

**THE EFFECT OF ORGANIC AND MINERAL FERTILIZERS ON SOIL
MICROBIAL COMMUNITIES IN KABETE LONG-TERM EXPERIMENT, KENYA**

Musyoki Mary Kamaa B. Sc. Environmental Science

N50/11298/04

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF MASTER OF ENVIRONMENTAL
SCIENCE OF KENYATTA UNIVERSITY**

May 2011

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

Musyoki Mary Kamaa

N50/11298/04

Signature _____ Date _____

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

Supervisors

Prof. James B. Kung'u
Department of Environmental Science
School of Environmental Studies and Human Sciences
Kenyatta University

Signature _____ Date _____

Prof. Eliud N.M. Njagi
Department of Biochemistry and Biotechnology
School of Pure and Applied Sciences
Kenyatta University

Signature _____ Date _____

Dr. Didier Lesueur
Tropical Soil Biology and Fertility (TSBF) Institute of CIAT
P.O. Box 30677 - 00100 Nairobi

Signature _____ Date _____

DEDICATION

This thesis is dedicated to my dear parents and siblings. Their vision for a better tomorrow is unrivalled by the best educators in my life.

ACKNOWLEDGEMENT

I thank my supervisors: Prof James B Kung'u, Prof Eliud NM Njagi and Dr Didier Lesueur for the guidance and assistance that was offered willingly and tirelessly during the study. Special appreciations go to the director of Tropical Soil Biology and Fertility Institute (TSBF), Dr Sanginga for giving me the chance to work as an intern-student in TSBF and finally sponsoring my master's study. I am also grateful to Dr Catherine N Kibunja of Kenya Agricultural Research Institute (KARI), for allowing me to work on the Kabete Long-term Experiment. Special thanks go to my colleagues from Kenyatta University and Moi University for their encouragement and valuable technical discussions during the study.

The financial expenses related to this study was generously given by the MICROBES project through TSBF. I sincerely thank them for the early recognition that below ground biodiversity loss is an emerging problem in various agro ecosystems that needs to be addressed for sustainable soil fertility management.

.

TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENT	iv
TABLE OF CONTENTS	v
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
ABBREVIATIONS AND ACRONYMS	ix
ABSTRACT	x
CHAPTER ONE.....	1
INTRODUCTION.....	1
1.1 Background information	1
1.2 Statement of the problem and justification	3
1.3 Research hypotheses	4
1.4 Objectives of the study.....	4
1.5 Research questions.....	5
1.6 Significance and conceptual framework of the study	6
1.7 Definition of terms.....	6
CHAPTER TWO.....	7
LITERATURE REVIEW	7
2.1 Role of organic inputs in soil fertility management and microbial activity	7
2.2 Role of mineral fertilizer inputs in soil fertility management and microbial activity	9
2.3 Effects of combining organic and mineral fertilizer inputs on soil fertility and microbial activity.....	11
2.4 Role of soil biota in soil fertility improvement.....	12
2.4.1 The role of bacteria in soil	15
2.4.2 Role of Fungi in the soil.....	16
2.4.3 Denitrification	18
2.5 Factors affecting microbial diversity	21
2.6 Influence of organic and mineral fertilizers on soil microbial communities	22
2.7 Molecular techniques for soil microbial diversity Analyses.....	24
2.7.1 Restriction Fragment Length Polymorphism (RFLP).....	25
2.7.2 Random Amplified Polymorphic DNA (RAPD)	26
2.7.3 Low-Molecular-Weight (LMW) RNA fingerprinting	27
2.7.4 Temperature Gradient Gel Electrophoresis (TGGE)	27
2.7.5 Denaturing Gradient Gel Electrophoreses technique.....	28
CHAPTER THREE.....	31
MATERIALS AND METHODS	31
3.1 Site description.....	31
3.2 Experimental design.....	31
3.3 Soil sampling	32
3.4 Laboratory analysis	33

3.4.1 Soil analysis	33
3.4.2 Deoxyribonucleic Acid (DNA) extraction from soil samples.....	36
3.4.3 Denaturing Gradient Gel Electrophoresis	40
3.5 Data analysis	41
CHAPTER FOUR	43
RESULTS AND DISCUSSION	43
4.1 Soil characterization for selected treatments at Kabete long-term experiment.....	43
4.2 Total bacterial DGGE-finger prints as affected by organic and mineral fertilizers.....	45
4.2.1 Cluster analysis of total bacterial communities	47
4.2.3 Statistical analysis of Shannon-Weaver indices of total bacterial communities	49
4.3 Effect of organic and inorganic inputs on fungal communities	50
4.3.1 Cluster analysis of fungal communities	51
4.3.2 Statistical analysis of Shannon-weaver indices of fungal communities	53
4.4 Effects of organic and inorganic resources on denitrifying bacteria	54
4.4.1 Cluster analysis of denitrifying bacteria communities.....	57
4.4.2 Statistical analysis of Shannon-Weaver indices of the denitrifying bacteria communities	59
CHAPTER FIVE	63
CONCLUSIONS AND RECOMMENDATIONS.....	63
REFERENCES	65
Appendices	76
Appendix 1: Experimental layout for the Kabete long-term experiment	76
Appendix 2: Soil sampling design	77
Appendix 3: Analysis of variance Bacterial communities data	77
Appendix 4: Analysis of variance fungal communities data	78
Appendix 5: Analysis of variance denitrifying bacteria	79

LIST OF TABLES

Table 3.1: Selected Treatments at the Kabete Long- term Experiment	32
Table 3.2: Primer pairs used for PCR-DGGE.....	38
Table 4.2: Means of Shannon Weaver indices for the Kabete long-term trial study on the total bacterial.....	49
Table 4.3: Means of Shannon Weaver indices of the fungal communities in Kabete long-term trial	54
Table 4.4: Means of Shannon Weaver index for the Kabete long-term trial study on the nirK and nirS denitrifying communities' diversity	60

LIST OF FIGURES

Figure 3.1: Flow diagram of the application of PCR-DGGE analysis	37
Figure 4.1: DGGE profiles of 16S rDNA gene in representative replicates of soil samples from Kabete long-term experiment.....	46
Figure 4.2: Cluster analysis of 16S rDNA gene in representative replicates of soil samples from Kabete long-term experiment.....	48
Figure 4.3: DGGE profiles of representative 28S rDNA gene analysis of soil samples from Kabete long-term experiment.	51
Figure 4.4: Cluster analysis of 28S rDNA gene of soil samples from Kabete long-term experiment.....	52
Figure 4.5: DGGE profiles of nir K rDNA gene from Kabete long-term experiment. ...	55
Figure 4.6: DGGE profiles of nir S rDNA gene Kabete long-term experiment.....	56
Figure 4.7: Cluster analysis of nir K rDNA gene of soil samples from Kabete long-term experiment.....	58
Figure 4.8: Cluster analysis of nir S rDNA gene of soil samples from Kabete long-term experiment.....	59
Figure 4.9: Relationship between nir K and nir S denitrifying bacteria community.....	61

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
C	Carbon
CAN	Calcium Ammonium Nitrate
DAP	Di-ammonium phosphate
DNA	Deoxy Ribonucleic Acid
DGGE	Denaturing Gradient Gel Electrophoreses
FYM	Farmyard manure (cattle and goat manure)
ha	hectare
ICRAF	International Centre for Research in Agro forestry
ISFM	Integrated Soil Fertility Management
KARI	Kenya Agricultural Research Institute
M	Molar
N	Nitrogen
NARL	National Agricultural Research Laboratories
P	Phosphorus
PCR	Polymerase Chain Reaction
R	Residue (Maize Stover)
RCBD	Randomized Complete Block Design
SAP	Structural Adjustment Programme
SSA	Sub- Saharan Africa
TSBF	Tropical Soil Biology and Fertility Institute

ABSTRACT

Soil micro-organisms play a key role in determining soil quality. Over 70% of small holder farmers in the central high lands of Kenya are using crop manure, animal wastes and mineral fertilizers to increase their farms fertility and subsequent productivity. The dilemma with these practices is that less is known on the impact of these resources on the below ground biodiversity particularly the microbial communities. A field study was conducted at a site in Kabete where a long-term experiment that had been established in 1976 to investigate the effects of various organic and mineral fertilizers on total bacterial communities, fungal communities and denitrifying bacterial community structures. The objectives of this study were to determine the impact of continuous cropping with repeated applications of organic and mineral inputs on soil microbial communities. Denaturing gradient gel electrophoresis (DGGE) molecular technique of polymerase chain reaction-amplified 16S rRNA genes, 28S rRNA genes, nir K rRNA genes and nir S rRNA genes were used. The study comprised of two organic inputs; maize stover (R) all harvest returned to soil and farmyard manure (FYM) applied at 10 t ha⁻¹ with or without 120 kg N, 52.8 kg P (N+P) mineral fertilizer, a virgin land (fallow) that had not been cultivated for the thirty two years and a complete control (C) with neither organic nor mineral fertilizer. Soil samples from the top soil (0-10cm depth) were analyzed for total bacterial, denitrifying bacteria and fungal communities' diversity as well as soil pH, carbon (C), nitrogen (N), and nitrates (NO₃⁻). DGGE gels were then converted to graphs using the Total lab software and cluster analysis done to assess the similarity of communities between treatments. Shannon weaver index of diversity (H) was used to calculate the various microbial diversities. All data was analyzed with the Anova procedure of the GENSTAT statistical software and treatment means separated using least significant difference at (P≤0.001). Application of mineral fertilizer along with farmyard manure or maize stover had increased both fungal and total bacterial communities as well as the nir S denitrifying bacteria diversity. Sole application of mineral fertilizers (N+P) negatively affected microbial communities as a decrease in bacterial and fungal communities was noted. Application of organic inputs alone had a lower diversity of microbial communities compared to combination of both organic and mineral fertilizers though a relatively higher diversity was noted compared to sole application of mineral fertilizers. Soil pH, N, C, and NO₃⁻ influenced the diversity of microbial communities as indicated by the low diversity where these values were low. It is concluded that application of mineral fertilizer along with farmyard manure or maize stover is an effective way of enhancing soil organic matter hence increased microbial diversity and activity in the central highlands of Kenya. This study is an important contribution to the debate surrounding the sustainability of organic agriculture as the findings not only scores a beneficial point for organic agriculture, but give credibility to the middle-ground approach of integrated soil fertility management which uses both organic and mineral fertilizers.

CHAPTER ONE

INTRODUCTION

1.1 Background information

The Sub-Saharan Africa's (SSA) economic growth and quality of life is largely dependent on the agricultural sector. However, the region has been experiencing a declining per capita food production for the past decade (Buresh & Tian, 1997) consequently contributing to increased levels of poverty and environmental degradation (Bationo et al., 2004). In large parts of Kenya, food crop yields are very low due to declining soil fertility which is as a result of continuous cropping with little or no replenishment of nutrients removed through either crop harvests or other losses such as leaching and soil erosion (Kibunja et al., 2003). The Eastern and Central highlands small-holder farmers in particular, have been experiencing declining soil and crop productivity. This has compelled them to rely mainly on organic inputs to maintain or improve soil fertility with small or no additions of mineral fertilizers.

Within the integrated soil fertility management (ISFM) framework, it is recognized that both organic and mineral fertilizers are necessary in enhancing crop yields without deteriorating the soil resource base (Kazombo-Phiri, 2005). This recognition has an important practical dimension because either of the two inputs is hardly available in sufficient quantities, and also has an important resource management dimension as there are added benefits created by positive interactions between both inputs when applied in combination. The processes of land conversion and agricultural intensification are a significant cause of biodiversity loss including the below ground biodiversity (BGBD), with consequent negative effects both on the environment, ecosystem services and the sustainability of agricultural production. Documentation of BGBD is an essential component of the information required for

assessment of environment-agriculture interactions as is the evaluation of the impact of agricultural management on resource base, particularly that of soil. Soil organisms contribute a wide range of essential services to sustainable function of agroecosystems. The combination of physio-chemical and biological soil fertility management approaches is likely to be a unique opportunity to exploit synergies for the benefit of crop productivity.

The soil biota provides means for regulating the transformation of organically and inorganically bound nutrients into plant-available forms through mineralization (Vanlauwe et al., 1996; Lavelle et al., 1994; Tian Kang & Brussard., 1995). Besides this, some of the bacteria have a protective function, of “soaking up” and de-toxifying the soil environment from pollutants (Murphy et al. 2007). For instance, in denitrifiers, it has been proposed that cytochrome c’ has a role in protecting the cells from the potentially toxic effects of NO, a gas which is formed in large amounts during the process of denitrification in the nitrogen cycle (Ref). However, as an important biological resource that contributes to plant production and maintenance of a variety of ecosystem services, soil biodiversity has been significantly neglected in agricultural research (Swift et al. 1989).

Although organic inputs on farms have been shown to have slightly higher levels of organic matter and carbon than neighboring mineral fertilizer managed farms (Drinkwater, Wagoner & Sarrantonio 1998), only limited research has investigated the structures and compositions of microbial communities following a switch to organic and mineral fertilizers farming practices. Farmyard manure (cattle and goat) have traditionally been used as the main nutrient source during maize production in the central highlands of Kenya for several years. Another important source of nutrients during maize production has traditionally been maize stover that is returned to the field rather than burnt or removed. However, in the recent years, there has

been a large increase in the use of mineral fertilizers with a decrease in the use of cattle or goat manure. In particular, there is concern that this change is leading to a decrease in the size of soil microbial community (Tu et al., 2006). Against this background, this study aimed to assess the impact of organic and mineral fertilizers on soil microbial functional diversity communities in Kabete long-term experiment in Kenya by characterizing the bacterial and fungal communities through Denaturing Gradient Gel Electrophoresis (DGGE) technique.

1.2 Statement of the problem and justification

The role of soil micro-organisms in the decomposition of organic matter and thus nutrient release, soil structure and soil-water relations is well documented. However, their functioning and biological process is poorly understood. Ever since the Rio summit in 1992 on biodiversity, heightened interest in the effect that microbial diversity has on ecological function in soil ecosystems has emerged. However, the existing theories on terrestrial ecosystems have been developed from above ground observations whereas comparatively few studies have been made in soil (Wardle & Griller, 1996; Griffiths et al., 2001).

Rather than applying mineral fertilizers alone, their application along with farmyard manure or maize stover could provide a compromise between traditional and modern production systems, which would maintain both below ground biodiversity and high yields. Although many studies since late 1970s up to date have been done in Central Kenya, most of these studies have been on the interaction between organic and mineral resources quality, aggregate turnover, and agro-ecosystem nitrogen and carbon cycling (Vanlauwe et al., 2006). Further, few of these studies were dedicated on the assessment of the macro-fauna thus less is known about the effects of these applications on soil microbial communities. Therefore, limited data exists on the changes of soil microbial communities under different fertilization schemes in

the prevailing maize-based cropping systems. A long-term (32 years) maize cropping system experiment in the central highlands of Kenya was investigated to evaluate the effects of organic fertilizers, mineral fertilizers, and combination of both inputs on soil microbial communities. This study focused on the soil bacterial and fungal communities since they contribute a greater percentage of microorganisms in the soil. The study aimed to fill the existing knowledge gaps by assessing the long-term impacts of organic and mineral fertilizer applications on soil microbial functional diversity community in Kabete long-term experiment through DGGE analysis, which is a new technique in molecular biology.

1.3 Research hypotheses

The research tested the following hypotheses:

- (i) The application of organic fertilizers does not significantly increase soil microbial functional community diversity.
- (ii) The application of mineral fertilizers does not significantly decrease soil microbial functional community diversity.
- (iii) The combination of organic and mineral fertilizers does not significantly increase soil microbial functional diversity as compared to the application of either organic or mineral fertilizers alone.

1.4 Objectives of the study

The main objective of the study was to assess the impact of organic and mineral fertilizers application on soil below ground biodiversity in a maize cropping system. To achieve this, the following specific objectives were pursued:

- (i) To determine the effect of organic fertilizers on total bacteria, denitrifying bacteria and fungal functional communities' diversity in a long-term maize cropping field experiment.
- (ii) To determine the effect of mineral fertilizers on total bacteria, denitrifying bacteria and the fungal functional communities' diversity in a long-term maize cropping field experiment.
- (iii) To determine the effect of combining organic and mineral fertilizers on the functional bacterial (denitrifying and total bacteria) and fungal communities' diversity in a maize cropping field experiment.

1.5 Research questions

The study sought to answer the following questions:

- (i) How does organic fertilizer application affect soil microbial community diversity (total bacteria, denitrifying bacteria and fungal communities)?
- (ii) How does mineral fertilizer application affect soil microbial community diversity (total bacteria, denitrifying bacteria and fungal communities)?
- (iii) What is the effect of combining both organic and mineral fertilizer resources on microbial community diversity (total bacteria, denitrifying bacteria and fungal communities)?
- (iv) How do soil chemical and physical characteristics influence soil microbial community diversity (total bacteria, denitrifying bacteria and fungal communities)?

1.6 Significance and conceptual framework of the study

Soil fertility decline is a major limiting factor in food production in the central highlands of Kenya. Most of the smallholder farmers are not endowed with resources to enable them to apply the recommended rates of mineral fertilizers to curb the decline in soil fertility. Consequently, use of both organic and mineral fertilizers is a common practice among smallholder farmers. There is therefore need to determine the effect of these resources on below ground biodiversity which are key role players in soil chemical and physical characteristics.

1.7 Definition of terms

Denaturing Gradient Gel Electrophoreses (DGGE) – A molecular fingerprinting method that is employed for the separation of double-stranded DNA fragments (polymerase chain reaction (PCR)-generated DNA products) that are identical in length, but, differ in sequence.

Functional Diversity – Refers to the diversity of species that maintain ecological processes such as nutrient retention and recycling in the ecosystem.

Polymerase Chain Reaction (PCR) - A molecular biology technique for enzymatically replicating DNA without using a living organism, such as *Escherichia coli* or yeast.

Integrated Soil Fertility (ISFM) - A holistic approach to soil fertility research that embraces the full range of driving factors and consequences of soil degradation right from the biological, chemical, physical, social, and economic to the political aspects of soil fertility.

CHAPTER TWO

LITERATURE REVIEW

2.1 Role of organic inputs in soil fertility management and microbial activity

Improving soil fertility is essential for intensification and diversification of cropping systems and the recuperation of degraded lands. Farmers in Africa particularly in the East African region rely mainly on organic inputs to maintain or improve soil fertility with small or no additions of inorganic fertilizers (Bationo et al., 2004). Within the ISFM framework, it is now recognized that both organic and mineral inputs are necessary to enhance crop yields without deteriorating the soil resource base (Vanlauwe et al., 2002).

Many cropping systems use the waste products of livestock enterprises as part of their fertilization strategy either as solid materials (manures) or in liquid form (slurry). Other organic materials for example sewage sludge, food processing wastes and compost of various materials are also applied to soils depending on local availability (Bationo et al., 2004). Organic matter concentration largely determines the total cation exchange, pH and aluminium toxicity of soils. Thus application of organic materials tends to either stimulate or depress the microbial biomass directly and substantially unlike applications of mineral fertilizers (Wardle & Giller 1992). Further, organic fertilizers add C, N and other nutrients simultaneously satisfying components of the microbial population which are (usually) otherwise C-limited (Murphy et al., 2007).

Continuing release of nutrients as the materials are slowly decomposed in soils can also sustain the microbial biomass population for longer periods as compared to the impact of mineral fertilizers (Wardle & Giller, 1992). For example, the maintenance of a higher population of nitrifiers where farmyard manure rather than mineral fertilizers are applied has

been reported (Mendum et al., 1999). Other positive effects of farmyard manure application are increased soil porosity and aggregate stability, increased water infiltration and water holding capacity, decreased soil losses, increased soil organic matter (SOM), pH, CEC and nutrient availability (Powell et al., 1999).

Achieving synchrony between crop demand and nutrient supply is very difficult (Myers, van Noordwijk & Vityakon., 1997) particularly where nutrients are supplied solely through microbially mediated chemical transformations and decomposition of diverse and variable range of organic materials. However, optimum efficiency of nutrient use seems to be achieved in many situations where mineral fertilizers and organic materials are used together in fertilization strategies for cropping systems (Palm, Myers & Nandwa., 1997). There is some evidence that the mineral N pool applied in manures is more efficiently used for plant uptake than mineral N fertilizer (Stockdale, Rees & Davies 1995). In addition, a large proportionate increase in both microbial P and the conventionally measured forms of available P is measured when farmyard manure is applied over the long-term. In soils which strongly fix inorganic P, combined use of soluble phosphate fertilizers with manure stimulates the uptake of P by the biomass, thus protecting it from immediate fixation and significantly increasing crop yield (Twomlow et al., 1999). Incorporation of the mineral N and P added in manure into the microbial biomass through immobilization may protect these nutrients from loss before crop roots are fully developed and release of nutrients through microbial biomass turnover and predation may be more closely matched to crop demand.

On the contrary, application of manures and/or sewage sludge to soils can significantly increase the heavy metal loading in soils. For example, Brookes & McGrath (1984) demonstrated that heavy metals derived from sewage sludge substantially reduced microbial

biomass even twenty years after application. The reduced microbial activity where organic materials are repeatedly applied leads to extreme accumulation of organic matter in the soil (Chander & Brookes, 1991). Heavy metals also decrease microbial diversity as a result of species extinction due to a lack of tolerance to the imposed stress and/or the competitive advantage of certain species that predominate in the presence of the heavy metal stress (Giller et al., 1998). Heavy metal content of organic materials therefore has significant implications for the use and management of such materials in agricultural systems.

2.2 Role of mineral fertilizer inputs in soil fertility management and microbial activity

Mineral fertilizers (simply inorganic salts) are primarily used to overcome the nutrient limitations to plant growth, which occur in many farming systems (Murphy et al., 2007). They may also be used to optimize product quality. The response of the microbial biomass to fertilizer amendments depends critically on whether the nutrient applied is limiting microbial activity or growth (Murphy et al., 2007). Additionally at high rates of fertilizer addition, osmotic effects may occur especially in zones close to fertilizer granule or liquid injection points (Murphy et al., 2007). However, such effects are only temporary and often only at rates of fertilizer addition well in excess of normal farm rates (Murphy et al. 2007). High concentrations of soluble nutrients in the soil have been shown to discourage the activity of some symbiotic microorganisms. For example, N₂ fixation by rhizobia is significantly reduced in soils with high concentrations of mineral N. Similarly high concentrations of soluble P in soil solution suppress the infectivity of mycorrhizal fungi to their host plants (Murphy et al., 2007).

Mineral N applications has been shown to either increase or decrease microbial biomass. However, around half of all studies published show only very slight effects (Wardle 1992).

For instance, application of anhydrous ammonia initially kills many soil microorganisms: bacteria and actinomycete populations recover within one to two weeks (Doran & Werner, 1990). However, the fungal population may take as long as seven weeks to recover (Doran & Werner, 1990). Long-term applications of ammonium fertilizer to grassland has also been shown to cause a significant decrease in methane oxidation rates but the application of nitrate for the same length of time does not (Willison et al., 1995). The use of ammonium fertilizer may also favour the development of a population of soil nitrifiers which outcompete methanotrophs for niche environments within the soil (Bedard & Knowles, 1989).

By maintaining added N in the ammonium form for an extended period, losses can be reduced; plant uptake efficiency increased and microbial immobilization of applied N may be increased (Murphy et al., 2007). Mineral fertilizers can also have indirect effects on soil microorganisms through effects on plant growth (Murphy et al., 2007). For example, optimization of potassium (K) fertilization can have a stimulatory effect on microbial activity through increases in root exudation, which supply energy to the microbial biomass. The use of fertilizers increases plant yield and thus increases the return of C to soil in above-and below-ground plant residues, which increase soil microbial activity (Dick, 1992). Long-term inorganic N application in an experiment at Broad balk (started in 1843) resulted in an increase in soil organic matter, heterotrophic microbial activity and microbial biomass compared to plots that had never received N fertilizer (Dick, 1992). On the contrary, studies by, Bationo and Buerkert (2001) have shown that the use of mineral fertilizers on weakly buffered soils may lead to rapid decreases in SOM and pH. Long-term application of mineral fertilizers needs to be combined with the application of organic matter to compensate for the higher SOM turnover rates on mineral fertilized plots.

2.3 Effects of combining organic and mineral fertilizer inputs on soil fertility and microbial activity

Although mineral fertilizers application is the most efficient way to reverse soil nutrient depletion, many reports in the literature (Bationo et al., 2004) have shown that continuous use of sole organic fertilizers may lead to shortage of nutrients not supplied by the chemical fertilizer and while on the contrary use of mineral fertilizers may also lead to chemical soil degradation (Bationo et al., 2004). Moreover, sole application of organic fertilizers is constrained by low availability of N to current crop (Hagggar et al., 1993), low or imbalanced nutrient content, unfavorable quality and high labour demands for transporting bulky materials (Palm, Myers & Nandwa., 1997). The alternative has been to combine application of organic and mineral fertilizers so that improved crop yields are maintained without degrading soil fertility status (Swift et al., 1994). Further, with the rise of the Green Revolution paradigm, the need for the combined use of organic and mineral fertilizers for the improvement of soil fertility in SSA arose on the realization that sole dependence on mineral fertilizers alone could not meet the food needs of Africa (Bationo et al., 2004).

When combined, mineral resources act as a nutrient source for plants while organic fertilizer serves as a precursor of soil organic matter (SOM) which maintains the physical and chemical attributes that contribute to soil fertility such as cation exchange capacity (CEC) and soil structure (Vanlauwe et al., 2002). There is substantial evidence demonstrating gains in crop productivity from nutrient additions through mixtures of organic and mineral sources of nutrients compared with inputs alone (Swift et al., 1994). Supplementation of 5 t ha⁻¹ of manure with 40 kg N ha⁻¹ (mineral fertilizer) for instance in Zimbabwe resulted in higher yields than sole manure treatment (Murwira & Kirchmann, 1993). Studies by Murwira et al. (2002) showed that synchrony between N release and crop uptake was best achieved by applying combination of manure and mineral N and having it in such a way that the N is

applied a little later. This is because late application of mineral N reduced the amount of N lost through leaching.

Similar results were reported by Kibunja et al. (2003) in the Kabete long-term experiment where supplementation of 10 t ha⁻¹ of farmyard manure with 120 kg N ha⁻¹ and 52.8 kg P ha⁻¹ continually gave the highest yields after the first ten years of implementation. This could be attributed to P addition from inorganic fertilizers or N which may not be supplied in sufficient amounts by organic inputs alone leading to better synchrony of nutrient release and uptake. Organic and mineral amendments can be expected to increase the biomass therefore providing more carbon and energy source for the microbes.

2.4 Role of soil biota in soil fertility improvement

In soils, microorganisms occur in great numbers and variety. They are essential for maintaining soil fertility and plant growth as they play an important role in nutrient cycling and availability (Fritze et al., 1994). Microorganisms, being a part of all natural and man-made ecosystems, compose biocenoses, which are significant, and essential biochemical elements responsible for the entirety of biogenic element transformation in the soil environment which exert critical effects on biochemical activity and ecological stability, as well as biological productivity of many fields such as forest, agricultural and grassland ecosystems. Soil organisms mediate both the synthesis and decomposition of soil organic matter and therefore influence cation exchange capacity, the soil N, S, and P reserve, soil acidity and toxicity; and soil water holding capacity (Lavelle & Spain 2001). Soil microbes are involved in biochemical transformations of mineral fertilizers, particularly NPK fertilizers, synthesis of biologically active substances (amino acids, vitamins, antibiotics,

toxins) and nitrogen fixation from the atmosphere (Kennedy & Gewin, 1997). Generally, they regulate element circulation in soil environment and make them assimilable for plants.

Soil microbes regulate most of the processes essential for plant growth such as nutrient availability, defenses against disease, soil health (soil structure and agrochemical degradation) and sustained productivity (development and maintenance of physical and chemical soil properties) (Ritz and Griffiths 2001). Soils have the ability to suppress soil-borne root diseases to some extent through the activity of soil microbes. For instance, studies of changes in the severity of *Rhizoctonia* root rot at Avon, South Africa, showed that disease suppression levels could be modified over time to the point where complete control of the disease could be achieved (Ritz and Griffiths 2001). Improved disease suppression was related to increased carbon inputs (stubble, roots) to the soil from higher yielding crops and increased cropping frequency. Increased carbon inputs result in changes to the composition and activity of the soil microbial community over time. These changes result in more competition for soil resources, which, along with predation and inhibition of pathogens, lead to increased suppression of many soil-borne fungal diseases (Murphy et al., 2007).

Increased soil microbial activity can improve carbon turnover, increase crop nutrient supply and produce a more stable soil structure. Soil microbes improve soil structure by stabilizing the aggregation of soil particles through the production of organic 'glues' and fungal hyphal networks (Martens, 2001). No-till systems can enhance these effects as there is less mechanical disruption of the aggregates. The disruptive effect of tillage in intensive stubble retention cropping systems could be reduced significantly by the associated increase in microbial activity (Martens, 2001).

Increased soil microbial activity further results in improved carbon turnover and nutrient supplying potential of soils (Murphy et al., 2007). Soil microbes can help prevent aggressive plant pathogens taking hold and improve the plant's ability to withstand disease (Murphy et al., 2007). They also reduce the loss of inorganic fertilizers through erosion and leaching by short-term immobilization of the nutrients from the inputs. Further, soil microbes play a key role in the stabilization of soil structure as well as a reduced reliance on agrochemicals and reduced persistence of pesticides in soil therefore lessening off-site impacts of agrochemicals (Hopkins, 2004).

The two main soil microbial groups, fungi and bacteria often play different roles in nutrient cycling and are affected differently by changes in soil pH, moisture, C:N ratio and substrate type (West, 1986). A change in their distribution can influence the flow of nutrients through the ecosystem and hence the health and growth of vegetation. It is thus important to evaluate soil biodiversity and activity under various arable land uses in order to determine soil fertility. Due to the key role of soil biota in nutrient cycling and pest control, it has been argued that the build-up of a large and active soil microbial biomass is critically important for sustaining the fertility of soils especially in organic farming systems (Tu, Ristaino, & Hu, 2006). Some experimental evidence shows that changes in decomposer communities have implications for litter decomposition (Cookson et al., 1998). For instance Cookson, Beare, & Wilson., (1998) showed that continuous use of a particular residue altered microbial community composition and litter decomposition rates with significantly faster decomposition of wheat (*Triticum aestivum L.*) residues compared with barley (*Hordeum vulgare L.*) and white lupin (*Lupinus albus L.*) residues in soil previously amended with wheat residues.

In general, the complex microbial and faunal communities in the soil is fundamental for many ecosystem processes on which crop production depends although the relationship between soil biodiversity or community composition and biological processes is not well understood. Organic and mineral amendments can be expected to increase the biomass and activity of decomposer organisms whereas the influence on decomposer community composition and function is more uncertain (Murphy et al., 2007). It is against this known background that this study sort to understand the influence of different organic and mineral inputs in a maize cropping system on the functional diversity of microbial communities.

2.4.1 The role of bacteria in soil

Bacteria play a number of transformations and biochemical reactions in soil thereby directly or indirectly help in nutrition of biological fixation of nitrogen-symbiotic and non-symbiotic fixation; decomposition of carbohydrates and lignins; decomposition of proteins with the liberation of ammonia or ammonification, nitrification and denitrification, the transformation of carbon (C), nitrogen (N), phosphorus (P), sulphur (S), iron (Fe) and manganese (Mn) among others (Van Straaten, 2007). Bacteria also stabilize soil micro aggregates by binding polysaccharides and inorganic materials. All these processes play a significant role in plant nutrition. The process of conversion of molecular nitrogen into complex proteins through the agency of biological organisms is known as biological nitrogen fixation. Bacteria can be classified mainly into two groups:

- (i) **Nitrogen transforming bacteria:** - nitrogen is utilized by micro-organisms and higher plants in inorganic form as nitrate or ammonium. The complex proteinaceous and nitrogenous organic compounds are broken down to produce ammonia through a microbiological process known as ammonification and the microbes responsible for this are ammonifiers or ammonifying bacteria. In nitrification, first, nitrites are formed by

nitrite forming bacteria (*Nitrosomonas*) and then to nitrate by nitrifying bacteria or nitrifier (*Nitrobacter*). The immobilized nitrogenous dead bodies of the organisms are again converted by microbes into inorganic forms; ammonium or nitrate which can be utilized by plants and micro-organisms (Cavigelli & Robertson 2001).

- (ii) Denitrifying bacteria or denitrifiers: - Denitrification is the process by which nitrates are reduced to oxides of nitrogen and even to gaseous nitrogen. The bacteria which are responsible for this transformation are known as denitrifying bacteria or denitrifiers such as *Pseudomonas*, *Bacillus* and *Paracoccus*. These microbes are aerobic, however, denitrification takes place mostly under anaerobic conditions ref.

2.4.2 Role of Fungi in the soil

Fungi are heterotrophic plants larger than the bacteria. Some live on the dead tissues of organic substances (saprophytes). Fungi may be regarded as the scavengers that decompose almost anything in soil of organic nature that bacteria cannot tackle and many of them serve as food for the bacteria. Fungi play a number of important roles in ecosystem dynamics. The fungal mycelium has been described as a “stick string bag” because it entangles particles within the hyphae network and cements particles together through extracellular polysaccharides production (Oades & Water, 1991). Fungi, as saprophytes, are among the primary decomposers of substances and are essential for recycling nutrients to plants.

Fungi are also essential as mutualists. Mycorrhizal fungi are involved in nutrient capture and expand the resources available to plants. Some fungi achieve this goal through increasing surface area explored in the soil while others increase activities through active decomposition (Cairney & Burke, 1998; Dighton, Mascarenhas & Arbuttle-Keil, 2001) or parasitism

(Klironomos & Hart, 2001). Pathogenic fungi are also essential components of ecosystems, and play key roles such as maintaining biodiversity and mediating competition (Winder & Shamoun, 2006). Various studies have shown how important fungi are for maintenance of sustainable soil system (Klironomos & Hart, 2001). For example, Wardle (2006) and Moore et al. (1990) in studying fungal responses to tillage practices identified significant linkages of fungi to processes associated with the dynamics of soil organic matter (SOM). In addition, investigations have corroborated the importance of fungi in the hierarchical model of soil structure (Tisdall & Oades, 1982; Oades, 1984) by demonstrating structural role of hyphae and the annealing properties of the polysaccharides and glycoproteins that they exude to form and maintain a stable aggregate structure (Miller & Jastrow, 1990; Tisdall, 1991; Degens 1997, Rillig & Mummey, 2006).

In acid soils, fungi are the main decomposers of cellulose since bacteria and actinomycetes become inactive under acidic conditions. In these conditions, *Penicillium* and *Trichoderma* take part in cellulose decomposition whereas in other non-acidic soils, fungi species include *Aspergillus* and *Fusarium*. Certain species of fungi such as the humus forming fungi such as *Alternaria* and *Aspergillus* produce substances similar to humic substances in soil and may be important in the synthesis of soil humus (Rillig & Mummey, 2006).

Actinomycetes have characteristics, which are transitional between bacteria and fungi and are sometimes called fungi-like bacteria. Actinomycetes are more abundant in dry soil than in wet soils and more in grassland and pasture soils than in the cultivated soils. This is because of their unique structure and physiology that make them sensitive and resilient in the face of natural disturbance. They are responsible for the decomposition of the more resistant organic matter of soil (Winder & Shamoun, 2006).

Although there are many studies concerned with management practices in agriculture and forestry, a neglected area of research is the integration of fungal responses to various inputs used by farmers yet these responses relate to soil structure, nutrient cycling and organic matter in soil.

2.4.3 Denitrification

There is increasing concern over the production of nitrous oxide (N_2O) from intensive arable crop production systems. Biological denitrification is central to the nitrogen cycle. It is a dissimilatory process whereby nitrate (NO_3^-) and nitrite (NO_2^-) are used as alternative electron acceptors and reduced to gaseous nitric oxide (NO), nitrous oxide (N_2O) and molecular nitrogen (N_2) (Zumft, 1992). Facultative anaerobic microorganisms under anaerobic conditions carry out the stepwise dissimilatory reduction process. These microorganisms belong to phylogenetically diverse groups of bacteria and Archea (Philippot & Hallin, 2005), although the majority of denitrifiers isolated from soil are *Pseudomonas* and *Alcaligenes* species. Denitrification is important in the loss of N from the soil system and in removing unwanted nitrogenous pollutants from agricultural soils.

The denitrification process is regulated by various environmental factors including soil water content, temperature, soil pH, redox potential, nitrogen oxide concentrations, NO_3^- availability, oxygen and availability of carbon (Firestone & Davidson, 1989). Besides the fertilizer type, NH_4^+ concentration and external C source are other important factors that affect denitrifying activities as well as nir K and nir S community structure. Season and temporal variation also plays an important role in the denitrifying communities as they come

along with changes in soil water content and soil temperature, which are some of the factors affecting denitrifiers' communities' diversity (Wolsing & Prieme, 2004).

Most denitrifying bacteria are heterotrophs and, therefore, C availability is an important factor controlling denitrification (Knowles, 1982; Beauchamp, Trevors & Paul, 1989). Increased C availability increases denitrification directly by increasing the energy and electron supply to denitrifiers, and indirectly through enhanced microbial growth and metabolism thereby stimulating high O₂ consumption (Beauchamp, Trevors & Paul, 1989; Garcia-Montiel et al., 2003) and creates conditions favorable for denitrification. Increasing C addition from simple sugars (Weier et al., 1993; Murray et al., 2004) or complex (crop residues or manure) (McKenney & Wang, 1993; Chantigny, Angers & Rochett, 2002) C sources increases the denitrification rate.

Increased soil NO₃⁻ concentrations can also result in increased denitrification and N₂O emissions (Smith et al. 1998). However, other studies have reported no significant effect of NO₃⁻ addition on denitrification (Blackmer & Bremner, 1978; De Klein & Van Logtestijn, 1996; Rillig & Mummey, 2006). Although the influence of aeration, C availability and soil NO₃⁻ concentration on denitrification rate and partitioning of gaseous N losses from denitrification is generally understood, the influence of organic and mineral inputs on denitrifier microbial communities under field conditions within specific cropping systems is not well understood. Further, literature reviews have suggested that the composition and density of soil denitrifier communities may be factors affecting denitrification (Philippot & Hallin, 2005), with studies reporting that the denitrifier community differs in response to environmental conditions that indirectly control the rate of denitrification (Cavigelli & Robertson, 2000; Holtan-Hartwig, Dorsch & Bakken., 2002). Therefore, there is need to understand the community dynamics of denitrifiers and the environmental factors influencing

the abundance of the denitrifiers in agricultural soils to determine if the denitrifying community may play a role in the increasing levels of nitrous oxides in the atmosphere.

2.4.3.1 Nir K / Nir S communities

Nitrite reductase is the defining enzyme in denitrification because it catalyses the conversion of soluble NO_2^- to gaseous form of N (Zumft, 1992). The gene responsible for encoding nitrite reductase is *nir* and is one of the common molecular markers for denitrifier communities (Braker, Fesefeldt & Witzel, 1998). The *nir* gene is found in two different forms, which are structurally different but functionally and physiologically equivalent (Zumft, 1997). Nir K translates to a copper-containing enzyme nitrite reductase and *nir* S translates to a nitrite reductase enzyme containing cytochrome cd^1 (heme c and heme d^1) (Zumft, 1997).

The nitrite reductase genes are mutually exclusive in a given bacterial species (Braker et al. 2001). The *nir* K genes are predominantly found in α -proteobacteria and *nir* S genes in β -proteobacteria whereas both *nir* K and *nir* S are equally found in γ -proteobacteria (Wolsing & Prieme, 2004). Coenye et al. (2003) found that *nir* S predominates in the denitrifying bacteria but *nir* K has greater variation. Wolsing & Prieme (2004) found higher richness as well as higher diversity of *nir* S gene than *nir* K in soils. Braker, Fesefeldt & Witzel. (1998) reported higher percentage of *nir* S gene than *nir* K in an activated sludge.

Although quantification of soil denitrifier gene copy numbers has been reported previously in ecological studies, few studies have analyzed the response of denitrifier community diversity in agricultural soils following a switch to organic and mineral fertilizer treatments. Furthermore, few studies have assessed the diversity of denitrifying bacteria in the different soil fertility replenishment regimes in Kabete long-term experiment. This study therefore

focused on the variability of denitrifying community by using nir K and nir S nitrite reductase genes by taking advantage of the clay soils in Kabete long-term experiment.

2.5 Factors affecting microbial diversity

Microbial diversity is a general term used to include genetic diversity which means the amount and distribution of genetic information within microbial species (Nannipieri et al., 2003). Soil microbial diversity reflects the mix and populations of diverse living microorganisms in the soil. Functional diversity of the microbial communities is the variation in community structure, complexity of interactions, number of trophic levels and number of guilds. In microbial terms, it describes the number of different types (species) and their relative abundance in a given community in a given habitat or as the number of species present in the system (Nannipieri & Badalucco, 2003). In molecular-ecological terms, it can be defined as the number and distribution of different sequence types present in the DNA extracted from the community in the habitat (Garbeva, Veen & Van Elsas, 2004). To determine how to manage biological processes controlled by microbes, it is important to understand patterns, causes and consequences of microbial diversity (Murphy et al., 2007). The right balance between the physical, chemical and biological components of the soil system is necessary for long-term sustainability of crop production, soil health and consequently soil microbial diversity. In most soils, microbes are concentrated in a thin layer of surface soil which is prone to environmental extremes such as lack of moisture, high temperatures and erosion. In addition, the distribution of biological activity in soils is patchy, for example, it is concentrated in 'hot spots' such as decomposing crop residues (especially in the absence of living plants) and the root zone (Murphy et al., 2007).

To allow soil microbes to function at their best, the soil needs to be suitable in terms of adequate pH, habitable pore space and oxygen concentration as well as optimum

environmental conditions such as temperature and moisture level (Murphy et al., 2007). Further, the function of soil microbes in cropping soils is regulated mainly by the amount of available carbon. Soil microbes need carbon as a source of energy. This means carbon inputs have a significant influence on soil biological activity. Carbon inputs are supplied via crop residues or from the roots of growing plants. This therefore means that soil biota dynamics needs to be well understood for its proper management.

2.6 Influence of organic and mineral fertilizers on soil microbial communities

Microbial communities are affected by soil moisture, pH, temperature, C:N ratio and availability and quality of organic substrates (Houston, Visser & Lautenschlager 1998). Arnold et al., (1999) noted that soil microbial biomass and diversity are influenced by seasonal patterns of soil temperature, moisture and substrate availability.

In agricultural soils, C can be supplied to below ground communities from litter fall and roots of the growing crop and organic amendments. Organic amendments such as green and animal manures influence soil biota immediately through increased food supply, and indirectly through changes in soil chemical and physical variables (Kautz, Lopez-Fando & Ellmer, 2006). Direct effects of litter inputs include an increase in biomass compared with the biomass supported by living plants alone and changes in the trophic structure and dynamics of the decomposer food webs whereas indirect effects such as alterations in habitats may also alter community structures by supporting some species and inhibiting others (Moore et al., 2004). In addition green manures or crop residue inputs have been shown to increase the size, biodiversity and activity of soil microbial populations (Bolton et al., 1985; Martens, Johanson & Frankeberger, 1992; Kirchner, Wollum & King, 1993, Fauci & Dick, 1994, Kautz, Wirth & Ellmer, 2004). Maize stover residues for instance contain various amounts of

polysaccharides, simple sugars, amino acids, proteins, phenols, and waxes. Some of these compounds decompose quickly, others slowly; some are transformed into other compounds while some become biological 'glue' from microbes or earthworm feces that combine mineral and organic particles into larger aggregates thus contributing to soil aggregation. For example, a study of maize stover residues reported about 29% soluble organic compounds, 27% hemicellulose, 28% cellulose, 6% lignin, 9% ash, and 10% nitrogen (Buyanovsky, Brown & Wagoner, 1997). Although only few studies exist on the specific effects of crop residues and green manures on soil fauna (Thorup-Kristensen, Magid & Jensen, 2003), these inputs can be expected to increase the abundance of soil fauna.

Further changes in substrate quality can affect the relative abundance of bacteria and fungi. Materials of high C:N ratios favour colonisation by fungi while more labile materials with low C:N ratios favour bacteria (Moore et al., 2004). With these changes come shifts in the abundances of bacteria and fungi (Moore et al., 2004). It is reported that under organic farming regimes with lower inputs of mineral fertilizers, there is greater soil microbial activity and more soil meso-fauna and earthworms than under higher input of mineral inputs (Edwards, 1998). Results from studies on the effects of added mineral N on microbial activity in combination with organic residues-amended soils have generally ranged from stimulatory, no effects to depressive effects. Microbial N uptake has often been reported to be faster than plant uptake and therefore addition of high C:N ratio on organic residues can induce temporary N deficiency in plants (TSBF, 1999). This is mainly attributed to increased soil microbial biomass build up in response to more available C (Palm, Myers & Nandwa, 1997).

Despite the recognized role of soil microbial communities in nutrient dynamics of soils, there are few published literature regarding soil microbial diversity measurements in sub-Saharan

Africa (Henrot & Robertson, 1994). This is because only a few studies have been conducted on the microbiology of tropical soils and most of these are confined to culture dependent techniques of which only a small percentage of the microorganisms is culturable (10%). Ayuke (2000) has investigated the soil biodiversity of Kabete long-term experiment, however, this study only concentrated on the macro organisms such as the termites, earthworms, beetles and weevils thus little information is available on the soil micro organisms in relation to agricultural management. Moreover, to a large extent there is still a lot that is unexplored on the impact of the organic and mineral inputs that farmers use on the below ground microbial communities.

2.7 Molecular techniques for soil microbial diversity Analyses

While analysis of microbial diversity in soil is one of the most important aspects of microbial ecology, knowledge about structure and dynamics of microbial communities in soil has been limited because only a small fraction of the communities' diversity is accessible to culturable methods (Giovannoni et al., 1990). It is known that the portion of microbial diversity estimated through conventional culture techniques amounts to only 0.1 to 10 % of the diversity, indicating that techniques based on laboratory cultivation known as "great plate count phenomenon" might be biased (Giovannoni et al., 1990).

These problems have resulted in the popularity of molecular approaches for studying soil microbial populations under the emerging discipline of molecular microbial ecology which uses molecular biology to investigate the ecology of microorganisms bypassing the cultivation techniques and uses. However, molecular techniques have largely been superceded by polymerase chain reaction (PCR) technology due to its high sensitivity and specificity. PCR-based fingerprinting methods give high resolution and provide information

about changes in the whole microbial community structure in the soil. Some of these techniques include; Restriction fragment length polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Low-Molecular-Weight (LMW) RNA fingerprinting, Temperature Gradient Gel Electrophoresis (TGGE) and Denaturing Gradient Gel Electrophoresis (DGGE).

2.7.1 Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is a molecular method of genetic analysis that allows individuals to be identified based on unique patterns of restriction enzymes cutting in specific regions of DNA. The term polymorphism refers to the slight differences between individuals in base pair sequences of common genes. Even though all members of a species have essentially the same genetic makeup, these slight differences account for variations in phenotype (that is appearance and metabolism) between individuals (Lee & ward 1990).

In RFLP, 16S rDNA fragments are generated by PCR using general primers digested with restriction enzymes, electrophoresed in agarose or acryl amide gels, and stained with ethidium bromide or silver nitrate. The number of fragments and relative sizes is then determined (Massol-Deya et al., 1995). The pattern of fragment sizes differs for each individual tested. Advantages of the technique are (1) highly robust methodology with good transferability between laboratories, (2) codominantly inherited and, as such, can estimate heterozygosity, (3) no sequence information required, (4) highly recommended for phylogenetic analysis between related species, and (5) well suited for constructing genetic linkage maps Locus-specific markers which allow synteny studies. The major disadvantages of estimating microbial diversity by RFLP are: large amount of DNA is required, low levels of polymorphism in some species is experienced and it is time consuming especially with

single-copy probes. Furthermore, the technique is costly and moderately demanding technically.

2.7.2 Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA (RAPD) markers are DNA fragments from polymerase chain reaction (PCR) amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence. RAPD has been used to follow the response of different soil microbial communities to the application of 2, 4-D (Xia Bollinger & Ogram., 1995).

Unlike traditional PCR analysis, RAPD does not require any specific knowledge of the DNA sequence of the target organism. However, the technique uses short (5-10 nucleotides) random primers which anneal at different sites of the genomic DNA generating PCR products of various lengths (Williams et al., 1990). The identical 10-mer primers will or will not amplify a segment of DNA depending on positions that are complementary to the primers' sequence. For example, no fragment is produced if primers annealed too far apart or 3' ends of the primers are not facing each other. Therefore, if a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced resulting in a different pattern of amplified DNA segments on the gel. Selecting the right sequence for the primer is very important because different sequences will produce different band patterns and possibly allow for a more specific recognition of individual strains. Reproducibility is the main problem of this technique; mismatches between the primer and the template may result in the total absence of PCR product as well as in a merely decreased amount of the product. Thus, the RAPD results can be difficult to interpret. Further differences in the quantity of template DNA and in the concentration of $MgCl_2$ and primer may result in different patterns (Hadrys, Balick, & Schierwater, 1992). Furthermore, no

phylogenetic information can be obtained from the bands. However, RAPD analysis is fast, highly variable and relatively inexpensive. The conditions for direct amplification of DNA in natural habitats by RAPD techniques must apparently be optimized.

2.7.3 Low-Molecular-Weight (LMW) RNA fingerprinting

Low-Molecular-Weight (LMW) RNA is one of the molecular techniques that has been used for more than a decade in profiling of low molecular weight RNA. In this technique, total RNA is extracted from an environmental sample and separated by high-resolution polyacrylamide gel electrophoresis. Subsequently, the profiles are scanned and stored in an electronic database for comparison. LMW RNA profiling has been used to monitor bacterial population dynamics in a set of freshwater mesocosms after addition of non-indigenous bacteria and culture medium (Hofle 1992).

2.7.4 Temperature Gradient Gel Electrophoresis (TGGE)

To understand TGGE, there are two fundamental points. The first is how the structure of DNA changes with temperature and the second is how these changes in structure affect the movement of DNA through a gel. At room temperature, in the presence of at least a mM of salt, the double stranded form is quite stable and the molecule can be considered to be two strings tightly wrapped about each other so that there are effectively two ends. DNA is a negatively charged molecule (anion) and in the presence of an electric field, it moves to the positive electrode (Muyer & Smalla, 1998). A gel is a molecular mesh with holes roughly the same size as the diameter of the DNA string. In the presence of the electric field, the DNA moves through the mesh and for a given set of conditions, the speed of movement is roughly proportional to the length of the DNA molecule — this is the basis for size dependent

separation in standard electrophoresis. As the temperature raises, the two strands of the DNA start to come apart; this is known as melting. At some high temperature, the two strands completely separate. However, at some intermediate temperature, the two strands are partly separated with part of the molecule still double stranded and part single stranded. TGGE is useful in that the mobility of the DNA molecule through the gel decreases drastically when these partially melted structures are formed, and, most important, the exact temperature at which this occurs depends on sequence. Thus, D/TGGE offers a "*sequence dependent, size independent method*" for separating DNA molecules. TGGE uses a uniform concentration of denaturant in the gel and temperature is increased uniformly with time throughout the electrophoresis (Muyer & Smalla, 1998).

One of the strongest points of TGGE is that the bands can be excised from the gel and subsequently sequenced to reveal the phylogenetic affiliation of the community members. Within short time, TGGE and DGGE have become very popular in microbial ecological studies and they are now routinely used in many laboratories. However, it is not always possible to separate fragments with different sequences because of similar melting behavior of the fragments (Muyer & Smalla, 1998).

2.7.5 Denaturing Gradient Gel Electrophoreses technique

Denaturing gradient gel electrophoresis (DGGE) works by applying a small sample of DNA (or RNA) to an electrophoresis gel that contains a denaturing agent. DGGE is based on the principle that increasing denaturants melts double-stranded DNA in distinct domains. When the melting temperature (T_m) of the lowest domain is reached, the DNA partially melts creating branched molecules and reducing its mobility in a polyacrylamide gel (Miller et al., 1999). The denaturing environment is created by a uniform run temperature between 50 and

65° C and a linear denaturant gradient formed with urea (ranging from 0-7 M) and Formamide.

Complete strand separation is prevented by the presence of a high melting domain which is usually artificially created at one end of the molecule by incorporation of a GC clamp. This is accomplished during PCR amplification using a PCR primer with a 5' tail consisting of a sequence of 40 GC.

During DGGE, PCR products of different sequence encounter increasing higher concentrations of chemical denaturants as they migrate through a polyacrylamide gel. Upon reaching a threshold denaturant concentration, the weaker melting domains of the double-stranded PCR product begin to denature at which time migration slows dramatically. Double-stranded DNA fragments migrate better in the acrylamide gel while denatured DNA molecules become effectively larger and slow down or stop in the gel (Muyzer, Waal & Uitterlinden, 1993). In this manner, DNA fragments of differing sequence can be separated in the acrylamide gel.

Differing sequences of DNA (from different bacteria) denature at different denaturant concentrations resulting in a pattern of bands. Each band theoretically representing a different bacterial population present in the community. Once generated, fingerprints can be assessed to determine microbial structural differences between environments or among treatments. With the breadth of PCR primer available, DGGE can also be used to investigate broad

phylogenies or specific target organisms such as soil bacteria and fungi or pathogens (Muyer & Smalla., 1998)

Despite the fact that DGGE produces results that are more accurate and reliable than previous gel electrophoretic techniques, the reality is that there are a number of limitations inherent in this technique. "Chemical gradients such as those used in DGGE are not as reproducible, they are difficult to establish and often do not completely resolve heteroduplexes (Muyer & Smalla., 1998). However, DGGE is perhaps the most commonly used among the culture-independent fingerprinting techniques due to its advantages which include: high detection rate and sensitivity, an overview of the diversity, opportunity to get some more sequence information by excision of bands, with DGGE multiple samples can be tested simultaneously and the method is simple and involves non-radioactive detection.

The only disadvantages of DGGE are that the purchase of DGGE equipment is expensive and that PCR fragments of over 400bp are less successful thus limiting the design of probes for hybridization analysis (Fisher & Lerman, 1983).

Although many studies have been done on the role of microorganisms in soil chemical and physical properties, few of these studies have been based in the tropics and more specially in sub Saharan Africa. More over the few studies that have been carried out have been based on culture-based techniques. This study therefore assessed the long-term effects of organic and inorganic inputs by use a molecular technique.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site description

In order to assess the effect of management strategies on soil properties and microbial diversity, a study was superimposed on the on-going long-term field experiment at Kabete. The field experiment was established in 1976 to investigate the effect of continuous application of farmyard manure, crop residues and nitrogen (N) and phosphorus (P) fertilizers in a maize-bean rotation. The trial site is located at the National Agricultural Research Laboratories (NARLs) station at Kabete, Kenya. It is located at 36° 46'E and 01° 15'S and at an altitude of 1650 m above sea level (Kimetu, 2002). The soils are mainly humic Nitisols (FAO, 1990) that are deep and well weathered. The mean annual rainfall is about 950 mm received in two distinct annual rainy seasons; the long rains (LR) received between mid-March and June, and the short rains (SR) received between mid-October and December. The average monthly maximum and minimum temperature is 23.8° C and 12.6° C, respectively.

3.2 Experimental design

The long-term experiment was established in 1976 as a randomized complete block design (RCBD) with eighteen treatments (Appendix 1) replicated four times. Since this study was evaluating the effects of organic and mineral fertilizer application on the functional diversity of the soil microbial communities, only seven treatments in the original experiment receiving farmyard manure at 10 t ha⁻¹, maize stover all residue returned, mineral fertilizer N at 120 kg ha⁻¹ and P and a control (without any treatment) were monitored. The selection of these treatments was based on the highest crop yields from the KARI yields database (Kibunja et al., 2002).

Table 3.1: Selected Treatments at the Kabete Long- term Experiment

Treatment	Fertilizer treatment	Organic amendments
1. Control	0 kg N, 0 kg P	None
2. Maize Stover (R)	0 kg N, 0 kg P	Maize Stover (all harvest returned)
3. FYM	0 kg N, 0 kg P	10 t / ha farmyard manure
4. N+P	120 kg N, 52.8 kg P	None
5. N+P + R	120 kg N, 52.8 kg P	Maize Stover(all harvest returned)
6. FYM + N+P	120 kg N, 52.8 kg P	10 t / ha farmyard manure
7. Fallow	Fallow	Fallow

Key: R = Maize stover; N = Nitrogen; FYM = Farmyard manure; P = Phosphorus

3.3 Soil sampling

Soil samples were collected six weeks after planting following the ‘W’ design (Appendix 3), across the plots receiving treatments of interest by pushing a sterile soil auger to a depth of 0-10 cm. Six soil cores were taken from each replicate plot and pooled to make a composite sample. The soils were then stored in sterile zip lock plastic bags and transported in cooler boxes to the laboratory where they were stored at -80°C for laboratory analysis. Physico-chemical analysis was done after soil drying, grinding and successive sieving at 0.25 mm sieve.

3.4 Laboratory analysis

3.4.1 Soil analysis

3.4.1.1 Total Nitrogen in the soil

Total nitrogen was analyzed following the Kjeldahl method (Anderson & Ingram, 1993). About 0.4 g of soil was ground to pass 0.3mm mesh and then digested with 7.5mL of the digestion mixture (selenium powder + concentrated sulphuric acid + salicylic acid) and let to stand overnight. The following day the solution was heated at 100°C for one hour and then allowed to cool, then 1 mL aliquots of 30% hydrogen peroxide was added to the solution and the temperature raised to 250°C, heated for one hour, then raised to 330°C and heated for two hours. The solution was allowed to cool and then 70 mL of deionized water added and allowed to settle overnight. 5.0 mL of reagent N1 (Sodium salicylate 68 g + 50 g Sodium citrate + 50 g Sodium tartrate + 0.24 g Sodium nitroprusside mixed with deionised water to make 2 litres) was added to 0.2 mL of the digested solution in a test tube and left for 15 minutes. 5.0 ml of reagent N2 (60 g Sodium hydroxide + 20 mL Sodium hypochlorite + deionized water to make 2 liters) was added to the solution, mixed well and left for one hour for colour development. The absorbance reading was recorded at 655 nm using a spectrophotometer. The total N was calculated as:

$$\text{N concentration in soil (N \%)} = \frac{(\text{NCONC} - \text{NBLNK}) \text{ NVOL}}{\text{NSOLWT}} * 0.0001$$

where:

NCONC means N concentration in soil digest (mg/L)

NBLNK means N concentration in blank digest (mg/L)

NVOL means Total volume of diluted digest (ml)

NSOLWT means Soil sample weight (g)

3.4.1.2 Determination of soil pH

Ten milligrams of soil was scooped and added into a 60 mL bottle, then 25 mL distilled water was added with a dispenser. The solution was then stirred for 10 minutes and then let to stand for 20 minutes. Immediately before measuring the pH of each sample, the sample was stirred for 5 seconds with a glass rod. The soil was allowed to settle for 30 seconds, then the electrode immersed into 60mL bottle with soil, and pH reading was recorded after the readings stabilized (about 30 seconds to 1 minute). The electrode was then removed from the bottle and rinsed with distilled water. All samples were handled the same way.

3.4.1.3 Determination of total soil carbon

About 1.0 g soil sample ground to pass through a 0.3 mm mesh was weighed and put in a test tube and 2 mL deionized water added into the soil sample. Ten mL 5% potassium dichromate solution and 5 mL concentrated sulphuric acid were then added consecutively and the mixture was then digested at 150°C for 30 minutes. The mixture was then allowed to cool and 50 mL 0.4% barium chloride added to it; this was then shaken and allowed to stand overnight. The concentration was then read at 600 nm on the spectrophotometer and total organic carbon was the calculated as:

Total soil organic carbon (C %) =

$$\frac{(CCONC-CBLNK)(0.1)}{CSOLWT}$$

CSOLWT

where:

CCONC means carbon content of sample (mgC)

CBLNK means carbon content of blank (mgC)

CSOLWT means dry weight of soil sample (g)

3.4.1.4 Extractable soil nitrate

Twenty (20) g of field moist soil was extracted using 100ml 2 N KCl by shaking for one hour at reciprocations per minute. This was followed by gravimetric filtration through a pre-washed Whatman No. 5 paper. The resulting extract was analyzed for extractable nitrate using cadmium reduction with subsequent calorimetric determination (Dorich & Nelson, 1984; Anderson & Ingram, 1993). 1 mL concentrated ammonium chloride was added into the Cd column and 3 mL of sample pipetted into the column and the solution was then drained (into a test tube containing 5 mL sulphanilic acid reagent) almost to the top of the granules, leaving 2 mm of the solution on top. The reservoir was then rinsed with approximately 2 mL dilute ammonium chloride solution and again drained into the test tube almost to the top of granules leaving 2mm solution on top. Forty five (45) mL of dilute ammonium chloride was then added to the reservoir and drained within 25-30 seconds leaving approximately 2mm of the solution on top of the column. The test tube was then removed, stoppered and shaken well and let to stand for at least 5 minutes after which 5 mL of 5-amino-2-naphthalene sulphanilic acid (5-2 ANSA) solution was added, stoppered and well shaken. The solution was let to stand for 30 minutes and the absorbance read at 525 nm using a spectrophotometer. The soil extractable nitrate was then calculated using the formula:

Nitrate –N content of soil (kg N/ha) (EXNNKGA):

$$\text{EXNNMGKG (BD) SD/10}$$

where:

EXNNMGKG (Nitrate concentration in soil, mg/N/kg) =

$$((\text{EXNCONC} - \text{EXNBLNK}) (100 + (\text{EXNSLWT} - \text{EXNDSWT}))) / \text{EXNDSWT}$$

where: EXNNMGKG = Nitrate concentration in soil (mg/N/kg)

EXNCONC = Nitrate concentration for sample (mg N/L)

EXNBLNK = Nitrate concentration for blank (mg N/L)

BD= bulk density g/cm³)

SD = Depth of soil layer (cm).

3.4.2 Deoxyribonucleic Acid (DNA) extraction from soil samples

Total genomic DNA was extracted from all soil samples by use of direct lysis extraction procedure (Laurent et al., 2001). A subsample of 250 mg of soils from each treatment was taken. To this sample, 0.5 g glass beads (106µm diameter, Biospec Products) and 1 mL lysis buffer (100mM Tris-HCl (pH 8.0), 100mM NaCl, 1% (w/v) polyvinylpolypyrrolidone (PVPP), and 2% (w/v) sodium dodecyl sulfate (SDS) was added in a 2 ml Eppendorf tube (Figure 1). The samples were then homogenized for 30 seconds at 1600 rpm in a mini-bead beater cell disruptor after which the samples were centrifuged at 14,000 g for 1 min at 4° C. The supernatant was then collected and incubated in ice for ten minutes with 1/10 volume of 5M sodium acetate and centrifuged at 14,000 g for 5 minutes. After precipitation with one volume of ice-cold isopropanol, the nucleic acids were washed with 70 % ice-cold ethanol and dissolved in double distilled water (Laurent et al., 2001). The quality and the size of DNA were checked by electrophoresis on 1 % agarose gels. The DNA was stored at -20° C for further analysis.

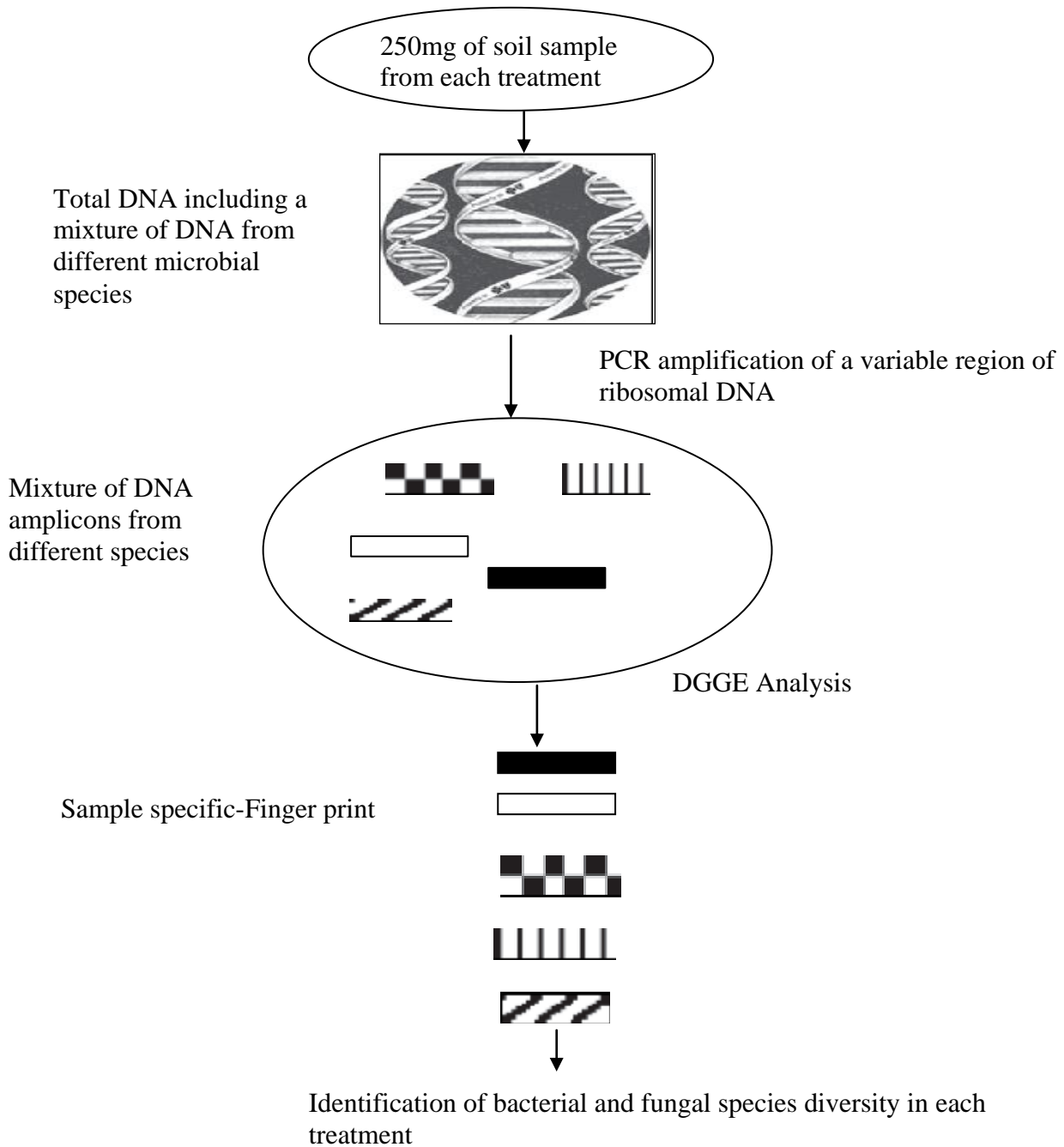


Figure 3.1: Flow diagram of the application of PCR-DGGE analysis

Source; Ercolini et al., 2004

3.4.2.1 DNA amplification by Polymerase Chain Reaction (PCR)

The PCR process, which provides an extremely sensitive means of amplifying small quantities of DNA, was used to make copies of DNA in order to produce enough DNA for DGGE analysis (Figure 1). The DNA template obtained (as described above) was amplified by using primer pairs (Table 2). For total bacterial studies a standard touchdown PCR procedure was used as described by Komi (2006) while for the denitrifying community amplification, a different procedure as described by Throback et al. (2004) was used. For fungal studies, a different PCR procedure was used as described by Sigler et al. (2002). A Primus 96^{plus} PCR system (MWG AG BIOTECH) thermal cycler was used for all the PCR procedures. PCR products were then visualized under UV illumination using the BIORAD Gel Documentation System 2000.

Table 3.2: Primer pairs used for PCR-DGGE

Primer	Target	Sequence (5'-3')
Gc-338F	Bacteria	5'ACT CCT ACG GGA GGC AGC AG 3'
518 R		5'ATT ACC GCG GCT GCT GG 3'
403 F	Fungi	5'-GTG AAA TTG TTG AAA GGG AA-3'
Gc-662 R		5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GGA CTC CTT GGT CCG TGT T-3'
nirK R cu-Gc	Denitrifiers	5'GGC GGC GCG CCG CCC GCC CCG CCC CCG TGC-3'
nirK Flacu		5'ATC ATG GT5 CTG CCG CG-3'
nir S Rcd-Gc	Denitrifiers	5'GGC GGC GCG CCG CCC GCC CCG CCC CCCG TCG CCC GA5 TTC GGR TG5-3'
nir Scd3af		GT5 AAC GT5 AAG GAR AC5 GG-3'

3.4.2.2 PCR amplification of bacterial 16S rDNA gene

PCR amplifications were performed using the forward primer 338f with a GC clamp and reverse primer 518r (Table 3. 2). The total reaction mixture (25 μ l) contained 2 μ l of pure total DNA extract, 1X freeze dried bead (Ready-to-Go PCR beads, Pharmacia Biotech) containing 1.5 U of Taq polymerase, 10mM Tris-HCl, (pH 9 at RT), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M of each dNTP and 1.0 μ M of each primer and sterile distilled water. Two replicates were performed for each sample. A Primus 96^{plus} PCR system (MWG AG BIOTECH) thermal cycler was used for PCR amplification with 2 min at 94°C followed by 30 cycles of 30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C. The first 20 cycles had an annealing temperature of 65°C, which decreased every cycle until a touchdown at 55°C. The primer extension was carried out at 72°C for 15 min. The PCR product (approximately 180 bp) was checked on a 1% agarose gel stained with ethidium bromide.

3.4.2.3 PCR amplification of fungal 28S rDNA gene

PCR amplifications were performed using the forward primer 662f with a GC clamp and reverse primer 314r (Table 2). PCR amplifications were carried out in 25- μ l reaction volumes with a Primus 96^{plus} PCR system (MWG AG BIOTECH). The reaction mixture was slightly modified by reducing the quantity of template DNA from 2.0 μ l to 1.0 μ l. Cycling conditions were as follows: initial denaturation at 95°C for 10 minutes followed by 49 cycles of denaturation at 95°C for 1 minute, annealing at 50°C for 1 minute, and extension at 72°C for 2 minute followed by a final extension phase at 72°C for 10 minutes. The PCR product (approximately 348 bp) was checked for size on a 1% agarose gel stained with ethidium bromide.

3.4.2.4 PCR amplification of nitrite reductase (nir K and nir S) gene

Primer pairs, cd3aF:R3cd and F1aCu:R3Cu were used for amplifying the Nir S and nir K genes respectively (Table 2). The total reaction mixture (25 µl) contained 1.5 µl of pure total DNA extract, 1X freeze dried bead (Ready-to-Go PCR beads, Pharmacia Biotech) containing 1.5 U of Taq polymerase, 10mM Tris-HCl, (pH 9 at RT), 50 mM KCl, 1.5 mM MgCl₂, 200 µM of each dNTP and 1.0 µM of each primer and sterile distilled water for nir S. Two replicates were performed for each sample. For nir K the reaction mixture was slightly modified by reducing the quantity of template DNA from 2.0 µl to 0.5 µl. A Primus 96^{plus} PCR system (MWG AG BIOTECH) thermal cycler was used for PCR amplification. All primer pairs amplifying gene fragments of nir S and nir K were run with initial denaturation of the DNA at 94°C for 2 minutes, followed by 35 cycles of 30 S at 94 °C, 1 minute at 57°C and 1 minute at 72°C. The reaction was completed after 10 minutes at 72°C.

3.4.3 Denaturing Gradient Gel Electrophoresis

Denaturing Gradient Gel Electrophoresis (DGGE) was done using the DCodeTM Universal Mutation System (*Bio-Rad*). Sixteen ml of each low (40%) and high (60%) denaturing solutions were prepared with urea and formamide denaturants mixed with acrylamide solution. In the acrylamide gel, the denaturing conditions were provided by urea (CH₄N₂O) and formamide (CH₃NO). The 32 ml solutions were poured with the aid of the gradient maker to the top of the glass plates. After the gel was poured, combs were carefully inserted without trapping air bubbles under the wells. The gel was then left for one hour to set.

The PCR amplification products (23 µl) were loaded onto the 8% (w/v) bisacrylamide gels. The gels were electrophoresed in 1 x TAE buffer consisting of (0.2w/v Tris base; 0.06v/v glacial acetic acid; 0.2v/v EDTA 0.5M pH 8.0). The temperature of the DCodeTM Universal

Mutation System (*Bio-Rad*) was set to 60°C and run at 20V for 10 minutes, then run at 75V for 16 hours. The gels were stained for 30 minutes in 100 ml of sterile deionized distilled water containing 10 µl of 10M ethidium bromide and destained in 100 ml of sterile deionized distilled water. The gels were visualized under UV illumination using the BIORAD Gel Documentation System 2000.

3.5 Data analysis

The DGGE patterns were transformed to graphs by the Bio-Rad Quantity One™ software where each resolved band of the gel lanes was converted to a trait whose intensity is related to the amount of the corresponding DNA fragment. Total Lab 120 version 2006 software (Nonlinear Dynamics Ltd) was used to calculate the percentage of similarity among lanes taking into account the migration distance and the relative intensity of all bands. The DGGE profiles were compared and unweighted-pair group method with mathematical averages (UPGMA, Eichner et al., 1999) and cluster analysis method was used to produce the dendograms. Dendograms were then used to characterize shifts in microbial population under the different organic and mineral inputs. Microbial diversity was determined according to the Shannon diversity indexes (H') (Shannon & Weiner, 1949) using the following equation:

$$H' = -\sum p_i \log p_i$$

Where:

$$p_i = n/\sum n$$

n = the volume of a single band per lane.

$\sum n$ = total volume of all bands per lane.

The Shannon indexes were then used to perform ANOVA analysis using GENSTAT statistical software and treatment means separated using LSD at ($p \leq 0.0001$). Data on soil

properties was also subjected to ANOVA using GENSTAT 5 for windows (Release 4.1) computer package.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Soil characterization for selected treatments at Kabete long-term experiment

The soil properties at the start of the experiment in 1976 (Qureshi, 1987) and 32 years later (2002) were compared. The soil pH in all treatments was below 5.5 (Table 4.1), which was the pH of the soil in 1976 at the on set of the experiment (Qureshi 1987). The fallow had a slightly higher pH of 6.31 compared to all other treatments. The control and the N+P-treated soils had the lowest pH of 5.07 and 5.09, respectively (Table 4.1). This shows that soils were generally acidic which could be attributed to loss of cations as a result of continuous maize harvesting with no returns for the control plot. The low pH in the N+P could be attributed to the crops, absorbing more ammonia nitrogen than nitrified nitrate and in the process, roots of 4 regards to N content, highest N content was found in the fallow and in N+P+FYM treated soils with 0.22 % and 0.18 %, respectively while lowest N content was found in the N+P treated soils and the control (Table 4.1). Highest C content on the other hand, was also found in the fallow and the N+P+FYM treated soils with 2.8 % and 3.0 % C, respectively. The control had the lowest C and N content of 1.8% and 0.14 % respectively. Application of different organic sources positively influenced the physicochemical properties of soil over the inorganic fertilizers (Table 4.1). Combination of farmyard manure with nitrogen and phosphorus fertilizers increased the nutrient availability. Among the different organic and inorganic sources, application of farmyard manure with nitrogen and phosphorus fertilizers recorded maximum organic carbon (0.78%) over the inorganic fertilizers. It might be due to higher humus content of this treatment compared to others.

Table 4.1: Soil characterization of selected treatments at Kabete long-term experiment

Soil properties	2008							
	1976†	Control	Maizestover	FYM	N+P	N+P+R	N+P+FYM	Fallow
pH (1:2 in H ₂ O)	5.5	5.07	5.16	5.41	5.09	5.23	5.45	6.31
SOC (%)	2.0	1.8	2.0	2.0	1.9	2.0	3.0	2.8
Total N (%)	0.16	0.14	0.15	0.16	0.14	0.16	0.18	0.22
NO ₃	na	8.95	8.48	7.75	11.28	9.27	10.15	23.6
C:N	na	13.0	13.6	12.5	13.4	13.1	12.6	12.6
Soil texture (%)	Sand	clay	clay	clay	clay	clay	clay	clay

† Source of 1976 data: Qureshi, 1987.

Control –No input, FYM-farmyard manure at 10 t ha⁻¹, N+P-Chemical fertilizer at 120 kg N ha⁻¹ and 26.4 kg P ha⁻¹, N+P+ FYM-chemical fertilizer at 120 kg N ha⁻¹ and 26 P ha⁻¹ and farmyard manure at 10 t ha⁻¹, na- not available

The C:N ratio was similar in almost all treatments and it ranged from 12.5 to 13.6. The high C and N concentrations observed with the N+P+FYM treatment could be attributed to the long-term application of manure in the Kabete long-term experiment. This is because nutrient release from manure is low but can persist through several seasons. This could also be attributed to the high percent carbon in manure treatments (Table 4.1). Olsen (1986) observed high maize yield under manure treatment suggesting that the release of nutrients from manure persists throughout the growing period. The findings are also in agreement with those of Kavoo (2008) who reported higher N concentration in manure treated soils while the control had the least N content under similar site conditions. Kang (1993), Schoroth et al. (1995) and Mugendi et al. (1999) reported a general reduction in soil pH, total N and total P after application of fertilizers and continuous cropping over time.

The decline in soil pH in all treatments with an exception of the fallow which has not been cultivated for over thirty years could be due to export of crop products, nitrification, and nitrate leaching which leads to soil acidification (Helyer 1989). Helyar et al. (1989) reported that export of crop products, nitrification and nitrate leaching can lead to soil acidification. The greater soil pH decrease in the sole application of mineral inputs (N+P) compared to the integration of organics and mineral fertilizers could be as a result of the additions of H⁺ ions on the cation exchange sites of soils from the mineral fertilizers. Tisdale et al. (1993) reported that mineral fertilizers add H⁺ ions to the exchange sites of the soil leading to lower soil pH. (Drinkwater, Wagoner & Sarrantonio.,1998) and Werner (1997) observed that changes in soil properties under organically and conventionally managed farming systems are more variable which could be due to differences in climate, crop rotation, soil type, or length of time a soil has been under a particular management.

4.2 Total bacterial DGGE-finger prints as affected by organic and mineral fertilizers

PCR-DGGE profiles of the total bacterial communities for each of the selected treatments at the Kabete long-term experiment are shown in Figure 2. Analysis of the four replicates of each treatment showed good reproducibility of DGGE banding patterns; however, only two replicates of each treatment are shown in the DGGE profiles (Figure 4.1). DGGE banding patterns were quite similar among all treatments for the bacterial communities except one dominant band that was observed in the N+P+R-treated soil. On the other hand, few visible bands were noted in plots treated with farmyard manure alone while the control had the least number of bands (Figure 2). Farmyard manure amendments produced considerable changes in bacterial community structure as illustrated in Figure 2. Treatments with farmyard manure and maize stover indicated disappearance of some bands and appearance of others as compared to the control and the fallow (Figure 4.1).

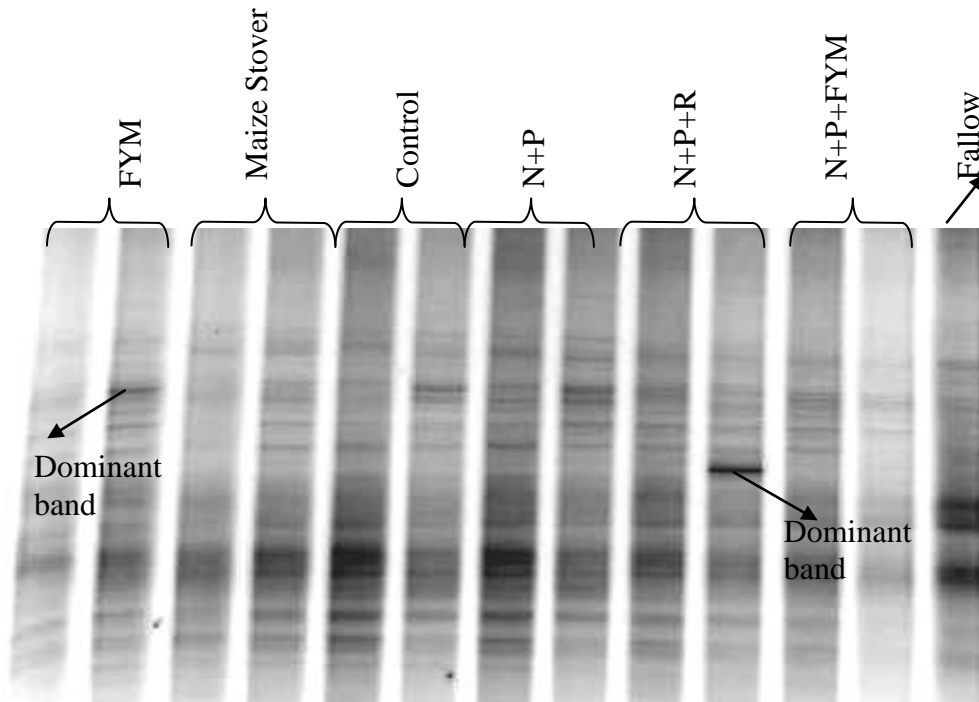


Figure 4.1: DGGE profiles of 16S rDNA gene in representative replicates of soil samples from Kabete long-term experiment.

The different profiles in the treatments with farmyard manure suggested that shifts induced in the bacterial community structure were mainly due to the amendment of the soil with new community members that originated from the FYM. The high number of bands in farmyard manure could have been a direct effect from the manure as an important source of nutrients for microorganisms, its addition to the soil generally enhanced the development of microfauna and microflora communities and microbial activity of the soils hence reactivating the dormant microbial species in the soil (Bailey & Lazarovitis, 2003).

The different behaviors in the two organic residues (farmyard manure and maize stover) could be attributed to slow decomposition of farmyard manure at short term and mainly

acting as a long-term source of nutrients. On the contrary, maize stover, which is a medium quality organic residue, decomposed slowly because of a wider C: N ratio and high lignin and polyphenols contents that lead to lack of metabolites for microbial utilization and activities such as reproduction because of limiting N. This could have resulted to few dominant bacterial communities that are able to decompose lignins and polyphenols. Low N content of maize stover may have led to the lower number of bands (bacterial communities) as N limiting conditions reduced microbial activities. The results are in agreement with Tian et al. (1992) who observed that N limitation leads to lower bacterial communities due to reduced microbial activities in soils.

Generally, genetic finger printing by DGGE of eubacteria 16S rDNA amplified fragment showed few strong dominating bands and a number of faint unresolved bands (Figure 2). This could be attributed to the higher heterogeneity of bacterial communities than that of fungal communities. The change of bacterial community structure by organic inputs has been observed in several long-term field experiments (Marshner, Kandeler, & Marshner 2003; Sun, Deng & Raun 2004) which are in agreement with the findings of this study.

4.2.1 Cluster analysis of total bacterial communities

To assess the microbial diversity based on the DGGE patterns of the 16S rDNA total bacteria genes, two different methods were used: (i) cluster analysis, and (ii) the Shannon- Weaver index of diversity, (H). Cluster analysis of the DGGE patterns for total bacteria is shown in Figure 4.2.

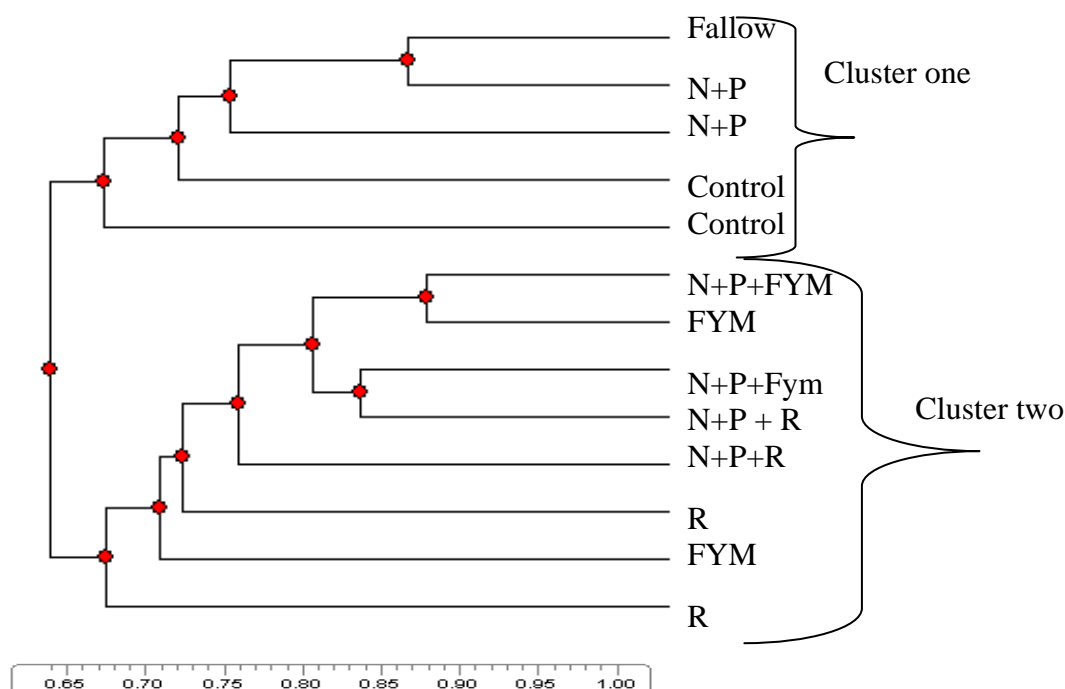


Figure 4.2: Cluster analysis of 16S rDNA gene in representative replicates of soil samples from Kabete long-term experiment.

The analysis showed great similarity (65%) with two major clusters: cluster one were those with (i) mineral fertilizers (ii) the control and (iii) the fallow. Cluster two had the treatments treated with organic inputs combined with mineral fertilizers of the organic inputs alone (Figure 4.2). This type of clustering was attributed to the differences in the soil pH, C, N, and C:N ratios. Within cluster one, the control and the fallow treatments further grouped away indicating a change in bacterial community structure. This could be attributed to the changes in the soil pH thus communities that were adapted to acidic and alkaline conditions as per the pH results of these two treatments ended up forming similar communities. A previous study by Duineveld et al. (1998) concluded that a similarity of more than 18% in cluster analysis indicates a significant change in community structure.

4.2.3 Statistical analysis of Shannon-Weaver indices of total bacterial communities

Statistical analysis of Shannon indices indicated that there were no significant differences between N+P+R, N+P+FYM and fallow treatments for total bacterial communities (Table 4.2). A significant ($P < 0.001$) difference was however noted between N+P+R and N+P+FYM treatments and N+P, control, maize stover and FYM with highest bacterial diversity found in the fallow followed by the N+P+FYM, while lowest diversity was found in the N+P treated soils. This may be attributed to the N+P + FYM and the fallow containing higher amount of growth promoting substances such as carbon, available nitrogen and improved physical and chemical soil properties which in turn increased the bacterial microbial population. This study is in agreement with that of Kannan et al., (2005) where application of different organic N sources had significantly increased the microbial build up of soil. In their study compost treated soils had five times higher bacterial populations compared to hundred percent urea treated soils.

Table 4.2: Means of Shannon Weaver indices for the Kabete long-term trial study on the total bacterial

Treatment	Bacterial community
Maize stover	1.007 ^b
Control	1.030 ^b
Fallow	1.245 ^d
FYM	1.045 ^{bc}
N+P	0.860 ^a
N+P+R	1.152 ^{cd}
N+P+FYM	1.192 ^d
Grand mean	1.076
SED(P<0.05)	0.0509
LSD(P<0.05)	0.0107

NB: Means followed by the same letter are not significantly different from each other (Duncan Multiple Range Test ($P < 0.001$)).

4.3 Effect of organic and inorganic inputs on fungal communities

Most of the fungal 28S rDNA gene bands were less numerous but distinct (strong) and well resolved as compared to the PCR-DGGE amplification of 16S rDNA gene of the total bacterial communities (Figure 4.3). Some bands for fungal communities were common to all treatments while others disappeared in some treatments and others appeared in others (Figure 4.3).

Sole application of inorganic inputs gave the lowest number of bands (Figure 4.3) while a combination of organic (maize stover and FYM) and inorganic inputs gave higher number of bands. Compared to all other treatments the fallow soils had the highest number of bands. Appearance of less numerous but strong bands in the fungal DGGE profiles would substantiate the hypothesis that a limited number of dominant and ecologically well-adopted fungal types were present in the long-term experiment.

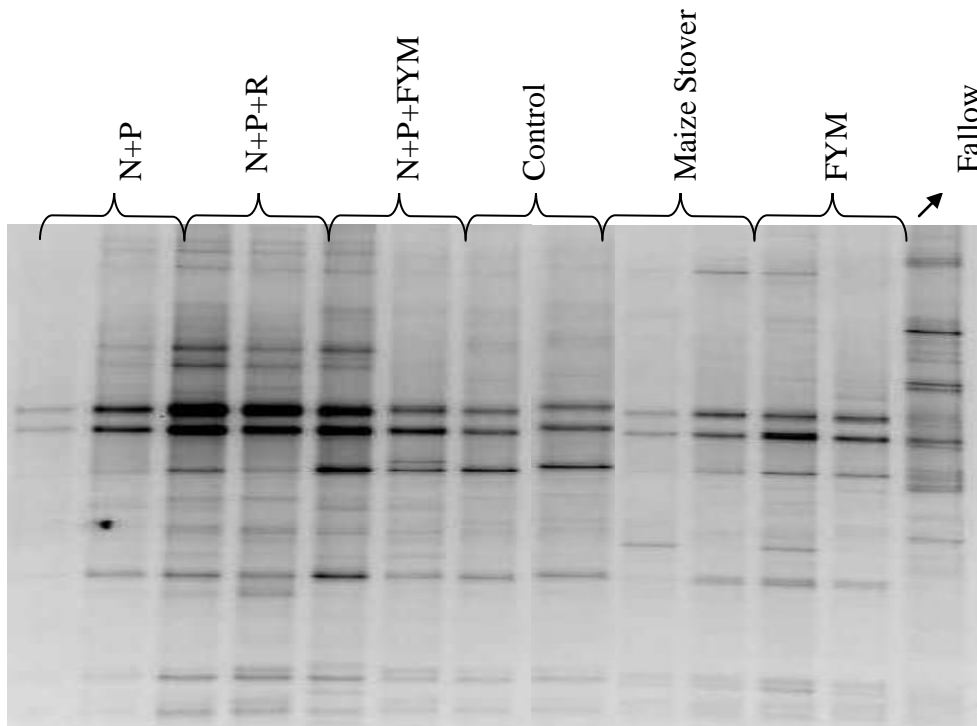


Figure 4.3: DGGE profiles of representative 28S rDNA gene analysis of soil samples from Kabete long-term experiment.

The high number of bands in the fallow could be attributed to the fact that the fallow had not been subjected to any sort of agricultural disturbance such as tillage and agrochemicals hence their high prevalence in the fallow land. Research on the impacts of disturbance such as tillage on agricultural soils suggests that soil disturbance decreases fungal biomass (McGonigle et al., 1996; Denef et al., 2001; Rillig et al. 2). Sieverding (1991) also found that in monoculture agroecosystems with conventional tillage and high application of soluble P and N, the number of fungal species decreased more than 50% compared to the native ecosystems. This is in agreement with the findings of this study.

4.3.1 Cluster analysis of fungal communities

Two major clusters were formed for the fungal communities (Figure 4.4) with a moderately lower percentage (55%) compared to the bacterial communities. Cluster one consisted of the

control, N+P+FYM, N+P+R and N+P with the sole mineral treated soils further clustering away from the main cluster. Cluster two consisted of FYM and maize stover-treated soils. This showed that organically treated soils clustered away from those with combined inorganic and organic inputs (Figure 4.4).

This kind of clustering showed that fungal community structures were positively affected by both organic and inorganic inputs. The difference in the clustering between the organically treated and inorganically treated soils suggested shifts induced in the fungal community structure were mainly due to the amendments of the soil with new community members originating from the farmyard and adopted to maize stover residues.

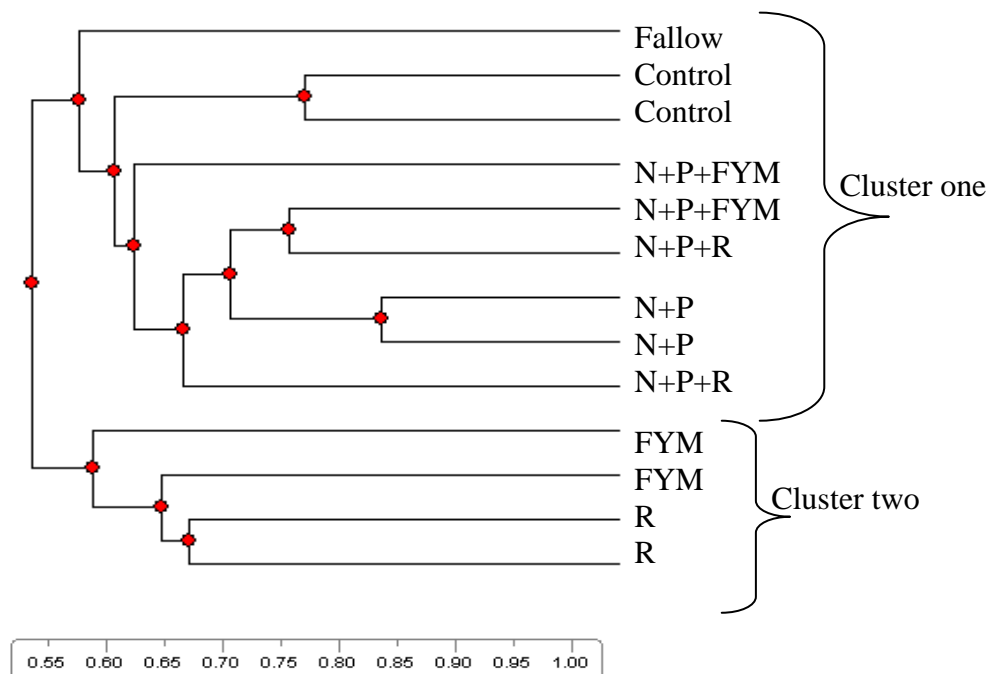


Figure 4.4: Cluster analysis of 28S rDNA gene of soil samples from Kabete long-term experiment.

Organic inputs are an important source of nutrients usable by decomposers especially the fungi communities. Bailey and Lazarovilis (2003) observed that FYM and maize stover amendments generally enhanced the development of soil microfauna and microflora hence

increasing microbial activity of the soils. Shifts in fungal and bacterial community structure following addition of different inputs have been reported in other studies (Peacock et al., 2001, Smith et al., 2001).

4.3.2 Statistical analysis of Shannon-weiver indices of fungal communities

No significant differences ($P < 0.05$) were found between N+P+R and N+P+FYM treatments on fungal communities (Table 4.3). Significant differences were however observed between fallow, control, maize stover, FYM and N+P (Table 4.3). These observations suggested that both organic and inorganic application affected fungal communities. Fungal communities' diversity was positively influenced by the combination of organic and inorganic resources since highest diversity (H) was found in these combinations. On the contrary, the control, maize stover and the fallow gave a low fungal diversity. This could be attributed to the low C and N available in the soils for the control and the maize stover treated soils. The low diversity in the fallow could be attributed to the theory that few fungal communities got adopted to the long fallow system hence only those that were persistent to long fallows without cultivation were able to survive and colonize the habitat. The findings of this study are also in accordance with those obtained by Parham et al. (2002) on the evaluation of the culturable fungal populations where high diversity of culturable fungal communities was found in the soils treated with a combination of organic and mineral inputs.

Table 4.3: Means of Shannon Weaver indices of the fungal communities in Kabete long-term trial

Treatment	Fungal community
Maize stover (R)	0.545 ^a
Control	0.565 ^a
Fallow	0.580 ^a
FYM	0.627 ^b
N+P	0.705 ^c
N+P+R	0.835 ^d
N+P+FYM	0.852 ^d
Grand mean	0.673
SED (p<0.05)	0.017
LSD (p<0.05)	0.365

Means followed by the same letter are not significantly different from each other (Duncan Multiple Range Test (P<0.05)).

4.4 Effects of organic and inorganic resources on denitrifying bacteria

The fingerprints analysis revealed that the composition of the denitrifying community (nir S and nir K) were clearly different for each treatment (Figures 4.5 and 4.6). However, the nir K and nir S DGGE fingerprints displayed similar patterns among the four replicate field plots within treatments. Different patterns by DGGE analysis of nir K genes were distinguished from the seven treatments. Plots treated with N+P, N+P+FYM and N+P+R exhibited similarities (Figure 4.5) while plots fertilized with FYM and maize stover exhibited different patterns. This was attributed to the introduction of chemical fertilizers since denitrifyer communities have different physiological sensitivity. The fallow plot had the most different pattern visible with the naked eyes.

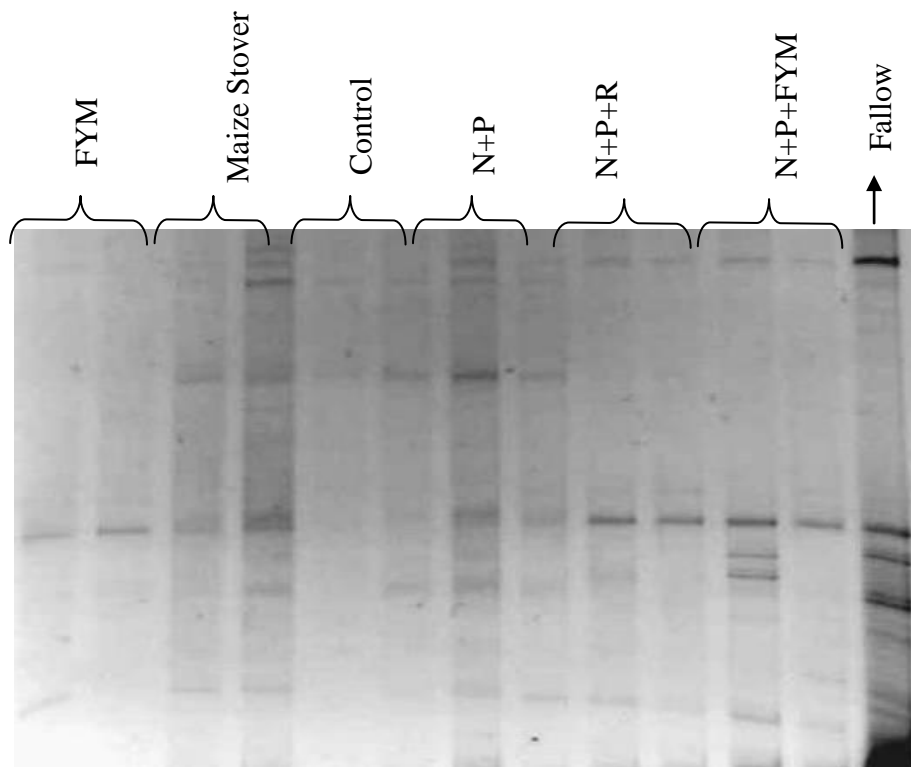


Figure 4.5: DGGE profiles of nir K rDNA gene from Kabete long-term experiment.

The different pattern in the fallow could be associated to the high soil NO_3^- in the fallow compared to all other treatments. Soil NO_3^- is an important substrate for denitrification hence increased soil NO_3^- can result in increased denitrifying communities (Smith et al., 1998).

In contrast to nir K, DGGE analysis of partial nir S genes resulted in only a few bands with one dense band dominating in the top of the gel (Figure 4.5). The few bands could be an indication of a less denitrifying community of this particular species in the soils. Unlike the nir K genes, the nir S gene gels were not apparent thus, only a few bands were visible by eye. There were no significantly different pattern types exhibited by the DGGE profiles as most of the treatments gave similar banding patterns with an exception of the FYM treated soils that gave a different profile (Figure 4.5). The higher number of bands in the FYM treated soils

was attributed to the high total organic carbon concentration in this treatment. The differences in the DGGE profiles of the two genes of the denitrifying bacteria could be because different populations have contrasting physiological characteristics, such as growth kinetics or sensitivity to oxygen (Cavigelli & Holtan-Hartwig, 2002).

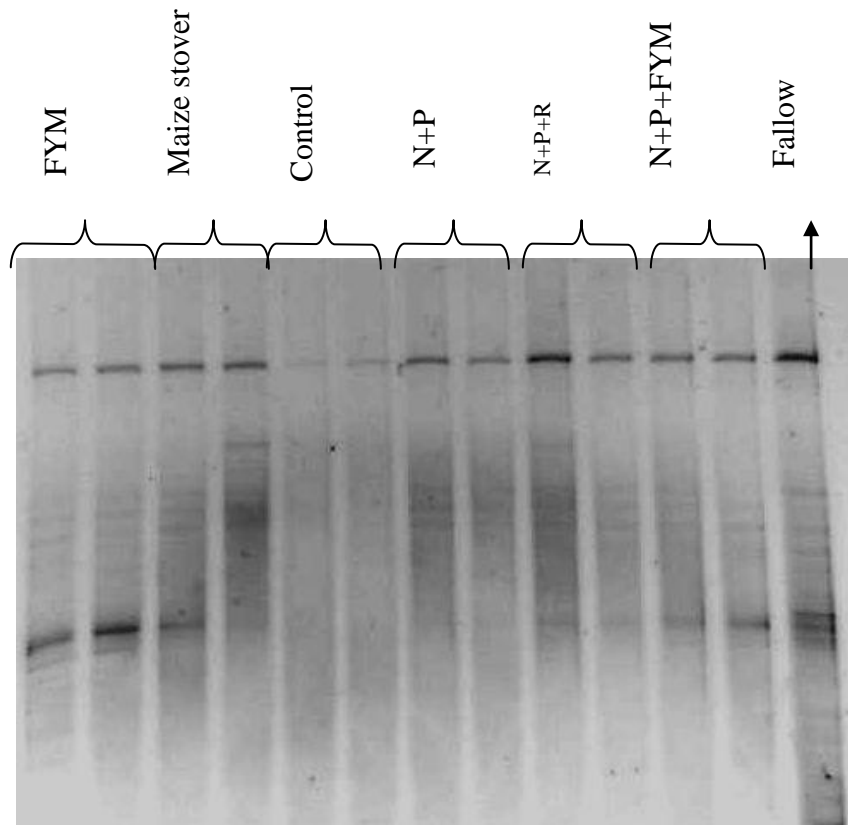


Figure 4.6: DGGE profiles of nir S rDNA gene Kabete long-term experiment

Rochette et al. (2000) observed a stimulation of the denitrifying enzyme activity in soil after a long-term application of organic fertilizers, which is in agreement with the findings of this study. Further most denitrifying bacteria are heterotrophs and therefore, carbon availability is an important factor in controlling denitrification hence the difference in the FYM treated soils and the fallow.

4.4.1 Cluster analysis of denitrifying bacteria communities

Cluster analysis of PCR-DGGE patterns of the nir S genes denitrifying bacteria community showed low similarity (40%) with five major clusters (Figure 4.7). N+P+FYM, N+P+R, and N+P were in one cluster; however, in this cluster, the N+P and N+P+R treatments further formed a sub cluster of their own. This indicated that N+P and maize stover treatments had a stronger effect on the denitrifying communities. This could be attributed to the low pH and input type as observed by Wolsing and Prieme (2004). Their study reported variations in the denitrifying community, which was attributed to fertilizer use and type. Similarly, the low pH in these two treatments may have contributed to the formation of similar communities hence the formation of the subcluster.

In contrast, the control and the fallow clustered separately on the two far ends of the dendograms (Figure 4.7). The FYM treatment was also on a cluster of its own an indication that it also had a strong effect on the structure of the denitrifying communities; this may be explained by the high available carbon in this treatment.

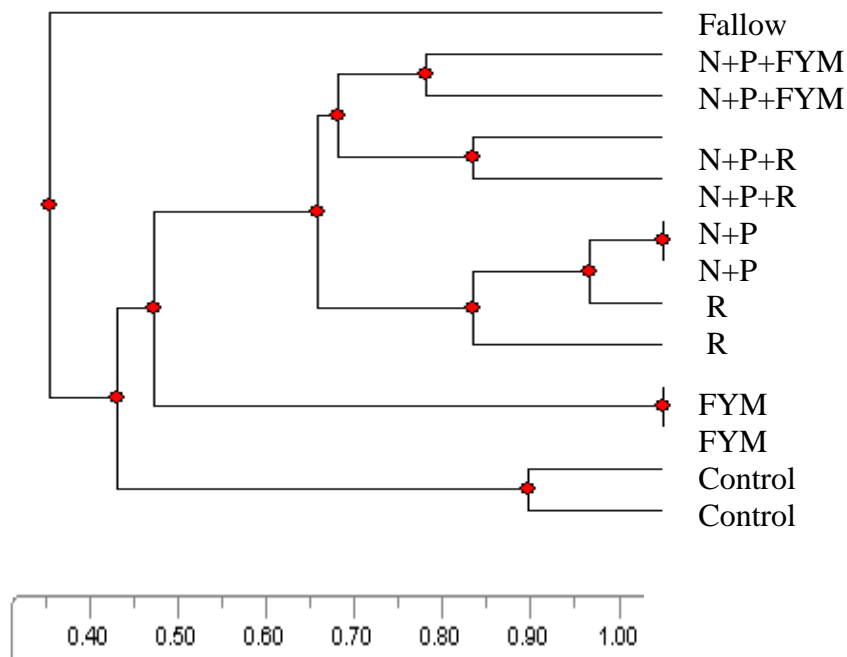


Figure 4.7: Cluster analysis of nir K rDNA gene of soil samples from Kabete long-term experiment.

In the nir S genes a low percentage (40%) similarity was also observed (Figure 4.8). Four major clusters were formed for these genes of the denitrifying bacteria. The fallow, N+P, and the control were each in different clusters an indication that each had different effects on the denitrifying communities. The N+P and the control treatments clusters could be explained by the low pH in these treatments leading to communities that are adapted to low pH. On the other hand, N+P+FYM, N+P+R, FYM and maize stover were on the same cluster. Within this cluster, three sub clusters were formed that were N+P+FYM, FYM and N+P+R and maize stover. The difference in the clustering of the nir K and nir S genes of the denitrifying bacteria could be attributed to contrasting physiological characteristics of different denitrifying bacteria populations such as growth kinetics or sensitivity of enzymes to oxygen (Cavigelli & Robertson, 2000; Holtan-Hartwig, Dorsch & Bakken ., 2002).

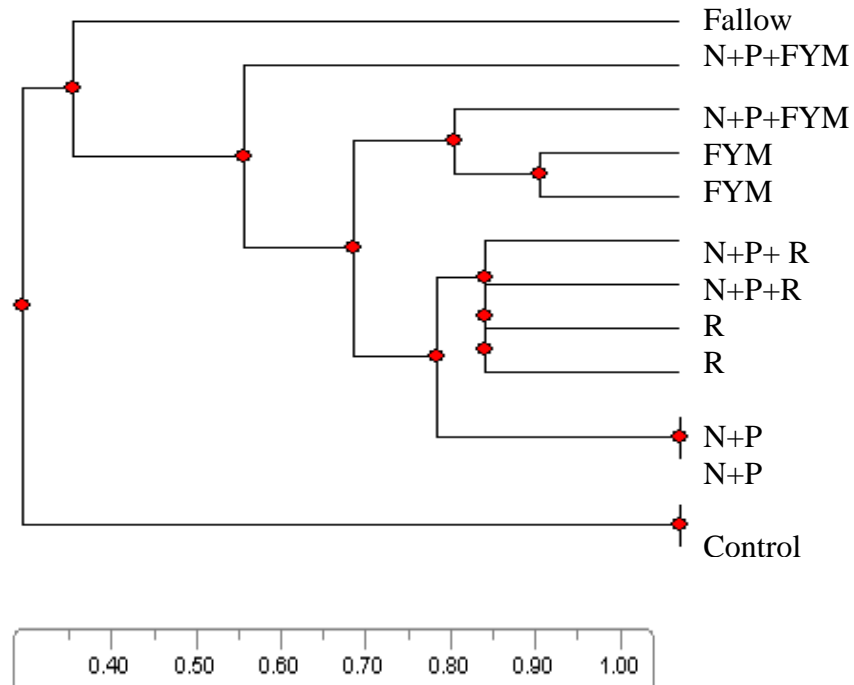


Figure 4.8: Cluster analysis of nir S rDNA gene of soil samples from Kabete long-term experiment.

4.4.2 Statistical analysis of Shannon-Weaver indices of the denitrifying bacteria communities

The highest diversity ($H=0.8$) of nir S was found in the N+P+R while the lowest diversity ($H=0.3$) was found in the control (Table 4.4). Generally, nir S diversity was low as compared to that of nir K (Table 4.4). On the contrary, highest diversity ($H=0.7$) was found in the FYM treatment while lowest diversity was found in the N+P+R and N+P+FYM treatments with $H=0.49$ and $H=0.47$, respectively, for the nirK genes. For nir K, N+P, fallow, maize stover and the control were not significantly different ($P<0.05$) an indication that they inhabited similar denitrifying communities (Table 4.4). FYM treatment was significantly different ($P<0.05$) from all other treatments. This was attributed to the high organic carbon in FYM compared to other treatments. For the case of nir S, N+P+R was significantly different from all other treatments while fallow, FYM, NP+FYM and maize stover treatments were not significantly different (Table 4.4). N+P treatment had a significant effect compared to the fallow, FYM, N+P+FYM and maize stover. The significance of N+P treatment indicated that

chemical fertilization alone with no organic inputs had an effect on the composition of the denitrifying community and specifically on the nir S genes community. Long-term fertilization effect was partially attributed to an indirect effect by soil acidification, which has resulted in a selection of bacteria adapted to low pH for the nir S. This agrees with the results reported by Parkin et al. (1985) on denitrification activity after twenty years of fertilization with acid generating ammonium salts who found that pH can affect the composition of the denitrifying communities in soil.

The high diversity indices in the N+P+R and FYM for nirS and nirK species, respectively, could be attributed to the high total organic C concentration provided by the additional organic inputs. A study by Hallin & Lindgren (1999) reported external carbon source affect both nirK and nirS community composition. Similarly, Rochette et al. (2000) observed a stimulation of the denitrifying enzyme activity in soil after a long-term application of organic fertilizers and denitrification was generally correlated to the soil organic C.

Table 4.4: Means of Shannon Weaver index for the Kabete long-term trial study on the nirK and nirS denitrifying communities' diversity

Treatment	Denitrifying community genes	
	nirK	nirS
N+P+FYM	0.475 ^a	0.613 ^{bc}
N+P+R	0.493 ^{ab}	0.870 ^d
Control	0.697 ^{abc}	0.367 ^a
N+P	0.706 ^{abc}	0.666 ^c
Fallow	0.741 ^{abc}	0.426 ^{ab}
Maize stover(R)	0.767 ^{bc}	0.638 ^{bc}
FYM	0.776 ^c	0.587 ^{bc}
Grand mean	0.664	0.595
SED(P<0.05)	0.098	0.085
LSD (P<0.05)	0.02058	0.179

Means followed by the same letter are not significantly different from each other (Duncan Multiple Range Test (P<0.05))

The diversity of nirK was inversely proportional to that of nirS community (Figure 4.9) and this could be linked to contrasting physiological characteristics of the two genes of the different denitrifying bacteria (Cavigelli & Robertson, 2000). Similarly another study that focused on NO_3^- contaminated ground water showed that the diversity of nirK and nirS community were inversely proportional but the diversity indices of communities were not related to a single geochemical characteristic (Yan et al., 2003).

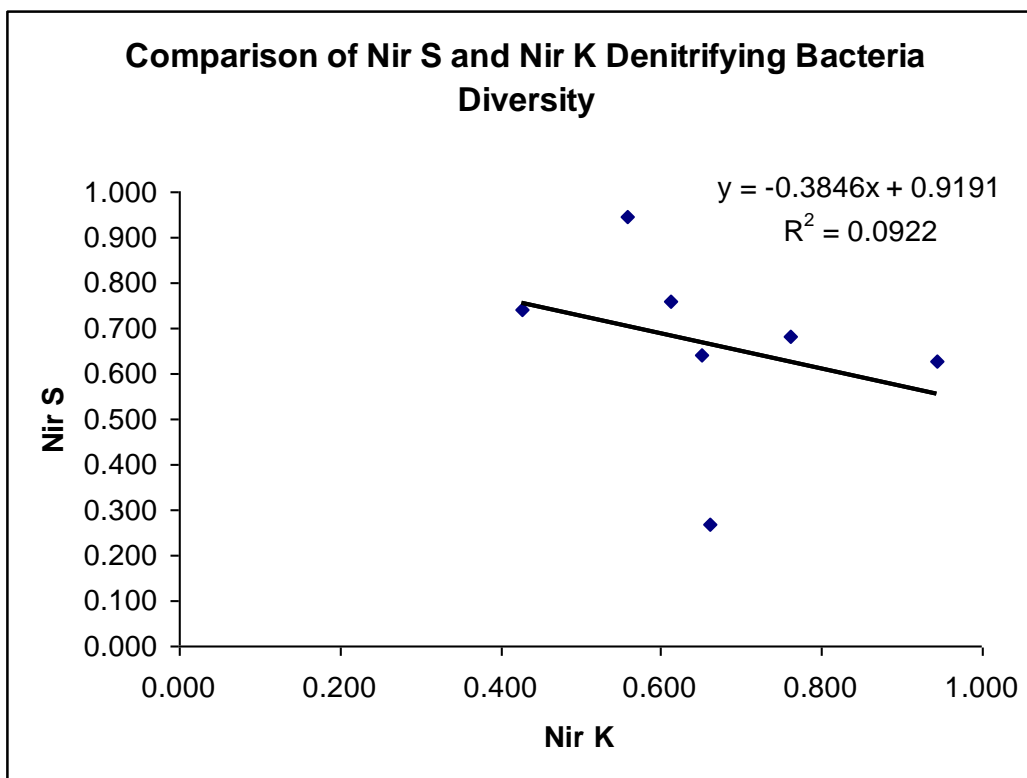


Figure 4.9: Relationship between nir K and nir S denitrifying bacteria community

A long-term fertilization regime can differently affect the composition of denitrifying community depending on their enzyme structure. Denitrifiers reduce nitrates and nitrites to gaseous compounds (NO_2 , N_2O and N_2) which are released to the atmosphere, leading to loss of plant available N from soils. The findings of this study indicated a low diversity of nirK genes of the denitrifying bacteria in the N+P+R and N+P+FYM an indication of low N losses

in these treatments for this particular gene of denitrifying bacteria. However, simultaneous assessment of denitrifying communities' composition and ecological functionality including identification of species population is still in its infancy and much of denitrifying communities is poorly understood.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

The main objective of the study was to assess the impact of organic and inorganic inputs on microbial communities. Results from this study indicate that combination of both organic and inorganic inputs (N+P+R or N+P+FYM) increases soil microbial diversity as the microbial diversity for all microbes studied tended to be higher in the treatments with N+P+FYM and N+P+R with an exception of the denitrifying communities. The high microbial diversity for the total bacteria and fungal communities in the N+P+FYM and N+P+R treatments demonstrates the positive effect that the combination of both organic and inorganic inputs have on belowground biodiversity. Combination of both organic and inorganic inputs provided more substrate for utilization by the soil microbes and reduced competition for mineral nutrients from chemical fertilizers by plants and microbes in the soil. This was evidenced from the higher total bacterial and fungal communities in the N+P+R and N+P+FYM treatments.

Secondly, application of either FYM or maize stover alone gave a comparatively low diversity compared to combination of both organic and inorganic inputs. However, compared to sole application of inorganic fertilizers (N+P), organic inputs alone had a positive effect on the microbial communities while inorganic inputs (N+P) alone seemed to have a negative effect on the bacterial and fungal communities. The extent to which an organic material will enhance microbial diversity however depends on its quality and other factors such as climatic factors and site characteristics.

Further, combination of organic and inorganic inputs gave the lowest diversity of denitrifying bacteria (nir K genes) indicating that the negative effect of denitrification through available N

loss and environmental pollution from the potent green house gas nitrous oxide (N₂O) from arable soils was at a reduced rate.

The following recommendations can be drawn based on the findings from this research:

- Farmers need to adopt the integrated soil fertility management which embraces the combination of organic and inorganic inputs together since it conserves the below ground biodiversity which are key role players in cycling of soil nutrients.
- Other studies should investigate on the specific communities that are affected by the chemical fertilizer as well as those that are promoted by the combined use of organic and inorganic inputs by sequencing and indentifying the individual species as this would be more specific than general.
- More research needs to be carried out on more organic inputs as this study only looked at farmyard manure and maize stover of which maize stover is classified as a low quality organic matter resource. Further research is therefore needed on other organic matter resources such as *Tithonia diversifolia* (Hemsley) residues, *Senna Spectabilis* and *Calliandra calothyrsus* residues as they are also commonly used by farmers as organic inputs.
- There is need for more research in order to understand the forces shaping denitrifying communities since it is critical to link these communities to ecosystem processes and sustainable ecosystem management.

REFERENCES

- Anderson, J M & Ingram, J S L (1993). *Tropical Soil Biology and Fertility: A Handbook of Methods*, 2nd Edition .CAB International, Wallingford, U.K.
- Andren, O & Balandreau, J (1999). Biodiversity and soil functioning – from black box to can of worms? *Applied Soil Ecology*. 13: 105–108.
- Ayuke, O.F. (2000). Diversity, Abundance and Function of soil Invertebrate Fauna in relation to quality of organic residues. Ph. D. Thesis, Moi University.
- Bailey, K L, and Lazarovits, G, (2003). Suppressing soil-borne diseases with residue management and organic amendments. *Soil and Tillage Research* 72, 169–180
- Bardgett, R & Shine, A (1999). Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. *Soil Biology & Biochemistry*. 31: 317–321.
- Bationo, A, Kimetu, J, Ikerra, S, Kimani, S, Mugendi, D, Odendo, M, Silver, M, Swift, & Sanginga, N. (2004). The African Network for Soil Biology and Fertility: New challenges and opportunities. In: Bationo, A. (ed) *managing nutrient cycles to sustain soil fertility in Sub Saharan Africa*. Academy Science Publishers (ASP) Nairobi, Kenya.
- Bationo, A & Burkert A, (2001) Soil organic carbon management for sustainable land use in Sudano-sahelian west Africa, nutrient cycling in agroecosystems. 76:2-3.
- Beauchamp, E G, Trevors, J T & Paul, J W (1989). Carbon sources for bacterial denitrification. *Advanced soil science* 10: 113-142.
- Bedard, C and Knowles, R (1989) Physiology, biochemistry and specific inhibitors of CH₄, NH₄⁺ and CO oxidation by methanotrophs and nitrifiers. *Microbiological reviews* 53: 68-84.
- Blackmer, A M & Bremner, J M (1978) Inhibitory effect of nitrate on reduction of N₂O to N₂ by soil microorganisms. *Soil biology and biochemistry*.10:187-191.
- Bolton, H, Elliott, L, Papendick, R & Bezdicek, D (1985). Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. *Soil Biology & Biochemistry*. 17: 297-302.
- Braker, G., Fesefeldt, A. & Witzel, K.-P. (1998) Development of PCR primer systems for amplification of nitrite reductase genes (nirK and nirS) to detect denitrifying bacteria in environmental samples. *Applied Environmental Microbiology* 64, 3769–3775.
- Braker, G. L., Ayala –del-Rio, A.H.Devol, A.Fesefeldt, and J.M Tiedje.(2001). Community structure of denitrifiers, bacteria, and archaea along redox gradients in Pacific Northwest marine sediments by terminal restriction fragment length

polymorphism analysis of amplified nitrate reductase (*nirS*) and 16S rRNA genes. *Applied Environmental Microbiology*. 67:1893-1901.

- Brookes, P & McGrath, S (1984). Effects of metal toxicity on the size of the soil microbial biomass. *Journal of soil science*. 35:341-346.
- Buresh, R & Tian, G. (1997). Soil improvement by trees in sub-Saharan Africa. *Agroforestry Systems*. 38: 51-76.
- Buyanovsky, G A, Brown, J R, Wagner, G H, (1997). Sanborn Field: effect of 100 years of cropping on soil parameters. In: Paul, E A, Paustian, K, Elliott, E T, Cole, C V (Eds.), *Soil Organic Matter in Temperate Ecosystems: Long-Term Experiments in North America*. CRC Press, New York, pp. 205–226.
- Dick R P, (1992) A review –long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agricultural Ecosystems and Environment*. 40:25-36.
- Cairney, J & Merharg, A (1999). Influence of anthropogenic pollution on mycorrhizal fungal communities. *Environmental pollution* 106:169-182.
- Cairney, J W G & Burke, R M (1998). Extra cellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (read) Korf & Kernan: their likely roles in decomposition of dead plant tissue in soil. *Plant and soil* 205:181-192
- Cavigelli, M & Robertson, G (2002). The functional significance of denitrifier community in a terrestrial ecosystem. *Ecology*. 81:1402-1414.
- Cavigelli, M. A & Robertson, G P (2001). The functional significance of denitrified community composition in a terrestrial ecosystem. *Ecology* 81:1402-1414.
- Chander, K & Brookes, P (1991). Effects of heavy metals from past applications of sewage sludge on microbial biomass and organic matter accumulation in a sandy loam and silty loam UK soil. *Soil Biology & Biochemistry*. 23:927-932.
- Chantigny, M H, Angers, D A & Rochette, P (2002). Fate of carbon and nitrogen from animal manure and crop residues in wet and cold soils. *Soil Biology and Biochemistry*. 34:509-517.
- Cookson, W, Beare, M, & Wilson, P (1998). Effects of prior crop residue management on microbial properties and crop residue decomposition. *Applied Soil Ecology*. 7: 179-188.
- De Klein, C A M & Van Logtestijn, R S P (1996) denitrification in grassland soils in the Netherlands in relation to irrigation, N application rate, soil water content and soil temperature. *Soil Biology and Biochemistry*. 28:231-237.
- Denef, K, Six, J, Bossuyt, H, Frey, S, Elliot, E, Merckx, R, & Paustin, K (2001). Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic

- matter, and microbial community dynamics. *Soil Biology & Biochemistry* 33:1599-1611.
- Dick, R P (1992). A review –long term effects of agricultural systems on soil biochemistry and microbial parameters. *Agricultural Ecosystem & environment* 40:25-36.
- Dighton, J, Mascarenhas, M, & Arbuttle-Keil G (2001). Changing resources: Assessment of leaf litter carbohydrate resource change at a microbial scale of resolution. *Soil Biology & Biochemistry* 33:1429-1432.
- Doran, J W & Werner, M R (1990). Management and soil biology. In: *sustainable Agriculture in Temperate Zones*. C A Francis, C B Flora, L D King (eds.) pp.151-177. Wiley. New York
- Dorich, R A and Nelson (1984). Evaluation of manual cadmium reduction methods for determination of nitrate in potassium chloride extracts of soil. *Soil Science Society of American Journal*
- Drinkwater, L, Wagoner, D, & Sarrantonio, M. (1998). Legume-based cropping system having reduced carbon and nitrogen losses. *Nature*. 396: 262-264.
- Duineveld, B M, Rosado, A S, van Elsas & van Veen, J A (1998). Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. *Applied Environmental microbiology*. 64:4950-4957.
- Ercolini, D. (2004). PCR-DGGE fingerprinting; novel strategies for detection of microbes in food. *Journal of Microbiology. Methods* 56: 297-314.
- Edwards, C A (1998). *Earthworm ecology*. Soil and Water Conservation Society. St. Lucie Press, Ankeny, Iowa, 389p.
- Fauci, M F & Dick, R P (1994). Soil microbial dynamics-short-term and long-term effects of inorganic and organic nitrogen. *Soil Science Society of America Journal*. 58: 801–806.
- Firestone, M K and Davidson, E A (1989). Microbiological basis of NO and N₂O production and consumption in soil. Pages 7-21 in Andreae, M O and Schimel, D S eds. *Exchange of trace gases between terrestrial ecosystems and the atmosphere*. John Wiley and sons, New York, NY
- Fisher, S G & Lerman, L S (1983). DNA fragments differing by single base pair substitutions are separated in denaturing gradient gels: correspondence with melting theory. *Proceedings National Academics in Science Society of America*. 80: 1579–1583.
- Fritze, H A, Smolander, T, Levula, V & Kitunen, E 1994. Wood-ash fertilization and fire treatments in a Scots pine forest stand: effects on the organic layer, microbial biomass and microbial activity. *Biology & Fertility of Soils*. 17: 57-63.

- FAO (1990). FAO-UNESCO Soil Map of the World Revised Legend. Soil Bulletin 60 Food Agriculture Organization: Rome.
- Garbeva, J A, Veen, V & Van Elsas, J D (2004). Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressive ness. *Annual Review Phytology*. 42: 243-270.
- Garcia-Montiel, D C, Melillo, J M, Steudler, P A, Cerri, C C & Piccolo, (2003). Carbon limitations to nitrous oxide emissions in ahumid tropical forest of the Brazilian amazon. *Biology and fertility of soils* 38:267-272.
- Giller, K E, Witter, E & McGrath, S P (1998). Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. *Soil Biology and Biochemistry* 30:1389-1414.
- Giovannoni, S J, Britschgi, T B, Moyer, C L & Field, K G (1990). Genetic diversity in Sargasso Sea bacterioplankton. *Nature (London)*: 345, 60– 63.
- Griffiths, B S, Ritz, K, Wheatley, R E, Kuan, H L, Boag, B, & Christensen, S (2001). An examination of the biodiversity–ecosystem function relationship in arable soil microbial communities. *Soil Biology & Biochemistry*. 33: 1713–1722.
- Hadrys, H, Balick, M, Schierwater B (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol Ecol* 1: 55-63.
- Haggar, J P, Tanner, E U, Beer, J W & Kass, D C L (1993). Nitrogen dynamics of tropical agroforestry and annual cropping systems. *Soil Biology & Biochemistry*. 25: 363-1378.
- Hallin, S. and Lindgren, P.-E. (1999) PCR detection of genes encoding nitrite reductase in denitrifying bacteria. *Applied Environmental Microbiology* 65, 1652–1657.
- Helyar K R and Porter W M (1989). Soil acidification, its measurements and the processes involved. In: Robson A D (ed.). *Soil acidity and plant growth*. Pp 61-101. Academic Press, Sydney.
- Henrot, J & Robertson, G P (1994). Vegetation removal in two soils of the humid tropics: effect on microbial biomass. *Soil Biology & Biochemistry*. 26:111-116.
- Hofle M (1992) Bacterioplankton community structure and dynamics after large-scale release of non-indigenous bacteria as revealed by low molecular weight RNA analysis. *Appl Environ Microbiol* 58:3387-3394.
- Holtan-Hartwig, Dorsch, L P & Bakken, L R (2002). Comparison of denitrifying communities inorganic soils: kinetics of NO₃ and N₂O reduction. *Soil Biology and Biochemistry*. 32:833-843

- Houston, A P C, Visser, S & Lautenschlager, R A (1998). Microbial processes and fungal community structure in soils from clear cut and unharvested areas of two mixed wood forests. *Canadian Journal of Botany*. 76: 630-640.
- Kang (1993). Alley cropping: achievements and future directions. *Agrof. Syst* 23:141-155
- Kautz, T, Wirth, S & Ellmer, F (2004). Microbial activity in a sandy arable soil is governed by the fertilization regime. *European Journal of Soil Biology*. 40: 87–94.
- Kautz, T, López-Fando, C & Ellmer, F (2006). Abundance and biodiversity of soil microarthropods as influenced by different types of organic manure in a long-term field experiment in Central Spain. *Applied Soil Ecology*. 33: 278–285.
- Kazombo-Phiri, S F M (2005). Main causes of declining soil productivity in Malawi: Effects on agriculture and management technologies employed to alleviate the problems. 59-66: In: Omany, G. O. and Pastern D. (eds 2005). *Sustainable Agriculture systems for the dry lands. Proceedings of the International Symposium for sustainable dry land agriculture systems, 2-5 December 2003, Niamey, Niger.*
- Kennedy, A C & Gewin, V L (1997). Soil microbial diversity: Present and Future considerations. *Soil Science* 162:607-167.
- Kibunja, C N, Mugendi, D N, Mwaura, F B, Kitonyo, E M & Salema, M P (2003). Fate of Applied Nitrogen in Long-term Maize-Bean Cropping System in Kenya. In Mugendi, D N, Kironchi G, Gicheru, P T, Gachene, C K K, Macharia, P N, Mburu, M, Mureithi, J G & Maina, F (eds). *Proceedings of the 21st Annual Conference 1st -5th December. 2003. Soil Science Society of East Africa, Nairobi Kenya.*
- Kimetu, J M (2002). Nitrogen fertilizer equivalency values for organic materials of contrasting quality base on maize performance at Kabete, Kenya. Msc. Thesis, Kenyatta University, Kenya.
- Kirchner, M J, Wollum, A G & King, L D (1993). Soil microbial populations and activities in reduced chemical input agro ecosystems. *Soil Science Society of America Journal*. 57: 1289–1295.
- Klironomos, J N & Hart, M M (2001). Food-web dynamics-animal nitrogen swap for plant carbon. *Nature* 410:651-652.
- Knowles, R (1982), Denitrification. *Microbiology Review* 46:43-70.
- Komi, A (2006). Training course manual in molecular Biology TSBF-CIAT Nairobi 27th February-2nd March pp 5-28.
- Laurent, F M, Philippot, L, Hallet, S, Chaussod, R, Germon, J C, Soulas, G & Catroux, G (2001). DNA Extraction from Soils: Old bias for new microbial diversity analysis methods. *Applied Environmental Microbiology*. 67: 354–359.

- Lavelle P and Spain A V (2001) Soil Ecology. Kluwer Academic Publishers. Dordrecht/Boston/London. pp 654.
- Lavelle, P, Gilot, C, Fragoso, C, Eschnebrenner, V, Hernandez, D L, Pashasi, B & Brussard, L (1994). The relationship between soil macro-fauna and tropical soil fertility. In: Wooper, P L & Swift, M J (eds). The Biological Management of Tropical Soil Fertility. pp 136–169. A Wiley-Sayce publication.
- Marschner, P., Kandeler, E., & Marschner, B., (2003). Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology & Biochemistry 35, 453–461.
- Martens D A (2001). Nitrogen cycling under different soil management systems. Advances in Agronomy 70:143-192.
- Martens, D A, Johanson, J B & Frankeberger J R, W T (1992). Production and persistence of soil enzymes with repeated additions of organic residues. Soil Science. 153: 53–61.
- Massol-Deya A A, Odelson D A, Hickey RF, Tiedje J M (1995) Bacterial community fingerprinting of amplified 16S and 16S-23S ribosomal DNA gene sequences and restriction endonuclease analysis (ARDRA). Molecular Microbiology Ecology Manual 3.3.2:1-18
- McGonigle, T P & Miller, M H (1996). Development of fungi below ground in association with plants growing in disturbed and undisturbed soils. Soil Biology & Biochemistry 28:263-269
- McKenney, D J, Wang, S W, Drury, C F, & Findlay, W I (1993). Denitrification and mineralization in soil amended with legume, grass, and corn residues. Soil Science Society of American journal 57: 1013-1020.
- Mendum, T A, Sockett, R E & Hirsch, P R (1999). Use of molecular and isotopic techniques to monitor the response of autotrophic ammonia-oxidizing population of the β subdivision of the class *Proteobacteria* in arable soils to nitrogen fertilizer. Applied and Environmental Microbiology 65: 4155-4162.
- Miller, R M & Jastrow, J D (1990). Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil biology and Biochemistry 22:579-584.
- Moore- Landecker E (1990) Fundamentals of the fungi. Prentice Hall, New Jersey.
- Moore, J C, Berlow, E L, Coleman, D C, de Ruiter, P C, Dong, Q, Hastings, A, Johnson, N C., McCann, K S, Melville, K, Morin, P J, Nadelhoffer, K, Rosemond, A D, Post, D M, Sabo, J L, Scow, K M, Vanni, M J & Wall, D H (2004). Detritus, trophic dynamics and biodiversity. Ecology Letters. 7: 584-600.

- Mugendi D N, Nair P K R, Mugwe J N, O'Neill M K, & Woomer P L (1999). Calliandra and Leuceana alley cropped with maize. Part 1: soil fertility changes and maize production in the sub-humid highlands of Kenya .*Agrof. Syst*, 46: 39-50.
- Murphy, V.D, Stockdale, A E, Brookes, C P & Goulding, W T K. (2007). Impact of microorganisms on chemical transformations in soil. In: Abbott, K L. and Murphy, D (eds), *Soil biological fertility: A key to sustainable land use in agriculture*.pp. 37-59. Springer; Netherlands.
- Murry, P J, Hatch, D J, Dixon, E R, Stevens, R J, Laughlin, R J and Jarvis, S C (2004)
- Murwira, H.K & Kirchmann, H. (1993). Nitrogen dynamics and maize growth in a Zimbabwean sandy soil under manure fertilization. *Communications soil science plant Annual review*. 24: 2343-2359.
- Murwira, H. K., Mutuo, P., Nhamo, N., Marandu, A. E., Rabeson, R., Mwale, M. & Palm, C. A (2002). Fertilizer equivalent values of organic materials of differing quality. In: Vanlauwe, B, Diels, J, Sanginga, N & Merckx, R (eds), *integrated plant and nutrient management in Sub-Saharan Africa: From Concept to Practice*. CAB International, Wallingford, pp. 113-122.
- Muyzer & Smalla K (1998). Application of denaturing gradient gel electrophoresis and temperature gradient gel electrophoresis in microbial ecology. *Antonie van Leeuwenhoek*, 73:127-141.
- Muyzer, G, De Waal, E C & Uitterlinden, A G (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied Environmental Microbiology*. 59: 695– 700.
- Myers, R J K, van Noordwijk M & Vityakon, P (1997). Synchrony of nutrient release and plant demand: Plant litter quality, soil environment and farmers management options. In: *Driven by nature: Plant litter quality and decomposition* Cadisch and KE Giller (eds.) pp.215-212.CAB international. Wallingford, Oxon, UK.
- Nannipieri, P & Badalucco, L (2003). Biological processes. In: *Processes in the Soil–Plant System: Modeling concepts and applications* (eds) Bembli, D K & Nieder, R). The Haworth Press, Binghamton, NY, in press.
- Oades, J M (1984). Soil organic matter and structural stability: mechanisms and implications for management. *Plant Soil* 76:319-337.
- Peacock, A D, Mullen, M D, Ringel berg, D B, Tyler, D d, Hedrick, D B, Gale, P M, White, D C (2001). Soil microbial community responses to dairy manure or ammonium nitrate applications. *Soil Biology and Biochemistry* 33,1011-1019.
- Palm, C A, Myers, R J K & Nandwa, S M (1997). Combined use of organic and inorganic nutrient sources for soil fertility maintenance and replenishment. In: Buresh, R J, Sanchez, P A & Calhoun, F (eds).*Replenishing soil Fertility in Africa*. Soil Science Society of America (SSSA). Special Publication No.51, USA.

- Parkin, T A, Sextone J & Tiedje, J M (1985). Adaptation of denitrifying populations to low soil pH. *Applied Environmental microbiology*. 49:1053-1056.
- Parham, J. A., S. P. Deng, H. N. Da, H. Y. Sun, and W. R. Raun. (2003). Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure. *Biology and Fertility of Soils* 38:209-215.
- Philippot, L & Hallin S (2005). Finding the missing link between diversity and activity using denitrifying bacteria as a model functional community. *Curr. Opin. Microbiol.*8:234-239.
- Powell, J M, Ikpe F N and Somda Z C (1999). Crop yield and the fate of nitrogen and phosphorus following application plant material and feces to soil. *Nutrient cycling in Agroecosystems*.54;215-226.
- Qureshi J N (1987). The cumulative effects of N-P fertilizers, manure and crop residues on maize grain yields, leaf contents and some soil chemical properties at Kabete. Pp.12 . National Maize Agronomy Workshop, Nairobi, 17-19.
- Rillig, M C & Mummey, D L 2006. Mycorrhizas and soil structures. *New Phytology* 171:41-53.
- Ritz K and Griffiths B S (2001). Implications of soil bioersivity for sustainable organic matter management. *In Sustainable management of soil organic matter*. R M Rees B C ball, C D Campbell and C A Watson (eds) pp. 343-356. CAB international Wallingford, Oxon, UK.
- Rochette, P E, van Bochove, D, Prevost, D A, Angers D,Cote & Bertrand N (2000). Soil carbon and nitrogen dynamics following application of pig slurry for the 19th consecutive year. II. Nitrous oxide fluxes and mineral nitrogen. *Soil science society of American Journal* 64:1396-1403.
- Schroth, G R, Oliver P, Balle, G M, Gnahoua , N, Kanchanakanti, B Ledue, B Mallet, B Peltier and W Zech (1995). Alley cropping with *Gliricidia sepium* on high base status soil following forest clearing: effects on soil conditions, plant nutrition and crop yields .*Agrof. Syst* 32: 261-276.
- Shannon & Weiner (1949). Measurement of diversity indices. In Magurran, A E 1988. *Ecological diversity and its measurements*. Cambridge University Press, Great Britain, 179p.
- Sieverding, E (1991). Vesicular-Arbuscular Mycorrhiza management in Tropical Agrosystems. *Deutsche Gesellschaft für Technische Zusammenarbeit, GTZ No. 224*. Eschborn 371 p.
- Sigler, V, William, Ronald, F & Turco (2002). The impact of chrothalnonil application on soil bacterial and fungal populations as assessed by denaturing gradient gel electrophoresis. *Applied Soil Ecology*, 21, 107-118.

- Stenberg, B M, Johansson, M, Pell, K, Sjudahi-Svenssen, Stentrem, J & Torstensson, L (1998). Microbial biomass and activities in soil as affected by frozen and cold storage. *Soil Biology & Biochemistry*. 30:393-402.
- Stockdale, E A, Rees R M and Davies M G (1995) Nitrogen supply for organic cereal production in Scotland .In: soil management in sustainable agriculture H F Cook and H C Lee (eds) pp.254-264.Proceeding of third international conference on sustainable agriculture. wye college press. Ash ford, UK
- Swift, M J, Seward, P D, Frost, P G H, Qureshi, and J N & Muchena, F N (1994) Long term experiments in Africa: developing a database for sustainable land use under global change. In: Leigh, R. A, Johnson, A. E. (eds), Long term Experiments in Agriculture and Ecological Sciences, pp 229-251.
- Swift, M J & Woomer, P (1992). Organic matter and the sustainability of agricultural systems: definition and measurement. In: Merckx, R. and Mulongoy, K (eds), Dynamics of soil organic matter in relation to the sustainability of agricultural systems. pp 3-18. John Wiley, Chichester.
- Swift, M J, Frost, P G H, Campell, B M, Hatton, J C & Wilson, K B (1989). Nitrogen cycling in farming systems derived from savanna: Perspectives and challenges. In: Clarholm, M & Bergstrom, L (eds), Ecology of Arable Land, Kluwer: Dordrecht, Netherlands.
- Throback, I N, Enwall K, Jarvis A & Hallin (2004). Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiol. Ecol.*49:401-417.
- Thorup-Kristensen, K, Magid, J & Jensen, S (2003). Catch crops and green manure as biological tools in nitrogen management in temperate zones. *Advances in Agronomy*: 79 227-302.
- Tian, G, Kang, B T & Brussard, L (1995). Breakdown of plant residues with contrasting chemical compositions under humid tropical conditions; Effects of earthworms and millipedes. *Soil Biology & Biochemistry*. 27: 277-280.
- Tsidale S L, Nelson W L , J D Beaton & J L Halvin (1993).Soil fertility and fertility.5th ed, Macmillan publication Company, New York.
- Tropical Soil Biology Fertility institute of CIAT (1999). First Draft Report. International workshop on Tropical Soil Biology: Opportunities and Challenges for African Agriculture.
- Tsidall, J M, (1991). Fungal hyphae and structural stability of soil. *Australian Journal of soil Resources* 29:729-743.
- Tu, C, Ristaino, J B & Hu, S J (2006). Soil microbial biomass and activity in organic tomato farming systems: Effects of organic inputs and straw mulching. *Soil Biology & Biochemistry*. 38: 247-255.

- Twomlow S, Riches C, O'Neill D, Brookes P and Ellies –Jones J (1999) Sustainable dry land smallholder farming in sub-Saharan Africa. *Annals of Arid Zone* 38: 93-135.
- Vanlauwe, B, Six, J, Wangechi, H, Nhamo, P, Kavoo, A, Yeboah, E, Mugendi, D & Mapfumo, P (2006). Interaction between organic resource quality, aggregate turnover and agro- ecosystem nitrogen and carbon cycling. *Nutrient cycling in Agro ecosystems*, (in press).
- Vanlauwe, B, Diels, J, Aihou, K, Iwouafor, E N O, Lyasse, O, Sanginga, N & Merckx, R (2002). Direct interactions between N fertilizer and organic matter: evidence from trials with ¹⁵N labeled fertilizer. In: Vanlauwe B, Diels J, Sanginga N, and Merckx R, (Eds). *Integrated Plant Nutrient Management in Sub-Saharan Africa: From concept to practice* CAB International Wallingford, UK, p173-84.
- Vanlauwe, B, Dendooven, L, Merckx, R, Vanlangenhove, G, Vlassak, K & Sanginga, N (1996). Residue quality and decomposition under controlled and field conditions. *International Institute of Tropical Agriculture (IITA) Research highlights and annual report*. 12: 1-6.
- Van Straaten, P (2007). Phosphorus. In: *Agro geology: The use of rocks for crops*. Pp: 125-145. Enviroquest ltd.
- Waswa, B S, Mugendi, D N, Vanlauwe, B & Kungu, J B (2007). Changes in soil organic matter as influenced by organic residue management regimes in selected experiments in Kenya. In Bationo, A, Waswa, B, Kihara, J & Kimetu, J (eds). *Advances in integrated soil fertility management in Sub-Saharan Africa: Challenges and opportunities*. Springer. Netherlands. 457– 469.
- Wardle D A (1992). A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biological reviews* 67:321-355.
- Wardle, D A & Giller, K E (1996). The quest for a contemporary ecological dimension to soil Biology – Discussion. *Soil Biology & Biochemistry*. 28:1549–1554.
- Weier, K L, Doran, J W , Power, J F and Walters, D T 1993. Denitrification and the dinitrogen to nitrous oxide ratio as affected by soil water, available carbon and nitrate. *Soil science Society of America Journal* 57:66-72.
- Werner MW (1997). Soil quality characteristics during conversion to organic orchard management .*Applied Soil Ecology*.5: 151-167.
- West, A W, (1986). Improvement of the selective respiratory inhibition technique to measure eukaryote: prokaryote ratios in soils. *Journal of Microbial Methods*. 5:125-138.
- Willison T W, Webster C P, Goulding K W T & Powelson D S (1995) methane oxidation in temperate soils: effects of land use and the chemical form of nitrogen fertilizer .*Chemosphere* 30: 539-546.
- Winder R S, Shamoum, S F (2006).Forest pathogens: friend or foe to biodiversity? *Canadian Journal of plant Pathology* 28:S221-S227.
- Wolsing, M & Prieme (2004). Observation of high seasonal variation in community structure of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle manure by determining T-RFLP of nir gene fragments. *FEMS microbiol.Ecol.*48:261-271.

- Yan, T, Fields, M W, Wu, L, Zu, Y, Tiedje, J M. & Zhou, J. (2003) Molecular diversity and characterization of nitrite reductase gene fragments (nirK and nirS) from nitrate- and uraniumcontaminated groundwater. *Environmental Microbiology*. 5; 13–24.
- Zumft, W G (1997) Cell biology and molecular basis of denitrification. *Microbiology Molecular Biology R* 61, 533–616.
- Zumft, W G (1992). The denitrifying prokaryotes, p. 554–582. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), *The prokaryotes*. Springer-Verlag, New York, N.Y.

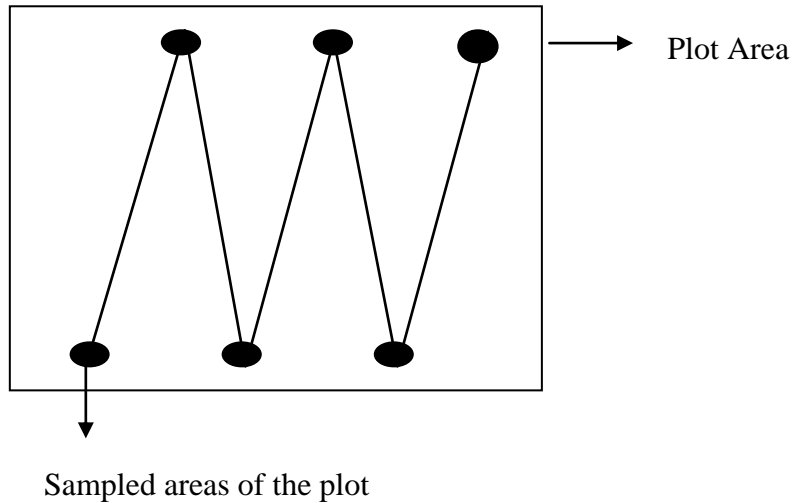
Appendices

Appendix 1: Experimental layout for the Kabete long-term experiment

	Block 1	Block 2	Block 3	Block 4
	7.0m			
4.5m	↔			
↑	1. N ₂ P ₂ FYM ₂ R	19. N ₁ P ₁	37. N ₂ P ₂ FYM ₁ R	55. N ₁ P ₁ FYM ₁ R
	2. N ₁ P ₁ FYM ₁	20. N ₂ P ₂ *	38. N ₂ P ₂ FYM ₂ R	56. N ₁ P ₁ FYM ₂ R
	3. FYM ₁ R	21. N ₂ P ₂ FYM ₁	39. N ₁ P ₁ FYM ₁	57. N ₂ P ₂ FYM ₂ *
	4. N ₁ P ₁ FYM ₂	22. N ₁ P ₁ FYM ₂	40. N ₂ P ₂ R*	58. N ₂ P ₂ FYM ₁
	5. FYM ₂ R	23. Nil*	41. N ₂ P ₂ *	59. FYM ₂ R
	6. N ₂ P ₂ FYM ₁	24. NilR*	42. FYM ₁	60. N ₁ P ₁ FYM ₁
	7. N ₂ P ₂ R*	25. N ₂ P ₂ FYM ₂ R	43. NilR*	61. FYM ₁ R
	8. N ₂ P ₂ *	26. N ₂ P ₂ FYM ₁ R	44. N ₁ P ₁ FYM ₁ R	62. N ₁ P ₁ FYM ₂
	9. FYM ₂ *	27. N ₁ P ₁ FYM ₁ R	45. N ₁ P ₁ R	63. NilR*
	10. N ₁ P ₁	28. N ₁ P ₁ FYM ₁	46. N ₁ P ₁ FYM ₂	64. N ₂ P ₂ *
	11. Nil+R*	29. FYM ₁ R	47. N ₂ P ₂ FYM ₂ *	65. N ₁ P ₁
	12. N ₁ P ₁	30. FYM ₂ R	48. FYM ₁ R	66. FYM ₁
	13. N ₂ P ₂ FYM ₁ R	31. N ₁ P ₁ FYM ₂ R	49. FYM ₂ R	67. FYM ₂ *
	14. N ₁ P ₁ FYM ₂ R	32. N ₂ P ₂ R*	50. N ₁ P ₁	68. N ₂ P ₂ FYM ₂ R
	15. N ₂ P ₂ FYM ₂ *	33. FYM ₂ *	51. Nil*	69. N ₂ P ₂ FYM ₁ R
	16. N ₁ P ₁ FYM ₁ R	34. N ₁ P ₁ R	52. N ₂ P ₂ FYM ₁	70. N ₂ P ₂ R*
	17. FYM ₁	35. FYM ₁	53. FYM ₂ *	71. N ₁ P ₁ R
	18. Nil*	36. N ₂ P ₂ FYM ₂ *	54. N ₁ P ₁ FYM ₂ R	72. Nil*
	Fallow*	Fallow*	Fallow*	Fallow*

Legend * selected treatments for this study at Kabete site.

Appendix 2: Soil sampling design



Appendix 3: Analysis of variance Bacterial communities data

Analysis of variance *****

Variate: bacterial communities

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.022871	0.007624	1.47	
Rep.*Units* stratum					
Treatment	6	0.408743	0.068124	13.12	<.001
Residual	18	0.093429	0.005190		
Total	27	0.525043			

***** Tables of means *****

Grand mean 1.076

Treatment

FALLOW	FYM	N+P	N+P+FYM	N+P+R	Control	Maize stover
1.245	1.045	0.860	1.193	1.153	1.033	1.008

*** Standard errors of means ***

Table	Treatment
rep.	4
d.f.	18
e.s.e.	0.0360

*** Standard errors of differences of means ***

Table	Treatment
rep.	4
d.f.	18
s.e.d.	0.0509

*** Least significant differences of means (5% level) ***

Table	Treatment
rep.	4
d.f.	18
l.s.d.	0.1070

***** Stratum standard errors and coefficients of variation *****

Variate: bacterial

Stratum	d.f.	s.e.	cv%
Rep	3	0.0330	3.1
Rep.*Units*	18	0.0720	6.7

Appendix 4: Analysis of variance fungal communities data

Analysis of variance *****

Variate: fungal

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0032286	0.0010762	1.77	
Rep.*Units* stratum					
Treatment	6	0.3930214	0.0655036	107.96	<.001
Residual	18	0.0109214	0.0006067		
Total	27	0.4071714			

***** Tables of means *****

Grand mean 0.6729

Treatment

Fallow	FYM	N+P	N+P+FYM	N+P+R	Control	Maize stover
0.5800	0.6275	0.7050	0.8525	0.8350	0.5650	0.5450

*** Standard errors of means ***

Table	Treatment
rep.	4
d.f.	18
e.s.e.	0.01232

*** Standard errors of differences of means ***

Table	Treatment
rep.	4
d.f.	18
s.e.d.	0.01742

*** Least significant differences of means (5% level) ***

Table	Treatment
rep.	4
d.f.	18
l.s.d.	0.03659

***** Stratum standard errors and coefficients of variation *****

Variate: fungal

Stratum	d.f.	s.e.	cv%
Rep	3	0.01240	1.8
Rep.*Units*	18	0.02463	3.7

Appendix 5: Analysis of variance denitrifying bacteria

***** Analysis of variance *****

Variate: Nir_K

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.16929	0.05643	2.94	
Rep.*Units* stratum					
Treatment	6	0.38496	0.06416	3.34	0.022
Residual	18	0.34544	0.01919		
Total	27	0.89969			

Stratum	d.f.	s.e.	cv%
Rep	3	0.0898	13.5
Rep.*Units*	18	0.1385	20.9

***** Analysis of variance *****

Analysis of variance *****

Variate: Nir_S

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.04925	0.01642	1.13	
Rep.*Units* stratum					

Treatment	6	0.65427	0.10905	7.48	<.001
Residual	18	0.26251	0.01458		
Total	27	0.96603			

***** Tables of means *****

Variate: Nir_S

Grand mean 0.595

Treatment	Fallow	FYM	N2P2	N2P2+FYM	N2P2+R	Nil	Nil+R
	0.425	0.587	0.666	0.613	0.870	0.367	0.638

*** Standard errors of means ***

Table	Treatment
rep.	4
d.f.	18
e.s.e.	0.0604

*** Standard errors of differences of means ***

Table	Treatment
rep.	4
d.f.	18
s.e.d.	0.0854

*** Least significant differences of means (5% level) ***

Table	Treatment
rep.	4
d.f.	18
l.s.d.	0.1794

***** Stratum standard errors and coefficients of variation *****

Variate: Nir_S

Stratum	d.f.	s.e.	cv%
Rep	3	0.0484	8.1
Rep.*Units*	18	0.1208	20.3

