Microbial community structure as an indicator of soil health in apple orchards

Oluwafemi James Caleb

Thesis presented in partial fulfillment of the requirements for the degree of Masters of Science (Department of Microbiology) at Stellenbosch University

Supervisor: Prof K. Jacobs
Co-supervisor: Dr K. du Plessis

March 2010

DECLARATION

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

March 2010

Copyright © 2009 Stellenbosch University

All rights reserved

Table of contents

Page

Acknowledgements vi

Summary vii

Opsomming ix

CHAPTER 1

Introduction

1.1. Commercial farming practices with emphasis on apple production 2

1.2. Apple production in South Africa 4

1.3. Land management in South Africa 5

2.0. Soil 6

2.1.Soil as a microhabitat 6

2.2.Significance of soil microbial communities in soil processes 9

3.0. Concept of soil quality/soil health 10

3.1. Soil quality: indicator(s) of sustainable management 11

3.2. Qualitative indicators 11

3.3. Quantitative indicators 11

3.3.1. Physical indicators 13

3.3.1.1. Bulk density 14

3.3.1.2. Soil texture 14

3.3.1.3. Water infiltration rate 15

3.3.1.4. Soil and rooting depth 15

3.3.1.5. Water holding capacity 16

3.3.2. Chemical indicators 17

3.3.2.1. Soil pH 17

3.3.2.2. Electrical conductivity 17

3.3.2.3. Nutrient availability 18

3.3.2.4. Organic matter 19

3.3.3. Biological indicators 20

3.3.3.1. Microbial biomass 20

3.3.3.2. Potentially mineralizable N 20

3.3.3.3. Soil respiration 21

4.0. Assessments of soil microbial communities 22

4.1. **Culture-dependent methods for assessing microbial diversity** 22

4.1.1. Viable cell count 22

4.1.2. Sole carbon source utilization (SCSU) 23

4.2. Culture-independent methods for assessing microbial diversity 24

4.2.1. Biochemical methods 25

4.2.2. Polymerase chain reaction (PCR)-based methods 26

4.2.2.1. Denaturing gradient gel electrophoresis (DGGE)/
Temperature gradient gel electrophoresis (TGGE) 26

4.2.2.2. Single strand conformation polymorphism (SSCP) 28

4.2.2.3. Restriction fragment length polymorphism (RFLP) 29

4.2.2.4. Terminal restriction fragment length polymorphism (T-RFLP) 29

4.2.2.5. Ribosomal intergenic spacer analysis (RISA) and
Automated ribosomal intergenic spacer analysis 31

5. Purpose of study 33

References 34

CHAPTER 2

Materials and Methods

2.1. Site description 67

2.2. Sampling and Storage 68

2.3. Soil characteristics 69

2.4. Molecular characterization

2.4.1. Genomic DNA extraction 69

2.4.2. Polymerase Chain Reaction (PCR) 69

2.4.3. Automated Ribosomal Intergenic Spacer analysis (ARISA) 70

2.5. Microbial community diversity 71

2.6. Variation within treatment plots 71

2.7. Variation between replicated plots 72

2.8. Microbial community structure among treatments 72

2.9. Effect of different soil treatments on microbial diversity 72

3.0. Relationship between soil microbial diversity and soil physicochemical properties 73

4.0. Relationship between apple yield and soil microbial diversity 73

References 74

CHAPTER 3

Results

3.1. Soil characteristics 77

3.2. Molecular characterization 79

3.2.1. DNA extraction 79

3.2.2. PCR amplification 79

3.2.3. Automated Ribosomal Intergenic Spacer Analysis (ARISA) 80

3.3. Microbial community diversity 81

3.4. Variation within treatment plots 84

3.5. Variation between replicated control plots 85

3.6. Microbial community structure among treatments 86

3.7. Effect of different soil treatments on microbial diversity 89

3.8. Relationship between soil microbial diversity and soil physicochemical properties 101

3.9. Relationship between apple yields and soil microbial diversity 103

CHAPTER 4

DISCUSSION

Introduction 105

4.1. Soil characteristics 105

4.2. Microbial community diversity 106

4.3. Variation in microbial diversity with sampled depth within plots 107

4.4. Variation in microbial diversity between replicated treatments 108

4.5. Microbial community structure amongst treatments 108

4.6. Effect of different soil treatments on microbial diversity 109

4.7. Relationship between soil microbial diversity and soil properties 111

4.8. Relationship between apple yield and soil microbial diversity 112

References 114

Chapter 5

CONCLUSION AND FUTURE STUDY

5.1. Conclusion and future study 126

Addendum 127

Acknowledgements

Unto God almighty for His grace, love, mercy and kindness, unto Him I give all praise and adoration. To my parents and my family for believing in me and most importantly for their support at all times even when it was tough on us all. Most importantly, to Prof K. Jacobs and Dr K. du Plessis under whom this study was conducted for their guidance and support during the entire study, and the Department of Microbiology, University of Stellenbosch, for the privileges and support throughout my study.

Thanks also due to Mr. C. J. van Heerden and staff of the DNA sequence facility, Department of Genetics for their assistance, and Mr. J. Harvey of the Centre for Statistical Consultation, Stellenbosch University, for the assistance with data analysis and interpretation. Also appreciated are the Agricultural Research Council and Deciduous Fruit Producer Trust, for the financial support for this study. To Mr. A. Meyer and Ms. M. Joubert from ARC Infruitec-Nietvoorbij for the experimental site and data on apple yield. Finally, to my colleagues and friends for your patience, understanding, and encouragement throughout my study and stay in Stellenbosch.

Summary

The relationship between various land management practices, soil properties and the soil microbial communities are complex and little is known about the effect of these interactions on plant productivity in agricultural systems. Although it would be advantageous to have a single organism or property that can be used as a measure of soil health, it may not be possible. Soil organisms which include both the microorganisms as well as soil fauna are subjected to the effect of their immediate environment. This microenvironment in turn is determined by the soil properties as well as above ground flora and their interactions. Most soil indicators interact with each other, and these interactions can modify or influence the soil properties. The complexities of the interactions between critical soil indicator values often preclude its practical use by land managers and policy makers. However, soil microbial communities (e.g. diversity and structural stability) may serve as a relative indicator of soil quality. These communities are sensitive to land management practices and changes in the microenvironment.

The objective of this study was to gain an understanding of the complex relationships by investigating the effect of conventional, integrated and organic apple production systems on the physical, chemical and biological (particularly soil microbial diversity) properties of the soil. Automated Ribosomal Intergenic spacer analysis (ARISA) was used to characterise fungal (F-ARISA) and bacterial (B-ARISA) communities from soil samples obtained from an experimental apple orchard in Elgin, Grabouw. The intergenic spacer (ITS) region from the fungal rRNA operon was amplified using ITS4 and fluorescently FAM (6-carboxylfluorescein) labelled ITS5 primers. Similarly, the 16S-23S intergenic spacer region from the bacterial rRNA operon was amplified using ITSR and FAM-labelled ITSF primers.

The sensitivity of the technique allowed us to discriminate between the soil microbial communities of the different treatments. From our results we observed significant increase ($p < 0.05$) in the fungal community diversity between the February and April samples, while the bacterial community diversity was consistent ($p > 0.05$). Also, treatments with mulch showed a significantly higher microbial diversity than the other treatments at a 5 % significance level. Fungal communities showed significant correlation with the potassium concentration in the soil, while bacterial communities depicted a significant correlation with the soil phosphorous concentration.

Based on the results we concluded that different management practices have a significant effect on the soil microbial communities and that these communities are particularly sensitive to small changes in the environment. However, there is still a need to determine what the composition of the soil microbial communities are to be able to correlate our observations with soil health.

Opsomming

Die verhouding tussen verskillende landboubestuurspraktyke, grondeienskappe en die mikrobiese gemeenskappe in grond is kompleks en weinig is bekend oor die uitwerking van hierdie interaksies op die produktiwiteit van landboustelsels. Alhoewel dit voordelig sou wees om ‘n enkele organisme of eienskap te kan hê wat die gesondheid van grond kan meet, sal dit dalk nie moontlik wees nie. Grondorganismes wat die mikroörganismes sowel as die grondfauna insluit, is onderworpe aan die invloed van hulle onmiddelike omgewings. Hierdie mikro-omgewings op hulle beurt word weer beïnvloed deur die grondeienskappe sowel as die die oppervlak flora en hulle wisselwerkinge. Meeste van die grondaanwysers toon ook wisselwerkinge met mekaar, en hierdie wisselwerkinge kan die grondeienskappe beïnvloed or selfs verander. Die kompleksiteit van die wisselwerkinge tussen kritiese grond aanwysers is meestal die rede waarom dit nie deur grondbestuurders en beleidsmakers gebruik word nie. Dit is ongeag die feit dat grond mikrobiese gemeenskappe (bv. diversiteit en stukturele stabiliteit) mag dien as ‘n relatiewe aanwyser van grondkwaliteit. Hierdie gemeenskappe is sensitief vir bestuurspraktyke en veranderinge in die mikro-omgewing.

Die doel van die studie was om die ingewikkelde verhoudings in die grondgemeenskappe te bestudeer en die uitwerking van konventionele, geïntegreerde en organiese appel produksie sisteme op die fisiese, chemiese en biologiese eienskappe (veral die grond mikrobiologiese diversiteit) te bepaal. Geoutomatiseerde Ribosomale Intergeniese Spasie Analise (ARISA) is gebruik om die fungus (F-ARISA) en bakteriese (B-ARISA) gemeenskappe van grondmonsters wat vanaf ‘n proef appelboord in Elgin (Grabouw) verkry is, te bepaal. Die intergeenspasie (ITS) area van die fungus rDNA operon is vermeerder deur die ITS4 en fluoresserende FAM (6-karboxylfluorescein) gemerkte ITS5 inleiers te gebruik. Soortgelyk is die 16S-23S intergeenspasie area van die bakteriese rDNA operon vermeerder deur ITSR en FAM-gemerkte ITSF inleiers te gebruik.

Die sensitiwiteit van die tegniek laat ons toe om te onderskei tussen die grond mikrobiese gemeenskappe vanaf verskillende grondbehandelings. Vanuit die resultate kon ons aflei dat daar ‘n toename ($p < 0.05$) in die fungus gemeenskap diversiteit vanaf Februarie to April was terwyl die bakteriese gemeenskap ‘n konstante diversteit getoon het ($p > 0.05$). Behandelings met grondbedekking het ook ‘n beduidend hoër mikrobiese diversiteit getoon as ander behandelings. Fungus gemeenskappe het beduidende korrelasies getoon met kalium

konsentrasies in die grond, terwyl bakteriese gemeenskappe ‘n beduidende korrelasie getoon het met grond fosfor konsentrasies.

Gebaseer op die resultate kon ons aflei dat verskillende bestuurspraktyke ‘n uitwerking kan hê op die grond mikrobiese gemeenskappe en dat hierdie gemeenskappe sensitief is vir klein veranderinge in die omgewing. Dit sal egter nog nodig wees om die spesifieke samestelling van die grond mikrobiese gemeenskappe te bepaal voor ons hierdie waarnemings kan korreleer met grondgesondheid.

Chapter 1

INTRODUCTION

INTRODUCTION

1.1. Commercial farming practices with emphasis on apple production

Sustainability of agricultural systems has become an important issue in both developed and developing countries. Globally there has been a tremendous increase in the number of commercial farmers and the total land area practicing organic and integrated farm management systems in apple (*Malus domestica* Borkh.) orchards, to meet the increasing demands of consumers for healthier and more environmentally sustainable agricultural products (Glover *et al*., 2000; Peck *et al*., 2005). Organic and integrated farm management practices for apple production offer an alternative approach compared to the conventional farm management systems, which involve the use of synthetic pesticides, herbicides and fertilizer inputs (Conacher and Conacher, 1998; Peck *et al.,* 2006; Tu *et al*., 2006). According to Korsaeth (2008), an ideal cropping system should maximize the production of human nutrients per unit area, while minimizing the impact on the environment, thus resulting in a low ratio between emitted pollutants and food produced. Both organic and integrated management systems strive towards this ideal state, by improving soil quality and minimizing environmental degradation while maximizing economic returns and productivity (Reganold *et al*., 2001).

Organic farming is becoming a major consideration for sustaining soil quality damaged by intensive use of synthetic chemicals to enhance crop production (Srivastava *et al*., 2007). It relies on recycling and organic input for nutrient supply, and concentrates on biological control for pest management and cropping system design (Rigby and Cáceres, 2001). Well-established organic systems have been shown to reduce incidence and severity of phyto-pathogenic infections caused by soil borne pathogens compared to conventional systems (van Bruggen and Termorshuizen, 2003). Similarly, increased biodiversity, microbial biomass and enzymatic activity have been reported under organic farming systems (Tiquia *et al*., 2002). A comparative study of organic and conventional arable farming systems was conducted by van Diepeningen *et al.* (2006), to determine the effect of management practices on soil biological and chemical properties, as well as on soil health. They observed among others, that soils from organically managed farms had significantly lower levels of total soluble nitrogen and nitrate in the soil, and a higher number of bacteria of different trophic groups. Species richness of bacteria and nematode communities were equally higher in the organic soils

sampled compared to the conventional soils. The organic soil was also more resilient to drying–rewetting disturbances (van Diepeningen *et al.*, 2006).

Conventional management practices on the other hand, also referred to as industrial agriculture, relies mostly on inputs of off-farm products such as pesticides, herbicides and fertilizers (Horrigan *et al*., 2002; Tu *et al*, 2006). Although this management practice has played a major role in the improvement of fibre and food quality as well as productivity, practices employed have raised numerous public health and environmental concerns (Horrigan *et al.,* 2002). Studies have shown that current conventional management practices have an adverse effect on biodiversity (Moffat, 1998), soil microbial biomass and activities (Doran and Zeiss, 2000), agricultural ecosystems and its immediate environment (Aigner *et al*., 2003), agricultural workers and their relatives (Curl *et al*., 2002) and the safety and health of consumers (Curl *et al*., 2003).

On the other hand, integrated farm management systems, utilize both conventional and organic production systems in an effort to optimize both economic profit and environmental quality (Glover *et al*., 2000). The integrated farming approach has been successfully adopted in some of the major apple farming regions in Europe (Sansavini, 1997). Studies have demonstrated that microbial (carbon and nitrogen) biomass was significantly higher in an integrated farming system compared to organically and conventionally farmed plots (Gunapala and Scow, 1998; Glover *et al*., 2000).

A long-term study carried out by Peck *et al*. (2006) compared the orchard productivity and fruit quality of apples under organic, conventional and integrated farm management systems. In the first year of their study, organic crop yields were two thirds of the conventional and almost half of the integrated yields. During the two year study conventional treatments had a larger yield than the organically managed farm. The organic farm yield was inconsistent, which was attributed to higher pest and weed pressure, limited satisfactory crop load, lower nitrogen levels in leaves and fruit tissue, and deficiency of zinc in leaf tissue (Peck, 2004). Despite all production difficulties encountered with organic farming. They observed that organic apples had the highest flesh firmness compared to conventional and integrated apples after storage treatments in 2002 and 2003. Similarly the total antioxidant activity was highest in organic apples. For 200g of apple, organic apples had 10% to 15% greater total antioxidant

activity in its edible portion than conventional apples and 8% to 25% more than integrated apples (Peck, 2004).

Reganold *et al*. (2001) investigated the effect of organic and conventional farming systems on energy efficiency, environmental and soil quality, orchard profitability, and horticultural performance. They observed no significant difference in the cumulative yields for all three systems. However, from the soil quality assessment organic and integrated systems were significantly higher than those for the conventional system. This observation was attributed to the earlier addition of organic matter in the form of mulch and compost. Organic matter is known to have a significant impact on soil quality, increasing water infiltration and storage and enhancing soil fertility and structure (Brady and Weil, 1999). Furthermore, from their assessment of the impact of the three production systems on the environment using a rating index, the total environmental impact rating was highest for conventional farming systems compared to organic and integrated systems, while, organic systems were the most energy efficient based on the cumulative energy inputs and outputs over the six year study period.

1.2. Apple production in South Africa

Apple production in South Africa dates back to 1652 when the first apple farm was established (Crouch, 2003). Presently, approximately 20,736 hectares of land is under apple production with the main growing area in the Western Cape (DFPT, 2009). Apples are one of the most important deciduous fruit exported from South Africa. Constituting about 30 % of the total deciduous crop produce in South Africa on the basis of volume produced (NDA, 2000) (Fig. 1). Furthermore, within the deciduous fruit industry, apple farming generated about 28,068 employments as at year 2003 (OABS, 2003) (Table 1). In 2008, the apple industry contributed more than R1.2 billion within the local market and approximately R1.8 billion in export earnings (DFPT, 2009; NDA, 2008; OABS, 2008; PPECB, 2008), with over 26 million cartons of apples exported (PPECB, 2008). With such financial and employment benefits, an adequate and appropriate land management practice is required for the sustainability of apple production in South Africa.

Table 1. Deciduous fruit production: Land in use, job created, and dependent population.

Type	Area (ha)	Employments (Farm workers)	Dependents (persons)
Apples	22,454	28,068	112,272
Pears	12,912	16,140	64,558
Table grapes	20,643	35,093	140,371
Plums	4,962	6,699	26,796
Peaches	9,575	11,490	45,959
Nectarines	1,379	1,724	6,896
Apricots	4,751	5,226	20,904
Total	**76,676**	**104,439**	**417,756**

Source: OABS, 2003

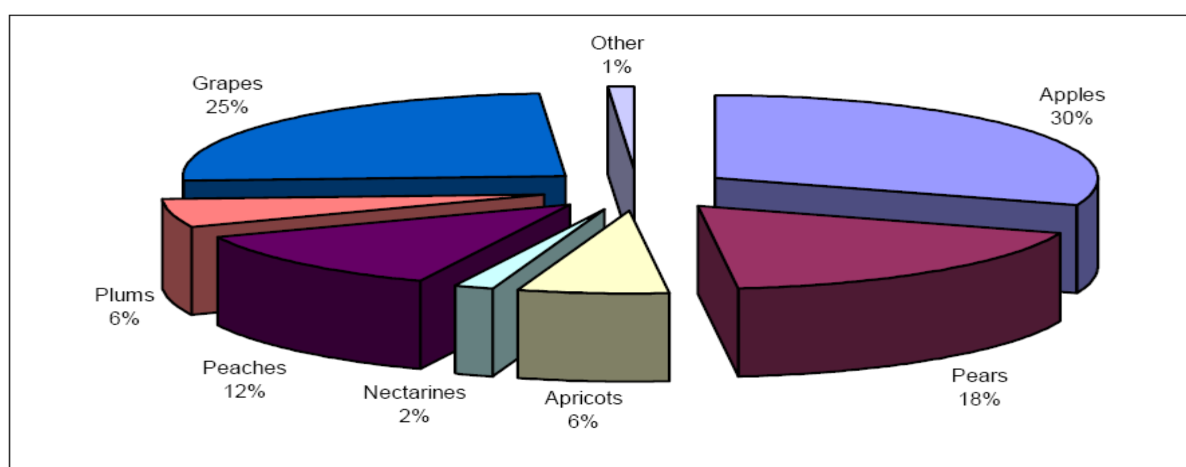

Figure 1. Land area used in the production of deciduous crops in South Africa (NDA, 2000).

1.3. Land management is South Africa

Historically, soil conservation and land management practices in South Africa, has focused more on preventing soil loss through erosion rather than soil quality (Mills and Fey, 2003). Since 1923, policies and bodies have been set up to mitigate erosion in agricultural farmlands in South Africa. In 1928 the Drought Investigation Commission stressed the alarming rate of soil erosion across the country. This was followed in 1930 by the Soil Erosion Advisory Council. However, soil depletion became more pronounced resulting in wide spread erosion and desiccation. The implementation of the Soil Conservation Act (No. 45 of 1946) and various polices was effective enough to control erosion in many parts of the country (Donaldson, 2002). The sustainability of apple production systems in South Africa is of great importance, both economically and environmentally, to be able to meet the demands of the

ever-increasing global population and maintain the global ecosystem. Similarly, understanding the ecology, physiology and biochemistry of soil microorganisms and their interactions, will enable us to adequately comprehend their role in the soil and in all the biogeochemical processes in the soil.

2. Soil

Soil plays a crucial role in the survival of terrestrial life, and serves as a habitat for a wide range of organisms (Doran *et al*., 1996). The biological, chemical, and physical properties of soil give it a unique characteristic that enhances or influences its overall biodiversity. These properties also vary with time and space, resulting in various microhabitats or micro-niches with soil organisms exhibiting spatial and aggregated distribution patterns (Ettema and Wardle, 2002). In addition, the ability of the soil to absorb important biological molecules such as extracellular enzymes and nucleic acids helps to prevent these bio-molecules from degradation and enable their uptake by competent microbial populations (Nannipieri *et al*., 2003). This section explores the role of soil as a microhabitat and the significance of microorganisms in the ecosystem.

2.1. Soil as a microhabitat

Soil is the foundation of natural and agricultural plant communities. The thin layer of soil covering the earth surface represents the difference between survival and extinction for most terrestrial life (Doran *et al*., 1996). Soil is a structured, heterogeneous, and discontinuous system. It is generally poor in energy sources and nutrients (compared to optimal growth conditions *in vitro*). The different components of its solid fractions (organic matter, clay, sand and silt content) provide a myriad of different microhabitats (Stotzky, 1997). Higher organisms range over wide territories of habitat which may be on the scale of a landscape or watershed and beyond. On the other hand, microorganisms' habitat occurs on a micro-scale. They occupy less than 5% of the overall available space in the soil (Ingham *et al*., 1985; Voroney, 2007). These microhabitats or micro-zones support an enormous biomass, with approximately 2.6×10^{29} prokaryotic cells, and a gram of soil contains about a kilometre of fungal hyphae and over 10^9 bacterial cells (Voroney, 2007).

Biological, chemical and physical properties of these microhabitats vary in both time and over space (Nannipieri *et al*., 2003). This spatial characteristic of the soil resources is an important contributor to the coexistence of species in the soil microbial communities because of better resource partitioning (Giller, 1996; Ettema and Wardle, 2002). This enhances overall soil biodiversity by promoting the persistence of individual populations (Ellner, 2001). Soil organisms usually occur in predictable spatial and aggregated patterns over wide scales ranging from square millimetres to hectares (Fig. 2) (Ettema and Wardle, 2002), in contrast to the aboveground biota (Wardle *et al*., 2004).

Several ecological factors (abiotic or biotic) can influence the activity, ecology and population dynamics of microorganisms in soil. Associated with biodiversity of the soil is the soil resilience to endure disturbance (Nannipieri *et al*., 2003) and an increase in the microbial diversity of the soil increases its resilience capacity (Arias *et al*., 2005). Abiotic factors include pH, oxidation-reduction potential, mineral nutrients, ionic composition, the availability of water and carbon, temperature, pressure, composition of air, and electromagnetic radiation (Pardue *et al*., 1988; Killham *et al*., 1993; Chenu *et al*., 2001; McLean *et al*., 2001; Singh *et al*., 2003). Biotic factors include the genetics of the microorganisms and the interactions between these organisms (Nannipieri *et al*., 2003).

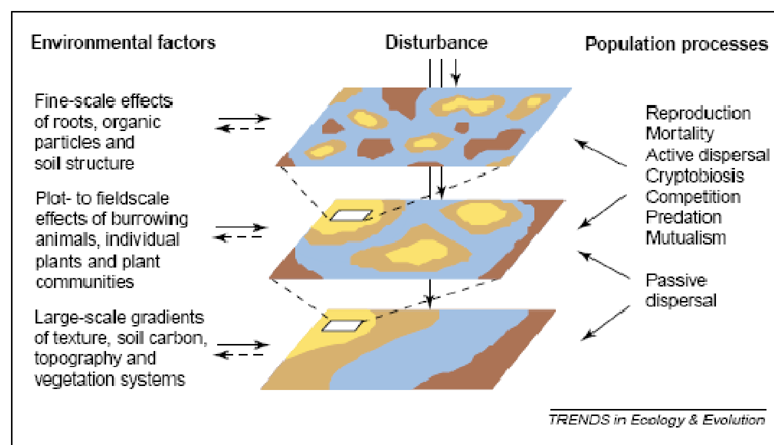

Figure 2. Predictable spatial distribution of soil organisms on nested scales (Ettema and Wardle, 2002).

Spatial heterogeneity in soil organisms is influenced by environmental factors, disturbance and population processes (Fig. 2). Disturbance plays a crucial role at all scale levels and can be a major stimulator of spatial heterogeneity (Ettema and Wardle, 2002). The complexity of

interaction between spatial patterns of soil organisms' activity and environmental activity are represented with dotted arrows in Figure 2. In addition to the spatial patterns and ecological factors of the soil as a microhabitat, the ability of the soil solid fractions to absorb important biological molecules such as nucleic acids, proteins and organic compounds plays an important role in the maintenance of genetic information (Nannipieri *et al*., 2003; Huang *et al.,* 2005; Levy-Booth *et al*., 2007). Extracellular enzymes are some of the biological molecules entrapped by humic molecules or absorbed by clay minerals, and they become more resistant to extreme pH and high thermal denaturation, heavy metal deposition, and microbial degradation (Huang and Shindo, 2000; Nannipieri *et al*., 2002; Klitzke and Lang, 2007). Studies have been carried out to characterize activities and absorption of some important biological molecules on pure clay minerals, intercalated clay minerals by metal ions, and clay-organic compound complexes (Cai *et al*., 2007; Helassa *et al*., 2009). Helassa *et al*. (2009) investigated the absorption and desorption of monomeric Bt (*Bacillus thuringiensis*) Cry1 Aa toxin on montmorillonite and kaolinite. The absorption isotherm obtained for sodium saturated clay were low, but suggested that an optimal condition is required for maximal adsorption.

In another report, Cai *et al*. (2007) observed that nucleic acid, Deoxyribonucleic acid (DNA) adsorption on soil colloids and clay minerals was enhanced in the presence of Ca^{2+}. They compared organic-mineral complexes (organic clays) and fine clays (< 0.2 mm). Kaolinite (organic clay) exhibited the highest adsorption affinity for DNA among the examined soil colloids and clay minerals. The presence of minerals and soil colloids was shown to provide protection to DNA against degradation by DNase I, and montmorillonite, organic clays and fine clays showed stronger protective effects for DNA than inorganic clays and coarse clays. The efficient adsorption of nucleic acid materials on soil colloids and minerals lower the chances of degradation and enhance transformation of extracellular DNA in soils (Cai *et al*., 2007; Levy-Booth *et al*., 2007). Therefore, extracellular or naked DNA released into the soil via sloughing-off of the root cap cells or pollen dispersal of transgenic plants (de Vries *et al*., 2003), decomposing crop residues (Ceccherini *et al*., 2003), pathogens colonising plant roots (Kay *et al*., 2002), and soil microorganisms (Backert and Meyer, 2006) can be transformed into the genetic populations. The natural transformation of extracellular or foreign DNA through their uptake by competent microbial population through horizontal or lateral gene transfer in the soil is an important component of prokaryotic evolution (Levy-Booth *et al*.,

2007) and acquisition of various resistance genes (Kay *et al*., 2002). This extracellular DNA cycle in soil is an open system, which serves as a source of energy and nutrient for microorganisms and plants in nutrient deficient soils. It also help to maintain the genetic pool of information carried in DNA molecules via their natural transformation (Levy-Booth *et al*., 2007).

2.2. Significance of soil microbial communities in soil processes

About 80-90% of all the biogeochemical processes carried out in the soil are reactions mediated by microorganisms (Nannipieri and Badalucco, 2003). Due to their high surface area-to-volume ratio, microorganisms have more intimate interactions with their immediate environment (Douglas and Beveridge, 1998; Ledin, 2000), compared to higher organisms (Gömöryová *et al*., 2009). Soil microorganisms respond rapidly to changes, hence they adapt to environmental conditions (Nielsen and Winding, 2002), and the microorganisms that are best adapted will be most dominant. This adaptive character allows microbial analyses to be discriminating in soil health assessment, and changes in microbial populations and activities may, therefore, function as an excellent indicator of change in soil health (Kennedy and Papendick, 1995; Pankhurst *et al.,* 1995). In some instances, changes in microbial community structure or function can precede detectable changes in soil chemical and physical properties, thus providing an early sign of soil improvement or an early warning of soil degradation (Pankhurst *et al.,* 1995).

Extracellular enzymes of soil microorganisms help to break down complex polymers of soil organic matter into monomeric units, which are readily available to other microbes that can break it down further into simple compounds (Wolf and Wagner, 2005). This is a classic illustration of metabiosis, and interspecies metabolism of soil organic complexes (Waid, 1999). The decomposition of soil organic matter such as plant litter, polymers and humic substances releases nutrients to the soil, which is essential for the survival of the above ground biomass. This also helps to stabilize the net carbon equilibrium of the terrestrial ecosystem (Liski *et al*., 2003). Under anaerobic conditions, carbon dioxide is used as an electron acceptor while reduced organic compounds serve as the donor (Fuhrmann, 2005). The anaerobic respiration process enables anaerobic and fermentative bacteria (methanogens)

to breakdown complex organic substrates into simple substrates that are subsequently mineralized releasing methane (Tate, 2000).

Soil microorganisms also play a crucial role in the bioremediation of toxic organic waste. Bioremediation involves the use of plants and naturally occurring soil microorganisms in processes such as biostimulation, bioaugmentation, biopiling, bioventing, bioreactors and land farming, to degrade organic waste into less toxic forms (Vidali, 2001; Bento *et al*., 2005; Marin *et al*., 2005). Xenobiotic compounds including petroleum hydrocarbons, nitro-aromatic compounds, aromatic and aliphatic compounds, polychlorinated biphenyls (PCBs), pesticides, and surfactants. These compounds are wide-spread environmental pollutants in the soil, which can be degraded by soil microorganisms and soil microbial processes (Zhang and Bennett, 2005; Lambo and Patel, 2007; Rein *et al*., 2007; Fallgren and Jin, 2008; Nitu and Banwari 2009; Tigini *et al*., 2009).

Soil microorganisms have a profound effect on the transformation of other biogeochemical cycles such as nitrogen (N), phosphorus (P) and sulphur (S), as well as various micronutrients and heavy metals (Stevenson and Cole, 1999; Rawlings, 2002; Morton and Edwards, 2005; Robertson and Groffmann, 2007). They also serve as a strong integrator of the various elemental cycles in the soil. Carbon, nitrogen, sulphur, phosphorus and other metal element cycles are integrated through the selection of alternative electron acceptors under different redox conditions by soil microbes and the stoichiometry of biomass production (Bottomley and Myrold, 2007). An example of this is the autotrophic facultative anaerobe *Thiobacillus denitrificans*, which is capable of oxidizing sulphide to elemental sulphur using nitrate as its electron acceptor and carbon dioxide as carbon source under anoxic conditions (Kelly and Wood, 2000). Schink and Friedrich (2000) reported a process of phosphite oxidation by sulphate reduction observed among the strain FiPS-3 genera *Desulfobacter*, *Desulfobacula*, *Desulfospira*, and other representatives of major lineages of the δ-subclass of Proteobacteria.

3.0. Concept of soil quality and soil health

Various definitions of soil quality have been suggested over the last decade, which embody similar elements (Arshad and Martin, 2002). The most general accepted is that by the Soil Science Society of America Ad Hoc Committee on soil quality (S-581). They define soil

quality as the capability of a specific type of soil to function, within managed or natural ecosystem boundaries, to be able to sustain biological productivity, enhance or maintain air and water quality as well as support human habitation and health (Karlen *et al.,* 1997). However, the term soil health is most preferred by some researchers because it describes the soil as a living entity with a dynamic system. The soil functions are controlled by its biological diversity and require maintenance for sustainability (Doran *et al*., 1996, 1998). Soil health in a broader concept, identifies the functionality of a soil to promote environmental quality, preserve plant and animal health, and sustain biological productivity, while the term soil quality is associated with the fitness of the soil for a specific purpose (Doran and Zeiss, 2000).

3.1. Soil Quality: indicator(s) of sustainable management

According to Doran (2002), good soil quality is a requirement for the conservation of water resources as well as the basis for a sustainable agricultural production and the improvement of soil ecosystem functions. Thus, there should be a balance in the relationship between soil function and quality for optimal production of agricultural products. This requires a sustainable soil management approach as well as a dynamic indicator to monitor changes. These indicators must be sufficiently diverse in order to give a descriptive representation of the chemical, biological and physical processes and properties of the soil (Snakin *et al*., 1996; Karlen *et al*., 2003). The indicators for characterizing the quality of soil are grouped into two major categories: qualitative (descriptive), and quantitative (analytical) indicators (Arshad and Cohen, 1992).

3.2. Qualitative indicators

The importance of qualitative soil quality information is not often covered in scientific literature (Arshad and Cohen, 1992). They are generally considered of limited value and soft by technical experts and other natural scientists (Harris and Bezdicek, 1994). Information obtained with the qualitative component is considered soft because they are associated with basic visual and morphological indicators that are inherently qualitative and subjective (Harris and Bezdicek, 1994; Dang, 2007). Farmers often describe soil quality based on their

perception of its smell, look, feel and taste (Harris and Bezdicek, 1994). Farmers' experience and indigenous knowledge offer a simple approach to characterising the status of a healthy soil and to monitor observable changes in soil quality (Romig *et al*., 1995). However, its potential has not been fully explored in both developed and developing countries (Pawluk *et al*., 1992).

Arshad and Cohen (1992) proposed that qualitative data and information should form an essential part of soil quality monitoring programs. The data and information indicate morphological and visual observations, which can be used by both farmers and scientists in the field to identify decline in soil quality and health. This qualitative data include: (i) soil crusting, reduced aggregation, and surface sealing as indicators of loss of organic matter; (ii) observation of rills, gulleys, stones on surface, uneven topsoil, and exposed roots as indicators of water erosion; (iii) ripple marks on topsoil, sand against plant stems, and damaged plants as indicators of wind erosion; (iv) growth of salt-tolerant plants and salt crusting as indicator of soil salinization; (v) growth of acid-tolerant plants and lack of plant response to fertilizer application as indicators acidification and chemical degradation of the soil; and (vi) water stagnation and poor and patchy crop stands as indicators of poor drainage and compact-hardpan structure of the soil (Arshad and Cohen, 1992).

Dang (2007) conducted a survey with 42 randomly selected farmers with at least 15 years working experience on tea farms, where soil samples were collected. From the information gathered, the farmers ranked soil organic matter content, soil compaction and fertility as the key indicators of soil health and quality. Their assessments of some of these indicators were in agreement with data obtained with a quantitative approach. The criteria used by the farmers to evaluate changes in soil quality are described in Table 2.

Table 2. Farmers' perceptions of selected soil quality indicators, adapted from Dang (2007).

Indicators	Description used by farmers
Soil organic matter	Soil feels good to touch and dark in colour
Soil chemical fertility	Based on observable plant growth and yeild response
Soil acidity	Observation of the presence of specific weed species in the field
Soil compaction	Soil feels tough or hard when hoeing or ploughing
Soil moisture	Observing the leaves at noon and evening and soil feels moist when touched
Surface (A horizon) thickness	The depth of dark coloured soil when hoeing or ploughing
Soil erosion	Observable changes in the soil surface after rain and year to year comparison of topsoil depth when ploughing at upper and lower slope positions
Soil structure	Based on observable changes during hoeing or ploughing
Earthworm population	Based on observed earthworm casts at the soil surface in the morning or after rainfall
Weed incidence	Based on evidence of weed species and communities in the field

3.3. Quantitative indicators

Analytical indicators are quantitative and precise, using specific units as descriptors. Quantitative indicators of soil quality are based on measurable diagnostic properties (Harris and Bezdicek, 1994). Therefore, analytical indicators are more accepted by scientists and technical experts (Harris and Bezdicek, 1994). However, due to the fact that it is not possible to measure all inherent properties and attributes that influence soil quality, Larson and Pierce (1991) suggested a basic minimum data set (MDS). This data set consists of soil physical, chemical and biological properties for assessing the quality of soils, which can be of practical use to farmers, scientists as well as to policymakers.

Subsequently, Doran and Parkin (1994) proposed a specific set of criteria to guide farmers, scientists and policymaker in the choice of indicators. According to Doran and Parkin (1994) indicators of soil quality should integrate soil physical, chemical, and biological processes and properties, which include ecosystem processes and relate to process-oriented modelling. Furthermore, it must be accessible to many users and applicable to field conditions and most importantly, it must be sensitive to variations in management and climate, and where possible, soil indicators should be components of existing soil databases. Table 3 summarizes sets of basic indicators of soil quality that meet the criteria of Larson and Pierce (1991) and Doran and Parkin (1994). These indicators are grouped into physical, chemical and biological indicators.

Table 3. Key soil analytical indicators of soil quality.

Physical	Chemical	Biological
Bulk density	pH	Microbial biomass
Soil Texture	Electrical conductivity	Potentially mineralizable N
Water infiltration rate	Nutrient availability	Soil respiration
Soil and rooting depth	Organic matter	
water holding capacity		

Doran and Parkin, 1994; Larson and Pierce, 1991.

3.3.1. Physical indicators

Soil physical properties such as texture, bulk density, soil depth, water infiltration rate and holding capacity can serve as indicators of healthy soils. The roles of several of the physical

indicators are influenced by other parameters or inherent properties of the soil. For instance, water infiltration rate can influence chemical properties such as pH, electrical conductivity and nutrient availability.

3.3.1.1. Bulk density

Bulk density is used as an indicator to investigate soil compaction or loosening, which is directly related to soil porosity. This parameter expresses the relationship between dry soil mass and its bulk volume (Grossman and Reinsch, 2002). Likewise, it enables gravimetric moisture content to be put in terms of volumetric moisture (Are *et al*., 2009). This, in turn, gives insight into the water storage profile, structural condition and compactness of the soil (Hernanz *et al*., 2000). However, optimal and critical limits of soil bulk density are dependent on the soil texture, particle size, management practices and organic matter content (Etana *et al*., 1999; Are *et al*., 2009; Reichert *et al*., 2009).

Bulk density has great influence on the soil structure, the movement of air and water, as well as mechanical resistance of the soil. Various cultivars and crops respond differently to variation in soil bulk density or its degree of compactness (Stirzaker *et al*., 1996; Guimarães *et al*., 2002). At critical bulk density the growth of plant roots is inhibited or crop yield is reduced (Stizaker *et al*., 1996; Beutler and Centurion, 2004; Beutler *et al*., 2004; Secco *et al*., 2004).

3.3.1.2. Soil Texture

Soil texture refers to the relative amounts of sand, silt, and clay in a specific type of soil (Gee and Bauder, 1986). Soil textural properties vary in relation to initial mineralogy parent weathering material and weathering rates. Thus, soil texture tends to vary at local scales along topographic gradients and at regional or landscape scales, in association with changes in parent material or the rate at which weathering occurs (Silver *et al*., 2000). In total, there are twelve generic soil textural classes, based on the United State Department of Agriculture (USDA) classification, however, soil texture can be classified under three major size classes namely clay (< 0.002 mm), silt (0.05 -0.002 mm), and sand (2.0-0.05 mm) (Gee and Bauder, 1986).

Several studies have shown that soil texture influences characteristics of the soil microenvironment. Campbell *et al*. (1996) reported a positive correlation between clay and soil organic matter (SOM) content with greater SOM observed in non-tilled soil than in the conventional tillage soils at three sites in western Canada. Similarly, soil texture influences soil aggregation (Schlecht-Pietsch *et al*., 1994; Lado *et al*., 2004; Mamedov *et al*., 2007) in such a way that increased clay content was associated with increased soil aggregation. Increasing soil aggregation directly affects soil carbon storage by occluding organic materials, making them inaccessible to enzymatic or microbial degradation (Plante *et al*., 2006).

3.3.1.3. Water infiltration rate

Water retention and flow dynamics in soil is a major stimulant of crop growth, nutrient cycling, and transportation of contaminants (Haws *et al*., 2004). Infiltration is one of the most important processes in the water cycle. It controls the soil-water available for plants, the transportation of nutrients and pesticides as well as the amount of runoff and soil erosion (Haws *et al*., 2004; Lado *et al*., 2005). Increased organic matter is known to correlate with an increase in soil infiltration and water-holding capacity. This has a major impact on soil water management. Under this condition, organic matter (crop residues) reduces the rate of runoff water and facilitates infiltration via macropores, plant root holes and earthworm channels (Edwards *et al*., 1988).

Studies have shown that water infiltration is faster in soils with earthworms than in soils without earthworms (Willoughby *et al*., 1997). Soil organic matter primarily helps to stabilize soil aggregates, and the extent of aggregation within soil influences porosity of the soil and its capacity to retain plant-available water (Karlen and Stott, 1994). Nonetheless, agricultural management practices, especially soil tilling, disrupts soil surface aggregates, resulting in various degrees of crusting, increased runoff and a subsequent increase in soil erosion (Agassi *et al*., 1981; Karlen and Stott, 1994; Ben-Hur and Assouline, 2002; Assouline, 2004; Gregory *et al*., 2005; Lado *et al*., 2005). A crust develops when soil aggregates disintegrate and its fine particles block pore spaces in the soil.

3.3.1.4. Soil and rooting depth

Soil depth provides a direct measure of the ability of a specific soil to support plants (Singer and Ewing, 1999). The distance from the soil surface to restrictive layers can be referred to as the effective soil depth (ESD). Most soil processes that affect soil quality are confined within this depth (Rhoton and Lindbo, 1997). An effective soil depth provides adequate zones for plant roots to explore for nutrient and has greater capacity to retain water and plant nutrients compared to shallow soils (Singer and Ewing, 1999; Troeh and Thompson, 2005). For instance, plants can survive a long period of drought when they grow on soils with effective depth, due to the ability of the soil to retain more water (Troeh and Thompson, 2005).

The depth of a plant rooting system can also serve as a good indicator of soil quality. Plant roots are mostly restricted to the zone of stored water in the soil (Rhoton and Lindbo, 1997), and their growth is influenced by soil compactness (Aggarwal *et al*., 2006). Aggarwal *et al*. (2006) investigated the variation in soil strength and rooting properties of wheat in relation to soil management systems. They observed a significantly higher root volume density and root surface area density in a bed planting system compared to that of the conventional method. Likewise, the root length density of 0-30 cm soil layers in bed planting was about 22 % higher than conventional flat planting system. These observed differences were attributed to the tilling of the soil, which reduced the degree of soil's compactness, improved soil depth, and enabled more root growth or root penetration (Aggarwal *et al*., 2006).

3.3.1.5. Water holding capacity

The water holding capacity of a soil is the volume of water that can be stored in a form accessible or available for plants use. Often, most soil profiles are able to store between 2.0 to 10.0 inches of available water (Troeh and Thompson, 2005). The ability of a specific type of soil to hold or retain water depends greatly on the texture of the soil. The finer the soil's texture, the higher its ability to hold or retain water for plant use (Lavelle and Spain, 2001; Troeh and Thompson, 2005). Fan *et al.* (2005) investigated the long-term effect of fertilizer application, and available-water on soil chemical properties as well as cereal yield in Northwest China. Water availability was reported to have had a great influence on wheat and corn grain yields over the six years. However, the addition of organic matter to some of the treatments resulted in an improved water-holding capacity and improved grain yield.

3.3.2. Chemical indicators

Soil chemical indicators hold a crucial link between the physical properties and fertility or productivity of soil. The various chemical reactions that maintain soil pH, electrical conductivity, nutrient availability, and organic matter content are indispensable for sustaining soil quality. Similar to the physical indicators, soil chemical indicators are interdependent. One indicator can modify or influence other indicators.

3.3.2.1. Soil pH

Soil pH has been identified as the principal indicator of the chemical characteristic of a particular soil (Sinsabaugh *et al*., 2008). It plays a significant role in all biogeochemical processes, as well as in microbial and enzymatic activity in the soil (Brady and Weil, 2002; Pietri and Brookes, 2008a; Sinsabaugh *et al*., 2008). Soil pH influences the solubility of soil macronutrients, micronutrients or essential trace elements including aluminium (Al), that can be potentially toxic to plants at elevated concentrations (Gramss and Bergmann, 2007; Naramabuye and Haynes, 2007). Acidification of the soil results in leaching of nutrients and releases aluminium in solubilized forms from its insoluble state (Marschner, 1995), which, in turn affects plant's uptake of cations, induces organic acid secretion, and inhibits cell division and growth in the roots (Minocha and Minocha, 2005). This change in pH invariably affects the availability of plant nutrients, microbial processes in the soil (Plante, 2007; Pietri and Brookes, 2008b), as well as the rate of organic compound decomposition within the soil (Leifeld *et al*., 2008; Yao *et al*., 2009).

3.3.2.2. Electrical conductivity

Electrical conductivity (EC) of a soil is a measure of the number of ions or dissolved salts present in the soil solution (Arias *et al*., 2005). A salty or saline soil will have a very high electrical conductivity (Troeh and Thompson, 2005). Increased soil salinity suppresses plant growth, reduces crop yield, and the soil-water balance (Fitter and Hay, 1981; De Pascale and Barbieri, 1997; Ahmed, 2009). Salinity of soil reduces water up-take by plants due to reduction in the osmotic potential. This may cause an upset in the nutritional balance or result

in ionic toxicity (Fitter and Hay, 1981; Corwin and Lesch, 2003). Furthermore, the composition of the salt in soil water affects the soil's cation exchange capacity. This, in turn, influences soil tilth and permeability, depending on the exchangeable cation composition and the level of the soil salinity (Corwin and Lesch, 2003).

3.3.2.3. Nutrient availability

Generally, there are seventeen essential elements associated with plant growth and productivity, of which carbon, hydrogen, and oxygen are obtained by plants through water and air (Troeh and Thompson, 2005). The other elements are further divided into six macronutrients and eight micronutrients. The macronutrients are the elements required in large quantities by plants, and include nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur (Troeh and Thompson, 2005; Naramabuye and Haynes, 2007). The micronutrients are those elements required in trace amounts by plants and include boron, chlorine, copper, iron, manganese, molybdenum, nickel and zinc (Troeh and Thompson, 2005).

Nutrient availability is a crucial soil property. It influences plant productivity, water quality and can serve as an indicator of soil health (Soltanpour and Delgado, 2002; de Rouw and Rajot, 2004; da Silva *et al*., 2008). For instance, grape production and the quality of wine are directly affected by nutrient availability. Elements such nitrogen (N) affects both the production and quality of berries; boron (B) influences the size and number of berries; and zinc (Zn) favours the retention of bunches onto the branches (da Silva *et al*., 2008). Furthermore, in a three year field experiment conducted by De Rouw and Rajot (2004), on a pearl millet field with different farming systems, they observed a reduction in nutrient levels and grain yield over time, with the exception of the fields exposed to dung treatment. Dung input maintained a yield of 350 Kg ha^{-1} and a stable nutrient supply under prolonged cropping of 10 to 17 years. The observable differences in nutrient levels and grain yield was attributed to dung application early in the season. It protected the soil mechanically, by reducing crusting and trapping mobile soil during storms.

3.3.2.4. Organic matter

Soil organic matter plays a crucial role in the functioning of agricultural ecosystems, ecosystem productivity and in the global C cycle (Loveland and Webb, 2003; Weil and Magdoff, 2004; Pan *et al*., 2009). It comprises organic materials, such as tissues of living organisms, altered plant and animal organic residues, and decomposed plant and animal tissues. Soil organic matter is subdivided into three groups, humic acid, fulvic acid, and humin substances (Wander, 2004). Humic acid occurs in soluble state in alkali solvent, but is precipitated on acidification of the alkaline extract. Fulvic acid is the humic fraction which remains in solution after the acidification of the alkaline extract and it is soluble in both alkaline and acidic dilute, while, humin, is the fraction of the humic substances that can not be extracted from the soil by alkaline or acidic solvent (Schnitzer, 1978). However, a descriptive approach can also be used to group soil organic matter into pools or stages; this includes active, intermediate and passive pools (Wander, 2004). This division is dependent on the biological, physical, and chemical processes taking place within the soil organic matter. The functional importance of soil organic matter of different ages varies, with the youngest organic matter being most biologically active and intermediate age influences the physical status of soil (Wander, 2004).

The role of soil organic matter in the quality and health of soil is significant because it influences other important physical, chemical, and biological properties of soil (Rahimi *et al*., 2000; Oorts *et al*., 2003; Abu-Zahara and Tahboub, 2008), as well as crop productivity (Pan *et al*., 2009). Rahimi *et al*. (2000) investigated the effect of varying amounts of organic matter on soil electrical conductivity (EC) and its sodium adsorption ratio (SAR). They observed among others a significant correlation between the soil tensile strength and the amount of organic matter input. The soil with a higher amount of organic matter showed greater tensile strength and had a higher EC and SAR compared to soil with low input of organic matter. The study confirms the role and influence of organic matter on soil chemical properties. Similarly, Oort *et al.* (2003) reported a significant contribution of soil organic matter to the cation exchange capacity (CEC) of a tropical soil. In their study, soil samples were collected from a twenty year old arboretum established on a Ferric Lixisol with seven multipurpose tree species. Upon chemical analysis of the soil pH and CEC, they observed about 85 % variation among soil samples and this was associated with carbon content to which organic matter content plays a major role.

3.3.3. Biological indicators

Biological indicators or soil organisms are sensitive to anthropogenic disturbance, and climate change. Indicators such as microbial biomass, potentially mineralized nitrogen, and soil respiration are sensitive to various induced stress factors over a period of time, and have served as a good measure of the quality and health of soil (Ritz and Wheatley, 1989; Dai *et al*., 2004; Kruse *et al*., 2004).

3.3.3.1. Microbial biomass

Soil microbial biomass is the active component of soil organic pool (Henrot and Robert, 1994). It plays a crucial role in organic matter decomposition as well as in nutrient transformation and consequently influences ecosystem productivity (Maithani *et al*., 1996; Franzluebbers *et al*., 1999). According to Insam (2001), microbial biomass is an important indicator of soil productivity and its evaluation is invaluable in soil ecological studies. The knowledge acquired is also fundamental to sustaining the environment and productivity. Studies have shown that soil microbial biomass is often influenced by soil depth, seasonal fluctuation, pH, heavy metal deposition and land management practices (Dai *et al*., 2004; Calbrix *et al*., 2007; Vásquez-Murrieta *et al*., 2007). High concentrations of heavy metals are known to affect the morphology, metabolism and growth of microorganisms in soils (Giller *et al*., 1998), as they disrupt the integrity of their cell membranes and cause protein denaturation (Leita *et al*., 1995). Furthermore, microbial biomass has been reported to correlate positively with yield in organic farming compared to conventional farming systems (Mäder *et al*., 2001).

3.3.3.2. Potentially mineralizable nitrogen (N)

Nitrogen (N) is an essential plant nutrient, and significantly influences agricultural productivity (Picone *et al*., 2002). In most soils, a significant ratio of available N is derived from mineralization of the soil organic matter (Cabrera *et al*., 1994; Kerek *et al*., 2003). Soil organic N is trapped in an heterogeneous mixture of components which includes stable humic substances, microbial metabolites adsorbed to soil colloids, microbial biomass, as well as

animal and crop residues (Campbell, 1978). Microorganisms play a part in the mineralization or release of the organic N.

Potentially mineralizable nitrogen is the amount of soil organic nitrogen that is mineralized and available during plant growth (Stevenson and Braids, 1968). Other than organic matter residues, factors such as soil pH, heavy metal deposition, temperature, water content, and excessive fertilizer N input affects N mineralization (Higby and Bell, 1999; Khan and Scullion, 2002; Kruse *et al*., 2004). Changes in soil water content due to drying and rewetting have been reported to affect N mineralization. In a study conducted by Kruse *et al*. (2004), they observed after 185 days that cotton (*Gossypium hirsutum* L.) leaves decomposing in continuously moist soils resulted in mineralization of 30 % of the applied N. In contrast, cotton leaves subjected to a 14 day drying-rewetting cycle after 185 day resulted in reduced N mineralization. Research has also shown that an increase input of fertilizer N increases the amount of N mineralized from soil organic matter (Higby and Bell, 1999).

3.3.3.3. Soil respiration

Soil respiration involves the oxidation of organic matter to the eventual production of carbon-dioxide (CO_2) and water as end products. The oxidation process is mostly mediated by soil aerobic microorganisms, which makes use of oxygen as electron acceptor. Thus, the metabolic activities of soil microbial communities can be quantified by measuring the amount of carbon-dioxide produced or oxygen (O_2) consumed in a given soil (Nannipieri *et al*., 1990). Soil respiration can be subdivided into basal respiration and substrate-induced respiration (Alef, 1995). Basal respiration refers to respiration that occurs without the addition of organic substrate to the soil (Ritz and Wheatley, 1989; Vanhala *et al*., 2005), while substrate-induced respiration refers to respiration that occurs in the presence of added substrate (Ritz and Wheatly, 1989; Alef, 1995). The measurement of soil respiration rates has been used in the assessment of the side effects of heavy metals and pesticide accumulation, and various amendments such as, addition of sewage sludge or other forms of substrates in the soil (Doelman and Haanstra, 1979; Debosz *et al*., 1985; Ritz and Wheatley, 1989; Prasad *et al*., 1994; Lin and Brookes, 1999; Fernandes *et al*., 2005).

4.0. Assessments of soil microbial communities

Understanding the response of soil microbial community composition to agricultural management practices over time will help to evaluate the effect of the practices on soil quality. However, the qualitative and quantitative description of soil microbial communities is one of the most difficult challenges facing microbial ecologists (Crecchio *et al*., 2007). Thus, there is the need for accurate and reliable mechanisms to study soil microorganisms before we can focus on how changes in microbial community structure affect ecosystem functions (Kirk *et al*., 2004). Several microbiological and molecular methods have been adopted over time to study microbial diversity in agricultural soil (Turco *et al*., 1994; Ibekwe and Kennedy, 1998; van Elsas *et al*., 1998; Muyzer and Smalla, 1999; Classen *et al*., 2003; Keer and Birch, 2003), and can be grouped into culture-dependent and –independent methods.

4.1. Culture-dependent methods for assessing microbial diversity

Culture-based techniques currently are insufficient to answer all questions posed by microbial ecologists. It is often observed that direct microscopic counts exceed the total viable cell counts by several orders of magnitude. One gram of soil may contain more than 10^{10} bacteria as counted under a fluorescence microscope after staining with fluorescent dyes (Torsvik *et al*., 1990). Culturing conditions often select a distinct subpopulation of the microbial community, which only accounts for 1-10% of total microbial communities (Torsvik *et al*., 1998).

4.1.1. Viable cell count

Traditionally, analysis of soil microbial diversity depends on the ability of cells to grow and form visible colonies on solid media. This involves the use of a wide array of selective media and direct viable cell counts on plates (van Elsas *et al*., 1998; Keer and Birch, 2003). This approach is fast, inexpensive and can provide basic information about the active, heterotrophic microbial diversity within the population (Kirk *et al*., 2004). However, under certain circumstances the number of viable microorganisms is often under-represented by this

method. For instance the fastidious unculturable section of the population (Ward *et al*., 1990; Trevors, 1998), sub-lethally damaged organisms (Blackburn and McCarthy, 2000), and viable cells that have lost their ability to form colonies under the culturing conditions (Keer and Birch, 2003) will not be detected. Growth conditions such as light, pH, temperature, lowering level of oxygen concentration, substrate concentration, as well as depth of media in plates equally influence the outcome of viable cell counts (Xenopoulos and Bird, 1997; Olson *et al*., 2000; Bussmann *et al*., 2001; Keer and Birch, 2003).

4.1.2. Sole carbon source utilization (SCSU)

Sole carbon source utilization technique or community-level physiological profiles (CLPP) was developed originally for the characterization of clinical bacterial isolates, using commercially available ninety-six well microtitre gram-negative (GN) and gram-positive (GP) bacterial plates (Garland and Mills, 1991). Subsequently, the Eco-plate system was introduced by Biolog (Hayward, CA, USA) containing three replicas of thirty-one different environmentally important carbon sources and one control well per replicate (Choi and Dobbs, 1999). The indicator substrate, tetrazolium salt changes colour as the carbon source is metabolized. However, many fungal species are not able to reduce this indicator salt. Hence, fungal specific plates SFN2 and SFP2 without the tetrazolium were developed by Biolog (Dobranic and Zak, 1999; Classen *et al*., 2003).

In addition, substrate utilization in fungal plates is measured turbidimetrically (Buyer *et al*., 2001), and antibiotics are added to the inoculating media to reduce the impact of bacteria on the fungal substrate utilization pattern (Dobranic and Zak, 1999; Buyer *et al*., 2001). The inoculated microbial populations are monitored over time for their ability to utilize the carbon source and the speed at which the carbon source is utilized. The data generated is subjected to multivariate analysis and relative differences between soils functional diversity can be inferred (Kirk *et al*., 2004).

Sole carbon source utilization technique, has been used successfully to characterize potential metabolic diversity of microbial communities in soil treated with herbicides (Lupwayi *et al*., 2009a; Mijangos *et al*., 2009), soil amended with calcium cyanamide ($CaCN_2$) and other forms of fungicides (Lupwayi *et al*., 2009b; Shi *et al*., 2009), soil contaminated with heavy

metal (Harris-Hellal *et al*., 2009) and in compost biofilters (Grove *et al*., 2004) among others. Lupwayi *et al*. (2009a) used the Biolog Ecoplate® with enzyme-linked immunosorbent assay (ELISA) plate reader to characterize soil microbial community response to herbicides applied to glyphosate-resistant canola. They observed significant differences in the functional structure of the bacteria community.

Sole carbon source utilization has advantages over plate counts in that, it can differentiate between microbial communities (Grove *et al*., 2004; Harris-Hellal *et al*., 2009; Shi *et al*., 2009). It is relatively easy to use, and a large volume of data can be generated reflecting the potential metabolic characteristics of the soil metabolic diversity (Garland and Mills, 1991; Zak *et al*., 1994). However, the methods still rely only on culturable microorganisms with their ability to grow under experimental conditions (Garland and Mills, 1991). It is sensitive to microbial or inoculum load (Garland, 1996), and often favours fast growing microbial communities (Yao *et al*., 2000).

Sole carbon source utilization gives an idea of the potential metabolic diversity and not the real metabolic diversity *in situ* (Garland and Mills, 1991). For instance, the carbon sources may not be an adequate representative of the carbon sources available in the soil (Yao *et al*., 2000). Furthermore, species representing only a minority in the microbial community population *in situ* may possess a competitive edge within the Biolog well and the data obtained may overestimate the contribution of this species in the soil (Kirk *et al*., 2004). This questions the interpretation and reliability of the data and the information. Nonetheless, sole carbon source utilization is useful when investigating the functional diversity of soils and is a valuable tool especially when used together with other methods, such as the combination of Biolog Ecoplates and PCR-DGGE technique (Mijangos *et al*., 2009).

4.2. Culture-independent methods for assessing microbial diversity

Culture-independent methods such as phospholipid fatty acid analysis (PLFA), and fatty acid methyl ester (FAME) analysis, and various advancements in molecular biology, have enhanced our ability to investigate the unculturable soil microbial communities. Culture-independent approaches give a greater resolution of microbial diversity and are more sensitive compared to the culture-dependent methods. The various culture-independent

approaches provide more information on the soil microbial community structure in comparison to culturing techniques (Muyzer *et al*., 1993; Ibekwe and Kennedy, 1998; Schwieger and Tebbe, 2000; Donegan *et al*., 2001).

4.2.1. Biochemical methods

Phospholipid fatty acids make up a relatively constant proportion of cell biomass of organisms in naturally occurring communities (Lechevailer, 1989). Each taxonomic group possesses a unique fatty acid or signature fatty acid, which serves as a marker to differentiate a taxonomic group from other groups within a population. Thus, a change in the phospholipid fatty acid pattern in a soil sample would indicate a change in microbial population of that soil sample (Ibekwe and Kennedy, 1998; Kirk *et al*., 2004). This technique in principle does not rely on culturing of soil microbes, but provides information on the soil microbial community structure based on grouping of fatty acid profiles (Ibekwe and Kennedy, 1998).

There are two approaches to this technique, namely fatty acid methyl ester (FAME) or phospholipid fatty acid (PLFA) analysis. FAME profiles are based on all fatty acids extracted, which include both polar and non-polar fatty acids. With FAME analysis, fatty acids are extracted directly from soil samples, methylated and quantified by gas chromatography (Ibekwe and Kennedy, 1998; Zelles, 1999; Buyer, 2006). In PLFA profiles, the polar phospholipids are separated from the non-polar lipids via exchange columns (Bååth *et al*., 1995).

Fatty acid methyl ester analyses have been used to compare microbial community structures and populations of different soil types, such as soil contaminated with heavy metals (Ellis *et al*., 2001), chemically perturbed soil (Zelles *et al*., 1994; Kozdrój and van Elsas, 2001) as well as soil exposed to different agricultural practices (Ibekwe and Kennedy, 1998; Steger *et al*., 2003). Ellis *et al*. (2001) combined community fatty acid methyl ester (C-FAME), dehydrogenase enzyme activity measurements, CLPP, and plate counts to investigate the impact of long term heavy metal contamination on soil microbial communities. Community fatty acid methyl ester (C-FAME) analysis revealed a distinct difference between sampling stations and these results were correlated well with other techniques used in the study.

FAME analysis has shown relative success in the study of microbial diversity composition. However, the technique is burdened with limitations. For example, cellular fatty acid composition may be influenced by factors such as availability of nutrients and temperature (Graham *et al*., 1995). Fatty acids extracted from soil samples may also include that of dead microorganisms, plant residues and roots or other soil organisms (Jandl *et al*., 2005), resulting in a complex FAME profiles. In addition, when studying fungal diversity, fungal biomass may be underestimated due to the limited number of signature fatty acids for fungi (Marschner, 2007). FAME profiles have no taxonomic significance because individual fatty acids cannot be used to represent specific species and microbial populations can have similar fatty acids (Bossio *et al*., 1998).

4.2.2. Polymerase Chain Reaction (PCR)-based methods

Molecular-based approaches for ecological studies initially relied on cloning of target genes isolated from environmental samples (Muyzer and Smalla, 1999), which is a tedious and time consuming routine. Advancement in the field of molecular biotechnology, has aided the development of cutting-edge methodology in the field of microbial ecology. These molecular approaches are generally based on PCR or real time (RT)-PCR, targeting generic or specific rRNA (16S and 18S) subunits, internal transcribed spacer (ITS) regions or their rDNA genes which serves as useful molecular markers for prokaryotes and eukaryotes.

4.2.2.1. Denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE)

Denaturing gradient gel electrophoresis (DGGE) was originally developed by the medical community to investigate point mutations in DNA sequences (Lerman *et al*., 1984; Borresen *et al*., 1988). However, Muyzer *et al*. (1993) extended the use of DGGE to study genetic diversity in microbial populations. This approach is based on separation of PCR-amplicons via electrophoresis in polyacrylamide gel containing a linearly increasing gradient of denaturants urea and formamide. DNA fragments of similar length but different base-pair sequences can be separated based on the melting point of their double-stranded DNA (Lerman *et al*., 1984; Miller *et al*., 1999; Muyzer, 1999). A GC clamp is added to the PCR-

amplicons during amplification process to ensure that part of the DNA remains double stranded. Thereby, improving the quality of bands obtained on the gel. Upon denaturation the DNA fragment melts at specific points, and moves differentially through the gel (Torsvik *et al*., 1998; Muyzer, 1999; Shishido *et al*., 2008). Temperature gradient gel electrophoresis (TGGE) works on the same basic principle as DGGE, but the gradient denaturant is temperature and not chemical (Muyzer, 1999).

These techniques have been successfully applied in microbial ecology, to characterize genetic diversity of microbial communities in the rhizosphere of soil exposed to diverse anthropogenic disturbances such as herbicide applications (Mijangos *et al*., 2009), agricultural practices (Liu *et al*., 2007; Postma *et al*., 2008; Shishido *et al*., 2008), and soil contaminated or amended with heavy metal or sludge (Sheppard *et al*., 2005; Anderson *et al*., 2008; Harris-Hellal *et al*., 2009).

DGGE or TGGE have various advantages over the culture-base methods. It is a rapid and reproducible technique. Multiple samples can be run concurrently, making it possible to follow shifts in microbial community composition (Muyzer, 1999). After electrophoresis visible bands on the gel can also be excised and sequenced (Yang and Crowley, 2000; Nakatsu *et al*., 2005; Joynt *et al*., 2006). This reduces the ambiguity of band identification, and help to identify specific populations potentially responsible for observable changes (Nakatsu *et al*., 2005; Joynt *et al*., 2006).

However, there are several limitations in using PCR-DGGE for microbial community assessments. The biggest challenge when performing DGGE is gel to gel comparisons. It is very difficult to accurately reproduce gel gradients, and a reproducible result can only be achieved by minimizing variation between gel gradients (Fromin *et al*., 2002). This can be achieved by the use of computer-assisted characterization of the banding patterns and the application of statistical approach to data obtained (Fromin *et al*., 2002). DGGE markers often have an insufficient number of bands to span the whole gradient, making gel-to-gel comparison difficult (Nakatsu, 2007). Therefore, sufficient diversity of organisms most be chosen when making standard markers, to ensure that marker bands will span the whole gradient (Nakatsu, 2007).

Being a PCR-based approach, DGGE is influenced by PCR biases such as the choice of primers, numbers of cycles, annealing temperatures, formation of chimaeric PCR products,

and inhibition of the polymerase enzyme by humic substances within the reaction mix (von Wintzingerode *et al*., 1997). This can result in inconsistent variation in copy numbers and the inclusion of artefacts that can influence data interpretations of DGGE profile. Another limitation of DGGE is the inability of the technique to resolve complex microbial communities. For example, a complex microbial community such as that found in soils may contain numerous populations of approximately 10^{10} colony forming units (Torsvik *et al*., 1990). This results in a smear which makes it difficult to identify individual bands on the gel (Nakatsu *et al*., 2000). It is impossible for DGGE to detect the entire microbial diversity in a given soil sample. Similarly, the number of observable bands in a profile cannot be interpreted to be the exact numbers of populations in a community. Studies have revealed that a single microbial isolate can produce multiple banding patterns on DGGE (Nübel *et al*., 1996; Satokari *et al*., 2001). In addition, variation in DNA extraction efficiency can influence the banding patterns on DGGE gels (Maarit-Niemi *et al*., 2001).

4.2.2.2. Single strand conformation polymorphism (SSCP)

Similar to DGGE/TGGE, the single strand conformation polymorphism (SSCP) technique was initially developed to detect point mutations in DNA (Orita *et al*., 1989a, 1989b). SSCP has been used extensively in the medical field (Walsh *et al*., 1995; Donnelly *et al*., 2002; Jeffery *et al*., 2007), and in the identification of plant-pathogenic fungi (Kumeda and Asao, 1996; Kong *et al*., 2003). SSCP is a gel-based approach and relies on electrophoretic separation of DNA fragments. A single stranded DNA is separated on a polyacrylamide gel based on differences in mobility due to folded secondary structure (Nishigak *et al*., 1986; Lee *et al*., 1996). This technique has been extended to study microbial diversity such as rhizosphere microbial community structure (Schwieger and Tebbe, 2000; Dohrmann and Tebbe, 2005), microbial communities' population and genetic dynamics in contaminated soils (Klenistenber *et al*., 2006; Witzig *et al*., 2006), and bacterial community changes during milk and cheese production (Duthoit *et al*., 2003; Delbés *et al*., 2007).

The high rate of reannealing of DNA strands after an initial denaturation during electrophoresis is one of the major limitations of SSCP for the analysis of microbial communities (Selvakumar *et al*., 1997). This is crucial if high concentrations of DNA might be required for analysis of high-diversity communities, and loaded onto the gels. Another

disadvantage of SSCP is the appearance of multiple bands from a double stranded PCR product after electrophoresis. Characteristically, three bands are detected; two single strands and one double stranded DNA molecule (Schwieger and Tebbe, 1998), and multiple conformations of one single stranded may coexist in one gel (Tiedje *et al*., 1999). SSCP also has the same setbacks associated with DGGE or TGGE (Kirk *et al*., 2004).

4.2.2.3. Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP), also referred to as amplified ribosomal DNA restriction analysis (ARDRA) is another molecular technique use in the study of microbial diversity. This technique, which relies on rDNA fragment length polymorphisms, involves the digestion of the amplicons or rDNA sequences obtained via PCR using restriction enzymes. Different restricted fragment lengths obtained are separated on agarose or non-denaturing polyacrylamide gel electrophoresis (Ranjard *et al*., 2000; Kirk *et al*., 2004). The technique has been used to characterize changes in microbial community structure as a result of changes in environmental conditions and different soil types (Donegan *et al*., 2001; Sheppard *et al*., 2005; Soares *et al*., 2006; Matsuyama *et al*., 2007), as well as, exposure of soil to toxic compounds (Donegan *et al*., 1995; Smit *et al*., 1997).

Similar to DGGE, RFLP bands can be used for the taxonomic identification of microbial communities. Either by hybridization of the RFLP profile to specific probes (Ranjard *et al*., 2000) or by excising, purifying and re-amplification of bands from gels (Soares *et al*., 2006). However, it does not measure community diversity (Liu *et al*., 1997), and banding patterns in a highly diverse population may become too complex to analyse (Tiedje *et al*., 1999). In addition the choice of restriction enzymes is crucial for obtaining optimal resolution of rDNA fragment lengths. Preliminary test on enzyme(s) of choice must be conducted to ensure that optimal resolution is obtained in detecting shifts in microbial communities (Ranjard *et al*., 2000).

4.2.2.4. Terminal restriction fragment length polymorphism (T-RFLP)

Terminal restriction fragment length polymorphism (T-RFLP) follows the same principle as RFLP/ARDRA, except that fluorochrome-labelled primers, such as 6-FAM (phosphoramidite

fluorochrome 5-carboxyfluorescein), HEX (5-hexachloroflourescein) or TET (4, 7, 2', 7'-tetrachloro-6-carboxyfluorescein)-labelled primers are used instead of non-labelled primers. This makes it possible to detect the labelled terminal restriction fragment (Liu *et al*., 1997; Tiedje *et al*., 1999; Tiquia *et al*., 2002). The banding profiles obtained are simpler based on the numbers of bands, as the use of labelled primers limits the analysis to only the terminal fragments of the digestion (Marsh, 1999). This enables the analysis of complex communities and provides information on community diversity as each visible band represents an operational taxonomic unit (OTU) or ribotype (Liu *et al*., 1997; Ranjard *et al*., 2000). A sequence database of the 16SrDNA terminal restriction fragment for specific restriction enzymes on bacterial species has been generated and this makes it possible for profile to profile comparison of the bands in a given community profile (Marsh, 1999).

The T-RFLP approach has been automated, through the use of automated sequencing systems with laser detection of fluorescently labelled DNA fragments. This allows a high-throughput in which a large number of samples can be processed simultaneously (Osborn *et al*., 2000; Forney *et al*., 2004). The automated approach has significantly greater resolution and sensitivity compared to the electrophoretic systems of DGGE or SSCP (Marsh, 1999).

Like every other PCR-based method, T-RFLP is not exempted from biases associated with PCR reactions and products (von Wintzingerode *et al*., 1997). Often amplification of numerically dominant species may be favoured more than other species, due to the large quantity of available DNA template and in some instances different species have different genes in multiple copies (Liu *et al*., 1997). T-RFLP outcome can equally be influenced by DNA extraction techniques (Maarit-Niemi *et al*., 2001). They observed variation in band profile and resolution due to different DNA extraction and clean-up techniques. Similarly, a decrease in concentration of the template DNA can result in poor band resolution and a decline in the complexity of fragments obtained in the T-RFLP community profile (Osborn *et al*., 2000).

The choice of universal primers is not as universal as initially believed, they are based on 16S rRNA, 18S rRNA, or ITS database which until recently, contained only sequences obtained from culturable microorganisms (Baker *et al*., 2003; Forney *et al*., 2004). For instance, Baker *et al*. (2003) investigated the specificity of primers and the total number of 16S ribosomal database sequences available, which are complementary to primers developed for the

amplification of bacterial 16S rRNA genes. They observed that 16S rDNA library does not completely represent the true biodiversity of prokaryotes, and most of the primers were non-specific for Eubacteria. Therefore, data obtained may not be representative of the true microbial diversity in environmental sample (Liu *et al*., 1997; Baker *et al*., 2003; Forney *et al*., 2004), which results in underestimation of community diversity. In addition, incomplete restriction enzyme digest could result in an overestimation of microbial diversity (Osborn *et al*., 2000). Nonetheless, T-RFLP has been used to investigate the changes in soil microbial communities in response to agricultural amendments (Tiquia *et al*., 2002), to measure the impact of biotic and abiotic interactions of soil microbial community structure (Singh *et al*., 2007, 2009), and to distinguish between bacterial communities of two contrasting forest soils treated with lincomycin (Čermák *et al*., 2008).

4.2.2.5. Ribosomal intergenic spacer analysis (RISA) and Automated ribosomal intergenic spacer analysis (ARISA)

Ribosomal intergenic spacer analysis (RISA) is a PCR-based approach similar to RFLP and T-RFLP. It involves PCR amplification of the intergenic spacer (IGS) region between the small (16S) and large (23S) subunit rRNA operon with oligonucleotide primers. The region between the 16S-23S subunit rRNA, may encode tRNAs which display significant heterogeneity in both length and nucleotide sequence depending on the bacterial species (Fisher and Triplett, 1999). In RISA, the length heterogeneity of the intergenic spacer is detected using polyacrylamide gel electrophoresis, and the DNA fragments or bands are visualized by silver staining. This generates a complex banding pattern that provides a microbial community-specific profile and each DNA band represents one organism assemblage or operational taxonomic unit (OTU) (Fisher and Triplett, 1999).

RISA has been used to explore microbial diversity in soils, such as the impact of rhizodeposition on the spatial and temporal variation of bacterial communities along maize roots (Baudoin *et al*., 2002), the effect of seasonal fluctuations on the diversity of bacterial communities in agricultural soil (Meier *et al*., 2008), and the influence of heavy metal deposition on soil microbial communities in forest soil (Hartmann *et al*., 2005). Meier *et al*. (2008) were able to confirm a T-RFLP profile using RISA. Nonetheless, RISA is a very cumbersome, time-consuming, and relatively cost efficient. The preparation of the

polyacrylamide gel takes time and requires technical know-how. Also a large amount of PCR products is required for analysis in order to obtain a good resolution on the polyacrylamide gel, and silver staining is relatively insensitive in the detection of DNA fragments (Fisher and Triplett, 1999).

ARISA overcomes the some of limitations existing with the use of RISA. It involves the use of a fluorescence-labelled oligonucleotide primer for PCR amplification, and the electrophoresis step is subsequently automated using laser detection (Fisher and Triplett, 1999; Ranjard *et al*., 2001; Mora *et al*., 2003). The high resolution of the gels and sensitivity of the laser detection of fluorescence fragments makes the number of peaks detectable by ARISA much higher compared to RISA profile. Also, differences in the intensity of the bands can be estimated accurately with ARISA, which enables a better profile to profile comparison (Ranjard *et al*., 2001). From the profile obtained each band or peak represents different fragment sizes in base pairs, and are each regarded as operational taxonomic unit (OTU) (Fisher and Triplett, 1999; Hewson and Fuhrman, 2006).

ARISA as a fingerprinting tool has been used in various sectors of microbial ecology. These include the characterization of bacterial communities in aquatic environments (Arias *et al*., 2006; Fisher and Triplett, 1999), comparison of fungal communities from different salt marsh plants (Torzilli *et al*., 2006), investigation of the effect of heavy metal deposition on soil microorganisms (Gleeson *et al*., 2006; Ranjard *et al*., 2008), and to assess the effect of agricultural practices or land amendments on soil microbial community structure (Peixoto *et al*., 2006).

As a molecular tool that depends on amplification of DNA fragments, ARISA is also not exempted from familiar biases introduced by DNA extraction procedures and PCR amplification (von Wintzingerode *et al*., 1997; Maarit-Niemi *et al*., 2001). ARISA may underestimate microbial diversity by grouping similar ribotypes of unrelated origin together because of the similarity in length of the intergenic spacer (ITS) region (Fisher and Triplett, 1999; Crosby and Criddle, 2003). Similarly, the microbial genome may consist of multiple copies of the ITS region this results in an overestimation of microbial diversity, when a single organism contributes more than one peak to the ARISA profile (Nagpal *et al*., 1998; Crosby and Criddle, 2003). Despite all the shortcomings stated above, ARISA has proven to be a

relatively cost efficient, rapid, sensitive, and highly reproducible technique (Fisher and Triplett, 1999; Ranjard *et al*., 2001; Arias *et al*., 2006; Ranjard *et al*., 2008).

5.0. Purpose of study

Biodiversity is often associated with soil resilience to endure disturbance, and increase in the soil microbial community diversity has been reported to increase soil resilience capacity. The aim of this study is to evaluate the effect of various soil management practices on the soil microbial communities in apple orchards. This will be achieved by assessing the direction of change in the soil microbial community in relation to soil management practices. The crucial goal is to gain an understanding of the relative microbial diversity patterns of the different soil management systems in relation to their physical and chemical properties.

Referrences

Abu-Zahara, T. R. and Tahboub, A. B., 2008. Effect of organic matter sources on chemical properties the soil and yield of strawberry under organic farming conditions. *World Applied Sciences Journal* **5**, 383-388.

Ahmed, S., 2009. Effect of soil salinity on the yield and yield components of mungbean. *Pakistan Journal of Botany* **41**, 263-268.

Agassi, M., Shainberg, I. and Morin, J., 1981. Effect of electrolyte concentration and soil sodicity on the infiltration rate and crust formation. *Soil Science Society of America Journal* **45**, 848-851.

Aggarwal, P., Choudhary, K. K., Singh, A. K. and Chakraborty, D., 2006. Variation in soil strength and rooting characteristics of wheat in relation to soil management. *Geoderma* **136**, 353-363.

Aigner, D. J., Hopkins, J. and Johansson, R., 2003. Beyond compliance: sustainable business practices and the bottom line. *American Journal of Agricultural Economics* **85**, 1126-1139.

Alef, K., 1995. Soil respiration. In: Alef, K. and Nannipier, P. (eds.), 1995. Methods in applied soil microbiology and biochemistry. Academic Press, London, pp. 214-219.

Anderson, I. C., Parkin, P. I. and Campbell, C. D., 2008. DNA- and RNA-derived assessments of fungal community composition in soil amended with sewage sludge rich in cadmium, copper and zinc. *Soil Biology and Biochemistry* **40**, 2358-2365.

Andrews, S. S., Karlen, D. L. and Cambardella, C. A., 2004. The soil management assessment framework: A quantitative soil quality evaluation method. *Soil Science Society of America Journal* **68**, 1945-1962.

Are, K. S., Oluwatosin, G. A., Adeyolanu, O. D. and Oke, A. O., 2009. Slash and burn effect on soil quality of an Alfisoil: Soil physical properties. *Soil and Tillage Research* **103**, 4-10.

Arias, C. R., Abernathy, J. W. and Liu, Z., 2006. Combined use of 16S ribosomal DNA and automated ribosomal intergenic spacer analysis to study the bacterial community in catfish ponds. *Letters in Applied Microbiology* **43**, 287-292.

Arias, M. E., González-Pérez, J. A., González-Vila, F. J. and Ball, A. S., 2005. Soil health-a new challenge for microbiologists and chemists. *International Microbiology* **8**, 13-21.

Arshad, M. A. and Coen, G. M., 1992. Characterization of soil quality: Physical and chemical criteria. *American Journal of Alternative Agriculture* **7**, 25-32.

Arshad, M. A. and Martin, S., 2002. Identifying critical limits for soil quality indicators in agro-ecosystems. *Agriculture, Ecosystems, and Environment* **88**, 153-160.

Assouline, S., 2004. Rainfall-induced soil surface sealing: A critical review of observations, conceptual models and solutions. *Vadose Zone Journal* **3**, 570-591.

Backert, S. and Meyer, T. F., 2006. Type IV secretion systems and their effectors in bacterial pathogenesis. *Current Opinion in Microbiology* **9**, 207-217.

Bååth, E., Frostegård, A., Pennanen, T. and Fritze, H., 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood ash fertilized, clear-cut or burned coniferous forest soils. *Soil Biology and Biochemistry* **27**, 229-240.

Baker, G. C, Smith, J. J. and Cowan, D. A., 2003. Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods* **55**, 541-555.

Baudoin, E., Benizri, E. and Guckert, A., 2002. Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. *Applied Soil Ecology* **19**, 135-145.

Bento, F. M., Camargo, F. A. O., Okeke, B. C. and Frankenberger, W. T., 2005. Comparitive bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology* **96**, 1049-1055.

Ben-Hur, M. and Assouline, S., 2002. Tillage effects on water and salt distribution in a vertisol during effluent irrigation and rainfall. *Agronomy Journal* **94**, 1295-1304.

Beutler, A. N. and Centurion, J. F., 2004. Effect of soil compaction in root development and soybean yield. *Brazilian Journal of Agricultural Research* **39**, 581-588.

Beutler, A. N., Centurion, J. F., Silva, A. P., Roque, C. G. and Ferraz, M. V., 2004. Soil compaction and least limiting water range in dry-land rice yield. *Brazilian Journal of Agricultural Research* **39**, 575-580.

Blackburn, C. W. and McCarthy, J. D., 2000. Modifications to methods for the enumeration and detection of injured Escherichia coli O157:H7 in foods. *International Journal of Food Microbiology* **55**, 285-290.

Blume, E., Bischoff, M., Reichert, J. M., Moorman, T., Konopka, A. and Turco, R. F., 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. *Applied Soil Ecology* **20**, 171-181.

Borresen, A. L., Hovig, E. and Brogger, A., 1988. Detection of base mutations in genomic DNA using denaturing gradient gel-electrophoresis (DGGE) followed by transfer and hybridization with gene-specific probes. *Mutation Research* **202**, 77-83.

Bossio, D. A., Scow, K. M., Gunapala, N. and Graham, K. J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology* **36**, 1-12.

Bottomley, P. J. and Myrold, D. D., 2007. Biological N inputs. In: Paul E. A. (ed.), 2007. Soil microbiology, ecology and biochemistry, 3rd ed., Academic press, UK, pp. 365-388.

Brady, N. C. and Weil, R. R., 1999. The nature and properties of soils. Prentice Hall, Upper Saddle River, New Jersey, pp. 468-469.

Brady, N. C. and Weil, R. R., 2002. The Nature and Properties of Soil, 13th ed. Springer Netherlands, pp. 249.

Bussmann, I., Philipp, B. and Schink, B., 2001. Factors influencing the cultivability of lake water bacteria. *Journal of Microbiological Methods* **47**, 41-50.

Buyer, J. S., 2006. Rapid and sensitive FAME analysis of bacteria by cold trap injection gas chromatography. *Journal of Microbiological Methods* **67**, 187-190.

Buyer, J. S., Roberts, D. P., Millner, P. and Russek-Cohen, E., 2001. Analysis of fungal communities by sole carbon source utilization profiles. *Journal of Microbiological Methods* **45**, 53-60.

Cabrera, M. L., Vigil, M. F. and Kissel, D. E., 1994. Potential nitrogen mineralization: Laboratory and field evaluation. In: Havlin, J. L. and Jacobsen, J. S. (eds.), 1994. Soil testing: Prospects for improving nutrient recommendations. Soil Science Society of America Special Publication No. 40, Madison, WI, pp. 15-30.

Cai, P., Huang, Q., Chen, W., Zhang, D., Wang, K., Jiang, D. and Liang, W., 2007. Soil colloids-bound plasmid DNA: Effect on transformation of *Escherichia coli* and resistance to DNase I degradation. *Soil Biology and Biochemistry* **39**, 1007-10103.

Calbrix, R., Barray, S., Chabrerie, O., Fourrie, L. and Laval, K., 2007. Impact of organic amendments on the dynamics soil microbial biomass and bacterial communities in cultivated land. *Applied Soil Ecology* **35**, 511-522.

Campbell, C. A., 1978. Soil organic carbon, nitrogen and fertility. In: Schnitzer M. and Khan, S. U. (eds.), Soil Organic Matter. Elsevier, Amsterdam, pp. 173-271.

Campbell, C. A., McConkey, B. G., Zentner, R. P., Selles, F. and Curtin, D., 1996. Long-term effect of tillage and crop rotations on soil organic C and total N in a clay soil in southwestern Saskatche-wan. *Canadian Journal of Soil Science* **76**, 395-401.

Ceccherini, M., Poté, J., Kay, E., Van, V. T., Marechal, J., Pietramellara, G., Nannipieri, P., Vogel, T. M. and Simonet, P., 2003. Degradation and transformability of DNA from transgenic leaves. *Applied and Environmental Microbiology* **69**, 673-678.

Centre for International Agricultural Marketing and Development (CIAMD), 2001. Key deciduous fruit statistics.

Chenu, C., Hassink, J. and Bloem, J., 2001. Short-term changes in the spatial distribution of microorganisms in soil aggregates as affected by glucose addition. *Biology and Fertility of Soils* **34**, 349-356.

Choi, K. –H. and Dobbs, F. C., 1999. Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. *Journal of Microbiological Methods* **36**, 203-213.

Classen, A. T., Boyle, S. I., Haskins, K. E., Overby, S. T. and Hart, S. C., 2003. Community level physiology profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soils. *FEMS Microbiology Ecology* **44**, 319-328.

Coleman, N. T. and Thomas, G. W., 1967. The basic chemistry of soil acidity. In: Pearson R. W. and Adams F. (eds.), 1967. Soil acidity and liming. 1st ed. Madison, Wisconsin, pp. 1-41.

Conacher, J. and Conacher, A., 1998. Organic farming and the environment, with particular reference to Australia: a review. *Biology Agriculture Horticulture* **16**, 145-171.

Corwin, D. L. and Lesch, S. M., 2003. Application of soil electrical conductivity to precision agriculture: Theory, principles, and guidelines. *Agronomy Journal* **95**, 455-471.

Crecchio, C., Curci, M., Pellegrino, A., Ricciuti, P., Tursi, N. and Ruggiero, P., 2007. Soil microbial dynamics and genetic diversity in soil under monoculture wheat grown in different long-term management systems. *Soil Biology and Biochemistry* **39**, 1391-1400.

Crosby, L. D. and Criddle, C. S., 2003. Understanding the bias in microbial community analysis due to rrn operon copy number heterogeneity. *BioTechniques* **34**, 790-802.

Crouch, I., 2003. Post harvest apple practises in South Africa. Proceeding: Washington tree fruit postharvest conference. 2nd and 3rd December, 2003, Wenatchee, Washington.

Curl, C. L., Fenske, R. A. and Elgethum, K., 2003. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. *Environmental Health Perspectives* **111**, 377-382.

Curl, C. L., Fenske, R. A., Kissel, J. C., Shirai, J. H., Moate, T. F., Griffith, W., Coronado, G. and Thompson, B., 2002. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and children. *Environmental Health Perspectives* **110**, A787-792.

Çakir, Ş. and Sarikaya, R., 2005. Genotoxicity testing of some organophosphate insecticides in the Drosophila wing spot test. *Food and chemical Toxicology* **43**, 443-450.

Čermák, L., Kopecky, J., Novotná, J., Omelka, M., Parkhomenko, N., Plháčková, K. and Ságová-Marečková, M., 2008. Bacterial communities of two contrasting soils reacted differently to lincomycin treatment. *Applied Soil Ecology* **40**, 348-358.

Da Silva, M. A. G., Pavan, M. A., Muniz, A. S., Tonin, T. A. and Pelizer, T., 2008. Nutrient availability in soil and its absorption, transport, and redistribution in vines. *Communications in Soil Science and Plant Analysis* 39, 1507-1516.

Dai, J., Becquer, T., Rouiller, J. H., Reversat, G., Bernhard-Reversat, F. and Lavelle, P., 2004. Influence of heavy metals on C and N mineralisation and microbial biomass in Zn-, Pb-, Cu-, and Cd-contaminated soils. *Applied Soil Ecology* **25**, 99-109.

Dang, M. V., 2007. Quantitative and qualitative soil quality assessments of tea enterprises in Northern Vietnam. *African Journal of Agricultural Research* **2**, 455-462.

Debosz, K., Babich, H. and Stotzky, G., 1985. Toxicity of lead to soil respiration: mediation by clay, minerals, humic acids and compost. *Bulletin Environmental Contamination and Toxicology* **35**, 517-524.

DECIDUOUS FRUIT PRODUCERS TRUST (DFPT), 2009. The key deciduous fruit statics-2009.

De Pascale, S. and Barbieri, G., 1997. Effect of soil salinity and top removal on growth and yield of broadbean as a green vegetable. *Scientia Horticulturae* **71**, 147-165.

De Rouw, A. and Rajot, J. –L., 2004. Nutrient availability and pearl millet production in Sahelian farming systems based on manuring or fallowing. *Agriculture, Ecosystems and Environment* **104**, 249-262.

De Vires, J., Heine, M., Harms, K. and Wackernagel, W., 2003. Spread of recombinant DNA by roots and pollen of transgenic potato plants, identified by highly specific biomonitoring using natural transformation of an *Acinetobacter* sp. *Applied and Environmental Microbiology* **69**, 4455-4462.

Delbés, C., Ali-Mandjee, L. and Montel, M. –C., 2007. Monitoring bacterial communities in raw milk and cheese by culture-dependent and independent 16S rRNA gene-based analyses. *Applied and Environmental Microbiology* **73**, 1882-1891.

Dobranic, J. K. and Zak, J. C., 1999. A microtitre plate procedure for evaluating fungal functional diversity. *Mycologia* **91**, 756-765.

Doelman, P. and Haanstra, L., 1979. Effect of lead on soil respiration and dehydrogenase activity. *Soil Biol*ogy and *Biochemistry* **11**, 475-479.

Dohrmann, A. B. and Tebbe, C. C., 2005. Effect of elevated tropospheric ozone on the structure of bacterial communities inhabiting the rhizosphere of herbaceous plants native to Germany. *Applied and Environmental Microbiology* **71**, 7750-7758.

Donaldson, J. S., 2002. Biodiversity and conservation farming in the agricultural sector. In: Pierce, S. M., Cowling, R. M., Sandwith, T. and MacKinnon, K. (eds.), 2002. Mainstreaming biodiversity in development. Case studies from South Africa. The World Bank Environmental Department, Washington, D.C., pp. 43-55.

Donegan K. K., Palm C. J., Fieland V. J., Porteous L. A., Ganio L. M., Schaller D. L., Bucao L. Q. and Seidler R. J., 1995. Changes in level, species and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin. *Applied Soil Ecology* **2**, 111-124.

Donegan, K. K., Watrud, L. S., Seidler, R. J., Maggarda, S. P., Shiroyamab, T., Porteous, L. A. and DiGiovanni, G., 2001. Soil and litter organisms in Pacific northwest forests under different management practices. *Applied Soil Ecology* **18**, 159-175.

Donnelly, J. G., Detombe, S. and Hindmarsh, J. T., 2002. Single-strand conformational polymorphism and denaturing gradient gel electrophoresis in screening for variegate porphyria: Identification of two new mutations. *Annals of Clinical and Laboratory Science* **32**, 107-113.

Doran, J. W., 2002. Soil health and global sustainability: translating science into practice. *Agriculture, Ecosystems and Environment* **88**, 119-127.

Doran, J. W. and Parkin, T. B., 1994. Defining and assessing soil quality. In: Doran, J. W., Coleman, D. C., Bezdicek, D. F. and Stewart, B. A. (eds.), 1994. Defining soil quality for a sustainable environment. Soil Science Society of America special publication no. 35, Madison, WI, pp. 3-21.

Doran, J. W. and Zeiss, M.R., 2000. Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology* **15**, 3-11.

Doran, J. W., Liebig, M. A., and Santana, D. P., 1998. Soil health and global sustainability. In: Proceedings of the 16th World Congress of Soil Science, 20-26 August 1998. Montepellier, France.

Doran, J. W., Sarrantonio, M. and Liebig, M., 1996. Soil health and sustainability. In: Sparks, D. L. (ed.), 1996. *Advance in Agronomy*, Vol. 56, Academic Press, San Diego, CA, USA, pp. 1-54.

Douglas, S. and Beveridge, T. J., 1998: Mineral formation by bacteria in natural microbial communities. *FEMS Microbiology Ecology* **26,** 79-88.

Duthoit, F., Godon, J. –J. and Montel, M. –C., 2003. Bacterial community dynamics during production of registered designation of origin salers cheese as evaluated by 16S rRNA gene single-strand conformation polymorphism analysis. *Applied and Environmental Microbiology* **69**, 3840-3848.

Edwards W. M., Shipitalo M. J. and Norton L. D., 1988. Contribution of macroporosity to infiltration into a continuous corn no-tilled watershed: implications for contaminant movement. *Journal of Contaminant Hydrology* **3**, 193-205.

Ellis, R. J., Neish, B., Trett, M. W., Best, J. G., Weightman, A. J., Morgan, P. and Fry, J. C., 2001. Comparison of microbial and meiofaunal community analyses for determining impact of heavy metal contamination. *Journal of Microbiological Methods* **45**, 171-185.

Ellner, S. P., 2001. Habitat structure and population persistence in an experimental community. *Nature* **412**, 538-543.

Etana, A., Håkansson, I., Zagal, E. and Bucas, S., 1999. Effects of tillage depth on organic carbon content and physical properties in five Swedish soils. *Soil and Tillage Research* **52**, 129–139.

Ettema, C. H. and Wardle, D. A., 2002. Spatial soil ecology. *TRENDS in Ecology and Evolution* **17**, 177-182.

Fallgren, P. H. and Jin, S., 2008. Biodegradation of petroleum compounds in soil by a solid-phase circulating bioreactor with poultry manure amendments. *Journal of Environmental Science and Health Part A* **43**, 125-131.

Fan, T., Stewart, B. A., Payne, W. A., Yong, W., Luo, J. and Gao, Y., 2005. Long-term fertilizer and water availability effects on cereal yield and soil chemical properties in Northwest China. *Soil Science Society of America Journal* **69**, 842-855.

Fernandes, S. A. P., Bettiol, W. and Cerri, C. C., 2005. Effect of sewage sludge on microbial biomass, basal respiration, metabolic quotient and soil enzymatic activity. *Applied Soil Ecology* **30**, 65-77.

Fisher, M. M. and Triplett, E. W., 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Applied and Environmental Microbiology* **65**, 4630-4636.

Fitter, A. H. and Hay, R. K. M., 1981. Environmental physiology of plants. Academic Press, London, UK. pp. 200-231.

Forney, L. J., Zhou, X. and Brown, C. J., 2004. Molecular microbial ecology: land of the one-eyed king. *Current Opinion in Microbiology* **7**, 210-220.

Franzluebbers, A. J., Haney, R. L. and Hons, F. M., 1999. Relationships of chloroform fumigation-incubation to soil organic matter pools. *Soil Biology and Biochemistry* **31**, 395-405.

Freitag, T. E., Chang, L., Clegg, C. D. and Prosser, J. I., 2005. Influence of inorganic nitrogen-management regime on the diversity of nitrite oxidizing bacteria in agricultural grassland soils. *Applied and Environmental Microbiology* **71**, 8323-8334.

Fromin, N., Hamelin, J., Tarnawski, S., Roesti, D., Jourdain-Miserez, K., Forestier, N., Teyssier-Cuvelle, S., Gillet, F., Aragno, M. and Rossi, P., 2002. Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. *Environmental Microbiology* **11**, 634-643.

Fuhrmann, J. F., 2005. Microbial metabolism: In: Sylvia, D. M., Hartel, P. G., Fuhrmann, J. J. and Zuberer, D. A. (eds.), 2005. Principles and applications of soil microbiology, 2nd ed., Pearson Prentice Hall, pp. 54-83.

Garland, J. L., 1996. Analytical approaches to the characterization of samples of microbial communities using pattern of potential C source utilization. *Soil Biology and Biochemistry* **28**, 213-221.

Garland, J. L. and Mills, A. L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community level sole carbon source utilization. *Applied and Environmental Microbiology* **57**, 2351-2359.

Gee, G. W. and Bauder, J. W., 1986. Particle-size analysis. In: Klute, A. (ed.), 1986. Methods of soil analysis. Part 1 2nd ed. Agronomy Monograph 9. ASA and SSSA, Madison, WI. p. 383-411.

Giller, K. E., Witter, E. and McGrath, S. P., 1998. Toxicity of heavy metals to micro-organisms and microbial processes in agricultural soils: a review. *Soil Biology and Biochemistry* **30**, 1389-1414.

Giller, P. S., 1996. The diversity of soil communities, the ‘poor man’s tropical rainforest’. *Biodiversity and Conservation* **5**, 135-168.

Gleeson, D., McDermott, F. and Clipson, N., 2006. Structural diversity of bacterial communities in a heavy metal mineralized granite outcrop. *Environmental Microbiology* **8**, 383-393.

Glover, J. D., Reganold, J. P. and Andrews, P. K., 2000. Systematic methods for rating soil quality of conventional, organic, and integrated apple orchards in Washington State. *Agriculture, Ecosystems and Environment* **80**, 29-45.

Graham, J. H., Hodge,N. C. and Morton, J. B., 1995. Fatty acid methyl ester profiles for characterization of Glomalean fungi and their endomycorrhizae. *Applied and Environmental Microbiology* **61**, 58-64.

Gramss, G. and Bergmann, H., 2007. Microbial competition, lack in macronutrients, and acidity as main obstacles to the transfer of basidiomycetous ground fungi into (organically or heavy-metal contaminated) soils. *Journal of Basic Microbiology* **47**, 309-316.

Gregory, M. M., Shea, K. L. and Bakko, E. B., 2005. Compairing agroecosystems: Effects of cropping and tillage patterns on soil, water, energy use and productivity. *Renewable Agriculture and Food Systems* **20,** 81-90.

Grossman, R. B. and Reinsch, T. G., 2002. Bulk density and linear extensibility: Core method. In: Dane, J. H. and Topp, G. C. (eds.), 2002. Methods of soil analysis. Part 4: physical methods. SSSA, Inc., Madison, WI, pp. 208-228.

Grove, J. A., Kautola, H., Javadpour, S., Moo-Young, M. and Anderson, W. A., 2004. Assessment of changes in the microorganism community in a biofilter. *Biochemical Engineering Journal* **18**, 111-114.

Gömöryová, E., Vass, D., Pichler, V. and Gömöry, D., 2009. Effect of alginate amendment on microbial activity and soil water content in forest soils. *Biologia* **64**, 585-588.

Guimarães, C. M., Stone, L. F. and Moreira, J. A. A., 2002. Soil compaction in a bean crop. II. Effect on root and shoot development. *Revista Brasileira de Engenharia Agrícola e Ambiental* **6**, 213-218.

Gunapala, N. and Scow, K. M., 1998. Dynamics of soil microbial biomass and activity in conventional and organic farming systems. *Soil Biology and Biochemistry* **30**, 805-816.

Harris-Hellal, J., Vallaeys, T., Garnier-Zarli, E. and Bousserrhine, N., 2009. Effect of mercury on soil microbial communities in tropical soil of French Guyana. *Applied Soil Ecology* **41**, 59-68.

Harris, R. F. and Bezdicek D. F., 1994. Descriptive aspects of soil quality/health. In: Doran J. W., Coleman, D. C., Bezdicek, D. F. and Stewart, B. A. (eds.), 1994. Defining soil quality

for a sustainable environment. SSSA Special Publication Number 35, Wisconsin, USA. pp. 23-36.

Hartmann, M., Frey, B., Kölliker, R. and Widmer, F., 2005. Semi-automated genetic analyses of soil microbial communities: comparison of T-RFLP and RISA based on descriptive and discriminative statistical approaches. *Journal of Microbiological Methods* **61**, 349-360.

Haws, N. W., Liu, B., Boast, C. W., Rao, P. S. C., Kladivko, E. J. and Franzmeier, D. P., 2004. Spatial variability and measurement scale of infiltration rate on an agricultural landscape. *Soil Science Society of America Journal* **68**, 1818-1826.

Helassa, N., Quiquampoix, H., Noinville, S., Szponarski, W. and Staunto, S., 2009. Adsorption and desorption of monomeric Bt (*Bacillus thuringiensis*) Cry1Aa toxin on montmorillonite and kaolinite. *Soil Biology and Biochemistry* **41**, 498-509.

Henrot, J. and Robertson, G. P., 1994. Vegetation removal in two soils of the humid tropics: Effect on microbial biomass. *Soil Biology and Biochemistry* **8**, 209-213.

Hernanz, J. L., Peixoto, H., Cerisola, C. and Sánchez-Girón, V., 2000. An empirical model to predict soil density profiles in field conditions using penetration resistance, moisture content and soil depth. *Journal of Terramechanics* **37**, 167-184.

Hewson, I. and Fuhrman, J., 2006. Improved strategy for comparing microbial assemblage fingerprints. *Microbial Ecology* **51**, 147-153.

Higby, J. R. and Bell, P. F., 1999. Low soil nitrate levels from golf course fairways related to organic matter sink for nitrogen. *Communications in Soil Science and Plant Analysis* **30**, 573-588.

Horrigan, L., Lawrence, R. S. and Walker P., 2002. How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environmental Health Perspectives* **110**, 445-456.

Horwath, W., 2007. Carbon cycling and formation of soil organic matter. In: Paul E. A. (ed.), 2007. Soil microbiology, ecology and biochemistry, 3rd ed., Academic press, UK, pp. 303-337.

Huang, Q. and Shindo, H., 2000. Effect of copper on the activity and kinetics of free and immobilized acid phosphate. *Soil Biology and Biochemistry* **32**, 1885-1892.

Huang, Q., Liang, W. and Cai, P., 2005. Absorption, desorption and activities of acid phosphates on various colloidal particles from an Ultisol. *Colloids and surfaces B: Biointerfaces* **45**, 209-214.

Ibekwe, A. M. and Kennedy, A. C., 1998. Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. *FEMS Microbiology Ecology* **26**, 151-163.

Ingham, R. E, Trofymow, J. A., Ingham, E. R. and Coleman, D. C., 1985. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecological Monographs* **55**, 119-140.

Insam, H., 2001. Development in soil microbiology since the mid. *Geoderma* **100**, 389-402.

Jandl, G., Leinweber, P., Schulten, H. R. and Ekschmitt, K., 2005. Contribution of primary organic matter to fatty acid pool in agricultural soils. *Soil Biology and Biochemistry* **37**, 1033-1041.

Jeffery, N., Gasser, R. B., Steer, P. A. and Noormohammadi, A. H., 2007. Classification of *Mycoplasma synoviae* strains using single-strand conformation polymorphism and high-resolution melting-curve analysis of the *vlhA* gene single-copy region. *Microbiology* **153**, 2679-2688.

Jensen, M. A., Webster, J. A. and Straus, N., 1993. Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. *Applied Environmental Microbiology* **59**, 945-952.

Johnson, S. P., 1993. The earth summit: The United Nations Conference on Environment and Development (UNCED) Graham and Trotman/ Martinus Nijhoff, London.

Joynt, J., Bischoff, M., Turco, R. F., Konopka, A. and Nakatsu, C. H., 2006. Microbial community analysis of soils contaminated with lead, chromium and organic solvents. *Microbial Ecology* **51**, 209-219.

Karlen, D. L., Andrews, S. S., Doran, J. W. and Wienhold, B. J., 2003. Soil quality-humankind's foundation for survival. *Journal of soil water conservation* **58**, 171-179.

Karlen, D. L. and Stott, D. E., 1994. A framework for evaluating physical and chemical indicators of soil quality. In: Doran, J. W., Coleman, D. C., Bezdicek, D. F. and Stewart, B. A. (eds.), 1994. Defining soil quality for a sustainable environment. Soil Science Society of America, Inc., Madison, Wisconsin, USA, pp. 53-72.

Karlen, D. L., Mausbach, M. J., Doran, J. W., Cline R. G., Harris, R. F. and Schuman, G. E., 1997. Soil quality: A concept, definition and framework for evaluation. *Soil Science society of America Journal* **61**, 4-10.

Kay, E., Vogel, T. M., Bertolla, F., Nalin, R. and Simonet, P., 2002. In situ transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. *Applied and Environmental Microbiology* **68**, 3345-3351.

Keer, J. T. and Birch, L., 2003. Molecular methods for the assessment of bacterial viability. *Journal of Microbiological Methods* **53**, 175-183.

Kelly, D. P. and Wood, A. P., 2000. Confirmation of *Thiobacillus denitrificans* as a species of the genus *Thiobacillus*, in the beta-subclass of the Proteobacteria, with strain NCIMB 9548 as the type strain. *International Journal of Systematic and Evolutionary Microbiology* **50**, 547-50.

Kennedy, A. C. and Papendick, R. I., 1995. Microbial characteristics of soil quality. *Journal of Soil and Water Conservation* **50**, 243-248.

Kerek, M., Drijber, R. A. and Gaussoin, R. E., 2003. Labile soil organic matter as a potential nitrogen source in golf greens. *Soil Biology and Biochemistry* **35**, 1643-1649.

Khan, M. and Scullion, J., 2002. Effects of metal (Cd, Cu, Ni, Pb, or Zn) enrichment on sewage-sludge on soil micro-organisms and their activities. *Applied Soil Ecology* **20**, 145-155.

Killham, K., Amato, M. and Ladd, J. N., 1993. Effect of substrate location in soil and soil pore water regime on carbon turnover. *Soil Biology and Biochemistry* **25**, 57-62.

Kirk, J. L., Beaudette, L. A., Hart, M., Moutoglis, P., Klironomos, J. N., Lee, H. and Trevors, J. T., 2004. Methods of studying soil microbial diversity. *Journal of Microbiological Methods* **58**, 169-188.

Kleinsteuber, S., Riis, V., Fetzer, I., Harms, H. and Müller, S., 2006. Population dynamics within a microbial consortium during growth on diesel fuel in saline environments. *Applied and Environmental Microbiology* **72,** 3531-3542.

Klitzke, S. and Lang, F., 2007. Hydrophobicity of soil colloids and heavy metal mobilization: Effects of drying. *Journal of Environmental Quality* **36**, 1187-1193.

Kong, P., Hong, C., Richardson, P. A. and Gallegly, M. E., 2003. Single-strand-conformation polymorphism of ribosomal DNA for rapid species differentiation in genus *Phytophthora*. *Fungal Genetics and Biology* **39**, 238-249.

Koop, H. –P. and Pommerening-Röser, A., 2001. Distribution and ecophysiology of nitrifying bacteria emphasizing cultured species *FEMS Microbiology Ecology* **37**, 1-9.

Korsaeth, A., 2008. Relations between nitrogen leaching and food productivity in organic and conventional cropping systems in a long-term field study. *Agriculture, Ecosystems and Environment* **127**, 177-188.

Kozdrój, J. and van Elsas, J. D., 2001. Structural diversity of microorganisms in chemically perturbed soil assessed by molecular and cytochemical approaches. *Journal of Microbiological Methods* **43**, 197-212.

Kruse, J., Kissel, D. E. and Cabrera M. L., 2004. Effects of drying and rewetting on carbon and nitrogen mineralization in soils and incorporated residues. *Nutrient Cycling in Agroecosystems* **69**, 247-256.

Kumeda, Y. and Asao. T., 1996. Single-strand conformation polymorphism analysis of PCR-amplified ribosomal DNA internal transcribed spacers to differentiate species of *Aspergillus* section *Flavi*. *Applied and Environmental Microbiology* **62**, 2947-2952.

Lado, M., Ben-Hur, M. and Assouline, S., 2005. Effects of effluent irrigation on seal formation, infiltration, and soil loss during rainfall. *Soil Science Society of America Journal* **69**, 1432-1439.

Lado, M., Ben-Hur, M. and Shainberg, I., 2004. Soil wetting and texture effects on aggregate stability, seal formation, and erosion. *Soil Science Society of America Journal* **68**, 1992-1999.

Lambo, A. J. and Patel, T. R., 2007. Biodegradation of polychlorinated biphenyls in Aroclor 1232 and production of metabolites from 2, 4, 4'-trichlorobiphenyl at low temperature by psychrotolerant *Hydrogenophaga* sp. strain IA3-A. *Journal of Applied Microbiology* **102**, 1318-1329.

Larson, W.E. and Pierce, F.J., 1991. Conservation and enhancement of soil quality. In: Dumanski, J. (ed.), 1991. Evaluation for Sustainable land management in the developing World, Vol. 2: Technical papers. Proceedings of International Workshop, Chaning Rai, Thailand, 15–21 September 1990. Int. Board Soil Res. Manage., Bangkok, Thailand, pp. 175–203.

Lavelle, P. and Spain A. V., 2001. Soil ecology. Kluwer Academic Publishers, Dordrecht / Boston / London, pp. 65-73.

Lechevalier, M. P., 1989. Lipids in bacterial taxonomy. In: O'Leary W. M. (ed.), 1989. Practical handbook of microbiology. CRC, Boca Raton, Fla., pp. 455-561.

Ledin, M., 2000. Accumulation of metals by microorganisms – processes and importance for soil systems. *Earth-Science Reviews* **51**, 1-31.

Lee, D. –H., Zo, Y.,-G. and Kim, S.-J., 1996. Nonradioactive method to study genetic profiles of natural bacterial communities by PCR-single strand conformation polymorphism. *Applied and Environmental Microbiology* **62**, 3112-3120.

Leifeld, J., Zimmermann, M. and Fuhere, J., 2008. Simulating decomposition of labile soil organic carbon: Effect of pH. *Soil Biology and Biochemistry* **40**, 2948-2951.

Leita, L., De Nobili, M., Muhlbachova, G., Mondini, C., Marchiol, L. and Zerbi, G., 1995. Bioavailability and effects of heavy metals on soil microbial biomass survival during laboratory incubation. *Biology and Fertility of Soils* **19**, 103-118.

Lerman, L. S., Fischer, S. G., Hurley, I., Silverstein, K. and Lumelsky, N., 1984. Sequence-determined DNA separations. *Annual Review of Biophysics and Bioengineering* **13**, 399-423.

Levario-Carrillo, M., Olave, M. E., Corral, D. C., Alderete, J. G., Gagioti, S. M. and Bevilacqua, E., 2004. Placental morphology of rats prenatally exposed to methyl parathion. *Experimental and Toxicologic Pathology* **55**, 489-496.

Levy-Booth, D. J., Campbell, R. G., Gulden, R. H., Hart, M. M., Powell, J. R., Klironomos, J. N., Pauls, K. P., Swanton, C. J., Trevors, J. T. and Dunfield, K. E., 2007. Cycling of extracellular DNA in the soil environment. *Soil Biology and Biochemistry* **39**, 2977-2991.

Lin, Q. and Brookes, P. C., 1999. Comparison of substrate induced respiration, selective inhibition and biovolume measurements of microbial biomass and its community structure in unamended, ryegrass-amended, fumigated and pesticide-treated soils. *Soil Biology and Biochemistry* **31**, 1999-2014.

Liski, J., Nissinen, A., Erhard, M. and Taskinen, O., 2003. Climatic effects on litter decomposition from arctic tundra to tropical rainforest. *Global Change Biology* **9**, 575-584.

Liu, B., Gumpertz, M. L., Hu, S. and Ristaino, J. B., 2007. Long-term effects of organic and synthetic soil fertility amendments on soil microbial communities and the development of southern blight. *Soil Biology and Biochemistry* **39**, 2302-2316.

Liu, W. -T., Marsh, T. L., Cheng, H. and Forney, L. J., 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Applied Environmental Microbiology* **63**, 4516-4522.

Loveland, P. and Webb, J., 2003. Is there a critical level of organic matter in the agricultural soils of temperate regions: a review. *Soil and Tillage Research* **70**, 1-18.

Lupwayi, N. Z., Harker, K. N., Clayton, G. W., O'Donovan, J. T. and Blackshaw, R. E., 2009a. Soil microbial response to herbicides applied to glyphosate-resistant canola. *Agriculture, Ecosystems and Environment* **129**, 171-176.

Lupwayi, N. Z., Harker, K. N., Dosdall, L. M., Turkington, T. K., Blackshaw, R. E., O'Donovan, J. T., Cárcamo, H. A., Otani, J. K. and Clayton G. W., 2009b. Changes in functional structure of soil bacterial communities due to fungicide and insecticide applications in canola. *Agriculture, Ecosystems and Environment* **130**, 109-114.

Maarit-Niemi, R., Heiskanen, I., Wallenius, K. and Lindstrom, K., 2001. Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. *Journal of Microbiological Methods* **45**, 155-165.

Maithani, K., Tripathi, R. S., Arunachalam, A. and Pandey, H. N., 1996. Seasonal dynamics of microbial biomass C, N and P during regrowth of a disturbed subtropical humid forest in north-east India. *Applied Soil Ecology* **4**, 31-37.

Mamedov, A. I., Beckmann, S., Huang, C. and Levy, G. J., 2007. Aggregate stability as affected by polyacrylamide molecular weight, soil texture and water quality. *Soil Science Society of America Journal* **71**, 1909-1918.

Marin, J. A., Hernandez, T. and Garcia, C., 2005. Bioremediation of oil refinery sludge by landfarming in semiarid conditions: Influence on soil microbial activity. *Environmental Research* **98**, 185-195.

Marschner, H. (ed.), 1995. Mineral nutrition of higher plants. 2nd ed., Academic Press, London. pp. 313-404.

Marschner, P., 2007. Soil microbial community structure and function assessed by FAME, PLFA and DGGE-Advantages and limitations. In: Varma, A. and Oelmüller, R. (eds.), 2007. Advanced techniques in soil microbiology. Springer-Verlag Berlin Heidelberg, Soil Biology, Vol. 11, 181-200.

Marsh, T. L., 1999. Terminal restriction fragment length polymorphism (T-RFLP); an emerging method for characterising diversity among homologous populations of amplification products. *Current Opinion in Microbiology* **2**, 323-327.

Matsuyama, T., Nakajima, Y., Matsuya, K., Ikenaga, M., Asakawa, S. and Kimura, M., 2007. Bacterial community in plant residues in a Japanese paddy field estimated by RFLP and DGGE analyses. *Soil Biology and Biochemistry* **39**, 463-472.

McLean, K. L., Swaminathan, J. and Stewart, A., 2001. Increasing soil temperature to reduce sclerotial viability of *Sclerotium cepivorum* in New Zealand soils. *Soil Biology and Biochemistry* **33**, 137-143.

Meier, C., Wehrli, B. and van der Meer, J. R., 2008. Seasonal fluctuations of bacterial community diversity in agricultural soil and experimental validation by laboratory disturbance experiments. *Microbial Ecology* **56**, 210-222.

Mijangos, I., Becerril, J. M., Albizu, I., Epelde, L. and Garbisu, C., 2009. Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and –independent methodologies. *Soil Biology and Biochemistry* **41**, 505-513.

Miller, K. M., Ming, T. J., Schulze, A. D. and Withler, R. E., 1999. Denaturing gradient gel electrophoresis (DGGE): a rapid and sensitive technique to screen nucleotide sequence variation in populations. *BioTechniques* **27**, 1016-1030.

Mills, A. J. and Fey, M. V., 2003. Declining soil quality in South Africa: effects of land use on soil organic matter and surface crusting. *South African Journal of Science* **99**, 429-436.

Minocha, R. and Minocha, S. C., 2005. Effects of soil pH and aluminium on plant respiration. In: Lambers, H. and Ribas-Carbo, M. (eds.), 2005. Plant respiration. Springer, The Netherlands, pp. 159-176.

Moffat, A. S., 1998. Global nitrogen overload problem grows critical. *Science* **279**, 988-989.

Mora, D., Ricci, G., Guglielmetti, S., Daffonchio, D. and Fortina, G. M., 2003. 16S-23S rRNA intergenic spacer region sequence variation in *Streptococcus thermophilus* and related dairy streptococci and development of a multiplex ITS-SSCP analysis for their identification. *Microbiology* **149**, 807-813.

Morton, S. C. and Edwards, M., 2005. Reduced phosphorus compounds in the environment. *Critical Reviews in Environmental Science and Technology* **35**, 333-364.

Muyzer, G., 1999. DGGE/TGGE a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology* **2**, 317-322.

Muyzer, G., Waal, E. C. D. and Uitterlinden, A. G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* **59**, 695-700.

Muyzer, G. and Smalla, K., 1999. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis in microbial ecology. *Antonie van Leeuwenhock* **73**, 127-141.

Mäder, P., Flieβbach, A., Dubois, D., Gunst, L., Fried, P. and Niggli, U., 2002. Soil fertility and biodiversity in organic farming. *Science* **296**, 1694-1697.

Nagpal, M. L., Fox, K. F. and Fox, A., 1998. Utility of 16S-23S rRNA spacer region methodology: how similar are interspace regions within a genome and between strains for closely related organisms. *Journal of Microbiological Methods* **33**, 211-219.

Nakatsu, C. H., 2007. Soil microbial community analysis using denaturing gradient gel electrophoresis. *Soil Science Society of America Journal* **71**, 562-571.

Nakatsu, C. H., Carmosini, N., Baldwin, B., Beasley, F., Kourtev, P. and Konopka, A., 2005. Soil microbial community response to additions of organic carbon substrates and heavy metals (Pb and Cr). *Applied and Environmental Microbiology* **71**, 7679-7689.

Nakatsu, C. H., Torsvik, V. and Øvreås, L., 2000. Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. *Soil Science Society of America Journal* **64**, 1382-1388.

Nannipieri, P. and Badalucco, L., 2003. Biological processes. In: Bembi, D. K. and Nieder, R. (eds.), 2003. Processes in the soil-plant system: Modelling concepts and applications. The Haworth Press, Binghamton, New York, pp. 57-82.

Nannipieri, P., Ascher, J., Ceccherini, M. T., Landi, L., Pietramellara, G. and Renella, G., 2003. Microbial diversity and soil functions. *European Journal of Soil Science* **54**, 655-670.

Nannipieri, P., Grego, S. and Ceccanti, B., 1990. Ecological significance of the biological activity in soil. In: Bollag, J. –M. and Stotzky, G. (eds.), 1990. Soil Biochemistry. Volume 6, Marcel Dekker, New York, pp.293-355.

Nannipieri, P., Kandeler, E. and Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In; Burns, R. G. and Dick, R. (eds.), 2002. Enzymes in the environment. Marcel Dekker, New York, pp. 1-33.

Naramabuye, F. X. and Haynes, R. J., 2007. The liming effect of five organic manures when incubated with an acid soil. *Journal of Plant Nutrient Soil Science* **170**, 615-622.

NATIONAL DEPARTMENT OF AGRICULTURE (NDA) 2000**.** *Abstract of Agricultural Statistics.* Pretoria.

NATIONAL DEPARTMENT OF AGRICULTURE (NDA) 2008**.** *Abstract of Agricultural Statistics.* Pretoria.

Nielsen, M. N. and Winding, A., 2002. Microorganisms as indicators of soil health. National Environment Research Institute (NERI), Technical Report No. 388. Ministry of the Environment, NERI, pp. 15.

Nishigaki, K., Husimi, Y. and Tsubota, M., 1986. Detection of differences in higher order structure between highly homologous single-stranded DNAs by low-temperature denaturant gradient gel electrophoresis. *Journal of Biochemistry* **99**, 663-671.

Nitu, S. and Banwari, L., 2009. Isolation of a novel yeast strain Candida digboiensis TERI ASN6 capable of degrading petroleum hydrocarbon in acidic conditions. *Journal of Environmental Management* **90**, 1728-1736.

Nübel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R. I., Ludwig, W. and Backhaus, H., 1996. Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *Journal of Bacteriology* **178**, 5636-5643.

Olson, J. B., Lord, C. C. and McCarthy, P. J., 2000. Improved recoverability of microbial colonies from marine sponge samples. *Microbial Ecology* **40**, 139-147.

Oorts, K., Vanlauwe, B. and Merckx, R., 2003. Cation exchange capacities of soil organic matter fractions in a ferric lixisol with different organic matter inputs. *Agricultural, Ecosystems and Environment* **100**, 161-171.

OPTIMAL AGRICULTURAL BUSINESS SYSTEMS (OABS), 2003. Oorsig van die 2002/2003 vrugteseisoen en die impak van watertekorte op toekomstige groei in die bedryf. Paarl.

OPTIMAL AGRICULTURAL BUSINESS SYSTEMS (OABS), 2008. Annual report briefing 2008/09.

Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. and Sekiya, T., 1989a. Detection of polymorphisms of human DNA by gel electrophoresis as single strand conformation polymorphisms. *Proceedings of the National Academy of Sciences* **86**, 2766-2770.

Orita, M., Suzuki, Y., Sekiya, T. and Hayashi, K., 1989b. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* **5**, 874-879.

Osborn, A. M., Moore, E. R. B. and Timmis, K. N., 2000. An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. *Environmental Microbiology* **2**, 39-50.

Pan, G., Smith, P. and Pan, W., 2009. The role of soil organic matter in maintaining the productivity and yield stability of cereals in China. *Agriculture, Ecosystems and Environment* **129**, 344-348.

Pankhurst, C. E., Hawke, B. G., McDonald, H. J., Kirkby, C. A., Buckerfield, J. C., Michelsen, P., O'Brien, K. A., Gupta, V. V. S. R. and Doube, B. M.,1995. Evaluation of soil biological properties as potential bioindicators of soil health. *Australian Journal of Experimental Agriculture* **35**, 1015-1028.

Pardue, J. H., Delaune, R. D. and Patrick Jr., W. H., 1988. Effect of sediment pH and oxidation-reduction potential on PCB mineralization. *Water, Air, and Soil Pollution* **37**, 439-447.

Pawluk, R. R., Sandor, J. A. and Tabor, J. A., 1992. The role of indigenous knowledge in agricultural development. *Journal of Soil and Water Conservation* **47**, 298-302.

Peck, G. M., 2004. Orchard productivity and apple fruit quality of organic, conventional, and integrated farm management systems. Master of Science thesis, Washington State University, Pullman. pp. 62-72.

Peck, G. M., Andrews, P. K., Reganold, J. P. and Fellman, J. K., 2006. Apple orchard productivity and fruit quality under organic, conventional and integrated management. *HortScience* **41**, 99-107.

Peck, G. M., Andrews, P. K., Richter, C. and Reganold, J. P., 2005. Internalization of organic fruit market: The case of Washington State's organic apple exports to the European Union. *Renewable Agriculture and Food Systems* **20**, 101-112.

Peixoto, R. S., Coutinho, H. L. C., Madari, B., Machado, P. L. O. A., Rumjanek, N. G., van Elsas, J. D., Seldin, L. and Rosado, A. S., 2006. Soil aggregation and bacterial community structure as affected by tillage and cover cropping in the Brazilian Cerrados. *Soil and Tillage Research* **90**, 16-28.

PERISHABLE PRODUCTS EXPORT CONTROL BOARD (PPECB), 2008. Annual report briefing 2008/09.

Picone, L. I., Cabrera, M. L. and Franzluebbers, A. J., 2002. A rapid method to estimate potentially mineralizable nitrogen in soil. *Soil Science Society of American Journal* **66**, 162-170.

Pietri, J. C. A. and Brookes, P. C., 2008(a). Nitrogen mineralisation along a pH gradient of a silty loam UK soil. *Soil Biology and Biochemistry* **40**, 797-802.

Pietri, J. C. A. and Brookes, P. C., 2008(b). Relationships between soil pH and microbial properties in a UK arable soil. *Soil Biology and Biochemistry* **40**, 1856-1861.

Plante, A. F., 2007. Soil biochemical cycling of inorganic nutrients and metals. In: Paul, E. A. (ed.), 2007. Soil microbiology, ecology, and biochemistry, 3rd ed., Academic Press, Oxford, UK, pp. 389-432.

Plante, A. F., Conant, R. T., Stewart, C. E., Paustian, K. and Six, J., 2006. Impact of soil Texture on the distribution of soil organic matter in physical and chemical fractions. *Soil Science Society of America Journal* **70**, 287-296.

Postma, J., Schilder, M. T. and van Leeuwen-Haagsma, W. T., 2008. Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. *Soil Biology and Biochemistry* **40**, 2394-2406.

Prasad, P., Basu, S. and Behera, N., 1994. A comparative account of the microbiological characteristics of soil under natural forest, grassland and cropfield from Eastern India. *Plant and Soil* **175**, 85-91.

Rahimi, H., Pazira, E. and Tajik, F., 2000. Effect of soil organic matter, electrical conductivity and sodium adsorption ration on tensile strength of aggregates. *Soil and Tillage Research* **54**, 145-153.

Ranjard, L., Nowak, V., Echairi, A., Faloya, V. and Chaussod, R., 2008. The dynamics of soil bacterial community structure in response to yearly repeated agricultural copper treatments. *Research in Microbiology* **159**, 251-254.

Ranjard, L., Poly, F. and Nazaret, S., 2000. Monitoring complex bacterial communities using culture-independent molecular techniques: application to soil environment. *Research in Microbiology* **151**, 167-177.

Ranjard, L., Poly, F., Lata, J. –C., Mougel, C., Thioulouse, J. and Nazaret, S., 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: Biological and methodological variability. *Applied and Environmental Microbiology* **67**, 4479-4487.

Rawlings, D. E., 2002. Heavy metal mining using microbes. *Annual Review of Microbiology* **56**, 65-91.

Reichert, J. M., Suzuki, L. E. A. S., Reinert, D. J., Horn, R. and Hakansson, I., 2009. Reference bulk density and critical degree-of-compactness for non-till crop production in subtropical highly weathered soils. *Soil and Tillage Research* **102**, 242-254.

Reganold, J. P., Glover, J. D., Andrews, P. K. and Hinman, H. R., 2001. Sustainability of three apple production systems. *Nature* **410**, 926-930.

Rein, A., Fernqvist, M. M., Mayer, P., Trapp, S., Bittens, M. and Karlson, U. G., 2007. Degradation of PCB congeners by bacterial strains. *Applied Microbiology and Biotechnology* **77**, 469-481.

Rhoton, F. E. and Lindbo, D. L., 1997. A soil depth approach to soil quality assessment. *Journal of Soil and Water Conservation* **52**, 66.

Rigby, D. and Cáceres, D., 2001. Organic farming and the sustainability of agricultural systems. *Agricultural systems* **68**, 21-40.

Ritz, K. and Wheatly, R. E., 1989. Effect of water amendment on basal and substrate-induced respiration rates of mineral soils. *Biology and Fertility of Soils* **8**, 242-246.

Robertson, G. P. and Groffman, 2007. Nitrogen transformation. In: Paul, E. A. (ed.), 2007. Soil microbiology, ecology and biochemistry, 3rd ed., Academic press, UK, pp. 341-364.

Romig, D. E., Garlynd, M. J., Harris, R. F. and Mc Sweeney, K., 1995. How farmers assess soil health and soil quality. *Journal of Soil and Water Conservation* **50**, 229-235.

Sansavini, S., 1997. Integrated fruit production in Europe: research and strategies for a sustainable industry. *Scientia Horticulturae* **68**, 25-36.

Satokari, R. M., Vaughan, E. E., Akkermans, A. D. L., Saarela, M. and de Vos, W. M., 2001. Bifidobacterial diversity in human feces detected by genus-specific PCR and denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology* **67**, 504-513.

Schlecht-Pietsch, S., Wagner, U. and Anderson, T. –H., 1994. Changes in composition of soil polysaccharides and aggregate stability after carbon amendments to different textured soils. *Applied Soil Ecology* **1**, 145-154.

Schink, B. and Friedrich, M., 2000. Phosphite oxidation by sulphate reduction. *Nature* **406**, 37.

Schnitzer, M., 1978. Humic substances: Chemistry and reactions. In: Schnitzer, M. and Khan, S. U. (eds.), 1978. Developments in soil science 8: Soil organic matter. Elsevier scientific publishing company, The Netherlands, pp. 1-2.

Schwieger, F. and Tebbe, C., 1998. A new approach to utilize PCR-single strand conformation polymorphism for 16S rRNA gene-based microbial community analysis. *Applied and Environmental Microbiology* **64**, 4870-4876.

Schwieger, F. and Tebbe, C. C., 2000. Effect of field inoculation with *Sinorhizobium meliloti* L33 on the composition of Bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*)-Linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. *Applied and Environmental Microbiology* **66**, 3556-3565.

Secco, D., Reinert, D. J., Reichert, J. M. and Da Ross, C. O., 2004. Implications of soil management and compaction state on soil physical properties and soybean yield. *Brazilian Journal of Soil Science* **28**, 797-804.

Selvakumar, N., Ding, B. C. and Wilson, S M., 1997. Separation of DNA strands facilitates detection of point mutations by PCR-SSCP. *BioTechniques* **22**, 604-606.

Sheppard, S. K., McCarthy, A. J., Loughnane, J. P., Gray, N. D., Head, I. M. and Lloyd, D., 2005. The impact of sludge amendment on methanogens community structure in an upland soil. *Applied Soil Ecology* **28**, 147-162.

Shi, K., Wang, L., Zhou, Y. -H., Yu, Y. –L. and Yu, J. –Q., 2009. Effects of calcium cyanamide on soil microbial communities and *Fusarium oxysporum* f. sp. *cucumberinum*. *Chemosphere* **75**, 872-877.

Shishido, M., Sakamoto, K., Yokoyama, H., Momma, N. and Miyashita, S. –I., 2008. Changes in microbial communities in an apple orchard and its adjacent bush soil in response to season, land-use, and violet root rot infestation. *Soil Biology and Biochemistry* **40**, 1460-1473.

Siemann, S., Schneider, K., Oley, M. and Müller, A., 2003. Characterization of a Tungsten-Substituted Nitrogenase Isolated from *Rhodobacter capsulatus*. *Biochemistry* **42**, 3846-3857.

Silver, W. L., Neff, J., McGroddy, M., Veldkamp, E., Keller, M. and Cosme, R., 2000. Effect of soil texture on belowground carbon and nutrient storage in a lowland Amazonian forest ecosystem. *Ecosystem* **3**, 193-209.

Singer, M. J. and Ewing, S. A,. 2000. Soil quality. In: Sumner, M. E. (ed.), 2000. Handbook of soil science. CRC Press Inc., Boca Raton, FL. pp. G271-G298.

Singh, B. K., Dawson, L. A., Macdonald, C. A. and Buckland, S. M., 2009. Impact of biotic and abiotic interaction on soil microbial communities and functions: A field study. *Applied Soil Ecology* **41**, 239-248.

Singh, B. K., Munro, S., Potts, J. M. and Millard, P., 2007. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *Applied Soil Ecology* **36**, 147-155.

Singh, B. K., Walker, A., Morgan, J. A. W., and Wright, D. J., 2003. Effect of soil pH on the Biodegradation of Chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. *Applied and Environmental Microbiology* **69**, 5198-5206.

Sinsabaugh, R. L., Lauber, C. L.,Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., Contosta, A. R., Causack, D., Frey, S., Gallo, M. E., Gartner, T. B., Hobbie, S. E., Holland, K., Keeler, B. L., Powers, J. S., Stursova, M., Takacs-Vesbach, C., Wallenstein, M. D., Zak, D. R. and Zeglin, L. H., 2008. Stoichiometry of soil enzyme activity at global scale. *Ecology Letter* **11**, 1252-1264.

Smit, E., Leeflang, P. and Wernars, K., 1997. Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. *FEMS Microbiology Ecology* **23**, 249-261.

Snakin, V. V., Krechetov, P. P., Kuzovnikova, T. A., Alyabina, I. O., Gurov, A. F. and Stepichev, A. V., 1996. The system of assessment of soil degradation. *Soil Technology* **8**, 331-343.

Soares R. A., Roesch, L. F. W., Zanatta, G., Camargo, F. A. O. and Passaglia, L. M. P., 2006. Occurrence and distribution of nitrogen fixing bacterial community associated with oat (*Avena sativa*) assessed by molecular and microbiological techniques. *Applied Soil Ecology* **33**, 221-234.

Soltanpour, P. N. and Delgado, J. A., 2002. Profitable and sustainable soil test-based nutrient management. *Communications in Soil Science and plant analysis* **33**, 2557-2583.

Srivastava, R., Roseti, D. and Sharma, A. K., 2007. The evaluation of microbial diversity in a vegetable based cropping system under organic farming practices. *Applied Soil Ecology* **36**, 116-123.

Steger, K., Jarvis, Å., Smårs, S. and Sundh, I., 2003. Comparison of signature lipid methods to determine microbial community structure in compost. *Journal of Microbiological Methods* **55**, 371-382.

Stevenson, F. J. and Braids, O. C., 1968. Variation in the relative distribution of amino sugar with depth in some soil profiles. Soil Science Society of America Proceedings 32, 590-598.

Stevenson, F. J. and Cole, M. A., 1999. "Cycle of soil: Carbon, nitrogen, phosphorus, sulphur, micronutrient." 2nd ed., John Wiley and Sons Inc., New York, pp. 427.

Stotzky, G., 1997. Soil as an environmental for microbial life. In: van Elsas, J. D., Trevors, J. T. and Wellington, E. M. H. (eds.), 1997. Modern soil microbiology. Marcel Dekker, New York, pp. 1-20.

Stirzaker, R. J., Passioura, J. B. and Wilms, Y., 1996. Soil structure and plant growth: Impact of bulk density and biopores. *Plant and Soil* **185**, 151-162.

Tate, R. L., 2000. Soil Microbiology, 2nd ed., John Wiley and Sons, Inc., New York, pp. 59-61.

Tiedje J. M., Asuming-Brempong S., Nusslein K., Marsh T. L. and Flynn S. J., 1999. Opening the black box of soil microbial diversity. *Applied Soil Ecology* 13, 109-122.

Tigini, V., Prigione, V., Di Toro, S., Fava, F. and Varese, G. C., 2009. Isolation and characterization of polychlorinated Biphenyl (PCB) degrading fungi from a historically contaminated soil. *Microbial Cell Factories* **8**, 1-14.

Tiquia, S. M., Lloyd, J., Herms, D. A., Hoitink, H. A. J. and Michel, F. C., 2002. Effect of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial

community structure determined by analysis of TRFLPs of PCR-amplified 16S rRNA genes. *Applied Soil Ecology* **21**, 31-48.

Tisch, M., Schmer, P., Faulde, M., Groh, A. and Maier, H., 2002. Genotoxicity studies on permethrin, DEET and diazinon in primary human nasal mucosal cells. *European Archives of Oto-Rhino-Laryngology* **259**, 150-153.

Torsvik, V., Daae, F. L., Sandaa, R. –A. and Øvreås, L., 1998. Novel techniques for analysing microbial diversity in natural and perturbed environments. *Journal of Biotechnology* **64**, 53-62.

Torsvik, V., Goksøyr, J. and Daae, F. L., 1990. High diversity in DNA of soil bacteria. *Applied and Environmental Microbiology* **56**, 782-787.

Torzilli, A. P., Sikaroodi, M., Chalkley, D. and Gillevet, P. M., 2006. A comparison of fungal communities from four salt marsh plant using automated ribosomal intergenic spacer analysis (ARISA). *Mycologia* **98**, 690-698.

Trevors, J. T., 1998. Bacterial biodiversity in soil with an emphasis on chemically contaminated soils. *Water, Air, and Soil Pollution* **101**, 45-67.

Troeh, F. R. and Thompson, L. M., 2005. Soil water. In: Troeh, F. R. and Thompson, L. M. (eds.), 2005. Soil and soil fertility. 8th ed. Blackwell Publishing. pp. 81.

Tu, C., Louws, F. J., Creamer N. G., Mueller, J. P., Brownie, C., Fager, K., Bell, M. and Hu, S., 2006. Responses of soil microbial biomass and N availability to transition strategies from conventional to organic farming systems. *Agriculture, Ecosystems and Environment* **113**, 206-215.

Turco, R. F., Kennedy, A. C. and Jawson, M. D., 1994. Microbial indicators of soil quality. In: Doran, J. W., Coleman, D. C., Bezdicek, D. F. and Steward, B. A. (eds.), 1994. Defining soil quality for a sustainable environment. Soil Science Society of America Journal Special Publication No. 35, Madison, WI, pp. 73-90.

van Bruggen, A. H. C. and Termorshuizen, A. J., 2003. Integrated approaches to root disease management in organic farming systems. *Australasian Plant pathology* **32**, 141-156.

van Diepeningen, A. D., de Vos, O. J., Korthals, G. W. and van Bruggen,A. H. C., 2006. Effects of organic versus conventional management on chemical and biological parameters in agricultural soils. *Applied Soil Ecology* **31**, 120-135.

van Elsas, J. D., Duarte, G. E., Rosado, A. S. and Smalla, K., 1998. Microbiological and molecular methods for monitoring microbial inoculants and their effects in the environment. *Journal of Microbiological Methods* **32**, 133-154.

Vanhala, P., Tamminen, P. and Fritze, H., 2005. Relationship between basal soil respiration rate, tree stand and soil characteristics in boreal forest. *Environmental Monitoring and Assessment* **101**, 85-92.

Vásquez-Murrieta, M. S., Govaerts, B. and Dendooven, L., 2007. Microbial biomass C measurements in soil of the central highlands of Mexico. *Applied Soil Ecology* **35**, 432-440.

Vidali, M., 2001. Bioremediation. *Pure and Applied Chemistry* **73**, 1163-1172.

von Wintzingerode, F., Gobel, U. B. and Stackebrandt, E., 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiology Reviews* **21**, 213-229.

Voroney, R.P., 2007. The soil habitat. In: Paul E. A. (ed.), 2007. Soil microbiology, ecology and biochemistry, 3rd ed., Academic press, UK, pp. 25-49.

Waid, J. S., 1999. Does soil biodiversity depend upon metabiotic activity and influences? *Applied Soil Ecology* **13**, 151-158.

Walsh, T. J., Francesconi, A., Kasai, M. and Chanock, S. J., 1995. PCR and single-strand conformational polymorphism for recognition of medically important opportunistic fungi. *Journal of Clinical Microbiology* **33**, 3216-3220.

Wander, M., 2004. Soil organic matter fractions and their relevance to soil function. In: Magdoff, F. and Weil, R. R. (eds.), 2004. Soil organic matter in sustainable agriculture. CRC Press, Boca, Raton, FL. USA, pp. 67-102.

Ward, D. M., Weller, R. and Bateson, M. M., 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature* **345**, 63-65.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H. and Wall, D. H., 2004. Ecological linkages between aboveground and belowground biota. *Science* **304**, 1629-1633.

Weil, R. R. and Magdoff, F., 2004. Significance of soil organic matter to soil quality and health. In: Magdoff, F. and Weil, R. R. (eds.), 2004. Soil Organic Matter in Sustainable Agriculture. CRC Press, Boca Raton, FL. USA, pp. 1-43.

White, D. C., Davis, W. M., Nickels, J. S., King, J. D. and Bobbie, R. J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* **40**, 51-62.

Willoughby, G. L., Kladivko, E. J. and Savabi, M. R., 1997. Seasonal variations in infiltration rate under no-till and conventional (disk) tillage systems as affected by *Lumbricus terrestris* activity. *Soil Biology and Biochemistry* **29**, 481-484.

Witzig, R., Junca, H., Hecht, H. –J. and Pieper, D. H., 2006. Assessment of Toluene/Biphenyl dioxygenase gene diversity in benzene-polluted soils: Links between benzene biodegradation and genes similar to those encoding Isopropylbenzene dioxygenases. *Applied and Environmental Microbiology* **72**, 3504-3514.

Wolf, D. C. and Wagner, G. H., 2005. Carbon transformation and soil organic matter formation: In: Sylvia, D. M., Hartel, P. G., Fuhrmann, J. J. and Zuberer, D. A. (eds.), 2005. Principles and applications of soil microbiology, 2nd ed., Pearson Prentice hall, pp. 285-331.

Xenopoulos, M. A. and Bird, D. F., 1997. Effect of acute exposure to hydrogen peroxide on the production of phytoplankton and bacterioplankton in a mesohumic Lake. *Photochemistry and Photobiology* **66**, 471-478.

Yang, C. H. and Crowley, D. E., 2000. Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. *Applied and Environmental Microbiology* **66**, 345-351.

Yao, H., Bowman, D., Rufty, T. and Shi, W., 2009. Interaction between N fertilization, grass clipping addition and pH in turf ecosystems: Implications for soil enzyme activities and organic matter decomposition. *Soil Biology and Biochemistry* **41**, 1425-1432.

Yao, H., He, Z., Wilson, M. J. and Campbell, C. D., 2000. Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. *Microbial Ecology* **40**, 223-237.

Zak, J. C., Willig, M. R., Moorhead, D. L. and Wildman, H. G., 1994. Accelerated paper: functional diversity of microbial communities: a quantitative approach. *Soil Biology and Biochemistry* **26**, 1101-1108.

Zelles, L., 1999. Fatty acid pattern of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. *Biology and fertility of soils* **29**, 111-129.

Zelles, L., Bai, Q. Y., Ma, R. X., Rackwitz, R., Winter, K. and Beese, F., 1994. Microbial biomass, metabolic activity and nutritional status determined from fatty acid patterns and polyhydroxybutyrate in agriculturally-managed soils. *Soil Biology and Biochemistry* **26**, 439-446.

Zhang, C. and Bennett, G. N., 2005. Biodegradation of xenobiotics by anaerobic bacteria. *Applied Microbiology and Biotechnology* **67**, 600-618.

Chapter 2

MATERIALS AND METHODS

2.1. Site description

Samples were collected from an apple orchard on the Agricultural Research Council (ARC) Infruitec-Nietvoorbij experimental farm in Elgin, Western Cape, South Africa (34º 11' S; 19º 19' E). The 0.7 ha experimental apple orchard is planted with Cripps pink apple trees. Eight different land management treatments were applied within different experimental plots. The treatment plots were set up in the orchard using a randomized block design with four replications for each treatment (Fig. 1). Each treatment plot of 9 by 4.5 m^2 consisted of a single row of nine trees (tree row) and two working rows (Fig. 1). The treatment plots were separated by buffer zones. The land management treatments consisted of different combinations of chemical control, slashing of weed, use of cover crops and mulching along the working rows and bench rows (Table 1). Herbicides (glyphosate) were sprayed in the chemical control plots, while weeding was done by mechanical slashing of the weed in the working rows when overgrown. Mulch treatments were replaced each year during bud-break by applying a mixture of compost and wheat straw of approximately 50 mm thick and 1.5 m wide on the tree row. Wheat and legumes were planted in alternative years as cover crops.

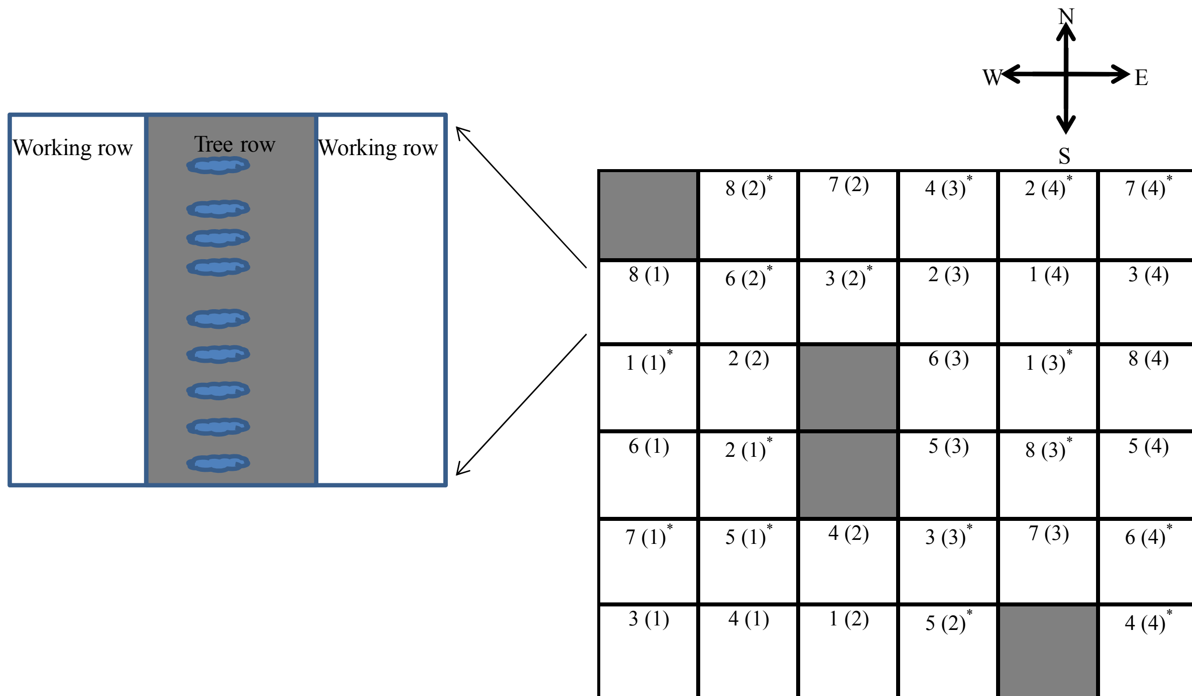

Figure 1. Experimental design of the apple orchard indicating the randomized pattern for soil treatments numbered 1 to 8, and replicated 4 times across the experimental field with buffer zone between treatments. *Sampled plots.

Table 1. Land management practice adopted on experimental field.

Treatment	Working row: Growing season	Working row: Winter season	Bench/Tree row
1	Slash weeds when necessary	Slash weeds when necessary	Chemical control
2	Chemical control of cover crop in spring & growing	Cover crop	Chemical control
3	Chemical control of cover crop in spring & growing	Cover crop	Mulch
4	Mulch	Mulch	Mulch
5	Flatten cover crop in spring; slash weed when necessary	Cover crop	Mulch
6	Flatten cover crop in spring; slash weed when necessary	Cover crop	Mulch, spray EM*
7	Slash weeds when necessary	Slash weeds when necessary	Mulch
8	Slash weeds when necessary	Slash weeds when necessary	Mulch, spray EM*

*Spray EM: Spray effective microorganisms. Treatment 1 was the control plot in this study.

2.2. Sampling and Storage

Soil samples were collected in February and April 2008. Samples were collected from three specific points (A-I) along working row 1 (W1), working row 2 (W2), and between apple trees in the bench/tree rows (TR). Samples were taken at depths of 10 cm and 20 cm. Samples obtained from each of the rows (W1, W2 and TR) was combined separately for each of the rows into 10 cm and 20 cm respectively (Fig. 2). For plots treated with mulch, the mulch layer was carefully removed from the soil surface by hand to prevent the contamination of core samples with surface organic matter. Soil samples were homogenized and sieved with a 2 mm mesh sieve (United wire test sieves, Nigel, South Africa) to remove plant material and large pieces of debris. Homogenised soil samples were kept in cold storage at 4^0C for 3 months, during which genomic DNA were extracted.

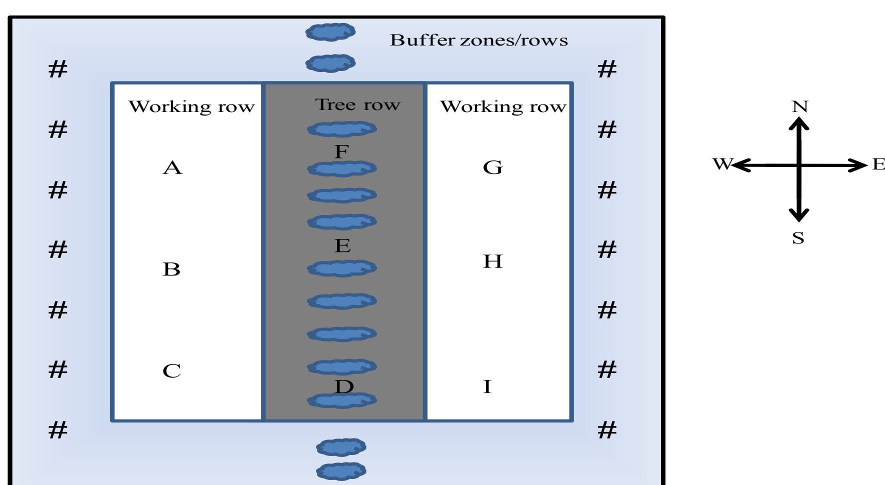

Figure 2. Description of the experimental plot sampling pattern indicating the working rows, tree rows, and the buffer zones.

2.3. Soil characteristics

The physical and chemical properties of the homogenized and sieved soil samples for each of the sampled blocks were conducted by Bemlab, Somerset West, South Africa. The soil properties investigated were, soil pH, resistance, percentage volume of rock, hydrogen (H), phosphorous (P), and potassium (K) concentrations, exchangeable cations of sodium (Na), K, calcium (Ca), and magnesium (Mg), carbon (C) concentration, % base saturation of Na, K, Ca, and Mg, as well as the T-value.

2.4. Molecular Characterisation

2.4.1. Genomic DNA extraction

DNA was extracted from 0.30 g of soil from each pooled sample using the ZR soil microbe DNA kit (Zymo Research, U.S.A) according to the specifications of the manufacturer. Extracted DNA was separated on a 1% (w/v) agarose gel stained with ethidium bromide and visualized using ultraviolet light. The electrophoretic separation was run with a 10kbp ladder (Hyperladder 1, Bioline).

2.4.2. Polymerase Chain Reaction (PCR)

The ITS region of the genomic DNA was amplified using fungal and eubacterial specific primer sets. For fungal-ARISA (F-ARISA), the genomic DNA was amplified using fungal specific ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and fluorescently FAM (6-carboxylfluorescein) labelled ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') primers (White *et al.,* 1990). Similarly, for bacterial-ARISA (B-ARISA) the sequences located between the 16S and 23S rRNA subunit for the bacterial genomic DNA were amplified with eubacterial specific primers ITSReub (5'-GCCAAGGCATCCACC-3') and FAM-fluorescence labelled 5'-end of ITSF (5'-GTCGTAACAAGGTAGCCGTA-3') (Mora *et al.,* 2003; Cardinale *et al*., 2004).

PCR reactions were carried out using GeneAmp PCR system 2400 (Applied Biosystems, USA). The reagent mix contained 1µl of DNA, 500 nM of each of the primers, 5 µl of KapaTaq Readymix (Kapa Biosystems, South Africa) and 3.5 µl of double distilled water, in

a total volume of 10 µl. The PCR conditions for F-ARISA included an initial denaturing step of 3 min at 95 ºC followed by 40 cycles of extension [95 ºC, for 45 sec, 54 ºC for 30 sec and 72^0C for 70 sec] and a final extension reaction at 72^0C for 5 min. For B-ARISA an initial denaturing step of 3 min at 95 ºC followed by 40 cycles amplification [95 ºC, for 45 sec, 55 ºC for 30 sec and 72^0C for 80 sec] and a final extension reaction at 72^0C for 5 min. A negative control sample containing double distilled water was included in each set of reactions. After amplification, the PCR reactions were kept at 4 ºC and aliquots were run on a 1% (w/v) agarose gel containing ethidium bromide. Three different 10 µl reactions were prepared for every sample and then pooled, in order to increase the resolution of low copy number operational taxonomic units (OTU) (Jones and Thies, 2007).

2.4.3. Automated Ribosomal Intergenic Spacer analysis (ARISA)

Products for each sample were run on ABI 3010xl Genetic analyser. The ARISA-PCR fragments were separated via capillary electrophoresis according to different fragment lengths and fluorescent intensities. LIZZ 600 and ROX 1.1 were run as internal size standards for the F-ARISA and B-ARISA respectively (Slabbert, 2008). The ARISA profiles obtained were analysed using the GeneMapper 4.1 software (Applied Biosystems, USA). GeneMapper 4.1 converts fluorescent data into electropherograms using a bin size of 3 for all analysis. Bin size of 3 was adopted based on previous studies, where a bin size of 3 had the highest number of shared peaks in comparison to other bin sizes, and an increase in bin size above 3 resulted in a decrease in the total number of operational taxonomic units (Slabbert, 2008). The length of each amplicon or fragment is registered as a peak and each peak represents a theoretical operational taxonomic unit. For further analysis, peak heights were preferred over peak size, because peak sizes for larger peaks may be inconsistent due to variation in the area. Outputs from GeneMapper were transferred to Excel software (Microsoft Corporation) for further analysis. Peak heights less than 0.5 % of total fluorescence intensity were discarded as they were regarded as background noise according to Hewson and Fuhrman (2004).

2.5. Microbial community diversity

For the microbial community diversity analysis, the Shannon diversity index (H') was calculated for each sample point at depths 10 and 20 cm within each plot. The index takes into consideration the evenness and the abundance of species, and assumes that individual species are randomly sampled from an infinitely large population (Price, 2004). It is defined as the negative sum of each species (OTU's) proportional abundance (p_i) multiplied by the logarithmic value of the same proportional abundance (Hill *et al.*, 2003). A higher value of Shannon index correlates to higher species diversity in the sample analysed (Magurran, 2004).

$$H' = -\sum_{i=1}^{n} p_i \log(p_i)$$

where, pi represents the fraction of each peak (OTU's) of the total integrated peak area (Hill et al., 2003; Legendre and Legendre, 1998). The Shannon diversity index was tested for normality using the Shapiro-Wilk test for normality.

Furthermore, Simpson's index (D) was calculated for each sample point within each plot for the sampled depths. The Simpson's index reflects the influence of the most abundant species on the overall diversity within a sample (Price, 2004). This index measures the probability that two species selected at random from a sample population, will be the same species (Legendre and Legendre, 1998; Hewson and Fuhrman, 2004; Hewson *et al.*, 2007). The higher the Simpson's index the larger the probability that two species randomly selected will be the same.

$$D = \sum_{i=1}^{n} (p_i)^2$$

2.6. Variation within treatment plots

The influence of sampling depth on microbial diversity was investigated for the treatment plots. The difference between the microbial community (as measured by the Shannon index) at depth of 10 and 20 cm was tested by performing an Analysis of variance (ANOVA) on

STATISTICA ver. 8 (Statsoft). This was done separately for the two treatment plots. The Kruskal-Wallis test, which is a non-parametric approach, was used to confirm the ANOVA results, where deviations from normality were observed.

2.7. Variation between replicated plots

The possibility that the diversity and structure of the replicated control treatment plots (T1R1 and T1R3) should have similar microbial community diversity was tested, as both experimental plots were exposed to the same treatment. The difference between the microbial communities between the replicated plots was tested using the Shannon index values by performing an ANOVA in STATISTICA.

2.8. Microbial community structure amongst treatments

To investigate the patterns of similarity between all plots, Whittaker similarity index (S_w) was calculated in Microsoft Excel, comparisons were separately done for the fungal and bacterial profiles and for each sampling date. Whittaker similarity index reflects the association between samples with indices scaled from 0 (being completely dissimilar) to 1 (being completely identical).

$$S_w = 1 - \sum_{i=1}^{n} \frac{|b_{i1} - b_{i2}|}{2}$$

The variables b_{i1} and b_{i2} are fractions of the peak value of profile samples 1 and 2 being compared (Hewson and Fuhrman, 2004). The S_w index was used to generate a distance matrix and cluster analysis was conducted with STATISTICA. The distance linkage between treatment plots was illustrated via the complete linkage clustering analysis using the 1-Pearson r value of the Whittaker similarity indices.

2.9. Effect of different soil treatments on microbial diversity

A 3-way ANOVA was performed to observe the main effects and the interactions of the treatments, rows and sampling depths as factors that influence the microbial diversity

observed. Least significance difference (LSD) and Tukey tests were performed to identify specific differences in factor levels.

3.0. Relationship between soil microbial diversity and soil physicochemical properties.

The possible relationship between the soil microbial diversity and soil properties was tested by Pearson correlation coefficient (PCC) analysis. The PCC measures the strength of association between two variables. The correlation coefficient (r) value could be either positive, zero, or negative, and r can never be greater than 1.0 nor less than -1.0 (Zar, 1999). A positive correlation indicates that for an increase in the value of one of the variables, the other variable also increases in value, while, a negative correlation implies that an increase in value of one of the variables is followed by a decrease in the value of the other variable. If $r = 0$, this denotes that there is no linear relationship between the magnitudes of the two variables (Zar, 1999). However, Pearson correlation assumes normality of values and it is sensitive to outliers. The soil physical and chemical properties assessed in relations to microbial diversity were soil pH, resistance, exchangeable cations (Na, K, Ca, and Mg), percentage saturation of C, Na, K, Ca, and Mg, as well as the T-value. The Spearman Rank order correlation test, which is a non-parametric analysis, was used to confirm the PCC results, in cases where deviations from normality were observed or outliers suspected.

4.0. Relationship between apple yield and soil microbial diversity

To relate fungal and bacterial community diversity to apple yield, the relationship between the microbial diversity index and the mass-ton yield of apples (provided by ARC Infruitec-Nietvoorbij) for each treatment plot was determined. A multiple correlation coefficient was performed to measure the strength of association, with the assumption that the yield of apples is functionally dependent on both fungal and bacterial diversity.

References

Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A. M., Rizzi, A., Zanardini, E., Sorlini, C., Corselli, C. and Daffonchio, D., 2004. Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. *Applied and Environmental Microbiology* **70**, 6147-6156.

Hewson, I. and Fuhrman, J. A., 2004. Richness and density of bacterioplankton species along an estuarine gradient in moreton bay, Australia. *Applied Environmental Microbiology* **70**, 3425-3433.

Hewson, I., Jacobson/Meyers, M. E. and Fuhrman, J. A., 2007. Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, southern California borderlands. *Environmental Microbiology* **9**, 923-933.

Hill, T., C. J., Walsh, K. A., Harris, J. A. and Moffett, B. F., 2003. Using ecological diversity measures with bacterial communities. *FEMS Microbiology Ecology* **43**, 1-11.

Jones, C. M. and Thies, J. E., 2007. Soil microbial community analysis using two-dimensional polyacrylamide gel electrophoresis of the bacterial ribosomal internal transcribed spacer regions. *Journal of Microbiology Methods* **69**, 256-267.

Legendre, P. and Legendre, L., 1998. Numerical ecology, Vol. 1. Elsevier, Amsterdam, The Netherland.

Magurran, A., 2004. Measuring biological diversity. Blackwell Publishing Company, Malden, pp. 106.

Mora, D., Ricci, G., Guglielmetti, S., Daffonchio, D. and Fortina, G. M., 2003. 16S–23S rRNA intergenic spacer region sequence variation in *Streptococcus thermophilus* and related dairy Streptococci and development of a multiplex ITS-SSCP analysis for their identification. *Microbiology* **149**, 807–813.

Price, D. L., 2004. Species diversity and seasonal abundance of scarabaeoid dung bettles (Coleoptera: Scarabaeidae, Geotrupidae, and Trogidae) attracted to cow dung in central New Jersey. *Journal of the New York Entomological Society* **112**, 334-347.

Slabbert, E., 2008. Microbial diversity of soils of the sand fynbos. M. Sc Thesis, Stellenbosch University, South Africa. pp. 77-93.

White, T. J., Burn, T. D., Lee, S. and Taylor, J. W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A. (ed.), 1990. PCR protocol: A guide to methods and applications, Academic press, New York. pp. 315-322.

Zar, J. H., 1999. Biostatistical analysis. 4th ed., Prentice Hall, Upper Saddle River, New Jersey, pp. 377-380.

Chapter 3

Results

3.1. Soil characteristics

The soil physical and chemical properties of the pooled soil sample for each treatment plot are presented in Table 1. The pH across all treatments ranged from pH of 4.7 to 6.2, with the highest pH value of 6.2 recorded for the mulch treated plot T4R3 and lowest in chemically treated plot T1R1. Bulk soil resistance ranged from 1080 to 2750 Ohms across treatments. Mulch treated plot T4R3 recorded the lowest penetration resistance value of 1080 Ohms, while the highest penetration resistance was on plots exposed to mechanical flattened cover crops (T5R2). Similarly, T4R3 had the highest value of phosphorus (mg/kg), exchangeable cations [sodium (Na), potassium (K), and calcium (Ca)]. However, different values were obtained from the replicated plot sampled for treatment 4 (T4R4).

Table 1. Measured physical and chemical properties of soil samples from the different treatment plots.

							(d)EC (cmol/Kg)				(%)					
Plots	pH(KCl)	(c)Res.(Ohm)	H (cmol/kg)	Rock (Vol %)	P (mg/kg)	K (mg/kg)	Na	K	Ca	Mg	C	Na	K	Ca	Mg	T-Value (cmol/kg)
(a)**T1** (b)**R(1)**	4.7	2060	1.77	46	15	164	0.15	0.42	5.33	1.41	2.45	1.69	4.6	58.68	15.55	9.09
T1 R(3)	5.3	2450	1.07	38	25	168	0.14	0.43	5.98	1.01	2.11	1.63	4.98	69.29	11.71	8.64
T2 R(1)	5	2450	1.29	38	39	105	0.14	0.27	5.29	1.03	2.36	1.73	3.37	66	12.8	8.01
T2 R(4)	5.5	1870	0.8	40	40	166	0.16	0.42	8.98	1.31	2.96	1.37	3.64	76.9	11.24	11.68
T3 R(2)	5.1	2160	1.07	43	62	185	0.12	0.47	5.39	1	2.19	1.46	5.89	66.99	12.37	8.05
T3 R(3)	5.2	2150	0.96	50	23	174	0.17	0.45	5.27	0.88	2.11	2.22	5.77	68.17	11.43	7.73
T4 R(3)	6.2	1080	0	32	47	346	0.19	0.89	9.54	0.71	2.5	1.69	7.82	84.25	6.24	11.32
T4 R(4)	5.3	1930	0.91	38	29	267	0.09	0.68	4.62	0.62	2.12	1.31	9.83	66.72	9.01	6.93
T5 R(1)	5.4	2640	0.8	36	26	209	0.12	0.53	5.9	1.06	2.23	1.45	6.35	70.1	12.59	8.41
T5 R(2)	5	2750	1.13	55	25	113	0.12	0.29	3.63	0.63	2.07	2.1	4.99	62.54	10.89	5.8
T6 R(2)	4.8	1800	1.55	58	41	300	0.12	0.77	5.17	0.95	2.97	1.36	8.97	60.44	11.1	8.55
T6 R(4)	4.9	3060	1.18	51	46	136	0.13	0.35	4.06	0.74	2.04	1.99	5.37	62.9	11.45	6.45
T7 R(1)	5.3	2610	0.91	56	43	194	0.12	0.5	6.14	1.08	2.42	1.36	5.67	70.21	12.36	8.75
T7 R(4)	5.4	2100	0.91	68	25	214	0.13	0.55	7.22	1.09	3.03	1.35	5.53	72.91	11.02	9.9
T8 R(2)	5.5	1410	0.7	56	32	280	0.21	0.72	7.09	1.33	2.09	2.09	7.12	70.56	13.26	10.05
T8 R(3)	5	2330	1.18	41	23	241	0.18	0.62	6.74	0.84	2.1	1.86	6.47	70.55	8.76	9.55

(a) : Treatment; (b): Replicate(s); (c): Resistance; (d): Exchangeable cations

3.2. Molecular characterization

3.2.1 Genomic DNA extraction

High molecular weight DNA above 100ng/nl was obtained for all the samples extracted (Fig. 1), using the soil DNA extraction kit (Zymo Research, U.S.A).

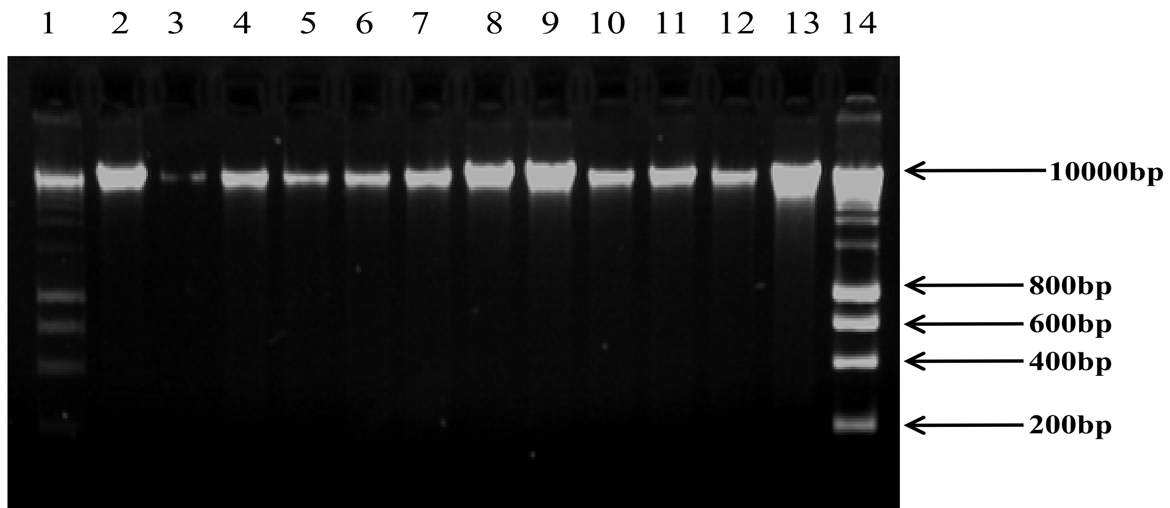

Figure 1. Extracted genomic DNA ran on 1 % agarose gel electrophoresis directly from the soil using the ZR soil microbe DNA kit (Zymo research, U.S.A). Lane 1 and 14: 10 kbp ladder (Hyperladder 1); lane 2-13: soil genomic DNA extracts from different soil sample points and treatment plots.

3.2.2. PCR amplification

All PCR reactions resulted in smears. For the fungal specific primers a smear of approximately 300 to 1200 bp was observed (Fig. 2), while the bacterial specific primers produced a smear within the range of 200 bp to 1500bp (Fig. 3).

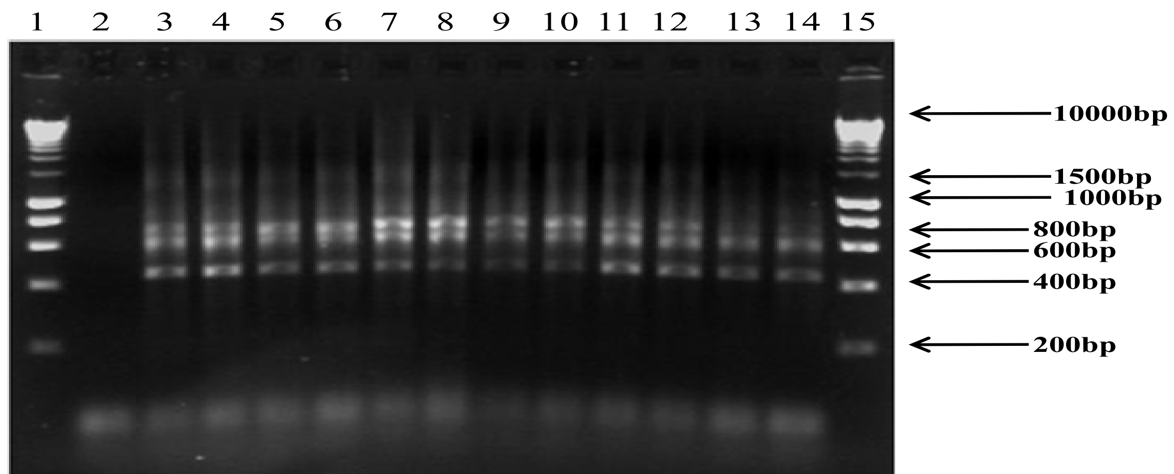

Figure 2. Fungal-ARISA PCR products obtained from genomic DNA extracted from the soil sample using fungal ITS4 and FAM-labelled ITS5 primers. Lane 1 and 15: 10 kbp ladder (Hyperladder 1); Lane 2: negative control; lanes 3-14: amplicons from genomic DNA extracts for one of the treatment blocks.

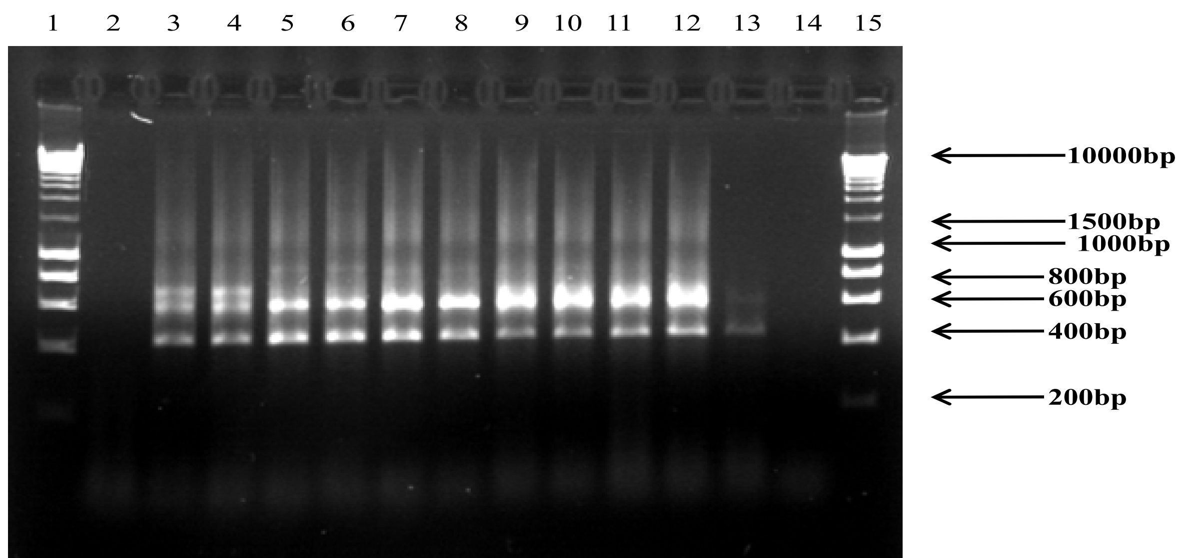

Figure 3. Bacterial-ARISA PCR products obtained from genomic DNA extracted from the soil sample using ITSF and FAM-labelled ITSReub primers. Lane 1 and 15: 10 kbp ladder (Hyperladder 1); Lane 2 and 14: negative control; lanes 3-13: amplicons from genomic DNA extracts for one of the treatment blocks.

3.2.3. Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Fungal ARISA electropherograms resulted in peaks ranging from 150 to 800 bp as shown by the GeneMapper LIZZ 600 size standard. For the bacterial ARISA electropherogram peaks

ranges from 100 to 900 bp as shown by the GeneMapper ROX 1.1 size standard. The difference in fragment sizes observed on gel and those of the electropherograms is as a result of the limited size range of the internal size standards.

3.3. Microbial community diversity

The diversity index for the fungal community ranged from 0.60 to 1.20, the lowest value of 0.60 was obtained in working row exposed to chemical (herbicide) control of cover crops and the highest diversity index were in plots treated with mulch and cover crops, respectively (Table 2, Addendum). Based on observations from the data show in Table 2, the working row 2 (W2) had a consistently higher fungal diversity index compared to working row 1 (W1) in February. Investigating the influence of sampling dates on microbial diversity, the variation in the bacterial diversity index between the sampling dates was not significant ($p < 0.05$) (Fig. 4), compared to the fungal community composition for both sampled dates. In April a significant increase ($p = 0.005$) in fungal community composition was observed (Fig. 5). The bacterial diversity index was consistently higher than fungal diversity across all treatments, in the range of between 1.20 and 1.80.

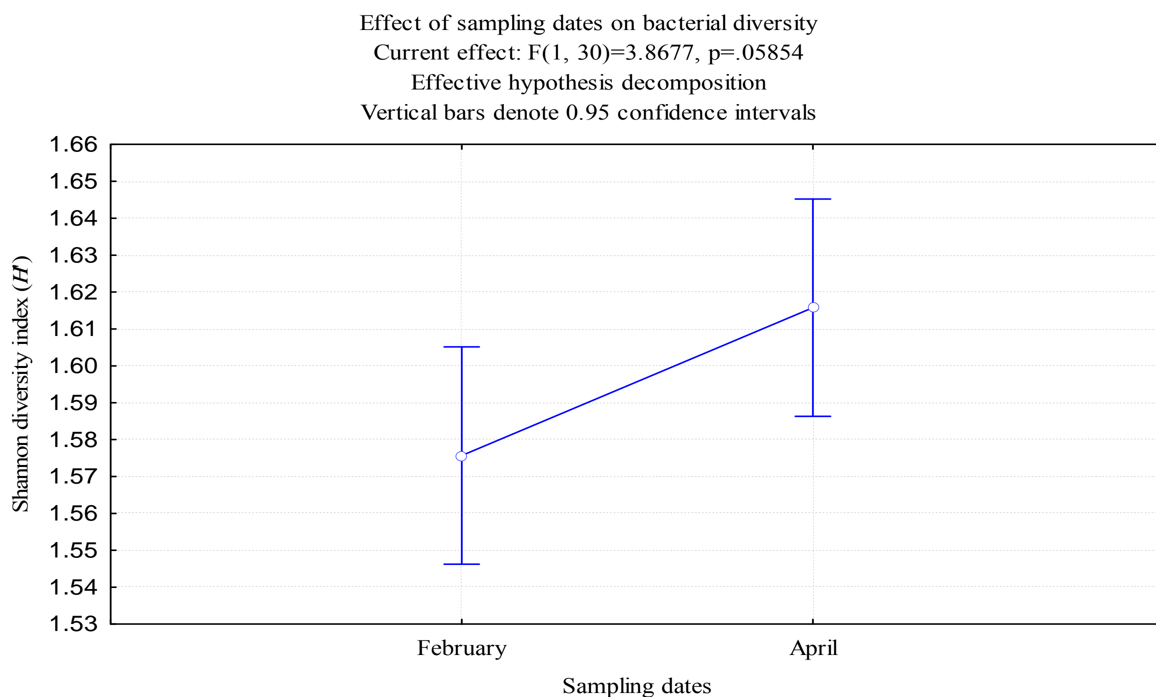

Figure 4. The effect of sampling seasons on bacterial diversity, as indicated by the Shannon diversity index obtained from the February and April 2008 sample.

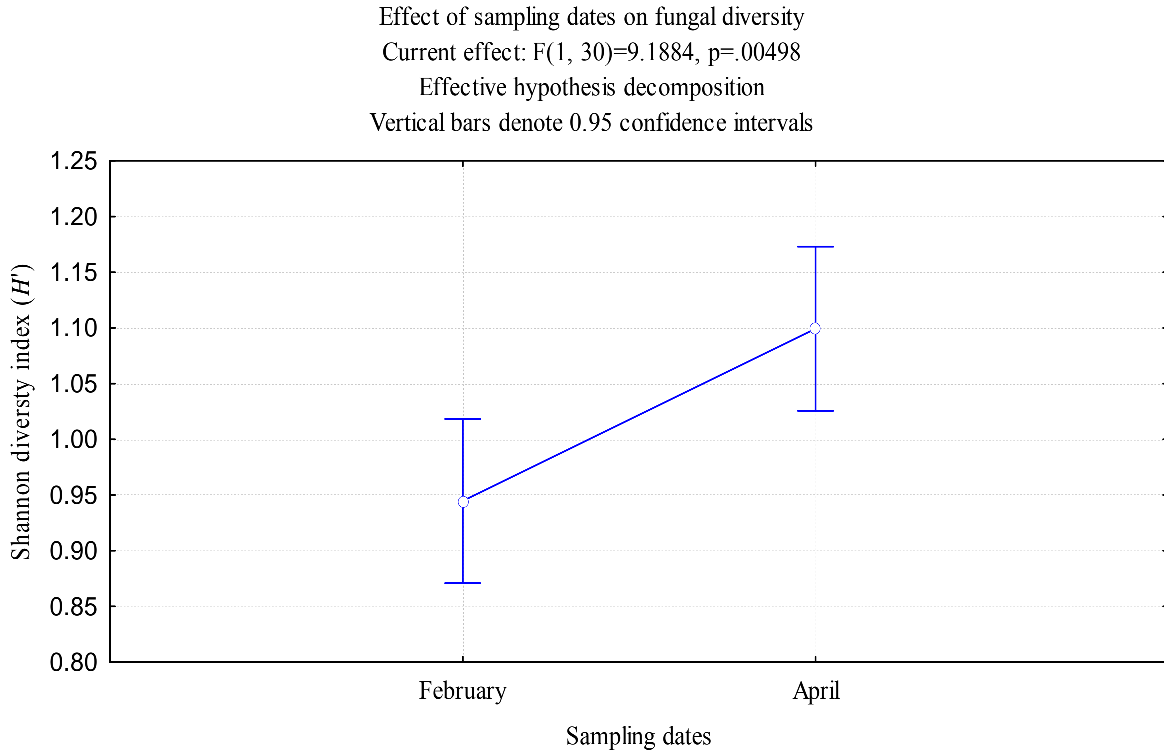

Figure 5. The effect of sampling seasons on fungal diversity as indicated by the Shannon diversity index obtained from the February and April 2008 sample.

The Simpson's index measures the probability that two species selected at random from a sample population, will be the same species (Ambinakudige and Sathih, 2009). Therefore, the higher the Simpson's index (D) the larger the probability that two species randomly selected will be the same. From the summary reported in Table 3 (Addendum), the Simpson's index for bacterial community were consistently lower than that of fungal community in the February and April profile. This observation further supported the Shannon index result, where in the bacterial community diversity were consistently higher than the fungal community diversity. The bacterial community diversity was consistent with no significant difference between the February and April index (Fig. 6). Simpson's index for fungal community diversity was significantly higher in the February sample than that of April ($p = 0.0013$) (Fig. 7). This results confirms the observations obtained from the Shannon diversity index.

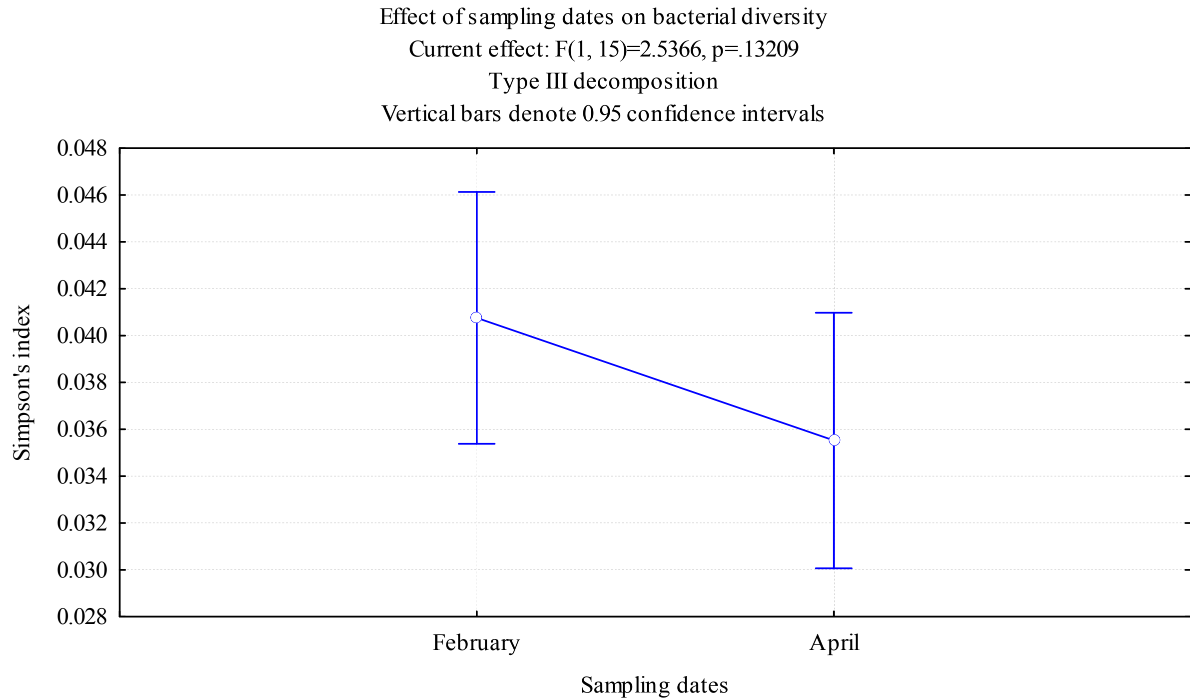

Figure 6. The effect of sampling seasons on bacterial diversity, as indicated by the Simpson's index obtained from the February and April 2008 sample.

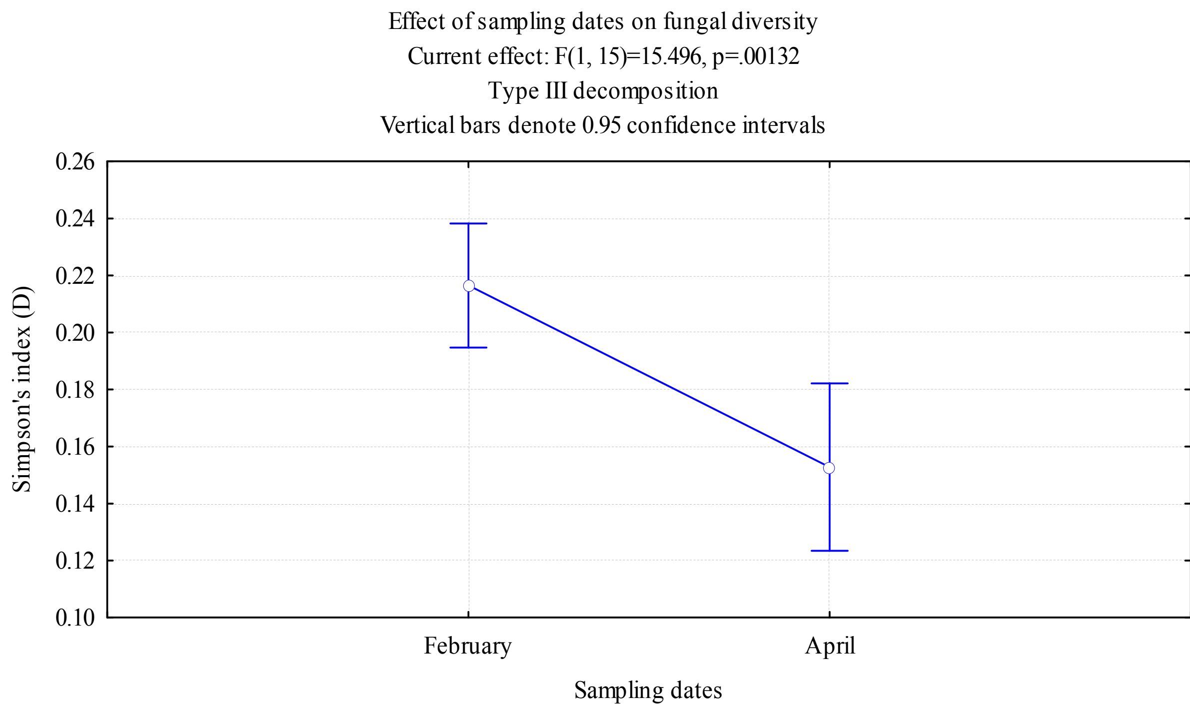

Figure 7. The effect of sampling seasons on fungal diversity, as indicated by the Simpson's index obtained from the February and April 2008 sample.

3.4. Variation within treatment plots

On the sampled plots, the effect of different sampling depths does not seem to have any effect on microbial community diversity. The fungal diversity in T1R3 for April had a marginal p-value of 0.0502 (Fig. 8, 9). However, the Kruskal-Wallis test confirmed the ANOVA result observed in T1R3 for fungal diversity to be significant (p = 0.049).

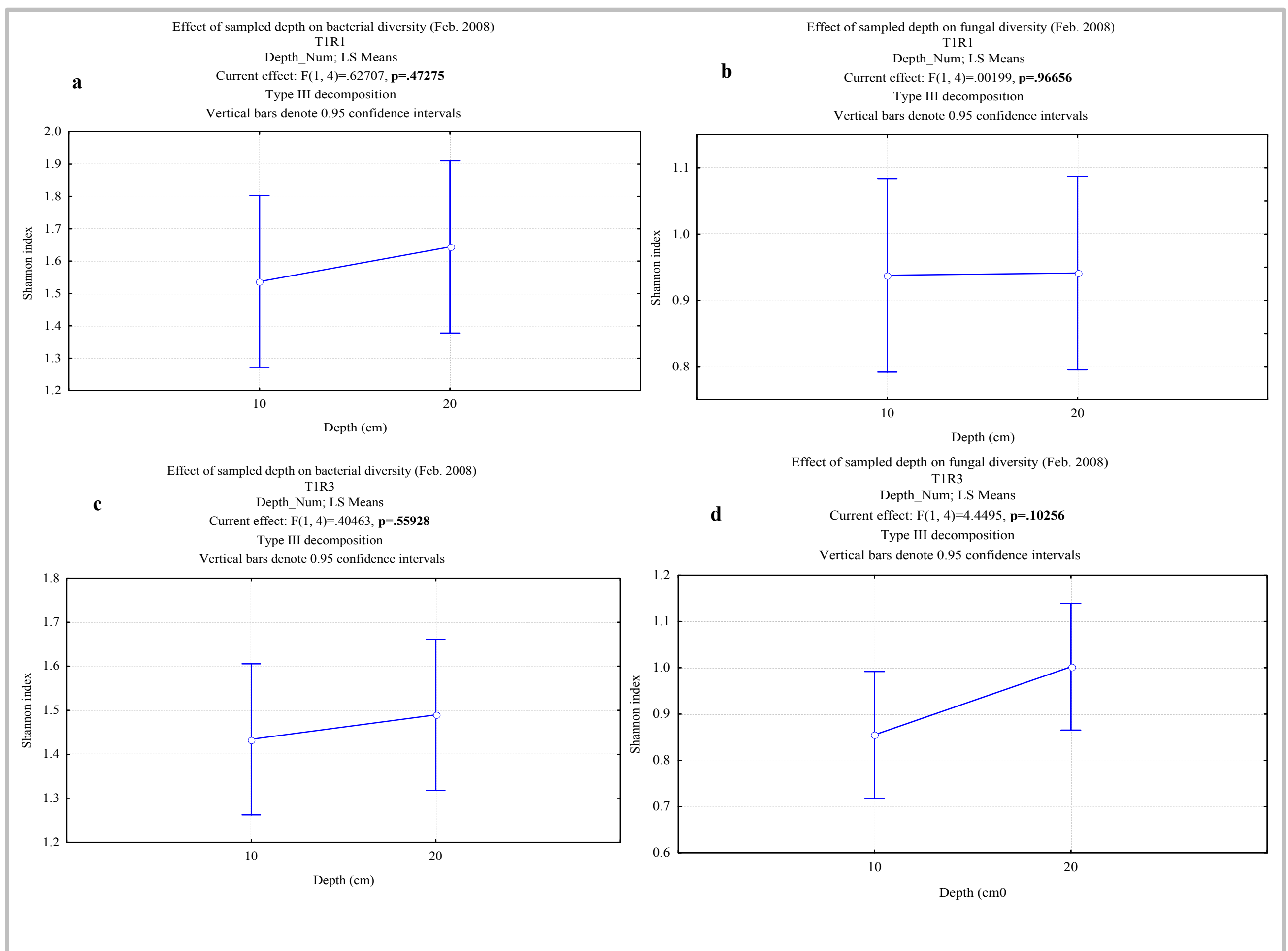

Figure 8. ANOVA comparison of Shannon diversity index at sample depth 10 and 20 cm for (T1R1 and T1R3) control plots for February 2008. Graphs; a: bacterial diversity in T1R1; b: fungal diversity in T1R1; c: bacterial diversity in T1R3; d: fungal diversity in T1R3.

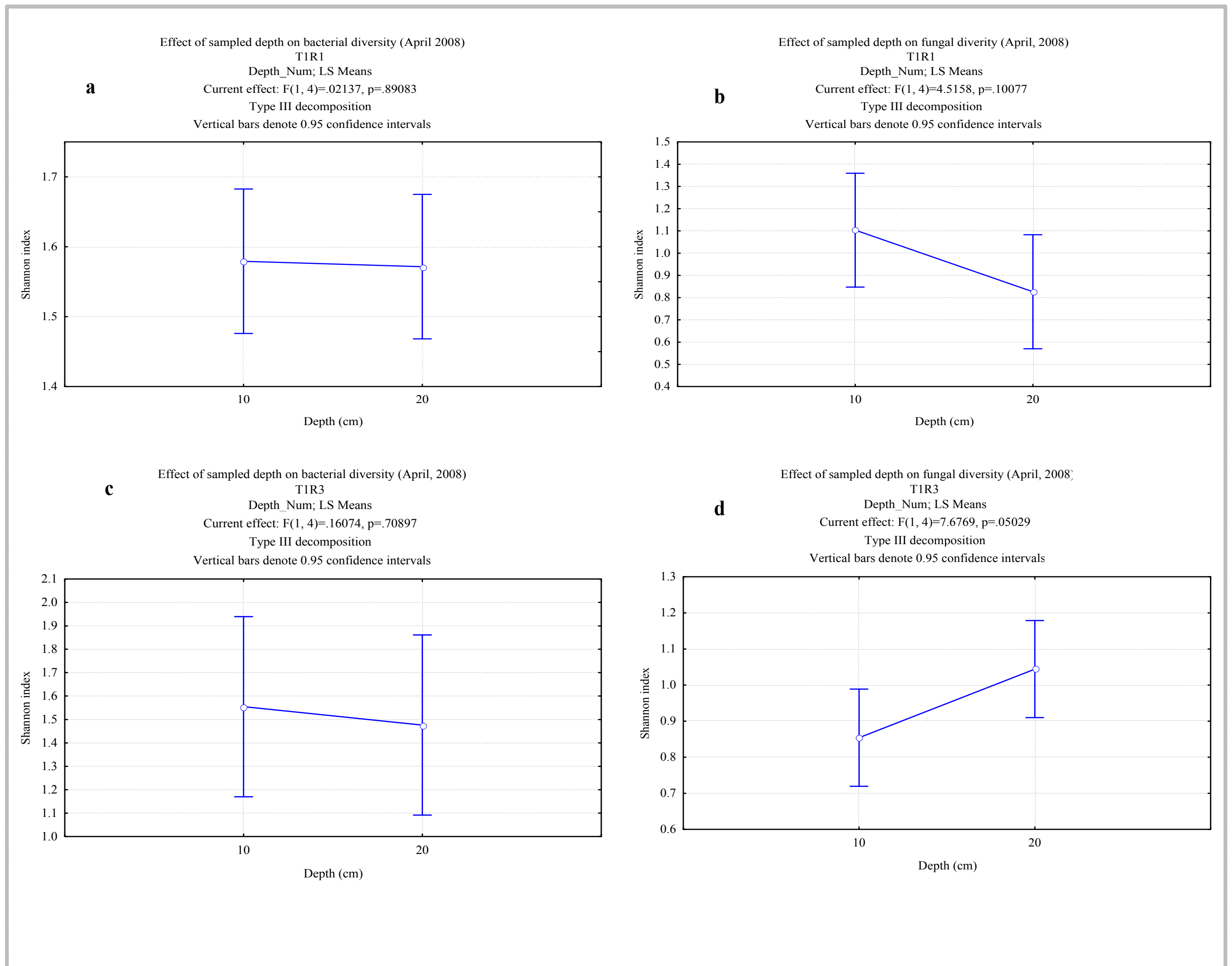

Figure 9. Comparative Shannon diversity index at sample depth 10 and 20 cm for (T1R1 and T1R3) control plots for April 2008. Graphs; a: bacterial diversity in T1R1; b: fungal diversity in T1R1; c: bacterial diversity in T1R3; d: fungal diversity in T1R3.

3.5. Variation between replicated control plots

Comparing the replicates T1R1 and T1R3, for the February and April samples from the ANOVA of their Shannon index, there was no significant difference ($p < 0.05$) in the fungal and bacterial diversity (Fig. 10).

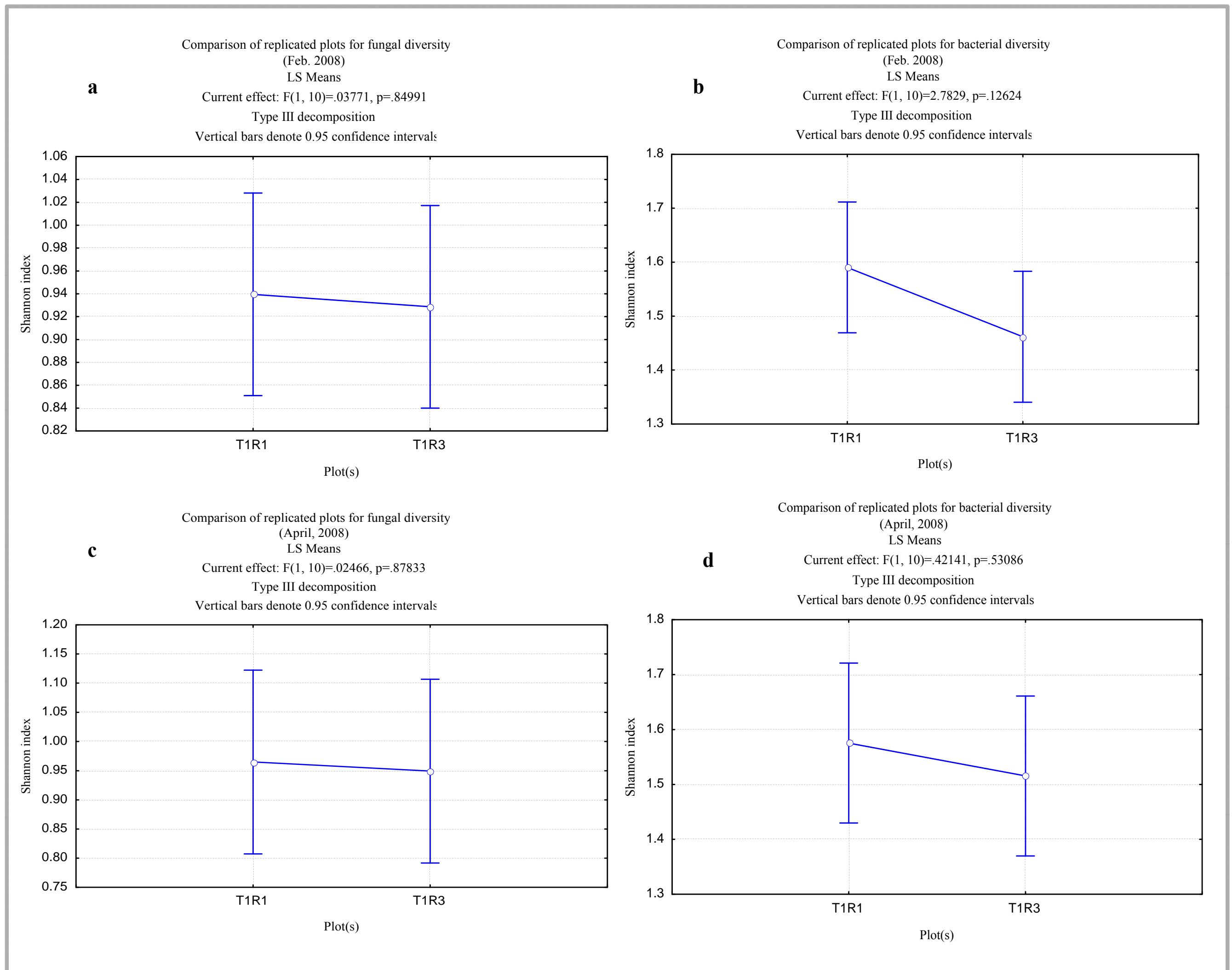

Figure 10. Comparative Shannon diversity index of the control treatment plots for February and April. Graphs; a: fungal diversity in February 2008; b: bacterial diversity in February 2008; c fungal diversity in April 2008; d: bacterial diversity in April 2008.

3.6. Microbial community structure amongst treatments

Cluster analysis was performed to illustrate the distance or relationship between the fungal and bacterial communities based on the profile obtained from the GeneMapper analysis of the occurrence of operational taxonomic units. The dendogram obtained displayed no consistent similarity pattern between replicated plots, with the exception of treatment 1 and treatment 7 for the fungal community structure in February and treatment 1, 3, 7 and 5 combined with 6 in April. For the bacterial community structure treatment 4 clustered in the February profile and treatment 3, 5 and 6 in April (Fig. 11-14).

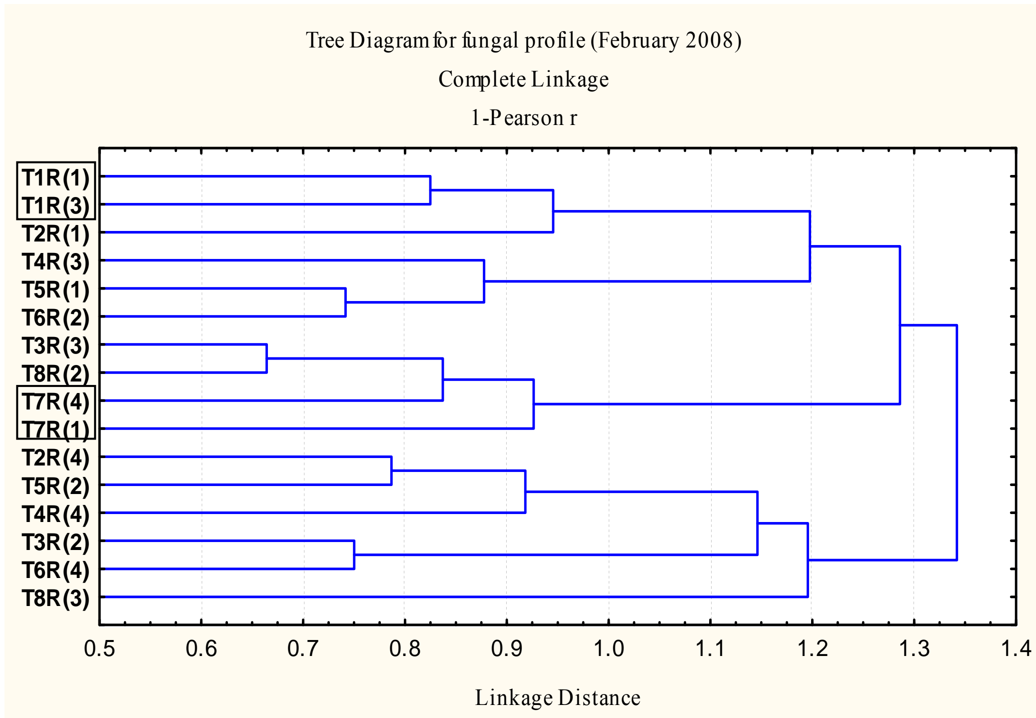

Figure 11. The dendogram of the sixteen experimental plots based on the Whittaker similarity analysis for the fungal profile in February 2008.

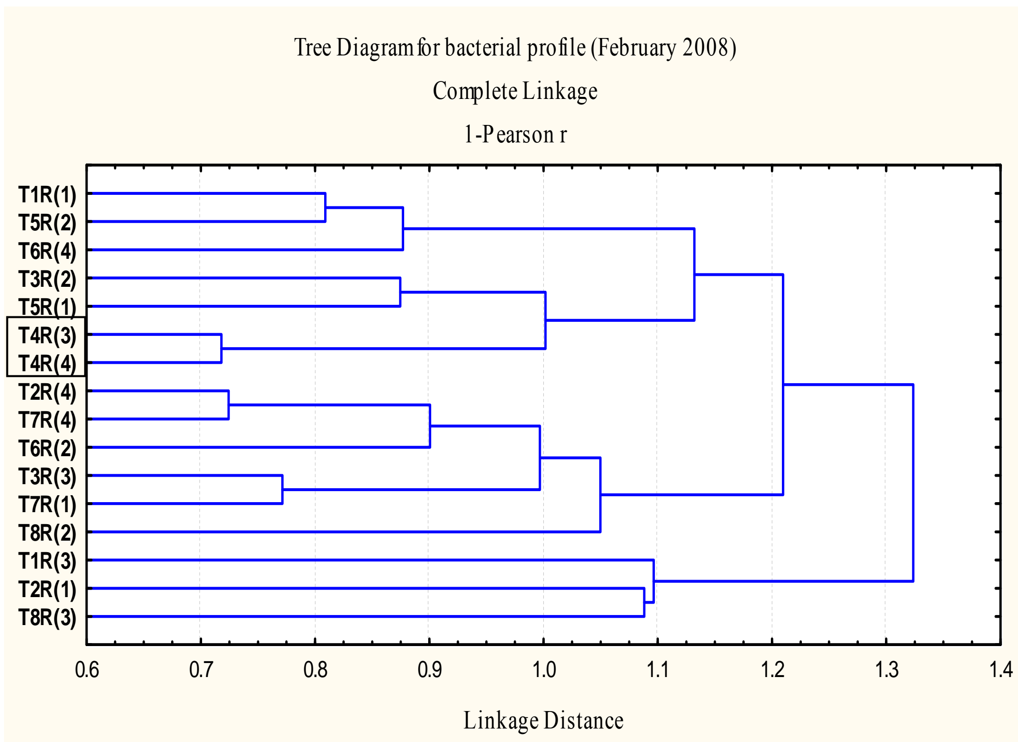

Figure 12. The dendogram of the sixteen experimental plots based on the Whittaker similarity analysis for the bacterial profile in February 2008.

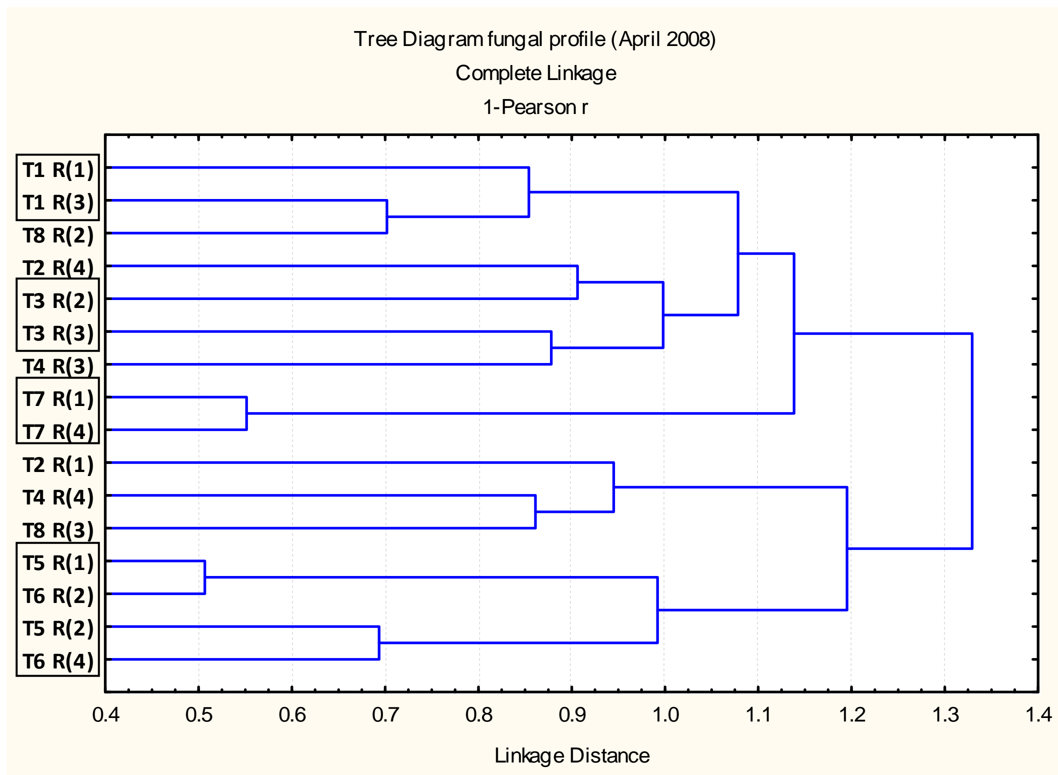

Figure 13. The dendogram of the sixteen experimental plots based on the Whittaker similarity analysis for the fungal profile in April 2008.

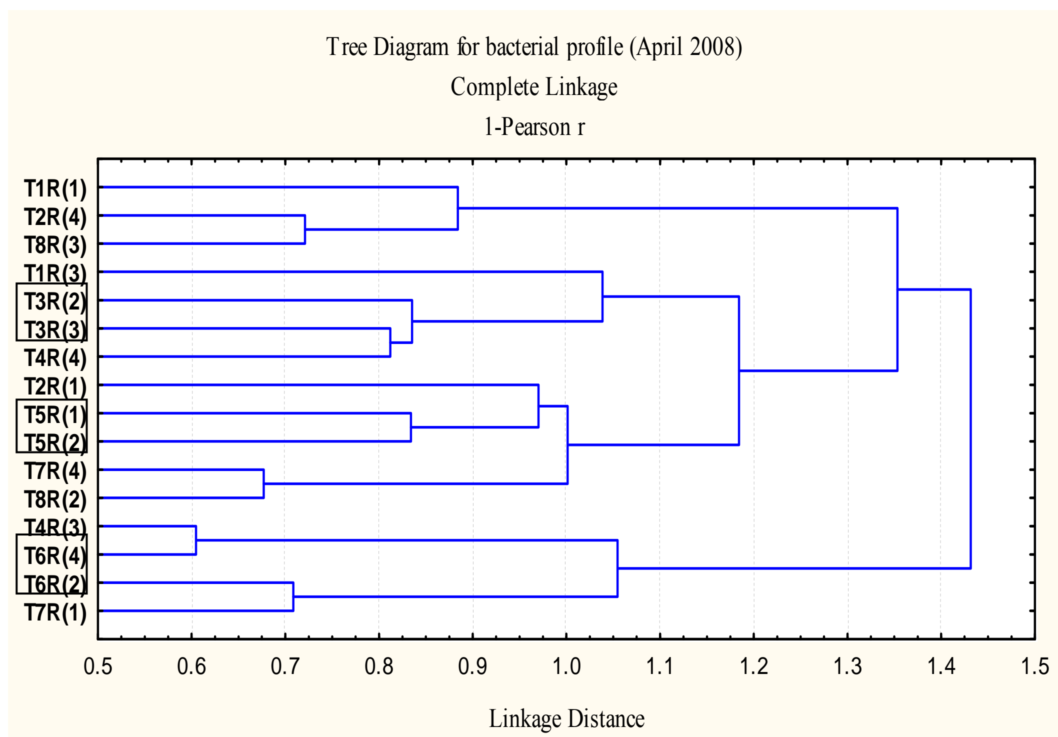

Figure 14. The dendogram of the sixteen experimental plots based on the Whittaker similarity analysis for the bacteria profile in April 2008.

3.7. Effect of different soil treatments on microbial diversity

Table 6 summarizes the results from the 3-way ANOVA performed for the February samples to investigate the effect of treatments, rows, sampling depth and their interactions on the soil microbial diversity. The interaction between treatment and rows had a significant influence on fungal diversity (p= 0.0195), while other interactions and main effects had no significant effect on the fungal diversity (Fig. 15-18). The Post-hoc (LSD and Tukey) test performed confirmed this result and identified the specific significant interactions. Also, the test of treatment as one of the main effects on fungal diversity gave a marginal p-value of 0.059 (Fig. 19). For the bacterial diversity index, out of the four possible interactions investigated, only the interaction between treatments and row had a significant influence on the microbial diversity with p value of 0.047 (Fig. 20, 21, 22, 23). From the three main effects, treatments and depth had a significant effect on the bacterial diversity with p values of 0.036 and 0.015, respectively (Fig. 24, 25). Although rows independently had no significant effect on bacteria diversity, combinations of treatment(s) with row(s) have a significant effect on bacteria diversity (Figure 20). Similarly, Post-hoc test performed confirmed this result and also identified significant interactions.

Table 6. Summary of the effects of soil treatments, rows, and depths, with their interactions on fungal and bacterial diversity in February 2008.

Shannon index	Effect	SS	Degree of Freedom	MS	F	p
Fungal	Intercept	77.32360	1	77.32360	5840.626	**0.000000**
	Treatments	0.19203	7	0.02743	2.072	0.059377
	Rows	0.04727	1	0.04727	3.570	0.063348
	Depths	0.00016	1	0.00016	0.012	0.911559
	Treatment(s)*Row(s)	0.24215	7	0.03459	2.613	**[0.019465]**
	Treatment(s)*Depth(s)	0.19222	7	0.02746	2.074	0.059128
	Row(s)*Depth(s)	0.00096	1	0.00096	0.073	0.788299
	Treatment(s)*Row(s)*Depth(s)	0.12264	7	0.01752	1.323	0.253937
	Error	0.84729	64	0.01324		
Bacterial	Intercept	210.8511	1	210.8511	20739.71	**0.000000**
	Treatments	0.1652	7	0.0236	2.32	**[0.035590]**
	Rows	0.0108	1	0.0108	1.06	0.307149
	Depths	0.0631	1	0.0631	6.21	**[0.015319]**
	Treatment(s)*Row(s)	0.1553	7	0.0222	2.18	**[0.047418]**
	Treatment(s)*Depth(s)	0.0085	7	0.0012	0.12	0.996726
	Row(s)*Depth(s)	0.0285	1	0.0285	2.81	0.098695
	Treatment(s)*Row(s)*Depth(s)	0.0219	7	0.0031	0.31	0.948227
	Error	0.6507	64	0.0102		

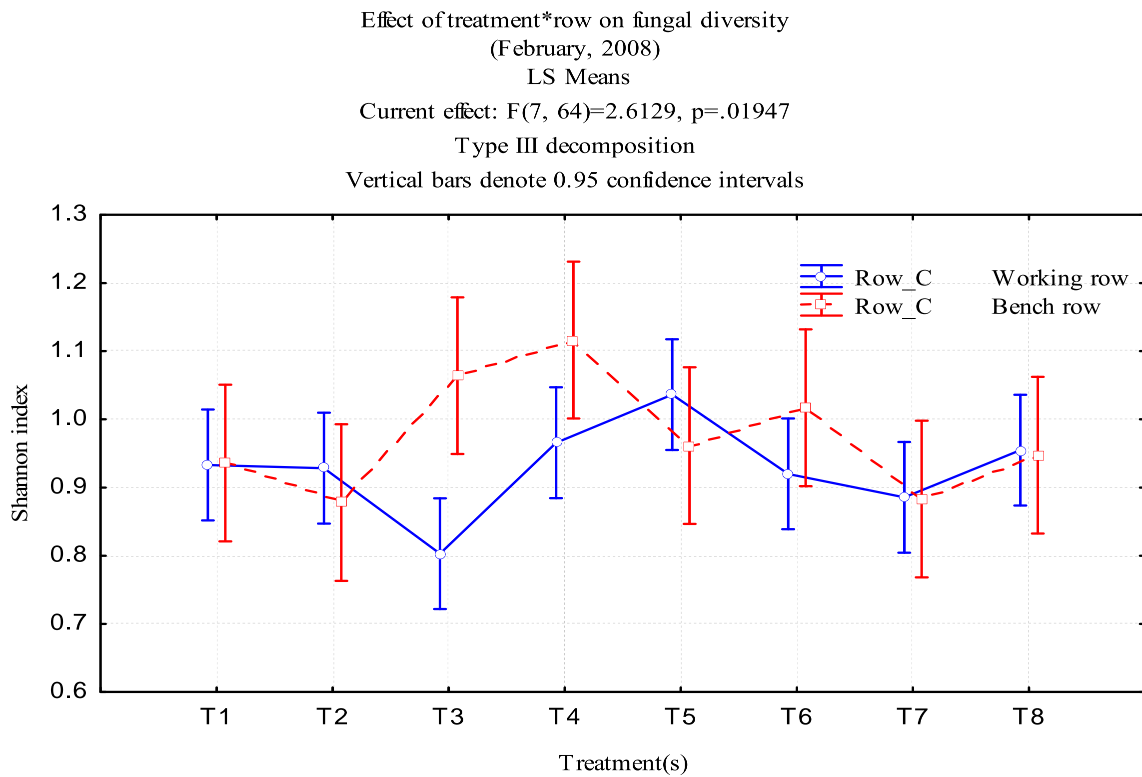

Figure 15. The effect of treatments combined with rows on fungal diversity (February, 2008).

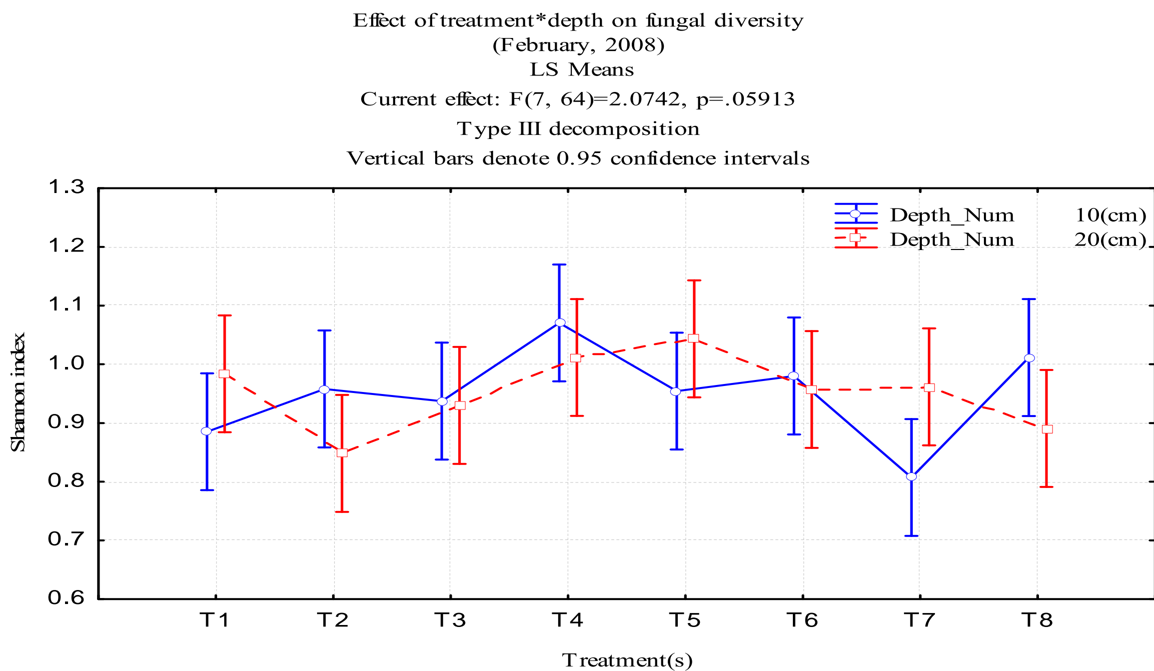

Figure 16. The effect of treatments combined with depths on fungal diversity (February, 2008).

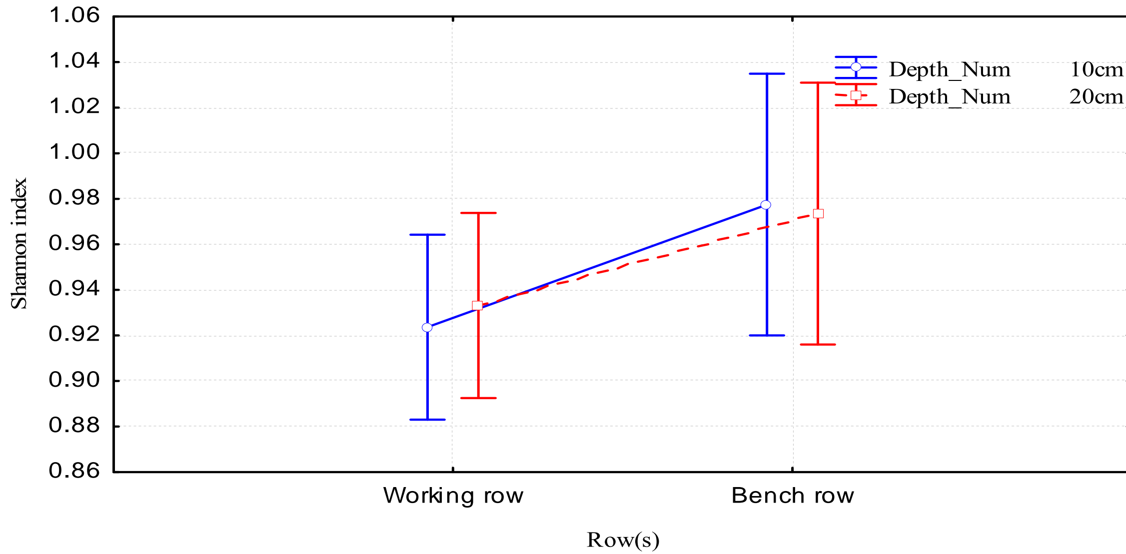

Figure 17. The effect of rows combined with depth on fungal diversity (February, 2008).

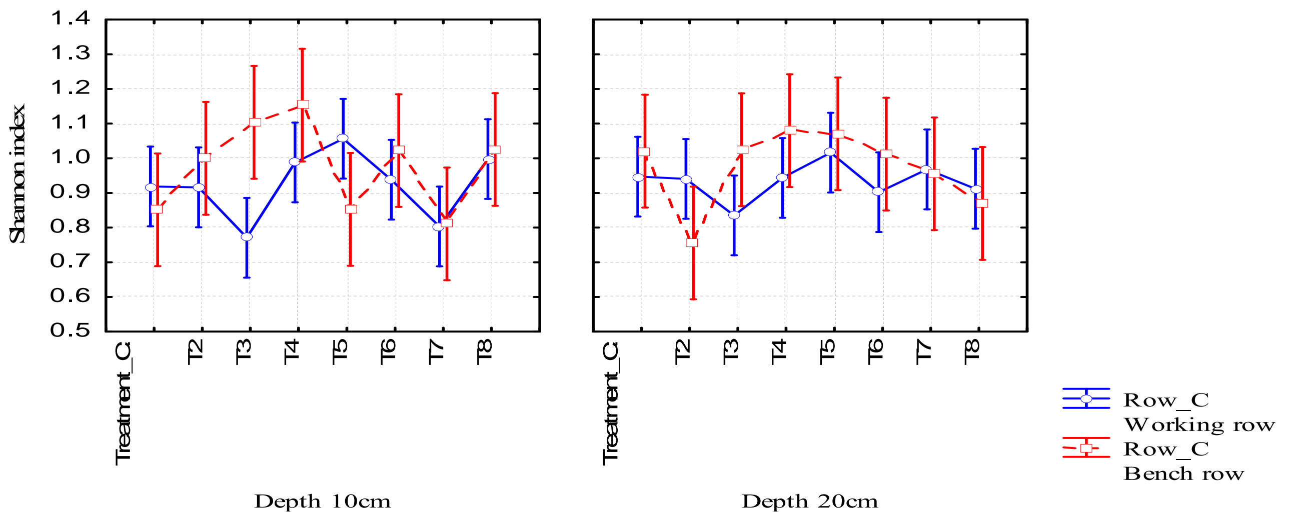

Figure 18. The effect of treatments, rows combined with depths on fungal diversity (February, 2008). Treatment_C (T1)

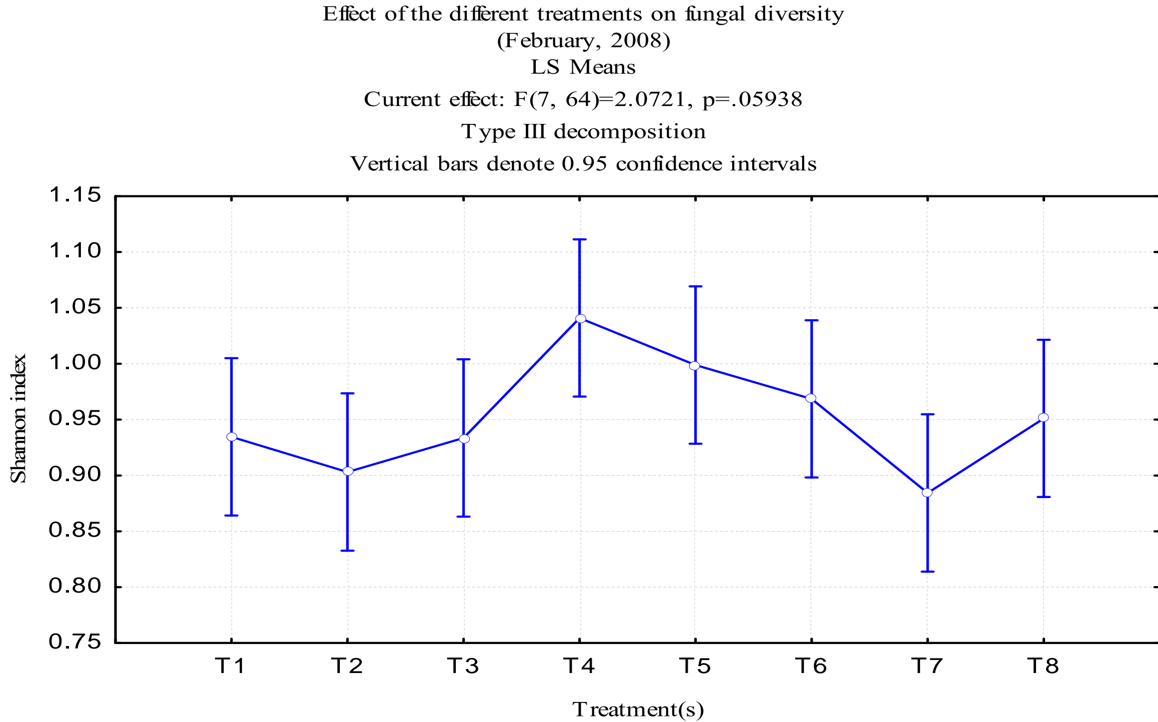

Figure 19. The effect of the different treatments fungal diversity (February, 2008).

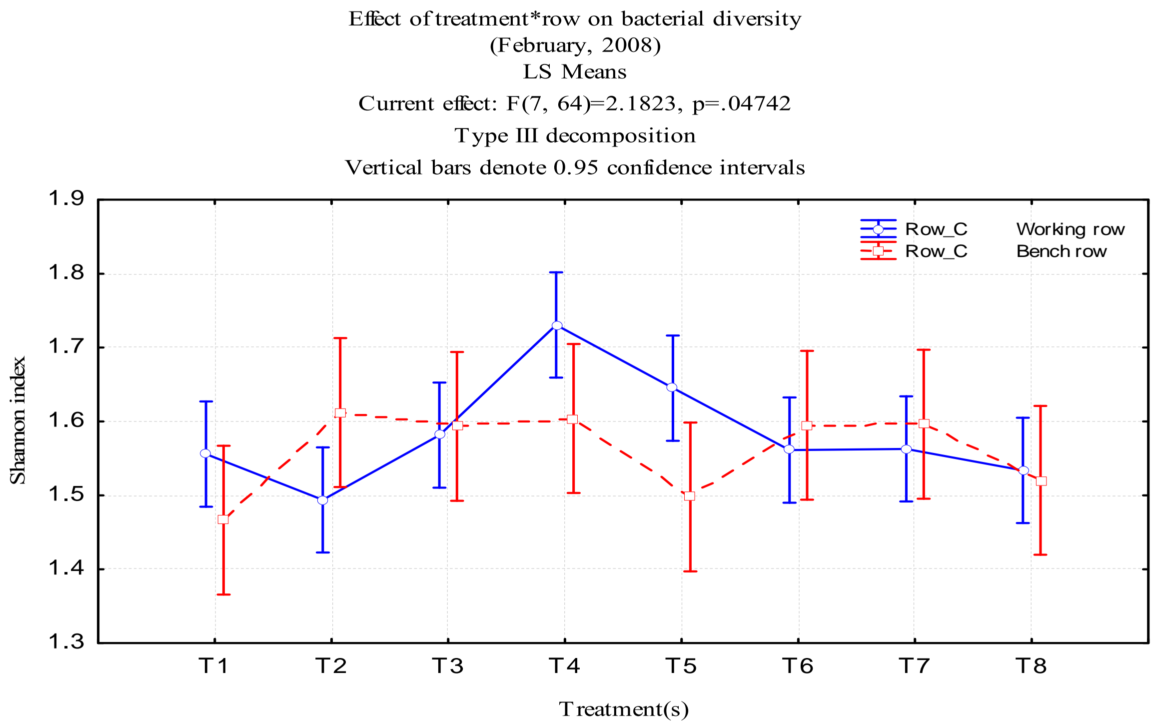

Figure 20. The effect of treatments combined with rows on bacterial diversity (February, 2008).

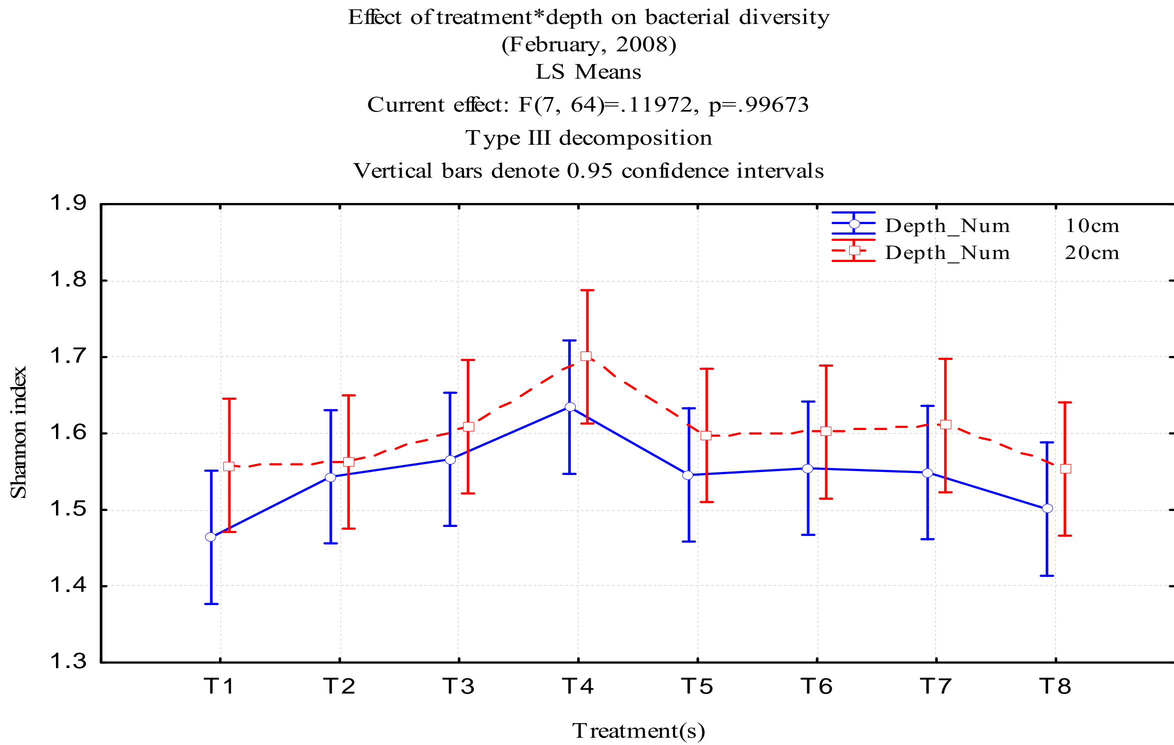

Figure 21. The effect of treatments combined with depths on bacterial diversity (February, 2008).

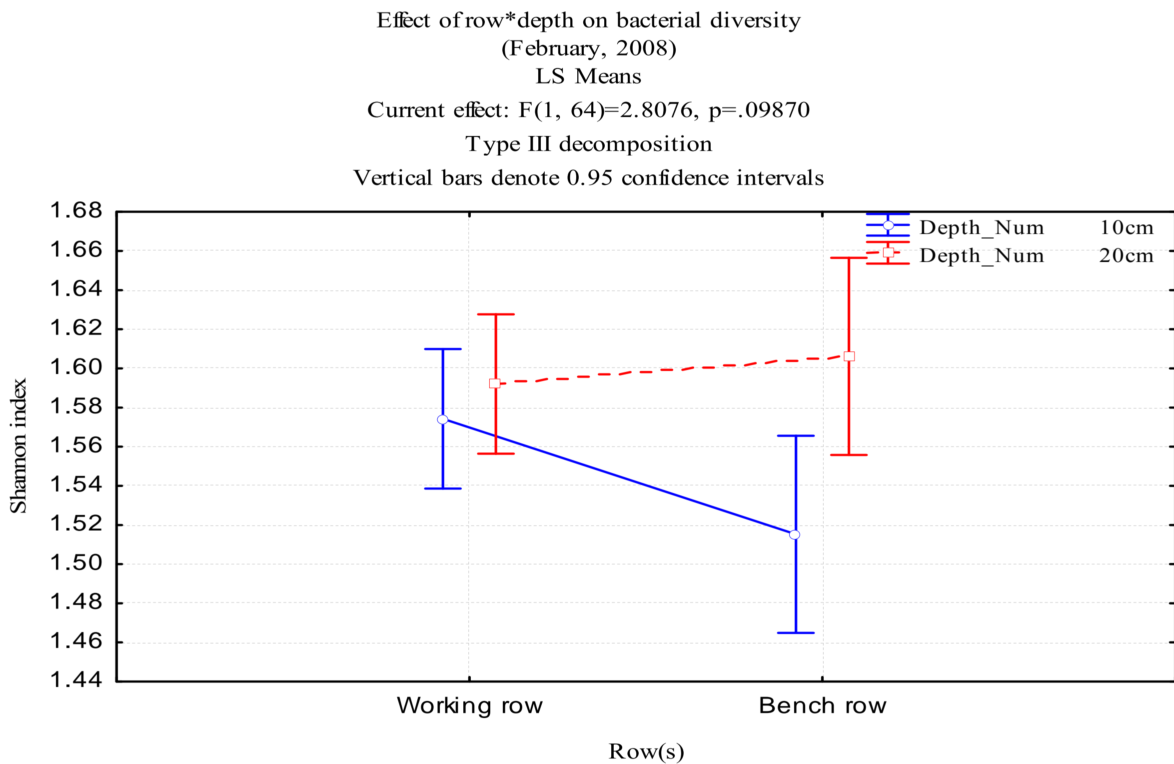

Figure 22. The effect of rows combined with depths on bacterial diversity (February, 2008).

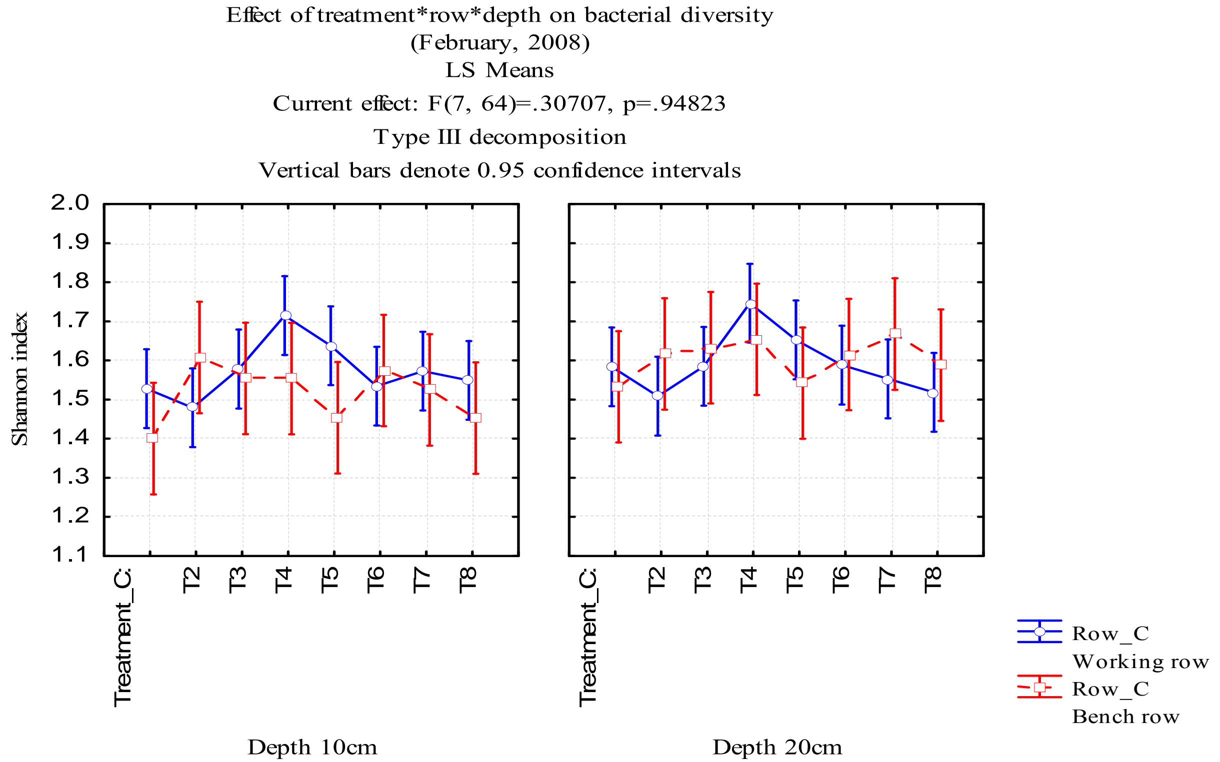

Figure 23. The effect of treatments, rows combined with depths on bacterial diversity (February, 2008).

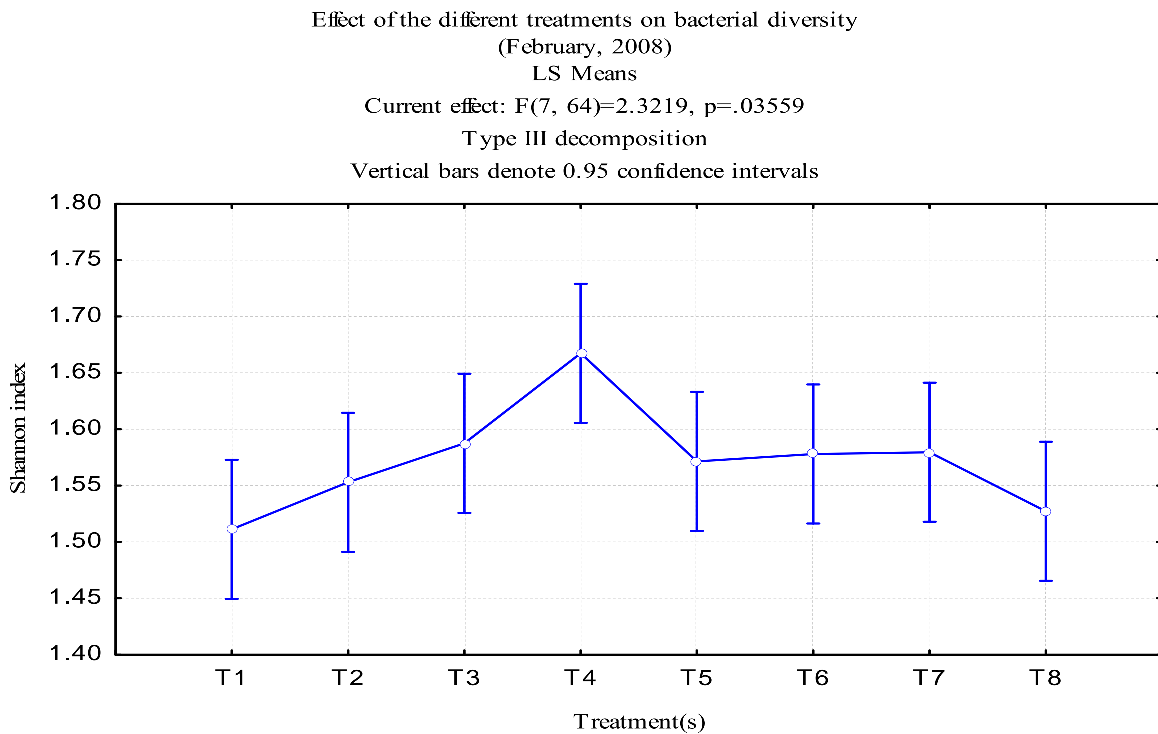

Figure 24. The effect of the different treatments bacterial diversity (February, 2008).

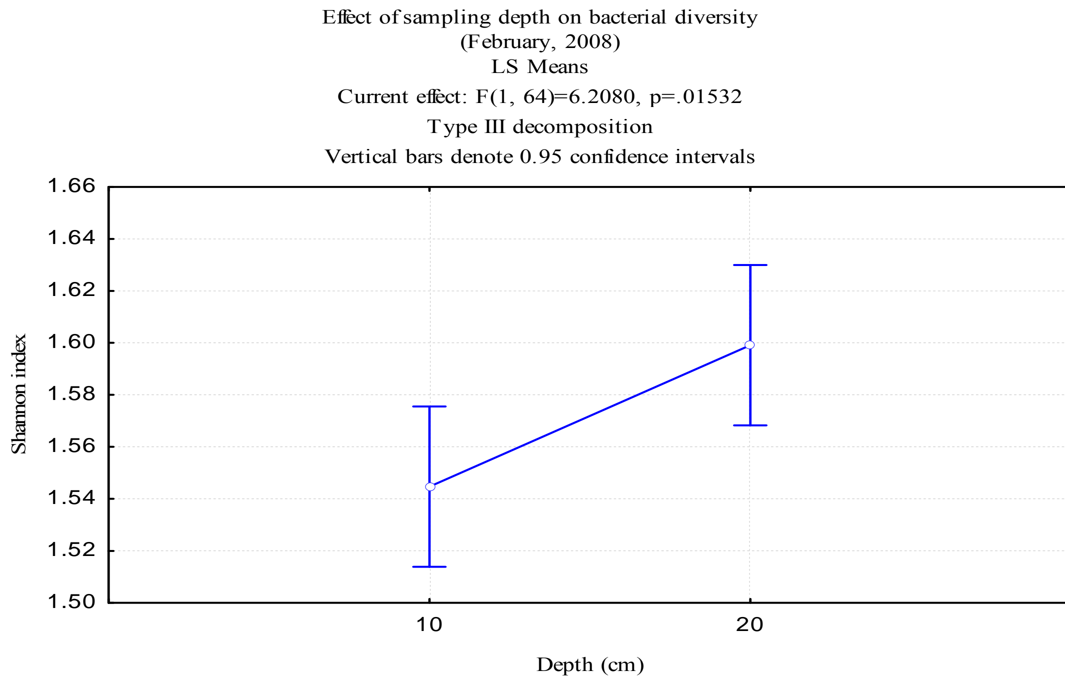

Figure 25. The effect sampling depths on bacterial diversity (February, 2008).

The April sampling (Table 7), showed that none of the interactions investigated had a significant influence on fungal diversity (Fig. 26-29). Treatment as a main effect, had a significant influence on fungal diversity with a p-value of less than 0.0000, and treatment 5 and 6, were observed to be significantly different from the other treatments (Fig. 30). The post-hoc tests performed confirmed the ANOVA result of the interactions on fungal diversity. For the bacterial diversity, only the interaction between treatments and rows had a significant influence on the microbial diversity with p value of 0.01 (Fig. 31-34). Post-hoc tests confirmed this result and also identified those interactions with ($p < 0.05$). None of the main effects investigated had a significant effect on the bacterial diversity ($p > 0.05$).

Table 7. Summary of the effects of soil treatments, rows, and depths with their interactions on fungal and bacterial diversity in April 2008.

Shannon index	Effect	SS	Degree of Freedom	MS	F	p
Fungal	Intercept	104.1032	1	104.1032	2905.247	**0.000000**
	Treatments	2.7226	7	0.3889	10.854	**[0.00000]**
	Rows	0.0213	1	0.0213	0.596	0.443120
	Depths	0.0003	1	0.0003	0.009	0.926442
	Treatment(s)*Row(s)	0.1144	7	0.0163	0.456	0.862356
	Treatment(s)*Depth(s)	0.1109	7	0.0158	0.442	0.871924
	Row(s)*Depth(s)	0.0540	1	0.0540	1.508	0.223950
	Treatment(s)*Row(s)*Depth(s)	0.1593	7	0.0228	0.635	0.725154
	Error	2.2933	64	0.0358		
Bacterial	Intercept	222.3555	1	222.3555	22167.03	**0.000000**
	Treatments	0.0755	7	0.0108	1.08	0.389484
	Rows	0.0018	1	0.0018	0.18	0.675359
	Depths	0.0068	1	0.0068	0.68	0.413122
	Treatment(s)*Row(s)	0.2048	7	0.0293	2.92	**[0.01032]**
	Treatment(s)*Depth(s)	0.0972	7	0.0139	1.38	0.227103
	Row(s)*Depth(s)	0.0077	1	0.0077	0.77	0.383250
	Treatment(s)*Row(s)*Depth(s)	0.0530	7	0.0076	0.76	0.626446
	Error	0.6420	64	0.0100		

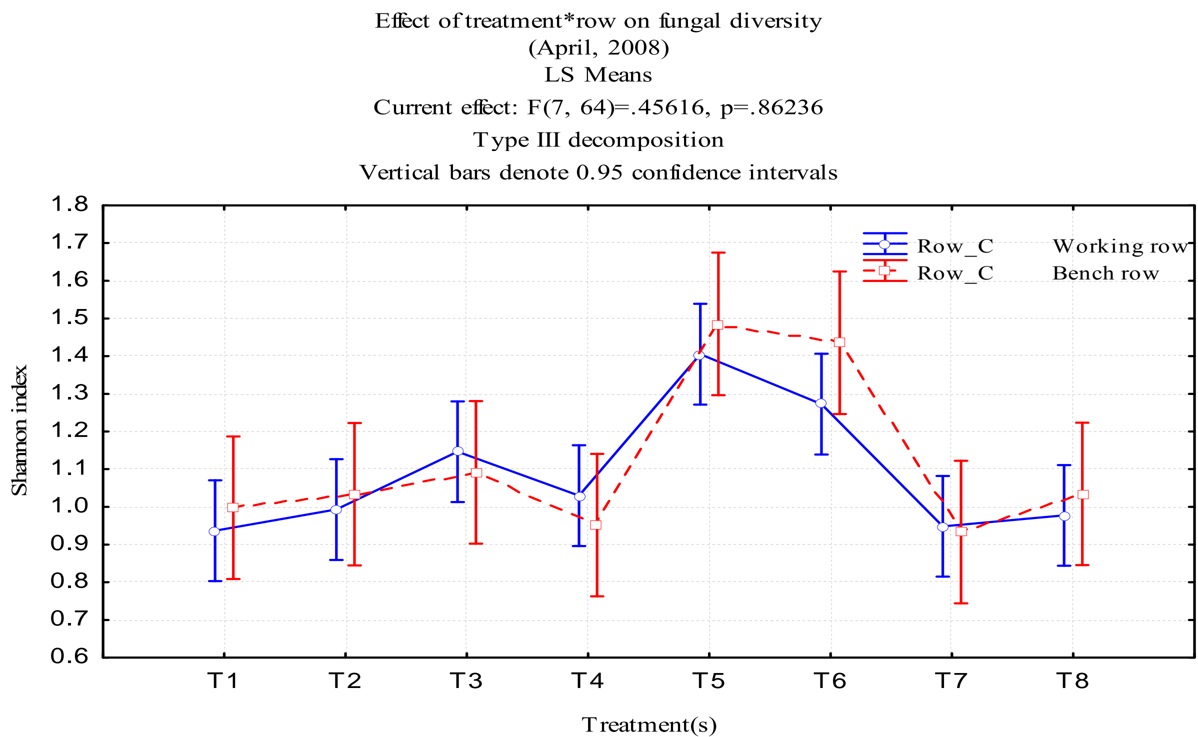

Figure 26. The effect of treatments combined with rows on fungal diversity (April, 2008).

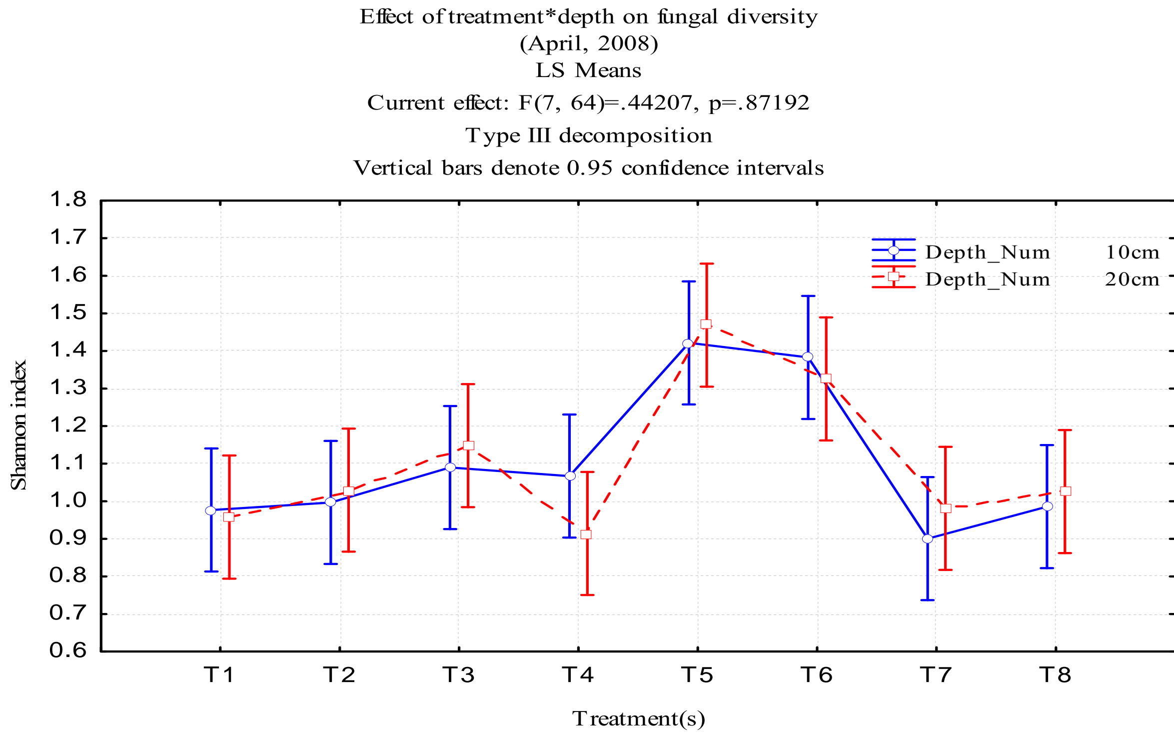

Figure 27. The effect of treatments combined with depths on fungal diversity (April, 2008).

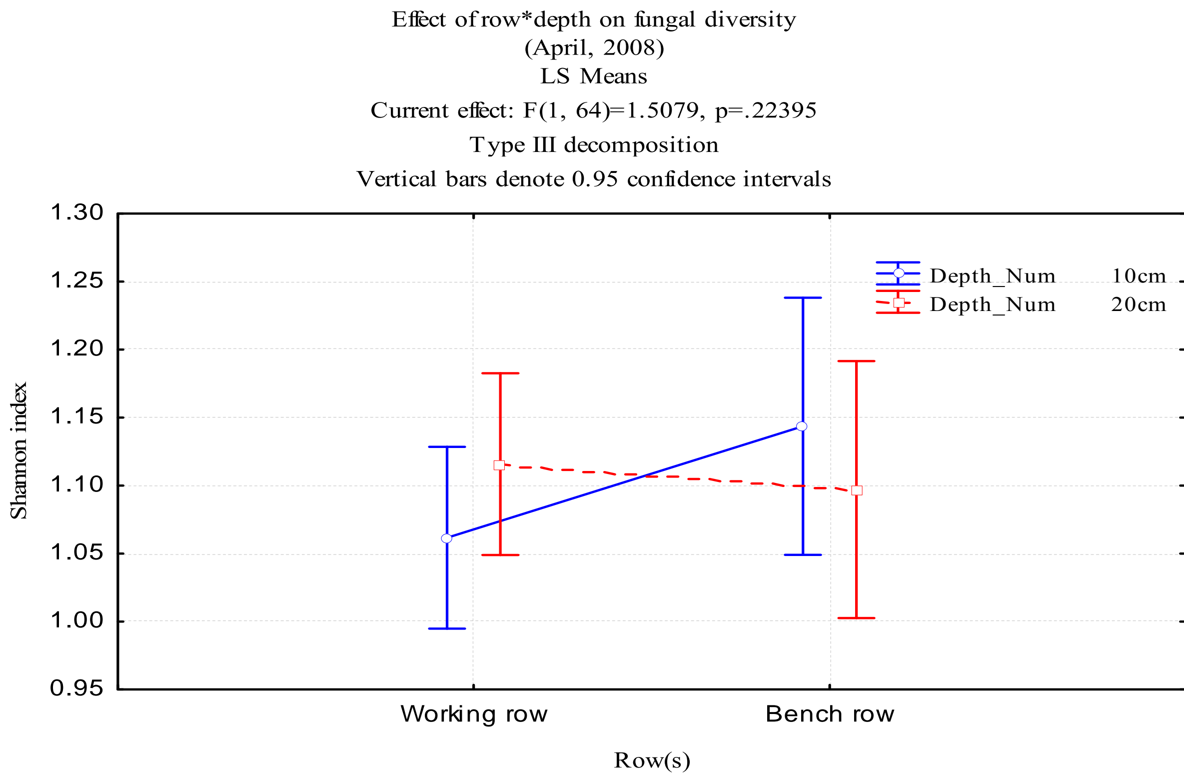

Figure 28. The effect of rows combined with depths on fungal diversity (April, 2008).

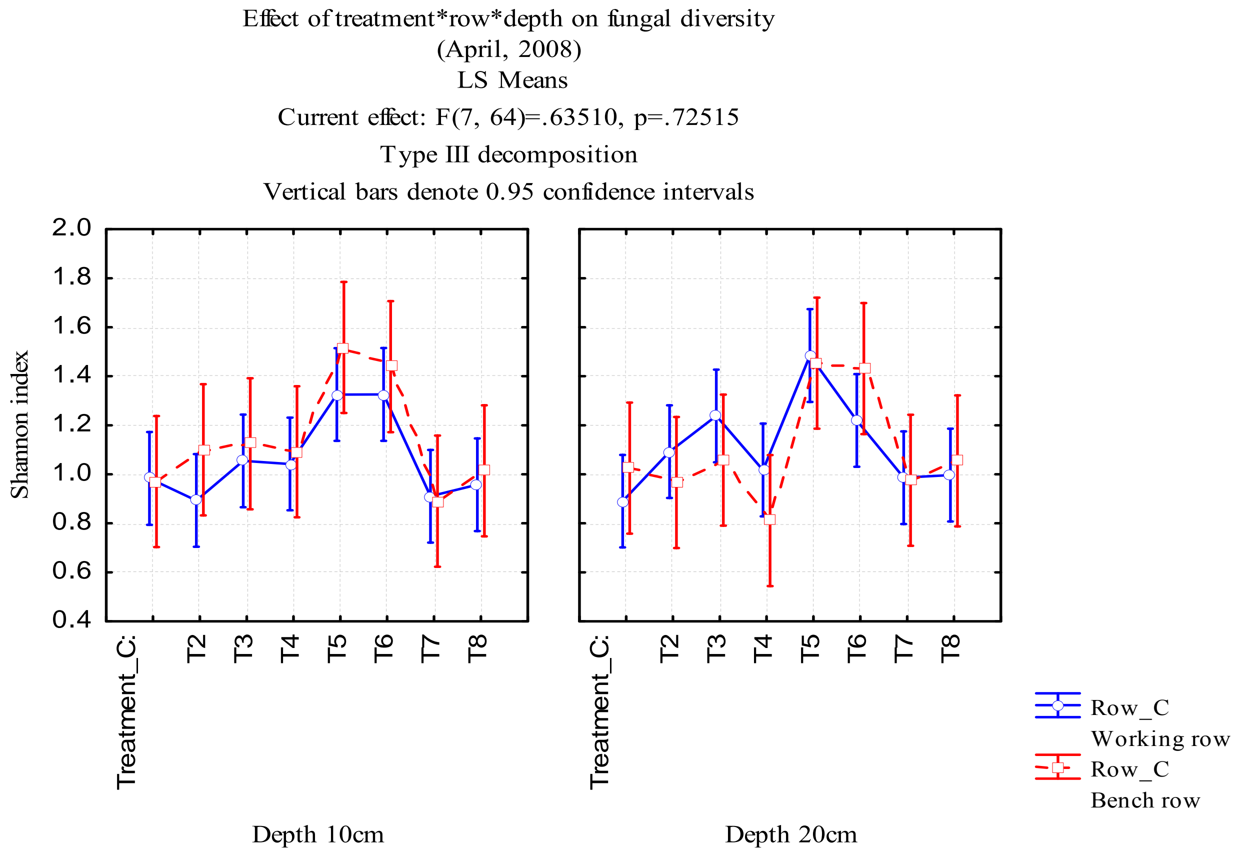

Figure 29. The effect of treatments, rows combined with depths on fungal diversity (April, 2008).

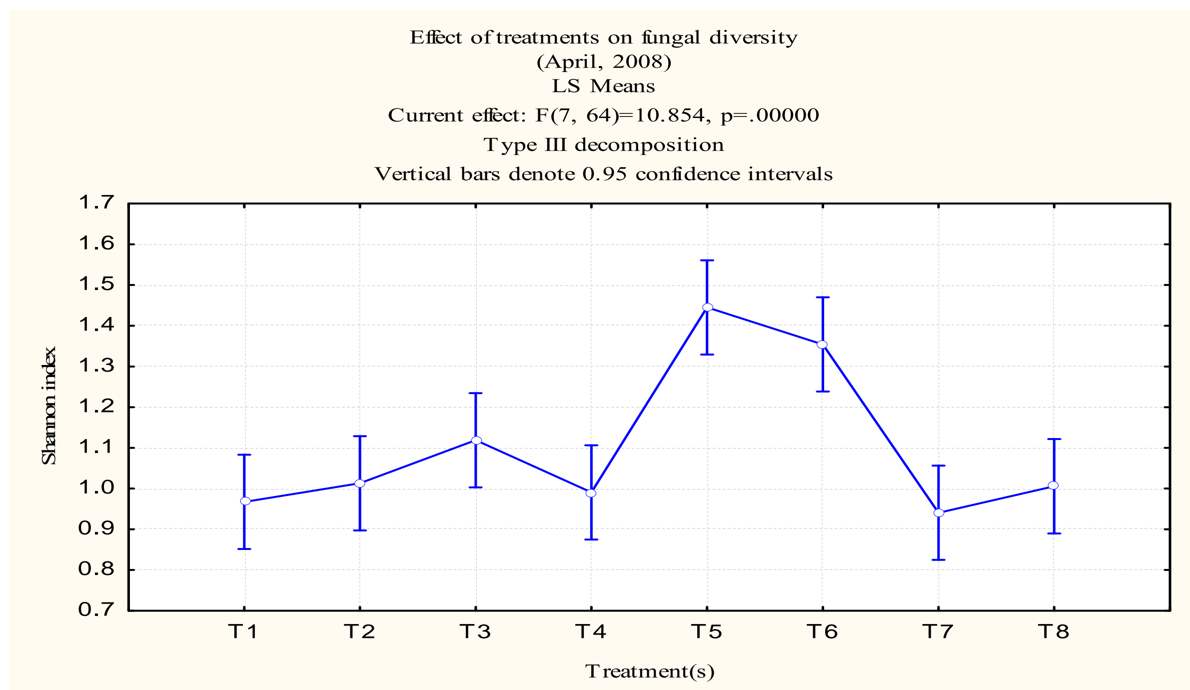

Figure 30. The effect of treatments on fungal diversity (April, 2008).

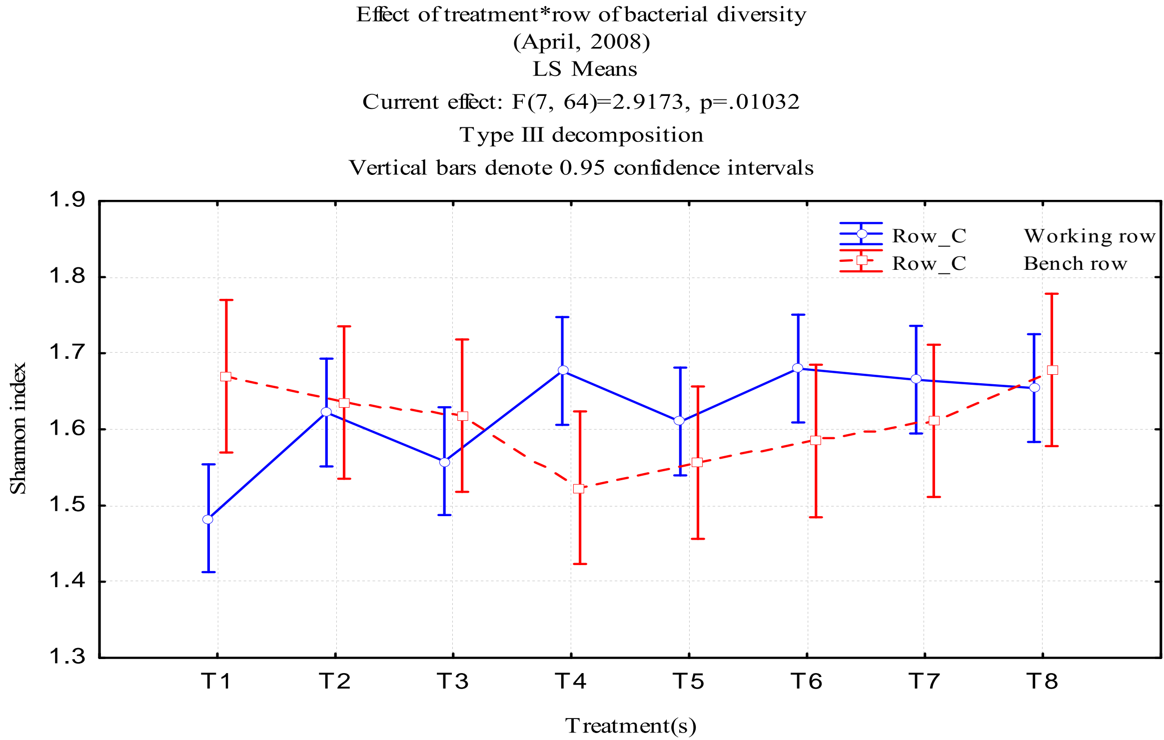

Figure 31. The effect of treatments combined with rows on bacterial diversity (April, 2008).

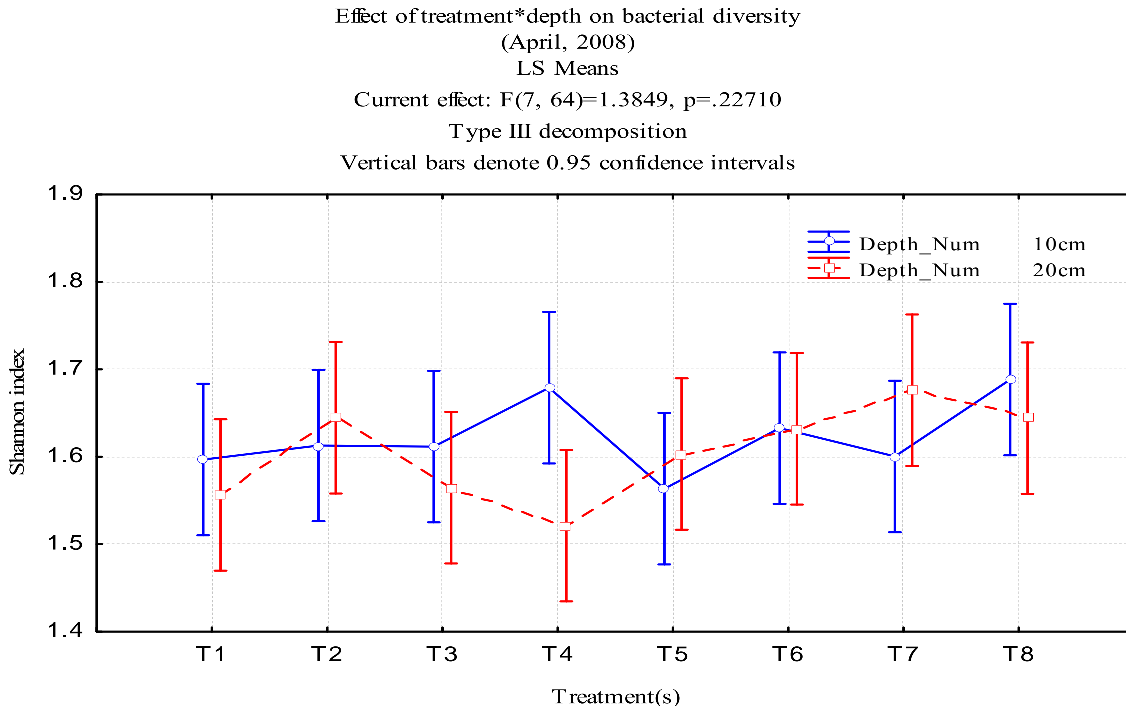

Figure 32. The effect of treatments combined with depths on bacterial diversity (April, 2008).

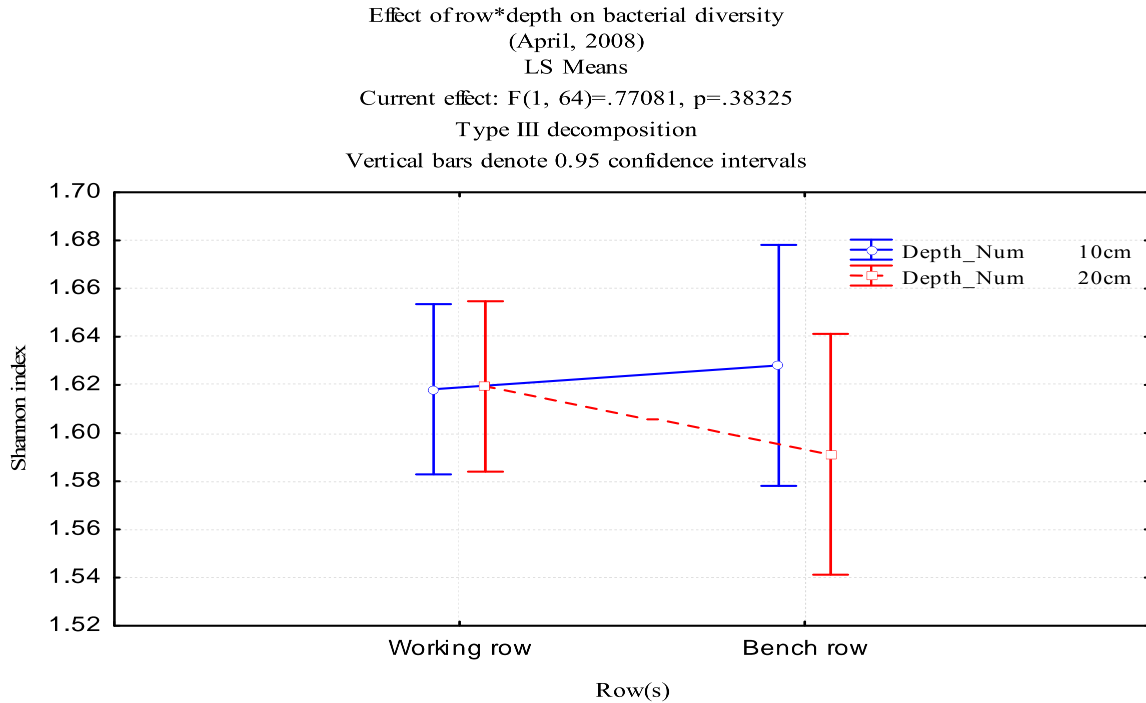

Figure 33. The effect of rows combined with depths on bacterial diversity (April, 2008).

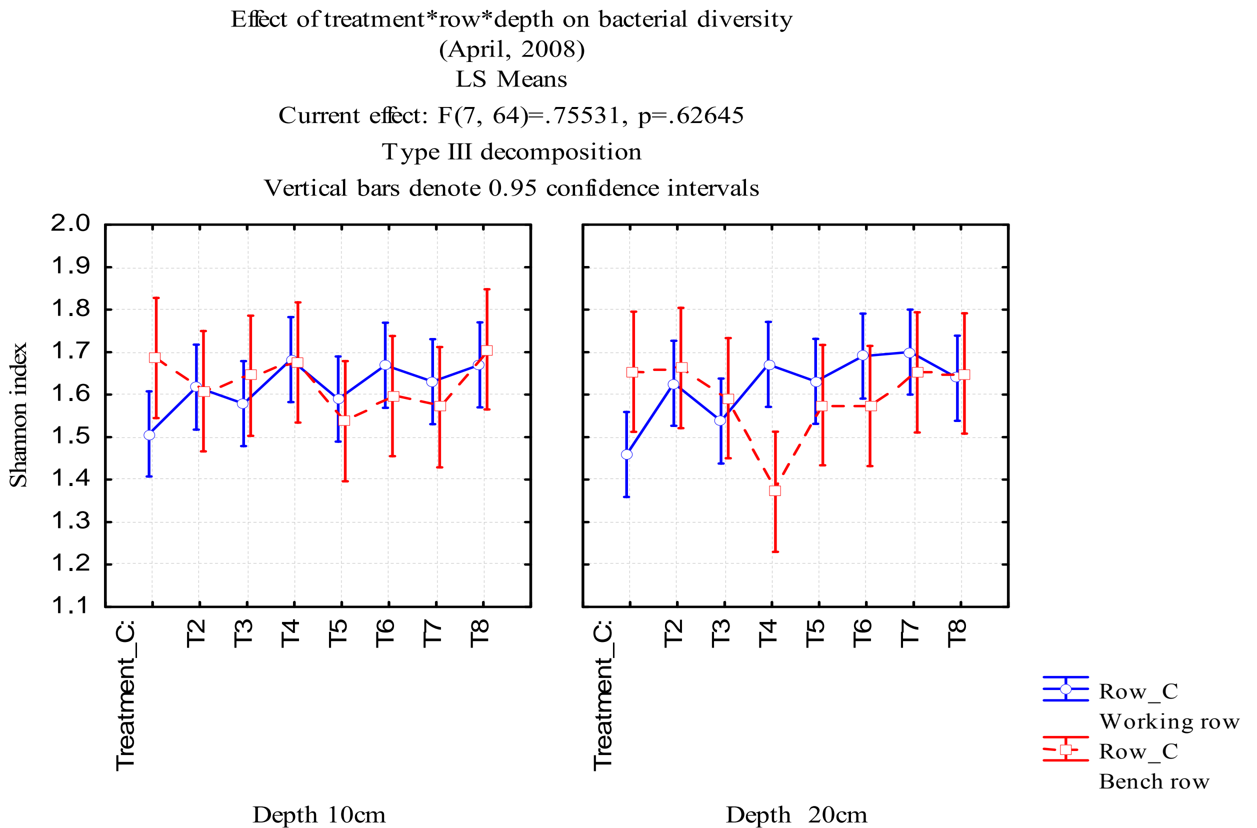

Figure 34. The effect of treatments, rows combined with depths on bacterial diversity (April, 2008).

3.8. Relationship between soil microbial diversity and soil physicochemical properties

The Pearson correlation coefficient (PCC) analysis reflects a weak relationship between the soil microbial diversity and the soil physical and chemical properties investigated with a few exceptions (Table 8). The potassium concentration in the soil was observed to have significant relationship with the fungal diversity ($p < 0.05$). As potassium concentration increases, an increase in fungal diversity was also observed (Fig. 35). Similarly, the bacteria diversity depicted a significant linear correlation with the phosphorous concentration (p-value 0.013) (Fig. 36). Ca and soil penetration resistance correlated negatively with fungal and bacterial diversity, while a positive correlation was observed between the soil microbial communities and soil pH.

Table 8. Pearson correlation coefficient analysis between soil properties, fungal and bacteria diversity.

Soil characteristic(s)	Fungal diversity		Bacterial diversity	
	r-values	p-values	r-values	p-values
pH(KCl)	0.012	0.965	0.379	0.148
Res.(Ohm)	-0.269	0.314	-0.271	0.309
H (cmol/kg)	-0.107	0.694	-0.426	0.099
Rock (Vol %)	-0.011	0.968	0.235	0.382
P (mg/kg)	0.212	0.429	0.605	**[0.013]**
K (mg/kg)	0.236	0.379	0.095	0.728
Na (cmol/kg)	-0.067	0.805	0.004	0.989
K (cmol/kg)	0.069	0.705	0.028	0.879
Ca (cmol/kg)	-0.301	0.241	0.144	0.596
Mg (cmol/kg)	-0.474	0.064	-0.066	0.807
C %	-0.354	0.179	0.172	0.524
Na %	0.291	0.274	-0.038	0.889
K %	0.505	**[0.046]**	0.096	0.724
Ca %	-0.192	0.475	0.267	0.318
Mg %	-0.241	0.368	-0.118	0.663
T-value	-0.383	0.143	0.04	0.882

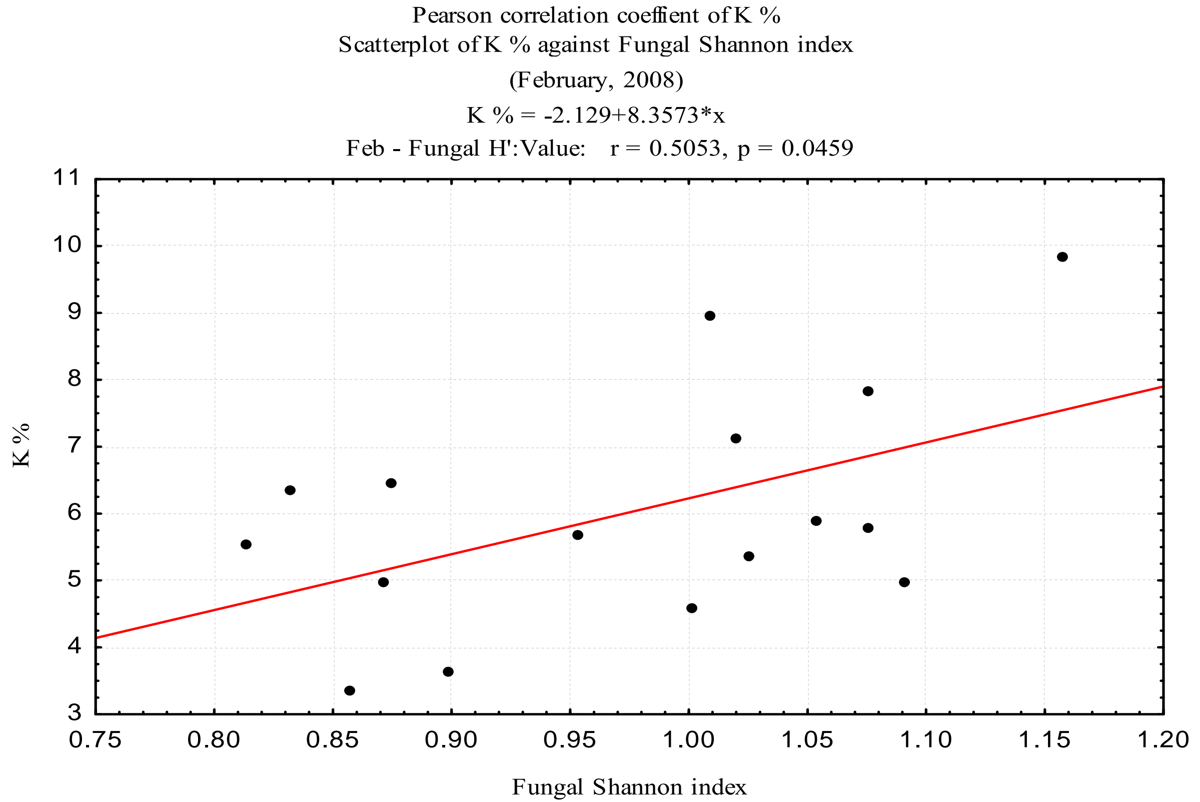

Figure 35. Graph of Pearson correlation coefficient of K % against fungal diversity.

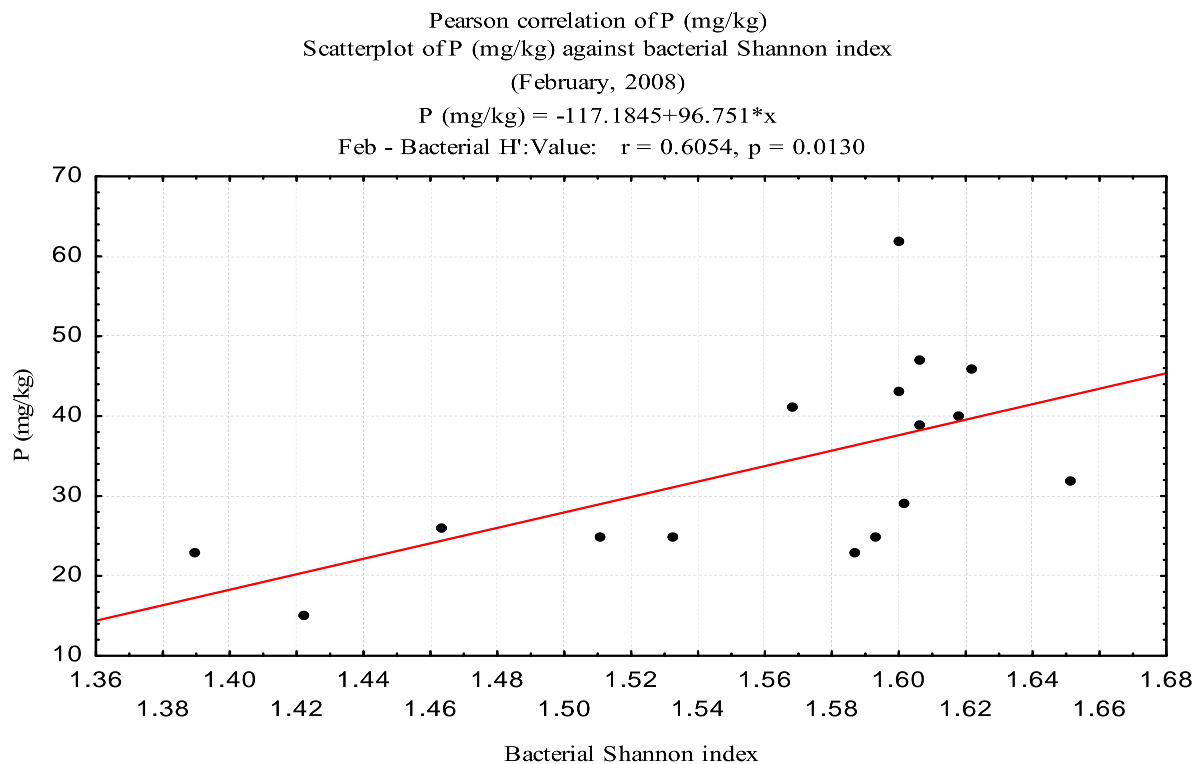

Figure 36. Graph of Pearson correlation coefficient of P (mg/kg) against bacterial diversity.

3.9. Relationship between apple yield and soil microbial diversity.

The multiple correlation coefficient analysis (Fig. 37) depicted a significantly negative relationship between apple yield and fungal diversity ($p < 0.05$) across all treatments. As the fungal diversity index increased there was a decrease in mass-ton apple yield. However, this result should be interpreted with caution due to the short term of study. Furthermore, the relationship between apple yield and bacterial diversity index reflected weak negative correlation with r-value of -0.061.

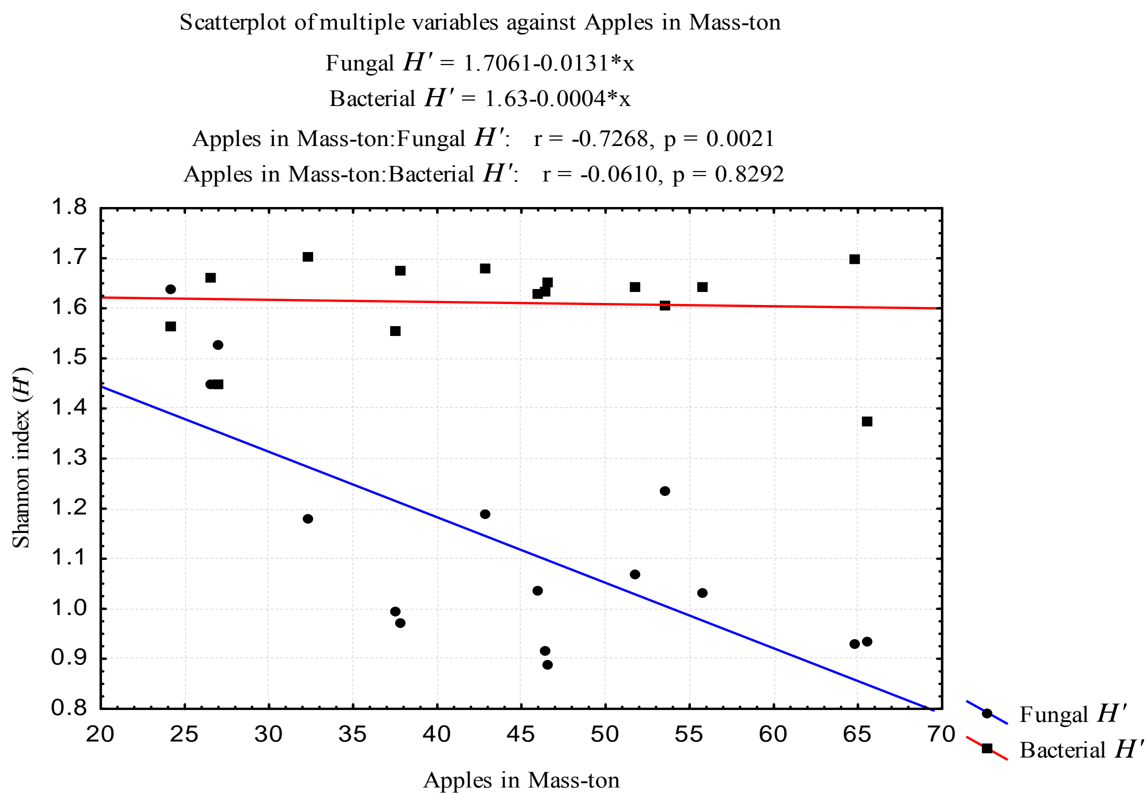

Figure 37. Graph of multiple correlation coefficient of mass-ton of apple yield against fungal and bacterial diversity.

Chapter 4

Discussion

Introduction

Soil microorganisms respond rapidly to anthropogenic disturbances (Zhong and Cai, 2007; Acosta-Martínez *et al*., 2008; Shishido *et al*., 2008). Hence they adapt to environmental conditions and the microbial communities that are best adapted will be most dominant (Nielsen and Winding, 2002). This adaptive characteristic allows microbial analyses to be discriminating in soil health assessment, and changes in microbial populations and activities may, therefore, function as an excellent indicator of change in soil health (Kennedy and Papendick, 1995; Pankhurst *et al.,* 1995). In some instances, changes in microbial community structure or function can precede detectable changes in soil chemical and physical properties, thus providing an early sign of soil improvement or an early warning of soil degradation (Pankhurst *et al.,* 1995).

4.1. Soil characteristics

Most agricultural soils have a pH in the range of 5.5 to 8.0 (Kyveryga *et al*., 2004). The optimal pH range for most crops is 6.0 to 7.5, while apple trees thrive best at pH of 5.6 to 6.9 (Raese, 1995; 1992). However under different treatments or perturbations of agricultural soils, the pH values either increase or reduce (Tisdale *et al*., 1993; Iles and Dosmann, 1999; Ayansina and Oso, 2006). In our study, plots treated with a chemical control showed the lowest pH, while the mulch treated plots had the highest pH values. These results agree with previous studies that observed a reduction in pH in soil treated with herbicides (Ayansina and Oso, 2006). Mulching also has an influence on pH of soils. Non-mulched plots show lower pH values compared to mulched plots (Iles and Dosmann, 1999). The increased pH observed under mulch treatments could have been due to the leaching of basic cations (NH_4^+) from decomposing organic matter into the soil (Tisdale *et al*., 1993). Therefore, it is advised that mulch replacement should be regular, because the soil pH will decrease as ammonia is oxidized to nitrate by nitrifying bacteria within the soil.

Soil pH is known to influence the solubility of soil macronutrients, micronutrients or essential trace elements (Gramss and Bergmann, 2007; Naramabuye and Haynes, 2007). Macronutrients and micronutrients are readily solubilized in soils within the pH range of 5.5 to 7.0. High pH levels in the soil result in leaching of nutrients and releases aluminium in

solubilized forms from its insoluble state (Marschner, 1995). This, in turn affects the plant's uptake of cations, induces organic acid secretion, and inhibits cell division and growth in the roots (Minocha and Minocha, 2005). Mulch treatment has been reported in literature to stabilize soil pH and increase nutrient availability (Buerkert *et al*., 2000; Tiquia *et al*., 2002). This could be attributed to the organic matter decomposition process (Tisdale *et al*., 1993). This was consistent with the findings in our study, where mulch treated plots recorded higher levels of phosphorus and potassium (mg/kg), exchangeable cations (Na, K and Ca), and base saturation in comparison to other treatments.

An optimal bulk density or soil compactness in agricultural soils is of great importance to the growth of plant roots as well as crop yield (Beutler and Centurioun, 2004; Secco *et al*., 2004; Stizaker *et al*., 1996), and this also influences the penetration resistance of the soil (Stizaker *et al*., 1996). In this study flattening of cover crops was adopted as treatment and recorded highest soil penetration resistance, while the mulched plot recorded the lowest resistance value. This is consistent with literature as mulched plots have been reported to reduce soil penetration resistance by about 20 % below the plant rooting zone compared to other soil management practices (Edwards *et al*., 2000). The plots with the highest soil resistance value could be as a result of the use of heavy agricultural machinery in the flattening of the cover crops. In previous studies, the use of heavy agricultural machineries has been associated with increased compaction and resistance of the soil (Canarache *et al*., 1984; Kovačević *et al*., 2004). Increased soil compaction is known to be one of the factors that impede water infiltration (Assouline, 2004; Lado *et al*., 2005).

4.2. Microbial community diversity

Managed ecosystems have less diverse microbial communities compared to the natural ecosystems (Torsvik *et al*., 2002). The decline in soil microbial diversity in managed ecosystems has been ascribed to reductions in soil organic C (Degens *et al*., 2000), and to various anthropogenic disturbances (Fox and MacDonald, 2003; Zhong and Cai, 2007; Acosta-Martínez *et al*., 2008; Shishido *et al*., 2008). For example, conventional farming systems, where pesticides, herbicides, and fertilizers are used (Horrigan et al., 2002), have been reported in literature to reduce biodiversity in soils (Lupwayi *et al*., 2001; Oehl *et al*.,

2004). In organically managed farms soil microbial diversity and functional diversity is enhanced (Bucher and Lanyon, 2005; Tu *et al*., 2006).

Similar to reports in previous studies, our treatment plots exposed to chemical control had the lowest fungal diversity and mulch treated plots had the highest fungal diversity. This observed difference was attributed to the choice of herbicide glyphosate used on this study. Glyphosate is known to inhibit protein synthesis via the "shikimate acid pathway", a biochemical route present in microorganisms (Bentley, 1990), and have been reported to inhibit the fungal mycelial growth in *in-vitro* studies (Meriles *et al*., 2006). On the contrary, glyphosate has repeatedly been found to be inoffensive to soil bacterial communities (Stratton and Steward, 1992; Busse *et al*., 2001; Weaver *et al*., 2007), and even stimulates bacterial activity (Haney *et al*., 2000; Busse *et al*., 2001; Araújo *et al*., 2003; Mijangos *et al*., 2009). Furthermore, the increased fungal diversity under mulch treatments could be as a result of response to added carbon source (Wardle, 1992), conserved soil moisture and moderate soil temperature (Robinson, 1988; Hoitink and Boehm, 1999). The variation in fungal community composition between the working rows, suggests the possible impact of slope position or landscape gradient across the experimental plots, as fungal diversity was consistently higher in working row 2 (the lower end of the gradient) than in working row 1. Slope position and elevation have been reported to influence soil fungal community composition (Maggi *et al*., 2005), however, the degree of slope needed for this observed change has not been investigated.

4.3. Variation in microbial diversity with sampled depth within plots

Soil microbial diversity has been shown to be lower in managed soil systems with the increase in sampling depth (Niemi *et al*., 2005; Yao *et al*., 2006). Soil organic matter may be a prime determinant of the reduction in microbial community diversity. As the reduction in soil organic matter was correlated with low soil carbon (Yao *et al*., 2006). From our study, no significant decrease in microbial diversity occurred within treatments in respect to the sampled depth. This finding is at odds with those reported by Niemi *et al.* (2005) and Yao *et al*. (2006). These authors stated that soil microbial community diversity is lower with increase in sample depth, Niemi *et al*. (2005) sampled at 30 cm and 40 cm, while in Yao *et al*. (2006) study soil samples were obtain from depth 5 to 15 cm. The insignificant shift

observed in this study could be as a result of a mono-cropping system with consistent soil treatments, while the study by Niemi *et al.* (2005) and Yao *et al*. (2006) involved soils exposed to alternating soil management practices. Furthermore, it is believed that a minimal number of species is essential for ecosystem functioning under steady conditions, while a larger number of species is critical in maintaining stable processes in a changing environment (Loreau *et al*., 2001). However, elucidating more on the soil microbial functional diversity could give a better resolution into the influence of sampling depth on the microbial diversity and changes in dominant microbial species (Aon and Colaneri, 2001; Griffiths *et al*., 2003; Niemi *et al*., 2005).

4.4. Variation in microbial diversity between replicated treatments

It is expected that soil microbial communities will respond in similar pattern to same treatment. However, it is not impossible to rule out the occurrence of hot spots (zones with a high concentration of nutrients) and the effect of spatial and temporal variation on soil microbial communities (Sexstone *et al*., 1985; Peterson *et al*., 1996). From this study, the comparison of replicated treatments showed no significant variation in microbial diversity between replicated plots (Fig. 10, Chapter 3).

4.5. Microbial community structure amongst treatments

Microbial community structures of distinct environmental samples, agricultural samples or soil sample from different locations when compared pair-wise using similarity indices, tend to assemble into distinct clusters in correlation to the sample source (Fisher and Triplett, 1999; Hewson and Fuhrman, 2004; Cao *et al*., 2008; Slabbert, 2008). This suggests the possibility that the observed relationship between the microbial communities profile can not be attributed to chance alone. However, from our ARISA fingerprint analysis of the February profiles, no distinct assemblage of replicate treatments was observed with the exception of treatment 1 and 7 for the fungal community profile (Fig. 11, Chapter 3), and treatment 4 for the bacterial community profile (Fig. 12, Chapter 3). This suggests that the soil microbial composition in February was fairly homogeneously dispersed amongst the treatment plots. Furthermore, the microbial communities could have become stable at the time of sampling,

which was the peak of the summer without rainfall. Cruz-Matínez *et al*. (2009) demonstrated that microbial community composition was consistently stable over a five year rainfall manipulation experiment. The clustered replicates, suggests the probable involvement of a local selective factor such as presence of weeds or mulching as the cause of their assemblages.

In April, cluster analysis (Fig. 13 and 14, Chapter 3) revealed distinct assemblages among treatments in the soil fungal and bacterial communities. This suggests that microbial communities seem to be sensitive to certain changes in their extrinsic factors (e.g. moisture). The April samples were collected few weeks after rainfall. For instance, from the study conducted by Cruz-Matínez *et al*. (2009) they only observed a shift in microbial composition when watering or rainfall was intensified to mimic the ambient climate.

4.6. Effect of different soil treatments on microbial diversity

In February, the interaction between treatment (mulch) and rows (working row and bench row) in treatment 3 and 4 for fungal and treatment 4 and 5 for bacterial profile, had a significantly higher diversity compared to other amendments ($p < 0.05$) (Fig. 15 and 20, Chapter 3). This supports previous studies in which increased soil microbial diversity was observed with the input of organic mulch (Huang *et al*., 2008). Mulch treatment has been proven to have a significant influence on soil respiration, organic matter content, the concentration of essential plant nutrients, soil pH, exchangeable cations and soil microbial biomass (Tiquia *et al*., 2002). While, other land management approaches such as weed slashing and flattening of cover crops have been reported to disrupt soil faunal communities, degrade soil structure, increase soil compaction, as well as accelerate the loss of organic matter (Canarache *et al*., 1984; Kovačević *et al*., 2004; Hoagland *et al*., 2008).

The interaction between treatments and depths, rows and depths as well as the interaction of treatments, rows and depths, had no significant effect on both bacterial and fungal diversity (Fig. 16, 17, 18, 21, 22 and 23, Chapter 3) in February. This shows that either combination of treatment or rows with sampled depth had no influence on the microbial diversity and observable changes are due to treatments on rows. Furthermore, using treatments as a main effect it had a marginal p-value of 0.059 on the fungal diversity and a significant effect on the

bacterial diversity with treatment 4 (mulch) having the highest Shannon index values (Fig. 19 and 24, Chapter 3). The marginal p-value observed for the fungal diversity could be attributed to the large standard deviation (SD) from the LS means, due to small sampled data points. It is believed that with more data points, the SD will be reduced and no overlapping SD-bars would have been obtained.

In April, interaction between treatments and rows, as well as treatments and depth, fungal diversity was higher in treatment 5 and 6 (which involved the use of cover crops) but insignificant (Fig. 26-29, Chapter 3). Suggesting that combination of treatment with other factors had no significant influence on fungal diversity. However, applying treatments as the main effect the observed increase in treatment 5 and 6 was significant (Fig. 30, Chapter 3). This observation suggests a possible relationship between the cover crops and the fungal community. A previous study on cropping systems found that the variation in fungal fatty acid biomakers was higher in soil with cover cropping system compared to other practices (Schutter *et al*., 2001). Furthermore, cover crops have been reported to form mutualistic symbiosis with arbuscular mycorrhizal fungi (Cheng and Baumgartner, 2004). This could probably explain the surge in the fungal community diversity at the time when there was an improved growth of the cover crops in April.

For the bacterial community in April, only combinations of treatments with rows had a significant influence on the bacterial diversity (Fig. 31, Chapter 3), although, bacterial diversity was greater in the mulch treatments (Treatment 4). This was consistent with previous studies where various soil management practices (cover cropping and chemical control) were shown to have no significant effect on bacterial diversity (Peixoto *et al*., 2006; Lupwayi *et al*., 2009). Mulch treatment has been reported to increase soil microbial biomass. Tiquia *et al*. (2002) showed that mulching with composted yard waste significantly enhanced the rhizosphere bacterial community.

Furthermore, the observed significant effect of treatment combined with rows, identifies with treatments T1 and T4. The higher bacterial diversity observed in the tree row of T1 could be attributed to the increase in microbial activity due to the utilization of the herbicide glyphosate as an available carbon source. Glyphosate has been reported in literature to stimulating bacterial activity (Haney *et al*., 2000; Busse *et al*., 2001; Araújo *et al*., 2003; Mijangos *et al*., 2009). The significant decrease in bacterial diversity on the tree row,

suggests a possible dominance of a few microorganisms in the rhizosphere community. It has been shown in the case of monoculture fruit trees such as apple, that root exudates and antagonistic relations in the rhizosphere may lead to the dominance of saprophytic phytotoxic microorganisms, which negatively affect the microbial equilibrium of the soil (Čatská, 1993).

4.7. Relationship between soil microbial diversity and soil properties

Various studies on both managed and natural ecosystem have reported diverse relationships between soil microbial communities and soil physical and chemical properties (Staddon *et al*., 1998; Anand *et al*., 2003; Pande and Tarafdar, 2004; Schipper and Lee, 2004; Fierer and Jackson, 2006; De Vries *et al*., 2007; Zhong and Cai, 2007; Slabbert, 2008). Contrary to previous studies (De Vries *et al*., 2007) we observed a significantly positive correlation between potassium (K) concentrations and soil fungal diversity (Fig. 35, Chapter 3). This observation suggests that soil K, a chemical analogue of cesium and an essential macronutrient (Gyurica *et al*., 2008), plays a crucial role in the growth of fungi (Yuan *et al*., 2005). As our mulch treated plots had the highest concentrations of available potassium and fungal diversity.

Furthermore, bacterial communities showed a significant correlation to available phosphorus in soil (Fig. 36, Chapter 3). Our result agrees with previous studies, wherein microbial count correlated with soil phosphorous (Zhong and Cai, 2007). Phosphorus is one of the essential macronutrients for plants, and a large portion of the soluble inorganic phosphate applied to the soil as synthetic fertilizer is immobilized rapidly and becomes unavailable to plants (Goldstein, 1986). Soil bacterial communities are known to convert insoluble forms of phosphorous into an accessible form. This promotes bacterial community growth through the mutualistic symbiosis with the plant and improves phosphate availability to plants (Glick, 1995; Chen *et al*., 2006). Adequate availability of phosphate, invariably improves plant growth and yield (Goldstein *et al*., 1999; Fasim *et al*., 2002).

Fierer and Jackson (2006) reported a positive correlation between soil microbial communities and the pH in agricultural soils. Their findings, agrees with that obtained from this study, as soil pH was found to correlate positively with bacterial diversity, although ours was not significant. The correlation between soil pH and microbial community could be a result of

soil pH integrating other variables e.g. nutrient availability (Plante, 2007). However, soil pH could be an independent driver of soil diversity, as the intracellular pH of most microorganisms is often within 1 pH unit of neutral (Madigan *et al*., 1997), and suboptimal pH, in various environments has been shown to have a significant effect microbial diversity (Schnittler and Stephenoson, 2000; Hornstrom, 2002; Bååth and Anderson, 2003).

Furthermore, we observed a negative correlation between soil penetration resistance and soil microbial diversity. This observation suggests the possible impact of soil penetration on microbial diversity. Increased penetration resistance of the soil is influenced by the degree of its compactness or bulk density (Guimarães *et al*., 2002). At critical bulk density the growth of plant roots in inhibited (Stizaker *et al*., 1996; Beutler *et al*., 2004; Secco *et al*., 2004), and plant root inhibition could affect the rhizosphere community (Nannipieri *et al*., 2007). Moreover, increase in soil compaction is shown to impede water infiltration (Assouline, 2004), and this could affect the transportation of much needed nutrients (Haws *et al*., 2004).

From this study, soil available calcium (Ca) correlated negatively with fungal diversity and positively with bacterial diversity. This is consistent with previous studies. Anand *et al*. (2003), showed a positive correlation between soil Ca and bacterial diversity, and Pande and Tarafdar (2004), reported a negative correlation between fungal diversity and Ca in agroforestry systems. The negative correlations observed with Ca could be due to the ability of calcium carbonate to alter root morphology and root differentiation by enhancing lignification and suberization of root endodermis, thereby affecting possible arbuscular mycorrhizal root infections (Dehne and Schonbeck, 1997).

4.8. Relationship between apple yield and soil microbial diversity

Changes in rhizosphere microbial communities are among the numerous factors that contribute to improve apple growth (Yao *et al*., 2006; Rumberger *et al*., 2007). However, microbial diversities have not been directly linked to apple yield (Rumberger *et al*., 2004; Yao *et al*., 2006). From our study, neither the increase in fungal community diversity nor the weak negative bacterial community correlation translated into improved yield or productivity. This implies that other extrinsic factors play a crucial role in the productivity and quality of apples. Other production practices, such as thinning and pruning practices have been

identified to contributing to the yield and quality of apples (Glover *et al*., 2000). Furthermore, rootstock genotype and orchard planting location are other dominant factors influencing apple tree performance (Foote *et al*., 2001; Rumberger *et al*., 2004). After planting, rootstocks have been shown to modify their soil microenvironment and influence the microbial community composition making it more suitable for their own growth and development (Gu and Mazzola, 2003; Mazzola *et al*., 2004).

References

Acosta-Martínez, V., Acosta-Mercado, D., Sotomayor-Ramírez, D. and Cruz-Rodríguez, L., 2008. Microbial communities and enzymatic activities under different management in semiarid soils. *Applied Soil Ecology* **38**, 249-260.

Anand, M., Ma, K. –M., Okonski, A., Levin, S. and McCreath, D., 2003. Characterising biocomplexity and soil microbial dynamics along a smelter-damaged landscape gradient. *The Science of the Total Environment* **311**, 247-259.

Aon, M. A. and Colaneri, A. C., 2001. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied Soil Ecology* **18**, 255-270.

Araújo, A. S. F., Monteiro, R. T. R. and Abarkeli, R. B., 2003. Effect of glyphosate on the microbial activity of two Brazilian soils. *Chemosphere* **52**, 799-804.

Assouline, S., 2004. Rainfall-induced soil surface sealing: A critical review of observations, conceptual models and solutions. *Vadose Zone Journal* **3**, 570-591.

Ayansina A. D. V. and Oso, B. A., 2006. Effect of two commonly used herbicides on soil microflora at two different concentrations. *African Journal of Biotechnology* **5**, 129-132.

Bååth, E. and Anderson, T. H., 2003. Comparison of soil fungal/bacterial ration in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry* **35,** 955-963.

Bentley, R., 1990. The Shikimate pathway-a metabolic tree with many branches. *Critical Reviews in Biochemistry and Molecular Biology* **25**, 307-384.

Beutler, A. N. and Centurion, J. F., 2004. Effect of soil compaction in root development and soybean yield. *Brazilian Journal of Agricultural Research* **39**, 581-588.

Bucher, A. E. and Lanyon, L. E., 2005. Evaluating soil management with microbial community-level physiological profiles. *Applied soil Ecology* **29**, 59-71.

Buerkert, A., Bationo, A. and Dossa, K., 2000. Mechanisms of residue mulch-induced cereal growth increases in west Africa. *Soil Science Society of America Journal* **64**, 346-358.

Busse, M. D., Ratcliff, A. W., Shestak, C. J. and Powers, R. F., 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. *Soil Biology and Biochemistry* **33**, 1777-1789.

Canarache, A., Colibas, J., Horonbeanu, J., Patru, V., Simota, H. and Trandafilescu, T., 1984. Effect of induced compaction by wheel traffic on soil physical properties and yield of maize in Romania. *Soil and Tillage Research* **4**, 199-213.

Cao, L., Shen, G. and Lu, Y., 2008. Combined effects of heavy metal and polycyclic aromatic hydrocarbon on soil microorganism communities. *Environmental Geology* **54**, 1531-1536.

Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. –A. and Young, C. C., 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology* **34**, 33-41.

Cruz-Martínez, K., Suttle, K. B., Brodie, E. L., Power, M. E., Andersen, G. L. and Banfield, J. F., 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlaying grassland. *International Society for Microbial Ecology* **3**, 738-744.

Čatská, V., 1993. Fruit tree repalnt problem and microbial antagonism in soil. *Acta Horticulturae* **324**, 23-33.

Degens, B. P., Schipper, L. A., Sparling, G. P. and Vojvodic-Vukovic, M., 2000. Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. *Soil Biology and Biochemistry* **32**, 189-196.

Dehne, H.W. and Schonbeck, F., 1979. Untersuchungen zum Einfluss der endotrophen Mykorrhiza auf Pflanzenkrankheitein. I. Ausbreitung von *Fusarium oxyporum* f. sp. lycopercisi in Tomaten. *Phytopathologische Zeitschrift* **95**, 105-110.

De Vries, F. T., Bloem J., Van Eekren, N., Brussard, L. and Hoffland, E., 2007. Fungal biomass in pastures increases with age and reduced N input. *Soil Biology and Biochemistry* **39**, 1620-1630.

Edwards, L., Burney, J. R., G. Richter, G. and MacRae, A. H., 2000. Evaluation of compost and straw mulching on soil-loss characteristics in erosion plots of potatoes in Prince Edward Island, Canada. *Agriculture, Ecosystems and Environment* **81**, 217-222.

Ellingose, P. and Johnsen, K., 2002. Influence of soil sample sizes on the assessment of bacteria community structure. *Soil Biology and Biochemistry* **34**, 1701-1707.

Ettema, C. H. and Wardle, D. A., Spatial soil ecology. *TRENDS in Ecology and Evolution* 17, 177-183.

Fasim, F., Ahmed, N., Parson, R. and Gadd, G. M., 2002. Solubilization of zinc salts by a bacterium isolated from air environment of a tannery. *FEMS Microbiology Letter* **213**, 1-6.

Fierer, N. and Jackson, R. B., 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Science* **103,** 626-631.

Fisher, M. M. and Triplett, E. W., 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Applied and Environmental Microbiology* **65**, 4630-4636.

Foote, K. F., Tustin, D. S., Campbell, J. M., Palmer, J. W. and Wunsche, J. N., 2001. Effect of soil treatment, rootstock and tree density on the establishment phase of apple high density plantings on a replant site. *Acta Horticulturae* **557**, 343-345.

Fox, C. A. and MacDonald, K. B., 2003. Challenges related to soil biodiversity research in agroecosystems-issues within the context of scale of observation. *Canadian Journal of Soil Science* **83**, 231-244.

Glick, B. R., 1995. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* **41**, 109-117.

Glover, J. D., Reganold, J. P. and Andrews, P. K., 2000. Systematic methods for rating soil quality of conventional, organic, and integrated apple orchards in Washington State. *Agriculture, Ecosystems and Environment* **80**, 29-45.

Goldstein, A.H., 1986. Bacterial solubilization of mineral phosphates: historical perspectives and future prospects. *America Journal of Alternative Agriculture* **1**, 57-65.

Goldstein, A. H., Braverman, K., and Osorio, N., 1999. Evidence for mutualism between a plant growing in a phosphate-limited desert environment and a mineral phosphate solubilizing (MPS) bacterium. *FEMS Microbiology Ecology* **3**, 295-300.

Gramss, G. and Bergmann, H., 2007. Microbial competition, lack in macronutrients, and acidity as main obstacles to the transfer of basidiomycetous ground fungi into (organically or heavy-metal contaminated) soils. *Journal of Basic Microbiology* **47**, 309-316.

Griffiths, R. I., Whiteley, A. S., O'Donnel, A. G. and Bailey, M. J., 2003. Influence of depth and sampling time on bacterial community structure in an upland grassland soil. *FEMS Microbiology Ecology* **43**, 35-43.

Griffiths, R. I., Whiteley, A. S., O'Donnel, A. G. and Bailey, M. J., 2003. Physiological and community responses of established grassland bacterial populations to water stress. *Applied Environmental Microbiology* **69**, 6961-6968.

Gu, Y. H. and Mazzola, M., 2003. Modification of fluoresecent pseudomonad community and control of apple replant disease induced in a wheat cultivar-specific manner. *Applied Soil Ecology* 24, 57-72.

Guimarães, C. M., Stone, L. F. and Moreira, J. A. A., 2002. Soil compaction in a bean crop. II. Effect on root and shoot development. *Revista Brasileira de Engenharia Agrícola e Ambiental* **6**, 213-218.

Gyuricza, V., de Boulois, D. H. and Declerck, S., 2008. Effect of potassium and phosphorus on the transport of radiocesium by arbuscular mycorrhizal fungi. *Journal of Environmental Radioactivity*, In Press.

Haney, R. L., Senseman, S. A., Hons, F. M. and Zuberer, D. A., 2000. Effect of glyphosate on soil microbial activity and biomass. *Weed Science* **48**, 89-93.

Haws, N. W., Liu, B., Boast, C. W., Rao, P. S. C., Kladivko, E. J. and Franzmeier, D. P., 2004. Spatial variability and measurement scale of infiltration rate on an agricultural landscape. *Soil Science Society of America Journal* **68**, 1818-1826.

Hoagland, L., Carpenter-Boggs, L., Granatstein, D., Mazzola, M., Smith, J., Peryea, F. and Reganold, J. P., 2008. Orchard floor management effects on nitrogen fertility and soil

biological activity in a newly established organic apple orchard. *Biology of Fertile Soils* **45**, 11-18.

Hoitink, H. A. J. and Boehn, M. J., 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annual Review of Phytopathology* **37**, 427-446.

Horrigan, L., Lawrence, R. S. and Walker P., 2002. How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environmental Health Perspectives* **110**, 445-456.

Hörnström, E., 2002. Phytoplankton in 63 linked lakes in comparison with the distribution in 500 untreated lakes with varying pH. *Hydrobiologia* **470**, 115-126.

Huang, Z., Xu, Z., Chen, C. 2008. Effect of mulching on labile soil organic matter pools, microbial community functional diversity and nitrogen transformations in two hardwood plantations of subtropical Australia. *Applied Soil Ecology* **40**, 229-239.

Iles, J. K. and Dosmann, M. S., 1999. Effect of organic and mineral mulches on soil properties and growth of Fairview flame red maple trees. *Journal of Arboriculture* **25,** 163-166.

Kang, S. and Mills, A. L., 2006. The effect of sample size in studies of soil microbial community structure. *Journal of Microbiological Methods* **66**, 242-250.

Kennedy, A. C. and Papendick, R. I., 1995. Microbial characteristics of soil quality. *Journal of Soil and Water Conservation* **50**, 243-248.

Kovačević, D., Denčić, S., Kobiljski, B., Momirović, N., Oljača, S. and Dolijanović, Z., 2004. Effect of farming systems on soil compaction, weed synuzia and yield of winter wheat. *Acta Herbologica* **13**, 385-392.

Kyveryga, P. M., Blackmer, A. M., Ellsworth, J. W. and Isla, R., 2004. Soil pH effects on nitrification of fall-applied anhydrous ammonia. *Soil Science Society of American Journal* **68**, 545-551.

Lado, M., Ben-Hur, M. and Assouline, S., 2005. Effects of effluent irrigation on seal formation, infiltration, and soil loss during rainfall. *Soil Science Society of America Journal* **69**, 1432-1439.

Lee, S. -Y., Bollinger, J., Bezdicek, D. and Ogram, A., 1996. Estimation of the abundance of an uncultured soil bacterial strain by a competitive quantitative PCR method. *Applied and environmental Microbiology* **62**, 3787-3793.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D. and Wardle, D. A., 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* **294**, 804-808.

Lupwayi, N. Z., Harker, K. N., Clayton, G. W., O'Donovan, J. T. and Blackshaw, R. E., 2009. Soil microbial response to herbicides applied to glyphosate-resistant canola. *Agriculture, Ecosystems and Environment* **129**, 171-176.

Lupwayi, N. Z., Monreal, M. A., Clayton, G. W., Grant, C. A., Jhonston, A. M. and Rice, W. A., 2001. Soil microbial biomass and diversity respond to tillage and sulphur fertilizers. *Canadian Journal of Soil Science* **81**, 577-589.

Madigan, M., Martinko, J. and Parker, J., 1997. Brock Biology of Microorganisms. Prentice Hall, Upper Saddle River, NJ.

Maggi, O., Persiani, A. M., Casado, M. A. and Pineda, F. D., 2005. Effect of elevation, slope position and livestock exclusion on microfungi isolated from soils of Mediterranean grasslands. *Mycologia* **97**, 984-995.

Marschner, H. (ed.), 1995. Mineral nutrition of higher plants. 2nd ed., Academic Press, London.

Martin-Laurent, F., Philippot, L., Hallet, S., Chaussod, R., Germon, J. C., Soulas, G. and Catroux, G., 2001. DNA extraction from soil: Old bias for new microbial diversity analysis methods. *Applied and Environmental Microbiology* **67**, 2354-2359.

Mazzola, M., Funnell, D. L. and Raaijmakers, J. M., 2004. Wheat cultivar-specific selection of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* species from resident soil populations. *Microbial Ecology* **48**, 338-348.

Meriles, J. M., Gil, S. V., Haro, R. J., March, G. J., and Guzman, C. A., 2006. Glyphosate and previous crop residue effect on deleterious and beneficial soil-borne fungi from a peanut-corn-soybean rotations. *Journal of phytopathology* **154**, 309-316.

Mijangos, I., Becerril, J. M., Albizu, I., Epelde, L. and Garbisu, C., 2009. Effect of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and –independent methodologies. *Soil Biology and Biochemistry* **41**, 505-513.

Minocha, R. and Minocha, S. C., 2005. Effects of soil pH and aluminium on plant respiration. In: Lambers, H. and Ribas-Carbo, M. (eds.), 2005. Plant respiration. Springer, The Netherlands, pp. 159-176.

Mumy, K. L. and Findlay, R. H., 2004. Convenient determination of DNA extraction efficiency using an external DNA recovery standard and quantitative-competitive PCR. *Journal of Microbiological Methods* **57**, 259-268.

Nannipieri, P., Ascher, J., Ceccherini, M. T., Landi, L., Pietramellara, G., Renella, G. and Valori, F., 2007. Microbial diversity and microbial activity in the rhizosphere. *Ciencia del Suelo* **25**, 89-97.

Naramabuye, F. X. and Haynes, R. J., 2007. The liming effect of five organic manures when incubated with an acid soil. *Journal of Plant Nutrient Soil Science* **170**, 615-622.

Nielsen, M. N. and Winding, A., 2002. Microorganisms as indicators of soil health. National Environment Research Institute (NERI), Technical Report No. 388. Ministry of the Environment, NERI, pp. 15.

Niemi, R. M., Vepsäläinen, M., Wallenius, K., Simpanen, S., Alakukku, L. and Pietola, L., 2005. Temporal and soil depth-related variation in soil enzyme activities in root growth of red clover (*Trifolium pratense*) and timothy (*Phleum pratense*) in the field. *Applied Soil Ecology* **30**, 113-125.

Oehl, F., Sieverding, E., Mäder, P., Dubois, D., Ineichen, K., Boller, T. and Wiemken, A., 2004. Impact of long-term conventional and organic farming on diversity of arbuscular mycorrhizal fungi. *Oecologia* **138**, 574-583.

Pande, M. and Tarafdar, J. C., 2004. Arbuscular mycorrhizal fungal diversity in neem-based agroforestry systems in Rajasthan. *Applied Soil Ecology* **26**, 233-241.

Pankhurst, C. E., Hawke, B. G., McDonald, H. J., Kirkby, C. A., Buckerfield, J. C., Michelsen, P., O'Brien, K. A., Gupta, V. V. S. R. and Doube, B. M.,1995. Evaluation of soil biological properties as potential bioindicators of soil health. *Australian Journal of Experimental Agriculture* **35**, 1015-1028.

Peixoto, R. S., Coutinho, H. L. C., Madari, B., Machado, P. L. O. A., Rumjanek, N. G., Van Elass, J. D., Seldin, L. and Rosado, A. S., 2006. Soil aggregation and bacterial community structure as affected by tillage and cover cropping in the Brazilian Cerrados. *Soil Tillage Research* **90**, 16-28.

Peterson, S. O., Nielsen, T. H., Frostegård, Å. and Olesen, T., 1996. O_2 uptake, C metabolism and denitrification associated with manure hot-spots. *Soil Biology and Biochemistry* **28**, 341-349.

Plante, A. F., 2007. Soil biochemical cycling of inorganic nutrients and metals. In: Paul, E. A. (ed.), 2007. Soil microbiology, ecology, and biochemistry, 3rd ed., Academic Press, Oxford, UK, pp 389-432.

Raeses, J. T., 1995. Effect of fertilizers on soil pH and apple trees grown in different soils in the greenhouse. *Communication in Soil Science and Plant Analysis* **23**, 2365-2381.

Raeses, J. T., 1995. Effect of low soil pH from different fertilizers on performance of apple and pear trees. In: Date, R. A., Grundon, N. J., Rayment, G. E. and Probert, M. E. (eds.), 1995. Plant soil interactions at low pH: Principles and management. Kluwer Academic publisher, The Netherlands, pp. 803-807.

Ranjard, L., Lejon, D. P. H., Mougel, C., Schehrer, L., Merdinoglu, D. and Chaussod, R., 2003. Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. *Environmental Microbiology* **5**, 1111-1120.

Robinson, D. W., 1988. Mulches and herbicides in ornamental plantings. *Horticultural Science* **23**, 547-552.

Rumberger, A., Merwin, I. A. and Thies, J. E., 2007. Microbial community development in the rhizosphere of apple trees at a replant disease site. *Soil Biology and Biochemistry* **39**, 1645-1654.

Rumberger, A., Yao, S., Merwin, I. A., Nelson, E. B. and Thies, J. E., 2004. Rootstock genotype and orchard replant position rather than soil fumigation or compost amendment determine tree growth and rhizosphere bacterial community composition in an apple replant soil. *Plant and Soil* **264**, 246-260.

Sanchez, F. G., Carter, E. A. and Leggett, Z. H., 2009. Loblolly pine growth and soil nutrient stocks eight years after slash incorporation. *Forest Ecology and Management* **257**, 1413-1419.

Schipper, L. A. and Lee, W. G., 2004. Microbial biomass, respiration and diversity in ultramafic soils of West Dome, New Zealand. *Plant and Soil* **262**, 151-158.

Schnittler, M. and Stephenson, S. L., 2000. Myxomycete biodiversity in four different forest types in Costa Rica. *Mycologia* **92,** 626-637.

Schutter, M. E., Sandeno, J. M. and Dick, R. P., 2001. Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. *Biology of Fertile Soils* **34**, 397-410.

Secco, D., Reinert, D. J., Reichert, J. M. and Da Ross, C. O., 2004. Implications of soil management and compaction state on soil physical properties and soybean yield. *Brazilian Journal of Soil Science* **28**, 797-804.

Sexstone, A. J., Revsbeach, T. B., Parkin, T. B. and Tiedje, J. M., 1985. Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Science Society of America Journal* **49**, 645-651.

Shishido, M., Sakamoto, K., Yokoyama, H., Momma, N. and Miyashita, S. –I., 2008. Changes in microbial communities in an apple orchard and its adjacent bush soil in response

to season, land-use, and violet root rot infestation. *Soil Biology and Biochemistry* **40**, 1460-1473.

Slabbert, E., 2008. Microbial diversity of soils of the sand fynbos. M. Sc Thesis, Stellenbosch University, South Africa. pp. 117-118.

Staddon, W. J., Trevors, J. T., Duchensne, L. C. and Colombo, C. A., 1998. Soil microbial diversity and community structure across a climatic gradient in western Canada. *Biodiversity and Conservation* **7**, 1081-1092.

Stirzaker, R. J., Passioura, J. B. and Wilms, Y., 1996. Soil structure and plant growth: Impact of bulk density and biopores. *Plant and Soil* **185**, 151-162.

Stotzky, G., 1997. Soils as an environment for microbial life. In: van Elsas, J. D., Trevors, J. T. and Wellington, E. M. H. (eds.), 1997. Morden soil microbiology. Marcel Dekker, New York, pp. 1-20.

Stratton, G. W. and Steward, K. E., 1992. Glyphosate effects on microbial biomass in a coniferous forest soil. *Environmental Toxicology and Water Quality* **7**, 223-236.

Tien, C. C., Chao, C. C. and Chao, W. L., 1999. Methods for DNA extraction from various soils: a comparison. *Journal of Applied Microbiology* **86**, 937-943.

Tiquia, S. M., Lloyd, J., Herms, D. A., Hoitink, H. A. J. and Michel Jr., F. C., 2002. Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure determined by analysis of TRFLPs of PCR-amlified 16S rRNA genes. *Applied Soil Ecology* **21**, 31-48.

Tisdale, S. L., Nelson, W. L., Beaton J. D. and Havlin, J. L., 1993. Soil fertility and fertilizers. MacMillan, New York, NY, pp. 634.

Torsvik, V., Øvreås, L. and Thingstad, T. F., 2002. Prokaryotic diversity-magnitude, dynamics, and controlling factors. *Science* **296**, 1064-1066.

Tu, C., Louws, F. J., Creamer, N. G., Mueller, J. P., Brownie, C., Fager, K., Bell, M., and Hu, S., 2006. Responses of soil microbial biomass and N availability to transition strategies from conventional to organic farming systems. *Agriculture, Ecosystems and Environment* **113**, 206-215.

Wardle, D. A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biological Reviews* **67**, 321-358.

Weaver, M. A., Krutz, L. J., Zablotowicz, R. M. and Reddy, K. N., 2007. Effect of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. *Pest Management Science* **63**, 388-393.

Yao, H., Bowman, D. and Shi, W., 2006. Soil microbial community structure and diversity in a turfgrass chronosequence: Land-use change versus turfgrass management. *Applied Soil Ecology* **34**, 209-218.

Yuan, L., Huang, J., Christle, P. and Li, X., 2005. Influence of potassium supply on growth and uptake of nitrogen, phosphorus, potassium by three ectomycorrhizal fungal isolates in vitor. *Journal of Plant Nutrition* **28,** 271-284.

Yao, S., Merwin, I. A., Abawi, G. S. and Thies, J. E., 2006. Soil fumigation and compost amendment alter soil microbial community composition but do not improve tree growth or yield in an apple replant site. *Soil Biology and Biochemistry* **38**, 587-599.

Zhong, W. H. and Cai, Z. C., 2007. Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a paddy soil derived from quaternary red clay. *Applied Soil Ecology* **36**, 84-91.

Zhou, J., Bruns, M. A. and Tiedje, J. M., 1996. DNA recovery from soils of diverse composition. *Applied Environmental Microbiology* **62**, 316-322.

Chapter 5

Conclusion and Future Research

5.1. Conclusion and Future research

This study highlights the possibility that soil microbial communities could serve as a good indicator of soil health, as shown from this study that the soil microbial community structure and composition of the apple orchard showed considerable variance to the land management practices and the landscape gradient. Furthermore, the soil microbial communities correlated positively to some essential soil properties, which could be linked to the land management practices adopted on those experimental plots. However, no correlation between microbial diversity and apple yield was established. This establishes the possibility that other extrinsic factors influence productivity. Future research should monitor the direction of shift in the microbial communities' structures and compositions over a long-term cropping system, as well as a progressive evaluation of the soil properties. To increase the resolution of data obtained it is advised that sampling points should be increased, to reduce the standard deviations such as obtained in this study.

Our results indicate that Automate Ribosomal Intergenic Spacer Analysis (ARISA) technique can be effectively used to estimate microbial community composition. Most importantly for comparative purposes and to detect microbial community shifts due to anthropogenic disturbances. However, ARISA can be further improved to investigate the dynamics of specific phylogenetic groups, by employing phylum-level oligonucleotide primers. Analysis of specific taxonomic groups such as methanotrophic bacteria, and the arbuscular mycorrhiza rather than the entire microbial communities, would give more insight into the functional diversity of the soils. Furthermore, to elucidate more on the complex interaction between soil microbial communities, soil properties and land management practices a polyphasic approach should be employed. For instance, a combination of T-RFLPs with ARISA could improve identification of specific taxonomic groups. Similarly, ARISA approach could be combined with the sole carbon source utilization (Biolog Ecoplates) which is useful when investigating the functional diversity of soils.

Addendum

Table 2. Summary of the Shannon diversity index February and April samples for bacterial and fungal profile.

Treatment	Row and depth	Shannon index (February)		Shannon index (April)	
		Fungal	Bacterial	Fungal	Bacterial
T1R(1)	W1**(10)**	0.8896	1.7018	0.8812	1.5824
	W1**(20)**	0.8238	1.7231	0.8173	1.5297
	BR**(10)**	0.9388	1.2875	1.1893	1.6509
	BR**(20)**	1.0629	1.5574	0.9461	1.6328
	W2**(10)**	0.9851	1.6207	1.2399	1.5046
	W2**(20)**	0.9369	1.6515	0.7159	1.5524
T1R(3)	W1**(10)**	0.8219	1.5288	0.9089	1.3531
	W1**(20)**	0.9717	1.4825	1.0725	1.1515
	BR**(10)**	0.7641	1.5128	0.7512	1.7212
	BR**(20)**	0.978	1.5081	1.1044	1.6744
	W2**(10)**	0.9789	1.2606	0.9027	1.5899
	W2**(20)**	1.0568	1.4784	0.9559	1.6026
T2R(1)	W1**(10)**	0.8843	1.5993	0.9719	1.6401
	W1**(20)**	0.8421	1.5053	1.1254	1.5759
	BR**(10)**	1.0005	1.6118	1.0486	1.6712
	BR**(20)**	0.7139	1.6006	1.0212	1.5882
	W2**(10)**	0.9877	1.1476	0.9106	1.4197
	W2**(20)**	1.0638	1.5425	1.1137	1.6627
T2R(4)	W1**(10)**	0.7532	1.6137	0.8931	1.6723
	W1**(20)**	0.8624	1.3943	1.0552	1.6896
	BR**(10)**	0.999	1.6033	1.1519	1.5447
	BR**(20)**	0.798	1.6326	0.912	1.7372
	W2**(10)**	1.0396	1.5558	0.7981	1.7386
	W2**(20)**	0.9937	1.5925	1.0752	1.5783
T3R(2)	W1**(10)**	0.6546	1.5941	1.2928	1.5311
	W1**(20)**	0.6902	1.6334	1.0164	1.5445
	BR**(10)**	1.0809	1.5434	1.3239	1.6941
	BR**(20)**	1.0255	1.6567	1.0568	1.6637
	W2**(10)**	0.6842	1.5637	0.9909	1.6093
	W2**(20)**	0.8166	1.6203	1.6913	1.5499
T3R(3)	W1**(10)**	0.9482	1.5378	1.0135	1.5667
	W1**(20)**	0.9435	1.3815	1.0715	1.6602
	BR**(10)**	1.1264	1.5648	0.9256	1.5947
	BR**(20)**	1.0241	1.6087	1.0594	1.5195
	W2**(10)**	0.7966	1.6164	0.9223	1.6078
	W2**(20)**	0.8901	1.7047	1.1728	1.3967
T4R(3)	W1**(10)**	0.8737	1.6979	1.0634	1.7336
	W1**(20)**	0.9166	1.8071	1.0099	1.6672
	BR**(10)**	1.1197	1.5589	1.1616	1.6838
	BR**(20)**	1.0314	1.6535	0.7795	1.6651
	W2**(10)**	1.1367	1.7645	0.7758	1.7568
	W2**(20)**	1.0006	1.7435	0.7613	1.7314
T4R(4)	W1**(10)**	0.8178	1.6483	1.0696	1.5816
	W1**(20)**	0.9108	1.7196	1.1179	1.6149
	BR**(10)**	1.1865	1.5489	1.022	1.6676
	BR**(20)**	1.1277	1.6546	0.8436	1.0775
	W2**(10)**	1.1231	1.7487	1.2604	1.6575
	W2**(20)**	0.9464	1.7157	1.1812	1.6706

Table 2. Continues

Treatment	Row and depth	Shannon index (February)		Shannon index (April)	
		Fungal	**Bacterial**	**Fungal**	**Bacterial**
T5R(1)	W1(**20**)	0.8756	1.6054	1.6502	1.6765
	BR(**10**)	0.7219	1.4703	1.3877	1.4017
	BR(**20**)	0.942	1.4565	1.6621	1.4971
	W2(**10**)	1.1417	1.6533	1.1812	1.5768
	W2(**20**)	0.9753	1.6334	1.6705	1.6376
T5R(2)	W1(**10**)	1.1172	1.6027	1.2001	1.5635
	W1(**20**)	1.1359	1.6874	1.5363	1.5822
	BR(**10**)	0.9833	1.4367	1.6478	1.6731
	BR(**20**)	1.1988	1.6277	1.2443	1.6534
	W2(**10**)	1.0559	1.6534	1.2669	1.5283
	W2(**20**)	1.0782	1.6844	1.0812	1.6274
T6R(2)	W1(**10**)	0.7714	1.4945	1.6406	1.5544
	W1(**20**)	0.7162	1.7194	1.5596	1.7093
	BR(**10**)	1.0898	1.5559	1.6493	1.5505
	BR(**20**)	0.9273	1.5802	1.6254	1.5816
	W2(**10**)	0.947	1.5425	1.3024	1.6975
	W2(**20**)	1.0761	1.5405	0.9532	1.6835
T6R(4)	W1(**10**)	0.9731	1.4951	1.213	1.6817
	W1(**20**)	0.8868	1.4608	1.1424	1.7349
	BR(**10**)	0.9544	1.5929	1.2294	1.6422
	BR(**20**)	1.0967	1.6504	1.2372	1.5647
	W2(**10**)	1.0603	1.6055	1.1476	1.7428
	W2(**20**)	0.9843	1.6318	1.2232	1.6348
T7R(1)	W1(**10**)	0.6191	1.5669	0.8245	1.6277
	W1(**20**)	1.0207	1.6504	0.9278	1.6775
	BR(**10**)	0.8915	1.5257	0.8233	1.5476
	BR(**20**)	1.0149	1.6736	1.0755	1.6277
	W2(**10**)	0.7728	1.4725	1.011	1.5612
	W2(**20**)	0.9291	1.5734	1.1638	1.6858
T7R(4)	W1(**10**)	0.8911	1.5913	0.8539	1.6454
	W1(**20**)	0.8858	1.5731	0.9083	1.673
	BR(**10**)	0.7307	1.5241	0.9579	1.5931
	BR(**20**)	0.8952	1.6616	0.8756	1.6767
	W2(**10**)	0.9302	1.6605	0.9521	1.6873
	W2(**20**)	1.0366	1.415	0.9459	1.7642
T8R(2)	W1(**10**)	0.7663	1.5593	0.8398	1.6667
	W1(**20**)	0.9927	1.4391	0.8451	1.6553
	BR(**10**)	1.0479	1.6258	1.1356	1.7688
	BR(**20**)	0.9923	1.6772	1.2233	1.6358
	W2(**10**)	1.0389	1.5512	0.9271	1.6774
	W2(**20**)	0.7637	1.6245	0.8222	1.6512
T8R(3)	W1(**10**)	1.0795	1.5148	1.0325	1.6078
	W1(**20**)	0.8758	1.4482	1.2062	1.5799
	BR(**10**)	1.0028	1.2798	0.892	1.6443
	BR(**20**)	0.7467	1.4989	0.8866	1.6639
	W2(**10**)	1.1056	1.571	1.0312	1.7283
	W2(**20**)	1.016	1.5628	1.1147	1.6672

Table 3. Summary of the Simpson's index February and April samples for bacterial and fungal profile.

Treatment	Row and depth	Simpson's index (February)		Simpson's index (April)	
		Fungal	Bacterial	Fungal	Bacterial
T1R(1)	W1**(10)**	0.2764	0.0243	0.1858	0.0383
	W1**(20)**	0.2836	0.0259	0.286	0.046
	BR**(10)**	0.1656	0.1007	0.1066	0.0299
	BR**(20)**	0.2514	0.0366	0.2264	0.0268
	W2**(10)**	0.2176	0.0324	0.0917	0.0553
	W2**(20)**	0.1615	0.0269	0.3657	0.0412
T1R(3)	W1**(10)**	0.2538	0.0529	0.1743	0.0966
	W1**(20)**	0.2137	0.0554	0.1346	0.1045
	BR**(10)**	0.3028	0.0516	0.2722	0.022
	BR**(20)**	0.1418	0.0453	0.1608	0.0245
	W2**(10)**	0.2382	0.1159	0.1997	0.0363
	W2**(20)**	0.1823	0.0548	0.1967	0.0316
T2R(1)	W1**(10)**	0.2648	0.0344	0.1835	0.0298
	W1**(20)**	0.2485	0.0449	0.1452	0.0372
	BR**(10)**	0.1864	0.0292	0.1676	0.0244
	BR**(20)**	0.3988	0.0303	0.1594	0.0377
	W2**(10)**	0.1821	0.1187	0.1923	0.0934
	W2**(20)**	0.1631	0.0401	0.1185	0.0259
T2R(4)	W1**(10)**	0.3501	0.0368	0.2056	0.0241
	W1**(20)**	0.2578	0.0866	0.1288	0.0262
	BR**(10)**	0.1654	0.0308	0.1496	0.0406
	BR**(20)**	0.3535	0.0283	0.1779	0.0213
	W2**(10)**	0.1916	0.0364	0.2114	0.0203
	W2**(20)**	0.2353	0.0332	0.1519	0.0359
T3R(2)	W1**(10)**	0.4002	0.0329	0.1022	0.0454
	W1**(20)**	0.4057	0.0299	0.1812	0.0404
	BR**(10)**	0.1459	0.0449	0.0611	0.0227
	BR**(20)**	0.1678	0.0273	0.1225	0.0263
	W2**(10)**	0.3909	0.0344	0.1704	0.0374
	W2**(20)**	0.3179	0.0316	0.0239	0.0385
T3R(3)	W1**(10)**	0.2262	0.0486	0.1626	0.0397
	W1**(20)**	0.1948	0.0579	0.1585	0.0273
	BR**(10)**	0.1404	0.0428	0.265	0.0322
	BR**(20)**	0.1444	0.0296	0.1292	0.0405
	W2**(10)**	0.2492	0.0317	0.1918	0.0305
	W2**(20)**	0.2393	0.0232	0.1317	0.0707
T4R(3)	W1**(10)**	0.2152	0.0234	0.1715	0.0213
	W1**(20)**	0.2131	0.0178	0.1586	0.0258
	BR**(10)**	0.1285	0.0414	0.1122	0.0238
	BR**(20)**	0.1474	0.0287	0.2535	0.0259
	W2**(10)**	0.1385	0.0202	0.2282	0.0192
	W2**(20)**	0.1615	0.021	0.2298	0.0221
T4R(4)	W1**(10)**	0.3429	0.0292	0.1428	0.0419
	W1**(20)**	0.2221	0.0231	0.1257	0.0293
	BR**(10)**	0.1	0.0401	0.1637	0.0264
	BR**(20)**	0.1169	0.0277	0.2275	0.2184
	W2**(10)**	0.1378	0.0218	0.0798	0.0303
	W2**(20)**	0.2536	0.0231	0.1175	0.0258

Table 3. Continues

Treatment	Row and depth	Simpson's index (February)		Simpson's index (April)	
		Fungal	Bacterial	Fungal	Bacterial
T5R(1)	W1**(10)**	0.2089	0.0286	0.0263	0.0248
	W1**(20)**	0.2563	0.0385	0.0281	0.0269
	BR**(10)**	0.3304	0.0638	0.0661	0.0651
	BR**(20)**	0.2202	0.0635	0.0256	0.0487
	W2**(10)**	0.1274	0.0289	0.143	0.0393
	W2**(20)**	0.1834	0.0296	0.0252	0.0271
T5R(2)	W1**(10)**	0.1084	0.0388	0.0995	0.0402
	W1**(20)**	0.1132	0.0248	0.0387	0.0357
	BR**(10)**	0.1763	0.0569	0.0307	0.0291
	BR**(20)**	0.0919	0.0301	0.0731	0.0279
	W2**(10)**	0.1441	0.0301	0.0763	0.0406
	W2**(20)**	0.1551	0.0247	0.1318	0.0291
T6R(2)	W1**(10)**	0.3522	0.0538	0.0317	0.0373
	W1**(20)**	0.3966	0.0234	0.0357	0.0242
	BR**(10)**	0.1732	0.0355	0.0304	0.0343
	BR**(20)**	0.2288	0.0365	0.0347	0.0374
	W2**(10)**	0.2012	0.0434	0.0832	0.0244
	W2**(20)**	0.116	0.0389	0.1943	0.0262
T6R(4)	W1**(10)**	0.2114	0.0707	0.0963	0.0346
	W1**(20)**	0.2177	0.0532	0.1151	0.0205
	BR**(10)**	0.1784	0.0391	0.0798	0.0319
	BR**(20)**	0.1169	0.0283	0.0763	0.0428
	W2**(10)**	0.1333	0.0444	0.1164	0.0232
	W2**(20)**	0.1807	0.0285	0.0981	0.0303
T7R(1)	W1**(10)**	0.4296	0.0358	0.283	0.0287
	W1**(20)**	0.1588	0.0276	0.2274	0.0264
	BR**(10)**	0.1844	0.0422	0.2318	0.0328
	BR**(20)**	0.1781	0.0267	0.1801	0.0289
	W2**(10)**	0.3324	0.0477	0.2108	0.0361
	W2**(20)**	0.2027	0.0449	0.1378	0.0235
T7R(4)	W1**(10)**	0.2485	0.0334	0.2598	0.0307
	W1**(20)**	0.2295	0.033	0.2305	0.0271
	BR**(10)**	0.311	0.0561	0.2259	0.0398
	BR**(20)**	0.203	0.0284	0.2296	0.0249
	W2**(10)**	0.2113	0.0259	0.1779	0.0237
	W2**(20)**	0.1712	0.0573	0.1986	0.0194
T8R(2)	W1**(10)**	0.3212	0.0359	0.2444	0.0257
	W1**(20)**	0.1721	0.0503	0.259	0.0254
	BR**(10)**	0.1567	0.0277	0.1248	0.01946
	BR**(20)**	0.1601	0.0253	0.0812	0.0286
	W2**(10)**	0.1473	0.04	0.2177	0.0262
	W2**(20)**	0.2726	0.0307	0.2336	0.0263
T8R(3)	W1**(10)**	0.1735	0.0619	0.2009	0.0364
	W1**(20)**	0.2007	0.0534	0.1171	0.0385
	BR**(10)**	0.1769	0.1206	0.2143	0.0304
	BR**(20)**	0.3141	0.0465	0.2162	0.0282
	W2**(10)**	0.1585	0.0444	0.1698	0.0239
	W2**(20)**	0.1578	0.0368	0.1325	0.0248