 30 cm, pH (KCl) values averaged 6.1, total soil carbon 1.9% and stone 48%. The third site (Monteith) consisted of 'Royal Gala' apple trees on M793 rootstock planted in 2006 at a spacing of 4.0 m x 1.5 m (within row). The soil was a clay loam (20% clay, 26% silt and 54% sand) and at orchard establishment, in the top 30 cm, pH (KCl) values averaged 6.0, total soil carbon 1.8%, and stone 55%. The experimental layout for all trials was a randomised complete block design with either five or six treatments, applied to plots consisting of nine trees, replicated in eight blocks. Plots were separated by two guard trees. Irrigation in all three sites was supplied through a micro sprinkler system and scheduling done using specific crop factors and a standard class-A pan. The Graymead site received a total of 4562 $m^3.ha^{-1}$ water for the 2008/2009 season. Monteith received 5548 $m^3.ha^{-1}$ and Eikenhof approximately 6000 $m^3.ha^{-1}$, respectively. Furthermore, all treatments at each site were treated equally in terms of fertiliser, pesticide and herbicide application, as per the standard orchard practice of that specific site. Glyphosate ($3L.ha^{-1}$) was applied at the Graymead and Eikenhof sites, twice a year, to control weeds. At the Montieth site Gramoxone (Paraquat) ($2L.ha^{-1}$) was initially applied to young trees and from the third growing season Glyphosate ($3L.ha^{-1}$) was applied. In the spring of 2008 all treatments from the Monteith site was treated with a single fenamiphos application ($2.5\ ml.m^{-2}$), due to high levels of lesion nematode (*Pratylenchus*).

4.2.2 Treatment application

Biological amendment treatments consisted of compost application in combination with soil inoculants and included the following:

1) Untreated control plots, managed as per the standard orchard practice.

2) Methyl bromide treated plots fumigated (300 g per running m) the autumn before planting.

3) Compost application in combination with Biostart® (Microbial Solutions, Kya Sand, SA). This *Bacillus* inoculant was applied as described in Chapter 2, with an activator and in combination with a low dosage humic acid product (Superguard®, Microbial Solutions, Kya Sand, SA). In adition, the first applications of each season consisted of a single strain *Bacillus* (DPress®, Microbial Solutions) applied at $1L.ha^{-1}$, with antagonistic activity against various soilborne pathogens, applied in combination with a fulvic acid product (MS® Humate Liquid, Microbial Solutions, Kya Sand, SA) at $10\ L.ha^{-1}$.

4) Compost application in combination with an aerobically produced compost extract (ExtractA). This extract was prepared by adding 1000 L of water to 50 kg of re-composted compost (Bioearth, Stellenbosch, SA) and actively aerating the suspension for 48 h, with no additional additives.

5) Compost application in combination with an aerobically produced compost extract (ExtractB). This extract was produced from a mixture of the same compost, molasses, fish extract and kelp.

6) The Eikenhof site had an additional treatment consisting of a combination of compost, the *Bacillus* programme and ExtractA.

Compost was applied at 20 ton.ha^{-1} as a top dressing with planting. Surface application of compost was repeated annually in spring at 20 ton.ha^{-1}. Turned aerobic windrow composting was used to prepare the compost. Compost consisted of aerobically composted peat (15%), straw (15%), wood shavings (5%), chicken manure (30%) and 35% of pre-composted, inoculated green garden waste material, used as a starter (same compost as CompostA in Chapter 3). Soil inoculants were applied as a drench with planting and thereafter monthly throughout the growing season. Properties of the compost and compost extracts used are shown in Appendix A.

4.2.3 Tree performance evaluation

Trees were permanently marked 20 cm above the graft union and trunk circumference measured at planting and every year during winter. Total shoot growth was measured at the end of the first growing season. After the second growing season total extension growth of one scaffold branch was used as an indication of vigour and after the third growing season total extension growth of two scaffold branches and leader growth were measured. Trees were allowed to bear fruit in the Graymead site from the third growing season, but for the other two sites fruit were removed in the second and third growing seasons to improve growth. Annual yield was recorded as the average yield (kg.tree^{-1}) of the centre six trees from each plot after three seasons of biological applications. Yield efficiency was calculated as yield per trunk cross section area. The number of harvested fruit was determined for each tree in order to calculate average fruit mass per plot for each treatment replicate. Fruit quality was evaluated from 35 fruit sampled at harvest and parameters measured included fruit size, fruit firmness, total soluble solids (TSS) background colour and red colour development. Percentage starch conversion was also measured as an indication of ripeness at harvest.

4.2.4 Leaf nutrient analyses

Leaf nutrient analyses were done for all treatments. A combined 50 leaf sample of mature leaves in the mid shoot section of the current year's growth was collected at the end of January from the six trees in each plot for the 2008 and 2009 season, to allow for at least two seasons of biological amendment application. Samples were prepared and analysed by a commercial laboratory (Bemlab, Strand, SA), as described in Chapter 2).

4.2.5 Soil sampling and analyses

Soil samples were taken in autumn 2008 and 2009 for soil chemical analyses of the Graymead and Eikenhof sites and only in 2008 for the Monteith site, due to economic constraints. Microbial analyses were performed on the 2008 samples of all the sites. Soil was sampled within the root zone of the top soil where microbial activity is expected to be greatest, at a depth of 0-25 cm. Samples were taken at a distance of 30-40 cm from the tree base, from two holes beneath four trees in each plot and composite samples prepared for each treatment from the eight sub-samples in each of the five experimental plots. Soil were analysed for chemical soil properties and prepared for soil microbial analyses as described in Chapter 2.

4.2.6 Soil microbial analyses

4.2.6.1 Soil enzyme activity. Acid phosphatase and β-glucosidase activity were determined based on the release and spectrophotometric detection of *p*-nitrophenol (Tabatabai and Bremner, 1969; Tabatabai, 1982). Urease hydrolysing activity was determined by the non-buffered method of Kandeler and Gerber (1988). Controls were performed for all enzymes assayed by the addition of the substrate after incubation, but prior to analysis of the reaction product.

4.2.6.2 Substrate utilization profiles. Soil microbial community function within each of the soil samples was determined by generating community level physiological profiles (CLPPs) from commercially available Biolog® EcoPlates (Biolog® Inc., Hayward, USA) containing different carbon sources, according to a modified procedure of Buyer and Drinkwater (1997), described in Chapter 2.

4.2.7 Statistical analysis

A standard analysis of variance (ANOVA) was performed on tree performance data, soil enzyme activity, as well as soil physiochemical characteristics using the general linear means (GLM) procedure of SAS Statistical Software (SAS, 2002-2003). Trunk circumference measurements over the trial period were analysed as repeated measurements by comparing the slopes (b values) of linear regressions fitted to the data (R^2 between 0.94 and 0.99) in an ANOVA. Student's t-LSD was calculated at a 5% significance level to compare the treatment means. Profiles of substrate utilisation were statistically analysed by principal component analysis (PCA) (Garland and Mills, 1991; Buyer and Drinkwater, 1997; Palojärvi et al., 1997; Larkin, 2003), using the correlation matrix (Rencher, 2002). Stepwise discriminant analysis (SDA) was used to select a subset of variables from the initial group of variables from all three sites, including leaf nutrient content and soil chemical and biological parameters. The subset of variables contained those variables which best differentiate or discriminate between the soil amendments and were used for canonical discriminant analysis (CDA). A PCA bi-plot was constructed on data from the Graymead site where yield was measured, using XLStat. This plot illustrates the relationship between the variables and their association to the different soil treatments.

4.3 RESULTS

4.3.1 Tree performance

Graymead. Trunk circumference measurements showed that all trees were of a similar size at planting (Table 1). Trunk circumference growth tempo over the trial period was only significantly increased by fumigation, and effects were significant compared to the biological amendments, as well as the control. Biological amendments resulted in no significant effect on trunk circumference growth compared to the control. After the first growing season, fumigation significantly increased total shoot growth compared to all other treatments. Compost with ExtractB was the only biological soil treatment that improved total growth significantly when compared to the control, but not compared to other biological amendments. Shoot extension growth with compost application in combination with Biostart® after two growing seasons

was significantly more compared to the control, but growth was still more favourable on trees from MeBr fumigated plots compared to all other treatments. After three growing seasons, shoot extension growth for all biological treatments were similar to that of controls and MeBr was the only treatment showing a significant increase. The addition of compost extracts induced more shoot growth after three growing seasons than Biostart application, but results did not differ significantly.

Trees from MeBr treated plots yielded significantly more fruit than all other treatments, more than doubling fruit number compared to control plots (Table 2). Despite higher yield, fumigation also resulted in the biggest fruit, but fruit were only significantly bigger compared to compost extract treated trees. When taking into account tree size, trees from fumigated plots showed highest yield efficiency and results were significant compared to all other treatments. Fruit quality parameters at harvest showed little significant differences between the soil treatments (Table 3). Fruit size was significantly increased in trees from fumigated plots compared to all other treatments. Fruit TSS levels were significantly lower for fruit from trees treated with MeBr compared to all treatments except compost with Biostart. Fruit firmness at harvest was highest for control fruit compared to both biological treatments and fumigation, which was in agreement with higher starch conversion with these treatments compared to control fruit, but results were not significant. No significant differences in background colour, or red colour development were found.

Eikenhof. Trunk circumference measurements showed that all trees were of a similar size at planting (Table 4). Trunk circumference growth tempo over the trial period showed no significant differences between the various soil treatments, although growth tempo was highest for the compost, ExtractA and Biostart combination. Total growth after the first growing season was significantly increased by fumigation, as well as compost with the two treatment combinations including Biostart, when compared to the control. Shoot growth from these treatments also did not differ significantly from each other (Table 4). Shoot extension growth after the second growing season was significantly more with fumigation, as well as all biological treatments when compared to control trees. After the third growing season none of the soil treatments resulted in significant differences in shoot growth. However, similar to shoot growth effects with biological amendments after the first season of establishment, treatments where Biostart was applied showed more growth compared to all treatments, including fumigation.

Monteith. Trunk circumference measurements showed that all trees were of a similar size at planting (Table 5). Trunk circumference growth tempo over the trial period showed significantly higher growth tempo with fumigation, as well as all biological amendments compared to the control. Furthermore, there were no significant differences on growth tempo between the biological amendments and fumigation. Total growth in the first growing season, showed significantly more shoot growth on trees from fumigated plots compared to all treatments, except where Biostart was applied. There was also a significant improvement in first season growth with both the compost extract treatments when compared to the control, and growth with ExtractB compared favourably to that of the fumigation treatment. Shoot extension growth in the

second growing season was only significantly increased by fumigation and compost with ExtractB, compared to the rest of the treatments and the amount of growth was similar for these two treatments. Shoot growth in the third growing season showed no significant differences and was similar for all treatments.

4.3.2 Soil chemical properties

Graymead. Extractable soil nutrients were generally higher in soils where compost was applied for both the 2008 and 2009 season (Table 6). Soil pH was significantly increased by biological treatments compared to fumigation, as well as control soil, except with ExtractA application in 2008. The only significant difference measured in soil chemical properties between control and fumigated plots was for soil resistance. Fumigated soil showed highest soil resistance, significantly higher than all treatments, with control plots showing significantly higher resistance compared to the various biological amendments. There was no significant difference in total C% of the top 0-25 cm soil layer between the various treatments in 2008 or 2009. However, total C% for the top 0-5 cm soil was higher with biological amendments, although significant differences were only recorded with the addition of ExtractB when compared to controls and fumigated plots, but not compared to other biological amendments. Total soil N content (%) was significantly higher with biological amendments compared to both control and fumigated plots in the 2008 season, and a similar trend was noted in 2009, although not significant. In both seasons, soil extractable P, and K were significantly higher with biological amendment application compared to control and fumigated plots. Results were similar for soil Ca, with the exception of ExtractA in 2008, where treatment did not result in significant increased in soil Ca compared to control and fumigated plots. Mg showed no significant differences between the treatments for either of the years.

Soil micronutrients Zn, Mn and B were generally also higher with compost application in both 2008 and 2009, although results were not always significant. Soil Cu was significantly lower with biological amendments in both years compared to controls. Soil Mn was significantly higher in 2009 with compost applications compared to treatments not receiving compost. In 2008, only compost with Biostart or ExtractB significantly increased soil Mn. Soil B was low in the 2008 season in all treatments and no significant differences were found. In 2009 soil B levels increased, but were still low in control and fumigated plots compared to soil where biological amendments were applied. However, only compost with either ExtractA or B significantly increased soil B. Cation exchange capacity (CEC) was lowest with fumigated and control plots, but results were not significant (Table 6).

Eikenhof. Treatment effects on soil chemical properties were very similar to effects found at the Graymead site. Soil pH was significantly higher in soil where compost was applied and for 2009 soil pH of MeBr treated soil was also significantly lower compared to controls (Table 7). Soil resistance levels were significantly lower with compost application in both years and results for 2009 were similar to that found for soil pH. Total soil C% showed no significant differences in 2008. In 2009, soil C% was higher with

biological amendments and effects were significant with the application of ExtractA when compared to fumigated and control plots. Total C% in the top 0-5 cm soil, showed lowest C% in fumigated soil and significantly higher C% for all soil treated with biological amendments, but compared to the controls results were only significant for compost with ExtractB or Biostart. In contrast to the Graymead site, soil extractable P showed no significant treatment differences, although it was noted that biological amendments showed highest soil P levels in the 2008 season, as well as the 2009 season, with the exception of compost with Biostart. Total soil N% and soil extractable K and Ca were consistently higher with compost application although differences were not always significant compared to the control for all biological amendments. Similar to the Graymead site, soil Mg levels showed no significant differences between the treatments.

Soil treatments in 2008 did not result in significant differences in soil Cu, although compost application resulted in higher soil Cu. However, in 2009 soil Cu was lower with these amendments and significantly lower compared to controls for compost and ExtractA, as well as compost and Biostart treatment. No significant differences in soil Zn or Mn were recorded in 2008 or 2009. Soil extractable B was increased with compost application and highest in 2008 and 2009 for both treatments where ExtractA was applied, although results were only significant in 2008. In 2009 all biological treatments showed significantly higher soil B levels than fumigated soil. Cation exchange capacity showed no significant treatment differences (Table 7).

Monteith. This site showed less significant treatment effects with compost application and for some nutrients fumigation resulted in a decrease in soil extractable content when compared to control plots. There were no significant differences found in soil pH and soil resistance (Table 8). However, lowest soil resistance was found in control soil, which is in contrast to the other sites showing lowest resistance with compost applications. Total C% was highest with the addition of ExtractA, but differences between the treatments were not significant. No significant differences in macronutrient content were found between the treatments, although soil extractable K and total soil N was lower in fumigated soil compared to controls. Soil micronutrient levels only showed significant effects for soil extractable Zn, for which fumigation resulted in significantly lower soil Zn levels compared to the control and compost with Biostart. Soil extractable B was highest with compost and Biostart application, although results were not significant. Bulk density and water holding capacity showed no significant treatment differences (Table 8).

4.3.3 Leaf nutrient analyses

Graymead, Leaf nutrient concentrations related to the various treatments was not consistent for the 2008 and 2009 season. In 2008, no significant treatment effects were found for leaf N and P (Table 9). All treatments increased leaf K significantly compared the control. Furthermore, leaf K was significantly higher for MeBr treatment, compost with ExtractB and compost with Biostart, compared to compost applied with ExtractA. The opposite trend was found for leaf Mg, showing significantly higher

concentrations for MeBr treatment, compost with ExtractB and compost with Biostart, compared to the control and compost with ExtractA. Leaf Ca levels were significantly higher in plants treated with compost and ExtractA compared to all other treatments. In 2008 leaf micronutrient levels of Mn, Zn and B were also significantly higher for methyl bromide, compost with ExtractB and compost with Biostart treatment, compared to control plots, as well as soil treated with compost and ExtractA. Leaf Cu levels showed a similar trend, except for Biostart treatment which only differed significantly compared to the control.

In the 2009 season leaf N content was significantly higher with compost application when compared to the control and also significantly higher for the combination with Biostart, when compared to both controls and fumigated plots. No significant differences in P and K content were observed with treatment application. Leaf Ca content was significantly higher for fumigated soil compared to all other treatments. Leaf Mg content was generally lower with compost application and significantly lower with Biostart application compared to controls and fumigated plots. Micronutrient levels were generally high for all treatments in 2009 and Mn levels very high compared to industry norms (Kotzé, 2001). However, no significant treatment effects were observed with Mn, Cu and Zn. Leaf B content was significantly lower in fumigated soil compared to all treatments, except compost with ExtractB.

Eikenhof. Leaf N content was highest for methyl bromide treated trees in 2008, and significantly lower compared to all other treatments for compost with ExtractA application (Table 10). This was not consistent with 2009 when compost with ExtractA and B showed significantly higher N content compared to controls and fumigated plots. ExtractB application also resulted in significantly higher N levels compared to Biostart treatment, as well as the combination of Biostart with ExtractA. No significant differences in leaf P content were found in either of the seasons. In 2008 soil extractable K was significantly higher for all treatments when compared to the control and compost with ExtractA. However, in the 2009 season all biological amendments increased leaf K content significantly compared to fumigated plots and ExtractB, showing highest leaf K in 2008, was the only biological treatment that did not significantly increased leaf K compared to controls. Few significant differences were found in leaf Ca content over the two seasons. In 2008 Ca was lowest in plants where the combination of compost, Biostart and ExtractA was applied and results were significant compared to the control and compost with ExtractB. Similar to results from the Graymead site in 2008, leaf Mg content in 2008 showed an opposite trend to leaf K, with leaf Mg found to be higher in controls and with compost and ExtractA application. However, results were not significant. In 2009, leaf Mg also showed the opposite trend of leaf K in that season, with all biological treatments showing significantly lower leaf Mg content compared to fumigated and control plots.

Leaf Na content differed significantly only in the 2008 season and MeBr treatment, as well as compost with ExtractB showed significantly higher leaf Na compared to all other treatments. Leaf micronutrient levels were very high in both seasons compared to industry norms (Table 10). No significant treatment

differences in micronutrient contents were found in either of the seasons. In 2009 a trend was observed with compost with ExtractB treatment showing highest leaf Mn, Cu and Zn levels.

Monteith. Results were not consistent for the 2008 and 2009 season. There were no significant differences in N, P and Ca in either of the seasons (Table 11). Leaf K also did not show significant differences in 2008, however lowest levels were found with MeBr treatment. In 2009, leaf K content was significantly lower in trees treated with MeBr and compost with Biostart compared to all other treatments. In 2008 leaf Mg levels were significantly lower with compost and ExtractB application compared to all treatments except ExtractA. In the 2009 season compost treatment resulted in significantly lower leaf Mg content compared to plants from fumigated plots, but not the control.

Few significant differences in micronutrients were found. Leaf Cu was higher with compost application, but only for the 2008 season and results were not significant. In 2008, Zn levels were significantly lower in control soils compared to all treatments except compost with ExtractA. Leaf Zn concentrations was significantly higher with fumigation compared to all treatments except compost with ExtractB. In 2009, leaf B content of plants subjected to compost with Biostart treatment showed lowest leaf B content and results differed significantly from all treatments except fumigation.

4.3.4 Soil microbial analyses

4.3.4.1 Enzyme activity.

Urease activity in fumigated soil was either lowest or similar to the control levels for all three trials (Figure 1). Differences were not significant in the Monteith site possibly due to great variation in urease activity across the trial area. For the Eikenhof site, urease activity was significantly higher for all biological soil applications compared to the control and MeBr treated plots. Urease activity was highest for the combination of compost, Biostart and ExtractA, but within the biological treatments only differed significantly from compost with ExtractB application. The Graymead site showed significantly lower urease activity with fumigation compared to al other treatments, but no significant effect of the biological treaments were observed compared to the control. β-Glucosidase activity was similar for fumigated soil compared to the controls in the three sites (Figure 2). The Eikenhof site again showed that the combination of compost, Biostart and ExtractA resulted in highest enzyme activity, but results were not significant. Differences were again not significant in the Monteith site, although ExtractA showed highest β-glucosidase activity. Enzyme activities of the control plots of the Graymead site were high compared to other treatments, and β-glucosidase activity was significantly lower with ExtractA, as well as compost with Biostart compared to the control. Phosphatase activity showed very similar effects than β-glucosidase activity for all three sites (Figure 3). Results from the Eikenhof trial showed a significant increase in phosphatase activity with the compost, Biostart and ExtractA treatment combination compared to all other treatments. There was no indication of a negative effect of fumigation with MeBr on phosphatase activity in any of the trials.

4.3.4.2 Substrate utilisation profiles

Average well colour development (AWCD) values of the 31 carbon substrates on the Biolog Ecoplates were analysed separately for each site by using PCA to generate CLPPs of microbial communities associated with the selected soil treatments.

Incubation for 24 h. Incubating the inoculated plates for 24 h, revealed that CLPPs of the biological soil treatments differed from profiles of the microbial communities of untreated control plots (Figure 4A-C). In the Graymead (Figure 4A) and Monteith (Figure 4C) site, CLPPs of the untreated plots also differed from CLPPs of microbial communities associated with fumigated soil. The Graymead site also showed distinct CLPPs for the biological soil treatments compared to CLPPs of microbial communities associated with fumigated soil. In the Eikenhof site (Figure 4B), CLPPs of microbial communities of soil treated with biological amendments, also differed from CLPPs of communities from fumigated soil. In both the Graymead and Eikenhof sites, biological treatments showed similar CLPPs, except for profiles of soil microbial communities subjected to compost with Biostart treatment, which for the Eikenhof site showed a distinct profile compared to the other biological treatments. In the Monteith site, CLPPs of microbial communities from soil with biological amendments generally showed distinct profiles, although profiles from microbial communities subjected to ExtractA treatment were similar to CLPPs from microbial communities of fumigated soil. The percentage of total variation accounted for by PC 1 and 2 were very low in all sites (25-37%). This may be due to the execution of the PCA on data including block replicates, in order to calculate standard deviations.

Incubation for 38 h. After incubation of the inoculated plates for 38 h, CLPPs of microbial communities from the Graymead and Eikenhof sites were remarkably similar (Figures 5A and B). Community level physiological profiles from microbial communities of fumigated soil, as well as control soil were different from CLPPs from microbial communities subjected to biological treatments. At Graymead, compost with Biostart showed distinct CLPPs, with the two compost extract applications resulting in similar CLPPs. At the Eikenhof site substrate utilisation with compost and ExtractB was distinct from all other treatments. Compost with either ExtractA or Biostart, as well as the combination of all three these treatments, showed similar substrate utilisation profiles by the microbial communities associated with them. In the Monteith site (Figure 5C) differences in CLPPs were less clear between microbial communities from the various soil treatments. Soil microbial communities subjected to compost with ExtractA showed most distinct CLPPs. Soil treated with compost and Biostart also resulted in microbial communities with different CLPPs compared to most treatments, except compost treated with ExtractB. The percentage of total variation accounted for by PC 1 and 2 were 30-35%.

4.3.5 Correlations and regressions

Principal component analysis (PCA) was performed on the soil biological and chemical parameters, as well as leaf nutrient content and yield parameters measured for the Graymead site. The PCA bi-plot illustrating

the relationship between the various parameters measured and their association to the different soil treatments are presented in Figure 6. The percentage accounting for total variability in the data was 78%. Soil treatments showed distinct differences between fumigated soil, untreated soil (controls), and soil treated with biological amendments. The biological treatments could not be distinguished from each other based on the variables measured. Tree performance parameters, as well as leaf Cu and leaf Ca content were closely associated with the fumigation treatment. Biological amendments were closely associated with soil chemical properties measured, as well as leaf N, Mn and Zn content. There was no clear association of microbial parameters measured with any of the treatments.

Stepwise discriminant analyses (SDA) was performed on combined variables of the three sites. Nine discriminant elements (variables) which had the most discriminatory powers for subsequent analysis were identified. These included clay %, pH, soil resistance, soil extractable P and K, soil Zn and Mn, as well as trunk circumference growth from planting till 2008 and shoot growth in 2007. The selected variables were subjected to CDA analysis to establish whether discrimination between the various soil treatments could be achieved. Canonical variants 1 and 2 explained 85% of the total dispersion (canonical variant 1 explained 57% of the variation, while canonical variant 2 explained the remaining 28% of the variation) (Figure 7). Soil treatments from the three sites could be separated from each other and the Graymead and Eikenhof sites were more closely associated. There was no clear separation of the biological treatments within each soil. However, for the Monteith site control soils were separate from most other treatments, while in the Graymead and Eikenhof site, fumigated soil could be distinguished from all other treatments.

4.4 DISCUSSION

4.4.1 Effect of soil treatments on microbial communities

Two years after MeBr fumigation, enzyme activity was significantly reduced only for urease activity and only in one of the three trial sites. However, this is in agreement with a previous study, also in an ARD orchard (Chapter 3), where urease activity was negatively affected in the first sampling date by fumigation and plastic sheeting. Negative effects on enzyme activity were also reported in strawberry field plots over a trial period of 37 weeks (Stromberger et al., 2005), but results varied with different fumigants and enzymes and recovery of activity after 37 weeks was not recorded. Wada et al. (2008) suggested that soil microbial function recovered significantly during cropping and the majority of literature has showed few persistent effects of fumigation on broad-scale properties such as total culturable bacteria, microbial biomass and soil respiration (Ridge, 1976; Sinha et al., 1979; Toyota et al., 1999; Stromberger et al., 2005; Yao et al., 2006). However, in the current study fumigation still affected substrate utilisation (CLPPs) of microbial communities from inoculated biolog plates after two years, compared to functioning of the microbial community of control soil, as well as those subjected to biological treatments. Similar results were found in the previous chapter three years after fumigation. Yao et al. (2006) found persistent effects of soil

fumigation on soil microbial community composition and diversity of bacterial communities up to 22 months. Our results indicate a persistent effect on soil functioning, even after three years of cropping.

With the application of biological amendments, it was clear that the treatment combination of compost, ExtractA and Biostart showed highest soil microbial activity, based on enzyme activity measurements. A high degree of specificity is exhibited by various microbial isolates towards controlling different pathogens, as well as producing plant growth promoting substances (Bent et al., 2001; Jeon et al., 2003; Martin, 2003; Zahir et al., 2004). The addition of multiple microorganisms to a given system therefore appears attractive, considering the multifaceted beneficial effects of various rhizosphere organisms (Avis et al., 2008). Furthermore, compost and other substrates serve as carriers to augment survival and function of introduced inoculants (Hoitink and Boehm, 1999). In our study, metabolic function of the community as measured through CLPPs, generally showed distinct substrate utilisation profiles for the biological treatments compared to control soils, as well as fumigated soils. Distinction between the various biological amendments was less clear in the Graymead and Eikenhof sites. It is therefore suggested that compost was the dominant factor affecting microbial community function in these two sites. In the Monteith site CLPPs from microbial communities in soil treated with ExtractA differed from the other biological treatments. However, the CDA showed little separation between soil and growth parameters of soil treated with biological amendments at the three different sites. Drenovsky et al. (2005) in a study on replanted peach orchards found that compost application and not additional amendments, was the main factor that influenced microbial communities. They suggested that the application of labile carbon masked the effect of the inoculants and also that in relation to the compost the diversity of the inoculant community was low, therefore not affecting overall function or diversity of the soil microbial community. However, in the previous study (Chapter 3) compost extract application was the dominant factor affecting soil microbial parameters. It is therefore clear that the combination of resident soil microbes, microbes in the compost, microbes in the inoculant, as well as site specific abiotic effects will determine ultimate changes in soil microbial properties.

Results from the CDA further showed that treatments from the different sites grouped together. Soil chemical properties, soil clay percentage and to a lesser extent effects on growth, were mostly responsible for the separation. Pérez-Piqueres et al. (2006) in their study indicated that changes in community structure induced by compost, related both to soil and type of organic amendment. Few composts are therefore universally effective and specific compost properties, as well as soil chemical and microbial properties play a role in the effect of compost on tree performance. Therefore, results can be expected to be site-specific and site-specific knowledge is needed to predict potential effects.

4.4.2 Relation to tree performance and mechanisms involved

In all three sites, application of biological amendments generally increased growth already in the first year after establishment, when compared to untreated control plots. However, despite initial positive effects on

growth with compost and ExtractB at the Graymead site, fumigation was the only treatment that showed increased shoot growth, as well as yield after three seasons when compared to untreated sites. Hoestra (1968) stated that ARD does not affect all apple soil equally and that growth increases with fumigation compared to untreated soil can be used as an indication of ARD severity. Apple replant disease seemed to be most severe in the Graymead orchard, which showed the biggest improvement in growth with MeBr treatment (68% growth increase). This may explain the lack of positive results with biological amendments at this site. Furthermore, abiotic factors can aggravate ARD effects. Irrigation scheduling at all these sites was done with the use of an evaporation pan and not directly measuring soil moisture by probes. The Graymead site received a total amount of water applied of 4563 $.ha^{-1}$, which is lower than the average of 6000-7000 applied generally. This may indicate that trees in this site were subjected to water stress, showing a more negative effect on biological treatments where root system development was probably shallower due to organic material applied to the soil surface. The PCA-biplot from the Graymead site also showed no association between biological treatments and changes in soil microbial properties.

In the Eikenhof and Monteith sites, growth improvement with biological treatments provided similar effects than broad spectrum fumigation. There was also a trend for trunk growth tempo to be higher than all other treatments, including fumigation, where compost+ExtractA+Biostart was applied. In the Monteith site shoot extension growth was similar for all treatments after the third growing season. This could be due to the effect of the nematicide application obscuring growth difference between the various treatments for that season. However, in this site both fumigation, as well as all the biological treatments significantly increased trunk growth tempo to a similar extent, compared to the control. The importance of large trees to initial apple yield has been indicated by various researchers. Addition of different compost extracts or Biostart to compost, resulted in similar growth effects and showed no clear indication of which amendment in addition to compost resulted in the best tree performance. The exception was possibly where all three amendment types were combined, but this treatment was only applied in one of the sites. In a similar study in peach replant orchards, Drenovsky et al. (2005) found that compost application and not additional amendments, was the main factor that influenced microbial communities, however these amendments had little effect on tree growth and vigour. Few other studies have combined compost with soil inoculant application.

Compost applied on its own were reported to be mainly ineffective in controlling ARD either when applied as an orchard floor management practice (Neilsen et al., 2004), worked into the top soil, (Granatstein and Mazzola, 2001; Wilson et al., 2004) or applied as a pre-plant treatment (Rumberger et al., 2004; Leinfelder and Merwin, 2006). However, it was clear from our results that combining compost with a diverse microbial inoculant, such as compost extract, or a mixture of various *Bacillus* strains, could significantly improve growth in ARD orchards. Results from Chapter 3 also suggested that monthly application of soil microoganisms and labile nutrients in combination with organic material, showed promise in managing ARD, although fumigation was still the treatment that resulted in the most significant yield improvement.

Furthermore, Hoitink et al. (1997) maintained that inoculation of composts with specific microbial organisms is a procedure that can induce more consistent effects, especially regarding disease suppression. Additionally, positive results from our studies compared to other studies, can be explained by the site-specific etiology of ARD, combined with general and specific disease suppression. General suppression is mainly related to high microbial activity and biomass of many types of organisms and plays a role in the suppression of *Pythium*, and *Phythophtora* (Hoitink et al., 1997). However, only a narrow group of microorganisms are capable of eradicating pathogens such as *Rhizoctonia*, in which case specific suppression is needed. In the USA, Mazzola and co-authors reported the aggressive pathogen *Rhizoctonia solani* AG 5 to play a major role in ARD disease development in various sites (Mazzola, 1998; 1999; Gu and Mazzola, 2003). However, in a study on ARD etiology in South Africa conducted in the same orchards as our study (Tewoldemedhin, 2008, personal communication), soilborne pathogens isolated mainly included *Cylindrocarpon*, *Pythium*, and *Phytophthora*. These pathogens can more easily be controlled by general increases in microbial activity.

Mechanisms through which plant performance is affected by biological amendments can not be easily separated and result from a number of direct and indirect effects. These include changes in soil chemical and physical properties, as well as microbial populations, which through disease suppression and production of biologically active substances affect root proliferation and nutrient availability and uptake. The Graymead and Eikenhof site, both sandy soils, showed increased soil extractable nutrients with compost application. This probably resulted from a combination of the chemical and biological properties of the compost applied. The Monteith site, a soil consisting of higher percentage clay, showed less significant effects of compost on soil chemical properties. This may be due to already high inherent nutrient retention of soil containing more clay particles. At all three sites there were few significant changes with inoculant application on soil chemical properties in addition to compost. However, these inoculants can significantly effect the possible utilisation of these nutrients by the plant (Glick, 1995; Jones et al., 1998; Zahir et al., 2004). Although similar differences in chemical parameters were induced with compost amendment at the Graymead and Eikenhof site, MeBr was the treatment that performed best in the Graymead site, but performed similar to biological treatments in the Eikenhof, as well as the Monteith site. Leaf nutrient content did not reflect the differences found in soil extractable nutrients. Furthermore, treatment effects on leaf nutrient content were inconsistent over the two seasons and between the three orchards, and could not be related to improved growth or yield. Leaf Ca was higher with MeBr treatment in one of the sites, which was also in agreements with results from the previous study (Chapter 3). In this study leaf Ca was significantly correlated to yield in both cultivars studied.

Since ARD has mainly been ascribed to biological causes, biological control mechanisms may have caused increased growth in some of these ARD affected sites. Although similar pathogens were isolated from all the trial sites (Tewoldemedhin, 2008, personal communication), their association with roots was not

quantified. It is therefore possible that disease pressure was higher in the Graymead orchard, increasing with cropping in non-fumigated soils and affecting growth to a similar extent than control soils after three seasons. Lesion nematode levels also showed sporadic high counts in this orchard. In the Eikenhof site, the combination of compost, ExtractA and Biostart had the most significant effect on tree vigour after three seasons. This site had no nematode problems and it is possible that disease incidence was low. Increased microbial activity with compost, ExtractA and Biostart application could therefore have shown positive effects on growth by aiding in nutrient uptake from increased soil extractable nutrients from compost application, or production of plan growth hormones (Arshad and Frankenberger, 1998). Zahir et al. (2004) stated that application of a mixture of beneficial organisms can have more significant influence on plant performance due to a reduction in variability and effectiveness with a wider range of microorganisms. The Monteith site also showed improvement in growth with biological amendments. Apple roots from this site showed high levels of lesion nematode infestation and it is possible that plants were protected from nematode attack or soilborne pathogens by changes in the soil microbial populations with biological amendment application.

Drenovsky et al. (2005) in peach replant orchards found that, although organic carbon amendments increased microbial biomass and influenced microbial community composition, they had little effect on tree growth and vigour. Yao et al. (2006) found that growth and yield of apple trees were not improved by compost treatments in ARD sites, although community composition was altered to some extent. However, they concluded that fungal and pseudomonad populations in the rhizosphere of different rootstocks were an important factor influencing tree growth and yield in ARD. In this study, orchard sites showing similar substrate utilisation and possibly microbial function, resulted in significantly different effects on tree performance. This illustrates the importance of site-specific effects when applying organic amendments, as well as differences in ARD severity.

4.5 CONCLUSION

In the site where ARD symptoms were most severe, and fumigation showed the most significant response, biological amendments showed little improvement in initial yield when compared to untreated plots. In the other two replant sites biological amendment application from orchard establishment improved tree growth to the same extent as fumigation over three growing seasons. Although it is believed that any advantage given to the young tree at establishment could be significant throughout the life of the orchard, effects on yield in these two orchards need to be assessed to provide evidence of long-term improvement in tree performance. However, results showed no clear indication of which amendment in addition to compost resulted in the best tree performance, except possibly the treatment where compost application was combined with compost extract treatment and *Bacillus* inoculants combined with humic substances. This treatment was also the only biological amendment treatment that showed increased enzyme activity.

However, at these sites compost seemed to be the dominant factor in affecting microbial community substrate utilisation (CLPPs), as well as soil extractable nutrients.

Results suggest that in replant disease conditions improvement in microbial activity or changes in microbial community function can not be used to predict effects on tree performance. The site-specific etiology and different degrees of ARD severity experienced at the three sites can possibly explain different effects found with soil treatments at the various sites.

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Table 1. Effect of biological soil amendments in comparison with methyl bromide fumigation on growth over three growing seasons of 'Fuji' apple trees planted on M793 in 2006 at Graymead, an apple replant disease site (loamy sand soil). Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	Trunk circumference (cm)		Total growth (cm) 2007	Shoot growth** (cm) 2008	Shoot growth (cm) 2009
	At planting (2006)	Growth tempo*			
Control	5.25	2.75 b	349 c	248.8 c	116.3 b
Methyl bromide	5.11	4.36 a	588 a	274.8 a	406.8 a
Compost+ExtractA	5.14	2.80 b	416 bc	252.3 bc	153.7 b
Compost+ExtractB	5.31	2.99 b	434 b	255.7 bc	164.1 b
Compost+Biostart	5.22	2.73 b	430 bc	258.6 b	116.9 b
Treatment (P value)	0.5555	<0.0001	<0.0001	<0.0001	<0.0001

* Slope (b value) of linear regressions (R^2 between 0.94 and 0.99) fit to trunk circumference measured from 2006 to 2009, indicating growth tempo.

**Measured as total extension growth of one scaffold branch in 2008, and the sum of total extension growth of two scaffold branches and leader growth in 2009

Table 2. Effect of biological soil amendments in comparison with methyl bromide fumigation on yield parameters in 2009 of 'Fuji' apple trees planted on M793 in 2006 at Graymead, an apple replant disease site (loamy sand soil). Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same letter are not significantly different.

Treatment	Fruit number	Fruit mass (g)	Yield ($kg.tree^{-1}$)	Yield Efficiency ($kg.cm^{-2}$)
Control	22.9 b	160 ab	3.30 b	0.333 b
Methyl bromide	72.5 a	173 a	12.29 a	0.779 a
Compost+ExtractA	33.8 b	147 b	4.79 b	0.437 b
Compost+ExtractB	32.4 b	142 b	4.54 b	0.386 b
Compost+Biostart	30.4 b	153 a	4.56 b	0.420 b
Treatment (P value)	<0.0001	<0.0001	0.0389	<0.0001

Table 3. Effect of biological soil amendments in comparison with methyl bromide fumigation on fruit quality parameters measured in 2009 with harvest of 'Fuji' apple trees planted on M793 in 2006 at Graymead, an apple replant disease site (loamy sand soil). Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same letter are not significantly different. Where no significant treatment differences were found with Student's t-LSD, no letters are indicated following the treatment means.

Treatment	Fruit size (mm)	Firmness ($kg.cm^{-2}$)	TSS (%)	Starch conversion (%)	Background Colour	Red Colour
Control	70.24 b	7.43	16.53 a	82.3	3.14	2.5
Methyl bromide	75.10 a	7.03	15.87 b	90.3	3.14	2.2
Compost+ExtractA	69.76 b	7.11	16.60 a	94.6	2.79	3.9
Compost+ExtractB	69.41 b	7.13	16.96 a	91.4	3.14	3.3
Compost+Biostart	70.14 b	7.13	16.42 ab	93.4	2.79	3.1
Treatment (P value)	0.0002	0.1368	0.0251	0.0838	0.8952	0.1821

Table 4. Effect of biological soil amendments in comparison with methyl bromide fumigation on growth over three growing seasons of 'Fuji' apple trees planted on M7 in 2006 at Eikenhof, an apple replant disease site (sandy soil). Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	Trunk circumference (cm)		Total growth	Shoot growth**	Shoot growth
	At planting (2006)	Growth tempo*	(cm) 2007	(cm) 2008	(cm) 2009
Control	4.75	3.40	574 c	227.1 b	356.0
Methyl bromide	4.76	3.70	711 a	249.6 a	410 8
Compost+ExtractA	4.78	3.72	599 bc	239.5 a	426.6
Compost+ExtractB	4.72	3.78	606 abc	239.3 a	444.7
Compost+Biostart	4.78	3.60	648 ab	243.1 a	476.0
Comp+Biost+ExtA	4.70	4.12	685 ab	241.7 a	468.1
Treatment (P value)	0.9534	0.1166	0.0131	0.0045	0.2521

* Slope (b value) of linear regressions (R^2 between 0.94 and 0.99) fit to trunk circumference measured from 2006 to 2009, indicating growth tempo.

** Measured as total extension growth of one scaffold branch in 2008, and the sum of total extension growth of two scaffold branches and leader growth in 2009

Table 5. Effect of biological soil amendments in comparison with methyl bromide fumigation on growth over three growing seasons of 'Ruby Gala' apple trees planted on M793 in 2006 at Monteith, an apple replant disease site (sandy clay loam). Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same letter are not significantly different. Where no significant treatment differences were found with Student's t-LSD, no letters are indicated following the treatment means.

Treatment	Trunk circumference (cm)		Total growth (cm) 2007	Shoot growth[**] (cm) 2008	Shoot growth (cm) 2009
	At planting (2006)	Growth tempo[*]			
Control	4.38	3.59 b	311.5 c	258.6 b	446.2
Methyl bromide	4.24	4.12 a	485.5 a	273.3 a	465.5
Compost+ExtractA	4.33	3.92 a	399.4 b	258.0 b	472.4
Compost+ExtractB	4.51	3.89 a	410.9 ab	273.8 a	455.4
Compost+Biostart	4.37	3.95 a	383.9 bc	263.6 b	478.1
Treatment	0.1276	0.0020	0.0040	0.0381	0.8238

[*] Slope (b value) of linear regressions (R^2 between 0.94 and 0.99) fit to trunk circumference measured from 2006 to 2009, indicating growth tempo.

[*]Measured as total extension growth of one scaffold branch in 2008, and the sum of total extension growth of two scaffold branches and leader growth in 2009

Table 6. Soil chemical properties from fumigated soil, as well as soil amended with biological applications at the Graymead site (loamy sand soil). Soil samples were taken in May 2008 and 2009 in the top 0-25 cm soil layer. Total soil carbon % was also measured in the top 0-5 cm. Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5% significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	pH (KCl)		Resistance (ohm)		Total C (%)			Total N (%)		P BrayII (mg.kg^{-1})		K (cmol.kg^{-1})	
	2008	2009	2008	2009	2008	2009 (0-25 cm)	2009 (0-5 cm)	2008	2009	2008	2009	2008	2009
Control	6.40 bc	6.18 b	1615 b	2076 b	1.61	1.40	1.72 bc	0.143 b	0.132	46.5	38.2 b	0.27 b	0.318 b
MeBr	6.22 c	6.14 b	2036 a	2646 a	1.66	1.34	1.39 c	0.141 b	0.116	38.6	44.0 b	0.24 b	0.250 b
Comp+ExtA	6.68 ab	6.76 a	832 c	1302 c	1.58	1.56	2.19 ab	0.165 a	0.169	57.2	104.8 a	0.63 a	0.738 a
Comp+Biost	6.92 a	6.78 a	1038 c	1394 c	1.56	1.49	2.06 ab	0.168 a	0.166	92.2	103.2 a	0.62 a	0.696 a
Comp+ExtB	6.86 a	6.86 a	1008 c	1342 c	1.57	1.62	2.22 a	0.177 a	0.161	91.6	101.4 a	0.58 a	0.722 a
P-Value	0.0028	<0.0001	<0.0001	<0.0001	0.9428	0.2355	0.0118	0.0025	0.1013	0.0545	0.0016	<0.0001	<0.0001

Treatment	Ca (cmol.kg^{-1})		Mg (cmol.kg^{-1})		Cu (cmol.kg^{-1})		Zn (cmol.kg^{-1})		Mn (cmol.kg^{-1})		B (cmol.kg^{-1})		CEC* (cmol.kg^{-1})
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2009
Control	5.08 b	5.39 b	0.843	1.008	3.50 a	4.01 a	9.60	12.26	11.0 b	13.16 b	0.47	0.772 c	11.32
MeBr	4.78 b	5.31 b	0.856	0.984	3.46 ab	4.09 a	11.88	14.22	10.5 b	13.80 b	0.42	0.784 c	10.31
Comp+ExtA	5.69 b	7.62 a	0.756	1.022	2.57 bc	2.50 b	13.03	18.04	11.7 b	19.10 a	0.42	1.056 ab	12.55
Comp+Biost	7.04 a	7.32 a	0.862	0.956	2.29 c	2.31 b	12.72	18.16	14.9 a	19.30 a	0.47	0.914 bc	12.71
Comp+ExtB	6.93 a	7.54 a	0.880	1.010	2.22 c	2.11 b	12.84	18.20	14.7 a	19.60 a	0.48	1.144 a	12.26
P-Value	0.0023	0.0013	0.1854	0.9184	0.0160	<0.0001	0.2114	0.1385	0.0088	0.0102	0.3782	0.0116	0.2812

* CEC = Cation exchange capacity

Table 7. Soil chemical properties from fumigated soil as well as soil amended with biological applications at the Eikenhof site (sandy soil). Soil samples were taken in May 2008 and 2009 in the top 0-25 cm soil layer. Total C% was also measured in the top 0-5 cm. Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	pH (KCl)		Resistance (ohm)		Total C %			Total N %		P BrayII (mg.kg^{-1})		K (cmol.kg^{-1})	
	2008	2009	2008	2009	2008	2009 (0-25 cm	2009 (0-5cm)	2008	2009	2008	2009	2008	2009
Contro1	6.18 b	6.25 b	2528 a	2055 b	1.79	1.81 b	2.35 bc	0.159 c	0.140 bc	136.8	173.8	0.282 c	0.203 b
MeBr	5.88 b	5.78 c	2706 a	2565 a	2.05	1.80 b	2.06 c	0.179 bc	0.129 c	157.6	182.0	0.260 c	0.183 b
Comp+ExtA	6.58 a	6.68 a	1140 b	1063 c	2.00	2.35 a	2.64 ab	0.200 bac	0.184 ab	205.4	271.8	0.970 a	0.663 a
Comp+ExtB	6.60 a	6.68 a	1288 b	1100 c	2.00	2.05 ab	2.67 a	0.236 a	0.161 abc	182.2	202.5	0.964 ba	0.625 a
Comp+Biost	6.74 a	6.93 a	1234 b	1140 c	2.03	2.24 ab	2.69 a	0.220 ba	0.208 a	213.8	173.8	0.808 b	0.620 a
CompBioExtA	6.74 a	6.58 ab	1288 b	1143 c	1.99	2.37 ab	2.64 ab	0.238 a	0.189 ab	222.0	256.3	0.918 ba	0.578 a
P-Value	0.0006	0.0004	<0.0001	<0.0001	0.8204	0.0366	0.0019	0.0158	0.0478	0.2244	0.2638	<0.0001	0.0007

Treatment	Ca (cmol.kg^{-1})		Mg (cmol.kg^{-1})		Cu (cmol.kg^{-1})		Zn (cmol.kg^{-1})		Mn (cmol.kg^{-1})		B (cmol.kg^{-1})		CEC* (cmol.kg^{-1}
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2009
Contro1	6.08 b	7.38 bc	1.08	1.39	1.86	6.81 ab	3.04	21.9	8.04	22.4	0.610 c	0.723 ab	11.04
MeBr	6.22 b	6.24 c	1.20	1.25	1.84	7.72 a	3.06	13.0	16.82	13.1	0.590 c	0.455 b	11.08
Comp+ExtA	8.73 a	10.89 a	1.21	1.50	2.51	2.61 c	4.60	14.2	12.36	16.3	0.836 ab	1.003 a	13.49
Comp+ExtB	8.44 a	9.15 ab	1.20	1.27	2.52	3.63 bc	5.00	16.3	11.70	19.9	0.774 bc	0.833 a	12.26
Comp+Biost	9.36 a	11.55 a	1.25	1.57	2.30	2.80 c	2.62	14.8	9.10	17.2	0.706 bc	0.935 a	12.62
CompBioExtA	9.67 a	10.10 ab	1.37	1.43	2.41	5.28 abc	4.86	20.9	12.16	27.9	1.020 a	0.988 a	11.31
P-Value	0.0020	0.0180	0.5868	0.6351	0.4531	0.0115	0.6694	0.1665	0.4044	0.0597	0.0055	0.0171	0.1038

* CEC = Cation exchange capacity

Table 8. Soil chemical properties from fumigated soil as well as soil amended with biological applications at the Monteith site (sandy clay loam). Soil samples were taken in May 2008 in the top 0-25cm soil layer. Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	pH (KCl)	Resistance (ohm)	N (%)	C (%)	P (BrayII) ($mg.kg^{-1}$)	K ($cmol.kg^{-1}$)	Mg ($cmol.kg^{-1}$)	Ca ($cmol.kg^{-1}$)
Control	6.05	542	0.222	1.99	69.8	0.645	1.820	7.94
MeBr	6.05	922	0.185	1.85	47.0	0.457	1.665	7.99
Comp+ExtractA	6.15	708	0.225	2.37	83.0	0.610	1.847	8.17
Comp+Biostart	6.17	1005	0.204	1.84	85.8	0.615	1.777	7.22
Comp+ExtractB	6.07	847	0.180	1.68	61.7	0.647	1.647	6.77
P value	0.8931	0.1182	0.3489	0.2613	0.1423	0.4354	0.7979	0.5588

Treatment	Cu ($cmol.kg^{-1}$)	Mn ($cmol.kg^{-1}$)	Zn ($cmol.kg^{-1}$)	B ($cmol.kg^{-1}$)	BD* $kg.L^{-1}$	Water holding capacity		
						kPa 10	kPa 100	mm/m
Control	1.89	16.27	13.25 a	0.692	1.162	15.45	9.93	70.85
MeBr	1.54	11.85	7.63 c	0.762	1.182	13.68	8.51	75.90
Comp+ExtractA	2.03	16.02	11.00 abc	0.792	1.130	20.56	13.14	58.15
Comp+Biostart	1.92	14.05	11.82 ab	1.076	1.140	17.99	11.58	65.05
Comp+ExtractB	2.09	13.43	9.26 bc	0.783	1.140	16.12	10.59	71.13
P value	0.7826	0.1033	0.0470	0.2330	0.4127	0.2850	0.2685	0.2190

* BD = Bulk density

Table 9. Leaf nutrient analyses of the various soil treatments from the Graymead site for samples taken in January 2008 and 2009. Results are expressed as % for macronutrients and mg.kg^{-1} DW for micronutrients. Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	N (%)		P (%)		K (%)		Ca (%)		Mg (%)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Control	2.720	2.824 c	0.188	0.160	1.342 c	1.406	0.918 b	1.377 b	0.245 a	0.306 ab
Methyl bromide	2.705	2.847 bc	0.180	0.156	1.827 a	1.319	1.003 b	1.651 a	0.190 b	0.3100 a
Comp+ExtractA	2.695	2.939 ab	0.193	0.153	1.490 b	1.459	1.182 a	1.483 b	0.240 a	0.294 abc
Comp+Biostart	2.825	2.964 a	0.167	0.137	1.908 a	1.417	0.982 b	1.441 b	0.185 b	0.271 c
Comp+ExtractB	2.743	2.940 ab	0.193	0.146	1.807 a	1.437	0.987 b	1.431 b	0.182 b	0.286 bc
P value	0.5102	0.0203	0.3690	0.3256	<0.0001	0.5659	0.0016	0.0252	<0.0001	0.0143

Treatment	Na (mg.kg^{-1})		Mn (mg.kg^{-1})		Cu (mg.kg^{-1})		Zn (mg.kg^{-1})		B (mg.kg^{-1})	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Control	144.7	203.9 a	147.3 b	574.7	17.2 c	7.286	51.0 b	163.8	45.7 b	44.3 a
Methyl bromide	155.0	128.0 c	205.8 a	545.7	23.7 a	7.571	71.2 a	178.7	50.8 a	39.4 b
Comp+ExtractA	143.5	167.0 b	131.5 b	592.7	18.3 bc	6.857	45.7 b	179.1	45.2 b	42.4 a
Comp+Biostart	140.5	168.1 b	187.2 a	642.6	21.8 ab	7.000	64.7 a	192.6	51.3 a	43.1 a
Comp+ExtractB	130.3	162.1 b	200.2 a	615.0	23.7 a	6.857	69.0 a	174.3	50.0 a	41.7 ab
P value	0.4035	0.0004	0.0007	0.1301	0.0107	0.7625	0.0005	0.5043	0.0027	0.0231

Footnotes: Kotzé (2001) norms: N (2.1-2.6%), P (0.14-0.19%), K (1.2-1.4%), Ca (1.45-1.60%), Mg (0.30-0.40%), Na (500 mg.kg^{-1}), Mn (20-90 mg.kg^{-1}), Fe (80-150 mg.kg^{-1}), Cu (5-10 mg.kg^{-1}), Zn (30-50 mg.kg^{-1}), B (30-35 mg.kg^{-1}). Each leaf sample consisted of 50 leaves. Samples were analysed by a commercial laboratory (Bemlab®, Strand, SA) using an inductively coupled plasma-optical emission spectrometer and a nitrogen analyzer.

Table 10. Leaf nutrient analyses of the soil treatments from the Eikenhof site for samples taken in January 2008 and 2009. Results are expressed as % for macronutrients and mg.kg^{-1} DW for micronutrients. Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	N (%)		P (%)		K (%)		Ca (%)		Mg (%)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Control	2.637 b	2.638 c	0.192	0.218	1.477 c	1.368 bc	1.102 ab	1.407	0.310	0.283 a
Methyl bromide	2.800 a	2.630 c	0.190	0.218	1.767 a	1.363 c	1.025 bc	1.413	0.252	0.298 a
Compost+ExtractA	2.497 c	2.777 ab	0.180	0.232	1.477 c	1.608 a	1.062 abc	1.310	0.312	0.255 b
Compost+Biostart	2.702 ab	2.708 bc	0.182	0.222	1.608 b	1.613 a	1.058 abc	1.347	0.275	0.238 bc
Compost+ExtractB	2.707 ab	2.807 a	0.180	0.218	1.843 a	1.555 ab	1.153 a	1.420	0.293	0.223 c
Compost+ Biost+ExtrA	2.708 ab	2.682 bc	0.175	0.232	1.735 a	1.612 a	1.000 c	1.313	0.255	0.247 bc
P value	<0.0001	0.0036	0.8645	0.9942	<0.0001	0.0146	0.0469	0.3599	0.0665	0.0001

Treatment	Na (mg.kg^{-1})		Mn (mg.kg^{-1})		Cu (mg.kg^{-1})		Zn (mg.kg^{-1})		B (mg.kg^{-1})	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Control	96 bc	222.5	370	327.7	79	38.0	133	198.2	46	65.2
Methyl bromide	124 a	233.7	408	305.3	84	39.0	144	180.5	44	64.7
Comostp+ExtractA	96 bc	196.0	366	315.8	70	40.3	130	187.7	45	66.5
Compost+Biostart	96 bc	224.2	345	314.8	76	39.0	119	197.3	45	67.8
Compost+ExtractB	117 a	184.0	372	355.8	76	45.0	127	215.5	46	64.5
Compost+Biost+ExtrA	94 c	201.8	363	328.7	82	42.3	1125	191.3	44	65.5
P value	<0.0001	0.1722	0.4354	0.3278	0.3348	0.0879	0.4227	0.4009	0.7243	0.7750

Footnotes: Kotzé (2001) norms: N (2.1-2.6%), P (0.14-0.19%), K (1.2-1.4%), Ca (1.45-1.60%), Mg (0.30-0.40%), Na (500 mg.kg^{-1}), Mn (20-90 mg.kg^{-1}), Fe (80-150 mg.kg^{-1}), Cu (5-10 mg.kg^{-1}), Zn (30-50 mg.kg^{-1}), B (30-35 mg.kg^{-1}). Each leaf sample consisted of 50 leaves. Samples were analysed by a commercial laboratory (Bemlab®, Strand, SA) using an inductively coupled plasma-optical emission spectrometer and a nitrogen analyzer.

Table 11. Leaf nutrient analyses of the soil treatments from the Monteith site for samples taken in January 2008 and 2009. Results are expressed as % for macronutrients and mg.kg^{-1} DW for micronutrients. Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Treatment	N (%)		P (%)		K (%)		Ca (%)		Mg (%)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Control	2.782	2.517	0.196	0.150	1.802	1.728 a	0.908	0.923	0.298 a	0.278 ab
Methyl bromide	2.633	2.583	0.215	0.138	1.660	1.505 b	0.890	0.867	0.298 a	0.292 a
Compost+ExtractA	2.742	2.453	0.208	0.165	1.793	1.727 a	0.904	0.840	0.273 ab	0.263 b
Compost+Biostart	2.705	2.605	0.223	0.153	1.858	1.598 b	0.895	0.793	0.287 a	0.258 b
Compost+ExtractB	2.653	2.323	0.195	0.155	1.838	1.723 a	0.873	0.883	0.243 b	0.265 b
P value	0.4994	0.1034	0.4739	0.3711	0.1653	0.0198	0.7960	0.2565	0.0141	0.0353

Treatment	Na (mg.kg^{-1})		Mn (mg.kg^{-1})		Cu (mg.kg^{-1})		Zn (mg.kg^{-1})		B (mg.kg^{-1})	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Control	187	239.0	90	165.8	15.6	82.7	18.0 c	142.7	34.0	32.5 a
Methyl bromide	194	235.7	153	168.5	17.3	86.8	29.7 a	146.7	33.8	31.2 ab
Compost+ExtractA	176	264.2	128	168.7	20.2	83.0	23.3 bc	153.0	34.3	33.2 a
Compost+Biostart	180	262.2	135	153.2	22.2	82.0	24.0 b	140.0	34.3	29.3 b
Compost+ExtractB	147	196.3	134	163.3	22.3	89.3	25.0 ab	149.8	32.3	31.8 a
P value	0.6970	0.6138	0.0787	0.6274	0.5010	0.8400	0.0078	0.5941	0.9358	0.0241

Footnotes: Kotzé (2001) norms: N (2.1-2.6%), P (0.14-0.19%), K (1.2-1.4%), Ca (1.45-1.60%), Mg (0.30-0.40%), Na (500 mg.kg^{-1}), Mn (20-90 mg.kg^{-1}), Fe (80-150 mg.kg^{-1}), Cu (5-10 mg.kg^{-1}), Zn (30-50 mg.kg^{-1}), B (30-35 mg.kg^{-1}). Each leaf sample consisted of 50 leaves. Samples were analysed by a commercial laboratory (Bemlab®, Strand, SA) using an inductively coupled plasma-optical emission spectrometer and a nitrogen analyzer.

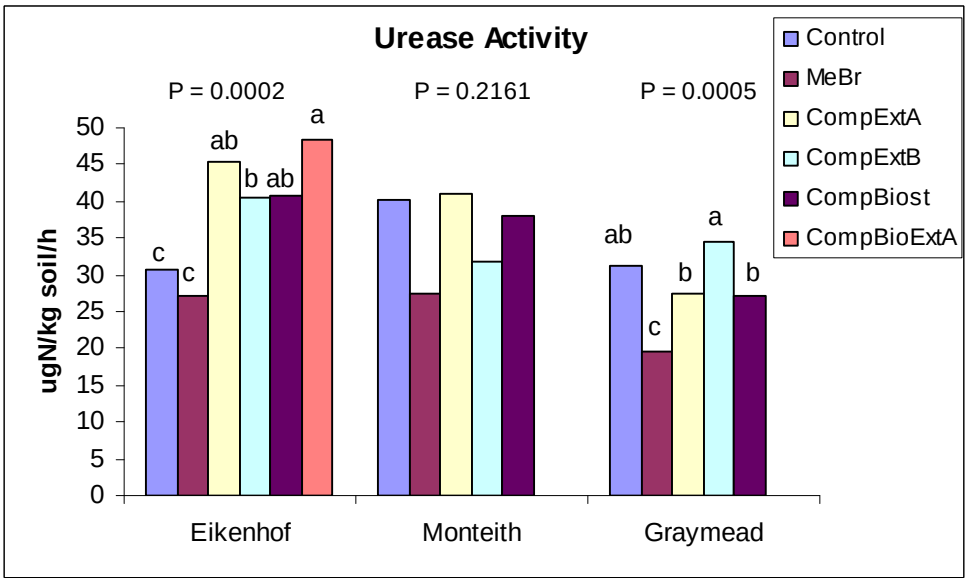


Figure 1. Effect of biological soil amendments and methyl bromide fumigation on soil urease activity at three replant disease sites, Eikenhof, Monteith and Graymead. Soil samples were taken in May 2008. Probability values from the standard ANOVA are shown at the top of each replant site's graph. Bars within replant sites topped by the same or no letter are not significantly different according to Student's t-LSD at a 5 % significance level.

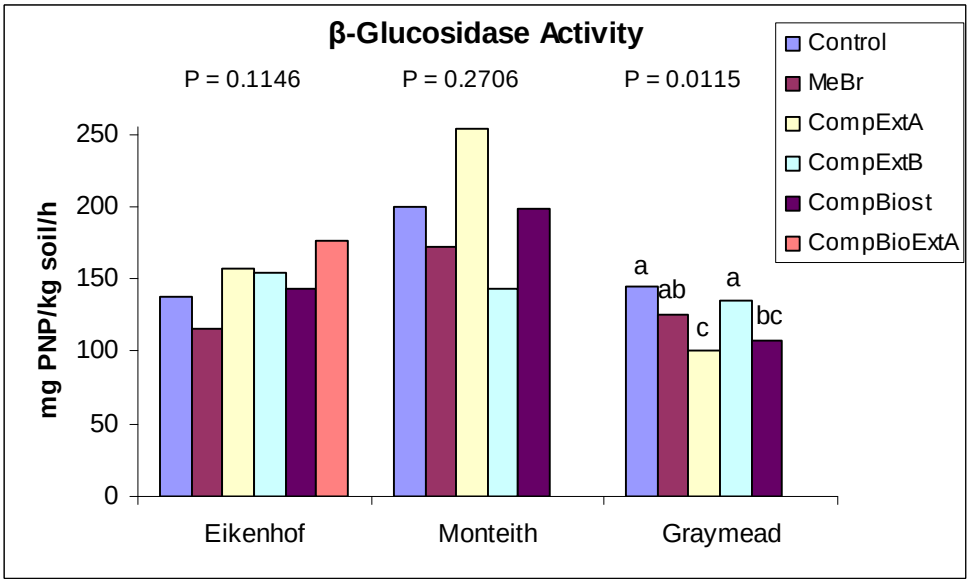


Figure 2. Effect of biological soil amendments and methyl bromide fumigation on soil β-glucosidase activity at three replant disease sites Eikenhof, Monteith and Graymead. Soil samples were taken in May 2008. Probability values from the standard ANOVA are shown at the top of each replant site's graph. Bars within replant sites topped by the same or no letter are not significantly different according to Student's t-LSD at a 5 % significance level.

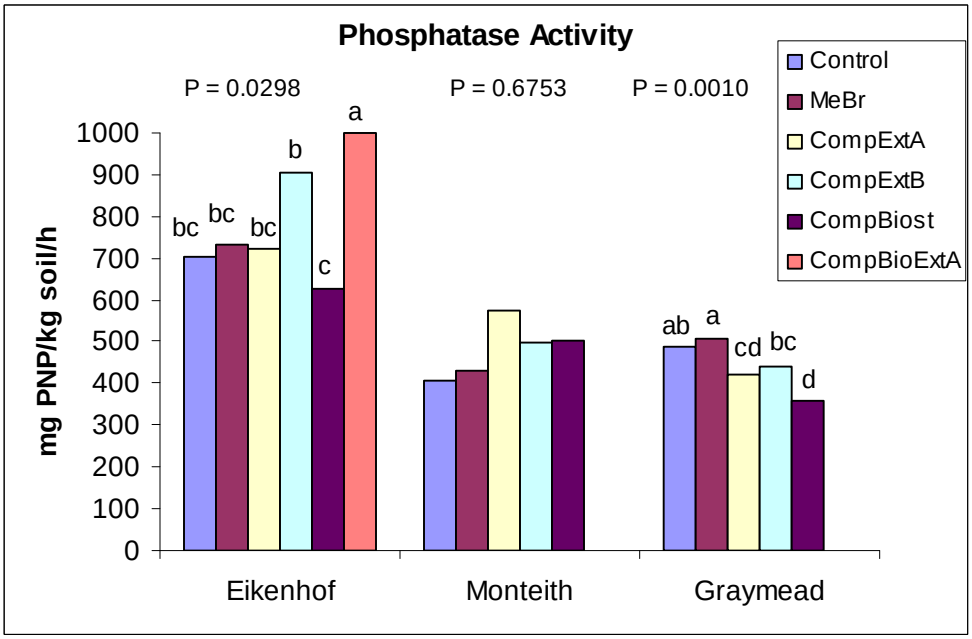
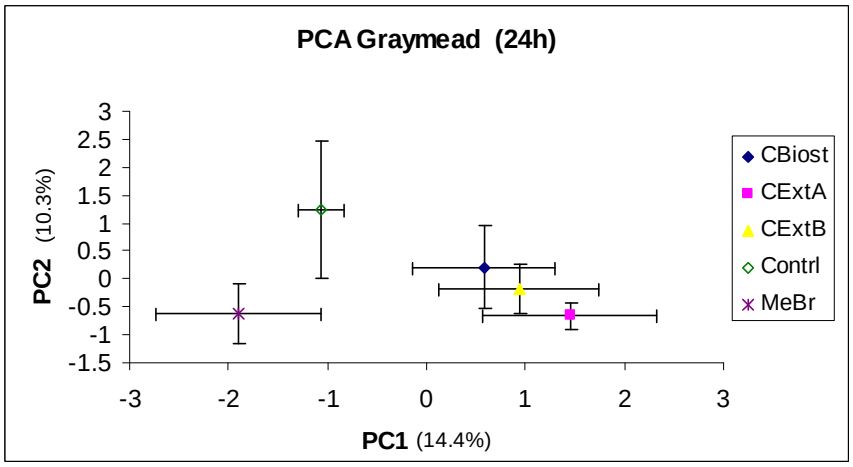


Figure 3. Effect of biological soil amendments and methyl bromide fumigation on soil phosphatase activity at three replant disease sites Eikenhof, Monteith and Graymead. Soil samples were taken in May 2008. Probability values from the standard ANOVA are shown at the top of each replant site's graph. Bars within replant sites topped by the same or no letter are not significantly different according to Student's t-LSD at a 5 % significance level.



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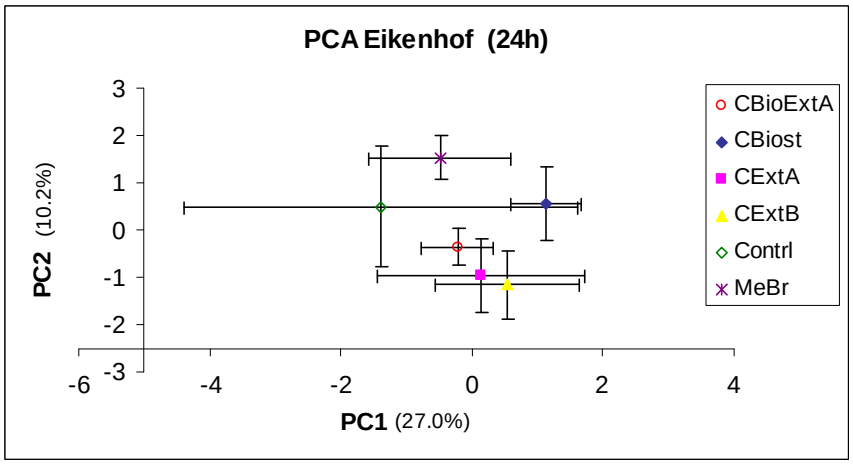

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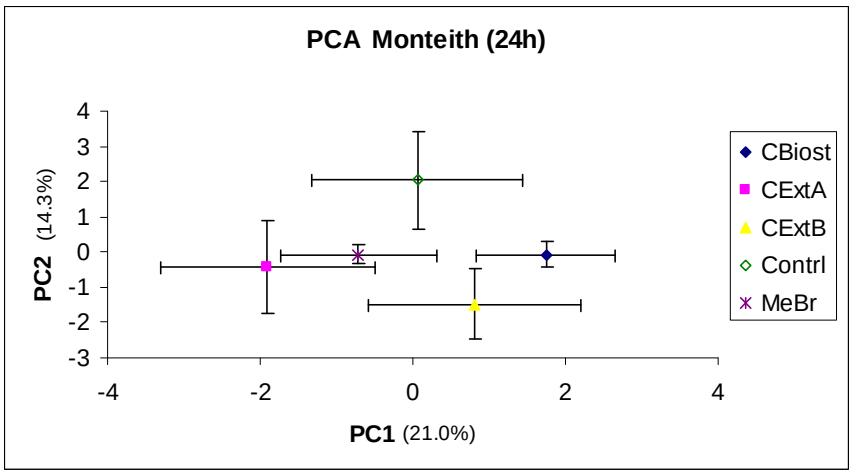

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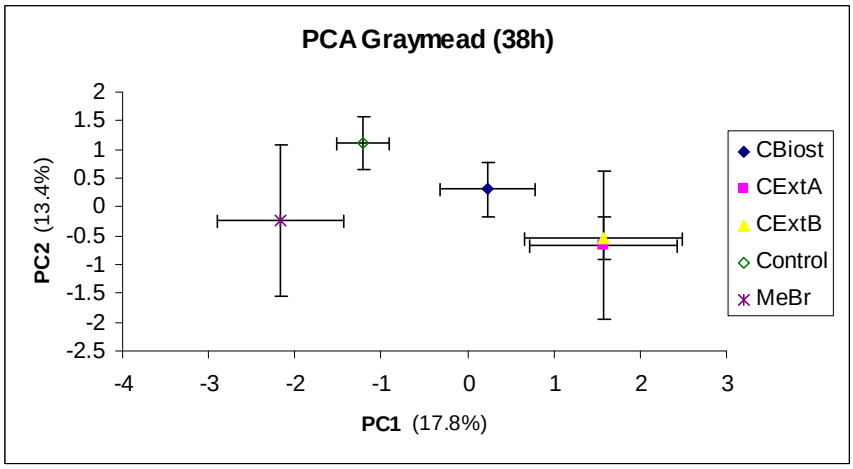
Figures 4A-C. Ordination plots of principal components (PCs) 1 and 2 from community level physiological profiles of soil treated with biological amendments and methyl bromide. Principal component analysis was conducted on 24 h incubation data from Biolog EcoPlates for samples taken in autumn 2008 from three orchard sites, (A) Graymead, (B) Eikenhof, (C) Monteith. CBiost: compost with Biostart, CExtA: compost with ExtractA, CExtB: compost with ExtractB, MeBr: methyl bromide fumigation. Error bars represent ±1 standard error of the mean. Values in brackets indicate the percent of total variation accounted for by each principal component axis.



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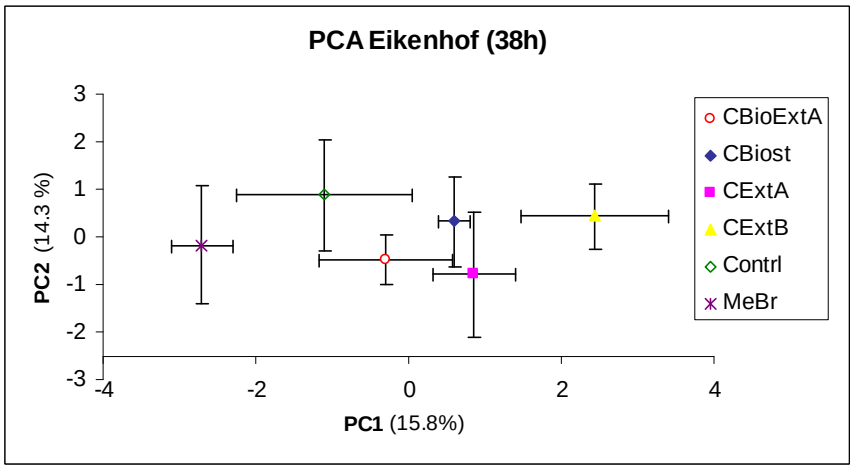

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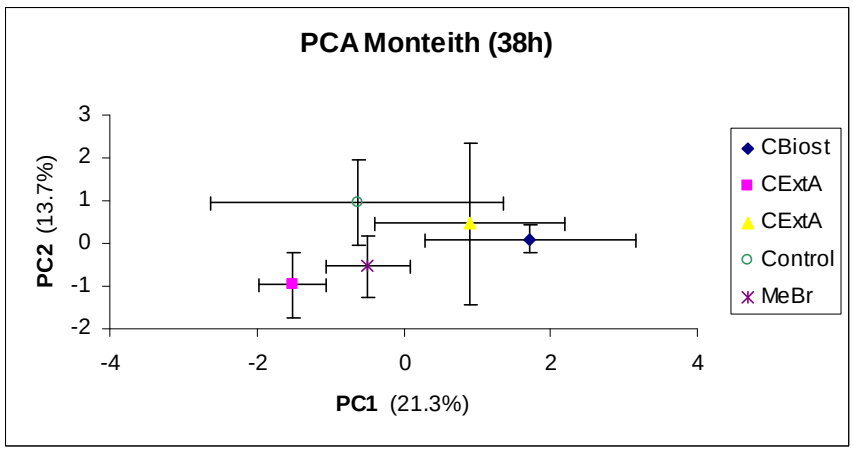

C

Figures 5A-C. Ordination plots of principal components (PCs) 1 and 2 from community level physiological profiles of soil treated with biological amendments and methyl bromide. Principal component analysis was conducted on 38 h incubation data from Biolog EcoPlates for samples taken in autumn 2008 from three orchard sites, (A) Graymead, (B) Eikenhof, (C) Monteith. CBiost: compost with Biostart, CExtA: compost with ExtractA, CExtB: compost with ExtractB, MeBr: methyl bromide fumigation. Error bars represent ±1 standard error of the mean. Values in brackets indicate the percent of total variation accounted for by each principal component axis.

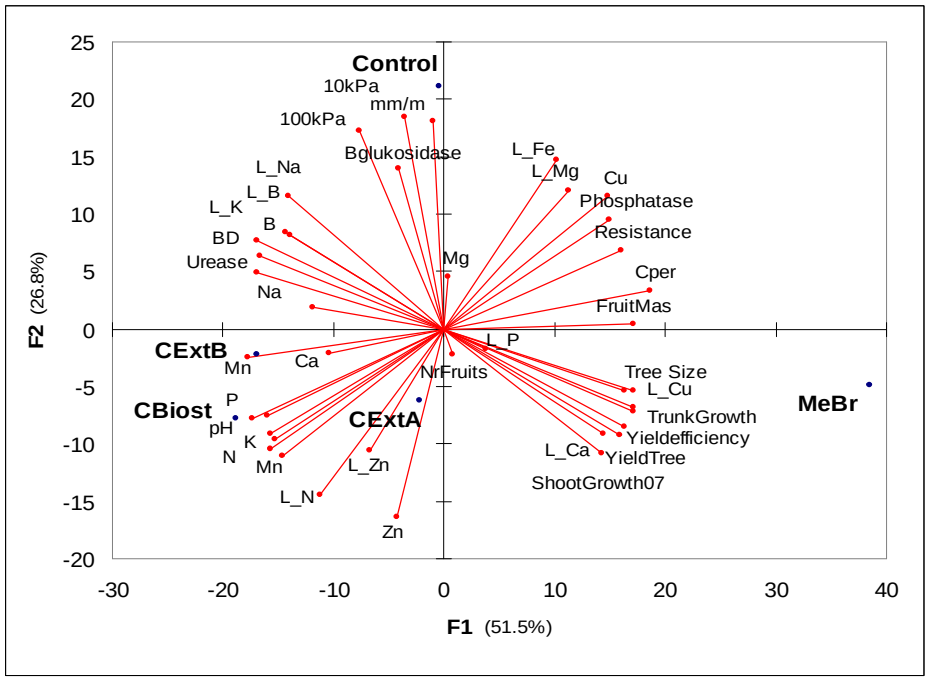

Figure 6. Principal component analysis (PCA) Bi-plot of the different variables (chemical and biological soil properties, tree performance parameters)measured at the Graymead site in relation to the various soil treatments. Values in brackets indicate the percent of total variation accounted for by each principal component axis. CBiost: compost with Biostart, CExtA: compost with ExtractA, CExtB: compost with ExtractB, MeBr: methyl bromide fumigation. L_ indicates leaf nutrient content.

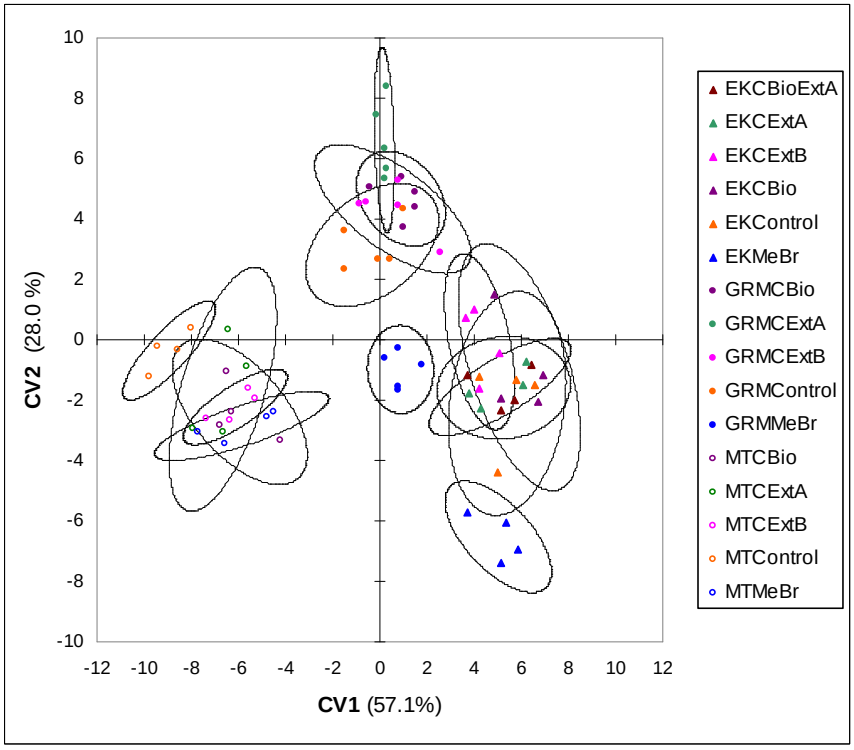

Figure 7. Plots of the first two canonical variables (CVs) from a canonical discriminant analysis (CDA), showing separation of the various sites and soil treatments. Values in brackets indicate the percent of total dispersion explained by each CV. EK: Eikenhof, GRM: Graymead, MT: Monteith, CBio: compost with Biostart, CExtA: compost with ExtractA, CExtB: compost with ExtractB, MeBr: methyl bromide fumigation.

CHAPTER 5

EFFECT OF ORGANIC MATERIAL AND BIOLOGICAL AMENDMENTS ON SOIL ENZYME ACTIVITY AND TREE PERFORMANCE IN AN OPTIMALLY MANAGED APPLE ORCHARD

ABSTRACT

Organic material (compost, wood chips) and biological amendments (compost extract, humates) were applied in an optimally managed high density orchard, to determine possible complimentary effects on tree performance with use of these biological management practices in a controlled fertigation system. It was also investigated whether the concentration of nitrogen (N) in the nutrient solution can be reduced with the use of organic mulches and biological amendment application without affecting tree performance negatively. The study site was a commercial 'Brookfield Gala' apple orchard in second leaf, planted on M793 rootstock at 2000 trees.ha^{-1} in the Greyton region (34° 03' S; 019° 37' E) Western Cape Province, South Africa. The trial consisted of three N levels (main treatments) and nine biological orchard practices (sub treatments). Organic material was applied with trial establishment, biological amendments from third leaf and N levels adjusted after the first harvest. Changes in selected soil chemical properties, as well as leaf nutrient content were measured at the end of the four year trial period. Furthermore, the effect of biological management practices on soil enzyme activity was determined as an indicator of soil quality. Results showed a significant interaction between biological amendments and the inorganic N levels applied. With continued application of the biological amendments, humate treatment in combination with compost application significantly increased yield when applied with the full N application (N100). Furthermore, a significant increase in yield was also found with compost extract applied in combination with compost at the lowest N application. Application of the N85 regime possibly resulted in the best balance between root growth, shoot growth, bud quality and fruit set as indicated by showing highest yield in 2008, as well as cumulative yield. Compost treatments showed positive effects on soil extractable nutrients. Effects on enzyme activity were not significant, although some tendencies were noted. The significant interactions found between inorganic and organic applied nutrients need further investigation.

Keywords: *compost, compost extract, fertigation, humates, mulch, nitrogen, wood chips*

5.1 INTRODUCTION

In response to environmental concerns and escalations in production costs there has been renewed interest in the integration of biological soil amendments into standard agricultural management systems. The application of organic material and biological amendments also need to be investigated in combination with the more intensive cultivation practices. In high density orchards the controlled application of fertiliser directly through the irrigation system (fertigation) is becoming increasingly popular. Fertigation offers potential to synchronise nutrient application with plant requirements, as influenced by plant age, physiological stage and environmental conditions (Stassen et al., 1999). Lebese (2008) found a 80% increase in the dry weight of fine and medium roots and a 26% increase in yield of apple trees using a daily drip fertigation system compared to a conventional micro-irrigation system. However, Neilsen et al. (1999) stated that fertigation through drip systems concentrates root development in smaller soil volumes, increasing tree reliance on applied nutrients and requiring optimum soil quality in the root zone. Application of organic and biological amendments could possibly aid in improving soil conditions in the root zone of fertigated trees.

The importance of soil biological processes in maintaining plant health and yield has largely been neglected in agricultural systems. Since microbial activity is generally carbon-limited in agricultural soil (Campbell, 1989; Magarey, 1999; Bünemann et al., 2006) it is widely accepted that management practices providing a range of organic compounds on a regular basis, will tend to maintain an active and diverse microbial population (Kennedy and Gewin, 1997; Magdoff and Weil, 2004). The development of soil structure (Tisdall and Oades, 1982; Gupta and Germida, 1988; Beare, 1997; ; Wright and Upadhyaya, 1998; Miller and Jastrow, 2000), soil fertility and plant nutrition, (Jenkinson and Ladd, 1981; Glick, 1995; Jeffries et al., 2003; Zahir et al., 2004), as well as disease suppression (Baker and Cook, 1974; Bowen and Rovira, 1999; Whipps, 2001) are regulated by the interactions of a highly diverse and complex web of soil flora and fauna that is sustained by the influx of organic matter into the soil (Alexander, 1977; Larson and Pierce, 1991; Tisdall, 1996; Murphy et al., 2003; Davet, 2004; Magdoff and Weil, 2004).

Improvement of tree performance in deciduous fruit was found after organic amendment, either in the form of mulch application or incorporated into the top soil (Hogue and Neilsen, 1987; Autio et al., 1991; Kotzé and Joubert, 1992b; Reganold et al., 1993; Marsh et al., 1996; Pinamonti, 1998; Neilsen et al, 2003a, 2007; Van Schoor et al., 2009). Mulching conditions provide a favourable environment for microbial activity and fine feeder root development, especially in surface soil and can reduce leaching, and improve nutrient uptake (Boynton and Oberly, 1966; Kotzé and Joubert, 1992a; Pinamonti, 1998). Furthermore, if in addition to organic amendments, nutrients can be applied regularly through fertigation (Neilsen et al., 2007), competition for nutrients between the plant and microorganisms can be minimised (Hogue and Neilsen, 1987; Lipecki and Berbec, 1997). Drinkwater et al. (1995) concluded that differences between agroecosystems with and without

organic matter input suggested that biological processes can compensate for reductions in the use of synthetic fertilisers through enhanced nutrient cycling.

The addition of biostimulants, such as humic substances (HS), is widely advocated in biological management systems (Chen and Aviad, 1990). Humic substances comprise a major part of soil organic matter (SOM) and are classified into humic acids (HA), fulvic acids (FA) and humin on the basis of their solubility in water as a function of pH (Swift, 1999). Commercial HS products are mostly derived from brown coal (leonordite or lignite), or peat and applied as humates, which are the salts of humic acids that hold ions, such as K or Na. Their most important role is the chelation of ions, increasing their availability to organisms, including plants. Humic substances can also directly stimulate plant biomass production, especially root growth (Vaughan and Malcolm, 1985; Visser, 1985a, Chen and Aviad, 1990; Nardi et al., 2002). The stimulatory effect of HS on plant nutrition and growth might, at least in part, be explained on the basis of both a direct action of low molecular weight (LMW) humic molecules on plasma membrane H^+-ATPase activity (Varanini et al., 1993; Canellas et al., 2002) and specific modification of cell membrane permeability (Vaughan et al., 1985). Furthermore, various studies have shown positive effects of HS on soil microbial activity and function (Visser, 1985 a, b; Vallini et al., 1993; Valdrighi et al., 1996; Lizarazo et al., 2005).

Application of soil inoculants was shown to improve plant health and uptake of nutrients (Glick, 1995; Zahir and Arshad, 1996; Rodriguez and Fraga, 1999; Zahir et al., 2004), as well as increase yield and crop quality (Kloepper, 1994; Dobbelaere et al., 2001; Esitken et al., 2003; Orhan et al., 2006; Aslantas et al., 2007). Furthermore, the use of compost extracts or compost tea, have been advocated as microbial inoculants that can stimulate and enhance the soil microflora (Ingham, 1999; Litterick et al., 2004). These inoculants are used locally in organic agriculture to a wide extent and since virtually no scientific literature is available on their use in deciduous fruit production it is important to establish their value in terms of improving tree performance.

Biological amendments can affect plant nutrition directly by supplying bulk nutrients (Ashworth and Harrison, 1983; Roe, 1998; Neilsen et al., 2003a) or indirectly through increasing the availability and uptake of nutrients (Jenkinson and Ladd, 1981; Chen and Aviad, 1990; Glick, 1995; Ferris et al., 1998) or preventing leaching (McKenzie et al., 2001; Davet, 2004; Ball, 2006). Microbial activity in the rhizosphere is a major factor that determines the availability of nutrients to plants and has a significant influence on plant health and productivity (Jeffries et al., 2003). It is therefore possible that the application of inorganic fertilizer can be reduced when applied in combination with these amendments.

The objective of this study was to determine whether the integration of biological amendments into a optimally managed drip fertigation system can lead to complimentary improvement in tree performance. It was also investigated whether the concentration of nitrogen (N) in the nutrient solution can be reduced with the use of

organic mulches and biological amendment application without affecting tree performance negatively. Furthermore, the effect of these amendments on soil enzyme activity was determined as an indicator of soil quality.

5.2 MATERIALS AND METHODS

5.2.1 Orchard study site and experimental design

The experiment was conducted in a young commercial 'Brookfield Gala' apple orchard on M793 rootstock. The orchard was planted in 2003 at a spacing of 4.0 m x 1.25 m (within row) on a virgin loamy sand soil (clay 3%, silt, 11%, sand 86%), in the Greyton region (34° 03' S; 019° 37' E) in the Western Cape Province, South Africa. Trees were trained to a central leader spindle with lateral shoots bent horizontally according to the solaxe principle, and implementing both summer and winter pruning. With trial establishment in October 2004, pH (KCl) values averaged 6.6, total soil carbon 1.2% and the soil contained 0% stone. The experimental layout was a split-plot design consisting of three main treatments and nine sub treatments, blocked three times, with an experimental unit consisting of three trees. Plots were separated by two guard trees. A drip fertigation system was used to supply a nutrient solution to the trees once or twice daily. Drippers were spaced 600 mm apart and had a discharge rate of 2.3 $L.h^{-1}$. Annual macro- and micronutrient requirements were based on a local study by Stassen and North (2005), and divided into five phenological periods. Water requirements were calculated using long term evaporation data from two nearby weather stations and locally developed apple crop factors (Kotzé, 2001). Watermark sensors and C-probes were used to adapt the predicted water requirements into actual water requirements according to plant available soil water. A total amount of water between 3800 and 4000 $m^3.ha^{-1}$ was applied for the various seasons. A glyphosate herbicide (3-4 $L.ha^1$) was applied in autumn and a paraquat (3-5 $L.ha^1$) containing herbicide in spring to all treatments.

5.2.2 Treatment application

The trial was established in October 2004, when trees were going into third leaf. Main treatments consisted of three various nitrogen (N) regimes; 70%, 85% and 100% of the standard N in the nutrient solution applied. Application of the various N levels only commenced in February 2006. Subplot treatments consisted of organic material application, commencing in October 2004. Soil inoculants and biostimulants were applied from the 2005/2006 season. The subplot treatments included the following combinations:

1) Untreated control plots, managed according to the standard orchard practices used at this site.

2) Compost extract (BioEarth, Stellenbosch, SA) applied at 500 $L.ha^{-1}$, diluted 50:1 and sprayed onto the tree row with each application. The compost extract was prepared by adding 1000 L of water to 50 kg of compost and actively aerating the suspension for 48 h, with no additional additives. Compost extracts were applied monthly throughout the growing season in all treatments.

3) Compost extract in combination with commercial compost applied at 30 ton.ha^{-1} as a mulch. Compost application was repeated annually in spring. The same commercial compost was used as CompostA in Chapter 3.

4) Compost extract applied in combination with a 10 cm thick pine wood chip mulch. Wood chips were applied with trial establishment in 2004 and again in spring 2006.

5) A potassium humate product was applied anually, at 75 L.ha^{-1} split into a spring and an autumn application.

6) Humate application as in treatment 5, in combination with compost applied annually at 30 ton.ha^{-1} as a mulch.

7) Humate application as in treatment 5, in combination with pine wood chip mulch of 10 cm thick.

8) Compost extract in combination with the humate product, as well as commercial compost applied at 30 ton.ha^{-1} as a mulch (CE+H+C).

9) Compost extract in combination with the humate product, as well as a wood chip mulch (CE+H+W).

Properties of the compost and compost extracts used are shown in Appendix A.

5.2.3 Tree performance evaluation

Trees were permanently marked 20 cm above the graft union and trunk circumference measured with trial establishment in 2004 and from then on every year during winter. Pruning mass was recorded as a further indication of tree vigour in 2006. Annual yield was recorded on a per tree basis in February 2006, 2007 and 2008. Yield efficiency was calculated by dividing yield (kg.tree^{-1}) by trunk cross-sectional area as measured with harvest. Fruit quality parameters included fruit firmness, total soluble solids (TSS), total titratable acids (TTA), starch conversion and fruit colour. Due to economic reasons, these parameters were only evaluated for selected treatments at harvest, after 12 weeks storage at -0.5 ºC under regular atmosphere (RA), and then after 7 days at room temperature (21-24 ºC) (shelf life period) in 2006 and 2008. For each evaluation 35 fruit from each treatment and block combination were analysed.

5.2.4 Leaf nutrient analyses

Leaf nutrient analyses were conducted for control treatments, where compost was applied in the treatment combination, as well as humates and compost extract applied on its own. This was due to economic constraints. A combined 50 leaf sample of mature leaves in the mid shoot section of the current years growth was collected at the end of January 2008 from the three trees in each plot. Leaf samples were prepared and analysed as described in Chapter 2.

5.2.5 Soil sampling and analyses

Control treatments, as well as treatments with biological amendments showing the most potential based on yield parameters in 2008 were sampled. Soil was sampled within the root zone of the top soil where microbial activity is expected to be greatest, at a depth of 0-25 cm. Samples were taken at a distance of 30-40 cm from

the tree base, between the two drippers, from two holes beneath the three trees in each plot and composite samples prepared for each treatment from the six sub-samples in each of the three block replicates. Soil samples were taken in May 2008 one month after the last application of the season. Samples were analysed for selected soil chemical properties using the methods described in Chapter 2. Field moist sub-samples were sieved through a 2 mm mesh screen for microbial analyses. Visible root pieces and un-decomposed organic matter were removed and soil stored at 4 ºC for no more than two weeks before analyses.

5.2.6 Soil enzyme activity analyses

Acid phosphatase, β-glucosidase and arylsulfatase activity were determined based on the release and spectrophotometric detection of *p*-nitrophenol (Tabatabai and Bremner, 1969; Tabatabai, 1982). Urease hydrolysing activity was determined by the non-buffered method of Kandeler and Gerber (1988). Controls were performed for all enzymes assayed by the addition of the substrate after incubation, but prior to analysis of the reaction product.

5.2.7 Statistical analysis

A standard split-plot analysis of variance (ANOVA) was performed on the data using, SAS Statistical Software (SAS, 2002-2003). Trunk circumference measurements over the trial period were analysed as repeated measurements by comparing the slopes (b values) of linear regressions fitted to the data (R^2 between 0.98 and 0.99) in an ANOVA. Student's t-LSD (least significant difference) was calculated at a 5% significance level to compare the treatment means.

5.3 RESULTS

5.3.1 Growth measurements

Trunk circumference measurement at the start of the trial period showed that all trees were of similar size. There was no significant interaction between the different rates of N application and the various biological treatments for trunk circumference growth tempo over the trial period, or pruning mass measured in 2006 (Table 1). Furthermore, biological amendments and various levels of N application did not result in a significant increase in trunk growth tempo, although compost extract with wood chips and humates with compost showed the highest growth tempo over the trial period. Pruning mass was measured in the 2006 season in September, as an indication of tree vigour and although there were no significant differences between the various treatments, some tendencies were noted. Highest shoot mass was found for trees treated with the CE+H+C, as well as trees only treated with humates and compost (Table 1).

5.3.2 Yield and fruit quality

The first yield was recorded in February 2006, after two seasons of organic material application and only one season of compost extract and humate application. Furthermore, application of the various N levels only commenced in February 2006 after harvest. Therefore no significant differences in yield were expected between trees in the various N regimes in the 2006 yield. There were no significant differences in yield between trees treated with organic material and biological amendments (Table 2). However, yield and yield efficiency was highest with the humate and compost treatment. In the 2007 season yield was very low. This was due to abnormal cold and wet weather conditions experienced in this area during the fruit set period. Furthermore, there was considerable variation between trees of the same plot and no significant treatment effects were found.

There was a significant interaction between the N levels and the biological treatments for yield in 2008, as well as cumulative yield (Table 2). Compost extract applied with compost increased yield significantly under N70 fertigation compared to all treatments except CE+H+W. Yield was also significantly higher compared to compost applied with compost extract under N100 fertigation. Yield was generally highest at the N85 level for all treatments, although results were not always significant. Application of CE+H+C at N85 significantly increased yield compared to applications at N100. Results were similar for compost extract applied in addition to wood chips. Furthermore, there was no significant effect of the biological amendments compared to the control under N85 fertigation. With the N100 application humates with compost resulted in significantly higher yields than the control, compost extract with wood, as well as the combinations CE+H+C and CE+H+W. Humates applied with wood chips, as well as humates applied on its own, at the N70 level showed significantly lower yield compared to application in the N85 level. In contrast to this, CE+H+W applied at the N100 level, showed significantly lower yield compared to the lower N levels. Results for cumulative yield were similar to yield effects in 2008. However, CE+H+C, compost extract with wood and compost extract with compost, showed no significant differences between the N levels although trends were similar compared to yield in 2008. Yield efficiency in 2008 showed no significant treatment effect, but there was a clear tendency that yield efficiency was highest for treatments with compost and also compost extract applied on its own (Table 2).

Fruit quality data for 2006 are presented in Table 3 only for sub treatments, since the different N levels were only introduced after the harvest of 2006. Only selected treatments were sampled due to economic constraints. No significant treatment differences were found in fruit size and TSS levels for any of the evaluations. In general, differences in fruit quality were most significant for the evaluation at harvest. Based on fruit firmness and starch conversion, there was an indication that treatments receiving compost was more mature at harvest than control fruit. Fruit firmness was significantly lower with compost applications when compared to the control, as well as humate with wood chips. Starch breakdown was significantly lower in fruit from control plots compared to all other treatments and where compost was applied starch breakdown was highest.

However, this effect was not consistent for TTA measured at harvest. Treatments receiving compost extract in combination with either wood chips or compost, as well as humate with wood chips, had significantly higher acidity (TTA) levels compared to the control. At harvest, no significant differences in colour were found between the treatments. There were no significant differences between the treatments for any of the parameters measured after storage evaluations.

In 2008, there was no significant interaction between the main and sub treatments (Table 4). Furthermore, no significant differences were found for the biological treatments or between the three nitrogen regimes at any of the evaluation times (Table 4). However, it was observed that fruit size was bigger with the two treatment combinations including both humates and compost. Results found in 2008 were not consistent with results found for compost application at harvest in 2006.

5.3.3 Soil enzyme activity analyses

Treatments that showed a favourable effect on yield in the 2008 season were selected for enzyme analyses in order to relate increased tree performance with microbial activity. There were no significant interaction between the different N regimes and biological amendments (Table 5) and only main effects will be discussed. No significant differences were found between the treatments for urease, phosphatase, β-glucosidase or arylsulfatase activity. This may be attributed to a small number of replicates when evaluating only selected biological treatments. In general, differences between the N100 and N85 applications were more pronounced than for the various biological amendments. Application of the full N level consistently showed higher enzyme activity for all four enzyme analyses. The combination of CE+H+C generally resulted in highest enzyme activity, except for β-glucosidase activity which was highest with humate and compost application when applied without the extract. It was also noted that phosphatase activity was highest for soil treated with compost. Humate application on its own showed lowest soil enzyme activity for all enzyme assays.

5.3.4 Soil chemical properties and leaf nutrient analyses

Soil chemical parameters measured in May 2008 for the top 0-25 cm soil are presented in Table 6. The same treatments sampled for enzyme activity analyses were also analysed for soil chemical properties. Application of the lower N level (N85) showed significantly lower total soil N, as would be expected, but no other significant differences were found between soil properties of the two N levels sampled. There was a significant interaction between main and sub treatments for C%, as well as soil extractable P (Table 6). At the N100 level, although there was no significant treatment differences compared to the control, CE+H+C treatment resulted in highest C% and results were significant compared to compost with humate treatment. At the N85 level, treatment differences were also not significant compared to the control, but compost with humate treatment and compost with extract treatment showed highest C% and results were significant compared to CE+H+C and compost extract applied on its own. This was in contrast to results found with N100. Soil C% in the N100

system was also significantly higher with compost extract application on its own, compared to its application in the N85 system. The interaction for soil P showed that extractable P levels were significantly higher with the CE+H+C combination when applied with the full N level (N100), than when applied with the lower N level (N85). Furthermore, this treatment resulted in highest soil P under N100 fertigation, compared to lowest soil P under N85 application. In the N100 system, compost extract applied on its own, humate applied with compost, as well as the combination of these treatments (CE+H+C) significantly increased soil extractable P compared to the control, and for the treatments including humates also compared to humates applied on its own. In the N85 system, compost application significantly increased soil P, except for the CE+H+C combination. However, soil extractable P was high in general, including soil from the control plots.

Compost application had the most significant effect on soil chemical properties for the main effects (Table 6). Soil pH, K, as well as total soil N% and C% were highest with compost application, while soil resistance was lowest. However, differences were not always significant compared to the control. Soil pH with compost extract treatment on its own was significantly lower than both treatments where compost extract was applied in combination with compost. The same results were found with humate application on its own. Soil extractable K was significantly higher for all compost treatments compared to the control, as well as treatments not receiving compost. No significant effects were found for Soil N%, Ca, Mg, Zn, or Cu, although similar trends were found with application of compost, with the exception of soil Cu. Soil extractable B was also higher with compost application, but results were only significant compared to humate applied on its own.

Few significant differences were found in leaf nutrient content between the different treatments in 2008 (Table 7). Few significant differences were also found between leaf nutrient content of trees subjected to the different N levels, although results did show a slight decrease in leaf N content with lower levels of N application. Leaf Cu concentrations were significantly higher for the N100 and N85 application, compared to the lowest N application. There was a significant interaction between N applications and the sub treatments for Leaf P content. At N100 level, compost extract application significantly increased leaf P compared to all other treatments. With both the lower N applications, leaf P levels in control trees were highest and significantly higher than all treatments except when compost extract was applied on its own, or in combination with compost only. Leaf K content was significantly higher compared to untreated controls with application of all biological treatments, except compost extract applied on its own. Leaf Mg content was significantly lower with application of compost extract on its own compared to the control, humate applied on its own, and CE+H+C. There were no differences in leaf micronutrient content between the biological amendment treatments (Table 7).

5.4 DISCUSSION

5.4.1 Effect of organic material, biological amendments and various N levels on tree performance

Results from the study showed limited significant effects on tree performance with initial application of organic material and biological amendments in a fertigated apple orchard. Results were more favourable in the 2008 season (five year old trees) with continued application of the biological amendments and there was a significant interaction between the sub and main treatments for yield. There were no significant treatment differences with the N85 levels and yield was generally in the higher range for all treatments. The application of 85% of the standard N concentration showed the most positive effect on cumulative yield over three seasons, as well as yield efficiency in the 2008 season. Application of the N85 regime possibly resulted in the best balance between root growth, shoot growth, bud quality and fruit set, thereby showing optimal yield effects.

The effect of humate with compost was significant at the full N application, increasing cumulative yield from 80 ton.ha^{-1} for the control, to 108 ton.ha^{-1}. However, at the lower N levels this treatment did not significantly increase yield. The effects of humate application in fruit production have received relatively little attention, although some positive effects on yield have been found in citrus (Webb and Bings, 1988) and grapevine (Reynolds et al., 1995; Zachariakis et al., 2001) with the application of commercial humates. In previous studies on pome fruit (Chapters 2, 3 and 4) high concentrations of applied HS products negatively influenced tree performance (Chapter 3). However, low dosages applied in combination with a *Bacillus* inoculant, was suggested to be an important factor in positively affecting tree performance (Chapter 2 and 3). Furthermore, application of *Bacillus* inoculant, low dosage HS and compost, significantly improved tree growth in apple replant disease orchards (Chapter 4). A significant increase in cumulative yield from 84 ton.ha^{-1} in the control to 109 ton.ha^{-1}, was also found with compost extract applied in combination with compost at the lowest N application, but not the higher N levels. This is in agreement with positive effects found with compost and compost extract application in previous studies (Chapter 2, 3 and 4). Furthermore, trees treated with compost showed higher yield efficiency in the 2008 harvest season.

In 2008, with the higher N applications, humates with wood chips, as well as humates applied on its own, showed a more positive effect on yield than with N70 application. In material with a high C:N ratio, such as wood chips, N can be temporarily immobilised in microbial biomass which can create N-deficient conditions at critical stages in plant development. The higher levels of N fertiliser in combination with the wood chips possibly compensated for N-immobilisation (Hogue and Neilsen, 1987; Geiger et al., 1992; Lipecki and Berbec, 1997).

No consistent treatment effects were found on fruit quality and no negative effects on fruit colour or firmness were observed with the full N application (N100). Additional K uptake in compost treated trees did not show lower leaf Ca levels, however Ca levels was in the lower range of the norm (Kotzé, 2001) and possible negatives effects on fruit Ca uptake can not be excluded and may result in future problems with fruit quality.

5.4.2 Relation of soil chemical properties and leaf nutrient concentrations to tree performance

Fertigation leads to the development of a more restricted root zone (Bravdo, 1993; Neilsen et al., 1999), possibly making it easier to manage effects in the root zone. It was hypothesised that the application of organic material and biological amendments to these restricted root volumes may lead to conditions that improve nutrient uptake, as well as microbial activity and thereby affect yield positively. Significant interaction was found between the application of these biological management practices and different N levels on yield, soil C% and extractable P, as well as leaf P concentrations. At N100 levels, soil extractable P was significantly increased with compost application when applied to humates, but not when applied to compost extract. Compost extract application on its own also significantly increased soil P levels when compared to the control. This indicates direct effects on soil extractable P from both the compost and the compost extracts. These amendments contain available P and additionally its microbial content, although not measured, can contribute to P solubilisation, also increasing plant available P. In combination with N85 application, compost treatment showed the dominant effect on soil P and compost extract resulted in no significant effects. This interaction between lower N and compost extract can not be easily explained. However, soil C% with compost extract application did show significantly lower C% at the lower N level. A positive relationship has generally been found between the microbial biomass and soil organic carbon levels (Fraser et al., 1988; Houot and Chaussod, 1995; Burgos et al., 2002; Magdoff and Weil, 2004). Soil organic carbon has therefore become an important indicator of soil quality and lower soil C% may therefore reflect decreased microbial activity and diversity, which can affect soil P status.

Furthermore, leaf P concentrations showed significantly higher P uptake from trees treated with compost extract on its own, but not when combined with other amendments. This was again only found when applied in combination with the N100 level. Since all treatments including compost extract showed similar soil P levels, these results indicate improved uptake of P in soil treated with compost extract on its own. This could be an indication of microbial effects, improving root proliferation and affecting P uptake (Glick, 1995; Moore-Gordon et al., 1996; Jones et al. 1998; Pinamonti, 1998; Yao et al., 2005; Forge et al., 2008). Results are in agreement with effects found in Chapter 3, where application of compost extract to compost resulted in increased leaf P levels. In the current study, leaf P concentrations was significantly lower than in control plots for soil treated with the combination of humates and compost, when applied with the lower N levels. Results are in contrast to increased nutrient uptake generally reported with HS application (Chen and Aviad, 1990).

However, soil and leaf P was generally high for all treatments when compared to industry norms and therefore probably explains the lack of tree performance effects, despite significant changes.

In this study, soil extractable macronutrients were mainly within the suggested range for apple production, except for soil K which was low when compost was not applied. However, K uptake was sufficient and leaf K content relatively high for all treatments, as well as controls. Increased soil extractable K with compost application also resulted in increased K uptake and higher leaf K content. Increased leaf K concentration is one of the most frequently recognized consequences of mulching with organic materials (Boynton and Oberly, 1966; Kotzé and Joubert, 1992a; Merwin et al., 1994; Marsh et al., 1996; Smith et al., 2000; Neilsen et al., 2003a; Neilsen et al., 2007). However, too much available K may adversely affect Ca and Mg uptake (Klein, 1992; Callan and Westcott, 1996). Leaf Ca and Mg in our study was in the lower range of the norms for apple (Kotzé, 2001) in all treatments, but additional K uptake in compost treated trees did not seem to have a negative effect on Ca and Mg leaf nutrient concentrations. Leaf nutrient analyses also showed that leaf Cu, Zn, Mn and B content were within industry norms. Nevertheless, although differences in soil extractable nutrients and leaf nutrient concentrations could not directly be related to improved tree performance, it may show positive effects in future, or under stress conditions. Neilsen et al. (2003a, 2007) found increased yield with application of biosolids and paper mulch in a high density fertigated apple orchard, but in a different study concluded that these organic amendments may be ineffective in orchards with high fertility and good nutrient management (Neilsen et al., 2004). The effects of organic matter application may therefore have not affected yield dramatically due to already favourable nutrient and soil moisture conditions due to the fertigation management system. Furthermore, optimal nutrient application and favourable conditions for uptake are most critical in the first year after planting in order to improve establishment (Neilsen et al., 1990). In our study trees were already in their second leaf when applications were started. It is also possible that more significant effects will only be seen with continued application over a longer period than 3 years, since the tree will take time to adjust to changes in management practices.

5.4.3 Relation of soil microbial activity to tree performance

Soil enzyme systems are associated with organic residue management, and therefore affect the rate at which nutrients become available to the crop and other soil organisms (Tabatabai, 1982; Perrucci et al., 1984). In our study the activity of various enzymes important in nutrient cycling was used as an indicator of soil quality (Dick, 1994, 1997; Fließbach and Mäder, 1997; Garcia et al., 1997; Pascual et al., 2001; Caldwell, 2005). In general, an increase in various soil enzyme activities have been reported with application of organic amendments in long-term field experiments (Martens et al., 1992; Masciandaro et al., 1997; Albiach et al., 2000; Garcia-Gill et al., 2000; Ros et al., 2003; Bastida et al., 2008). The lack of significant effects found on soil enzyme activities with organic material and biological amendments in our study was probably a result of large variation observed between measurements of different samples of the same treatment (sub-plot error

variation, as a percentage of the total variation of the model, accounted for more than 57%). However, a clear trend was noted with the combination of C+CE+HS, which consistently showed highest enzyme activity, with the exception of β-glucosidase. This treatment also showed highest soil C% in the N100 system. Furthermore, phosphatase activity was higher with compost application. These P-hydrolysing enzymes play a major role in the mineralisation of organic P in soil (Rodriques and Fraga, 1999). The extra-radical hyphae of AM fungi also have phosphatase activity associated with their cell walls (Joner et al., 2000). This may have resulted in increased soil extractable P indicated with compost application. Enzyme activity was higher with the full N application, indicating increased microbial activity in this treatment. However, yield was generally more positively affected by N85 application. Bünemann et al. (2006) in a review on the impacts of agricultural inputs on soil organisms, found variable effects of mineral fertiliser on soil organisms.

Our results, in agreement with result from previous chapters, and various other studies in fruit trees (Renagold et al., 2001; Neilsen et al., 2003b; Varga et al., 2004; Yao et al., 2005; Hoagland et al., 2008), confirm the difficulty of relating specific soil properties to fruit tree performance. However, in other studies it was shown that microbial community composition of the rhizosphere show close relations to yield (Porter et al., 2005; Yao et al., 2006). Although variable results with enzyme activity were found in our study when sampling in the root zone, it is possible that effects of organic material and biological amendments were more significant in the rhizosphere. Furthermore, effects on enzyme activity can not be related to soil microbial diversity or community composition. Changes in microbial community composition with biological amendment application could have affected yield positively either through improved uptake and availability of nutrients or changes in plant growth hormone levels by exogenous production or affecting translocation in the plant. In our study, increased extractable P was shown with compost, humates and compost extract application. Compost extract also resulted in improved P uptake. However, the significant interactions found between inorganic and organic applied nutrients need further investigation in order to explain varied effects on yield.

5.6 CONCLUSION

There was a significant interaction between organic material application and biological amendments and various inorganic N levels applied. Humate treatment in combination with compost application significantly increased yield when applied with the full N application (N100). Furthermore, a significant increase in yield was also found with compost extract applied in combination with compost at the lowest N application. Application of the N85 regime possibly resulted in the best balance between root growth, shoot growth, bud quality and fruit set as indicated by highest yield in 2008, as well as cumulative yield.

Compost treatments showed positive effects on soil extractable nutrients. It was also indicated that compost extract application improved uptake of P. Effects on enzyme activity were not significant, although some tendencies were noted. It is clear that future research should focus on effects in the rhizosphere, since root

exudates are the main factor determining soil microbial community composition and can possibly better relate to effects on tree performance.

It is possible that due to the high fertility regime required for tree establishment, effects of these biological management practices will be more significant when applied with or even before orchard establishment. Furthermore, effects on tree performance may be more pronounced under conditions of stress.

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Table 1. Effect of organic material, compost extract and humate application on tree vigour of 'Brookfield Gala' apples planted on M793 rootstock in 2003 (loamy soil) at 2000 trees.ha^{-1} under fertigation. The trial was established in October 2004 when trees were between second and third leaf. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. No significant differences are indicated by ns following the treatment means.

Main Treatment[z]	Trunk circumference (cm)		Pruning mass
Sub Treatment[y]	October 2004	Growth tempo[*]	2006 (kg)
N100	10.82 ns	3.83 ns	0.914 ns
N85	10.49	3.72	0.773
N70	10.53	3.76	0.794
Control	10.45 ns	3.66 ns	0.807 ns
CExtract+Compost	10.64	3.77	0.807
CExtract+Wood	10.54	4.11	0.854
CExtract	10.57	3.77	0.824
Humate+Compost	10.86	3.99	0.911
Humate+Wood	10.60	3.67	0.683
Humate	10.57	3.69	0.886
CE+H+C[x]	10.58	3.50	1.040
CE+H+W	10.67	3.76	0.854
P value			
Main Treatment	0.1850	0.6049	0.7501
Sub Treatment	0.8571	0.2149	0.3722
Main x Sub	0.3485	0.1053	0.2754

[z] Differential nitrogen applications commenced in February 2006, after harvest.
[y] Organic matter application commenced from October 2004, and biological amendment application in 2005.
[x] CE+H+C = Compost extract + Humates + Compost, and CE+H+W = Compost extract+ Humates + Wood chips
[*]Slope (b value) of linear regressions (R^2 = 0.99) fit to trunk circumference measured from 2004 to 2008, indicating growth tempo.

Table 2. Effect of organic material, compost extract and humate application on yield and yield efficiency of 'Brookfield Gala' apples planted on M793 rootstock in 2003 (loamy soil) at 2000 trees/ha under fertigation. The trial was established in October 2004 when trees were between second and third leaf. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different.

Main Treatment[z]	Sub Treatment[y]	Yield (kg.tree^{-1})			Yield efficiency (kg.cm^{-2})		Cumulative yield 2008
		2006	2007 *	2008	2006	2008	(kg.tree^{-1})
N100		16.16	4.46	-	0.993	0.876	-
N85		17.51	3.33	-	1.100	1.172	-
N70		16.55	3.51	-	1.041	0.922	-
Control	N100**	16.77	4.05	19.69 hij	1.050	0.964	40.11 c-f
	N85			26.56 a-h			51.73 abc
	N70			23.36 c-j			41.81 b-f
CE+Comp	N100	16.79	4.15	22.64 e-j	1.034	1.070	43.01 a-f
	N85			27.40 a-g			43.41 a-f
	N70			32.14 a			54.39 ab
CE+Wood	N100	16.25	4.88	20.19 g-j	1.030	0.912	39.73 cdef
	N85			29.99 a-d			51.81 abc
	N70			22.99 d-j			47.29 abcd
CExtract	N100	16.65	3.68	25.72 b-j	1.033	1.041	43.53 a-f
	N85			30.93 ab			50.08 abc
	N70			23.83 b-j			47.01 a-e
H+Comp	N100	18.07	4.27	29.77 a-e	1.124	1.040	53.88 ab
	N85			30.42 abc			51.62 abc
	N70			23.44 c-j			46.17 a-e
H+Wood	N100	15.88	2.73	23.81 b-j	1.004	0.948	44.69 a-f
	N85			29.28 a-f			51.01 abc
	N70			18.82 j			34.47 ef
Humate	N100	17.60	4.46	23.55 c-j	1.093	0.964	51.95 abc
	N85			28.88 a-f			49.07 abc
	N70			18.04 j			35.03 def
CE+H+C[x]	N100	15.49	3.26	20.33 g-j	0.971	1.019	42.46 a-f
	N85			28.62 a-f			47.01 a-e
	N70			22.01 f-j			44.61 a-f
CE+H+W	N100	17.09	2.43	16.79 j	1.058	0.959	32.48 f
	N85			31.68 a			54.82 a
	N70			26.43 a-h			47.21 a-e
P values							
Main Treatment		0.8241	0.1909	0.1479	0.6988	0.0859	0.4179
Sub Treatment		0.8615	0.3150	0.2667	0.8902	0.8218	0.2242
Main x Sub		0.5984	0.0578	0.0311	0.8164	0.2578	0.0312

[z] Differential nitrogen applications commenced in February 2006, after harvest.
[y] Organic matter application commenced from October 2004, and biological amendment application in 2005.
[x] CE+H+C = Compost extract + Humates + Compost, and CE+H+W = Compost extract+ Humates + Wood chips
* Poor weather conditions during fruit set
** Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments

Table 3. Effect of organic material, compost extract and humate application on fruit quality parameters of selected treatments for 'Brookfield Gala' apples planted on M793 rootstock in 2003 (loamy soil) at 2000 trees/ha under fertigation. Evaluation was done in the 2006 season at harvest, after cold storage (at -0.5 ºC for 8 weeks), as well as cold storage and a shelf life period of 7 days at room temperature (21-24 ºC). The trial was established in October 2004 when trees were between second and third leaf. Probability values shown at the bottom of the table are according to a standard ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means. Treatment means in a column followed by the same or no letter are not significantly different. (TSS = Total soluble solids and TTA = Total titratable acids).

Treatment[y]	Evaluation at harvest						After storage			After shelf life		
	Fruit size (mm)	TSS (% Brix)	TTA (%)	Firmness ($kg.m^{-2}$)	Colour	Starch breakdown (%)	TSS (% Brix)	TTA (%)	Firmness ($kg.m^{-2}$)	TSS (% Brix)	TTA (%)	Firmness ($kg.m^{-2}$)
Control	68.7	13.40	0.63 d	8.24 a	6.03	49.3 c	14.07	0.67	6.47	13.67	0.52	5.72
CExtract+Compost	70.1	13.10	0.76 ab	7.67 bc	6.63	59.3 ab	13.78	0.63	6.26	13.83	0.52	5.32
CExtract+Wood	69.6	13.33	0.78 a	7.97 abc	6.67	57.9 b	13.92	0.61	6.41	13.85	0.51	5.59
Humate+Compost	70.3	12.98	0.67 cd	7.59 c	6.27	65.0 ab	13.48	0.61	6.04	13.40	0.53	5.43
Humate+Wood	69.4	13.37	0.71 bc	8.05 ab	6.28	58.5 ab	14.10	0.63	6.41	13.77	0.54	5.67
CE+H+C[x]	68.9	13.37	0.66 cd	7.55 c	5.97	65.7 a	13.90	0.64	6.35	13.75	0.51	5.70
P values	0.0705	0.5981	0.0011	0.0218	0.4313	0.0024	0.1252	0.4301	0.1424	0.5033	0.8372	0.1917

[y] Organic matter application commenced from October 2004, and biological amendment application in 2005.
[x] CE+H+C = Compost extract + Humates + Compost

Table 4. Effect of organic material, compost extract and humate application on fruit quality parameters of selected treatments for 'Brookfield Gala' apples planted on M793 rootstock in 2003 (loamy soil) at 2000 trees/ha under fertigation. Evaluation was done in the 2008 season at harvest, after cold storage (at -0.5 ºC for 8 weeks), as well as cold storage and a shelf life period of 7 days at room temperature (21-24 ºC). The trial was established in October 2004 when trees were between second and third leaf. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means Treatment means in a column followed by the same or no letter are not significantly different. (TSS = Total soluble solids and TTA = Total titratable acids).

Treatment	Evaluation at harvest					After storage		After shelf life	
Main	Fruit size	TSS	Firmness	Colour	Starch	TSS	Firmness	TSS	Firmness
Sub	(g)	(% Brix)	(kg .m^{-2})		breakdown	(% Brix)	(kg.m^{-2})	(% Brix)	(kg.m^{-2})
N100	127.3	12.31	7.48	7.81	65.30	12.44	6.48	12.20	5.77
N85	124.9	12.13	7.48	10.32	60.17	12.44	6.19	12.51	5.96
N70	126.4	12.03	7.40	9.00	70.83	12.49	6.19	12.33	5.87
Control	125.7	12.10	7.43	10.83	66.89 ab	12.39	6.13	12.31	5.49
CExtract+Compost	123.8	12.02	7.62	9.89	59.56 b	12.30	6.87	12.22	5.69
CExtract	125.5	12.21	7.34	8.39	71.11 a	12.58	6.48	12.54	6.20
Humate+Compost	131.5	12.16	7.28	9.07	65.89 ab	12.54	6.21	12.30	5.98
Humate	120.5	12.19	7.71	8.17	57.33 b	12.27	5.71	12.10	5.89
CE+H+C[x]	130.2	12.78	7.34	7.63	71.89 a	12.67	6.34	12.60	5.94
P values									
Main	0.2847	0.4305	0.8334	0.4970	0.0683	0.9756	0.6414	0.4175	0.7672
Sub	0.0602	0.8138	0.0917	0.7494	0.0192	0.5056	0.1320	0.1401	0.1995
MainxSub	0.5181	0.1116	0.5863	0.4326	0.4029	0.9855	0.8543	0.6043	0.1758

[z] Differential nitrogen applications commenced in February 2006, after harvest.
[y] Organic matter application commenced from October 2004, and biological amendment application in 2005.
[x] CE+H+C = Compost extract + Humates

From each treatment and block combination 35 fruit were analysed per evaluation.

Table 5. Effect of organic material, compost extract and humate application on soil enzyme activity associated with selected treatments for 'Brookfield Gala' apples planted on M793 rootstock in 2003 (loamy soil) at 2000 trees/ha under fertigation. The trial was established in October 2004 when trees were between second and third leaf. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means No significant differences are indicated by ns following the treatment means.

Main Treatment Sub Treatment	Urease[z] (ug N/g soil/2h)	Phosphatase[y] (mgPNP/ kg soil/h)	β-Glucosidase (mgPNP/ kg soil/h)	Arylsulfatase (mgPNP/ kg soil/h)
N100	25.0 ns	342.5 ns	55.6 ns	57.7 ns
N85	20.5	274.7	42.7	43.2
Control	22.2 ns	297.3 ns	48.0 ns	50.2 ns
CExtract	23.9	281.5	48.1	47.8
CExtract+Compost	23.0	324.6	47.3	51.9
Humate	20.1	275.5	39.3	41.1
Humate+Compost	22.2	323.2	58.9	51.6
CE+H+C[x]	24.9	349.4	52.5	59.9
P values				
Sub Trt	0.8670	0.7211	0.4970	0.5875
Main Trt	0.0691	0.0580	0.1781	0.1528
Main x Sub	0.8904	0.8837	0.4094	0.8242

[z] Urease hydrolysing activity was determined by the non-buffered method of Kandeler and Gerber (1988).
[y] Acid phosphatase, β-glucosidase and arylsulfatase activity were determined based on the release and spectrophotometric detection of *p*-nitrophenol (Tabatabai and Bremner, 1969; Tabatabai, 1982).
[x] CE+H+C = Compost extract + Humates

Table 6. Effect of biological management practices in combination with different nitrogen regimes on soil chemical properties for 'Brookfield Gala' apples planted on M793 rootstock in 2003 (loamy soil) at 2000 trees/ha under fertigation. Soil was sampled from the top 0-25 cm in May 2008 at the end of the trial period. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means Treatment means in a column followed by the same or no letter are not significantly different.

Main treatment		pH	Resist.	P (BrayII)	K	Ca	Mg	Zn	B	Cu	N	C
SubtTreatment		(KCl)	(ohm)	$mg.kg^{-1}$	$cmol.kg^{-1}$	$cmol.kg^{-1}$	$cmol.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	%	%
N100		6.56	1752.4	-	0.294	5.44	0.955	0.91	0.328	1.144	0.113 a	-
N85		6.62	2008.9	-	0.389	4.70	0.870	2.94	0.285	0.897	0.082 b	-
Control	N100[z]	6.57 ba	2135.0 a	103.0 cd	0.218 b	5.19	0.952	1.45	0.298 ab	0.763	0.098	0.747 abc
	N85			112.0 bcd								0.737 abc
Cextract	N100	6.52 bc	2083.3 a	145.7 abc	0.168 b	4.82	0.805	1.82	0.286 ab	1.310	0.089	0.900 ab
	N85			183.3 a								0.487 c
CE+Comp	N100	6.70 a	1838.3 ab	154.3 ab	0.507 a	5.56	0.977	2.20	0.366 a	0.822	0.106	0.793 abc
	N85			99.3 cd								1.013 ab
Humate	N100	6.36 c	2210.0 a	167.3 a	0.118 b	4.05	0.782	1.55	0.213 b	0.865	0.083	0.837 abc
	N85			166.7 a								0.617 bc
H+Comp	N100	6.65 ab	1591.7 b	108.7 bcd	0.530 a	5.45	1.017	2.35	0.333 a	1.088	0.108	0.653 bc
	N85			88.3 d								1.133 a
CE+H+C	N100	6.70 a	1501.7 b	178.0 a	0.508 a	5.36	0.943	2.18	0.340 a	1.222	0.101	1.140 a
	N85			85.3 d								0.563 c
Main Trt		0.4062	0.0749	0.4446	0.6910	0.2490	0.0597	0.1287	0.1630	0.4673	0.0270	0.3620
Sub Trt		0.0021	0.0116	0.0010	<0.0001	0.1027	0.2819	0.8129	0.0340	0.6764	0.4067	0.5873
Main x Sub		0.2684	0.1577	0.0085	0.3108	0.0599	0.2085	0.0639	0.1740	0.2976	0.3663	0.0185

[z]Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments

Table 7. Leaf nutrient analyses of trees sampled in January 2008 as affected by the various biological management practices and nitrogen regimes. Results are expressed as g.kg^{-1} DW for macronutrients and mg.kg^{-1} DW for micronutrients. Probability values shown at the bottom of the table are according to a split-plot ANOVA. Student's t-LSD was used at a 5 % significance level to compare the treatment means Treatment means in a column followed by the same or no letter are not significantly different.

Main treatment[z]	Sub treatment[y]	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg.kg^{-1})	Mn (mg.kg^{-1})	Fe (mg.kg^{-1})	Cu (mg.kg^{-1})	Zn (mg.kg^{-1})	B (mg.kg^{-1})
N100		2.103	-	1.774	1.143	0.340	148	201	249	5.8 a	31.3	34.2
N85		2.054	-	1.674	1.224	0.333	154	221	241	5.6 a	32.6	33.2
N70		2.027	-	1.835	1.258	0.316	145	229	221	5.2 b	33.8	34.1
Control	N100[*]	2.078	0.210 def	1.509 b	1.254	0.353 ab	154	225	238	5.3	34.7	33.8
	N85		0.317 b									
	N70		0.315 bc									
Cextract	N100	1.978	0.425 a	1.688 ab	1.114	0.286 c	146	238	213	5.7	36.0	33.5
	N85		0.230 b-f									
	N70		0.265 b-f									
CE+Comp	N100	2.073	0.220 b-f	1.932 a	1.190	0.307 bc	149	228	242	5.4	35.8	34.8
	N85		0.287 b-e									
	N70		0.217 c-f									
Humate	N100	2.043	0.210 def	1.798 a	1.202	0.390 a	150	181	235	5.7	27.0	36.0
	N85		0.300 bcd									
	N70		0.190 ef									
H+Comp	N100	2.064	0.243 b-f	1.813 a	1.310	0.310 bc	152	268	238	5.4	38.5	32.1
	N85		0.185 f									
	N70		0.190 ef									
CE+H+C	N100	2.109	0.220 b-f	1.840 a	1.144	0.334 b	143	162	247	5.6	23.8	33.0
	N85		0.185 f									
	N70		0.215 def									
Main trt		0.4261	0.8243	0.1107	0.3251	0.6720	0.2598	0.5283	0.5211	0.0275	0.5930	0.8800
Sub trt		0.6678	0.0218	0.0128	0.5198	0.0075	0.9757	0.1278	0.9625	0.8831	0.1104	0.4442
Main x Sub		0.4134	0.0078	0.2194	0.5439	0.7356	0.9385	0.8887	0.2331	0.7725	0.6743	0.2345

Footnotes: Kotzé (2001) norms: N (2.1-2.6%), P (0.14-0.19%), K (1.2-1.4%), Ca (1.45-1.60%), Mg (0.30-0.40%), Na (500 mg.kg^{-1}), Mn (20-90 mg.kg^{-1}), Fe (80-150 mg.kg^{-1}), Cu (5-10 mg.kg^{-1}), Zn (30-50 mg.kg^{-1}), B (30-35 mg.kg^{-1}). Each leaf sample consisted of 50 leaves. Samples were analysed by a commercial laboratory (Bemlab®, Strand, SA) using an inductively coupled plasma-optical emission spectrometer and a nitrogen analyzer.

[z] Differential nitrogen applications commenced in February 2006, after harvest.

[y] Organic matter application commenced from October 2004, and biological amendment application in 2005.

[x] CE+H+C = Compost extract + Humates

[*] Were significant interaction was found treatment means are shown for the interaction between the N levels and the biological amendment treatments

CHAPTER 6

CRITICAL ASSESSMENT OF THE ROLE OF BIOLOGICAL AMENDMENTS ON POME FRUIT CULTIVATION SYSTEMS

6.1 INTRODUCTION

There has been tremendous interest in the application of more sustainable, biologically orientated management practices over the past decade as a result of increased sensitivity to environmental issues among fruit producers. The role of biodiversity in ensuring crop production and soil fertility and the importance of soil microbial functions in agricultural systems has become more evident. Since microbial activity is generally carbon-limited in agricultural soil, it is widely accepted that management practices providing a range of organic compounds on a regular basis will tend to maintain an active and diverse microbial population. Although there has been a vast increase in the number of scientific publications on this subject in the past five years, clear guidelines on the use of biological management practices are lacking. The wide scope of the field, and the very variable nature of amendments and different orchard conditions make the subject extremely complex. Furthermore, biostimulants such as seaweed extracts and humic substances, as well as microbial inoculants in the form of compost extracts and effective microorganisms (EM) are used locally to a wide extent with limited scientific literature available on their effects on deciduous fruit crops. The evaluation of biological amendments under local, field conditions is of extreme importance, since extrapolation of results from greenhouse or laboratory studies to the field are often inconsistent.

Apple replant disease (ARD) is a disorder associated with the poor growth of young apple trees planted on previous apple or pear sites and is one of the major impediments in establishing an economically viable apple orchard and maintaining a successful local industry. It is becoming an increasingly important problem since apple producers are forced to replant old orchard soil due to limited availability of suitable virgin soil sites. The problem is further exacerbated by the market demand for new cultivars and the release of improved rootstocks which necessitates new plantings. Although the etiology of ARD is still not fully understood, it is mainly a problem of biological origin involving a shift in the microbial community composition towards pathogens dominating the soil microbial profile. Apple replant disease has been controlled successfully in most cases by the application of methyl bromide, a broad spectrum fumigant. However, due to its impeding phase-out, biological alternatives are needed.

The objective of this study was to investigate the long-term effect of continued applications of organic material, various microbial inoculants and biostimulants on tree performance in conventional management systems. For this purpose, field trials were established under various orchard conditions. A trial was conducted in a pear orchard established on BP1 rootstock that generally suffers from poor root

development in the initial years after planting. It was also investigated whether application of biological amendments in an optimally managed orchard could lead to complimentary improvement in tree performance. Furthermore, trials were established to investigate the potential use of biological soil amendments as an alternative management practice to reduce the effects of ARD under South African conditions. The use of compost, compost extracts, a *Bacillus* inoculant and humates were investigated intensively. In all trials, the effect of amendments on nutrient availability and uptake was investigated in order to aid in the interpretation of tree performance effects. Furthermore, to improve our understanding of soil biological functioning and adapt current soil management practices to be more sustainable, it is important to know the effect of management practices on the soil microbial community. However, although literature is abundant and various methods are available, no single method has been widely accepted, since each method has its own limitations. In this study we applied a combination of simple, practical methods that have been used extensively in literature, to establish the effect of amendments on soil microbial properties. Methods used included conventional culture-based plate counts of actinomycetes and *Bacillus* bacteria, measurement of the activity of various enzymes important in nutrient cycling, as well as carbon source substrate utilisation as an indication of soil microbial community function. Effects on soil microbial properties were also related to tree performance.

6.2 GENERAL DISCUSSION

6.2 1 Effect of fumigation and inorganic fertilizer application on soil microbial community

It is generally perceived that use of chemicals in fruit production has negative effects in terms of sustainable production practices and must therefore be replaced with more natural, biological amendments. However, complete loss of soil function due to conventional management practices is unlikely and if inorganic fertilizer is not excessively applied, its effects on microbial activity can be stimulating. Many producers embrace the move towards more biological management systems, while others are forced to use alternatives due to environmental legislation. The phase-out of methyl bromide, because of its impact on the ozone layer, has caused major concern due to its very effective control of ARD. Therefore, an important part of this study was to evaluate biological alternatives in managing ARD. Improved yield with MeBr is usually ascribed to its broad spectrum biocidal activity, resulting in improved root growth and plant health, possibly due to pathogen control and reduced competition for resources from microbes during the establishment phase of the orchard. Achieving the latter through biological management practices has proved to be a major challenge. Various studies in literature show few persistent effects of fumigation on soil microbial properties over the long term. In our study effects on soil enzyme activity was not significant three years after fumigation, however, long term effects on soil microbial community substrate utilisation were found. Appe replant disease studies in Washington State (USA), has shown that cultivation of apples induces microbial communities capable of inciting ARD within two to three cropping years. Therefore, although MeBr does provide consistent reduction in ARD over a wide scope of soil types and orchard conditions, the repeated use of fumigants is necessary when the next orchard is to be established.

6.2.2 Effect of biological management practices on microbial parameters

Results from this study showed that application of biological amendments will not necessarily result in increased microbial activity or function. However, application of labile organic matter (straw mulch and compost) showed the most significant effect on microbial parameters and generally resulted in significant changes in soil microbial function and activity. Additionally, compost extract application resulted in increased numbers of *Bacillus* bacteria, as well as activity of various soil enzymes. The effect of Biostart applications was generally less significant, with increased enzyme activity only observed in one of the trials. Effective use of soil inoculants strongly depend on soil physical and chemical factors, environmental conditions, as well as the plant type involved. The inoculant organism must not only survive, but establish itself and dominate in the soil or rhizosphere. Therefore, results with inoculants containing a diverse group of organisms may be more beneficial. In three ARD sites, compost and not inoculants, showed the dominant effect on soil microbial function, while a combination of compost, compost extract and Biostart with humic substances was the only treatment to show significant effects on soil enzyme activity. Under controlled fertigation conditions, it was also clear that the combination of compost, compost extract and humates consistently showed highest enzyme activity.

6.2.3 Effect of soil applications on tree performance

Regular application of compost extract in combination with compost was the biological amendment showing most significant effect in improving tree performance in commercial pome fruit orchards under various conditions. In a conventionally managed pear orchard planted on BP1 rootstock, application of compost extract in addition to compost for five growing seasons improved yield, as well as yield efficiency and resulted in a 51% increase in cumulative yield over two harvest seasons. In a high density (2000 trees.ha^{-1}) optimally managed apple orchard, results showed a significant interaction between biological amendments and inorganic N levels applied. In this orchard the application of compost extract applied in combination with compost at 75% of the standard N application significantly increased yield. Other biological amendments which showed positive effects on yield were application of *Bacillus* inoculants (Biostart®) in combination with a labile C source and a low dosage humate product, as well as a combination of compost and humates.

Biological amendments also showed improved growth in orchards suffering from stunted growth symptoms typical of ARD. In an ARD site, application of Biostart soil inoculant in combination with a labile C-source and low dosage of humate, as well as compost extract applied with compost, over a five year period, showed significant improvement in tree performance compared to untreated plots. Compost extract application also showed a possible benefit in increasing fruit size. In three additional ARD trials, it was noted that effects on yield with these biological amendments were mostly significant in orchards showing mild ARD effects and not in cases of severe ARD. Furthermore, there was no clear indication of which amendment in addition to compost resulted in the best tree performance, except for the trial where

compost application was combined with compost extract treatment and the application of Biostart combined with humic substances.

Application of humates without labile organic material generally showed no effect on tree performance in any of the trials.

6.2.4 Use of soil parameters measured as indicators of yield

The use of microbial measures as indicators of soil health and soil quality is becoming increasingly popular. However, although certain microbial parameters may indicate the production potential of soil, the difficulty of relating soil properties to fruit tree performance has been recognised throughout this study, as well as in literature. Crop productivity represents the outcome of complex interactions among plant, soil and management practices. In our study, yield of trees from soils receiving biological amendments could not always be distinguished from those of conventionally managed controls. Although for some specific treatments, increased microbial numbers and activity may have resulted in improved tree performance, in general, changes in culture-based plate counts, soil enzyme activity and carbon utilisation profiles could not be used as an indicator of yield. Recent literature suggests a more dominant effect of microbial community dynamics in the soil rhizosphere. In our study, soil was sampled from soil in the root zone, but not specifically the rhizosphere. It is therefore possible that better correlations with yield can be found by investigating microbial properties in the rhizosphere.

From the various soil management practices applied in the different trials, compost was the dominant factor affecting nutrition and consistently resulted in increased soil extractable macronutrients in the more sandy soils. Although this did not show a direct relationship with yield, these increased levels of soil extractable nutrients are available to the plant and therefore have potential benefits to plant performance.

6.2.5 Use of biological strategies in managing apple replant disease

Although biological amendments showed promise in managing ARD symptoms, fumigation was still the treatment that showed the most significant and consistent response in terms of tree performance in ARD sites. This could be ascribed to the broad-spectrum activity of methyl bromide and its effectiveness therefore under various soil biological conditions. Effects with fumigation are dramatic and immediate. In contrast to this, effects with biological amendments are more gradual and long term and require regular use in management systems. With continued application, long term changes are induced in soil microbial communities, making it more difficult for soilborne pathogens to dominate. This can possibly have more positive implications for the next orchard to be established. However, effects with these biological amendments are site-specific and many issues remain to be addressed before they can be relied on for use in ARD control. Furthermore, a trade-off between short and long term advantages and/or disadvantages must be considered. As with other soilborne diseases, it seems that orchards showing severe ARD symptoms need intensive treatment and that a combination of biological strategies can play a positive role in inducing soil suppressive effects.

6.2.6 Possible mechanisms involved in effects with biological amendments

The mechanism through which biological amendments affect plant growth is through either direct or indirect effects on root development and soil microbial communities, leading to improved plant nutrition, crop protection against pests and diseases or changes in plant hormonal balances. Limited research has been conducted on the mechanisms through which compost extracts can improve plant performance. The majority of research has focused on the use of compost extracts in disease suppression and their role in biological control. However, it is also possible that improved synchronisation of nutrient release and plant uptake may play an important role in improving tree performance with application of compost extracts. It makes sense that synchronisation should be easier with regular application of solubilised nutrients readily available for plant uptake, as occurs with monthly compost extract applications.

Changes in phytohormone levels mainly control growth and developmental processes in plants. Plants may, under certain conditions, for example stress, not have the capacity to synthesize sufficient endogenous phytohormones for optimal plant growth and they then respond favourably to exogenous sources. Various plant growth promoting rhizobacteria (PGPR), including *Bacillus* strains, have been found to produce phytohormones and even promote growth and yield of plants. Hormone-like activity has also been suggested for several humic fractions. The exogenous supply of plant hormones can in addition to providing supplemental quantities to the plant's endogenous levels, stimulate endogenous changes in plant phytohormone levels through long distance messaging in plants, as well as local signaling. Furthermore, exogenous supply of plant hormones can affect the sensitivity of the plant to phytohormones, or affect plant growth indirectly through modifying the rhizosphere environment.

Since root meristems are possibly the main site of cytokinin (CK) production, this phytohormone plays an important role in linking root growth proliferation and effects on plant performance. In deciduous fruit, CK plays a crucial role in regulating lateral bud burst and development, the quality of fruiting spurs, fruit set and controlling the balance of roots and shoots. Furthermore, there are several reports suggesting that the accumulation level of CK and export by the roots is closely correlated with the nutritional status of the plant. The production of CK by *Bacillus* bacteria has been well documented. It is possible that phytohormones, especially CK, may play an important role in the mechanism of action of biological amendments.

6.3 RECOMMENDATIONS

Based on results from this study, as well as recent findings in literature, the following recommendations are made:

- Optimal nutrient application and favourable conditions for nutrient uptake are most critical in the first year after planting in order to improve establishment. Furthermore, protection of roots from

soil pathogens and parasites can improve early root proliferation. Therefore, application of biological amendments is recommended with or even before orchard establishment. A high degree of specificity is exhibited by various microbial isolates towards controlling different pathogens, as well as producing plant growth promoting substances.

- Application of a mixture of beneficial organisms in combination with organic material serving as a carbon source to augment survival and function of introduced inoculants, can have more significant influence on plant performance due to reduction in variability and increased effectiveness with a wider range of microorganisms.
- However, practical, as well as economic considerations such as transportation, labour costs and availability of organic material need to be taken into account. Therefore ways need to be investigated to improve plant biomass production in the orchard to use as organic material.
- Furthermore, much more research is needed on the effects of compost extracts and the mechanisms involved in generating positive functioning in a field environment in order to develop management strategies that can be applied in commercial agricultural systems. These inoculants are easy to apply and not very costly.
- The integration of chemicals with a less toxic effect on non-target organisms and biological management systems also needs intensive investigation to control severe soilborne problems.
- The importance of rhizosphere conditions to plant growth and development needs to be recognised. Recently, research has focused on modification of resident soil rhizosphere communities through the use of cover crops, as well as different rootstocks. These management practices can be combined with biological amendment application to form part of an integrated management strategy.
- The role of microbially produced phytohormones in affecting tree performance in these biological systems needs more attention and changes in phytohormone levels may provide better correlation to yield than broad-scale microbial properties. Plant response is regulated by the net balance of exogenous and endogenous phytohormones. Since endogenous levels of plant phytohormones vary with different stages in plant development, responses with biological amendments would depend on the time of release or production of these phytohormones, possibly explaining variable results found with biological amendment application.
- Lastly, the effects of biological amendments on root morphology and proliferation of root tips can provide valuable information in relating effects to tree performance. Actively growing root tips are an important source of cytokinin (CK) production and translocation. In deciduous fruit production, CK plays a crucial role in regulating lateral bud burst and development, the quality of fruiting spurs, fruit set, delay of senescence, and controlling the balance between roots and shoots.

6.4 GENERAL CONCLUSION

Applciation of biological amendments can improve tree performance under various conditions, including orchards suffereing from mild ARD symptoms. Results with compost extract in combination with compost are encouraging and the beneficial effects on soil extractable nutrients are clear. Furthermore, the combined application of a diverse group of microbial inoculants in combination with organic material seems to result in more consistent positive effects on pome fruit tree performance. However, in some cases, application of biological amendments did not result in improved yield. Effects with chemicals are predictable and consistent, while effects with biological amendments are gradual and long term, requiring intensive management and increased knowledge of the plant-soil system. In this study, results were based on four to five year trial periods. Long term studies are needed to determine eventual benefits. Furthermore, no simple relationship could be shown between the broad-scale microbial properties measured in this study, and tree performance. The high degree of specificity of beneficial microorganisms towards controlling different fungi and improving nutrient uptake and plant growth, as well as influences of soil type and environmental conditions, makes more research essential for application of these biological management strategies with confidence.

Appendix A1. Selected physical and chemical properties of commercial composts used in the various trials in Chapters 2-5. Average values for compost analyses conducted each year are shown.

Compost	**pH**	**Resist.**	**Moist.**	**Density**	**N**	**C**	**P**	**K**	**Ca**	**Mg**	**Na**	**Mn**	**Cu**	**Zn**	**B**
	KCl	**Ohm**	**%**	**kg/m^3**	**%**	**%**	**%**	**%**	**%**	**%**	**Mg/kg**	**Mg/kg**	**Mg/kg**	**Mg/kg**	**Mg/kg**
Pear trial (Chapter 2)	7.2	85	28.4	651	0.84	17.5	0.24	0.50	1.16	0.16	950	102	2.55	76.5	9.25
CompostA (Chapters 3-5)	7.3	70	25.3	549	1.15	20.0	0.40	0.96	1.28	0.27	1973	186	2.94	120	9.75
CompostB (Chapter 3)	7.0	90	25.6	411	0.89	21.7	0.22	0.52	0.95	0.15	1252	87	3.08	118	7.55

Appendix A2. Macro- and micronutrient content of the different compost extracts used in Chapter 4. Average values for compost extract analyses performed each year are shown.

Extract	**N**	**P**	**K**	**Ca**	**Mg**	**Na**	**Mn**	**Fe**	**Cu**	**Zn**	**B**
	mg.L^{-1}										
ExtractA[z]	427	7.53	76.6	17.7	7.3	27	0.151	9.47	0.29	0.1709	1.35
ExtractB[x]	348	8.62	94.8	15.2	6.7	32	0.105	7.45	0.34	0.109	0.78

[z] ExtractA was used in Chapters 2-5

[x] Extract B was anly used in Chapter 4 and contained additives, including molasses, kelp and fish extract.