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EDWARD NDERITU KARANJA

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MICROBIAL COMMUNITY DIVERSITY AND STRUCTURE WITHIN ORGANIC AND CONVENTIONAL FARMING SYSTEMS IN CENTRAL HIGHLANDS OF KENYA

EDWARD NDERITU KARANJA (MSc)

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DECLARATION

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

Signature……. Date…….……..

Edward Nderitu Karanja
Department of Biological Science
B801/147/2015

This thesis has been submitted for examination with our approval as the University Supervisors

Signature……. Date…….…….

Prof. Romano Mwirichia
Department of Biological science
University of Embu (UoEm), Kenya

Signature……. ……. Date…….……

Dr. Andreas Fliessbach
Department of Soil Science
Research Institute of Organic Agriculture - FIBL, Switzerland

DEDICATION

I dedicated to my family; my wife Anne Kelly Kambura, my children; Shawn Karanja, Melissa Wangithi, Joseph Munyuithia, Shayne Koome and Ann Wanjiku, my parents; Mr. Samuel Karanja and Mrs. Agnes Wangithi, my siblings, Ruth Wairimu, Juliet Muthoni, Alex Ngochi, James Karuma and Nelly Njoki. I appreciate the support you have accorded me during my studies. Your inspiration and backing in this journey made it easier to manage all challenges encountered.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAFC	Agriculture and Agri-Food Canada
ANOSIM	Analysis of Similarity
ARDRA	Amplified ribosomal DNA restriction analysis
BLASTn	Basic Local Alignment Search Tool (Nucleotide)
BWA	Burrows Wheeler aligner
CDD	Conserved Domain Database
DGGE	Denaturing and temperature gradient gel electrophoresis
DNA	Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
EAOPS	East African Organic Products Standard
FAO	Food and Agricultural Organization
FiBL	Research Institute of Organic Agriculture
GDP	Gross domestic product
icipe	International Centre for Insect Physiology and Ecology
IFPRI	International Food Policy Research Institute
ITS	Internal Transcribed Spacer
KALRO	Kenyan Agricultural and Livestock Research Organization
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIOF	Kenya institute of Organic Farming
KO	KEGG Orthology
KOFA	Kenya Organic Farmers Association
KOPA	Kenya Organic Producers Association
LED	Liechtenstein Development Service
LTE	Long Term Experiment
MEGAN	Metagenome Analyzer
NAST	Nearest Alignment Space Termination
NCBI	National Centre for Biotechnology Information
NGS	Next Generation Sequencing
NMDS	Non-Metric Dimensional Scaling
NR	Non-Redundant
ORF	Open Reading Frames

OTUs	Operational Taxonomic Units
PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen
PyNAST	Python Nearest Alignment Space Termination
QIIME	Quantitative Insights into Microbial Ecology
RNA	Ribonucleic Acid
SDC	Swiss Agency for Development and Cooperation
SDS	Sodium Dodecyl Sulphate
SSA	Sub Saharan Africa
SSCP	Single Strand Confirmation Polymorphism
SSU	Small Sub-Unit
TGGE	Temperature Gradient Gel Electrophoresis
T-RFLP	Terminal Restriction Fragment Length Polymorphism
UCLUST	Universal Clustering

ABSTRACT

Microbial diversity and function in agro-ecosystems is influenced by various aspects linked to soil and agronomic practices for example, tillage, irrigation, crop rotation and application of organic and inorganic inputs. Farming systems practices may affect the dynamic interactions existing between soil, plant and microorganisms in different agricultural biomes. Due to limitations associated with conventional microbial cultivation strategies, only a fractional number of cultivable species has been extensively studied. This study explored the effects of conventional versus organic farming systems on microbial communities. Soil samples were collected from an ongoing long-term farming system comparison trials established in 2007 at Chuka and Thika in Kenya. Illumina sequencing technology and analysis of 16S rDNA, 16S rRNA cDNA amplicons, fungal ITS and mRNA transcripts were used to determine the diversity, structure and function of bacteria, archaea and fungal communities within conventional and organic farming systems. Grouping of sequences into operational taxonomic units at 97% similarity was done using QIIME2 pipeline and taxonomy assigned via BLASTn against SILVA 128 and UNITE ITS database, and a curated database extracted from GreenGenes, RDPII and NCBI. Transcriptomes were analysed using Parkinson lab pipeline (https://github.com/ParkinsonLab/Metatranscriptome-Workshop). Statistical analysis was done using R programming language version 3.1.5 and Vegan Community Ecology Package version 2.5.2. R. The total number of OTUs obtained per dataset included 4,916 OTUs (16S rDNA), 530 OTUs (16S rRNA cDNA) and 1,128 OTUs (fungal ITS) at 97% genetic distance datasets, respectively. The most notable bacterial phyla within farming systems were *Proteobacteria, Actinobacteria, Firmicutes Acidobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Fusobacteria, Gemmatimonadetes, Planctomycetes and Verrucomicrobia.* Farming systems in both sites were dominated by unassigned fungal phyla. The known fungal phyla revealed included *Ascomycota*, Basidiomycota, *Chytridiomycota, Glomeromycota, Calcarisporiellomycota*, Kickxellomycota and *Mortierellomycota*. *Ascomycota* was most abundant in organic farming systems while *Chytridiomycota* was dominant in conventional farming systems in both sites. Conventional farming systems had a higher species richness and diversity when compared with organic farming systems. Factors such as pH, C, N, Zn, Fe, Al, B and micro-aggregates were found to be the major drivers of microbial diversity within farming systems in both sites. Major metabolic pathways within the farming systems in both sites comprised of carbohydrates and energy metabolism, biodegradation and metabolism of xenobiotics and secondary metabolites biosynthesis. This shows that microbes in the farming systems utilize diverse carbon sources for survival, as revealed by metabolic processes and genes responsible for specific pathways. These findings indicate integration of organic and inorganic inputs, not only affect the soil chemistry but also the microbial population dynamics and their functional roles.

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Soil is a complex and dynamic biological ecosystem which acts as the habitat of a diverse array of organisms. Therefore, the interaction between biodiversity and ecosystem functioning is a fundamental subject in ecological research (Hooper *et al.*, 2005, Tilman *et al.,* 2006). Soil communities are extremely complex and diverse. They range from microscopic prokaryotes (bacteria and archaea), eukaryotes (fungi), larger organisms for example ants, earthworms and moles. Scientific knowledge of this unobserved biodiversity is limited, when compared to what is well-known about aboveground diversity. Below-ground biodiversity significantly contributes to the maintenance of soil ecosystem functioning and defining aboveground biodiversity (Bardgett, *et al.*, 2014).

Microorganisms in soil play key role in soil fertility by carrying out biochemical transformations thereby doubling as a source and sink of mineral nutrients (Jenkinson and Ladd, 1981). Bacteria and fungi play diverse roles in regulating soil microbiological activities such as specific enzymatic activities and soil microbial biomass (Nannipieri *et al*., 2003, Reeve *et al*., 2016), mineralize complex organic substances (Brussaard *et al*., 1997), control the cycling of nutrients and carbon storage in soils (Bardgett *et al.*, 2014).

The diversity of soil microorganisms as well as their activity is immensely affected by climate change and human activities (Castro *et al.,* 2010). Human driven ecosystem simplification has underlined questions on how the number of species influence ecosystem functions. In different agricultural ecosystems there is a dynamic interaction between plant and microorganisms (Wei *et al*., 2018). Plant-associated microbes colonize both exterior and interior plant surfaces, while the surrounding soil is a key resource for these microbes (Zarraonaindia *et al*., 2015). It has been postulated that variations in soil microbial community structure and diversity due to effects of soil management practices could reflect possible environmental impacts (Sachs *et al*., 2010). This means that intensive farming practices tend to undermine the welfare of natural habitats and soils which may lead to disruption of ecosystem services (Sun *et al*., 2004). Organic

agriculture becomes an alternative to conventional agriculture and it aims to minimize impact on the environment by using organic manure instead of synthetic fertilizers, limited amounts of chemical pesticides, crop rotation and pathogen-resistant cultivars (Li *et al*., 2013). However, there is still some controversy on the beneficial effect of organic agriculture on microbial diversity and plant-associated microorganisms (Hole *et al.*, 2005; Granado *et al.,* 2008; Crowder *et al.*, 2010). Understanding how major changes in land management affect soil microbial community structure, may well provide an important index for evaluating the relative ability of soils' response to future disruptions (Ruimy *et al.,* 2010, Nacke *et al*., 2011). High throughput sequencing of both DNA and RNA has proven to be a powerful tool that provides valuable insights about the structure, functions and interactions of different microbial communities (Helgason *et al.,* 2010; Kaiser *et al*., 2016).

In this study, next generation sequencing and analysis of amplicons generated from 16S rDNA, 16S rRNA cDNA, Fungal ITS and mRNA were used to create high resolution taxonomic and functional profiles of soil microbial communities. Soil samples were collected from the on-going long-term farming systems comparison (SysCom; www.system-comparison.fibl.org) trials in Kenya. The study sites were initiated by the Research Institute of Organic Agriculture (FiBL) and their local partners; International Centre for Insect Physiology and Ecology (*icipe*) and Kenyan Agricultural and Livestock Research Organization (KALRO) to compare the performance of organic and conventional farming systems in the tropics on farm productivity, profitability and sustainability.

1.2 Statement of the problem

Effects of agricultural farming systems such as organic and conventional farming systems on soil microbiome is an area that is not well understood. While the economic benefits of intensive agricultural management practices remain clear, the debate on ecological benefits of organic and conventional systems is still uncertain. Microbes associated with plants play a significant role in soil ecosystem services and intensive farming practices may undermine the welfare of natural habitats leading to disruption of ecosystem

services, posing substantial challenges in maintaining sustainable agricultural production systems.

Knowledge on how the structure of soil microbial community is affected by major changes in land management may perhaps provide an imperative index for assessing the relative ability of the soils to respond to future disruptions (Helgason *et al.,* 2010; Lopes *et al.,* 2011). Long-term experiments, particularly when compared to medium and/or short-term experiments can yield important information to predict the dynamics of the soil microbial community with time. Therefore, a clearer understanding of the structure, functions and interactions of different microbial communities would be obtained by use of novel high throughput sequencing techniques that target both total and active members of the microbial communities.

1.3 Justification

Climate change has adverse effects on agricultural productivity in the developing countries. In addition, global population is projected to increase from 7.3 billion in 2015 to 9.5 billion by 2050 (Pocket, 2017), with an approximate 800 - 925 million people being under-nourished by 2020 (Federoff, 2015, http://www.fao.org/3/a-i4646e.pdf). Therefore, food security is a major global challenge with key question involving how agriculture can provide enough food to feed everyone using current practices (ISAAA Infographic 1. 2016. www.isaaa.org). Sustainable agricultural practices need to be expanded by at least 70% by 2050 as a fundamental action in meeting future world's food demands. Microorganisms play critical roles in soil health, nutrient cycling, plant pathology and nutrition. Therefore, understanding the role of soil microbiome can help improve plant health, productivity, nutrient availability, and defense to diseases. In this study, we applied metagenomic (DNA) and metatranscriptomic (RNA) analysis of environmental nucleic acids extracted from soil to determine diversity and function of bacterial, archaeal and fungal communities within the on-going long-term farming systems comparison trials in the central highlands of Kenya.

1.4 Hypotheses

1. Agricultural inputs affect soil microbial diversity within conventional and organic farming systems.
2. There is no difference in the microbial community structure between organic and conventional farming systems.

1.5 Objectives

1.5.1 General Objective

To study microbial diversity within conventional and organic farming systems using metagenomic and metatranscriptomic analysis.

1.5.2 Specific Objectives

2. To assess the total bacterial, archaeal and fungal diversity within conventional and organic farming systems.
3. To analyze the active microbial communities within the two farming systems using Illumina sequencing of cDNA libraries generated from rRNA.
4. To determine the influence of organic/conventional inputs on metabolic function of microbial communities.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 General background of farming systems

About 90 % of the world's poor population live in rural countryside areas with majority relying on agriculture as their core source of food, employment and income (Collier, 2007; African Development Bank, 2010). Africa's 30-40 % gross domestic product (GDP) and 60 % of its exports is from agriculture (International Food Policy Research Institute (IFPRI), 2004). In sub-Saharan Africa, small scale farmers practice mixed/multispecies farming for both subsistence and commercial purposes (Vandermeer *et al*., 1998; Dixon *et al*., 2001). The major agricultural systems identified in Sub Saharan Africa (SSA) include agro-pastoral, highland perennial, mixed cereal-root crops, root and tuber crops (Dixon *et al*., 2001; Aurich *et al*., 2014).

Agricultural yields have increased in the recent past due to improved crop varieties, use of mineral fertilizers, pesticides and fungicides in management of pests and diseases (Vitousek *et al*., 2009; FAO, 2013; Zhang *et al*., 2013; Robertson *et al*., 2014). However, loss of soil organic matter and biodiversity is real and it has been corelated to land use intensification (FAO, 2013). Increased land use intensification in most parts of the world has led to different degrees of ecological pollution (Li *et al*., 2013). The environmental damage is mainly reflected in; loss of key nutrients such as nitrogen and phosphorus through leaching into water bodies and groundwater causing eutrophication; food pollution by chemical compounds like fertilizers and pesticides; reduced buffering capacity of soils against adverse conditions and; destruction of habitat for many plant and animal species (Lal, 2004; Bot and Benites, 2005; Foley *et al*., 2005; De Vries *et al*., 2013). This in turn has led to enhanced sensitivity to extreme weather patterns, pest and pathogen outbreaks, invasive species outbreaks coupled with greenhouse gasses emissions (Li *et al*., 2013).

Low crop productivity per unit area of land in mixed farming in East and Southern Africa has led to persistence of rural poverty within the region (Jaetzold *et al*., 2006; Akinnifesi *et al*., 2010). This has been attributed to low soil fertility and long-term soil degradation through loss of soil organic carbon and nitrogen, compaction and acidification (Amede,

2003; Henao and Baanante, 2006; Folberth *et al*., 2014). These changes are as a result of continuous and intensified cropping systems with insufficient replacement of soil nutrients, clearing of forest areas and overgrazing (Henao and Baanante, 2006; Sileshi *et al*., 2010), in addition to low adoption rates of ecologically sustainable resource management approaches (Omotayo and Chukwuka, 2009). Hence it is imperative to reverse soil fertility decline and preservation of soil and water biodiversity. This can be achieved through improvements of conventional agriculture and diversification of agricultural production systems (Dixon *et al*., 2001; Folberth *et al*., 2014).

In most countries in SSA, the potential of conventional agriculture that is based on monocultures, mechanization and the use of synthetic fertilizers, pesticides and fungicides (Beus and Dunlap, 1990) is limited by the high cost of production investment that is associated with the inputs. Other limiting factors include diverse soils, high phosphorous fixation capacity in soils leading to low P availability to crops (Nziguheba *et al*., 1998; Kwabiah *et al*., 2003) coupled with large within-farm soil fertility gradients (Vanlauwe *et al*., 2006). This raises serious concerns on how productivity and sustainability of yields in SSA can be enhanced by use of conventional agricultural farming system (Rigby and Càceres, 2001).

Organic agriculture has been proposed as a solution to counteract loss of soil organic matter, soil biodiversity and stabilization of associated ecosystem services (Mäder *et al*., 2002; Tsiafouli *et al*., 2014; Robertson *et al*., 2014). However, crop productivity in organic agriculture has been lower than in conventional agriculture (De Ponti *et al*., 2012; Seufert *et al*., 2012; Ponisio *et al*., 2015) hence raising apprehensions on its potential to offer a sustainable solution towards increasing food, animal feed, and biomass production required to sustain the growing population (Trewavas, 2001).

2.2 Sustainable and conventional agricultural systems

Sustainable agriculture (Organic) started mainly in economically developed countries. This was after realizing the negative effects associated with soil degradation and environmental pollution from synthetic chemical based farming practices that were widely practiced and promoted in the era of Green Revolution around 1960s. Currently,

there is growing concern from world population on the deterioration of world land resources and capacity to produce food for the ever-increasing inhabitants. And hence, sustainable agriculture is not about achieving maximum possible production for a certain period but rather having production for a longer sustainable period (Kimemia and Oyare, 2006). Sustainable agriculture respects the biological relationship that exists in nature thereby encouraging natural resource and environmental conservation as defined by Kenyan Organic Agriculture Stakeholders.

Accurate information on throughput and lucrativeness of organic agricultural systems is frequently deficient while progression in the organic sector has been limited further by lack of organic seeds, bio-fertilizers, bio-pesticides and other inputs (Niggli *et al*., 2017). Organic sector is largely dependent on external markets and necessitates additional investigation on organic food systems and their sustainability. The question on whether organic farming can feed the world lingers and how it influences the soil microbiome.

Conventional farming systems involve usage of high amounts of synthetic inputs and hybrid crops to increase crop productivity (Stinner and Blair, 1990; Aune and Coulibaly, 2015). This has contributed to environmental pollution, loss of indigenous/local crop diversity and increased health risks among farmers. Chemicals unquestionably provide a quick fix, but they are not sustainable (Altieri *et al*., 2017). Seeing the ill adverse effects of these chemicals has taken many years in temperate countries. In tropical regions where soils are much degraded and deficient of nutrients, the effects of chemical inputs associated with conventional farming systems have been more destructive. Conventional agriculture is also a costly venture to operate sustainably, especially in developing countries. This is due to the demand created to increase quantities of chemical inputs while trying to achieve a higher yield productivity output that is cost-effective (Bello, 2008a). Due to increased costs of inputs that farmers must invest in, coupled with low economic returns, many don't break even and they become trapped in poverty caused by the system, its commercial pressures and marketing framework.

2.3 Organic resources used in agro-ecosystems

Organic resources are key in both short-term supply of nutrients and long-term build-up of soil organic carbon (Palm *et al*., 2001). Their use as a nutrient source depends on

quantities available and their chemical composition (Palm *et al*., 2001). Organic resources such as crop residues, green manure, agroforestry prunnings and biomass transfer have been intensely assessed for use in agricultural production (Sanginga and Woomer, 2009). Most crop residues are low in nutrient content and have competing alternative uses such as feed and fuel (Sanginga and Woomer, 2009). Green manures are useful in nutrient uptake from deep soil layers making them available to the crop once they are incorporated into farming systems (Fageria, 2007). However, green manures are infrequently adopted by resource-poor farmers mainly due to small land parcel holdings and high labour requirement (Sanginga and Woomer, 2009).

Manure is a key source of nutrients to crops among resource poor farmers and most of the farming systems revolve around manure use and management (Rufino *et al*., 2007; Zingore *et al*., 2008). However, the quality of manure is low due to poor animal housing and manure storage while in pastoral areas, collection and transportation is a major hindrance to manure use (Lekasi *et al*., 2003; Muriuki *et al*., 2013). Thus, focus on improving the quality of manure would be an appropriate intervention to improve nutrient content (Harris, 2002). Agro-industrial by products such as coffee husks, sugarcane bagasse, rice husks have also been tested for their use as sources of nutrient (Kifuko *et al*., 2007; Sanginga and Woomer, 2009). Their use is, however, limited by transportation to farms (Sanginga and Woomer, 2009). Majority of the organic based resources available to farmers are deficient in nutrient content and they fail to sufficiently supply required nutrient amounts as demanded by crops under cultivation (Vanlauwe and Giller, 2006; Vanlauwe *et al*., 2006).

2.4 Long-term farming system comparison in the tropics

Long-term farming system comparison in the tropics (SysCom) was initiated in 2007 with the aim of enhancing knowhow on opportunities and challenges associated with different farming systems. The trials are in three different locations in the tropics namely Kenya, India and Bolivia. In Kenya, the trials compare conventional and organic farming systems at two input levels i.e. at recommended N and P levels and at the levels applied at small scale farms. To this end, the trials which are on the 4th (2019-2022) phase (4-year

phases) seek science-based know-how on the comparative performance of organic and conventional farming systems and foster sustainable agricultural production systems.

Previous results indicate maize and bean yields in organic and conventional systems as similar (Adamtey *et al*., 2016) while yields of potato, leafy vegetables were lower in organic systems possibly due to pest pressure (Unpublished data). Nitrogen uptake and use efficiency was reported not comparable in organic and conventional farming systems under maize but it was lower in organic systems compared to conventional systems under potato and leafy vegetables (Musyoka *et al*., 2017). In addition, N balance was only positive in organic high input system and negative in all the other systems (Musyoka *et al*., 2018). Soil physicochemical properties increased in organic high input system but there was a drop in organic carbon over the years in both systems (unpublished data). After nine years of treatment application, a trend of positive soil quality indicators improvement was highest in Org-High while Conv-High preserved soil quality indicators but showed trends of acidification (von Arb *et al*., 2020). In addition, termite incidence and abundance was higher in organic farming system compared to the conventional farming system (Anyango *et al*., 2020). In regard to maize and baby corn injury and damage by termites, organic farming system was significantly affected more than conventional farming system (Anyango *et al*., 2019).

2.5 Soil microbial ecology

Soil habitat embodies a remarkably heterogeneous environment for the existing microbiomes with different solid fractions such as silt, clay, sand and organic matter which create different microhabitats (van Elsas and Trevors, 1997). The microbiota inhabiting stable soil systems are exposed to diverse abiotic and nutritional conditions with each microhabitat being inhabited by organisms that can colonize the niche and establish (Garbeva *et al*., 2004). The scale of microbial diversity in soil is critical in maintenance of soil health, quality and function in both undisturbed and disturbed soils. Microbes play key roles in soil processes such as soil structure formation, organic matter breakdown, removal of toxins; and the cycling of nitrogen, carbon and phosphorus (van Elsas and Trevors, 1997). In addition, microorganisms are known to suppress soil borne plant diseases through various antagonistic mechanisms hence promoting healthy plant growth (Ortíz-Castro *et al*., 2009).

Soil microbes ranging from free-living bacteria to single fungi are diverse groups in terms of taxonomy, structure, and function (Harris, 2009). They gradually release macro and micronutrients from organic matter for use by plants through decomposition processes. Microbial communities in soil are involved in nutrient cycling and organic matter decomposition (Schimel, 1995; Bergkemper *et al*., 2016). They regulate plant productivity and community dynamics (Wardle *et al*., 2004, Van der *et al*., 2008) as well as soil structural generation (Feeney *et al*., 2006). Soil nutrient availability can be increased through mineralization of soil organic matter and solubilization of soil minerals by microbes (Lee and Parkhurst, 1992; Sparling, 1994; Bender *et al*., 2016). Microbial community rapid growth and turnover potential in a terrestrial ecosystem is greatly influenced by external stresses than plants and animals (Panikov, 1999). Dynamics in microbial communities can be used to assess positive and negative impact created by organic and conventional farming practices in an agricultural ecosystem (Bending *et al*., 2000; Van-Bruggen and Semenov, 2000; Poudel *et al*., 2002; Sharma *et al*., 2010; Jacoby *et al*., 2017).

Various agricultural practices such as cropping systems, crop rotational cycles, tillage practices, soil-water management practices, applications of fertilizers and agrochemicals can significantly affect microbes present in the soil (Hengeveld, 1996; Tony *et al*., 2020). Soil microbes are vulnerable to alterations in soil (Schirmer and Sonnletner, 1996; Zhen *et al*., 2014; Jacoby *et al*., 2017) and microbial population has been shown to shift after fertilization regimes (Hyman *et al*., 1990). Application of fertilizer inputs directly promote growth of microbes due to nutrients supplied and this may shift the composition of various individual microbes within the soil (Khonje *et al*., 1989; Bargaz, *et al*., 2018). Chemical fertilizer input generally improves crop production but cause severe environmental problems and therefore they do not ensure sustainability on a long-term basis (Mader *et al*., 2002; Chandini *et al*., 2019).

The use of organic based inputs such as crop residues, animal and green manures has been found to increase soil nutrient status, productivity potential of soil and microbial activities while the use of only inorganic based inputs in the cropping system has occasioned limited microbial activity and reduced soil productivity (Kang and Akinifessi,

2000). Organic farming has potential benefits towards promoting soil structure formation and stabilization (Reganold *et al*., 1987; Pulleman et al., 2003; Ayuke *et al*., 2019), boosting soil biodiversity (Doles *et al*., 2001; Mader *et al*., 2002; Oehl *et al*., 2004; Brussaard, 2012), lessening environmental stresses (Horrigan *et al*., 2002; Macilwain, 2004; Paul *et al*., 2013), as well as improving quality and safety of food and feed (Giles, 2004).

In highly intensive conventional farms, the roles played by microbes in nutrient cycling are minimal since most nutrients in inorganic fertilizers are readily available for the plants and do not require degradation or mineralization. However, with reduced tillage, use of agrochemicals and inorganic fertilizers, it is generally thought that the role of soil microbes in decomposition and mineralization of complex organic compounds and reduction of plant pathogens may increase (Schnürer *et al*., 1986; Lebbink *et al*., 1994; McCaig *et al*., 1999; Adnan *et al*., 2017; Bargaz, *et al*., 2018). A previous study on functional and structural microbial diversity in organic and conventional viticulture showed that plant protection in vineyards changed the structure and function of grape associated fungi (Schmid *et al*., 2011). Overall, variations in bacterial and fungal communities between organic and conventional systems were limited to the specific farming system (Foissner, 1992; Wander *et al*., 1995; Yeates *et al*., 1997; Shannon *et al*., 2002; Girvan *et al*., 2003; Zhang *et al*., 2019; Fernandez *et al*., 2020). However, a general trend towards elevated bacterial (Fraser *et al*., 1988; Bossio *et al*., 1998; Gunapala and Scow, 1998) and fungal activity under organic systems (Fraser *et al*., 1988; Yeates *et al*., 1997; Shannon *et al*., 2002), with a microbial biomass 10 – 26 % greater was observed. Inclusion of green and animal manures in the organic farm was alluded as the key factor, that supplied a significantly greater amounts of organic carbon, thus augmenting bacterial populations (Fraser *et al*., 1988; Bossio *et al*., 1998; Gunapala and Scow, 1998).

2.6 Prokaryotic organisms in soil ecosystems

Soil is an abundant, complex and valuable natural product on Earth and it is a habitat for prokaryotic and eukaryotic organisms. Biodiversity of belowground and aboveground soil organisms is greatly influenced by soil. Although soil covers most of Earth's land surface (about 30 %), fertile and healthy soil are considered as a 'threatened species'

(Kaiser, 2004; Drohan and Farnham, 2006; Lehman *et al*., 2015). Soil degradation is happening more rapidly than reclamation (Quinton *et al*., 2010; Stockmann *et al*., 2014). On a microscale (< 1 mm) soil is considered as highly heterogeneous, offering numerous microhabitats per gram of soil. It is this spatial micro heterogeneity that drives community assembly and functional roles of soil microbes. The most dominant and diverse form of life in soil are prokaryotes (bacteria and archaea). They are important in soil ecology and Earth system processes and that is the reason they are considered as unseen majority (Whitman *et al*., 1998) whose cells are independent entities that perform life processes independently of other cells (Madigan *et al*., 2010). Approximately 2.5 x 10^{29} prokaryotic cells occur in soil and Earth hosts $>10^{30}$ of these cells (Whitman *et al*., 1998). One gram of soil may harbor from 10^{8} (bulk soil) up to 10^{11} (rhizosphere) prokaryotic cells (Torsvik *et al*., 1990; Portillo *et al*., 2013; Regan *et al*., 2014) and an approximate species diversity of 4 x 10^{3} (Torsvik *et al*., 1990) to 8 x 10^{6} species (Gans *et al*., 2005). Prokaryotes are considered as the main drivers of various ecological functions in soils (Prosser *et al*., 2007; Treseder *et al*., 2012). In terms of distance and occupation, a distance of 1 mm for a bacterium is comparable to a distance of 1 km for humans and the occupation of 1 g of sterile soil is comparable to the occupation of the Earth's globe by humans (Prosser, 2012). Microbes sustain life on this planet because of their myriad associations and biogeochemical processes. Without microbes, most biogeochemical cycling would cease, human and animal waste would accumulate rapidly in the world. It would be difficult to find living food sources in absence of microbes (Gilbert and Neufeld, 2014). Majority of ruminant livestock cannot survive without microbial symbionts, and plants would rapidly deplete nitrogen and cease photosynthesis. Without microbes, a complete societal collapse would occur within a year or so, due to catastrophic breakdown of the food supply chain (Gilbert and Neufeld, 2014).

Cultivation of prokaryotic organisms using classical microbiology methods developed by Robert Koch and Joseph Lister in 1873, and numerous sophisticated ways that have been developed to culture bacteria (Overmann, 2013) have enabled their accessibility to the scientific community. This has resulted in 12, 604 effectively described and validly named species (as of January 2015; http://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up to date) (Sikorski, 2015). This represents about 0.001 % to 0.1 % of all

bacterial species (Epstein, 2013; Overmann, 2013) because the not-yet cultured prokaryotes most probably exhibit a physiology that does not match the addressed cultivation methods (Overmann, 2013). In addition, many microorganisms can enter the reversible state of dormancy, which is a bet-hedging strategy to overcome unfavorable environmental conditions (Jones and Lennon, 2010). Dormant individuals become members of a seed bank, which has the potential to substantially shape the structure of microbial (soil) communities (Lennon and Jones, 2011).

2.7 Microbial ecology in farming systems

Soil microorganisms are extremely sensitive to changes in soil physicochemical properties that arise from various agricultural practices introduced in farming systems (Acosta-Martínez *et al*., 2008; Schipanski and Drinkwater, 2012; Vasseur *et al*., 2013). Intensive agriculture cultivation techniques such as deep tillage, monocropping, export of crop and other plant material residues without incorporation into the soil and inorganic fertilizer application reduces soil fertility and alter soil microbial community structure (Mäder *et al*., 2002). Tillage breaks up soil aggregates, hastens organic matter decomposition and also compacts the soil (Ussiri and Lal, 2009). Removal of crop residue reduces soil organic matter content (Fuentes *et al*., 2009) while extensive inorganic fertilizer applications increases salt content and electrolytic conductivity thereby lowering soil fertility (Rezapour, 2014).

Soil microorganisms play an important role in soil fertility by acting as a source and sink of mineral nutrients (Jenkinson and Ladd, 1981; Jacoby *et al*., 2017). Modern farming practices undermine the wellbeing of microbial communities in many ways. For instance, huge regions of natural habitats, including their ecosystem services, have been destroyed (Sachs *et al*., 2010). An alternative ecological friendly farming system to conventional agriculture is offered by organic farming system. This system aims to minimize impact on the environment by introducing crop rotation, intercropping, pathogen-resistant cultivars, limited amounts of chemical pesticides, incorporation of crop residues back into the soil and organic fertilizers instead of synthetic fertilizers. However, the beneficial effect of organic agriculture on microbial diversity and plant-associated microorganisms

is still debatable (Hole *et al*., 2005; Granado *et al*., 2008; Crowder *et al*., 2010; Ruimy *et al*., 2010).

2.8 Fungal communities in soil habitats

Fungal communities are an essential constituent of soil microbial biomass that is involved, and/or linked to processes of carbon and nitrogen cycles, organic matter decomposition, as well as nitrogen mineralization and immobilization (Bloem *et al*., 1995; Bååth and Anderson, 2003; Wall *et al*., 2012; Berthrong *et al*., 2013; Milner, 2014; Fierer, 2017). On the other hand, fungi which constitute one of the largest groups of eukaryotes, play key role in nutrient cycling as symbionts, mutualists, pathogens and free-living saprotrophs (Barea *et al*., 2005; Gadd, 2007; Lindahl *et al*., 2007; McLaughlin and Spatafora, 2014). They are also involved in soil aggregation, enhancing soil water holding capacity, promoting plant growth and suppression of phytopathogens (Sommermann *et al*., 2018). For example, mutualistic root endophytes are known to induce systemic resistance in host plants thereby increasing plant tolerance levels to biotic and abiotic stress factors (Lahlali *et al*., 2010). Therefore, they are a key component of sustainable soil-plant systems that govern major plant nutrient cycles hence sustaining the vegetation cover and ecosystem services (Schreiner *et al*., 1997; Dighton, 2003; Johansson *et al*., 2004).

Soil fungal community composition is influenced by soil physicochemical properties, plant populations and geo-climatic conditions (Tkacz *et al*., 2015). However, in agro-ecosystems, they are exposed to added influencing factors associated with soil and crop agronomic management practices. To date, only few studies have delved to determine the effects of tillage practices, fertilization regimes, cropping systems and crop rotation cycles on fungal diversity despite there being trends that indicate different agricultural management regimes have an impact on fungal communities (Lentendu *et al*., 2014). Little information is available concerning the effect of cultivation systems on fungal diversity and the level of fungal diversity between different crops in the same farm (Lentendu *et al*., 2014; Lopes *et al*., 2014; Kazeeroni and Al-Sadi, 2016). The fungal diversity ecosystem is still undefined; though, Wang et al. (2008) reported that about 5-13 % of the total estimated global fungal species have been described. Since many fungi

are unculturable and rarely produce visible sexual structures, molecular techniques have become widely used for taxonomic detection of species to understand shifts in their richness and composition along environmental gradients (Persˇoh 2015; Balint *et al*., 2016; Tedersoo and Nilsson 2016; Tedersoo *et al*., 2018). It is still not understood how fungal communities respond to different inputs within organic and conventional farming systems (Hartmann *et al*., 2015; Wang *et al*., 2017).

2.9 Effect of land use on microbial diversity

Changes in microbial community composition due to seasonal and temporal variations in nutrient or physical conditions are slow and gradual, making it difficult to interpret the data and obtain conclusive results (Amann *et al*., 1995; Smit *et al*., 2001). Previous studies have demonstrated perturbation of microbial community equilibrium populations by changes in environmental conditions and soil management practices (Peacock *et al*., 2001; Smit *et al*., 2001). Microbial community diversity in cultivated areas may change depending on variation in environmental factors, such as nutrient availability and pH (Jesus *et al*., 2009). Previous comparisons between agricultural and grassland soils indicated a decrease in microbial species richness (Steenwerth *et al*., 2003), while other studies found that conversion of the Amazon to cultivation resulted in an increase of microbial diversity (Rodriges *et al*., 2012). It was confirmed that changes in microbial community structure is a function of pH and other factors (Hartman *et al*., 2007; Jesus *et al*., 2009). However, different ecosystems respond differently. For instance, wetlands are more strongly affected by pH than they are by soil carbon or nutrient inputs (Hartman *et al*., 2007). Understanding how major changes in land management affect the structure of the soil's microbial community could provide an important index for assessing the relative ability of the soils to respond to future disturbance (Helgason *et al*., 2010; Lopes *et al*., 2011).

2.10 Use of molecular techniques in microbial ecology

Soil microbiomes are often difficult to fully characterize, primarily because of their vast phenotypic and genotypic diversity, heterogeneity and crypticity and most of these cells are mostly unculturable via classical microbiological approaches. Molecular techniques became essential in microbial ecology in the early 90s (Pickup, 1991; Stackebrandt *et al*.,

1993; Amann *et al*., 1995; Holben and Harris, 1995). Previously, determination of DNA sequences was far too expensive for the analysis of numerous samples and alternative molecular methods were developed. DNA analysis has been applied to analyze whole communities, individual isolates, and clones of genes. Low resolution and broad scale analysis of community DNA like DNA reassociation, allow assessment of total diversity of microbial communities (Torsvik *et al*., 1996). Denaturing and temperature gradient gel electrophoresis (DGGE and TGGE), single- strand confirmation polymorphism (SSCP), amplified ribosomal DNA restriction analysis (ARDRA) and terminal restriction fragment length polymorphism (T-RFLP) techniques characterize the sequence diversity of 16S rRNA gene PCR amplicons obtained from soil DNA without sequencing (Kirk *et al*., 2004). The PhyloChip, based on Affymetrix GeneChip microarray technology, categorizes with high reproducibility all known bacteria and archaeal Operational Taxonomic Units (OTU, typically defined at 97 % 16S rRNA gene similarity) into over 50,000 taxa using probes that target variations in the 16S rRNA gene (Hazen *et al*., 2010).

Next-generation high-throughput (HTP) sequencing methods involve sequencing of the entire genetic material in a habitat (Daniel, 2005; Council, 2007; Thomas *et al*., 2012). This enables discovery of interactions between microorganisms and the environment, and assignment of ecosystem functions to various communities (Hugenholtz, 2002; Handelsman, 2004; Lopez-Garcia and Moreira, 2008). Metatranscriptome studies target environmental RNA, hence the functional part of the environmental community can be assessed (Moran *et al*., 2013). Functional genes of uncultured organisms can be linked to phylogenetic groups by cloning and sequencing of large genomic DNA fragments (Sjöling and Cowan, 2008; Carola and Rolf, 2009). This enables assessment of dominant biosynthetic pathways and primary energy sources (Biddle *et al*., 2008; Frias-Lopez *et al*., 2008; Carola and Rolf, 2009). RNA extracted from environmental samples provides more valuable information than DNA in revealing active microbial communities versus dormant microbial communities (Torsvik and Øvreås, 2002). Several genes, e.g., ammonia oxidation, nitrogen fixation, denitrification and sulfate reduction, have been amplified from DNA/RNA isolated from microbial communities to obtain insights into key microbial processes (Hansel *et al*., 2008). Combining metatranscriptomic approaches

with new sequencing methods has been demonstrated as a powerful approach in the study of microbes in diverse habitats (Frias-Lopez *et al*., 2008; Segata *et al*., 2013). These novel next generation sequencing technologies generate large volumes of data in a reasonably short time and in a cost-effective way (Elahi and Ronaghi, 2004; Kozarewa *et al*., 2009; Creer *et al*., 2010). By directly sequencing DNA or cDNA, possible cloning bias is avoided in large-scale studies (Adams *et al*., 2009).

To resolve fungal community structures, primers targeting the Internal Transcribed Spacer regions ITS1 and ITS2, which are located between rRNA genes in eukaryotes, are routinely applied for amplicon generation. Several studies have compared the information content of ITS1 and ITS2 sequences, but results are ambiguous (Bazzicalupo *et al*., 2013). The ITS2 region was suggested to be more variable than ITS1 (Bazzicalupo *et al*., 2013), but many studies include both ITS regions to avoid under estimation of diversity in the sampled communities (Blaalid *et al*., 2013; Monard *et al*., 2013). An established method to examine fungal diversity is paired-end sequencing of PCR amplicons on the Illumina MiSeq platform and it reliably reflects fungal diversity from environmental samples. Former studies analyzed mycobiomes from plants, soil (Xu *et al*., 2011), decaying organic material as well as aquatic and marine environments (Nagahama and Nagano, 2012). For taxonomic assignment of fungal OTUs, sequences are analyzed using BLASTn based on comprehensive databases such as UNITE ITS Reference Database (Kojalg *et al*., 2005; Koljalg *et al*., 2013), a curated database derived from GreenGenes, RDPII and NCBI (www.ncbi.nlm.nih.gov; http://rdp.cme.msu.edu). Molecular techniques have the potential to offer a comprehensive picture of soil microbial community diversity and structure, since both culture grown and non-culture grown components of a community can be surveyed, thus enabling analysis of the entire microbial community in an ecosystem. The main objective of this study was to understand how different agricultural management practices influence diversity and function of soil microbial populations as well as linking the structure and function of microbial communities within the ongoing long-term farming systems comparison trials in the central highlands of Kenya using metagenomic and metatranscriptomic analysis based on both DNA and RNA.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

This study was carried out within the ongoing long-term farming systems comparison trials (SysCom; www.system-comparison.fibl.org) in Kenya (Adamtey *et al*., 2016). The trials were established in 2007 at two locations: Chuka and Thika in the Central Highlands of Kenya **(Figure 3.1)**. These experimental sites were established based on Food and Agricultural Organization (FAO) world reference system of soil classification (IUSS Working Group WRB, 2006). The Central highlands of Kenya lie between Nairobi and Mt Kenya region (1500-2000 meters above sea level). They are endowed with a high potential for agricultural production due to the abundant rainfall (1000 - 2000 mm) and fertile soils that can support a wide range of crops within two cropping seasons and livestock (Place *et al*., 2006a). Common farmers practice in nutrient management is mainly integrated use of mineral fertilizers combined with use of fresh manure (Mucheru-Muna *et al*., 2007; Musyoka, 2007). Manure production in this area range from 4-13t yr^{-1} (fresh weight) with each household having an average of 2.3 cows (Place *et al*., 2006a).

The sites have a bimodal rainfall pattern with long rains (LR) occurring between March and June and short rains (SR) occurring between October and December. Chuka site is situated in the upper midland 2 agro ecological zone, also referred to as the coffee zone (Jaetzold *et al*. 2006a). Thika site is situated in the upper midlands agro-ecological zone 3 (UM3), also referred to as the sunflower maize zone (Jaetzold *et al*., 2006b). The soils at Chuka site are classified as Humic Nitisols and those at Thika as Rhodic Nitisols (Adamtey *et al*., 2016) in the FAO World reference base for soil resources (IUSS Working Group WRB, 2006). The site characteristics are as summarized on **Table 3.1**.

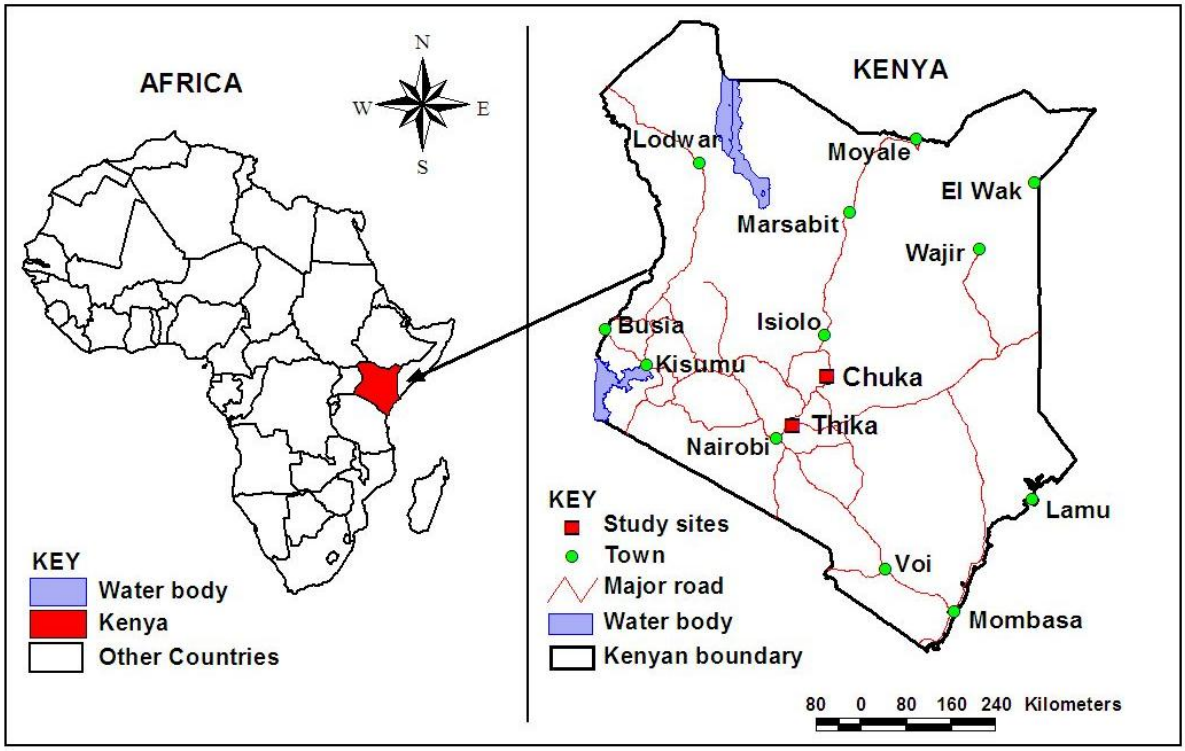


Figure 3.1: Long-term farming systems comparison trial sites in Kenya

Table 3.1: Long term experiment trial sites characteristics

Site	Coordinates	Agro ecological Zone	Altitude	Rainfall pattern	Temperature Range	Cropping Seasons	Cropping Period
Thika	01° 0.231' S, 37° 04.747' E	UM 3	1518 m	840 mm	19.5 - 20.7 °C	Long Rain (LR)	March - June
						Short Rain (SR)	October - December
Chuka	0° 20.864' S, 37° 38.792' E	UM 2	1458 m	1373 mm	19.2 - 20.6 °C	Long Rain (LR)	March - June
						Short Rain (SR)	October - December

*UM 2 – Main Coffee Zone; *UM 3 – Sunflower and Maize Zone

3.2 Farming systems

Conventional (Conv) and organic (Org) farming systems were compared at low input levels (Conv-Low and Org-Low), where nitrogen and phosphorous application rates mirrored small-scale farmers' practices in the region; and at high input levels (Conv-High and Org-High), which represented the recommended nitrogen and phosphorous input

levels used in market-oriented and large-scale production systems. In Conv-High system, nutrients were applied in the form of synthetic fertilizers (diammonium phosphate, triple super phosphate, calcium ammonium nitrate) and decomposed cow manure. Nutrient application rate was based on recommendations by Muriuki and Qureshi, 2001, while in Org-High system, nutrients were applied in form of compost, green manure, plant tea and phosphate rock (IFOAM, 2013) at the same nutrient levels for Phosphorus and Nitrogen as in Conv-High system. The high input systems received supplementary irrigation during the dry period and pest and disease were controlled based on a scouting program (Adamtey *et al*, 2016). In the low input conventional and organic farming systems, nutrients were applied in form of synthetic fertilizers and fresh farmyard manure (Conv-Low) and decomposed manure, biomass of *Tithonia diversifolia* and low amounts of phosphate rock (Org-Low) (**Table 3.2**).

Table 3.2: Inputs in convention and organic farming systems in the LTE trials

Treatment	Cropping Year	Season	FYM (t/ha)	Compost *(t/ha)	DAP (kg/ha)	PR (kg/ha)	TSP (kg/ha)	CAN (kg/ha)	Tithonia ** t/ha	Tithonia (LM) t/ha	Total N applied (kg/ha)	Total P applied (kg/ha)
Conv Low	1	1	5		50						37	12
		2	1				50	60			20	13
	2	1	5		50						31	18
		2	0		0						0	0
	3	1	5		50						31	18
		2	2		100						27	25
Conv High	1	1	7.5		200			100			96	54
		2	15				200	300			145	64
	2	1	11.3		200			100			113	60
		2	11.3		200			100			113	60
	3	1	11.3		200			100			113	60
		2	11.3				300	200			103	83
Org Low	1	1		5		100			1.36		31	18
		2		1		90			1.2	1.2	20	13
	2	1		5		100			1.36		31	18
		2		0		0			0		0	0
	3	1		5		100			1.36		31	18
		2		2		200			2.72		27	26
Org High***	1	1		7.5		364			5.4	3.9	96	54
		2		15		400			6	6	147	70
	2	1		11.3		364			5.4	3.9	113	59
		2		11.3		364			5.4	3.9	113	59
	3	1		11.3		364			5.4	3.9	113	59
		2		11.3		581			8.2		105	83

*Compost preparation starts with the indicated amount of Fresh FYM; **Tithonia mulch is applied after crop germination as starter N; ***Organic high treatment also receives maize stover residues at 2t/ha during the short rain season. The plots are also intercropped with Mucuna during the first season and the mucuna biomass is applied during the short rain season. French bean biomass is also incorporated during the next baby corn season. FYM = Farm Yard Manure; DAP = Diammonium Phosphate; TSP = Triple Super Phosphate; CAN = Calcium Ammonium Nitrate; N = Nitrogen; P = Phosphorous

In both sites, the four farming systems were randomly replicated four times in a Randomized Complete Block Design (RCBD) with plot sizes of 8 x 8 m (net plot size of 6 x 6 m). At Chuka, the replicates were designated as; Conv-High (plots 3C, 6C, 12C and 14C), Conv-Low (plots 2C, 7C, 11C and 16C), Org-High (plots 4C, 8C, 9C and 15C) and Org-Low (plots 1C, 5C, 10C and 13C) (Figure 3.2 a).

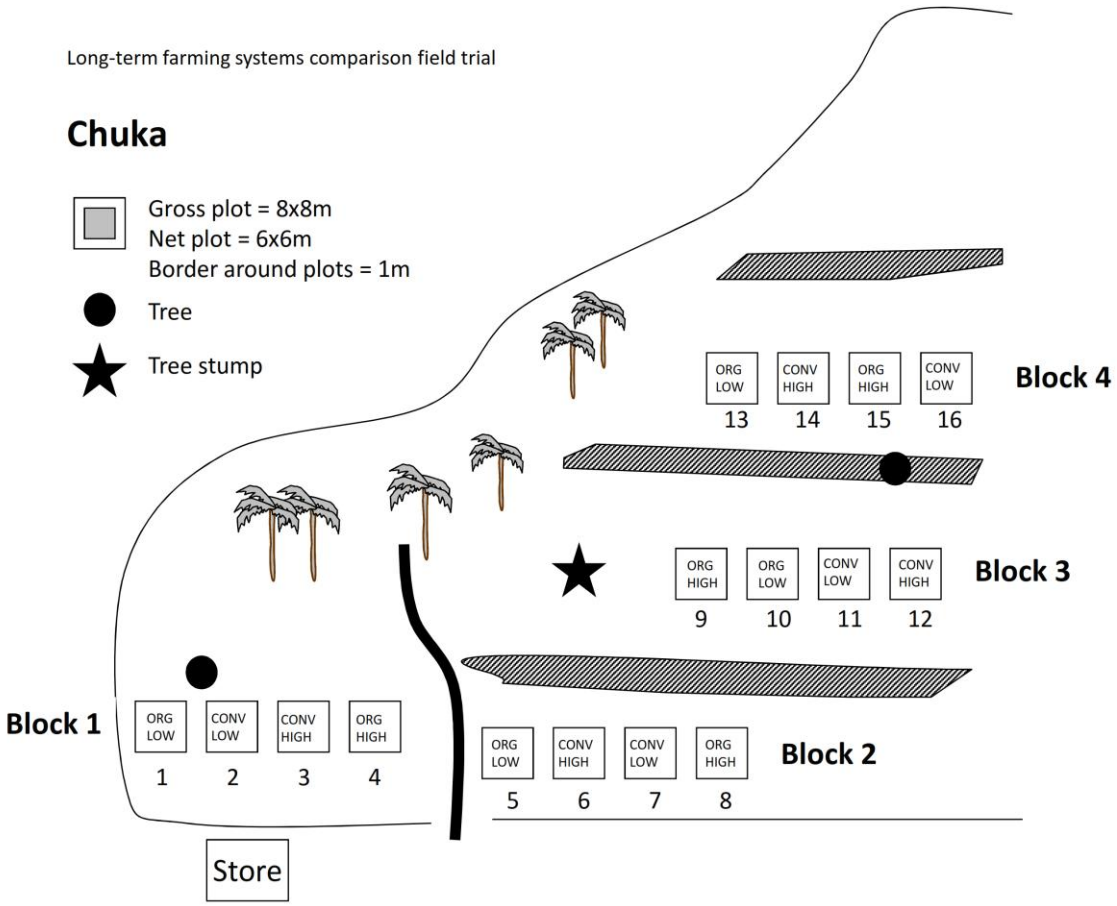


Figure 3.2a: Chuka long-term farming system comparison experiment field trial layout

At Thika, the replicates were designated as; Conv-High (plots T2, T7, T9 and T20), Conv-Low (plots T1, T6, T12 and T18), Org-High (plots T3, T8, T11 and T17) and Org-Low (plots T4, T5, T10 and T19) (Figure 3.2 b).

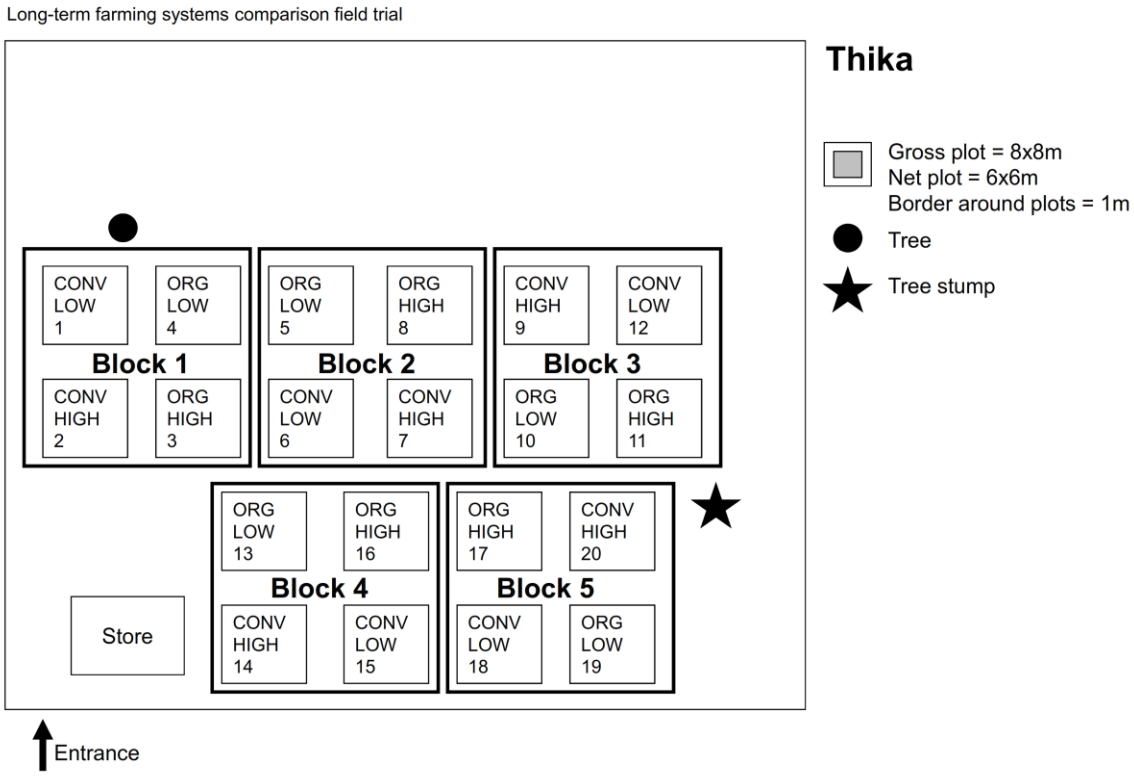


Figure 3.2b: Thika long-term farming system comparison experiment field trial layout

The Long-term Experiment trial is based on a two-season-three-year crop rotation as shown in **Table 3.3**. Selection of crops for the high input systems (Conv-High and Org-High) and low input systems (Conv-Low and Org-Low) were based on reports by Székely (2005), Musyoka (2007) and MOA/JICA (2000). Crop rotations were based on farmers' practices in the area and the principle of crop rotation recommended by the Kenyan Institute of Organic Farming (Székely, 2005).

Table 3.3: Long-term Farming Systems Comparison trial crop rotation

		Year 1		Year 2		Year 3	
Input level	**System type**	**Season 1**	**Season 2**	**Season 1**	**Season 2**	**Season 1**	**Season 2**
Low	CONV	Maize	Kales and spinach	Maize and Beans	Beans	Maize and Beans	Potatoes
Low	ORG	Maize	Kales and spinach	Maize and Beans	Beans	Maize and Beans	Potatoes
High	CONV	Maize	Cabbage	Baby corn	French beans	Baby corn	Potatoes
High	ORG	Maize/ Mucuna	Cabbage	Baby corn/ *Mucuna p.*	French beans	Baby corn/ Mucuna	Potatoes

3.3 Soil sampling

Soil sampling was done before land preparation in March 2015. Surface organic materials were removed, and a composite soil sample collected from 12 single cores within topsoil (0-20 cm depth) which is the root zone of majority crops grown in the trial sites. Two batches of sixteen (16) composite samples from each site were packed in sterile 500 g containers. Samples for metatranscriptome analysis were sub-sampled by pooling the four replications of each farming system into one sample resulting into four (4) samples per site. Samples for molecular analysis were preserved on dry ice and transported to the laboratory at *icipe* for preservation at -80 ^{o}C whilst the batch of samples for physicochemical analysis were transported to the laboratory at *icipe* and preserved at room temperature. Soil physicochemical parameters were analyzed using methods summarized in **Table 3.4.**

Table 3.4: Soil physicochemical parameters that were analyzed and their respective methods

Parameter	Method
pH and Electrical conductivity (EC)	Potentiometric (Okalebo *et al*., 2002)
Cation exchange capacity (CEC), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulphur (S), Sodium (Na), Copper (Cu), Boron (B), Zinc (Zn) and Iron (Fe)	Mehlich 3 (Mehlich, 1984)
Exchangeable Aluminium (Exch. Al)	Spectrophotometry (Kennedy and Powell, 1986)
Organic Carbon (OC)	Wet oxidation (Anderson and Ingram, 1993)
Total Nitrogen (N)	Kjeldahl acid digestion (Gupta, 1999)
Total Phosphorous (P),	Olsen (Okalebo *et al.*, 2002)
Soil moisture and Temperature	Soil Moisture Meter (IMKO GmbH – Germany)
Aggregate size separation (Small macro-aggregates and micro-aggregates)	Wet sieving (Six *et al*, 1998)
Soil mineralogy	Diffraction (Shepherd, 2010)

3.4 Microbial community analysis

3.4.1 Total DNA extraction

Total community DNA was extracted from 0.2 g of the soil samples in triplicates as described by Sambrook et al. (1989). Solution A, 500 µl (50 mM Tris pH 8.5, 50 mM EDTA pH 8.0 and 25 % sucrose solution) was added to each tube and homogenized and centrifuged at 13,000 revolutions per minute for one minute to remove exopolysaccharides from soil samples. The supernatant was discarded and the sample re-suspended in 200 µl of solution A, 5 µl of Lysozyme (20 mg/ml) and 5 µl of RNAse A (20 mg/ml) were added, gently mixed and incubated at 37 ^{o}C for one hour. Following incubation, 600 µl of solution B (10 mM Tris pH 8.5, 5 mM EDTA pH 8.0 and 1 % SDS) was added and mixed by inverting the eppendorf vial several times. Ten (10) µl of Proteinase K (20 mg/ml) was added, mixed gently and incubation at 50 ^{o}C for 1 hour. Phase separation was achieved by adding equal volumes of phenol: chloroform to the cell lysate followed by centrifugation for 15 minutes at 13000 revolutions per minute. Phenol was cleaned from the crude DNA using an equal volume of Chloroform: Isoamyl alcohol

(24:1). The aqueous phase was carefully transferred into a new tube and precipitation done overnight at -80 ºC using an equal volume of isopropanol and 0.1 volumes of 3M NaCl. The DNA pellets were washed twice using 70% Ethanol and air-dried at room temperatures for 20 minutes and thereafter stored at -20 ºC.

3.4.2 Total RNA Extraction

Total RNA was extracted from 0.25 g of soil samples in triplicates using Trizol RNA extraction protocol (Chomczynski and Sacchi, 1987). Succinctly, 750 µl of Trizol LS and 250 µl of each sample were added to a 2 ml eppendorf vial and vortexed for 5 seconds. The samples were incubated at room temperature for 10 minutes to allow complete lysis of the cells and then centrifuged for 30 seconds to get the liquid down the tube. Two hundred (200) µl of chloroform (Molecular grade) was added to the sample supernatant and vortexed to get the phases mixed. The samples were incubated at room temperature for 10 minutes and then centrifuged at 12,000 revolutions per minute for 10 minutes at 4 ºC. The aqueous phase (500-550 µl) was transferred to a new 1.5 ml eppendorf tube. One (1) µl of glycogen and 500 µl of isopropanol (Molecular grade) were added, vortexed for 30 seconds and then centrifuged at 12,000 revolutions per minute for 10 minutes at 4 ºC. At this stage, RNA precipitate formed a gel-like pellet on side/bottom of the tube. The supernatant was removed and discarded. Five hundred (500) µl of 75 % ethanol was added to the RNA precipitate and the tube was inverted gently, centrifuged for 2 minutes at 12000 rounds per minute at 4 ºC and the supernatant was removed and discarded. RNA extracted from the triplicate samples was pooled during the precipitation stage, the pellets air dried at room temperature for 10 minutes and stored at –80 ºC awaiting cDNA synthesis.

3.4.3 Synthesis of cDNA from 16S rRNA

Complimentary (c) DNA synthesis, amplification and sequencing were performed at Molecular Research DNA Lab (www.mrdnalab.com, Shallowater, TX, USA). The quality of total RNA was assessed using gel electrophoresis. The extracted RNA was dissolved in RNase-free water and subsequently treated to remove DNA contaminants using the Amplification Grade DNase I Kit (Sigma, MO) according to manufacturer's instructions. Complimentary (c) DNA first-strand and second-strand synthesis was done

using the Superscript III First-Strand Synthesis SuperMix (Invitrogen, CA) and the Second-strand cDNA Synthesis Kit (BeyoTime, Jiangsu, China), respectively, following manufacturer's instructions. Single-strand reverse transcription was done to provide template for amplicon libraries using Superscript III (Invitrogen) according to the manufacturer's protocol, random hexamer primed and with subsequent RNAse H digestion. The Double stranded cDNA synthesis was carried out as described by as described by Urich et al. (2008).

3.4.4 16S rRNA amplicon library preparation and sequencing

PCR amplification of the 16S rRNA gene V4 variable region was carried out from extracted DNA and cDNA generated from rRNA, using bacteria/archaeal primers 515F (GTGCCAGCMGCCGCGGTAA) that had barcode and 806R (GGACTACHVGGGTWTCTAAT) (Caporaso *et al*., 2012). Amplification proceeded in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with initial denaturation heating at 94 °C for 3 minutes, followed by 28 cycles of denaturation at 94 °C for 30 seconds, annealing at 53 °C for 40 seconds and extension at 72 °C for 1 minute, and a final elongation at 72 °C for 5 minutes. The quality of PCR products was assessed on 2 % agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples, tagged with different barcodes, were pooled in equimolar ratios based on their DNA concentrations from the gel images. Pooled samples were purified using calibrated Ampure XP beads (Beckman Coulter) for use in library preparation. The pooled and purified PCR products were used to prepare 16S rDNA and cDNA library by following Illumina TruSeq DNA library preparation protocol (Yu and Zhang 2012). Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq 2x300bp Version 3 following the manufacturer's guidelines.

3.4.5 Prokaryotic bioinformatic sequence processing, taxonomic identification and statistical analysis

Amplicons were analyzed using QIIME2 pipeline (Bolyen *et al*., 2018) whereby QIIME2 pipeline input file was created using "convert_fastaqual_fastq.py" script on QIIME v1.9 (Caporaso *et al*., 2010). The script combines "FASTA" and "QUALITY" files into composite FASTAQUALITY (FASTQ) files. The barcode sequences with

demultiplexing information that linked each sequence to its respective sample were extracted into a sample metadata file. The FASTQ sequences were demultiplexed using barcode information, checked for quality and construction of feature tables done using dada2 software (Callahan *et al*., 2016). Precisely, dada2 software was used to denoise sequences, remove chimeras, create OTU table, pick representative sequences and calculate denoising statistics. Sequences which were < 200 base pairs after phred20- base quality trimming, with ambiguous base calls, and those with homopolymer runs exceeding 6bp were removed. Representative sequences were aligned using MAFFT and highly variable regions were masked to reduce the noise in phylogenetic analysis (Kazutaka and Daron, 2013). Phylogenetic trees for use in phyloseq analysis were created and rooted at midpoint (Price *et al*., 2010). Taxonomic classification of representative sequences obtained from the OTU clustering was done using QIIME feature-classifier classify-sklearn based on SILVA 128 16S classifier at 97 % level of similarity using default settings as implemented in QIIME2 (Bolyen *et al*., 2018). The 16S rDNA and 16S rRNA cDNA sequences have been deposited at National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number PRJNA523239 (https://www.ncbi.nlm.nih.gov/sra/PRJNA523239) and SRA accession: PRJNA523223 (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA523223) for 16S rDNA and 16S rRNA cDNA datasets, respectively.

Microbial diversity analysis was carried out using Vegan Community Ecology Package version 2.5.2 (Oksanen *et al*., 2016) while microbiome census was analyzed using phyloseq version 1.24.2 in R (R Development Core Team, 2016). Alpha diversity measures (Richness - S' and Shannon - H') were used to test significant differences within high and low input farming systems. Rarefaction curves were generated, plotted and customized using Vegan Community Ecology Package in R (Oksanen *et al*., 2016). Community and environmental distances were compared using Analysis of similarity (ANOSIM) (Clarke, 1993) while significance was determined at 95 % confidence interval ($P<0.05$). Calculation of Bray-Curtis dissimilarities between datasets and hierarchical clustering were carried out using Vegan package in R (Oksanen *et al*., 2016). Diversity between farming systems (β diversity) was estimated by computing the

Principal Component Analysis (PCA) of soil physicochemical characteristics versus prokaryotic taxa in R (R Core Team 2016).

In order to understand the influence of farming systems on soil physicochemical characteristics, analysis of variance was performed at $P < 0.05$, 0.01 and 0.001 using a linear mixed-effect model with *lmer* function from lme4 package (Bates *et al*., 2013) with system and site as fixed effects, while replication was used as random effect. Computation of least mean squares was done using *lsmeans* package. Means were separated using Tukey's *ad hoc* method implemented using *cld* from *multicomp* package as developed by (Piepho, 2004) in R software version 3.1.5 (R Development Core Team, 2018).

3.4.6 Fungal amplicon DNA library preparation and sequencing

Polymerase Chain Reaction (PCR) amplification of ITS region was done using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) barcode primers (White *et al*., 1990 and Ihrmark et al., 2012). Amplification proceeded in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with initial heating at 94 °C for 3 minutes, followed by 28 cycles of denaturation at 94 °C for 30 seconds, annealing at 53 °C for 40 seconds and extension at 72 °C for 1 minute, after which a final elongation step at 72 °C for 5 minutes was performed. PCR products were visualized on 2 % agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples tagged with different barcodes, were pooled together in equal proportions based on their molecular weight and DNA concentrations during sequencing. The pooled samples were purified using calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA) and used to prepare DNA library by following Illumina sequencing protocol (Yu and Zhang, 2012). Sequencing was performed at Molecular Research DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq 2x300bp Version 3 platform following the manufacturer's guidelines.

3.4.7 Fungal Sequence analysis

Generated amplicons were analyzed using QIIME2 pipeline (Bolyen *et al*., 2018). The FASTQ sequences were demultiplexed, quality checked, and a feature table constructed

using dada2 software in QIIME2 (Callahan *et al*., 2016). Taxonomic classification of representative sequences obtained from the OTU clustering was done using QIIME feature-classifier classify-sklearn based on UNITE ITS Reference Database (Kojalg *et al*., 2005; Koljalg *et al*., 2013) and a curated database derived from GreenGenes, RDPII and NCBI (www.ncbi.nlm.nih.gov; http://rdp.cme.msu.edu) at 97 % level of similarity using default settings as implemented in QIIME2 (Bolyen *et al*., 2018). Diversity analysis were carried out using Vegan Community Ecology Package version 2.5.2 as explained in **section 3.4.5** above. The Fungal ITS sequence reads were deposited at National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number PRJNA532741
(https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA532741).

3.4.8 cDNA synthesis from mRNA, library construction and sequencing

Complimentary (c) DNA synthesis, library construction and short gun sequencing were performed at Molecular Research DNA Lab (www.mrdnalab.com, Shallowater, TX, USA). The quality of total RNA was assessed using gel electrophoresis. The extracted RNA was dissolved in RNase-free water and subsequently treated to remove DNA contaminants using the Amplification Grade DNase I Kit (Sigma, MO) according to the manufacturer's instructions. The cDNA first-strand and second-strand synthesis was done using the Superscript III First-Strand Synthesis SuperMix (Invitrogen, CA) and the Second-strand cDNA Synthesis Kit (BeyoTime, Jiangsu, China), respectively, following the manufacturer's instructions. Single-strand reverse transcription was done to provide template for amplicon libraries using Superscript III (Invitrogen) according to the manufacturer's protocol, random hexamer primed and subsequent RNAse H digestion. The double stranded cDNA synthesis was carried out as described by Urich et al. (2008). Polymerase Chain Reaction enrichment was done to add the barcode to the cDNA library using Phusion® high fidelity *Taq* polymerase enzyme (NEB, USA). The library concentration was determined using Quant-iT dsDNA HS assay kit and a Qubit fluorometer (Invitrogen GmbH, Karlsruhe, Germany) as recommended by the manufacturer. The library was then concentrated using Ampure beads, eluted in 10 µl TE buffer and used as template for Illumina Sequencing following manufacturer's

instructions (Yu and Zhang, 2012). Sequencing was done at the Molecular Research DNA Lab on an Illumina HiSeq 2000 platform without amplification.

3.4.9 Transcriptome sequence analysis

Metatranscriptomic data processing was done through a pipeline developed by the Parkinson lab using a tutorial that was produced by Mobolaji Adeolu, John Parkinson and Xuejian Xiong (https://github.com/ParkinsonLab/Metatranscriptome-Workshop). Quality control was done by checking the read quality using FastQC Version 0.11.9, a quality control tool for high throughput sequence data (Batut *et al*., 2018; Batut, 2020) to show basic statistics such as total number of reads, read length and Guanine Cytosine (GC) base content; Per base sequence quality, Per Base Sequence Content and Adapter Content. The adapter sequences and vector contamination (adapter, linker, and primer) were removed using the Burrows Wheeler aligner (BWA), version 0.6 (Li and Durbin, 2009). Low quality bases and sequencing reads were trimmed using Trimmomatic software, Version 0.39 (Bolger *et al*., 2014). The duplicate reads were removed using the software tool CD-HIT, Version 4.8.1 (Limin *et al*., 2012). The quality reads were assembled into larger contigs using SPAdes version 3.14.1 (Antipov *et al*., 2019) genome assemblers' transcript assembly algorithm, to significantly increase the ability to annotate them to known genes through sequence similarity searches and improve annotation quality.

Prediction of Open Reading Frames (ORF) was done using Prodigal (Hyatt *et al*., 2010) and MetaGeneAnnotator (Noguchi *et al*., 2008). A series of similarity searches were performed to select optimal gene annotations using UniProt, NCBI's NR, NCBI's Conserved Domain Database (CDD), Kyoto Encyclopedia of Genes and Genomes (KEGG) database and Interproscan. To help interpret the metatranscriptomic datasets from a functional perspective, the data was mapped to functional networks such as metabolic pathways and maps of protein complexes. After the pathway maps and molecular functions were drawn, each box was given a KEGG Orthology (KO) identifier called K number (Kanehisa *et al*., 2016 a and b). To infer the taxonomic origin of mRNA reads, a reference based short read classifier was used. Kaiju, a program for the taxonomic classification of high-throughput sequencing reads,

(https://github.com/bioinformatics-centre/kaiju) was used to generate taxonomic classifications for mRNA reads based on a reference database. Reads were directly assigned to taxa using the NCBI taxonomy and a reference database of protein sequences from microbial and viral genomes (Menzel *et al*., 2016).

CHAPTER FOUR

RESULTS

4.1 Diversity and structure of prokaryotic communities within organic and conventional farming systems in central highlands of Kenya

4.1.1 General sequence analysis

After demultiplexing, quality filtering, denoising, and removal of potential chimeras, 476,103 and 632,573 high quality sequences were obtained from 16S rDNA and 16S rRNA cDNA datasets, respectively at Chuka site. These were clustered into 4,916 and 530 OTUs at 97% genetic distance in 16S rDNA and 16S rRNA cDNA datasets, respectively. The 16S rDNA OTUs were further classified into 29 phyla, 96 classes and 166 orders while 16S rRNA cDNA OTUs were assigned to 14 phyla, 30 classes and 52 orders. At Thika site, 931,400 and 937,810 high quality sequences were obtained from 16S rDNA and 16S rRNA cDNA datasets, respectively. These were clustered into 10,082 and 648 OTUs at 97 % genetic divergence in 16S rDNA and 16S rRNA cDNA datasets, respectively. The 16S rDNA OTUs were assigned to 35 phyla, 123 classes and 229 orders while 16S rRNA cDNA OTUs were assigned to 14 phyla, 35 classes and 57 orders within prokaryotic domain (**Table 4.1.1**). Composition and diversity assessment of prokaryotic communities within sites and farming systems displayed Thika site to harbor more unique OTUs as compared to Chuka site. For instance, at Thika site, Conv-High (2,444) and Org-Low (1,633) systems had the highest number of unique OTUs within 16S rDNA dataset.

Table 4.1.1: Distribution of high-quality sequences, OTUs, diversity indices and prokaryotic taxa at Chuka and Thika sites sorted as per total number of OTUs

	Site	System	Raw sequences	High quality sequences	OTUs	Unique OTUs	Richness	Shannon (H)	Phyla	Classes	Orders	Unknown orders	Most abundant taxa (Order level)
16S rDNA	Thika	Conv-High	335706	319678	3193	2444	877.2	6.26	19	97	170	81	*Solirubrobacterales*
		Org-High	191546	182931	2314	1565	757.5	6.09	27	87	151	68	Uncultured *Chloroflexi*
		Org-Low	216335	207067	2307	1633	823.4	6.12	29	87	144	62	*Burkholderiales*
		Conv-Low	232797	221724	2268	1594	728.6	6.09	27	83	154	66	Uncultured *Chloroflexi*
	Chuka	Conv-Low	115027	108652	1737	1400	407	5.29	23	77	120	45	*Gaiellales*
		Conv-High	121078	115842	1497	1210	358	4.74	21	64	110	36	*Sphingomonadales*
		Org-Low	152796	145520	862	525	405.5	5.33	23	72	119	46	*Acidimicrobiales*
		Org-High	111198	106089	820	533	350.25	5.08	23	71	111	41	*Acidimicrobiales*
16S rRNA cDNA	Thika	Org-High	240682	230728	174	75	81	2.56	12	25	41	7	*Corynebacteriales*
		Conv-High	254276	242725	164	65	72.6	1.68	13	29	49	12	*Rhizobiales*
		Conv-Low	187704	181506	160	73	76	2.66	12	24	43	11	*Corynebacteriales*
		Org-Low	294489	282851	150	63	65	1.77	12	26	42	9	*Corynebacteriales*
	Chuka	Conv-Low	162110	156088	144	67	62	2.4	11	23	40	7	*Enterobacteriales*
		Org-Low	201174	193582	136	59	58	1.55	11	22	37	6	*Rhizobiales*
		Org-High	128482	122091	126	63	54.75	2.05	11	22	37	4	*Rhizobiales*
		Conv-High	168053	160812	124	61	55.75	2.03	11	19	35	6	*Rhizobiales*

Bacterial domain was the most abundant within datasets at both sites. The top 10 most abundant classes of bacteria comprised *Alphaproteobacteria*, *Actinobacteria*, *Thermoleophila*, Unknown phylum, *Bacillus*, *Blastocatellia*, *Betaproteobacteria*, *Acidimicrobia*, *Solibacteres* and *Gammaproteobacteria*. Archaeal domain was represented by *Thaumarchaeota* and *Euryarchaeota*. The most predominant phyla within each dataset are on **Figure 4.1.1** while distribution of high-quality sequences, OTUs, diversity indices and prokaryotic taxa are summarized in **Table 4.1**.

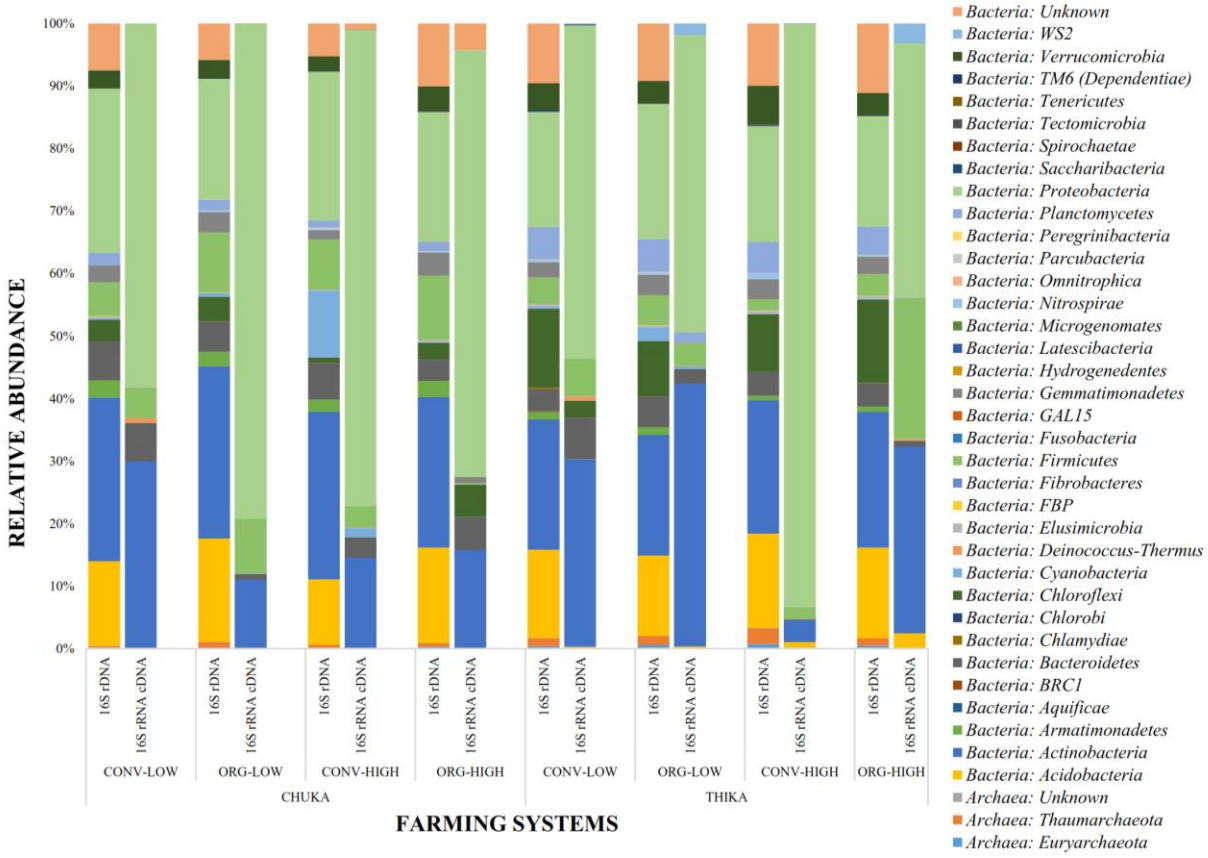
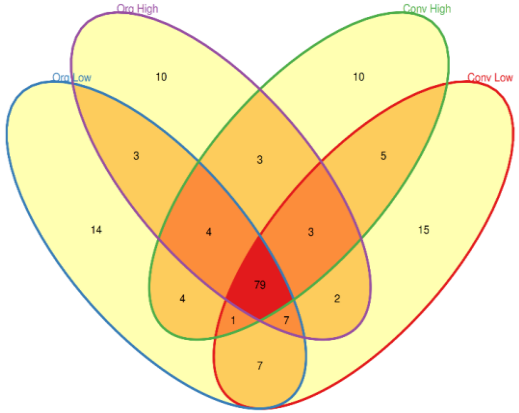


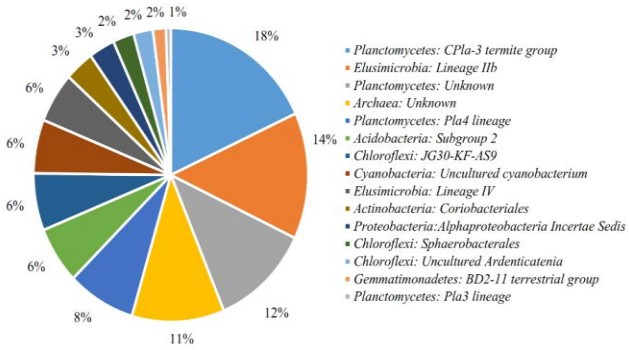
Figure 4.1.1: Relative abundance of the most predominant phyla in both datasets at Chuka and Thika sites.

Comparison of prokaryotic diversity at order level within 16S rDNA, revealed 79 and 115 shared orders across all farming systems at Chuka and Thika sites respectively. The number of unique taxa within each farming system are indicated in (**Figure 4.1.2a**) at

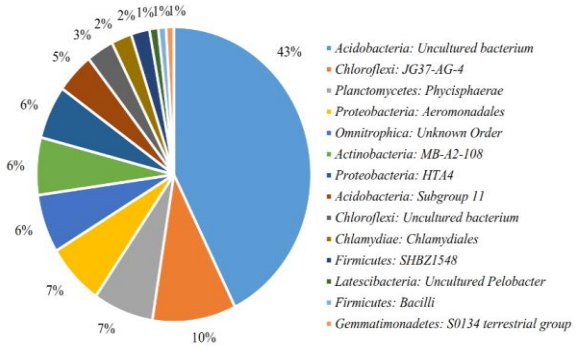
Chuka site and (**Figure 4.1.2b)** at Thika site. Twenty one (21) and 35 prokaryotic orders were shared across all farming systems at Chuka (**Figure 4.1.2a)** and Thika (**Figure 4.1.2b**) sites respectively, within 16S rRNA cDNA dataset. Unique taxa within 16S rRNA cDNA dataset are shown in (**Figure 4.1.3a)** at Chuka and (**Figure 4.1.3b)** at Thika sites. Mean abundances of the most notable bacterial and archaeal orders in each farming system indicated *Proteobacteria* orders (*Caulobacterales, Rhizobiales, Burkholderiales, Sphingomonadales, Pseudomonadales* and *Enterobacteriales*); *Actinobacteria* orders (*Acidimicrobiales, Corynebacteriales, Solirubrobacterales* and *Gaiellales*); and *Firmicutes* (*Bacillales* and *Lactobacillales)* as key drivers of biological processes.



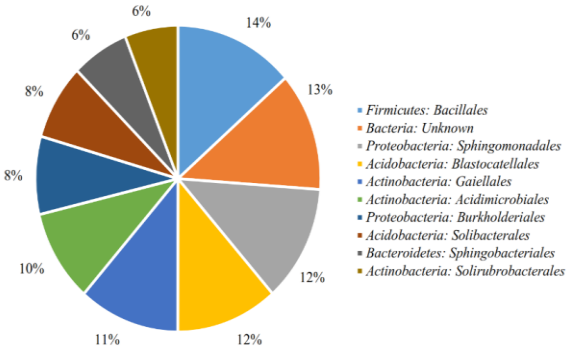
Chuka 16S rDNA



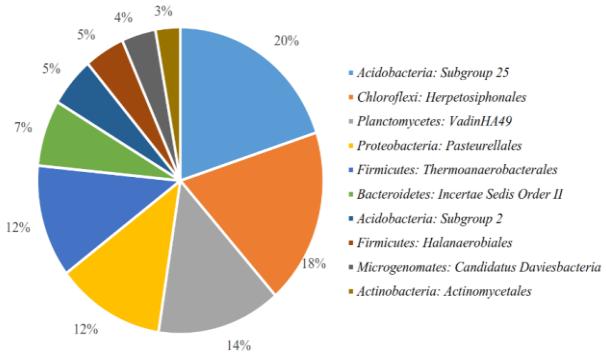
Chuka 16S rDNA Conv Low



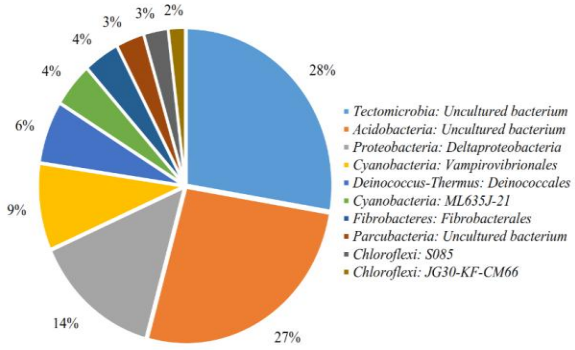
Chuka 16S rDNA Org Low



Chuka 16S rDNA shared taxa

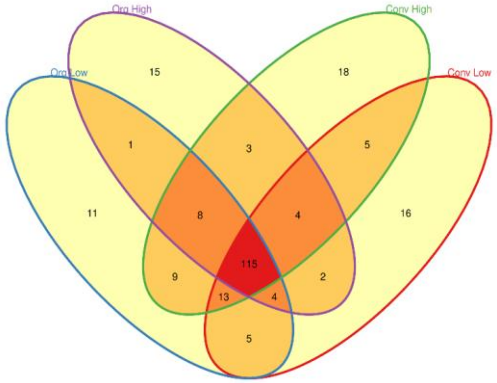


Chuka 16S rDNA Conv High

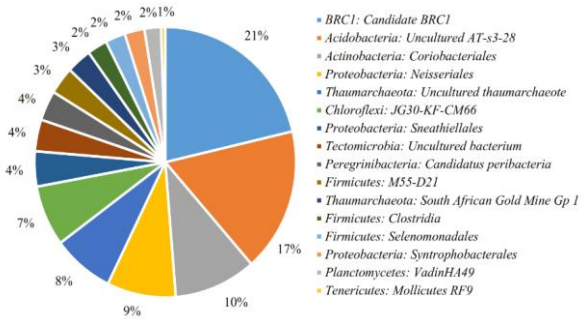


Chuka 16S rDNA Org High

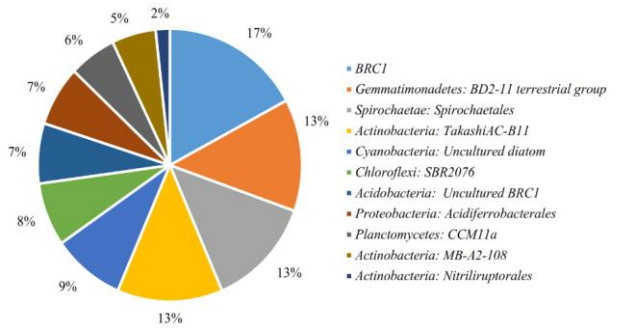
Figure 4.1.2a: Shared and unique prokaryotic taxa in 16S rDNA at Chuka. The Venn diagram show number of shared and unique taxa at order level within farming systems. The pie diagrams show most abundant and unique taxa across farming systems.



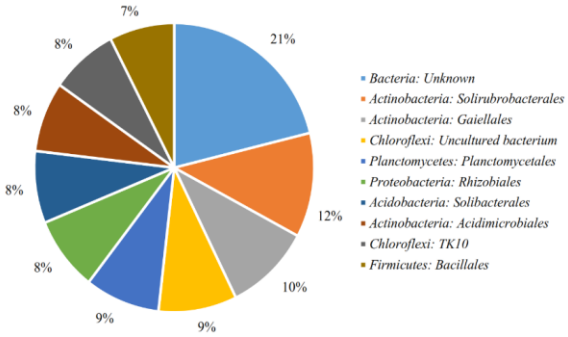
Thika 16S rDNA



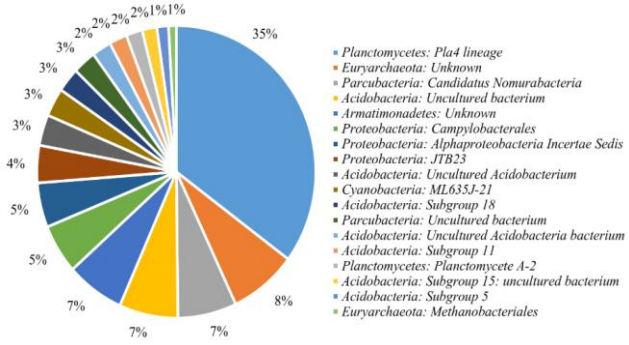
Thika 16S rDNA Conv Low



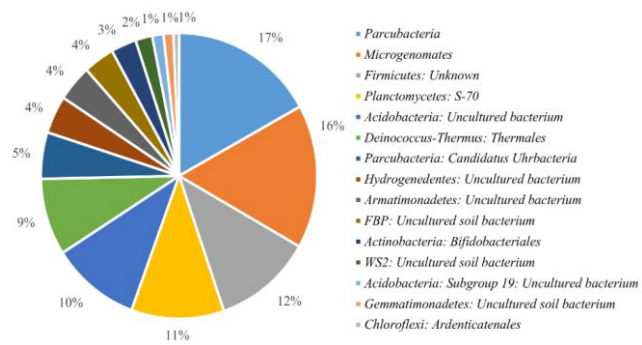
Thika 16S rDNA Org Low



Thika16S rDNA shared taxa

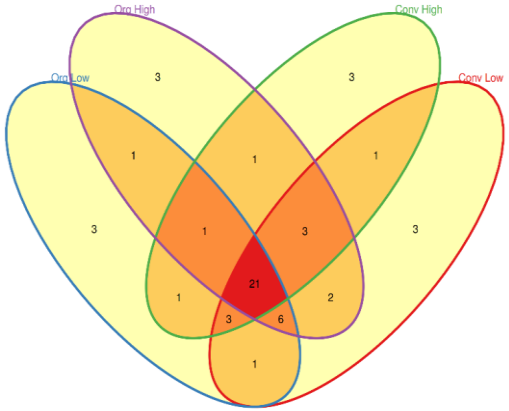


Thika 16S rDNA Conv High

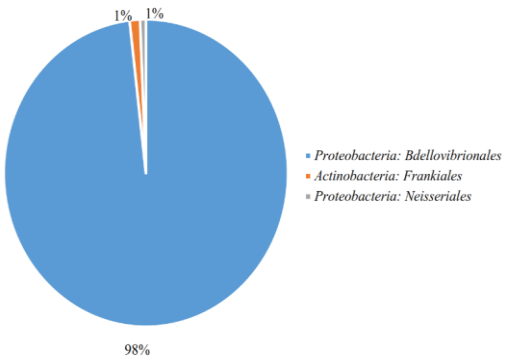


Thika 16S rDNA Org High

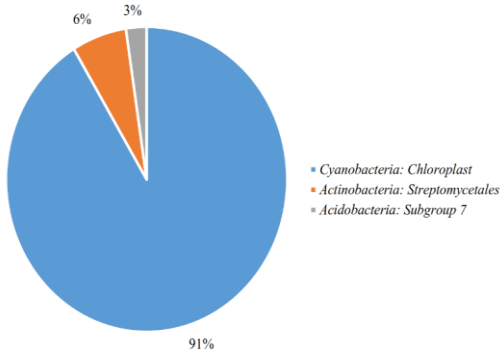
Figure 4.1.2b: Shared and unique prokaryotic taxa in 16S rDNA at Thika. The Venn diagram show number of shared and unique taxa at order level within farming systems. The pie diagrams show most abundant and unique taxa across farming systems.



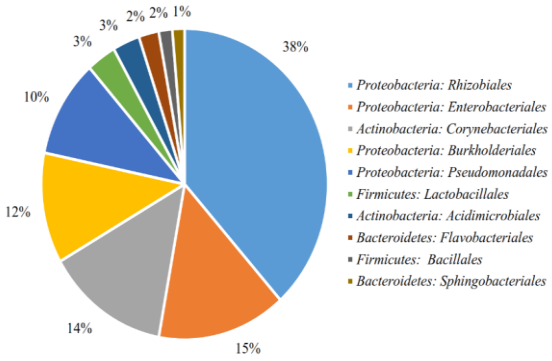
Chuka 16S rRNA cDNA



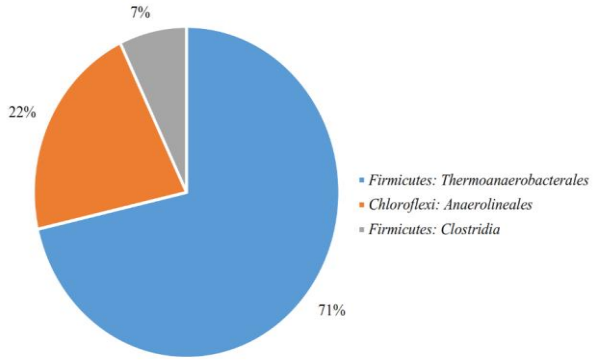
Chuka 16S rRNA cDNA Conv Low



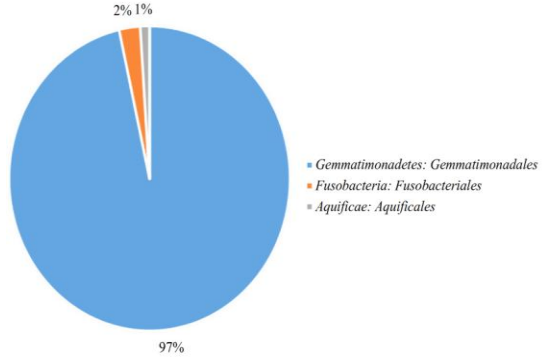
Chuka 16S rRNA cDNA Org Low



Chuka 16S rRNA cDNA shared taxa

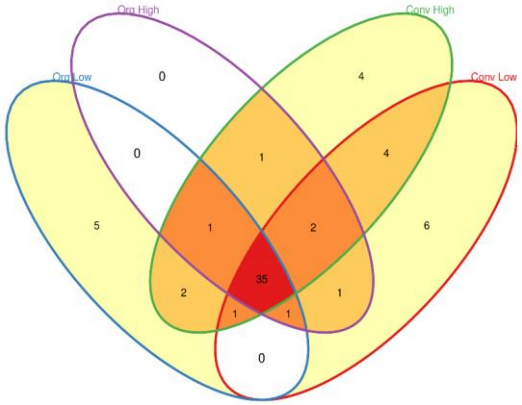


Chuka 16S rRNA cDNA Conv High

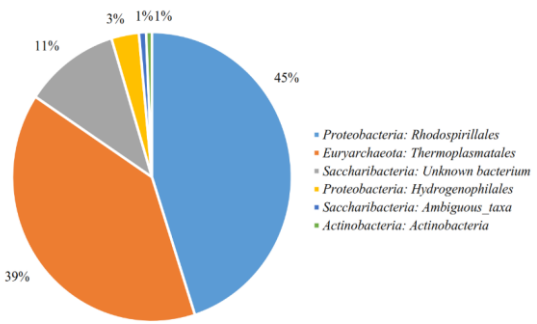


Chuka 16S rRNA cDNA Org High

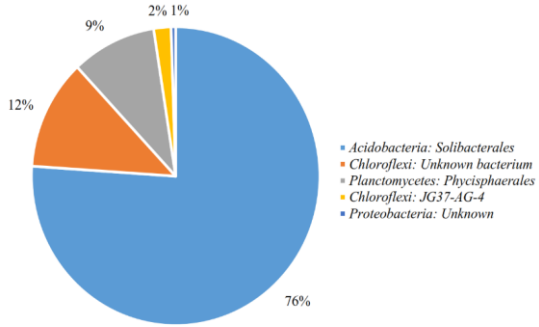
Figure 4.1.3a: Shared and unique prokaryotic taxa in 16S rRNA cDNA at Chuka. The Venn diagram show number of shared and unique taxa at order level within farming systems. The pie diagrams show most abundant and unique taxa across farming systems.



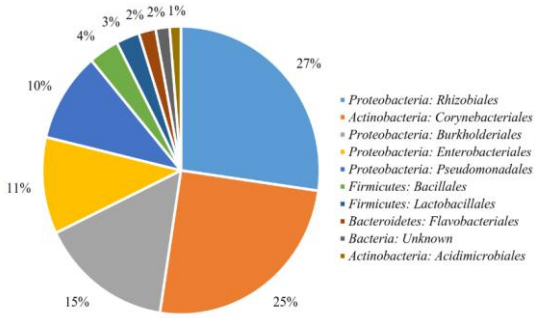
Thika 16S rRNA cDNA



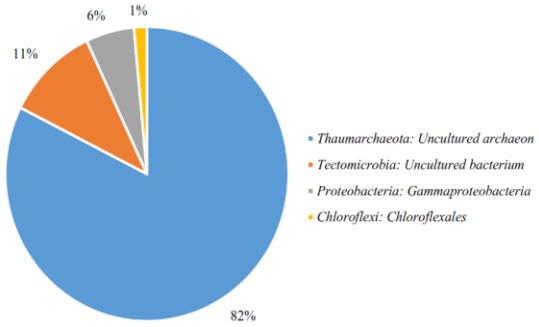
Thika 16S rRNA cDNA Conv Low



Thika 16S rRNA cDNA Org Low



Thika 16S rRNA cDNA shared taxa



Thika 16S rRNA cDNA Conv High

Figure 4.1.3b: Shared and unique prokaryotic taxa in 16S rRNA cDNA at Thika. The Venn diagram show number of shared and unique taxa at order level within farming systems. The pie diagrams show most abundant and unique taxa across farming systems.

4.1.2 Diversity indices of soil prokaryotic communities

Alpha diversity indices within each sample showed that Richness (S) and Shannon index (*H'*) differed between sites and farming systems. At Thika there was a higher species richness and the communities were more diverse (*H*) compared to Chuka (**Table 4.1.1**). At Chuka site, low input farming systems were found to exhibit higher total species richness (Conv-Low = 407.00 and Org-Low = 405.50) as compared to high farming systems (Conv-High = 358.00 and Org-High = 350.25). At Thika, Conv-High had higher total species richness (877.2) and diversity (H = 6.26) but Org-High and Conv-Low exhibited higher active species richness (81) and active species diversity (H = 2.66) respectively. Analysis of similarity pointed to significant differences between OTUs within high and low input farming systems ($P<0.001$) at Chuka site. However, there were no significant differences observed at Thika site (ANOSIM $P<0.672$ and 0.241 within 16S rDNA and 16S rRNA cDNA datasets respectively). The prokaryotic taxa on each farming system were visualized using rarefaction curves. A steep slope that flattened to the right was observed in the rarefaction curves indicating that a reasonable number of prokaryotic groups had been sequenced and more intensive sampling was likely to yield only a few additional species. The sampling curves tended to be asymptotic, denoting that prokaryotic communities were relatively deeply sampled (**Figure 4.1.4 a-d**).

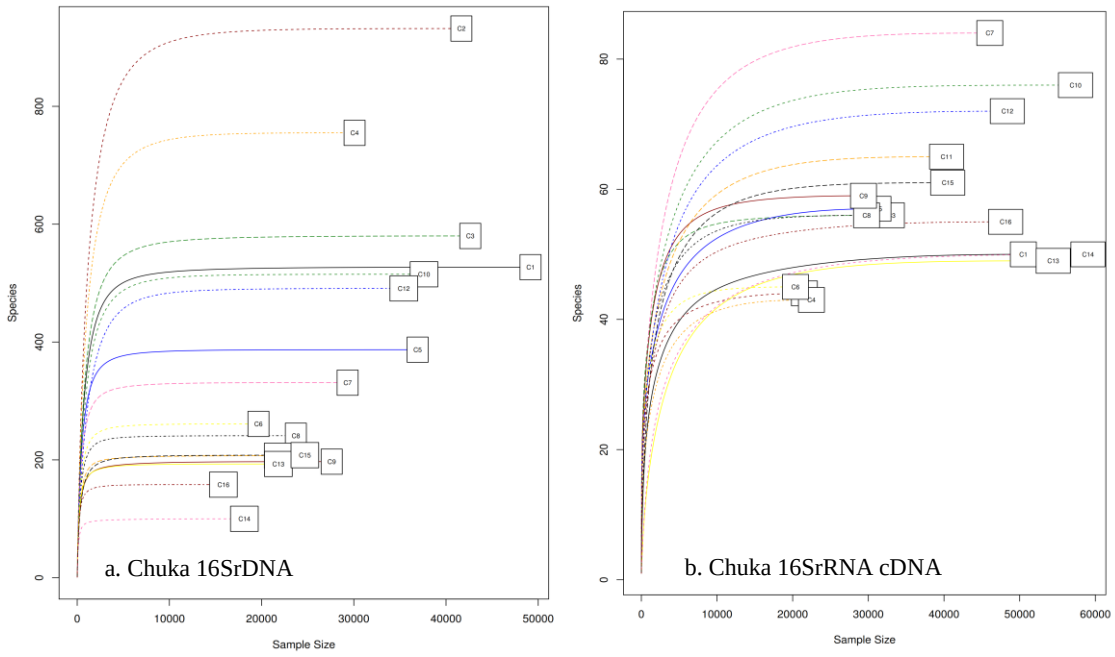


Figure 4.1.4 a-b: Rarefaction curves indicating level of sequence coverage within 16S rDNA and 16S rRNA cDNA at Chuka.

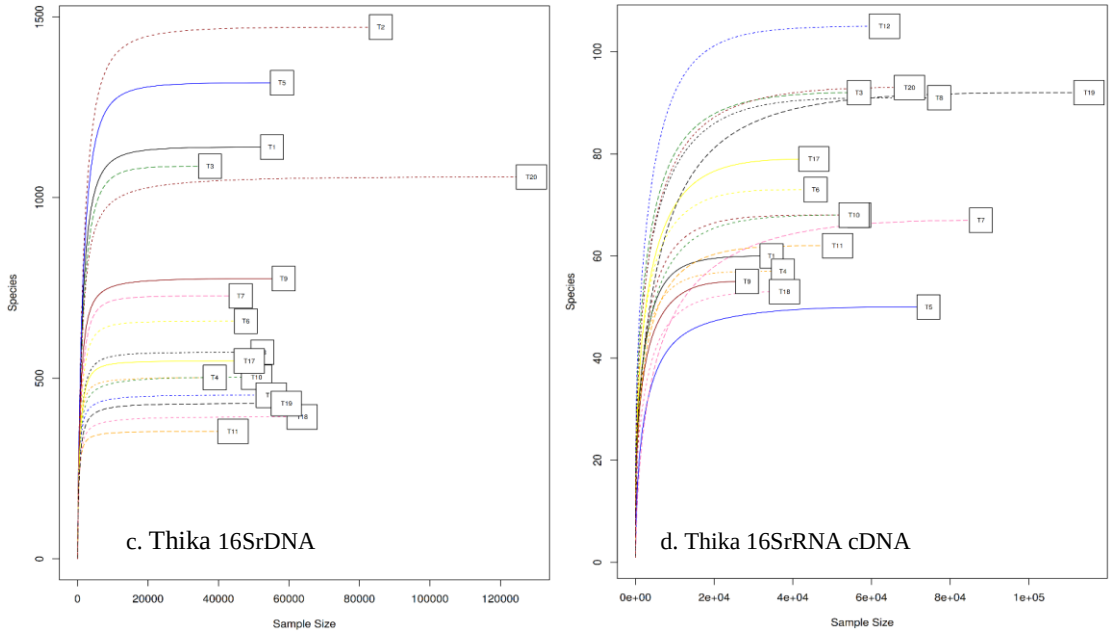


Figure 4.1.4 c-d: Rarefaction curves indicating level of sequence coverage within 16S rDNA and 16S rRNA cDNA at Thika.

4.1.3 Soil physicochemical properties for the different sites

In this study, prokaryotic community composition was assessed in 32 soil samples collected from long-term farming system comparison trials at Chuka and Thika in Kenya. The physicochemical characteristics for the samples analysed are presented (**Table 4.1.2)**. Tukey's separation of means revealed a trend in the means of soil pH, P, K, Ca, Mg, B and small macro-aggregates that were found to be significantly higher ($P<0.05$) in Org-High farming system. Higher means of Fe and micro-aggregates were recorded in Conv-High and Conv-Low systems respectively (**Table 4.1.2**). Soils from Chuka contained as much as 59.4 % primary clay minerals and 40.6 % secondary clay minerals, while soils from Thika were characterized by high primary minerals (78.3 %) and low secondary clay minerals (21.7 %). Congruently, the rate of formation and stabilization of macro aggregates was found to be higher at Thika than Chuka site.

Table 4.1.2: Soil physicochemical characteristics as influenced by farming systems.

Parameters	Farming Systems				System x Site								Source of variation	
					Chuka				Thika				System	System x Site
	Conv-High	Org-High	Conv-Low	Org-Low	Conv-High	Org-High	Conv-Low	Org-Low	Conv-High	Org-High	Conv-Low	Org-Low		
pH	5.68^{a}	6.61^{ab}	5.43^{a}	5.87^{a}	5.64^{ab}	6.50^{bc}	5.58^{ab}	5.75^{ab}	5.72^{ab}	6.71^{c}	5.23^{a}	5.98^{abc}	***	ns
EC.S (uS/cm)	85.75^{a}	113.75^{a}	60.13^{a}	75.50^{a}	48.50^{a}	74.00^{ab}	46.50^{a}	48.50^{a}	123.00^{bc}	153.50^{c}	73.75^{ab}	102.50^{abc}	ns	ns
OC (%)	2.29^{a}	2.52^{a}	2.29^{a}	2.34^{a}	2.60^{cd}	2.89^{d}	2.78^{d}	2.51^{bcd}	1.97^{ab}	2.16^{abc}	1.79^{a}	2.16^{abc}	ns	ns
N (%)	0.19^{a}	0.205^{a}	0.185^{a}	0.196^{a}	0.208^{cde}	0.223^{e}	0.203^{bcde}	0.215^{de}	0.173^{ab}	0.188^{abcd}	0.168^{a}	0.178^{abc}	ns	ns
S (ppm)	16.37^{a}	8.00^{a}	15.59^{a}	14.04^{a}	10.09^{ab}	1.22^{a}	9.80^{ab}	8.10^{ab}	22.65^{b}	14.78^{ab}	21.39^{b}	19.97^{b}	ns	ns
P (ppm)	30.80^{ab}	42.31^{b}	16.97^{a}	20.18^{a}	35.75^{a}	39.08^{a}	14.55^{a}	19.23^{a}	25.86^{a}	45.55^{a}	19.38^{a}	21.14^{a}	**	ns
K (ppm)	472.63^{a}	1077.25^{b}	453.13^{a}	541.63^{a}	339.00^{a}	994.25^{bc}	334.75^{a}	366.00^{a}	606.25^{ab}	1160.25^{c}	571.50^{a}	717.25^{ab}	***	ns
Ca (ppm)	1462^{a}	2086^{b}	1438^{a}	1539^{a}	1765^{ab}	2315^{b}	1598^{ab}	1695^{ab}	1159^{a}	1858^{ab}	1279^{a}	1384^{a}	**	ns
Mg (ppm)	248^{a}	342^{b}	260^{a}	245^{a}	250^{ab}	344^{c}	237^{a}	235^{a}	246^{a}	340^{bc}	283^{abc}	256^{abc}	***	ns
Na (ppm)	21.63^{a}	32.73^{a}	18.03^{a}	18.34^{a}	7.17^{ab}	9.29^{ab}	4.48^{a}	5.70^{ab}	36.10^{bc}	56.18^{c}	31.58^{abc}	30.98^{abc}	ns	ns
Exch. Al (meq/ 100g)	0.07^{a}	0.04^{a}	0.19^{a}	0.11^{a}	0.78^{ab}	0.12^{a}	0.53^{ab}	0.04^{a}	0.06^{ab}	0.07^{ab}	0.33^{b}	0.17^{ab}	ns	ns
B (ppm)	0.58^{a}	0.96^{b}	0.55^{a}	0.68^{a}	0.54^{a}	0.93^{ab}	0.53^{a}	0.58^{a}	0.63^{ab}	0.99^{b}	0.58^{a}	0.78^{ab}	***	ns
Mn (ppm)	434^{a}	443^{a}	446^{a}	429^{a}	567.50^{b}	533.50^{b}	575.75^{b}	553.75^{b}	300.50^{a}	353.25^{a}	315.25^{a}	303.75^{a}	ns	*
Fe (ppm)	89.25^{b}	70.19^{a}	83.70^{b}	77.33^{ab}	97.93^{c}	72.76^{ab}	89.63^{bc}	83.78^{abc}	80.58^{ab}	67.60^{a}	77.75^{ab}	70.88^{a}	**	ns
Zn (ppm)	8.89^{a}	10.51^{a}	7.19^{a}	8.06^{a}	12.23^{de}	12.80^{e}	9.55^{cd}	10.80^{cde}	5.49^{ab}	8.23^{bc}	4.82^{a}	5.32^{ab}	ns	ns
Small Macro-aggregate (g)	48.11^{ab}	52.15^{b}	42.17^{a}	42.28^{a}	46.09^{b}	48.56^{bc}	36.53^{a}	36.76^{a}	50.15^{bc}	55.75^{c}	47.82^{bc}	47.80^{bc}	**	ns
Micro-aggregate (g)	21.15^{ab}	17.43^{a}	28.66^{b}	27.13^{b}	25.58b^{c}	22.29^{b}	34.22^{c}	33.81^{c}	16.72^{ab}	12.58^{a}	23.10^{b}	20.46^{ab}	*	ns

a) Letters designate significant differences at $P \leq 0.05$. b) Means followed by the same letter are not significantly different.

ns= not significant; *$P \leq 0.05$; ** $P \leq 0.01$ and *** $P \leq 0.001$.

4.1.4 Key environmental drivers of prokaryotic communities

To assess how environmental variables shaped soil prokaryotic community structure, PCA was performed on soil physicochemical characteristics within farming systems and prokaryotic taxa at species level. Each characteristic was assessed on its ability to influence diversity positively or negatively within sites and farming systems. At Chuka, pH, OC, N, Zn, Fe and Al were found to be the major drivers of prokaryotic diversity within farming systems while at Thika, key properties displayed were pH, EC, OC, N, K, Fe, Zn, B and micro-aggregate (MA) as shown on **Figure 4.1.5 a-d**.

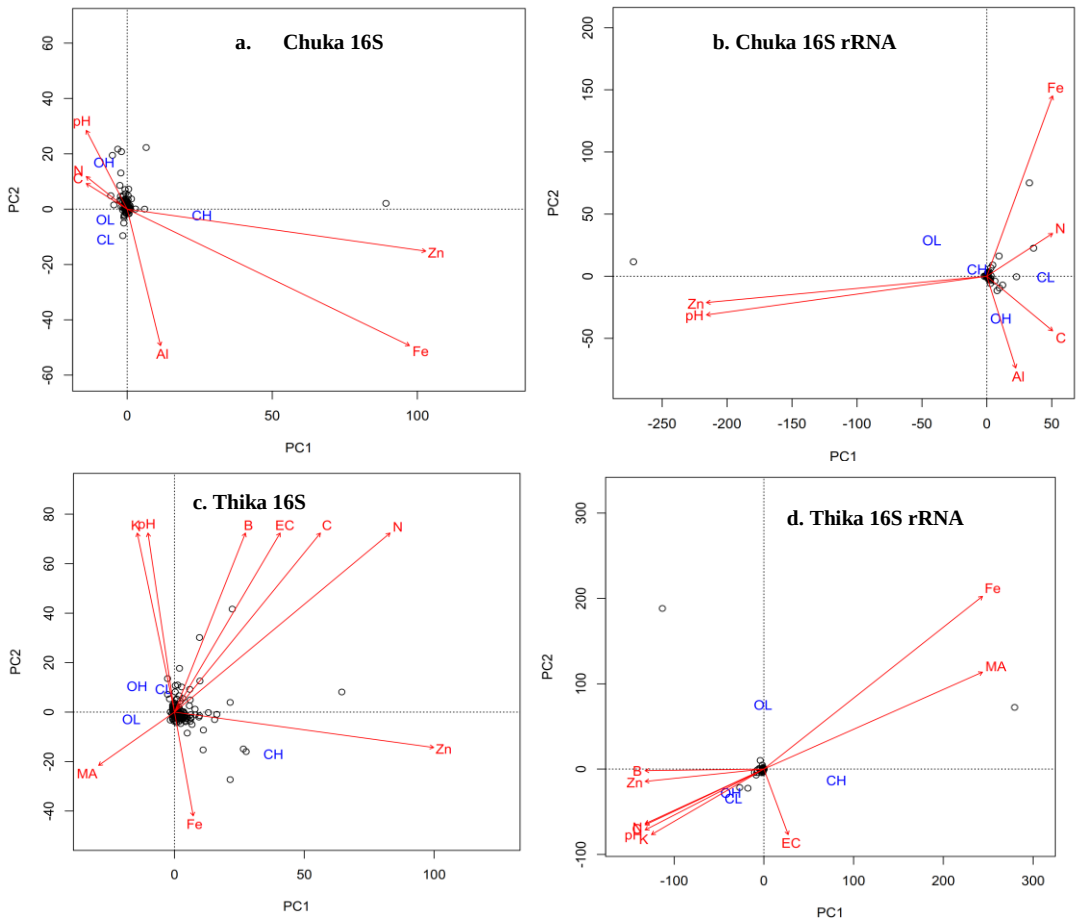
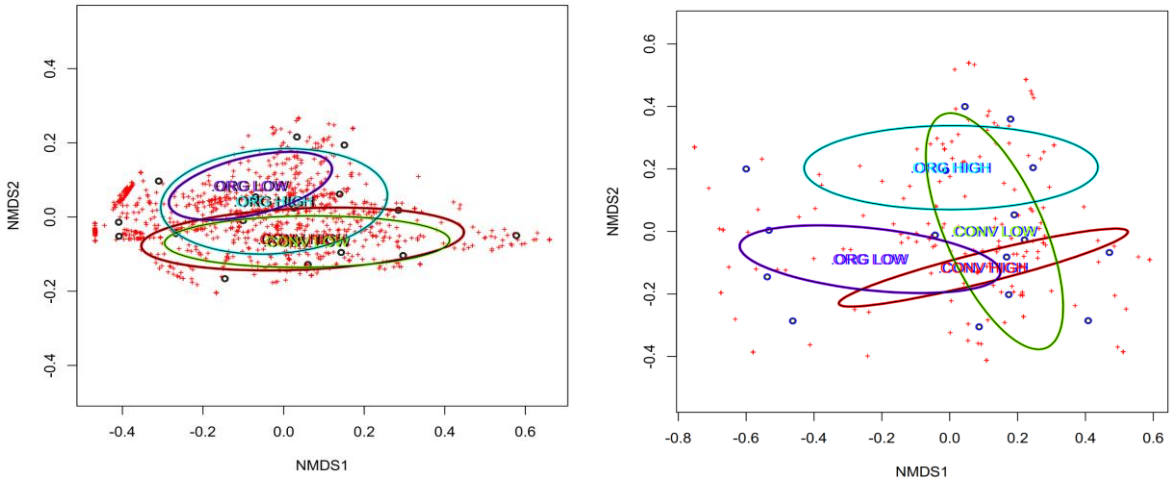


Figure 4.1.5 a-d: Principal component analysis of soil physicochemical characteristics that drive diversity within farming systems. OH, CH, OL and CL represents Org-High, Conv-High, Org-Low and Conv-Low farming systems.

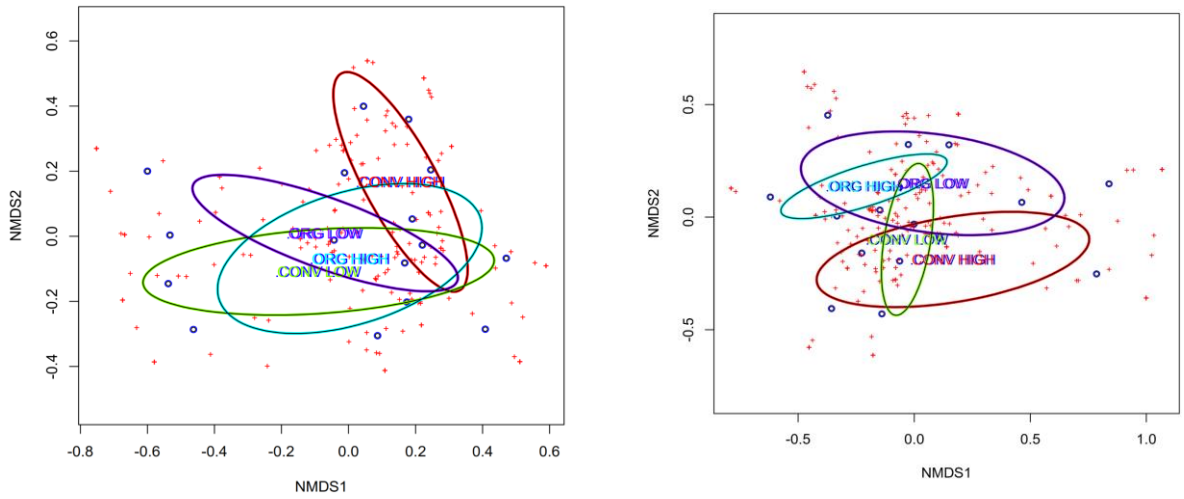
4.1.5 Effect of farming systems on beta diversity of prokaryotic communities

Beta diversity analysis was used to evaluate differences in OTU composition among the farming systems. Beta diversity analysis was based on non-metric multidimensional scaling and Hierarchical clustering. β-diversity, analyzed by community comparison of the non-metric multidimensional scaling plot indicated the four different ellipses formed by each farming system. There was an overlap of ellipses between farming systems indicating that some OTUs were shared across farming systems; while numerous OTUs appeared outside the ellipses, signifying that the prokaryotic taxa revealed were highly diverse (**Figure 4.1.6 a** and **b)**.



16SrDNA **16SrRNA cDNA**

Figure 4.1.6a: Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities between prokaryotic OTUs grouped according to farming systems at Chuka site.



16SrDNA **16SrRNA cDNA**

Figure 4.1.6b: Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities between prokaryotic OTUs grouped according to farming systems at Thika site.

The relationship between most predominant phyla within both datasets in the two study sites and farming systems was analyzed using hierarchical clustering. Heatmaps revealed clustering of sites into two major groups while farming systems clustered into four sets on the dendrogram, representing the two sites, each with four farming systems under investigation. There was an indication that farming systems in both sites harbored prokaryotic taxa within active diversity dataset which possibly interacted with one another to perform essential ecological functions as shown on **Figure 4.1.7 a** and **b**.

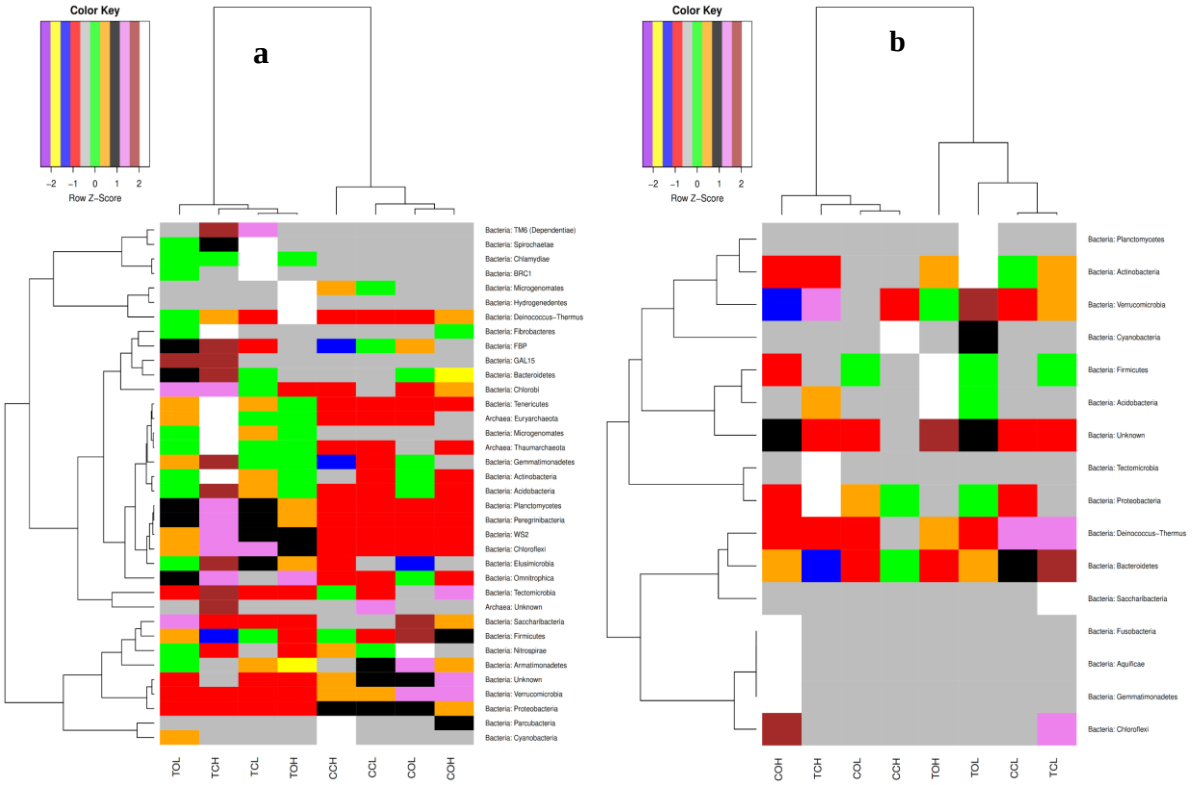


Figure 4.1.7 a and **b:** Hierarchical clustering of the most predominant prokaryotic taxa at phylum level within each farming system of 16S rDNA and 16S rRNA cDNA datasets in both sites. X-axis indicates the replicates within each system while the Y-axis indicates the taxonomic relationships. Total and active prokaryotic diversity is represented by **a** and **b** respectively.

CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.2 Fungal diversity within organic and conventional farming systems in Central Highlands of Kenya

4.2.1 Soil physicochemical properties in the two sites

In this study we assessed the fungal community composition in 32 soil samples collected from long-term farming system comparison trials at Chuka and Thika in Kenya. The physicochemical characteristics of soils were as shown in Table 2. Tukey's separation of means revealed a trend in the means of soil pH, P, K, Ca, Mg, B and small macro-aggregates that were found to be significantly high ($P<0.05$) in organic farming systems. Fe and micro-aggregates were high in conventional farming systems. Soils from Chuka contained 59.4 % primary clay minerals and 40.6 % secondary clay minerals, while soils from Thika were characterized by high primary minerals (78.3 %) and low secondary clay minerals (21.7 %) (Adamtey et al., unpublished results). Congruently, the rate of formation and stabilization of small macro aggregates was found to be higher at Thika than Chuka site.

4.2.2 Operational Taxonomic Unit analysis within the farming systems

After denoising and demultiplexing, a total of 556,135 and 466,053 high quality sequences were obtained from Chuka and Thika sites respectively. At Chuka site, the sequences were clustered into (Conv-high = 143, Org-high = 113, Conv-low = 161 and Org-low = 155) OTUs while at Thika, sequences were grouped into (Conv-high = 144, Org-high = 128, Conv-low = 168 and Org-low = 116) OTUs at 97 % genetic distance. The distribution of OTUs between sites and farming systems is shown on (**Figure 4.2.1**).

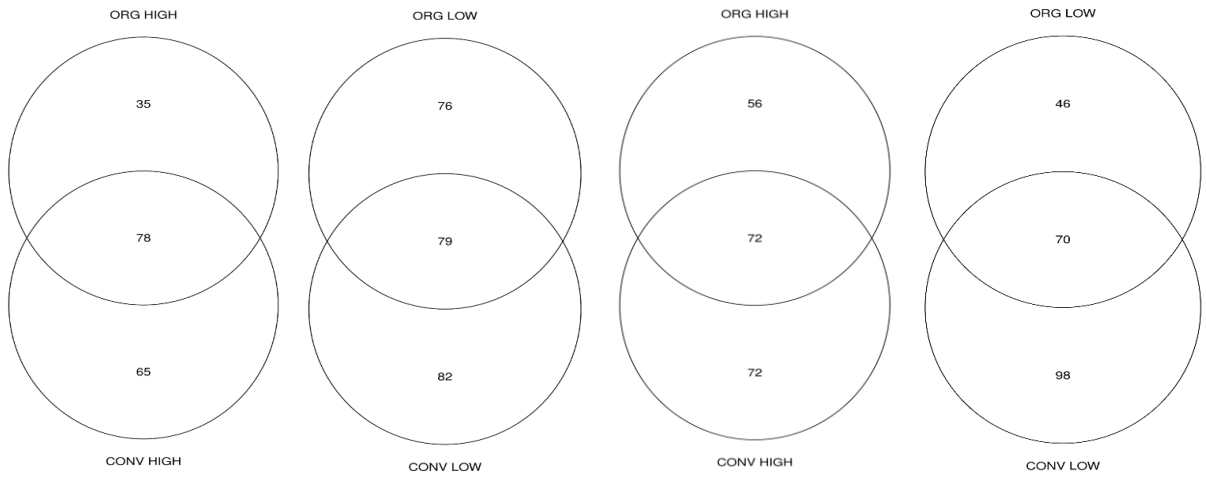


Figure 4.2.1: Two way Venn diagrams showing the distribution of unique and shared fungal OTUs of each farming system within the two sites.

4.2.3 Fungal sequence coverage analysis within farming systems

After denoising and demultiplexing, a total of 556,135 and 466,053 high quality sequences were obtained from Chuka and Thika sites respectively. Rarefaction analysis of the extent of diversity captured in each farming system and the level of sequence coverage visualized using rarefaction curves showed a steep slope that plateaued to the right in some of the replications within farming systems (**Figure 4.2.2a** and **b**). This indicated that a good proportion of the fungal diversity had been captured within the represented farming systems and an increase in the number of sequences extracted would only marginally increase the number of OTUs obtained. However, rarefaction curves of some replications within farming systems displayed a steep slope, denoting that more intensive sampling within the replicate plots was likely to yield more fungal communities for further classification (**Figure 4.2.2a** and **b**).

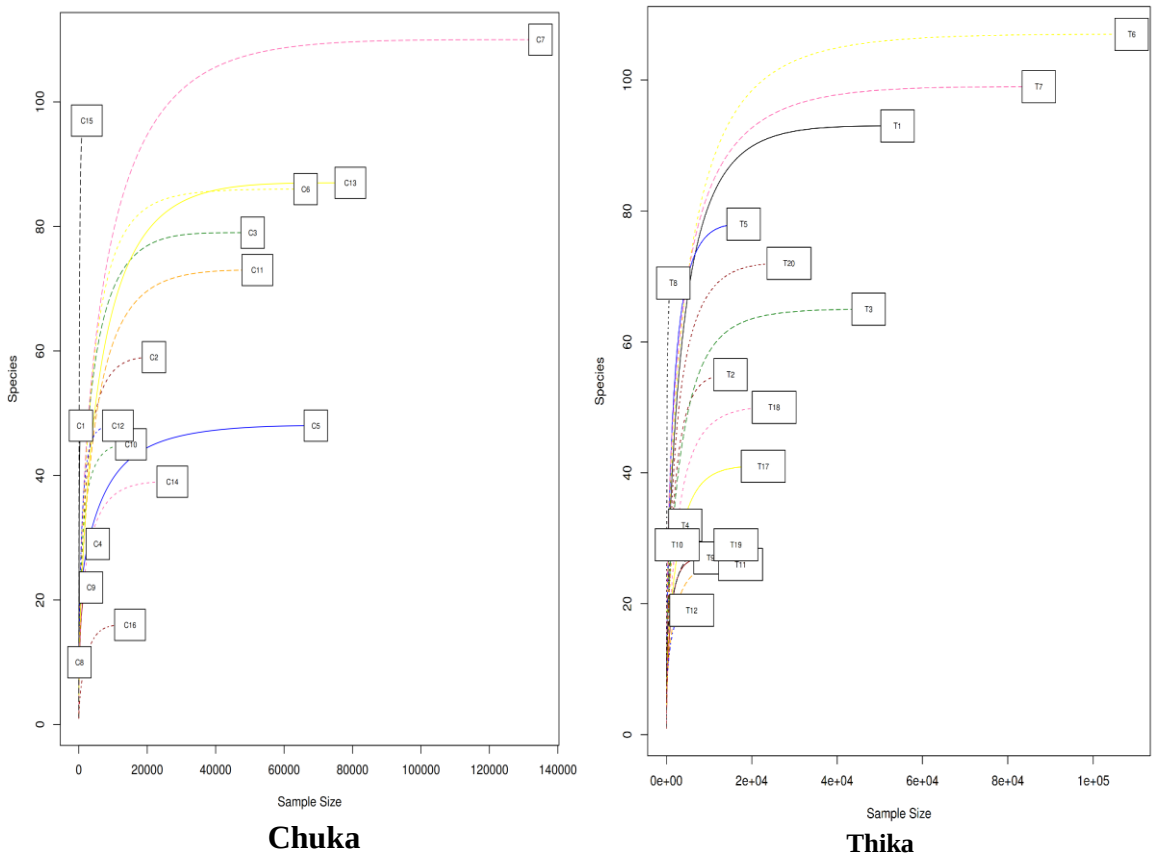


Figure 4.2.2: Rarefaction curves of each farming system replication indicating the level of fungal ITS sequence coverage at Chuka and Thika sites.

The curves labelled C3, C6, C12 and C14 represents Conv-High; C2, C7, C11 and C16 represents Conv-Low; C4, C8, C9 and C15 represents Org-High; C1, C5, C10 and C13 represents Org-Low. T2, T7, T9 and T20 represents Conv-High; T1, T6, T12 and T18 represents Conv-Low; T3, T8, T11 and T17 represents Org-High; C1, C5, C10 and C13 represents Org-Low.

4.2.4 Taxonomic composition and relative abundance analysis of soil fungi

Sequences obtained from the fungal dataset were assigned to 1,128 OTUs at 97 % genetic distance. Conventional systems were found to harbor more (both total and unique) OTUs as compared to organic farming systems (**Table 4.2.1**). Taxonomic classification of final OTUs based on UNITE ITS Reference Database and a curated database derived from GreenGenes, RDPII and NCBI grouped the OTUs into a total of eight (8) phyla. Farming systems were dominated by unassigned fungal phyla with low input farming systems in both sites scoring the highest relative abundance. Notably, known fungal taxa revealed included *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota*, *Calcarisporiellomycota*, *Kickxellomycota* and *Mortierellomycota*. *Ascomycota* was most abundant in organic systems in both sites while *Chytridiomycota* was dominant in conventional systems in both sites. *Basidiomycota* was dominant in conventional systems at Chuka site whilst *Kickxellomycota* and *Calcarisporiellomycota* phyla were present in all organic systems in both sites, but relative abundances were too low to allow their view in **Figure 4.2.3**.

Table 4.2.1: Distribution of high-quality sequences, OTUs, diversity indices and fungal taxa at Chuka and Thika sites. The farming systems have been sorted as per total number of OTUs in each site.

Site	System	Raw sequences	High quality sequences	OTUs	Unique OTUs	Richness (S)	Shannon (H')	Phyla	Classes	Orders	Family	Genus	Species
Chuka	Conv-Low	251706	224073	161	82	64.5	1.15	8	18	21	103	134	204
	Org-Low	202955	164528	155	76	57.0	1.53	8	19	21	103	131	201
	Conv-High	176457	155879	143	65	63.0	1.53	8	18	21	96	129	196
	Org-High	14496	11655	113	35	39.5	2.05	8	16	19	92	124	185
Thika	Conv-Low	222100	194317	168	98	67.3	1.43	8	19	24	101	147	224
	Conv-High	158825	141355	144	72	63.3	1.44	8	21	24	101	141	213
	Org-High	104555	89075	128	56	50.3	2.00	8	20	24	101	134	200
	Org-Low	50254	41306	116	46	42.0	1.49	8	17	20	94	124	189

In the high input systems, uncharacterized fungal phylotypes, *Basidiomycota* and *Chytridiomycota* were the fungal groups that showed the greatest relative abundance in conventional systems, whereas in the organic systems *Ascomycota* and *Glomeromycota* were the prevalent groups, in both sites. The *Kickxellomycota* phyla occurred more strongly in the Org-High system in Thika site, the same occurred for *Mortierellomycota* phyla in Org-High system in Chuka site (**Figure 4.2.3**).

In the low input systems, uncharacterized fungal phylotypes and *Chytridiomycota* were more abundant in conventional systems in both sites. In organic systems, uncharacterized fungal phylotypes, *Basidiomycota* and *Ascomycota* were the most abundant groups, in both sites. In addition, *Chytridiomycota*, *Glomeromycota* and *Calcarisporiellomycota* phyla were abundant in Org-Low system at Thika site (**Figure 4.2.3**).

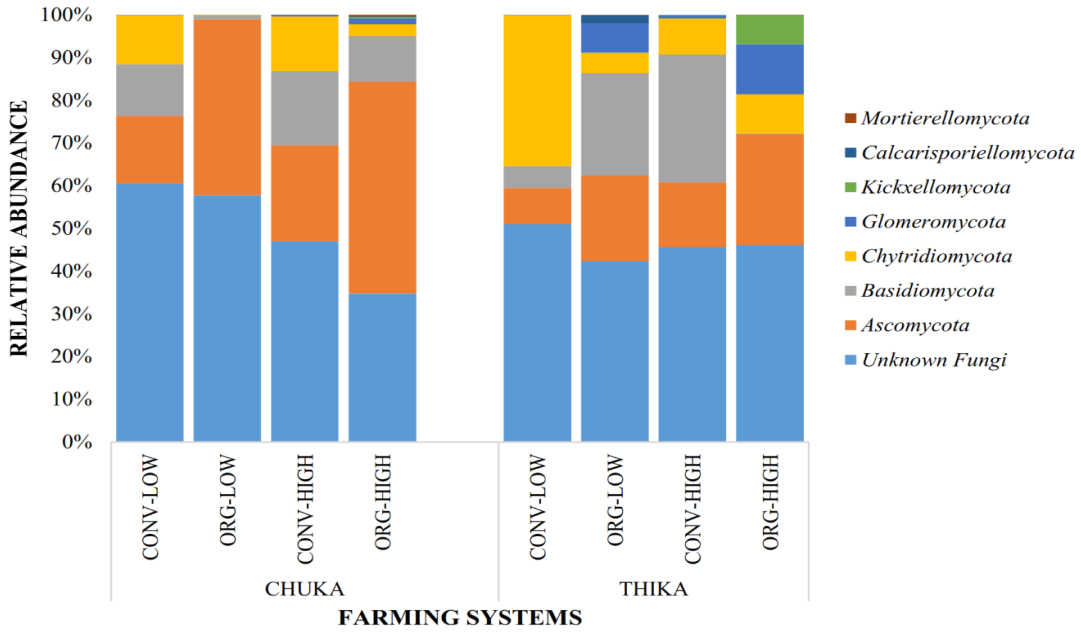


Figure 4.2.3: Relative abundance of fungal taxa at phylum level as revealed at Chuka and Thika sites.

Taxonomy assignment at genus level revealed the most abundant genera within farming systems to include; at Chuka site, *Gnomonia*, *Sporobolomyces*, *Saccharomyces* and *Exophiala* in Conv-Low; *Minimedusa*, *Pluteus*, *Macrophomina*, *Leucoagaricus* in Org-Low; *Penicillium*, *Malassezia*, *Aspergillus* and *Marasmius* in Conv-High; and *Alternaria*, *Marasmius*, *Harknessia* and *Laetisaria* in Org-High farming systems. At Thika site, the

most abundant genera within farming systems included *Alternaria*, *Spizellomyces*, *Rhizophlyctis* and *Conocybe* in Conv-Low, *Leucoagaricus*, *Marasmius*, *Rhizophagus* and *Mortierella* in Org-Low; *Lepiota*, *Penicillium*, *Phialemonium* and *Conocybe* in Conv-High; and *Racocetra*, *Tomentella*, *Spizellomyces* and *Ramicandelaber* in Org-High farming systems (**Figure 4.2.4**). The distribution of various fungal OTUs and taxonomic groups within farming systems in both sites are as shown in **Table 4.2.1**.

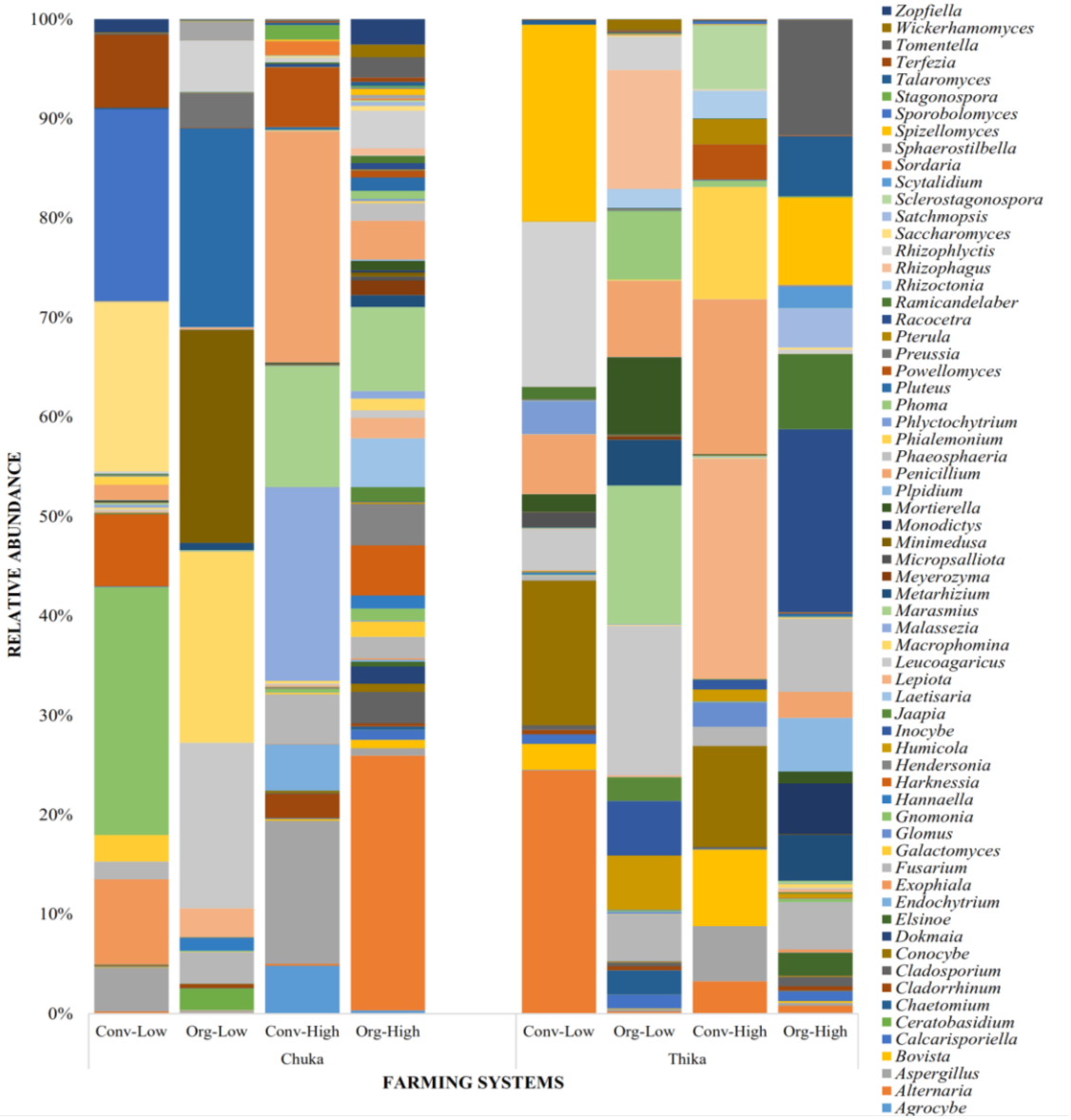


Figure 4.2.4: Relative abundance of the most predominant fungal taxa at genus level as revealed in the long-term comparison trials at Chuka and Thika sites.

4.2.5 Diversity indices of soil fungal communities as influenced by farming systems

Alpha diversity was used to analyze species diversity in each farming system. In both sites, there was a higher species richness in conventional farming systems. For instance, at Chuka site, species richness was: Conv-High = 63, Org-High = 39.5, Conv-Low = 64.5 and Org-Low = 57; while at Thika site, the species richness was Conv-High = 63.3, Org-High = 50.3 Conv-Low = 67.3 and Org-Low = 42. However, fungal communities within organic farming systems were more diverse (H) as compared to conventional farming systems (**Table 4.2.1**).

At Chuka site, Analysis of Similarity (ANOSIM) of fungal diversity within farming systems indicated significant differences between fungal community OTUs within high and low input farming systems at 95 % level of confidence (P value = 0.05 and R = 0.115). However, there were no significant differences observed at Thika site (P value = 0.17 and R=0.066).

4.2.6 Effect of farming systems on beta diversity of fungal communities

Beta diversity analysis was used to evaluate differences in species complexity among the different farming systems. Beta diversity was based on non-metric multidimensional scaling and Hierarchical clustering. β-diversity, analyzed by community comparison of the Non-metric multidimensional scaling plot indicated the four different ellipses formed by each farming system. There was an overlap of ellipses between farming systems indicating that some fungal taxa were shared across farming systems; while numerous taxa appeared outside the ellipses, signifying that the fungal taxa revealed were highly diverse (**Figure 4.2.5)**. At Chuka site, diversity was higher in Org-High system while at Thika, Org-Low system revealed the highest diversity of fungal communities as shown by Shannon index (H') (**Table 4.2.1**).

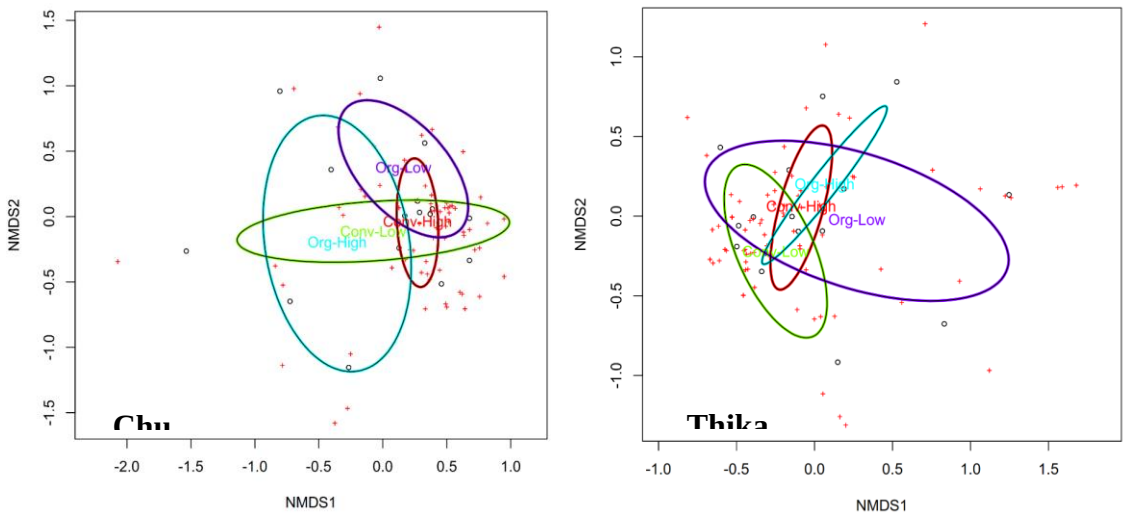


Figure 4.2.5: Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities between fungal taxa at species level grouped according to farming systems.

Hierarchical clustering analysis was done to compare the similarity and dissimilarity of most abundant fungal taxa at family level as well as clustering of the four farming systems in each site. The hierarchical heatmap of fungal community was generated based on bray–curtis distance indices, displaying the relative abundances of fungal communities across farming systems. The dendrogram revealed two main groups within farming systems; first group in Org-High systems in both sites and second group; Conv-Low systems in both sites while Chuka Conv-High and Thika Org-Low systems were shown to cluster together. Thika Conv-High and Chuka Org-Low systems were outliers within the second group on the dendrogram as shown in **Figure 4.2.6**. Although some farming systems were shown to cluster together, they harbored different fungal taxa, an indication that soil ecosystem supports a diverse group of microorganisms.

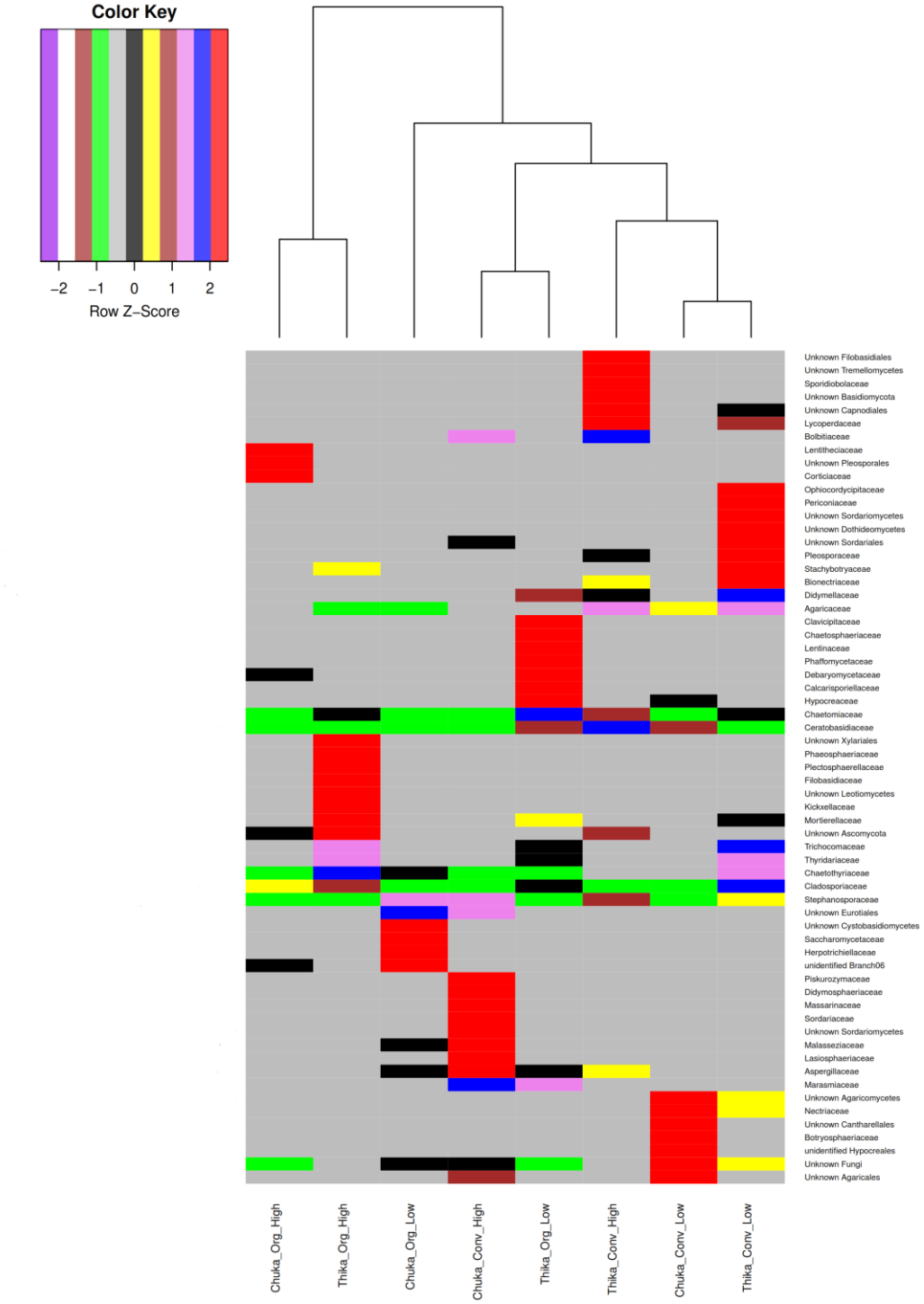


Figure 4.2.6: Hierarchical clustering of most predominant fungal taxa at family level in both sites. X-axis indicates the farming systems at Chuka and Thika.

4.2.7 Key environmental drivers of fungal community diversity and structure

To assess how environmental variables shaped soil fungal community composition, Principal Component Analysis (PCA) was performed on soil physicochemical characteristics within farming systems and fungal taxa at species level. Each characteristic was assessed on its ability to influence diversity positively or negatively within sites and farming systems. At Chuka, pH, C, N, Zn, Fe and Al were designated as major drivers of fungal diversity within farming systems while at Thika, key properties displayed were pH, EC, C, N, K, Fe, Zn, B and micro-aggregate (MA) as shown on **Figure 4.2.7**. Aluminum (Al) was shown to have a negative influence on fungal diversity at Chuka site.

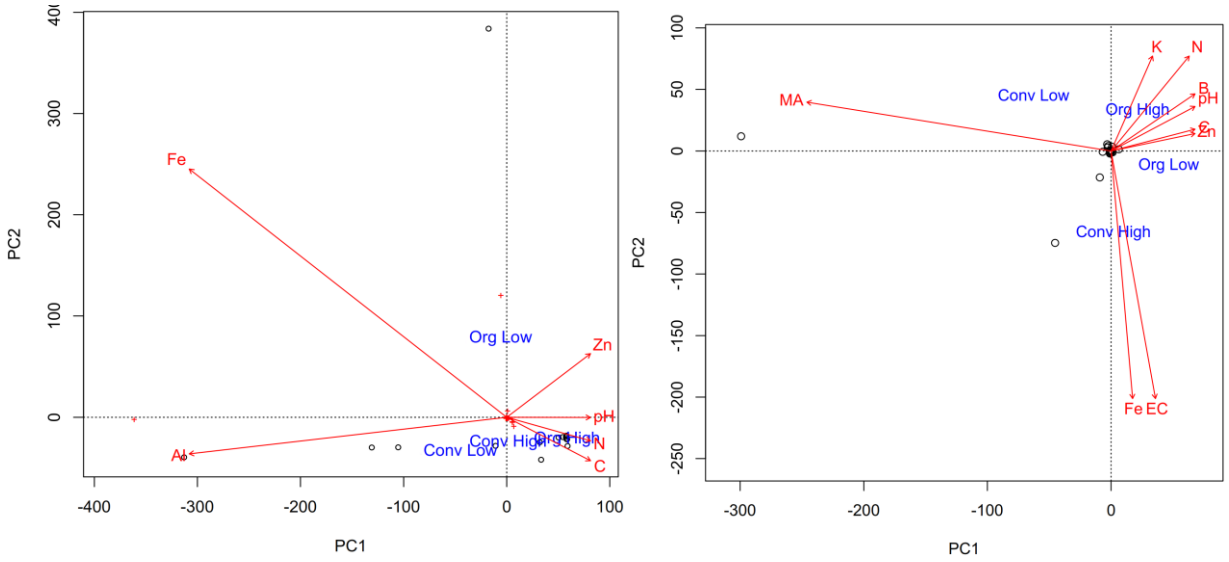


Figure 4.2.7: Principal component analysis of soil physicochemical characteristics that drive diversity within farming systems.

4.3 Metabolic potential of the microbial communities within organic and conventional farming systems in central highlands of Kenya

4.3.1 General characteristics of the soil metatranscriptome dataset

Metatranscriptome sequencing and quality filtering was done for eight (8) soil samples (covering the farming systems; Conv-High, Conv-Low, Org-High and Org-Low in two sites). The number of reads ranged between 6.4 – 9.5 million high-quality reads with approximately 8.3 million reads on average per sample (**Table 4.3.1**). The average read length per paired-end read, Base pairs (before and after quality filtering), and average Guanine Cytosine content were as shown in **Table 4.3.1**. The high-quality sequences obtained after quality filtering were clustered into 13, 907 OTUs at 97 % genetic distance.

Table 4.3.1: Sequence counts of all samples before and after quality filtering

Farming System	Forward read length (before QF)	Reverse read length (before QF)	Read count (before QF)	Base pairs (before QF)	GC content (before QF)	Forward read length (after QF)	Reverse read length (after QF)	Read count (after QF)	Base pairs (after QF)	GC content (after QF)
CCH	142	142	10435572	1487646663	46.9	131	128	9570650	1245395745	46.7
COH	144	144	10223128	1477776867	46.3	128	125	8774942	1115220307	45.4
CCL	142	141	10549958	1499151472	47.6	126	122	9015834	1125374298	46.4
COL	141	141	10287786	1455132220	55.4	132	127	9011916	1173214903	55.0
TCH	143	143	8637048	1239198114	46.0	130	127	7580854	977954931	44.8
TOH	144	143	9218686	1328639897	46.7	134	131	8358158	1113785168	46.2
TCL	142	142	7614478	1083574496	42.7	127	123	6421848	805833065	41.6
TOL	144	144	8574578	1238102371	40.7	135	133	7770070	1045566962	39.9

CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.2 KEGG Orthology of transcriptomes analysed from farming systems

Molecular functions represented in terms of functional orthologs within transcriptomes from farming systems were sorted into categories. Approximately, 50,176 KEGG Orthologs (KO) were obtained. About 40 % were categorized as Brite functional hierarchies incorporating different types of relationships including: genes and proteins, compounds and reactions, drugs, diseases, organisms and cells. The major hierarchies within farming systems transcriptomes were; RNA family, Protein families and Genetic information processing. Twenty-four (24) % of KO obtained from farming systems transcriptomes were associated with cellular processes within organisms. These include transport and catabolism, cell motility, cell growth and death and cell community in both prokaryotes and eukaryotes. Fifteen (15) % of KO were classified as being associated with metabolic activities within farming systems. The major metabolic pathways include amino acid metabolism, biosynthesis of secondary metabolites, carbohydrate metabolism, energy metabolism, glycan biosynthesis and metabolism, lipid metabolism, metabolism of co-factors and vitamins, metabolism of other amino acids, metabolism of terpenoids and polyketides, nucleotide metabolism and; xenobiotic biodegradation and metabolism.

Other major functional orthologs included human diseases (15 %), organismal systems (10 %), environmental information processing (10 %), unclassified groups that were not included in brite or pathways (5 %), genetic information processing (3 %) and poorly characterized proteins whose functions were unknown (1 %) which comprised general function prediction and unknown function. Human disease pathways comprised cancers, cardiovascular diseases, drug resistance, endocrine and metabolic diseases, immune diseases, bacterial, parasitic and viral infectious diseases, substance dependence and neurodegenerative diseases. Environmental information processing pathways comprised signal transduction mechanisms, membrane transport, signaling molecules and interaction. Genetic information processing encompassed transcription, translation, replication and repair (non-homologous end joining, mismatch repair, homologous recombination, DNA replication, base excision and repair), folding, sorting and degradation.

4.3.3 Diversity of metabolic pathways within farming systems

Approximately, 15 % of KO within farming systems microbiome were affiliated to metabolism at level one (1) of functional classification. These include pathways that participate in the metabolism of carbohydrates (17.3 %), amino acids (12.8 %), terpenoids and polyketides (11.3 %), energy (10.5 %), biodegradation and metabolism of xenobiotics (8.7 %), lipids (8.4 %), biosynthesis of secondary metabolites (8.1 %), metabolism of cofactors and vitamins (7.9 %), nucleotide metabolism (6.1 %), glycan biosynthesis and metabolism (5.6 %) and metabolism of other amino acids (3.1 %) at level two (2) of functional classification. The diversity of major metabolic pathways as revealed in the transcriptomes are shown in **Figure 4.3.1**.

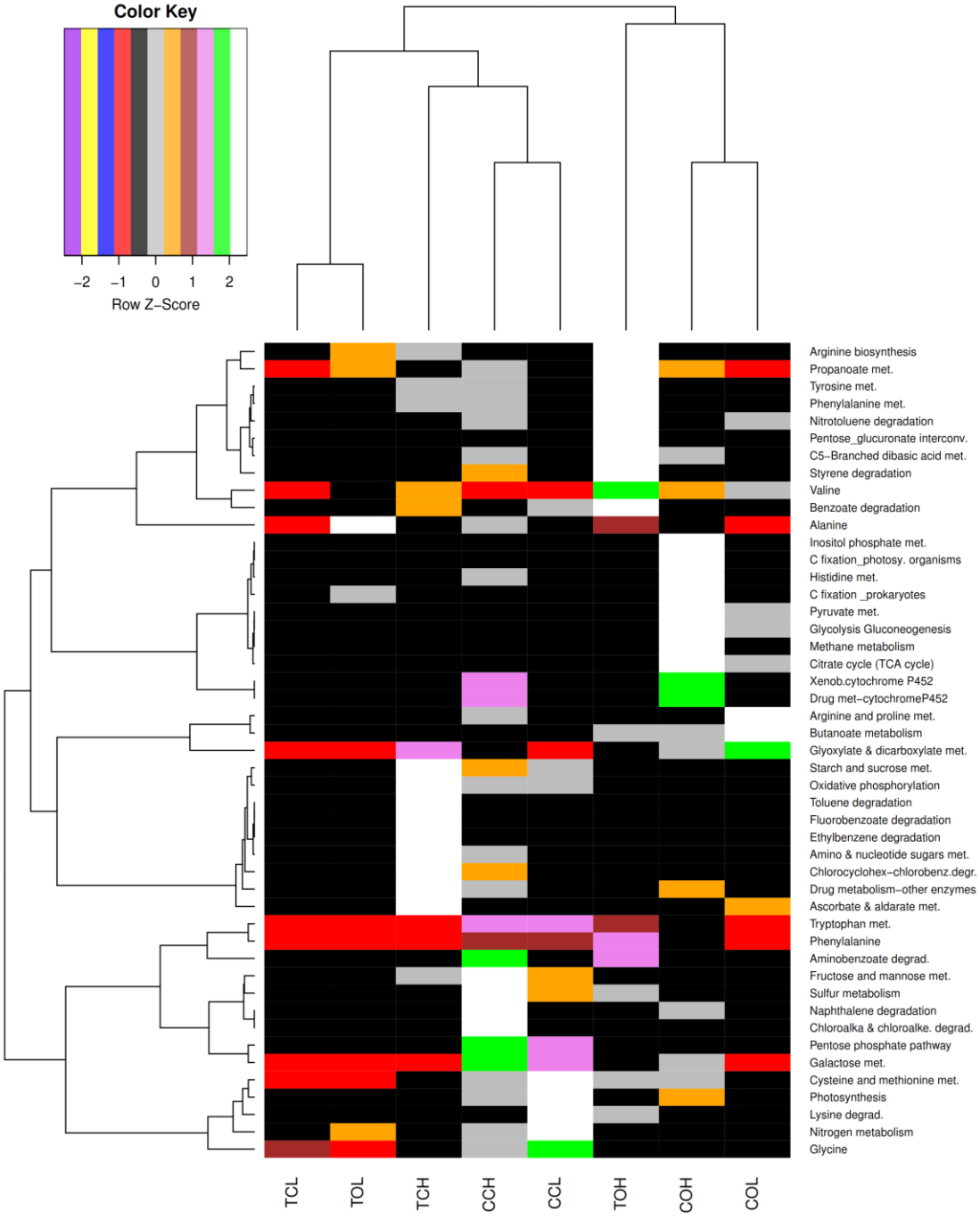


Figure 4.3.1: Diversity of major metabolic pathways as revealed in the transcriptomes within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

The most important pathways in agricultural systems were energy metabolism, carbohydrate metabolism and xenobiotics biodegradation and metabolism.

4.3.3.1 Energy metabolism

Energy metabolism pathways revealed within farming systems included; carbon fixation in photosynthetic organisms, carbon fixation pathways in prokaryotes, methane metabolism, nitrogen metabolism, oxidative phosphorylation, photosynthesis and sulfur metabolism. The major carbon fixation pathway modules in photosynthetic organisms included; reductive pentose phosphate cycle (Calvin cycle), Crassulacean acid metabolism (CAM) light and dark reactions, C4-dicarboxylic acid cycle, NADP and NAD (malic enzyme type), and C4-dicarboxylic acid cycle (phosphoenolpyruvate carboxykinase type). Major carbon fixation pathway modules in prokaryotes included; reductive citrate cycle, 3-Hydroxypropionate bi-cycle, hydroxypropionate-hydroxybutylate cycle, dicarboxylate-hydroxybutyrate cycle, reductive acetyl-CoA pathway, phosphate acetyltransferase-acetate kinase pathway and incomplete reductive citrate cycle. Methane metabolism pathway modules comprised of; reductive citrate cycle, 3-Hydroxypropionate bi-cycle, hydroxypropionate-hydroxybutylate cycle, dicarboxylate-hydroxybutyrate cycle, reductive acetyl-CoA pathway, phosphate acetyltransferase-acetate kinase pathway and incomplete reductive citrate cycle.

At Chuka site, Org-High farming systems revealed significantly higher abundance of carbon fixation functions in photosynthetic organisms, prokaryotes and methane metabolism (**Figure 4.3.2**). Methane metabolism was also shown to be higher in organic systems in both sites as compared to conventional systems. At Thika site, carbon fixation pathways in prokaryotes were dominant in organic systems while methane metabolism was higher in Org-High **(Figure 4.3.2)**.

Nitrogen metabolism pathways in soils within farming systems comprised nitrogen fixation, assimilatory nitrate reduction, dissimilatory nitrate reduction, denitrification and nitrification. Nitrogen metabolism was found to be higher in conventional systems at Chuka site. This was also observed in soils sampled from conventional systems at Thika

site **(Figure 4.3.2)**. In addition, oxidative phosphorylation (the metabolic pathway in which cells use enzymes to oxidize nutrients and release the chemical energy of molecular oxygen), and sulfur metabolism (oxidation, assimilatory and dissimilatory sulfate reduction), were found to be higher at Chuka site when compared to farming system counterparts at Thika site **(Figure 4.3.2)**. The enzymes that were found to catalyze various energy metabolic processes within farming systems are as enlisted in **Appendix 3.**

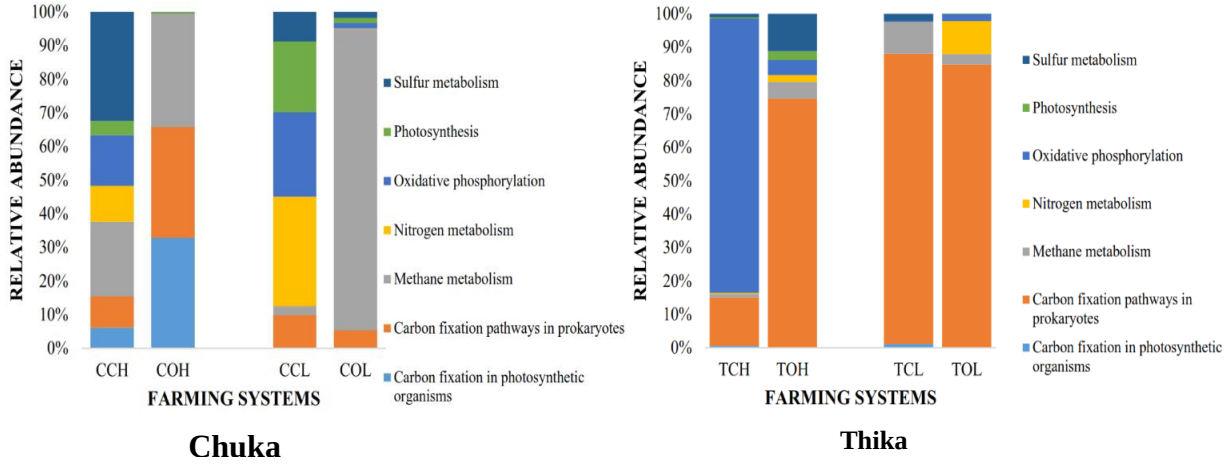


Figure 4.3.2: Diversity of Energy metabolic pathways as revealed in the transcriptomes within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.3.2 Carbohydrate metabolism

In this study, the major carbohydrate metabolic pathways within farming systems included amino sugar and nucleotide sugar metabolism, ascorbate and aldarate metabolism, butanoate metabolism, C5-branched dibasic acid metabolism, TCA cycle, fructose and mannose metabolism, galactose metabolism, glycolysis gluconeogenesis, glyoxylate and dicarboxylate metabolism, inositol phosphate metabolism, pentose and glucuronate interconversions, pentose phosphate pathway, propanoate, pyruvate, starch and sucrose metabolism.

At Chuka site, amino sugar, nucleotide sugar, ascorbate, aldarate fructose, mannose, galactose, starch and sucrose metabolism; and pentose phosphate pathway were highly expressed in conventional farming systems. The functions that highly expressed in organic systems included glycolysis gluconeogenesis, TCA cycle, butanoate, C5-branched dibasic acid metabolism, glyoxylate and dicarboxylate metabolism, pentose and glucuronate interconversions and pyruvate metabolism. These pathways were most abundant in Org-High.

A similar scenario was observed at Thika site except for glycolysis gluconeogenesis, TCA cycle, glyoxylate and dicarboxylate metabolism which were highly expressed in Conv-High systems **(Figure 4.3.3)**. Carbohydrate metabolism was catalyzed by a wide range of enzymes including dehydrogenases, transferases, kinases, isomerases, synthetases, reductases, carboxylases, phosphatases and synthases among others. The enzymes that were found to catalyze various metabolic processes within farming systems are as enlisted in **Appendix 4.**

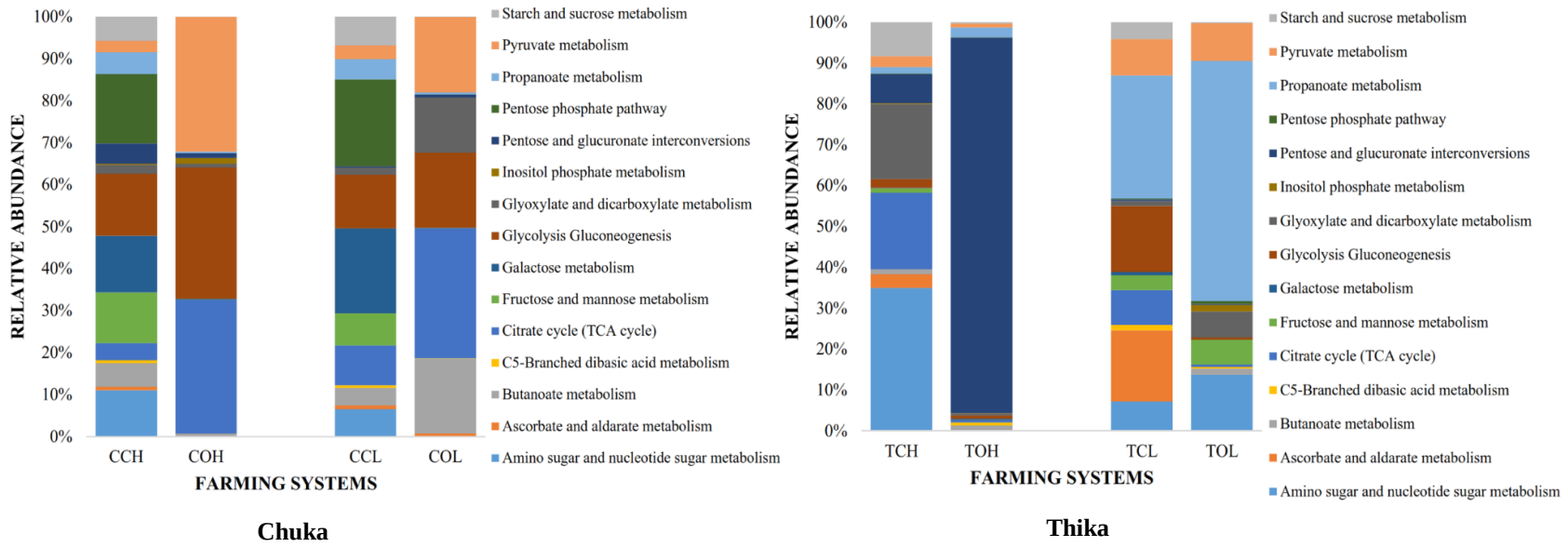


Figure 4.3.3: Diversity of carbohydrate metabolic pathways as revealed in the transcriptomes within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.3.3 Xenobiotics biodegradation and metabolism

In this study, major xenobiotics biodegradation and metabolic pathways were associated with conventional farming systems. They comprised benzoate degradation, chloroalkane and chloroalkene degradation, chlorocyclohexane and chlorobenzene degradation, fluorobenzoate degradation, toluene degradation and nitrotoluene degradation. However, at Thika site, the functional genes responsible for styrene degradation and aminobenzoate degradation were expressed in organic farming systems. The diversity of xenobiotics biodegradation and metabolic pathways within farming systems were as shown in **Figure 4.3.4.** The enzymes that were found to catalyze various xenobiotics biodegradation and metabolic processes within farming systems are as enlisted in **Appendix 5.**

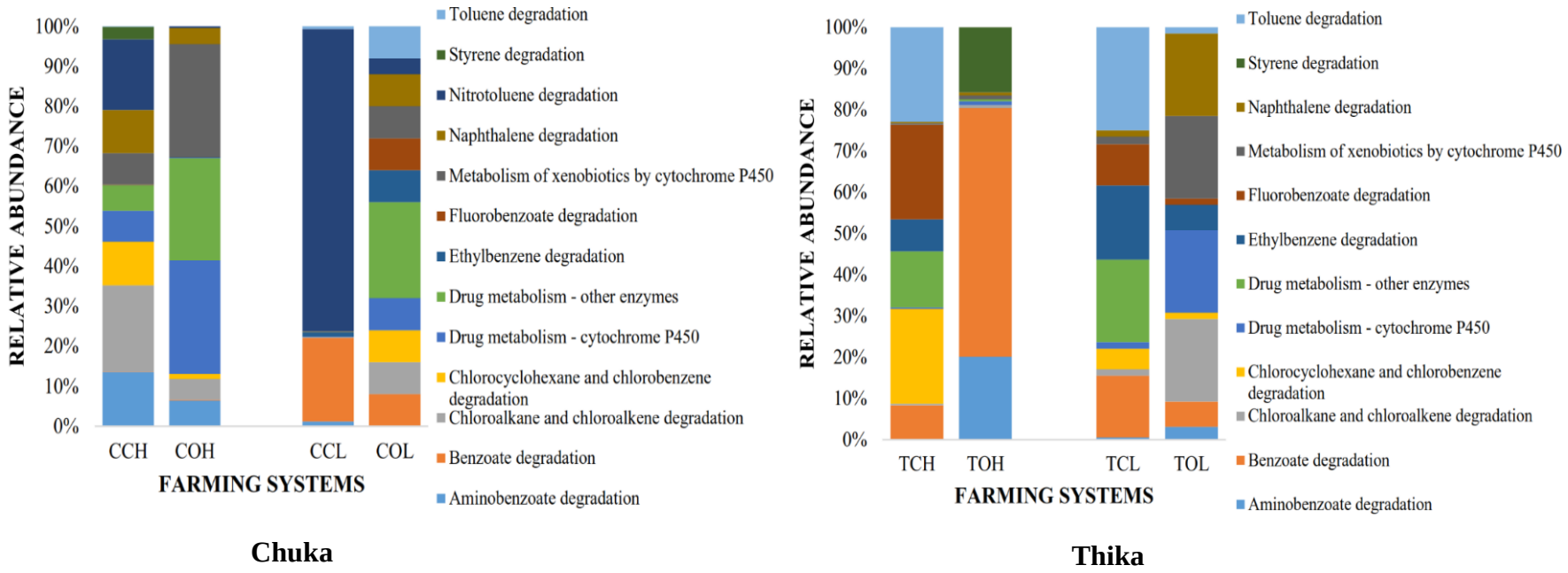


Figure 4.3.4: Diversity of Xenobiotics biodegradation and metabolism pathways as revealed in the transcriptomes within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.4 Diversity of functional genes highly expressed within farming systems

Classification of the top twenty (20) most highly expressed functional genes within farming systems transcriptomes at level 5 of Gene ontology indicated that majority belonged to Brite hierarchies Protein families (genetic information processing, signaling and cellular processes and metabolism), Human diseases (Cancer, infectious diseases - Bacterial, Parasitic, and viral) and disease resistance) and unclassified functional groups of genes. The distribution of most abundant genes present and active at the time of sampling were dominated by unclassified genes which were not included in Pathway or Brite (**Figure 4.3.5**).

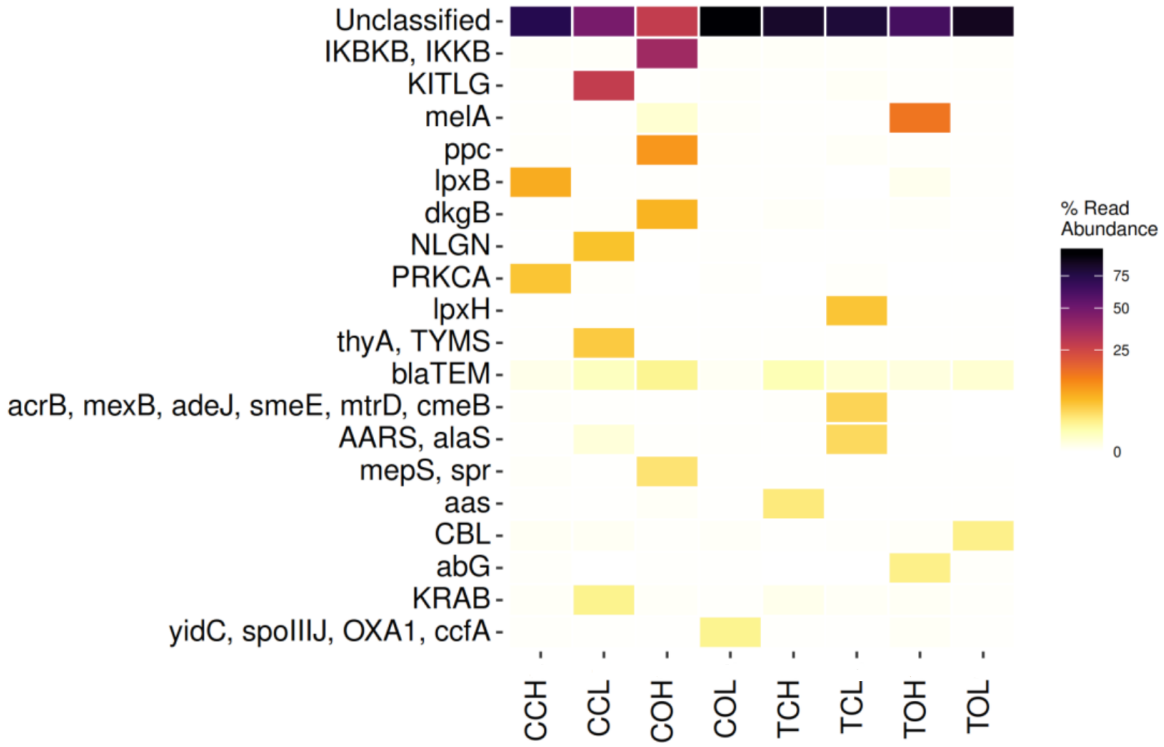


Figure 4.3.5: Diversity of the top 20 functional genes involved in various metabolic pathways as revealed in the transcriptomes within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.5 Key metabolic pathways and their associated genes

Functional groups covering genes involved in nutrient cycling within farming systems included nitrogen metabolism, methane metabolism, carbon fixation and sulphur metabolism. However, their abundance was shown to be very low possibly due to the reduced microbial activity since sampling was conducted at the end of cropping season. The outstanding nutrient cycles comprised sulphur metabolism, nitrogen metabolism and carbon metabolism.

4.3.5.1 Sulphur metabolic pathway

The most abundant sulphur metabolism related genes sulphur metabolic pathways included cysD found in CCH, TOH and TOL, cysN observed within CCH and COH and; TST, MPST and SSeA observed within TOL farming system (**Figure 4.3.6**).

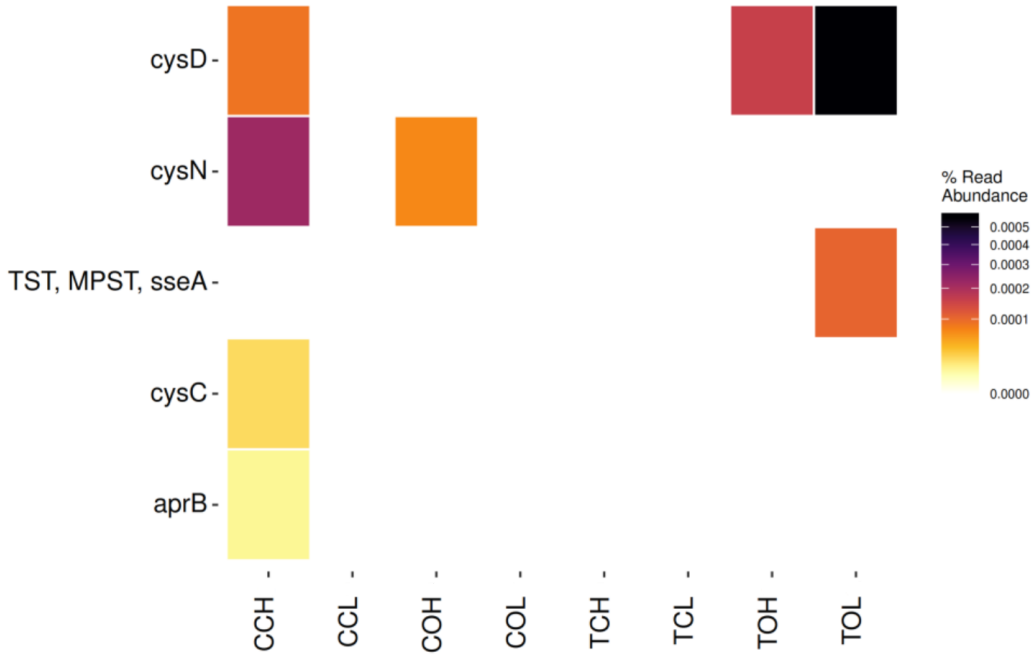


Figure 4.3.6: Diversity of genes involved in Sulphur metabolism as revealed in the transcriptomes within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.5.2 Nitrogen cycle

The most abundant nitrogen metabolism related genes included glnA and GLUL; found in CCH, COH, TOH and TCH; narG, narZ and nxrA genes found within CCH, TCH, TOH and TOL. Other genes present in low abundance included GLUD1 2, gdhA, E1.4.1.4, gdhA, nif D, nif H and napA among others as shown in **Figure 4.3.7**.

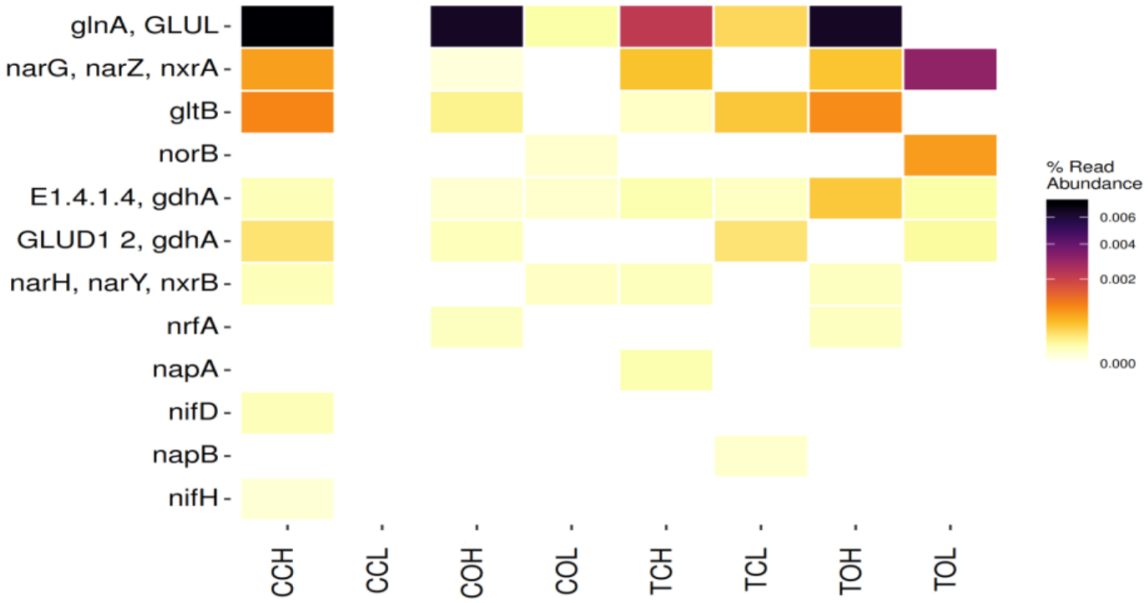


Figure 4.3.7: Diversity of genes involved in Nitrogen cycle as revealed in the transcriptomes within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.5.3 Carbon cycle

The most abundant genes involved in carbon metabolism included COX1 and COX3 in CCH, coxA and ctaD in TOH. Other genes scoring low abundance included coxL, cutL, PRK, prkB, coxM, CutM, KorA, oorA, and offor A as shown in **Figure 4.3.8**.

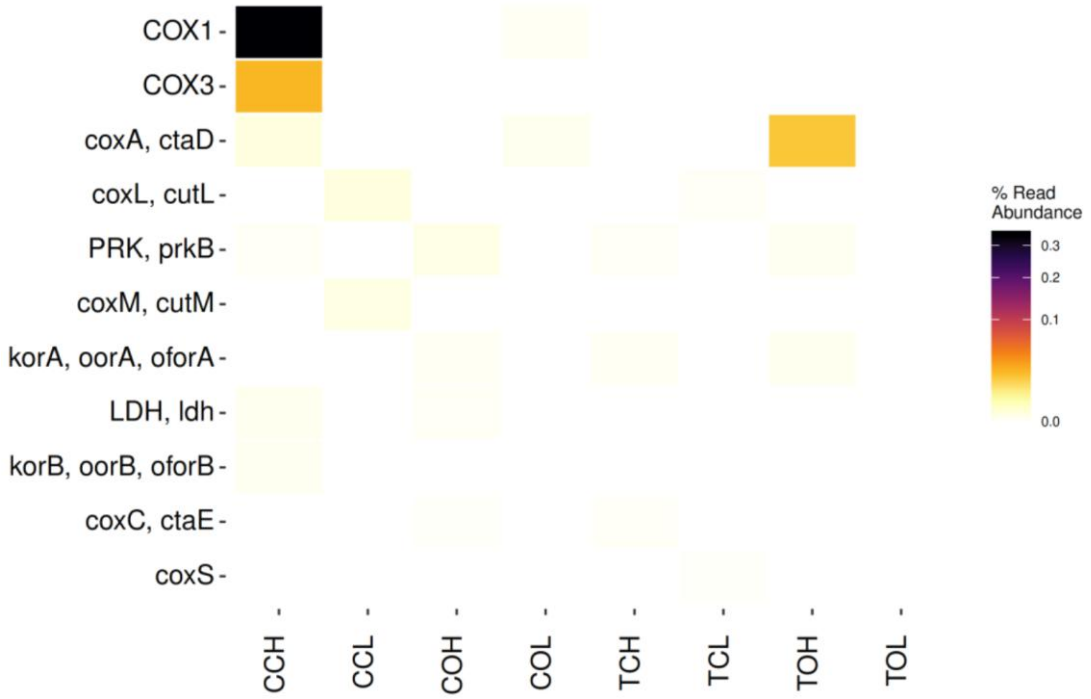


Figure 4.3.8: Diversity of genes involved in Carbon cycle as revealed in the transcriptomes within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.6 Microbial communities within farming systems and their role in nutrient cycling

To infer the origin of mRNA reads, they were directly assigned to taxa using the NCBI taxonomy and a reference database of protein sequences from microbial and viral genomes. Microbial classification comprised cellular organisms, fungi and viruses across farming systems. Cellular organisms included the super kingdom bacteria and archaea while viruses included unclassified phages, ssDNA viruses, dsDNA viruses, Retro-transcribing viruses, dsRNA viruses and ssRNA viruses. The soil microbiome within farming systems was dominated by bacteria followed by fungi. The bacterial dominance was recorded at 69.5 % of total reads, distributed among twenty two (22) phyla. Approximately, 23 % of total reads were clustered as Eukaryota within which; 29 % comprised fungi spread among seven (7) phyla. The other 71 % of Eukaryotic groups

included *Annelida*, *Apicomplexa* and *Arthropoda*. The remaining phylotypes represented were classified as viruses (3.1 %) comprising 62 families since most of the viral taxonomic classification various levels was unknown. Two (2) % of the total phylotypes were mapped to Archaea, distributed among nine (9) phyla while the remaining 1.8 % of the total phylotypes were unclassified groups.

4.3.7 Bacterial taxonomic composition

Out of the observed twenty two (22) bacterial phyla, there were four (4) major predominant phyla namely; *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* were found to dominate farming systems. At Chuka site, the sequenced transcriptomes revealed seventy eight (78) orders, sixty (60) of which were shared across all farming systems. Three (3) orders were unique to Conventional High farming system. On the other hand, seventy six (76) orders were observed at Thika site, sixty two (62) of which were shared across all farming systems. Two (2) orders were unique to Conventional Low system while Organic High and Organic Low systems displayed one (1) unique bacterial order. Some of the dominant orders shown within *Actinobacteria* phylum comprised *Micrococcales*, *Streptomycetales*, *Corynebacteriales*, *Streptosporangiales*, *Bifidobacteriales* and *Glycomycetales* while *Proteobacteria* phylum was dominated by *Xanthomonadales*, *Sphingomonadales*, *Burkholderiales*, *Rhodobacterales*, *Oceanospirillales* and *Rhizobiales*. Key *Firmicutes* orders comprised *Lactobacillales*, *Clostridiales*, *Tissierellales*, *Selenomonadales* and *Halanaerobiales* while *Bacteroidetes* were dominated by *Sphingobacteriales*, *Cytophagales* and *Flavobacteriales* (**Figure 4.3.9)**.

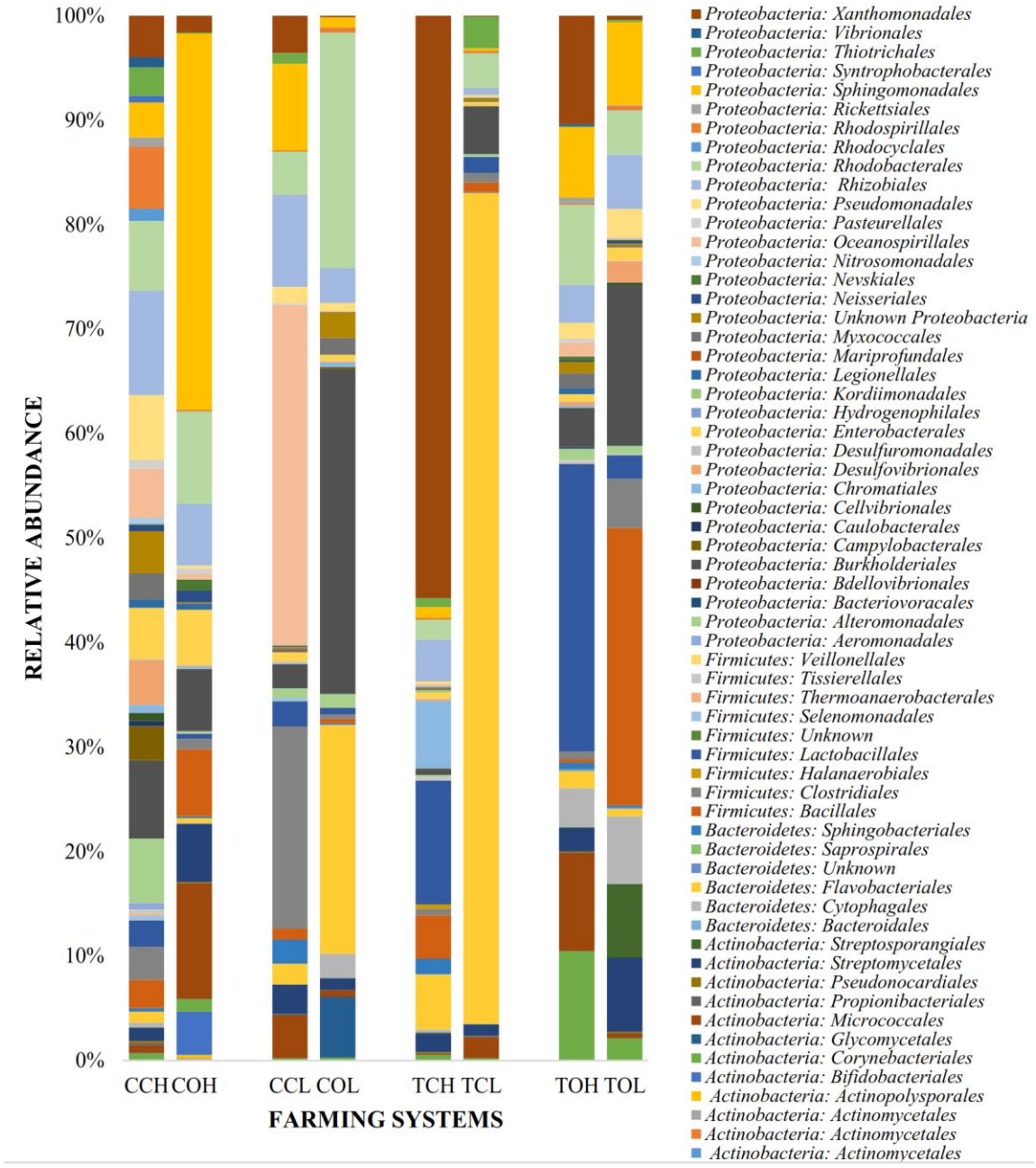


Figure 4.3.9: Taxonomic assignment of the most predominant bacterial taxa at order level as revealed in the transcriptomes of long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

The major taxa responsible for most gene expression, whose members were affiliated to *Actinobacteria* phylum comprised of *Streptomyces*, *Curtobacterium*, *Nocardia*, *Cryobacterium*, *Nocardiopsis*, *Microbacterium*, *Bifidobacterium* and *Corynebacterium* whereas most predominant *Proteobacteria* genera accountable for most gene expression included *Sphingobium*, *Methylibium*, *Pseudomonas*, *Xanthomonas*, *Paraburkholderia*, *Pseudophaeobacter*, *Paracoccus*, *Halomonas*, *Caballeronia* and *Sulfitobacter* among others. *Bacteroidetes* phylum genera that were key drivers of metabolic activities encompassed *Chryseobacterium*, *Pedobacter*, *Winogradskyella*, *Marivirga*, *Capnocytophaga* and *Flammeovirga* while *Firmicutes* most predominant genera comprised of *Bacillus*, *Christensenella*, *Dolosigranulum*, *Hungateiclostridium*, *Lactobacillus*, *Leuconostoc*, *Paenibacillus*, *Staphylococcus*, *Streptococcus* and *Weissella* among others.

Other functional drivers of metabolic activities within farming ecosystems were members of *Verrumicrobia*, *Chloroflexi*, *Chlamidae*, *Cyanobacteria* and *Tenericutes* phyla among others as shown in **Figure 4.3.10**.

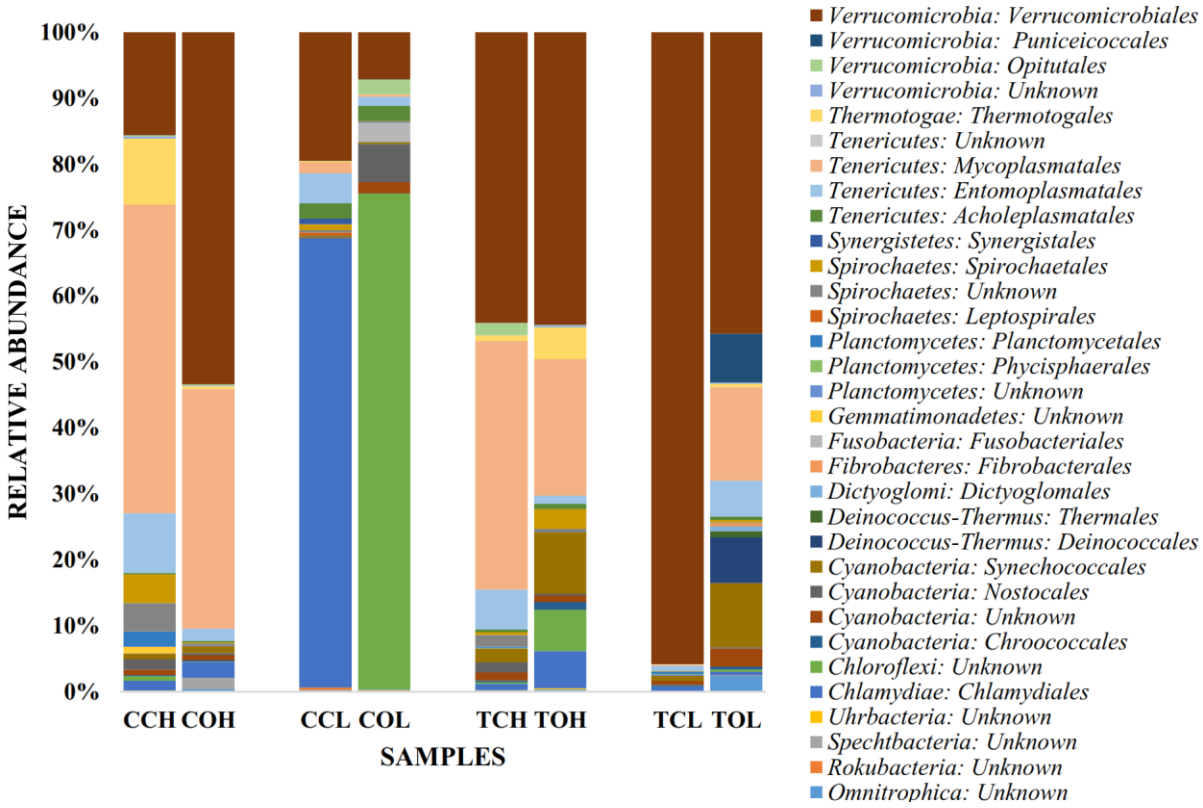


Figure 4.3.10: Taxonomic classification of other taxa at class level revealed in the transcriptomes as functional drivers of metabolism within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.8 Archaeal taxonomic composition

In this study, nine (9) archaeal phyla were represented within the transcriptomes. These comprised *Crenarchaeota*, *Euryarchaeota*, Thaumarchaeota, *Nanoarchaeota*, *Candidatus Bathyarchaeota*, *Candidatus Micrarchaeota*, *Candidatus Odinarchaeota*, *Candidatus Thorarchaeota*, *Candidatus Woesearchaeota* and unclassified archaea. These were further classified into eleven (11) classes namely; *Thermoprotei*, *Thermococci*, *Thermoplasmata*, *Nitrososphaeria*, *Methanomicrobia*, *Methanopyri*, *Methanobacteria*, *Methanococci*, *Candidatus Thalassoarchaea*, *Hadesarchaea* and *Archaeoglobi*. At Chuka site, the sequenced transcriptomes revealed twenty two (22) orders, thirteen (13) of which were shared across all farming systems. One (1) order was unique to Organic High

farming system. On the other hand, 19 orders were observed at Thika site, fourteen (14) of which were shared across all farming systems. Some of the dominant orders shown within archaeal phyla comprised twenty one (21) archaeal taxa as shown in **Figure 4.3.11**.

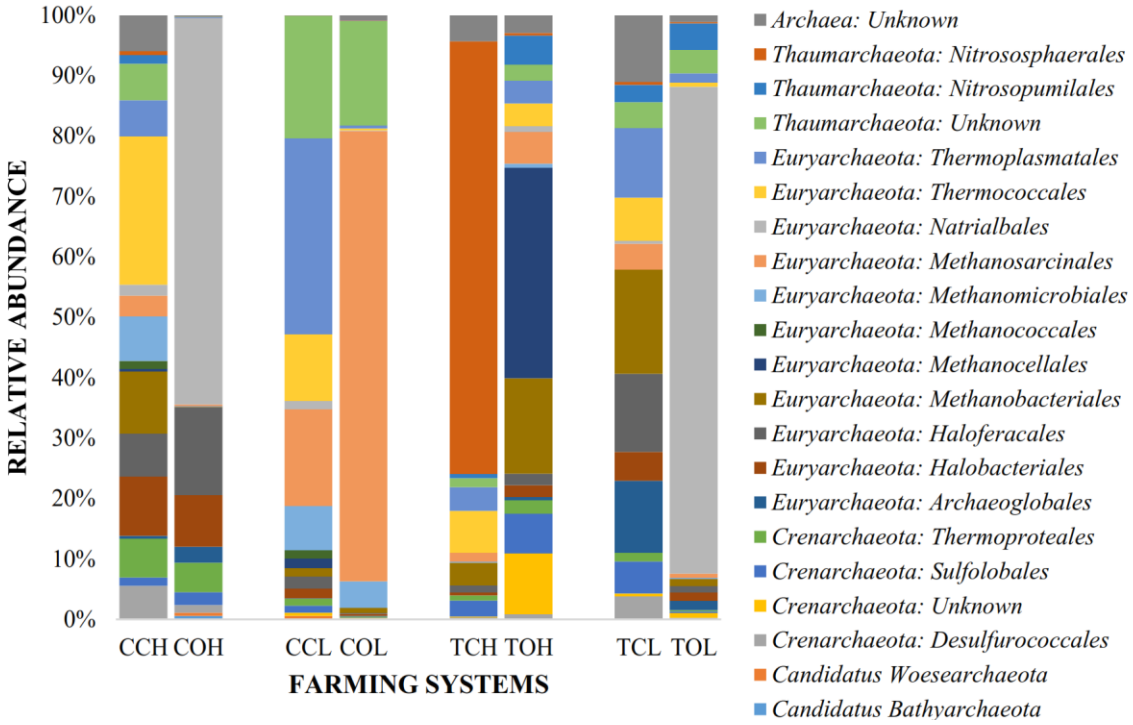


Figure 4.3.11: Taxonomic classification of the most predominant Archaeal taxa at order level of revealed in the transcriptomes as functional drivers of metabolism within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.9 Fungal taxonomic composition

In this study, seven (7) fungal phyla were represented within the farming system transcriptomes. These included; *Ascomycota*, *Bacillariophyta*, *Basidiomycota*, *Blastocladiomycota*, *Chytridiomycota*, *Mucoromycota* and *Zoopagomycota*. Due to the high number of unknown orders within the fungal diversity gene transcripts, the classification of fungi was presented at class level. At Chuka site, the sequenced transcriptomes revealed forty five (45) classes, twenty three (23) of which were shared

across all farming systems. Eight (8) classes were unique to Org-High farming system, while tow (2) classes were unique to Org-Low and one (1) class unique to Conv-Low farming systems. On the other hand, thirty eight (38) classes were observed at Thika site, twenty five (25) of which were shared across all farming systems. The distribution of most abundant fungal classes within each phylum were as shown in **Figure 4.3.12**. *Dothideomycetes* class of *Ascomycota* phylum was presented as the most dominant class revealed from the Eukaryotic gene transcripts.

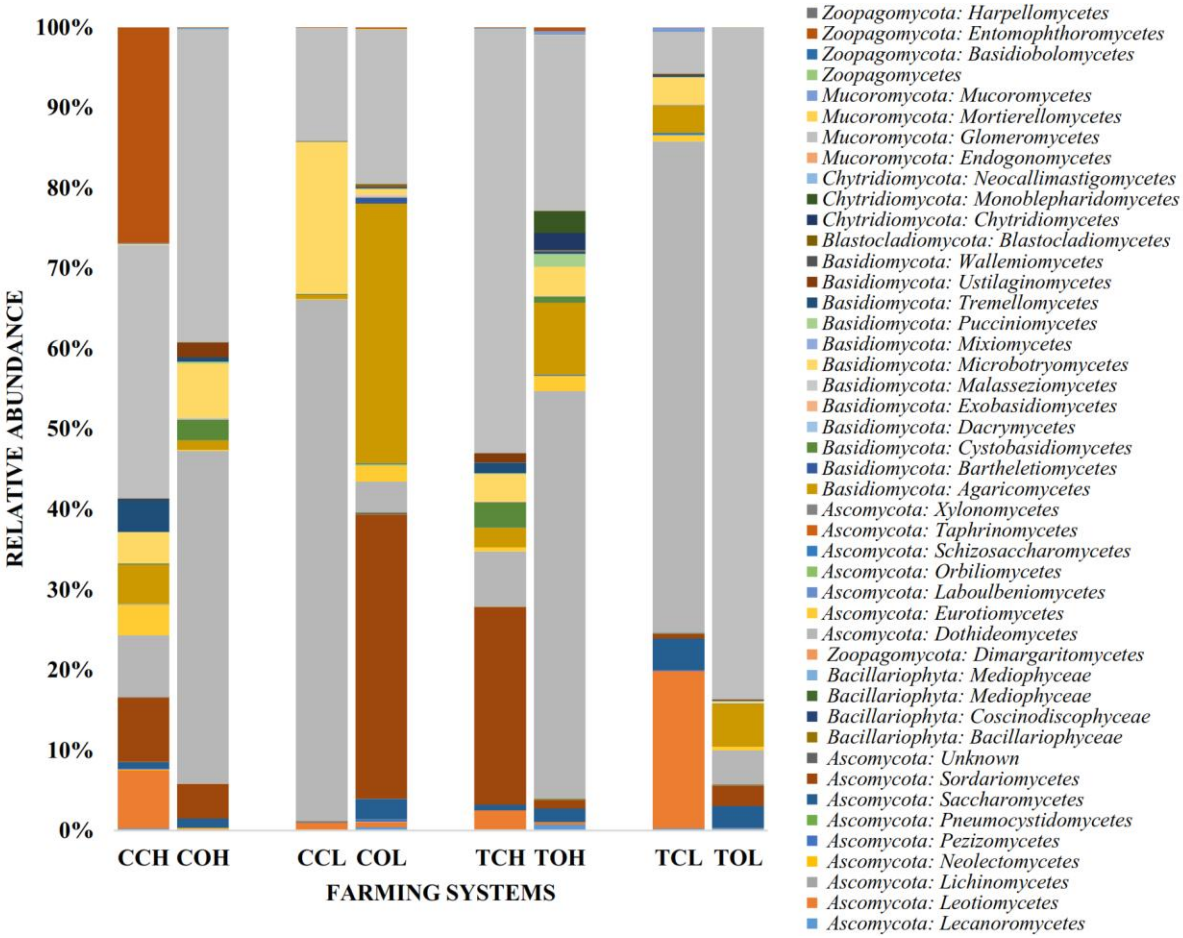


Figure 4.3.12: Taxonomic classification of the most predominant fungal taxa at class level as revealed in the transcriptomes as functional drivers of metabolism within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

4.3.10 Viral taxonomic composition

Several lineages of viruses were represented in the mRNA transcripts from various farming systems. These include *ssDNA viruses*, *ssRNA viruses*, *dsDNA viruses*, *Retro-transcribing viruses*, *unclassified phages*, *unclassified viruses* and *unassigned viruses***.** The presentation of viral classification was based at family level, showing sixty two (62) families within the viral mRNA transcripts. At Chuka site, the sequenced transcriptomes revealed thirty nine (39) families, thirty two (32) of which were shared across all farming systems. One (1) family was unique to Org-High and Conv-Low farming systems respectively. On the other hand, thirty six (36) families were observed at Thika site, thirty one (31) of which were shared across all farming systems. One (1) family was unique to Org-High and Org-Low farming systems respectively. The top five most predominant families comprised *Phenuiviridae*, *Phycodnaviridae*, *Myoviridae*, *Flaviviridae* and *Closteroviridae* within the farming systems. The distribution of viral families' functional classification within the farming systems were as shown in (**Figure 4.3.13**).

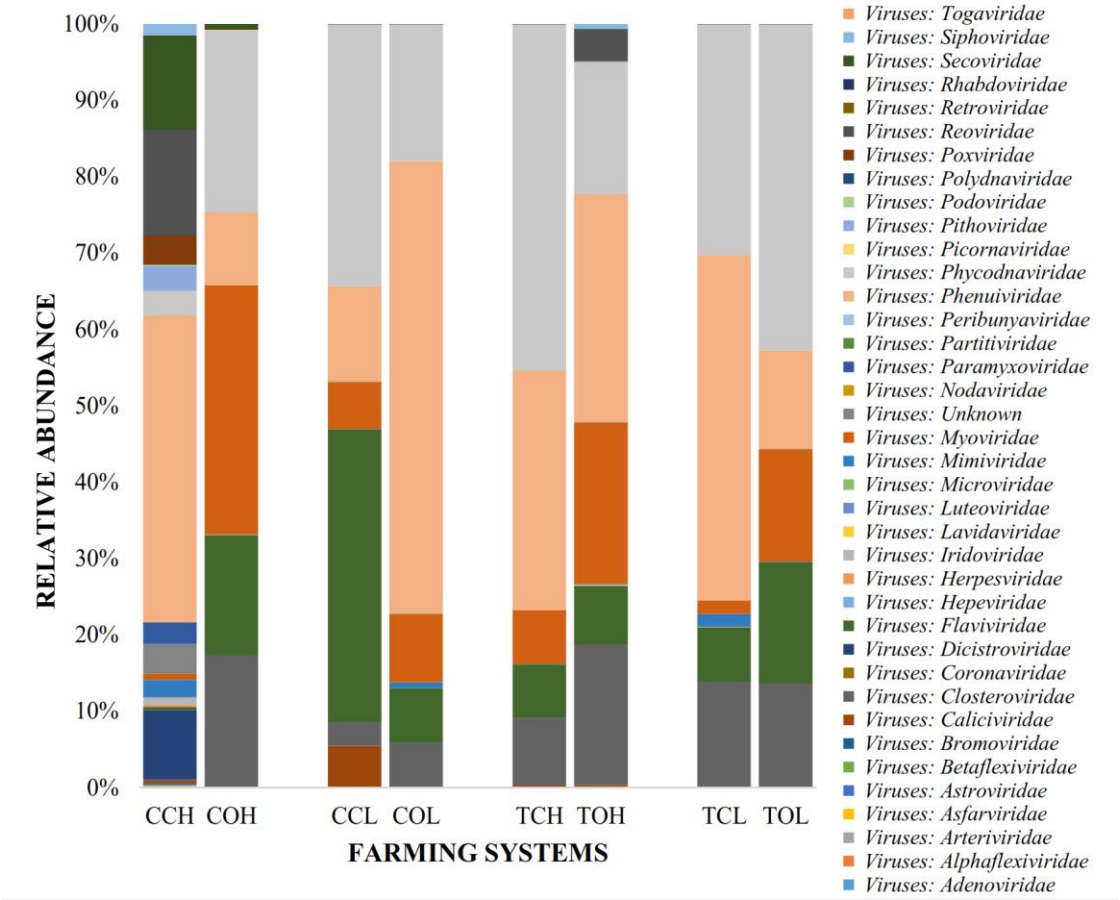


Figure 4.3.13: Taxonomic classification of the most predominant viral taxa at family level as revealed in the transcriptomes as functional drivers of metabolism within long-term comparison trials at Chuka and Thika sites. CCL = Chuka Conv-Low; COL = Chuka Org-Low; CCH = Chuka Conv-High; COH = Chuka Org-High and TCL = Thika Conv-Low; TOL = Thika Org-Low; TCH = Thika Conv-High; TOH = Thika Org-High.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In this study, high-throughput sequencing of 16S rDNA, 16S rRNA cDNA amplicons as well as metatranscriptomes was used to demonstrate that farming inputs whether organic or conventional have an immense influence on the prokaryotic community structure and presumptively function. Abundance of phylotypes affiliated to *Acidobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Fusobacteria, Gemmatimonadetes, Planctomycetes and Verrucomicrobia* were observed in this study. Members of these phyla are major contributors to soil biogeochemical processes and have been reported in other studies (Chaudhry *et al*., 2012; Shange *et al*., 2012; Pershina *et al*., 2015). This study describes the taxonomic composition of microbial community established in soil following long-term exposure to conventional and organic farming systems. Within the soil ecosystem, different groups perform varied functions hence a shift in the diversity and abundance due to inputs effect on the soil and plant health. Major families within *Proteobacteria* comprised *Rhodospirillaceae, Beijerinckiaceae, Burkholderiaceae* and *Bradyrhizobiaceae*. Some representatives of these families (e.g. *Burkholderiaceae*) are known to degrade recalcitrant organic matter in soil while other groups (e.g. *Beijerinckiaceae*) fix atmospheric nitrogen in the soil (Werner *et al*., 2005; Li *et al*., 2012). At high relative abundance, these microbial groups could increase available nitrogen in organic farming system without fertilizer supplementation. *Actinobacteria* are known to play a major role in organic matter turnover and carbon cycling. They can decompose recalcitrant carbon sources like cellulose and chitin and degrade herbicides and pesticides (Jenkins *et al*., 2010; Li *et al*., 2012). In this study, Prokaryotic community composition and diversity analysis within sites and farming systems displayed Thika site to harbor more shared and unique OTUs compared to Chuka site. This is a factor attributed to soil aggregate composition and mineralogy. In both sites, conventional farming systems supported significantly higher species richness. This was ascribed to integration of farmyard manure and inorganic fertilizer into the systems, promoting copiotropic prokaryotic groups to thrive due to high

nutrient availability within the cropping season. On the other hand, low nutrient levels at the end of cropping season enhanced high abundance of unique prokaryotic groups observed in conventional systems. Analysis of the 16S rRNA cDNA gives an indication of active microbial diversity at the time of sampling which explains the low OTU numbers in both sites (Chuka - 390 and Thika - 501 OTUs) as compared to16S rDNA dataset. These could have been the communities carrying out the various biological processes within farming systems at the time. The low number of OTUs affiliated to active microbial diversity was attributed to lack of cropping activities within farming systems at the time of sampling. In this dataset, the most abundant phylotypes were affiliated to the classes *Alphaproteobacteria*, *Actinobacteria*, *Gammaproteobacteria*, *Betaproteobacteria*, *Acidimicrobia*, *Bacilli* and *Unknown bacterial phyla*. The unknown groups could form the basis for further studies to reveal their role within the farming systems.

Soil microbial activity has been reported to affect soil carbon dynamics by releasing carbon in form of carbon dioxide back into the atmosphere through respiration and is accountable for 80 – 95 % of carbon mineralization (Hassink *et al*., 1994). The presence of a higher number of unique OTUs and low organic carbon levels at Thika site as compared to Chuka site may be an indicator that higher species richness may eventually lead to carbon depletion through increased metabolic activities. Furthermore, Thika soils were found to contain higher sand content, a property that exposes soil organic carbon to heightened microbial activity (Chivenge *et al*., 2007). High amounts of organic carbon detected in samples from Chuka confirms findings of a previous study that indicated the soils found in humid regions contain more organic carbon than soils within drier regions (Lal, 2007). After six (6) years of continuous cropping within the trial sites, (Adamtey et al. unpublished results) pointed towards organic carbon build-up at Chuka and organic carbon depletion at Thika sites.

Clay minerals and oxides associated with Fe and Al significantly influence adsorption of dissolved organic carbon (Singh *et al*., 2016; 2017b). Since Thika soils contained high Fe levels coupled with high primary clay minerals, this may have created a stable environment for microbes to thrive. Chuka soils have been reported to contain the highest

phyllosilicate clay minerals, especially kaolinite, involved in dissolved organic carbon preservation (Feng *et al*., 2005), making it unavailable for microbial attack and hence its build up at the site. In some occurrences within the current study, low input systems were found to harbor more OTUs than high input systems. This could be due to differences in soil macro-aggregates (> 250 – 2000 µM) and micro-aggregates (< 53 – 250 µM) (Adamtey et al. unpublished results). High macro-aggregates may have provided unique environmental partitioning for soil microbiome which was isolated from its surroundings. Macro-aggregates are considered as massively concurrent incubators that allow enclosed microbial communities to pursue their own independent progression (Rillig *et al*., 2017), hence creating more unique habitats for microbial colonization within these farming systems. Organic inputs not only carry various types of organic compounds, but also indigenous prokaryotes that remain in soil for a certain period (Hartmann *et al*., 2015). Besides, incorporation of *Tithonia diversifolia* leaves and leaf extracts as well as *Lantana camara* leaves during composting and as starter N in organic farming systems could have lowered microbial diversity. These plants have been shown to contain anti-microbial properties resulting from steroids, saponins, tannins, polyphones and alkaloids which might be responsible for broad anti-bacterial activity (Barreto *et al*., 2010; Gutierrez *et al*., 2015). A significant prokaryotic community structuring based on farming systems was observed, probably reflecting variations in agricultural input amounts and management practices. This observation suggests a high degree of agro ecosystem microbiomic endemism and implies that each farming system harbors some degree of unique soil prokaryotic genetic resource. This result has significance in maximizing microbial functions in agroecosystems which has become a promising approach for the future of global agriculture. The data creates a better understanding in application of the benefits of soil microorganisms for resource uptake, plant growth, development, and health, on agricultural production systems.

Fungal diversity within organic and conventional farming systems in Central Highlands of Kenya study, combined high-resolution power of Illumina sequencing technology and analysis of fungal ITS amplicon sequences. This was to assess the effects of organic and conventional farming systems on the diversity and composition of fungi and generate a

taxonomic profile within long-term experiment trial sites in the central highlands of Kenya. The number of OTUs and alpha diversity analysis gives a glimpse of the resident fungal diversity. Eight (8) fungal phyla (*Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota*, *Calcarisporiellomycota*, *Kickxellomycota*, *Mortierellomycota* and unknown fungal phyla) were identified at Thika and Chuka sites. Taxonomic composition analysis indicated unknown fungal phyla, *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Glomeromycota* as the most predominant phyla within both sites and farming systems. *Ascomycota* and *Basidiomycota* are important decomposers in carbon cycle. They break down organic substances such as cellulose, lignocellulose, and lignin within plant residues into micro-molecules hence, promoting the carbon cycle in soil (Purahong *et al*., 2016). At family level, unique families to Chuka site included; *Unknown Pleosporales*, *Lentitheciaceae*, *Unknown Eurotiales* and *Unknown Cystobasidiomycetes* while at Thika site, unique families included *Didymellaceae*, *Periconiaceae*, *Phaeosphaeriaceae*, *Thyridariaceae*, *Chaetosphaeriaceae*, *Plectosphaerellaceae*, *Clavicipitaceae*, *Ophiocordycipitaceae*, *Unknown Sordariomycetes, Unknown Xylariales*, *Lentinaceae*, *Filobasidiaceae*, *Unknown Filobasidiales, Unknown Tremellomycetes* and *Mortierellaceae*.

At genus level, potentially phytopathogenic genera (Sharma-Poudyal *et al*., 2017; Fraç *et al*., 2018) were revealed and they included *Alternaria* (scored up to 92 % relative abundance at Chuka Org-High and 87 % at Thika Conv-Low), *Epicoccum (*1.4 % relative abundance at Chuka Org-High and 0.1 % at Thika Org-Low), *Fusarium* (17 % relative abundance at Chuka Conv-High and 17 % at Thika Org-High and Org-Low), *Olpidium* (0.4 % relative abundance at Chuka Org-High and 20 % at Thika Org-High), *Phoma* (2.3 % relative abundance at Chuka Org-High and 26.3 % at Thika Org-Low), *Rhizoctonia* (0.2 % relative abundance at Chuka Org-High and 10.7 % at Thika Conv-High), and *Stagonospora* (5.4 % relative abundance at Chuka Conv-High and 0.4 % at Thika Org-High). Other major putative pathogenic groups revealed included members of *Nectriaceae, Ceratobasidiaceae, Bionectriaceae, Phaeosphaeriaceae* and *Mycosphaerellaceae* families.

Potentially plant beneficial fungal genera (Madi *et al*., 1997; Harman *et al*., 2004; Fraç *et al*., 2018) were revealed within the farming systems. These include: *Glomus* (scored up to 0.2 % relative abundance at Chuka Org-High and 9.7 % at Thika Conv-High), *Trichoderma* (0.5 % relative abundance at Chuka Org-High and 0.3 % at Thika Org-Low) and *Talaromyces* (1.5 % relative abundance at Chuka Org-High and 22.1 % at Thika Org-High). *Glomus species* have plant endosymbiotic properties especially arbuscular mycorrhizal fungi which form symbiotic relationships with plant roots (Harman *et al*., 2004). The species within *Glomus* genus consisted of *Glomus cerebriform, Rhizophagus intraradices, Rhizophagus diaphanum* and unknown *Glomus species*. *Trichoderma* and *Talaromyces* are prominent biocontrol agents with antagonistic potential and mycoparasitic lifestyle (Harman *et al*., 2004). *Trichoderma* genus included *Hypocrea lixii, Hypocrea koningii*; while *Talaromyces* genus included *Talaromyces islandicum*, *Talaromyces rotundus* and unknown *Talaromyces* species. Plant inoculation with *Epicoccum nigrum* and *Trichoderma atroviride* has been reported to protect potato against *Rhizoctonia solani* (Lahlali and Hijri, 2010). In this study, *Epicoccum nigrum* and *Epicoccum sorghi* were among the fungal species found within farming systems. The presence of potential phytopathogens, biocontrol agents, mycoparasites, plant beneficial fungi and endosymbiont fungal groups within farming systems was similar to a previous study carried out to analyze profiles of fungal communities in agricultural soils within a long-term field trial under different fertilization, tillage and crop rotation regimes (Sommermann *et al*., 2018). The study revealed eight genera with potential phytopathogenic roles, namely *Alternaria*, *Bionectria*, *Epicoccum, Fusarium, Olpidium, Phoma, Rhizoctonia, Stagonospora, Ophiosphaerella* and *Verticillium*. Among the biocontrol agents identified were *Trichoderma sp*., *Coniothyrium minitans* and *Talaromyces* some of which have designated efficacy against phytopathogens (Sommermann *et al*., 2018).

A few groups of fast-growing soil-inhabiting saprophytic fungi and root colonizers such as *Humicola* (Family *Chaetomiaceae*), *Mortierella* (Family *Mortierellaceae*) and *Exophiala* (Family *Herpotrichiellaceae*) were revealed. Some species within these genera are potential pathogens while others are considered potential biocontrol agents and may

benefit plant health (Sommermann *et al*., 2018). Also common within the farming systems were *Penicillium* and *Aspergillus* (Family *Trichocomaceae*), common cellulolytic colonizers of soil and plant residues (Sharma-Poudyal *et al*., 2017).

Fungal diversity in all farming systems were to a large extent dependent on the flow of nutrients within the soil. Composition and diversity assessment of fungal communities within sites and farming systems displayed Thika site to harbor more OTUs when compared to Chuka site. This could be linked to the presence of higher quantities of small macro-aggregates that provided unique environmental habitats for soil fungi. Macro-aggregates have been considered as massively concurrent incubators that allow enclosed microbial communities to pursue their own independent progression (Rillig *et al*., 2017), hence creating more unique habitats for microbial colonization within these farming systems. Chuka soils contained high primary and secondary clay minerals, while Thika soils were characterized by high primary minerals and low secondary clay minerals. Clay minerals and oxides of Fe and Al have been exhibited to play important roles in adsorbing dissolved organic carbon (Singh *et al*., 2016; 2017b). Since Thika soils contained high Fe levels coupled with high primary clay minerals, this may have created a stable atmosphere for fungal groups to thrive. At Chuka site, fungal diversity was also negatively influenced by high Al levels, hence low OTU numbers obtained. However, in both sites, Conv-Low had the highest number of OTUs (161 and 168 OTUs at Chuka and Thika), respectively. This could be due to use of undecomposed farmyard manure as input component in the system during planting. The fungal diversity within farming systems is influenced by complex interactions between a wide range of soil properties and agronomic inputs, thus signifying that fungi within the soils are exceptionally diverse. These inputs change soil properties and microbial diversity, and the microbial community in turn manipulates nutrient cycling processes altering soil fertility, plant productivity and environmental sustainability.

In the diversity of metabolic pathways and functional genes of microbial communities within farming systems study, some of the gene clusters revealed were affiliated to nutrient cycling within farming systems (carbon fixation, methane, nitrogen and sulfur metabolism pathways) demonstrating a high level of different genes contained within the

organisms present in the agricultural ecosystems. The results had similar trend to a recent investigation on effects of rainforest change over various land use systems (Berkelmann *et al*., 2020). A high level of pathway completeness suggested their possible adaptive importance within the environmental conditions created by soil interference through crop production in various farming systems. For instance, KEGG Orthologs categorized as metabolism comprised genes affiliated to pathways that participate in energy, carbohydrate and amino acid metabolism. Global and overview metabolic pathways included: carbon metabolism, biosynthesis of amino acids, oxocarboxylic acid metabolism, degradation of aromatic compounds, metabolism and biodegradation of xenobiotics among others. Other major pathways include butanoate metabolism, nitrogen metabolism, unsaturated fatty acids synthesis and lipid metabolism. The extensiveness of these pathways and carbohydrate transport and metabolism obtained suggest that some of the microbes' present were largely heterotrophic prokaryotes that depended heavily on extracellular aromatic compounds as well as other sources of organic carbon.

At Chuka site, the high methane metabolism revealed in organic farming systems could be attributed to the presence of manures obtained from zero grazing livestock production sources (animal droppings and other plant materials) while, manure used at Thika site was Masai cow manure (animal droppings only). These manures harbor methane oxidizing prokaryotic microbes that play key roles in cycling of methane in soils. The microbial groups are capable of either producing or removing greenhouse gas methane (CH_4), that is the second most important greenhouse gas in the atmosphere and substantially contributes to global warming (Le Mer and Roger, 2001). Methane uptake in soils is mainly facilitated by aerobic methane oxidizing bacteria that utilize CH_4 as their sole source of carbon and energy through oxidation of CH_4 to produce carbon dioxide and water (Hanson and Hanson, 1996).

High abundance of carbon fixation pathways in photosynthetic organisms and prokaryotes within organic systems could be attributed to the use of compost made from farm yard manure and other organic materials, incorporation of *Mucuna pruensis* and maize stovers; in addition to use of *Tithonia diversifolia* and rice straw mulch. Nitrogen and Sulfur metabolic pathways were highly expressed in conventional systems at Chuka

site and vice versa at Thika site. High nitrogen and sulfur metabolic pathways in conventional systems are because of nitrogen availability from organic and inorganic sources in addition to higher microbial diversity richness in these systems. This difference could be due to higher carbon and nitrogen ratio of applied compost manure and low available mineral nitrogen in the soil as a source for denitrification. Higher soil temperature coupled with high moisture holding capacity in organic systems at Thika, also promoted increased metabolism.

Xenobiotic compounds that contribute to ecological pollution and are persistent in the environment include herbicides, chemical fertilizers, pesticides, insecticides, dioxins, dyes, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), trinitrotoluene, trichloroethylene and nitroglycerine (Eapen *et al*., 2007; Agarwal *et al*., 2009; Fatta-Kassinos *et al*. 2011). The fate of synthetic organic pesticides, herbicides and insecticides commonly added to the soil in large amounts yearly include complete biodegradation, or stabilization of the parent compound, or some metabolite of the compound in soil. Exposure of living organisms to such pollutants impose dangerous toxicity threats. High xenobiotic concentrations create a biological imbalance in soil leading to surface and groundwater pollution. They interfere with the functional groups of biologically important molecules such as enzymes, transport system of polynucleotides and nutrient ions within plants (Godheja *et al*., 2016). The contamination of agricultural soil with PAHs is a serious threat to human food chain. These PAHs gain entry into humans mainly by inhalation of particulates carrying PAHs, alimentary consumption of contaminated food products, and direct association with polluted soils (Steffan *et al*., 2018). Different microbial species such as bacteria, fungi, yeast, and algae have an ability to breakdown xenobiotic compounds and biodegrade them (El-Sheekh and Mahmoud, 2017; Ijoma *et al*., 2017). Bioprospecting of bacterial groups such as *Flavobacterium*, *Xanthobacter*, *Arthrobacter*, *Sphingobium* and *Pseudomonas* with biodegradation potential has been done (Chowdhury *et al*., 2008; Varsha *et al*., 2012). These bacterial groups are capable of degrading chemical compounds such as benzene, ethylbenzene, toluene and xylene among others. One of the persistent organic xenobiotics is benzene and it has a thermodynamically stable ring in its structure. Recalcitrancy of these

compounds is due to the presence of chloro, methyl, amino, nitro and sulfonyl groups in benzene ring (Dıaz and Prieto 2000; Chandra and Singh, 2015). Hazardous xenobiotic compounds are transformed into harmless or less hazardous forms such as water, carbon dioxide, nitrogen and methane by microorganisms. Enzymes and organic acids that degrade recalcitrant compounds into simpler forms are produced by different groups of microbes (Singh *et al*., 2016). In this study, high functional abundance was revealed in conventional systems. This could be attributable to continued use of synthetic fertilizers and pesticides which contain various recalcitrant chemical compounds.

Studies in microbial ecology have helped researchers to appreciate and recognize the extent of diversity owing to the recent awareness created by metagenomic studies of components within the interactive assemblage of microorganisms, most of which are not achievable by use of culture dependent techniques (Woese *et al*., 1990; Head *et al*., 1998; Handelsman, 2004). Most prokaryotic and eukaryotic taxa revealed in this study are presumed to be involved in metabolic processes such as nutrient (carbon, sulfur and nitrogen) cycling within the farming systems.

The study revealed presence of various microbial groups that are known to take part in primary production within farming systems for instance, non-sulfur purple bacteria from the family *Rhodobacteraceae* and purple sulfur bacteria from the family *Ectothiorhodospiraceae*. *Rhizobiales* and *Burkholderiales* orders found in farming systems are known for their role in various processes related to nitrogen metabolism and bacterial chemotaxis as previously identified in rainforest soils (Tang *et al*., 2018). Species that possess an extensive range of metabolic functions which include photosynthesis, respiration, lithotrophy, aerobic and anaerobic nitrogen fixation and production of tetrapyrroles, chlorophylls, heme, and vitamin B12 comprised members of alpha-proteobacteria genus *Rhodobacter*. The most studied photosynthetic organism in terms of the structural and functional light reactions is *Rhodobacter sphaeroides.* The metabolisms of each species generate great interest within research community, especially in regard to renewable energy sources (Puskas, 1997; Owusu *et al*., 2016). On the other hand, members of *Rhodanobacter* genus were found to efficiently reduce heavy

metals and nitrates in environments with low pH (Green *et al*., 2012) while *Sulfitobacter* are key in organic matter decomposition.

Actinobacterial taxa such as *Streptomyces, Nocardia, Cryobacterium* and *Nocardiopsis* revealed in this study, are abundant in endophytic communities. These microbial organisms are known to produce biologically active secondary metabolites that are of industrial importance (Chen *et al*., 2019). The genus *Nocardiopsis* consist of biotechnologically important bacterial taxa that are adaptable to a wide range of ecosystems. These groups have been reported as producers of various bioactive compounds such as tumor inducers, anticancer substances, antimicrobial agents, immunomodulators and toxins (Bennur *et al*., 2015). In addition, they secrete novel extracellular enzymes such as proteases, cellulases, amylases, xylanases, chitinases, inulinases and β-glucanases (Bennur *et al*., 2015). Previously, analysis of sequences that targeted *Curtobacterium* genus from around the globe indicated the genus to be a diverse terrestrial taxon whose isolated strains were mainly from soil habitats and plants. Some species of this Actinobacteria phyla have been shown as effective microbial agents that are capable of improving photosynthetic efficiency, modulation of osmolytes and antioxidative enzymes, development of induced systemic tolerance and alleviating salt stress in paddy plants (Vimal *et al*., 2019).

Members of genus *Rhizobia*, found to inhabit the soils within all farming systems are diazotrophic common prokaryotic symbionts forming root nodules that fix nitrogen in leguminous plants after establishment. Recently, other bacteria that form symbiotic association with legume plants have been shown to nodulate and fix nitrogen. For instance, *Devosia* were found to form a unique symbiotic relationship with nitrogen fixing root nodules of *Neptunia natans*, an aquatic legume (Raul *et al*., 2002). *Paraburkholderia,* a genus of Proteobacteria present within farming systems under investigation colonize endophytic tissues of cross breed (*Picea glauca* x *engelmannii*) and lodgepole pine capable to carry out biological nitrogen fixation and promote plant growth (Puri *et al*., 2018 and 2020). *Paracoccus* genus is biochemically versatile with various metabolic roles in degradation of diverse compounds hence, has applications in bioremediation (Rzeszcz *et al*., 2018). *Paracoccus* genera present in this study for

example, *Paracoccus denitrificans* are indicators for denitrification in which nitrate is reduced to dinitrogen under anaerobic conditions. Denitrification is significant in greenhouse gas emission, soil fertility, waste management and waste water treatment processes. In addition, these denitrification characteristics of *Paracoccus denitrificans* are presumed to highly contribute to loss of nitrogen fertilizers within agricultural soils (Kelly *et al*., 2006). Other groups associated with Nitrogen cycle within the farming systems include *Methylobacterium*, *Ochrabactrum*, *Phyllobacterium*, *Burkholderia*, *Ralstonia* and *Cupriavidus* (Willems, 2006; Sprent, 2009).

The mRNA transcripts in this study revealed other prokaryotes previously shown to participate significantly in soil element and material recycling such as genus *Dyella* normally isolated from soil and rhizosphere. Probiotics such as *Bifidobacteria* that help the body in metabolic functions such as staving off harmful bacteria and digestion (Hills *et al*., 2019) were similarly found to colonize soils within farming systems. Some bacteria belonging to the genera *Bacillus, Azospirillum, Pseudomonas, Acetobacter, Azotobacter, Klebsiella, Burkholderia,* and *Serratia* have been documented to be capable of promoting plant growth (Glick, 1995; Jones *et al*., 2007). Amongst numerous species of plant growth-promoting bacteria, *Bacillus* and *Pseudomonas* spp. have been recognized as the major communities (Kang *et al*., 2015a). Some of the plant growth promoting bacteria have been commercialized owing to their persistence within a diverse range of abiotic and biotic environments. Some members of the genus *Bacillus* inhibit growth of pathogenic microorganisms in soil and/or in plant tissues besides harmful effects of the pathogens in plants (Jamil *et al*., 2017). For instance, pathogenic bacteria such as *Pseudomonas savastanoi, Ralstonia solanacearum,* and *Xanthomonas axonopodis* infect plants and cause diseases. Genus *Kordiimonas* that have been described as essential constituents of biofilms with potential to degrade several polycyclic hydrocarbons (Xu *et al*., 2014) was present in the farming systems. *Kordiimonas gwangyangensis* strains possess alkane hydroxylase enzymes and are of prospective interest in bioremediation (Wang *et al*., 2010).

Affiliates of genus *Variovorax* present in farming systems have been found in the surrounding soil and plant root rhizosphere. This genus is associated with species with

diverse metabolic abilities that facilitate degradation of a wide assortment of recalcitrant organic pollutants including aliphatic polycarbonates, polychlorinated biphenyls and 2, 4-dinitrotoluene (Barbara *et al*., 2012). *Variovorax* species catabolic capabilities have been explored in numerous plant species with implications of mechanisms promoting growth comprising; increase in nutrient availability, inhibiting growth of plant pathogenic microorganisms and reduction of plant stress (Han *et al*., 2011). A study conducted in the rhizosphere of *Pisum sativum* displayed *V. paradoxus* to increase plant growth and yield by degrading 1-aminocyclopropane-1-carboxylate (ACC), the ethylene precursor molecule by means of a secreted enzyme, ACC deaminase (Belimov *et al*., 2009). *V. paradoxus* strains capable of degrading N-acyl homoserine-lactones, the microbial signaling molecules in quorum sensing (Leadbetter *et al*., 2000). It is postulated that this ability may possibly provide protection of the host plant from pathogenic infection, with the influence of quorum quenching to reduce virulence in present pathogenic strains. Other *Variovorax* species applications include their role in cycling various inorganic elements including sulfur, manganese, arsenic and rare earth elements in various ecosystems (Manjiroh *et al*., 1998; Macur *et al*., 2003; Nogueira, *et al*., 2007; Schmalenberger *et al*., 2008). Members of genus *Sphingobium* found in this study have been reported to have xenobiotic degrading ability. These microbial groups are efficient degraders of a wide range of chlorinated and aromatic hydrocarbons, and may possess roles in bioremediation (Verma *et al*., 2014).

Genus *Vitreoscilla* present in the farming systems has been previously found to have a wide array of biological and industrial applications some of which have been exploited as potential benefits in biotechnology industries. These include cell growth promotion, protein synthesis, enhanced metabolism and metabolite productivity, increased respiration, cellular detoxification, fermentation for ethanol production and biodegradation (Stark *et al*., 2011). *Bacillus* and *Pseudomonas* genera secrete several metabolites that trigger plant growth and prevent pathogen infection, hence are the prominent plant growth-promoting bacteria (Radhakrishnan *et al*., 2017).

This study also revealed some prokaryotic taxa that are most associated with pathogenic infections in crops, livestock and humans. Some of these include *Xanthomonas*,

Stenotrophomonas, *Microbacterium*, *Sphingomonas*, *Erwinia*, *Pseudomonas*, *Halomonas*, *Flavobacterium*, *Dolosigranulum*, *Staphylococcus*, *Streptococcus* and *Chryseobacterium* among others. For instance, *Xanthomonas* which includes several pathovars that are mainly plant pathogens produce extracellular protease enzymes and Type II secretion systems primarily to help these bacteria in colonizing their host plant. Members of genus *Halomonas* are extremely versatile with ability to effectively grow in a variation of temperature and pH conditions. This adaptability is significant and may enable these microorganisms to substitute the exploitation of starch-derived raw materials (Okamoto *et al*., 2004).

Several studies on soil viruses have intentionally concentrated on autecology of specific viruses in the environment conducted from perspectives of crop production and epidemiology. The multiplication, inactivation, existence and fate of particular viruses has been the main concerns. For instance, special focus has been directed towards studying viruses infecting useful and undesirable microorganisms such as *rhizobia* and plant pathogens respectively, in agriculture (Kimura *et al*., 2008). Over the last 5 decades, agronomic benefits have constantly been the drive for carrying out research on soil viruses. During this period, several studies have examined and elucidated *rhizobiphage* populations, r*hizobiphages* host range among *Rhizobium spp*., and the effects of *rhizobiphages* lysogenic and lytic infection on stimulation of growth by arbuscular fungus, formation of nodules and yield of leguminous plants (Abebe *et al*., 1992; Novikova and Limeschenko, 1992; Novikova *et al*., 1993; Hussein *et al*., 1994; El Didamony and Abdel-Fattah, 1998). In this present study, *Myoviridae* (a family of bacteriophages within which bacteria and archaea serve as natural hosts), was among the most predominant viral taxa. *Myoviridae* family comprises 434 species, divided among five subfamilies and 168 genera. Most *Myoviridae* are lytic phages and have been investigated for possible use as a remedy in management of bacterial diseases in humans and other animals (Rosanna *et al*., 2007). The effects of viruses on soil-borne plant pathogens and beneficial bacteria have also been comprehensively explored (Gross *et al*., 1991; Toyoda *et al*., 1991; Hashem *et al*., 1996; Ezuka and Kaku, 2000; McKenna *et al*., 2001). In this study, *Closteroviridae* viruses' family comprise of 56 species, divided

among 4 genera and have plants serving as their natural hosts. Yellowing and necrosis diseases that particularly affect the phloem are associated with *Closteroviridae* family (ICTV, 2014). A wide diversity of viruses observed could be credited to the fact that some viruses exist in association with prokaryotic groups either as hosts or in symbiotic relationships. Several viruses are also common in the environment and due to their fast growth rates; they could be potential candidates for utilization during cloning. Conversely, the extent to which they control the diversity, composition and structure of microbial community within these agricultural environments remains unknown.

The diversity of eukaryotes observed in this study which included; Fungi and higher eukaryotes such as *Annelida*, *Apicomplexa* and *Arthropoda* play key roles at various trophic levels in the food web within farming ecosystem. Fungi are successful soil inhabitants, attributable to their high capacity and plasticity to adopt abundant forms in response to hostile environments (Sun *et al*., 2005). They regulate the balance of carbon and nutrients by producing a range of extracellular enzymes which enables them to break down all kinds of organic matter thus, decomposing soil components (Žifčáková *et al*., 2016). Fungi regulate the dynamics of physiological processes in soil environment and are responsible for soil structure formation and modification of habitats for other organisms. Biological controlling fungi regulate pathogens, pests and growth of other organisms (Bagyaraj and Ashwin, 2017). For instance, *Mycorrhiza* fungi increase the uptake of nutrients and protect plants against pathogens as biological agents thus influencing soil health (Frąc *et al*., 2015; Bagyaraj and Ashwin, 2017). Fungi participate in biological control against root pathogens, hormone production, nitrogen fixation, and protection of plants against drought (Jayne and Quigley, 2014; Baum *et al*., 2015; El-Komy *et al*., 2015). Moreover, they are vital in decomposition of residues and stabilization of soil organic matter (Treseder and Lennon, 2015). Fungal populaces are remarkably influenced by the diversity and composition of plant community. In response, this affects plant growth through fungal effect on nutrient cycling, availability, mutualism and pathogenicity (Wardle, 2002; Wagg *et al*., 2014; Hannula *et al*., 2017).

5.2 Conclusion

- It was evident that microbial diversity within the farming systems was influenced by complex interactions between a wide range of soil physicochemical properties and agricultural inputs, demonstrating that microorganisms within farming systems are remarkably diverse. These inputs amend soil properties and microbial diversity, which in turn manipulates nutrient cycling processes altering soil fertility, plant productivity and environmental sustainability.
- Conventional farming systems were shown to support more diverse microbial communities compared to organic farming systems, possibly due to the integration of organic and inorganic inputs into conventional farming systems which enhanced nutrient availability for fungal proliferation, thus increasing their diversity.
- The study on diversity of metabolic pathways and functional genes of microbial communities within farming systems revealed dissimilarities in composition of microorganisms and metabolism pathways amongst the farming systems transcriptomes; as well as microbial taxa and metabolic pathways previously un-observed.
- Some of the microorganisms detected are relatives of viral lineages that could be causing microbial mortality within the farming systems.
- The results provide evidence that microbes existing within the farming systems depended heavily on organic carbon, aromatic compounds, heterocyclic compounds, amino acids and structural sugars within the surrounding environment.
- Some of the major sugar metabolism pathways across the samples were represented by TCA cycle, glycolysis and Pentose phosphate pathway among others. The most important pathways in energy metabolism included nitrogen, oxidative phosphorylation, methane and; carbon fixation pathways in prokaryotes and photosynthetic organisms. These are important in supporting various metabolic pathways and enhancing microbial survival within the agricultural ecosystem.

- The study gives an insight of possible metabolic processes within the farming systems and the genes possibly responsible for specific pathways.
- The unassigned microbial species and KEGG Orthologs within the various functional classifications points towards a need for a comprehensive survey of microbial communities to establish an actual picture of various pathways within the farming systems and how they occur.

5.3 Recommendations

- Future studies should endeavor to build knowledge on soil and plant microbial biodiversity. This is in relation to common agronomic practices in different crop growth stages within farming systems, unravelling functional relations of soil-plant microbe interactions as well as developing strategies and tools for sustainable soil/plant management.
- The aim for future agricultural practices will be to safeguard agro-biodiversity by applying microbiome science in order to improve plant health, productivity, nutrient availability, and defense to diseases for a sustainable agriculture and environment.
- Fungal diversity composition results offer a baseline for further studies on regulation of quality and quantity of farming inputs and could provide guidance for selecting the best farming system model to protect soil ecology.
- The study on diversity of metabolic pathways and functional genes of microbial communities within farming systems provides novel insights into the composition of prokaryotic, eukaryotic and viral communities within these agro-ecosystems as well as their possible ecological function. This data could serve as a basis for development of culture dependent techniques for yet uncultivated microorganisms and unrecognized species.
- Integration of data from metagenomics, metatranscriptomics, proteomics, and classical microbiology can guide in development of protocols for culturing uncultured organisms from these environments with the aim of manipulating them for practical applications in biotechnology as well as day to day life.
- More studies should be done on fungi viruses and other eukaryotes from farming systems to reveal their diversity and functional role within the habitat.
- A keen follow-up study should be done to unearth the gene expression patterns when different crops are at different stages of growth.

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APPENDICES

Appendix 1: DNA Extraction Reagents

- Solution 1
    - 50 mM Tris pH 8.5
    - 50 mM EDTA pH 8.0
    - 25 % Sucrose solution

- Solution 2
    - 10 mM Tris pH 8.5
    - 5 mM EDTA pH 8.0
    - 1 % SDS
- Lysozyme 20 mg/ml
- RNase A 20 mg/ml
- Proteinase K 20 mg/ml
- Phenol
- Chloroform
- Absolute ethanol.
- 3 M NaCl
- Isopropanol

Appendix 2: RNA Extraction Reagents

- TRIZOL LS reagent
- Chloroform
- Isopropanol
- Ethanol
- Glycogen or GlycoBlue
- RNAse free water

Appendix 3: Energy Metabolism Enzymes

Carbon fixation in photosynthetic organisms	Malate dehydrogenase, malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+), glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, pyruvate, orthophosphate dikinase, phosphoenolpyruvate carboxylase, ribulose-bisphosphate carboxylase large chain, phosphoenolpyruvate carboxykinase (ATP), ribose 5-phosphate isomerase A and ribose 5-phosphate isomerase B
Carbon fixation pathways in prokaryotes	Malate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase fumarate reductase, flavoprotein subunit, methylenetetrahydrofolate reductase (NADPH), acetyl-CoA C-acetyltransferase, acetate kinase, pyruvate orthophosphate dikinase, methylenetetrahydrofolate dehydrogenase (NADP+) methenyltetrahydrofolate cyclohydrolase, phosphoenolpyruvate carboxylase, fumarate hydratase, class II, aconitate hydratase, aconitate hydratase 2 2-methylisocitrate dehydratase, acetyl-CoA synthetase, succinyl-CoA synthetase alpha subunit, succinyl-CoA synthetase beta subunit, acetyl-CoA carboxylase, biotin carboxylase subunit, acetyl-CoA carboxylase carboxyl transferase subunit alpha, pyruvate-ferredoxin/flavodoxin oxidoreductase, acetyl-CoA synthase, malonyl-CoA/succinyl-CoA reductase (NADPH), putative phosphotransacetylase, ATP-citrate lyase beta-subunit, NADH-dependent fumarate reductase subunit C, 3-hydroxypropionyl-CoA synthetase (ADP-forming),biotin carboxyl carrier protein, succinate dehydrogenase fumarate reductase, subunit D and putative succinate dehydrogenase fumarate reductase, subunit D
Methane metabolism	Malate dehydrogenase, D-3-phosphoglycerate dehydrogenase 2-oxoglutarate reductase, S-(hydroxymethyl)glutathione dehydrogenase alcohol dehydrogenase, formate dehydrogenase iron-sulfur subunit, formate dehydrogenase subunit gamma, dimethylamine/trimethylamine dehydrogenase, glycine hydroxymethyltransferase, phosphoserine aminotransferase, acetate kinase, phosphoserine phosphatase,

	phosphoenolpyruvate carboxylase, acetyl-CoA synthetase, sulfopyruvate decarboxylase subunit alpha, (4-(4-[2-(gamma-L-glutamylamino)ethyl]phenoxymethyl)furan-2-yl)methanamine synthase, methane/ammonia monooxygenase subunit A, methanogen homocitrate synthase, phosphate acetyltransferase.
Nitrogen metabolism	2-phospho-L-lactate guanylyltransferase, methylamine dehydrogenase light chain, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase, monomethylamine corrinoid protein, 6-phosphofructokinase 2, methylamine-glutamate N-methyltransferase subunit B, heterodisulfide reductase subunit C1, nitronate monooxygenase, carbonic anhydrase, nitrate reductase (NAD(P)H), hydroxylamine dehydrogenase, nitrate/nitrite transport system substrate-binding protein, nitrate/nitrite transport system ATP-binding protein fungal nitric oxide reductase, hydrazine synthase subunit, hydrazine dehydrogenase and vanadium-dependent nitrogenase alpha chain
Oxidative phosphorylation	Succinate dehydrogenase (ubiquinone) membrane anchor subunit, succinate dehydrogenase fumarate reductase, flavoprotein subunit, NADH-quinone oxidoreductase subunit H, NADH-quinone oxidoreductase subunit N, ubiquinol-cytochrome c reductase cytochrome b subunit, cytochrome bd ubiquinol oxidase subunit I, inorganic pyrophosphatase, F-type H+-transporting ATPase subunit a, b and c, F-type H+-transporting ATPase subunit alpha, F-type H+-transporting ATPase subunit delta, F-type H+-transporting ATPase subunit c, cytochrome c oxidase subunit 1, cytochrome c oxidase subunit 2, cytochrome c oxidase subunit 3, cytochrome c oxidase subunit I, cytochrome o ubiquinol oxidase subunit I, cytochrome o ubiquinol oxidase subunit III, menaquinol-cytochrome c reductase cytochrome b/c subunit, NADH dehydrogenase (ubiquinone) Fe-S protein 1, NADH dehydrogenase (ubiquinone) Fe-S protein 3, NADH dehydrogenase (ubiquinone) flavoprotein 2, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 8, NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 1, NAD(P)H-quinone oxidoreductase subunit 4L,

	nucleosome-remodeling factor 38 kDa subunit and succinate dehydrogenase fumarate reductase, subunit D
Photosynthesis	F-type H+-transporting ATPase subunit a, b and c, F-type H+-transporting ATPase subunit alpha, F-type H+-transporting ATPase subunit delta, photosystem I P700 chlorophyll a apoprotein A2, photosystem II P680 reaction center D1 protein, photosystem II Psb28-2 protein, photosystem I subunit V, light-harvesting complex II chlorophyll a/b binding protein 1 and R-phycocyanin alpha-cysteine-84 phycourobilin lyase/isomerase
Sulfur metabolism	Phosphoadenosine phosphosulfate reductase, 3'(2'), 5'-bisphosphate nucleotidase, cysteine synthase, sulfate transport system substrate-binding protein, alkanesulfonate monooxygenase, sulfite dehydrogenase, adenylyl-sulfate reductase (glutathione), thiosulfate reductase polysulfide reductase chain A, cysteine synthase O-phosphoserine sulfhydrylase cystathionine beta-synthase, cystathionine gamma-lyase homocysteine desulfhydrase, sulfur reductase FeS subunit, sulfur-oxidizing protein SoxX and sulfhydrogenase subunit beta (sulfur reductase)

Appendix 4: Carbohydrate Metabolism Enzymes

Amino sugar and nucleotide sugar metabolism	UDP-N-acetylmuramate dehydrogenase, UDP-N-acetylglucosamine 1-carboxyvinyltransferase, fructokinase, galactokinase, beta-N-acetylhexosaminidase, UDP-N-acetylglucosamine 2-epimerase (non-hydrolysing), mannose-6-phosphate isomerase, UDP-galactopyranose mutase, PTS system, sugar-specific IIA component, PTS system, mannose-specific IID component, PTS system, N-acetylglucosamine-specific IIA component, phosphoglucosamine mutase, hexosaminidase, bifunctional chitinase/lysozyme, alpha-1,4-galacturonosyltransferase
Ascorbate and aldarate metabolism	L-xylulokinase, galactarate dehydratase, L-ribulose-5-phosphate 4-epimerase, 3-dehydro-L-gulonate-6-phosphate decarboxylase, L-ribulose-5-phosphate 3-epimerase
Butanoate metabolism	Succinate dehydrogenase fumarate reductase, flavoprotein subunit, acetyl-CoA C-acetyltransferase, formate C-acetyltransferase, 3-oxoacid CoA-transferase subunit B, acetate CoA/acetoacetate CoA-transferase alpha subunit, acetate CoA/acetoacetate CoA-transferase beta subunit, acetolactate synthase I/II/III large subunit, pyruvate-ferredoxin/flavodoxin oxidoreductase and acetaldehyde dehydrogenase alcohol dehydrogenase
C5-Branched dibasic acid metabolism	3-isopropylmalate dehydrogenase, acetolactate synthase I/II/III large subunit, 3-isopropylmalate/(R)-2-methylmalate dehydratase large subunit, succinyl-CoA synthetase alpha subunit and succinyl-CoA synthetase beta subunit
Citrate cycle (TCA cycle)	Malate dehydrogenase, isocitrate dehydrogenase, pyruvate dehydrogenase E1 component alpha subunit, 2-oxoglutarate dehydrogenase E1 component, succinate dehydrogenase (ubiquinone) membrane anchor subunit, succinate dehydrogenase fumarate reductase, flavoprotein subunit, dihydrolipoamide dehydrogenase, 2-oxoglutarate dehydrogenase E2 component (dihydrolipoamide succinyltransferase), phosphoenolpyruvate carboxykinase (ATP),

	citrate synthase, fumarate hydratase, class II, aconitate hydratase, aconitate hydratase 2 2-methylisocitrate dehydratase, succinyl-CoA synthetase alpha subunit, succinyl-CoA synthetase beta subunit and pyruvate-ferredoxin/flavodoxin oxidoreductase
Fructose and mannose metabolism	Mannitol-1-phosphate 5-dehydrogenase, L-fuculokinase, allose kinase, rhamnulose-1-phosphate aldolase, ribose 5-phosphate isomerase B, mannose-6-phosphate isomerase, PTS system, fructose-specific IIA component, PTS system, glucitol/sorbitol-specific IIB component, PTS system, mannose-specific IID component, mannosyl-3-phosphoglycerate phosphatase, 6-phosphofructokinase 2, D-allulose-6-phosphate 3-epimerase
Galactose metabolism	Galactokinase, 2-dehydro-3-deoxygalactonokinase, aldose 1-epimerase, UDP-galactopyranose mutase, galactosamine-6-phosphate isomerase, alpha-galactosidase, PTS system, galactosamine-specific IID component, evolved beta-galactosidase subunit alpha, evolved beta-galactosidase subunit beta and 6-phosphofructokinase 2
Glycolysis Gluconeogenesis	S-(hydroxymethyl)glutathione dehydrogenase alcohol dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase, pyruvate dehydrogenase E1 component alpha subunit, dihydrolipoamide dehydrogenase, pyruvate kinase, phosphoglycerate kinase, 6-phospho-beta-glucosidase, 6-phospho-beta-glucosidase, phosphoenolpyruvate carboxykinase (ATP), aldose 1-epimerase, acetyl-CoA synthetase, PTS system, sugar-specific IIA component, pyruvate-ferredoxin/flavodoxin oxidoreductase, alcohol dehydrogenase, acetaldehyde dehydrogenase alcohol dehydrogenase, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase and 6-phosphofructokinase 2
Glyoxylate and dicarboxylate metabolism	Malate dehydrogenase, formate dehydrogenase subunit gamma, glycine dehydrogenase, dihydrolipoamide dehydrogenase, glycine hydroxymethyltransferase, ribulose-bisphosphate carboxylase large chain, citrate synthase, aconitate hydratase, acetyl-CoA synthetase, L(+)-tartrate dehydratase beta subunit, catalase, glycolate oxidase FAD

	binding subunit and glycolate oxidase iron-sulfur subunit
Inositol phosphate metabolism	1-phosphatidylinositol-3-phosphate 5-kinase, 3-phytase, myo-inositol-1(or 4)-monophosphatase and synaptojanin
Pentose and glucuronate interconversions	Fructuronate reductase, 2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase, L-xylulokinase, pectinesterase, rhamnulose-1-phosphate aldolase, glucuronate isomerase, 4-deoxy-L-threo-5-hexosulose-uronate ketol-isomerase, L-ribulose-5-phosphate 4-epimerase, 3-dehydro-L-gulonate-6-phosphate decarboxylase, L-ribulose-5-phosphate 3-epimerase, L-gulonate 5-dehydrogenase, mannonate dehydratase, xylonate dehydratase and 2-dehydro-3-deoxy-D-pentonate aldolase
Pentose phosphate pathway	6-phosphogluconate dehydrogenase, glucose-6-phosphate 1-dehydrogenase, ribose-phosphate pyrophosphokinase, ribose 5-phosphate isomerase A, ribose 5-phosphate isomerase B, ribose 1,5-bisphosphokinase, 6-phosphofructokinase 2
Propanoate metabolism	Dihydrolipoamide dehydrogenase, formate C-acetyltransferase, acetate kinase, propionate kinase, acetate CoA/acetoacetate CoA-transferase alpha subunit, acetate CoA/acetoacetate CoA-transferase beta subunit, 2-methylcitrate synthase, 2-methylcitrate dehydratase, acetyl-CoA synthetase, succinyl-CoA synthetase alpha subunit, succinyl-CoA synthetase beta subunit, acetyl-CoA carboxylase, biotin carboxylase subunit, acetyl-CoA carboxylase carboxyl transferase subunit alpha, methylisocitrate lyase, 3-hydroxyisobutyryl-CoA hydrolase and methylglyoxal reductase
Pyruvate metabolism	Malate dehydrogenase, malate dehydrogenase (oxaloacetate-decarboxylating), malate dehydrogenase (oxaloacetate-decarboxylating)(NADP+), pyruvate dehydrogenase (quinone), pyruvate oxidase, pyruvate dehydrogenase E1 component alpha subunit, dihydrolipoamide dehydrogenase, formate C-acetyltransferase, acetate kinase, pyruvate, orthophosphate dikinase,

	phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxykinase (ATP), 2-isopropylmalate synthase, lactoylglutathione lyase, acetyl-CoA synthetase, acetyl-CoA carboxylase, biotin carboxylase subunit, acetyl-CoA carboxylase carboxyl transferase subunit alpha, pyruvate-ferredoxin/flavodoxin oxidoreductase, D-lactate dehydrogenase (quinone) and acetaldehyde dehydrogenase alcohol dehydrogenase
Starch and sucrose metabolism	Glycogen phosphorylase, trehalose 6-phosphate synthase, 4-alpha-glucanotransferase, trehalose 6-phosphate phosphatase, alpha-amylase, 6-phospho-beta-glucosidase, 6-phospho-beta-glucosidase, PTS system, cellobiose-specific IIA component, PTS system, cellobiose-specific IIC component, PTS system, sugar-specific IIA component, PTS system, trehalose-specific IIB component, beta-glucosidase, beta-glucosidase and dextranase

Appendix 5: Xenobiotic Biodegradation and Metabolism Enzymes

Aminobenzoate degradation	Monooxygenase, nitrile hydratase subunit alpha, nitrile hydratase subunit beta, Amidase, nitrilase, acylphosphatase, benzoate-CoA ligase, mandelamide amidase, mandelate racemase, (S)-mandelate dehydrogenase, benzoylformate decarboxylase, benzaldehyde dehydrogenase (NAD), benzoate 4-monooxygenase, 4-hydroxybenzoate-CoA ligase, 3-hydroxybenzoate/4-hydroxybenzoate--CoA ligase, 4-hydroxybenzoyl-CoA reductase subunit gamma, 4-hydroxybenzoyl-CoA reductase subunit alpha, 4-hydroxybenzoyl-CoA reductase subunit beta, phenol 2-monooxygenase, 4-hydroxybenzoate decarboxylase subunit C, vanillate/4-hydroxybenzoate decarboxylase subunit C, vanillate/4-hydroxybenzoate decarboxylase subunit D, flavin prenyltransferase, 2-aminobenzoate-CoA ligase, anthraniloyl-CoA monooxygenase, 2,3-dihydroxybenzoate decarboxylase, anthranilate 1,2-dioxygenase (deaminating, decarboxylating) large subunit, anthranilate 1,2-dioxygenase (deaminating, decarboxylating) small subunit, anthranilate 1,2-dioxygenase reductase component, anthranilate 1,2-dioxygenase large subunit, anthranilate 1,2-dioxygenase small subunit, anthranilate 1,2-dioxygenase ferredoxin component, anthranilate 1,2-dioxygenase ferredoxin reductase component, 2-nitrobenzoate nitroreductase, 2-hydroxylaminobenzoate mutase, naphthalene 1,2-dioxygenase subunit alpha, naphthalene 1,2-dioxygenase subunit beta, naphthalene 1,2-dioxygenase ferredoxin component, naphthalene 1,2-dioxygenase ferredoxin reductase component, 2-nitrobenzene nitroreductase, 2-Hydroxyamino benzene mutase, 2-aminophenol/2-amino-5-chlorophenol 1,6-dioxygenase subunit alpha, 2-aminophenol/2-amino-5-chlorophenol 1,6-dioxygenase subunit beta, 5,5'-dehydrodivanillate O-demethylase, OH-DDVA oxygenase, OH-DDVA meta-cleavage compound hydrolase, 5-carboxyvanillate decarboxylase, vanillin dehydrogenase, vanillate monooxygenase, vanillate monooxygenase ferredoxin subunit, protocatechuate 4,5-dioxygenase, alpha chain, protocatechuate 4,5-dioxygenase, beta chain, gallate dioxygenase, 2-hydroxy-4-

	carboxymuconate semialdehyde hemiacetal dehydrogenase, 2-pyrone-4,6-dicarboxylate lactonase, syringate O-demethylase, 3-O-methylgallate 3,4-dioxygenase, vanillate/3-O-methylgallate O-demethylase, acetate CoA/acetoacetate CoA-transferase alpha subunit, acetate CoA/acetoacetate CoA-transferase beta subunit, acetate CoA-transferase, enoyl-CoA hydratase, enoyl-CoA hydratase long-chain 3-hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase 3-hydroxyacyl-CoA dehydrogenase 3,2-trans-enoyl-CoA isomerase, enoyl-CoA hydratase, unspecific monooxygenase, cytochrome P450 NADPH-cytochrome P450 reductase, 4-nitrophenyl phosphatase, 4-nitrocatechol/4-nitrophenol 4-monooxygenase, 4-nitrophenol 2-monooxygenase 4-nitrocatechol 4-monooxygenase, oxygenase component, 4-nitrophenol 2-monooxygenase 4-nitrocatechol 4-monooxygenase, reductase component, 3-(hydroxyamino)phenol mutase, 4-sulfomuconolactone hydrolase
Benzoate degradation	Benzoate/toluate 1,2-dioxygenase subunit alpha, benzoate/toluate 1,2-dioxygenase subunit beta, benzoate/toluate 1,2-dioxygenase reductase component, dihydroxycyclohexadiene carboxylate dehydrogenase, catechol 1,2-dioxygenase, muconate cycloisomerase, muconolactone D-isomerase, 3-oxoadipate enol-lactonase, 3-oxoadipate enol-lactonase 4-carboxymuconolactone decarboxylase, 3-oxoadipate CoA-transferase, alpha subunit, 3-oxoadipate CoA-transferase, beta subunit, acetyl-CoA acyltransferase, 3-oxoadipyl-CoA thiolase, benzene/toluene/chlorobenzene dioxygenase subunit alpha, benzene/toluene/chlorobenzene dioxygenase subunit beta, benzene/toluene/chlorobenzene dioxygenase ferredoxin component, benzene/toluene/chlorobenzene dioxygenase ferredoxin reductase component, cis-1,2-dihydrobenzene-1,2-diol dehydrogenase, phenol hydroxylase P0 - P5 proteins, 2,3-dihydroxybenzoate decarboxylase, catechol 2,3-dioxygenase, catechol 2,3-dioxygenase, 2,3-dihydroxy-p-cumate/2,3-dihydroxybenzoate 3,4-dioxygenase, HCOMODA/2-hydroxy-3-carboxy-muconic semialdehyde decarboxylase, 2-hydroxymuconate-semialdehyde hydrolase, 2-keto-4-pentenoate

	hydratase, 2-oxopent-4-enoate/cis-2-oxohex-4-enoate hydratase, 4-hydroxy 2-oxovalerate aldolase, 4-hydroxy-2-oxovalerate/4-hydroxy-2-oxohexanoate aldolase, acetaldehyde dehydrogenase, acetaldehyde/propanal dehydrogenase, aminomuconate-semialdehyde/2-hydroxymuconate-6-semialdehyde dehydrogenase, 2-aminobenzenesulfonate 2,3-dioxygenase subunit alpha, 2-aminobenzenesulfonate 2,3-dioxygenase subunit beta, 4-oxalocrotonate tautomerase, 2-oxo-3-hexenedioate decarboxylase, gamma-resorcylate decarboxylase, resorcinol 4-hydroxylase (FADH2), resorcinol 4-hydroxylase (NADPH), resorcinol 4-hydroxylase (NADH), hydroxyquinol 1,2-dioxygenase, maleylacetate reductase, protocatechuate 3,4-dioxygenase, alpha subunit, protocatechuate 3,4-dioxygenase, beta subunit, 3-carboxy-cis,cis-muconate cycloisomerase, 4-carboxymuconolactone decarboxylase, carboxy-cis,cis-muconate cyclase, protocatechuate 4,5-dioxygenase, alpha chain, protocatechuate 4,5-dioxygenase, beta chain, 2-hydroxy-4-carboxymuconate semialdehyde hemiacetal dehydrogenase, 2-pyrone-4,6-dicarboxylate lactonase, 4-oxalomesaconate tautomerase, 4-oxalmesaconate hydratase, 4-oxalomesaconate hydratase, 4-hydroxy-4-methyl-2-oxoglutarate aldolase, benzoate 4-monooxygenase, 4-methoxybenzoate monooxygenase (O-demethylating), p-hydroxybenzoate 3-monooxygenase, 3-hydroxybenzoate 4-monooxygenase, 3-hydroxybenzoate 6-monooxygenase, 4-hydroxybenzoate-CoA ligase, 3-hydroxybenzoate/4-hydroxybenzoate--CoA ligase, 4-hydroxybenzoyl-CoA thioesterase, 4-hydroxybenzoyl-CoA reductase subunit gamma, 4-hydroxybenzoyl-CoA reductase subunit alpha, 4-hydroxybenzoyl-CoA reductase subunit beta, benzoate-CoA ligase, benzoyl-CoA reductase subunit C, benzoyl-CoA reductase subunit B, benzoyl-CoA reductase subunit A, benzoyl-CoA reductase subunit D, benzoyl-CoA reductase subunit BamB, benzoyl-CoA reductase subunit BamC, cyclohexa-1,5-dienecarbonyl-CoA hydratase, 6-hydroxycyclohex-1-ene-1-carbonyl-CoA dehydrogenase, 6-oxocyclohex-1-ene-carbonyl-CoA hydrolase, cyclohex-1-ene-1-

	carbonyl-CoA dehydrogenase, cyclohexane-1-carbonyl-CoA dehydrogenase, cyclohexanecarboxylate-CoA ligase, cyclohexanecarboxyl-CoA dehydrogenase, cyclohex-1-ene-1-carboxyl-CoA hydratase, 2-hydroxycyclohexanecarboxyl-CoA dehydrogenase, 2-ketocyclohexanecarboxyl-CoA hydrolase, pimeloyl-CoA dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase enoyl-CoA hydratase 3-hydroxybutyryl-CoA epimerase, 3-hydroxyacyl-CoA dehydrogenase enoyl-CoA hydratase 3-hydroxybutyryl-CoA epimerase enoyl-CoA isomerase, 3-hydroxyacyl-CoA dehydrogenase, glutaryl-CoA dehydrogenase (non-decarboxylating), glutaconyl-CoA decarboxylase, glutaryl-CoA dehydrogenase, enoyl-CoA hydratase, enoyl-CoA hydratase, 3-hydroxybutyryl-CoA dehydrogenase, acetyl-CoA C-acetyltransferase, benzoyl-CoA 2,3-epoxidase subunit A, benzoyl-CoA 2,3-epoxidase subunit B, 3,4-dehydroadipyl-CoA semialdehyde dehydrogenase, benzoyl-CoA-dihydrodiol lyase
Chloroalkane and chloroalkene degradation	Haloalkane dehalogenase, alcohol dehydrogenase, propanol-preferring alcohol dehydrogenase, alcohol dehydrogenase S-(hydroxymethyl)glutathione dehydrogenase, acetaldehyde dehydrogenase alcohol dehydrogenase, aldehyde dehydrogenase (NAD+), alpha-subunit of trans-3-chloroacrylic acid dehalogenase, beta-subunit of trans-3-chloroacrylic acid dehalogenase, cis-3-chloroacrylic acid dehalogenase, malonate semialdehyde decarboxylase, acetylene hydratase, nitrogenase delta subunit, nitrogenase molybdenum-iron protein beta chain, nitrogenase iron protein NifH, nitrogenase molybdenum-iron protein alpha chain, tetrachloroethene reductive dehalogenase, tetrachloroethene reductive dehalogenase membrane anchor, alkene monooxygenase alpha subunit, alkene monooxygenase beta subunit, alkene monooxygenase coupling protein, alkene monooxygenase reductase, alkene monooxygenase alpha subunit, alkene monooxygenase beta subunit, alkene monooxygenase gamma subunit, alkene monooxygenase ferredoxin subunit, alkene monooxygenase effector subunit, alkene monooxygenase ferredoxin reductase component, 2-hydroxypropyl-

	CoM lyase, soluble epoxide hydrolase lipid-phosphate phosphatase, benzene/toluene/chlorobenzene dioxygenase subunit alpha, benzene/toluene/chlorobenzene dioxygenase subunit beta, benzene/toluene/chlorobenzene dioxygenase ferredoxin component, benzene/toluene/chlorobenzene dioxygenase ferredoxin reductase component, dichloromethane dehalogenase, glutathione-independent formaldehyde dehydrogenase, formaldehyde dismutase methanol dehydrogenase, formaldehyde dismutase, methanol dehydrogenase (cytochrome c) subunit 1, methanol dehydrogenase (cytochrome c) subunit 2, alcohol dehydrogenase (cytochrome c), haloacetate dehalogenase and 2-haloacid dehalogenase
Chlorocyclohexane and chlorobenzene degradation	Haloalkane dehalogenase, benzene/toluene/chlorobenzene dioxygenase subunit alpha, benzene/toluene/chlorobenzene dioxygenase subunit beta, benzene/toluene/chlorobenzene dioxygenase ferredoxin component, benzene/toluene/chlorobenzene dioxygenase ferredoxin reductase component, cis-1,2-dihydrobenzene-1,2-diol dehydrogenase, phenol hydroxylase P0 - P5 proteins, gamma-hexachlorocyclohexane dehydrochlorinase, biphenyl-2,3-diol 1,2-dioxygenase, catechol 1,2-dioxygenase, chloromuconate cycloisomerase, carboxymethylenebutenolidase, 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase 1, 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase 2, 2,5-dichlorohydroquinone reductive dechlorinase, hydroquinone 1,2-dioxygenase, pentachlorophenol monooxygenase, tetrachlorobenzoquinone reductase, tetrachloro-p-hydroquinone reductive dehalogenase, 2,6-dichloro-p-hydroquinone 1,2-dioxygenase, maleylacetate reductase, 2,4,5-trichlorophenoxyacetic acid oxygenase1, 2,4,5-trichlorophenoxyacetic acid oxygenase 2, chlorophenol-4-monooxygenase component 1, chlorophenol-4-monooxygenase component 2, hydroxyquinol 1,2-dioxygenase, alpha-ketoglutarate-dependent 2,4-dichlorophenoxyacetate dioxygenase, 2,4-dichlorophenol 6-monooxygenase, muconate cycloisomerase, catechol 2,3-dioxygenase, catechol 2,3-dioxygenase, chlorocatechol 1,2-

	dioxygenase, 2-haloacid dehalogenase and haloacetate dehalogenase
Drug metabolism - cytochrome P450	Cytochrome P450 family 2 subfamily D polypeptide 6, cytochrome P450 family 2 subfamily C polypeptide 9, cytochrome P450 family 3 subfamily A polypeptide 4, dimethylaniline monooxygenase (N-oxide forming), cytochrome P450 family 2 subfamily C polypeptide 19, cytochrome P450 family 2 subfamily B polypeptide 6, cytochrome P450 family 3 subfamily A polypeptide 5, glutathione S-transferase, glutathione S-transferase kappa 1, prostaglandin-H2 D-isomerase glutathione transferase, alcohol dehydrogenase 1/7, alcohol dehydrogenase 4, S-(hydroxymethyl)glutathione dehydrogenase alcohol dehydrogenase, alcohol dehydrogenase 6, alcohol dehydrogenase, propanol-preferring, alcohol dehydrogenase, aldehyde dehydrogenase (NAD(P)+), monoamine oxidase, aldehyde oxidase, glucuronosyltransferase, cytochrome P450 family 1 subfamily A polypeptide 2, cytochrome P450 family 2 subfamily E polypeptide 1, cytochrome P450 family 2 subfamily C polypeptide 8, cytochrome P450 family 2 subfamily A polypeptide 6 and hypoxanthine phosphoribosyltransferase
Drug metabolism - other enzymes	IMP dehydrogenase, GMP synthase (glutamine-hydrolysing), thiopurine S-methyltransferase, xanthine dehydrogenase/oxidase, inosine triphosphate pyrophosphatase, carboxylesterase 1, carboxylesterase 2, glucuronosyltransferase, beta-glucuronidase, cytochrome P450 family 3 subfamily A polypeptide 4, cytidine deaminase, thymidine phosphorylase, dihydropyrimidine dehydrogenase (NADP+), dihydropyrimidinase beta-ureidopropionase, cytochrome P450 family 2 subfamily A polypeptide 6, uridine phosphorylase, uridine kinase, thymidine kinase, uridine monophosphate synthetase, UMP-CMP kinase, ribonucleoside-diphosphate reductase subunit M1, ribonucleoside-diphosphate reductase subunit M2, nucleoside-diphosphate kinase, dUTP pyrophosphatase, catalase-peroxidase, myeloperoxidase, arylamine N-acetyltransferase, cytochrome P450 family 2 subfamily E polypeptide

	1 and glutathione S-transferase
Ethylbenzene degradation	Naphthalene 1,2-dioxygenase subunit alpha, naphthalene 1,2-dioxygenase subunit beta, naphthalene 1,2-dioxygenase ferredoxin component, naphthalene 1,2-dioxygenase ferredoxin reductase component, ethylbenzene hydroxylase subunit alpha, ethylbenzene hydroxylase subunit beta, ethylbenzene hydroxylase subunit gamma, (S)-1-phenylethanol dehydrogenase, acetophenone carboxylase, benzoylacetate-CoA ligase, acetyl-CoA acyltransferase, ethylbenzene dioxygenase subunit alpha, ethylbenzene dioxygenase subunit beta, ethylbenzene dioxygenase ferredoxin component, 2,3-dihydroxyethylbenzene 1,2-dioxygenase and 2-hydroxy-6-oxo-octa-2,4-dienoate hydrolase.
Fluorobenzoate degradation	Benzoate/toluate 1,2-dioxygenase subunit alpha, benzoate/toluate 1,2-dioxygenase subunit beta, benzoate/toluate 1,2-dioxygenase reductase component, dihydroxycyclohexadiene carboxylate dehydrogenase, catechol 1,2-dioxygenase, chloromuconate cycloisomerase, carboxymethylenebutenolidase, muconate cycloisomerase, bromoxynil nitrilase, nitrile hydratase subunit alpha, nitrile hydratase subunit beta, pentachlorophenol monooxygenase, maleylacetate reductase, 2-halobenzoate 1,2-dioxygenase large subunit, 2-halobenzoate 1,2-dioxygenase small subunit, 2-halobenzoate 1,2-dioxygenase electron transfer component, 4-chlorobenzoate-CoA ligase and 4-chlorobenzoyl-CoA dehalogenase.
Metabolism of xenobiotics by cytochrome P450	Mytochrome P450 family 1 subfamily A polypeptide 1, cytochrome P450 family 2 subfamily C polypeptide 9, cytochrome P450 family 3 subfamily A polypeptide 4, cytochrome P450 family 1 subfamily B polypeptide 1, glutathione S-transferase, glutathione S-transferase kappa 1, prostaglandin-H2 D-isomerase glutathione transferase, microsomal epoxide hydrolase, cytochrome P450 family 2 subfamily B polypeptide 6, bile-salt sulfotransferase, cytochrome P450 family 1 subfamily A polypeptide 2, cytochrome P450 family 2 subfamily A polypeptide 6, cytochrome P450 family 2 subfamily E polypeptide 1,

	cytochrome P450 family 2 subfamily F, cytochrome P450 family 2 subfamily S polypeptide 1, 20alpha/3alpha-hydroxysteroid dehydrogenase dihydrodiol dehydrogenase, dihydrodiol dehydrogenase D-xylose 1-dehydrogenase (NADP), cytochrome P450 family 2 subfamily A polypeptide 13, cytochrome P450 family 2 subfamily D polypeptide 6, corticosteroid 11-beta-dehydrogenase isozyme 1, carbonyl reductase 1, carbonyl reductase 2, carbonyl reductase 3, glucuronosyltransferase, cytochrome P450 family 3 subfamily A polypeptide 5, aflatoxin B1 aldehyde reductase, aldehyde dehydrogenase (NAD(P)+), alcohol dehydrogenase 1/7, alcohol dehydrogenase 4, S-(hydroxymethyl)glutathione dehydrogenase alcohol dehydrogenase, alcohol dehydrogenase 6, alcohol dehydrogenase, propanol-preferring and alcohol dehydrogenase.
Naphthalene degradation	Naphthalene 1,2-dioxygenase subunit alpha, naphthalene 1,2-dioxygenase subunit beta, naphthalene 1,2-dioxygenase ferredoxin component, naphthalene 1,2-dioxygenase ferredoxin reductase component, cis-1,2-dihydro-1,2-dihydroxynaphthalene/dibenzothiophene dihydrodiol dehydrogenase, 1,2-dihydroxynaphthalene dioxygenase, 2-hydroxychromene-2-carboxylate isomerase, trans-o-hydroxybenzylidenepyruvate hydratase-aldolase, salicylaldehyde dehydrogenase, salicylate 5-hydroxylase large subunit, salicylate 5-hydroxylase small subunit, salicylate hydroxylase, alcohol dehydrogenase, propanol-preferring, alcohol dehydrogenase, alcohol dehydrogenase, S-(hydroxymethyl)glutathione dehydrogenase alcohol dehydrogenase, acetaldehyde dehydrogenase alcohol dehydrogenase, naphthyl-2-methylsuccinate synthase alpha subunit, naphthyl-2-methylsuccinate synthase beta subunit, naphthyl-2-methylsuccinate synthase gamma subunit, naphthyl-2-methylsuccinate CoA transferase subunit, naphthyl-2-methylsuccinate CoA transferase subunit, naphthyl-2-methylsuccinyl-CoA dehydrogenase, naphthyl-2-hydroxymethylsuccinyl-CoA hydratase, naphthyl-2-hydroxymethylsuccinyl-CoA dehydrogenase BnsC subunit, naphthyl-

	2-hydroxymethylsuccinyl-CoA dehydrogenase BnsD subunit, naphthyl-2-oxomethyl-succinyl-CoA thiolase subunit, naphthyl-2-oxomethyl-succinyl-CoA thiolase subunit and 2-naphthoate monooxygenase
Nitrotoluene degradation	Nitroreductase, nitroreductase dihydropteridine reductase, N-ethylmaleimide reductase, hydrogenase large subunit, hydrogenase small subunit, anaerobic carbon-monoxide dehydrogenase catalytic subunit, anaerobic carbon-monoxide dehydrogenase iron sulfur subunit, pyruvate ferredoxin oxidoreductase alpha subunit, pyruvate ferredoxin oxidoreductase beta subunit, pyruvate ferredoxin oxidoreductase gamma subunit, pyruvate ferredoxin oxidoreductase delta subunit, dissimilatory sulfite reductase alpha subunit, dissimilatory sulfite reductase beta subunit, arylamine N-acetyltransferase, naphthalene 1,2-dioxygenase subunit alpha, naphthalene 1,2-dioxygenase subunit beta, naphthalene 1,2-dioxygenase ferredoxin component, naphthalene 1,2-dioxygenase ferredoxin reductase component and 4-methyl-5-nitrocatechol 5-monooxygenase
Styrene degradation	Styrene monooxygenase, styrene monooxygenase reductase component, styrene-oxide isomerase, phenylacetaldehyde dehydrogenase, phenylacetate 2-hydroxylase, homogentisate 1,2-dioxygenase, maleylacetoacetate isomerase, fumarylacetoacetase, fumarylacetoacetate (FAA) hydrolase, phenylacetaldoxime dehydratase, nitrile hydratase subunit alpha, nitrile hydratase subunit beta, amidase, nitrilase, 3-hydroxyphenylacetate 6-hydroxylase, cis-1,2-dihydrobenzene-1,2-diol dehydrogenase, catechol 2,3-dioxygenase, catechol 2,3-dioxygenase, 2-hydroxymuconate-semialdehyde hydrolase, aliphatic nitrilase, glutaconate CoA-transferase, subunit A, glutaconate CoA-transferase, subunit B, lactoyl-CoA dehydratase subunit alpha and lactoyl-CoA dehydratase subunit beta
Toluene degradation	Propionate CoA-transferase, benzylsuccinate synthase,

	benzylsuccinate CoA-transferase BbsE subunit, benzylsuccinate CoA-transferase BbsF subunit, (R)-benzylsuccinyl-CoA dehydrogenase, E-phenylitaconyl-CoA hydratase, 2-[hydroxy(phenyl)methyl]-succinyl-CoA dehydrogenase BbsC subunit, 2-[hydroxy(phenyl)methyl]-succinyl-CoA dehydrogenase BbsD subunit, benzoylsuccinyl-CoA thiolase BbsA subunit, benzoylsuccinyl-CoA thiolase BbsB subunit, phenol hydroxylase P0 - P5 proteins, benzene/toluene/chlorobenzene dioxygenase subunit alpha, benzene/toluene/chlorobenzene dioxygenase subunit beta, benzene/toluene/chlorobenzene dioxygenase ferredoxin component, benzene/toluene/chlorobenzene dioxygenase ferredoxin reductase component, cis-1,2-dihydrobenzene-1,2-diol dehydrogenase, toluene monooxygenase system protein A, toluene monooxygenase system protein B toluene monooxygenase system ferredoxin subunit, toluene monooxygenase system protein D, toluene monooxygenase system protein E, toluene monooxygenase electron transfer component, phenol 2-monooxygenase, toluene methyl-monooxygenase, toluene methyl-monooxygenase electron transfer component, aryl-alcohol dehydrogenase, benzaldehyde dehydrogenase (NAD), 4-cresol dehydrogenase (hydroxylating) flavoprotein subunit, 4-cresol dehydrogenase (hydroxylating) cytochrome subunit, 4-hydroxybenzaldehyde dehydrogenase (NADP+), 4-hydroxyisophthalate hydroxylase, 2-aminobenzenesulfonate 2,3-dioxygenase subunit alpha, 2-aminobenzenesulfonate 2,3-dioxygenase subunit beta, catechol 1,2-dioxygenase, muconate cycloisomerase, p-toluenesulfonate methyl-monooxygenase oxygenase component TsaM, p-toluenesulfonate methyl-monooxygenase reductase component TsaB, 4-(hydroxymethyl)benzenesulfonate dehydrogenase, 4-formylbenzenesulfonate dehydrogenase, chloromuconate cycloisomerase, carboxymethylenebutenolidase and maleylacetate reductase