EFFECTS OF MANAGEMENT PRACTICES ON SOIL ORGANIC MATTER CONTENT, SOIL MICROBIAL ACTIVITY AND DIVERSITY IN THE KWAZULU-NATAL MIDLANDS

BY

DONAT NSABIMANA

Submitted in fulfilment
of the academic requirements for the degree of
Master of Science in Agriculture

IN THE DISCIPLINE OF MICROBIOLOGY
SCHOOL OF APPLIED ENVIRONMENTAL SCIENCES
FACULTY OF SCIENCE AND AGRICULTURE
UNIVERSITY OF NATAL
PIETERMARITZBURG

2002

ABSTRACT

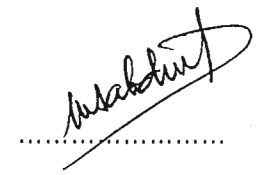
The objective of this study was to investigate the effects of land use and management practice on the soil organic matter content and the size, activity and diversity of the microbial biomass. These effects were investigated using samples taken from the top (0-10 cm) layer of the soils from long-term agricultural managements including natural grassland, maize under conventional (maize CT), maize under zero tillage (maize ZT), annual ryegrass, *Eucalyptus*, *Pinus,* and permanent kikuyu pasture. The natural grassland was used as a control since records indicated that no agricultural activity had ever been exerted on the soil. The measurements used to investigate these effects included soil organic C, total N, soil pH, microbial biomass C, basal respiration rate, microbial quotient, metabolic quotient, dehydrogenase activity, fluorescein diacetate (FDA) hydrolysis, arginine ammonification rate, arylsulphatase activity and acid and alkaline phosphatase activities. The microbial functional diversity was measured using the Biolog Eco plate and catabolic response profiles methods.

Soil organic C and total N were lowest under maize CT, followed by maize ZT and annual ryegrass and were higher under natural grassland, *Eucalyptus and Pinus* plantations while permanent kikuyu pasture had the highest values. The other analyses, namely microbial biomass C, basal respiration rate, FDA hydrolysis, arginine ammonification rate and arylsulphatase activity also followed the same pattern. Annual cultivation was responsible for a decrease in microbial biomass C, basal respiration rate and enzyme activity, principally because there was an appreciable decrease in soil organic matter content. Conversely, permanent pasture, *Eucalyptus* and *Pinus* plantations increased appreciably the amount of organic C and consequently, promoted the size and activity of the microbial biomass in the soils.

The principle component scores showed that management practices affected the microbial functional diversity because different treatments were found in separate zones of the principle component spaces. The regression analysis showed that the variation in the PC1 and PC2 scores was correlated with the variation in soil organic C, exchangeable acidity, extractable P and exchangeable K and Mg. In addition, richness, evenness, Shannon, and Simpson diversity indices showed that any management practice affects the dynamics of soil microbial diversity.

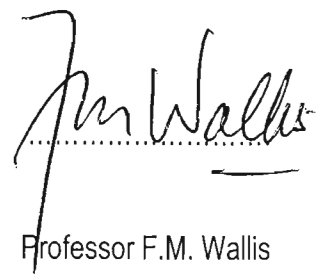
DECLARATION

I hereby declare that the research reported in this thesis was conducted at the University of Natal, Pietermaritzburg, under the supervision of Professors F.M. Wallis and R.J. Haynes. The results presented are from my own investigation except where otherwise indicated in the text and have not been submitted for a higher degree in any other university.



D. NSABIMANA

We certify that the above statement is correct.



Professor F.M. Wallis

Professor R.J. Haynes

ACKNOWLEDGEMENTS

My sincere appreciation and grateful acknowledgement to all people and organisations who have given their resources and time, especially the following:

My supervisors, Professor F.M. Wallis and Professor R.J. Haynes, for their unforgettable guidance and advice throughout this study. May God Almighty thank them forever.

The National University of Rwanda and the government of the Republic of Rwanda, for financial assistance during my studies.

Miss I.F. Schlosser, Miss D. Fowlds (Microbiology discipline) and Mr Essack Abib (Soil Science discipline) for their technical assistance.

Students Marlene Graham and Richard Finn for their assistance with some soil analyses and statistical analysis, and Esther Ugoji for her assistance with grammatical corrections.

Many thanks also to my wife Mrs Christine Mukantwali and my daughter Carrie Anne Lamienne for their unceasing encouragements.

The Almighty, Alpha and Omega for He has strengthened me physically and intellectually for the accomplishment of this study. He was my help according to his holy word: "If Yahweh does not build a house in vain do its builders toil" (Psalm 127:1).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
AWCD	average well colour development
C	carbon
CFE	chloroform fumigation extraction
CFI	chloroform fumigation incubation
$CHCl_3$	chloroform
CLPP	community level physiological profiling
cm	centimetre
C_{mic}	microbial biomass C
CO_2	carbon dioxide
CO_3^{2-}	carbonate
CRPs	catabolic response profiles
CT	conventional tillage
Eco	ecology
FDA	fluorescein diacetate
Fe^{3+}	ferric iron
g	gram
ISCP	*in situ* catabolic potential
KCl	potassium chloride
K_2SO_4	potassium sulfate
L	litre
LSD	least significant difference
M	molar
m	meter
mL	millilitre
mm	millimetre
N	nitrogen
N_2	molecular nitrogen

NAD^+, NADH	nicotinamide adenine dinucleotide
NaOH	sodium hydroxide
NH_3	ammonia
NO_3^-	nitrate
NPKS	nitrogen, phosphorus, potassium, sulphur
O_2	molecular oxygen
PC	principal component
PCA	principal component analysis
pH	potential hydrogen
PLFA	phospholipid fatty acid
qCO_2	metabolic quotient
r	correlation coefficient
SIR	substrate induced respiration
SO_4^{2-}	sulfate
TPF	triphenyl formozan
TTC	2,3,5-triphenyltetrazolium chloride
ZT	zero tillage

GENERAL INTRODUCTION

Soil is a dynamic, living resource that plays many key roles in terrestrial ecosystems (Pankhurst, Doube and Gupta, 1997) and its use for agriculture, pasture, forestry, and urbanization can cause soil degradation. The biological composition of soil is a very sensitive measurement of soil degradation (Sims, 1990). A number of authors have considered the size and activity of the soil microbial community as one indicator of soil health, soil quality and fertility and a key to sustainable agriculture (Doran and Parkin, 1994; Kennedy and Papendick, 1995; Warkentin, 1995; Sparling, 1997). This is because soil microbial communities are responsible for many processes in soil related to soil fertility. They are responsible for organic matter decomposition, humus formation, nutrient cycling, soil aggregation, nitrogen fixation, symbiotic association with plant roots, and degradation of toxic residues in soil (Alexander, 1977; Kirchner, Wollum and King, 1993; Kennedy and Papendick, 1995; Sparling, 1997).

In South Africa, soil degradation has resulted in low fertility (Harrington and Grace, 1997), low organic matter content, soil acidity and soil salinity (Scotney and McPhee, 1991; Beukes, 1995) and, therefore, low crop productivity. The microbial status of South African soils is not well documented. In addition, little research has considered the functional diversity of microbial communities in managed soils and little is known regarding the microbiological activity in arable, pasture and forest agro-ecosystems in South Africa. Because microbiological indicators of soil quality are responsive to management practices, quantifying these indicators through long-term monitoring may lead to an understanding of the effects that land management practices and human-caused disturbances have on the soil component of ecosystems.

In this research, the effects of management practices on soil microbial activity and diversity were evaluated. Following the general introduction, this thesis is subdivided in 4 chapters. The first chapter is a review of organic matter and microbiological characteristics of soils and the main changes that occur due to pasture, cultivation, fertilizer and pesticide application. Chapter 2 is an assessment of organic matter content and the size and activity of microbial biomass in soils under different agricultural systems; namely natural grassland (control), maize under zero tillage, maize under conventional tillage, annual ryegrass, permanent kikuyu (P*enisetum clandestinum)* pasture, *Pinus* and *Eucalyptus* plantations. Chapter 3 the effects of management on the functional diversity of soil microbial communities are investigated under the above land uses. Chapter 4 will be general conclusions.

CHAPTER 1

SOIL ORGANIC MATTER AND SOIL MICROBIAL CHARACTERISTICS: A REVIEW OF LITERATURE

1.1 INTRODUCTION

Soil quality is a topic of interest to soil scientists, agriculturists, biologists, and agricultural and environmental policy makers (Warkentin, 1995; Karlen, Mausbach, Doran, Cline, Harris and Schuman, 1997). Soil quality has been differently characterized. Some authors have suggested that soil quality may be simply related to the yield of crop produced (Romig, Garlynd, Harris and McSweeney, 1995). Others have emphasized the importance of demonstrating how soil quality affects feed and food quality (Hornick, 1992), or how soil quality affects the habitat provided for a wide array of biota (Warkentin, 1995). Aldo Leopold cited by Steinhardt (1995) suggested that land evaluations should be based on the number and type of plant or animal species inhabiting that land (Romig *et al.*, 1995; Warkentin, 1995; Karlen *et al.*, 1997). Scientists used descriptive and analytical aspects and defined soil quality as "the capacity of a specific soil to function, within natural or managed ecosystem, to sustain plant and animal productivity, maintain or enhance water and air quality, resist erosion, and support human health and habitation" (Doran, Sarrantonio and Liebig, 1996; Karlen *et al.*, 1997; Karlen, Rosek, Gardner, Allan, Alms, Bezdicek, Flock, Huggins, Miller and Staben, 1999; Islam and Weil, 2000).

For example, soil quality parameters that may be used to evaluate how soil accepts, retains, and transmits water to crops could include measurement of soil structure, pore space size, aggregate

stability, saturated hydraulic conductivity, particle bonding, or retention mechanisms (Karlen *et al.*, 1997). Soil physical, chemical and biochemical properties were suggested as being sensitive indicators of agro ecosystem changes (Kennedy and Papendick, 1995; Islam and Weil, 2000). This review outlines the role of organic matter in the soil and characterizes the size, activity, and diversity of the microbial biomass in the soil. Following that, the effects of management practices on soil organic matter and microbiology are discussed.

1.2.1 SOIL ORGANIC MATTER

Soil organic matter was defined by Schnitzer (2000) and Smith, Peterson and Needelman (2000) as "*a heterogeneous mixture of living, dead, decomposing organic materials in soil and substances synthesized microbiologically and/or chemically from the breakdown of products*". It includes plant, animal and microbial residues, water-soluble organics, carbohydrates, amino acids, aliphatic fatty acids and humic substances.

Soil organic matter is viewed as the most important factor in evaluating the effects of soil management systems on soil quality (Bayer, Neto, Mielniczuk and Ceretta, 2000) because of its impact on other biological, chemical and physical indicators of soil quality (Reeves, 1997). Soil organic matter is an important nutrient reservoir and energy source for soil biota and nutrient source for plants. It also improves soil structure and water-holding capacity, increases the cation exchange capacity and reduces the toxicity of toxic substances (Haynes and Beare, 1996; Stott, Kennedy and Cambardella, 1999; Smith *et al.*, 2000) and is often a good indicator of soil fertility (Swift and Woomer, 1991; Stott *et al.*, 1999).

The amount of soil organic matter is greatly influenced by management, increasing under pastoral management and decreasing under arable cropping. For example, in temperate agricultural soils, organic C ranges from 3 to 6% in pastoral soils (Haynes and Beare, 1996) and between 1 and 4% soil organic matter under continuous arable crops (Schnitzer, 2000). The rate of decomposition of organic matter is dependent on soil type, texture and mineralogy. For example, in the tropic zone, soil organic matter decomposes five times faster than in temperate regions (Bayer *et al.*, 2000).

1.3 SOIL MICROBIAL BIOMASS

1.3.1 Definition

Soil microbial biomass is defined as the living microbial component of the soil (Wardle, 1992) and usually accounts for 1-5% of total soil organic C, and 1-6% of total soil organic N (Sparling, 1997). Although the microbial biomass represents a small portion of total soil organic matter, it responds more quickly to changes in management practices than does the total soil organic matter (Powlson, Brookes and Christensen, 1987; Anderson and Domsch, 1989; Haines and Uren, 1990; Campbell, Biederbeck, Zentner and Lafond, 1991; Sparling, 1995; Lovell, Jarvis and Bardgett, 1995; Franzluebbers and Arshad, 1997). For this reason, soil scientists often measure the microbial biomass as an indicator of soil quality and soil fertility (Doran and Parkin, 1994; Karlen *et al.*, 1997; Yao, He, Wilson and Campbell, 2000).

1.3.2 Role of soil microbial biomass

Soil microorganisms contribute to the maintenance of soil quality because they control many important processes in the soil (Kennedy and Papendick, 1995). Microorganisms are responsible for organic

matter decomposition, humus formation, nutrient cycling, nitrogen fixation, and symbiotic association with plants (Alexander, 1977; Kirchner *et al.*, 1993; Kennedy and Papendick, 1995; Pankhurst *et al.*, 1997) and therefore, a labile source of nutrients (Carter, 1991; Dalal, 1998). They also play a major role in the formation of good soil structure. For example, bacterial mucigel and the hyphae of fungi and actinomycetes bind the soil particles together (Haynes and Beare, 1996). Microbial aggregation reduces erosion, facilitates water infiltration, and maintains adequate aeration of the soil (Kennedy and Papendick, 1995; Carter, Gregorish, Angers, Beare, Sparling, Wardle and Voroney, 1999). Their key roles in soil formation and pollutant degradation are also well known (Paul and Clark, 1989; Gewin, Kennedy and Miller, 1999; Prescott, Harley and Klein, 1999). In biological control, microbes have been used to control insects, pathogens and weeds because of their ability to lower the populations of target organisms (Kennedy and Papendick, 1995; Dalal, 1998). The above-mentioned traits make soil microorganisms one of the most sensitive indicators of soil quality (Turco, Kennedy and Jawson, 1994; Kennedy and Papendick, 1995; Franzluebbers, 1999).

1.3.3 Variability of soil microbial biomass

The amount of microbial biomass in the soil depends on carbon and nitrogen contents, residues and nutrient amendment, root biomass, soil pH, concentration of heavy metals and pesticides, and soil physical factors (clay content, moisture, temperature) (Carter, 1991; Wardle, 1992). The microbial biomass is enhanced around the rapidly growing portion of the root, and decreases as roots age and with increasing distances from the root (Paul and Clarck, 1989). Soil pH exerts a dominating influence on the incorporation of organic matter into the microbial biomass, and is probably at least as important a factor as soil carbon and nitrogen and an increased acidification of a soil reduces the microbial biomass values (Wardle, 1992). Microbial biomass increases with annual precipitation but decreases with an

increase of annual temperature (Dalal, 1998). Seasonal fluctuations of microbial biomass are common due to changes in amounts of substrate, temperature and moisture (Dalal, 1998 citing Wardle and Parkinson, 1990).

Microbial biomass is a function of soil management practices (Carter, 1991; Pankhurst *et al.*, 1997). Its size declines when soils under forest and grassland vegetation are brought under cultivation (Dalal, 1998). The quantity and quality of plant litter returned to the soil also greatly influences the size of the microbial biomass (Carter *et al.*, 1999). The effects of management on microbial biomass are discussed later in section 1.6.

1.3.4 Microbial quotient

Microbial quotient is the ratio of microbial biomass C to total organic C (Anderson and Domsch, 1989). Many authors have suggested that the microbial quotient indicates changes in soil processes and soil health, and is a more useful measure than microbial C or organic C measured individually (Sparling, 1995) because the ratio avoids the problems of working with absolute values when comparing soils with different organic matter contents. Lower microbial quotients are found in soil under intensive agriculture and they tend to be greater under permanent pastures (Sparling, 1997; Haynes and Tregurtha, 1999). Microbial quotient was observed to be lower under monocropping than that under multicropping (Anderson and Domsch, 1989).

1.3.5 Measurement of microbial biomass

Several methods have been used to estimate microbial biomass in soil. These include *chloroform fumigation incubation* (CFI), *chloroform fumigation extraction* (CFE), *substrate induced respiration* (SIR), *adenosine triphosphate analysis* and analysis of *phospholipid fatty acids* (Turco *et al.*, 1994; Martens, 1995; Dalal, 1998; Franzluebbers, Haney, Hons and Zuberer, 1999). Other biochemical methods for measuring microbial biomass have been developed such as ninhydrin reactive-extraction, rehydration-extraction, microwave irradiation-extraction, and hot water extraction (Franzluebbers, 1999). Results of soil microbial biomass determinations can be influenced by sieve size, intensity of sieving of soil samples, soil water content and storage condition prior to treatment [e.g. temperature and duration of storage and soil moisture] (Martens, 1995).

1.4 SOIL MICROBIAL ACTIVITY

Soil microbial biomass is a measure of intact, active or dormant microbial cells inhabiting the soil. Measurement of microbial activity in soil is an estimation of active biomass. Microbial activity includes all biochemical reactions catalysed by microorganisms. In this review, microbial respiration and enzyme activities in soil are discussed.

1.4.1 Microbial respiration

Soil microorganisms need to oxidize compounds to meet their energy requirements for metabolism, growth and reproduction (Schlegel, 1993). Heterotrophic microflora gain energy from the transformation of organic compounds such as cellulose, proteins, nucleotides and humified compounds. These

reactions are typically oxidation-reduction reactions. Respiration expresses the process in which different substrates are broken down with a release of electrons to produce energy. In this process oxygen acts as a terminal electron acceptor for aerobic microorganisms, whereas anaerobic microorganisms utilize other electron acceptors such as nitrate (NO_3^-), ferric iron (Fe^{3+}), sulfate (SO_4^{2-}), carbonate (CO_3^{2-}), and even certain organic compounds. Nitrifying bacteria also show preference for CO_2 as terminal electron acceptors (Schlegel, 1993; Prescott *et al.,* 1999). Anaerobic processes such as denitrification and sulfate-reduction occur in anaerobic microsites of the soil by anaerobic bacteria such as *Clostridia* (Paul and Clark, 1989).

The end products of respiration are carbon dioxide and water. Measurement of CO_2 production or O_2 consumption can express the metabolic activity of soil microorganisms (Nannipieri *et al.*, 1990 cited by Alef, 1995b). The measurement of microbial respiration without addition of organic substrates to soil is termed **basal respiration**, whereas in **substrate-induced respiration** (SIR) the soil is amended with glucose, amino acids, etc. (Alef, 1995b; Martens, 1995). The relative metabolic activity of bacteria and fungi can be performed by addition of antibiotics to soil, which inhibit one target group. The well-used antibiotics are streptomycin, as an inhibitor of bacteria and cycloheximide for inhibiting fungi (Beare, Neely, Coleman and Hargrove, 1990; Neely, Beare, Hargrove and Coleman, 1991).

In the laboratory, before measuring soil microbial respiration, the soil is bulked, sieved and mixed. These operations remove macrofauna and plant parts; therefore the microorganisms present become central for the measurement of soil respiration (Anderson, 1982). The procedures available for determining basal respiration and substrate-induced respiration are described by Alef (1995b) and Sparling (1995).

Microbial respiration is a function of moisture, temperature, structure, aeration, the availability of nutrients in soil, management practices, and the season as well as the size of the microbial community. It decreases with soil depth and correlates with soil organic matter content (Singh and Gupta, 1977; Alef, 1995b: Haynes and Tregurtha, 1999).

Microbial respiration was used to quantify microbial activities in soils (Heinemeyer, Insam, Kaiser and Walenzik, 1989; Beare *et al.*, 1990; Nay, Mattson and Bormann, 1994; Alef, 1995b). For example, a significant correlation between soil respiration and abundance of soil hyphae was found (Vose, Elliott, Johnson, Walker, Johnson and Tingey, 1995). Soil microbial respiration has also been used to assess the effects of pesticides and heavy metals on the soil (Alef, 1995b) and to measure the metabolic activity of heterotrophic microorganisms (Rochette, Ellebert, Gregorish, Desjardins, Pattey, Lessard and Johnson, 1997).

Microbial respiration may be expressed as a ratio of the amount of CO_2-C produced per unit of microbial biomass C. This ratio is termed **metabolic quotient**, **respiratory quotient** or **specific respiratory rate** (Anderson and Domsch, 1993; Sparling, 1995). Metabolic quotient has been used to investigate microbial activity in response to soil development, substrate quality, soil disturbance, microbial stress and field management (Anderson and Domsch, 1990, 1993; Wardle and Ghani, 1995). The increases in metabolic quotient indicate the stress or unfavourable conditions for microorganisms. In such conditions, microorganisms maintain their life by repairing damage due to disturbance by increasing the rate of respiration per unit of microbial biomass (Anderson and Domsch, 1993). For example, metabolic quotient was found to be higher in agricultural soils and lower in less disturbed soils (Anderson and Domsch, 1990; Wardle and Ghani, 1995). Metabolic quotient was suggested to be higher in young microbial communities than that from matured sites (Anderson and Domsch, 1989).

1.4.2 Soil enzyme activities

Soil enzymes are the mediators and catalysts of important soil functions that include decomposition of organic inputs, transformation of native soil organic matter, release of organic nutrients for plant growth, detoxification of xenobiotics, N_2 fixation, nitrification and denitrification (Dick, 1997). Enzyme activities in soils are derived from exoenzymes released from living cells, endoenzymes released from disintegrating cells and enzymes bound to cell constituents (Tabatabai and Minhong, 1992). Exoenzymes may be associated with enzyme-substrate complexes, adsorbed to clay minerals or associated with humic colloids (Tabatabai, 1982; Alef and Nannipieri, 1995). Measurement of enzymes in the soil may provide information on the microbial activity in the soil. Dehydrogenases, phosphatases and arylsulfatases are a few of the enzymes that may be helpful to assess soil quality (Dick, 1994; Kennedy and Papendick, 1995) as well as the measurement of the rate of enzymes involved in the process of arginine ammonification and FDA hydrolysis (Haynes and Tregurtha, 1999).

1.4.2.1 Role of soil enzymes

Enzymes excreted by microorganisms into the soil solution could have three main roles: (i) hydrolysis of substrates that are too large or insoluble to be taken up directly by cells; (ii) detoxification of the surrounding environment; and (iii) creation of a favourable environment for the survival of the organisms (Dick, 1997).

Soil enzymes are of ecological importance because they can be used as an index of soil fertility and indicators of soil pollution. They may be useful to assess ecosystem status and the impact of land use. For example, Metting (1993) and Dick and Tabatabai (1993) noted a close relationship between nutrient

availability, soil fertility and enzyme activities. Thus, enzymatic approaches can play a significant role in reclamation management (Klein, Sorensen and Redente, 1985) and are considered as sensitive indicators of management-induced changes in soil properties (Bergstrom, Monreal and King, 1998). Enzyme measurements answer qualitative questions about specific metabolic processes and in combination with other measurements (CO_2 evolution, etc.), may increase understanding of the effect of agrochemicals, cultivation practices, and environmental and climatic factors on the microbiological activity of soil (Alef and Nannipieri, 1995). Soil enzyme activities can be measured to assess ecosystem function (Kulinska, Kamargo and Drozdowicz, 1982; Bergstrom *et al.*, 1998). Enzymes have been useful in determining the impact of severe perturbations (e.g. open cast mining) on soil health and to evaluate the success of remediation activities (Pankhurst *et al.*, 1997). They have also been used to assess the effects caused by acid rain, heavy metals, pesticides and other industrial and agricultural chemicals (Dick and Tabatabai, 1993; Dick, 1997). Dick (1997) considered soil enzyme activities as sensitive indicators of soil health. Many researchers have used enzyme activities to study the dynamics of the microbial population in various ecosystems and to assess the effects of land management on microbial activity (Dick, 1984; Deng and Tabatabai, 1997; Perucci *et al.*, 1997; Bandick and Dick, 1999; Haynes, 1999; Acosta-Martinez and Tabatabai, 2000).

1.4.2.2 Groups of soil enzymes

Research into soil enzymes has increased over the past 30 years. New approaches and methods have been introduced and information on various enzyme reactions in soil has been collected (Alef and Nannipieri, 1995). These methods have to be interpreted with caution because the data represent the maximum potential rather than the actual enzyme activity and the incubation conditions of enzyme

essays are chosen to ensure optimum rates of catalysis. The concentration of substrate is in excess and optimal values of pH and temperature are selected so as to permit the highest rate of enzyme activity, and the volume of the reaction mixture is such that it allows free diffusion of substrate (Alef and Nannipieri, 1995; Dick, 1997).

According to Pankhurst *et al.* (1997) methods for measuring over 50 different enzymes are available. Alef and Nannipieri (1995) and Tabatabai (1982; 1994) describe some of the methods. These methods use different biochemical reactions involved in nutrient cycling (Martinez and Tabatabai, 2000), especially those involved in organic residue decomposition and those that show a correlation with organic C, such as proteases, phosphatases, arylsulfatases, glutaminase, catalase, and urease (Alef and Nannipieri, 1995). The following sections describe some of the enzyme activities that can be measured in soils, namely dehydrogenase activity, fluorescein diacetate hydrolysis, arginine ammonification rate, phosphatases, sulphatases, urease and proteases.

(i) Dehydrogenase activity

Dehydrogenases are a group of enzymes involved in all living cells and take part in many reactions involving the transfer of pairs of electrons. In catabolic reactions, dehydrogenases catalyse the transfer of electron pairs from some substrate to NAD^+ forming NADH, which transfers electrons to another compound. As dehydrogenases take part in the electron transfer system of aerobic organisms, the activity of these enzymes is a measure of respiration (Alef, 1995a; Papper, Gebra and Brendecke, 1995). Because dehydrogenase is a specific intracellular enzyme, there is a correlation between dehydrogenase activity and oxygen uptake (Turco *et al.*, 1994). Because of its role in the respiratory chain, dehydrogenase activity could be used to measure the total oxidative activities of the microbial

population in a soil (Turco, *et al.,* 1994). Dehydrogenase activity has been widely studied because it is an intracellular enzyme and exists only in viable cells (Dick, 1997).

The most frequently used assay involves incubation of soil mixed with a solution of the competitive NAD^+ inhibitor, 2,3,5-triphenyltetrazolium chloride (TTC), which in respiration serves as the ultimate electron acceptor. Nearly all microorganisms reduce TTC to triphenyl formozan (TPF), which is estimated colorimetrically (Alef, 1995a; Papper *et al.*, 1995).

Several treatments may affect dehydrogenase activity such as toluene and $CHCl_3$, which can inhibit its activity. Inhibition of up to 70% of the original activity may occur in soil treated with 3% chloramphenicol. Because of this inhibition effect, bactericidal and bacteriostatic compounds are not included in the reaction mixture for essay of dehydrogenase in soil (Tabatabai, 1982).

(ii) Fluorescein diacetate hydrolysis

Microbial hydrolysis of fluorescein diacetate (FDA) into fluorescein can be used as an indicator of total microbial activity. A good correlation was found between FDA hydrolysis and respiration rate (Schnürer and Rosswall, 1982). The rate of fluorescein diacetate hydrolysis in soils is considered an index of overall microbial activity because different enzymes such as proteases, lipases and esterases carry out FDA hydrolysis (Haynes and Tregurtha, 1999).

The method for estimating the hydrolysis of fluorescein diacetate involves the incubation of soil with FDA, which will be converted into fluorescein as the end product by microbial activity. Fluorescein is visualized within cells by fluorescence microscopy or quantified by spectrophotometry (Schnürer and

Rosswall, 1982; Alef, 1995c). This method has the advantage of being simple, rapid, sensitive, and can prove useful in comparative studies of microbial activities in natural habitats (Schnürer and Rosswall, 1982).

(iii) Arginine ammonification

Soil microorganisms take up amino acids released during extracellular proteolysis. In the cells the amino acids are deaminated and amino group parts are excreted as ammonia. Since most heterotrophs possess ammonifying capacity (Alef and Kleiner, 1995), arginine ammonification has been proposed as a general indicator of microbial activity (Turco *et al.*, 1994) and its activity has been correlated with microbial activity in laboratory studies (Haynes and Tregurtha, 1999). The method of assay of arginine ammonification is based on the determination of ammonium concentration after the incubation of soil mixed with arginine solution for 3 h at 30°C (Alef and Kleiner, 1995).

(iv) Phosphatases

Phosphatases are enzymes with relatively broad specificity that catalyse the hydrolysis of phosphate esters and anhydrides of phosphoric acid (Deng and Tabatabai, 1997; Martinez and Tabatabai, 2000). Phosphomonoesterases play an important role in plant nutrition because they catalyse the hydrolysis of organic phosphomonoesters to inorganic phosphorus, which can then be absorbed by plants (Alef and Nannipieri, 1995). Acid and alkaline phosphatases are the commonly measured enzymes in the soil (Tabatabai, 1982; 1994) and play an important role in plant nutrition because they catalyse the hydrolysis of organic phosphomonoester to inorganic phosphorus, which can be taken up by plants (Alef and Nannipieri, 1995). These enzymes are usually extracellular (Kang and Freeman, 1999).

The method for estimating acid and alkaline phosphatase activities is based on the determination of p-nitrophenol released after the incubation of soil mixed with p-nitrophenyl phosphate for 1 h at 37°C, using a modified universal buffer pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase (Alef and Nannipieri, 1995).

(v) Sulphatases

Sulphatases catalyse the hydrolysis of organic sulphate esters and are important for the mineralization of sulphur-containing compounds in soils (Alef and Nannipieri, 1995). The main group of sulphatases are arylsulphatases, alkylsulphatases, steroid sulphatases, glucosulphatases, chondrosulphatases, and myrosulphatases (Tabatabai, 1994) and arylsulphatase is the commonly measured in soils (Tabatabai, 1994). These enzymes are usually extracellular (Kang and Freeman, 1999).

The determination of arylsulphatase activity involves the measurement of p-nitrophenol released after the incubation of soil sample mixed with a buffered p-nitrophenyl sulphate solution and toluene at 37°C for 1 h (Tabatabai and Bremner, 1970a cited by Tabatabai, 1994).

(vi) Urease

Urease is an enzyme that catalyses the degradation of urea to CO_2 and NH_3 and catalyses also the hydrolysis of hydroxyurea, dihydroxyurea and semicarbazid. This enzyme is very widely distributed in nature and is present in microbial, plant and animal cells (Alef and Nannipieri, 1995). The estimation of

urease activity in soils involves the determination of ammonium released after incubation of soil with a buffered urea solution (Tabatabai, 1994; Alef and Nannipieri 1995, citing Tabatabai and Bremner, 1972).

(vii) Proteases

Proteases are enzymes that catalyse the hydrolysis of proteins to polypeptides and oligopeptides to amino acids. These enzymes are present in living, active, and dead cells as free enzymes, adsorbed to organic, inorganic or organomineral particles. Under laboratory conditions, significant correlation was found between protease activity and arginine ammonification, substrate-induced respiration, nitrogen mineralization and adenosine triphosphates (Alef and Nannipieri, 1995).

Protease activity is estimated by the determination of amino acids released after incubation of soil with sodium caseinate for 2 h at 50°C using folin-ciocalteu (Ladd and Butler, 1972, cited by Alef and Nannipieri, 1995).

1.5 SOIL MICROBIAL DIVERSITY

Soil contains a vast diversity of microorganisms (Roper and Gupta, 1995). A single gram of soil can contain more than 10 000 different species (Turco *et al.*, 1994). Many of them are unknown because they are nonculturable (Beare, Coleman, Crossley, Hendrix and Odum, 1995). Soil microbial diversity provides for extensive physiological capabilities and can be studied according to nutritional, functional, systematic, or genetic relationships (Sims, 1990; Zak, Willig, Moorhead and Wildman, 1994).

Nutritional groups among microorganisms are based on carbon source, energy source and hydrogen or electron source (Metting, 1993, Prescott *et al.*, 1999). Organisms that assimulate C from inorganic

sources are termed *autotrophic*, whereas *heterotrophs* require organic C sources. *Phototrophs* use sunlight as their energy source and *chemotrophs* obtain energy from the oxidation of reduced organic or inorganic compounds. *Lithotrophs* use reduced inorganic substances as their electron source whereas *organotrophs* extract electrons or hydrogen from organic substances. Photoautotrophic, chemoautotrophic, photoheterotrophic, and chemoheterotrophic organisms result from the combination of the above different nutritional requirements (Metting, 1993; Schlegel, 1993; Prescott *et al.*, 1999).

Soil microorganisms are systematically grouped into eubacteria, cyanobacteria, actinomycetes, archaebacteria, fungi, algae, protozoa, viruses, and some nematodes (Paul and Clark, 1989; Sims, 1990; Roper and Gupta, 1995). Their number and their collective biomass vary according to soil type. For example, the number and biomass of five major groups of microorganisms in a temperate soil in North America is shown in Table 1.1.

Table 1.1 Number and biomass of microorganisms in a fertile soil in North America (Metting, 1993; Miller, 1990, cited by Pankhurst, 1997)

Microorganisms	Number g^{-1} soil	Biomass (kg ha^{-1})
Bacteria	10^8-10^9	300-3000
Actinomycetes	10^7-10^8	300-3000
Fungi	10^5-10^6	500-3000
Microalgae	10^3-10^6	10-1500
Protozoa	10^3-10^5	5-200

Bacteria are most numerous in soil (Paul and Clark, 1989) because of their small size, but fungi, with their greater biomass, are of more importance in many soils because of their intimate association with plant roots and their saprophytic competence with larger detritus and complex compounds (Metting, 1993). These values (Table 1.1) are not necessarily absolute because some microorganisms in soil are viable but non-culturable and do not grow on laboratory media (Sims, 1990; Turco *et al.*, 1994; Bakken, 1997). For example, Marilley, Vogt, Blanc and Aragno (1998) estimated that only 0.3-20% of soil bacteria are culturable. Turco *et al.* (1994) indicated that 99.5 to 99.9 % of the soil bacteria, observed by fluorescence microscopy, cannot be isolated or cultured on laboratory media. For this reason, novel methods (See section 1.5.6) have been developed to circumvent problems with culturability.

The soil microbial community is not evenly distributed within the surface soil. For example, a plate count on a natural grassland soil showed a decrease with depth and soil around the roots showed a greater number of microorganisms than the bulk soil (Paul and Clark, 1989) because living roots release many types of organic materials into the rhizosphere, which stimulate the growth of microorganisms (Tisdall, 1994). Paul and Clark (1989) observed that the total number of microbes in the rhizosphere increased by 10- to 50-fold compared with the bulk soil.

1.5.1 Bacteria

Bacteria have many varied functions in the soil. The majority of soil inhabiting bacterial species is chemoorganotrophic (Paul and Clark, 1989; Bakken, 1997). They decompose animal, plant and microbial residues. However, their degree of substrate selectivity varies greatly from one species to another. Chemoautotrophic bacteria in soil consist of the nitrifiers and the sulphur oxidizers. Nitrification in soil is carried out largely by the chemoautotrophic bacteria (*Nitrosomonas, Nitrosococcus,*

Nitrosocystis), which oxidize ammonium to nitrite and *Nitrobacter, Nitrosinus,* etc., which oxidize nitrite to nitrate (Paul and Clark, 1989). Sulphur oxidation in soil is largely performed by species of the aerobic genus *Thiobacillus* (Paul and Clark, 1989; Killham and Foster, 1994).

The heterotrophic bacterial community of the soil has a number of additional functions other than decomposition of organic material. Non-symbiotic nitrogen fixation is carried out by species of the genera, *Azotobacter, Azomonas, Beijerinkia, Clostridium* and *Bacillus. Azotobacter, Azomonas* and *Beijerinkia* are obligatory aerobic N_2-fixers, whereas *Clostridium* is obligately anaerobic and *Bacillus* is facultatively anaerobic. Species of the genus *Rhizobium* live in soil and incite nodule formation on leguminous plant roots as aerobic saprophytes. Other groups of heterotrophic bacteria in soil are plant pathogens, which are the causal agents of many plant diseases. These include *Agrobacterium* (causing gall diseases), *Pseudomonas* and *Erwinia* (Paul and Clark, 1989; Killham and Foster, 1994). In soil, the well known genera of bacteria are *Arthrobacter* (40%), *Streptomyces*, *Pseudomonas*, *Bacillus* (5-20%), *Clostridium*, N_2-fixing bacteria (*Azotobacter*, *Rhizobium*), *Nitrosomonas* and *Nitrobacter* which are chemolithotrophs, and *Lactobacillus*, a fermentative organotroph (Paul and Clark, 1989).

The actinomycetes are now considered as bacteria. Most soil inhabiting actinomycetes are free-living saprophytes, able to decompose very large molecular mass substrates such as chitin, cellulose and hemicellulose, particularly under high soil pH. A few soil actinomycetes are also important plant pathogens, for example *Streptomyces scabies*, the causal agent of potato scab. Many soil actinomycetes exude antibiotics such as streptomycin. This group predominates in soil under high pH, high water stress, or high temperature (Killham and Foster, 1994).

1.5.2 Fungi

Fungi dominate the soil microbiota in terms of their biomass and are quantitatively more important in acidic and forest soils. They also dominate in well-aerated temperate soils, rich in carbon and nitrogen (Thorn, 1997; Metting, 1993). Fungi are heterotrophic in metabolism and the great majority are obligate aerobes. The most numerous genera are *Phytophthora, Pythium, Mucor, Rhizopus, Glomus, Gigaspora* and *Acaulospora* (Paul and Clark, 1989).

The most important ecological role of fungi in soil is the decomposition of organic components such as lignin and complex soil humic substances. Because of the greater fungal tolerance to acidity, the decomposition of organic matter in more acidic soils is predominantly a fungal process. The role of soil fungi in forming symbiotic, mycorrhizal associations with most plant roots is important in terms of regulating nutrient uptake [N and P have been best studied], disease resistance, water relations, and growth of the plant partner in the association (Paul and Clark, 1989; Killham and Foster, 1994; Thorn, 1997).

Several fungi have been investigated as potential agents of biological control in agricultural systems because of their role as parasites, predators, and antagonists of plant pathogens. Fungi are used for bioremediation of anthropogenic pollutants, including persistent pesticides, aromatic hydrocarbons, benzene, toluene, ethylbenzene, dyes and others (Thorn, 1997).

1.5.3 Algae

Four groups of algae are recognized in soil. These include green (chlorophyta), blue-green (cyanophyta or cyanobacteria), yellow-green, and diatoms (bacillariophyta) (Paul and Clark, 1989). Soil algae are photoautotrophs. They have no dependence on pre-formed organic matter in soil and, therefore, play key role as primary colonizers on volcanic and desertic soils that are exposed to sunlight. Algae also produce large amounts of extracellular polysaccharides, which can act as soil aggregating agents at the soil surface. Some algae have an ability to carry out non-symbiotic and symbiotic nitrogen fixation using the enzyme nitrogenase. Non-symbiotic N_2-fixation by blue-green algae may be of considerable agronomic significance as a major source of nitrogen to the rice plants under paddy cultivation (Paul and Clark, 1989; Killham and Foster, 1994).

1.5.4 Protozoa

Soil contains a rich variety of protozoa, which are largely restricted to the top 15-20 cm of the soil profile. The better-known protozoa are *Euglena, Amoeba, Vorticella* and *Negleria.* Soil protozoa are predominantly heteroptrophs (phagototrophic nutrition). Some protozoa are involved in organic matter decomposition (Paul and Clark, 1989; Killham and Foster, 1994).

The role of protozoa in soil systems has been summarized as regulation and modification of the size and composition of the microbial community; acceleration of the turnover of microbial biomass, soil organic matter and nutrients; and direct excretion of nutrients (Bardgett and Griffiths, 1997). Predation by protozoa can decrease bacterial population densities both in the rhizosphere of crop plants and in the bulk soil. For example, protozoa can consume 10^3 to 10^5 bacterial cells per division cycle (Anderson,

1988). In temperate, arable soil protozoa could consume between 150 and 900 g bacteria/m^2/year, equivalent to between 15 and 85 times the standing crop of bacteria (Anderson, 1988).

1.5.5 Viruses

Viruses may be present in the soil environment in dormant form. Human enteric viruses, and viruses that infect bacteria, algae, fungi and protozoa are the better known ones. Soils will also contain viruses of vascular plants and viruses whose natural hosts are vertebrate or invertebrate animals that exist in terrestrial environments (Paul and Clark, 1989; Hurst, 1997).

1.5.6 Methods of quantifying microbial diversity in soil

Microbiological diversity indices have been used to describe the status of microbial communities and the effects of natural or human disturbances. These indices can function as bio-indicators by showing community stability and describing the ecological dynamics of a community and impacts of stress on that community (Turco *et al.*, 1994). A number of methods have been used to describe the diversity and structure of soil microbial populations. These include cultural methods, microscopic methods, phospholipid fatty acid analysis and functional diversity using substrate utilization patterns (Bending, Putland and Rayns, 2000).

1.5.6.1 Cultural methods

This method is based on the suspension and serial dilution of a soil sample followed by incubation of the cells on appropriate growth media. During incubation under suitable conditions, each cell develops a

colony that can be seen, counted and characterized. This method is appropriate for studies requiring identification or characterization of individual isolates or for population genetics studies of particular species (Ogram and Feng, 1997). This may be the most sensitive approach available for enumeration and characterization of specific groups, if a suitable selective medium is available. However, the number of colonies detected is low relative to the true number and types of microorganisms present in the natural undisturbed soil. The limitation of this approach is thus that many species are nonculturable on common media, and many strains of interest may not be included in the analysis (Zak *et al.*, 1994; Ogram and Feng, 1997).

1.5.6.2 Microscopic methods

The number and structure of whole soil microorganisms may be studied by direct observation by light, fluorescence or electron microscopy (Bakken, 1997). Epifluorescence microscopy is a major technique for direct enumeration of the soil microbial population. Soil is homogenized in sterilized water and the soil suspension is then treated with formaldehyde to fix bacterial cells and fungal hyphae. The formaldehyde-fixed soil suspension is placed on a glass slide. After air-drying, bacteria on the slide are stained with a fluorescent dye which binds to proteins in their cell walls and cytoplasm. The bacteria are counted using an epifluorescence microscope. For determination of fungal populations the soil suspension is filtered through a membrane filter. The total number of fungal hyphae and/or hyphal length or mycelial fragments on the filter surface is estimated using an epifluorescence microscope (Bloem, Bolhuis, Veninga and Weiringa, 1995).

1.5.6.3 Phospholipid fatty acid analysis

Phospholipid fatty acids (PLFAs) are specific components of cell membranes that are only found in viable cells. A range of various PLFAs is indicative of major microbial group. These fatty acid patterns can be analysed to provide taxonomic information at the species level (Ogram and Feng, 1997; Yao *et al.*, 2000). Changes in the PLFA profiles represent changes in the total soil microbial community and these have been used to determine how land use alters microbial community structure (Yao *et al.*, 2000) and to differentiate microbial communities under different tree plantations (Priha *et al.*, 2001).

Phospholipids are extracted and purified from soil using the Bligh and Dyer extraction procedure and silicic acid chromatography. The ester-linked fatty acids are then transesterified to methyl esters by mild alkaline methanolysis, and the fatty acid methyl esters are analysed by capillary gas chromatography (Tunlid and White, 1992; Yao *et al.*, 2000).

1.5.6.4 Functional diversity

The measurement of microbial diversity based on the capability of members of a mixed population to utilize a number of carbon sources has recently been developed and is variously termed: community level physiological profiling (CLPP) (Yao *et al.*, 2000; Priha *et al*, 2001), substrate utilization patterns (Konopka *et al.*, 1998), *in situ* catabolic potential (ISCP) (Degens, 1998a, b) and catabolic response profiles (CRPs) (Degens and Vojvodic-Vukovic, 1999).

The CLPP approach is a characterization of the heterotrophic bacterial community based on inoculation of samples into Biolog plates (Garland and Mills, 1991). These plates consist of multiple carbon

substrates each contained in a separate well to which are added a minimal growth medium and tetrazolium, a redox indicator dye (Choi and Dobbs, 1999). The original Biolog plates contained 95 C sources including amino acids, carbohydrates, carboxylic acids, polymers, miscellaneous compounds and a blank well (Garland and Mills, 1991). Soil extracts are inoculated into Biolog plates and the rate of utilization of the C sources is indicated by the reduction of tetrazolium, which changes from colourless to purple (Garland and Mills, 1991; Bending *et al.*, 2000; Yao *et al.*, 2000). Classification of microbial communities is based on multivariate analysis of average well colour development, AWCD (Choi and Dobbs, 1999). Zak *et al.* (1994) utilized the Biolog plates with 128 carbon compounds to study the microbial community of soils under different vegetation. Nowadays, the Biolog Inc. Company produces ecology plates (ECO plates), which contain a triplicate of 31 substrates. These showed their ability to distinguish among heterotrophic microbial communities similarly to Biolog Gram Negative and Biolog Gram Positive (Choi and Dobbs, 1999).

However, Biolog microplate systems have some limitations. They may assess the activity of only the species that can grow in the microplate's media; therefore they may not provide an accurate indication of changes in the diversity of the greater microbial community (Degens and Harris, 1997; Yan, McBratney and Copeland, 2000) and they are unable to determine fungal activity (Zak *et al.*, 1994; Yan *et al.*, 2000).

To circumvent these problems, substrate-induced respiration method has been used to measure the metabolic activity of soil microbial communities. Many authors have used glucose as amendment to soil. In this approach soil is incubated with glucose, which is broken down by soil microorganisms. The carbon dioxide end product is quantified by titration (Beare *et al.*, 1990; Stamatiadis, 1990; Neely *et al.*, 1991; Alef, 1995b; Sparling, 1995; Bardgett, Hobbs and Frostegard, 1996).

The catabolic response profiles (CRPs) approach was developed as a modification of the substrate-induced respiration with a range of amendments. Degens and Harris (1997) used substrate-induced respiration with a wide range of carbon sources (83 substrates) to study the metabolic activity of microorganisms inhabiting arable, pastoral and forest soils. Their results showed a greater SIR in pasture than in a cropped soil. They concluded that the SIR response to glucose is related to the total microbial biomass in a wide range of soils but the patterns of SIR responses to different carbohydrate compounds were not greatly different between soils in comparison with the patterns of SIR for other groups of organic substrates. Degens (1998a) studied the microbial functional diversity of arable soils using 36 substrates. Recently, the catabolic diversity of soil microbial communities from pasture, forests, horticultural cropping and cereal cropping was characterized using only 25 selected substrates which were the most responsive in the previous studies (Degens and Vojvodic-Vukovic, 1999; Degens *et al*, 2000).

1.6 EFFECTS OF MANAGEMENT ON SOIL ORGANIC MATTER AND MICROBIAL STATUS

It is difficult to separate the effects of individual agricultural management practices on soil organisms because many factors are interdependent (Sims, 1990). Management practices affect soil organic matter in two ways, by altering the annual inputs and by altering the rate of decay (Haynes and Beare, 1996). The following sections discuss effects of agricultural management on soil organic matter content and soil microbial activity in arable, pastoral, grassland and forest soils.

1.6.1 Arable soils

In agricultural land, cultivation is the major cause of soil organic matter loss (Smith, Papendick, Bezdicek and Lynch, 1993; Beauchamp and Hume, 1997; Wander, Bidart Bouzat and Aref, 1998; Duiker and Lal, 1999; Gewin *et al.*, 1999). For example, Sparling (1992) showed a decline in organic C and microbial biomass C caused by continuous cultivation of maize in New Zealand soils (Table 1.2). Haynes and Tregurtha (1999) reported similar effects with a most pronounced decline occurring during the first 10 years of cultivation.

Table 1.2 Effect of continuous cropping with maize on organic matter levels in the 0-20 cm soil layer (From Sparling, 1992)

Number of years continuous maize	Organic C (%)	Microbial biomass C ($\mu g\ g^{-1}$)
0	4.18	941
1	3.26	522
2	3.89	721
4	2.59	378
6	3.71	559
12	3.34	463

A native sample collected in 1910 contained 3.0% soil organic matter but a cultivated sample from the same field, collected in 1990 contained only 2.2% soil organic matter. The decline in soil organic C was also associated to a decrease of enzyme activity levels. For example, the activity of dehydrogenase dropped by 60%, that of acid phosphatase by 77.5%, and that of urease by 82% (Schnitzer, 2000).

Decreased soil organic C due to intensive cultivation also results in a decrease of microbial biomass C, arginine ammonification, FDA hydrolytic activity and basal respiration (Haynes and Tregurtha, 1999).

The decline in organic matter in cultivated soils is the results of a number of different factors. These include a decrease of organic materials returned to soil due to tillage intensity (Grace, Ladd and Skjemstad, 1994), a rapid release of organic C due to an increase in the accessibility of organic matter to microorganisms caused by the aggregates being broken up by mixing and greater aeration and erosion of top soil rich in organic matter (Smith *et al.*, 1993; Beauchamp and Hume, 1997; Wander *et al.*, 1998; Duiker and Lal, 1999; Gewin, 1999).

(i) Tillage

Tillage method can greatly affect the amount and distribution of soil organic matter and microbial activity in the soil. The main effects are summarized below.

- No tillage (zero tillage), reduced tillage (minimum tillage) and stubble retention methods reduce the number of operations on soil and maintain plant residues on the soil surface, whereas conventional tillage incorporates plant residues and weeds and only the desired crops are allowed to grow (Ones, Allen and Unger, 1990).

- Plant residues maintained on the soil surface increase organic matter level and water holding-capacity of the soil, which decreases erosion and provides a conducive habitat for microbial growth and activity (Papendick *et al.*, 1990, Smith *et al.*, 1993; Arshad, 1999)

- Conventional tillage affects the vertical distribution of soil organic carbon and nitrogen by mixing soil, by distributing crop residues throughout surface soil and by promoting good aeration of the soil, which increases the oxidation and loss of soil organic matter (Etana *et al.*, 1999; Yang and Wander, 1999). By contrast, no tillage promotes higher concentrations of organic carbon, total nitrogen and mineralizable nitrogen in surface soil than does conventional tillage (McCallister and Chien, 2000).

The respective numbers of aerobic microorganisms, facultative anaerobes and denitrifiers in the surface (0-7.5 cm) of a soil were found to be 1.14, 1.58 and 7.31 times higher in no-tilled than in tilled soil (Doran, 1980). Phosphatase and dehydrogenase activities and organic C were also significantly higher in the surface layers of no-till soils than in those undergoing conventional tillage. Below the 7.5 cm level, microbial populations decreased rapidly under no tillage. At the 7.5-15 cm depth counts of aerobic microorganisms and nitrifiers were 1.32-1.82 times higher in the conventionally tilled soil (Doran, 1980). Schenk, Smith, Mitchell and Gallaher (1982) reported lower root colonization, spore levels, and vesicular-arbuscular mycorrhizal fungal species diversity with conventional tillage than with minimum tillage for sorghum (*Sorghum bicolor*), soybean (*Glycin max*), and maize (*Zea mays*). Yocom, Larsen and Boosalis (1985) also reported a lower biomass of vesicular-arbuscular mycorrhizal fungi in tilled than in no-till fields under winter wheat.

Dick (1984) studied the enzyme activities in soil (0-7.5 cm) under continuous maize and found that the activities of acid phosphatase, alkaline phosphatase, Arylsulphatase, invertase, amidase and urease were higher under zero tillage compared to conventional tillage practices (Table 1.3). The activity of these enzymes was positively correlated with organic C content of the soil.

Table 1.3 Enzyme activities in soil (0-7.5 cm) under zero tillage and conventional tillage practices and continuous maize cropping systems (Dick, 1984)

Enzyme activities	Zero tillage	Conventional tillage
Alkaline phosphatase ($\mu g\ g^{-1}h^{-1}$)	83	69
Acid phosphatase ($\mu g\ g^{-1}h^{-1}$)	211	132
Arylsulphatase ($\mu g\ g^{-1}h^{-1}$)	77	49
Invertase ($\mu gg^{-1}h^{-1}$)	161	97
Amidase ($\mu g\ NH_3\ 3g^{-1}\ 24\ h^{-1}$)	18.7	7.5
Urease ($\mu g\ NH_3\ g^{-1}\ 4h^{-1}$)	201	110

Papendick *et al.* (1990) and Reicosky, Reeves, Prior, Runion, Rogers and Raper (1999) noted the influence of crop residues in increasing soil organic matter level, soil fertility and productivity and in minimizing the impact of the environment on soil. Gupta, Roper, Kirkegaard and Angus (1994) studied the effects of long-term stubble retention and reported an increase in microbial biomass C and N and microbial activity in soil with increasing stubble levels. Islam and Weil (2000) also concluded that a soil under conservation tillage had a larger and more active microbial biomass, higher assimilation, greater accumulation of organic C, lower specific respiration and higher aggregate stability than did soil under conventional tillage.

In arable soils, other practices may be associated to tillage. These involve application of manures, fertilizers, herbicides and other amendments (Sims, 1990; Beauchamp and Hume, 1997; Arshad, 1999). The following sections describe some of their effects.

(ii) Effect of fertilizers and manures

Fertilizers affect the soil microbial biomass by increasing crop growth and thus root biomass, root exudates and crop residues, thus providing increased substrate for microbial growth (Kirchner *et al.*, 1993). The size and activity of the microbial biomass are reduced if fertilizer applications cause a decrease in soil pH, but if the environmental conditions remain unchanged, there is an increase in microbial biomass (Roper and Gupta, 1995). For example, application of nitrogen can result in increases in the numbers of nitrifying and denitrifying bacteria (Focht and Verstraete, 1977). Similarly, sulphur application results in an increase in the number and activities of sulphur-oxidizing microorganisms (Lawrence, Gupta and Germida, 1988).

Long-term use of organic amendments (manures) results in an increase in microbial biomass C and N because they not only add nutrient but also increase the amount of C in soil, which is a source of energy for microorganisms (Fauci and Dick, 1994). Similarly, Kirchner *et al.* (1993) found incorporation of leguminous green manure increased microbial biomass and enzyme activities compared with soil that had been inorganically fertilized.

Fertilizers can act by either stimulating or inhibiting soil enzyme activities (Dick, 1997). For example, application of NPKS fertilizers increased soil enzyme activities whereas phosphorus fertilization under field conditions has been shown to depress phosphatase activity (end product repression) in agricultural and forest systems (Dick, 1997).

(iii) Pesticides and herbicides

Pesticides are used in agriculture because they are toxic to some organisms (Sims, 1990). The effects of different herbicides on various microbial groups and biological processes in soils depend upon the nature of the chemical, the dose and the method of application, soil type, temperature and moisture regimes, crop residues and soil management practices (Camper, Moherek and Huffman, 1973; Edwards, 1989). Nitrifying bacteria are the most sensitive to herbicide applications (Edwards, 1989; Paul and Clark, 1989). The most common indicator of the effects of pesticides on microbial activity is a change in respiration levels. Pesticides designed for antimicrobial action, such as fungicides, exhibit the most pronounced effects on respiration (Sims, 1990).

1.6.2 Pastoral soils

The amount of organic matter, microbial biomass and microbial activity is typically high under permanent pastures. Increases in organic matter in soils under pasture arise from senescing plants, exudation of organic compounds from roots, large turnover of microbial biomass and return of ingested plants by grazing animals (Haynes, 2000).

For example, Sparling (1992) noted an increase of organic C in soil under pasture compared with forest or arable soils. Organic C levels were higher in fertilized pasture than in grazed and permanent pastures, whereas microbial biomass was greater in permanent pasture than in other types of pasture (Table 1.4). Haynes, Swift and Stephen (1991) also found that soil microbial biomass C increased significantly after 4 years of pasture, while total organic carbon remained relatively unchanged. Kandeler and Murer (1993) noted a high amount of microbial biomass C and N, dehydrogenase activity and substrate-induced

respiration in pasture than in arable soil. Kahn (1975) studied the density of vesicular-arbuscular mycorrhizal spores in soil (0- 8 cm) and found spore density in pasture soils was twice that under arable cultivation.

Table 1.4 Size of organic matter in 0-5 cm of New Zealand soils (Sparling, 1992)

Pasture type	Organic C (%)	Microbial biomass C ($\mu g\ g^{-1}$)
Fertilized pasture	6.8	1055
Grazed pasture	5.2	851
Permanent pasture	6.13	1176

1.6.3 Soils under native forest and grassland

The soil organic carbon contents are generally higher in virgin soils under grass or forest vegetation than in arable soil (Haynes and Beare, 1996). Organic matter inputs result from large amounts of litter residues, root exudates and high turnover rate of microbial biomass (Lovell *et al.*, 1995). Conversion of grassland and forestland to cropland leads to losses of soil organic carbon. Grassland and forest soils tend to loose from 20 to 50% of the original soil organic carbon content in the zone of cultivation within 40 to 50 years after conversion to arable land (Haynes and Williams, 1992; Haynes and Beare, 1996; Bruce, Frome, Haites, Janzen, Lal and Paustian, 1999). Such a decline was also demonstrated under long-term vegetable production by Haynes and Tregurtha (1999). There was a decline of organic C from 65 to 15 g kg^{-1}. Microbial biomass and activities declined proportionately (Haynes and Tregurtha, 1999).

In general, nitrogen- fixing plants support a higher microbial biomass. This is demonstrated in Table 1.5 where the nitrogen-fixing plant (*Ulex europaeus*) supported a higher soil microbial biomass and organic C than other species.

Table 1.5 Organic C and microbial biomass C contents of soil (0-5 cm) under different types of forest (Modified from Sparling, 1992)

Vegetation species	Organic C (%)	Microbial biomass C ($\mu g\ g^{-1}$)
Nothofagus truncata (Native forest)	6.0	818
Pinus radiata (Plantation)	5.4	649
Ulex europaeus	7.2	920

Fire is commonly used as a management tool in forests and after clearing forests for cultivation of field crops. Use of fire to reduce ground cover and the litter layer in forests can result in a significant reduction of soil organic matter and soil water holding capacity (Phillips, Foss, Buckner, Evans and FitzPatrick, 2000). Such a decline is also associated with a decline in microbial populations immediately following a fire (Sims, 1990).

1.7 CONCLUSIONS

This review discussed the status of organic matter, nutrients, microbial biomass, microbial activities and the diversity of microbial communities under pasture, cultivated, and forest soils in different parts in the world. Annual arable production increases the oxidation of organic matter in the soil and consequently

the size and activity of microbial communities is also decreased. Systems with large organic matter inputs (forest, grassland and pasture) have a good soil structure, moisture holding capacity and higher microbial biomass. In South Africa and other African countries such information is largely not available and is surely needed. In the following chapters of this thesis the effects of various long-term management practices on agricultural soils in the Kwazulu-Natal midlands (South Africa), particularly with respect to their organic matter content and microbial characteristics, are investigated.

CHAPTER 2

EFFECTS OF MANAGEMENT PRACTICES ON SOIL ORGANIC MATTER CONTENT AND THE SIZE AND ACTIVITY OF THE MICROBIAL BIOMASS

2.1 INTRODUCTION

Soil organic matter content is important in relation to nutrient availability for soil biota and plants, energy source for soil microorganisms, soil structure, air and water infiltration, water retention, erosion and the transport or immobilization of pollutants (Troeh and Thompson, 1993; Knoepp *et al.,* 2000; Smith *et al.,* 2000) and it is often indicative of soil fertility (Swift and Woomen, 1991; Scott *et al.,* 1999). Soil nitrogen is present mainly in organic form and it is an important nutrient for microflora and plants and often determines crop productivity (Forster, 1995). Soil pH is important in influencing microbial activity since concentration of H^+ and Al^{3+} influences the ionisation and solubility of enzymes, substrates and cofactors, and governs the activity of soil microorganisms (Tabatabai, 1994; Forster, 1995).

Soil microorganisms are responsible for many transformations in soil related to plant nutrition such as organic matter decomposition, humus formation and nutrient cycling including nitrogen fixation, nitrification, denitrification, and nitrogen mineralization (Turco *et al.*, 1994; Beare *et al.,* 1995; Forster, 1995; Kennedy and Papendick, 1995). Microorganisms help to aggregate the soil, which reduces soil erosion, increases water infiltration, and maintains adequate aeration of the soil (Kennedy and Papendick, 1995).

Due to the dynamic nature of microorganisms in soils, soil microbiological properties can be used as sensitive indicators to assess changes in soil quality resulting from changing management practices. The size and activity of the soil microbial biomass in response to changes in agricultural practices have been studied in terms of total number and/or mass of microorganisms, total respiration rate, and enzyme activities (Anderson and Domsch, 1975; Dick, 1984; Lovell *et al.*, 1995; Bardgett *et al.*, 1996; Crovetto, 1998). Significant changes in microbial biomass and enzyme activities have been associated with changes in tillage and stubble management (Frankenberger and Dick, 1983; Haynes and Knight, 1989; Carter, 1991; Gupta *et al.*, 1994; Bergstrom *et al.*, 1998; Acosta-Martinez and Tabatabai, 2001), pastoral managements (Sparling and West, 1988; Bristow and Jarvis, 1991; Ross *et al.*, 1995; Haynes and Tregurtha, 1999), forest and grassland managements (Priha and Smolander, 1997; Piao, Liu, Wu and Xu, 2001).

In this study, effects of management practices on soil organic matter content and the size and activity of the microbial biomass were monitored. The hypothesis was that microbial communities in South African soils will be greatly affected by management changes. To date very little is known regarding the microbial activity in South African soils or the effect of land use on soil fertility. Since soil microbial characteristics may respond to changes in soil management more quickly than other soil variables, microbiological monitoring can also help in increasing the awareness of soil degradation as a serious problem in South Africa.

2.2 MATERIALS AND METHODS

2.2.1 Site

The experimental soils sampled were from seven fields with long-term application of different agricultural practices such as maize under conventional tillage (maize CT), maize under zero tillage (maize ZT), *Eucalyptus* and *Pinus* plantations, natural grassland, annual ryegrass and permanent kikuyu (*Penisetum clandestinum)* pasture. The sites were all located at the Cedara Agriculture Research Station near Pietermaritzburg, South Africa (29°32'S, 30°17' E). At Cedara, the main annual rainfall is 874mm and mean monthly temperatures range from a maximum of 19.9°C in January (maximum = 25.0°C, minimum 14.7°C) to a minimum of 11.3°C in June (maximum = 19.0°C, minimum = 3.6°C). Soils were classified as Hutton form (Farmingham series) (Soil Classification Working Group, 1991) or as Rhodic Ferisols (FAO). These soils have a clay content of 55 to 65% and the mineralogy is dominated by kaolinite plus halloysite and there are also appreciable amounts of crystalline sesquioxides, gibbsite and interlayered chlorite.

The maize CT and maize ZT experiments were initiated in 1982. Conventional tillage consisted of winter discing to 150 mm depth followed by spring mouldboard ploughing to 250 mm depth and discing to 150 mm depth. Before annual cultivation of maize, these two systems were fertilized with 450 kg ha^{-1} NPK (30 kg N, 45 kg P and 60 kg K ha^{-1}) and additional nitrogen was broadcast in the form of LAN (28%) at a rate of 350 kg ha^{-1} five weeks after planting. The herbicides and insecticides applied were paraquat, atrazine, terbuthylazine, cyanazine, carbofuran, sodiumfluosilicate, deltamethrin and monocrotophos (Lawrance, Prinslo and Berry, 1999). The annual ryegrass experiment was initiated 35 years ago and

the main agricultural applications were chemical fertilization and overhead irrigation. The permanent pasture consisted of plots of kikuyu herbage that was planted in 1940 and used for cow grazing. The *Pinus* and *Eucalyptus* plantations are approximately 30 years old. To evaluate the effects due to the above management practices, native grassland was sampled as a control because no agricultural activity had ever been exerted on the soil. To eliminate the influence of soil type on microbial characteristics, the systems chosen were all in the same geographic location.

2.2.2 Sampling

Samples were collected on 20-22 June 2001. To provide replication, 4 plots were selected in each of the systems. These plots were approximately 120 m long and 30 m wide. A total of forty cores was randomly collected from each plot, twenty from 0-5 cm and twenty from 5-10 cm soil depth. The 20 cores from each depth were bulked to give one sample per plot per soil depth, so that a total of 4 samples per system per depth was collected. In the laboratory, the bulked samples were sieved (< 2 mm) and divided into two sub-samples: one (1000 g) for microbiological analyses and another (500 g) for chemical analyses. Samples for microbiological analyses were stored as moist soil in plastic bags at 2-4°C and were brought to room temperature 24 h before each microbiological essay. Their water content was about 32.5 %. Samples for chemical analysis were air-dried at room temperature.

2.2.3 Analyses

Organic C was determined by the Walkley and Black oxidation procedure (Nelson and Sommers, 1982). Total soil nitrogen was determined by the modified Kjeldhal procedure with colorimetrical determination

of ammonium concentration (Forster, 1995). Soil pH was measured in a 1:2.5 soil: water or soil: 1 M KCl solution ratio using a glass electrode. Microbial biomass C was measured by the fumigation-extraction method based on the difference between C extracted with 0.5 M K_2SO_4 from chloroform-fumigated and unfumigated soil samples using a K_c factor of 0.38 (Vance, Brooks and Jenkinson, 1987; Voroney, Winter and Beyaert, 1993; Joergensen, 1995). The microbial quotient was calculated by expressing microbial biomass C as a percentage of total organic C. Basal respiration was determined by placing 50 g of soil into 50 mL beakers and incubating the samples in the dark at 25°C in 1L, airtight sealed jars along with 25 mL of 0.05 M NaOH. The CO_2 evolved was measured after 5 days by titration (Alef, 1995b). The Metabolic quotient (qCO_2) was calculated as a basal respiration rate (μg CO_2-C h^{-1}) per mg^{-1} of microbial biomass C. Dehydrogenase activity was based on colorimetric estimation of triphenylformozan (TPF), the end product of 2,3,5-triphenyltetrazolium (TTC) oxidation after incubation of the soil samples at 30°C for 24 h (Alef, 1995a; Perucci *et al.*, 1997). The rate of fluorescein diacetate (FDA) hydrolysis was estimated as described by Alef (1995c). Arginine ammonification rate was measured by the method described by Alef and Kleiner (1995). The activity of arylsulphatase was assayed by the method outlined by Alef and Nannipieri (1995). The activities of acid and alkaline phosphatase were determined by the method of Tabatabai (1994). Statistical analyses of data were conducted using analysis of variance (ANOVA) and mean separations between treatments were based on the least significant difference (LSD) at the 5% level of probability using Genstat 5.2.

2.3 RESULTS

2.3.1 Soil organic C and N

Organic C was lowest under maize CT, followed by maize ZT and annual ryegrass. Values determined for natural grassland, *Eucalyptus* and *Pinus* plantations were approximately double that for maize CT. The highest values were observed under permanent kikuyu pasture (Figure 2.1a), and were approximately 3.3 times the amount detected under maize CT. A uniform distribution of organic C within the top 10 cm of soil was observed under maize CT and annual ryegrass pasture, whereas a decline was noted with increasing soil depth under the other treatments (Figure 2.1a).

In relation to native grassland, the disturbances due to cultivation or land use resulted in losses of organic C amounting to 8.6% for *Eucalyptus* plantation, 23.8% for annual ryegrass, 35.8% for maize ZT and 44.3% for maize CT. On the other hand, organic C was increased in the less disturbed systems: 6.1% under *Pinus* plantation and 60% under permanent kikuyu pasture. The zero tillage system resulted in a 15% higher organic C content than under conventional tillage.

Similar trends to those observed for organic C, were observed for total soil nitrogen (Figure 2.1b). The table of analysis of variance for organic C and total N contents showed a very significant difference ($P < 0.001$) between treatments and between soil depth except for maize CT and annual ryegrass (See appendix A).

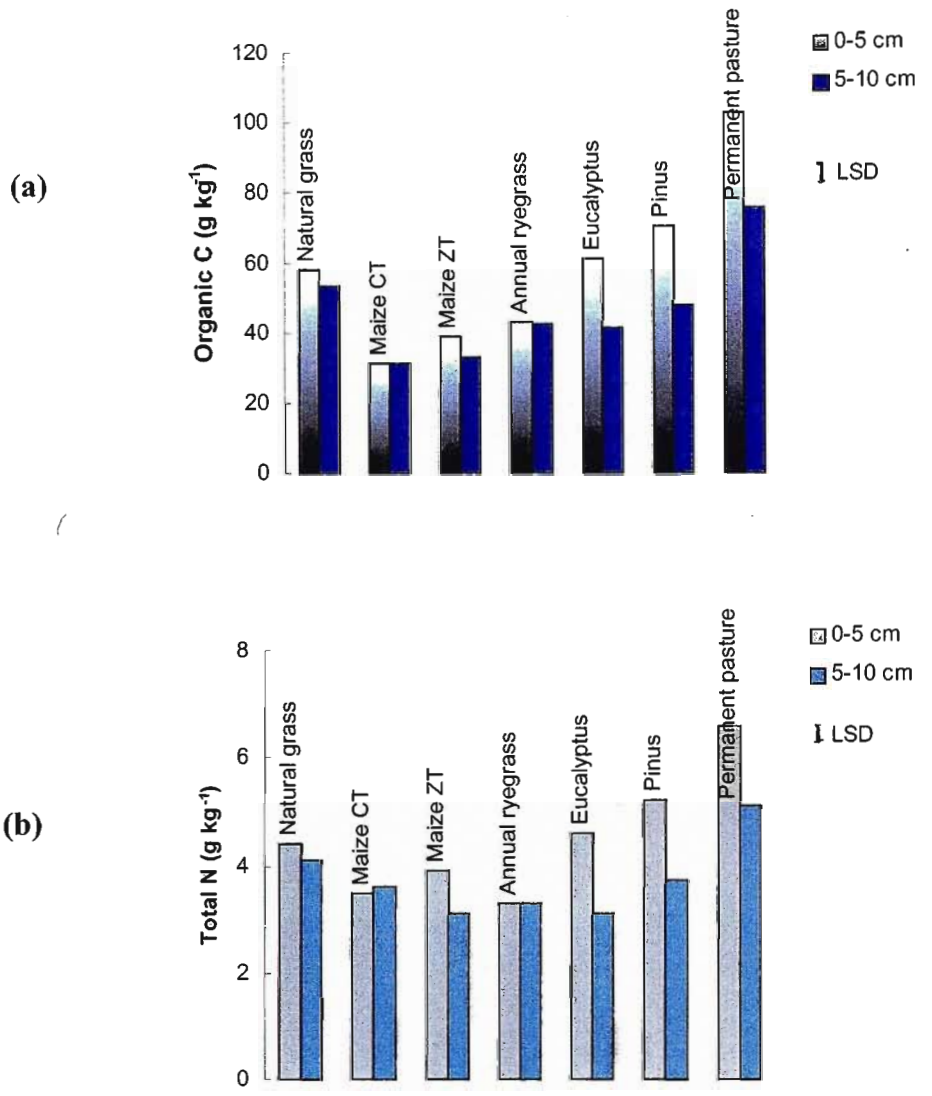


Figure 2.1 Land management effects on the amount of organic C (a) and total N (b) in the 0-5 and 5-10 cm soil depth. Least significant difference (LSD) at $P \leq 0.05$ shown.

2.3.2 Soil pH

Soils under annual ryegrass, *Pinus* and *Eucalyptus* plantations were highly acidic (pH_{KCl} 4.1), whereas soils under natural grassland, maize ZT, maize CT and permanent pasture were moderately acid with pH_{KCl} 4.5 – 4.9 (Figure 2.2). Soil pH in water and that in KCl showed the same trends with approximately one unit pH higher in water than in KCl solution. The analysis of variance of these data showed a very large difference ($P < 0.001$) between treatments whereas the relationship of pH to soil depth was much less significant ($P < 0.026$) [See appendix B].

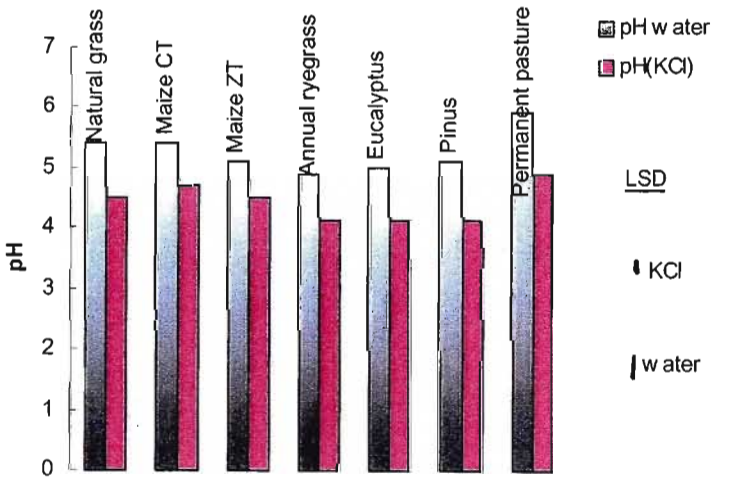


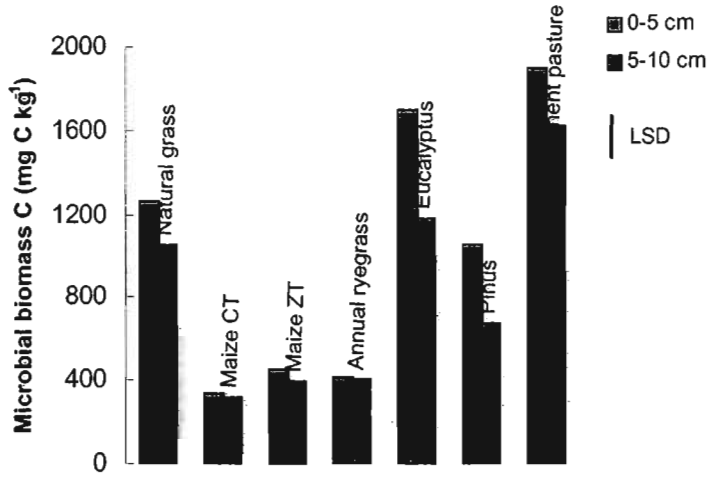
Figure 2.2 Effects of management practices on soil pH measured in water and 1 M KCl. LSD at $P \leq 0.05$ shown.

2.3.3 Microbial biomass C and microbial quotient

The size of the microbial biomass at the different sites is presented in Figure 2.3a. Microbial biomass C ranged from 324 under maize CT to 1754 mg kg^{-1} in the permanent kikuyu pasture (mean values of 0-5 and 5-10 cm layers). In comparison to native grassland, maize CT, maize ZT, annual ryegrass and *Pinus* plantation had lower values, whereas *Eucalyptus* plantation and permanent kikuyu pasture had higher values. Microbial biomass C values were higher in the surface 0-5 cm depth and decreased in the 5-10 cm (Figure 2.3a). The decrease in microbial biomass C with soil depth was not significant in maize CT, maize ZT, and annual ryegrass but was significant in the other treatments. The rate of microbial biomass decrease with increasing soil depth was estimated as: *Pinus* plantation 37%, *Eucalyptus* plantation 31%, natural grassland 17% and permanent pasture 15%. The test of analysis of variance of these data showed that these treatments were largely different ($P < 0.001$) between them and with soil depth (See appendix C).

Values for the microbial quotient at the 0-5 and 5-10 cm soil layers are presented in Figure 2.3b and show that only the *Eucalyptus* plantation had a higher value than native grassland. All the other land uses had substantially lower values than the natural grassland, especially the annual ryegrass, maize ZT and maize CT treatments.

(a)



(b)

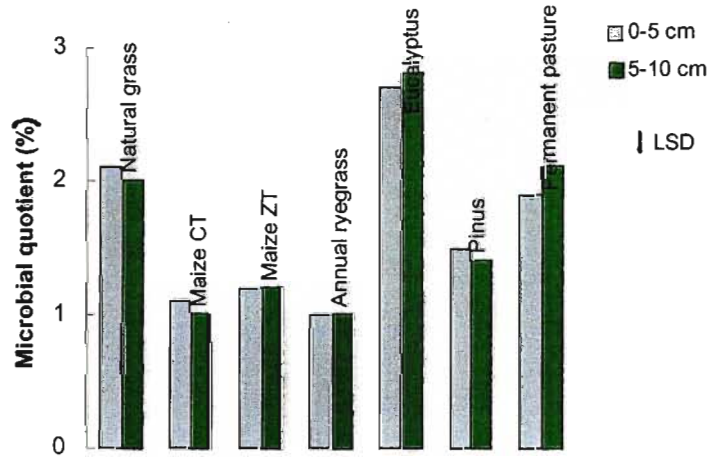


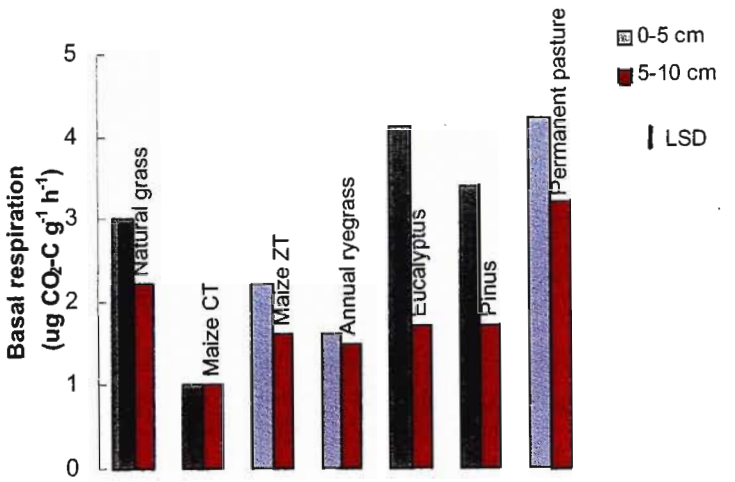
Figure 2.3 Effects of agricultural management on microbial biomass C (a) and microbial quotient (b) in soil at 0-5 and 5-10 cm soil depth. LSD at $P \leq 0.05$ shown.

2.3.4 Basal respiration and metabolic quotient

The basal respiratory rate was greatly influenced by soil management and followed the sequence: maize CT < annual ryegrass < maize ZT < natural grassland < *Pinus* plantation < *Eucalyptus* plantation < permanent pasture (Figure 2.4a). For the 7 systems investigated, only *Eucalyptus* and *Pinus* forests and Kikuyu pasture had values greater than the native grassland at the 0-5 cm soil depth. The decrease in basal respiration between 0-5 and 5-10 cm soil layers was least significant for maize CT and annual ryegrass, but increased appreciably in the other treatments. The analysis of variance of these data shows a very large difference between treatments and with soil depth ($P < 0.001$; See appendix C).

The metabolic quotients (qCO_2) were presented in Figure 2.4b. This ratio was highest under maize ZT followed by annual ryegrass, maize CT; *Pinus* plantation, all of which were higher than that under native grassland. Only *Eucalyptus* plantation and permanent pasture had lower quotients than the control. In general low metabolic quotients were associated with the high microbial biomasses and the high microbial quotients.

(a)



(b)

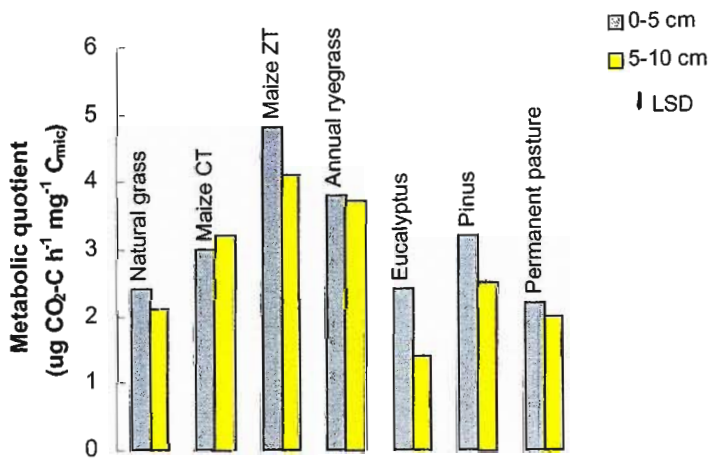


Figure 2.4 Effects of agricultural management on basal respiration (a) and metabolic quotient (b) in soil at 0-5 and 5-10 cm soil depth. LSD at $P \leq 0.05$ shown.

2.3.5 Enzyme activities

Figures 2.5 and 2.6 show enzyme activity levels in the different treatments. The permanent kikuyu pasture showed the highest enzyme activity for dehydrogenase (Figure 2.5a), FDA hydrolytic activity (Figure 2.5b), arginine ammonification (Figure 2.6a) and arylsulphatase (Figure 2.6b). Enzyme activities were intermediate under natural grassland, *Eucalyptus* and *Pinus* plantations. Enzyme activities were generally lowest under maize CT, maize ZT and annual ryegrass. In general, the levels of enzyme activity decreased from the 0-5 cm to 5-10 cm soil layers with the exception of the annual ryegrass and maize CT treatments (Figures 2.5 and 2.6).

As shown in Figure 2.5a, the treatments under study may be placed in three categories with increasing dehydrogenase activity rates: (1) lowest rate for maize ZT and maize CT; (2) intermediate rate for annual ryegrass, *Pinus* and *Eucalyptus* plantations; and (3) highest rate for natural grassland and permanent pasture, approximately 8 times the rate measured in maize under zero and conventional tillage. A decline of dehydrogenase activity was noted with increasing soil depth in all treatments except for maize CT (Figure 2.5a). The activity of dehydrogenase was linearly correlated with pH_{water}, organic C, microbial biomass C, basal respiration, FDA hydrolytic activity, arginine ammonification and arylsulphatase activity ($P < 0.001$; Table 2.1).

From FDA hydrolytic activity levels (Figure 2.5b), the treatments may be grouped in two subgroups based on enzyme reaction rate: (i) a lower FDA hydrolysis rate (0.1-0.17 μmoles $g^{-1}h^{-1}$) for maize CT, maize ZT and annual ryegrass and (ii) a higher rate (0.22-0.37 μmoles $g^{-1}h^{-1}$) for *Pinus* and *Eucalyptus* plantations, natural grassland and permanent pasture. The levels of FDA hydrolytic activity in permanent

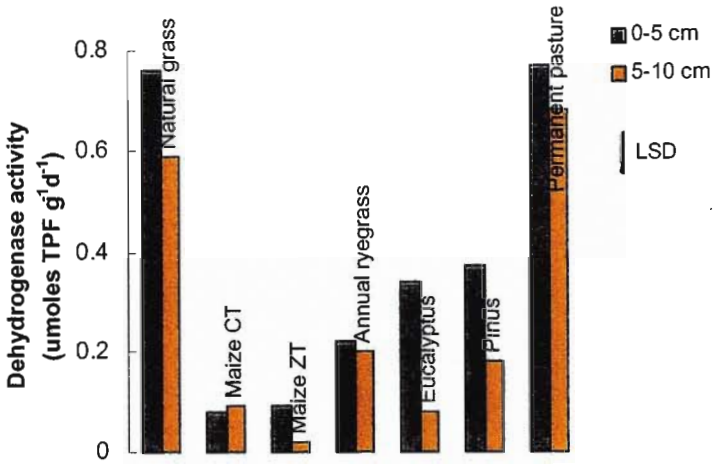
pasture and *Pinus* were approximately four times those for maize CT and maize ZT. The rate of FDA hydrolysis showed a positive correlation with organic C, microbial biomass C and other enzyme activities (Table 2.1).

The rate of arginine ammonification was lowest under maize CT, and increased approximately 1.8 times under maze ZT, annual ryegrass and natural grassland and approximately 2.5 times for *Pinus*. Arginine ammonification levels for *Eucalyptus* and permanent pasture were nearly 4 times that for maize CT (Figure2.6a). The rate of arginine ammonification was positively correlated with other measures such as organic C ($r = 0.91$), microbial biomass C ($r = 0.85$), basal respiration ($r = 0.81$), dehydrogenase activity ($r = 0.64$), FDA hydrolytic activity ($r = 0.79$) and arylsulphatase activity ($r = 0.96$) (Table 2.1).

Arylsulphatase activity (Figure 2.6b) was lowest under maize CT (2 μmoles p-NP g^{-1} h^{-1}), followed by annual ryegrass and maize ZT. The level of arylsulphatase activity was approximately 8 μmoles p-NP g^{-1} h^{-1}under natural grassland and *Pinus.* The higher arylsulphatase activities were detected in soils under *Eucalyptus* plantation and permanent pasture, and were approximately 10 times the amount found under maize CT. Arylsulphatase activity showed the highest correlation with microbial biomass C ($r = 0.93$) and basal respiration ($r = 0.86$) (Table 2.1).

The activities of alkaline and acid phosphatases are not presented because they were not comparable to any other previous findings. The results are appended to this thesis simply to serve as a base line for a future research, which will be needed for verification (See appendix D). Therefore, these results will not be discussed. In addition, the data on enzyme activities were statistically largely different ($P < 0.001$; See appendix E).

(a)



(b)

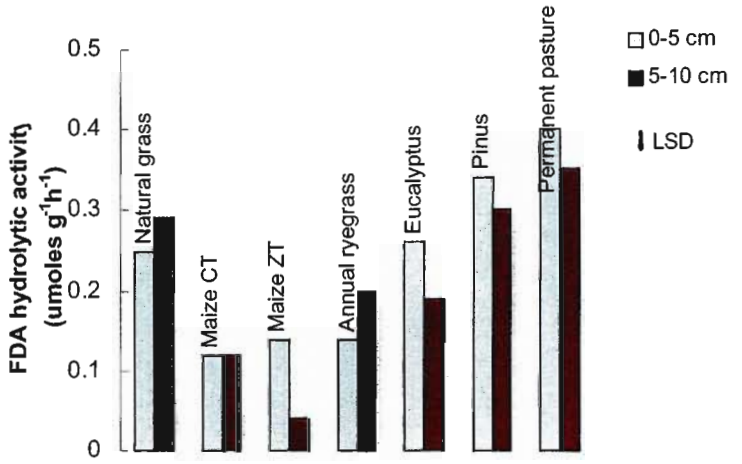
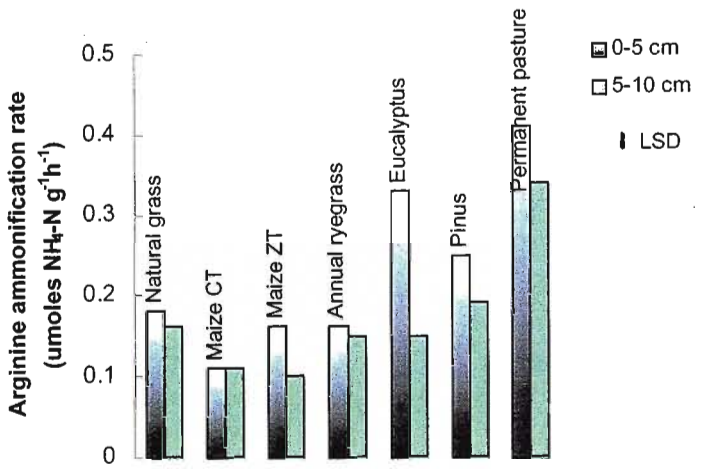


Figure 2.5 Dehydrogenase (a) and FDA hydrolytic (b) activities in soils at 0-5 and 5-10 cm depths as affected by agricultural practices. LSD at $P \leq 0.05$ shown.

(a)



(b)

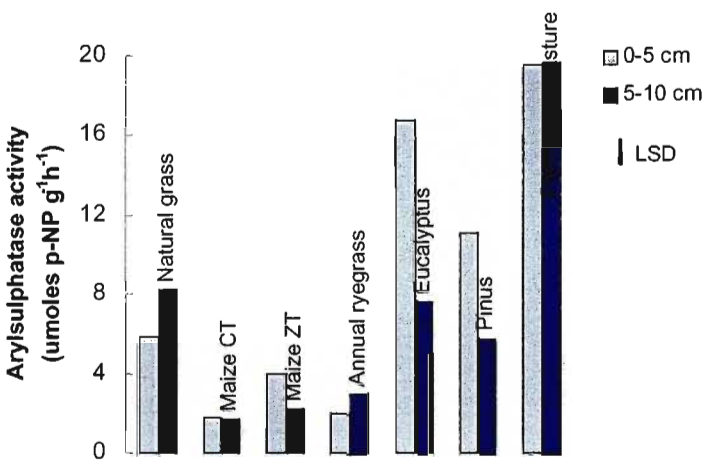


Figure 2.6 Distribution of arginine ammonification rate (a) and arylsulphatase activity (b) in the 0-5 and 5-10 cm soil profiles as affected by management practices. LSD at $P \leq 0.05$ shown.

Table 2.1 Correlation coefficients (r) between soil chemical and biochemical properties

Soil properties	Organic carbon	Microbial biomass C	Basal respiration	Dehydrogenase	FDA hydrolytic activity	Arginine ammonification	Arylsulphatase
pH $_{water}$	0.57***	0.43*	0.45*	0.70***	0.52**	0.50**	0.53**
Organic carbon		0.82***	0.81***	0.77***	0.88***	0.91***	0.91***
Microbial biomass C			0.85***	0.69***	0.75***	0.85***	0.93***
Basal respiration				0.67***	0.73***	0.81***	0.86***
Dehydrogenase					0.79***	0.64***	0.65***
FDA hydrolytic activity						0.79***	0.78***
Arginine ammonification							0.96***

Statistical significance: * Significant ($P \leq 0.05$)

** = Highly significant ($P \leq 0.01$)

*** = Very highly significant ($P \leq 0.001$).

2.4 DISCUSSION

2.4.1 Effects of management practices on soil organic C and N

Effects of tillage and no-tillage cultivation methods on soil organic C have been extensively studied (Johnston, 1986; Gupta *et al.*, 1994; Riezebos and Loerts, 1998). Conventional tillage is generally associated with greater reduction in soil organic C compared to no-tillage (Grace *et al.*, 1994). For example Haynes and Beare (1996) noted an annual rate of decline in soil organic C content of 1.8 t ha^{-1} under zero tillage and 2.6 t ha^{-1} under conventional tillage when a 5-year grass/clover pasture was

converted to arable crops. In the present study (Figure 2.1a), differences between maize CT and maize ZT systems may be explained by the fact that after harvesting, zero tillage leaves crop residues *(Zea mays*) on the soil surface whereas conventional tillage incorporates them into the ploughed layer. Under no-tillage, residues accumulate in the topsoil, and are more exposed to desiccation, resulting in a slower rate of decomposition (Alvarez, Alvarez, Grigera and Lavado, 1998). Such decline was also noted by Grace *et al.* (1994) when grassland soils were converted to continuous cultivation. In addition, under zero tillage organic matter accumulates close to the soil surface. Since in the present study soils were sampled from the surface 0-5 and 5-10 cm, higher values under maize ZT are to be expected.

In general, there is an increased rate of decomposition of organic material due to ploughing and secondary tillage (Grace *et al.,* 1994). In fact, tillage exposes inaccessible soil organic matter to microorganisms and leads to its decomposition (Haynes and Beare, 1996). In addition, ploughing aerates the soil and leads to a more oxidative microbial metabolism. Ploughing also increases contact between soil and residues and promotes decomposition of plant residues (Haynes, 1999). According to Riezebos and Loerts (1998), these higher decomposition rates are due to the enhancement of biological activity caused by soil mixing and higher temperature from increased soil exposure.

The largest soil organic C content was encountered under permanent pasture (Figure 2.1a) suggesting that pastoral management contributes to increased soil organic matter content. In fact, under pasture large organic matter inputs occur from senescent plants, root exudates, return of excreta by grazing animals in the form of dung and urine and turnover of the large microbial biomass in the pasture rhizosphere (Lovell *et al.*, 1995; Haynes and Beare, 1996). Results presented here confirm those of Riezebos and Loerts (1998) who found a substantial loss of soil organic matter under arable production compared to natural forest.

Total N is a measure of organic matter content (Carter, 1991; Perucci *et al.*, 1997). Since organic nitrogen is the predominant form of soil nitrogen, the increase of organic matter content in soils results in a relatively large increase of total nitrogen (Bristow and Jarvis, 1991). Therefore, the above-mentioned factors that affect the variation of organic C may also explain the variation of total soil nitrogen under different treatments.

2.4.2 Effects of management practices on soil pH

In South Africa, soil acidity is a serious problem and is one of the greatest limiting factors to productivity. Acid soils occur in the Western and Eastern Cape, KwaZulu-Natal, the Eastern and Northern Provinces, where 5 million ha have a $pH_{KCl} < 4.5$ and a further 11 million ha a pH of 4.5-5.5 (Beukes, 1995).

Beukes (1995) evoked three main causes of soil acidification in South Africa: (1) that attributed to cultivation, which enhances aeration and promotes a more rapid bacterial oxidation of soil organic matter, resulting in the production of acids; (2) nitrification after application of ammoniacal fertilizers and (3) forest plantations which acidify the soil by accumulation of basic cations in the forest biomass. This was demonstrated by Du Toit (1993) who studied the effects of *Pinus*, *Eucalyptus* and *Acacia* plantations on soil acidification in KwaZulu-Natal. He found that afforestation decreased the pH of soils to $pH_{KCl} = 4$ in comparison with adjacent grassland of pH 4.3.

In this study, similar results were found where pH under *Pinus* and *Eucalyptus* plantations was 4.1, whereas under natural grassland (control) it was 4.5. The pH of the soils under natural grassland, maize ZT and maize CT were not significantly different (Figure 2.2). Haynes and Knight (1989) also found no significant difference in soil pH between conventional tillage and no-tillage in New Zealand. In the

permanent pasture treatment, the pH tended to be high (pH_{KCl} = 4.9) and this is probably the result of heavy lime applications that are routinely applied to such pastures.

2.4.3 Effects of management practices on soil microbial biomass C, basal respiration, microbial quotient and metabolic quotient

Microbial biomass and basal respiration are useful measurements for estimating the rate of change in organic matter status of the soil due to land management. The derived indices, microbial and metabolic quotients, provide additional information relating to microbial processes in soil (Pankhurst *et al.*, 1997). Microbial biomass, basal respiration and metabolic quotient have all been proposed as indicators of stress or disturbance induced by land management, and as early indicators of changes in soil organic matter due to various management practices (Carter, 1991; Wardle, 1992; Priha and Smolander, 1997; Dalal, 1998; Haynes, 1999). Basal respiration and metabolic quotient may provide information on the physiological state of the microbial community (Alvarez *et al.*, 1998). Microbial biomass C, basal respiration, microbial and metabolic quotients are suitable variables that characterize C availability and soil quality (Knoepp *et al.*, 2000).

Similar to the findings of Haynes (1999), the greatest microbial biomass C was accumulated under permanent pasture (1754 mg C kg^{-1}) and this highlights the importance in pastoral management of maintaining high soil microbial biomass and activity. The high microbial biomass C in permanent pasture, *Eucalyptus* plantation and natural grassland is associated with high organic C contents. These results are in accordance with the findings of other researchers who have observed that microbial biomass C in soils is closely correlated with organic C content (Anderson and Domsch, 1989; Carter,

1991; Haynes and Tregurtha, 1999). In the present study a linear relationship was also found between organic C and microbial biomass C contents in the soils (r = 0.82; Table 2.1). In addition, these results correspond to what was indicated by Bandick and Dick (1999), Kramer and Green (2000) that the rhizosphere is a zone of increased microbial biomass.

Although soil organic C was higher in *Pinus* than in *Eucalyptus* plantations, the microbial biomass C was 1.7 times higher under *Eucalyptus*. This is probably attributable to forest litter quality, since easily decomposable litter results in a higher microbial biomass C (Carter *et al.*, 1999). The results obtained are comparable to those of Priha and Smolander (1997) who studied microbial biomass in soil under different tree species (*Pinus sylvestris, Picea abies* and *Betula pendula*). They found lower soil microbial biomass under pines than under the other trees. The explanation may be that the high concentrations of phenolic compounds in pine needle litter inhibit microbial activity.

The low microbial biomass in the soils under maize CT and maize ZT is attributable to low organic C contents (Alvarez *et al.*, 1998). In addition, it is possible that the application of pesticides and herbicides to these treatments for 20 years (Lawrance *et al.*, 1999) may have exerted negative effects on the microbial biomass C. For example, Wardle (1992) and Dalal (1998) observed a reduction in microbial biomass and alteration of microbial diversity due to the application of some insecticides.

Reports in the literature show a wide range of microbial quotients (0.27 to 7.0%) which are due to differences in soils, vegetation cover, management, variation in sampling time, soil water content and the analytical methods used (Anderson and Domsch, 1989; Carter *et al.*, 1999). In the present investigation, the low microbial quotient (1 to 1.2 %) under annual ryegrass, maize ZT and maize CT is indicative of high exploitation of the available C resources (Sparling, 1997). Similar values (e.g. 1.1%) were found in soil under arable management in New Zealand (Haynes and Tregurtha, 1999). The higher microbial

quotients in permanent pasture 2%, natural grass 2.1%, and *Eucalyptus* plantation 2.8% are attributable to the high organic C content in these soils. High values are indicative of environmental conditions conducive to the establishment of large and diverse microbial populations. These stabilize soil structure and contribute to the liberation of various nutrients for macro- and micro- organisms and plant growth (Roper and Gupta, 1995; Haynes and Beare, 1996).

The metabolic quotient (qCO_2) can be used: to investigate ecosystem development and response to ecosystem stress or disturbance (Anderson and Domsch, 1993; Sparling, 1997; Alvarez *et al.*, 1998); to compare field management (Anderson and Domsch, 1990); and to evaluate the efficiency of soil microbial populations in utilizing organic compounds (Dilly and Kutsch, 2002). Anderson and Domsch (1989) suggested metabolic quotients in young ecosystems are usually larger than those in mature sites. In general, greater metabolic quotients are associated with soils with low microbial biomass contents (Sparling, 1997) because the microbial quotient is a ratio of basal respiration to microbial biomass.

In the present paper, high qCO_2 values were obtained under maize ZT 4.4, annual ryegrass pasture 3.8 and maize CT 3.1 $\mu g\ CO_2\text{-}C\ h^{-1}\ mg^{-1}\ C_{mic}$ (Figure 2.4b). Similarly, Haynes (1999) found higher microbial metabolic quotients in treatments under tillage than those under undisturbed management. A high qCO_2 is generally an indicator of a microbial community under stress (Anderson and Domsch, 1993). Under the above three land uses a shortage of available C is the most likely stress factor.

The highest metabolic quotient determined (maize ZT) may be the result of an additional stress response due to pesticide and herbicide application. Indeed it was noted by Sims (1990) that increased microbial respiration rate is a common indicator of pesticide application. High qCO_2 may also arise from

the prevalence of zymogenous organisms (r-strategists) in contrast to autochthonous species (K-strategists), which arise in agricultural soils (Dilly and Munch, 1998).

In the *Pinus* plantation, qCO_2 was high and this may be because pine litter is difficult to decompose since it contains phenolic compounds (Wardle and Ghani, 1995; Priha and Smolander, 1997). The reasons for low metabolic quotients in *Eucalyptus* plantation (1.9), permanent pasture (2.1), and natural grass (2.2) may be that they are undisturbed ecosystems. Under such condition competition for the available C source favours microorganisms requiring the least amounts of energy for maintenance and growth and also they release less CO_2 (Sakamoto and Oba, 1994). For these sites with relatively low qCO_2, less organic carbon is lost by respiration and more carbon is assimilated into the microbial biomass. The microorganisms are expending relatively less energy for basic survival and devoting greater resources to growth (Islam and Weil, 2000). As a result, the microbial biomass C increases.
Some workers have concluded that the functional relationship between the parameters discussed above is not yet fully understood and that there is no universal equilibrium constant (Anderson and Domsch, 1989; Sakamoto and Oba, 1994). For example, Dilly and Kutsch (2002) noted that microbial biomass and metabolic quotient values seem to contradict each other because high microbial biomass suggests high-energy requirements, high organic matter degradation and thus a high metabolic quotient. The present results and previous findings illustrate the contradiction. This may be the reason why Wardle and Ghani (1995) failed to distinguish between stress and disturbance and suggested that the metabolic quotient does not decline predictably as ecosystems develop, or along successional gradients. However, it may be concluded that intensive management practices lead to a more active microbial biomass, increased organic matter oxidation and consequently a decline in microbial biomass C.

2.4.4 Effects of management practices on dehydrogenase activity

Intracellular dehydrogenases have been measured as an estimation of overall microbial activity in soils (Alef, 1995a; Perucci *et al.*, 1997) and have been used to compare soils under different management systems (Beyer *et al.*, 1993). In the present study, management changes affected the activity of dehydrogenase (Figure 2.5a). Similar results to those shown in Figure 2.5a and Table 2.1 have been noted in previous studies (Beyer *et al.*, 1993; Haynes, 1999).

Although dehydrogenase is the most widely studied enzyme indicator of soil biological activity (Dick, 1997), recent literature suggests that dehydrogenase activity is an inappropriate measurement to characterize effects of soil management on microbial activity since it has more influence on soil specific reactions or soil type than on microbial biomass (Beyer *et al.*, 1993; Perucci *et al.*, 1997). Indeed, in the present study dehydrogenase activity was respectively similar under maize ZT and maize CT, natural grass and permanent pasture, while other assays showed these practices to produce significantly different effects. Similarly, in the Haynes and Williams's work (1999) animal camping showed no effect on the dehydrogenase activity in the soil whereas arginine ammonification and FDA hydrolysis indicated clear differences.

2.4.5 Effects of management practices on FDA hydrolytic activity

Hydrolysis of fluorescein diacetate (FDA) is used as an index of microbial activity in soil because it is carried out by active cells producing a variety of enzymes including esterases, lipases and proteases (Schnürer and Rosswall, 1982; Alef, 1995c; Haynes and Tregurtha, 1999). In the present study, all

treatments showed differences in the ability of the resident microbial population to utilize fluorescein diacetate substrate (Figure 2.5b).

The above results confirm the findings of other researchers. For example, higher FDA hydrolysis, microbial biomass C and organic C were found in soils under pasture compared to arable and annual grass in New Zealand (Haynes, 1999; 2000). The rate of FDA hydrolysis was also higher in maize ZT than under annual ryegrass or maize CT (Haynes and Tregurtha, 1999). Perucci *et al.* (1997) noted a higher FDA hydrolysis rate in a field where crop residues had been incorporated than where residues had been removed from the land. Positive correlations between FDA hydrolytic activity and other parameters suggest that the high organic C and microbial biomass C in undisturbed treatments was translated into high FDA hydrolysis rates.

2.4.6 Effects of management practices on the rate of arginine ammonification

The arginine ammonification assay is a relatively recent method used to study microbial activities in soils. It was proposed by Alef and Kleiner (1986; 1987) who concluded that arginine ammonification was carried out by living microorganisms because its rate was strongly related to respiration and the number of microorganisms present. Recently, arginine ammonification was suggested to be a rapid and good index of gross N mineralization in agricultural soils (Bonde, Nielsen, Miller and Sorensen, 2001). This assay has been applied to study the microbial activity of the soils under grass and crop management (Haynes, 1999; Haynes and Tregurtha, 1999).

In the present study, arginine ammonification was used to monitor the effects of long-term management practices on soil microbial activities. The results suggest that arginine ammonification rate increased as

increased the size of the microbial population in the soil. These results are comparable to previous findings (Alef and Kleiner, 1987; Haynes and Tregurtha, 1999) where arginine ammonification was higher in soil under pasture than arable cultivation.

2.4.7 Effects of management practices on the activity of arylsulphatase

Arylsulphatase is a specific enzyme involved in soil organic S mineralization and it catalyses the hydrolysis of ester sulphate bonds (Tabatabai, 1994; Deng and Tabatabai, 1997; Bandick and Dick, 1999). Arylsulphatase activity increased proportionately with organic C content and microbial biomass C (Table 2.1). Correlations between arylsulphatase activity and microbial biomass C ($r = 0.93$) reveal that microorganisms are the major producers (Bandick and Dick, 1999). The correlation between arylsulphatase activity and organic C ($r = 0.91$) suggests its integral role in nutrient cycling, especially in the S cycle, and the fact that soil organic matter stabilizes or protects soil enzymes. Arylsulphatase was also found to be weakly correlated with pH_{water} ($r = 0.52$). In previous studies arylsulphatase activity was found to be positively correlated with organic C and total N (Tabatabai, 1994; Alef and Nannipieri, 1995; Deng and Tabatabai, 1997; Haynes, 1999), microbial biomass C (Haynes, 1999) and soil pH (Deng and Tabatabai, 1997; Kang and Freeman; 1999).

In the present investigation, management practices were found to affect arylsulphatase activity. For example, arylsulphatase activity was lower under the more disturbed treatments (maize CT, maize ZT and annual ryegrass), which had low organic C and microbial biomass C contents. In previous research arylsulphatase activities were found to be lower under conventional than under zero tillage (Dick, 1984; Deng and Tabatabai, 1997; Bergstrom *et al.*, 1998).

On the other hand, the activity of arylsulphatase was higher in the less disturbed systems (natural grassland, *Pinus*, *Eucalyptus* and permanent pasture). In fact, arylsulphatase activity was positively and strongly related to organic C and microbial biomass C levels. In addition, both Bandick and Dick (1999) and Kramer and Green (2000) suggested that the rhizosphere is a zone of increased microbial and enzyme activities. Thus, in the presence of permanent vegetation with extensive root systems and lack of tillage arylsulphatase activity was high.

2.4.8 Effects of soil pH on enzyme activities

Soil pH influences the ionisation and solubility of enzymes, substrates, and cofactors and governs the activity of soil microorganisms (Tabatabai, 1994; Forster, 1995; Acosta-Martinez and Tabatabai, 2000). Low soil pH may cause irreversible inactivation of enzymes or modify the activity in soils systems by changing the interaction between enzymes and stabilizing substances, e.g. humus and clay (Kang and Freeman, 1999). Thus pH may be one of the factors controlling enzyme activities in soils. However, in this study although pH was strongly correlated with dehydrogenase activity, the correlation was weak with arginine ammonification rate, arylsulphatase activity and FDA hydrolysis activity (Table 2.1). The effects of pH were presumably masked by the large differences in organic matter and microbial biomass that occurred between the different land uses.

2.5 CONCLUSIONS

Soil fertility refers to the ability of a soil to supply essential nutrients for plant growth and is a function of two major factors. Firstly, soil organic matter, which is an important nutrient reservoir and nutrient source for plants. Secondly, soil microbial population, which decomposes soil organic matter and make available essential nutrients for plant growth. In addition, microbial aggregation reduces erosion, facilitates water infiltration and maintains adequate aeration of the soil.

The results of the present study show that the above-mentioned factors were greatly influenced by management practices. Annual cultivation is responsible for losses of soil organic matter due to the increased rate of oxidation and low organic returns and consequently, the size of the microbial biomass and basal respiration levels are low. The loss of soil fertility under arable cropping is also observed through the low enzyme activities, which are responsible for organic matter decomposition and release of nutrients for plant. Conversely, permanent pasture, forestry and native grassland managements conserve soil fertility. These practices stabilise soil organic matter and favour the establishment of a large, active, microbial population.

CHAPTER 3

MANAGEMENT PRACTICES AFFECT THE CATABOLIC DIVERSITY OF THE SOIL MICROBIAL COMMUNITIES

3.1 INTRODUCTION

Studies of microbial diversity have been concerned in three interrelated areas: the genetic, functional and taxonomic diversities (Garland and Mills, 1991; Zak *et al.*, 1994). The aspect of functional diversity seem to be the most important because it is more relevant to the understanding of the role of the microorganisms in the functioning of the soil ecosystem than the species diversity (Degens and Vojvodic-Vukovic, 1999). Knowledge regarding microbial diversity in soils is still scant due to a general lack of taxonomic information, limitation in methodology and the complex way in which genetic and taxonomic diversity may affect functional diversity (Zak *et al.*, 1994).

The classical approaches to the studies of microbial diversity involved the assessment of a species richness (abundance) and evenness (number of groups) in the microbial communities (Degens and Vojvodic-Vukovic, 1999 citing Trevors, 1998; Yan *et al.*, 2000). Newly developed technologies include the analysis of the ribosomal RNA gene sequences, the analysis of the microbial lipids (Konopka *et al.*, 1998) and the use of the substrate utilization patterns (Zak *et al.*, 1994). The later consist of the measurement of the diversity of the decomposition functions of the microbial communities based on the community-level physiological profile (CLPP) approach that was introduced by Garland and Mills (1991). The method succeeded to distinguish the differences among samples and to assess the functional

diversity of the microbes in the ecosystems. Garland and Mills (1991) indicated that the CLPP method could be a useful tool for classifying the microbial communities. This approach was developed in two methods: (1) the short-term utilization of a range of the substrates that have been added to soil (Degens and Harris, 1997; Degens, 1998a, b; Degens *et al.*, 2000) and (2) the Biolog Gram-negative and ECO microplates, which are used to investigate the soil microbial diversity on the basis of the sole carbon source utilization abilities by soil microorganisms (Garland and Mills, 1991; Zak *et al.*, 1994; Choi and Dobbs, 1999; Bending *et al.*, 2000; Liu *et al.*, 2000; Priha *et al.*, 2001). These two methods aim to measure the diversity of the decomposition functions performed by the soil heterotrophic microorganisms, which represent a major component of the soil microbial functional diversity (Degens *et al.*, 2000).

In the previous chapter, it was shown that management practices influence the soil microbial biomass, basal respiration rate and the enzyme activity levels. The purpose of this chapter was to determine the effects of land management on the catabolic diversity of the inhabiting soil microbial communities. The hypothesis was that changes in management will affect the amount, quality and diversity of substrates entering the soil. As a result, management could influence the catabolic diversity of the soil microorganisms by causing changes in their physiological status by eliminating or favouring a group of the microorganisms responsible for the degradation of one or more substrates.

3.2 MATERIALS AND METHODS

Soil samples were collected as described in chapter two. Soil chemical characteristics were analysed by the laboratory of soil fertility at CEDARA, Pietermaritzburg in South Africa. These were exchangeable acidity, extractable P, and exchangeable K, Ca and Mg. Other soil properties were analysed in chapter

2. The microbial functional diversity of the soils (0-5 cm) was determined by two different approaches. Firstly, the carbon utilization patterns were measured by the method described by Bending *et al.* (2000) using Biolog Eco plates (Biolog, Hayward CA 94545, USA). These contained three replicate wells of 31 carbon substrates and water (Choi and Dobbs, 1999). Colour development in wells was measured at 12 h intervals for 5 days using a Biotek EL 312 microplate reader at 490 nm. The number of positive wells was also counted after every 24 h. Analysis of the data included number of positive responses, the average well colour development (AWCD) and the principal component analysis (PCA) (Garland and Mills, 1991; Zak *et al.*, 1994). The PCA was performed on the transformed data of the AWCD (Garland and Mills, 1991). The relationships among samples were obtained by plotting first two principle component scores in two dimensions. The treatments were also separated along the PC1 and the PC2 axes by the analysis of the variance of the PC scores and on the basis of the least significant difference at 5% level of probability. The standardised data of AWCD were also used in calculation of richness, evenness, and diversity indices (Kennedy and Smith, 1995) using the Multi Variate Statistical Package.

The catabolic potentials of soil samples were investigated by measuring the short-term respiratory responses after the addition of the solutions of 36 organic substrates (Degens and Harris, 1997; Degens, 1998a, b; Degens *et al.*, 2000). These substrates were: two amines (glucosamine, glutamine), eight amino acids (alanine, arginine, asparagine, glutamic acid, histidine, lysine, serine, tyrosine), two aromatic chemicals (inosine, urocanic acid), four carbohydrates (galactose, glucose, lactose, mannose), eighteen carboxylic acids (acetic acid, ascorbic acid, citric acid, Na-citrate, Na-formate, fumaric acid, gluconic acid, α-ketobutyric acid, α-ketoglutaric acid, malic acid, malonic acid, oxalic acid, pantothenic acid, quinic acid, succinic acid, tartaric acid, uric acid, valeric acid) and two polymers (α-cyclodextrin, tween 80). The concentration of the solutions and the details of the method are outlined by Degens and

Vojvodic-Vukovic (1999) and Degens *et al.* (2000). Principal component analysis of the data was performed on the standardized catabolic response profiles (Degens, 1998a, b). The analysis of the variance (ANOVA) was also conducted on the PC1 and the PC2 scores (Degens and Vojvodic-Vukovic, 1999) and the treatments were separated on the basis of the least significant difference of means at 5% level of probability. In addition, richness, evenness, and diversity indices were calculated (Kennedy and Smith, 1995) using the Multi Variate Statistical Package.

3.3 RESULTS

Soil chemical properties are presented in Table 3.1. Soil properties varied greatly between land uses. Organic C ranged from 3.2% under maize CT to 10% under permanent kikuyu pasture. Soil acidification was evident under *Eucalyptus* and *Pinus* forests and under ryegrass pasture whilst the pH under maize and permanent pasture was greater than that under native grassland reflecting the regular lime applications that are made to these land uses. Extractable soil P was very low (5 mg L^{-1}) under natural grassland and was higher under all the other land uses; it was notably high under maize ZT and particularly permanent pasture. There was also a wide range of concentrations of exchangeable bases with exchangeable Ca being high in the maize ZT, *Pinus* forest and permanent pasture and exchangeable Mg and K being notably high under permanent pasture.

Table 3.1 Soil chemical characteristics at 0-5 cm depth under different land uses

Soil property	Treatments						
	Natural grassland	Maize CT	Maize ZT	Annual ryegrass	*Eucalyptus*	*Pinus*	Permanent pasture
Organic C (%)	5.8	3.2	3.9	4.3	6.2	7.1	10
K_2SO_4 Extractable C ($\mu g\ g^{-1}$ soil)	126	32	45	43	154	112	180
pH (KCl)	4.6	4.5	4.7	4.1	4.1	4.3	4.8
pH water	5.5	5.3	5.3	4.8	5.1	5.1	5.7
Exchangeable acidity (cmol/L)	0.61	0.4	0.23	2.23	1.93	0.7	0.15
Extractable P (mg/L)	5	12	42	24	6	19	124
Exchangeable K (mg/L)	205	128	143	203	177	195	1033
Exchangeable Ca (mg/L)	688	891	1324	635	408	1326	2018
Exchangeable Mg (mg/L)	320	175	252	157	197	310	515

Substrate richness, expressed as the number of different substrates in Eco plates that was metabolised by the microbial population inhabiting the different soil communities and the variation of the AWCD over time are presented in Figures 3.1 and 3.2. The evolution of the number of positive wells was highest for permanent pasture; followed by natural grassland, *Eucalyptus,* maize ZT and *Pinus.* Numbers were lowest for annual ryegrass and maize CT and amounted to approximately half of the number determined under permanent pasture (Figure 3.1). A similar trend was observed with the variation of the AWCD over time (Figure 3.2) except values for maize ZT was consistently greater than those for *Eucalyptus* and native grassland.

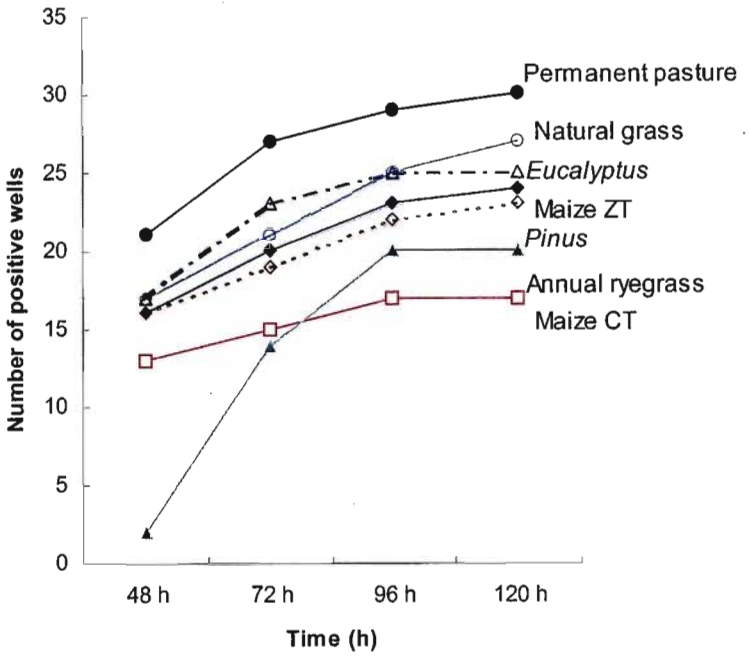


Figure 3.1 Evolution of the number of positive responses in the Biolog Eco plates after the inoculation with soil extracts of the different agricultural management.

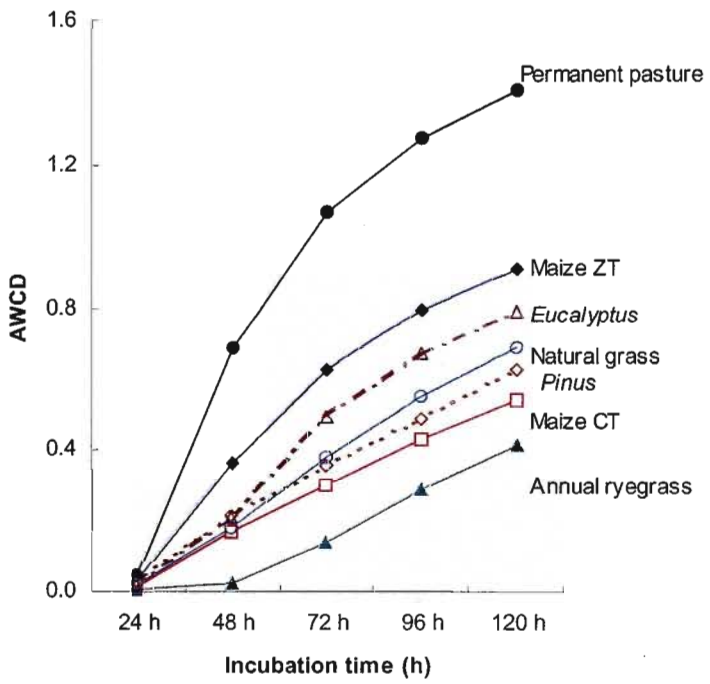


Figure 3.2 Variation in average well colour development (AWCD) over time in the Eco plates inoculated with soil extracts of the different management practices at 0-5 cm soil depth.

As the richness index has a temporal dimension that develops during the incubation (Zak *et al.*, 1994) the statistical analysis of the variation of the AWCD along the time, which expresses the catabolic capacity of the different microbial communities showed a very high difference in the increased AWCD along the time of the incubation ($P < 0.001$; See appendix F).

Confirmation of the differences in functional abilities of the soil microbial populations inhabiting the different treatments was demonstrated by their different positions in the plane of the first two principal components (Figure 3.3). A large difference in location was observed between permanent pasture, maize CT and annual ryegrass, whereas maize ZT, *Eucalyptus,* and natural grassland were closely located. The soil samples from the *Pinus* plantation showed a very large difference in their location. These differences were confirmed by the analysis of the variance of the PC scores ($P < 0.001$; See appendix G).

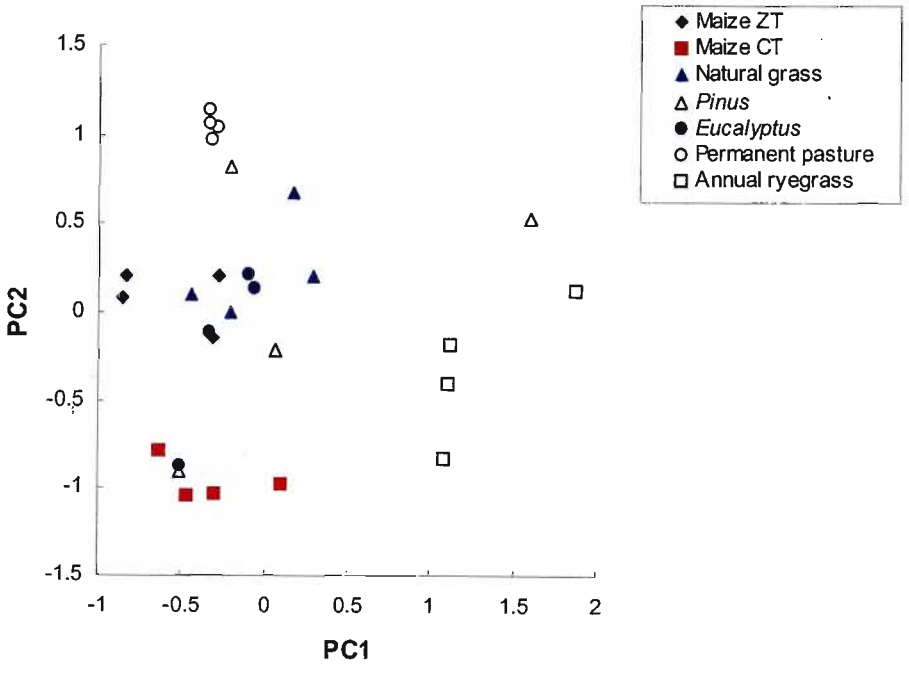


Figure 3.3 Plot showing the position of the different soils within PC1 and PC2 of a principal component analysis of the average well colour development at 120 h.

In addition, there were differences in the degree of utilization of substrates by the soil microorganisms in different managements as their first two principal component scores differed. The PC1 ranked between –1.16 and +1.15 whereas the PC2 ranked between –1.17 and 1.08 (Figure 3.4). Substrates most utilized by microorganisms under the different land uses are those found in the same position in the zones of the principal component spaces in Figures 3.3 and 3.4. For example, comparing the top of the two figures, it is evident that the microorganisms under permanent pasture utilized lactose, hydroxybutyric acid, α-cyclodextrin, glucose 1 phosphate and erythritol most effectively.

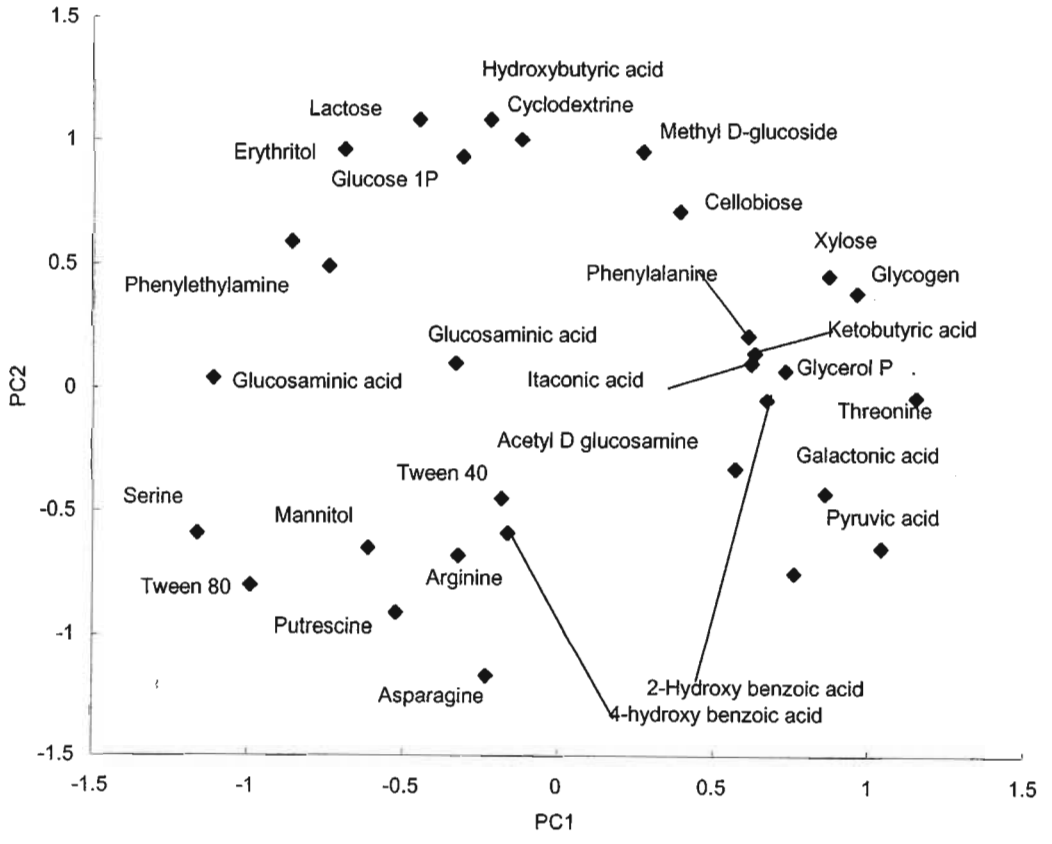


Figure 3.4 Plot of the position of 31 substrates within the plane of PC1 and PC2. Principal component analysis based on the transformed data of the average well colour development at 120 h of incubation.

The richness, evenness, Shannon and Simpson diversity indices for the Biolog Eco plates data are presented in Table 3.2. The greatest richness was observed under permanent pasture, whereas other treatments had lower and similar values. The species evenness was greater in permanent pasture, and intermediate for *Eucalyptus*, *Pinus* and natural grassland, and lower for annual ryegrass, maize CT and maize ZT. The Shannon and Simpson diversity indices followed the order: permanent pasture > natural grassland, *Pinus*, *Eucalyptus* and maize ZT > maize CT and annual ryegrass. The number of positive wells (Figure 3.1), the AWCD (Figure 3.2) and Shannon and Simpson diversity indices (Table 3.2) show the similar order.

Table 3.2 Richness, evenness and diversity indices for soil microbial populations from different agricultural management. Indices calculated using the transformed AWCD at 120h of incubation of Biolog Eco plates

Treatments	Richness	Evenness	Diversity	
			Shannon	Simpson
Natural grassland	29 a	0.94 b	1.37 b	0.95 b
Maize CT	29 a	0.88 a	1.29 a	0.94 a
Maize ZT	30 a	0.91 a	1.35 b	0.95 b
Annual ryegrass	29.3 a	0.87 a	1.28 a	0.93 a
Eucalyptus	29.8 a	0.92 b	1.35 b	0.95 b
Pinus	29 a	0.92 b	1.35 b	0.95 b
Permanent pasture	30.8 b	0.97 c	1.44 c	0.96 c

The letters following values indicate statistical differences. In each column, values followed by the same letter are not significantly different (LSD at P = 0.05).

Regression analysis (Table 3.3) suggested that the main factor separating Eco plate data in the PC1 axis was exchangeable acidity and in the PC2 axis it was unclear with organic C, extractable P and exchangeable Mg and K all seemingly important.

The substrate induced respiration responses of soils under different managements with various carbon source groups are presented in Figure 3.5. The largest SIR response was for carboxylic acids and lowest was for the amines and aromatic chemicals. For amino acids, aromatic chemicals, carbohydrates and polymers, the greatest CO_2 evolution was from permanent pasture. Conversely, for amino acids, amines, carbohydrates and polymers, the least CO_2 evolution was from maize under conventional tillage.

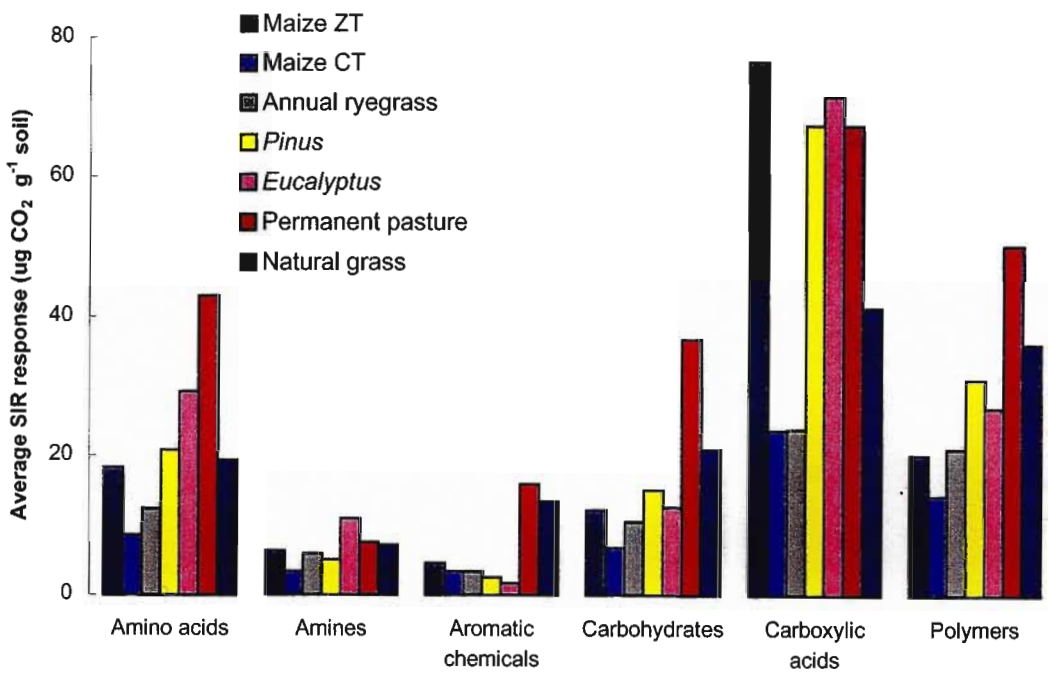


Figure 3.5 Average substrate induced respiration (SIR) responses of soils in different land-use types with the different carbon source groups.

The standardised substrate induced respiration responses were submitted to principal component analysis. The position of the different treatments within the PC1 and PC2 axes are shown in Figure 3.6 whereas the 36 substrates in the plane of the first two principal components, in relation to the rate at

which they were utilized by soil microorganisms under different management practices, are shown in Figure 3.7.

The values of the PC1 decreased from maize ZT to maize CT, annual ryegrass, natural grassland and permanent pasture. Natural grassland and annual ryegrass and *Eucalyptus* and *Pinus* respectively were similarly positioned on the PC1 and PC2 axes (Figure 3.6). In addition, the PC1 and PC2 scores of the different treatments were significantly different ($P < 0.001$; Appendix H).

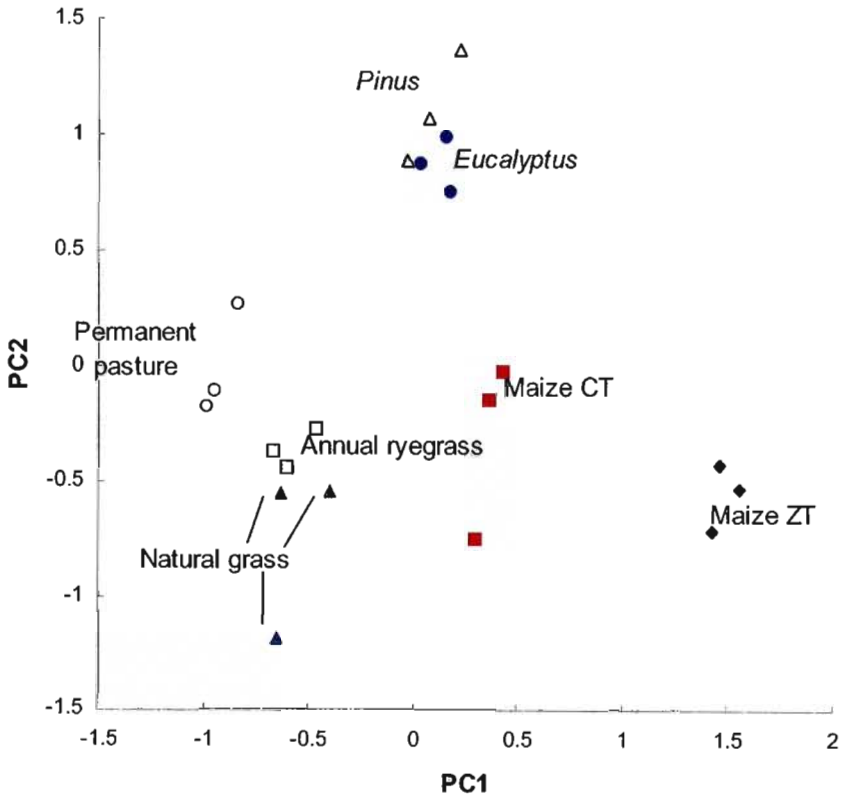


Figure 3.6 Plot of the position of the soils in the PC1 and PC2 axes. The principal component analysis based on the standardized substrate induced respiration responses of soils to 36 substrates.

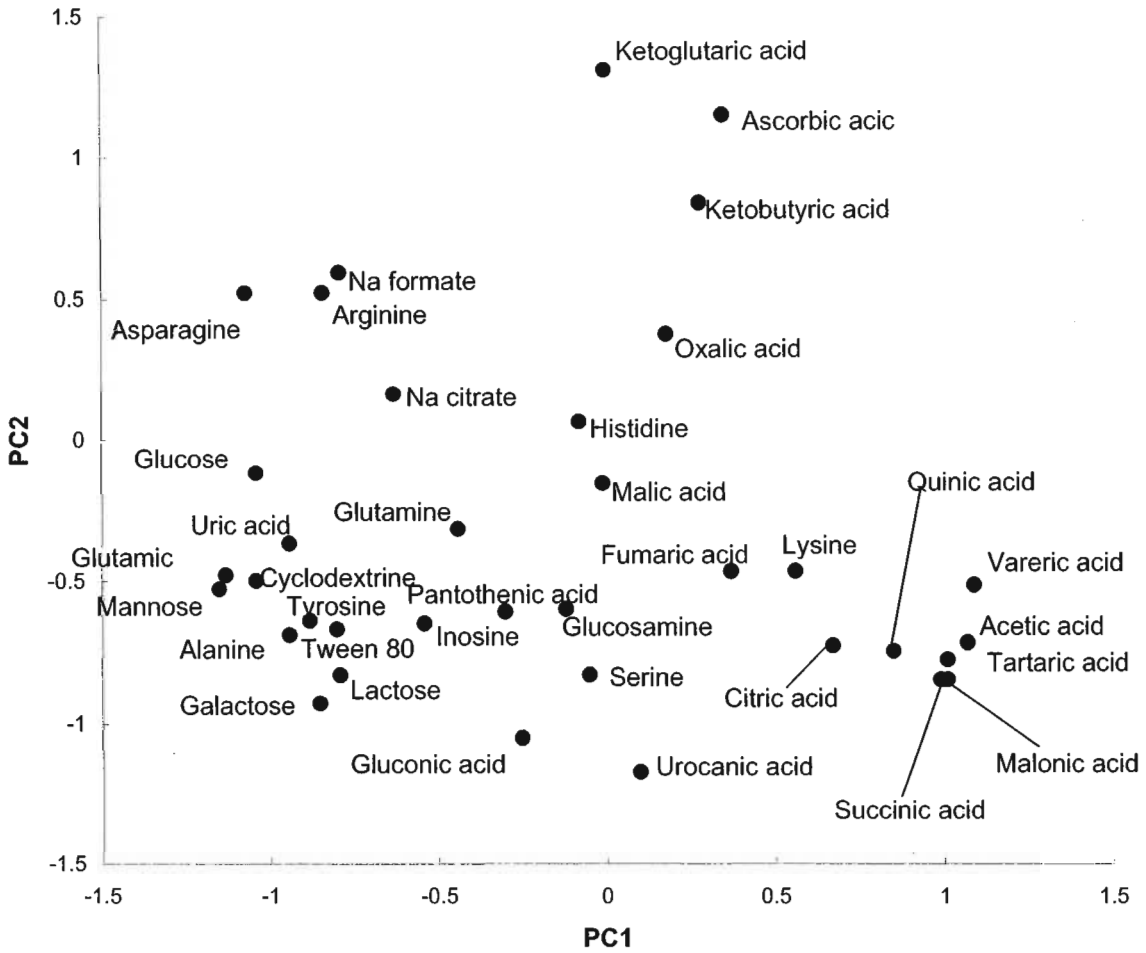


Figure 3.7 Plot showing the position of 36 substrates in the plane of the first two principle components. The principal component analysis is based on the standardized substrate induced respiration responses of soils to 36 substrates.

Results of regression analysis (Table 3.3) showed that none of the measured soil properties significantly explained a substantial proportion of the separation of catabolic response profile (CRPs) data in either the PC1 or PC2 axes.

Table 3.3: Relationship (r) between the PC1 and PC2 of the catabolic response profiles (CRPs) and ECO plate method and the soil chemical characteristics

Soil property	ECO		CRPs	
	PC1	PC2	PC1	PC2
Organic C	-0.18 NS	0.85**	-0.59 NS	0.41 NS
K_2SO_4- extractable C	-0.27 NS	0.74**	-0.54 NS	0.43 NS
pH (KCl)	-0.59 NS	0.54*	0.09 NS	-0.50 NS
pH water	-0.67 NS	0.62*	-0.16 NS	-0.29
Exchangeable acidity	0.75*	-0.33 NS	-0.24 NS	0.26
Extractable P	-0.14 NS	0.81*	-0.31 NS	-0.07
Exchangeable K	-0.12 NS	0.84**	-0.56 NS	0.01
Exchangeable Ca	-0.38 NS	0.69 NS	-0.06 NS	0.02
Exchangeable Mg	-0.36 NS	0.90**	-0.43 NS	0.03

Statistical significance shown: NS: non-significant, *P≤0.05, **P≤0.01

In addition, the catabolic response profiles were used to calculate richness, evenness and diversity indices (Table 3.4). The substrate richness is indicative of the number of substrates that were metabolised by different soil microbial populations using the 36 amendments. The treatments were grouped in two categories based on the substrate richness index. The greater richness was observed under maize ZT and natural grassland. The other treatments had lower richness indices and these values were not statistically different (P = 0.1).

The second component of the microbial diversity was species evenness, which indicates the distribution of the individuals within species designation (Kennedy and Smith, 1995). Species evenness indices followed the order: natural grassland > maize CT and maize ZT and permanent pasture > *Eucalyptus* > *Pinus* > annual ryegrass. The Shannon diversity index was highest for natural grassland, maize CT and maize ZT, annual ryegrass and permanent pasture, lowest for *Pinus* and intermediate for *Eucalyptus*. A similar order was obtained for the Simpson diversity index except natural grassland was significantly greater than the other land uses (Table 3.4).

Table 3.4 Richness, evenness and diversity indices of soils under the different agricultural management. Indices calculated using the transformed substrate induced respiratory responses of soils to 36 substrates

Treatments	Richness	Evenness	Diversity	
			Shannon	Simpson
Natural grassland	33.6 b	0.91 e	1.39 c	0.95 d
Maize CT	31 a	0.89 d	1.33 c	0.93 c
Maize ZT	35 b	0.87 d	1.35 c	0.93 c
Annual ryegrass	30.3 a	0.71 a	1.35 c	0.94 c
Eucalyptus	31.6 a	0.82 c	1.24 b	0.91 b
Pinus	29.6 a	0.77 b	1.13 a	0.87 a
Permanent pasture	32.6 a	0.87 d	1.33 c	0.93 c

The letters following values indicate statistical differences. In each column, values followed by the same Letters are not significantly different (LSD at P = 0.05).

3.4 DISCUSSION

The aim of this chapter was to assess the functional differences in soil microbial communities under different long-term management systems based on carbon source utilization patterns. Separation of samples along the principal component axes is related to the differences in the use of particular carbon sources (Garland and Mills, 1991). For Biolog Eco plates, the difference in the number of positive wells, variation in AWCD over time and the differences in positions in the planes of the principal components (Figures 3.1, 3.2 and 3.3) as well as the differences in diversity indices (Table 3.2) demonstrate that land management practices had indeed effected the functional diversity of the heterotrophic microorganisms in the soil.

Differences observed in Figure 3.3 may reflect differences between the grazed treatments (permanent pasture) and the tilled systems (maize CT and annual ryegrass). That is, they were located on the opposite extremes of PC2. Annual cultivation with a mouldboard plough may decrease the functional diversity of the microbial communities. Under annual cultivation there is a loss of soil organic matter and particularly that of readily metabolizable compounds (Degens *et al.*, 2000), inputs of organic residues to the soil are small and all from one plant species (maize). In addition, annual tillage has a disturbing and stressing effect on the microbial community. Thus, under these land uses the amount and diversity of organic materials present, and added to the soil is reduced and the consequence is a reduction in microbial diversity. Table 3.1 illustrates a lower concentration of nutrients in soil under maize CT, maize ZT and annual ryegrass compared to the amount of soil nutrients in permanent pasture.

Similarly, maize CT and annual ryegrass differed themselves because they were located on two extremes of the PC1. That difference may have occurred due to differences in irrigation practice. Annual

pastures are characteristically irrigated heavily over the spring-summer period in order to get rapid pasture establishment. Irrigation favours microorganisms that are resistant to anaerobic conditions in the waterlogged soils (Kang and Freeman, 1999). The remaining treatments (*Pinus*, *Eucalypus* and native grassland) were located in the middle of the PC2 axis, approximately equal to zero. That may be the result of a general lack of disturbance under these land uses. This is confirmed by the Shannon diversity indices (Table 3.2).

Because the Biolog method is a relatively new method for the investigation of the microbial functional diversity, especially for soil communities, the literature provides little comparable results. Nonetheless, Bending *et al.* (2000) showed that patterns of the substrate utilization by soil microbial communities were highly sensitive to management practice. Yan *et al.* (2000) also noted significantly larger substrate richness, and a higher rate and diversity of the substrate use in the uncultivated than in the cultivated sites. Yao *et al.* (2000) observed that multivariate analysis of the sole carbon source utilization patterns demonstrated that land use history and plant cover type had a significant impact on microbial community structure. The results in this study confirm their statements.

Because the Biolog Eco plate's method (Choi and Dobbs, 1999) failed to discriminate differences between the microbial functional diversity in some treatments such as *Eucalyptus*, *Pinus*, natural grassland and maize ZT (Figure 3.3 and Table 3.2), another method was also used. Moreover, the Biolog technique does not assess the functional diversity of the whole soil microbial community (Degens and Harris, 1997). It only assesses the diversity of culturable bacteria. Konopka *et al.* (1998) noted that the formation of the colour may represent the metabolism of the numerically dominant bacterial species, or could be a consequence of the rapid growth by a minor member of the community. Therefore, the method of catabolic response profiles (Degens, 1998a, b) was also used in order to circumvent the

problems of unculturability of the soil microorganisms when they are submitted to the artificial environment.

The results obtained using Degens's method (1998a, b) revealed its effectiveness in distinguishing the functional diversity of the microbial community in soils under different land-use types. That is because different treatments were found in separate zones of the principal component spaces in Figure 3.6. Because the influence of soil type was eliminated by collecting soils from the same geographic location, differences are the result of the management history.

In the previous chapter, management practices affected soil microbial characteristics in two directions: (1) annual cultivation decreased soil organic C and microbial biomass C contents, due to the increased oxidation of the organic residues on which the microorganisms would find their substrates; (2) the increase of organic inputs under the permanent pasture and the other less disturbed treatments which raised up the microbial biomass and activity. This relationship between soil organic C and soil microbial population conducted to the assessment of weather they may influence the microbial functional diversity in the soils. Therefore, the linear regression test was used to determine the main sources of the variation of the PC1 and PC2 of catabolic response profiles (CRPs) and substrate utilization in ECO plates within other soil chemical characteristics. The analysis revealed the results presented in Table 3.3

The variation in the PC1 and the PC2 was correlated to the variation in the soil organic C, exchangeable acidity, extractable P, exchangeable K, Ca, and Mg (Table 3.3). That happened because soil organic matter is a source of nutrients for soil microorganisms. This relates to the findings of Degens *et al.* (2000) in which a positive correlation ($r^2 = 0.76$) was found between catabolic diversity and soil organic C in which pastoral soil was compared with soil under cropping and pine forestry. These authors concluded

that land management that cause proportional decrease of organic C could also cause the same proportional losses of microbial catabolic diversity.

The richness, evenness, Shannon, and Simpson diversity indices (Table 3.4) provide more explanation of the effects of management practice on functional diversity of soil microbial populations. Because the native grassland was considered as a control, the richness index decreased in all treatments where annual cultivation, tree plantation and pastoral activities were applied except the increased richness in maize ZT. This finding suggests, for example, the impact of tillage and soil disturbance on the reduction of the microbial diversity (Bendig *et al.*, 2000 citing Lupwayi *et al.*, 1998). Liu *et al.* (2000) and Yan *et al.* (2000) also noted a decrease in soil microbial diversity following soil disturbances.

The fact that the substrate richness indices in different treatments were broadly similar (Tables 3.2 and 3.4) may be partly explained by metabolic redundancy in soil microbial communities. That is, a large change in species diversity could result a very small change in functional diversity because many species possess the genetic potential to metabolise different substrates (Konopka *et al.*, 1998).

Management practices also affected soil microbial diversity as measured by the Shannon and Simpson diversity indices (Table 3.4), which decreased from the native grassland control to the other managed systems especially the *Eucalyptus* and the *Pinus* plantations. The soils in these two treatments were highly acidic (Table 3.1), which may favour the acid tolerant microbial groups, such as fungi (Metting, 1993; Thorn, 1997). This was demonstrated by the negative correlation ($r = -0.59$ and -0.67) between PC1 in Eco plate technique and pH and between PC2 of CRPs and pH ($r = -0.50$) [Table 3.3]. The fact that Shannon and Simpson indices were similar under maize CT, maize ZT, annual ryegrass and permanent pasture may be related to the explanation of Pankhurst (1997) citing Wardle (1995) that there

is a minimal effect of tillage on species diversity within soil microbial communities. Conversely, the increased substrate richness in maize ZT may be explained by its highest metabolic activity (Figure 2.4b), which could be partly due to the application of pesticides and herbicides to that treatment (Wardle, 1992; Pankhurst, 1997 citing Wardle, 1995). For example, Roper and Gupta (1995) noted a negative impact of herbicides and pesticides on soil microbial population.

3.5 CONCLUSIONS

The objective of this chapter was to evaluate the effects of agricultural management on soil microbial diversity. The differences in the position of the treatments on the plots of the principle component scores (Figure 3.3 & 3.6) and the diversity indices (Table 3.2 and 3.4) demonstrate differences in functional capacity of the different microbial populations which were a result of management history. The different variables of cropping history that may influence the microbial diversity include the application of tillage, fertiliser, insecticides, herbicides and irrigation. Differences in soil organic matter content and in the amounts of organic residues returned to the soil will also be important. This was confirmed by Degens *et al.* (2000) that management practices affected the dynamics of soil microbial diversity principally through a decrease in soil organic matter and microbial biomass C content in cropped soils. In addition, because each site had its own vegetation, the phytodiversity can lead to the differences in the substrate quality.

Changes in microbial functional diversity in response to land use are likely to be the result of the complex interaction of the above factors. Changes in soil conditions may eliminate existing microbial niches but also create new ones and dormant species will be able to take advantage of the new niches created or the newly active populations will be able to increase and use the niche to its full capacity in a relatively short period of time (Yan *et al.*, 2000). For this reason, microbial diversity may not necessarily

be associated directly with the size of the microbial biomass, basal respiration or organic C contents (Degens and Vojvodic-Vukovic, 1999).

CHAPTER 4

GENERAL CONCLUSIONS

In the present study the specific objective was to determine whether long-term management practices have contributed to changes in soil organic matter content, soil microbial biomass, activity and diversity. In the previous studies (e.g., Carter, 1991; Lovell *et al.*, 1995; Bayer *et al.*, 2000; Haynes, 2000) soil organic C and microbial biomass contents were major indicators of changes due to management practices and that was confirmed by the present results.

Management practices that involved annual cultivation (e.g. maize under conventional tillage) were found to decrease the soil organic C due to a reduction in the quantity of residues returned to soil and an increased rate of organic matter decomposition. Zero tillage leaves organic residues on the soil surface after harvesting and the organic C is preserved from the rapid mineralization. As a result, organic matter content and nutrient availability were higher under maize ZT than maize CT (Table 3.1). Therefore, zero tillage may be preferred for the annual arable cultivation because it conserves the organic matter, which is a source of nutrients for soil microorganisms and which contribute to soil structure and soil fertility.

The organic C contents are maintained or increased in soils under forestry, but soil acidification can occur. This could limit microbial growth. Organic matter content was very high under permanent pasture and this is attributable to the return of the ingested plant material by grazing animals in the form of dung and the turnover of the large, ramified grass root system. The use of pastoral systems in arable rotations

could be promoted in order to stabilise the soil organic C, N and soil pH and consequently, promote the size and activity of the microbial community in the soil.

The size of the soil microbial biomass is greatly influenced by soil organic C content because the soil organic matter is the substrate and a nutrient source for heterotrophic microorganisms in the soil. The microbial biomass C was largest in soil under permanent pasture because that environment was found to be less oxidative (low metabolic quotient) and favoured the microbial proliferation. In general, soils under permanent vegetation were rich in microbial biomass C because of lack of tillage and the inputs of substantial amounts of above – and/or below - ground litter. On the other hand, soils under annual cultivation were associated with low microbial biomass C because microorganisms live under stress and utilize the limited supply of organic C for their maintenance (high metabolic quotient). Under such fields the low microbial biomass is probably principally the result of the low rate of the microbial reproduction.

The active microbial biomass was determined through the basal respiration rate and enzyme activity levels (dehydrogenase, FDA hydrolysis, arginine ammonification rate and arylsulphatase). These measurements reflected the physiological status of the microbial population under different managements. In general, microbial activity was, as expected, strongly related to the size of the microbial community.

In chapter 3, two approaches were used to assess the role of management on the microbial diversity in soils. Neither method separated land uses completely but separation was generally more complete for the substrate induced respiration method than for the Biolog Eco plate technique. The measured functional diversity indices (PC1 and PC2) were in positive correlation with soil properties namely soil organic C, exchangeable acidity, extractable P, exchangeable K and Mg (Table 3.3). The effects of

management practices were more obvious when the land uses were considered as either cultivated or uncultivated. The effects of management on soil microbial functional diversity were also demonstrated through the richness, evenness, and diversity indices (Tables 3.2 and 3.4). These indices showed that any agricultural management decreases the soil microbial diversity.

REFERENCES

Acosta-Martinez, V. and Tabatabai, M.A. 2000. Enzyme activities in a limed agricultural soil. *Biology and Fertility of Soils* 31, 85-91.

Acosta-Martinez, V. and Tabatabai, M.A. 2001. Tillage and residue management effects on arylamidase activity in soils. *Biology and Fertility of Soils* 34, 21-24.

Alef, K. 1995a. Dehydrogenase activity. In: Alef, K. and Nannipieri, P. (eds.). Methods in applied soil microbiology and biochemistry. Academic Press, London, pp. 228-231.

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

Alef, K. 1995c. Estimation of the hydrolysis of fluorescein diacetate. In: Alef, K. and Nannipieri, P. (eds.) Methods in applied soil microbiology and biochemistry. Academic Press, London, pp. 232-238.

Alef, K. and Kleiner, D. 1986. Arginine ammonification, a simple method to estimate microbial activity potentials in soils. *Soil Biology and Biochemistry* 18, 233-235.

Alef, K. and Kleiner, D. 1987. Applicability of arginine ammonification as indicator of microbial activity in different soils. *Biology and Fertility of Soils* 5, 148-151.

Alef, K. and Kleiner, D. 1995. Arginine ammonification. In: Alef, K. and Nannipieri, P. (eds.). Methods in applied soil microbiology and biochemistry. Academic Press, London, pp. 238-240.

Alef, K. and Nannipieri, P. 1995. Enzyme activities. In: Alef, K. and Nannipieri, P. (eds.). Methods in applied soil microbiology and biochemistry. Academic Press, London, pp. 311-373.

Alexander, M. (1977). Introduction to soil microbiology. John Wiley, New York.

Alvarez, C.R., Alvarez, M.S., Grigera, M.S. and Lavado, R.S. 1998. Associations between organic matter fractions and the active soil microbial biomass. *Soil Biology and Biochemistry* 30, 767-773.

Anderson, J.M. 1988. The role of soil fauna in agricultural systems. In: Wilson, J.R. (ed.). Advances in nitrogen cycling in agricultural ecosystems. Proceedings of the symposium on advances in nitrogen cycle in agricultural ecosystems, Brisbane Australia 11-15 May 1987. CAB International, pp. 89-112.

Anderson, J.P.E. and Domsch, K.H. 1975. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. *Canadian Journal of Microbiology* 21, 314-322.

Anderson, J.P.E. 1982. Soil respiration. In: Page, A.L. and Miller, R.H. (eds.). Methods of soil analysis, part 2. Chemical and microbiological properties. Soil Science Society of America, Madison, Wis, pp. 831-871.

Anderson, T.H. and Domsch, K.H. 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. *Soil Biology and Biochemistry* 21, 471-479.

Anderson, T.H. and Domsch, K.H. 1990. Application of eco-physiological quotients on microbial biomasses from soils of different cropping stories. *Soil Biology and Biochemistry* 22, 251-255.

Anderson T. H. and Domsch K. H. 1993. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of the forest soils. *Soil Biology and Biochemistry* 25, 393-395.

Arshad, M.A. 1999. Tillage and soil quality: Tillage practices for sustainable agriculture and environmental quality in different agroecosystems. *Soil and Tillage Research* 53, 1-2.

Bakken, L.R. 1997. Culturable and nonculturable bacteria in soil. In: Elsas, J.D., Trevors, J.T. and Wellington, E.M.H. (eds.). Modern soil microbiology. Marcel Dekker, New York, pp. 47-61.

Bandick, A.K. and Dick, R.P. 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochemistry* 31, 1471-1479.

Bardgett, R.D. and Griffiths, B.S. 1997. Ecology and biology of soil protozoa, nematodes and microarthropods. In: Elsas, J.D., Trevors, J.T. and Wellington, E.M.H. (eds.). Modern soil microbiology. Marcel Dekker, New York, pp. 129-163.

Bardgett, R.D., Hobbs, P.G. and Frostegard, A. 1996. Changes in soil fungal: bacteria biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils* 22, 261-264.

Bayer, C., Neto, L.M., Mielniczuk, J. and Ceretta, C.A. 2000. Effect of no-till cropping systems on soil organic matter in sandy clay loam acrisol from Southern Brazil monitored by electron spin resonance and nuclear magnetic resonance. *Soil and Tillage Research* 53, 95-104.

Beare, M.H., Neely, C.L., Coleman, D.C. and Hargrove, W.L. 1990. A substrate induced respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues. *Soil Biology and Biochemistry* 22, 585-594.

Beare, M.H., Coleman, D.C., Crossley, D.A., Hendrix, P.F. and Odum, E.P. 1995. A hierarchical approach to evaluating the significance of soil biodiversity to biochemical cycling. *Plant Soil* 170, 5-22.

Beauchamp, E.G. and Hume, D.J. 1997. Agricultural soil manipulation: the use of bacteria, manuring and plowing. In: Elsas, J.D., Trevors, J.T. and Wellington, E.M.H. (eds.). Modern soil microbiology. Marcel Dekker, New York, pp. 643-664.

Bending, G.D., Putland, C. and Rayns, F. 2000. Change in microbial community metabolism and labile organic matter fractions as early indicators of the impact of management on soil biological quality. *Biology and Fertility of Soils* 31, 78-84.

Bergstrom, D.W., Monreal, C.M. and King, D.J. 1998. Sensitivity of soil enzymes activities to conservation practices. *Soil Science Society of America Journal 62*, 1286-1295.

Beukes, D.J. 1995. Soil acidity in agriculture. ARC.LNR, p.11.

Beyer, L., Wachendorf, C., Elsner, D.C. and Knabe, R. 1993. Suitability of dehydrogenase activity assay as an index of soil biological activity. *Biology and Fertility of Soils* 16, 52-56.

Bloem, J., Bolhuis, P.R., Veninga, M.R. and Weiringa, J. 1995. Microscopic methods for counting bacteria and fungi in soil. In: Alef, K. and Nannipieri, P. (eds.). Methods in applied soil microbiology and biochemistry. Academic Press, London, pp. 162-173.

Bonde, T.A., Nielsen, T.H., Miller, M. and Sorensen, J. 2001. Arginine ammonification assay as a rapid index of gross N mineralization in agricultural soils. *Biology and Fertility of Soils* 34, 179-184.

Bristow, A.W. and Jarvis, S.C. 1991. Effects of grazing and nitrogen fertilizer on the soil microbial biomass under permanent pasture. *Journal of Science Food and Agriculture* 54, 9-21.

Bruce, J., Frome, M., Haites, E., Janzen, H., Lal, R. and Paustian, K. 1999. Carbon sequestration in soils. *Journal of Soil and Water Conservation* 54, 382-389.

Campbell, C. A., Biederbeck, V.O., Zentner, R.P. and Lafond, G.P. 1991. Effect of crop rotations and cultural practices on soil organic matter, microbial biomass and respiration in a thin Black Chernozem. *Canadian Journal of Soil Science* 71, 363-376.

Camper, N.D., Moherek, E.A. and Huffman, J. 1973. Changes in microbial populations in paraquat-treated soil. *Weed Research* 13, 231-233.

Carter, M.R. 1991. The influence of tillage on the proportion of organic carbon and nitrogen in the microbial biomass of medium-textured soils in a humid climate. *Biology and Fertility of Soils* 11, 135-139.

Carter, M.R., Gregorich, E.G., Angers, D.A., Beare, M.H., Sparling, G.P., Wardle, D.A. and Voroney, R.P. 1999. Interpretation of microbial biomass measurements for soil quality assessment in humid temperate regions. *Canadian Journal of Soil Science* 79, 507-520.

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.

Crovetto, C.C. 1998. No-till development in Chequen farm and its influence on some physical, chemical and biological parameters. *Soil and Water Conservation* 53, 194-199.

Dalal, R.C. 1998. Soil microbial biomass-what do the numbers really mean? *Australian Journal of Experimental Agriculture* 38, 649-665.

Degens, B.P. 1998a. Microbial functional diversity can be influenced by the addition of simple organic substrates to soil. *Soil Biology and Biochemistry* 30, 1981-1988.

Degens, B.P. 1998b. Decreases in microbial functional diversity do not result in corresponding changes in decomposition under different moisture conditions. *Soil Biology and Biochemistry* 30, 1989-2000.

Degens, B.P. and Harris, J.A. 1997. Development of physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biology and Biochemistry* 29, 1309-1320.

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.

Degens, B.P. and Vojvodic-Vucovic, M. 1999. A sampling strategy to assess the effects of land use on microbial functional diversity in soils. *Australian Journal of Soil Research* 37, 593-601.

Deng, S.P. and Tabatabai, M.A. 1997. Effect of tillage and residue management on enzyme activities in soil: III. Phosphatases and arylsulfatase. *Biology and Fertility of Soils* 24, 141-146.

Dick, W.A. 1984. Influence of long-term tillage and crop rotation combinations on soil enzyme activities. *Soil Science Society of America Journal* 48, 569-574.

Dick, R.P. 1997. Soil enzyme activities as integrative indicators of soil health. In: Pankhurst, C.E., Doube, B.M. and Gupta, V.V.S.R. (eds.). Biological indicators of soil health. CAB International, New York, pp. 121-149.

Dick, W.A. and Tabatabai, M.A. 1993. Significance and potential uses of soil enzymes. In: Metting, F. B. (ed.). Soil microbial ecology, application in agricultural and environmental management. Marcel Dekker, New York, pp. 95-127.

Dilly, O. and Munch, J. C. 1998. Ratio between estimates of microbial biomass contents and microbial activity in soils. *Biology and Fertility of Soils* 27, 374-379.

Dilly, O. and Kutsch, W.L. 2002. Rationale for aggregating microbiological information for soil and ecosystem research. [WWW document]. URL http:// www.pz-oekosys.uni-kiel.de/~olovier/paper/roma98pi.doc/ (accessed 2002, March 28).

Doran, J.W. 1980. Soil microbial and biochemical changes associated with reduced tillage. *Soil Science Society of America Journal* 44, 765-771.

Doran, J.W. and Parkin, T.B. 1994. Defining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezidicek, D.F. and Stewart, B.A. (eds.). Defining soil quality for a sustainable environment. *Soil Science Society of America Journal,* Publication No. 35, Madison, Wis., pp. 3-21.

Doran, J.W., Sarrantonio, M. and Liebig, M.A. 1996. Soil health and sustainability. *Advances in Agronomy* 56, 1-54.

Duiker, S.W. and Lal, R. 1999. Crop residue and tillage effects on carbon sequestration in Luvisol in central Ohio. *Soil and Tillage Research* 52, 73-81.

Du Toit, B. 1993. Soil acidification under plantations and the determination of the acid neutralizing capacity of soils. Thesis, University of Natal. p. 90.

Edwards, C. A. 1989. Impact of herbicides on soil microcosms. *Critical Review in Plant Science* 8, 221-253.

Etana, A., Hakanson, 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.

Fauci, M.F. and Dick, R.P. 1994. Microbial biomass as an indicator of soil quality: effect of long-term management and recent soil amendments. In: Doran, J.W., Coleman, D.C., Bezidicek, D.F. and Stewart, B.A. (eds.). Defining soil quality for a sustainable environment. *Soil Science Society of America Journal*, Publication No. 35, Madison, Wis., pp. 229-234.

Forster, J.C. 1995. Soil nitrogen. In: Alef, K. and Nannipieri, P. (eds.). Methods in applied soil microbiology and biochemistry. Academic Press, London, pp. 79-87.

Focht, D.D. and Verstraete, W. 1977. Biochemical ecology of nitrification and denitrification. *Advances in Microbial Ecology* 1, 135-214.

Frankenberger, W.T. and Dick, W.A. 1983. Relationships between enzyme activities and microbial growth and activity indices in soil. *Soil Science Society of America Journal* 47, 945-951.

Franzluebbers, A.J. 1999. Introduction to symposium microbial biomass: measurement and role in soil quality. *Canadian Journal of Soil Science* 79, 505-506.

Franzluebbers, A.J. and Arshad, M.A. 1997. Soil microbial biomass and mineralizable carbon of water-stable aggregates. *Soil Science Society of America Journal* 61, 1090-1097.

Franzluebbers, A.J., Haney, R.L., Hons, F.M. and Zuberer, D.A. 1999. Assessing biological soil quality with chloroform fumigation-incubation: why subtract a control? *Canadian Journal of Soil Science* 79, 521-528.

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 Environmental Microbiology* 57, 2351-2359.

Gewin, V.L., Kennedy, A.C. and Miller, B.C. 1999. Soil quality changes in eastern Washington with conservation reserve program (CRP) take-out. *Journal of Soil and Water Conservation* 54, 432-438.

Grace, P.R., Ladd, J.N. and Skjemstad, J.O. 1994. The effect of management practices on soil organic matter dynamics. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R. and Grace, P.R. (eds.). Soil biota. Management in sustainable farming systems. CSIRO, Melbourne, pp. 162-171

Gupta, V.V.S.R., Roper, M.M., Kirkegaard, J.A. and Angus, J. F. 1994. Changes in microbial biomass and organic matter levels during the first year of modified tillage and stubble management practices on a red earth. *Australian Journal of Soil Research* 32, 1339-1354.

Haines, P. J. and Uren, N. C. 1990. Effects of conservation tillage farming on soil microbial biomass, organic matter and earthworm populations, in north-eastern Victoria. *Australian Journal of Experimental Agriculture* 30, 365-371.

Harrington, L. and Grace, P. 1997. Research on soil fertility in South Africa: Ten awkward questions, NRG, Mexico.

Haynes R.J. 1999. Size and activity of the soil microbial biomass under grass and arable management. *Biology and Fertility of Soils* 30, 210-216.

Haynes, R.J. 2000. Labile organic matter as an indicator of organic matter quality in arable and pastoral soils in New Zealand. *Soil Biology and Biochemistry* 32, 211-219.

Haynes, R.J. and Beare, M.H. 1996. Aggregation and organic matter storage in meso-thermal, humid soil. In: Carter MR, Stewart BA (eds.). Advances in soil science. Structure and organic matter storage in agricultural soils. CRC Lewis, Boca Raton, pp. 213-262.

Haynes, R.J. and Knight, T.L. 1989. Comparison of soil chemical properties, enzyme activities, levels of biomass N and aggregate stability in the soil profile under conventional and no-tillage in Canterbury, New Zealand. *Soil and Tillage Research* 14, 197-208.

Haynes, R.J. and Tregurtha, R. 1999. Effects of increasing periods under intensive arable vegetable production on biological, chemical and physical indices of soil quality. *Biology and Fertility of Soils* 28, 259-266.

Haynes, R.J. and Williams P.H. 1999. Influence of stock camping behaviour on the soil microbiological and biochemical properties of grazed pastoral soils. *Biology and Fertility of Soils* 28, 253-258.

Haynes, R.J., Swift, R.S. and Stephen, R.C. 1991. Influence of mixed cropping rotations (pasture-arable) on organic matter content, water stable aggregation and clod porosity in a group of soils. *Soil and Tillage Research* 19, 77-87.

Heinemeyer, O., Insam, H., Kaiser, E.A. and Walenzik, G. 1989. Soil microbial biomass and respiration measurement: an automated technique based on infrared analysis. *Plant Soil* 116, 191-195.

Hornick, S.B. 1992. Factors affecting the nutritional quality of crops. *American Journal of Alternative Agriculture* 7, 63-68.

Hurst, C.J. 1997. Sampling viruses from soil. In: Hurst, C.J., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D. and Walter, M.V. (eds.). Manual of environmental microbiology. ASM Press, Washington, D.C, pp. 400-405.

Islam, K.R. and Weil, R.R. 2000. Soil quality indicator properties in mid-Atlantic soils as influenced by conservation management. *Journal of Soil and Water Conservation* 55, 69-78.

Joergenson, R.G. 1995. The fumigation extraction method. In: Alef, K. and Nannipierri, P. (eds.). Methods in applied soil microbiology and biochemistry. Academic Press, London, pp. 383-387.

Johnston, A.E. 1986. Soil organic matter, effects on soils and crops. *Soil Use Manage.* 2, 97-115.

Kahn, A.G. 1975. The effect of vesicular arbuscular mycorrhizal associations on growth of cereals. II. Effects on wheat growth. *Annual Applied Biology* 80, 27-36.

Kandeler, E. and Murer, E. 1993. Aggregate stability and soil microbial processes in a soil with different cultivation. *Geoderma* 56, 503-513.

Kang, H. and Freeman, C. 1999. Phosphatase and arylsulphatase activities in wetland soils: annual variation and controlling factors. *Soil Biology and Biochemistry* 31, 449-454.

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.

Karlen, D.L., Rosek, M.J., Gardner, J.C., Allan, D.L., Alms, M.J., Bezdicek, D.F., Flock, M., Huggins, D.R., Miller, B.S. and Staben, M.L. 1999. Conservation reserve program effects on soil quality indicators. *Journal of Soil and Water Conservation* 54, 439-444.

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

Kennedy, A.C. and Smith, K.L. 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil* 170, 75-86.

Killham, K. and Foster, R. 1994. Soil ecology. Cambridge University Press, Great Britain. p. 242.

Kirchner, M.J., Wollum, A.G. and King, L.D. 1993. Soil microbial population and activities in reduced chemical input agroecosystems. *Soil Science Society of America Journal* 57, 1289-1295.

Klein, D. A., Sorensen, D.L., and Redente, E. F. 1985. Soil enzymes: a predictor of reclamation potential and progress. In: Tate, R. L. and Klein, D. A. (eds.). Soil reclamation processes. Marcel Dekker, New York, pp. 141-171.

Knoepp, J.D., Coleman, D.C., Crossley, D.A., Clark, J.S. 2000. Biological indices of soil quality: an ecosystem case study of their use. *Forest Ecology and Management* 138, 357-368.

Konopka, A.L., Olivier, L., Turco, R.F. 1998. The use of carbon substrate utilization patterns in environmental and ecological microbiology. *Microbial Ecology* 35,103-115.

Kramer, S., and Green, D.M. 2000. Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in a semiarid woodland. *Soil Biology and Biochemistry* 32, 179-188.

Kulinska, D., Camargo, V.L.L. and Drozdowicz, A. 1982. Enzymes activities in 'Cerrado' soil in Brazil. *Pedobiologia* 24, 101-107.

Lawrance, K.F., Prinsloo, M.A. and Berry, W.A.J. 1999. Long and short term effects of tillage systems on grain yield, the incidence of diseases and production costs for maize. *South African Journal of Plant Soil* 16, 85-91.

Lawrence, J. R., Gupta, V. V. S. R. and Germida, J. J. 1988. Impact of elemental sulphur fertilization on agricultural soil. II. Effects on sulphur oxidizing populations and oxidation rates. *Canadian Journal of Soil Science* 68, 475-483.

Liu, X., Lindemann, W.C., Whitford, W.G. and Steiner, R.L. 2000. Microbial diversity and activity of disturbed soil in the northern Chihuahuan desert. *Biology and Fertility of Soils* 32, 243-249.

Lovell, R.D., Jarvis, S.C. and Bardgett, R.D. 1995. Soil microbial biomass and activity in long-term grassland: Effects of management changes. *Soil Biology and Biochemistry* 27, 969-975.

Marilley, L., Vogt, G., Blanc, M. and Aragno, M. 1998. Bacterial diversity in bulk soil and rhizosphere fractions of *Lolium perenne* and *Trifolium repens* as revealed by PCR restriction analysis of 16S rDNA. *Plant Soil* 198, 219-224.

Martens, R. 1995. Current methods for measuring microbial biomass C in soil: potentials and limitations. *Biology and Fertility of Soils* 19, 87-99.

Martinez, V.A. and Tabatabai, M.A. 2000. Enzyme activity in a limed agricultural soil. *Biology and Fertility of Soils* 31, 85-91.

McCallister and Chien, W.L. 2000. Organic carbon quantity and forms as influenced by tillage and cropping sequence. *Communication in Soil and Plant Analysis* 31, 465-479.

Metting, F.B. 1993. Structure and physiological ecology of soil microbial communities. In: Metting, F. B. (ed.). Soil microbial ecology, application in agricultural and environmental management. Marcel Dekker, New York, pp. 3-25.

Nay, S.M., Mattson, K.G. and Bormann, B.T. 1994. Biases of chamber methods for measuring soil CO_2 efflux demonstrated with laboratory apparatus. *Ecology* 75, 2460-2463.

Neely, C.L., Beare, M., Hargrove, W.L. and Coleman, D. C. 1991. Relationships between fungal and bacterial substrate induced respiration, biomass and plant residue decomposition. *Soil Biology and Biochemistry* 23, 947-954.

Nelson, D.W. and Sommers, L.E. 1982. Total carbon, organic carbon and organic matter. In: Page, A.L., and Miller, R.H. (eds.). Methods of soil analysis, part 2. Chemical and microbiological properties. *Soil Science Society of America Journal*, Madison, Wis. pp. 539-579.

Ogram, A. and Feng, X. 1997. Methods of soil microbial community analysis. In: Hurst, C.J., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D. and Walter, M.V. (eds.). Manual of environmental microbiology. ASM Press, Washington, D.C, pp. 422-430

Ones, O.R., Allen, R.R, and Unger, P.W. 1990. Tillage systems and equipment for dryland farming. *Advances in Soil Science* 13, 89-130.

Pankhurst, C.E. 1997. Biodiversity of soil organisms as an indicator of soil health. In: Pankhurst, C.E., Doube, B.M., and Gupta, V.V.S.R. (eds.). Biological indicators of soil health. Biddles, London, UK. pp. 297-324.

Pankhurst, C.E., Doube, B.M. and Gupta, V.V.S.R., 1997. Biological indicators of soil health: Synthesis. In: Pankhurst, C.E., Doube, B.M. and Gupta, V.V.S.R. (eds.). Biological indicators of soil health. Biddles, London, UK, pp. 418-435.

Papendick, R.I., Parr, J.F. and Meyer, R.E. 1990. Managing crop residues to optimize crop/livestock production systems for dryland agriculture. *Advances in Soil Science* 13, 253-272.

Papper, I.L., Gebra, C.P. and Brendecke, J.W. 1995. Dehydrogenase activity. In: Environmental Microbiology, a Laboratory Manual. Academic Press, San Diego, pp. 51-56.

Paul, E.A. and Clark, F.E. 1989. Soil microbiology and biochemistry. Academic Press, New York, p. 273.

Perucci, P., Bonciarelli, U., Santilocchi, R. and Bianchi, A.A. 1997. Effect of rotation, nitrogen fertilization and management of crop residues on some chemical, microbiological and biochemical properties of soil. *Biology and Fertility of Soils* 24, 311-316.

Phillips, D.H., Foss, J.E., Buckner, E.R., Evans, R.M. and FitzPatrick. 2000. Response of surface horizons in an Oak Forest to prescribed burning. *Soil Science Society of America Journal* 64, 754-760.

Piao, H.C., Liu, G.S., Wu, Y.Y. and Xu, W.B. 2001. Relationship of soil microbial biomass carbon and organic carbon with environmental parameters in mountainous soils of southwest China. *Biology and Fertility of Soils* 33, 347-350.

Powlson, D.S., Brookes, P.C. and Christensen, B.T. 1987. Measurement of microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biology and Biochemistry* 19, 159-164.

Prescott, L.M., Harley, J.P. and Klein, D.A. 1999. Microbiology. 4th ed., McGraw-Hill, Boston.

Priha, O. and Smolander, A. 1997. Microbial biomass and activity in soil and litter under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites. *Biology and Fertility of Soils* 24, 45-51.

Priha O., Grayston, S.J., Hiukka, T., Pennanen, T. and Smolander, A. 2001. Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. *Biology and Fertility of Soils* 33, 17-24.

Reeves, D.W. 1997. The role of soil organic matter in maintaining soil quality in continuous cropping system. *Soil and Tillage Research* 43, 131-167.

Reicosky, D.C., Reeves, D.W., Prior, S.A., Runion, G.B., Rogers, H.H. and Raper, R.L. 1999. Effect of residue management and controlled traffic on carbon dioxide and water loss. *Soil and Tillage Research* 52, 153-165.

Riezebos, H.T. and Loerts, A.C. 1998. Influence of land use and tillage practice on soil organic matter in southern Brazil and eastern Paraguay. *Soil and Tillage Research* 49, 271-275.

Rochette, P., Ellebert, B., Gregorich, E.G., Desjardins, R.L., Pattey, E., Lessard, R. and Johnson, B.G. 1997. Description of a dynamic closed chamber for measuring soil respiration and its comparison with other techniques. *Canadian Journal of Soil Science* 77, 195-203.

Romig, D.E., Garlynd, M.J., Harris, R.F. and McSweeney, K. 1995. How farmers assess soil health and quality. *Journal of Soil and Water Conservation* 50, 229-239.

Roper, M.M. and Gupta, V.V.S.R. 1995. Management practices and soil biota. *Australian Journal of Soil Research* 33, 321-339.

Ross, D. J., Speir, T.W., Kettles, H.A., Tate, K.R. and Mackay, D.A. 1995. Soil microbial biomass, C and N mineralization, and enzymes activities in hill pasture: influence of grazing management. *Australian Journal of soil Research* 33, 943-959.

Sakamoto, K. and Oba, Y. 1994. Effect of fungal to bacterial biomass ratio on the relationship between CO_2 evolution and total soil microbial biomass. *Biology and Fertility of Soils* 17, 39-44.

Schenk, N.C., Smith, G.S., Mitchell, D.J. and Gallaher, R.N. 1982. Minimum tillage effects on the incidence of beneficial mycorrhizal fungi on agronomic crops. Florida Scientist 45:8 (Abstr.).

Schlegel, H.G. 1993. General microbiology. 7th ed., Cambridge, Britain.p.655.

Schnitzer, M. 2000. A lifetime perspective on the chemistry of soil organic matter. *Advances in Agronomy* 68, 1-58.

Schnürer, J, and Rosswall, T. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied Environmental Microbiology* 43, 1256-1261.

Scotney, D.M. and McPhee, P.J. 1991. Our dwindling soil resources. In World Environment Day Symposium. 5 June 1991, Mintek Auditorium, 200 Hans Strydom Road, Randburg.

Sims, G. K. 1990. Biological degradation of soil. *Advances in Soil Science* 11, 289-330.

Singh, J.S. and Gupta, S.R. 1977. Plant decomposition and soil respiration in terrestrial ecosystems. *Botanical Review* 43, 449-528.

Smith, J.L., Papendick, R.I., Bezdicek, D.F. and Lynch, J.M. 1993. Soil organic matter dynamics and crop residue management. In: Metting, F.B. (ed.). Soil microbial ecology, application in agricultural and environmental management. Marcel Dekker, New York, pp. 65-94.

Smith, O.H., Peterson, G.W. and Needelman, B.A, 2000. Environmental factors of agroecosystems. *Advances in Agronomy* 69, 75-97.

Soil Classification Working Group, 1991. A Taxonomic system for South Africa. Soil and Irrigation Research Institute, Department of Agricultural Development, Pretoria.

Sparling, G.P. 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. *Australian Journal of Soil Research* 30, 195-207.

Sparling, G.P. 1995. The substrate- induced respiration method. In: Alef, K. and Nannipieri, P. (eds.). Methods in applied soil microbiology and biochemistry. Academic Press, London, pp. 397-404.

Sparling, G. P. 1997. Soil microbial biomass, activity and nutrient cycling as indicators of soil health. In: Pankhurst, C.E., Doube, B.M. and Gupta, V.V.R.S. (eds.). Biological indicators of soil health. CAB International, New York. pp. 97-119.

Sparling, G.P. and West, A.W. 1988. A direct method to estimate soil microbial C: calibration in situ using microbial respiration and 14C labelled cells. *Soil Biology and Biochemistry* 20, 337-343.

Stamatiadis, S., Doran, J.W., and Ingham, E.R. 1990. Use of staining and inhibitors to separate fungal and bacterial activity in soil. *Soil Biology and Biochemistry* 22, 81-88.

Steinhardt, G.C. 1995. Soil quality: a new idea that includes an old one. *Journal of Soil and Water Conservation* 50, 222.

Stott, D.E., Kennedy, A.C. and Cambardella, C.A. 1999. Impact of soil organisms and organic matter on soil structure. In: Lal, R. (ed.). Soil quality and soil erosion. CRC Press, Boca Raton, pp. 57-73.

Swift, M.J. and Woomer, P. 1991. Organic matter and the sustainability of agricultural systems: Definition and measurement. In: Mulongoy, K. and Merckx, R. (eds.). Soil organic matter dynamics and sustainability of tropical agriculture. John Wiley & Sons. New York.

Tabatabai, M.A. 1982. Soil enzymes. In: Page, A.L., and Miller, R.H. (eds.). Methods of soil analysis, part 2. Chemical and microbiological properties. *Soil Science Society of America Journal*, Wis., pp. 903-947.

Tabatabai, M A 1994. Soil enzymes. In: Weaver R.W., Angle J.S., Bottomley, P.S. (eds.). Methods of soil analysis, part 2, Microbiological and biochemical properties. Book Series No. 5, *Soil Science Society of America*, Madison, Wis., pp. 775-833.

Tabatabai, M.A. and Minhong, F. 1992. Extraction of enzymes from soils. In: Strotzky, G. and Bollag, J.M. (eds.). Soil Biochemistry. Vol. 7, Marcel Dekker, Inc, New York, pp. 197-227.

Thorn, G. 1997. The fungi in soil. In Elsas, J. D., Trevors, J.T. and Wellington, E. M. H. (eds.). Modern Soil Microbiology. Marcel Dekker, New York, pp. 63-127

Tisdall, J.M. 1994. Possible role of soil microorganisms in aggregation in soils. *Plant Soil* 159, 115-121.

Troeh, F.R. and Thompson, L.M. 1993. Soils and soil fertility. 5th ed. New York, Oxford University Press, p 462.

Tunlid, A. and White, D.C. 1992. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial community in soil. In: Stotzky, G. and Bollag, J.M. (eds.). Soil biochemistry. Marcel Dekker, Inc., New York, pp. 229-262.

Turco, R. F., Kennedy, A. C. and Jawson, M.D. 1994. Microbial indicators of soil quality. In: Defining soil quality for a sustainable environment. *Soil Science Society of America*, Special Publication No. 35, 73-89.

Vance, E.D., Brooks, F.C., and Jenkinson, D.S. 1987. An extraction method for measuring microbial biomass C. *Soil Biology and Biochemistry* 19, 703 - 707.

Voroney, R.P., Winter, J.P., and Beyaert, R.P. 1993. Soil Microbial Biomass C and N. In: Carter, M.R. (ed.). Soil sampling and methods of analysis. Lewis publishers, USA., pp. 277-286.

Vose, J.M., Elliott, K.J., Johnson, D.W., Walker, R.F., Johnson, M.G. and Tingey, D.T. 1995. Effect of elevated CO_2 and N fertilization on soil respiration from ponderosa pine (*Pinus ponderosa*) in open-top chambers. *Canadian Journal of Forestry Research* 25, 1243-1251.

Wander, M.M., Bidart Bouzat, G. and Aref, S. 1998. Tillage impacts on depth distribution of total and particulate organic matter in tree Illinois soils. *Soil Science Society of America Journal* 62, 1704-1711.

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

Wardle D.A. and Ghani A 1995. A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. *Soil Biology and Biochemistry* 27, 1601-1610.

Warkentin, B.P. 1995. The changing concept of soil quality. *Journal of Soil and Water Conservation* 50, 226-228.

Yan, F., McBratney, A.B. and Copeland, L. 2000. Functional substrate biodiversity of cultivated and uncultivated A horizons of vertisols in NW New South Wales. *Geoderma* 96, 321-343.

Yang, X. and Wander, M. M. 1999. Tillage effects on soil organic carbon distribution and storage in a silt loam soil in Illinois. *Soil and Tillage Research* 52, 1-9.

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.

Yocom, D.H., Larsen, H.J., and Boosalis, M.G. 1985. The effects of tillage treatments and fallow season on VA mycorrhizae of winter wheat. Proc. 6th North American Conference on mycorrhizae. Forest Research Lab., Oregon State University: Corvallis, OR.

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

APPENDICES

APPENDIX A **ANOVA** table of effects of agricultural management on organic C and **N** levels in soils

Parameter	Source of variation	Degree of freedom	Mean square	F value	Probability
Organic C	Treatment	6	2966.45	174.02	< 0.001***
	Depth	1	1845.75	108.28	< 0.001***
	Interaction	6	247.96	14.55	< 0.001***
	Residual	42	17.05		
Total N	Treatment	6	10.45	31.83	< 0.001***
	Depth	1	6.04	18.41	< 0.001***
	Interaction	6	0.57	1.76	0.131 NS
	Residual	42			

NS = non-significant (P > 0.05)

*** = Very highly significant (P≤ 0.001)

APPENDIX B ANOVA table of effects of management practices on soil pH with changes of soil depth

Parameter	Source of variation	Degree of freedom	Mean square	F value	Probability
$pH_{(H2O)}$	Treatment	6	0.873	18.44	< 0.001***
	Depth	1	0.086	1.82	0.184 NS
	Interaction	6	0.136	2.87	0.020*
	Residual	42	0.047		
$pH_{(KCl)}$	Treatment	6	0.796	21.68	< 0.001***
	Depth	1	0.194	5.29	0.026*
	Interaction	6	0.111	3.03	0.015*
	Residual	42	0.036		

NS = non-significant (P> 0.05)

* = Significant (P≤ 0.05)

*** = Very highly significant (P≤ 0.001)

APPENDIX C ANOVA table of microbial biomass C and basal respiration results

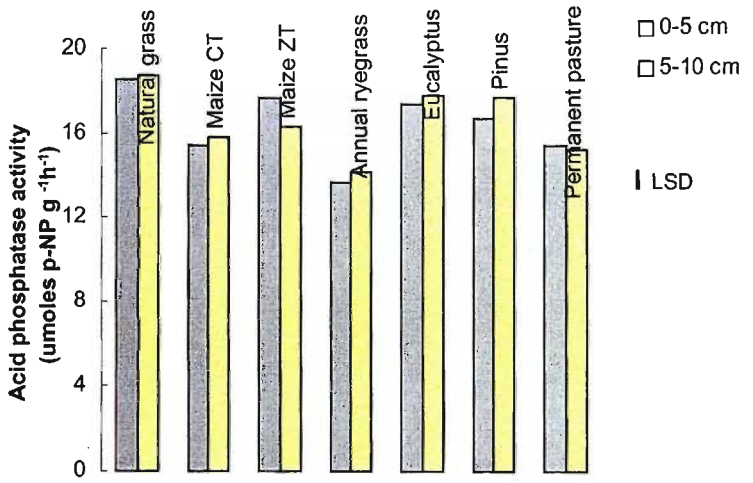
Parameter	Source of variation	Degree of freedom	Mean square	F value	Probability
Microbial biomass C	Treatment	6	2755768	181.4	< 0.001***
	Depth	1	557782	36.7	< 0.001***
	Interaction	6	58738	3.8	0.004**
	Residual	42	15184		
Basal respiration	Treatment	6	3631.7	42.7	< 0.001***
	Depth	1	9576.1	112.6	< 0.001***
	Interaction	6	908.5	10.69	< 0.001**
	Residual	42	85		

** = Highly significant (P≤ 0.01)

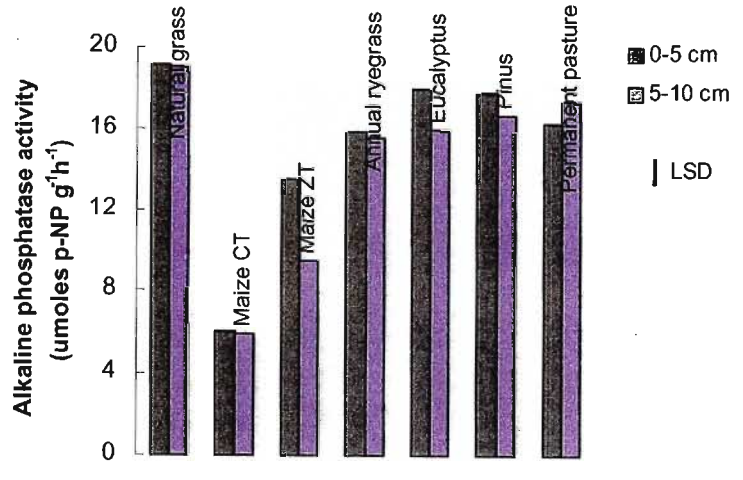
*** = Very highly significant (P≤ 0.001)

APPENDIX D Acid phosphatase (a) and alkaline phosphatase (b) activities at 0-5 and 5-10 cm soil depths as affected by agricultural practices. LSD at $P \leq 0.05$ shown

(a)



(b)



APPENDIX E ANOVA table of enzyme activities with statistical significance shown

Parameter	Source of variation	Degree of freedom	Mean square	F value	Probability
Dehydrogenase	Treatment	6	0.59	37.4	< 0.001***
	Depth	1	0.17	11.1	0.002**
	Interaction	6	0.018	1.2	0.32 NS
	Residual	42	0.015		
FDA hydrolysis	Treatment	6	0.083	36.8	< 0.001***
	Depth	1	0.008	3.7	0.06 NS
	Interaction	6	0.008	3.7	0.005**
	Residual	42	0.0022		
Arginine ammonification	Treatment	6	0.064	111.6	< 0.001***
	Depth	1	0.042	74.2	< 0.001***
	Interaction	6	0.007	12.7	< 0.001***
	Residual	42	0.0005		
Arylsulphatase	Treatment	6	326.7	209.4	< 0.001***
	Depth	1	47.5	30.4	< 0.001***
	Interaction	6	33.0	21.1	< 0.001***
	Residual	42	1.56		
Acid phosphatase	Treatment	6	20.4	38.0	< 0.001***
	Depth	1	0.15	0.28	0.6 NS
	Interaction	6	1.14	2.12	0.07 NS
	Residual	42	0.53		
Alkaline phosphatase	Treatment	6	165.7	129.9	< 0.001***
	Depth	1	11.79	9.25	0.004**
	Interaction	6	5.56	4.37	0.002**
	Residual	42	1.27		

NS = Non-significant (P> 0.05)
** = Highly significant (P≤ 0.01)
*** = Very highly significant (P≤ 0.001)

APPENDIX F ANOVA table of the AWCD evolution from 24-120 h of incubation

Incubation time	Degree of freedom	Mean of square	F value	Probability
24 h	6	0.0006	6.06	< 0.001***
48 h	6	0.1785	30.70	< 0.001***
72 h	6	0.3611	19.81	< 0.001***
96 h	6	0.3959	12.50	< 0.001***
120 h	6	0.4257	13.58	< 0.001***

APPENDIX G Table of analysis of variance of the principle component scores of the AWCD at 120 h of the incubation of the Biolog Eco plates after inoculation with soil extracts of different agricultural management

A. PC1 scores

Source of variation	Degree of freedom	Mean of square	F value	Probability
Treatments	6	1.52	7.74	< 0.001***
Residual	21	0.19		
Total	27			

LSD and means of treatments

LSD 5%	Maize ZT	Maize CT	Annual ryegrass	*Eucalyptus*	*Pinus*	Permanent pasture	Natural grass
0.65	-0.56	-0.32	1.28	-0.24	0.23	-0.31	-0.05

A. PC2 scores

Source of variation	Degree of freedom	Mean of square	F value	Probability
Treatments	6	1.45	9.06	< 0.001***
Residual	21	0.16		
Total	27			

LSD and means of treatments

LSD 5%	Maize ZT	Maize CT	Annual ryegrass	*Eucalyptus*	*Pinus*	Permanent pasture	Natural grass
0.58	0.07	-0.94	-0.31	-0.16	0.05	1.04	0.23

APPENDIX H Table of analysis of the variance of the first two principle component scores of the catabolic response profiles for soils under different agricultural management

A. PC1 scores

Source of variation	Degree of freedom	Mean of square	F value	Probability
Treatments	6	1.92	197.3	< 0.001***
Residual	14	0.009		
Total	20			

LSD and means of treatments

LSD 5%	Maize ZT	Maize CT	Annual ryegrass	*Eucalyptus*	*Pinus*	Permanent pasture	Natural grass
0.17	1.47	0.36	-0.57	0.12	0.09	-0.92	-0.56

A. PC2 scores

Source of variation	Degree of freedom	Mean of square	F value	Probability
Treatments	6	1.54	24.8	< 0.001***
Residual	14	0.06		
Total	20			

LSD and means of treatments

LSD 5%	Maize ZT	Maize CT	Annual ryegrass	*Eucalyptus*	*Pinus*	Permanent pasture	Natural grass
0.43	-0.55	-0.30	-0.36	0.87	1.10	-0.01	-0.75