FOREST DEGRADATION IMPACT ON SOIL PHYSICO-CHEMICAL PROPERTIES AND BACTERIA COMMUNITY STRUCTURE IN MOUNT KENYA FOREST ECOSYSTEM

BY
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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL BIOLOGY

MOI UNIVERSITY

2025

DECLARATION

Declaration by the Candidate

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To my son Gift Leone Kiplimo Kigen, who was born in midst of my studies and brought so much blessings and joy.

ACKNOWLEDGEMENT

First, I would like to thank God for the grace that has seen me through this work.

My heartfelt gratitude goes to all those who made the completion of this study possible, especially my supervisors, Dr. Jacqueline Makatiani and Prof. Mugatsia Tsingalia, for their invaluable support, guidance, and supervision.

I am sincerely thankful to my colleagues for their support during the course of this study particularly Dr. Fredrick Ogolla, Mr. Samson Chabari, who accompanied me to the forest for sample collection and assisted in laboratory analysis and Sioma Mulambula for his moral support, constant encouragement, and prayers. I also wish to acknowledge my family members, especially my grandmother, for her prayers, financial support and encouragement.

May you all receive immeasurable blessings!

ABSTRACT

The Mount Kenya forest ecosystem has experienced notable degradation in recent years largely due to uncontrolled anthropogenic land use activities such as deforestation, overgrazing and improper land management, and whose impact on soil microbial communities has not been fully elucidated. Hence, this study aimed to assess the effects of forest degradation on soil bio-physico-chemical properties within the Mt. Kenya Forest. The specific objectives were to: Assess the effects of forest degradation on selected soil physical properties; Analyze the effect of forest degradation on soil chemical properties; and evaluate the influence of forest degradation on soil bacteria community structure. Two contrasting and non-overlapping forest patches –undisturbed and degraded– were selected, and a 100×100 m plot established in each patch. Six sampling locations, with a spacing of 18 m between points, were established diagonally across each plot. To account for microvariability at each sampling location, sterile soil auger was used to collect soil samples in three replicates –one at the center and two others 1 m to the left and right. A total of 36 soil samples were transported in cool boxes and refrigerated prior to laboratory analysis. The dry combustion and core ring methods were used to assess the soil physical properties of organic matter and bulk density, respectively. Calcium, potassium and magnesium chemical properties were extracted using ammonium acetate and quantified by atomic absorption spectroscopy, while soil pH was measured in situ using a pH meter. Soil microbial abundance was determined using the pour plate method, while bacterial isolates were characterized based on morphological, biochemical, and molecular traits. Molecular identification involved DNA extraction, sequencing and BLAST analysis using MEGA X and NCBI GenBank. Data were analyzed using analysis of variance in SAS version 9.4, with LSD for post hoc comparisons. Results showed that undisturbed patch had significantly higher ($P<0.05$) soil organic matter, bulk density, calcium and potassium levels, but supported significantly lower (62.5±27.83; $F_{1, 94}=139.10$; $P<0.0001$) mean bacteria colony-forming units (CFUs) when compared to degraded patch with 152.74±48.74 CFUs ($F_{1, 94}=139.10$; $P<0.0001$), whose soil pH was more alkaline. Molecular analysis based on16SrRNA gene sequences showed distinct clustering of bacterial isolates with bootrap support values ranging from 69% to 100% with known strains in GenBank. Dominant bacterial isolates in undisturbed forest included *Bacillus aerius*, *Pseudochrobactrum saccharolyticum*, *Brucella pseudogrignonensis*, *Brevundimonas diminuta*, *Delftia tsuruhatensis*, *Stenotrophomonas maltophilia*, and *Pseudomonas fluorescens*, while degraded patch yielded *Vagococcus fluvialis*, *Achromobacter xylosoxidans*, *Cupriavidus* sp., and *Ochrobactrum* sp., thus indicating that forest land use degradation can affect soil bacterial communities through modifying soil physical parameters, nutritional conditions, and biological interactions. In conclusion, this study shows that forest land use changes can drastically alter the number of soil bacterial communities through modifying soil physical parameters, nutritional conditions, and biological interactions. This study recommends continuous monitoring of soil bio-physico-chemical properties that serve as indicators of forest soil health, and incorporating findings into forest management and restoration plans.

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ABBREVIATIONS AND ACRONYMS

CFU **:** Colony-Forming Unit

DNA **:** Deoxyribonucleic acid

RNA **:** Ribonucleic acid

UPF **:** Undisturbed primary forest

KFS **:** Kenya Forest Services

SRSD **:** Stratified Random Sampling Design

SOM **:** Soil Organic Matter

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Disturbances experienced in an ecosystem affect species dynamics by changing distribution, abundance and species composition, and this results in changes in community structure. It also causes fluctuation in population, increase or decrease in diversity and changes in competition and predator-prey dynamics. Forest degradation like logging significantly affects the disturbance of soil bacteria species and this often leads to shifts in microbial communities affecting ecosystem functions like nutrient cycling and in some instances increased microbial diversity (Bowd *et al.,*2022). Disturbances in the forest often causes shift in bacterial group which are dominant and, in many times, favours the fast-growing bacteria like certain Proteobacteria, utilizing readily available nutrients, while potentially affecting the presence of those bacterial which are involved in nutrient cycling.

Extensive research has focused on microbial communities in aquatic environments, including water reservoirs (Shilei *et al.,*2020). Estuaries (Crump & Bowen, 2024). rivers (Lu *et al.,* 2023), lakes and oceans (Huang *et al.,* 2024). However, fewer studies have examined bacterial diversity across altitudinal gradients in terrestrial ecosystems, particularly in high-altitude tropical montane forests (Looby *et al.,* 2020). There is limited understanding of how forest degradation impacts bacterial abundance and diversity in these ecosystems, which is a significant gap, especially in regions like Mount Kenya.

Soil microorganisms are fundamental to various ecological processes essential for terrestrial ecosystem function. They contribute to nutrient cycling, soil fertility, detoxification, and carbon sequestration (Basu *et al.,*2021). One of their critical roles is the decomposition of plant biomass, which recycles organic matter releasing nutrients to other organisms (Zhan, 2024). Soil microorganisms also play key roles in primary production and water purification. Through their role in biogeochemical cycles, soil bacteria significantly influence nutrient availability and cycling (Prasad *et al.,*2024)

Additionally, the diversity and community structure of soil organisms, including bacteria, impact ecosystem stability and functioning. Changes in microbial species richness and composition can affect ecosystem processes and stability, with the loss of key microbial species potentially disrupting ecosystem function even if overall microbial diversity remains high (Philippot *et al.,*2024).

Microbial communities are affected by the physical properties of soil, such as structure, bulk density, and moisture content which are influenced by forest disturbances. (Peng *et al.,*2023). Soil physical properties are crucial in shaping microbial habitats by influencing oxygen diffusion, nutrient availability, and water retention essential factors for microbial health and soil productivity (Syamsuri *et al.,*2024). For example, logging, burning, and land clearing associated with forest degradation can lead to soil compaction, reduced porosity, and impaired water retention, negatively impacting microbial diversity and activity. In intact forests, soil structure supports a diverse array of microbes by providing pores for oxygen and water exchange; however, these features are compromised in degraded soils (Hu *et al.,*2023).

Distribution of soil bacteria is influenced by factors such as soil depth, carbon and nitrogen content, vegetation cover, and soil properties. As soil depth increases, different bacterial groups exhibit depth-specific abundance patterns, shaped by environmental conditions like oxygen availability, moisture levels, and nutrient gradients (Murray *et al.,*2025). For example, copiotrophic bacteria, which thrive in nutrient-rich environments, are more abundant in the upper soil layers, where organic matter is more plentiful, while oligotrophic bacteria, adapted to low-nutrient conditions, dominate deeper horizons. Mixotrophic bacteria, which utilize both organic and inorganic carbon sources, exhibit varying abundance depending on the balance of nutrients available (Lei, *et al.,* 2024).

Forest degradation, through deforestation and land-use changes such as cutting of trees for timber, charcoal or firewood, conversion of forests into farmland and grazing liveso alters soil structure, moisture content, and nutrient availability, which in turn impacts microbial diversity and functional composition. These changes can shift bacterial communities, with a reduction in beneficial microorganisms involved in nutrient cycling and organic matter decomposition. In degraded soils, bacterial groups adapted to low-nutrient conditions may become dominant, while nitrogen-fixing bacteria could decrease due to nutrient imbalances; (Hu *et al.,*2023).

In high-altitude ecosystems like Mount Kenya, the effects of forest degradation on microbial communities across soil depths are not well understood, especially in comparison to intact ecosystems. Studies of other montane forests suggest that degradation has a more pronounced effect on deeper soil layers, where microbial communities are less resilient to changes in soil chemical and physical properties (Looby *et al.,* 2020). Variations in microbial community structure at different soil depths can influence critical processes, such as nutrient cycling and carbon sequestration, which are essential for soil fertility and ecosystem stability (Naylor *et al.,*2022).

However, little is known about how soil bacterial communities respond when forest conditions are altered through degradation, particularly in tropical montane environments such as the Mount Kenya Forest. Human-driven disturbances continually modify soil characteristics, yet the resulting impacts on microbial diversity and function remain poorly documented. To address this gap, this study investigated how different levels of forest degradation influence soil bio-physico-chemical properties and the composition of soil bacterial communities in the Mount Kenya ecosystem. By examining these interactions together, the study provided an integrated understanding of how degradation affects soil health and microbial ecology, offering evidence that can support more effective forest conservation and restoration efforts.

1.2 Statement of the Problem

The Mount Kenya Forest is a key ecological resource in Kenya, supporting exceptional biodiversity and contributing to climate regulation, water catchment protection, and ecosystem stability (Motuma *et al.,* 2022). Its soils, which contain high levels of organic matter and diverse microbial life, are fundamental to critical processes such as nutrient cycling, soil fertility maintenance, and carbon storage (Zhang *et al.,* 2022). When the forest remains intact, these microbial communities help sustain healthy soil functions. Soil health, in this context, refers to the soil's ability to function as a living, dynamic system capable of supporting plants, maintaining biological activity, and regulating essential ecological processes However, increasing forest disturbance through activities such as timber extraction, land conversion, and uncontrolled deforestation—is placing pressure on this ecosystem (Motuma *et al.,* 2022). These disturbances can modify important soil attributes, including its structure, moisture content, chemical balance, and nutrient profile. Such changes are likely to affect soil bacteria, which play an essential role in maintaining ecosystem productivity.

Despite the importance of these microorganisms, little is known about how degradation influences the composition and distribution of soil bacterial communities in the Mount Kenya Forest. In particular, there is limited information on how changes in soil properties interact with soil depth to shape microbial patterns in both disturbed and undisturbed forest areas. This lack of knowledge creates a significant gap, as understanding how bacterial communities respond to alterations in soil conditions is crucial for predicting the long-term impacts of forest degradation on soil health and ecosystem resilience.

This study aimed to address this gap by investigating how forest degradation affects soil physical and chemical characteristics and by examining how these changes influence bacterial diversity and abundance across different soil depths within the Mount Kenya Forest ecosystem. The results are expected to provide scientific evidence needed to support more effective forest conservation, restoration, and management practices.

1.3 Justification

The Mount Kenya Forest ecosystem ranked among Kenya's most ecologically and economically important landscapes, forming part of a UNESCO World Heritage Site and serving as a vital water tower for major rivers and agricultural zones (Motuma *et al.,* 2022). Over time, the forest experienced significant degradation due to human activities, including illegal logging, land encroachment, overgrazing, and conversion of land to agriculture or settlements. Despite its ecological and socio-economic significance, there was limited understanding of how such disturbances affected soil biological and physico-chemical processes that underpin ecosystem functioning. Investigating this ecosystem was therefore critical to generate evidence that could guide restoration, conservation planning, and sustainable management. Its rich biodiversity, varied vegetation zones, and gradients in climate and land-use intensity also provided a suitable natural setting for ecological and microbial studies (Kinyua *et al.,* 2021).

Soil depth played a key role in determining microbial distribution, nutrient availability, and organic matter dynamics. Surface soils (0–5 cm) typically contained higher organic matter and root biomass, while deeper layers (6–15 cm or more) represented slower nutrient turnover and more stable carbon pools (Zhang *et al.,* 2023). Forest degradation had the potential to alter these vertical patterns through changes in litter input, soil compaction, and water infiltration. Examining soil depth therefore allowed the study to assess whether disturbances were limited to the topsoil or also affected deeper, more stable layers, providing a better understanding of microbial community structure, nutrient fluxes, and carbon storage under both disturbed and intact forest conditions. Forest degradation was also associated with changes in soil bio-physico-chemical properties, including organic matter, moisture, texture, pH, and nutrient concentrations, which in turn influenced microbial communities (Motuma *et al.,* 2022; Zhang *et al.,* 2023). Bacteria, being highly responsive to environmental change, served as sensitive indicators of ecological disturbance. By integrating microbial community profiling with measurements of soil chemical and physical properties, the study provided a comprehensive assessment of soil health under different levels of forest degradation. This approach generated evidence that could inform effective restoration strategies, improve soil fertility management, and contribute to the conservation of biodiversity in the Mount Kenya Forest ecosystem.

1.4 Objectives

1.4.1 Broad Objective

The main objective of this study was to assess the impact of forest degradation on soil physical and chemical properties and on soil bacteria in the Mount Kenya forest ecosystem.

1.4.2 Specific Objectives

1. To determine the impact of forest degradation on selected soil physical properties in Mount Kenya Forest ecosystem.
2. To analyze the impact of forest degradation on selected chemical properties of soil in Mount Kenya Forest ecosystem
3. To measure the influence of forest degradation on soil microbial density by comparing bacterial counts in disturbed and intact forest sites.
4. To evaluate the impacts of the physico-chemical properties on forest soil bacteria

1.5 Null Hypotheses

1. There is no significant difference in selected soil physical properties (such as bulk density, soil texture, porosity, soil moisture content, and soil temperature) between degraded and non-degraded forest sites in the Mount Kenya Forest ecosystem.
2. Forest degradation has no significant impact on selected soil chemical properties (such as pH, organic carbon content, total nitrogen, available phosphorus, and cation exchange capacity) in the Mount Kenya Forest ecosystem.

3. There is no significant difference in soil bacterial counts between disturbed (degraded) and pristine (intact) forest sites in the Mount Kenya Forest ecosystem.
4. The physico-chemical properties of forest soil have no significant influence on soil bacterial abundance and distribution in the Mount Kenya Forest ecosystem.

1.6 Significance of the Study

This study is significant because it addresses gaps in understanding the impact of forest degradation on soil properties and microbial communities in the Mount Kenya Forest ecosystem. By studying the impacts of forest degradation on selected physical and chemical properties of soil, the study will provide insights into how land-use changes, such as deforestation and degradation, alter essential soil characteristics like texture, pH, and nutrient content. Understanding these alterations is crucial for developing impactive land management and restoration strategies aimed at preserving soil fertility and ecosystem health.

Furthermore, the study will examine the impact of forest degradation on total soil bacteria counts across different soil depths, which is essential for understanding how these microorganisms respond to changes in forest structure. Soil bacteria play a vital role in nutrient cycling and organic matter decomposition, and their abundance and diversity can be indicative of soil health. By comparing degraded and intact forest patches, this research will offer a clearer picture of how forest degradation influences microbial communities, which in turn affect broader ecological functions such as carbon sequestration and water filtration. Ultimately, the findings will inform conservation policies and restoration practices, helping to promote sustainable forest management and mitigate the ecological consequences of deforestation in tropical montane ecosystems like Mount Kenya Forest.

1.7 Scope of the Study

The study focused on assessing impacts of forest degradation on selected soil physico-chemical properties and bacterial communities in Mt Kenya Forest Ecosystem. The research was limited to comparing two main forest types degraded and intact forest patches within selected sites on the eastern side of Mt Kenya Forest. Soil samples were collected for predefined depth intervals to examine variations in soil physico-chemical properties and total bacterial populations across vertical depths. This study did not look at seasonal variations, fungal or other microbial groups, or the social economic drivers of forest degradation. The findings herein therefore apply specifically to the sampled sites and the measured parameters but they may provide understanding relevant to similar montane forest ecosystems.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter provides a literature review of what is known about soil microbiome. Specifically, it reviews research on the eimpacts of forest degradation on soil chemical and physical properties, impacts of forest degradation on soil pH, impacts of forest degradation on soil organic matter, impact of forest degradation on soil bulk density, impact of forest degradation on soil mineral nutrient, impact of forests degradation on abundance of soil bacteria in Mount Kenya forest, impact of forest species on bacterial community, impact of plant litter and deadwood on bacterial communities, impact of forests degradation on abundance of soil bacteria in mount Kenya forest, impact of forest species on bacterial community, soil depth impact on microorganisms.

Mount Kenya, a critical tropical montane rainforest, has witnessed accelerating degradation trajectories that profoundly reshape the microscopic architects of its soil fertility. These anthropogenic disturbances cascade through trophic networks, fundamentally altering vital ecological processes including litter decomposition dynamics, nutrient mobilization pathways, and the intricate tapestry of bacterial communities that orchestrate belowground biochemical transformations (Onyango, 2024). Deciphering the vertical stratification of microbial assemblages through soil depth profiles unlocks crucial insights into the temporal stability of these subterranean ecosystems, illuminating how deep soil microbial consortia contribute to carbon sequestration mechanisms that mitigate atmospheric carbon accumulation.

The chemical metamorphosis triggered by forest degradation, encompassing shifts in hydrogen ion concentration, organic carbon depletion, and diminished availability of essential base cations including potassium, calcium, and magnesium, exerts direct selective pressure on bacterial proliferation parameters and community architectural dynamics (Li *et al.,* 2024). Potassium plays an indispensable role in regulating microbial cellular homeostasis and catalytic enzyme functionality, while calcium provides critical structural reinforcement for bacterial cell walls and membrane integrity (Li., *et al.,* 2024). The progressive depletion of these elemental building blocks impedes fundamental microbial metabolic pathways, undermining soil fertility matrices and compromising ecosystem resilience mechanisms that buffer against environmental perturbations (Wang *et al.,* 2024).

The biochemical reconfiguration of soil environments through anthropogenic interventions triggers profound ripple impacts through bacterial community networks. Contemporary research has established unequivocal linkages between human-induced soil chemistry alterations and fundamental restructuring of bacterial diversity patterns and community organization principles (Kumar *et al.,* 2021). Nitrogen-rich agricultural runoff infiltrating degraded forest margins selectively amplifies nitrifying bacterial populations, creating microbial imbalances, while progressive calcium depletion inhibits beneficial decomposer bacteria essential for organic matter transformation and nutrient liberation (Hu *et al.,*2023).

Despite mounting evidence illuminating the intimate connections between soil biogeochemical parameters and microbial diversity metrics, a significant knowledge chasm persists regarding the specific ecological consequences of forest degradation on bacterial community dynamics within tropical montane forest ecosystems, particularly within Mount Kenya's unique ecological context (Gupta *et al.,* 2022;

Zhang *et al.,*2024). This critical research frontier demands urgent investigative attention to understand how bacterial communities respond to degradation pressures and how these responses might influence ecosystem recovery trajectories or further degradation cycles. Such insights would provide essential foundational knowledge for developing targeted conservation strategies that protect not only the visible forest components but also the invisible microbial diversity that sustains forest ecosystem functionality through biogeochemical regulation.

2.2 Impacts of Forest Degradation on Soil Chemical and Physical Properties

The soil physico-chemical and structural characteristics presents many microenvironments inhabited by diverse bacterial populations. Strong relationships between soil conditions and various bacterial taxa have been reported (Kenya, et al. 2022). Properties of surface soils such as pH, organic carbon concentration, salinity, texture as well as nitrogen concentration exhibit an enormous range with high variation brought about by factors that affect soil formation such as; climate, organism relief, parent material and time (Gebremedhin *et al.,*2022). In each soil profile, environmental conditions further vary considerably across the distinct microbial habitats found in soils (Philippot *et al.,* 2024). Thus, the relative abundance of bacteria classes appears to be influenced by many soil variables simultaneously (Medriano *et al.,*2023).

2.2.1 Impacts of Forest Degradation on Soil pH

Forest degradation tends to alter soil pH through changes in litter input, vegetation cover, and microbial activity. The removal of vegetation reduces the input of organic acids derived from leaf litter decomposition, which can lead to a shift in pH. In some degraded ecosystems, soil pH increases due to decreased acidifying inputs and enhanced mineralization of base cations (Musa *et al.,*2024). Conversely, prolonged degradation can result in acidification through leaching of basic nutrients and accumulation of acidic ions, especially in highly weathered tropical soils (Butterly *et al.,* 2022). These shifts in pH can have cascading impacts on microbial communities and nutrient availability. Since variations in pH leads to partitioning of microorganisms by creating favorable or unfavorable conditions for specific microorganisms it is thus a key determinant of primary and secondary soil biological activities (Wang & Kuzyakov, 2024). The relation between pH and bacterial diversity has been studied; higher diversity has been reported in neutral soils and lower in acidic and alkaline soils (Wang *et al.,* 2024). There exists a considerable shift in soil microbial community composition along pH gradients with enrichment of acidophilic bacteria in lower pH soils (Naz *et al.,*2022). Nonetheless, most ecosystems such as mountain forests have not been surveyed comprehensively to determine the relationship between pH variation and microbial diversity in the face of forest degradation. Soil acidification and pH variations if caused by both natural and anthropogenic processes affect soil bacterial diversity and community composition at different geographic scales (Naz *et al.,*2022).

2.2.2 Impacts of Forest Degradation on Soil Organic Matter

Soil organic matter is a critical component for maintaining soil fertility, structure, and microbial habitat. Zhang *et al.*(2022). Forest degradation leads to substantial reductions in soil organic matter due to decreased organic inputs (e.g., leaf litter, roots) and increased decomposition rates driven by higher temperatures and exposure (Gebremedhin *et al.,* 2022). Over time, this loss of soil organic matter contributes to soil compaction, reduced nutrient availability, and diminished soil biodiversity. The extent of soil organic matter decline is often proportional to the intensity and duration of the degradation process, with some studies reporting losses of up to 50% following forest conversion or intensive land use (Gebremedhin *et al.,* 2022). Soil organic matter is an important environmental quality indicator that signify changes as well as response to land management.

Due to its importance, soil organic matter maybe associated to different soil physical, chemical, and biological processes Wang & Kuzyakov (2024). The soil organic matter affects soil functional processes such as nutrient retention and storage, the capacity to hold water and soil stability in terms of forming aggregates Rotich *et al.,* (2023). Further soil organic matter is a significance soil fertility component (Zhang *et al.,* (2022).

According to Beillouin *et al.,* (2023) accumulation of organic matter in an area is determined by seasons, available plant species, soil temperatures, bioavailability of nutrients and prevailing soil pH among others. According to Bai *et al.,* (2023) amendments in forests may result into increased organic matter and minerals. However, the increased soil organic matter may not be sustained during forest recovery. Generally, where destruction occurs in the forest i.e., logging, long term reductions in organic matter content is likely to be experienced. Such sites are characterized by lower debris input to the soil as compared with intact forests. Low input of debris or organic matter may have implications on soil microorganisms such as fungi and bacteria in the soil (Raza *et al.,* 2023).

Forest degradation alters vegetation characteristics such as plant biomass, species, and canopy structure. As a result, soil properties suffer significantly (such as soil carbon elemental stoichiometry, and pH (Li *et al.,* 2021). A change in soil organic matter can have a significant impact on soil microbial attributes. According to studies, soil microbial abundance is related to both soil and climate factors (Bol & Tokuchi, 2018).

2.2.3 Impact of Forest Degradation on Soil Bulk Density

Soil bulk density is an important factor in determining soil structure, porosity, and water retention (Panagos *et al.,*2024). Forest degradation typically leads to an increase in soil bulk density, which can hinder root growth and water infiltration The removal of vegetation and the associated loss of organic matter and root structures can result in soil compaction (Houghton *et al.,* 2023). This leads to an increase in soil bulk density, particularly in tropical and subtropical forest ecosystems. A study found that deforestation and logging activities in Brazilian rainforests increased soil bulk density by up to 30%, reducing soil permeability and water retention. In temperate forests, both logging and grazing contribute to an increase in bulk density. Houghton *et al.,* (2023) found that in logged temperate forests, bulk density increased by 15–20% compared to undisturbed forest soils.

According to Houghton *et al.,* (2023) soil density is the mass per unit volume of the soil representing the ratio of the weight of the solids to the total volume of soil. Thus, soil density reflects the potential of the soil to facilitate movement of water and solute, give structural support and ensure soil aeration (Frene *et al.,* 2024). Soil density is their significance in ensuring ambient soil temperatures, maintenance of biological and chemical processes. To this end, soil density affects metabolic and other physiological activities of depended organisms such as microorganisms and plant as it determines formation of organo mineral complexes (Gaitán-Hernández *et al.,* 2022). Forest destruction activities such as logging cause untold impact on the forests' soil physical properties compaction of the soil (Frene *et al.,* 2024). During logging there are movement of people and equipment such as trucks which accelerate soil degradation and compaction (Panagos *et al.,* 2024). As Soil compaction occurs, it decreases soil porosity and water infiltration capacity while increasing increases soil density and strength (Frene *et al.,* 2024).

2.2.4 Impact of Forest Degradation on Soil Nutrients

The deterioration of forest ecosystems fundamentally disrupts belowground nutrient dynamics, particularly affecting essential base cations. As canopy cover diminishes and vegetation complexity simplifies, the intricate pathways supplying potassium, magnesium, and calcium to forest soils begin to unravel. The decline in litterfall quantity and diversity—coupled with diminished root biomass, creates a cascading impact that severs crucial biological conduits for nutrient cycling (Wang *et al.,* 2018). This nutrient deprivation manifests in stark contrasts between intact and degraded forest soils, where base cation concentrations often plummet in the latter (Widyati *et al.,* 2022). Forest degradation strips away the protective vegetative shield that moderates hydrological processes, leaving soils vulnerable to intensified rainfall impact and accelerated runoff. This hydrological disruption differentially affects base cations—potassium, being highly mobile, rapidly leaches through soil profiles, while magnesium and calcium experience a more gradual depletion through persistent erosion of nutrient-rich topsoil layers. Musa *et al.,* (2024), documented how potassium concentrations can diminish precipitously under degraded conditions, while magnesium and calcium exhibit a more protracted decline linked to erosion intensity and progressive base saturation depletion.

The progressive acidification of forest soils represents another critical consequence of degradation. As base-rich organic inputs diminish and acid-forming ions accumulate, soil chemistry undergoes a fundamental transformation. Calcium and magnesium ions, essential for maintaining soil structural integrity and plant physiological functions, become displaced from soil exchange sites by hydrogen and aluminium ions. This displacement not only compromises cation retention capacity but also creates hostile conditions for soil microbial communities that mediate nutrient transformations, ultimately hampering plant nutrient acquisition pathways (Zhou *et al.,* 2021). Musa *et al.,* (2024) further established connections between this acidification process and diminished soil fertility across multiple forest types.

The conversion of forested landscapes to agricultural or pastoral land uses represents perhaps the most severe form of degradation, triggering dramatic shifts in base cation dynamics. Such land-use transformations initiate nutrient extraction cycles without proportional return, creating systemic nutrient deficits (Mandah *et al.,* 2025). The magnitude of these losses proves particularly alarming in tropical and subtropical ecosystems, where exchangeable base cation pools can contract by half within remarkably brief timeframes following deforestation events (Dalal & Jayaraman, 2025).

Restoration efforts, whether through active reforestation or passive regeneration approaches, offer pathways toward rebuilding depleted soil nutrient reservoirs. However, the trajectory of recovery exhibits high context-dependency, influenced by degradation history, soil properties, and prevailing environmental conditions. While some research indicates partial restoration of potassium, magnesium, and calcium within two to three decades, severely degraded landscapes may require significantly longer timeframes to reestablish pre-disturbance nutrient status. Bai *et al.,* (2024) demonstrated that strategic selection of tree species and implementation of tailored management practices can substantially influence the rate and extent of nutrient accumulation during forest recovery phases.

2.2.5 Influence of Forest Degradation on Soil Bacteria Communities

The intricate dance between forest vegetation and soil bacterial assemblages unfolds through a complex network of biochemical exchanges centred around the decomposition of plant detritus. This process forms the cornerstone of ecosystem-level carbon sequestration and nutrient cycling dynamics (Bekele & Gebremedhin, 2024), orchestrating the biological rhythms that sustain forest productivity and resilience. The terrestrial litter layer, far from being a mere accumulation of dead material, represents a vibrant metabolic interface where forest biodiversity expresses itself through molecular signatures that profoundly shape belowground microbial landscapes. Forest species imprint their biochemical legacy upon the soil microbiome through distinctive litter profiles that vary dramatically in their structural and chemical architecture (Gao *et al.,* 2024). These plant-specific signatures manifest as unique stoichiometric ratios of essential elements particularly carbon and nitrogen, alongside specialized molecular compounds such as lignin, whose complex structure and recalcitrance create selective pressures on bacterial decomposer communities (Onet *et al.,*2025). Beyond these chemical dimensions, individual forest species generate characteristic patterns of litter biomass accumulation, create distinct pH microenvironments, and cultivate specialized relationships with a constellation of organisms including herbivores, mycorrhizal symbionts, and soil fauna—each interaction cascading through trophic networks to influence bacterial community composition and metabolic function (Xu *et al.,* 2023).

The interdependence between specific plant species and their associated microbial consortia creates vulnerability nodes within forest ecosystems (Lei *et al.,* 2024; Zhao *et al.,* 2018; Zhang *et al.,* 2024; Thotakuri *et al.,* 2024). When highly specialized plant-microbe relationships exist, the degradation or loss of particular forest species can sever critical nutrient pathways, triggering ripple impacts throughout bacterial networks that relied upon these botanical partners (Onet., *et al.,* 2025). This ecological disruption illustrates how forest species composition directly modulates the architectural complexity and functional capacity of soil bacterial communities. The remarkable synergistic impacts observed in multi-species forest systems reveal another dimension of plant-bacterial interactions. The litter matrices created by diverse indigenous forest communities consistently demonstrate accelerated decomposition rates compared to monospecific litter accumulations (García-Palacios *et al.,* 2023). This decomposition enhancement emerges from complementary biochemical profiles and inter-litter nutrient transfer mechanisms that create optimal conditions for diverse bacterial decomposer guilds (Zhang *et al.,* 2024). The microbial response to this botanical diversity is not merely quantitative but qualitative mixed indigenous forest litter fundamentally restructures bacterial community composition through selective pressure and niche diversification processes (Onet *et al.,*2025).

These botanical-bacterial relationships illustrate how forest species diversity functions as a master regulator of soil bacterial community assembly, highlighting the profound ecological consequences that forest composition changes exert on microbial diversity and associated ecosystem services. As forests face mounting anthropogenic pressures and compositional shifts, understanding these cross-kingdom interactions becomes increasingly crucial for predicting ecosystem responses and developing impactive conservation strategies.

2.2.6 Impact of Plant Litter and Deadwood on Bacterial Communities

The forest floor represents a metabolic theatre in which botanical afterlife fuels microbial prosperity. Dead plant biomass manifested as leaf litter and decomposing woody debris constitutes the premier carbon reservoir that sustains the complex microbial tapestry beneath the forest canopy. This botanical neuromas descends in prodigious quantities annually, carpeting the forest substrate with a biochemically diverse matrix that awaits microbial transformation (Shannon *et al.,* 2022). The subsequent decomposition odyssey orchestrates elemental redistribution essential for ecosystem functionality, perpetuating the biogeochemical choreography that underpins forest resilience and productivity.

The architectural and biochemical signatures embedded within plant litter—including structural density, hydrogen ion concentration, moisture retention capacity, and nitrogen content, function as selective filters that sculpt bacterial community assembly across forest ecosystems (Yang *et al.,* 2024). This decomposer microbiome exhibits remarkable taxonomic and functional diversity, mediating the progressive dismantling of complex plant polymers into simpler compounds that can re-enter the biological currency of forest systems. The metabolic capabilities of these microbial consortia demonstrate pronounced variation across different vegetation communities, reflecting co-evolutionary relationships between plant substrate quality and bacterial decomposition strategies (Yang *et al.,* 2024).

The biogeochemical alchemy of cellulose degradation reveals fascinating contrasts in bacterial resource utilization patterns across forest types. In coniferous ecosystems, bacterial assimilation of cellulose-derived carbon exceeds that of their fungal counterparts—challenging traditional assumptions about microbial niche partitioning in decomposition processes (Štursová *et al.,* 2012). Within these needle-dominated systems, cellulose-carbon incorporation concentrates particularly within specific bacterial lineages including Betaproteobacteria, Bacteroidetes, and Acidobacteria.

Deciduous forest litter harbors a different microbial landscape, where approximately one-tenth of resident bacteria demonstrate cellulolytic capacity, with Proteobacteria, Actinobacteria, Bacteroidetes, and Acidobacteria emerging as principal decomposition architects (Lladó, 2017). This taxonomic specialization represents merely one facet of a much broader bacterial involvement in litter decomposition, with numerous additional phyla contributing specialized enzymatic capabilities to this complex process. Vegetation composition exerts profound influence on bacterial community structure through modulation of soil physicochemical properties, particularly along pH gradients. The characteristically acidic edaphic conditions beneath coniferous stands selectively favour specific bacterial lineages, predominantly Proteobacteria, Acidobacteria, and Actinobacteria creating distinctive microbial signatures that reflect this biochemical environment (Jiao *et al.,*2024). By contrast, temperate deciduous forest litter cultivates bacterial assemblages with pronounced enrichment of Proteobacteria and Bacteroidetes, illustrating how vegetation-mediated soil conditions shape microbial community architecture (Liu *et al.,* 2022).

The botanical chemical diversity expressed through variable litter composition and rhizodeposition patterns constitutes a fundamental organizing principle for bacterial community assembly. Different tree species produce litter with distinctive molecular architecture and exude species-specific root metabolites, creating resource landscapes that directly mold bacterial communities through substrate-driven selection (Du *et al.,2024*).). The interspecific variation in litter quality parameters—including nutritional content, structural complexity, and carbon stoichiometry establishes the metabolic template that determines decomposition trajectories and bacterial succession patterns across forest ecosystems (Findlay, 2021). This intricate relationship between botanical chemistry and bacterial community structure underscores the profound ecological linkages connecting forest composition to soil microbial diversity and associated ecosystem functions.

2.3 Molecular Characterization of Soil Bacteria

Molecular characterization has become a central approach for understanding soil bacterial diversity, ecological functions, and responses to environmental disturbances. Traditional culture-based techniques capture only a fraction of soil microbial diversity, prompting the adoption of DNA-based tools such as 16S rRNA gene sequencing, BLAST homology searches, and phylogenetic analses to accurately identify and classify soil bacteria (Jiao *et al.,* 2023; Irsyadi *et al.,* 2024). The 16S rRNA gene remains the most widely used molecular marker because of its conserved and variable regions, which allow reliable differentiation among bacterial taxa (Taylor *et al.,* 2024). Studies comparing bacterial communities in disturbed and undisturbed forests have shown that molecular approaches reveal significant shifts in microbial composition, abundance, and functional guilds that are often undetectable with conventional microbiological methods (Bowd *et al.,* 2022; Zhang *et al.,* 2023). These techniques also support the detection of beneficial and ecologically important bacteria, including plant growth–promoting species, decomposers, and nitrogen fixers that contribute to soil fertility and nutrient cycling (Ahmad *et al.,* 2022; Nabi *et al.,* 2022).

Advancements in phylogenetics and metagenomics have further enhanced the ability to infer evolutionary relationships and ecological roles of soil microbes under different environmental conditions. High-resolution phylogenetic trees constructed using software such as MEGA allow researchers to compare environmental isolates with type strains, offering insight into taxonomic affiliations and potential functional traits (Xu *et al.,* 2023; Onyango *et al.,* 2023). Reference sequences from curated databases are essential in this process, ensuring accurate clustering and lineage placement (Philippot, *et al.,2021*). Recent studies in forest ecosystems demonstrate that molecular characterization can reveal how soil bacterial assemblages respond to factors such as forest degradation, soil depth, nutrient availability, and vegetation composition (Kumar *et al.,* 2023; Naylor, McClure, & Jansson, 2022). These approaches have also been used to identify novel or ecologically significant strains — for example, Bacillus, Ochrobactrum, and Brucella species isolated from forest soils— which contribute to biodegradation, carbon cycling, and plant-soil interactions (Baazeem *et al.,* 2022; Gaitán-Hernández *et al.,* 2022). Overall, molecular characterization provides a robust framework for understanding the diversity, taxonomy, and functional potential of soil bacteria, ultimately supporting conservation, restoration, and sustainable management of forest ecosystems.

2.4 Impact of Physico-Chemical Properties on Soil Bacteria

Forest soil bacterial communities are strongly influenced by a complex interaction of physico-chemical soil properties, vegetation types, and the degree of anthropogenic disturbance (Barbaccia *et al.,*2022) Key physical properties such as soil pH, bulk density, and organic matter content, along with chemical elements like calcium, potassium, and magnesium, are crucial in shaping microbial structure, activity, and diversity. These soil variables regulate the availability of nutrients and energy sources, modify soil structure and moisture dynamics, and influence the physiological functioning of microbes across forest ecosystems.

Lladó *et al.,* (2017) emphasized that soil pH is one of the most influential factors determining bacterial community composition and richness. Acidic soils, typically found in undisturbed or naturally regenerating forests, tend to support specialized microbial taxa adapted to low-pH environments, while neutral to slightly alkaline soils offer broader conditions for bacterial proliferation. Soil organic matter was also noted as a fundamental driver of microbial abundance due to its role as a primary carbon source. Furthermore, soil bulk density, which is typically elevated in disturbed areas due to compaction, can limit aeration and water infiltration, thereby reducing microbial activity and diversity.

Complementing these insights, Onyango *et al.,* (2023) investigated forest soils in the eastern slopes of Mount Kenya and found that physico-chemical properties varied significantly between disturbed and undisturbed forests, with direct consequences for bacterial abundance. Their findings showed that bacterial counts were consistently higher in undisturbed sites, which also had greater organic matter content, lower soil density, and more favorable pH ranges. Chemically, undisturbed soils were enriched in calcium and potassium, both of which positively correlated with bacterial population levels. These nutrients are essential for microbial enzymatic activity, cellular signaling, and structural stability. Conversely, magnesium levels did not significantly differ in their influence, suggesting a more limited role in bacterial community dynamics in the study area. Overall, the literature highlights that the quality and balance of physical and chemical soil properties are critical to sustaining microbial diversity and ecosystem functionality. Bacterial responses to factors such as pH, organic matter, and nutrient availability play a vital role in biogeochemical processes like nitrogen cycling, decomposition, and soil formation. Understanding how these properties interact under varying forest conditions provides an essential framework for biodiversity conservation, land rehabilitation, and sustainable forest management, especially in regions experiencing increasing degradation due to human activities and climate change (Lladó *et al.,* 2017; Onyango *et al.,* 2023).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter describes the study area and procedures used in the study. Experimental procedures in this chapter include; description of the study area, determination of the impacts of degraded forest types on total soil bacteria counts; the evaluation of the impacts of soil depth on soil bacteria counts in soil degraded forest patches; the evaluation of impacts of degraded forest on soil chemical properties in Mount Kenya Forest, the ethical consideration and methods of data analysis.

3.2 Study Area

This study was conducted in Mount Kenya Forest in Eastern Kenya (Figure 3.1). The forest is located about 16.5 km south of the equator, approximately 170 km north-northeast of the capital, Nairobi (Kenya Forest Service [KFS], 2014). It lies to the east of the Great Rift Valley, along latitude 0°10'S and longitude 37°20'E. Mount Kenya is the highest mountain in Kenya and the second highest in Africa (Kiteme *et al.,* 2008; KFS, 2014). The Mount Kenya Forest covers an area of 1,420 km^2 and has exceptional biodiversity, scenic, social, and cultural values, which justify its protected area status (UNESCO, 2013). The climate of Mt. Kenya region is largely determined by altitude (KFS, 2014). There are great climatic differences within short altitudinal distances. Average temperatures decrease by 0.6^0C for each 100 m increase in altitude. Soils of Mount Kenya show altitudinal variation (Rotich *et al.,* (2024). For instance, in the highest mountain area, above 4000 m the soils are shallow and consist of very stony dark loams with high organic matter and lower bulk density. Soils on the upper slopes between 2,400m and 4,000 m have dark horizons and lower bulk density and rich in organic matter. Lower slopes (below 2,600 m) soils are intensively red with considerable amount of clay. Soils on the western plains and to the northwestern of the mountain (grassland zone with lower rainfall) have dark top horizons and high proportions of clay minerals (Rotich *et al.,* 2024).

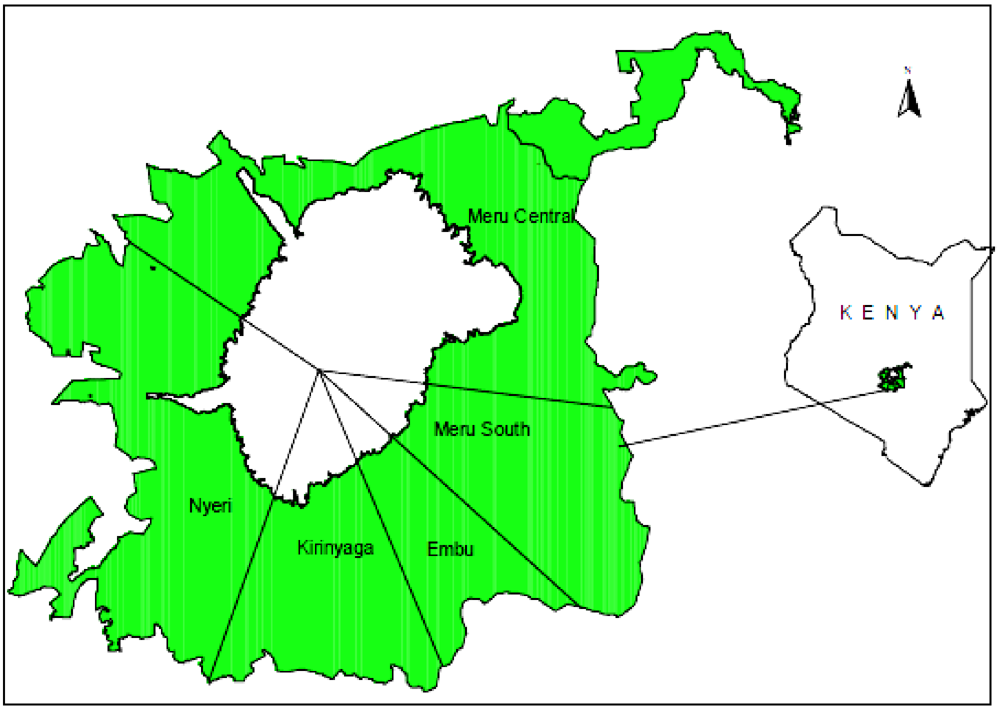

Figure 3.1 Map showing location of Mt. Kenya Forest Reserve -705km^2 (Source: KFS, 2014). The green section is the forest cover and the current study is at Meru South (Tharaka Nithi County)

Table 3.1 Geographical Location of Sampling Sites in Mount Kenya Forest

Sampling Site	Ecological Importance	Elevation (m)	Latitude	Longitude
1	Intact forest	1588	0°19'25"S	37°35'58"E
2	Degraded patch	1555	0°19'33"S	37°36'01"E

3.3 Study Design.

A stratified random sampling design (SRSD) was employed to guide the selection of sampling locations within the study area. This design was chosen to ensure representative sampling across different forest conditions, allowing for meaningful comparisons of soil and microbial characteristics between distinct forest types. Two primary sampling sites were identified based on the degree of forest disturbance. The first site represented an intact (undisturbed) forest patch, characterized as a relatively pristine, primary forest ecosystem. This site exhibited minimal to no evidence of recent anthropogenic disturbance and was dominated by mature trees and dense woody vegetation, indicative of a stable and minimally altered ecological state.

The second site was classified as a degraded forest patch, which exhibited clear signs of human-induced disturbance. This area had undergone structural and compositional changes due to activities such as logging or land clearance. Evidence of degradation included the presence of tree stumps, reduced canopy cover, and a heterogeneous assemblage of vegetation, including regenerating woody plants, gooseberries (*Physalis spp.*), grasses, and other herbaceous species. This stratification allowed for direct comparisons between ecosystems of differing integrity and helped in assessing the impacts of forest degradation on soil and microbial parameters.

3.4 Soil Sample Collection

Before collecting the soil samples, surface litter was first removed. Soil samples were collected from six (6) different sampling locations, as 1, 2, 3, 4, 5, and 6 as shown in Fig 3.2) within a sampling plot of 100 m x 100 m established for each forest sampling type. The distance between one sampling locations to the next was eighteen metres (18m) a part. Areas overlapping the degraded and intact sites were avoided to prevent edge impact on the soil samples. At the established sampling locations soil samples were collected in triplicate at different soil depths; 0-5 cm, 6-14 cm and 15-20 cm giving a total of 18 samples per soil depth from degraded site and 18 samples per soil depth from intact site. To achieve this, three points, one meter each across the marked location on the left and on the right were established (Figure 3.2). Using a sterile soil sampler, soil samples were collected and packed in sterile containers. The soil auger was cleaned and wiped with cotton socked in 70% alcohol after every collection to avoid cross contaminating the samples. Soil samples were labelled as per sampling site. Samples were then packed in cool boxed and taken to the laboratory analysis at Chuka University, Tharaka Nithi County, Kenya and stored in ziplock bags in a refrigerator awaiting analysis.

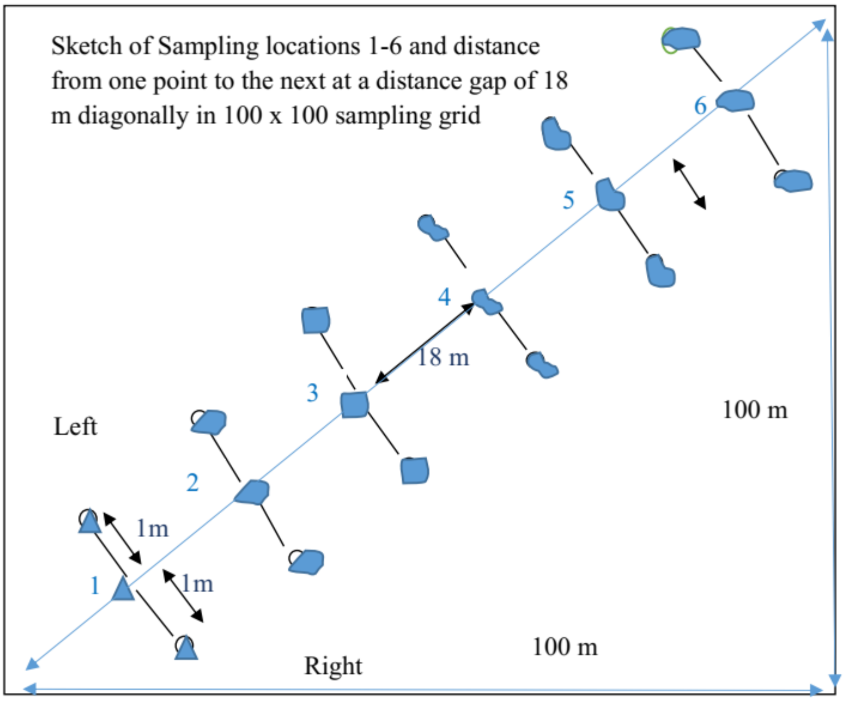

Figure 3.2: Sketch of sampling locations and pattern used in intact and degraded forest patches in a 100 x 100 m plot

3.5 Data Collection

3.5.1 Impacts of Forest Degradation on Selected Soil Physical Properties

3.5.1.1 Determination of Soil Organic Matter

The determination of soil organic matter (SOM) was carried out using the dry combustion method. An empty evaporating dish was weighed accurately to ensure precise measurement of the organic matter. Ten (10) grams of soil was placed into the dish, and the sample heated in an oven at 100°C for 24 hours to remove moisture content. The soil was then reheated at between 250°C -300 °C while stirring to ensure complete combustion of the organic matter. This heating process continued until there was no visible traces of organic material, leaving only ash. The dish and its contents were then allowed to cool in a desiccator to prevent the absorption of moisture from the air. The organic matter content was determined by calculating the difference between the mass of the original soil and the mass of the dried soil, expressed using the formula by Silfverberg (1957)

3.5.1.2 Determination of Soil Bulk Density

Soil bulk density was determined using the core ring method, following the procedure outlined by Panagos *et al.* (2024). A cylindrical core ring with a diameter of 8 cm and a height of 5 cm was used to collect soil samples. Prior to sampling, the top litter layer was carefully removed to ensure that only the underlying soil was sampled, avoiding the inclusion of surface organic material that could affect the results. Three soil samples were taken from each selected sampling point to ensure consistency, representing both degraded and intact forest areas. Each sample was labeled appropriately and transported to Chuka University Laboratory in a cool box to prevent moisture loss or contamination. Upon arrival, the samples were stored in a refrigerator until they were processed. In the laboratory, the soil samples were placed in a hot air oven at 105°C for 24 hours to remove all moisture content. The drying process continued until the soil reached a constant weight, indicating the complete removal of moisture. After drying, the oven-dried soil was weighed. The bulk density of the soil was calculated using the formula provided by Kumar *et al*., 2017.

$$Soil\ Bulk\ Density = \frac{WOS\ in\ grams}{VOS\ in\ ml}$$

Where: WOD = weight of soil in grams, VOS = Volume of the soil, Unit= g/ml

3.5.2 Impacts of Forest Degradation on Selected Soil Chemical Properties

3.5.2.1 Determination of Potassium, Magnesium and Calcium in Soil Samples

Analysis of soil chemical properties, specifically the concentrations of potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}), was conducted in the Chemistry Laboratory at Chuka University. Ten grams of dried soil were weighed and placed into a 500 ml beaker. To this, 250 ml of 1M ammonium acetate was added, ensuring the sample was completely covered. The beaker was sealed tightly with aluminium foil and placed in an orbital shaker, where it was left overnight to facilitate the extraction of cations from the soil particles into the solution. After the extraction period, the cation concentrations of calcium, magnesium, and potassium were determined using Atomic Absorption Spectrometry (AAS) (Singhal & Singh 2024). The results of the exchangeable cations were expressed in cmol/kg of soil (dry weight basis), a standard unit that facilitates comparison between samples.

The concentrations of exchangeable calcium (Ca), magnesium (Mg), and potassium (K) were calculated using the formulas by Antonangelo *et al.* (2024).

$$i.\ \text{Exchangable cations Ca} = \frac{(a-b)*20*mcf}{10*40.078*S} \quad \left[\frac{\text{cmol}}{\text{kgsoil}}\text{DW}\right]$$

$$\text{ii. Exchangable cations Mg} = \frac{(a-b)*20*mcf}{10*24.305*S} \quad [\text{cmol/kgsoil DW}]$$

$$\text{iii. Exchangable cations K} = \frac{(a-b)*20*mcf}{10*39.10*S} \quad [\text{cmol/kgsoil DW}]$$

Where: a= mg/l Ca, Mg, K, in extraction solution, b= ditto in blanks, s= air dry sample weight in gram, mcf = Moisture correction factor, 20 = ml of Ammonium used in extraction, 10 = Mass of the soil sample in grams, 20 represents the volume (in ml) of the ammonium acetate extract used in the extraction procedure (The atomic weights of the elements are Ca=40.078, Mg=24.305, K=39.098, respectively, which are used to convert concentrations to cmol/kg for standard reporting.

3.5.2.2 Determination of Soil Alkalinity

To assess soil alkalinity or acidity directly in the field, in-situ measurements of soil pH were conducted at stratified sampling plots within both intact and degraded forest areas of the Mount Kenya Forest. A total of thirty-six sampling points were selected using a stratified random sampling design based on forest condition and soil depth (0–5 cm, 6-15cm, and 16–20 cm). At each sampling point, a handheld portable pH meter equipped with a soil probe was used to measure the pH directly in the soil profile. The soil surface was cleared of litter, and a soil auger was used to expose the targeted depth layers.

The pH probe was then inserted into the moist soil at each depth, and the pH was recorded after allowing the probe to stabilize for approximately 60 seconds. The pH meter was calibrated daily before use with standard buffer solutions (pH 7.0 and pH 9.0) to ensure accuracy and consistency of the measurements. All readings were taken in the field at ambient temperature (approximately 20–25°C) to reflect natural soil conditions. This in-situ approach minimized sample disturbance and provided real-time pH values representative of the soil's natural state across different forest conditions and depths.

3.5.3 Evaluation of Impact of Soil Depth on Soil Bacteria Counts

3.5.3.1 Soil Sample Preparation, Media Preparation and Inoculation

Soil samples were collected from 18 sampling locations within Mount Kenya Forest, representing both intact and degraded forest types. Each sampling location provided samples from three distinct soil depths: 0-5 cm, 6-14 cm, and 15-20 cm. Ten (10) grams of soils from each depth was weighed and mixed with 100 ml of distilled water to create a homogeneous soil suspension. This process was repeated in triplicate for each depth and forest type to ensure consistency. The soil suspensions were then shaken thoroughly to facilitate the dispersion of soil particles and bacteria into the solution. These suspensions were stored at 4°C as stock cultures for subsequent inoculations. To ensure optimal growth conditions for the bacteria, Plate Count Agar (PCA) was prepared. Twenty-three and half (23.5) grams of PCA powder was dissolved in 1 liter of distilled water to create a 2.3% PCA solution. The solution was heated and stirred to ensure complete dissolution. Once dissolved, the solution was sterilized by autoclaving at 121°C for 15 minutes to eliminate any potential contaminants. The media was allowed to cool to approximately 45°C before use to prevent the agar from solidifying too quickly when poured into Petri dishes (this was done according to the manufacturers instruction). The cooled media was used immediately for inoculation.

From each stock soil suspension, a 10^{-4} serial dilution was prepared. The dilution process was performed in triplicate for each sample, with separate dilutions prepared for each depth (0-5 cm, 6-14 cm, 15-20 cm) and forest type. The serial dilutions ensured that bacterial cultures would be diluted to a manageable concentration for counting colony-forming units (CFUs). The prepared 10^{-4} dilutions were then used for bacterial inoculation. For each dilution, 1 ml of the inoculum was transferred into sterile Petri dishes. The pour plate method was adopted for inoculation, where 15 ml of cooled PCA was poured into each Petri dish. After the agar and inoculum were mixed by gently swirling the plates, the inoculated plates were allowed to solidify for

20 minutes at room temperature. This ensured that the agar was fully set and bacterial cells were evenly distributed throughout the medium. Once the plates were solidified, they were placed in an incubator (Memmert TYP INB200) at 37°C for 48 hours. The plates were incubated in an inverted position to prevent condensation from disrupting the growing bacterial colonies. During this period, bacteria in the inoculum grew into visible colonies on the surface of the agar.

3.5.3.2 Enumeration of Bacterial Colonies

After the 48-hour incubation period, the bacterial colonies were counted to determine the total number of colony-forming units (CFUs). The plates were carefully removed from the incubator and sorted according to the forest type (intact or degraded) and soil depth (0-5 cm, 6-14 cm, 15-20 cm). Each plate was visually inspected, and the number of bacterial colonies present was counted using a colony counter, which allowed for accurate enumeration of CFUs. Only well-isolated colonies were considered for counting to ensure accuracy. The total CFUs for each plate were recorded as colony-forming units per gram of soil (dry weight) for each sample. This calculation accounted for the dilution factor, where the soil suspension had been diluted by a factor of 10^{-4} prior to plating. The bacterial counts were then converted to CFUs per gram of soil by multiplying by the dilution factor. These counts were not averaged but were retained for statistical analysis.

3.5.3.3 Identification of Soil Bacteria Isolates Using Biochemical Methods

Bacteria isolates were identified based of their pigmentation and biochemical tests (Gram stain, catalase, oxidase, and indole tests (Ogolla *et al.*, 2020). Gram stain was performed on a thin smear from individual pure bacteria isolates on glass slide. One drop of normal saline on a slide a loop of pure colonies was added and mixed followed by air drying to fix the smear. The fixed smear was stained with crystal violet stain and washed off under running tap water. Gram's iodine was added to the smear and allowed to stand for 1 minute then rinsed with running water. The cells were then decolorized using 80% ethanol for 1 minute and rinsed. Safranin stain was then added to the smear for 1 minute then rinsed. Stained cells on the slide were viewed under the compound microscope using x100 lens and oil immersion. Gram positive bacteria stained blue and gram negative stained red (Ogolla & Neema, 2019).

Catalase test was done on 48 hr. old bacteria pure isolates. A loop-full of individual isolates were smeared on oil-free glass slide. Three drops of 3% hydrogen peroxide (H_2O_2) were then added on to the bacteria loop and allowed to stand for 30 seconds. The bacteria loop was then observed for production of gas and bubbles. Catalase positive bacteria produce gas and bubbles but catalase negative remained unchanged (Ogolla *et al.,* 2019). Oxidase test was done on moistened Whatman No. 2 filter paper treated with three drops of dimethyl p- phenylenediamine hydrochloride (Shields & Cathcart, 2010). Pure isolates of bacteria colony were smeared on the moistened paper and observed for reaction. Oxidase positive reaction turned purple in colour and negative reaction remained colourless.

The indole test was conducted following the procedure outlined by Ogolla et al. (2020). The indole test medium was prepared by dissolving 5 g of sodium chloride and 10 g of tryptophan in 100 ml of distilled water. The prepared media were dispensed into test tubes, autoclaved at 121°C, 15 psi for 15 minutes. Pure bacterial isolates were sub cultured into the prepared tryptophan broth in the test tubes. Dry blotting paper strips, impregnated with oxalic acid to prevent false positive or prevent background reactions by removing interfering ions from the medium were then suspended in the tubes, which were then incubated at 25°C for 14 days. The strips were checked every two days for the development of a positive pink color, while negative strips remained unchanged. A total of 69 bacterial isolates were randomly selected from a pool of 216 characterized isolates and further clustered into three main groups based on Euclidean distance as described by Liberti & Lavor (2017).

3.5.3.4 Molecular Identification of Selected Soil Bacteria

DNA extraction and Polymerase Chain Reaction (PCR) amplification were performed at the University of Nairobi's Center for Biotechnology and Bioinformatics Lab. The bacterial genomic DNA was isolated using the N-cetyl-N,N,N-trimethylammonium bromide (CTAB) method, as outlined by Irsyadi *et al.,* (2024). To extract the genomic DNA, 2 ml of overnight bacterial culture were centrifuged at 16,099 x g for 5 minutes.

The supernatant was discarded, and the remaining 2 ml of overnight culture was added to the same tube. The supernatant was removed again, leaving behind a pellet. This pellet, containing approximately 4 ml of culture, was resuspended by tapping or repeated pipetting (20 mg/ml) in 500 ml of Tris-EDTA buffer (100 mM Tris, 10 mM EDTA), followed by incubation for one hour at 37°C. Subsequently, 100 µl of 20% sodium dodecyl sulfate (SDS) buffer was added.

Gram-positive bacteria were cultured in a tube, and lysozyme was introduced to the mixture. The tube was gently inverted and left at room temperature for 5 minutes. Then, 200 µL of 5M NaCl buffer was added, and the contents were mixed by inverting the tube. The tube was incubated on ice for 10 minutes, followed by centrifugation at 11,180 x g for 5 minutes. The supernatant was carefully transferred to a fresh 2 mL tube. Next, 500 µL of phenol:chloroform:isoamyl alcohol (25:24:1) was added, and the mixture was thoroughly mixed by inversion before being centrifuged at 20,000 x g for 5 minutes. Without disturbing the middle white layer, the upper aqueous phase was transferred to a new 2 mL tube. An equal volume of chloroform and isoamyl alcohol (25:24:1) was added, and the centrifugation was repeated at 11,180 x g for 5 minutes. Again, the upper aqueous phase was transferred to a fresh tube without disturbing the white interface. To precipitate DNA, isopropanol was added in equal parts, and the solution was mixed by inversion. After allowing it to stand at room temperature, the mixture was centrifuged at 11,180 x g for 5 minutes. The tiny white pellet formed was left undisturbed while the supernatant was discarded. The pellet was washed with 500 µL of 70% ethanol, followed by centrifugation at 11,180 x g for 5 minutes. The supernatant was discarded without disturbing the pellet. To remove any remaining ethanol, the Eppendorf tubes were opened and placed inverted on tissue paper for 10 minutes to drain and evaporate the residual liquid. Depending on the pellet's appearance, it was dissolved in 50–100 µL of TE buffer. To remove RNA, 5 µL of RNase A was added to each tube, which was incubated at 37°C or room temperature for 30 minutes. The genomic DNA was then verified by electrophoresis on a 0.8% agarose gel, visualized under a UV transilluminator (BIO-RAD).

For 16S rDNA analysis, the primers used were 16S F (5'-AGAGTTTGATCCTGGCTCAG-3') and 16S R (5'-GTACGCTACCTTGTTACGAC-3'). The master mix was prepared by combining 0.625 U Taq DNA polymerase, 200 µM dNTPs, 2 mM MgCl2, 0.5 µL of each primer (10 µM), and 1 µL of DNA template (100 µg/mL). A 24.5 µL aliquot of the master mix was distributed into five PCR tubes. To each tube, 0.5 µL of the DNA sample was added, excluding the negative control. The contents of each tube were briefly mixed by vortexing and centrifuged at 1,112 x g for 10 seconds to concentrate the mixture at the bottom. The following PCR program was applied in a thermocycler

(Model C1000 Touch, Bio-Rad): initial denaturation at 94°C for 5 minutes, followed by 34 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 60 seconds. A final extension step at 72°C for 5 minutes was performed, followed by a holding step at 22°C until further processing. The PCR products were analyzed by electrophoresis on a 1% agarose gel, visualized using a transilluminator. A 1% agar rose gel stained with ethidium bromide was used to gel electrophorese PCR results for verification. The DNA amplicon (1:1) combined with loading dye was measured with a 1 kb 0 gene ruler with an initial reading of 75 kb. The DNA loading bands were observed using a gel documenter imager (Bio-Rad). In preparation for sequencer sequencing, the Gene Script firm provided a Rapid cleaning kit for use on positive DNA PCR results.

The BigDye Terminator v3.1 and a Senger sequencer (Model: ABI 3730 genetic analyzer) were used to sequence the DNA amplicons at the International Livestock Research Institute in Nairobi, Kenya. The sequences from the bacteria (from the 16S rRNA PCR fragment) were compared to the sequences from the NCBI/GenBank database(www.ncbi.nlm.nih.gov/blast/). Nucleotide multiple sequence alignment was performed utilizing the Multiple Sequence Comparison by Log-Expectation (MUSCLE) computer method in Mega X version 11.0 (Tamura *et al.,*2021). Using a neighbour-joining tool and bootstrapping with 1000 replications, a phylogenetic tree of the isolates’ sequences and those downloaded from the genebank was created to determine the stability of the branching (Segura-Alabart et al. 2024). The p-distance method was used to calculate the evolutionary distances between the species in the phylogenetic tree, and the results were provided in units of the number of base substitutions per site.

3.6 Ethical Consideration

Prior to commencement of the study research authorization clearance was obtained. The research permit was then obtained from the National Commission of Science, Technology, and Innovation (NACOSTI). The samples collected were deemed environmentally safe thus no special precaution was required. In order to prevent samples contamination, collected samples were aseptically collected and carried to Chuka University for further study. The research procedures steps described in the document were followed with fidelity. The results reported were based on finding of the field and laboratory analysis. Additionally, the policy regarding plagiarism was adhered to in the process of data collection, analysis and in document write up.

3.7 Data Analysis

Data were analyzed to address the study's objectives. Physical and chemical soil properties were visualized using PCA and analyzed with ANOVA and GLM in SAS, followed by mean separation with LSD. Bacterial abundance was similarly analyzed using PCA and ANOVA with LSD. Cluster and phylogenetic analyses of bacterial isolates were conducted using MUSCLE in Mega X, with bootstrapping to assess tree stability. This approach provided a comprehensive analysis of soil and bacterial characteristics across the forest patches.

3.7.1 Impacts of Forest Degradation on Selected Physical Properties of Soil in Mount Kenya Forest Ecosystem

Data on physical properties of soil (oil density, pH and soil organic matter) were visualized using Principal Component Analysis (PCA) and biplots constructed using the factor Minor package in R Studio version 4.2.2, with the first and second eigenvectors. This allowed for a visual representation of trends in the levels of these physical properties between degraded and intact forest patches. Further, the data were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) in SAS version 9.4. Statistically significant differences in the means were separated using Least Significance Difference (LSD).

3.7.2 Impacts of Forest Degradation on Selected Chemical Properties of Soil in Mount Kenya Forest Ecosystem

Data on the levels of soil nutrients (Calcium, Magnesium, and Potassium) in cmol/kg were also visualized using PCA and biplots constructed with the factor Minor package in R Studio version 4.2.2, using the first and second eigenvectors. This analysis helped observe trends in the chemical properties of soil in both degraded and intact forest patches. Similar to the physical properties, the data on soil nutrient levels were subjected to ANOVA using PROC General Linear Model (GLM) in SAS version 9.4 to determine statistical differences between forest patches. The means were separated using Least Significance Difference (LSD).

3.7.3 Impacts of Different Soil Depths on Total Soil Bacterial Counts in Degraded and Intact Forest Patches

Bacterial abundance, as determined by total plate count, was visualized using Principal Component Analysis (PCA) and biplots constructed using the factor Minor package in R Studio version 4.2.2, based on the first and second eigenvectors. This allowed for the observation of trends in bacterial abundance between different soil depths in degraded and intact forest patches. Additionally, the total CFUs (colony-forming units) were analyzed using Analysis of Variance (ANOVA) via PROC General Linear Model (GLM) in SAS version 9.4. Least Significance Difference (LSD) was used to separate statistically significant means.

3.7.3.1 Cluster Analysis and Phylogenetic Analysis of Bacteria Isolates

The cultural and biochemical traits of the bacterial isolates were subjected to cluster analysis, and a dendrogram was plotted using the factor Extra package in R Studio version 4.2.2. The bacterial characteristics used to plot the dendrogram included gram reaction (gram-positive or gram-negative), colony color (e.g., pale yellow, deep yellow, pink), catalase test results, oxidase test results, indole test results, colony edge types, and bacterial shapes. This analysis provided insights into the clustering of bacterial isolates based on shared traits. To further investigate the relationships between bacterial isolates, the Multiple Sequence Alignment (MSA) of nucleotide sequences from isolates and similar sequences obtained from the NCBI database was performed using the MUSCLE program in Mega X version 11.0. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method (Segura-Alabart *et al.,* 2024). with bootstrapping performed using 1000 replications to assess the stability of the branches. The evolutionary distances between species in the tree were calculated using the p-distance method, with results expressed as the number of base substitutions per site (Kumar *et al.,* 2018). A total of 31 nucleotide sequences were included in the analysis, and all ambiguous positions were removed using the pairwise deletion option. The final dataset included 2174 positions. The evolutionary analyses were conducted in Mega X and the evolutionary matrix analysis used the Maximum Composite Likelihood model (Kumar *et al.,*2018).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter contains results of laboratory analyses and discussion of the impacts of forest degradation on soil physical and chemical properties, the impacts of forest degradation on forest soils and total bacteria counts in Mount Kenya Forest. The results of the bacteria counts are based on the total number of the bacteria in samples collected. The chapter also contains the results of the impact of Undisturbed and disturbed forest patches on the selected soil physico-chemical properties that include, nutrients, soil pH, soil density and soil organic matter.

4.2 Impact of Forest Degradation on Selected Soil Physical Properties in Mount Kenya Forest

Table 4.1 presents the comparative impacts of disturbed and undisturbed forest conditions on selected soil physical properties; soil organic matter, soil density, and soil pH. The analysis integrates both raw and log-transformed data ($\log_{10}\{x + 3\}$) with post hoc comparisons using the Least Significant Difference (LSD) test to identify statistically significant differences at $p \leq 0.05$. Values in parentheses represent the transformed means, while the same letter within rows denotes non-significant differences.

Soil organic matter (SOM) content was significantly higher in undisturbed forests (11.68 ± 1.03 cmol/kg; log-transformed: 8.69 ± 0.39) than in disturbed ones (9.2 ± 1.08 cmol/kg; log-transformed: 6.23 ± 0.71). The LSD test confirmed a highly statistically significant difference ($F_{(1, 22)} = 111.83$; MSE =1.07; $p < .0001$; Appendix 7) This suggests that forest disturbance leads to a substantial decline in SOM, likely due to increased decomposition rates, reduced litter input, or soil erosion. The coefficient of variation (CV = 10.366%) indicates a moderate spread in the data, suggesting reliable trends despite inherent variability.

Soil density was significantly greater in disturbed forests (4.58 ± 1.09; log-transformed: 1.6 ± 0.4) compared to undisturbed areas (3.56 ± 1.22; log-transformed: 0.56 ± 0.08). The LSD value (1.047) confirmed a statistically significant difference. This variation was statistically significant ($F_{(1, 22)} = 131.34$; MSE = 1.068; $p < .0001$; Appendix 7) further highlighting the influence of disturbance on soil chemistry. This pattern implies that disturbance —potentially from logging, trampling, or machinery— results in soil compaction, reducing porosity and potentially impacting root penetration and water infiltration. The relatively high CV (4.04%) underscores some variation, yet the significance remains clear. A notable shift in soil acidity was observed between forest types, with disturbed areas exhibiting a more alkaline pH (6.99 ± 0.13) than undisturbed patches (5.7 ± 0.32). The LSD value of 0.186 confirm this difference as statistically significant ($F_{(1, 22)} = 131.34$; MSE = 0.268; $p < .0001$; Appendix 7). The higher pH in disturbed sites could be attributed to ash deposition from fire, reduced organic acid input, or alterations in microbial communities. A CV of 6.347% indicates strong consistency across replicates, reinforcing the reliability of this trend.

The results revealed a significant disparity in soil organic matter (SOM) content between disturbed and undisturbed forest patches, with the latter exhibiting markedly higher SOM levels. This aligns with the findings of Kooch *et al.* (2022) who demonstrated that forest degradation in a semi-arid ecosystem led to substantial losses in total organic matter. The observed decline in SOM within disturbed areas may be attributed to reduced litter deposition and the removal of surface residues through erosion agents such as wind and runoff. Organic matter plays a foundational role in soil function, acting as a primary reservoir of soil organic carbon. When enriched with organic compounds like amino and organic acids, it sustains microbial activity and enhances nutrient cycling (Zhang *et al.,*2022). Therefore, the degradation-induced reduction in SOM poses serious implications for soil fertility, structure, and long-term productivity (Kooch *et al.,* 2022).

Table 4.1: Impact of Undisturbed and Disturbed Forest Type on Selected Soil Physical Properties

Soil Physical Properties	Forest type		Means		LSD
	Disturbed	Undisturbed		CV (%)	(p<.05)
Soil Organic matter	(9.2±1.08 ^{a})	(11.68±1.03^{b})	10.366	2.893	1.053*
	6.23±0.71	8.69±0.39			
Soil Density	(4.58±1.09 ^{a})	(3.56±1.22 ^{b})	4.04	4.72	1.047*
	1.6±0.4	0.56±0.08			
Soil pH	(6.99±0.13 $^{a)}$	(5.7±0.32 ^{b})	6.347	4.23	0.186*

Means followed by the same letters in the rows are not significantly different at p≤0.05). The figures in parentheses are log transformed means.

The soil pH showed a clear contrast between forest types, with disturbed forests exhibiting more alkaline soils, while undisturbed forests maintained a relatively acidic profile. Interestingly, this finding diverges from previous studies such as Bol and Tokuchi (2018), who observed lower pH levels in logged forests. The acidity in undisturbed patches may be explained by the quality and accumulation of litter, as well as the composition of plant-derived organic anions, which influence the soil's acid-base balance (Zhou *et al.,* 2022). Conversely, the elevated pH in disturbed areas might result from reduced organic input or exposure of mineral soil layers that are inherently more alkaline. Moreover, the rhizosphere, the zone influenced by plant root activity, adds a further layer of complexity to pH regulation. Root exudates such as hydroxyl (OH^-), bicarbonate (HCO_3^-), and hydrogen ions (H^+) can significantly alter the local soil environment (de Graaff *et al.,* 2024). For example, roots may elevate pH by secreting bicarbonate ions or by absorbing more anions than cations, a phenomenon well-documented by *Poschenrieder et al.,* (2018). These chemical exchanges, mediated by both plant physiology and microbial processes, underscore the multifaceted mechanisms that shape soil pH and its influence on nutrient availability and biogeochemical cycling (Binkley & Fisher, 2019). A similar pattern of divergence emerged in soil density measurements, where disturbed forest sites exhibited significantly higher bulk density (1.654) compared to undisturbed forests (0.574). This finding echoes the work of Thakur *et al.,* (2024), who reported consistent increases in soil compaction linked to forest degradation. Elevated soil density is often a direct consequence of anthropogenic activities such as grazing, trampling, and mechanized logging, which physically compress the soil matrix (Frene *et al.,* 2024).

In the context of Mount Kenya Forest, the role of large mammals, particularly elephants, adds another ecological dimension. Their movement and foraging behaviour exert substantial pressure on the forest floor, contributing to localized compaction (Gaitán-Hernández *et al.,* 2022). Soil density, as an indicator of compaction, is critical to ecological function for it regulates porosity, gas exchange, water infiltration, and root penetration. Compacted soils restrict oxygen flow, reduce microbial activity, and hinder nutrient and water transport, factors that cumulatively impair plant growth and forest regeneration (Ramirez *et al.,* 2021). Consequently, changes in soil density linked to disturbance may ripple through forest ecosystems, affecting both above- and below-ground biodiversity and functionality.

4.3 Impacts of Forest Degradation on Selected Soil Chemical Properties

Table 4.3 presents the comparative analysis of key soil chemical nutrients, Magnesium (Mg), Calcium (Ca), and Potassium (K), between disturbed and undisturbed forest patches. The results are based on both log-transformed and untransformed data, with log values used to meet the assumptions of normality in the statistical tests.

Table 4.2: Impact of Disturbed and Undisturbed Forest on Selected Soil Chemical Properties

Soil Chemical Properties	Forest type		Means	CV (%)	LSD
	Disturbed	Undisturbed			(p<.05)
Magnesium	(7.3±0.53 ^{a}) 4.45±0.83	(7.6±0.3 ^{a}) 4.63±0.7	7.449	3.743	1.053
Calcium	(5.87±1.09 ^{a}) 2.95 ±0.53	(11.76±1.1 ^{b}) 8.88±0.81	8.309	4.34	1.066*
Potassium	(8.23±1.06 ^{a}) 5.26 ±0.96	(9.52±1.05 ^{b}) 6.58 ± 1.52	8.855	2.62	1.04*

Means followed by the same letters in the rows are not significantly different at $p \le 0.05$). Figures in parentheses are log transformed means.

Calcium concentrations were significantly higher in undisturbed forest patches (11.76 ± 1.1 cmol/kg) than in disturbed areas (5.87 ± 1.09 cmol/kg). The statistical comparison revealed a highly significant difference ($F_{(1, 22)}$ =516.01; MSE=1.096; $p < 0.0001$; LSD = 1.066), with an overall mean of 8.309 cmol/kg. This nearly twofold increase in calcium in intact forests suggests greater nutrient retention and cation exchange capacity, likely due to higher organic matter input and reduced leaching. Calcium plays a critical role in maintaining soil structure and buffering capacity. Its elevated presence in undisturbed patches indicates enhanced soil fertility and resilience against acidification.

A similar trend was observed for potassium, with significantly higher concentrations in undisturbed forests (9.52 ± 1.05 cmol/kg) compared to disturbed sites (8.23 ± 1.06 cmol/kg). The difference was statistically meaningful ($F_{(1,\ 22)}$ =58.25; MSE=1.06; $p < 0.0001$; LSD = 1.040, Appendix 6) with an overall mean of 8.855 cmol/kg. Potassium is an essential macronutrient for plant metabolism, especially in stress resistance and water regulation. The richer K pool in undisturbed forests underscores the positive impact of stable litter input and undisturbed root-soil interactions on nutrient cycling.

Unlike calcium and potassium, magnesium did not show a statistically significant difference between forest types (type ($F_{(1,22)}$ =2.55; MSE=1.078; $p = 0.1245$; LSD = 1.053 Appendix 6), although there was a slight numerical increase in undisturbed patches (7.60 ± 0.30 cmol/kg) compared to disturbed ones (7.30 ± 0.53 cmol/kg). The overall mean magnesium concentration stood at 7.449 cmol/kg. Magnesium, being central to chlorophyll structure and photosynthetic activity, may be more stable in soils due to lower susceptibility to leaching or anthropogenic impact. This stability across forest types suggests a degree of resilience, potentially moderated by soil mineralogy. The PCA biplot presented (Figure 4.1) summarizes the variation in soil physical and chemical properties between disturbed and undisturbed forest patches. The first two principal components (PC1 and PC2) explain a cumulative 80.8% of the total variance in the dataset. PC1 accounts for 63.9%, while PC2 explains 16.9%. This high percentage indicates that these two axes sufficiently capture the main patterns in the multivariate data. Interpretation of PC1.

PC1, the soil fertility disturbance axis, represents a gradient of disturbance-related soil change, with variables like calcium, potassium, and potentially organic matter or bulk density contributing strongly. There is clear separation along PC1 between disturbed (red) and undisturbed (blue) forest patches. The strong separation suggests PC1 may capture the cumulative impact of degradation on soil fertility and structure. PC2, the texture/moisture axis, explains 16.9% of the variance, which is meaningful but secondary. Since PC2 is orthogonal to PC1, PC2 likely captures variation not directly related to forest disturbance, possibly more subtle differences like moisture retention, soil texture, or specific nutrient ratios. The red cluster (disturbed forest) and blue cluster (undisturbed/intact forest) are distinctly separated along the PC1 axis. This clear separation suggests that the forest disturbance status strongly influences the overall soil property profile. The ellipses indicate confidence regions around group centroids, and minimal overlapping p between the ellipses suggests statistically meaningful differences in soil properties between forest types.

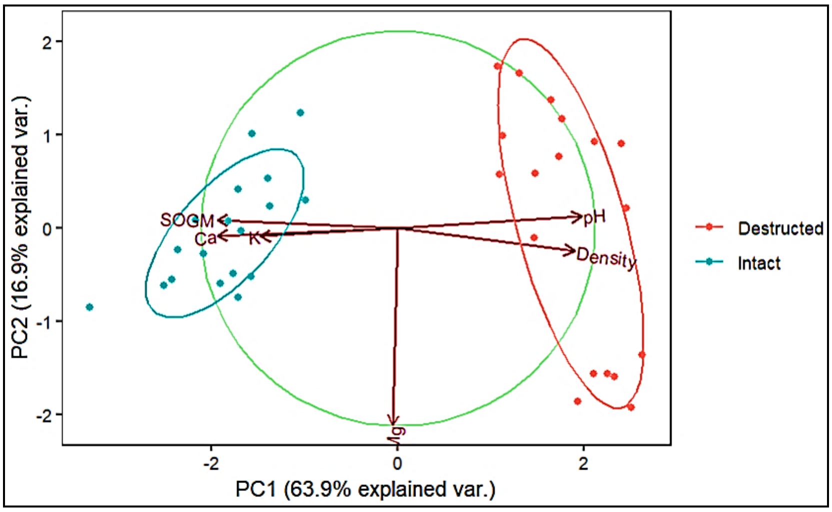

Figure 4. 1: Correspondence visualization Biplot for impact of forest degradation on soil bacteria in Mount Kenya Fores, where CPA = Component Principal Analysis, PC1= 1st principal component and PC2= 2nd principal component.

The length and direction of the arrows represent the strength and orientation of each variable's contribution to the principal components. Soil pH and Bulk Density have long vectors pointing positively along the PC1 axis, indicating that they are strongly associated with disturbed forest patches. Their direction suggests they are positively correlated with each other and play a major role in distinguishing disturbed sites.

Soil Organic Matter (SOGM), Potassium (K), and Calcium (Ca) have arrows pointing negatively along PC1, and are more associated with undisturbed forest patches. SOGM in particular is almost opposite in direction to pH and Density, indicating a strong negative correlation between organic matter and those variables. This supports earlier univariate results showing decreased SOM and increased pH and density in disturbed forests.

The PCA reveals two ecologically distinct soil environments. Undisturbed forests are characterized by higher levels of organic matter, calcium, and potassium, which are associated with better soil quality and nutrient retention. Disturbed forests are dominated by higher pH and bulk density, which may result from soil compaction, reduced organic inputs, and altered ionic composition due to vegetation loss or anthropogenic activity. It is clear from the findings that forest degradation not only alters individual soil properties but also reshapes the overall soil profile, pushing it into a different compositional space, quantitatively and qualitatively distinguishable from that of intact forests.

Results also show that forest degradation has a pronounced impact on soil nutrient concentrations, particularly calcium and potassium. This finding is consistent with previous research by Huntley (2023) who reported that disturbances in forest ecosystems often lead to significant reductions in essential base cations. The stark contrast in nutrient levels between undisturbed and disturbed forest areas can be largely attributed to altered forest floor dynamics resulting from degradation. The removal of the forest floor and increased soil compaction disrupt nutrient cycling and reduce organic matter inputs, thereby diminishing the concentrations of exchangeable cations such as Ca^{2+}, K^{+}, Mg^{2+}, and Na^{+} in the soil.

In forest ecosystems, plant litter serves as a critical reservoir and source of soil nutrients. Its decomposition releases base cations that enhance soil fertility. The quantity and quality of litter inputs are pivotal in determining the extent and rate at which nutrients become available in the soil (García-Palacios *et al.,* 2023), López-Merino *et al.,* (2023) further demonstrate that variations in litter quality among tree species influence the nutrient composition of the soil, with certain species contributing more calcium- and potassium-rich litter than others. The elevated calcium concentrations in undisturbed forest soils observed in this study may therefore be attributed to the long-term accumulation and decomposition of calcium-rich litter, as recently reaffirmed by López-Merino *et al.* (2023) This underscores the critical role of vegetation composition and litter dynamics in regulating soil nutrient availability. Consequently, forest degradation not only reduces biomass input but also alters the nutrient economy of the soil by interrupting these finely balanced ecological processes.

Overall, these findings emphasize the need to account for both the structural integrity of the forest floor and the biochemical quality of organic inputs when assessing the impacts of disturbance on soil nutrient dynamics. The degradation of forest ecosystems can have cascading impacts on soil fertility, which may, in turn, hinder natural regeneration and long-term ecosystem productivity.

4.4 Impact of Soil Depth and Forests Degradation on the Abundance of Soil Bacteria Isolates in Mount Kenya Forest

4.4.1 Impact of Soil Depth on Total Abundance of Soil Bacteria

Table 4.3 presents the variation in total soil bacterial abundance (expressed as colony-forming units per gram of soil, cfu/g) across different soil depths (0–5 cm, 6–14 cm, and 15–20 cm) in disturbed and undisturbed forest conditions.

The results demonstrate statistically significant differences ($p < 0.05$) in bacterial abundance between forest types at all soil depths.

Table 4.3: Impacts of Forest Type on Total Soil Bacteria Across Different Soil Depth

	Forest type				
Soil Depth	Disturbed forest	Undisturbed forest	Mean (cfu)	LSD (P<0.05)	CV (%)
0-5 cm	183.67 ± 23.36 ^{a}	86.38 ± 21.35 ^{b}	135.03	14.055	15.22
6-14cm	144.05 ± 29.29 ^{a}	65.27 ± 21.96 ^{b}	104.67	17.826	24.90
15-20 cm	130.5 ± 66.24 ^{a}	35.83 ± 10.56 ^{b}	83.167	27.673	48.65

* *Means followed by the same letters in the rows are not significantly different at p≤0.05.*

At the surface layer (0–5 cm), disturbed forest soils supported a significantly higher mean bacterial count (183.67± 23.36 cfu) compared to undisturbed forest soils (86.38±21.35 cfu). This difference in mean bacterial counts between forest types was statistically significant ($F_{(1,\ 27)} = 201.53$; MSE=20.557; $p < 0.0001$). This trend continued in the mid-depth layer (6–14 cm), where disturbed forests again recorded greater mean bacterial count (144.05±29.29 cfu) than their undisturbed counterparts (65.27±21.96 cfu). This difference was also statistically significant ($F_{(1,\ 27)} = 82.22$; MSE=26.063; $p < 0.0001$). The deepest layer (15–20 cm) also followed the same pattern, with disturbed forest soils exhibiting a mean bacterial count of 130.5±66.24 cfu, nearly four times higher than the mean bacterial count in undisturbed soils at the same depth (35.83±10.56 cfu; This difference was statistically significant $F_{(1,27)}$ =52.82; MSE=40.461; $p < 0.0001$). Post hoc LSD tests revealed significant differences in total soil bacterial counts between disturbed and undisturbed forest types at all soil depths (0–5 cm, 6–14 cm, and 15–20 cm), with disturbed forests consistently exhibiting higher bacterial counts. The Least Significant Difference (LSD) values reinforce the observation that the differences are statistically robust across all depth intervals (LSD at 0–5 cm = 14.055, LSD at 6–14 cm = 17.826, LSD at 15–20 cm = 27.673 respectively).

These findings are in agreement with those of Kumar *et al.* (2023). The unexpectedly higher bacterial abundance in disturbed forest soils may be attributed to several interacting factors, increased nutrient pulses from organic matter disturbance or decomposition of surface vegetation, greater aeration due to reduced canopy cover and litter accumulation, which could enhance microbial activity and alteration of microbial niches due to soil compaction, warming, and disturbance, favouring certain fast-growing or opportunistic bacterial taxa (Naylor *et al.,* 2022). Conversely, the lower bacterial abundance in undisturbed forests might reflect a more stable and oligotrophic (nutrient-limited) environment with less turnover of organic substrates, where microbial communities are less dense but potentially more diverse and functionally specialized (Naylor *et al.,* 2022). Furthermore, the general decline in bacterial abundance with depth across both forest types aligns with established ecological patterns, as microbial activity is highest near the surface due to greater organic matter inputs, root exudates, and oxygen availability.

Overall, these results underscore the sensitivity of microbial communities to forest disturbance and soil depth. The significantly higher bacterial counts in disturbed forests may indicate a microbial response to altered soil chemistry, structure, and organic input regimes. (Vietorisz *et al.,*2024) This microbial shift could have profound implications for soil nutrient cycling, carbon dynamics, and long-term forest soil health. Continued monitoring and deeper microbial community profiling (e.g., through DNA-based methods) would further elucidate the functional consequences of these shifts in microbial abundance (Pengshuai *et al.,*2023).

4.4.2 Variation in Soil Bacterial Abundance Across Forest Types and Soil Depths on The Basis of Colony Pigmentation

The abundance of soil bacteria, measured in colony-forming units (CFUs), varied significantly across soil depths and between disturbed and undisturbed forest patches. Bacterial colonies were categorized based on colour, creamish, pinkish, whitish, and yellow, which likely represent different bacterial taxa or physiological types.

Additionally, soil bacterial abundance varied significantly across soil depths between undisturbed and disturbed forest patches ($\alpha<0.05$; Appendix 4). Disturbed forests consistently exhibited higher bacterial colony counts across all phenotypic groups and soil depths, highlighting the impact of forest degradation on microbial community structure (Table 4.4).

At the 0–5 cm soil depth, the abundance of creamish colonies was significantly higher in disturbed forests (45.50 ± 6.06) than in undisturbed forests (18.89 ± 8.27) ($F_{(1, 27)} = 129.57$; MSE=7.013; $p < 0.0001$). Similarly, pinkish colonies ($\log_{10}(x + 4)$ transformed) were more abundant in disturbed sites (28.33 ± 6.47) compared to undisturbed ones (10.67 ± 2.28) ($F_{(1, 27)} = 104.37$; MSE=5.187; $p<0.0001$). Whitish colonies were significantly more prevalent in disturbed forests (73.83 ± 10.11) than in undisturbed forests (46.50 ± 12.34) ($F_{(1, 27)} = 76.62$; MSE=9.368; $p<0.0001$). The abundance of yellow colonies was also higher in disturbed forests (36.00 ± 6.76) than in undisturbed ones (10.56 ± 4.57) (F $_{(1, 27)}$ = 186.59; MSE=5.588; p<0.0001).

Table 4.4: Impact of Forest Degradation and Soil Depth on the Abundance of Soil Bacteria in Mount Kenya Forest

Soil Depth (cm)	Bacteria Colony Colour	Forest type		CV (%)	Means (CFU)	LSD (P<0.05)
		Disturbed	Undisturbed			
0-5	Creamish	(45.5±6.06^{a})	18.89 ±8.27^{b}	21.78	32.194	4.797 *
	Pinkish	(28.33±6.47^{a})	10.67±2.28^{b}	26.6	19.5	3.548 *
	Whitish	(73.83±10.11^{a})	46.5±12.34^{b}	15.57	60.16	6.407 *
	Yellow	(36±6.76^{a})	10.56±4.57^{b}	24.01	23.28	3.822 *
6-14	Creamish	(49.72±10.67^{a})	22.83±10.01^{b}	28.19	36.27	6.996*
	Pinkish	(19.83±4.29^{a})	(11± 4.43^{b})	27.11	15.42	2.86*
	Whitish	(61.33±12.87$^{a)}$	29.06±7.34^{b}	24.71	45.19	7.637*
	Yellow	(21.61±8.75^{a})	(10.44±4.44^{b})	42.54	16.03	4.663*
15-20	Creamish	60.72±27.47^{a}	14.06±3.92^{b}	47.51	37.39	12.149*
	Pinkish	(5.66±2.19^{a})	(4.11±0.47^{b}	28.135	4.88	0.941*
	Whitish	(70.3 ±37.19^{a})	(25.55 ±7.8^{b})	47.29	47.92	15.5*
		66.28±37.19	21.56±7.8			
	Yellow	(6.22±2.67^{a})	(4.06±0.24^{b})	32.405	5.138	1.139*
		2.22±2.27	0.056±0.24			

Means followed by the same letters in the rows are not significantly different at p≤0.05). Figures in parentheses are log transformed means.

At the 6–14 cm depth, disturbed forests again recorded significantly higher colony counts. Creamish colonies were more abundant in disturbed forests (49.72 ± 10.67) than in undisturbed forests (22.83 ± 10.01) (F $_{(1, 27)}$ = 62.19; MSE=10.229; p<0.0001). Pinkish colonies (log-transformed) showed a similar trend (19.83 ± 4.29 vs. 11.00 ± 4.43; F $_{(1, 27)}$ = 40.19; MSE=4.18; p<0.0001). Whitish colonies were significantly more abundant in disturbed areas (61.33 ± 12.87) than in undisturbed ones (29.06 ± 7.34) (F $_{(1, 27)}$ = 75.2; MSE = 11.166; p<0.0001). Yellow colonies (log-transformed) also followed this pattern (21.61 ± 8.75 vs. 10.44 ± 4.44; F $_{(1, 27)}$ = 24.14; MSE = 6.818; p<0.0001).

At 15–20 cm depth, the pattern persisted. Creamish colonies were significantly higher in disturbed forests (60.72 ± 27.47) than in undisturbed ones (14.06 ± 3.92) (F $_{(1, 27)}$ =

65.38; MSE=17.76; $p<0.0001$). Whitish colonies were also more abundant in disturbed forests (70.30 ± 37.19) compared to undisturbed areas (25.55 ± 7.80) ($F_{(1, 27)} = 65.53$; MSE=17.764; $p<0.0001$). Pinkish colonies (log-transformed) differed significantly (5.66 ± 2.19 vs. 4.11 ± 0.47; $F_{(1, 27)} = 11.81$; MSE=1.375; $p=0.0019$), as did yellow colonies (log-transformed: 6.22 ± 2.67 vs. 4.06 ± 0.24; $F_{(1, 27)} = 16.4$; MSE=1.665; $p=0.0004$).

Across all soil depths and bacterial colony types, disturbed forest patches consistently exhibited a higher abundance of bacterial colonies compared to undisturbed patches. This trend may be explained by disturbance-induced changes in soil physicochemical properties, such as enhanced nutrient availability, increased organic matter turnover, or altered microhabitats, that create more favourable conditions for bacterial growth. Soil depth also played a significant role, with the greatest bacterial abundance typically found in surface soils (0–5 cm), decreasing progressively with depth. However, exceptions to this pattern were observed in certain colony types, particularly the creamish and whitish colonies. The statistically significant differences, as indicated by LSD values at $p<0.05$ across most comparisons underscore the strong influence of both forest disturbance and soil depth on the abundance of culturable soil bacteria in this montane forest ecosystem.

4.4.3 Principal Component Analysis (PCA) of Bacterial Abundance Across Soil Depths

To explore patterns in bacterial colony abundance across varying soil depths, a Principal Component Analysis (PCA) was conducted. The first two principal components captured a substantial portion of the total variation in the dataset, with PC1 explaining 67.9% and PC2 accounting for 27.4%, cumulatively representing 95.3% of the variance (Figure 4.2). This indicates that most of the variation in bacterial colony composition across soil depths can be described by these two dimensions.

PC1 was predominantly influenced by the abundance of cream-coloured bacterial colonies, which exhibited a strong negative loading (eigenvector=–0.438) suggesting that variation along PC1 largely reflected the relative abundance or presence of cream-coloured colonies, with higher values on PC1 indicating a relative reduction in these colonies. PC2 was mainly driven by the abundance of pink-coloured bacterial colonies, showing a strong positive loading (eigenvector=0.497) implying that samples scoring high on PC2 were characterized by a greater presence of pink bacterial isolates.

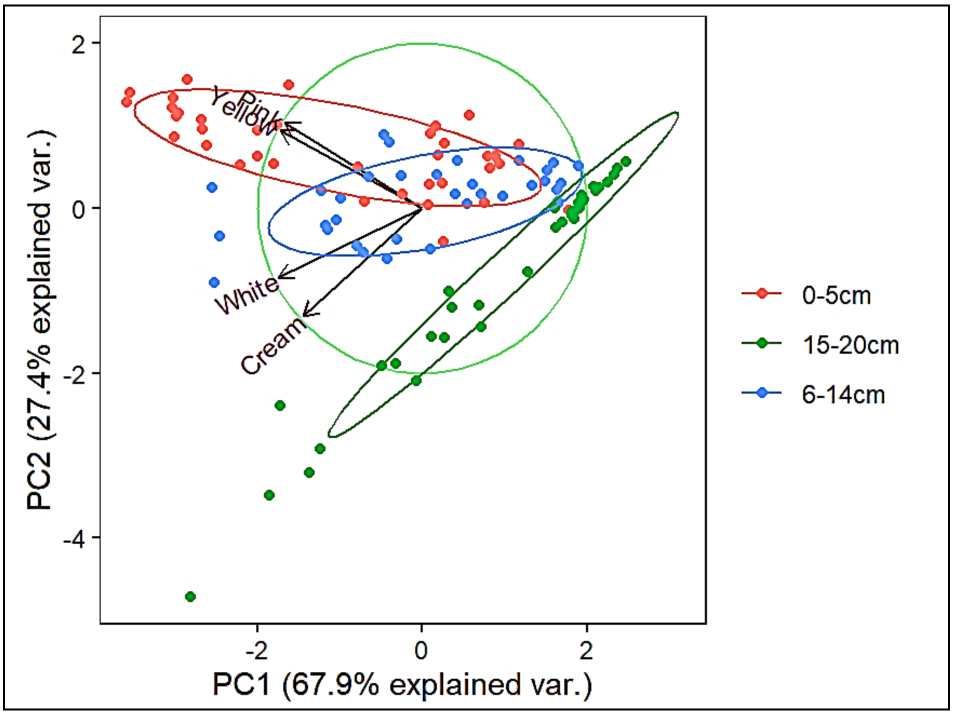

Figure 4. 2: PCA Biplot Showing the Ordination of Bacterial Communities Isolated from Three Soil Depth Intervals in Mount Kenya Forest. The arrows represent colony colour variables (cream, yellow, pink), and their directions indicate the influence on each principal component. Close proximity between points suggests similarity in bacterial composition. CPA = Component Principal Analysis.

The PCA biplot (Figure 4.2) revealed distinct patterns of bacterial community composition across the three sampled soil depths. Shallow (0–5 cm) and mid-depth (6–14 cm) soils displayed a high degree of overlap in bacterial community structure. These two depth ranges were closely associated with yellow and pink-coloured bacterial isolates, suggesting a shared or transitional community composition across the upper soil profile. In contrast, deep soil samples (15–20 cm) formed a more distinct cluster on the biplot, showing minimal overlap with the mid-depth layer. These deeper communities were more strongly aligned with cream-coloured bacterial colonies, supporting the idea of depth-specific microbial stratification. This analysis highlights clear vertical differentiation in bacterial colony composition, with colour-based morphological characteristics of colonies serving as strong indicators of depth-associated microbial patterns.

4.4.4 Phenotypical and Biochemical Identification of Soil Bacteria Isolates

The bacterial isolates showed notable variation in both their physical and biochemical traits, such as colony colour, shape, and edge morphology, as well as Gram stain, catalase, oxidase, and indole production. Four distinct bacterial colonies were identified from the two forest types, categorized by colour; yellow, white, cream, orange, and pink. (Plate 4.1). Further classification of the yellow isolates revealed three subgroups based on pigmentation intensity —pale yellow, deep yellow, and medium yellow— while the pink isolates had two distinct textures, watery and dry. The cream, white, and orange isolates exhibited consistent colony morphology, with ream-colored colonies having undulate or curled edges, white colonies displaying lobate or erose edges, and orange colonies showing erose edges. Isolates from the disturbed forest displayed a broader range of pigmentation, with both deep and pale-yellow colonies, while the undisturbed forest primarily had medium yellow colonies.

Additionally, pink and cream colonies were more prevalent in the disturbed forest. Biochemically, isolates from the disturbed forest showed more oxidase-positive reactions compared to those from the undisturbed forest. The isolates showed different biochemical reactions such as Gram reaction, catalase, oxidase, and indole production. (Appendix 9)

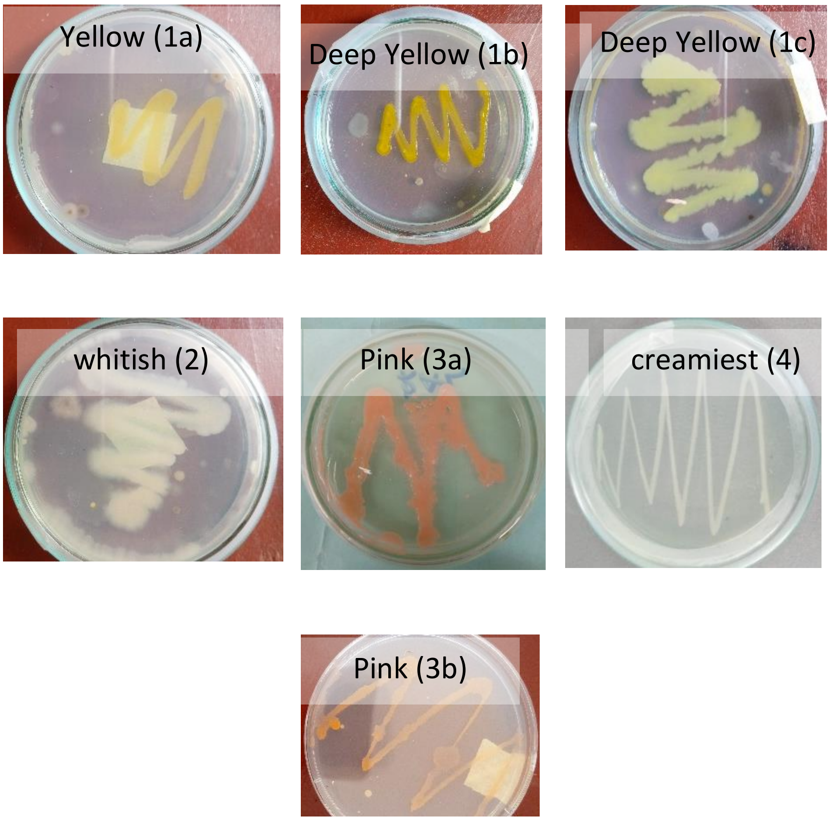

Plate 4.1 Images of different Bacteria Isolated in Both Undisturbed and Disturbed Forest patches. Isolate 1a, MTK14 – Yellow; Isolate1b MTK55 – Deep Yellow; Isolate 1c MTK8 – Light Yellow; Isolate 2 MTK31 – Whitish; Isolate3a, MTK16 – Pink; Isolate 3b, MTK22 – Orange; Isolate4, MTK31 – Creamish

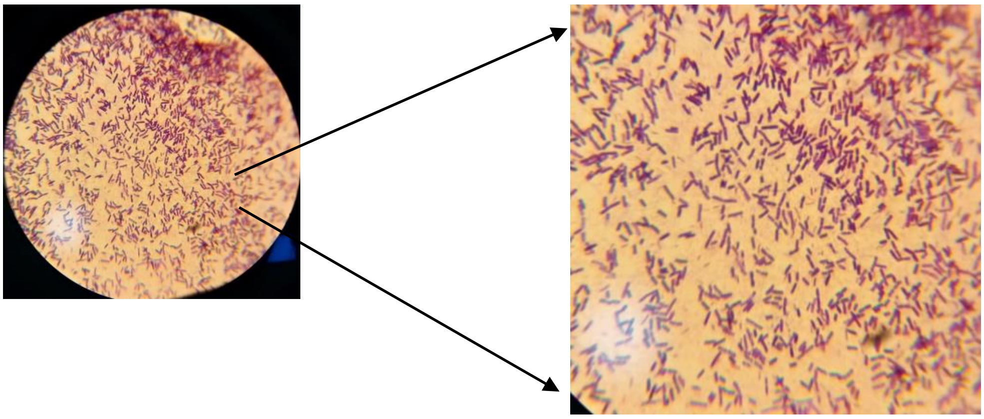

Plate 4.1: Gram Stain of Isolate MTK92, Gram Positive Rod-Shaped Bacterium (x1000)

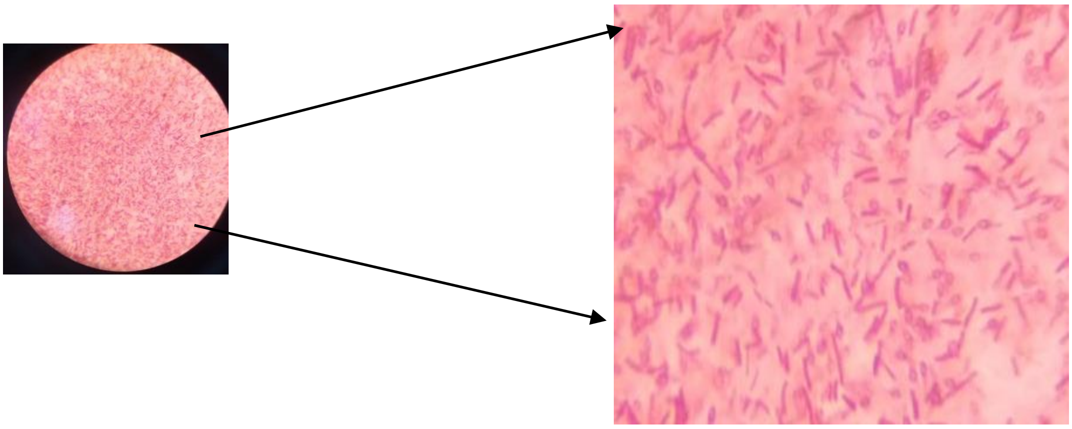

Plate 4.2: Gram Stain of Isolate MTK14, a Gram Negative Single Rod Bacterium (x1000)

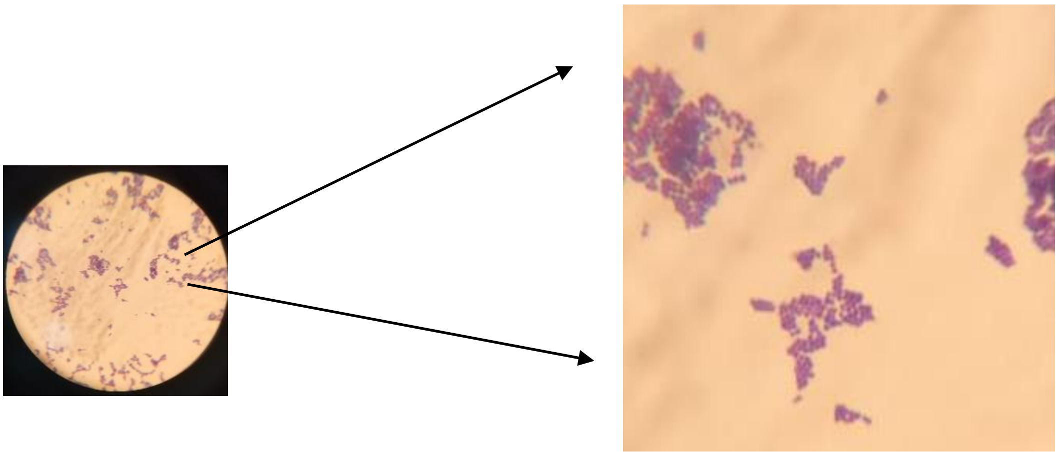

Plate 4.4: Gram Stain of Isolate MTK 25, a Gram-Positive Cocci Bacterium (x 1000

Sixty-nine isolates of bacteria were randomly selected from a total of 206 isolates. These were characterized and were further clustered in to three main groups based on Euclidean distances (Smith *et al.,*2025) utilizing the phenotypic characteristics. Cluster one which consisted of 21 bacteria isolates, was further subdivided into two sub-clades: a smaller sub-clade containing two isolates (MTK11 and MTK18) and a larger sub-clade comprising 19 isolates, which were distributed at varying distances from one another. Cluster Two comprised five bacteria isolates - MTK51, MTK77, MTK25, MTK48, and MTK16. Cluster Three included a total of 43 bacteria isolates, with the shortest sub-clade consisting of five isolates (MTK41, MTK15, MTK45, MTK63, and MTK46). The largest sub-clade within Cluster Three contained 17 bacteria isolates, while the remaining sub-clade consisted of 21 isolates (Figure 4.3).

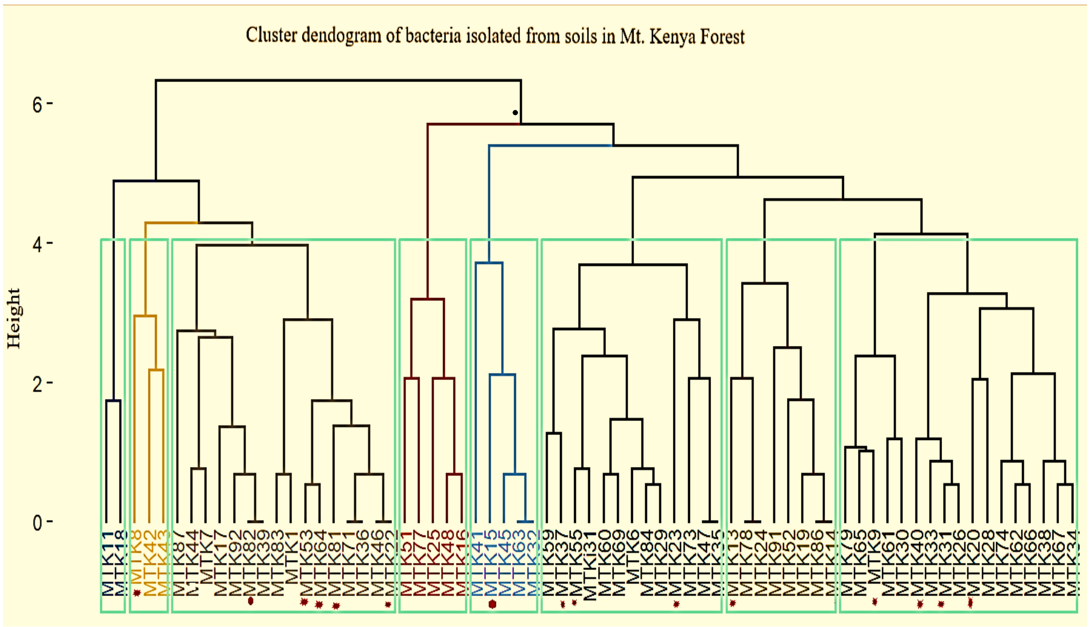

Figure 4.3 Cluster Membership of Bacteria Isolated from Mount Kenya Forest Based on Distance Matrix Computed. The isolates marked with red asterias were latter selected for molecular characterization. Isolates from undisturbed forest include; MTK 53, MTK64, MTK20, MTK8, MTK31, MTK9, MTK15, MTK55 while isolates from disturbed included MTK13, MTK81MTK23, MTK37, MTK40, MTK22, MTK82.

The broader range of pigmentation observed in isolates from the disturbed forest may be attributed to the bacteria's adaptation to environmental stressors, such as nutrient deficiencies, which may trigger specific pigment production pathways. Pigments like carotenoids, for instance, may play protective roles against oxidative damage, suggesting that bacterial isolates from disturbed environments develop mechanisms to cope with stress Moreover, the increased oxidase-positive reactions in disturbed forest isolates could indicate heightened oxidative stress in these environments.

4.4.1.1 Molecular Characterization of the Selected Bacteria Isolated from Both Disturbed and Undisturbed Forest Patches

By analyzing the 16S rRNA gene sequences of bacterial isolates using BLASTN searches against the NCBI GenBank database, the identities of various taxa were confirmed. Sequence similarities ranged from 56% to 100%, with *Bacillus aerius* and *Pseudochrobactrum saccharolyticum* representing the lowest and highest matches, respectively. Other identified species included *Brucella pseudogrignonensis*, *Vagococcus fluvialis*, and *Brevundimonas diminuta* (Table 4.8). These findings underscore the microbial diversity present across the forest degradation gradient and help link specific bacterial taxa to particular soil conditions, offering a foundation for understanding ecosystem functioning and resilience.

A total of 15 bacterial isolates, MTK53, MTK55, MTK64, MTK13, MTK81, MTK20, MTK23, MTK8, MTK37, MTK31, MTK9, MTK26, MTK15, MTK40, and MTK22. from various depths and forest conditions (disturbed and undisturbed) in Mount Kenya Forest were successfully identified using 16S rRNA gene sequencing (Table 4. 8). These isolates were carefully selected to represent a diverse array of pigmentation (e.g., creamish, deep yellow, medium yellow, pale yellow, orange, pink, and white), colony morphology (e.g., lobate, undulate, erose, curled), and biochemical traits (e.g., catalase and oxidase activities, indole production). The selection ensured a good coverage of observed variations in the soil bacterial community. The isolates showed varying degrees of similarity to known bacterial sequences in the GenBank database, with percentage identities ranging from 56% to 100%.

Table 4.5 Molecular Identification of Selected Soil Bacteria From Mount Kenya Forest

Isolates	Source & depth	Close match	ank Geenbank AccAccess No.	Percentage similarity	County
MTK53	I-05L	*Brucella pseudogrignonensis*	MH669291.1	99.28	USA
MTK55	I-06L	*Bacillus aerius*	NR_118439.1	84.75	China
MTK64	I-06L	*Brucella pseudogrignonensis*	KY880944.1	97.45	France
MTK13	D-06L	*Brucella pseudogrignonensis*	NR_042589.1	98.89	USA
MTK81	D-15L	*Vagococcus fluvialis*	NR_026489.1	98.25	USA
MTK20	I-05L	*P. saccharolyticum*	FJ652596.1	100	China
MTK23	D-06L	Bacterium strain	MW037593.1	98.09	NP
MTK8	I-05L	*Brevundimonas diminuta*	MF170840.1	98.26	China
MTK37	D-06L	*Stenotrophomonas* sp.,	MH769266.1	99.32	China
MTK31	I-06L	*Delftia tsuruhatensis*	MT040042.1	89.37	Russia
MTK9	I-06L	*Stenotrophomonas maltophilia*	ON357998.1	95.14	China
MTK82	D-15L	*Achromobacter xylosoxidans*	KP236299.1	96.90	NP
MTK15	I-05L	*Pseudomonas fluorescens*	OK136232.1	98.01	China
MTK40	D-06L	*Cupriavidus* sp	OP364587.1	98.73	China
MTK22	D-15L	*Ochrobactrum* sp	MN493898.1	99.95	NP

(where *P. saccharolyticum* = *Pseudochrobactrum saccharolyticum* and NP =County of origin not provided in the GenBank. Sources (I-05L= Undisturbed Forest at the depth (L) 0 – 5, I-06L= undisturbed forest at the depth (L) 6 – 14, I-15L= undisturbed forest at the depth (L) 15 – 20; D-05L= disturbed forest at the depth (L) 0 – 5, D-06L= disturbed forest at the depth (L) 6 – 14, D-15L= disturbed forest at the depth (L) 15 – 20).

Molecular identification of soil bacterial isolates from Mount Kenya Forest revealed a diverse assemblage of taxa with ecological and potential biotechnological relevance. This diversity reflects the influence of forest condition —whether disturbed or undisturbed— on soil microbial communities, supporting earlier findings that forest degradation alters microbial structure and function (Occhialini *et al.,* 2022). Sequence analysis of the 16S rRNA gene revealed varying degrees of similarity to known species, highlighting both common soil inhabitants and less-characterized taxa.

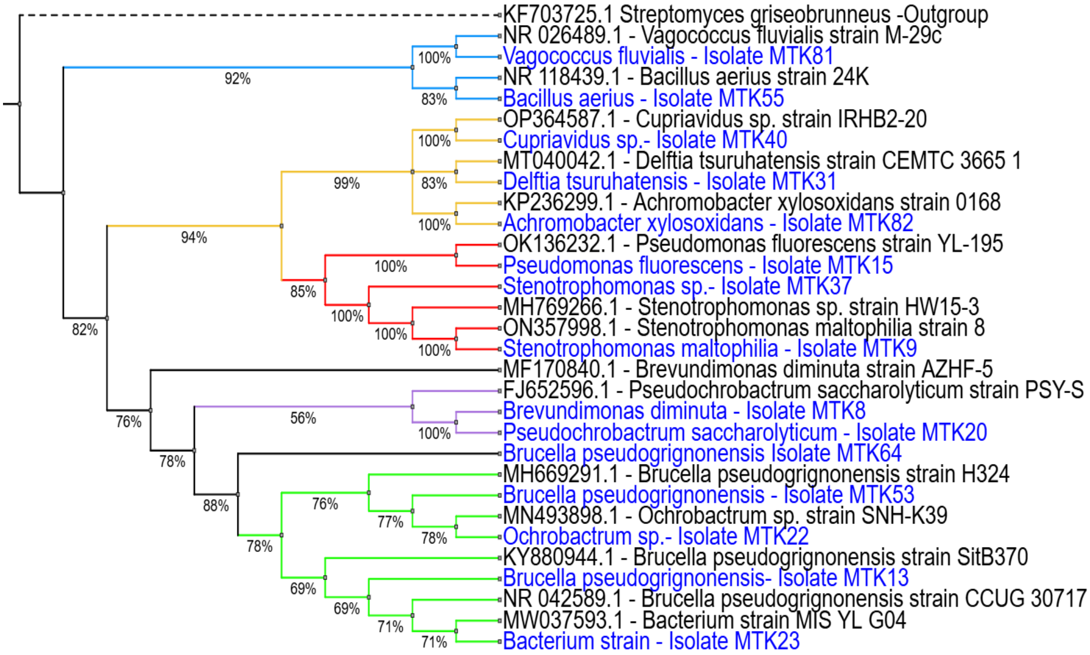

Figure 4.3: Phylogenetic Tree Based on 16S rRNA Gene Sequences of Bacterial Isolates from Mount Kenya Forest Soils. Blue names indicate bacterial isolates obtained from Mount Kenya Forest soils

This figure shows a phylogenetic tree based on 16S rRNA gene sequences, illustrating the evolutionary relationships among bacterial isolates obtained from the Mount Kenya Forest soils and their closest relatives from GenBank. The tree is rooted with *Streptomyces griseobrunneus* as an outgroup. *S. qriseolibruneus* was used because it is evolutionarily distant, taxonomically distinct, and provides a stable reference point for rooting the phylogenetic tree of your soil bacterial isolates. The isolates (labeled in blue, green, and other colors with codes such as MTK13, MTK16, MTK20) cluster with reference strains from diverse bacterial genera, including *Vagococcus*, *Bacillus*, *Curvibacter*, *Pseudomonas*, *Stenotrophomonas*, *Achromobacter*, *Pseudobrochothrix*, *Ochrobactrum*, *Brucella*, and *Bacterium* sp. Bootstrap values at the nodes (expressed as percentages) indicate the statistical support for each branching, with higher values (close to 100%) showing stronger confidence in the evolutionary relationship. The tree reveals that some isolates are closely related to well-known functional bacteria such as *Pseudomonas fluorescens*, *Bacillus aerisus*, and *Ochrobactrum* species, which are ecologically important in soil processes like nutrient cycling and bioremediation. The distinct clustering patterns suggest high bacterial diversity within the studied forest soils, reflecting different ecological niches and functional roles.

4.5 Impact of Forests Degradation on Abundance of Soil Bacteria in Mount Kenya Forest

4.5.1 Impact of Forests Type on Total Abundance of Soil Bacteria

A total of 216 bacterial isolates were obtained from both forest types. Of these, 153 isolates were from disturbed patches and 63 from undisturbed patches. The results indicated a significant impact of forest type on bacterial CFU counts ($F_{(1,\ 94)} = 139.10$; MSE=39.921; $p<0.0001$). The mean bacterial count in disturbed forests (152.74 ± 48.74 CFU) was significantly higher than in undisturbed forests (62.5 ± 27.83 CFU). The overall mean bacterial count across both forest types was 107.62 CFU (Table 4.10).

Table 4. 6: Impact of Undisturbed and disturbed forest Types on Soil Total Bacteria Count

Forest type	Bacteria CFU(**(Mean ± SD)**
Disturbed	152.74 ± 48.74 ^{a}
Undisturbed	62.5 ± 27.83 ^{b}
Mean	107.62
LSD (P<0.05)	15.254
CV (%)	37.094

Means followed by the same letters in the rows are not significantly different (p≤0.05)

Soil from disturbed forest had a significantly higher total bacterial count compared to the undisturbed forest. The high coefficient of variation (CV = 37.094%) suggests moderate variability in bacterial counts among replicates. The least significant difference (LSD = 15.254) confirms that the difference between the two means is statistically significant at the 5% level. The findings demonstrate that forest disturbance significantly enhances the abundance of culturable soil bacteria. These results align with previous studies (Gebremedhin *et al.,* 2022), which highlight the relationship between vegetation structure and soil microbial communities. Increased plant diversity and light penetration in disturbed forests may promote microbial proliferation by enriching the soil with varied organic substrates and root exudates. (Ma *et al.,*2022). As discussed Philippot *et al.,* 2024), soil microbial communities are tightly linked to plant cover, suggesting a feedback loop between aboveground vegetation and belowground biodiversity. Disturbed environments typically harbor a broader range of pioneer plant species with diverse chemical compositions, contributing to heterogeneity in soil microbial niches (Baazeem *et al.,*2023). Conversely, undisturbed forests often consist of dense canopies and limited undergrowth, leading to lower light penetration and reduced plant diversity. This homogeneity may restrict microbial diversity and abundance due to limited nutrient inputs and reduced niche differentiation (Tedersoo *et al.,* 2024). The impact of forest degradation on the abundance of soil bacterial isolates was further examined by comparing the mean colony-forming unit (CFU) counts across four visually distinguishable bacterial colony types, creamish, pinkish-orange, whitish, and yellow, between disturbed and undisturbed forest patches. The results are summarized in Table 4.7.

Table 4.7: Impact of Forests Degradation on Abundance of Soil Bacteria Isolates in Mount Kenya Forest

Types	Forest type		Means
	Disturbed	Undisturbed	(CFU)
Creamish	55.98±18.22 ^{a}	22.59±8.49 ^{b}	39.29 *
Pinkish-orange	19.27±11.91 ^{a}	9.92±5.64 ^{b}	14.6 *
Whitish	71.14±23.59 ^{a}	36.37±14 ^{b}	53.76 *
Yellow	22.61±15.35 ^{a}	9.68±5.66 ^{b}	16.15 *
CV (%)	45.18		
Means (CFU)	30.95		
LSD (P<0.05)	2.64		

^{a} *Means followed by the same letters in the rows are not significantly different (* - Significant at p≤0.05).*

Post hoc comparisons using the Least Significant Difference (LSD) test at the 5% significance level confirmed that forest degradation significantly influenced the abundance of bacterial isolates across all four colony types. For each bacterial morphotype (as indicated by colony colour), the mean CFU was significantly higher in disturbed forest patches than in undisturbed ones, as denoted by differing superscript letters (a and b). Creamish colonies had the largest disparity, with CFUs in disturbed forests (55.98 ± 18.22) more than double those in undisturbed sites (22.59 ± 8.49), a difference of 33.39 CFU. Whitish colonies similarly showed a large difference of 34.77 CFU, followed by yellow (12.93 CFU) and pinkish-orange colonies (9.35 CFU), all exceeding the LSD threshold of 2.64.

These results indicate a consistent pattern across different bacterial colony morphotypes, where disturbed forest conditions support higher bacterial abundance. The overall mean CFU of 30.95 further emphasizes the elevated microbial presence in disturbed patches. The increased abundance in disturbed forests may be attributed to several factors, including: enhanced nutrient cycling and organic matter decomposition due to increased litter turnover; greater plant species heterogeneity, providing a wider array of root exudates and substrates for microbial proliferation; altered microclimatic conditions such as increased light, temperature fluctuations, and moisture variability that can favour fast-growing bacterial taxa.

The coefficient of variation (CV) of 45.18% indicates a moderate level of variability in bacterial abundance, which is typical in heterogeneous natural environments and may reflect microhabitat variability or stochastic processes in microbial colonization. These findings reinforce the hypothesis that forest degradation modifies below ground microbial dynamics. Bacterial communities appear responsive to the physical and biological shifts induced by disturbance (Sveen *et al.,*2024). The marked differences among colony types suggest not only increased abundance but also potential shifts in bacterial community composition and functional diversity. Such changes could have broader ecological consequences for nutrient availability, soil structure, and plant-soil feedbacks (Pedrinho *et al.,* 2024). Conservation and restoration strategies should thus account for the microbiological implications of forest disturbance to maintain ecosystem health and function.

4.5.2 Principal Component Analysis of Bacterial Abundance and Distribution

Principal Component Analysis (PCA) was conducted to examine patterns in the abundance and distribution of bacterial isolates across varying soil depths and forest conditions within the Mount Kenya Forest ecosystem. The first two principal components (PCs) accounted for a cumulative 95.3% of the total variation in bacterial abundance, with PC1 explaining 67.9% and PC2 explaining 27.4% (Figure 4.5).

PC1 was primarily influenced by bacterial isolates with cream-colored colonies, which exhibited a high negative eigenvector loading (-0.438), suggesting their dominant or distinct presence in specific soil layers or forest conditions. In contrast, PC2 was driven by isolates with pinkish-orange colonies, marked by a strong positive loading (0.497), representing a separate gradient likely linked to microenvironmental changes such as disturbance or nutrient heterogeneity. The PCA biplot provides a visualization of the variation in soil bacterial community structure between disturbed) and undisturbed forest conditions in the Mount Kenya Forest ecosystem, based on colony morphology and abundance. The PCA biplot (Figure 4.5) reveals a considerable overlap in bacterial communities between disturbed and undisturbed forest soils, indicating minimal compositional differentiation based on forest condition alone. This overlap suggests either ecological resilience of bacterial taxa to disturbance or the presence of a stable core microbiome maintained by consistent soil properties or microclimatic conditions. PC1 (x-axis) explains 67.9% of the variation in the data while PC2 (y-axis) accounts for 27.4% of the variation. Together, these two components capture a cumulative 95.3% of the total variance, indicating that they impactively summarize the major patterns in the bacterial community dataset.

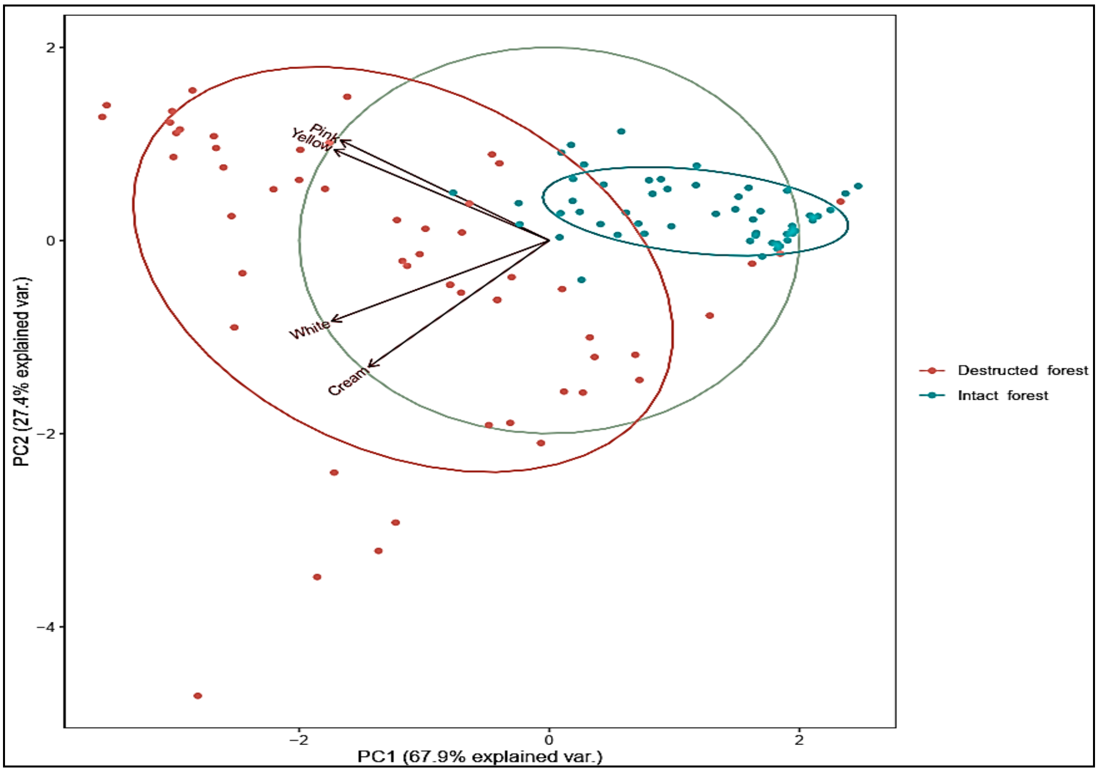

Figure 4.4: Correspondence Visualization Biplot of The Impacts of Forest Degradation on Soil Bacteria in Mount Kenya Forest Using Principal Component Analysis. Red dots represent bacterial isolates from the destructed forest. Green dots represent isolates from the intact forest. The ellipses around each group depict confidence regions, showing the clustering and dispersion of isolates within each forest type.

The undisturbed forest isolates (teal/blue) cluster more tightly and occupy a distinct region, suggesting a more homogeneous or stable bacterial community. The disturbed forest isolates (red) are more widely spread across the PCA space, indicating greater variability in bacterial communities due to disturbance. Some overlap exists between the two ellipses, implying a shared core microbiome or morphotypes that persist regardless of disturbance. The arrows represent colony colour traits (e.g., Cream, White, Yellow, Pink) and show how they correlate with the principal components. Cream-colored colonies load negatively on PC1, suggesting strong association with undisturbed forest conditions. Pink colonies show a positive correlation with PC2, more aligned with disturbed forest conditions. Yellow and white colonies fall in between, contributing to variation along both axes.

The spatial separation and dispersion of isolates suggest that forest disturbance affects bacterial community composition, likely due to shifts in microclimate, nutrient availability, or soil structure (Johnson *et al.,*2023). The observed core cluster in the intact forest may reflect microbial stability or resilience in less disturbed environments. The direction and length of vectors indicate how strongly each colony morphotype contributes to the overall variation, with longer vectors (e.g., Pink and Cream) indicating greater influence.

4.6 Impact of Soil Physical and Chemical Properties on Forest Soil Bacteria

4.6.1 Impact of Soil Physical Properties Forest on Soil Bacteria

The analysis of soil physical properties revealed significant differences between disturbed and undisturbed forest sites, which corresponded with marked variations in bacterial abundance. Bacterial counts were notably higher in disturbed soils (152.74 ± 39.63 cfu) compared to undisturbed soils (62.49 ± 17.29 cfu), suggesting that disturbance creates conditions conducive to rapid microbial proliferation. Increased aeration, exposure of organic substrates, and accelerated decomposition following canopy removal are likely drivers of the elevated bacterial numbers in disturbed areas. Soil organic matter (soil organic matter) content was significantly higher in undisturbed forests (11.68 ± 1.03%) compared to disturbed forests (9.2 ± 1.08%). Although soil organic matter generally provides a stable source of carbon and nutrients that supports microbial metabolism and diversity (Binkley & Fisher, 2019; Lladó *et al.,* 2017), the higher bacterial counts in disturbed soils indicate that short-term increases in substrate availability from disturbance can temporarily enhance bacterial proliferation, even under declining soil quality.

Bulk density also differed significantly, with disturbed soils showing higher compaction (4.58 ± 1.09 g/cm³) than undisturbed soils (3.56 ± 1.22 g/cm³). Higher bulk density typically reduces pore space, limits aeration, and constrains microbial activity. Despite this, disturbed soils exhibited greater bacterial abundance, suggesting that bacterial communities may shift toward disturbance-tolerant taxa capable of thriving under physically stressed conditions (Gao *et al.,* 2025).

Soil pH was higher in disturbed soils (6.99 ± 0.13) relative to undisturbed soils (5.7 ± 0.32). Since pH influences enzymatic activity, nutrient availability, and microbial cell function, the more neutral pH in disturbed soils may support a broader range of bacterial taxa, whereas acidic conditions in undisturbed soils favor more specialized microbial groups (Kumar *et al.,* 2023). Collectively, these results demonstrate that forest disturbance significantly alters key physical properties of soil; including soil organic matter, bulk density, and pH reshaping the microbial habitat and influencing bacterial abundance. While undisturbed forests maintain higher soil organic matter and lower compaction, disturbed soils support larger bacterial populations, likely due to increased resource turnover and altered microenvironmental conditions. These findings underscore the complex interplay between soil structure and microbial dynamics in forest ecosystems.

Table 4. 8: Impact of Soil Physical Properties on Forest Soil Bacteria

Parameter	**Disturbed Forest**	**Undisturbed Forest**
Bacterial Counts (cfu)	152.74 ± 39.63^{a}	62.49 ± 17.29^{b}
Soil Organic Matter (%)	9.2 ± 1.08^{a}	11.68 ± 1.03^{b}
Soil Density (g/cm³)	4.58 ± 1.09^{a}	3.56 ± 1.22^{b}
Soil pH	6.99 ± 0.13^{a}	5.7 ± 0.32^{b}

4.6.2 Impact of Soil Chemical Properties on Forest Soil Bacteria

The comparison of soil chemical properties between disturbed and undisturbed forest sites (Table 4.9) revealed nutrient shifts that help explain differences in soil conditions but do not directly mirror bacterial abundance patterns. Magnesium levels were statistically similar across the two forest types, indicating that this nutrient remained relatively stable despite environmental disturbance. Because magnesium did not vary significantly, it is unlikely to be a major contributor to the large difference observed in bacterial counts between the sites.

Table 4. 9: Impact of Soil Chemical Properties Forest on Soil Bacteria

Parameter	Disturbed Forest	Undisturbed Forest
Bacterial Counts (cfu)	152.74 ± 39.63^a	62.49 ± 17.29^b
Magnesium (cmol/kg)	7.3 ± 0.53^a	7.6 ± 0.30^a
Calcium (cmol/kg)	5.87 ± 1.09^a	11.76 ± 1.10^b
Potassium (cmol/kg)	8.23 ± 1.06^a	9.52 ± 1.05^b

Calcium showed the most pronounced contrast, with undisturbed soils containing nearly double the concentration recorded in disturbed areas. Higher calcium levels in intact forests typically support better soil structure, improved cation exchange capacity and greater stability of soil aggregates, all of which contribute to enhanced microbial functioning. However, despite the more favorable chemical environment in undisturbed soils, bacterial counts were substantially higher in disturbed sites. This suggests that factors linked to disturbance —such as increased exposure of organic substrates, enhanced aeration, and accelerated decomposition— may temporarily stimulate bacterial proliferation even when soil nutrient stocks are lower.

Potassium exhibited a similar pattern to calcium, with higher values in undisturbed soils. Potassium is important for microbial osmoregulation and energy balance, and its greater availability in intact forests likely contributes to a more functionally stable microbial community. Nevertheless, the elevated bacterial counts in disturbed soils again show that nutrient enrichment alone does not determine microbial abundance. Instead, disturbance appears to create short-term conditions that favor rapid microbial growth, even as overall soil quality declines. Overall, the results highlight a complex relationship between forest disturbance, soil nutrients and bacterial abundance. While undisturbed forests maintain higher levels of essential nutrients such as calcium and potassium, disturbed sites support larger bacterial populations, likely due to shifts in substrate availability and micro-environmental conditions following disturbance. These findings emphasize the importance of considering both chemical and ecological drivers when assessing how forest degradation shapes soil microbial communities.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study of soil properties in Mount Kenya Forest demonstrates that forest degradation has significant and measurable effects on soil health. Comparisons between disturbed and undisturbed areas revealed consistent differences across all measured parameters. Disturbed forests showed lower soil organic matter (9.2 vs 11.68 cmol/kg), higher bulk density (4.58 vs 3.56), and more alkaline soil pH (6.99 vs 5.7) compared to intact forests.

These changes have cascading ecological effects. Reduced organic matter limits the soil's ability to support microbial activity and nutrient cycling. Increased compaction restricts root growth, water movement, and gas exchange, while higher pH affects nutrient availability and may influence plant species composition. Overall, these shifts create less favourable conditions for forest regeneration and ecosystem stability.

Soil chemical properties were also significantly affected. Calcium and potassium concentrations were higher in undisturbed areas (Ca: 11.76 vs 5.87 cmol/kg; K: 9.52 vs 8.23 cmol/kg), while magnesium showed no significant change, suggesting resilience to disturbance. Principal Component Analysis identified two distinct soil environments: undisturbed sites with higher organic matter, calcium, and potassium, and disturbed sites characterized by higher pH and bulk density. Such differences likely create feedback loops that further hinder ecosystem recovery.

Forest disturbance also influenced soil bacterial communities. Disturbed soils consistently had higher bacterial counts across all depths (0–5 cm, 6–14 cm, 15–20 cm) and colony types (creamish, pinkish, whitish, yellow) than undisturbed soils. The higher abundance in degraded sites likely results from increased nutrient pulses, greater aeration, and selection for opportunistic bacteria. While elevated bacterial numbers may appear beneficial, they reflect altered community structure and potentially disrupted nutrient cycling, indicating reduced ecosystem resilience. Bacterial abundance generally decreased with soil depth, though some colony types showed unique distribution patterns.

Based on these findings, all four null hypotheses are rejected. There were significant differences in soil physical and chemical properties between degraded and intact forests, bacterial counts were higher in disturbed soils, and soil properties clearly influenced bacterial abundance and distribution. These results collectively demonstrate that forest degradation substantially alters both soil health and microbial dynamics in the Mount Kenya Forest ecosystem.

5.2 Recommendations

Forest degradation has long-term impacts on soil health, nutrient cycling, and overall ecosystem functioning. To mitigate these effects and promote effective ecosystem restoration, a set of integrated strategies is recommended. These strategies focus on improving soil properties, conserving intact forest patches, and supporting ecosystem processes critical for resilience and recovery.

Restoration efforts should prioritize rebuilding soil organic matter, which is essential for nutrient retention, microbial activity, and overall soil fertility. Measures to reduce soil compaction and lower bulk density in disturbed areas should also be implemented to improve aeration, water infiltration, and root growth. These interventions create favorable conditions for both plant and microbial recovery, which are crucial for restoring degraded forest soils.

Undisturbed forest patches act as critical nutrient reservoirs and models of healthy soil ecosystems. The creation and enforcement of buffer zones around these areas will prevent further degradation and safeguard essential ecosystem functions. Additionally, establishing long-term monitoring plots in both disturbed and intact sites will enable tracking of restoration progress and provide data to guide adaptive management practices.

Litter inputs play a pivotal role in nutrient cycling and soil fertility. Reforestation programs should prioritize tree species that produce nutrient-rich litter, particularly those high in calcium and potassium. Management practices should ensure continuous deposition and preservation of litter on the forest floor to support microbial communities and enhance soil recovery.

Maintaining undisturbed forest areas is critical for sustaining optimal soil chemical properties and nutrient cycling processes. Conservation of these patches supports biodiversity, strengthens soil microbial activity, and provides reference ecosystems that guide restoration in degraded areas. Protecting these intact forests ensures long-term ecological stability and resilience.

An integrated approach that combines soil restoration, buffer zone protection, litter management, and conservation of intact patches provides a comprehensive framework for ecosystem recovery. Long-term monitoring and adaptive management will help evaluate the effectiveness of interventions, improve understanding of soil and microbial responses, and inform sustainable restoration strategies for Mount Kenya Forest and similar tropical montane ecosystems.

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APPENDICES

Appendix 1 : Raw data for Total Bacteria Count in Mount Kenya Forest

Obs	Replication	Forest Type	*Surface (5 cm)*	Mid (15 cm)	*Lower depth* (20 cm)
1	1	Intact	80	61	43
2	1	Intact	90	47	51
3	1	Intact	70	60978	37
4	1	Intact	117	90	50
5	1	Intact	68	78	38
6	1	Intact	56	80	39
7	1	Intact	45	33	17
8	1	Intact	47	18	4
9	1	Intact	54	56	54
10	2	Intact	90	48	31
11	2	Intact	94	52	40
12	2	Intact	106	57	30
13	2	Intact	97	94	40
14	2	Intact	93	70	38
15	2	Intact	73	84	44
16	2	Intact	52	29	11
17	2	Intact	39	22	37
18	2	Intact	72	44	28
19	3	Intact	73	49	12
20	3	Intact	105	44	23
21	3	Intact	128	47	44
22	3	Intact	101	87	43
23	3	Intact	77	111	40
24	3	Intact	85	90	52
25	3	Intact	44	24	8
26	3	Intact	42	31	14
27	3	Intact	62	56	33
28	1	Destruct	218	194	124
29	1	Destruct	204	141	109
30	1	Destruct	180	130	110
31	1	Destruct	167	200	150
32	1	Destruct	190	137	200
33	1	Destruct	170	146	189
34	1	Destruct	120	286	200
35	1	Destruct	128	200	236
36	1	Destruct	170	210	192
37	2	Destruct	199	209	143
38	2	Destruct	200	124	137

Obs	Replication	Forest Type	*Surface (5 cm)*	Mid (15 cm)	*Lower depth (20 cm)*
40	2	Destruct	146	142	272
41	2	Destruct	132	151	220
42	2	Destruct	164	112	199
43	2	Destruct	156	250	210
44	2	Destruct	108	214	197
45	2	Destruct	158	226	186
46	3	Destruct	201	143	92
47	3	Destruct	189	122	99
48	3	Destruct	197	110	54
49	3	Destruct	188	130	71
50	3	Destruct	157	111	43
51	3	Destruct	190	140	19
52	3	Destruct	138	98	42
53	3	Destruct	140	106	29
54	3	Destruct	166	127	88

Appendix 2: Raw Data on Impact of Forest Type, Forest Site on Soil Nutrients, pH, Soil Density and Organic Matter

Obs	REP	Forest Type	Sit e	Magnesium	Calcium	Pottasium	Soil organic matter	Density	pH
1	1	1	1	4.54	8.87	5.56	9.11	0.46	5.6
2	1	1	2	5.23	10.01	7.83	9.24	0.67	5.7
3	1	1	3	3.97	9.32	5.76	8.47	0.84	5.2
4	1	1	4	5.43	7.54	9.75	9.03	0.48	5.0
5	1	1	5	3.37	8.41	6.32	8.57	0.59	5.8
6	1	1	6	4.85	12.47	6.77	8.36	0.60	6.0
7	1	1	7	6.09	7.58	4.46	8.32	0.57	5.7
8	2	1	1	5.24	8.00	6.30	9.08	0.40	5.9
9	2	1	2	5.20	11.07	7.41	9.01	0.54	5.9
10	2	1	3	4.33	8.86	5.49	9.00	0.71	5.9
11	2	1	4	4.92	7.06	7.34	9.86	0.38	5.7
12	2	1	5	3.11	7.53	6.13	8.03	0.63	6.0
13	2	1	6	4.39	10.27	6.06	8.76	0.67	5.2
14	2	1	7	6.25	8.06	8.46	8.68	0.52	6.3
15	3	1	1	5.55	8.48	6.00	9.04	0.43	5.9
16	3	1	2	5.29	9.52	7.01	8.77	0.57	6.0
17	3	1	3	4.00	9.51	5.20	8.26	0.44	5.8
18	3	1	4	5.20	7.62	7.69	7.88	0.46	6.1
19	3	1	5	4.19	5.39	6.05	8.04	0.72	5.5
20	3	1	6	4.57	9.83	5.77	7.92	0.57	5.4
21	3	1	7	6.44	7.07	7.96	8.84	0.81	6.0
22	1	2	1	6.47	2.05	4.22	5.76	1.32	6.8
23	1	2	2	6.39	3.00	5.33	5.20	1.65	7.2
24	1	2	3	4.29	1.22	5.14	6.49	1.77	7.4

Appendix 3: Raw Data on Impact of Forest Type, Forest Site on Soil Nutrients, pH, Soil Density and Organic Matter (Continued)

Obs	REP	Forest type	Site	Magnesium	Calcium	Pottasium	Soil Organic Matter	Density	pH
25	1	2	4	2.43	4.54	6.75	5.03	1.01	6.9
26	1	2	5	3.30	3.47	6.28	7.00	1.55	6.9
27	1	2	6	3.07	2.09	5.72	6.16	1.63	6.8
28	1	2	7	4.00	2.75	5.01	5.92	1.02	7.7
29	2	2	1	6.86	2.09	4.37	5.01	1.43	7.0
30	2	2	2	6.24	3.76	5.00	6.97	2.42	7.1
31	2	2	3	4.00	1.59	5.33	7.11	1.06	7.2
32	2	2	4	2.56	4.95	5.06	7.00	1.47	7.0
33	2	2	5	3.25	3.23	6.18	6.09	2.11	7.2
34	2	2	6	3.35	2.17	5.00	5.66	1.88	6.9
35	2	2	7	4.37	2.01	5.66	6.48	2.12	7.5
36	3	2	1	5.92	3.48	5.01	5.08	2.31	6.8
37	3	2	2	6.63	2.97	5.64	6.15	1.94	6.9
38	3	2	3	3.09	2.11	4.26	7.17	1.04	7.1
39	3	2	4	4.67	3.61	4.94	6.80	1.49	6.8
40	3	2	5	3.54	4.05	6.00	6.01	1.66	7.2
41	3	2	6	4.00	2.71	4.49	7.55	1.05	6.7
42	3	2	7	3.98	2.00	4.09	4.99	2.81	7.3

Appendix 4: ANOVA Result for the Impact of Intact and Degraded Forest Type on Total Bacteria Count in in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	237060.9534	18235.4580	11.44	<.0001
Error	94	149808.4818	1593.7073	94	
Corrected Total	35	0.16557990			

R-Square	Coeff Var	Root MSE	Log Magnesium Mean
0.612767	37.09452	39.92126	107.6204

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	7277.8210	3638.9105	2.28	0.1076
Forest Type	1	221682.1277	221682.1277	139.10	<.0001
Location	5	3124.2788	624.8558	0.39	0.8532
Forest *Location	5	4976.7259	995.3452	0.62	0.6814

Depth of 0-5 Total Count

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	90790.8889	11348.8611	26.86	<.0001
Error	27	11410.0833	422.5957	27	
Corrected Total	35	102200.9722			

R-Square	Coeff Var	Root MSE	Log Magnesium Mean
0.888356	15.22437	20.55713	135.0278

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	461.05556	230.52778	0.55	0.5858
Forest Type	1	85166.69444	85166.69444	201.53	<.0001
Location	5	5163.13889	1032.62778	2.44	0.0597

Depth of 6-14 Total Count

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	60301.11111	7537.63889	11.10	<.0001
Error	27	18340.88889	679.29218	27	
Corrected Total	35	78642.00000			

R-Square	Coeff Var	Root MSE	Log Magnesium Mean
0.766780	24.90118	26.06323	104.6667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	1814.00000	907.00000	1.34	0.2799
Forest Type	1	55853.44444	55853.44444	82.22	<.0001
Location	5	2633.66667	526.73333	0.78	0.5760

Depth of 15-20 Total Count

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	112941.5851	14117.6981	8.62	<.0001
Error	27	44201.4149	1637.0894	27	
Corrected Total	35	157143.0000			

R-Square	Coeff Var	Root MSE	Log Magnesium Mean
0.718719	48.65046	40.46096	83.16667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	25833.25180	12916.62590	7.89	0.0020
Forest Type	1	86470.25896	86470.25896	52.82	<.0001
Location	5	7644.35328	1528.87066	0.93	0.4748

Appendix 5: ANOVA Result for the Impact of Soil Depth on Total Bacteria Count in the Intact and Degraded Forest Patches in Mount Kenya Forest

a). ANOVA Result for the Impact of Soil Depth (1 cm) on Total Bacteria Count in the Intact and Degraded Forest Patches in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	118911.8889	39637.2963	52.03	<.0001
Error	50	38090.9259	761.8185		
Corrected Total	53	157002.8148			

R-Square	Coeff Var	Root MSE	Surface Mean
0.757387	22.41289	27.60106	123.1481

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	376.7037	188.3519	0.25	0.7819
Forest Type	1	118535.1852	118535.1852	155.60	<.0001

b). ANOVA Result for the Impact of Soil Depth (15 cm) on Total Bacteria Count in the Intact and Degraded Forest Patches in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	149206.2593	49735.4198	36.14	<.0001
Error	50	68810.1111	1376.2022		
Corrected Total	53	218016.3704			

R-Square	Coeff Var	Root MSE	Mid Mean
0.684381	34.11527	37.09720	108.7407

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	9363.5926	4681.7963	3.40	0.0412
Forest Type	1	139842.6667	139842.6667	101.61	<.0001

c). ANOVA Result for the Impact of Soil Depth (20 cm) on Total Bacteria Count in the Intact and Degraded Forest Patches in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	193937.4444	64645.8148	37.60	<.0001
Error	50	85964.9259	1719.2985		
Corrected Total	53	279902.3704			

R-Square	Coeff Var	Root MSE	Low Mean
0.692875	48.36024	41.46442	85.74074

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	45834.0370	22917.0185	13.33	<.0001
Forest Type	1	148103.4074	148103.4074	86.14	<.0001

Appendix 6: ANOVA Result Magnesium, Calcium and Potassium

a). Impact of Degraded and Intact Forest on Soil Mg^{2+} in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	0.14213596	0.01093354	10.26	<.0001
Error	22	0.02344394	0.00106563		
Corrected Total	35	0.16557990			

R-Square	Coeff Var	Root MSE	Log Magnesium Mean
0.858413	3.743120	0.032644	0.872108

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	0.00259544	0.00129772	1.22	0.3151
Forest Type	1	0.00271835	0.00271835	2.55	0.1245
Location	5	0.09655332	0.01931066	18.12	<.0001
Forest *Location	5	0.04026885	0.00805377	7.56	0.0003

b). Impact of Degraded and Intact Forest on Soil Ca^{2+} in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	0.94721538	0.07286272	45.67	<.0001
Error	22	0.03509875	0.00159540		
Corrected Total	35	0.98231413			

R-Square	Coeff Var	Root MSE	Log Calcium Mean
0.964269	4.343784	0.039942	0.919531

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	0.00004326	0.00002163	0.01	0.9865
Forest Type	1	0.82324543	0.82324543	516.01	<.0001
Location	5	0.02681889	0.00536378	3.36	0.0209
Forest *Location	5	0.09710779	0.01942156	12.17	<.0001

c).Impact of Degraded and Intact Forest on Soil Potassium in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	0.08499849	0.00653835	10.58	<.0001
Error	22	0.01359959	0.00061816		
Corrected Total	35	0.09859808			

R-Square	Coeff Var	Root MSE	Log Potasium Mean
0.862070	2.624938	0.024863	0.947180

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	0.00505193	0.00252597	4.09	0.0310
Forest Type	1	0.03600700	0.03600700	58.25	<.0001
Location	5	0.02999273	0.00599855	9.70	<.0001
Forest *Location	5	0.01394683	0.00278937	4.51	0.0056

Appendix 7: ANOVA Result for the Intact and Degraded Forest Types on Soil Alkalinity, Organic Matter and Density in Mount Kenya Forest

a). Impact of Degraded and Intact Forest on Soil Organic Matter in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	0.11026391	0.00848184	9.82	<.0001
Error	22	0.01899688	0.00086349		
Corrected Total	35	0.12926079			

R-Square	Coeff Var	Root MSE	Soil Organic Mean
0.853034	2.893363	0.029385	1.01561

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	0.00083268	0.00041634	0.48	0.6238
Forest Type	1	0.09656874	0.09656874	111.83	<.0001
Location	5	0.00307712	0.00061542	0.71	0.6204
Forest *Location	5	0.00978537	0.00195707	2.27	0.0834

b). Impact of Degraded and Intact Forest on Soil Density in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	0.12038637	0.00926049	11.32	<.0001
Error	22	0.01799094	0.00081777		
Corrected Total	35	0.13837731			

R-Square	Coeff Var	Root MSE	Log Soil Density Mean
0.869986	4.716165	0.028597	0.606355

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	0.00050227	0.00025113	0.31	0.7387
Forest Type	1	0.10740435	0.10740435	131.34	<.0001
Location	5	0.00678233	0.00135647	1.66	0.1865
Forest *Location	5	0.00569742	0.00113948	1.39	0.2652

b).Impact of Degraded and Intact Forest on Soil Alkalinity in Mount Kenya Forest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	15.86361111	1.22027778	16.93	<.0001
Error	22	1.58611111	0.07209596		
Corrected Total	35	17.44972222			

R-Square	Coeff Var	Root MSE	pH Mean
0.909104	4.230306	0.268507	6.347222

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replication	2	0.12055556	0.06027778	0.84	0.4467
Forest Type	1	15.080278	15.0802778	209.17	<.0001
Location	5	0.42805556	0.08561111	1.19	0.3473
Forest *Location	5	0.23472222	0.04694444	0.65	0.6637

Appendix 8: Soil Bacterial Isolates from Mount Kenya Forest and Their Corresponding 16S rRNA GenBank Reference Sequences

Ochrobactrum sp. Isolate MTK22, [2] MN493898.1 *Ochrobactrum* sp. Strain SNH-K39, [3] *Brucella pseudogrignonensis* – Isolate MTK53, [4] MH669291.1 -*Brucella pseudogrignonensis* strain H324, [5] Bacillus aerius – Isolate MTK55, [6] NR_118439.1 – *Bacillus aerius* strain 24K, [7] NR 118439.1 – *Bacillus aerius* strain 24K, [8] *Brucella pseudogrignonensis* Isolate MTK64, [9] KY880944.1- *Brucella pseudogrignonensis* strain SitB370, [10] *Brucella pseudogrignonensis*- Isolate MTK13, [11] NR_042589.1 – *Brucella pseudogrignonensis* strain CCUG 30717, [12] *Vagococcus fluvialis* - Isolate_MTK81, [13] NR_026489.1 – *Vagococcus fluvialis* strain M-29c, [14] *Pseudochrobactrum saccharolyticum* – Isolate MTK20, [15] FJ652596.1 – *Pseudochrobactrum saccharolyticum* strain PSY-S, [16] Bacterium strain - Isolate MTK23, [17] MW037593.1 - Bacterium strain MIS YL G04, [18] *Brevundimonas diminuta* – Isolate MTK8, [19] MF170840.1 – *Brevundimonas diminuta* strain AZHF-5, [20] *Stenotrophomonas* sp.- Isolate MTK37, [21]MH769266.1 – *Stenotrophomonas* sp. strain_HW15-3, [22] *Delftia tsuruhatensis* – Isolate MTK31, [23] MT040042.1 – *Delftia tsuruhatensis* strain CEMTC 3665_1, [24]*Stenotrophomonas maltophilia* – Isolate MTK9, [25] ON357998.1 – *Stenotrophomonas maltophilia* strain 8, [26] *Achromobacter xylosoxidans* – Isolate MTK26, [27]*Achromobacter xylosoxidans* – Isolate MTK82 , [27] KP236299.1 – *Achromobacter xylosoxidans* strain 0168, [28] *Pseudomonas fluorescens* – Isolate MTK15, [29] OK136232.1 – *Pseudomonas fluorescens* strain YL-195, [30] *Cupriavidus* sp.- Isolate_MTK40, [31]OP364587.1 – *Cupriavidus* sp. Strain IRHB2-20

Appendix 9: Phenotypical Biochemical Reaction of Soil Bacteria Isolates

Isolates	Source	Colony colour	Colony edge	Gram stain	Shape	Catalase	Oxidase	Indole
MTK53	Mt. Kenya forest	Whitish	Lobate	+ve	short chain rods	-ve	-ve	-ve
MTK55	Mt. Kenya forest	Deep yellow	undulate	-ve	short chain rods	-ve	+ve	+ve
MTK64	Mt. Kenya forest	Medium yellow	Lobate	+ve	short chain rods	-ve	-ve	-ve
MTK13	Mt. Kenya forest	Pale yellow	Erose	-ve	short chain rods	+ve	+ve	-ve
MTK81	Mt. Kenya forest	Medium yellow	Erose	+ve	single rods	-ve	-ve	-ve
MTK20	Mt. Kenya forest	Deep yellow	Erose	-ve	short rod chain	+ve	+ve	+ve
MTK23	Mt. Kenya forest	Whitish	undulate	-ve	single rods	-ve	-ve	-ve
MTK8	Mt. Kenya forest	Orange	Erose	+ve	short chain rods	+ve	+ve	+ve
MTK37	Mt. Kenya forest	Cream	undulate	-ve	short chain rods	-ve	+ve	+ve
MTK31	Mt. Kenya forest	Whitish	Erose	-ve	short chain rods	+ve	+ve	-ve
MTK9	Mt. Kenya forest	Pale yellow	Curled	-ve	single rods	+ve	-ve	+ve
MTK26	Mt. Kenya forest	Cream	Erose	-ve	short chain rods	+ve	+ve	-ve
MTK14	Mt. Kenya forest	Pale yellow	undulate	-ve	Short chain rods	+ve	+ve	+ve
MTK40	Mt. Kenya forest	Pink	Erose	-ve	single rods	+ve	+ve	-ve
MTK22	Mt. Kenya forest	Orange	Erose	+ve	short chain rods	-ve	-ve	-ve
MTK 52	Mt. Kenya forest	Whitish	Erose	-ve	single cocci	-ve	+ve	-ve
MTK 18	Mt. Kenya forest	Whitish	undulate	+ve	short chain rods	-ve	-ve	+ve
MTK 59	Mt. Kenya forest	Orange	Lobate	-ve	short chain rods	-ve	+ve	+ve
MTK 92	Mt. Kenya forest	Cream	Curled	+ve	short chain rods	+ve	-ve	-ve
MTK 60	Mt. Kenya forest	Medium yellow	Curled	-ve	single rods	-ve	+ve	+ve
MTK 78	Mt. Kenya forest	Pale yellow	Erose	-ve	cocci single	+ve	-ve	-ve
MTK 6	Mt. Kenya forest	Medium yellow	Erose	-ve	rod chain	-ve	+ve	+ve
MTK 24	Mt. Kenya forest	Pale yellow	Erose	-ve	single rods	+ve	-ve	-ve
MTK 42	Mt. Kenya forest	Pink	Curled	+ve	short chain rods	-ve	+ve	-ve
MTK 33	Mt. Kenya forest	Cream	Curled	-ve	short chain rods	+ve	+ve	-ve
MTK 17	Mt. Kenya forest	Cream	lobate	+ve	short chain rods	+ve	-ve	-ve
MTK 37	Mt. Kenya forest	Whitish	undulate	-ve	single rods	+ve	-ve	+ve
MTK 44	Mt. Kenya forest	Pink	lobate	-ve	single rods	+ve	-ve	-ve
MTK 28	Mt. Kenya forest	Whitish	lobate	-ve	cocci chain	+ve	+ve	+ve
MTK 91	Mt. Kenya forest	Deep yellow	Erose	-ve	single rods	-ve	-ve	-ve
MTK 43	Mt. Kenya forest	Cream	Erose	+ve	short chain rods	+ve	+ve	-ve
MTK 46	Mt. Kenya forest	Cream	Erose	+ve	short chain rods	-ve	-ve	-ve
MTK 32	Mt. Kenya forest	Pale yellow	Filamentous	-ve	cocci cluster	+ve	+ve	+ve
MTK 1	Mt. Kenya forest	Pink	Curled	+ve	short chain rods	-ve	-ve	+ve
MTK 74	Mt. Kenya forest	Orange	Curled	-ve	single rods	+ve	+ve	+ve

MTK 36	Mt. Kenya forest	medium yellow	undulate	-ve	single rods	-ve	+ve	+ve
MTK 79	Mt. Kenya forest	Deep yellow	Erose	-ve	rod chain	+ve	-ve	+ve
MTK 14	Mt. Kenya forest	Pale yellow	Curled	-ve	cocci single	-ve	+ve	-ve
MTK 48	Mt. Kenya forest	Pink	undulate	+ve	cocci cluster	+ve	-ve	-ve
MTK 47	Mt. Kenya forest	Whitish	Curled	-ve	short chain rods	-ve	+ve	-ve
MTK 39	Mt. Kenya forest	Cream	Erose	+ve	short chain rods	+ve	-ve	-ve
MTK 66	Mt. Kenya forest	Pink	Erose	-ve	single rods	+ve	+ve	+ve
MTK 55	Mt. Kenya forest	Whitish	Erose	-ve	cocci double	+ve	-ve	-ve
MTK 29	Mt. Kenya forest	Whitish	Erose	-ve	rod double	-ve	+ve	+ve
MTK 7	Mt. Kenya forest	Cream	lobate	-ve	short chain rods	+ve	-ve	-ve
MTK 71	Mt. Kenya forest	Cream	Curled	+ve	short chain rods	-ve	-ve	-ve
MTK 63	Mt. Kenya forest	Pale yellow	Filamentous	-ve	cocci cluster	+ve	+ve	+ve
MTK 11	Mt. Kenya forest	Pink	Erose	+ve	short chain rods	-ve	-ve	+ve
MTK 34	Mt. Kenya forest	Orange	lobate	-ve	single rods	+ve	+ve	+ve
MTK 84	Mt. Kenya forest	medium yellow	Erose	-ve	single rods	-ve	+ve	+ve
MTK 65	Mt. Kenya forest	Deep yellow	Erose	-ve	rod chain	+ve	-ve	+ve
MTK 86	Mt. Kenya forest	Pale yellow	Erose	-ve	cocci single	-ve	+ve	-ve
MTK 16	Mt. Kenya forest	Pink	lobate	+ve	cocci cluster	+ve	-ve	-ve
MTK 35	Mt. Kenya forest	Whitish	Curled	-ve	short chain rods	-ve	+ve	-ve
MTK 82	Mt. Kenya forest	Cream	Erose	+ve	short chain rods	+ve	-ve	-ve
MTK 38	Mt. Kenya forest	Pink	undulate	-ve	single rods	+ve	+ve	+ve
MTK 77	Mt. Kenya forest	Whitish	undulate	-ve	cocci double	+ve	-ve	-ve
MTK 41	Mt. Kenya forest	Whitish	undulate	-ve	rod double	-ve	+ve	+ve
MTK 36	Mt. Kenya forest	Cream	Curled	+ve	short chain rods	-ve	-ve	-ve
MTK 45	Mt. Kenya forest	Pale yellow	Erose	-ve	cocci cluster	+ve	+ve	+ve
MTK 83	Mt. Kenya forest	Pink	Erose	+ve	short chain rods	-ve	-ve	+ve
MTK 62	Mt. Kenya forest	Orange	Erose	-ve	single rods	+ve	+ve	+ve
MTK 60	Mt. Kenya forest	Medium yellow	lobate	-ve	single rods	-ve	+ve	+ve
MTK 61	Mt. Kenya forest	Deep yellow	undulate	-ve	Short chain rod	+ve	-ve	+ve
MTK 14	Mt. Kenya forest	Pale yellow	Erose	-ve	cocci single	-ve	+ve	-ve
MTK 25	Mt. Kenya forest	Pink	Erose	+ve	cocci cluster	+ve	-ve	-ve
MTK 73	Mt. Kenya forest	Whitish	Filamentous	-ve	short chain rods	-ve	+ve	-ve
MTK 87	Mt. Kenya forest	Cream	Filamentous	+ve	short chain rods	+ve	-ve	-ve
MTK 67	Mt. Kenya forest	Pink	lobate	-ve	single rods	+ve	+ve	+ve

Appendix 10: Plagiarism Awareness Certificate

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