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Measurement of microbial numbers, activity, biomass and diversity as a response to different methods of treatment of Tanzanian soil

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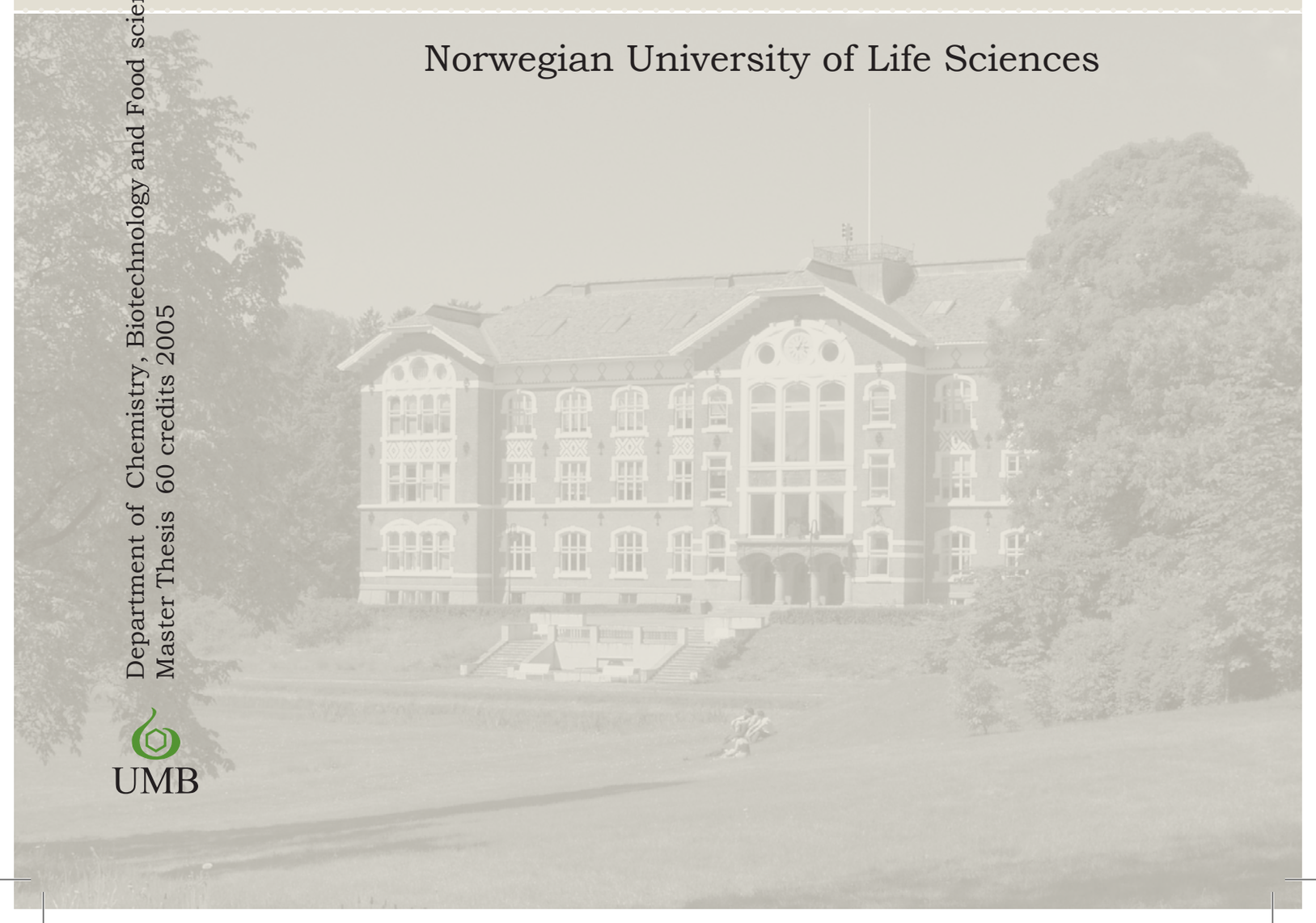

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UMB

Foreword

The thesis work in microbiology was done at the Department of Chemistry, Biotechnology and Food sciences at the Norwegian University of Life Sciences in Ås, and at the Soil Science Department at Sokoine University of Agriculture in Morogoro, Tanzania. Fieldwork and research in laboratory was done in Tanzania from February to June 2004. Further laboratory work, and writing was done in Norway from August 2004 to February 2005.

I thank Professor Rolf Arnt Olsen for his support, and incredibly nice talks about everything. I would also like to thank Dr. Ernest Semu and his family for giving me a warm welcome into a warm continent.

The guys at Suasa Shop, who were very patient listeners to me slamming away on a guitar.

My family who have been supporting me, and especially my grandmother Helen Finne who made this thesis readable!

Céline for always being there, even when you were not.

Lars Kåre Grimsby

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Abstract

Microbial biomass, numbers, activity and diversity were measured in tropical agricultural soil in Tanzania throughout the growth season of maize (*Zea maize*). Four farmers each had three plots that were subject to three different methods of treatment to improve soil fertility: 1) Removal of plant residues and other organic material, tilling to 10 cm, and adding nitrogen (60 kg pr ha) and phosphorus (40 kg pr ha). 2) Tilling organic plant material, such as maize residues, 10 cm into the soil, without adding fertilizer. 3) Collecting residues in heaps and burning them. No added fertilizer and no tillage. During the growth season, the total biomass and activity of the microflora increased as a response to higher soil water levels due to the rainy season. The microflora was also investigated to see whether there was any difference between the four farmers' soil types, and it was found that the total microbial biomass were in correlation with the organic carbon content in each of the four soil types. It was also found that the ratio between fungi, actinomycetes and bacteria was the same for all the four soils. The measurement of microbial activity by soil respiration was influenced by the different soil types' texture. When comparing the three different methods of treatment, it was found that burning organic material on the soil without tilling gave the greatest numbers and biomass. The combination of removing residues, tilling and fertilizing with inorganic fertilizer decreased the soil microflora most. And tilling and incorporating plant residues gave numbers and biomass between the two extremes.

Definitions

cm	centimeter
M	molar
g	gram
mg	milligram
µg	microgram
ng	nanogram
nm	nanometer
L	litre
ml	millilitre
^{o}C	Celsius
pmol	picomole
dw	dry weight

1 Introduction

Large scale agricultural development is supported by the application of mineral fertilizers. Some fertilizers, for example Urea, are acidic and may cause increased soil acidity if applied continuously over a long period of time. The effects of these acidic compounds after continuous application to soil may be: Leaching of soil nutrients (basic cations) from top to sub soils, and an increase of toxic elements, particularly aluminium and manganese. Acidity also affects the life of microbial organisms in soils by providing an unfavorable environment, which can be seen in the reduction of biological nitrogen fixation in such soils (Majule, 2004).

Small scale farming is the livelihood of 90 % of the Tanzanian population. The most profitable way of farming on a small scale is based on clearing forest and using whatever nutrients that were stored in the soil to grow crops (”slash and burn”), but this is only productive until the nitrogen reservoir is depleted (Hossner and Juo, 1999). The nitrogen reservoir found in tropical forest soil is based to a large extent on being held in living and dead biomass in the soil, and being cycled between them parallelly to the carbon cycle. When the plants on the soil are removed to give land to crop farming, an important part of the nitrogen- and carbon-cycle is reduced. The microbial biomass that is active in the nutrient cycling is largely heterotrophic, basing its nutrient intake on the organic matter that was removed by deforestation. A secondary effect following the removal of living plant biomass is a rapid leaching of nitrogen.

Soil organic matter (OM) is viewed as an important factor affecting soil quality and long-term sustainability of agriculture. Decrease in OM leads to a decline in the cation exchange capacity of soils, soil aggregate stability and crop yield. Besides being a source and sink of nutrients for plants, OM plays an important role in the carbon cycle, as it accounts for the major terrestrial pool of this element (Freixo et al., 2002).

1.1 Microbial response to altered soil environment

Much research has been done on how plants respond to different methods of soil treatment to improve soil fertility in the tropics, especially methods involving residue management that will improve the soil environment by ecologically and economically feasible means (Palm et al., 2001). But so far little is known about how the microorganisms in tropical soils respond to such methods of improving maize yield.

High microbial activity as a result of the high temperature in tropical areas removes the organic matter rapidly from soil by heterotrophic microorganisms mineralizing it to CO_2. It is generally agreed that in natural tropical ecosystems autotrophic plants quickly reincorporate the CO_2 into the carbon cycle, the growth rate of plants being much higher in tropical climates than in temperate climates. But on agricultural land plant biomass is relatively scarce compared with that of a tropical forest, resulting in a microbial depletion of the organic carbon reserves in the soil, since little of the CO_2 is fixed by plants and recycled into the soil.

Even though there may quickly be a relatively low content of organic carbon available to microorganisms in tropical agricultural soil, nitrogen is usually the growth limiting factor, and is therefore rapidly depleted in soil by soil microorganisms and plants. Theoretically, autotrophic plants would benefit more from addition of mineral fertilizers than heterotrophic microorganisms if the soil was depleted of nutrients, since the latter depend on organic matter to grow. The balance between which nutrients the plants take and which nutrients the microorgansims take is stabilized in ”normal” soil, but when there is no available carbon in the soil heterotrophic organisms will not be able to utilize any added mineral nitrogen. When competing for N in the rhizosphere, plant roots seem to be in control by feeding the bacteria C-substrate to make them immobilize N (Wang and Bakken, 1997).

It is generally agreed that large scale monoculture farming with mineral fertilizing in temperate climate has shown a decrease in the quantity of fungi in the soil, and has also caused the diversity of microorganisms to decrease in agricultural land subject to this treatment. The decline in microbial life is suspected to be a result of the continuous working of the soil, destroying aggregates and stopping efficient gas flux. Decrease in content of soil organic matter may also be a contributing factor, since heterotrophic microorganisms depend on this as a carbon source.

Continuous and extensive use of mineral fertilizers may decrease pH. A lowered pH may repress the activity of microorganisms involved in nitrification and N fixation (Maier et al., 2000), but materials increasing pH may also affect the microbial soil community structure. Certain groups are more prone to be affected by altering pH. There is a general understanding that fungi are more competitive at lower pH, and actinomycetes more competitive at higher pH.

Table 1.1: pH characteristics for bacteria, actinomycetes and fungi (Maier et al., 2000).

Characteristic	Bacteria	Actinomycetes	Fungi
Optimum pH	6-8	6-8	6-8
Competitive pH	6-8	>8	<5

Frostegård (Frostegard et al., 1993a) investigated how the microbial community structure changed as a result of increasing pH by liming and ashing soils. Changes in phospholipid fatty acid (PLFA) patterns indicated that the increased pH caused a shift in the bacterial community to more Gram-negative and fewer Gram-positive bacteria, while the amount of fungi was unaffected. There was also an indication of an increase in the amount of actinomycetes.

Majule and Nortcliff (2001) indicate that the composition of the added plant residues is important for whether the pH increases or decreases in the tropical soil subject to this treatment. In the study cashew leaves were found to contain high amounts of low molecular weight organic acids, whereas maize residues are heavily degradable and do not contain such amounts of organic acids. Instead, maize residues contain fairly high contents of basic cations (Ca^{2+}, Mg^{2+}, K^{+}, and NH^{4+}) which may increase pH. Another study found that increased fertility by the incorporation of relatively alkaline plant residues such as maize, also depended on the availabiltity of H_2PO_4 in the plant material and on the plant material's potential to release mineral N (Sakala et al., 2004).

1.2 The soil environment

According to a land report for Tanzania, the soil types in both Mikese and Michungwani are Rhodic ferrasols (Majule, 2004). These tropical soils, found in Tanzania in the coastal region, are characterized by high levels of oxidized metals and of the clay type kaolinite, and, as shown in a study of soils in Morogoro district, often with traces of smectite and mica/illite (Machado et al., 2003). Soils containing smectite present some problems of workability due to their firm moist-consistence and hard, to very hard, dry-consistence. However, in terms of fertility they are generally more fertile, having higher CEC than the highly weathered kaolinitic soils (Hossner and Juo, 1999; Msanya et al., 2003). These specifications are a result of the age and history of the African continent: The ground is built on a very old sea floor.

The negative charge on the clay particles binds a layer of cations, and these two layers make an electrical double layer. Outside this double layer, negatively charged bacteria may bind (Maier et al., 2000). The amount of clay varies drastically from place to place. It is very easily washed away

with hard rains on irrigated and deforested lands, as can be seen in the amount of particles in flooding rivers around deforested areas in Africa.

Table 1.2: Physical parameters of sand, silt and clay (Atlas and Bartha, 1997).

Soil component	Diameter (mm)	No. of particles/g	Surface area (cm^2/g)
Sand	2.00-0.05	90	11
Silt	0.05-0.002	$5.78x10^6$	454
Clay	0.002	$9.03x10^{10}$	8.000.000

There is a general understanding that clay behaves in Africa as the organic matter does in temperate zones: As an important factor in the binding of water and macronutrients. African soils are subject to higher temperatures than those in temperate climates. This not only increases microbial activity, but plants also grow faster, supporting the general perception that more of the organic matter is above the soil surface than below. Microbial respiration, as can be measured by CO_2-flux from the soil, should therefore be much higher than in cooler climates. But this activity also depends on the availability of water, and the clay content determines this to a certain extent.

1.2.1 Soil moisture's effect on microorganisms

The availability of soil moisture is important for microbial activity. Water potentials lower than about -0.5 MPa typically inhibit many bacterial activities due to physical constraints on substrate transport, cell movement and the thickness of films available for bacterial immersion. But water may also be a basis for natural selection among microorganisms. Based on their responses to, or tolerance of, water stress, a number of microbes have been assigned to one of five groups defined loosely by optimum and minimum water potentials for growth (Griffin, 1981). Three of these groups are most relevant to soils:

Group 1: Optimum -0.1MPa; minimum about -2.0 MPa. This group contains some fungi and a variety of Gram-negative bacteria.
Group 2: Optimum about -1.0 MPa; minimum -5.0 MPa. This group contains many phycomycete fungi, actinomycetes and Gram-negative bacteria.
Group 3: Optimum about -1.0 MPa; minimum -10 to -15 MPa. This group contains a variety of ascomycete and basidiomycete fungi, actinomycetes and Gram-positive bacteria.

1.2.2 Cation exchange capacity in clay

There are high contents of kaolinitic clay, measured to be up to 59 %, in areas in the Morogoro district (Msanya et al., 2003). Kaolinitic soils generally have a low effective cation exchange capacity, i.e. less than 12 meq/100g of clay (Hossner and Juo, 1999). Highly charged small cations such as Al^{3+} have high adsorption affinities compared with larger ions and ions with less charge; an isomorphic substitution happens when one cation changes place with another on the clay particle. Ionization occurs in hydrooxyl groups from metal oxides, oxyhydrides, and hydroxides that are exposed on the lattice surface, also resulting in the formation of a negative charge:

$$Al\text{-}OH \leftrightarrow Al\text{-}O^- + H^+$$

These are known as broken-edge bonds. Ionizations such as these are pH dependent and increase as the pH increases (Maier et al., 2000). An increase in soil pH from the addition of lime or ashed organic material should increase ionization of macro- and micro-nutrients necessary for plants and microorganisms.

The level of bound phosphate is high when the pH is low. A challenge is to free the phosphate for plant nourishment. This can be done by increasing the pH, for example by adding types of organic matter that is low on organic acids and has high levels of basic cations, such as maize (Majule and Nortcliff, 2001). An increase in pH can also be achieved by adding ashed plant material.

1.3 Physical properties of maize residues

Lowered pH caused by the addition of plant residues is mainly due to the presence of low molecular weight, soluble organic acids in the leaves. In contrast, a high pH resulting from the incorporation of plant residues may be due to large amounts of base-forming cations (Ca^{2+}, Mg^{2+}, K^+, and NH_4^+, table) (Majule and Nortcliff, 2001). Maize has proved to be a suitable organic material for adding to soil since its pH is relatively high, and it has relatively large amounts of basic cations. A contributing factor to the high pH may be that much of the organic plant material is found as lignin and cellulose, and not low molecular organic compounds.

Table 1.3: Mean pH of maize residue and major ions present in the organic material, from studies by Majule and Nortcliff (Majule and Nortcliff, 2001).

Parameter	Maize
pH (1:10)	7.10
Total ions ($mmol_c\ kg^{-1}$)	
Ca^{2+}	135.00
Mg^{2+}	148.30
K^{+}	346.40
Na^{+}	3.60
NH_4^{+}	0.31
SO_4^{2-}	131.57
Cl^{-}	80.50
$H_2PO_4^{-}$	23.55
NO_3^{-}	4.23

Table 1.4: Contents of organic C, N, lignin, and soluble polyphenol in maize residues (Majule and Nortcliff, 2001).

Parameter	Maize
Total C	44.20 %
Total N	0.55 %
Total lignin	7.98 %
Soluble polyphenol	1.73 %

According to Palm, Giller et al (2001) plant residues containing ”nitrogen<2.5 %, lignin<15 % and polyphenol<4 %” are defined as organic material of intermediate to low quality. This category implies that when the organic material is applied to soil it has a low nitrogen supplying capacity, and the organic residues will be responsible for a short term immobilization of whatever nitrogen was mineralized in the soil. Common for this category of organic material is also that its application has little effect on total SOM.

Table 1.5: Mean values of organic carbon to nitrogen (C:N) and lignin to nitrogen (L:N) (Majule and Nortcliff, 2001).

Parameter	Maize
C:N	80:1
L:N	15:1

1.4 The environment

The average air temperature in Tanzania is 24^{o}C. The climatic situation is different for the two locations Mikese and Michungwani (see Figure). The Michungwani area is defined as being dry

sub-humid to semi-arid (moisture index –10 to –30). According to a report (Majule, 2004) the land is not of forest potential but of high agricultural potential. Regular burning is common. Farmers in Mikese are in a semi-arid agroecological zone (moisture index –30 to –42). These are lands of marginal agricultural potential, mainly sisal, and dry form of woodlands is common. The annual rainfall in both villages is 800 – 1000 mm (Majule, 2004).

Most regions in Tanzania experience a bimodal rainfall pattern characterized by two rainfall peaks in a year with a definite dry season separating the short and the long rains. The short rainy season is from October to December while the long rainy season starts in February and ends in April. The seeds are usually planted in the beginning of the long rainy season, ”Masika”, normally starting the first weeks of February and continuing to some time in April. The temperature is at its peak around December, and falls during the rainy season. When the maize is harvested in July, the climate is cool and dry. The stem stops growing in May, when the cobs develop and ripen. In July the seeds on the cobs are ripe and the plants die. This is the time for harvesting the cobs, and the residues are usually left standing until next year. When next season comes, most farmers choose to pile all the dry stems and leaves, and burn them. This is a simple method of treatment, requiring little labour compared with tilling the dry stalks into the soil.

1.5 Outline of the research

This research project was the microbial part of a larger project where the aim was to investigate whether there are alternatives to using only mineral fertilizers for improving crop yield. It is titled “Evaluation of the effect of nitrogen and phosphorus application, tillage and residue management on physical and chemical characteristics of soil, weed, microbial population and on yield of maize”. It involved six different methods of treatment of 6 different plots. Plots 4, 5 and 6 were chosen for microbial analysis. Microbial numbers, activity, biomass and diversity for these three plots are analysed, compared and discussed in this paper.

Plot 1: Tilling in organic plant material, such as maize residues, to 10 cm depth. Add (60 kg pr ha) and phosphorus (80 kg pr ha).

Plot 2: Tilling in organic plant material, such as maize residues, to 10 cm depth. Add (60 kg pr ha) and phosphorus (40 kg pr ha).

Plot 3: Burning organic material, such as maize residues, on the plot. Till to 10 cm. Add (60 kg pr ha) and phosphorus (80 kg pr ha).

Plot 4: Removal of plant residues and other organic material, and tilling to 10 cm. Adding nitrogen (60 kg pr ha) and phosphorus (40 kg pr ha).

Plot 5: Tilling in organic plant material, such as maize residues, to 10 cm depth. No added fertilizer.

Plot 6: Collecting residues in heaps and burning them. No added fertilizer and no tilling.

1.6 Methods for improving soil fertility

4. Addition of mineral fertilizers will increase soil fertility on a short term basis.

But many fertilizers may lower the pH, and the content of organic matter may decrease as a secondary effect of increased microbial activity. Also, this alternative works to increase plant growth where there is access to fertilizers, but small scale farmers cannot afford it. An eventual increase in soil acidity will affect the cation exchange capacity, making minerals more inaccessible by binding them harder to clay particles.

In theory, the content of microorganisms should decrease as a result of treatment with this method. There may also be a decrease in the content of actinomycetes relative to fungi.

5. Addition of organic matter will increase the content of organic matter, it will increase the content of nutrients, and perhaps the pH.

It is probable that when maize residues are added, this causes an increased growth of microorganisms, because they must digest the heavily degradable cellulose and lignin to make it

available to the plants. The competition between microorganisms and plants must be finely adjusted to fit everyone's needs.
The microbial counts and biomass should increase, and there may be an increase in actinomycetes relative to fungi.

6. Addition of ashed organic matter may increase nutrient levels and pH slightly. No tilling may conserve the microflora's high diversity and biomass.
Maize residues burned may be useful for avoiding the need for mineral nitrogen, because mineral nitrogen is probably needed for the microorganisms to be able to degrade the heavily degradable maize material. But when the cellulose and lignin has already been burned, there is no carbon source for the heterotrophic bacteria. Instead there are easily available basic cations, ready to be utilized directly by plants. The pH may increase slightly from addition of ashed material, preventing some of the effects of acidity, and increasing ionization of bound inorganic nutrients.
Theoretically, the microorganisms should have more available macronutrients, increasing growth slightly, but organic carbon should be the growth limiting factor. Soil which has not been tilled is known to contain higher levels of carbon and stable microbial communities.

1.7 Methods for identifying changes in the microbial community as a response to the three methods of treatment

The classical way of measuring biomass and numbers of bacteria in soil is by counting colony-forming units on agar medium. The principle is to take advantage of the knowledge that a single bacteria has the potential to grow into a colony, which is visible to the human eye. This method was one of the first methods of analysis of microbial life, and is still in use. One of its benefits is that the result gives you the number of viable organisms in soil - the organisms that are vital enough to be able to reproduce. The challenge is to find a medium on which they are able to reproduce. Because of the enormous variety of preferences for substrates for bacteria in soil, it is very difficult to make all the different species form colonies. Some grow slowly, some need a specific medium, some are aerobic and some anaerobic etc.
A more recent method of counting bacteria is by counting the total amount of bacteria by staining them, and counting them directly in soil samples in a microscope. The most commonly used procedure for direct counting of bacteria is by a filtration method where bacterial cells are trapped

on 0.2-μm pore size polycarbonate membrane filters after being stained with nucleic acid dyes such as SYBR-green I. They can then be enumerated by fluorescence microscopy (Hobbie et al., 1977). This gives the total number of bacteria, both live and dead (Noble and Fuhrman, 1998), and is usually more accurate than the viable counts method.
Many bacteria are active, but not able to reproduce. The inability to reproduce is seen in the stationary phase of bacterial growth, when the bacteria start autolysis by eating their own enzymes, and thereby complicate the process of reproduction. The two methods above show the amount of bacteria able to divide, and the total number of bacteria, live and dead. Other information which might be of interest when it comes to analysing the microbial response to different treatments of soil, is the amount of actively respiring bacteria. The method follows the same procedure as for the total counts, except that the staining chemical (CTC) is a fluorochrome that reacts with redox-products instead of with DNA as in staining with SYBR-green I. Staining with the tetrazolium-salt CTC will make it possible to visualize active bacteria in the microscope (Rodriguez et al., 1992).

Although microbial counts are important for analysing microflora in soil samples, microbial activity may be the quantitative measurement of most interest when comparing soil samples from the three differently treated test plots, since microbial counts may not correlate directly with the rate of degradation of organic matter and other nutrients in the soil. There are several different methods for measuring soil microbial activity, but the one chosen for these experiments is perhaps the one that gives the most direct answer, because it is based on microbial aerobic respiration, done by measuring CO_2-flux in situ (Maier et al., 2000).

Much of the primary decomposition of organic matter in soil is done by fungi. It is fairly difficult to count fungi or measure their biomass in soil. Lengths of hyphae can be counted through a microscope, and fungal biomass may be calculated on the basis of a formula, but this is difficult and a very inaccurate method for measuring fungal biomass. The method which is often chosen is to grow the spores on agar medium, potentially showing one colony-forming unit per spore.

The classical methods of microbial analysis described above are used for measuring microbial biomass, numbers and activity. They can say something about the development of the soil microflora during the five months of the growth season of maize, but they do not say much about

the community structure. Several molecular and biochemical methods have been developed over the past decade that are useful for studying the great diversity of microorganisms in soil, most the microorganisms being unknown and unculturable (Torsvik et al., 1990). For example, analyses of microbial DNA and phospholipid fatty acids (PLFA) have proven extremely useful for describing the general structure of soil and aquatic microbial communities. Analysis of PLFAs has been used to monitor changes in microbial community structure in response to several factors, such as agricultural management activities (Frostegard and Baath, 1996; Zelles et al., 1992) and heavy metal contamination (Frostegard et al., 1996). But so far there have been no known PLFA-analyses on comparing microbial community structure as a response to different methods of treatment of tropical soil, and the PLFA composition in tropical soil has not yet been studied extensively (Burke et al., 2003).

1.8 The microbial part of Project 044, TARPII-SUA

The research project was led by Thomas Ikerra at Dakawa research station, Tanzania, and funded by NORAD through TARP II – SUA. The title of Project 044 is "Evaluation of the effect of nitrogen and phosphorus application, tillage and residue management on physical and chemical characteristics of soil, weed, microbial population and on yield of maize". This paper is the microbial part of Project 044, and is Lars Kåre Grimsby's Masters Degree thesis.

The aim of this research was to compare the microbial activity, biomass, numbers and diversity as a response to three different methods of improving soil fertility.

2 Materials

2.1 Chemicals

Table 2.1:

Chemical	**Producer**
Acetone	Merck eurolab
Acetic acid	Merck
Agar	Remel
Aluminum sulphate, $Al_2(SO_4)_3$	*[1]
Ammonium nitrate, NH_3NO_3	Merck eurolab
Boric acid, H_3BO_3	*[1]
Citric acid	Merck
Chloroform	Merck eurolab
Cobalt nitrate hydrate, $Co(NO_3)_2x6H_2O$	*[1]
Copper sulphate hydrate, $CuSO_4x5H_2O$	*[1]
5-cyano-2,3-ditolyl tetrazolium chloride (CTC)	Sigma
Cycloheximide	Sigma
Diphenylamine	*[3]
Ferrous sulphate hydrate, $FeSO_4xH_2O$	Merck eurolab
Fructose	Sigma
Glucose	BDH laboratory supplies
Hexane	Merck eurolab
Litium chloride, LiCl	*[1]
Magnesium sulphate hydrate, $MgSO_4xH2O$	Prolabo
Malt extract	Merck eurolab
Mangane chloride hydrate, $MnCl_2x4H_2O$	*[1]
Mangane sulphate hydrate, $MnSO_4xH_2O$	Imported by Einar D Fineide AS
Methanol	Merck eurolab
Nickel sulphate hydrate, $NiSO_4x6H_2O$	*[1]
Nitrogengas	AGA
Peptone	Merck eurolab
Phosphoric acid	Merck
Potassium bromide, KBr	*[1]
Potassium chloride, KCl	*[2]
Potassium dichromate, K_2CrO_4	Merck
Potassium dihydrogen phosphate, KH_2PO_4	Merck eurolab
di-Potassium hydrogen phosphate, K_2HPO_4	Merck eurolab
Potassium hydroxide, KOH	Eletrokemiska aktiebolaget
Potassium iodide, KI	*[1]
Sodium-azide, Na-azide	*[2]
Sodium citrate	JT Baker Chemicals
Sodium chloride	Merck eurolab
di-Sodium hydrogen phosphate hydrate, $Na_2HPO_4x12H_2O$	*[2]
Sodium hydroxide, NaOH	Merck eurolab

Sodium succinate	Koch-Light Laboratories Ltd
Sulphuric acid, H_2SO_4	*[3]
SYBR-green I	Molecular probes
Streptomycin B	Sigma
Tin chloride hydrate, $SnCl_2x2H_2O$	*[1]
Titaniumoxide, TiO_2	*[1]
Toluene	Merck eurolab
Xylose	Norsk Medisinaldepot
Yeast extract	Merck eurolab
Zink sulphate, $ZnSO_4x7H_2O$	*[1]

*[1] Premade A-Z solution, IKBM, UMB.
*[2] Premade PBS, IKBM, UMB.
*[3] Premade Indicator solution, SSD, SUA.

2.2 Scientific equipment

Table 2.2:

Instrument	**Model**	**Producer**
CO_2-flux meter	EGM-1	PD Systems
Soil thermometer	Model 2000	Solexpress
pH-meter	Delta 320	Mettler
Oven (105°C)	Termaks	Termaks
Furnace (550°C)	Carbolite	Carbolite
Filtering unit	1225 Sampling Manifold	Millipore
Airpump (- 1 mPa)	KNF Laboport	Neuberger
Microscope	Laborlux 12	Ernst Leitz Wetzlar
Objective for fluorescence microscopy	PL Fluotar 100x	Leitz Wetzlar
Ocular for fluorescence microscopy	BCZ	Leitz Wetlzar
Light filter for fluorescence microscopy	I3 Excitation range: Blue Exciting filters: BP450-490 Supression filter: LP515	Leitz Wetzlar
Heatingblock with injectionappratatus for N_2	Inndampningsblokk	Arne Svendsen, UMB
Supelco filtering unit	Visiprep 24	Supelco
Centrifuge	Digifuge	Heraeus Christ
Gas chromatograph	Autosystem XL	Perkin Elmer
NDIR 2-Channel gas analyzer	Binos 100	Rosemount
2 thermostats	Heto labequipment DK	Heto
2 cooler units	Heto frig	Heto
2 thermostats	MS Lauda	MS Lauda
Anopore membrane filters	Anodisc 25, 0.2 µm, 25 mm	Whatman International Ltd.

Polycarbonate membrane filters	Nucleopore, 0.2 µm, 25 mm	Costar
Varian columns	Bond Elut	Varian
Syringefilters, 0.1 µm poresize	Millex-VV	Millipore
Teflon coated testtubes		

2.3 Computer programs

Table 2.3:

Program	**Producer**
Minitab 14.0	Minitab Inc.
Microsoft office XP	Microsoft
TableCurve3D	AISN Software Inc.
TurboChrom Navigator 6.2.0.0.0:B27	PerkinElmer Instruments

2.4 Media

WSA+NS (Olsen and Bakken, 1987)

2 ml	Nutrient stock (NS)
1200 ml	Winogradskys' solution
0.2 g	Ammonium nitrate
40 g	Agar
0.8 L	dH_2O
1 ml	Cycloheximide solution added to agar when agar is cooled to ~ 40°C

Hagem's medium

10 g	Malt extract
10 g	Glucose
1 g	Ammonium nitrate
1 g	Potassium hydrogenphosphate
1 g	Magnesium sulphate hydrate
30 g	Agar
2.0 L	dH_2O
1 ml	Streptomycin solution added to agar when agar is cooled to ~40°C

2.5 Solutions

Indicator solution for determining organic carbon (Black et al., 1965)

1 g	Diphenylamine
100 ml	Sulphuric acid (96 – 98%)

Winogradsky's solution

1.2 ml	A-Z solution
1.2 ml	Mangane sulphate hydrate
1.2 ml	Iron(III)sulphate hydrate
0.24 g	Ammonium nitrate
0.15 g	Sodium chloride
0.15 g	Magnesium sulphate hydrate
0.3 g	Potassium hydrogen phosphate
1.2 L	dH_2O

Hoagland's A-Z solution (Hoagland and Arnon, 1950)

0.05 g	LiCl
0.1 g	$CuSO_4 \times 5H_2O$
0.1 g	$ZnSO_4 \times 7H_2O$
1.1 g	H_3BO_3
0.1 g	$Al_2(SO_4)_3$
0.05 g	$SnCl_2 \times 2H_2O$
0.7 g	$MnCl_2 \times 4H_2O$
0.1 g	$NiSO_4 \times 6H_2O$
0.1 g	$Co(NO_3)_2 \times 6H_2O$
0.1 g	TiO_2
0.05 g	KI
0.05 g	KBr
1.8 L	dH_2O

Nutrient stock solution (Olsen and Bakken, 1987)

0.5 g	Sodium citrate
0.5 g	Sodium succinate
0.5 g	Glucose
0.5 g	Fructose
0.5 g	Xylose
0.5 g	Peptone
0.5 g	Yeast extract
50 ml	dH_2O

Cycloheximide solution
0.2 g Cycloheximide
20 ml dH_2O

Streptomycin B solution
0.2 g Streptomycin B
20 ml dH_2O

PBS (Phosphate buffer solution)
8.0 g Sodium chloride
0.2 g Potassium dihydrogen phosphate
2.9 g di-Sodium hydrogen phosphate hydrate
0.2 g Potassium chloride
0.2 g Sodium-azide
1.0 L dH_2O

Glycerol/PBS solution
100 ml Glycerol
100 ml PBS

Mounting solution
100 µl Phenylenediamine
10 ml Glycerol/PBS solution
Kept dark, and used within 12 hours

SYBR-green I staining solution
5 µl SYBR-green I
2 ml dH_2O
Kept dark, used within 1 hour

PS
1.0 g NaCl
100 ml dH_2O

CTC (5 mM)
0.01 g CTC
6.6 ml PS

CTC staining solution

8 ml	CTC (5 mM)
2 ml	PS

Citrate buffer (0.5 L, 0.15 M, pH 4.0)

14.41 g	Citric acid
0.5 L	dH_2O

pH 4.0 Adjusted with NaOH

HAc solution (1 M)

6 g	Acetic acid
94 ml	dH_2O

KOH in MeOH (0.2 M)

0.2 g	KOH
18 ml	Methanol

3 Methods

3.1 Preparations done preliminary to the fieldwork

The research plots to be analyzed had been established two years before by Thomas Ikerra at Dakawa research station, Tanzania, and this was Project 044 in the TARP II – SUA program. He had chosen to involve small scale farmers on two different locations in Tanzania, Mikese and Michungwani.

The towns have different climates, one lying on the plains closer to the sea, and the other lying closer to the mountains. In the two towns he had picked out farmers whose fields were situated on as different as possible soil types. Five farmers were chosen in each of the two villages, but only two from each village were picked for the microbial studies. The two farmers at Mikese are Chande and Lusonzo, and the two at Michungwani on the plain closer to the sea are Kajiti and Ahmadi.

The plots prepared by Ikerra and the farmers were 5 x 6 meters, with an empty space of one meter between them. Each farmer had 6 plots, but only the methods chosen for further microbial analysis are presented below.

- Plot 4: Removal of plant residues and other organic material, adding nitrogen (60 kg pr ha) and phosphorus (40 kg pr ha). Tilling to 10 cm.
- Plot 5: Tilling in organic plant material, such as maize residues, to 10 cm depth. No added fertilizer.
- Plot 6: Collecting residues in heaps and burning them. No added fertilizer and no tilling.

After the treatment of the respective plots as described above, and the first rains had fallen, the maize plants were sown, two to three seeds in each hole. Each hole was two feet from the next in the row, and the rows three feet from each other.

3.2 Preparations for the microbial analysis

Fieldwork, analysis of soil physical properties in the laboratory, and four different methods of analysis of the microbial life, were done from February to June 2004 in Tanzania. Fieldwork was done at farms situated in the vicinity of the towns Michungwani and Mikese, located in the coastal region of Tanzania. Laboratory work performed in Tanzania was done at the Soil Science Department laboratories at Sokoine University of Agriculture, in Morogoro. Further laboratory work on microbial community structure and mineralisation rate *in vitro*, was done from August to December 2004 in Norway, at the Department of Chemistry, Biotechnology and Food Sciences at the Norwegian University of Life Sciences (UMB) in Ås.

The period of time chosen for analysis of the microbial response to the different methods of treatment of the maize plots was that of a normal growth season for maize in Tanzania. The seeds are usually planted in the beginning of the long rainy season, normally starting in February. The cobs are harvested in July.

3.3 Sampling and measurements in situ

Fieldwork was done once every month. In Mikese around the 15th every month, and around the 30th in Michungwani. The samplings were done in the morning before lunch time, since the instruments' LCD displays would blacken out at high temperatures. Analysing and soil sampling for one farmer was done in 1.5 hour's time.

Procedure:

1. Preparation of instruments: EGM-1 CO_2-flux measuring instrument, and soil thermometer.
2. Measurements were done along the diagonal across the plots.
3. One CO_2-measurement for each of the four spots along the diagonal for each plot.
4. Temperature measured in air, at 5 cm depth, 15 cm and 25 cm depth.
5. From each plot a composite-sample was made; this was a mix of soil from the four spots, and was kept in a plastic bag. Approximately 0.5 kg was collected from each plot.
6. On returning from fieldwork the samples were immideately sieved with 2 mm a sieve, returned to the plastic bag, and kept in a fridge at 4^{o}C for analysis.
7. Part of the sieved material was collected in Falcon-tubes and frozen for analysis of soil-community at a later stage.

3.4 Soil physical parameters, analysed in Tanzania

Temperature and moisture were measured in situ because they are the factors affecting microbial respiration the most on a short term scale, influencing the mineralisation rate. There is usually a correlation between these two soil physical factors and the response to mineralisation rate, which was measured as CO_2-flux in these experiments. The soil respiration rate is measured by calculating grams CO_2 emanating from the soil per square meter per hour. The EGM-1 measures this directly in situ.

Several other factors than temperature and soil moisture in situ affect the microbial communities. pH, soil moisture, organic matter and organic carbon content are some of them.

Soil samples from the research plots had been brought to the lab at SUA in plastic bags, sieved with a 2 mm sieve, and had then been stored at 4^{o}C. Soil physical parameters not possible to do in situ were analyzed at the Soil Science Department lab at SUA.

- pH was measured by putting 5 g wet weight soil in a capped glass and adding 10 ml distilled water, which was then left standing for two hours before measuring pH. The glass was shaken and the pH was measured after some minutes when most of the particles had settled.

- Porcelain crucibles were weighed after drying at 105^{o}C. Then 10 g wet weight soil from each plot was added and the crucibles placed at 105^{o}C overnight for measuring dry weight of soil.
- The same crucibles were weighed after a little cooling in an exicator, and set at 550^{o}C over night. After this period of scorching the crucibles were put in an exicator till cooled, then weighed for measurement of organic matter content.
- Measuring content of organic carbon was done by titration with potassium di-chromate (Black et al., 1965). Weighed 1 g finely grained dry weight soil into a 500 ml conical flask. Pipetted 10 ml potassium dichromate to the soil. Added 20 ml 96-98% sulphuric acid using a dispenser. Swirled the flask carefully, and let it stand for 30 minutes. Added 200 ml water and allowed it to cool. Then added 10 ml 85% phosphoric acid using a dispenser. Added 2 ml Indicator solution (see Materials) and titrated with ferrous sulphate while stirring. The color changed from brown to purple to blue and finally green, the last change being very abrupt.

$$\text{Organic carbon} = \frac{(\text{meq } K_2Cr_2O_7 - \text{meq } FeSO_4) \times 0{,}003 \times f \times MCF}{\text{g soil}}$$

MCF = moisture correction factor
f = correction factor of the organic carbon not oxidized by the treament (normally 1.3)

3.5 Biological analysis done at SUA in Tanzania

The soil collected from the plots was stored at 4^{o}C until further analysis in the laboratory at SUA. The methods of analysis done in Tanzania were: counting actively respiring bacteria by staining with CTC; counting total counts of bacteria by staining with SYBR-green I; viable counts on WSA-NS medium; and viable fungal counts on Hagem's medium. To prepare the soil for these methods of analysis, it was dissolved in dilution series:

Overview:

1. Double distilled water filtered through a 0.05 µm syringe-filter, then bottled and autoclaved, was used to prepare dilution series for analysis in the lab. Two dilution series were made for each plot, so every month dilution series from 10^{-1} to 10^{-6} were prepared for 12 samples.
2. Hagem's and WSA-NS media (see procedure below) were prepared the day before the dilution series were made, to prevent the antibiotica from withering, and stored until use.
3. The dilution series were made by weighing 10 g wet weight soil and adding them to 90 ml blue-cap bottles (100 ml), and shaking vigorously for half a minute before transferring 1 ml to the next level of dilution. This was repeated to a 10^{-6} dilution of the soil samples.
4. Platespreads were done and incubated at 26°C for 5 days.
5. Total counts were done by filtering the 10^{-4} diluted soil onto 0.2 µm Anodisc filters and staining with SYBR-green I (see below), and then counting in a microscope.
6. 10^{-3} diluted soil was filtered onto 0.2 µm Anodisc filters and stained with CTC (procedure below). Actively respiring bacteria could then be counted in a microscope with a UV-light source.

3.5.1 Hagem's medium – Viable counts of fungi

Fungi thrive in soil, and grow on organic matter, decomposing it. To count the amount of fungal spores in soil, a very simple medium was used, Hagem's medium. It is rich in easily degradable nutrients, and the use of an antibiotic to avoid unwanted growth of bacterial colonies is necessary. Streptomycin B acts on the cell wall production in bacteria, and is produced by Streptomycetes.

Procedure:

1. All the ingredients of the Hagem's medium (see Materials) were then mixed together and heated close to boiling point on a hot plate with a magnetic stirrer.
2. The medium was poured into 100 ml bottles and autoclaved. These bottles were stored until use.
3. Before use, the bottles were heated in an autoclave and Streptomycin B solution (see Materials) prepared. 1 ml of this solution was added to each of the 100 ml bottles with agar, when the agar had cooled off to ~50°C.

4. The bottles were slightly shaken to mix the antibioticum and agar, and the agar poured into the dishes.
5. 24 glass petri dishes were prepared and 50 µl 10^{-4} dilution plated. The plates were incubated at 26^{o}C for 4 days.

3.5.2 WSA-NS – Viable counts of bacteria

Because of the vast array of different nutrient requirements found among bacteria in soil, it is impossible to find a medium on which all of them grow. Many different bacteria also grow at different rates, even if they all grow on a nutrient rich medium. A medium fairly similar to soil has been most successful so far, an entirely synthetic medium called WSA-NS. It is quite low on nutrients, and this will avoid a few bacteria outcompeting the others.

A dilution series of soil samples is made, and the dilutions which will give a number of colonies between 20 and 200 on the agar dish will be the most feasible to use. Too low dilution will give too many bacteria, and it will be difficult to avoid bacteria outgrowing each other. Too high dilution will give too few colonies, thereby making it more susceptible to errors.

Cycloheximide is a chemical that destroys the ribosome in eukaroyotic organisms. In the preparation of a medium for growing soil bacteria, Cycloheximide will prevent fungal growth. It is sensitive to high temperatures, which may cause its effect to decrease.

Procedure:

1. All the ingredients of the WSA-NS medium (see Materials) were then mixed together and heated almost to boiling point on a hot-plate with a magnetic stirrer.
2. The medium was poured onto 100 ml bottles and autoclaved. These bottles were stored until use.
3. Before use, the bottles were heated in an autoclave and Cycloheximide solution (see Materials) was prepared. 1 ml of this solution was added to each of the 100 ml bottles with agar, when the agar had cooled off to ~50^{o}C.
4. The bottles were slightly shaken to mix the antibiotic and agar, and the agar was poured onto the petri dishes.
5. 24 glass petri dishes and 24 plastic petri dishes were prepared.

6. The glass dishes used were for the 100 µl 10^{-5} dilution and the plastic dishes for 50 µl of the 10^{-4} dilution. The plates were counted after 5 days incubation at 26°C.

3.5.3 SYBR-green I staining – Total counts

SYBR-green I is a fluorochrome that binds to DNA (Noble and Fuhrman, 1998). When a soil sample is stained, it is possible to see the bacteria as green fluorescent in the microscope. These can be counted, and based on the dilution it is possible to calculate the total amount of bacteria in the soil. Since SYBR-green I binds to DNA, it will also bind to dead cells, and to whole DNA found in soil .

Procedure:

1. Anodiscs (0.2 µm pore size) were prepared by filtering 1 ml 10^{-4} dilution of the soil dilution-series onto the filter in a Millipore setup.
2. The filters were dried overnight in petri dishes on pieces of filterpaper.
3. The SYBR-green I staining solution (see Materials) was prepared.
4. For each filter to be stained a drop of 100 µl SYBR-green I staining solution was set in the petridish, with the Anodisc filter on top, and let stand for 15 to 20 minutes in darkness.
5. The filters were then moved from the droplet onto the filter paper pieces again, and let dry overnight in the dark.
6. For visualization a mounting solution (see Materials) was prepared. A drop of the mounting solution (35 µl) was put on the slide before the Anodisc filter was laid on it, then a drop of mounting solution was put on the Anodisc, and the cover slide on top.
7. Counting was done in a 100x objective and 10x ocular, with UV and immersion oil.
8. Each filter was counted 100 times, i.e. 100 different viewpoints were counted. Five squares in the BCZ ocular were counted.
9. The formula used to calculate the number of bacteria pr gram wet weight soil:

$$\text{Total counts per g wet weight soil} = \frac{N \times 2.01 \times 10^6}{w \times d}$$

N = average number of bacteria pr BCZ
d = dilution of soil sample
w = ml dilution filtered

2.01×10^6 = the magnification of the area of the object covered by one BCZ square in the ocular

3.5.4 CTC- Actively respiring bacteria

The CTC fluorochrome is based on a reaction as a result of first being oxidized by products from the redox reaction in active cells, and secondly being radiated by UV-light. The radiation by UV triggers fluorescence from the fluorochrome, which makes it possible to count the stained bacteria in a microscope (Rodriguez et al., 1992). Soil diluted to 10^{-3} was filtered onto 0.2 µm Anodisc filters, dried and stained to visualise the actively respiring cells.

Procedure:

1. Anodiscs (0.2 µm pore size) were prepared by filtering 1 ml 10^{-3} dilution of the soil dilution series onto the filter in a Millipore setup.
2. The filters were dried for 0.5 to 1 hour, and then put on a droplet of CTC staining solution (see Materials) lying in a petri dish.
3. The filters were left to stain in darkness for 2 hours, and were then removed from the swabs and let dry for 1 hour, also in darkness.
4. Counting was done in a fluorescence microscope after mounting the filters in mounting solution (see Materials).
5. Each filter was counted 100 times, i.e. 100 different viewpoints were counted. Five squares in the BCZ ocular were counted.
6. The formula used to calculate the number of bacteria pr gram wet weight soil is the same as that used for calculating total counts of bacteria with SYBR-green I.

3.6 Biological analysis done at UMB in Norway

3.6.1 PLFA – Phospholipid fatty acid analysis

From the sampling done in situ there were 60 plastic bags of soil from the 12 plots analysed over the period of 5 months. An extraction of the phospholipid fatty acids from all the samples was done, the samples were analyzed on a GC and the peaks were mapped in the computer program TurboChrom Navigator.

Method:

1. Prepared Citrate buffer (see Materials).
2. Extraction of PLFA from soil
    - 4 g wet weight soil was weighed and put in 50 ml Teflon coated test tubes.
    - The citrate buffer was added so that the sum of the soil's water content and the buffer equaled 2 ml.
    - 2.5 ml $CHCl_3$ and 5.0 ml MeOH was added, and vortexed for one minute.
    - Let stand for two hours at room temperature.
    - Centrifuged at 2500 rpm for 10 minutes.
    - Extracted the supernatant with glass pipettes.
    - Split the phases by adding 2.5 ml $CHCl_3$ and 2.5 ml citrate buffer, and vortexed for 1 minute. It was then left overnight for phases to separate.
    - Extracted from the lower phase, and 4 ml of this lipid-extract was transferred to small, burned test tubes and evaporated under a stream of nitrogen gas on a heating block at 40^oC.
3. Separating into different lipids
    - Varian columns were mounted on a Supelco suction unit, and activated with 5 ml $CHCl_3$.
    - The "pellets" in the test tubes were dissolved in 100 µl $CHCl_3$, vortexed for 1 minute, and added to the Varian column. The samples were washed twice with 100 µl $CHCl_3$. The whole lipid mass was stuck to the column, and to eluate the unwanted lipids before eluating the phospholipids, the neutral and glycolipids were extracted with 1.5 ml $CHCl_3$ and 6 ml acetone, respectively. Then the collection-

tubes were replaced with ones that were burned, and the phospholipids were washed out with 1.5 ml MeOH.

- The 1.5 ml MeOH containing the phospholipids was evaporated at 40^{o}C under nitrogen gas.

4. Transesterification
    - To separate the phosphate backbone from the lipids, alkaline methanolysis was done. A solution of KOH in MeOH (see Materials) was prepared for this.
    - 100 µl 10^{-2} dilution of a lipid 19:0, was added to all the samples as an internal standard. This was dissolved in 1 ml toluene:methanol (1:1) and vortexed.
    - Alkaline methane solution was added, and this was incubated at 37^{o}C for 15 min.
    - 2 ml hexane:$CHCl_3$ (4:1), 0.3 ml 1M HAc and 2 ml H_2O were added and vortexed 1 minute. pH in the lower phase should be approximately 6.
    - Centrifuged at 3000 rpm for 5 min.
    - The upper phase was transferred to a new burned test tube and evaporated under nitrogen without a heating block.
5. Programming the GC
    - A method in TurboChrom Navigator for recognizing peaks of 30 different PLFAs common in microflora was used.
    - The temperature program on the GC was adjusted to get a good separation of the fatty acid chains between 12 and 26 carbons in length.
6. Running on gas chromatograph
    - The pellet from the last step was dissolved in 100 µl hexane.
    - 50 µl was injected into GC vials, and the vials put in the GC for analysis.

3.6.2 Mineralisation

Mineralisation of maize residues in the four different farmers' soils was measured in vitro by CO_2-flux from soil samples stored at different temperature and moisture levels, with maize residues added. 32 jam jars were prepared with 100 g dry weight soil from the four farmers. The soil moisture was adjusted to 7.5 % or 17.%, and set at 10, 20, 30 or 40^{o}C. 0.4 g organic material was

added to all the jars, and the response was measured as CO_2 on the Rosemont instrument. The mineralisation measurements ended after three weeks of sampling.

Table 3.1: Setup of the jars and the temperature and moisture level they were subject to.

	10°C	20°C	30°C	40°C
Chande	7.5 %	7.5 %	7.5 %	7.5 %
	17.5 %	17.5 %	17.5 %	17.5 %
Lusonzo	7.5 %	7.5 %	7.5 %	7.5 %
	17.5 %	17.5 %	17.5 %	17.5 %
Kajiti	7.5 %	7.5 %	7.5 %	7.5 %
	17.5 %	17.5 %	17.5 %	17.5 %
Ahmadi	7.5 %	7.5 %	7.5 %	7.5 %
	17.5 %	17.5 %	17.5 %	17.5 %

Procedure:

1. Measured dry weight for the four different soil types by drying at 105°C overnight.
2. Weighed the amount of wet soil corresponding to 100 g dry weight soil into 0.5 L jars with rubber-septum. 8 jars for each of the four soil types. Adjusted moisture content to 7.5 % for four of the 8 jars from each soil type, and 17.5 % for the remaining four.
3. Four waterbaths were adjusted to 10, 20, 30 and 40 degrees Celsius, and the jars put into the baths.
4. Measurement of soil respiration was done by extracting 2 ml gas with a gas-syringe, through the rubber-septum. This sample was injected into the Rosemont, and data could be read after the gas had stabilized itself in the tube system. Measurements were done four times during the first week of incubation.
5. After one week the jars were opened to exchange the air inside for fresh air, and to add organic material. The organic material was maize leaves and stem, ground to a fine mash. 0.4 g was added to each of the 32 jars.
6. Gas samples were taken every day the first week, and then twice a week for two weeks. General maintenance of the water baths was done every day: adding water to the bath, checking temperature, and shaking the jars to make the evaporated water droplets fall down into the soil again to maintain the wanted soil moisture levels.

3.7 Calculations

The results from all the methods of quantification of microbial life were analysed separately with the same statistical function in Minitab 14. Main Effects Plot is used to plot data means when one has multiple factors. The points in the plot are the means of the response variable at the various levels of each factor, with a reference line drawn at the grand mean of the response data. The Main Effects Plot is used for comparing magnitudes of main effects, here being defined as:

"Plot" – The effect of method of treatment of the respective plot.

"Farmer" – Effect of soil type.

"Month" – Overall climatic effects on the microbial response variable.

Lusonzo's plots being set up for research, October 2003.

Lusonzo and Grimsby next to the plots, May 2004.

4 Results

4.1 Measurement of soil physical parameters

The soil physical parameters were analysed partly *in situ* at Mikese and Michungwani, and partly at the Soil Science Department at Sokoine University of Agriculture (SUA), in Tanzania. Temperature was measured in the field, and soil moisture, pH, percent content of organic matter (OM) and percent organic carbon (OC) in the soil were measured in the laboratory.

The four farmers are shortened to the first letter in their names, and the following number indicates the plot.

Tables 4.1 a, b, c, d, e: The five tables show soil physical parameters pH, soil moisture, organic carbon (OC), organic matter (OM), and soil temperature at 15 cm, measured for the plots 4, 5 and 6. Farmers are indicated by the first letter in their names: (C)hande, (K)ajiti, (L)usonzo and (A)hmadi.

	February				
	pH	**%H_2O**	**%OC**	**%OM**	**Temp (C^0)**
C4	7.8	8.7	1.9	8.5	30.2
C5	7.0	5.7	1.7	7.0	29.3
C6	7.3	9.8	1.6	7.4	30.5
L4	6.9	21.6	2.8	9.2	29.2
L5	7.6	15.7	2.4	7.6	28.8
L6	6.9	25.9	3.2	11.0	27.8
K4	6.4	17.6	3.5	14.2	29.1
K5	6.3	17.4	3.7	14.6	29.0
K6	6.8	17.1	3.8	15.1	29.7
A4	6.6	11.8	3.5	10.0	28.7
A5	6.4	14.2	3.7	9.6	28.8
A6	7.1	14.9	3.9	11.3	28.7

	March				
	pH	**%H_2O**	**%OC**	**%OM**	**Temp (C^0)**
C4	8.2	12.6	1.6	7.5	27.4
C5	6.9	12.7	1.4	5.8	28.8
C6	6.8	12.1	1.4	5.1	29.0
L4	6.7	17.2	2.2	8.0	29.5
L5	7.1	16.6	1.9	5.9	29.6
L6	7.2	17.0	2.4	7.2	29.3
K4	6.6	23.4	3.4	9.3	29.0
K5	6.5	22.5	3.5	9.8	29.0
K6	6.5	25.0	3.7	9.4	29.0
A4	6.2	20.6	3.3	6.2	29.0
A5	6.2	22.0	3.5	5.1	29.0
A6	6.2	22.6	3.5	6.6	29.0

	April				
	pH	**%H_2O**	**%OC**	**%OM**	**Temp (C^o)**
C4	8.0	18.9	1.6	6.7	27.1
C5	7.2	16.6	1.4	6.0	27.1
C6	7.6	17.0	1.7	5.1	27.1
L4	6.8	21.9	2.4	8.4	26.9
L5	7.7	18.6	2.2	6.6	26.9
L6	7.5	25.1	3.0	8.1	26.9
K4	6.4	7.4	3.5	9.1	25.4
K5	6.6	9.3	3.6	10.0	25.4
K6	6.9	8.2	3.9	10.6	25.4
A4	7.1	9.3	3.8	6.3	26.5
A5	6.7	10.0	3.6	7.1	26.5
A6	6.7	8.6	3.4	7.8	26.5

	May				
	pH	**%H_2O**	**%OC**	**%OM**	**Temp (C^o)**
C4	8.3	7.9	1.6	5.1	27.4
C5	7.2	8.0	1.5	5.3	27.4
C6	7.7	7.1	1.4	4.2	27.4
L4	7.1	7.6	2.7	5.6	26.6
L5	7.7	8.0	2.6		26.6
L6	7.4	7.5	3.2	7.6	26.6
K4	6.4	9.6	3.5	10.8	27.2
K5	6.5	14.7	3.5	9.6	27.2
K6	6.8	15.0	3.9	9.8	27.2
A4	6.5	5.9	3.4	8.3	27.3
A5	6.5	7.3	3.2	7.5	27.3
A6	7.2	4.5	3.4	9.5	27.3

	June			
	pH	**%H_2O**	**%OM**	**Temp (C^o)**
C4	8.0	7.3	6.7	23.0
C5	6.9	5.2	6.0	23.0
C6	6.8	5.7	6.0	23.0
L4	6.7	5.1	6.3	25.5
L5	7.4	5.3	6.1	25.5
L6	7.3	6.9	8.1	25.5
K4	6.6	21.2	11.1	23.8
K5	6.5	21.7	11.4	23.8
K6	6.7	22.5	10.6	23.8
A4	6.5	17.4	8.0	24.1
A5	6.1	17.7	7.4	24.1
A6	7.1	18.1	8.3	24.1

The pH in Chande's soil was higher than that of the other soil types, and Ahmadi's and Kajiti's had the lowest pH. pH is influenced by organic acids from organic material in the soil (Maier et al., 2000). Kajiti's soil, with Ahmadi's very close, had the highest measured percentage of organic carbon (OC); Chande's had the lowest. The soil water content fluctuated because of rainfalls, and the average soil temperature sank from 29^{o}C in February to 27^{o}C in June, during the rainy season.

Table 4.2: Average content of organic carbon (OC), organic matter (OM), OM:OC ratio, C:N ratio and pH for the four soil types.

	Chande	Lusonzo	Kajiti	Ahmadi
OC	1.6 %	2.6 %	3.6 %	3.5 %
OM	6.1 %	7.5 %	11.0 %	7.9 %
OM:OC	3.8	2.9	3.1	2.3
C:N	12	10	15	16
pH	7.5	7.2	6.6	6.6

Clay's high water potential may contribute to the high OM:OC ratio. Clay have a large surface area compared with sand and silt (see Table), and water is bound within the layers of the clay. Not all of this water evaporates at 105^{o}C when a soils dry weight is measured, but will dissapear when the samples are heated to 550^{o}C, altering the weight of the measured OM.

Table 4.3: Average pH and organic carbon (OC) content for the three plots based on all the results.

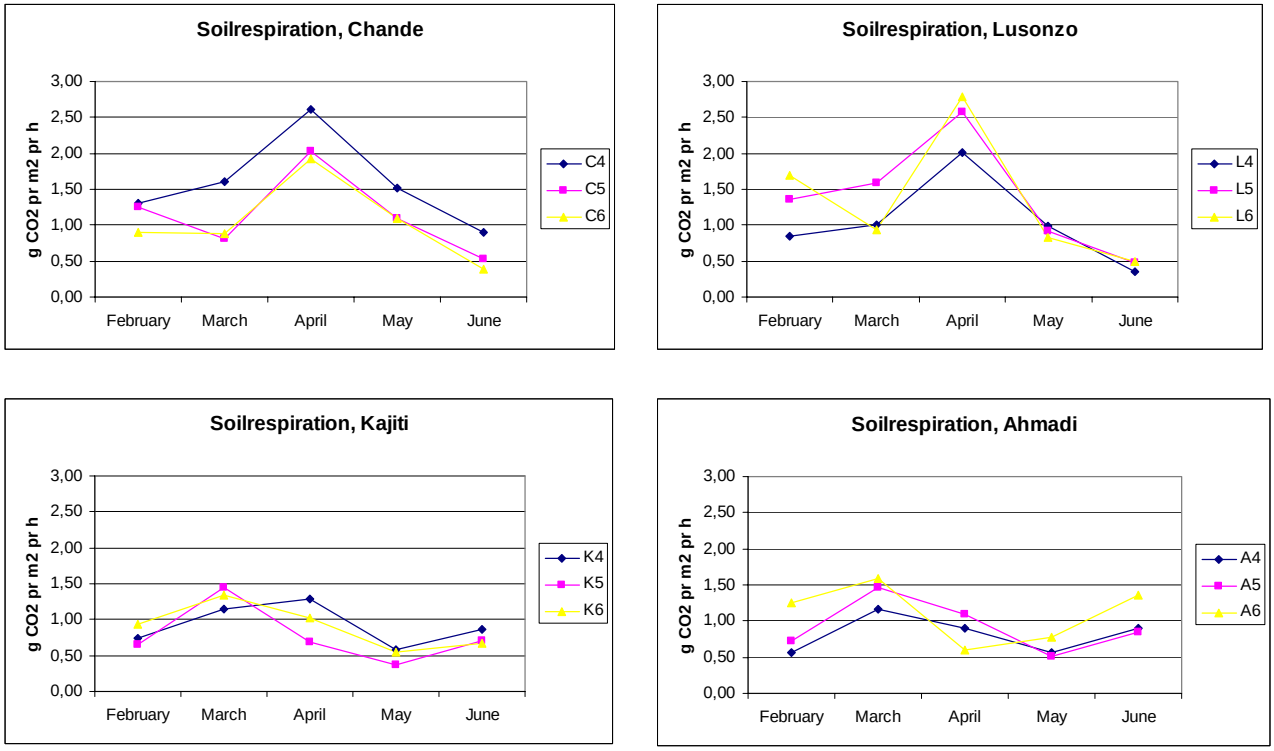
	Plot 4	Plot 5	Plot 6
pH	7.0	6.9	7.0
OC	2.8 %	2.7 %	3.0 %

An average based on all the numbers in table, was made for each of the three plots to compare the three methods of treatment.

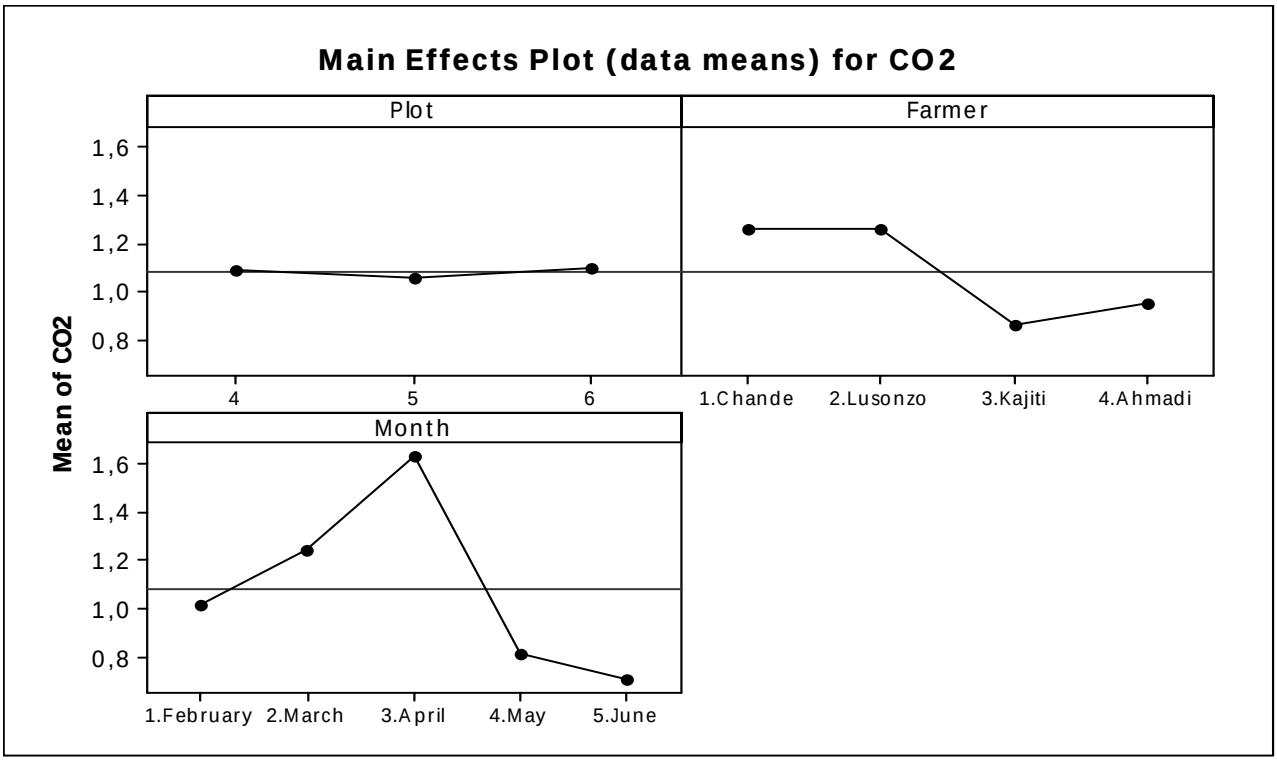
4.2 Measurement of microbial activity by soil respiration

The data are given in Appendix A.

Microbial activity was analyzed by measuring the soil respiration by quantifying CO_2 efflux. This information also gives the approximate amount of organic material being degraded in the soil at any given time. Measurement of soil respiration was done in situ and the results were given as g CO_2 $m^{2\ -1}$ h^{-1}.



Figures 4.1 a, b, c, d: The figures show soil respiration rate for the three plots from all the farmers, from February to June.



Figures 4.2 Plot, Farmer and Month: The three figures above show by comparison the effects of the method of treatment (”Plot”), the effect of soil type (”Farmer”) and the effect of the climate (”month”), on soil respiration rate.

The two first soil respiration graphs are from Mikese, the two latter from Michungwani. The curves for the two locations follow the same patterns, indicating that climatic conditions had an influence on soil microbial activity at each location. As indicated in the calculation of correlation between soil physical factors and CO_2-flux, this seemed to be mainly due to the soil water content (Calculation 4.1).

The Main Effects Plot of the different factors influencing soil respiration rate, shows what can also be seen in the soil respiration figures. The climate had the largest impact on the microbial activity, indicated by the difference in magnitude between CO_2-flux at the two locations Mikese and Michungwani. The latter effect can be seen on both the soil respiration figures, and the Main Effects Plot showing ”Farmer”; the average soil respiration rate was lower in Michungwani than Mikese.

The soil physical parameters given in the soil physical table (Table) are both effects of climate and effects of soil type. To get a better understanding of how the soil physical parameters influence, or are influenced by, microbial activity, a calculation of correlation between CO_2-flux and all the measured soil physical parameters was done in Minitab.

Calculation 4.1: The correlation between soil respiration (CO_2) and pH, soil moisture (H2O), organic matter (OM), organic carbon (OC) and temperature.

Correlations: pH; H2O; OM; Temp; OC; CO2

	pH	H2O	OM	Temp	OC
CO2	0,415	0,487	-0,223	0,211	-0,393
	0,001	0,000	0,087	0,105	0,006

Cell Contents: Pearson correlation
P-Value

The calulation of correlation above shows that there are significant correlations between CO_2-flux and pH, content of organic carbon and soil moisture. Based on the soil physical table, which shows the fluctuations in all the plots' parameters during the five months, it is clear that the content of water is the climatic factor that had changed the most. This indicates that rainfall may be the cause

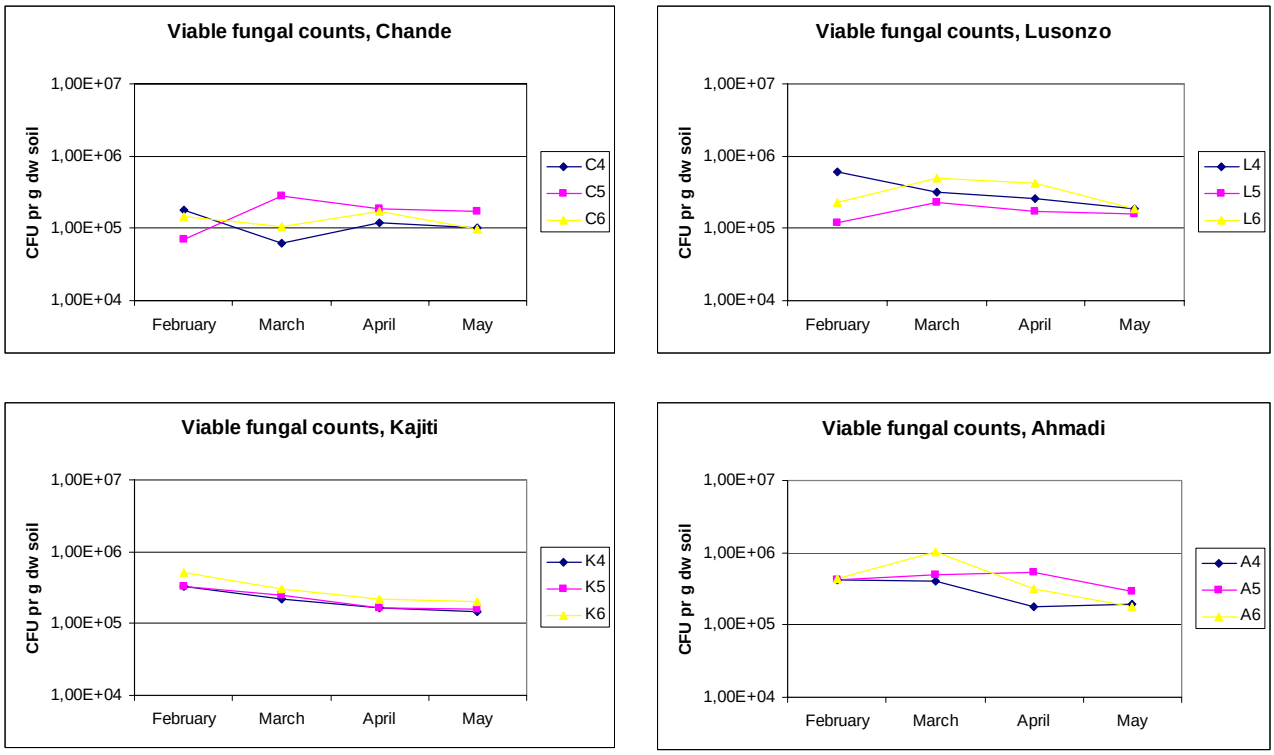
of the peak in mineralisation rate in April in Mikese, but it does not affect the Main Effects Plot showing ”Plot”.

4.3 Microbial counts

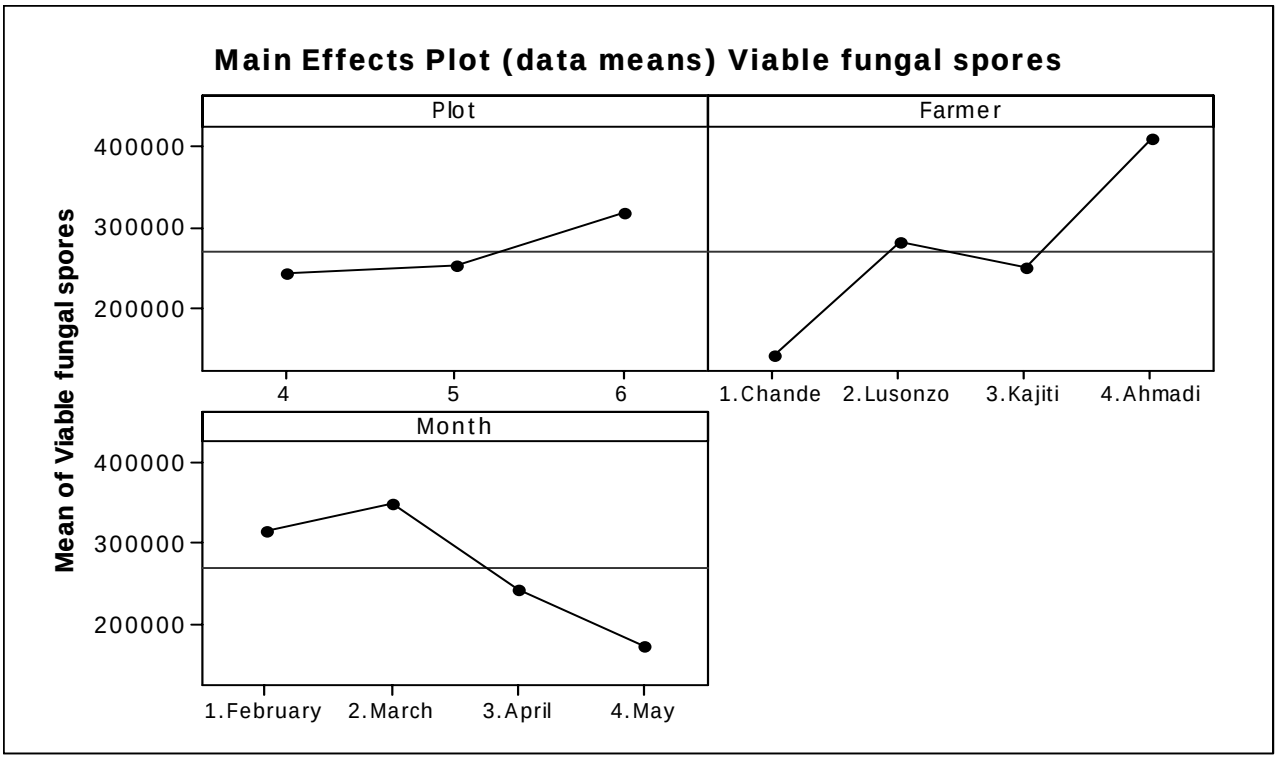
4.3.1 Viable fungal counts

The data are given in Appendix A.

Fungi contribute to the soil respiration rate, but little is known about their role in Tanzanian tropical soil. So far there are relatively few good methods for counting fungi, since they usually grow as hyphae or are present as spores. The method utilized in this analysis is based on counting colony forming units (CFU), which for soil fungi represent the number of viable spores.



Figures 4.3 a, b, c, d: A logarithmic presentation the viable fungal counts of every farmer’s plots from February to April.



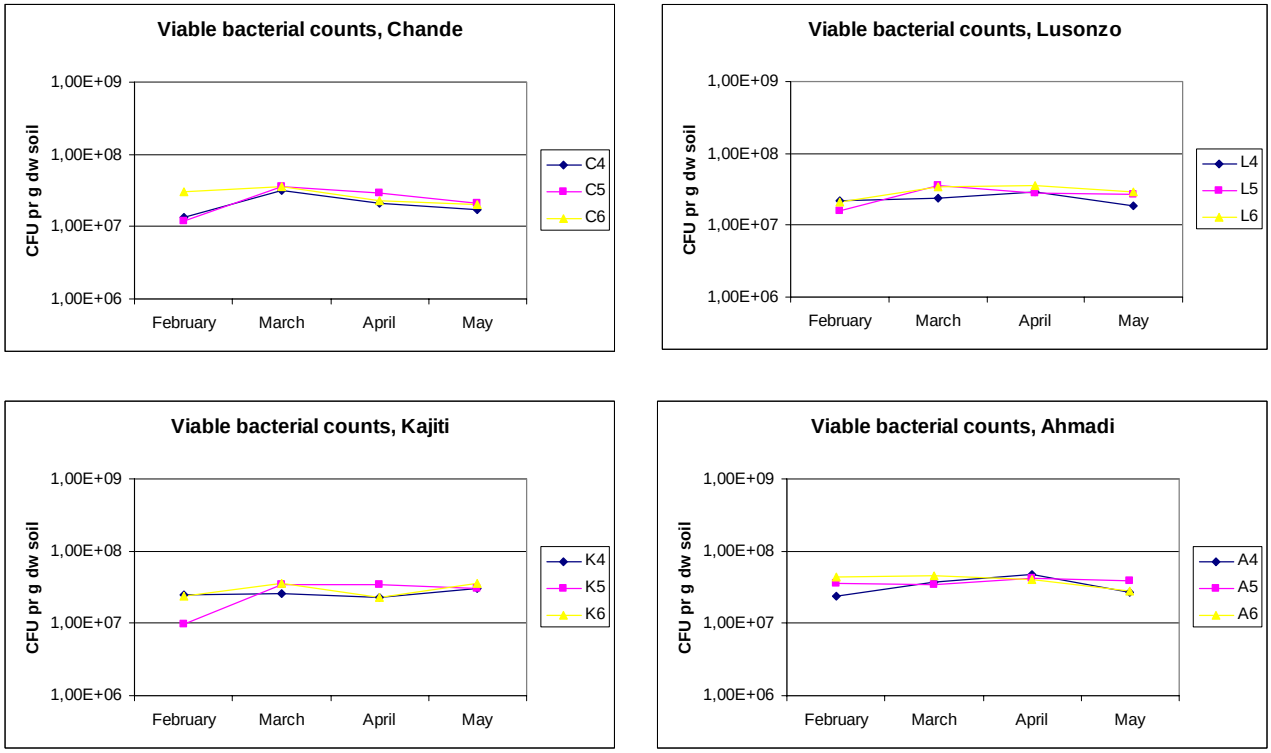
Figures 4.4 Plot, Farmer and Month: A Main Effects Plot where the effects of the different plots, farmers and months can be seen in separate graphs. The response on the y-axis is given as number of fungal CFUs pr g dw soil.

The number of viable spores was found to be about 10^5 spores per gram dry weight soil and decreased slightly during the growth season of maize.

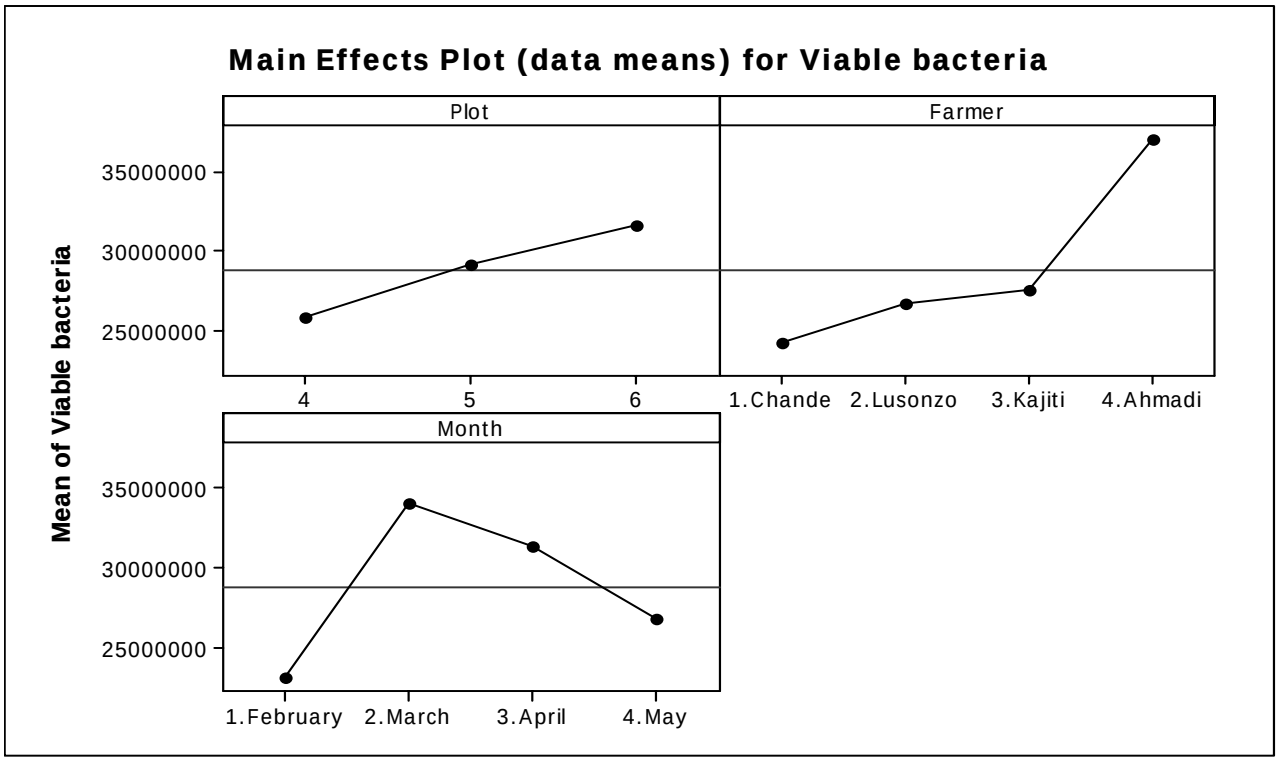
The Main Effects Plot enables comparison of the methods of treatment independent of the effects of soil or climate. The ”Month” plot shows that during the growth season, the numbers of viable fungal spores decreased. ”Farmer” shows that Chande’s relatively poor soil had fewer viable spores than the average, and that Ahmadi’s rich soil had more. The effect of methods of treatment, given as ”Plot”, do not show much influence on the amount of fungal spores, but it gives an indication that the treatment of plot 6 have given slightly increased amounts of fungal spores.

4.3.2 Viable bacterial counts

The data are given in Appendix A.



Figures 4.5 a, b, c, d: A logarithmic presentation of the viable bacterial counts of every farmer's plots from February to April.



Figures 4.6 Plot, Farmer and Month: A Main Effects Plot where the effects of the different

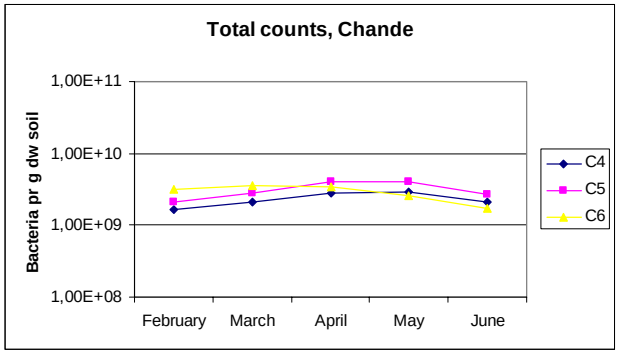
plots, farmers and months can be seen in separate graphs. The response on the y-axis is given as the number of viable bacteria pr g dw soil.

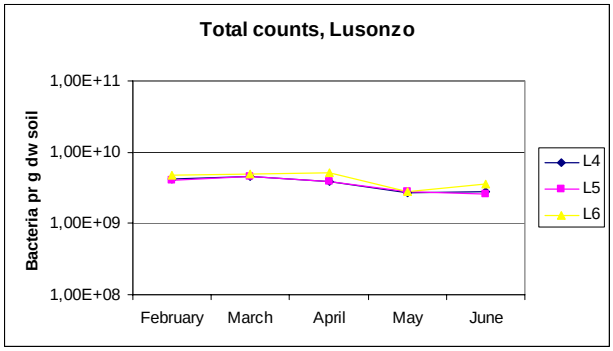
The figures showing the bacterial viable counts on a logarithmic scale give little reason to believe there is any difference even between the soil types, in bacterial counts.

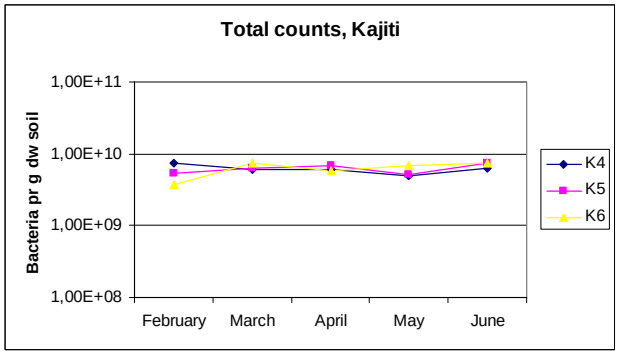
Climatic effects seemed to play an important role in the bacterial ability to reproduce, as shown in the ”Month” plot. Soil type also influenced bacterial viability, and Ahmadi’s rich soil supports this. The method of treatment affected the viability, and burning of maize residues on top of the plot gave the highest numbers of CFUs, whereas fertilizing with nitrogen and phosporus gave the lowest.

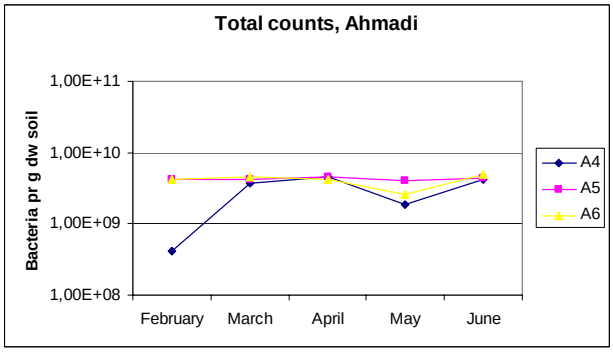
4.3.3 Total counts

The data are given in Appendix A.

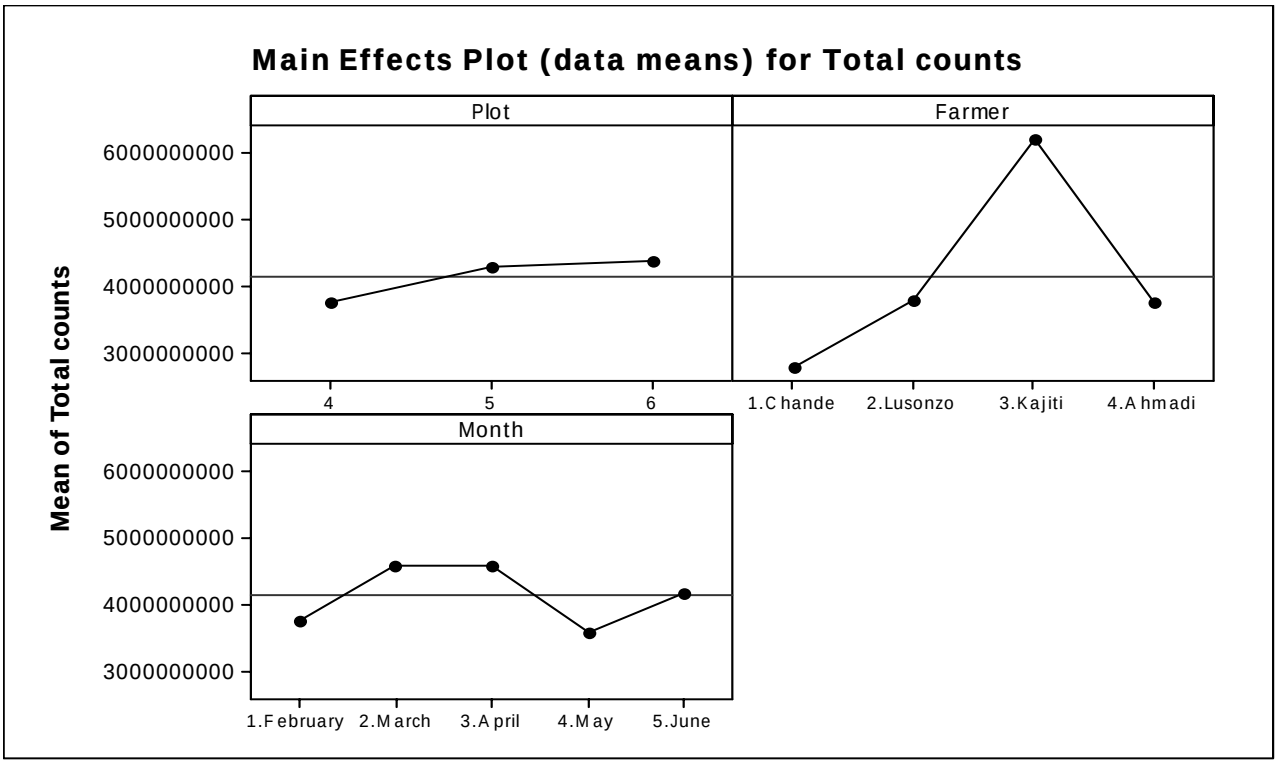








Figures 4.7 a, b, c, d: A logarithmic presentation of the viable bacterial counts of every farmer's plots from February to June.



Figures 4.8 Plot, Farmer and Month: A Main Effects Plot where the effects of the different plots, farmers and months can be seen in separate graphs. The response on the y-axis is given as the total number of bacteria pr g dw soil.

Changes in soil physical parameters caused some fluctuations in the number of counted bacteria, as seen in the "Month"-plot, but the strongest influence on total counts was by one of the soil types, Kajiti's. The treatment of the soil also had some effect on the total counts, and just as for the viable counts of bacteria, plot 6 gave the highest number total bacteria.

4.3.4 Actively respiring bacteria

The data are given in Appendix.

Bacteria that are not necessarily viable, but not dead either, are at least respiring. They maintain metabolism for some time, and if they are heterotrophic they will continue digesting organic

material, contributing to mineralisation. They can be counted by microscope when stained with the fluorochrome CTC, a tetrazolium salt wich reacts with products of the red-ox reaction in the cell.

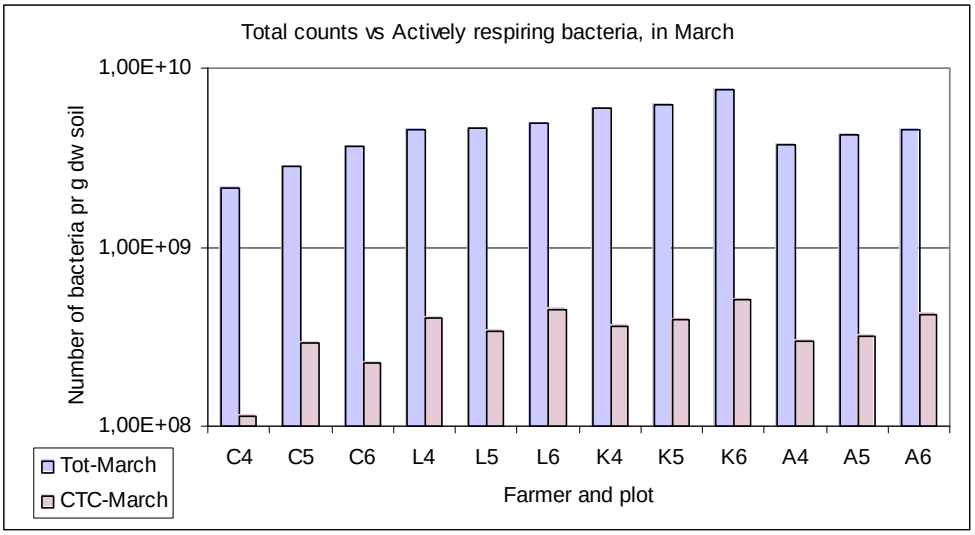


Figure 4.9: Total counted bacteria versus actively respiring bacteria on a logarithmic scale.

The measurement of actively respiring bacteria was only done in March, and these results are compared with the total counts for the same month to see whether there is a correlation between them.

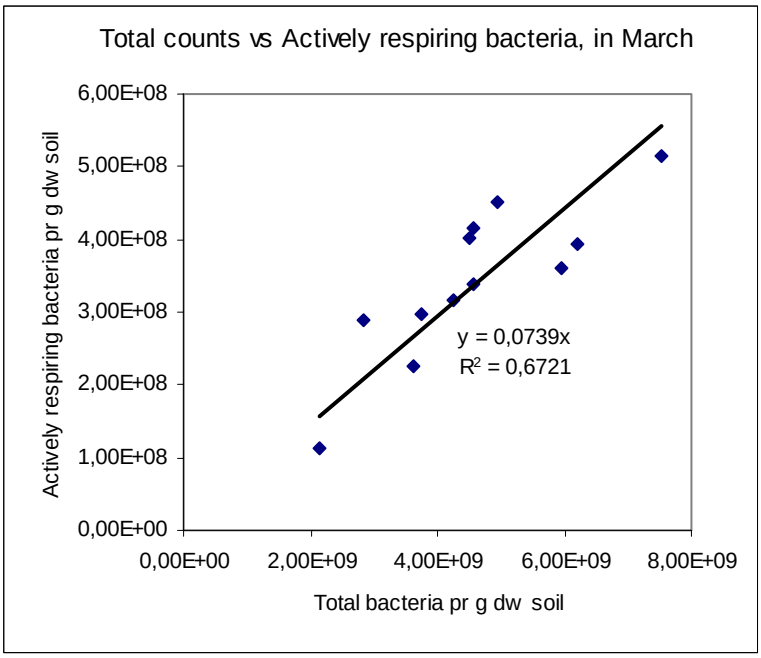


Figure 4.10: Actively respiring bacteria versus total counted bacteria in a regression plot.

There was a correlation between the total amount of bacteria and the number of actively respiring bacteria. The number of active bacteria was quite compared with the total counts, adding more to the relatively high level of microbial activity as measured by CO_2-flux. There is one anomaly when comparing these results; Kajiti's soil gave a high number of actively respiring bacteria, but a relatively low level of CO_2-flux.

4.4 Analysis of microbial community structure based on phospholipid fatty acids

Soil samples kept cool in Tanzania were transported to the Norwegian University of Life Sciences (UMB) for further analysis. The extraction of the phospholipid fatty acids from the soil samples, and the analysis of the composition by gas chromatography, were done at the Soil Science Department at UMB.

The phospholipid fatty acids were extracted and run on a gas chromatograph, and 30 peaks were identified by the TurboChrom Navigator program. Based on previous studies, some of these were chosen to represent bacteria (Frostegard and Baath, 1996). The methylated PLFAs represent

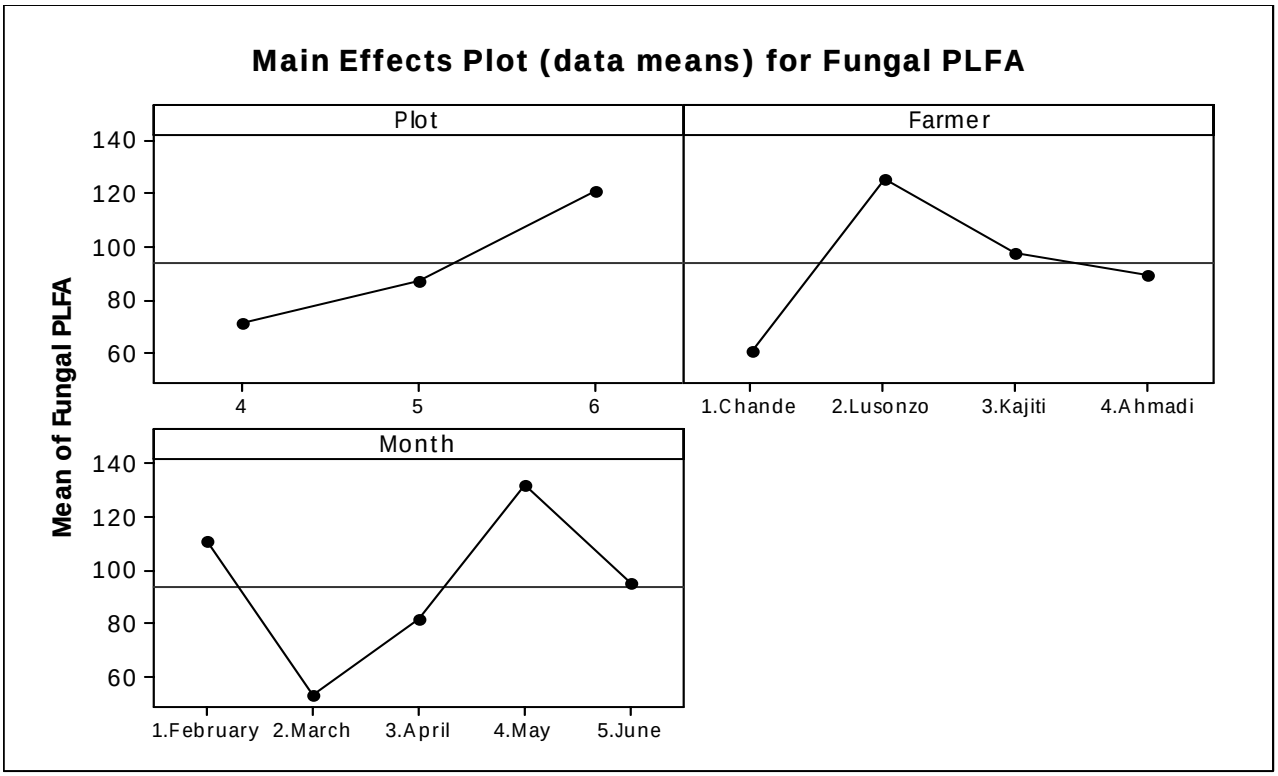
actinomycetes (Frostegard et al., 1993b), and one particular PLFA has been found to function as a marker for fungi (Frostegard and Baath, 1996).

Table 4.4: Phospholipid fatty acids and the organisms they can be found in.

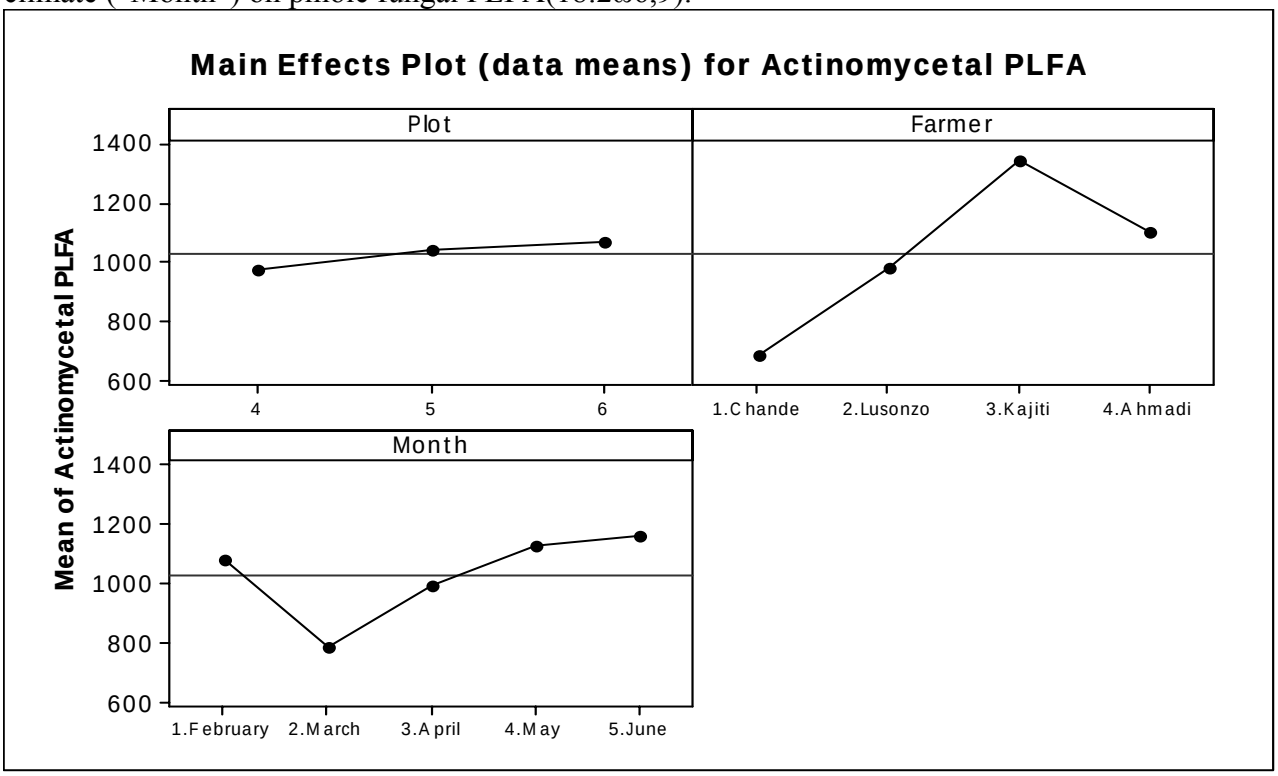
Organism	**PLFA**
Bacteria	i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7, cy19:0
Actinomycetes	10me16:0, 10me17:0, 10me18:0
Fungi	18:2ω6,9

Based on the results from the mapped peaks from the gas chromatograph, it was possible to do both quantitative and qualitative analysis of the soils' microbial communities. The PLFA composition was measured by summarizing the quantity of the peaks of interest, in this case being the ones represented in the table above.

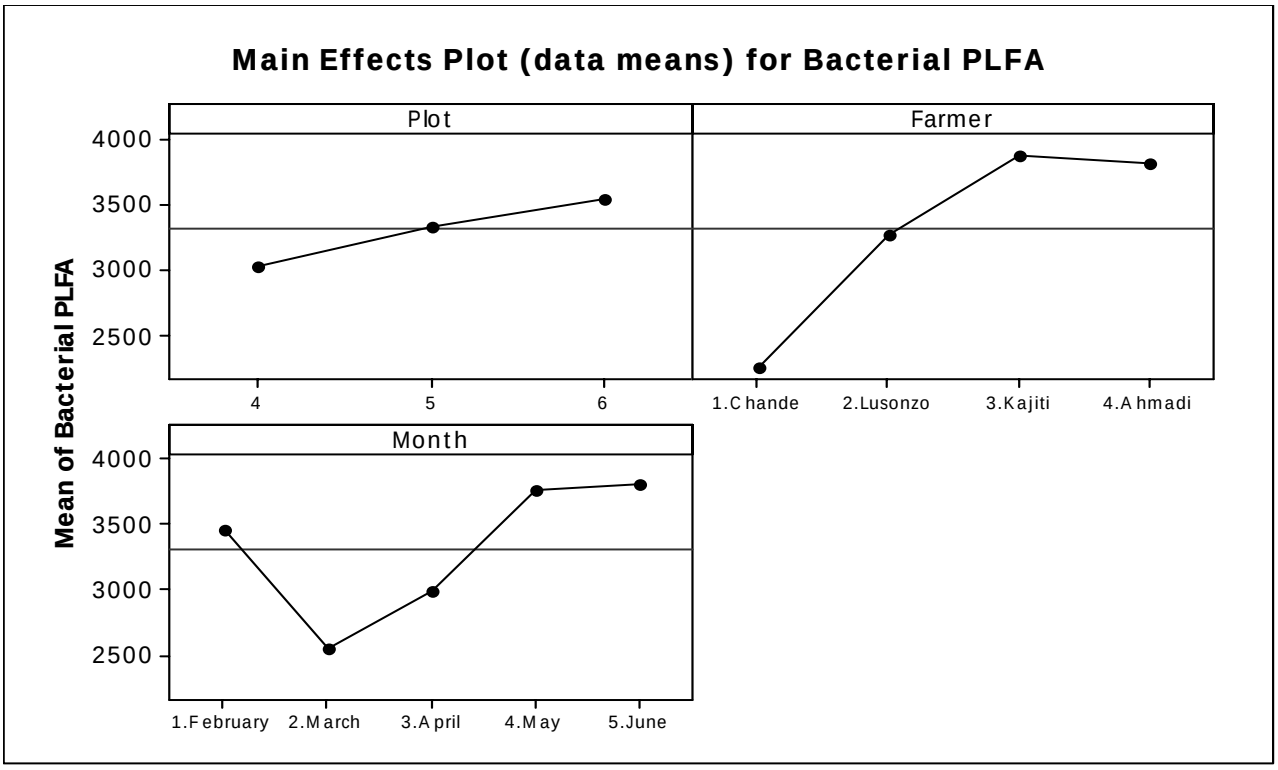
Based on the table above, it was possible to separate the measured PLFA into fungal, actinomycetal and bacterial. This gave information useful for mapping community changes as a response to methods of treatment of soil. The PLFA 18:2ω6,9 has been found almost exclusively in fungi, but little is known about its relative amount in the hyphal wall. The ratio between fungi and bacteria may therefore give results out of proportion, but the quantified 18:2ω6,9 does still say something about changes in the fungi. Actinomycetes, represented by the methylated PLFAs 10me16:0, 10me17:0 and 10me18:0, are important in degrading organic material in soil, since they are relatively drought resistant and are known to degrade complicated organic compounds. Bacteria are represented by a number of marker PLFAs, and are the most abundant of the three types of organisms.



Figures 4.11 Plot, Farmer and Month: Main effects of method (”Plot”), soil type (”Farmer”) and climate (”Month”) on pmole fungal PLFA(18:2ω6,9).



Figures 4.12 Plot, Farmer and Month: Main effects of method (”Plot”), soil type (”Farmer”) and climate (”Month”) on pmole actinomycetal PLFA (10me16:0, 10me17:0, 10me18:0).



Figures 4.13 Plot, Farmer and Month: Main effects of method (”Plot”), soil type (”Farmer”) and climate (”Month”) on pmole actinomycetal PLFA (see Table).

As seen in the other methods of quantifying bacteria, Chande seemed to have the soil with the lowest microbial biomass. Kajiti's soil had the highest amount of bacterial biomass, and Lusonzo's the highest amount of fungal biomass. There was also a tendency, as seen in the three analysed methods of treatment of the plots, that plot 6 had the largest microbial biomass and plot 4 the lowest.

Calculation 4.2: The correlation between pmol bacterial PLFA and the total number of bacteria.

```
Correlations: pmolBplfa; TOT

Pearson correlation of pmolBplfa and TOT = 0,336
P-Value = 0,009
```

The calculation above shows that there is a significant correlation between total counts of bacteria and the amount of bacterial PLFA in the samples.

Table 4.5: Content (pmol) and percent of fungal, actinomycetal and bacterial PLFA pr g dw soil for the four farmers.

	Chande	Lusonzo	Kajiti	Ahmadi
Fungal	61 (2 %)	125 (3 %)	98 (2 %)	90 (2 %)
Actinomycetal	686 (23 %)	983 (22 %)	1342 (25 %)	1103 (22 %)
Bacterial	2261 (75 %)	3264 (75 %)	3874 (73 %)	3821 (76 %)
Total	3008	4372	5314	5014

Table 4.6: Content (pmol) and percent of fungal, actinomycetal and bacterial PLFA pr g dw soil for every month.

	February	March	April	May	June
Fungal	111 (2 %)	54 (2 %)	82 (2 %)	132 (3 %)	96 (2 %)
Actinomycetal	1084 (23 %)	768 (23 %)	992 (24 %)	1128 (22 %)	1166 (23 %)
Bacterial	3468 (74 %)	2563 (76 %)	2994 (74 %)	3775 (75 %)	3818 (75 %)

Table 4.7: Content (pmol) and percent of fungal, actinomycetal and bacterial PLFA pr g dw soil for the three plots.

	Plot 4	Plot 5	Plot 6
Fungal	71 (2 %)	87 (2 %)	121 (3 %)
Actinomycetal	973 (24 %)	1040 (23 %)	1067 (23 %)
Bacterial	3030 (74 %)	3333 (75 %)	3549 (75 %)

4.5 Analysing mineralisation rate in vitro

The Main Effects Plots of fungal and bacterial counts and their biomass, showed that climatic effects were what influenced the microbial communities most, and there was also a correlation between several soil physical factors and CO_2-flux. A mineralisation experiment was set up at UMB were the microbial response to temperature and soil moisture was measured. The 3D plots below are factors soil moisture and temperature plotted against the total amount of CO_2 produced after three weeks of incubation.

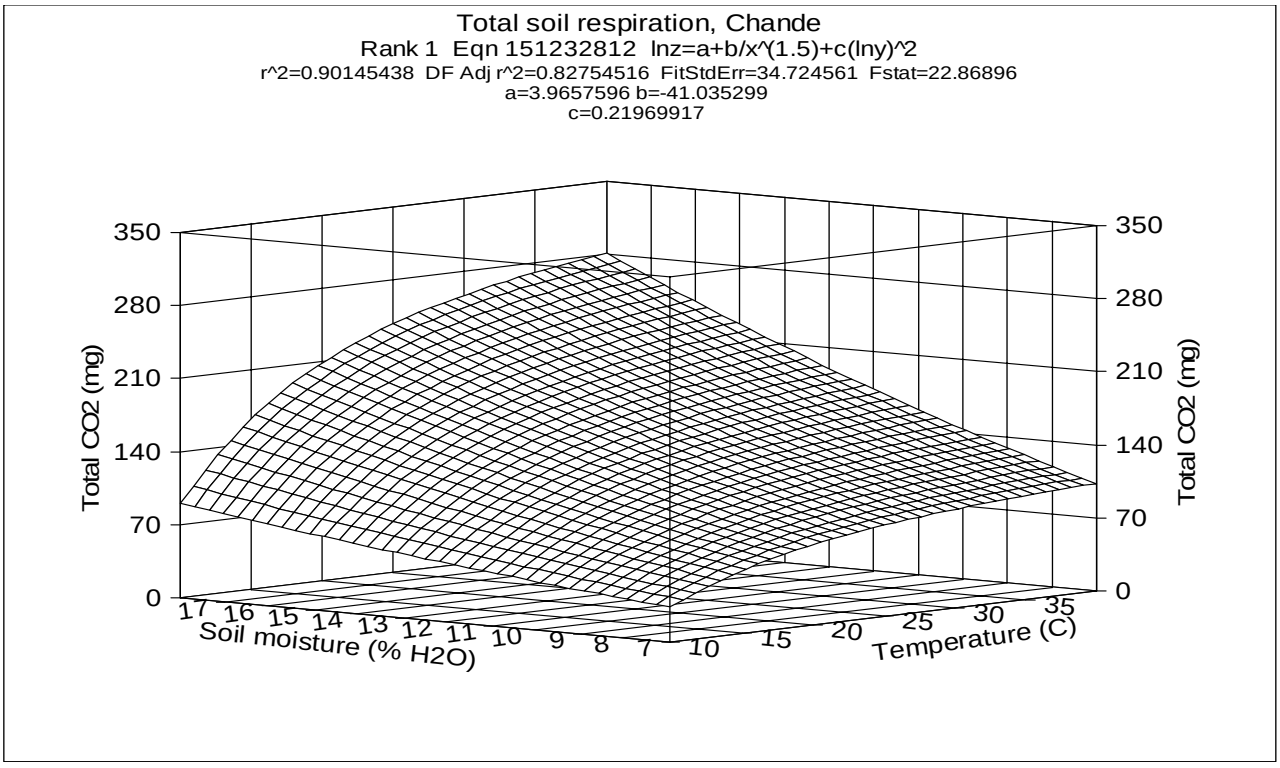


Figure 4.14: Chande's soil. CO_2-efflux measured against temperature and soil moisture.

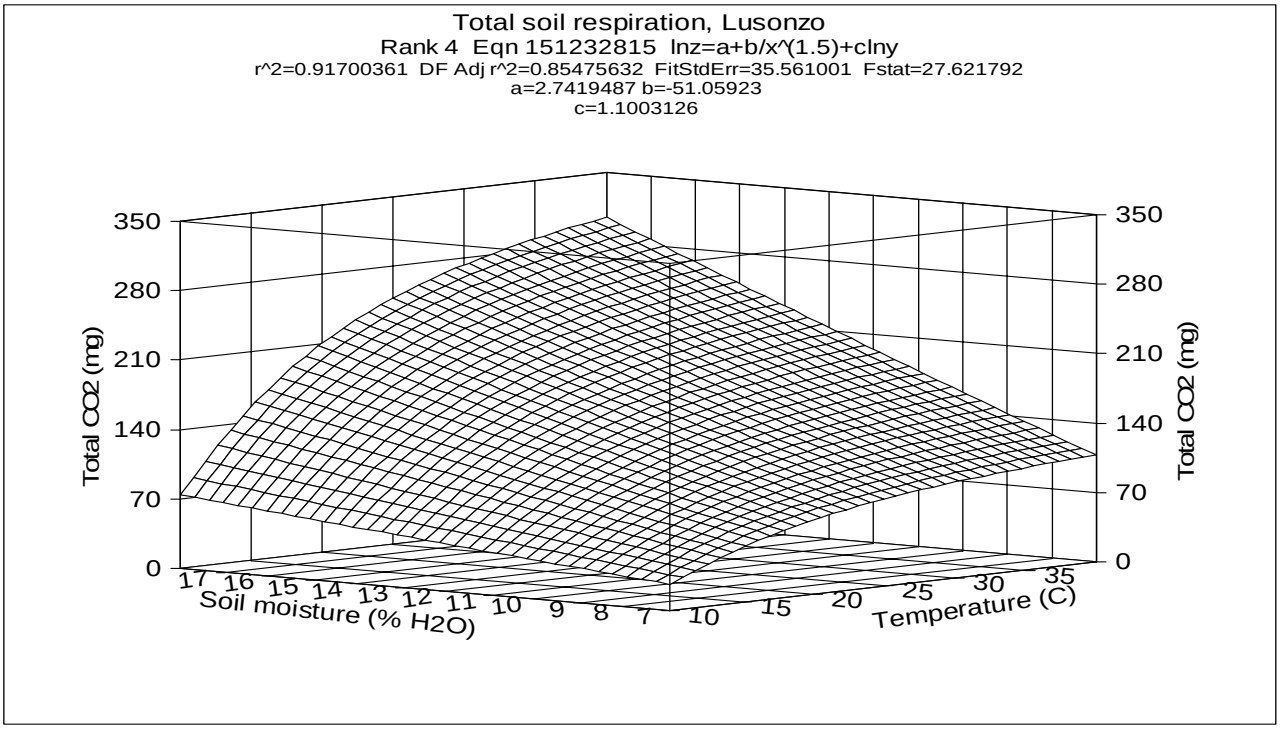


Figure 4.15: Lusonzo's soil. CO_2-efflux measured against temperature and soil moisture.

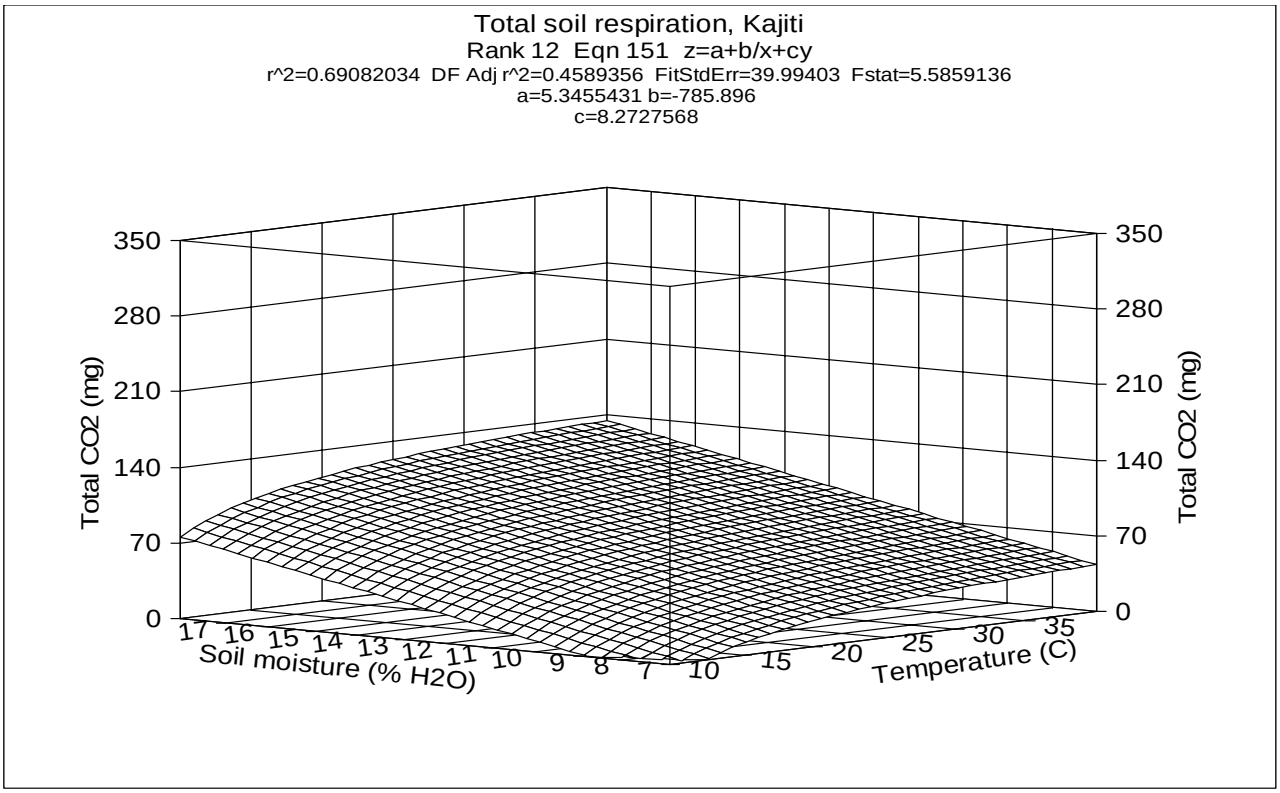


Figure 4.16: Kajiti's soil. CO_2-efflux measured against temperature and soil moisture.

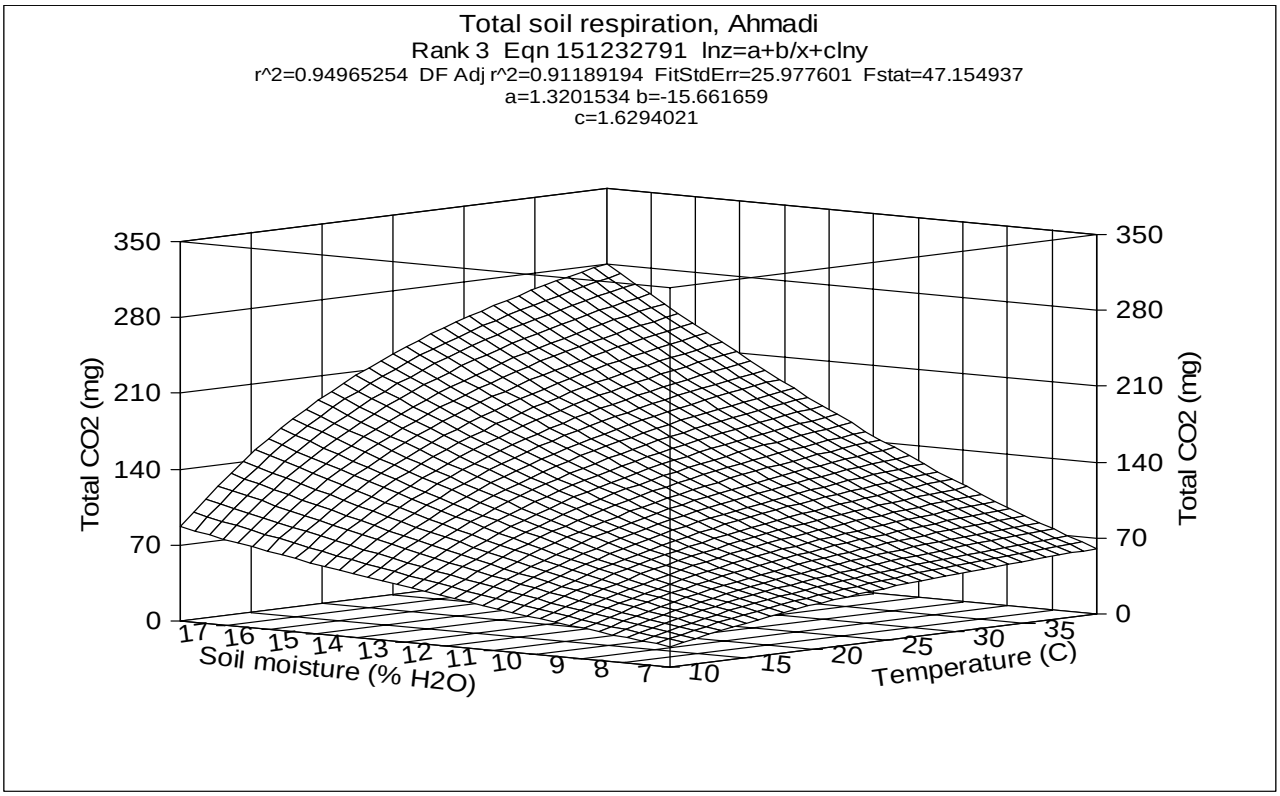


Figure 4.17: Ahmadi's soil. CO_2-efflux measured against temperature and soil moisture.

The two soils in Mikese, at Chande's and Lusonzo's farms, show the highest mineralisation rates *in vitro*. Kajiti's soil type shows the lowest soil respiration *in vitro*. These results correspond well with what was found when measuring CO_2-flux *in situ*.

5 Discussion

5.1 The microflora was different in the four soil types

The microbial activity (Figure 4.2 Farmer) showed that Chande's soil had the highest microbial activity (1.3 g CO_2 per m^2 per hour), and Kajiti's the lowest (0.9 g CO_2 per m^2 per hour). Kajiti's had the largest microbial biomass (5314 pmol bacterial PLFA per g dw soil), and Chande's the smallest (3008 pmol) (Table 4.5). Chande's soil also had the highest content of organic carbon (3.6 %), whereas Kajiti's soil had the lowest (1.6 %) (Table 4.2). These data support the theory that soil texture has a great impact on the measurement of CO_2-flux from soil respiration, because Kajiti's dense soil was rich in clay which may have stopped the CO_2 from flowing out of the soil, and Chande's soil was sandy, ensuring easy gas transport.

The same tendency was seen in the mineralisation experiment at different temperature and moisture constants *in vitro*; the activity in Kajiti's soil (Figure 4.16) was much lower than the activity in the Chande-soil (Figure 4.14). It is probable that this was mainly due to clay's ability to hold water, which leaves little available water for microorganisms and plants. Soil moisture content in the mineralisation experiment was adjusted to 7.5 % and 17.5 %, and Chande's soil got higher mineralisation rates than Kajiti's at both levels of moisture content.

Viable fungal counts (Figure 4.4 Farmer) were lowest in Chande's soil ($1.4x10^5$ fungal spores per g dw soil) and highest in Ahmadi's ($4.1x10^5$ fungal spores per g dw soil). This correlated well with the content of organic carbon in the two soils; Chande's having the lowest content (1.6 %) and Ahmadi's soil (3.5 %) close to the highest amount of organic carbon (Table 4.2).

Viable bacterial counts (Figure 4.6 Farmer) in the poorest soil, Chande's sandy loam, were $2.4x10^7$ viable bacteria per gram dry weight soil. Ahmadi's soil had the highest counts: $3.7x10^7$ viable bacteria per g dw soil. This corresponded well with the measurement of bacterial biomass as measured by amount of bacterial PLFA (Table 4.5): Chande's, the lowest, with 2261 pmol; Ahmadi's, the highest with 3821 pmol per g dw soil. Ahmadi's soil also had the highest content of organic carbon of the two (3.5 %) (Table 4.2).

Total counts (Figure 4.8 Farmer) showed the same tendency as the other methods of counting; soil poor in organic material had lower numbers. Chande's had $2.8x10^9$ total bacteria per g dw soil, and

Kajiti's $6.2x10^9$ total bacteria per g dw soil. Total counts of bacteria were in correlation with bacterial biomass, as measured by the amount of bacterial PLFA per gram dry weight soil (Calculation 4.2).
Kajiti's soil (1342 pmol per g dw soil) had twice the amount of actinomycetal PLFA as Chande's (686 pmol per g dw soil) (Table 4.5). This suggests Kajiti's soil, being rich in clay, may select for groups of microorganisms that can sustain soils with high water retention capacities. Actinomycetes are known to be tolerant to low water levels (Griffin, 1981).

The content of clay affected measurement of soil respiration, but it did not seem as if any of the other microbial parameters are affected significantly. The total amount of PLFA from microorganisms and the microbial counts corresponded to the content of organic carbon in the four different soils (Table 4.2), following the general assumption that the content of organic matter has a great impact on microbial life in soil.

5.2 The microflora fluctuated during the growth season of maize

The microbial respiration (Figure 4.2 Month) decreased from 1.0 to 0.7 g CO_2 per m^2 per hour during the growth season. The peak in April (1.6 g CO_2 per m^2 per hour) and fluctuations seen at each location, were mainly due to variation in soil water content. The strong correlation between soil water content and carbon dioxide flux also indicate this (Calculation 4.1). Another factor influencing soil respiration was the average temperature in the top layer soil (15 cm), which decreased from 29°C in February to 27°C in June.

The viable counts of fungi (Figure 4.4 Month) decreased from $3.2x10^5$ to $1.7x10^5$ per g dw soil during the growth season. This corresponded with the measured fungal biomass based on the content of 18:2w6,9 PLFA. The amount of 18:2w6,9 PLFA increased from 111 to 132 pmol PLFA per g dw soil (Figure 4.11 Month, Table 4.6), parallelly with the decrease in number of viable fungi, which may indicate that there was fungal sporulation. When fungal spores sporulate, the fungal biomass will increase, since the fungi enter a hyphal stage, but the number of measured viable fungi will decrease, since fungal hyphae are not known to be possible to grow on agar medium (Kendrick, 2000).

There seemed to be a higher amount of viable bacteria during the rainy season than before and after it. A relatively small increase from $2.3x10^7$ to $2.7x10^7$ viable bacteria per g dw soil from February to June, was peaked by $3.7x10^7$ viable bacteria in March (Figure 4.6 Month). Bacterial biomass peaked after the heaviest rainy season (3818 pmol; average 3313 pmol bacterial PLFA per g dw soil) (Figure 4.13 Month, Table 4.6), and this may have been partly due to the increased viability of the bacteria. Several factors may have contributed to the altered viability, but soil water content is suggested as that with the greatest impact (Maier et al., 2000).
The total number of bacteria (Figure 4.8 Month) remained relatively stable during the growth season (average $4.1x10^9$ bacteria per g dw soil), which was expected since the total number of soil bacteria is known to fluctuate little in response to environmental factors (Atlas and Bartha, 1997).
The amount of actinomycetal PLFA was low in the beginning of the growth season, but increased as the activity in the soil increased (Figure 4.12 Month, Table 4.6). The highest amount of actinomycetal PLFA was found in the soil in June.

The diversity of the soil microflora changed as a response to environmental parameters during the five months of growth season and rain. An increased fungal biomass could be seen, as the fungal spores sporulated in response to increased soil moisture. The activity and viability of the soil bacteria increased due to higher moisture levels, and when the viability increased, the increase in biomass followed. The actinomycetal biomass also increased as the soil moisture level increased.

5.3 The microflora responded to the methods of treatment

How the microflora responded to the three methods of improving soil fertility was measured as the average of the whole period from February to June, based on results from all the four farmers.

Plot 4: Removal of plant residues and other organic material, and tilling to 10 cm. Adding nitrogen (60 kg pr ha) and phosphorus (40 kg pr ha).
Plot 5: Tilling in organic plant material, such as maize residues, to 10 cm depth. No added fertilizer.
Plot 6: Collecting residues in heaps and burning them. No added fertilizer and no tilling.

Microbial activity (Figure 4.2 Plot) measured as CO_2-flux for plots 4, 5 and 6 indicated that there was no response to the methods of treatment of the plots; all the three plots show soil respiration close to the average (1.1 g CO_2 $m^{2\ -1}$ h^{-1}, ±0.02).

The highest viable fungal count (Figure 4.4 Plot) found when comparing the methods of treatment was $3.2x10^5$ per g dw soil (average $2.7x10^5$ viable fungi per g dw soil) found in plot 6. This treatment involved ashing of plant residues on top of the soil. The measured fungal biomass (PLFA) showed the same tendency as the fungal counts: plot 6 had 121 pmol 18:2w6,9 PLFA per g dw soil (average 94 pmol) (Table 4.7). The lowest viable fungal counts were in plot 4 ($2.4x10^5$ per g dw soil), and also the lowest amount of fungal biomass (71 pmol 18:2w6,9 PLFA per g dw soil).

Viable bacterial counts (Figure 4.6 Plot) indicated that the conditions most favourable for microbial life were found in plot 6 ($3.2x10^7$ viable bacteria; average $2.9x10^7$ per g dw soil). The bacterial PLFA (Figure 4.13 Plot, Table 4.7) in plot 6 (3459 pmol PLFA per g dw soil; average 3313 pmol) indicated the same as the viable counts. Plot 4 had the lowest bacterial biomass (3030 pmol PLFA per g dw soil), and the lowest viable counts ($2.6x10^7$ per g dw soil).

The total number of counted bacteria (Figure 4.8 Plot) varied little between the three plots, supporting the general perception that the total number of bacteria in soil is little affected by environmental factors. Yet the Main Effects Plot showed there was a slight tendency to total counts reflecting the viable counts of bacteria – Plot 4 giving lower counts than plot 5 and 6. But viability is more susceptible to environmental changes than are changes in the total amount of live and dead bacteria (Maier et al., 2000). The total counts correlated with the bacterial biomass (PLFA) (Calculation 4.2) (Frostegard et al., 1996), and the tendency indicating that plot 6 had the largest total number of bacteria, are supported by the same tendency in bacterial biomass.

Actinomycetal biomass was only slightly affected by the methods of treatment, but it still followed the same pattern, showing more actinomycetal growth in plot 6 than in the other two (Figure 4.12 Plot, Table 4.7).

All the methods of treatment of the soil showed the same tendency: Use of mineral fertilizer gave a lower measurement of microbial parameters than treatment by residue management, while treatment by ashing residues on the plots gave the best results.

5.4 Comparing the three methods of treatment

If inorganic fertilizer is added to a soil poor in organic matter, and carbon is the growth limiting factor for the microbial community (C:N < 20 in the soil), the heterotrophic bacteria should theoretically not benefit from the added nitrogen and phosphorus. Heterotrophic bacteria have optimal competitive growth at C:N 20, and fungi at a C:N ratio of 30 (Maier et al., 2000). In tropical soils, any easily degradable organic material added will be digested quickly by the microflora, leaving heavily degradable plant residues such as lignin behind. Fungi and actinomycetes are known to be more adapted to decomposing such compounds, than bacteria are in general. The C:N ratio in what was considered the poorest and richest soils were 12 and 16 in Chande's and Ahmadi's soils respectively (Table 4.2) – ratios that should leave the microflora with too little carbon relative to the amount of nitrogen. In plot 4, the added mineral fertilizer should therefore mostly be used by the autotrophic plants, and not be of any benefit to the microflora.

Based on the theory referred to when explaining the results showing lower microbial measurements in plot 4 than the in the other two plots, it is reasonable to assume that maize residues tilled into the soil in plot 5 would give the highest microbial measures since it contains both organic material and mineral nutrients, but apperantly this does not happen. According to Palm *et al* (2001), plant residues such as maize, containing "nitrogen<2.5 %, lignin<15 % and polyphenol<4 %" are defined as organic material of intermediate to low quality. This category implies that when the organic material is applied to soil it has a low nitrogen supplying capacity, and the organic residues will be responsible for a short term immobilization of whatever nitrogen was mineralized in the soil. Common for this category of organic material is also that application has little effect on total SOM. Table 4.2 shows that the average content of organic carbon is actually lowest in the plot where plant residues has been added.

It is suspected that the larger numbers and biomass of microorganisms in plot 6 than in the two other plots, was due to the increased pH caused both by the cations released from the burned maize residues, and by the basicity of the ashed plant material. An increased pH in soil should ionize more of the cations bound by clay particles by altering the cation exchange capacity (Maier et al., 2000). The macronutrients, mainly cations, will be more easily available to microorganisms and plants. Higher pH from the burned plant material will also alter cation exchange capacity by increasing isomorphic substitution. It is still difficult to answer why this should alter microbial

data, especially since the average pH in plots 4, 5 and 6 was 7.0, 6.9 and 7.0, respectively (Table 4.3).

Secondary effects from altered plant growth may help explain why the microflora responded as it did to the different methods of treatment. Wang and Bakken (1997) suggest that plant roots play a central role by two opposite effects. Root deposits sustain a substantial microbial population in the rhizosphere soil, and in return a considerable amount of N is immobilized. On the other hand, plants and microorganisms compete for the same available N by reducing reimmobilization.

It seems that plants control the relationship between them and the microorganisms, though this can be argued (Maier et al., 2000). Increased plant growth should therefore stimulate to increased microbial biomass. Plot 4 showed the best maize plant growth, but also the lowest microbial biomass. There may have been less stimulation to the growth of microorganisms by the plants because the plants did not need to ensure immobilization of N when nitrogen was added through the fertilizer and therefore was in abundance.

The results from the effect of soil type on the microbial numbers and biomass, clearly showed the impact content of organic carbon had on the microbial communities. Table 4.2 shows that plot 6 had the highest content of organic carbon, and it is likely that this is the major reason why plot 6 has more microbes than the other two. The one thing that really separates the treatment in plot 6 from the two other plots is the method of treatment: Plot 6 was not tilled. In soil which is tilled, the soil is areated, increasing heterotrophic activity. The bacteria will digest the organic carbon quicker in an aerobic environment (Maier et al., 2000). It is generally agreed that fungi are much affected by tilling of the soil, and Table 4.7 shows a slightly higher content of fungal PLFA than in the other two plots – which have been tilled. One of the suspected reasons why fungi dislike tilled soil, is because it tends to become very dense when it has been soaked with water, and dries out again. This as opposed to ”natural” soil, which maintains its aggregate structure, and thereby porosity. It is known that soil rich in clay is especially prone to becoming dense and airtight as a consequence of tilling, watering and drying.

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Appendix A:

Measured CO_2-flux for (C)hande, (L)usonzo, (K)ajiti and (A)madi, plot number indicated after capital letter.

	February	March	April	May	June
C4	1,31	1,61	2,61	1,51	0,91
C5	1,25	0,81	2,03	1,09	0,54
C6	0,90	0,88	1,93	1,10	0,39
L4	0,84	1,01	2,01	0,98	0,36
L5	1,36	1,58	2,59	0,92	0,48
L6	1,69	0,94	2,79	0,83	0,49
K4	0,75	1,14	1,30	0,58	0,87
K5	0,65	1,45	0,68	0,38	0,71
K6	0,94	1,35	1,02	0,54	0,67
A4	0,56	1,17	0,90	0,56	0,90
A5	0,72	1,46	1,09	0,51	0,85
A6	1,25	1,58	0,61	0,77	1,36

Viable fungal counts for (C)hande, (L)usonzo, (K)ajiti and (A)madi, plot number indicated after capital letter.

	February	March	April	May
C4	1,83E+05	6,10E+04	1,17E+05	1,03E+05
C5	7,07E+04	2,80E+05	1,86E+05	1,69E+05
C6	1,48E+05	1,06E+05	1,69E+05	9,69E+04
L4	5,95E+05	3,20E+05	2,62E+05	1,89E+05
L5	1,19E+05	2,32E+05	1,72E+05	1,58E+05
L6	2,25E+05	5,00E+05	4,27E+05	1,89E+05
K4	3,24E+05	2,15E+05	1,62E+05	1,44E+05
K5	3,23E+05	2,52E+05	1,65E+05	1,58E+05
K6	5,23E+05	3,07E+05	2,18E+05	2,06E+05
A4	4,16E+05	3,97E+05	1,82E+05	1,97E+05
A5	4,27E+05	4,87E+05	5,39E+05	2,97E+05
A6	4,31E+05	1,04E+06	3,12E+05	1,81E+05

Viable bacterial counts for (C)hande, (L)usonzo, (K)ajiti and (A)madi, plot number indicated after capital letter.

	February	March	April	May
C4	1,37E+07	3,13E+07	2,13E+07	1,72E+07
C5	1,18E+07	3,59E+07	2,97E+07	2,11E+07
C6	3,06E+07	3,53E+07	2,27E+07	1,99E+07
L4	2,17E+07	2,33E+07	2,94E+07	1,87E+07
L5	1,60E+07	3,64E+07	2,75E+07	2,64E+07
L6	2,09E+07	3,47E+07	3,64E+07	2,88E+07
K4	2,48E+07	2,53E+07	2,32E+07	3,03E+07
K5	9,69E+06	3,38E+07	3,47E+07	3,09E+07
K6	2,41E+07	3,58E+07	2,25E+07	3,58E+07
A4	2,40E+07	3,68E+07	4,66E+07	2,65E+07
A5	3,63E+07	3,46E+07	4,24E+07	3,86E+07
A6	4,39E+07	4,55E+07	4,05E+07	2,84E+07

Total counts for (C)hande, (L)usonzo, (K)ajiti and (A)madi, plot number indicated after capital letter.

	February	March	April	May	June
C4	1,65E+09	2,14E+09	2,80E+09	2,87E+09	2,13E+09
C5	2,10E+09	2,84E+09	3,99E+09	3,99E+09	2,72E+09
C6	3,19E+09	3,63E+09	3,40E+09	2,61E+09	1,72E+09
L4	4,27E+09	4,51E+09	3,82E+09	2,66E+09	2,75E+09
L5	3,97E+09	4,58E+09	3,90E+09	2,81E+09	2,53E+09
L6	4,70E+09	4,95E+09	5,21E+09	2,78E+09	3,54E+09
K4	7,48E+09	5,95E+09	6,14E+09	4,91E+09	6,23E+09
K5	5,39E+09	6,20E+09	6,93E+09	5,13E+09	7,34E+09
K6	3,76E+09	7,53E+09	5,70E+09	6,75E+09	7,56E+09
A4	4,18E+08	3,73E+09	4,52E+09	1,88E+09	4,22E+09
A5	4,18E+09	4,24E+09	4,48E+09	3,96E+09	4,36E+09
A6	4,17E+09	4,56E+09	4,15E+09	2,56E+09	4,87E+09

Actively respiring bacteria for (C)hande, (L)usonzo, (K)ajiti and (A)madi, plot number indicated after capital letter.

	March
C4	1,13E+08
C5	2,90E+08
C6	2,26E+08
L4	4,01E+08
L5	3,39E+08
L6	4,52E+08
K4	3,62E+08
K5	3,94E+08
K6	5,14E+08
A4	2,96E+08
A5	3,18E+08
A6	4,17E+08