MICROBIAL INDICATORS OF SOIL QUALITY UNDER INTEGRATED SOIL FERTILITY MANAGEMENT PRACTICE EFFECTS IN MADEYA, SIAYA COUNTY, KENYA

By

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DECLARATION

Declaration by the Student

I declare that this thesis is my original work and has not been submitted to any academic institution for award of any degree. No part of this thesis should be reproduced without prior approval from the author and/or Kenyatta University.

Signature …… Date ……

Peter Omondi Bolo

Declaration by the Supervisors

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as the University supervisors.

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DEDICATION

This thesis is dedicated to my beloved family (sons; Gilbert F. Mula Omondi, Job Hawi Omondi, and spouse Innocentiah Nduku; my parents George Simeon Bolo and Eunice Mito Bolo; and siblings) and my entire generations. May God's divine and supernatural provisions always locate you. May heavens always speak in your favour; Numbers 6:24-26 is your portion. This is my dedication to you, as inspired by my walk and completion of this study. Indeed, it is possible, believe! May this serve as a timeless beacon of hope. Above all, glory to God for He has done great things.

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ABBREVIATIONS AND ACRONYMS

ACP	Acid phosphatase enzyme
AGB	Above ground biomass yield of maize
ALP	Alkaline phosphatase enzyme
C	Carbon
CO_2	Carbon dioxide gas
DNA	Deoxyribonucleic acid
ECD	^{63}Ni-electron capture detector
FID	Flame ionization detector
FYM	Farmyard manure
GLU	β-Glucosidase enzyme
ISFM	Integrated soil fertility management
MBC	Microbial biomass carbon
N	Nitrogen
N_2O	Nitrous oxide gas
NAG	β-Glucosaminidase enzyme
OTUS	Operational Taxonomic unit
P	Phosphorus
SOC	Soil organic carbon
NMDS	Non-metric dimensional scaling

ABSTRACT

Decline in soil and crop productivity, following soil infertility and poor soil health and agronomic practices, has threatened food availability amidst the growing world human population. Integrated soil fertility management (ISFM) practices can greatly avert these problems by enriching soil health, fertility and increasing crop production. However, despite these benefits, there is scanty knowledge on the potential impacts of different ISFM practices on soil microbial community structure, abundance, enzyme activities and of greenhouse gas emissions in western Kenya. This study investigated the effects of different long-term ISFM practices on soil bacterial community composition; extracellular soil enzyme activities, nutrient mineralization, maize performance and greenhouse gas emissions in western Kenya. The five objectives of this study were to determine the effects of farmyard manure, inorganic fertilizers and residue application on: (i) soil microbial biomass and bacterial abundance; (ii) the potential activities of extracellular phosphomonoesterase, β-glucosidase, and β-glucosaminidase enzymes in soil; (iii) soil bacterial *pho*C and *pho*D genes abundance; (iv) greenhouse gas emissions; and (v) soil organic carbon (SOC) and chemical parameters, nitrogen (N) and phosphorus (P) nutrient mineralization and their monetary values and maize performance. The study was carried out under maize-tephrosia rotation and continuous maize systems in an existing long-term trial. The experiment was set up in randomized complete block design. Twelve treatments were selected, replicated thrice. Soil samples were taken at 0-15 cm depth. Three closed chambers, installed per plot, in three treatments (0N+45P; 90N+45P and 0N+45P+FYM), were used to assess the effects of phosphorus, nitrogen and farmyard manure, respectively on greenhouse gas (CO_2 and N_2O) emissions. Gas sampling was done once per week, with four gas samples taken at 15-minutes sampling intervals per plot, and later analyzed using Gas Chromatography. Samples for objectives (i), (ii) and (iii), respectively, were analysed using Illumina Miseq sequencing, high-throughput colorimetric assays and standard laboratory procedures. Normality test was conducted on all the data using Shapiro-Wilk test before any data analysis was done. Analysis on objective (i) for bacterial species data involved Shannon wiener diversity measure, Canonical correspondence analysis, non-metric dimensional scaling (NMDs) and one-way analysis of variation (ANOVA). Data on objectives ii, iii, iv and v were analyzed using one-way ANOVA and Pearson correlation tests. Tukey Honest Significant Difference (HSD) was used to separate the significantly different means at $P \leq 0.05$. Application of FYM increased bacterial abundance and diversity. Microbial biomass carbon (C) and *pho*D gene abundance were not significantly affected by treatments, but sole application of residue significantly reduced bacterial *pho*C gene abundance. Application of FYM, in combination with other inputs, increased soil extracellular enzymatic activities. Application of FYM significantly increased CO_2 and N_2O fluxes. Additionally, FYM increased the quantities of P mineralised and its equivalent monetary value, but reduced N mineralised and its monetary equivalent. Nitrogen fertilization reduced the quantity of N mineralized and its monetary equivalent. FYM addition significantly increased maize performance and grain prices. Thus, FYM addition, either alone or in combination with other inputs, can enhance soil quality and nutrient availability, microbial diversity and functions as well as overall maize performance.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The global population currently stands at approximately 7.3 billion people, with the possibility to exceed 9 billion by 2050 (Flies et al., 2018). The ability to meet future food demands for this increasing population depends on sustainable food production (Cirera & Masset, 2010). Soil infertility, characterised by progressive nutrient depletion, has become an increasingly urgent problem that constrains both crop yields and soil health in many regions in Sub-Saharan Africa (Hartemink, 2006; Sileshi et al., 2010; Kihara et al., 2016). For instance, in western Kenya region, approximately 80% of acid soils suffer from phosphorus (P) deficiency, with P nutrient losses estimated at 3-13 kg P ha^{-1} $year^{-1}$ (Jama & Van Straaten, 2006). Despite the reduction in crop yields caused by this problem, the situation has often been met with reduced nutrient amelioration interventions due to high inorganic fertilizer costs amongst resource constrained smallholder farmers (Tittonell et al., 2008b). This calls for embracing integrated soil fertility management (ISFM) practices that would improve the overall soil quality and increase crop productivity.

Soil quality/heath is a very integral aspect of sustainable agriculture (Bhatt et al., 2019). It directly influences both crop productivity as well as ecosystem health and processes (Kihara et al., 2020). Soil quality broadly entails physical, biological as well as chemical parameters, with soil microbial indicators often playing very fundamental roles in the assessment (Zhen et al., 2014). This is because they respond rapidly to changes occasioned by different soil management practices for example, ISFM. Integrated foil fertility management entails combined use of organic and inorganic inputs among other practices, in a synergistic manner, to enhance soil structure, fertility, health and crop productivity (Vanlauwe et al., 2010; Shah & Wu, 2019). Some of the most common microbial indicators encompass microbial biomass, microbial community composition and activities, enzymes activities and soil respiration. The microbes, involving bacteria and fungi, execute such soil functions involving decomposition of organic matter, nutrient mineralization,

nutrient cycling, aggregate formation and stabilization, suppression of plant disease and pathogens and bioremediation.

Soil microbes, together with their activities, serve as indicators of both soil health and its overall quality. They play vital roles in biogeochemical and ecosystem processes and functions relating to cycling of different nutrients, improvement of soil and degradation of soil pollutants (Gyaneshwar et al., 2002; Murphy et al., 2007; Singh & Dhar, 2010; Smith et al., 2015). Fungi and bacteria are the most predominant drivers of soil functions and processes and the fungal to bacterial ratio is indicative of the soil system's stability (Malik et al., 2016). Agronomic interventions promoting soil microbial properties can also augment soil health through spurring nutrient availability and enhancement of soil physio-chemical and biological characteristics.

Different ISFM practices can variably impact microbial indicators of soil quality (Nyamwange et al. 2021). Organic amendments, for example, farmyard manure, can increase microbial biomass, microbial community composition and activities by providing substrates necessary for microbial proliferation (Ye et al., 2021; Lee et al., 2023; Sayre et al., 2023). This can not only enhance soil nutrient availability and soil structure, but also promote a healthy soil ecosystem. Diverse microbial structures contribute to better nutrient cycling, organic matter decomposition, as well as overall soil fertility (Chen et al., 2022; 2023). Previous works suggested that different ISFM practices may improve soil health and stimulate microbial activities, with potentials to enrich soil fertility status alongside sustaining soil and crop productivity (Sommer et al., 2016; Kihara et al., 2018). Since soil fertility interventions should be sustainable, it is important to understand how different ISFM practices influence the soil fertility, microbial activities and greenhouse gas emissions in western Kenya. However, little data-driven evidences exist to support these in the present study region. The use of agricultural inputs has been ascribed to improved soil physio-chemical and biological conditions (Vanlauwe et al., 2010; Shah & Wu, 2019), but in western Kenya region, scanty knowledge exists on their effects and/or their interactions with other soil chemical variables, on soil microbial parameters involving microbial biomass, abundance and activities.

Nutrient mineralization in soil has been associated with increased nutrient availability (Santi et al., 2013). However, a dearth of information exists on the potential extent to which such microbial-mediated nutrient mineralization activities can bring financial relief to smallholder farmers in relation to their potential to substitute inorganic fertilizers for their agricultural activities under different agronomic management systems in the study region. Since the study region is predominantly characterised by soil P deficiency, quantification of the nutrients mineralised (specifically N and P) from such microbial responses is important to inform the most appropriate practices that would improve nutrient availability and lower the financial costs incurred in purchasing inorganic fertilisers. In addition, numerous soil extracellular enzymes contribute to nutrient cycling (Ekenler & Tabatabai, 2002; Piotrowska & Koper, 2010), but insufficient information exists regarding the effects of various soil fertility management practices on the potential activities these enzymes (especially on N, P and C cycling extracellular enzymes) and their associated gene abundances.

1.2 Problem Statement

In many regions in Sub-Saharan Africa, prolonged nutrient depletion and limitation, poor soil physio-chemical and biological conditions and abiotic factors have progressively constrained both sustainable crop yields and soil health (Sileshi et al., 2010; Mucheru-Muna et al., 2014; Kihara et al., 2016). Numerous ISFM practices have been established and implemented, but in most cases, much intensification has focused on food production but with scanty knowledge on the biological and ecological benefits (Sanginga & Woomer, 2009; Mucheru-Muna et al., 2014; Shah & Wu, 2019), especially in western Kenya. Soil microbes influence important soil biogeochemical processes and functions that increase soil fertility and crop productivity (Murphy et al., 2007; Singh & Dhar, 2010; Smith et al., 2015). However, how soil microbes are affected by different ISFM technologies in Western Kenya remains widely unknown. Some specific soil enzyme activities and functional genes are important for nutrient cycling (Ekenler & Tabatabai, 2002; Nannipieri et al., 2011; Tan et al., 2013; Chen et al., 2019). On the contrary, there is inadequate knowledge on how different ISFM technologies affect their potential activities and the genes that encode them, especially in western Kenya. In addition, although

nutrient mineralization is also an important soil microbial process that increases soil fertility and nutrient availability and crop yields, there is little information on how different ISFM technologies affect nutrient mineralization and the financial equivalence of the different quantities of nutrients mineralized under such ISFM technologies. Carbon sequestration and storage of nitrogen in different pools are important processes in global reduction of greenhouse gas emissions, yet, how N_2O together with CO_2 emissions are influenced by different agronomic interventions remains widely unexplored in western Kenya. In addition, there is paucity of knowledge on how greenhouse gas emissions relate to fungal to bacterial ratio under different agronomic systems in western Kenya.

1.3 Justification of the Study

Soil acidity, the first consideration for selecting the present study area, is a big problem for proper crop production. It negatively impacts soil nutrient availability and soil biodiversity, and considerably increases crop production costs in many resource-constrained farming communities. This warrants progressive and extensive research to explore various ways that can sustainably increase soil and crop productivity in such regions to bolster food security, alongside generating data-driven evidence that can inform relevant policies at different scales and levels in the entire sustainable soil management and crop production chains.

The soils in the study region are majorly acidic Ferralsols, characterised by high phosphorus fixation and reduced phosphorus availability. This makes the resource-constrained smallholder farmers continuously spend a lot of financial resources to purchase inorganic fertilisers, that are not only expensive and unsustainable but also not environmentally friendly. This study explored alternative, cheap and eco-friendly agronomic management systems that, if adopted by the smallholder farmers, would enhance bioavailability of phosphorus and nitrogen (the essential crop nutrients but inadequate in the region) for crop utilization. Hence, adoption of the agronomic practices suggested in the current study would complement crop nutrition and reduces dependence on inorganic fertilisers that otherwise have deleterious effects to the environment.

Secondly, this study was conducted in a long-term agricultural trial. The selection of the long-term trial over either a short-term trial or farmer practice was guided by the need to generate data to assess and understand the changes and implications that certain agronomic management practices imposed over long periods of time would have on soil health/quality related aspects. This would inform generation of more robust and generalizable evidence-based findings that may guide proper decision making to foster sustainability and provide frontiers for agricultural, soil health and related policy actions. Thirdly, the choice of the present study region was also informed by the fact that western Kenya region is one of the most integral food baskets in the country. Being an integral food basket, extensive agricultural related research has been previously conducted in this region and this study would not only add to the existing knowledge, but also provide new insights that would be critical in promoting environmental sustainability, driving economic growth and providing essential insights for policy makers related to agricultural and soil health frontiers. Moreover, the region also has good weather patterns evident through predictable and reliable rainfall, thus, providing for reduced climate-related risks that would have hindered the success of the study.

Furthermore, numerous research related to ISFM, previously conducted in the study area, have majorly focused on its adoption (Aura, 2016; Adolwa et al., 2019), and parameters related to its overall impacts on soil fertility and crop production (Tittonell et al., 2008a; Sommer et al., 2018). However, very few studies in the study area have explored the impacts of different ISFM practices on soil health related parameters focusing on soil microbial biodiversity, specifically microbial structure, diversity and functions (Margenot et al., 2017a; 2018; Bolo et al., 2021), with some studies reporting on macrofauna (Ayuke et al. 2009; 2019; Kihara et al., 2015; Mbau et al., 2015). This study builds on these past studies, providing additional microbial and molecular based evidences resulting from advanced molecular techniques like Illumina Miseq sequencing (Next Generation Sequencing Techniques) and High-throughput microbial assays. Presently, this is the first study in the region to explore the effects of ISFM practices on the abundance of bacterial *pho*C and *pho*D genes that are linked to extracellular phosphomonoesterase (acid and alkaline phosphatase) enzymes, that catalyse phosphorus cycling processes in the phosphorus deficient

soils of the study area. The current study also provides additional insights on the long-term responses that certain ISFM practices may have on greenhouse gas emissions in the field, the nitrogen and phosphorus nutrients mineralization potentials and the monetary equivalents of the quantities of the different nutrients mineralized, together with implications on maize performance.

1.4 Research Questions

The study aimed to answer the following inquiries:

i. How do farmyard manure and inorganic NPK fertilizer application influence soil microbial biomass and bacterial abundance under maize-tephrosia rotation and continuous maize systems?
ii. How does farmyard manure and inorganic NPK fertilizer application affect β-glucosidase, β-glucosaminidase and phosphomonoesterase extracellular soil enzyme activities under maize-tephrosia rotation and continuous maize systems?
iii. How does farmyard manure and inorganic NPK fertilizer application affect soil bacterial *pho*C and *pho*D gene abundance under maize-tephrosia rotation and continuous maize systems?
iv. How do farmyard manure and inorganic phosphorus fertilizer application influence greenhouse gas emissions?
v. How does farmyard manure and inorganic fertilizers influence soil organic carbon (SOC) and chemical parameters; nitrogen (N) and phosphorus (P) nutrient mineralization, their monetary values and maize performance under maize-tephrosia rotation and continuous maize systems?

1.5 Objectives of the Study

The primary goal of this study was to evaluate how various long-term integrated soil fertility management practices affect soil microbial communities, their activities, and their impact on nutrient mineralization and greenhouse gas emissions.

1.5.1 Specific Objectives

The research was structured around the following specific objectives:

i. To evaluate the effects of farmyard manure and inorganic NPK fertilizers on soil microbial biomass and bacterial abundance under maize-tephrosia rotation and continuous maize systems.
ii. To assess the effects of farmyard manure and inorganic NPK fertilizers on potential activities of extracellular phosphomonoesterase, β-glucosidase, and β-glucosaminidase enzymes under tephrosia-maize rotation and continuous maize systems.
iii. To determine the effects of farmyard manure and inorganic NPK fertilizer on soil bacterial *pho*C and *pho*D genes abundance under tephrosia-maize rotation and continuous maize systems.
iv. To evaluate the effects of application of farmyard manure and inorganic phosphorus fertilizer on greenhouse gas emissions under continuous maize systems.
v. To evaluate how farmyard manure and inorganic fertilizers affect soil organic carbon (SOC) and chemical parameters; N and P nutrient mineralization, their monetary values and maize performance under maize-tephrosia rotation and continuous maize systems.

1.6 Research Hypotheses

The study's guiding hypotheses were as follows:

i. Farmyard manure and inorganic NPK fertilizers significantly increase soil microbial biomass and bacterial abundance under maize-tephrosia rotation and continuous maize systems.
ii. Application of farmyard manure significantly increases soil microbial β-glucosidase, β-glucosaminidase and phosphomonoesterase extracellular soil enzyme activities than inorganic fertilizers and maize stover application under different cropping systems.
iii. Application of farmyard manure and inorganic NPK fertilizer significantly increase soil bacterial *pho*C and *pho*D genes abundance under maize-tephrosia rotation and continuous maize systems.
iv. Farmyard manure and phosphorus fertilizer application significantly increases greenhouse gas emissions under continuous maize cropping system.

v. Application of farmyard manure and inorganic fertilizers significantly affect soil organic carbon and chemical parameters; N and P nutrient mineralization, their monetary values and maize performance under maize-tephrosia rotation and continuous maize systems.

1.7 Significance of the Study

The findings from this study contributes to knowledge that would not only direct future scientific research and improve agricultural productivity, but also provide scientific guidance for formulating climate-smart agricultural policies necessary to boost food production and keep at pace with the increasing human population. In addition, the focus of this study could help farmers reduce soil nutrient load (plus associated costs) and develop more sustainable and eco-friendly agricultural interventions.

1.8 Conceptual Framework

Decline in soil fertility constrains crop yields and food security. The influence of different soil health parameters on numerous biogeochemical processes can greatly avert this problem (Hartemink, 2006; Sileshi et al., 2010; Kihara et al., 2016). Soil microbial biomass, abundance, diversity and functions can increase soil nutrient availability and organic carbon (Gyaneshwar et al., 2002; Murphy et al., 2007; Singh & Dhar, 2010; Aislabie et al., 2013). However, this greatly depends on the soil conditions (Lienhard et al., 2013) and agronomic management systems (Kihara et al., 2018). Thus, attainment of increased soil fertility, improved crop yields and food security depends on employment of agricultural interventions that would enhance all the parameters of soil health (Figure 1.1).

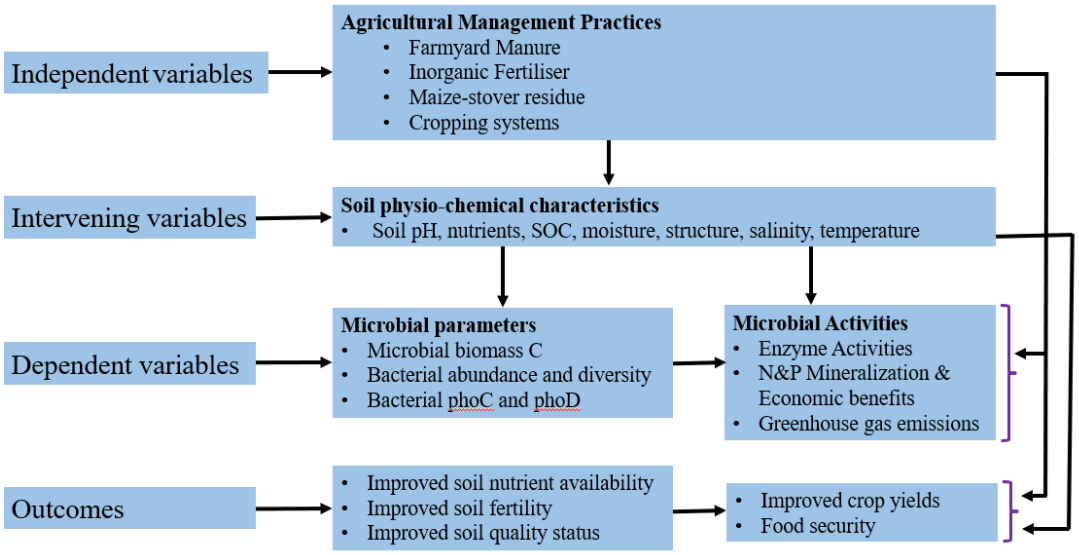

Figure 1.1: Conceptual framework depicting the interrelation between variables (Source: Author)

1.9 Definition of Terms

Acid phosphatase: Acid phosphatase is an important extracellular phosphomonoesterase enzyme that is responsible for the hydrolytic breakdown of organic phosphate compounds to inorganic forms. They are important indicators of P cycling and are majorly dominant in acidic environments.

Alkaline phosphatase: Alkaline phosphatases are important extracellular phosphomonoesterase enzymes that catalyse the hydrolysis of organic phosphates to inorganic forms. They are important indicators of P cycling and are mainly predominant in alkaline environments.

Beta-glucosidase: This is an important component of cellulase enzyme that catalyses the hydrolysis of cellulose into glucose, hence contributing to carbon cycling in the ecosystem.

Extracellular soil enzymes: These are enzymes produced from the cells but function outside the cells. They function as catalysis in soil biochemical processes involving nutrient cycling, nutrient mobilization, solubilization and mineralization, as well as decomposition of organic matter.

Greenhouse gas: Greenhouse gases are gases in the atmosphere that raise or increase the temperature of the atmosphere. These gases have the capability of absorbing different wavelengths of radiations that are emitted from different surfaces, including planets, potentially depleting the ozone layer, and resulting in greenhouse gas effect.

Integrated soil fertility management (ISFM): Is a set of agronomic management practices involving the use of organic inputs, inorganic inputs, crop rotation and improved germplasm to increase soil and crop productivity.

Nitrogen mineralization: This is the conversion of organic nitrogen into inorganic forms by soil microorganisms, making it available/accessible for plant absorption/uptake.

N-acetyl beta-glucosaminidase (NAG): This is an extracellular hydrolase enzyme that contributes to N cycling by catalysing hydrolysis of N-acetyl-glucosides.

***pho*C:** Soil bacterial gene responsible for encoding acid phosphatase enzyme.

***pho*D:** Soil bacterial gene responsible for encoding alkaline phosphatase enzyme.

Phosphatases: Enzymes that catalyse the process of phosphorus mineralization.

Soil microbial biomass: The collective/cumulative mass or weight of organisms within a specific/defined soil area or volume.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview

Globally, human population has been on an increasing trend, and the ability to meet food demands for this increasing population is constrained by reduced agricultural productivity (Cirera & Masset, 2010; Flies et al., 2018). Deline in agricultural yields to address the globally increasing food demand is exacerbated by poor soil fertility, declining soil health and employment of improper agronomic management practices, among others (Sileshi et al., 2010; Kihara et al., 2016). However, in the modern agriculture, agronomic management practices that observe proper soil health are critical to the realization of food security and environmental sustainability. The progressive decline in soil nutrient availability resulting from employment of poor agronomic management practices has occasioned exploration of the potentials of various integrated soil fertility management (ISFM) interventions, involving incorporation of organic and inorganic inputs, as promising measures to remedy the problem. Such ISFM practices have been advocated in some regions for their capability to abate some of these soil related problems and bolster crop productivity and food security due to their positive influences on soil physiochemical conditions, microbial abundance, diversity and activities that enhance soil quality and crop productivity (Tittonell et al., 2008b; Vanlauwe et al., 2010; Shah & Wu, 2019). For instance, soil microorganisms and their activities are important soil quality indicators due to their pivotal roles not only in nutrient cycling but also ecosystem stabilization, auguring well with improvement of the quality of soil as well as enhancing the productivity of crops (Kihara et al., 2020).

2.2 Effects of Farmyard Manure, Mineral Fertilizers and Residue Application on Soil Organic Carbon (SOC) and Soil Chemical Properties

Different agronomic management practices have numerous influences on soil organic carbon and soil chemical parameters, involving soil pH and nutrient availability. Organic inputs when applied either solely or together with other inputs, has previously been shown to impact soil physiochemical and biological properties (Mucheru-Muna et al., 2014; Kihara et al., 2018; Bolo et al., 2021). This is ascribed to the ability of the organic inputs to introduce organic materials and nutrients into

the soil upon decomposition, that stimulates nutrient availability as well as improve soil microbial life and functions involving nutrient cycling. In India, Rasool et al. (2008) stated that the SOC contents increased following application of FYM under maize-wheat cropping system. Mugwe et al. (2009) suggested that addition of FYM into the soil introduces organic materials and nutrients that can positively influence soil physiochemical properties. Similar assertions on the influences of FYM on soil parameters were also highlighted by Mucheru-Muna et al. (2014).

The previous reports have indicated that integration of organic and inorganic inputs is an effective strategy for enhancing SOC (Ndung'u et al., 2021) and other nutrients in soil in two ways. First, FYM combined with inorganic fertiliser inputs can offer benefits in enhancing carbon inputs by improving the amount of plant biomass produced, and secondly, the increased organic carbon contents that are directly added to the soil from the manure (Jiang et al., 2018; Gross & Glaser, 2021; Liang et al., 2023). Recently, Liang et al. (2023) noted that manure addition contributed the highest average rate of SOC increase in the soil, estimated at 684 kg C ha^{-1} yr^{-1}. Besides positive influences on SOC, application of FYM also improves soil pH, C.E.C, and nutrients (Bhatt et al., 2019). Recent studies reported that addition of FYM positively influenced soil pH due to introduction of organic matter into the soil that might have stimulated C.E.C and increased soil organic carbon fractions (Singh et al., 2023).

Soil quality is reliably assessed through the chemical and biophysical characteristics of the soil, as they collectively define the overall health of the soil, thus informing the status of nutrients within the soil (Bhatt et al., 2019). Application of organic inputs like residues and FYM in the soil has the capability to improve both the nutrient content and availability, as well as soil microbial activities relating to nutrient cycling in the soil. These simultaneously result to addition of, and cycling of, more nutrients in the soil comprising of macronutrients, secondary and micronutrients. Increase in soil pH, SOC, Zn, among other essential macronutrients and secondary nutrients following application of manure, either solely or combined with other inputs (either organic and inorganic inputs) has recently been observed in Kenya (Kihara & Bolo, 2021) and elsewhere in India (Patial et al., 2022).

In comparison to inorganic/mineral fertilisers, Dhaliwal et al. (2015) reported that application of organic inputs led to increase in levels of available potassium (K) in the soil. Similarly, long-term combined utilization of both inorganic inputs and organic amendments elevated the contents of soil available phosphorus (P) compared to sole application of NPK fertiliser (Ram et al., 2016). In soils, organic inputs have been shown to have the capacity to retain more soil moisture, enhance soil infiltration as well as improve crop yields (Mulumba & Lal., 2008). In India, Singh et al. (2016) stated that when FYM was added in a loam soil, it contributed to enhancing the capacity to hold water in the systems with FYM, in the range of 16-18%, compared the control treatments that were lacking FYM.

Application of inorganic inputs like mineral fertilisers may also have notable effects not only on soil chemical conditions but also SOC. Wu et al. (2020) suggested that excessive application of some inorganic fertilizers can disrupt the soil in soil nutrition balance by prompting SOC decline, aggravating reduction in soil fertility and occasioning soil acidification. Sole application of inorganic nitrogen fertilisers causes reduction in soil pH and this subsequently suppresses the health of soil, diminishes the availability of soil nutrients as well as reduces the plant productivity (Raza et al., 2020; Jia et al., 2022). Application of phosphorus-based fertilisers can increase the soil's phosphorus availability. Previous studies reported that continuous application of NP and NPK fertilisers, for 21 years, considerably stimulated the available P contents in the soil whereas the contents of soil N significantly declined (Li et al., 2012). Akin to this, large amounts of P in soils following fertilisation can prompt increased accumulation of P in organic pools as well as inorganic pools (Yan & Hou, 2018), and this can alter the soil P bioavailability and pose leaching risks (Yan et al., 2020).

2.3 Effects of Farmyard Manure, Mineral Fertilizers and Residue Application on Soil Microbial Biomass, Bacterial Community Structure, Abundance and Diversity

Soil microbial biomass, structure, diversity and activities are reflective of soil quality and its state of health (Alkorta et al., 2003). Healthy soils promote high microbial

biomass, microbial abundance, structure, diversity and functions (Chaparro et al., 2012). Improved microbial parameters indicate increased soil quality, fertility, stability and general productivity due to the myriad of roles, processes and functions executed by the different microbial structures (Alkorta et al., 2003; Schloter et al., 2003). For instance, soil microbes engage in nutrient cycling by breaking down organic matter and mineralizing nutrients, mobilizing and solubilizing recalcitrant nutrient sources, biological nitrogen fixation, nitrification, denitrification, immobilization of nutrients and general ecosystem services (Aislabie et al., 2013).

Soil microbial parameters are affected by agronomic different factors, either in isolation, or in synergy; with microbial responses to such culminating to shifts in soil quality, health, fertility and ecosystem stability (Van Horn et al., 2013; Li et al., 2018). Soil physio-chemical conditions involving temperature, moisture and nutrient contents, SOC and pH; disturbances and agronomic management practices are some of the immediate aspects that directly affect different microbial parameters (Lienhard et al., 2013; Bolo et al., 2021; 2023). In Kenya, omission of residues prompted 46.2% reduction in microbial biomass, and bacterial N and P nutrient cycler (Kihara et al., 2018). Elsewhere, long-term nitrogen fertilization decreased microbial biomass by 15% due to accumulation of large nitrogen load in the soil (Treseder, 2008).

Recent studies indicated that prolonged and excessive utilization of chemical fertilisers has prompted continual decline in soil quality parameters involving soil fertility, nutrient availability, and multiplicity of soil microbial parameters (Zhen et al., 2014). Cumulatively, these have negative effects on crop and soil health, and sustainable agricultural production. However, some reports on sole input application on soil microbes and their activities have been inconsistent, with some indicating suppressive, increase or no change (Lemanowicz, 2011; Kalembasa & Symanowicz, 2012; Yu et al., 2016). For instance, sole application of mineral nutrients suppressed important microbial parameters and enzyme activities (Zhou et al., 2012; Kihara et al., 2018) through inhibition or chemical imbalances. Omission of inorganic phosphorus, or its deficiency, increased phosphomonoesterase activities (Wang et al., 2011; Kihara et al., 2018; Margenot et al., 2018). Under maize-soybean rotation

in the Alfisols of sub-humid regions of China, Luo et al. (2015) reported that continuous application of fertilizers significantly increased bacterial and fungal diversity, as assessed using denaturing gradient gel electrophoresis (DGGE) methods.

The introduction of organic inputs can have a beneficial impact on various aspects of soil microorganisms, involving biomass, abundance, diversity and activities (Daquiado et al., 2016). This is due to the contribution of such organic inputs in introducing organic carbon and nutrients which are sources of food substrates that favour proliferation of soil microbes. Previous research highlighted that addition of organic amendments into the soil, alongside increasing soil fertility, also had the potential to stimulate the biomass and diversity of the soil microbial populations (Sun et al., 2014). Essentially, a greater diversity of soil microbial populations is beneficial for improvement of soil quality following the myriad roles they play in stabilization of soil structure, nutrient availability, pathogen suppression and bioremediation (Kihara et al., 2020; Basu et al., 2021; Li et al., 2021).

Organic amendments, like FYM, upon decomposition and mineralization, release essential nutrients like phosphorus and nitrogen that may promote the abundance of certain specialised microbial groups involving biocontrol agents and nutrient cyclers (Yin et al., 2019; Li et al., 2021; Semenov et al., 2021). Introduction of organic inputs such as manure influenced the microbial structure of nitrite oxidising bacteria resulting to increase in the rate of nitrification in the soil (Li et al., 2020). In addition, prolonged utilization of organic inputs like manure increased the abundance of phosphorus solubilizing microorganisms like *Aspergillus*, *Trichoderma* and *Flavobacterium*, with *Trichoderma* also coupling as a biocontrol agent (Chen et al., 2023). The shifts in microbial abundance and diversity following application of farmyard manure may be associated with manure effects in activating the indigenous soil microbiome community or introduction and/or survival of other soil microbes that are contained in the farmyard manure that is added (Semenov et al., 2021).

Besides, the increased carbon contents added to the soil following application of FYM may promote the growth, abundance, diversity and activities of certain soil

microbes involving copiotrophs that thrive well in carbon rich systems (Ranjan et al., 2015; Semenov et al., 2021), but simultaneously decreasing the abundance of oligotrophs that thrive well in carbon deficient systems (Fierer et al., 2007; Ho et al., 2017; Semenov et al., 2021). Apart from providing carbon and nutrients, FYM also moderates soil physical and chemical conditions that favour microbial proliferation (Niewiadomska et al., 2017). For instance, addition of FYM can improve soil moisture retention, soil structure as well as regulate soil temperature (Khan et al., 2010; Tadesse et al. 2013), thus creating enabling conditions that variably affect microbial colonization, growth and activities (Bolo et al., 2021; 2023).

In addition, soil pH is among the aspects that influence microorganisms in the soil (Muneer et al., 2022). Soil amendments with FYM improve soil pH (Opala et al., 2012), and this can variably influence the colonization, growth and activities of some microbial groups. The abundance, diversity and activities of some microbial groups may increase with increases in soil pH and reduce as soil pH reduces (Nicol et al., 2008; Bai et al., 2023). Previous studies indicated that the bacterial populations differed under different soil pH ranges, with some bacterial strains like *Actinobacteria* reducing with decline in soil pH or increasing as the pH increased (Wang et al., 2019). Conversely, some microbial strains may be favoured by low pH, and their abundance, diversity and activities may increase with reduction in soil pH and vice versa (Nicol et al., 2008; Wu et al., 2017; Wang et al., 2019; Bai et al., 2023). Wang et al. (2019) noted a considerable escalation in abundance of bacterial strains, involving *Acidobacteria* as well as *Proteobacteria*, that was consistent with reduction in soil pH, and vice versa.

Just like FYM, residue amendments are also characterised by increase in carbon contents that favour proliferation of microbes (Frasier et al., 2016; Chao et al., 2019; Wang et al., 2020). Organic inputs such as residues, upon decomposition, liberate soil organic carbon and nutrients contributing to carbon rich systems (Stella et al. 2019). Carbon rich systems often favour proliferation of copiotrophic microbes (Naylor et al., 2020). Besides, recent studies highlighted that application of plant residues with high nutritional quality (with high N content) greatly stimulated the soil microbial aspects whereas the residues with poor quality (with low N content)

depressed microbial community structure, diversity and activities (Almagro et al., 2021). However, Frasier et al. (2016) reported an escalation in the biomass of soil microorganisms regardless of the quality of residues applied. Within the soil microbiome, soil amendment with high quality residues is believed to favour the proliferation of bacterial groups whereas amendments with low quality residues promote the fungal microbial communities (Kramer et al., 2012).

2.4 Effects of Farmyard Manure, Mineral Fertilizers and Residue Application on Soil Extracellular Enzyme Activities

Microbes and plants exude soil extracellular enzymes whose activities are important in defining soil health, quality and nutrient availability. Different extracellular enzymes catalyze specific microbial mediated bio-chemical reactions and processes linked to soil nutrient (C, N and P) cycling (Ekenler & Tabatabai, 2002; Piotrowska & Koper, 2010; Adetunji et al., 2017). Phosphomonoesterases (acid and alkaline phosphatase) activities are vital in P cycling (Nannipieri et al., 2011), β-glucosaminidase (NAG) for N cycling (Ekenler & Tabatabai, 2002) and Beta-glucosidase for carbon cycling (Piotrowska & Koper, 2010). Plants, bacteria and fungi primarily exude acid phosphatase enzymes whereas alkaline phosphatase enzymes have their origins in soil microbes, particularly bacteria (Spohn & Kuzyakov, 2013). Soil extracellular enzymes are responsive to fluctuations in soil physiochemical conditions, nutrient availability, agronomic management systems and disturbances (Tan et al., 2013; Fraser et al., 2015; Ragot et al., 2015; 2017).

Agronomic management practices have the capacity/potential to modify both the physiochemical and biological properties of soil, but their influence on phosphomonoesterases have not been consistent. For instance, application of mineral nitrogen decreased potential alkaline phosphatase enzyme activity following reduction in soil pH (Liu et al., 2010; Chen et al., 2019) even though other studies reported contrary observations (Marklein & Houlton, 2012; Tan et al., 2013). However, other studies observed stimulation of alkaline phosphatase enzyme activity after manure application (Fraser et al., 2015) or following nitrogen application due to stimulation of plant and microbial production, thereby creating increased demand for phosphorus (Mandal et al., 2007; Tan et al., 2013).

Conversely, previous studies pointed towards nitrogen fertilization effects in reducing alkaline phosphatase while increasing acid phosphatase activities under fields where corn and wheat are cultivated (Lemanowicz, 2011). Similar sentiments were reported by (Kalembasa & Symanowicz, 2012). Integrated introduction of inorganic nitrogen and organic compost increased phosphatase activities more compared to sole application of inorganic/sythetic nitrogen fertilizer (Crecchio et al., 2004; Srivastava et al., 2012). On the other hand, previous reports indicated that application of phosphorus in soils characterised by phosphorus deficiency suppresses the activity of phosphomonoesterases (ALP and ACP), due to the inhibitory effects of phosphorus on such enzymes (Olander & Vitousek, 2000; Nannipieri et al., 2011).

The activities of different soil extracellular enzymes are affected by substrate availability, soil chemical conditions, as well as different agronomic management practices (Ning et al., 2020; Dong et al., 2022; Gonzalez et al., 2023). For instance, farmyard manure contains considerable quantities of organic matter nutrients that may influence the activities of different soil extracellular enzymes (Wang et al., 2020; Brichi et al., 2023). Previous studies indicated that incorporation of FYM into the soils can greatly improve microbial abundance, diversity and activities and this can influence the activities of different extracellular soil enzymes exuded by such microbes (Ye et al., 2021; Lee et al., 2023; Sayre et al., 2023). This is because addition of FYM into the soil introduces organic materials and nutrients upon decomposition (Mucheru-Muna et al., 2014) that promotes the proliferation, growth as well as production of different soil enzymes that perform different soil functions involving nutrient cycling.

Application of FYM into the soil can increase substrate availability for degradation and this has the potential to enhance the production of, and the associated roles of, Beta-glucosidase (GLU) enzyme that breaks down cellulose to glucose in the soil (Tiwari et al., 2021; Kumar et al., 2021). Conversely, FYM also contains nutrients like nitrogen, and its application into the soil may introduce these nutrients, potentially enhancing the production and activities of Beta-glucosaminidase (NAG) enzyme that degrades chitin in the soil (Moeskops et al., 2011; Brennan & Acosta-Martinez, 2019). Furthermore, the application of FYM in the soil can increase the

availability of organic phosphorus compounds that stimulate the production of, and activities of, acid phosphatase (ACP) extracellular enzymes together with alkaline phosphatase (ALP) enzymes, that hydrolyse the organic phosphates to inorganic forms (Lemanowicz et al., 2014; Nakayama et al., 2021). Recent studies highlighted that ACP, ALP, GLU and NAG enzyme activities significantly increased following application of FYM (Acosta-Martinez et al., 2011; Brennan & Acosta-Martinez, 2019; Liang et al., 2014; Chen et al., 2019). The above reported studies attributed these enzymatic responses to contribution of FYM in not only enhancing of soil organic matter, but also improving nutrient availability, as well as soil physiochemical characteristics.

Similar to FYM, crop residues may also provide organic substrates and nutrients upon decomposition that would variably influence the production of, and activities of, different soil extracellular enzymes (Rezgui et al., 2021; Tosi et al., 2022). However, the responses of some enzyme activities on residues may profoundly be impacted by either the quantity applied, or the type and nature of residues applied, in relation to the nature of their biochemical compositions (Prescott, 2010). For instance, residues that have higher lignin contents may pose variable rates of break down to simple molecules, or prompt nutrient immobilization (Yansheng et al., 2020), and this may suppress certain microbial activities and liberation of enzymes. Lignin content in the residues has been regarded as an important factor that influences the fate of the added carbon. Besides, Nicolardot et al. (2007) noted that the soluble carbon in residue is one of the factors that regulate the rate of breakdown/decomposition of the added residues (Hadas et al., 2004). Cumulatively, the soluble carbon and lignin contents in different residues can shift the microbial community structure, simultaneously influencing the production of, and activities of, certain soil enzymes. Recent studies highlighted that incorporation of residues reduced the activities of Beta-Glucosidase enzymes and increased nitrogen immobilization perhaps and this was associated to the shifts in microbial structure (Rezgui et al., 2021).

2.5 Effects of Farmyard Manure, Inorganic Fertilizers and Residues on *pho*C and *pho*D Gene Abundance

Bacterial *pho*C and *pho*D genes are the predominant biomarker genes that produce acid and alkaline phosphatase enzymes responsible for P cycling (Tan et al., 2013; Chen et al., 2019). Essentially, soil edaphic, biotic and management activities can variably affect *pho*C and *pho*D abundance (Ragot et al., 2015; 2017). Under different agronomic systems, various studies have documented conflicting reports on the effects of fertilizer inputs on *pho*C and *pho*D abundance, with some reporting increase (Luo et al., 2017), decrease (Chhabra et al., 2013; Ikoyi et al., 2018) or no change (Fraser et al., 2015). Addition of mineral N considerably reduced the abundance of bacterial *pho*D gene as well as alkaline phosphatase activity (Chen et al., 2019). Recent studies have indicated positive associations between the bacterial *pho*D gene abundance and activities of alkaline phosphatases (Fraser et al., 2015), calcium ions with alkaline phosphatase (Yamane & Maruo, 1978; Wu et al., 2007); and *pho*C abundance with acid phosphatase activity, carbon and macronutrient availability in acidic soils of China (Zheng et al., 2019).

Soil nutrients contribute a great deal in influencing the populations of soil microorganisms that harbour bacterial *pho*C and *pho*D genes (Zheng et al., 2019). Fertiliser application can also variably affect the bacterial *pho*D gene abundance by influencing the bacterial community that harbour *pho*D genes (Lu et al., 2023). Significant influences of fertilisation on bacterial *pho*D gene abundance owing to the contributions of fertilizers on soil properties that had an impact on the associated bacterial community structure and diversity are recently acknowledged (Ragot et al. 2015; Zheng et al., 2019). Fertilisers that can alter soil pH can concomitantly affect the *pho*D harbouring microbial community structure. Soil pH contributed a great deal, particularly poor soils characterised by acidity, in influencing the diversity of microbial communities that harbour bacterial *pho*D and *pho*C genes (Ragot et al. 2015; Zheng et al., 2019).

Akin to this, earlier studies noted a significant improvement in both soil pH, microbial abundance, together with diversity of soil microorganisms that harbour bacterial *pho*D genes following a long-term application of fertiliser with liming

properties (calcium phosphate) (Tan et al., 2013). The *pho*D gene harbouring microbial communities are also susceptible to declining soil pH, with a reduction in soil pH concomitantly reducing their abundance and diversity, and this can potentially impair or stifle the production of the *pho*D genes (Lu et al., 2023). Conversely, Richardson (2009) asserted the possibility of *pho*C harbouring microbial community to be stimulated in acidic soils characterised by low phosphorus levels owing to the capability of some phosphorus solubilizing microbes that harbour *pho*C to solubilize P enough to cater for their P nutritional requirements. Thus, agronomic practices with the potential to reduce soil pH, like application of synthetic nitrogen-based fertilisers can also depress the diversity of soil microorganisms that harbour bacterial *pho*D genes, hence influencing the expression of *pho*D genes. Recently, Chen et al. (2017) reported that prolonged utilization of inorganic nitrogen-based fertilizers negatively influenced soil pH, and this significantly suppressed the diversity of *pho*D harbouring microbial community, hence stifled the expression of *pho*D genes. Besides, Chhabra et al. (2013) and Tan et al. (2013) highlighted the possibility of addition of phosphorus fertilisers to either enhance or depress the *pho*D gene harbouring microbial diversity.

There is also notable evidence that nutrient management, involving application of organic inputs, has considerable influences bacterial *pho*C and *pho*D harbouring microbial communities (Tan et al. 2013; Fraser et al., 2017; Chen et al., 2017;2019). Addition of organic matter (manure) enhanced cycling of organic phosphorus, and this was also linked to stimulation of microbial communities that harbour both *pho*C and *pho*D genes (Bi et al., 2020). Recently, Guo et al. (2023) asserted that incorporation of organic manure, which acts as a strategy to ameliorate soil pH, significantly affected the structure of certain bacterial communities that harbour *pho*D genes.

2.6 Effects of Farmyard Manure and Inorganic Fertilizer Inputs on Greenhouse Gas Emissions

The three gases, namely CO_2, N_2O and CH_4, are potent greenhouse gases whose emissions into the atmosphere regulate C and N cycles, with increased likelihood to contribute to global warming (Signor & Cerri, 2013). Different agronomic

management systems, soil edaphic conditions and abiotic parameters may prompt variations in rates of microbial respiration, activities and processes causing liberation of these gases (Butterbach-Bahl et al., 2013; Signor & Cerri, 2013). Soil microorganisms, through their activities, can occasion nitrification and denitrification in different measures; magnitudes of which depend on such factors as environmental temperature variations, moisture availability and accumulation of nitrogen, together with organic carbon, in the soil (Bai et al., 2010). Nitrous oxide can be produced from different ways involving nitrification as well as denitrification processes commonly driven by the soil microbes (Wang et al., 2016). In most instances, the soil moisture content contributes a greater proportion in regulating the evolution of nitrous oxide, that is stimulated in agronomic systems that promote water retention in the soil (Ciarlo et al., 2007). Addition of organic inputs (involving FYM) in the soil has the capability to enhance water retention (Nath et al., 2023; Ndegwa et al., 2023), and this can enhance the evolution of nitrous oxide. Previously, Lesschen et al. (2011) suggested that the emission of nitrous oxide from agricultural fields, following addition of manure and fertilisers, can be directly or indirectly influenced by factors involving the type of organic inputs applied, prevailing environmental conditions alongside the subsequent agronomic management practices. Akin to this, recent studies noted that in arable soils, organic inputs contribute greatly to methane (Khosa et al., 2010).

The application of such organic amendments, like FYM, in arable soils can have variable effects on emission of different greenhouse gases, involving CO_2, N_2O as well as methane. These gases can cause significant changes in the atmosphere, involving depletion of ozone layer, as well as cause climate change. Addition of organic inputs in the agricultural systems may serve two important roles regarding the contributions to the complex dynamics of the greenhouse gas emissions. For instance, the organic inputs, like FYM, can play both the mitigation or exacerbating roles to greenhouse gases. Akin to the role of FYM in mitigating greenhouse gas emissions, FYM is characterised by rich organic matter with increased capabilities to enhance soil physiochemical and biological properties that favour both belowground and aboveground biodiversity (Chen et al., 2022; 2023). The increased belowground and aboveground biodiversity enhances carbon sequestration, with the

biodiversity acting as carbon sinks both in the soil, and aboveground, hence suppressing the emissions. Previous studies reported that application of FYM and/or inorganic fertilizers to cultivated fields had the potential to increase soil nutrient availability together with crop production (Sanginga & Woomer, 2009; Vanlauwe et al., 2010; Shah & Wu, 2019). Besides, the nutrients and organic matter contributed by the added organic inputs in the soil may stimulate the proliferation of special microbial structure called methanotrophs, organisms which utilize methane as their source of carbon, hence reducing the emission of methane into the system (Strong et al., 2015).

Even though, application of organic inputs like FYM in the soil may have potential benefits in mitigating the release of gases responsible for occasioning greenhouse effects (Gong et al., 2022; Holka et al., 2022), it is important to note that such organic inputs may also have the potential to cause or increase greenhouse gas emissions in the fields (Aguirre-Villegas et al., 2017). For instance, FYM contains organic elements which decompose upon introduction/incorporation into the soil, thereby releasing carbon compounds that further contribute to emissions of CO_2 (Pathak et al., 2003). Previous report indicated that manure contributes approximately 7% of the methane and CO_2 released in the arable lands (USEPA, 2006). Besides, the organic matter that is contained in the FYM is a source of food to the microbes, and this promotes increased belowground microbial abundance and diversity, characterised by increased respiration hence considerably releasing CO_2 (Khatoon et al., 2017; Fekadu et al., 2019; Wang et al., 2020; Bardgett et al., 2008). Akin to this, the organic compounds together with nutrients contributed by FYM upon decomposition can stimulate the abundance and diversity of special microbial groups called methanogens that can convert the organic as well as inorganic compounds into methane under anaerobia conditions (Ho et al., 2015). Recently, Buan (2018) described methanogens as microbes that grow and proliferate by producing methane gas whereas Enzmann et al. (2018) asserted that methanogens are the only microbial community with the capability of producing methane gas. Previous studies indicated that through introduction of methanogens, application of manure into the agricultural fields had the potential of increasing production of methane gas (Gattinger et al., 2007; Kim et al., 2014).

In arable soils, the quantity of synthetic/inorganic nitrogen fertiliser incorporated, either alone, or in combination with organic inputs, plays a vital role in influencing the production of N_2O gas (Stehfest & Bouwman, 2006; Wang et al., 2021). Besides, the amounts of nitrous oxide emitted following the incorporation of such organic and inorganic inputs is also greatly affected by the different agricultural management activities imposed, involving the type of inputs applied (both organic and inorganic), the rates of application of such inputs, as well as the techniques/methods employed in their application to the soils (Jäger et al., 2011; Wang et al., 2021). Relevant to the methods of application of inputs, previous studies indicated that broadcast application of the inputs, particularly manure, followed immediately by incorporation into the soil either through ploughing or any other method of cultivation, can greatly reduce emissions of NH_3 into the system (Sherman et al., 2023), thereby affecting N_2O emissions. On the contrary, some studies noted that the different methods/techniques employed in the application of such inputs into agricultural soils may either lack effects, or show inconsistent results, on N_2O emissions (Velthof et al., 2003; Webb et al., 2015).

Besides, the emissions of N_2O in agricultural fields may also be influenced by the availability of crop residues, especially in agricultural systems that receive nitrogen applications (Wang et al., 2021). In addition, the production of N_2O gas in the agricultural systems is also affected by availability of enough moisture and oxygen in such systems that directly influence such nitrogen transformation processes (Jäger et al., 2011; Wang et al., 2021). Increased moisture availability may prompt increased denitrification hence liberation of more N_2O while increased oxygen supply may drive nitrification processes from the soil. Residue and FYM application may improve moisture retention in the soil, and this may influence these processes. In addition, the application of organic inputs like FYM can also improve both the microbial abundance, as well as diversity of different microbial structures, involving nitrifiers and denitrifiers, hence influencing the liberation of N_2O gas in agricultural soils. Furthermore, application of organic inputs can also improve the soil physiochemical and biological properties, involving regulating temperature, soil pH, as well as increase nutrient availability thereby directly influencing the microbial diversity, activities and functions that relate to nitrification and denitrification.

Recent studies have acknowledged the contribution of FYM application, either alone or in combination with other inputs, on emission of different greenhouse gases. For instance, besides its benefits in enhancing soil nutrient status alongside improving crop production (Vanlauwe et al., 2010; Shah & Wu, 2019), the potential of the applied FYM and/or inorganic fertilisers to cultivated fields on increasing greenhouse gas emissions is previously acknowledged (Pelster et al., 2015; 2016; Rosenstock et al., 2016; Sommer et al., 2016; Ortiz-Gonzalo et al., 2018). Sommer et al. (2016) stated that the emissions of N_2O increased when FYM was applied together with inorganic N and residues in a maize-tephrosia rotation system. In pasture fields, Rosenstock et al. (2016) also stated that CO_2 emission was stimulated but N_2O and CH_4 emissions were depressed following manure application. In Eastern Africa, most studies have focused on the effects of combined inputs on greenhouse gas emissions (Sommer et al., 2016; Macharia, 2019), short term emissions on grasslands or small-holder/farmer managed sites (Rosenstock et al., 2013; Pelster et al., 2015; 2016; Rosenstock et al., 2016; Ortiz-Gonzalo et al., 2018) but least have assessed the effects of individual inputs on fluxes of different greenhouse gases under long-term researcher managed experiments.

2.7 Effects of Agronomic Management Systems on N and P Nutrient Mineralization and the Monetary Value of Quantities of N and P Nutrients Mineralized

Tillage associated disturbances can cause rapid mineralization of SOC resulting to high nutrient concentrations (Ghimire et al., 2019). Numerous soil microbes can mineralize organic and solubilize inorganic nutrient sources thereby stimulating nutrient availability (Nannipieri et al., 2011; Zhu et al., 2011). Microbes involving *Glomus*, *Pseudomonas*, *Aspergillus*, *Penicillium*, *Cyanobacteria* and *Rhizobia* and are important N and P solubilizers (Shridhar, 2012; Srinivasan et al., 2012). Agronomic management systems, like application of inputs, can influence soil microbial community thereby affecting nutrient mineralization. Application of lime and compost enriched soil pH, enhanced proliferation of microbial groups that solubilize phosphorus, alongside stimulating P availability (Zhang et al., 2014). Nutrient mineralization, and quantities of nutrients mineralized, may vary with different agronomic management systems (Zou et al., 2018). The quantity of

nutrients mineralized may partially substitute for the use of costly chemical fertilizer inputs, offering some financial relief to the farmers. However, there is a dearth of information on the quantity of nutrients that can be mineralized in different agronomic management systems and the economics of nutrient mineralization by the soil microbes.

Long-term nutrient amendments can increase soil fertility, but the quantities and financial value of nutrients mineralized following such is still unclear. In Germany, increased N availability was reported under continuously fertilized soils (Nett et al., 2010). However, Langmeier et al. (2002) reported that recent organic amendments in Alfisols of Switzerland did not increase net nitrogen mineralization in originally fertilized soils compared to those that lacked fertilization despite increased soil microbial biomass in the oils that received organic fertilization than non-fertilized soils. In Ethiopia, application of FYM at different rates resulted in 33.3% and 66.6% respective savings on usage of the recommended N and P fertilizers on potato tubers (Balemi, 2012).

2.8 The Role of Soil Microbes in Crop Production

Soil microorganisms assume several critical roles in crop production. They contribute to improvement of soil structure, microbes positively influence soil nutrient cycling, suppression of plant diseases, improvement of stress tolerance and promotion of plant growth (Rillig & Mummey, 2006; Sharma et al., 2011; Kalayu, 2019; Al-Maliki & Ebreesum). These directly influence plant growth, health together with yields. Soil microbes can make nutrients available for crop uptake through several ways involving decomposition of organic matter, mineralization, solubilization, mobilization and biological fixation (Rashid et al., 2016; Kalayu, 2019). Microbes like actinomycetes, bacteria and fungi are integral agents of decomposition of organic matter, culminating to conversion of complex organic compounds into simple inorganic forms that are either easily or readily available for plants utilization (Wang et al., 2024). The nitrogen-fixing microbes (diazotrophs), for example *Rhizobium*, *Frankia, Cyanobacteria*, *Azotobacter* and *Azospirillum* species, among others, convert atmospheric nitrogen into readily available forms of nitrogen for plant uptake (Bhat et al., 2015). Studies have shown that such

diazotrophs can fix about 20-300 kilograms of nitrogen per hectare in one year (Kumar et al., 2022). This implies that microbes can be important biofertilizers that improve soil nutrient availability for plant uptake thus resulting to higher crop yields.

Soil microbial community also serve as important reservoirs for phosphorus in the soil, influencing phosphorus availability through assimilation, solubilization among others. Phosphorus cycling microbes involving Arbuscular mycorrhiza, *Pseudomonas, Bacilli*, *Rhizobium* and *Azotobacter* species, among others, are capable of mineralizing, solubilizing and mobilizing phosphorus, in several ways, from a myriad of sources (Kalayu, 2019; Bolo et al., 2021). Recent studies indicated that soil microbial community can contribute different levels of phosphorus under different ecosystems. For instance, in arable lands, microbes contributed about 174-328 mg P kg^{-1} soil in a cover crop (Hallama et al., 2022). The microbial community can contribute about 0.5-7.5% of total phosphorus in both grassland and pasture topsoil ecosystems, while in the forests, they can contribute up to 26% of the total phosphorus (Mackey et al., 2019).

Nitrogen and phosphorus are amongst the most essential nutrients for plant growth, and their sustained availability at cost-effective and ecofriendly manner is vital for crop productivity (Jiaying et al., 2022). In particular, both nitrogen fixation and phosphorus solubilization by microbes, together with the cycling of other nutrients, are very important processes in nutrient deficient (particularly nitrogen and phosphorus-deficient) soils as they reduce the need for, and expenses incurred in, purchasing synthetic nitrogen and phosphorus fertilisers, promoting sustainable agriculture (Bolo et al., 2024). Moreover, soil microbes also cycle several other nutrients, including micronutrients (Sahu et al., 2018; Bolo et al., 2023). Furthermore, although plants directly absorb such nutrients from the soil, some microbes can directly transport the nutrients to the plant tissues, thereby improving the nutrient quality of the crop produce.

Besides influencing soil nutrient cycling, certain soil microbes also play significant roles in enhancing crop yields by promoting plant growth, suppressing plant pathogens, phytoremediation and improving their resilience against environmental stressors (Bravo et al., 2011; Saeed et al., 2021). Beneficial microorganisms such as

plant growth promoting rhizobacteria have the potential to produce vital phytohormones, together with other bioactive compounds, that not only stimulate plant growth but also enhance their defence mechanisms (Khoso et al., 2023; Nwachukwu et al., 2024). These improve plant vigour and enhance plant health, culminating to higher yields. Soil microbes involving *Bacillus thuringiensis*, *Trichoderma* spp. and *Beauveria bassiana*, among others, have widely been used as biocontrol agents, controlling pests and pathogens, significantly impacting crop production (Bravo et al., 2011; Sharma et al., 2011; Mascarin & Jaronski, 2016).

2.9 Gaps in Literature

From the literature above, it is evident that different agronomic management technologies can contribute to soil nutrient enrichment and influence soil microbial parameters. However, scientific evidence around some of the effects of different ISFM interventions on different soil microbial parameters are inconsistent, and majorly lacking in the study area. Wide knowledge gaps also exist on the relationship between different ISFM technologies with microbial community structure, soil extracellular enzyme activities and their associated gene abundances, microbial structure and emission of greenhouse gases in the study region. In addition, the influence of different ISFM practices, either practiced solely or in combination with other practices, on microbial biomass, diversity, abundance and functions and the interlink between these and the different soil chemical parameters remain widely unknown. Besides, the study region is characterised by increased phosphorus deficient soils. Phosphorus and nitrogen are major crop production constraints in the study region, commonly available through expensive fertilisers, but ecofriendly practices that promote their bioavailability are not yet adequately explored in the region. From the literature, there is a dearth on information on the studies that have associated the likely quantities or amounts of essential crop nutrients (nitrogen and phosphorus) that can potentially be mineralized under certain ISFM practices, together with their monetary equivalents and implications on crop yields in the study region. Yet such ecofriendly practices that promote bioavailability of nutrients can bring financial relief to the resource-constrained smallholder farmers amidst enhancing soil quality, productivity and crop yields. Furthermore, from the literature, it is evident that there exist very few studies associated with the long-term

responses of greenhouse gas emissions under different agronomic practices commonly practiced by the smallholder farmers in western Kenya region. Thus, more data-driven evidence is necessary to bridge these wide knowledge gaps in the study region and inform relevant soil health and related agricultural policies.

CHAPTER THREE: METHODOLOGY

3.1 Description of the Study Area

This research was conducted in Madeya, in an existing long-term experimental trial named INM3, situated in Siaya County, Kenya (Figure 3.1). The long-term experimental trial was initiated in 2003 by International Centre for Tropical Agriculture (CIAT). The trial is positioned between latitudes 0º 08' S and longitude 34º 24' E (Figure 3.1); under sub-humid climate characterised by biannual rainfall (1420-1730 mm), with annual temperatures averaging about 23 ºC (Kihara et al., 2012). Soils are mainly Ferralsols, characterized by low pH (4.75); with sand (26%), silt (18%), clay (56%), soil organic carbon (2.4%) and about 3.7 mg kg^{-1} of phosphorus (Kihara et al., 2012). Crop cultivation in the area mainly serves subsistence purposes, highly reliant on the rainfall and mostly undertaken within smallholder conventional farming systems (under approximately 0.5 acres of land). Mostly, cereals like maize and sorghum are intercropped with legumes involving beans, cowpeas and soybean. Maize is the predominant staple crop in the region.

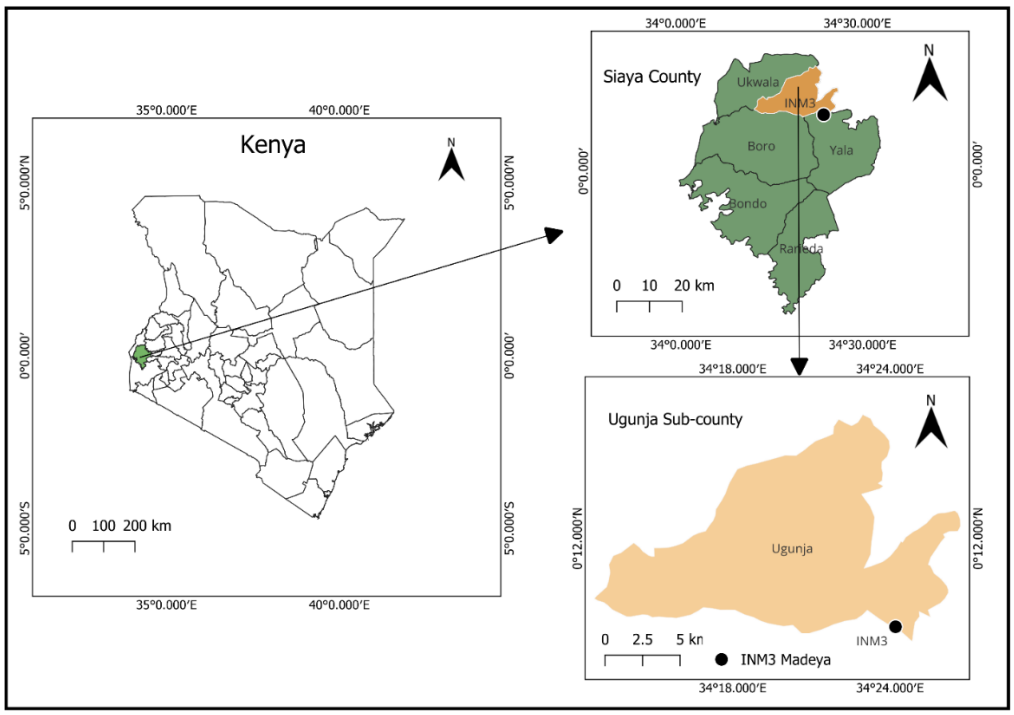

Figure 3.1. Map of the INM3-Madeya long-term experimental site (Source: Author)

3.1.1 Criteria for Selection of the Study Area

The selection of the study area was guided by several factors. First, due to soil acidity prevalent in the region, the soils in the study area are increasingly deficient of phosphorus, which is one of the essential nutrients for crop production. Soil acidity in the region seriously raises production costs by consistently impairing nutrient availability and affecting soil biodiversity. Besides, there was a need to conduct the present study in a long-term agricultural trial to understand the changes that agricultural management practices imposed over long periods of time may have on a range of soil quality parameters. The study area was also selected because it is one of the food baskets in the country, and together with previous research conducted, the present study would provide additional insights and evidence important for influencing policy decisions. Furthermore, the study area was chosen because it experiences good weather patterns evident through predictable and reliable rainfall necessary for successful crop production.

3.2 Land preparation and Trial Management

Land preparation is often done a few weeks before planting. During land preparation, the plots are often cleared of weeds and other vegetation by slashing. Thereafter, tilling often follows, and is normally done by hand hoeing to a depth of 15-20 cm. All the plots are normally tilled during the dry season, timed about two weeks before planting. At planting, maize are normally planting in the trail at a spacing of 25 cm x 75 cm. The same spacing used for planting maize is similar to the one employed for planting tephrosia. During planting, 2 maize seeds are placed in every planting station. However, after germination, the maize are normally thinned to one seedling per hill. On the other hand, soybean in the intercrops are always planted singly at a spacing of 5cm x 75cm. Weeding is often done in every plot by hand hoeing. Weeding is normally done twice or sometimes three times when the weed density is high. The first weeding is often done about four weeks after planting and the second weeding timed before tasselling. Topdressing is done with urea fertiliser, normally timed when the maize are about knee-high. Pests are often managed by spraying with appropriate chemicals when infestation incidences are noticed. The present study aligned with all the management practices as stipulated in the initial design.

3.3 Experimental Design

This research was carried out in Madeya, Siaya County, in an existing long-term experimental trial named INM3. The trial was established in a randomized complete block design, having four replications. The whole experiment contains 192 plots, each measuring 4.5 m x 6 m and testing different agronomic practices. The entire INM3 long-term experiment is composed of three distinct cropping systems. These involve maize planted as a monocrop (continuous/sole maize), maize planted as a rotation with tephrosia (*Tephrosia vogelii*) and maize planted as an intercrop with soybean. For the present study, only two cropping systems (continuous maize and maize-tephrosia rotation) were selected for the assessments. Besides, the entire trial also has two distinct residue (maize stover) management regimes. These involve plots where residue is either retained at 2 t ha^{-1} per season or plots where residues are totally removed (0 t ha^{-1}) from after harvesting. Additionally, the experimental trial also has two distinct organic input (FYM) management regimes. These involve plots where FYM is applied at either 0 or 4 t ha^{-1} per season.

3.3.1 Fertilizer Management in the Experimental Trial

Three different types of fertilisers are used every cropping season in the trial, and these were used in the present study. These include triple super phosphate (TSP), urea and muriate of potash (MOP). The fertilisers were applied at different timings, and at respective rates, based on the initial experimental design. Both MOP and TSP are often only applied once during planting. However, urea is often applied two times with the first application done at planting while the second application done during topdressing. During planting, MOP fertiliser was applied at 60 kg K ha^{-1} whereas TSP was applied at and 45 kg P ha^{-1}. On the other hand, urea application was done in two different splits. For instance, ⅓ of the quantity of urea was applied during planting while ⅔ was applied during topdressing. Generally, urea application is often targeted at four distinct rates of N (0, 30, 60 and 90 kg N ha^{-1}) in the entire trial, depending on the treatments, and in consistence with the initial experimental design. For nitrogen, all plots with maize and tephrosia rotations always receive either 0 and 30 or 60 kg N ha^{-1} while for phosphorus, they always receive either 0 or 60 kg P ha^{-1} per season. All plots with maize and soybean intercrops always receive 0 kg N ha^{-1} and 60 kg P per hectare per season. For continuous maize monocrop, all

plots often receive either 0, 30, 60 or 90 kg N ha^{-1} and P at 60 kg ha^{-1} per season. All plots always receive blanket application of K at 60 kg ha^{-1} per season in the entire experimental trial. The present research observed all the fertiliser input regimes in the respective treatments selected for the study in alignment with the initial trial design. The treatments selected for the study had either 0,60 or 90 kg N ha^{-1} for nitrogen and 0 or 60 kg ha^{-1} for phosphorus. All the fertilisers used and maize seeds planted were sourced and purchased by CIAT's procurement team from leading agrovets in Nairobi. Certified maize seed variety called DH-04 was used.

3.4 Selection of Treatments

Twelve treatments were selected for different assessments in this study. Eleven (11) of these were selected from the long-term trial and 1 treatment taken from undisturbed site chosen close to the trial site. Soil sampling was done only in three replications per treatment. A detailed description of the treatments studied, and the experimental design is given below (Table 3.1). The treatments selected for the study reflected the common practices normally done by the smallholder farmers in the region. These involve the modest retention of plant biomass in the field, use of manure and inorganic fertilisers, and/or no inputs. In addition, maize was chosen as the test crop in this study since it is a staple food crop in the region, and normally grown either alone or in rotations/intercrops with legumes.

The following were the characteristics of the composition of the manure that was used in the trial during the study period: Dry matter content (94.8%), electrical conductivity (4.04 mS/cm), pH (6.48), carbon (13.6%), nitrogen (1.18%), potassium (0.488%), phosphorus (0.29%), Calcium (0.803%), sulphur (0.11%), Magnesium (0.323%), Manganese (2319 ppm), Zinc (128.5 ppm), iron (48,050 ppm), boron (50.45 ppm), copper (42.975 ppm) and sodium (458.25 ppm). These attributes of manure are already published in Bolo et al. (2023).

Table 3.1. Description of the treatments selected to study the responses of soil microbial indices and greenhouse gas emissions on integrated soil fertility management systems

Treatment	Treatment (Input) description	CS	FYM	Residue	N	P
Control	No input	M-T	0	0	0	0
NPK	NP fertilizer	M-M	0	0	60	45
RK	Residue only	M-T	0	2	0	0
MK	FYM only	M-T	4	0	0	0
NPKR	NP fertilizer + residue	M-M	0	2	60	45
NPKMR	NP fertilizer + residue +FYM	M-M	4	2	60	45
NPKM	NP fertilizer + FYM	M-T	4	0	60	45
MRK	Residue + FYM	M-M	4	2	0	0
PK	P fertilizer	M-M	0	0	0	45
PKM	P + FYM	M-M	4	0	0	45
N*PK	90N+P fertilizer	M-M	0	0	90	45
Uns	Undisturbed natural site		-	-	-	-

CS=Cropping system; FYM = farmyard. The application of FYM was done at 4 t ha^{-1}. M-M= continuous sole maize; M-T= maize and tephrosia rotation. All treatments received a blanket potassium fertiliser application done at 60 kg K ha^{-1}.

3.5 Data Collection

The soil samples were taken at the top depths (0-15 cm). Sampling at this depth was important to capture the zones of active microbial populations (Marusenko et al., 2013). For objectives (i), (ii) and (iii), and in each treatment, sampling was done from five locations within each plot (in a W-shaped pattern) using soil augers. The soil samples collected from each plot were pooled in a bucket, thoroughly mixed and sub-sample taken. The sub-samples were placed in zip-lock bags, well stored and taken to the laboratory on the same day. The samples were transported under cool conditions. Upon reaching the laboratory, the soil samples were subjected to different processes with respect to the different analyses under study. Samples for assessment of soil chemical properties were air-dried, grinded and sieved to pass through 2mm mesh sieves and thereafter stored for further analyses. The samples for the assessment of biological parameters were sieved and stored in frozen state until extractions were done.

3.6 Laboratory Analysis

3.6.1 Analysis of Soil Chemical Parameters

The analysis of soil chemical parameters in the laboratory was done using airdried soil samples. Soil chemical parameters, involving soil organic carbon together with total nitrogen were assessed using Carbon and Nitrogen Elemental Analyzer. Other soil parameters like soil pH, N and Olsen P were assessed as per the conventional standards and techniques (Okalebo et al., 2002).

3.6.2 Determination of the Effects of Farmyard Manure and Inorganic Fertilizer Application on Soil Microbial Biomass and Bacterial Abundance

3.6.2.1 Assessment of Microbial Biomass Carbon (MBC)

MicroBiometer Soil Test kit (https://microbiometer.com) was used to assess MBC. Briefly, fresh soil samples were sifted. Using a calibrated syringe, 1 ml of samples were taken, compacted to 0.5 ml and the excess soil removed from the tip of the syringe. One sachet of the provided powder from the Kit was transferred into clean tube; water added and briefly mixed using a whisker. Soil samples were added, briefly mixed with the contents, whisked for 30 seconds to fully mix with the fluid and allowed to settle for 20 minutes. After every five minutes, the contents were briefly tapped to allow the floating debris to settle down the tube. After 20 minutes of settling time, the samples were extracted using pipette and 3 drops carefully applied to the reading card without wetting the surrounding of the card. The readings for microbial biomass carbon were thereafter taken by imaging the card using MicroBiometer App from Google Play Store.

3.6.2.2 Assessment of Bacterial Abundance

3.6.2.2.1 Soil Deoxyribonucleic Acid (DNA) Extraction

Fresh soil samples weighing 0.2g was in the process of extracting total DNA. The Phenol-Chloroform Isoamyl (PCI) Alcohol procedure that was previously described by Orwa et al. (2020) was used in the extraction of DNA from the soil samples. Soil DNA extraction was done in Embu University's microbiology laboratory. Briefly, 0.2 g fresh soil samples were weighed. This was followed by suspending the weighed samples in 200 µL of buffer solution and 5 µL of Lysozyme. The buffer

solution was made by mixing 100 µL Tris-HCl (pH 8.0) together with 100 mM EDTA (pH 8.0). The concentration of the lysozyme solution used was 20 mg/ml. The mixture was vortexed before incubation at 37 ℃ for 30 minutes. After vortexing the mixture, 400 µL of lysis buffer was added to the mixture. This was followed by incubation of the mixture for 10 minutes, with the incubation done at room temperature. The lysis buffer that was added in this step contained 60 mM EDTA (pH 8.0), 150 mM of sodium chloride, 400 mM Tris-HCl (pH 8.0) together with 1% sodium dodecyl sulfate. After incubation was complete, Guanidinium thiocyanate (10 µL) was added to the mixture. This was again followed by rei-incubation of the mixture. This mixture was incubated for two hours in a water bath whose temperature was maintained at 65 ℃ during the entire incubation period. After incubation, the mixture was subjected to phase separation that was achieved by addition of similar volume of the phenol chloroform isoamyl (PCI). The addition of the PCI was thereafter followed by centrifugation at 13,200 rpm, that proceeded for a total of 15 minutes, with temperatures maintained at 4 °C. thereafter, the supernatant that contained the crude DNA was carefully transferred to a new tube. This was then followed by recovery of the DNA from the supernatant, that was achieved by using the standard isopropanol. To check the DNA quality, 3 µL of the extracted DNA was separated on 1% agarose gel, checked against a 10 Kb marker. The resultant DNA pellets were thereafter lyophilized and stored at -80 °C until further analyses. The amplicon generation and sequencing of the DNA was done at MR DNA laboratory (www.mrdnalab.com, Shallowater, TX, USA).

3.6.2.2.2 Sequencing and Processing of the DNA extracted from the Soil Samples

The DNA generated was used in PCR amplification on the V4 variable region of 16S rRNA as described in Bolo et al. (2021). Briefly, the amplification was done using barcoded bacteria/archaeal primers 515/806 having barcode on the forward end as previously described by Caporaso et al. (2012). The process involved generating PCR amplicons first. This was done by HotStarTaq Plus Master Mix Kit (Qiagen, USA). This process of generating amplicons involved initial denaturation that was conducted at a temperature of 94 °C for a duration of 3 minutes. This was followed by 28 cycles done at temperatures maintained at 94 °C running for

30 seconds. This was then followed by primer annealing that was attained at temperatures of 53 °C for 40 seconds. The annealing of the primers was thereafter followed by extension of the amplicons at for 1 minute at temperatures maintained at 72 °C. This was proceeded by a final elongation of the amplicons at 72 °C for 5 min. Preparation of Illumina DNA library and sequencing followed the manufacturer's guidelines. The raw FASTQ sequences were uploaded in BaseSpace Sequence Hub under project number: Project 022020JKits (https://basespace.illumina.com/projects/163482319/samples).

The processing of the raw sequence reads was carried out using the sequencing facility's proprietary pipeline. Briefly, the original FASTQ files were demultiplexed. Thereafter, they were quality filtered (removal of barcodes, primers, adapters) followed by removal of reads containing ambiguous bases together with sequences having less than 150 base pairs. Paired reads were merged to obtain the full denoised sequences. This was followed by clustering the operational taxonomic units (OTUs). The clustering was done at 97% similarity. This was followed by taxonomic identification and classification of the clustered OTUs that was achieved by examining them using BLASTn. This was conducted using a carefully assembled database sourced from NCBI (www.ncbi.nlm.nih.gov) and RDPII (http://rdp.cme.msu.edu). Chimeric sequences were removed by Quantitative Insights into Microbial Ecology (QIIME).

3.6.2.2.3 Quantification of Extracellular Phosphomonoesterase Enzyme-linked *pho*C and *pho*D Gene Abundances

The quantification of the *pho*C and *pho*D gene abundances was done at the University of Guelph in Canada using the DNA previously extracted from the soil. All values of gene quantification were expressed in dry weight equivalent per gram of dry soil. The DNA amplification and quantification for both phosphomonoesterase enzyme-linked *phoC* gene and *phoD* genes followed the procedure previously reported (Fraser et al., 2015; 2017). Briefly, using CFX86 thermocycler (Bio-Rad), all the quantitative real-time polymerase chain reactions (*q*PCRs) were performed on 96 well plates. These were done using a Bio-Rad CFX86 thermocycler. Every qPCR was run in duplicates, each having total volumes

of 20 µL reactions. The 20 µL reactions consisted of different quantities and concentrations of mixtures of diluted DNA (1:25; 1 µL), nuclease-free water, primer (0.4 uM; 1 µL) and 2x SsoFast Evagreen Supermix (10 µL). The 2x SsoFast Evagreen Supermix used was a Bio-Rad product from Hercules, California, in the USA. The amplification of *pho*C and phoC gene were done in two steps. The conditions for amplification of *pho*C gene abundance involved the following steps; an initial 3-minute denaturation at 95°C. This was followed by 45 cycles of denaturation, each of 10 seconds at 95°C. This was proceeded by annealing and extension at 30 seconds (58°C). For amplification of *pho*D gene abundance, the conditions for the two-step process started with denaturation of 4 minutes at 94°C. This was followed by a melt phase of 40 cycles conducted for 30 seconds at 94°C and later proceeded by annealing and extension at 30 seconds. The amplification/elongation was thereafter followed by a melt step. This was to ensure the specificity of the reaction and was conducted at temperatures ranging from 65°C to 95°C, increasing by 0.5°C in each and every 5 seconds. Both the baseline as well as the threshold fluorescence values were calculated automatically using the Bio-Rad's CFX86 software.

3.6.3 Assessment of Effects of Manure, Fertilizer and Residue Addition on Extracellular Soil Enzyme Activities

Specific extracellular soil extracellular enzymes whose activities catalyse nitrogen cycling, phosphorus cycling and carbon cycling in the soil were assayed. These comprised N-acetyl-β-glucosaminidase (commonly referred as β-glucosaminidase), phosphomonoesterases (alkaline phosphatase and acid phosphatase), and β-glucosidase, respectively responsible for nitrogen, phosphorus and carbon cycling. High throughput colorimetric procedure previously developed by Tabatabai (1994) was employed in the laboratory in assaying the activities of the different extracellular enzyme (Plate 3.1).

Plate 3.1. Laboratory assay of extracellular soil enzyme activities

3.6.3.1 Acid Phosphatases Enzyme Activity Assay

Extracellular acid phosphatase (ACP) enzyme activities were assayed using high throughput colorimetric method (Tabatabai 1994) and as previously used by Luo and Gu (2016) and Kihara et al. (2018). Briefly, fresh soil were sieved to pass through 2-mm sieves. From the sieved soil samples, 1 gram was weighed and transferred into Erlenmeyer flasks each having 50 ml volumes. This was done for all the flasks except for the flasks that were to be used as control samples. After weighing of the samples, 4ml of modified universal buffer (MUB) was added to the contents. The MUB used for assaying acid phosphatase was at pH 6.5. Subsequently, toluene (0.25 ml) was added. After addition of toluene, 1ml of 115mM substrate (para-Nitrophenyl Phosphate (p-NPP)) solution to the contents in each flask. The flasks were stoppered, vortexed for 30 seconds to thoroughly mix the contents and incubated (37 ^{0}C) for a duration of 1 hour. Thereafter, CaCl (1 ml, 0.5 M) was added to the contents. NaOH (0.5M; 4ml) solution was then added. After termination of the reaction, the contents were centrifuged at 5000 revolutions per minute. Centrifugation was done for 10 minutes. This was followed by removal of the supernatants. The total quantity of p-NPP released per hour from one gram of dry

soil sample was used to express the potential ACP enzyme activity. Modified universal buffer (MUB) was prepared as described by Luo and Gu (2016).

3.6.3.2 Alkaline Phosphatases Enzyme Activity Assay

Extracellular alkaline phosphatase (ALP) enzyme activities was also assayed using high throughput colorimetric procedure following the protocol developed by Tabatabai (1994) and as adapted in previous studies (Luo & Gu, 2016; Kihara et al., 2018). This procedure was similar to the one used in assaying ACP except for the difference in pH of the universal buffer used. For ALP, the MUB used was pH at 11.0. Briefly, from the sieved soil samples, 1 gram was weighed and transferred into Erlenmeyer flasks each having 50ml volumes. This was done for all the flasks except for the flasks that were to be used as control samples. After weighing of the samples, 4ml of modified universal buffer (MUB) was added to the contents. The MUB used for assaying acid phosphatase was at pH 11.0. Subsequently, toluene (0.25 ml) was added. After addition of toluene, 1ml of 115mM substrate (para-Nitrophenyl Phosphate (p-NPP)) solution to the contents in each flask. The flasks were stoppered, vortexed for 30 seconds to thoroughly mix the contents and incubated (37^0C) for a duration of 1 hour. Thereafter, CaCl (1 ml, 0.5 M) was added to the contents. NaOH (0.5M; 4ml) solution was then added. After termination of the reaction, the contents were centrifuged at 5000 revolutions per minute. Centrifugation was done for 10 minutes and supernatants removed. The resultant concentrations were thereafter determined (at 400 nm) with a spectrophotometer. The total quantity of p-NPP released per hour from one gram of dry soil sample was used to express the potential ALP enzyme activity. Modified universal buffer (MUB) was prepared as described by Luo and Gu (2016).

3.6.3.3 β-Glucosidase Enzyme Activity Assay

Extracellular β-glucosidase enzyme activity (GLU) assay followed high throughput colorimetric method previously used by Luo and Gu (2016). The procedure of the assay was similar to the method used for assaying acid phosphatase enzyme activity, except for the use of para-Nitrophenyl–β-D-glucopyranoside (p-NPG) instead of p-NPP as substrate. In addition, Tris (hydroxyethyl) amino-methane (THAM) was used in terminating the reaction during β-glucosidase assay as opposed to using NaOH in ACP assay. Extracellular β-glucosidase enzyme activities were assayed

using high throughput colorimetric method following the protocol previously used by Luo and Gu (2016) and linked to Tabatabai (1994). Briefly, from the sieved soil samples, 1 gram was weighed into Erlenmeyer flasks having volumes of 50 ml. This was followed by adding 4ml of MUB. The MUB used was of pH 6.5. Thereafter, toluene (0.25 ml) and para-Nitrophenyl –β-D-glucopyranoside (p-NPG) solution (1 ml; 50 mM) were added to the contents in each flask (except for the control sample flasks that did not receive substrate). The flasks were stoppered and vortexed for 30 seconds. The vortexing was done to ensure the contents were thoroughly mixed. The vortexing was followed by incubating the contents for 60 minutes at temperatures maintained at 37 ^{0}C. This was thereafter followed by adding CaCl (1 ml, 0.5 M). After the addition of CaCl, 1 ml of THAM was added to terminate the reaction. The THAM buffer was of 0.1 M and pH 12. This is always the pH range of THAM buffer when it is made before its pH is altered). After termination of the reaction, the contents were centrifuged at 5000 revolutions per minute. Centrifugation was done for 10 minutes and supernatants removed. The resultant concentrations were thereafter determined (at 400 nm) with a spectrophotometer. The total quantity of p-NPG released per hour from one gram of dry soil sample was used to express the potential GLU enzyme activity. Modified universal buffer (MUB) was also prepared as described by Luo and Gu (2016).

3.6.3.4 N-acetyl-β-glucosaminidase (β-glucosaminidase; NAG) Enzyme Activity Assay

The assay of extracellular β-glucosaminidase activity, or sometimes called N-acetyl-β-glucosaminidase (NAG) enzyme followed the procedure previously used by Luo and Gu (2016); and as adapted from Parham and Deng (2000). This procedure is similar to the one for assaying GLU, with the only difference existing in the substrates and buffers used in the assay of NAG. Briefly, for NAG assay, the substrate used was 10mM para-Nitrophenyl-N-acetyl-β-D-glucosaminide (pNP-NAG). Acetate buffer (pH 5.5) having a concentration of 100 mM was used as the substrate buffer.

Briefly, 1 gram of sieved fresh soil sample from the field were weighed into Erlenmeyer flasks of 50 ml volumes. This was followed by adding 4ml of 100 mM Acetate buffer (pH 5.5), 0.25 ml of toluene and 1 ml of 10mM pNP-NAG solution

to the contents in each flask (except for the control sample flasks that did not receive substrate). The flasks were stoppered, vortexed for 30 seconds to ensure that the contents were thoroughly mixed. Afterwards, the contents were incubated for a duration of 1 hour at 37 ^{0}C. This step was followed by adding 1 ml of 0.5 M CaCl, followed by addition of 4 ml of THAM whose concentration was 0.1M and at pH 12 to ensure termination of the reaction. After the reaction was terminated, the contents were centrifuged at 5000 revolutions per minute. This step of centrifugation took 10 minutes and was followed by removal of the supernatant The resultant concentrations were thereafter determined (at 400 nm) with a spectrophotometer. The total quantity of pNP-NAG released per hour from one gram of dry soil sample was used to express the potential NAG enzyme activity.

3.6.4 Assessment of Effects of Manure, Phosphorus and Nitrogen Addition on Greenhouse Gas Emissions

The assessment of greenhouse gas emissions in the field was a short-term study conducted on three treatments (45 kg P ha^{-1} only; 45 kg P plus 4 t ha^{-1} FYM only; and 45 kg P ha^{-1} plus 90 kg N ha^{-1} only treatments (Table 3.1)) using closed chamber method (see Plate 3.2). Three gas chambers were installed in each plot before planting, but were removed and re-installed during and after completion of any land management activities involving weeding, manure and fertilizer applications. The base of each chamber (installed upto about 7 cm) measured 27 cm x 37.2 cm x 10 cm while the dimensions of the lids were 27 cm x 37.2 cm x 12.5 cm. During gas sampling, the chamber lids were mounted on the bases by tightly clipping them together using large metal binder clips. The chamber lids were uniquely fitted with vents, fans, sampling ports, digital probe thermometers and batteries. Gas sampling was done once per week during the normal days; or twice per week during the expected periods of high emission events like weeding, fertilizer application, manure application or heavy rains events). Gas sampling started in the morning (from 9 am) and ended by afternoon (around 1 pm) for every sampling day. During each gas sampling event, sampling chambers remained closed for 45 minutes. During gas sampling, 4 gas samples were taken from every plot. This was done at 15 minutes intervals (0, 15, 30 and 45 minutes). From each of the 3 gas chambers per plot, 20 ml gas samples were collected (using a needle and a 60 ml Luer lock propylene

syringe), gases pooled in the syringe by pumping repeatedly. 30 ml of the pooled gas samples were successfully transferred into pre-evacuated clean and well labelled gas vials by first displacing/flushing out 30 ml of the gas through a prior insertion of a small needle in the vial and removing when the 30 ml mark on the syringe is reached. The collected gas samples were sent to International Livestock Research Institute (ILRI) for analysis using Gas Chromatography.

In the laboratory, the concentrations of three gases comprising of CO_2, CH_4 together with N_2O gases were analyzed. The Gas Chromatography instrument, (8610C; SRI Instruments, Torrance, CA, USA) that was used was fitted with a ^{63}Ni-electron capture detector (ECD). The ECD was instrumental in determining N_2O concentrations. In addition, the instrument was also fitted with a flame ionization detector (FID). The FID was instrumental in the determination of CO_2. The instrument was also fitted with a methanizer. The methanizer played an integral role in the determination of CH_4. Nitrogen served as the carrier gas in the laboratory during the analysis. The nitrogen was released at a flow rate of 20 mL min^{-1}. Standards, consisting of four calibration gases, were used in the analysis. The peak areas for the different samples and standards were used in determining the concentrations of the different gas samples. Akin to this, depending on the peak areas of the different gases, concentrations of both CO_2 and CH_4 were determined using linear regression whereas that of N_2O were based on the power function. The rate at which the gas concentrations in the chamber headspace changed over time were used to calculate the emissions from the soil. In this calculation, ideal gas law was employed (see equation below), and involved the volume of the gas chamber, the total area of soil that was covered by the gas chamber, internal air temperature in the chamber and a correction of the fluctuations in the atmospheric pressure. Equation 1 was used in the calculation;

$$GHGF = (\partial conc/\partial time) * (mM/mV) * (Vh/A) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (i)$$

Where:

GHGF is the emission of the specific greenhouse gas from the plots, ∂conc/∂time denotes the change in the concentration of the particular gas emitted after given time. In addition, mM denotes or represents the molar mass of the particular element. For instance, for CO_2, as well as CH_4, the element in question is C, whereas for N_2O, the element is N_2. Moreover, mV denotes or represents the molar volume of gas. Furthermore, Vh denotes the

total volume of the entire headspace of the chamber. Finally, A denotes the entire area of soil that was covered in the field by the whole gas chamber.

Gas fluxes for CO_2 were reported in mg C m^{-2} h^{-1}. Similarly, gas fluxes for N_2O were reported in mg N m^{-2} h^{-1}. Linear regressions were fitted and coefficient of determination (R^2) determined for the gas concentrations. Where the coefficients of determination (R^2) for the CO_2 concentration fell below 0.90, it was deemed that the other flux measurements might not be dependable, probably following the assumption that the chambers might have malfunctioned following non-closure or leakages.

Plate 3.2. Setting up of chamber bases and in-field collection of greenhouse gases

3.6.5 Assessment and Quantification N and P Nutrient Mineralized, their Monetary Values and Maize performance under Select Input Management Practices

3.6.5.1 Preparation and Deployment of Ion Exchange Resin Bags In-situ

The assessment of microbial nutrient mineralization in the field involved the use of the in-situ resin core method. This procedure was recently utilized by Kihara et al. (2018). In short, an anti-insect net, a special net of 32-mesh size, was used to make special bags where the ion exchange resins would be contained (both cation and anion exchange resins). The nets were cut and customised to 5 x 6 cm, making bags

of approximately 30 cm^2. After making the bags, ion exchange resins (involving anion and cation) were separately weighed (5 grams) and transferred in the distinct bags. After every resin transfer into each bag was complete, the bags were tightly sealed. This was followed by leaching of the bags using 0.5 M HCl for 30 minutes. After leaching the bags already containing ion exchange resins, they were subsequently charged with 2 M NaCl for additional 30 minutes, followed by soaking in deionised water for 24 hours. After the elapse of 24 hours of soaking, the resin bags were removed and carefully packed in readiness for deployment into the field. Before deployment, the resin bags were tagged with different colours. The bags containing cation exchange resins were accorded red tags while the bags containing the anion exchange resins were tagged with blue colours. This tagging was necessary for ease of identification of the different ion exchange resins resin bags during deployment, and subsequently thereafter, during retrieval and further running the subsequent laboratory analyses. The resin bags were deployed in the fields and buried up to 15 cm depths.

When deploying the resin bags in the different plots, certain considerations were made. First, PVC pipes were used to protect the resin bags from damage by pests and also prevent lateral flows of nutrients that may be adsorbed by the buried ion exchange resins during the incubation period hence affecting the results. The PVC pipes used were of approximately 15 cm diameter (see Plate 3.3). The pipes were cut to a height of about 20 cm, so that approximately 15 cm would be inserted in the soil and about 5 cm protruding on the surface to prevent surface overflows from interfering with the buried resin bags. In addition, PVC plastics measuring approximately 20 x 20 cm were inserted a few centimetres beneath the PVC pipes to prevent upward flows of nutrients that may have interfered with the nutrients captured by the resin bags. Both lateral and upward nutrient flows were prevented to maximize on capturing only the nutrients mineralized from the top-soil up to the studied depth. The soil immediately surrounding the inserted PVC pipes were firmly pressed to ensure that the PVC pipe was strong, intact to prevent any likelihood of water soaking or saturating around the pipe that could interfere with the buried resin bags, and that the pipe remained upright throughout the entire period of resin incubation.

Plate 3.3 In-situ ion exchange resin incubation for nutrient mineralization assessment.

After deployment of the resin bags, they were incubated in the field for a total of 60 days. However, upon incubation, the first batch were retrieved after 30 days of incubation while the final batch were retrieved after 60 days of resin incubation. Careful considerations were observed to ensure that the retrieved anion exchange resin bags were not combined or put in contact with the cation exchange resins. For instance, upon retrieving each resin bag, the attached soils were carefully removed. Each bag was stored in separate zip-lock bags that were kept moist using distilled water to prevent the resins from drying up. The resins were thereafter taken to the laboratory for further processing under controlled temperatures (transported in cooler boxes) on the same day. In the laboratory, the retrieved resin bags were extracted and contents for different nutrients determined using colorimetric methods. The extracts for nitrates and ammonium were colorimetrically analysed using Salicylic acid method (Vendrell & Zupancic, 1990) and phosphates using Molybdate reagent method (Murphy & Riley, 1962). The concentrations were read using spectrophotometer at 400nm wavelength (for nitrates) and 880 nm (for phosphates).

The assessment of nutrient mineralization using in-situ resin core method was conducted in four treatments. These were selected from the list of 12 treatments presently studied. The four treatments included: i) A no-input control; ii) NP fertiliser (addition of 60 kg N + 45 kg P ha^{-1}, respectively); iii) addition of NP fertiliser combined with FYM (60 kg N ha^{-1} + 45 kg P ha^{-1} + FYM); and iv) addition of P only (45 kg P ha^{-1}). All these treatments received a baseline application of potassium fertiliser at 60 kg K $ha^{-1.}$ These treatments were selected to enable studying the effects of sole application of inorganic fertiliser inputs, combined application of fertiliser and organic inputs, and farmyard manure on nutrient mineralization. In the selection of the four treatments, a no-input control was necessary to assess how nutrient mineralization would respond following addition of sole P fertilizer. Except for the control, the rest of the treatments received uniform P fertiliser applications but different inputs. Hence, the uniform P applications in these treatments were assumed to have uniform effects in mineralization, and thus, the responses observed in such treatments would be ascribed to the other management factors embedded in such treatments over P application. In addition, topdressing was not done in any of these systems. This was necessary to limit any likelihood of the buried resins directly intercepting the nitrogen emanating from the added fertilisers from other.

3.6.5.2 Determination of Nitrogen and Phosphorus Mineralization from the Extracts from the Retrieved Anion and Cation Exchange Resin Bags

The resultant concentrations of the N and P from the spectrophotometer readings were therefore used in the calculation of the quantities of the nitrogen as well as phosphorus nutrients that were potentially mineralized per hectare. The bulk density, equivalent soil mass of the sampled depth (up to 0.15 m) in one hectare and nutrient concentrations were employed in quantifying the mineralized nitrogen and phosphorus contents from the assessment (Lee et al., 2009; Beesigamukama et al., 2021). This was done in four steps. The first step involved determination of the soil bulk density of the different samples within the fixed depth where the resin bags were buried. The second step involved determination of the volume of soil in the sampled depth per hectare basis. The volume of soil in one hectare was given by multiplying the area of one hectare (10,000 m^2) by the sampled depth (0.15 m) where

the resin bags were buried in the soil. The third step involved determination of the mass of soil up to the 0.15 m soil depth where the resin bags were buried, expressed on a per hectare basis. The mass of soil was calculated by multiplying the soil bulk density by the total soil volume of the sampled depth (0.15 m) on per hectare basis. The final step involved calculating the total amounts of nutrients mineralized per hectare basis. In this step, the total amount of nutrients mineralized per hectare were calculated by multiplying the mass of soil by the concentrations of either N or P from the analysis, borrowing from the previous formula (Lee et al., 2009; Beesigamukama et al., 2021). The total nutrients mineralised in one hectare within the depth where the resins were buried were determined using equation (ii).

$$Nutrient\ mineralised(kg\ ha-1) = \frac{nutrient\ concentration(mg\ kg-1)\ X\ mass\ of\ soil\ layer\ (kg)}{1{,}000{,}000} \quad \ldots\ldots\ldots\ldots$$

(ii)

*Where mass of soil layer (up to 0.15 m depth) per hectare = bulk density of the measured in kg m^{-3} **X** soil volume measured in m^3. The soil volume is from the soil layer (up to 15 cm depth) and was given by area of hectare (10000 m^2) multiplied by sampling depth (0.15m).*

3.6.5.3 Determination of Monetary Equivalent of the Quantities of Nitrogen and Phosphorus Mineralized during the Incubation Period

The monetary value of the resultant nutrient quantities mineralized were calculated on a per hectare basis. The equivalent prices were determined based on the cost of the equivalent amount of the nutrient available in a 50 kg bag of the commercial fertilizer that supply the particular nutrient within the trial. The two inorganic fertilisers used in these calculations were urea (for N) and triple superphosphate (TSP) for supplying P. These two inorganic fertilisers were selected because they were used as the source of nutrients that were applied, and also regularly used, in the agronomic trial where this study was conducted. The prevailing market price of a 50 kg bag of each of the inorganic fertilizer were attained. These prices were thereafter used in the calculations to determine the monetary values of the quantities of the nutrients mineralised following the equation below. From the calculations, the monetary values were calculated in Kenya shillings and expressed in dollars (USD) by dividing by 100. Monetary value (MV) of nutrients mineralized per hectare (USD ha^{-1}) was given by equation (iii):

MV = Nutrient mineralized (kg ha^{-1}) X {Price per kg} X % nutrient……..... (iii)

Where MV is the monetary value of the total nutrient mineralized per hectare (USD. ha^{-1}); NM is the nutrient mineralized per hectare; Price represents the cost (USD) of a 50 kg bag of the particular fertilizer providing the reference nutrient; and % Nutrient is the percentage nutrient content of the reference nutrient in the 50 kg fertilizer bag.

3.6.5.4 Determination of Maize Performance and Grain Prices

The maize yields (grain, stover and total aboveground biomass) and grain prices were determined as indicated in previous studies (Mupangwa et al., 2007; Bolo et al., 2024). Both grain yields and prices were expressed per hectare. The respective yeilds were determined (at 25% moisture content for maize grains) and expressed in tonnes per hectare using equation (iv).

$$Yield\ (t\ ha^{-1}) = \frac{\text{THFW}*\text{SHDW}*10}{\text{SHFW}*\text{Area}} \text{.....(iv)}$$

Where THFW represents the total fresh weight of particular plant part (either grains or stover) from the harvested net plot measured in kilograms; SHFW represents the fresh weight of the respective samples of particular plant part expressed in grams; SHDW represents the dry weight of the particular sample in grams; Area represents the area of the net plot expressed in square meters

The maize grain prices were expressed in USD on a per-hectare basis. A kilogram of the dry grains was estimated to cost approximately 0.4 USD (about 40 Kenyan Shillings) based on the prevailing local prices of the commodity during the cropping season.

3.7 Statistical Data Analysis

One-way analysis of variance (ANOVA) was used to determine the significantly different means of the different soil chemical and biological parameters per management practice (treatment) and means were seperated using Tukey HSD at $P \leq 0.05$. In addition, Pearson correlation was performed to estimate the relationships of bacterial community dynamics (richness, diversity and specific bacterial taxa) at the phylum level and environmental variables including soil pH, SOC, macronutrients, secondary nutrients and micronutrients; electrical conductivity (EC) and cation exchange capacity (C.E.C); together with the relationships that exist between particular soil chemical variables to each other.

Bacterial alpha-diversities were estimated based on the OTUs abundance matrices. Vegan package in R (Dixon, 2003) was utilised in calculating the OTUs number, species richness (observed and Chao1), relative abundances and Shannon diversity. Kruskal Wallis test was used to identify the significantly different ($P \leq 0.05$) bacterial phyla between three categories of management practices with addition of FYM (+FYM), with ommission of FYM (-FYM) and undisturbed natural site (Uns)] on a normalised data (log-relative transformed). The significantly different bacterial phyla were plotted using reshape and *ggplot2* libraries in R. Non-metric dimensional scaling (NMDS) was performed to evaluate the interrelatioships between bacterial community and the different input regimes. Cannonical correspondence analysis (CCA) was performed to identify the environmental variables that correlated with the total bacterial community structure at the phylum level. '*Anacor'* library and '*cca'* function in R was employed in running the CCA using Vegan packagec (Dixon, 2003). This was done on bacterial phyla that occurred in more than 90% of the total samples in the dataset. The CCA model results were further subjected to significance test using the *anova.cca* function in R, in order to assess whether there were notable differences that existed within the different management systems assessed. The overall significance of the relationship was assessed by ANOVA using the '*anova'* function in R, whereas the significant environmental variables were determined using the '*step'* function, with permutation test at 999 random permutations.

CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.1 Effects of Farmyard Manure, Inorganic Fertilizers and Residue Application on Soil Microbial Biomass Carbon, Bacterial Abundance and Diversity

The agronomic treatments significantly affected soil bacterial phyla ($P \leq 0.05$) but not microbial biomass carbon (MBC), in the study (Table 4.1). Alpha diversity indices for the bacterial phyla, for instance species richness (Chao1) and diversity (Shannon Index), significantly differed ($P \leq 0.05$) across the different management practices (Table 4.1; Figure 4.1b). *Acidobacteria, Actinobacteria, Firmicutes* and P*roteobacteria* were the bacterial phyla that exhibited the highest dominance across the different input management practices. Across the treatments, *Verrucomicrobia* was more abundant in undisturbed natural site (Uns) and least abundant under sole inorganic NPK fertilizer application (NPK). Long-term application of sole NPK fertilizer (NPK) significantly reduced abundance of *Bacteroidetes, Armatimonadetes* and *Fibrobacteres* relative to no input.

FYM addition, either alone (MK) or in combination with either inorganic NPK fertiliser (NPKM), or inorganic NPK fertiliser plus residue (NPKMR) significantly increased bacterial richness (Chao 1), diversity (Shannon), and abundance of *Proteobacteria, Verrucomicrobia, Fusobacteria, Armatimonadetes, Fusobacteria, Fibrobacteres, Nitrospinae, Bacteroidetes* and *Nitrospirae,* but in some instances, significantly decreased *Chloroflexi* and *Nitrospirae* abundance (Table 4.1; Figure 4.1a). Combined application of FYM with inorganic NPK fertilizer (NPKM) or NPK fertiliser plus residue (NPKMR) increased both bacterial species richness and diversity, and abundance of *Verrucomicrobia, Bacteroidetes, Nitrospirae, Armatimonadetes, Fusobacteria, Fibrobacteres* and *Nitrospinae* compared to sole application of NPK fertilizer. Long-term application of nitrogen at either 60 kg N ha^{-1} (NPK) or 90 kg N ha^{-1} (N*PK) respectively, significantly depressed bacterial richness, diversity and the abundance of *Verrucomicrobia, Armatimonadetes, Nitrospinae and Gemmatimonadetes;* but increased *Thaumarchaeota* and *Nitrospirae* abundance. Long-term sole addition of phosphorus (PK) significantly increased *Verrucomicrobia* but reduced *Thaumarchaeota* relative to no-input.

Residue addition, as a factor, increased the abundance of *Fusobacteria* and *Nitrospira*, respectively; but no significant effect was observed on the diversity indices (Chao 1 and Shannon) and the rest of the bacterial phyla. Compared to control, sole application of NPK fertilizer (both at 60 or 90 kg N ha^{-1}, respectively) reduced bacterial Shannon diversity and abundance of *Bacteroidetes, Nitrospirae, Armatimonadetes* and *Fibrobacteres.*

The bacterial species were generally more abundant in treatments applied with FYM (MK, NPKM, NPKMR, MRK, PKM) compared to treatments without FYM addition (Control, NPK, RK, PK and N*PK) (Table 4.1). For instance, sole incorporation of FYM (MK), or combined addition of FYM together with inorganic NPK fertilizers (NPKM, NPKMR, PKM) or residue (MRK) significantly increased the abundance of *Proteobacteria, Verrucomicrobia, Bacteroidetes, Nitrospira, Armatimonadetes, Fusobacteria, Fibrobacteres* and *Nitrospinae* relative to either long-term application of inorganic NPK fertilizer alone (NPK), NPK fertiliser combined with residue (NPKR); and/or no input (Control) addition.

Table 4.1. Microbial biomass carbon (MBC) and soil bacterial structure (phylum level) and diversity responses to long-term agronomic management practices in INM3 site

Variables	Control	NPK	RK	MK	NPKR	NPKMR	NPKM	MRK	PK	PKM	N*PK	Uns	*P*-value	LSD
MBC¥	418^{a}	573^{a}	470^{a}	480^{a}	357^{a}	410^{a}	390^{a}	353^{a}	336^{a}	454^{a}	409^{a}			276
Chao1	1892^{cd}	983^{d}	3217^{bc}	4642^{ab}	1750^{cd}	4525^{ab}	4275^{ab}	4633^{ab}	3392^{abc}	5108^{a}	2242^{cd}	4250^{ab}	***	
Shannon	6.92^{bc}	6.64^{d}	6.9^{bc}	7.18^{a}	6.74^{cd}	7.22^{a}	7.13^{ab}	7.32^{a}	6.94^{bc}	7.34^{a}	6.84^{cd}	7.19^{a}	***	0.2
*Proteobacteria**	90.1^{d}	93^{bcd}	90.5^{d}	99.4^{ab}	90.5^{d}	98.5^{abc}	99.1^{ab}	102.6^{a}	91.1^{cd}	99.7^{ab}	90.7^{cd}	97.7^{abcd}	***	7.9
*Actinobacteria**	70.5^{ab}	70.5^{ab}	70.8^{ab}	65.9^{b}	71.3^{ab}	66.6^{b}	68.2^{b}	65.7^{b}	71.2^{ab}	76.1^{a}	72.1^{ab}	76.5^{a}	***	7.2
*Firmicutes**	63.4^{ab}	73.9^{a}	66.1^{ab}	55.5^{b}	70.2^{ab}	61.2^{ab}	69.3^{ab}	56.8^{b}	66.5^{ab}	60^{ab}	73.2^{a}	55.5^{b}	***	14.8
*Acidobacteria**	61.2^{a}	63.2^{a}	61^{a}	57.6^{abc}	58.6^{ab}	61.4^{a}	58.3^{ab}	54.4^{bc}	61^{a}	57^{abc}	57.4^{abc}	51.4^{c}	***	6.5
*Gemmatimonadetes**	55.6^{abc}	59^{ab}	51.9^{cd}	56.9^{abc}	57.4^{abc}	54.5^{abcd}	52.5^{bcd}	55.5^{abc}	59.8^{a}	55^{abc}	52.7^{bcd}	48^{d}	***	6.7
*Chloroflexi**	57.5^{a}	56.2^{ab}	51.3^{abc}	44.2^{c}	55.8^{ab}	48.3^{abc}	47.5^{bc}	46.1^{c}	50.9^{abc}	49.8^{abc}	50.7^{abc}	43.8^{c}	***	9.3
*Planctomycetes**	43.8^{a}	40.2^{ab}	41.9^{ab}	38.2^{ab}	41.2^{ab}	39^{ab}	38.9^{ab}	36.3^{b}	39.3^{ab}	37.3^{b}	39.8^{ab}	39.5^{ab}	***	5.8
*Verrucomicrobia**	22.3^{c}	20^{c}	21.9^{c}	33.8^{b}	20^{c}	32.9^{b}	32.2^{b}	35.2^{b}	32^{b}	35.1^{b}	22.9^{c}	47^{a}	***	8.8
*Bacteroidetes**	29.6^{abcd}	21.7^{f}	27.4^{abcdef}	32.4a	26^{bcdef}	30.1^{abc}	28.1^{abcde}	30.9^{ab}	24.3^{def}	28.4^{abcde}	23.2^{ef}	24.8^{cdef}	***	5.7
*Thaumarchaeota**	18.2^{a}	18.5^{a}	15.6^{ab}	12.8^{ab}	17.5^{ab}	11.9^{ab}	13.9^{ab}	13.5^{ab}	11^{b}	10.5^{b}	15.9^{ab}	11.7^{ab}	***	7.1
*Nitrospirae**	12.5^{bcd}	16.4^{a}	10.7^{d}	11.8^{cd}	12.5^{bcd}	15^{ab}	10.5^{d}	16.9^{a}	15.3^{ab}	17^{a}	14.2^{abc}	17.1^{a}	***	3.1
*Armatimonadetes**	8.4^{ab}	6.8^{c}	6.9^{bc}	8.3^{abc}	6.9^{bc}	9.7^{a}	8.7^{a}	7.1^{bc}	8.5^{ab}	8.4^{ab}	6.8^{c}	5.1^{d}	***	1.6
*Fusobacteria**	2.6^{de}	1.6^{e}	3.4^{cde}	5.7^{ab}	4.2^{bcd}	6.9^{a}	5.8^{ab}	5.3^{abc}	2.8^{de}	5^{abc}	4.1^{bcd}	4.9^{bc}	***	1.9
*Fibrobacteres**	3.6^{ab}	0.9^{c}	3^{abc}	3.3^{ab}	1.6^{bc}	4.2^{a}	3.9^{a}	4.2^{a}	2.3^{abc}	3.8^{a}	2.7^{abc}	3.2^{ab}	***	2.1
*Nitrospinae**	1.9^{cde}	0.3^{e}	2.4^{cd}	3.6^{abc}	0.3^{e}	3.1^{bc}	3.3^{abc}	4.2^{ab}	3.5^{abc}	5^{a}	1.2^{de}	2.4^{cd}	***	1.8

Across every row, values that have similar letters do not have any significant differences. ¥ = mg kg^{-1}; * = square-root transformed counts; MBC = Microbial biomass carbon; Control = No input; NPK = P+N fertilizers only; RK = residue only; MK = + FYM only; NPKR = P+N + residue only; NPKMR = P+N +FYM+residue only; NPKM = P+N +FYM only; MRK = Residue and FYM only; PK = +P fertilizer only; PKM = P + FYM only; N*PK = P+90 kg N ha^{-1} only. *** = significant difference at $P \leq 0.001$; LSD = Least significant difference

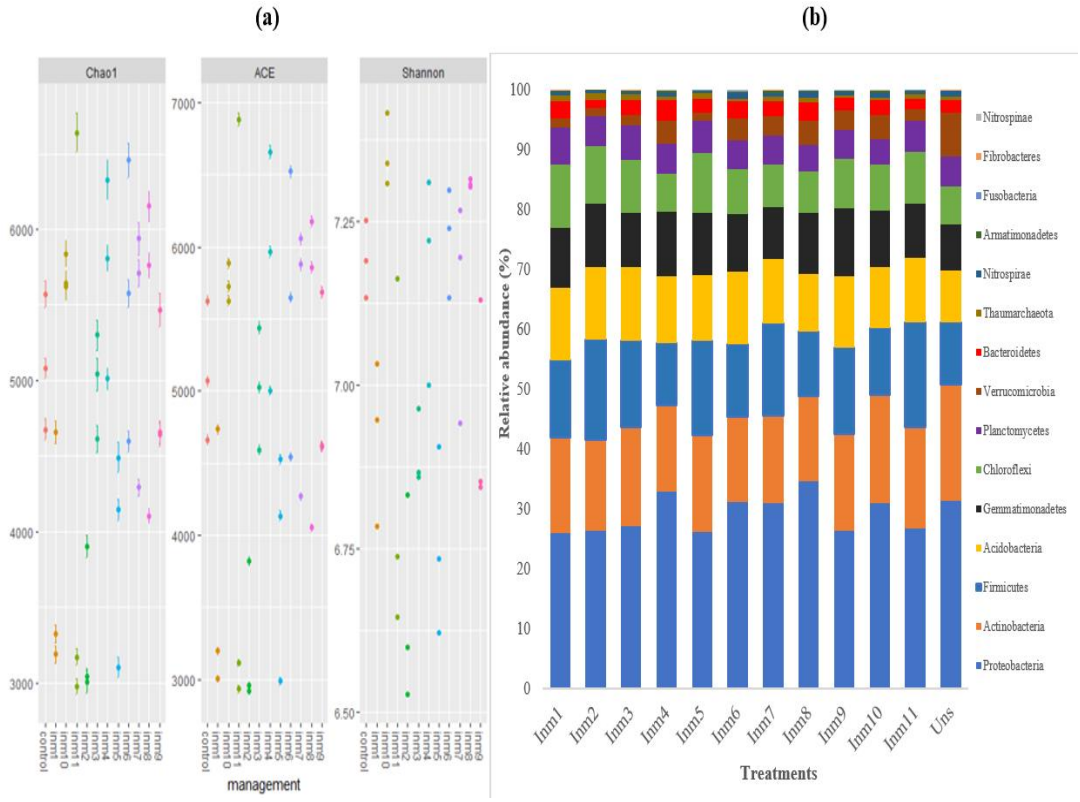

Figure 4.1. The overall diversity (a) and relative abundance (b (with ≥ 80% representation of data points per sample in the dataset)) of different bacterial communities under different agronomic practices in the study site.

Inm1 = No input; Inm2 = P+N fertilizers only; Inm3 = residue only; Inm4 = + FYM only; Inm5 = P+N + residue only; Inm6 = P+N +FYM+residue only; Inm7 = P+N +FYM only; Inm8 = Residue and FYM only; Inm9 = +P fertilizer only; Inm10 = P + FYM only; Inm11 = P+90 kg N ha^{-1} only; Uns = undisturbed natural site close to the trial.

Amongst the alpha diversity indices, both bacterial species richness (Chao1) and species diversity (Shannon index) significantly differed ($P \leq 0.05$) between the different management practices (Table 4.1; Figure 4.1a). Generally, bacterial diversity (Shannon index) reduced under treatment with sole incorporation of NPK fertilizer (NPK) in comparison to the other treatments. Bacterial richness (Chao1) and Shannon diversity increased when FYM was added; either solely (Inm4; MK) or when FYM was combined with fertilizer (Inm7; NPKM), residue (MR) or both fertilizer and residue (NPKMR) compared to the control (Table 4.1; Figure 4.1 a,b).

Bray-Curtis' similarity, based on hierarchical cluster analysis, revealed distinct grouping patterns of treatments relevant to the different agronomic management

practices implemented (Figure 4.2). Further results indicated that the soil bacterial species were clustered in two major clades based on either application or no application of farmyard manure. Specifically, the management practices having FYM addition were grouped in one major clade and those without FYM in a different clade. In Figure 4.2, the treatments with FYM addition appear as FYM+PN+R, FYM only, FYM+R, FYM+PN and P+FYM while the remaining treatments lack FYM. The clustering of the management practices with FYM addition had almost 91.4% similarity index (Bray-Curtis) while those lacking FYM addition had 90.0%. Management practices with combined application of inorganic NPK fertilizers (both P+N and P+N*) and residues (P+N+R) were similarly distinctly grouped, with 92.5% similarity index (Figure 4.2). This was the similar case observed under the treatments with no input application (None), sole application of either residue (R only) or P fertilizer (P only), all having 92.3% similarity.

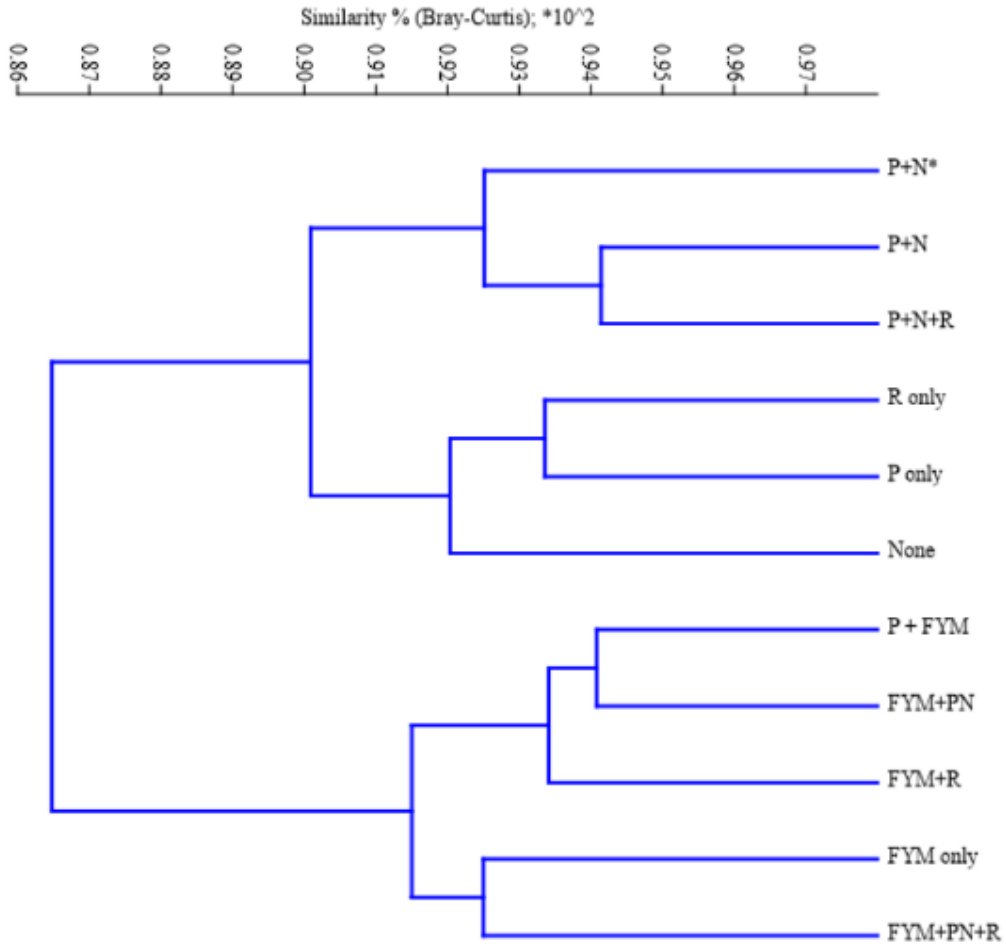

Figure 4.2. Bray-Curtis's similarity index plot based on hierarchical clustering of bacterial species under different soil fertility management systems in INM3 Madeya.

None = No input; P+N = P+N fertilizers only; R Only = residue only; FYM only = addition of 4 t ha^{-1} FYM only; P+N+R = P+N + residue only; FYM+PN+R = P+N +FYM+residue only; FYM+PN = P+N +FYM only; FYM+R = Residue and FYM only; P only = +P fertilizer only; P+FYM = P + FYM only; P+N* = P+90 kg N ha^{-1} only.

Evidence from Non-metric multidimensional scaling exhibited that distinct bacterial grouping patterns were formed under treatments applied with FYM relative to those lacking FYM or undisturbed natural site (Figure 4.3).

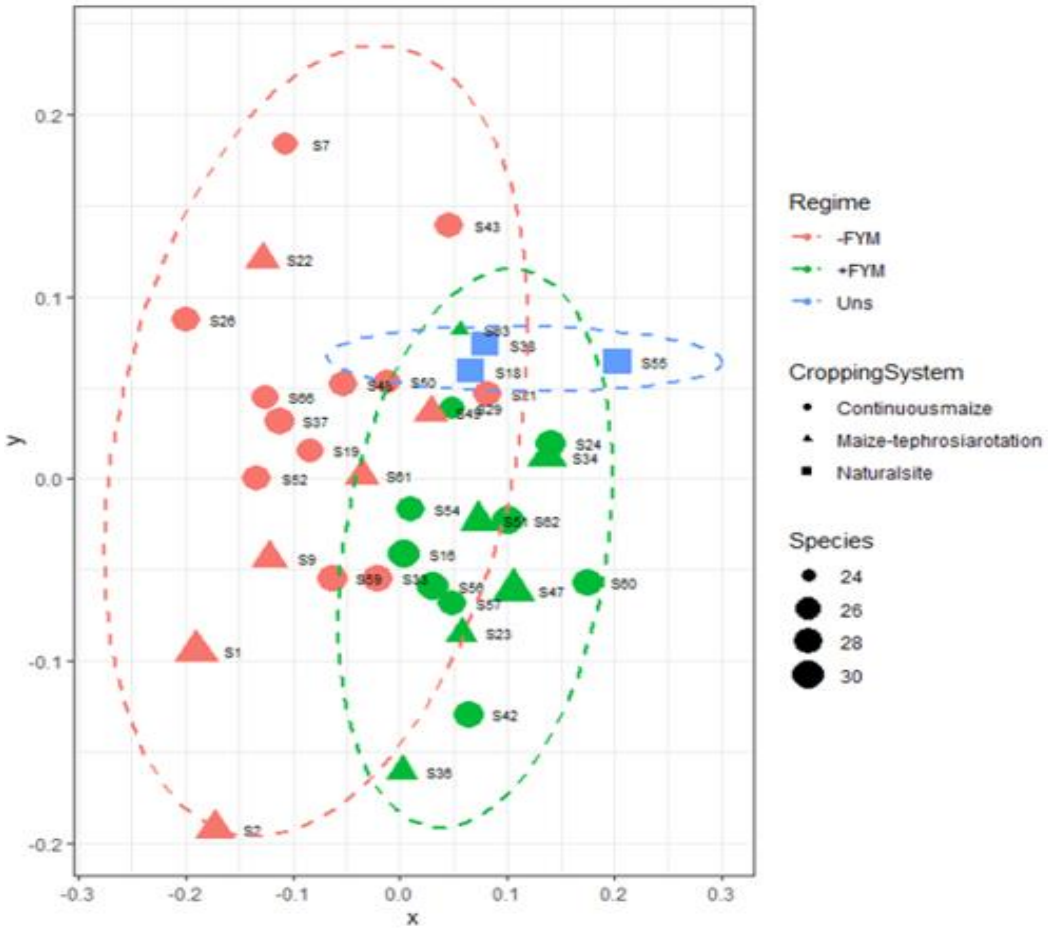

Figure 4.3. Distinct grouping patterns of bacterial phyla under three input regimes in the study site. S1-S66 represent specific samples per treatment.

The bacterial species were generally more abundant in treatments applied with FYM than those lacking FYM. In connection to this, cumulatively, twelve (12) bacterial phyla were significantly affected by either addition of FYM or omission of FYM. Amongst the 12, the population of 8 bacterial phyla significantly increased under treatments with FYM application relative to those lacking FYM while 4 bacterial phyla significantly increased in treatments lacking FYM relative to those with FYM applied (Figure 4.4).

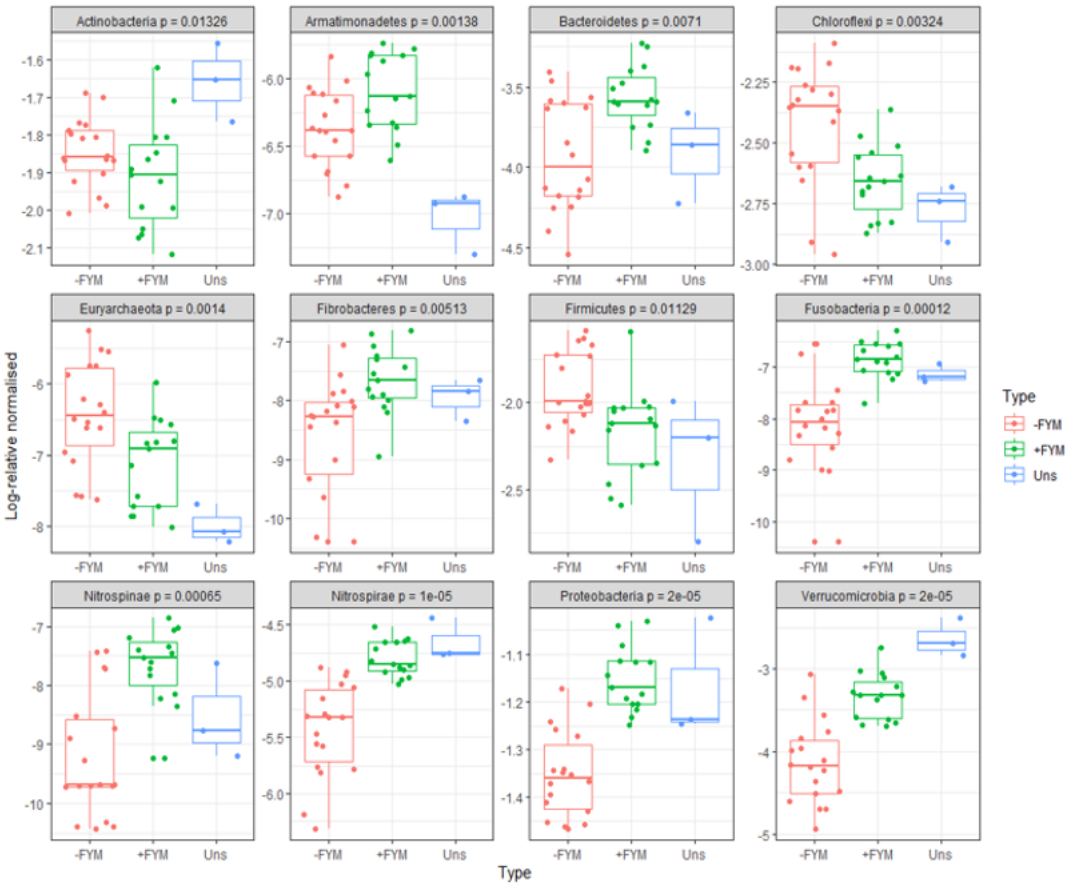

Figure 4.4. The specific bacterial phyla significantly affected by FYM application in the study site.

The outcomes from this study revealed that some bacterial communities were generally more dominant compared to the others across the different agronomic practices studied. The increased dominance of certain bacterial community structures like *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Acidobacteria* than the rest of the bacteria not only indicates the likelihood of some microbes to dominate across a wide range of systems, but also the possibility to survive under wide range of environmental conditions. Some of these microbes also act on a wide range of substrates, performing various roles related to nutrient cycling, but they can also be influenced by different management practices. The observed dominance of certain bacteria across the different systems can also be due to the nature of their nutritional lifestyles. Some bacteria often have nutritional preferences, with the copiotrophs preferring nutrient/carbon rich systems, oligotrophs (for example *Chloroflexi*) preferring nutrient/carbon deficient systems, whereas others may have complex nutritional adaptations that integrate both oligotrophic and copiotroph

lifestyles. Therefore, across the different agronomic systems studied, the practices characterised with more nutrient/carbon availability could prompt the dominance of copiotrophs involving *Proteobacteria* and *Actinobacteria* and depressed the oligotroph like *Chloroflexi*. The increased dominance of *Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria* and *Firmicutes* over the other groups across the different management systems is not unique to this study but consistent with the findings from other previous studies. For instance, recent studies reported that *Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria* and *Firmicutes* often dominate over other bacterial groups in most soils (Mendes et al., 2013; Deng et al., 2018). Besides, Bahram et al. (2018) and Mhete et al. (2020) reported that in most instances, the abundances of different microbial groups can be regulated by several aspects related to agronomic management interventions (like tillage, input addition, cropping systems), soil properties, nutrient availability, climate, among others. *Actinobacteria* and *Proteobacteria* were previously reported as copiotrophs (Ranjan et al., 2015; Semenov et al., 2021) while *Chloroflexi*, among others, as oligotrophs (Ho et al., 2017; Semenov et al., 2021).

The outcomes further revealed that both sole application of FYM, and combined incorporation of FYM with other inputs, increased both the abundance as well as diversity of the bacterial groups. The increase in bacterial abundance and diversity following addition of FYM could be due to the contribution of FYM in the improvement of soil health by enhancing soil physio-chemical conditions involving soil structure, pH, fertility, and nutrient status. Besides, the increase in microbial abundance with FYM can be linked to its influence in increasing soil organic carbon contents that provide food for microbes, as well as creation of enabling microclimate that favour microbial proliferation. The observed increase in bacterial abundance and diversity following FYM addition aligns with the outcomes reported in earlier studies (Ye et al., 2021; Lee et al., 2023; Sayre et al., 2023). Consistent with the present observations, Lee et al. (2023) noted that 13 bacteria positively correlated with addition of organic inputs. In addition, Sayre et al. (2023) noted that microbial abundance and diversity increased more with increased rate of application of manure, and this was attributed to the positive influences of organic inputs on different soil attributes. In Kenya, Nyamwange et al. (2021) also reported increased

bacterial abundance in treatments applied with organic inputs such as mulches. Previous studies suggested that manure contains humic substances that have the ability to stabilize soil structure, enhance soil porosity as well as stimulate formation of soil aggregates (Mugwe et al., 2009; Mucheru-Muna et al., 2014; Almendro-Candel et al., 2018). The aggregates would otherwise provide suitable ecological niches or microsites/habitats that harbour and favour the proliferation and interactions of different microbial communities (Chen et al., 2022; 2023). Besides, FYM application increases soil organic matter content that contributes to more SOC which is used as a source of food and energy by the soil microbes for growth and activities. As the population of the bacteria grows, they enhance nutrient availability by increasingly decomposing the added soil organic matter (FYM) and releasing more essential nutrients (Khatoon et al., 2017) that further support their growth, diversity and activities.

Furthermore, according to Fekadu et al. (2019), FYM has soil pH amelioration properties and provides more soil nutrients. Thus, it is likely that the FYM added could modify the soil pH conditions and increase nutrient availability, making it suitable for certain types of bacteria well adapted to thrive and proliferate, while the others (not adapted) to decline. Opala et al. (2012) stated that FYM, whether applied solely or incorporated in combination with other inputs, improved soil pH in two study sites. This this aligns with the observations in the current study whereby soil pH was significantly increased under treatments with FYM addition. The increase in soil pH, among other associated soil chemical properties, perhaps contributed to the increases observed in diversity and abundance of some bacterial strains involving *Proteobacteria, Verrucomicrobia, Bacteroidetes, Nitrospira, Armatimonadetes, Fusobacteria, Fibrobacteres* and *Nitrospinae* under systems applied with FYM (whether FYM was incorporated either solely or combined with inorganic NPK); and subsequent decreases in some bacterial strains involving *Chloroflexi* and *Nitrospirae* abundance, compared to the systems lacking FYM addition.

Further outcomes from the present study revealed that residue addition increased the abundance of certain bacterial strains involving *Fusobacteria* and *Nitrospira.* This observation, just like for FYM, can be attributed to the contribution of residues in improving the soil organic carbon content, nutrient status, enhancement of soil

physiochemical properties and creation of microclimate that favour microbial proliferation. This observation corroborates the outcomes reported in earlier studies (Wang et al., 2020). Increases in microbial parameters following residue application were previously documented in the study region (Bolo et al., 2021; 2023b) and elsewhere (Wang et al., 2020). These responses were attributed to the influences of organic inputs on soil organic carbon contents that act as important food sources for the microbial community. Previous literatures have acknowledged the positive contributions of organic inputs, involving residues, on soil organic carbon contents alongside microbial characteristics such as bacterial abundance and diversity (Wang et al., 2020; Brichi et al., 2023). This could explain the observed increase in abundance *Fusobacteria* and *Nitrospira* following residue application.

In the different management practices that were applied with inorganic nitrogen fertiliser, the outcomes indicated that bacterial community richness, abundance together with diversity were depressed following the addition of nitrogen. The decrease in these bacterial parameters following nitrogen addition could be attributed to contribution of nitrogen to alterations in soil pH, hence creating unfavourable conditions for microbial proliferation. This could elucidate the observed decrease in both the microbial abundance as well as bacterial diversity; and depressed abundance of certain bacterial strains involving *Verrucomicrobia, Armatimonadetes, Nitrospinae; Gemmatimonadetes*. However, some bacterial strains, like *Acidobacteria*, may be better adapted to, and thrive well in, acidic conditions as well as nitrogen-rich systems. Reduction of soil pH with nitrogen addition is previously documented (Tian et al., 2019). In this study, the observed reduction in some bacterial species abundance and diversity following nitrogen addition corroborates the findings from previous studies (Tian et al., 2019; Widdig et al., 2019; Bolo et al., 2021; Wang et al., 2021) who attributed the responses to contributions of nitrogen in modifying soil pH. Conversely, in this study, nitrogen addition also increased the abundance of some bacteria like *Thaumarchaeota* and *Nitrospirae*. These (*Thaumarchaeota* and *Nitrospirae)* are nitrogen-cycling bacterial community strains and therefore, the added nitrogen could provide additional substrate that would favour their proliferation and activities. This observation is consistent with the recent findings from a Luvisol in China where addition of

nitrogen provided substrate for a wide range of N-cycling bacteria resulting to increase in their abundance (Ibrahim et al., 2020).

Conversely, the current findings indicated that some bacteria, involving *Verrucomicrobia*, were more dominant in the undisturbed natural site compared to the management practices with NPK fertiliser addition. This could potentially be attributed to increased accumulation of organic matter and reduced soil disturbance in the undisturbed natural site compared to the NPK added treatments, which furthermore, were practiced in the tilled (disturbed) soils. According to West & Whitman (2022), soil disturbance sharply depresses the microbial community richness and diversity, and this could prompt the decrease in *Verrucomicrobia* abundance in the tilled (NPK added) site compared to the undisturbed natural site. Moreover, the increased accumulation of organic compounds in the undisturbed natural site could have increased the contents of soil organic carbon that would have favoured the proliferation of certain bacteria adapted to carbon rich systems. The higher abundance of *Verrucomicrobia* in undisturbed natural site compared to the NPK added (tilled) site in this study aligns with findings from earlier studies (Navarrete et al., 2015). Elevated soil microbial parameters (involving biomass, richness, diversity and activities) under agricultural systems with reduced soil disturbances are previously documented (Van Groenigen et al., 2010; Mbuthia et al., 2015; Kihara et al., 2018).

4.1.1 Relationship between Soil Chemical Properties and Bacterial Community Structure

Individual bacterial phyla and diversity indices (except richness) variably correlated with at least one soil chemical property across the management practices (Table 4.2). Both Pearson's correlation and CCA results showed that *Proteobacteria, Verrucomicrobia, Bacteroidetes, Nitrospirae, Fusobacteria, Nitrospinae, Cyanobacteria, Actinobacteria* and *Fibrobacteres* positively correlated ($P \leq 0.05$) with one or more soil properties such as pH, Mg, Ca, SOC, N, Fe, Cu, K, Zn and C.E.C (Table 4.2; Figure 4.5). *Firmicutes, Chloroflexi, Actinobacteria, Gemmatimonadetes, Planctomycetes, Deinococcota, Elusimicrobia* and *Spirochaetes* negatively correlated ($P \leq 0.05$) with (either and/or) Cu, pH, SOC, Mg, P, N, Ca, B, Zn, K and CEC.

Table 4.2. Relationship between soil chemical characteristics, bacterial diversity indices, and individual phyla in INM site

	pH	SOC	N	P	K	Ca	Mg	Mn	S	Cu	B	Zn	Fe	Na	EC	CEC
Actinobacteria	0.07	0.2	0.28	-0.08	-.49**	0.15	0.24	0.25	0.12	0.22	0.29	-0.32	-0.25	0.13	0.15	0.13
Proteobacteria	.54**	.42*	.45**	-0.09	.47**	.46**	.36*	0.26	-0.05	.47**	0.31	.60**	.35*	0.27	0.09	.61**
Firmicutes	-.57**	-.48**	-.46**	.40*	-.34*	-.34*	-.38*	-0.07	.35*	-.39*	-.36*	-.37*	-0.1	-0.13	-0.07	-.45**
Chloroflexi	-.63**	-.57**	-.57**	.39*	-0.24	-.58**	-.52**	-0.22	0.15	-.46**	-.50**	-.53**	-0.2	-0.22	-0.23	-.74**
Verrucomicrobia	.87**	.71**	.68**	-.40*	0.1	.85**	.87**	-0.001	0.11	.60**	.82**	.58**	-0.02	0.23	.54**	.82**
Bacteroidetes	0.26	0.06	0.08	-0.2	.60**	0.09	-0.03	-0.18	-0.27	0.16	-0.09	.38*	0.18	-0.03	-0.09	0.18
Nitrospirae	.76**	.56**	.57**	-0.23	.37*	.65**	.59**	-0.05	-0.11	.54**	.52**	.60**	0.27	.45**	0.24	.74**
Armatimonadetes	0.04	-.36*	-0.27	0.27	.35*	0.03	-0.28	-0.24	-0.16	-0.2	-0.32	0.28	.50**	0.12	-0.23	0.09
Fusobacteria	.44**	0.31	0.28	-0.01	.42*	.36*	0.32	0.09	-0.08	.46**	0.18	.64**	.54**	.36*	0.13	.62**
Fibrobacteres	0.32	0.06	0.14	-0.13	.44**	0.29	0.12	-0.28	-0.15	0.24	0.09	0.33	0.14	0.33	0.03	0.26
Nitrospinae	.53**	0.06	0.14	-0.07	.45**	.54**	0.18	-0.18	0.07	0.18	0.25	.48**	0.02	0.26	-0.01	.43**
Acidobacteria	-.37*	-.51**	-.53**	.35*	-0.05	-.35*	-.47**	0.2	-0.23	-.55**	-.43**	-0.16	0.22	-0.11	-.38*	-.37*
Gemmatimonadetes	-0.24	-.41*	-.41*	.47**	0.06	-0.25	-.44**	0.28	-0.18	-0.32	-0.3	-0.16	0.03	-0.2	-.38*	-0.26
Planctomycetes	-0.3	-0.11	-0.15	-0.03	-0.23	-.36*	-0.18	-0.09	0.03	-0.16	-0.2	-0.29	-0.22	-0.16	0.01	-.39*
Cyanobacteria	0.3	0.13	0.14	-0.28	.40*	0.16	0.06	-0.15	-0.16	0.05	0.06	0.28	-0.04	0	-0.09	0.07
Deinococcota	-.35*	-.47**	-.46**	0.32	0.08	-.38*	-.44**	-.47**	-0.11	-.34*	-.56**	-0.11	0.3	0	-0.16	-0.29
Elusimicrobia	-.45**	-.46**	-.42*	0.25	-0.17	-0.28	-.42*	0.03	0.07	-.41*	-.34*	-0.23	-0.14	-0.12	-0.11	-.40*
Spirochaetes	-.35*	-0.29	-0.27	0.01	-0.03	-.41*	-.33*	-0.03	0.05	-0.23	-0.3	-0.31	-0.27	-0.18	-0.2	-.47**

Values are correlation residuals of soil chemical properties and bacterial phyla. *CEC = Cation exchange capacity; EC= Electrical conductivity; SOC = Soil organic carbon; * represents significant correlation at P≤ 0.05 level; ** Represents significant correlation at P≤ 0.01; +* represents mg kg^{-1}; ₩ represents soil:water; 1:2.5.

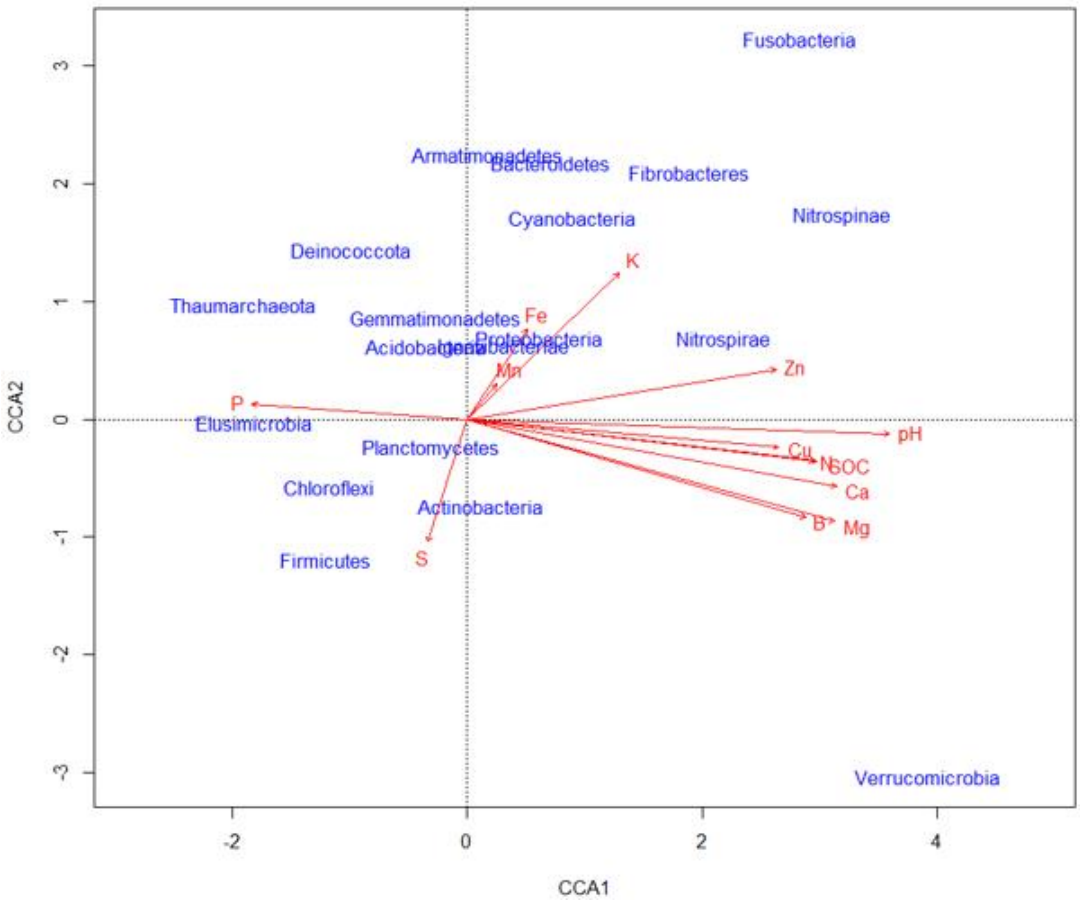

Figure 4.5. The relationship between soil chemical properties and bacterial phyla in the INM3 Madeya study site

The findings from Pearson correlations revealed that all the bacterial phyla correlated with at least one soil chemical property. For instance, the results from Pearson correlations (Table 4.2) and CCA (Figure 4.5) indicated that *Proteobacteria, Verrucomicrobia, Bacteroidetes, Nitrospirae, Fusobacteria, Nitrospinae, Cyanobacteria, Actinobacteria* and *Fibrobacteres* were appreciably enriched with increase in soil pH, K, SOC, Ca, N, Mg, Fe, Zn, Cu and CEC, signifying that agronomic management practices that promote soil nutrient status/availability would concomitantly boost soil microbial diversity. The results further depict the important contribution of soil fertility and pH in shaping microbial structure. These observations corroborate results from previous studies on related soil microbial parameters in the region (Kihara et al., 2012; Margenot et al., 2017b; 2018; Bolo et al., 2021) or elsewhere in Australia (Xue et al., 2018) and China (Cui et al., 2018). Soil microorganisms are vital catalysts in SOC and nutrient

transformations (Sahu et al., 2017). Some of these bacteria, for example, *Proteobacteria, Verrucomicrobia, Bacteroidetes, Nitrospirae, Fusobacteria, Nitrospinae, Actinobacteria* and *Fibrobacteres* were recently cited as copiotrophs that thrive well in nutrient rich environments (Ranjan et al., 2015; Semenov et al., 2021), explaining their positive response to different soil chemical properties.

On the other hand, some of the bacterial strains involving *Chloroflexi, Acidobacteria* and *Firmicutes* negatively correlated with soil pH and other soil nutrients. This perhaps indicates that some of these bacteria are well adapted to acidic environments (like *Acidobacteria*) or have preference for nutrient/carbon deficient systems. These observations are consistent with the recent findings (Leff et al., 2015; Yun et al., 2016). Previous research indicated that some bacterial strains like *Chloroflexi, Acidobacteria* and *Firmicutes* are attributed to oligotrophic lifestyles; preferring nutrient deficient systems (Fierer et al., 2007; 2012; Ho et al., 2017; Semenov et al., 2021). This likely reflects their observed negative correlation with soil pH, SOC, N, P, K, Ca, Mg, Cu, B, Zn and CEC. Furthermore, the significant effects of SOC, macronutrients (P and N) and micronutrients (Fe and Zn) on bacterial abundance and distribution indicates the fundamental role of nutrition in shaping and driving the microbial community distribution and abundance. Previous reports (Kihara et al., 2012, Koorem et al., 2014; Vukicevich et al., 2016; Lian et al., 2019) linked certain soil microbial parameters to nutrient enrichment and soil organic carbon accumulation, providing both food and energy.

4.2 Effects of Farmyard Manure, Inorganic Fertilizers and Residue Application on Potential Soil Extracellular Enzyme Activities involved in C, N and P Cycling

The results showed that combined application of FYM with residue (MRK) significantly increased acid phosphatase enzyme (ACP) activity relative to application of FYM only (MK) and/or its combination with residue and fertilizers (Table 4.3). Combined application of FYM, residue and fertiliser (NPKMR) significantly increased ACP activity relative to application of residue and fertiliser (NPKR). Briefly, the outcomes from the activities of the extracellular enzymes revealed two key insights. First, application of FYM greatly stimulates extracellular

soil enzymatic activities (nutrient cycling) especially when the amendment is done in combination with other organic matter. For example, application of FYM only (MK), combined application of FYM with residue (MRK) and combined application of FYM with P fertiliser (PKM) significantly increased beta-glucosidase (GLU) activity relative to; i) application of inorganic fertiliser only (NPK, PK, N*PK); ii) application of residue only (RK); iii) combined application of fertiliser with residue (NPKR); and iv) no input (Control). Secondly, the results from the enzyme activities further indicated that sole application of inorganic fertilizer, reduced alkaline phosphatase (ALP) and GLU activity relative to combining the inorganic fertilizers and residues. For instance, application of P only (PK) or inorganic fertilizer only (NPK, N*PK) significantly reduced ALP and GLU activity relative to combined application of FYM and P fertilizer (PKM).

Table 4.3. Responses of C, N and P cycling extracellular soil enzyme activities to long-term agronomic management practices in INM3 site

Treatment	ALP[#]	ACP[#]	GLU[#]	NAG[#]
Control	38.3^{ab}	126.2^{cd}	26.6^{b}	7.8^{a}
NPK	22.1^{b}	120.9^{cd}	20.3^{b}	8.1^{a}
RK	31.2^{b}	182.8^{ab}	26.8^{b}	9.5^{a}
MK	27.1^{b}	154.8^{bcd}	37.6^{a}	9.1^{a}
NPKR	29.2^{b}	119.7^{d}	22.1^{b}	11.4^{a}
NPKMR	32.4^{b}	158.3^{abc}	26.4^{b}	8.4^{a}
NPKM	44.3^{ab}	156.1^{abcd}	25.3^{b}	7.2^{a}
MRK	36.0^{b}	193.8^{a}	37.6^{a}	11.9^{a}
PK	39.6^{ab}	136.5^{cd}	22.0^{b}	9.3^{a}
PKM	61.2^{a}	153.9^{bcd}	46.3^{a}	12.5^{a}
N*PK	28.7b	135.7cd	24.9b	7.8a
Uns	119.1a	244.4a	82.9a	33.9a
P-value	***	***	***	***
LSD	26.31	36.59	20.05	7.26

If different letters are used within each column, it indicates that the means are significantly distinct from each other. # = unit representing μg P-nitrophenol (*p*NP) g-dry soil^{-1} hr^{-1}, Control = No input; NPK = P+N fertilizers only; RK = residue only; MK = + FYM only; NPKR = P+N + residue only; NPKMR = P+N +FYM+residue only; NPKM = P+N +FYM only; MRK = Residue and FYM only; PK = +P fertilizer only; PKM = P + FYM only; N*PK = P+90 kg N ha^{-1} only, Uns=undisturbed site. *** = significant difference at $P \leq 0.001$; LSD = Least significant difference

The findings from this research indicated that application of FYM increased beta-glucosidase and acid phosphatase extracellular enzyme activities. This can be

attributed to the contribution of FYM in increasing substrate availability and SOC that favour proliferation of microbes that harbour and exude these extracellular soil enzymes. Significant increases in the activities of ACP, ALP, GLU and NAG extracellular soil enzymes in response to addition of FYM have been documented in earlier studies (Acosta-Martinez et al., 2011; Brennan & Acosta-Martinez, 2019; Liang et al., 2014; Chen et al., 2019). These studies attributed the enzymatic activity responses associated with contribution of FYM to improvement of soil organic matter, nutrient availability, and soil physiochemical characteristics. Extracellular enzymes take part in catalysing the process of decomposition of organic matter in the soil, and their activity is influenced by substrate availability (Ning et al., 2020; Dong et al., 2022; Gonzalez et al., 2023). When organic resources like FYM are added to the soil, they contribute to improvement of the contents of soil organic matter and physiochemical parameters (Mugwe et al., 2009; Mucheru-Muna et al., 2014) thus increasing substrate availability which the extracellular enzymes act upon. This perhaps stimulated the extracellular GLU and ACP enzyme activities. On the other hand, it is also likely that when inorganic fertilisers are used alone, they contribute nutrients but do not directly increase the soil organic matter, thus impairing substrate availability for the enzymes to act upon. This perhaps depressed the activities of ALP and GLU enzymes in the systems applied with inorganic fertiliser without organic inputs.

4.2.1 The Relationship between the Different Soil Extracellular Enzyme Activities with Soil Parameters and Bacterial Diversity

The Pearson correlation results between the soil variables, microbial diversity and enzyme activities (GLU, ALP, ACP, NAG) also revealed interesting results. Soil pH, calcium, SOC, magnesium, total nitrogen, boron, zinc, potassium and CEC had positive correlation with bacterial richness and diversity, and activities of GLU and ACP **(**Table 4.4). In addition, ALP positively correlated with magnesium, calcium, soil pH and CEC.

Table 4.4. Relationship between soil chemical properties with potential C, N and P cycling enzyme activities and bacterial species richness and diversity

	GLU#	NAG#	ALP#	ACP#
SOC (%)	.73**	.11	.22	.51**
pH	.68**	.24	.35*	.55**
P	-.31	-.07	.07	-.45**
N (%)	.76**	.15	.17	.54**
K	.57**	.22	.12	.64**
Mg	.71**	.21	.42*	.41*
Mn	-.05	-.09	.07	-.26
Ca	.65**	.16	.43*	.42*
B	.59**	.22	.14	.40*
Cu	.25	.12	.44*	.24
Zn	.48**	.01	.13	.53**
S	.04	.14	.23	-.01
Fe	.04	-.25	.15	-.02
Na	-.031	-.12	.03	-.03
C.E.C	.58**	.07	.40*	.36*
EC	.16	.07	.18	-.02
Species richness	.63**	.14	.33	.55**
Shannon Diversity	.64**	.08	.31	.49**

Values are Pearson correlation residuals of soil chemical variables and biological properties. * denotes significance at $P \leq 0.05$, ** = $P \leq 0.01$; # represents enzyme activity units μg pNP g^{-1} (dry soil) h^{-1}**;** ALP = Alkaline Phosphatase; ACP = Acid Phosphatase; GLU = Beta-glucosidase; NAG = Beta-glucosaminidase

Soil available P significantly negatively correlated with ACP. This suggests that higher concentrations of soil available P have inhibitory effects on ACP activity. Consistent with these findings, negative correlations between soil available phosphorus concentrations and some extracellular phosphatase enzyme activities have previously been observed elsewhere (Olander & Vitousek, 2000). Other studies asserted that mineral phosphates can sometimes serve as competitive inhibitors that depress the activities of phosphatase enzymes (Olander & Vitousek, 2000; Nannipieri et al., 2011).

Besides, further findings from the Pearson correlations showed that soil pH, calcium, SOC, magnesium, total nitrogen, boron, zinc, potassium and CEC had positive relationships with the activities of GLU and ACP enzymes. This perhaps reflects the importance of nutrition or substrate availability in shaping microbial community structure, which perhaps favoured the proliferation of microbes that harbour and exude these extracellular soil enzymes. The availability of favourable soil pH and substrates might have favoured a richer and diverse microbial community that exude the ACP and ALP, resulting to the observed positive correlations. This was also confirmed by the concomitant significant positive correlations between these enzymes and bacterial richness and diversity. These results closely corroborate the findings from Zheng et al. (2019) who reported that ACP activity positively correlated with SOC and N, together with other soil variables, and this was attributed to substrate availability for microbial community that exude this soil extracellular enzyme. Bolo et al. (2021), working in the same region, reported that SOC and total N positively correlated with several phosphorus solubilizing microbes, most of which, exude the extracellular soil phosphatase enzymes.

4.3 Effects of Farmyard Manure, Inorganic Fertilizers and Residue Application on Bacterial *pho*C and *pho*D Gene Abundances

Bacterial *pho*D gene abundance remained largely unaffected by treatments (Table 4.5). However, although the results were not significantly different across the treatments for bacterial *pho*D gene abundance, their abundance showed a general increasing trend (not significant) under the treatments that were applied with farmyard manure. This was also similar to the results for *pho*C gene abundance. Highest *pho*D and *pho*C bacterial gene abundances were observed in the treatment applied with FYM only (MK). On the other hand, sole application of residue (RK) significantly reduced bacterial *pho*C gene abundance. In addition, bacterial *pho*D and *pho*C gene abundances also tended to reduce (not significant) with application of nitrogen fertiliser. For instance, the least bacterial *pho*C and *pho*D gene abundance was observed where sole NPK fertiliser was applied, particularly in the treatment where N was applied at 60kg ha^{-1} (NPK).

Table 4.5. Soil bacterial *pho*C and *pho*D gene abundance in INM experimental site.

Treatment	*pho*D ¥	*pho*C ¥
Control	5.16[a]	5[a]
NPK	4.76[a]	4.8[ab]
RK	4.96[a]	4.51[b]
MK	5.43[a]	5.15[a]
NPKR	5.28[a]	5[a]
NPKMR	5.24[a]	5.04[a]
NPKM	5.36[a]	5.01[a]
MRK	5.45[a]	5.08[a]
PK	5.16[a]	4.99[a]
PKM	5.17[a]	4.9[ab]
N*PK	5.39[a]	5.08[a]
Uns	5[a]	4.8[ab]
P-value	***	***
LSD		0.46

Values are means of bacterial *pho*C and *pho*D genes. If different letters are used within each column, it indicates that the means are significantly distinct from each other. Control = No input; NPK = P+N fertilizers only; RK = residue only; MK = + FYM only; NPKR = P+N + residue only; NPKMR = P+N +FYM+residue only; NPKM = P+N +FYM only; MRK = Residue and FYM only; PK = +P fertilizer only; PKM = P + FYM only; N*PK = P+90 kg N ha^{-1} only, ¥ = log_{10}-transformed bacterial gene counts, Uns=undisturbed site. *** = significant difference at $P \leq 0.001$; LSD = Least significant difference

The present study indicated that sole application of residue significantly reduced bacterial *pho*C gene abundance. Sole residue addition might have contributed to reduction of bacterial *pho*C gene abundance by either disrupting the availability of essential nutrients; or alteration of the soil chemical properties thereby depressing the growth of *pho*C-harbouring microbial populations; leading to reduced expression of *pho*C genes. Besides, it is also likely that the applied residues perhaps depressed soil nitrate availability by instigating nutrient immobilization, thus reducing the growth of *pho*C harbouring microbial communities. Studies have suggested that variations in soil properties, involving reduction in soil nitrate and phosphorus availability could affect the composition of phosphate solubilizing microbial community that harbours *pho*C genes (Ragot et a., 2017; Neal et al., 2018), and this could likely depress the *pho*C gene abundance. Previous studies reported that *pho*C harbouring microbial community abundance increased with increasing soil nitrate availability (Wei et al., 2016; Zheng et al., 2019). Bacterial *pho*C genes are primarily

harboured by phosphate solubilizing microbial community, and are vital in encoding acid phosphatases enzymes that contribute actively in the mineralization of organic phosphorus.

The results of this study also indicated that bacterial *pho*C and *pho*D gene abundances declined with application of NPK fertilisers. This can be explained by the effect of nitrogen in lowering soil pH and increasing acidity in soil, thereby creating unfavourable conditions for *pho*C and *pho*D harbouring microbial populations. These results are consistent with the findings from previous studies (Jorquera et al., 2014; Chen et al., 2019). Inorganic N application has been shown to lower the soil pH (Nakhro & Dkhar, 2010) which is key soil factor influencing the *pho*C and *pho*D-harbouring microbial populations. In addition, Chen et al. (2019) reported a reduction in bacterial *pho*D gene abundance following mineral nitrogen application and attributed the response to lowering of soil pH and reduction in soil microbial abundance and diversity. Different processes taking place in the soil rhizosphere, involving nitrogen input applications, have been reported to result in pH changes around the root zone (Rengel & Marschner, 2005; Philippot et al., 2013). These influence the soil chemical parameters and availability of soil nutrients which in turn influence the soil microbial populations (Rengel & Marschner, 2005; Philippot et al., 2013). The *pho*D and *pho*C harbouring bacteria and phosphatase enzyme activities are responsive to various physicochemical properties of the soil, which can be altered by diverse agricultural practices such as cropping systems, inorganic fertilizers and organic fertilizers application (Neal et al., 2017; Liu et al., 2018; Xiao et al., 2018). Cumulatively, these might have resulted to the reduction of the two specific genes reported in the current study.

The abundance of the bacterial *pho*C and *pho*D genes also showed an increasing trend following FYM application. The increase in soil organic carbon (SOC) and nutrient contents resulting from the application of FYM could be responsible for enhancing the abundance and diversity of both the bacterial *pho*D and *pho*C harbouring microbial populations. The SOC and nutrients contributed by the applied FYM are important sources of food for soil microbes, and this could have instigated improved microbial abundance and diversity. Moreover, the applied FYM could

have created favourable conditions that favour colonization and proliferation, and hence increased abundance of different soil microbes, including the *pho*D and *pho*C harbouring microbes. The observed increase in abundance of bacterial *pho*C and *pho*D gene following application of FYM corroborates outcomes from previous studies (Fraser et al., 2015; Luo et al., 2019; Wan et al., 2020). Increase in bacterial *pho*C gene abundance following application of organic manure was previously attributed to the increased SOC availability that promoted microbial population growth (Wan et al., 2020).

Besides, Luo et al. (2019) stated that the increase in abundance of *pho*D and *pho*C genes with organic manure amendments were attributed to the C, N and P added to the soil through the amendment, which feeds the micro-organisms. Long-term organic manure in Chilean extreme environments have also been reported to provide organic matter and organic P which serve as the primary substrates for microbes that exude phosphomonoesterase enzymes, consequently increasing the total relative abundance of bacterial *pho*D gene (Acuña et al., 2016). Combined addition of C and nutrients through cattle manure was reported to increase *pho*D gene abundance because the manure creates appropriate environmental conditions for the micro-organisms including the *pho*D-harbouring organisms (Hu et al., 2018, Liu et al., 2021).

4.4 Effect of Farmyard Manure and Nitrogen Fertilizer Application on CO_2 and N_2O Evolution

In arable fields, soil microbial activities can be inferred by the release of N_2O and CO_2 gases. The release of N_2O and CO_2 gases from the field was assessed by comparing three treatments. The three treatments included manure application, application of nitrogen at 90 kg N ha^{-1} and no-input application treatments. The three treatments were accompanied by uniform application of P and K fertilizers. The results showed that treatments significantly affected ($P \leq 0.05$) CO_2 and N_2O fluxes (Figure 4.6 a, b). For instance, application of FYM significantly increased CO_2 fluxes (48.7 mg C m^{-2} hr^{-1}) than both the input omission (FYM0N0; 33.9 mg C m^{-2} hr^{-1}) and the application of 90 kg N ha^{-1} (29.8 mg C m^{-2} hr^{-1}), respectively (Figure 4.6b). Likewise, application of FYM (FYM4N0) or nitrogen (FYM0N90)

significantly elevated N_2O fluxes (5.92 mg N m^{-2} hr^{-1} and 5.18 mg m^2 hr^{-1}, respectively) than the nutrient omission treatment (FYM0N0; 2.54 mg m^{-2} hr^{-1}) (Figure 4.6a).

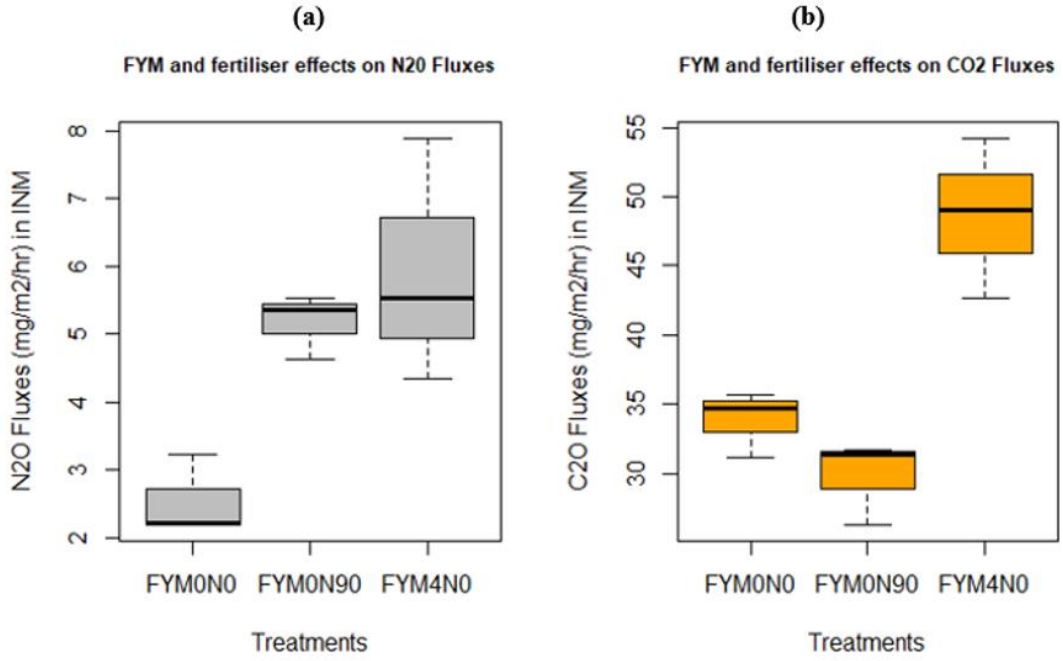

Figure 4.6. Soil N_2O (a) and CO_2 (b) fluxes observed under no-input, nitrogen and manure application treatments in INM3-Madeya trial.

FYM0N0 = P only; FYM0N90 = P+90 kg N ha^{-1} only; FYM4N0 = P + FYM only. All treatments had a uniform application of P (at 45 kg P ha^{-1}) and K (at 60 kg K ha^{-1}) fertilizers.

The results showed that CO_2 and N_2O emitted in the treatments that were applied with FYM (48.7 mg C m^{-2} hr^{-1} and 5.92 mg N m^{-2} hr^{-1}, respectively) were significantly higher than CO_2 and N_2O emitted in either the treatment with N added or no-input application. This can be attributed to the contribution of FYM in improving the SOC content and nutrient availability in the soil, being important substrates for improving microbial community, activities and respiration. Since emission of CO_2 and N_2O in the agricultural fields is a proxy of microbial respiration and other activities, it is likely that the application of FYM provided additional carbon substrate and nutrients that improved the microbial abundance, diversity and respiration; hence stimulating the release of the gases. Akin to this, further findings from the current study indicated that FYM positively correlated with SOC, N, microbial richness and diversity, but also, activities of extracellular GLU and NAG

enzymes that cycle C and N, respectively. These results align with previous findings (Dhadli & Brar., 2016; Seki et al., 2022) that suggested that application of FYM may stimulate microbial growth and activity hence hastening the breakdown of organic compounds, thereby liberating more CO_2. Lai et al. (2017) asserted that FYM addition increased CO_2 fluxes because it provided easily decomposable soil organic carbon that stimulates respiration. Besides, increased CO_2 fluxes were attributed to microbial responses following the stimulating effects of FYM (Seki et al., 2022). Previously in the same site, Sommer et al. (2016) also reported higher N_2O fluxes in the management practices with FYM addition.

Being an organic resource, application of FYM could increase microbial respiration and accelerate the microbial decomposition of organic matter available in the soil (Anjum & Khan, 2021), potentially stimulating release of CO_2. Besides, FYM as an organic resource provides organic carbon and other nutrient substrates which act as sources of food and energy required by microorganisms for growth (Khatoon et al., 2017; Fekadu et al., 2019; Wang et al., 2020). This could increase microbial activities thus concomitantly increasing fluxes of CO_2 and N_2O. Moreover, upon decomposition, FYM can improve availability and cycling of nutrients, involving nitrogen, that microorganisms depend on for their growth (increased abundance) and activities (including nitrification, denitrification, and respiration); hence stimulating release of both N_2O and CO_2.

Furthermore, the elevated N_2O and CO_2 fluxes observed in the FYM applied relative to either nitrogen or no input treatments can also be attributed to the positive influences of FYM on soil structure and physiochemical properties. FYM can improve soil pH and physical properties involving soil structure, porosity and moisture (Mucheru-Muna et al., 2007; Mugwe et al., 2009; Mucheru-Muna et al., 2014). These can concomitantly increase microbial proliferation, community diversity and activities involving decomposition rates and nutrient cycling, hence increasing liberation of the gases. For instance, in this study, soil pH significantly increased with FYM application. This increase in soil pH could stimulate increase in diverse microbes involving denitrifying bacteria and influence denitrification hence liberating more N_2O (Sun et al., 2012). Soil pH has a direct influence on the

microbial population, structure and activities, and this directly reflects increase in respiration and N_2O emissions (Tate et al., 2007).

4.5. Effect of Farmyard Manure, Inorganic Fertilizers and Residue Application on SOC, Soil Chemical Properties, Nitrogen and Phosphorus Nutrient Mineralization, the Monetary Value of the Mineralized N and P Nutrients and the Maize Performance

This section provides the outcomes on three sections. The first section provides the outcomes on the responses of soil chemical parameters to different treatments involving manure application, fertilizer application and residue application. The second section provides the outcomes on the effects of these treatments on nitrogen and phosphorus mineralization and the monetary value of the quantities of nitrogen and phosphorus nutrients mineralized. The third section details the effects of the different practices on maize performance (biomass, grain and total above ground yields) and grain prices.

4.5.1 Effect of Farmyard Manure, Inorganic Fertilizers and Residue Application on SOC, Soil Chemical Properties

Soil chemical properties significantly varied across the treatments (Table 4.6). The treatments where FYM were applied as manure only (MK), manure and residue only (MRK) or combined application of manure and other inorganic inputs (NPKMR, NPKM and PKM) had significantly higher soil pH, SOC and N relative to management practices lacking FYM. For instance, continuous incorporation of FYM led to significant increase in soil pH, SOC, N, Mg, Ca, Zn and Fe relative to systems lacking FYM addition, with SOC increases ranging between 9.4-21.8% noted. The treatments that lacked FYM addition comprised of no-input (control), NPK fertiliser only (NPK), residue only (RK), fertiliser and residue (NPKR) and P only (PK). In addition, combined application of FYM with NPK fertilizer (NPKM) rather than sole addition of NPK fertilizer (NPK) increased pH, SOC, N, Mg, Ca, Zn and Fe. Moreover, long-term addition of nitrogen at 60 kg N ha^{-1} (NPK) or 90 kg N ha^{-1} (N*PK) significantly reduced soil pH, Mg and Ca compared to application of P only (PK). Furthermore, long-term application of P only (PK) significantly increased soil

available P, Mg and Ca relative to no-input. Long-term addition of residue, as a factor, lacked any significant impacts on soil parameters.

Table 4.6. Responses of soil chemical characteristics to different management practices in INM3

Treatment	pH	SOC	N	P	Mg	Mn	Ca	Fe	Zn
	(1:2.5)	g kg^{-1}		(mg kg^{-1})					
Control	4.8^{de}	17.0^{cd}	1.3^{ef}	18.1^{e}	37.5^{d}	394^{ab}	382^{e}	118^{c}	1.0^{c}
NPK	4.5^{f}	16.4^{d}	1.2^{f}	79.0^{a}	37.5^{d}	431^{ab}	357^{e}	128^{bc}	1.3^{c}
RK	4.9^{de}	18.3^{bcd}	1.4^{def}	13.0^{e}	45.3^{d}	400^{ab}	356^{e}	117^{c}	1.2^{c}
MK	5.2^{abc}	20.5^{a}	1.7^{ab}	23.8^{de}	122^{ab}	396^{ab}	743^{bc}	143^{b}	2.6^{ab}
NPKR	4.7^{ef}	16.8^{cd}	1.3^{f}	71.2^{ab}	36.7^{d}	441^{a}	335^{e}	133^{bc}	1.3^{c}
NPKMR	5.1^{bcd}	19.6^{ab}	1.6^{bcd}	65.6^{ab}	139^{a}	397^{ab}	670^{cd}	169^{a}	3.6^{a}
NPKM	5.0^{cd}	18.6^{abc}	1.5^{cde}	55.9^{bc}	127^{ab}	366^{b}	818^{abc}	174^{a}	3.3^{a}
MRK	5.4^{a}	20.7^{a}	1.8^{a}	20.5^{e}	152^{a}	420^{ab}	939^{ab}	126^{bc}	3.5^{a}
PK	5.0^{cd}	17.0^{cd}	1.3^{ef}	56.7^{bc}	89.8^{bc}	389^{ab}	695^{cd}	126^{bc}	1.8^{bc}
PKM	5.3^{ab}	18.3^{bcd}	1.6^{bc}	59.2^{abc}	162^{a}	405^{ab}	998^{a}	139^{bc}	2.6^{ab}
N*PK	4.5^{f}	17.4^{cd}	1.4^{ef}	42.5^{cd}	55.4^{cd}	425^{ab}	463de	129^{bc}	1.6^{bc}
P-value	***	***	***	***	***	***	***	***	***
LSD	0.3	0	0.03	22.4	64.4	64.6	407	23.3	1.2

In each column, values that have similar letters do not have any significant differences. Control = No input; NPK = P+N fertilizers only; RK = residue only; MK = + FYM only; NPKR = P+N + residue only; NPKMR = P+N +FYM+residue only; NPKM = P+N +FYM only; MRK = Residue and FYM only; PK = +P fertilizer only; PKM = P + FYM only; N*PK = P+90 kg N ha^{-1} only. *** = significant difference at $P \leq 0.001$; LSD = Least significant difference

The study showed that FYM, when applied either solely or together with other inputs, improved the soil chemical properties, involving soil pH and nutrients. This observation could imply the contribution of FYM in enhancing soil organic matter content, stimulation of soil microbial parameters and improvement of nutrient availability upon decomposition and mineralization. These results corroborate previous studies that observed positive effects of continuous application of FYM on some physiochemical and biological characteristics of soils (Mugwe et al., 2009; Mucheru-Muna et al., 2014; Laub et al., 2022), where the observations were attributed to the FYM's contribution to the capacity of the organic matter to improve soil fertility and nutrient availability (evident in the current study through increased

soil pH, SOC, N, P, K, Mg, S, Cu, Zn and Fe) alongside creating favourable microclimate that promote microbial development and activities (Gautam et al., 2020; Tang et al., 2020). Recently, Ndung'u et al. (2021) asserted that application of organic matter improved soil carbon availability in Nitro-rhodic Ferralsols in Kenya.

Besides, the increased soil pH in response to FYM addition also likely reflects the characteristic liming effects of FYM; and aligns with the results reported in earlier studies (Li et al., 2015; Chen et al., 2017; Cui et al., 2018; Laub et al., 2022). Addition of FYM stimulates the accumulation of organic matter in the soil that provides multiple benefits on soil physiochemical and biological properties, involving soil fertility, pH and microbial diversity (Mucheru-Muna et al., 2007; 2014; Mugwe et al., 2009; Chen et al., 2022; 2023). These benefits may further be enhanced when FYM is applied in combination with other inputs like NPK fertilisers (Tadesse et al., 2013; Zerihun & Haile, 2017). This also perhaps explains the observed increases in soil chemical parameters following sole addition of FYM, or combined incorporation of FYM together with other inputs (NPK fertiliser). FYM, as a source of organic matter, can also increase pH over time by supplying calcium and magnesium (Tadesse et al., 2013).

Addition of nitrogen fertiliser contributed to reduction of soil pH as indicated in the findings from the present research. Nitrogen fertilisers are mostly made from ammonium or urea that are typically acidic in nature. The reduction in soil pH following prolonged application of nitrogen fertiliser likely indicates the acidifying effects of nitrogen in the soil. This observation reflects the findings from previous studies (Schroder et al., 2011; Tian & Niu, 2015; Daba et al., 2021) who also attributed the response of depressed pH to the acidifying effects following continuous use of nitrogen-based fertilizers in soil. Essentially, the degree of reduction of soil pH following continuous application of nitrogen fertilisers typically depends on the amount and nature of nitrogen-based fertiliser added into the soil (Buthelezi et al., 2022). For instance, use ammonium-based fertilisers have more potential to acidify soil than nitrate-based fertilisers (Buthelezi et al., 2022).

Amongst the macronutrients contained in fertilisers, nitrogen is often the main nutrient that may have negative effects on soil pH, and the nature of nitrogen-based fertiliser used can variably influence the soil pH.

4.5.2 Effects of Farmyard Manure and Fertilizer Application on N And P Nutrient Mineralization and Monetary Value of the Mineralized Nutrients

The overall trend (except for a few cases) indicated that higher levels of nitrogen mineralization and the monetary value of the mineralized nutrients were achieved after 60 days of incubation in comparison to the results attained after 30 days of incubation (Table 4.7). The highest quantities of P and N mineralized were obtained after 60 days of resin incubation under the integrated soil fertility management system in INM3. In the input omission treatment (Control) and sole P application treatment (PK), the amount of N mineralized (and its corresponding monetary value) in the initial period of resin incubation (after 30 days) was slightly higher than the amount of N mineralized in the subsequent period of resin incubation (after 60 days). The two respective treatments were the only treatments where there was no N application amongst the treatments studied for nutrient mineralization. For instance, in the treatment where only P was applied (PK), the amount of N mineralized were 2.7 and 1.8 kg N ha^{-1} respectively, for 30 and 60 days of resin incubation. Likewise, for the no-input control, the N mineralized were 20.6 and 14.7 kg N ha^{-1} for 30 and 60 days of resin incubation, respectively. However, in systems where N was applied (NPK and NPKM), the amount of N mineralized (and its corresponding monetary value) in the initial period of resin incubation was slightly lower than the amount mineralized in the final period of incubation. Conversely, P mineralized and its subsequent monetary value, was higher in the second than initial periods of resin incubation in all the systems with input application. For instance, after 30 days of resin incubation and 60 days respectively, the amount of N mineralized in the management system with fertilizer applied (NPK) were 8.0 and 10.7 kg N ha^{-1} while that mineralized under practice combining NPK and FYM (NPKM) were 7.6 and 9.4 kg N ha^{-1}, respectively. On the other hand, P mineralized were 0.4 and 0.6 kg P ha^{-1} (NPK) and 0.8 and 1.2 kg P ha^{-1} (NPKM), respectively for the 30 and 60 days of resin incubation, respectively.

In addition, combined application of NPK with FYM (NPKM) slightly reduced N mineralization and associated monetary value of the mineralized N compared to no-input control in both incubation periods. Akin to this, and compared to the other systems in INM3, the highest N mineralization and monetary value was observed in the no-input control while in the same treatment, P mineralization and its monetary value were at their lowest. Also, when FYM was considered as a factor (comparing NPKM with NPK) its application occasioned stimulation of P mineralization and monetary values of the quantities of P nutrients mineralised, both for the two incubation periods. The quantity of phosphorus mineralized increased by a minimum of 0.8 kg P ha^{-1} during the initial period of resin incubation. In addition, after 60 days, the amount of P mineralized increased by a minimum of 1.2 kg P ha^{-1}. Hence, addition of FYM as the only input conferred an equivalent monetary benefit of USD 3.02 and 4.76 ha^{-1} during the initial and final days of resin incubation, respectively. However, in both resin incubation periods, addition of FYM as only input slightly reduced the quantities of nitrogen mineralized and its equivalent monetary value. Conversely, when NPK fertiliser was applied in combination with FYM (NPKM) P mineralization and monetary value of the mineralised P increased relative to no-input system. However, when P was added as the only input (comparing PK with no-input control) the quantity of P mineralized and its monetary value increased, both during 30 and 60 days of resin incubation. For instance, the quantity of phosphorus that was mineralized during the initial (30 days) and final day of incubation respectively increased by 0.3 (USD. 1.13 ha^{-1}) and 1.2 kg P ha^{-1} (USD. 4.76 ha^{-1}).

Table 4.7. The quantities of N and P mineralized alongside their equivalent monetary values after incubating resins in the study site for 30 and 60 days

	Quantities of nutrients mineralized (kg ha^{-1})				Monetary values of nutrients mineralized (USD ha^{-1})			
	N		P		N		P	
	Number of days of resin incubation							
Treatment	**30**	**60**	**30**	**60**	**30**	**60**	**30**	**60**
Control	20.6^{a}	14.7^{a}	0.1^{b}	0.1^{a}	26.82^{a}	19.16^{a}	0.25^{b}	0.87^{a}
PK	2.7^{b}	1.8^{b}	0.4^{ab}	1.2^{a}	3.48^{b}	2.39^{b}	1.38^{ab}	4.85^{a}
NPKM	7.6^{ab}	9.4^{ab}	0.8^{a}	1.2^{a}	9.90^{ab}	12.31^{ab}	3.27^{a}	4.85^{a}
NPK	8.0^{ab}	10.7^{ab}	0.4^{ab}	0.6^{a}	10.45^{ab}	13.90^{ab}	1.60^{ab}	2.30^{a}

Means followed by different letters per column have significant differences between them. Control = no-input application treatment; PK = treatment with 45 kg P ha^{-1}, NPK = treatment with fertiliser applied at 60 kg N ha^{-1} and 45 kg P ha^{-1}, NPKM = treatment with combined application of FYM and fertiliser at 60 kg N ha^{-1} and 45 kg P ha^{-1}

Results from this study showed that more nitrogen was mineralised during the initial period of resin incubation (after 30 days) than the final period of incubation (after 60 days), and this also temporally translated to the monetary values. The higher amount of nitrogen mineralized (and subsequent monetary value) during the initial period of resin incubation (30 days) compared to 60 days perhaps could be attributed to "priming effect" phenomenon during the decomposition of the soil organic matter. Within the first 30 days of incubation, it is likely that there was a considerably larger pool/amount of easily decomposable organic matter available for microbial activity (decomposition), with more nitrogen perhaps released in the process.

However, as time progressed (at 60 days), the pool of easily degradable organic matter was either gradually depleted and microbial abundance and activity suppressed, or most of the available organic N were immobilised and complexed in stable recalcitrant organic forms that remained thereafter, hence resulting to lower rates of nitrogen mineralization and resultant low N mineralized and its monetary equivalent after the 60 days of resin incubation. These findings corroborate the previous research (Cordovil et al., 2005; Sharma et al., 2016; Maitlo et al., 2022) who reported that the amount of nitrogen that was mineralised in the initial days of incubation was higher than that mineralised at later stages of incubation. Cordovil et al. (2005) argued that in comparison to 35 days, after 57 days of incubation, most of

the organic N had formed complexes with the stable recalcitrant compounds, hence lowering the rates of mineralization. Besides, both Sharma et al. (2015) as well as Maitlo et al. (2022) also reported a reduction of nitrogen released or mineralized at later stages of incubation. They both alluded that this reduction in nitrogen released at later stages would signify the involvement or contribution of the persistent and less readily decomposable portion organic compounds which become available at later stages upon depletion of the pool of easily degradable organic compounds.

Addition of nitrogen-based fertilisers reduced nitrogen mineralization and the subsequent monetary value of mineralized N in this study. Reduction in the amount of nitrogen mineralised (and its subsequent monetary value) following fertiliser application likely indicates that the added fertiliser might have instigated reduction in microbial activity through alterations in soil pH, microbial community structure responsible for nutrient mineralization (nitrifiers) or altered soil conditions. This suggests that application of nitrogen fertilisers may lead to N-saturated soil, with continuous application likely to exceed microbial demand for N, reflecting the reduction in mineralised N with fertilisation. These results align with the findings from Mahal et al. (2019) who attributed this observation to the theory of microbial nutrient mining that alludes that when microbes mine nutrients, their activity often increases, to a larger extent, in nutrient poor substrates (addition of a particular nutrient increases its availability and lowers the microbial demand for that nutrient).

Furthermore, application of nitrogen-based fertilisers could have indirectly depressed the amount of nitrogen mineralised (and its equivalent monetary value) by affecting soil pH and altering the specific microbial community composition (nitrifiers) and activity. Consistent with observations made in recent studies, continuous incorporation of nitrogen fertilizer decreased soil pH (Wang et al., 2018; Bolo et al., 2021); but in contrast, soil pH positively correlated with several nitrogen cycling bacteria involving *Nitrospinae, Nitrospirae* and *Proteobacteria* among others, depicting the intricate complexity in soil-microbiome interactions. Still, in this study, soil pH significantly reduced with nitrogen fertiliser application. Therefore, as the pH reduces, the abundance and activity of certain bacterial community responsible for N cycling may also reduce, concomitantly stifling N

mineralisation. Besides reduction in their abundance, the induced alterations in soil conditions following addition of nitrogen-based fertilisers can also make some of the nitrogen-cycling microbial groups (nitrogen fixers, nitrifiers, denitrifiers and ammonifying bacteria), to be outcompeted by other microbial species that are more adapted to such environmental conditions; hence exhibiting reduced nitrogen mineralization activities. Previous studies reported that nitrogen fertilization suppressed nitrogen fixing bacteria community structure (Lüscher et al., 2000; Berthrong et al., 2014). Within terrestrial ecosystems, these group of bacteria are believed to have the capability of contributing approximately 97% of the nutrient input (Reed et al., 2011). Besides, Wang et al. (2018) stated that prolonged utilization of N fertiliser elevated denitrifying bacterial abundance, signifying increased potentials for nitrogen losses in the system.

This research also revealed that addition of FYM increased P mineralization (and subsequent monetary value of the amount of P mineralized) both after 30 days and 60 days of resin incubation. Increase in P mineralization following application of FYM could be attributed to the positive contribution of FYM to soil organic matter, stimulation of microbial activity, influence on soil pH, nutrient availability as well as production of beneficial organic acids (like citric and oxalic acids) that promote P availability. The increase in P mineralization following application of FYM is consistent with previous findings (Amin, 2018; Jamal et al., 2023). Besides, the increase in mineralized P (and associated monetary value of the P mineralized) observed in the current study is also consistent with the findings of Yan et al. (2016) who further attributed the elevated proportion of soil available P to conversion of the stable pool of phosphorus to labile or available form of P. Studies by Hill et al. (2015) suggested that FYM contain organic acids that are capable of forming complexes with P in soil and enhance its solubility, and this could perhaps result to the increased P mineralization following addition of FYM. FYM directly supplies all major nutrients to the soil, like P, hence can increase P availability (Tadesse et al., 2013). Besides, FYM also contains organic matter, which is a source of food (energy) for soil microbes, that when broken down and mineralized, releases more P into the soil, perhaps reflecting the increase in P mineralization and associated monetary benefits. It is also possible that sole addition of FYM, or combined

application of FYM together with other inputs (like P in this case), likely caused balanced soil nutrition that would support more plant biomass production and subsequent accumulation of organic compounds in the soil (through residues, leaf fall, dead roots and stubbles) that would further promote microbial growth and activities, reflecting the increased P mineralization and associated monetary value of the mineralized P.

In addition, the increase in P mineralised and its monetary value following application of FYM could also be ascribed to the effects of FYM in creating favourable soil conditions by improving physiochemical and biological properties (Khan et al., 2010). Akin to this, perhaps the enabling soil physiochemical conditions created by the added FYM stimulated proliferation and activities of phosphorus cycling microbial community. For instance, recent findings showed that certain soil enzymes linked to P cycling, like phosphatases, previously correlated with FYM addition (Kiboi et al., 2021). Still, Kumar et al. (2019) also reported that addition of FYM increased the activity of phosphatase enzymes responsible for P cycling perhaps due to the ability of the applied FYM to provide additional supply of carbon and nitrogen substrates that are essential for supporting different microbial parameters. Besides, Garg and Bahl (2008) and Bhatt et al. (2016) also observed increased activities of both alkaline phosphatase enzyme and acid phosphatase enzymes following FYM addition in combination with other inputs, and these perhaps justify the observed increase in P mineralization following incorporation of FYM, either alone or in combination with other inputs. On the other hand, FYM addition perhaps minimized further fluctuations in soil pH (created optimal soil pH thus reducing soil acidity) and this might have also favoured microbial abundance and activities, hence the increase in P mineralization. Increase in soil pH with FYM addition is recently acknowledged (Opala et al., 2012). Low soil pH decreased bacterial abundance (Rousk et al., 2010). However, microbial biomass and activities tended to increase with optimal soil pH ranges (Pietri et al., 2008). Amin (2018) reported that addition of FYM increased soil available P, and this also closely aligns with the increased P mineralization following FYM application as observed in this study.

4.5.3 Effects of Farmyard Manure and Fertilizer Application on maize performance (biomass, grain and aboveground yields) and grain prices

The agronomic management practices had a significant effect on maize performance and grain prices (Table 4.8). The treatment where FYM was applied in combination with inorganic fertiliser inputs (NPKM) had significantly higher maize grain, biomass and total aboveground biomass yields relative to management practices with sole inorganic fertiliser application (PK and NPK) or those lacking inputs (Control). Particularly, the maize biomass, grain, and total aboveground biomass yields significantly increased by 3.6, 2.7 and 6.7 t ha^{-1} respectively in NPKM treatment compared to Control. The maize biomass, grain and total aboveground biomass yields in NPKM treatment respectively conferred significant increases of 3.4, 2.2 and 5.9 t ha^{-1} relative to NPK fertiliser application; and 3.3, 3.1 and 6.8 t ha^{-1} respectively relative to application of PK fertiliser. The maize grain prices in the FYM applied treatment (NPKM) significantly increased by 1232, 1096 and 880 USD ha^{-1} relative to Control, application of inorganic PK and NPK fertilisers, respectively.

Table 4.8. The overall maize performance (biomass, grain and total aboveground yields) and grain prices under different management practices in INM3

Treatments	Maize yields (t ha^{-1})			Maize grain prices
	Stover	Grain	AGB	(USD ha^{-1})
Control	1.6^{b}	2.1^{b}	3.8^{b}	833^{b}
PK	1.9^{b}	1.7^{b}	3.7^{b}	697^{b}
NPKM	5.2^{a}	4.8^{a}	10.5^{a}	1929^{a}
NPK	1.8^{b}	2.6^{b}	4.6^{b}	1049^{b}

Means followed by different letters per column have significant differences between them. AGB = Above ground biomass; PK = +45 kg P ha^{-1}; NPK = +60 kg N+45 kg P ha^{-1}; NPKM = +FYM + 60 kg N + 45 kg P ha^{-1}

The outcomes from this study revealed that application of FYM combined with inorganic inputs (NPKM) increased maize performance compared to sole application of inorganic fertilisers or no input control. This was reflected by the significantly higher maize grain and biomass yields, and grain prices in the FYM added treatment than the others. The significant increases in maize yields under

FYM added treatment can be attributed to the holistic positive effects of FYM in soil that promoted maize performance. First, the added FYM might have improved soil pH, hence providing better conditions for maize growth. This was evident in the results from this study as soil pH among other chemical parameters significantly increased in the NPKM treatment. Previous studies showed that FYM could ameliorate soil, which often limits crop production (Opala et al., 2012). Besides, it is also likely that upon mineralization, the added FYM might have enhanced the microbial diversity, functions and increased the availability of essential nutrients for crop production, coinciding with the results of the soil chemical and microbial parameters obtained in the current study, and aligning with previous literature (Ge et al., 2018; Leroy et al., 2008). For instance, the results from the soil attributes showed that several vital nutrients involving N, P, K, SOC, Zn among others were significantly higher in the treatments with FYM applied, either alone or in combination with other treatments relative to the rest of the treatments. Perhaps maize yields increased due to the increased availability of these essential nutrients contributed by the added FYM. The increase in soil pH among other nutrient parameters, that could have increased maize performance, are reported in the previous studies (Mugwe et al., 2009; Opala et al., 2012; Mucheru-Muna et al., 2014). Recent studies reported that maize performance significantly improved under systems with FYM addition (Bhat et al., 2013; Ashenafi et al., 2023).

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The findings from this study indicated that addition of FYM, either alone, or in combination with other inputs increased soil chemical parameters. These involved soil pH, SOC, macronutrients, secondary nutrients as well as other micronutrients. This is because FYM contains these nutrients in organic pool, and upon decomposition, releases them into the soil. In particular, the following conclusions can be made from the current study:

i) Addition of FYM, either solely or combined with other inputs, improved microbial biomass, bacterial abundance, and diversity. This is ascribed to the improvement of SOC that serves as an integral food source to the soil microbes, hence stimulating their growth and proliferation. Besides, application of nitrogen-based fertilizers depressed the bacterial abundance and diversity, and this could imply the contribution of such nitrogen-based fertilizers in reduction of soil pH, thereby creating unfavorable conditions for proliferation of certain bacterial populations.

ii) Incorporation of FYM, either solely or combined with other inputs, stimulated bacterial abundance and diversity, extracellular soil enzymatic activities and phosphorus mineralization, but depressed nitrogen mineralization. The positive microbial responses to FYM addition suggest the potential of FYM to enhance soil microbial community, reflecting the possibility to improve nutrient cycling and soil health. Akin to this, the elevation of the activities of extracellular soil enzymes with application of FYM imply the likelihood that holistic agronomic management interventions that champion incorporation of different organic and inorganic inputs, could enhance soil biological functions, activities, and processes; hence resulting to increased nutrient availability.

iii) Application of FYM stimulated the abundances of bacterial *pho*C and *pho*D genes. These two bacterial genes are responsible for encoding acid phosphatase enzyme and alkaline phosphatase enzymes, respectively. Since

these genes and enzymes play roles in organic phosphorus mineralization in the soil, the results imply the possibility that addition of FYM in the soil stimulated microbial mediated phosphorus cycling.

iv) Addition of FYM increased emission of CO_2 and N_2O gases from the field more than fertilizer inputs. However, although application of FYM significantly increased CO_2 and N_2O fluxes, incorporation of FYM had additional benefits that would serve to counter the emission associated demerits, observing that the quantities of the two gases emitted were negligible to cause any significant environmental alarms. The added FYM increased the microbial community diversity and functions, soil nutrient availability, SOC, nitrogen and phosphorus mineralization, maize grain and biomass yields and grain prices, indicating the potential of manure in improving soil health, enhancing crop production, nutrient availability, carbon sequestration and accumulation.

v) Addition of FYM increased the amount of phosphorus mineralized and its corresponding monetary value; but reduced the amount of nitrogen mineralized and its corresponding monetary equivalent. This indicates that while FYM can be beneficial in improving phosphorus availability in the soil, it may limit soil nitrogen cycling. Furthermore, the findings revealed that application of inorganic nitrogen fertilizer reduced the quantity of nitrogen mineralized and its corresponding monetary value. This reflects the possible trade-off between nitrogen fertilization and soil nutrient cycling; and suggests that excessive use of nitrogen fertilizers may hinder the natural nutrient mineralization process. Additionally, application of FYM increased maize performance (yields) and grain prices likely due to the positive effects of FYM on improving soil fertility and nutrient availability.

5.2 Recommendations

The following recommendations can be appreciated from the findings of this study:

i) Farmyard manure should be regularly applied, either alone or in combination with other inputs to optimize the realization of a balanced soil ecosystem

with improved microbial biomass, abundance, diversity and functions. Manure applied should be of good quality to boost availability of soil organic carbon which acts as food for microbes. In addition, the use of nitrogen-based inorganic fertilisers should be limited as they tend to reduce soil pH and limit the availability of essential nutrients involving phosphorus, reducing microbial abundance, diversity and functions. The levels of soil pH should be regularly monitored and ameliorated by such practices involving addition of FYM, to ensure they remain within optimal ranges that are ideal for supporting a healthy, vibrant and diverse soil microbiome, as well as promote crop productivity.

ii) Farmyard manure should be applied in the soil either alone or in combination with other inputs to optimize its benefits in soil nutrient cycling and soil health by boosting the extracellular enzyme activities. FYM is a vital input in maintaining these activities at high levels culminating to enhanced breakdown or complex organic matter into simple plant-available nutrient compounds, thus increasing nutrient availability for optimum crop productivity. This is especially very key in nutrient deficient soils. Proper observation of such organic input regimes, like application of FYM in the crop fields, will enhance the activity of essential nutrient cycling enzymes (for example phosphatases, nitrogenases and glucosidases) thereby improving bioavailability of phosphorus, nitrogen and carbon, among other nutrients, alongside supporting a diverse and thriving soil microbial community.

iii) Application of FYM in agricultural farms should be widely adopted as it has the potential to stimulate the abundance of bacterial *pho*C and *pho*D genes which are biomarkers for acid and alkaline phosphatase enzymes, vital for biological-induced nutrient cycling in the soil. By increasing the abundance of *pho*C and *pho*D genes, together with their associated extracellular enzymes, FYM addition serves to maintain a balanced soil nutrient profile especially by optimizing the bioavailability of phosphorus for crop utilization through catalyzing the breakdown of complex organic compounds and mineralization of nutrients.

iv) Farmyard manure should be integrated into soil management practices to enhance microbial activities. However, this should be done with the consideration of its potentials in influencing greenhouse gas emissions. Although addition of FYM increased CO_2 and N_2O gas emissions in the present study, the quantities of the gases emitted were considerably negligible raise environmental alarms. Besides, the more benefits accrued from addition of FYM, involving increased nutrient availability, enhanced quantities and monetary equivalents of mineralized phosphorus, improved SOC availability, diverse soil microbiome community and activities, as well as enhanced maize yields and maize grain prices. These cumulatively offset the potential demerits associated with enhanced greenhouse gas fluxes observed in the treatments that received FYM. However, proper care should be taken reduce the amounts of gases released from such organic input (FYM) interventions. First, it is important to ensure that only well decomposed and mature FYM are used in the field. Additionally, the added manure should be incorporated into the soil rather than surface/broadcast application. Secondly, blanket applications of FYM should be discouraged and only targeted site-specific applications, guided by relevant analyses, should be embraced.

v) Farmyard manure should be regularly applied, either alone or in combination with other inputs to improve availability of key nutrients that are essential for optimum crop productivity. Nitrogen-based inorganic fertilisers should be used in moderation (preferably in combination with organic inputs) as they lower soil pH, impair availability of other important soil nutrients and suppress soil microbiome community and activities.

5.3 Areas for Further Research

The potential areas for further studies include:

i) Assessing the potential contributions of the soil microbes in crop/produce nutritional quality,

ii) Assessing the potential contributions of soil extracellular enzymes in plant nutrient acquisition and use,

iii) Optimizing specific soil microbes and enzymes to improve soil nutrient availability and input use efficiency under different agroecological zones, soil types, cropping systems and crop types,

iv) Investigating the ways in which FYM use can be optimized to benefit from the microbial influences alongside simultaneously addressing the greenhouse gas challenge.

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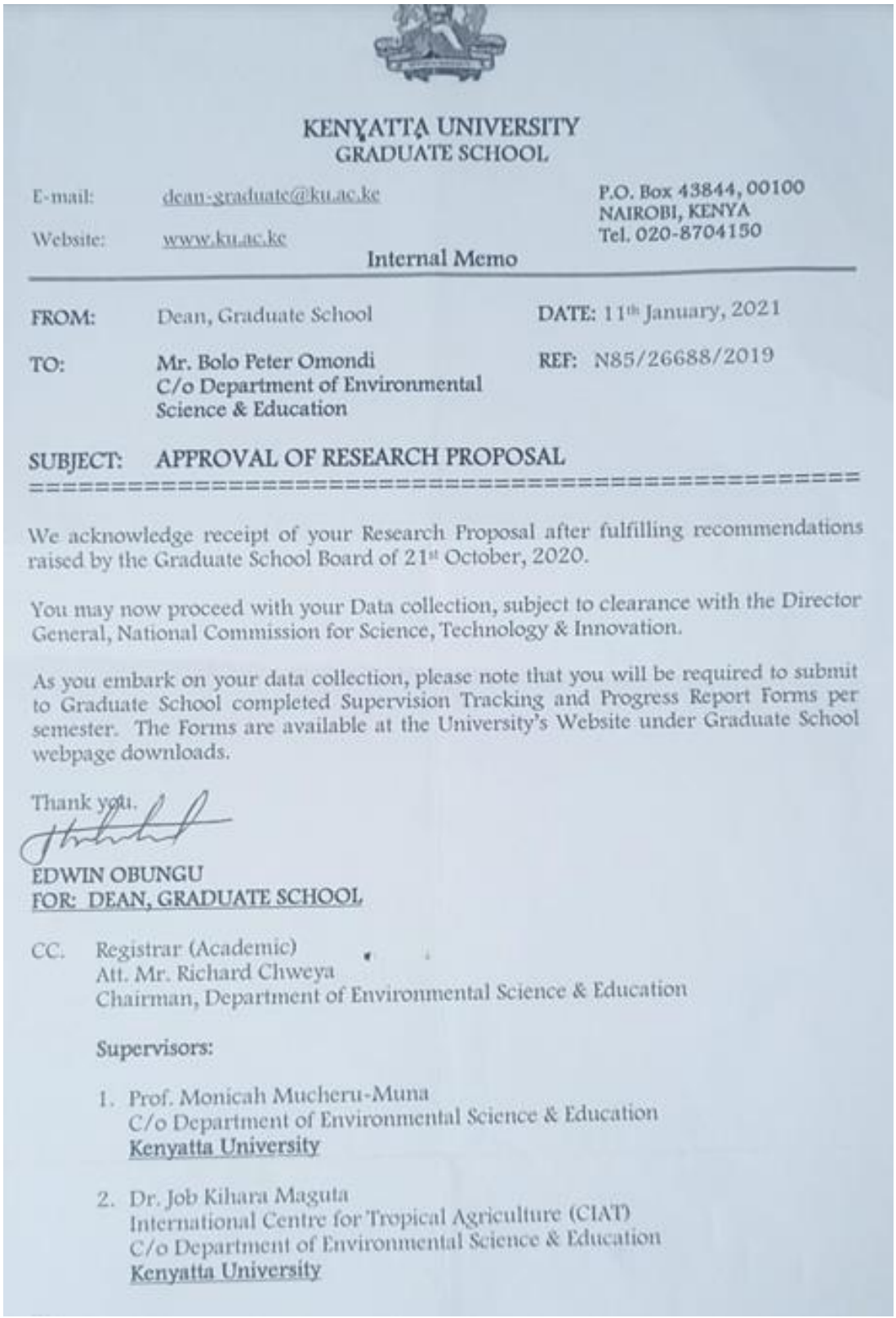
APPENDICES

Appendix I: Research Approval



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Internal Memo

FROM: Dean, Graduate School

DATE: 11th January, 2021

TO: Mr. Bolo Peter Omondi
C/o Department of Environmental Science & Education

REF: N85/26688/2019

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

We acknowledge receipt of your Research Proposal after fulfilling recommendations raised by the Graduate School Board of 21st October, 2020.

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The Forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

EDWIN OBUNGU
FOR: DEAN, GRADUATE SCHOOL

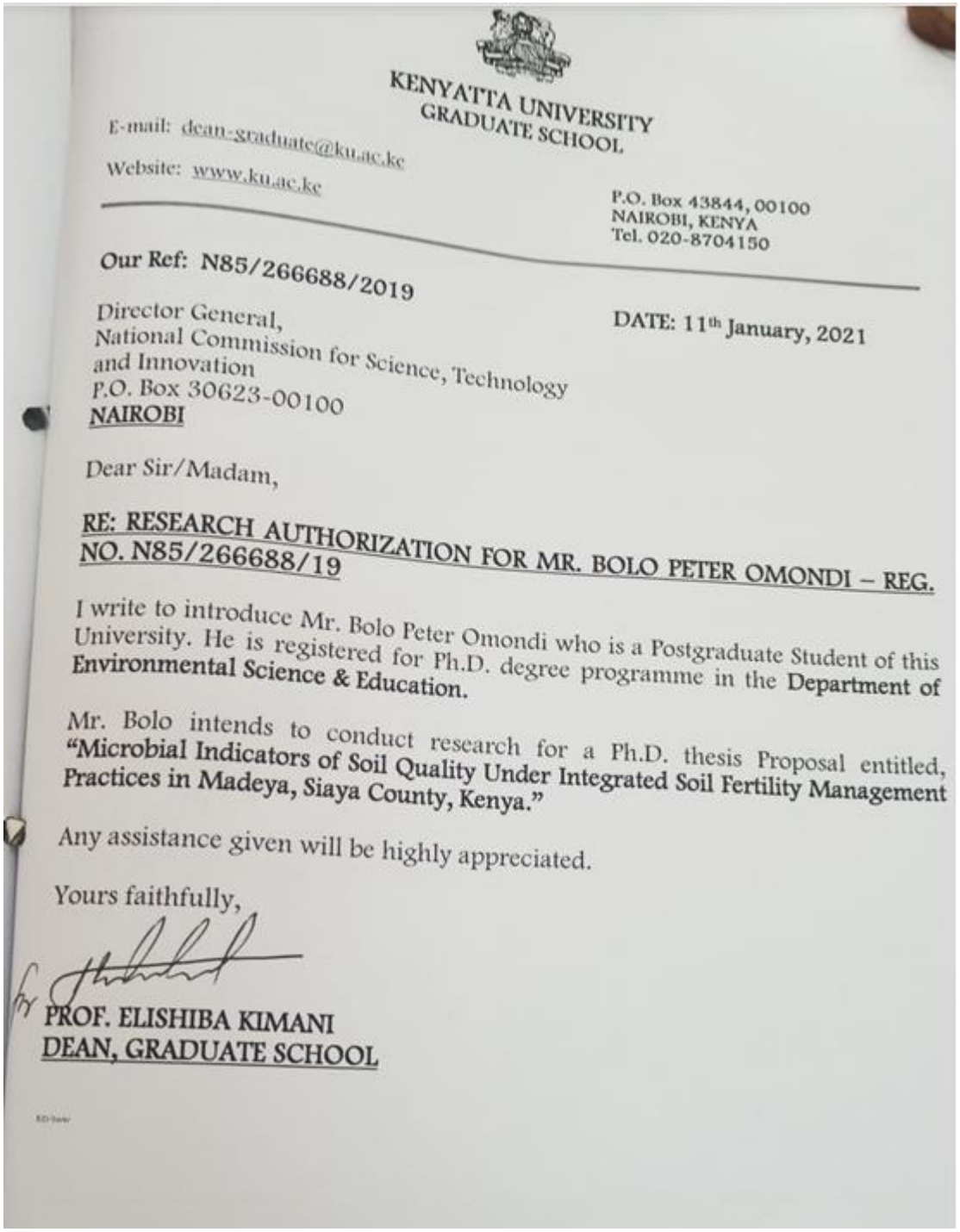
CC. Registrar (Academic)
Att. Mr. Richard Chweya
Chairman, Department of Environmental Science & Education

Supervisors:

1. Prof. Monicah Mucheru-Muna
   C/o Department of Environmental Science & Education
   Kenyatta University

2. Dr. Job Kihara Maguta
   International Centre for Tropical Agriculture (CIAT)
   C/o Department of Environmental Science & Education
   Kenyatta University

Appendix II: Research Authorizatoin



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Our Ref: N85/266688/2019

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

DATE: 11th January, 2021

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MR. BOLO PETER OMONDI – REG. NO. N85/266688/19

I write to introduce Mr. Bolo Peter Omondi who is a Postgraduate Student of this University. He is registered for Ph.D. degree programme in the Department of Environmental Science & Education.

Mr. Bolo intends to conduct research for a Ph.D. thesis Proposal entitled, "Microbial Indicators of Soil Quality Under Integrated Soil Fertility Management Practices in Madeya, Siaya County, Kenya."

Any assistance given will be highly appreciated.

Yours faithfully,

for

PROF. ELISHIBA KIMANI
DEAN, GRADUATE SCHOOL

Appendix III: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **741439**

Date of Issue: **20/December/2023**

RESEARCH LICENSE

This is to Certify that Mr.. Peter Omondi Bolo of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Siaya on the topic: MICROBIAL INDICATORS OF SOIL QUALITY UNDER INTEGRATED SOIL FERTILITY MANAGEMENT PRACTICES IN MADEYA, SIAYA COUNTY, KENYA for the period ending : 20/December/2024.

License No: **NACOSTI/P/23/31893**

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Applicant Identification Number

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