CGIAR MULTIFUNCTIONAL LANDSCAPES

PROJECT REPORT

Metagenomic Characterization of Soil Microbial Communities from Makueni and Kiambu Counties of Kenya

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About Multifunctional Landscapes

Multifunctional Landscapes is a CGIAR Science Program that aims to enhance the resilience, productivity, and sustainability of agricultural landscapes by integrating diverse land uses, ecosystem services, and livelihood strategies. The initiative supports evidence-based policies and innovations that balance food production with climate adaptation, biodiversity conservation, and social inclusion. By working with local communities, governments, and partners, it promotes landscape-level approaches to managing natural resources for long-term ecological and economic benefits.

Contents

Background

Soil microbiome and agricultural resilience

Soil is an inherently complex and dynamic system (Nannipieri, 2021), and its health is inextricably linked to the functioning of terrestrial ecosystems (Kibblewhite et al., 2007). Soil metagenomics is the study of all genetic material (DNA) from microbes in a soil sample, bypassing traditional culturing to reveal the vast, diverse microbial community and its functions, crucial for understanding nutrient cycles, plant health, bioremediation, and ecosystem services like soil fertility, by sequencing all DNA and using bioinformatics to identify organisms and potential metabolic roles (Daniel, 2005). This powerful molecular approach helps uncover previously unknown microbes and genes, transforming our knowledge of complex soil environments.

The soil microbiome is a key driver of ecosystem services. At the core of this functionality lies the soil microbiome: a diverse community of bacteria, archaea, fungi, and microfauna (Chen et al., 2024). This microbiome represents a delicate balance of microbes within the soil, acting as the key driver of essential ecosystem services critical for plant health and fertility. From an ecological function's perspective, soil microbes are crucial for:

- **Nutrient Cycling:** Driving biogeochemical processes such as nitrogen fixation, phosphorus solubilization, and sulfur oxidation (Chen et al., 2024).
- **Organic Matter Decomposition:** Breaking down residues to release bioavailable nutrients and form stable soil organic matter (Zhan, 2024).
- **Soil Structural Stability:** Microorganisms produce extracellular polymeric substances (EPS) that bind soil particles, creating stable aggregates that improve water infiltration and retention (Bargali, 2024).
- **Disease Suppression:** Providing a biotic shield against soil-borne pathogens through competition and antagonism.
- **Promoting Plant Growth:** Synthesizing hormones and increasing nutrient uptake efficiency (Chen et al., 2024)

The threat of agricultural intensification

The delicate balance of beneficial microbial communities is severely disrupted by conventional intensive agricultural practices, particularly overuse of synthetic fertilizers and pesticides, as well as intensive tillage. This disruption leads to:

- **Soil degradation:** Loss of microbial diversity, reduction in soil organic carbon, and impaired soil structure.
- **Reduced crop yields:** Decreased ability to buffer stress and efficiently acquire nutrients, leading to yield instability.
- **Increased environmental pollution:** Runoff of unused chemical inputs into water bodies, compromising future agricultural resilience.

Landscapes (B-REAL) initiative, which is designed to counteract this degradation by investigating and leveraging the power of the soil microbiome. Therefore, the overarching long-term goal of B-REAL was specifically meant to investigate and tap the benefits of the soil microbiome to develop sustainable and regenerative agricultural practices. This will demonstrably improve soil health by enhancing microbial diversity and biomass; crop resilience by improving stress tolerance (e.g., drought, disease); and input efficiency through the reduction of reliance on chemical inputs. It bolsters long-term agricultural resilience.

Research objectives and scope of microbiome study under B_REAL

The overall objective of this study was to conduct a detailed analysis of the soil microbial biodiversity within selected agricultural landscapes in selected counties of Kenya using advanced metagenomic techniques.

The specific objectives of this research were to identify and characterize the dominant microbial communities (taxonomic profiles) present in the agricultural soils of the study counties and compare microbial diversity and composition across different soil samples, specifically evaluating the differences between the distinct cropping systems found in Kiambu and Makueni counties.

In this study, microbiomes are the central focus of the project under different management practices, including a control plot, representing conventional or baseline practices, and a treatment plot where specific B-REAL interventions are applied, such as manure application (4 t/ha) to boost soil fertility; agroforestry (integrating mango, avocado, and citrus) to provide shade and extra income; water harvesting (Zai pits and plastic-layered holes) to combat water scarcity; and biopesticides (neem tree extracts) to reduce chemical dependency.

Methodology

Study locations and cropping systems

The study was conducted in two distinct agroecological zones in Makueni and Kiambu Counties in Kenya. The selection of these sites provides a critical contrast in climate, agricultural intensity, and dominant cropping systems, allowing for a robust comparison of microbial ecology. Mixed crop-livestock systems are the main farming systems of both study areas. Cereals, mainly maize intercropping with pigeon pea, integrated with citrus fruits, such as mangoes and oranges, are the major cropping system in Makunei County. In Kiambu County, in addition to intercropping of maize with fruit trees, different vegetables (e.g., cabbage, kale, lettuce, carrots, etc.) are also cultivated with maize as intercrops. Drip irrigation using water harvesting holes with plastic layers and zai pits are among the key practices in the study areas.

Agroecological principle is the main approach in agricultural practices, with the use of compost and manure as key inputs for fertilizing field crops and fruit trees, and the use of biopesticides (e.g., neem trees) for crop protection. Dairy and poultry production are major sources of manure for fertilizing farms. Makueni County, a predominantly semi-arid region in southeastern Kenya, is characterized by low and

unpredictable rainfall, high temperatures, and frequent droughts (Machio et al., 2025; Muia et al., 2024). The cropping systems are thus highly adapted and centered around drought tolerance (Machio et al., 2025), water conservation (Machio et al., 2025), and diversification (Riungu et al., 2024). In contrast, Kiambu County, situated in the central highlands (bordering Nairobi), boasts a humid to sub-humid climate and is characterized by a diverse, highly intensive agricultural system (Kuria et al., 2024), driven by its high potential and proximity to a major urban market. Land fragmentation is significant, with an average smallholder farm size of around 0.36 ha (Government of Kiambu County, 2013).

Table 1. Farming systems of the study areas

Makueni County	
Dominant cropping systems	**Key features**
Mixed crop-livestock systems	Integration of livestock (specifically goats, poultry, and dairy) provides manure for soil fertility and crop residues as animal feed. They also provide income for farmers.
Integrated fruit trees-crop-livestock (agroforestry)	Farmers usually incorporate fruit trees (e.g., mango, orange, avocado) alongside annual crops (e.g., maize/pigeon pea intercropping) and pearl millet. Trees contribute to soil and water conservation and microclimate regulation.
Kiambu County	
Dominant Cropping Systems	**Key Features**
High-Value Horticulture	Production of leafy vegetables (Cabbage, Spinach, Kale), carrots, and Irish potatoes.
Food Crops	Maize, Beans, Bananas, and Irish potatoes are commonly grown as major food crops or intercropped
Integrated Dairy and Poultry	Dairy and poultry Production at a small scale for home consumption and the local market

Soil sampling protocol and sampling approach

A total of 74 plots of farmland were randomly selected across both Makueni and Kiambu counties. The cropping systems of each farmland from which the samples were taken are presented in Annex 1. The sampling was carried out in collaboration with scientists from CIAT. An appropriate and standard soil sampling procedure was adopted from the NEON Soil Microbiome Sample Collection Protocol (https://www.protocols.io/view/soil-microbiome-sample-collection-protocol-adapted-yxmvmezk9g3p/v1

Soil samples were collected from the 0-20 cm soil depth using a hand-held spiral auger. To account for within-farm variability, three soil sub-samples were taken from each farm: S1 (close to the homestead), S2 (midway), and S3 (far from the homestead). Before homogenization, recognizable undecomposed plant material, rocks, and insects were removed using a pre-sterilized, gloved hand. The three sub-samples (S1, S2, S3) from each farm were mixed, homogenized, and about 0.5 kg of soil sub-sample was

transferred into a Ziplock plastic bag, properly labeled, and immediately stored in an icebox to maintain DNA integrity. Soil temperature was recorded during sampling. The samples were transported to the ICRISAT laboratory in Nairobi and stored in a deep freezer until DNA isolation.

DNA isolation and quality control

Initial trials with standard soil DNA isolation kits resulted in high co-extraction of humic acid impurities, which are known to interfere with downstream enzymatic processes like Polymerase Chain Reaction (PCR) and sequencing (as evidenced by initial gel imaging, Fig. 1).

Fig. 1. DNA gel image (note: the DNA was not successfully isolated from some of the samples)

To mitigate this issue, the extraction protocol was optimized by switching to the MP Biomedicals Fast DNA™ SPIN Kit for soil to specifically handle/remove high-humic acid content. Following optimization, total genomic DNA was successfully isolated as shown below in Fig. 2. The average DNA concentration achieved was (~20 µg/µl), which significantly exceeded the required quantity (1 µg/µl) of DNA per sample for the downstream analysis. The extracted DNA has been submitted to MrDNA (PCR and sequencing for selected genes; 16S rRNA and ITS region).

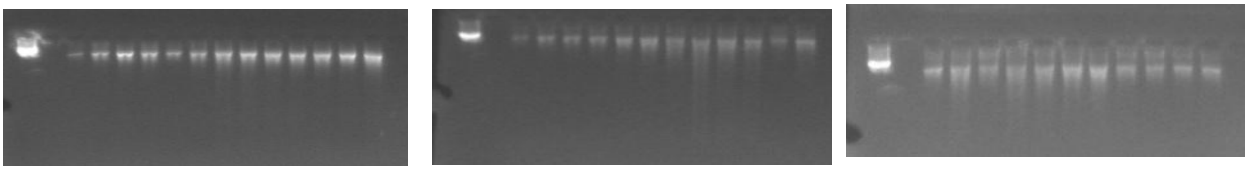

Fig. 2. DNA image on Agarose gel, showing that the DNA has been successfully isolated from the soil samples collected from the two counties.

16S rRNA and ITS amplicon sequencing

Amplicons and PCR conditions: Microbial community composition was characterized using domain-specific genetic markers. For the bacterial and archaeal domains, the 16S rRNA gene V4 variable region was targeted using primers 515F/806R. For the fungal domain, the Internal Transcribed Spacer (ITS) region was targeted using ITS1F/ITS2R primers. PCR amplifications were conducted using the AllTaq Master Mix Kit (Qiagen, USA). The 16S rRNA V4 region was amplified under the following conditions: initial denaturation at 95°C for 5 minutes; followed by 30 cycles of 95°C for 30 seconds, 53°C for 40 seconds, and 72°C for 1 minute; and a final elongation at 72°C for 10 minutes. Following the Mr DNA (Shallowater, TX, USA) standardized protocol for ITS amplicons, the PCR conditions were: initial denaturation at 95°C for 5

minutes; followed by 30 cycles of 95°C for 30 seconds, 53°C for 40 seconds, and 72°C for 1 minute; with a final elongation step at 72°C for 10 minutes.

Library preparation and sequencing: Following amplification, PCR products were visualized on a 2% agarose gel to verify successful amplification and assess band intensity. Samples were multiplexed using unique dual indices and pooled in equal molar proportions based on molecular weight and DNA concentration. The pooled samples were purified using calibrated Ampure XP beads to prepare the Illumina DNA library. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines. Sequence data were processed using the MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, sequences are joined, sequences < 150 bp were removed, and sequences with ambiguous base calls were removed. Sequences were quality filtered using a maximum expected error threshold of 1.0 and dereplicated. The dereplicated or unique sequences were denoised; unique sequences identified with sequencing and/or PCR point errors were removed, followed by chimera removal, thereby providing a denoised sequence or zOTU. The final zOTUs were taxonomically classified using BLASTn against a curated database derived from NCBI (www.ncbi.nlm.nih.gov). In total, our sample distribution included 30 samples from Kiambu and 44 samples from Makueni. The dataset also included extraction blanks and sterile water controls to ensure data quality.

Results and Discussion

To map the microbial architecture of Kiambu and Makueni, microbial community composition was characterized using domain-specific genetic markers. The 16S rRNA gene was employed for archaeal and bacterial classification, while the Internal Transcribed Spacer (ITS) region was sequenced for fungal identification. The analyses represent a high-depth of microbiome survey across two Counties. At a genus level, a total of 4,316,799 Zero-radius Operational Taxonomic Units (ZOTUs) were generated, which comprised bacteria (1,729,881 ZOTUs), Archaea (147,996 ZOTUs), and Fungi (2,234,424 total reads).

16S rRNA sequence analyses for the bacterial domain

The bacterial domain exhibited significantly higher diversity, accounting for 1,729,881 ZOTUs comprising 1,156 unique genera, of which only 154 genera represented more than 80% of the community. The top five most dominant genera across the entire dataset are *Brevitalea*, where a total of 99033 ZOTUs were identified with a relative abundance of 5.7%, followed by *Gaiella* (ZOTUs counts= 86,795, with a relative abundance of 4.98%), *Gemmatimonas* (ZOTUs counts= 85,791, with a relative abundance of 4.95%), *Rubrobacter* (ZOTUs count=65,925, with a relative abundance of 3.8%), and *Chthoniobacter* (ZOTUs count= 63,971, with a relative abundance of 3.69%), together accounting for 23.12% of the total bacterial community in the sampled soil.

Kiambu County comprised 685,456 ZOTUs, while Makueni County constituted 1,044,425 ZOTUs, implying that Makueni is the more diverse county in terms of total bacterial community composition. The top five genera identified in this study are globally prevalent soil bacteria, which play critical roles in nutrient cycling, soil structure, and carbon sequestration. Wüst et al. (2026) reported that members within the

genus *Brevitalea* are specialized for life in oligotrophic (nutrient-poor) soils and are often associated with acidic environments. Their ecological role is as primary degraders of complex plant polysaccharides, including cellulose and hemicellulose (Eichorst et al., 2018). They help break down organic matter into simpler forms that other microbes and plants can use, and their ability to thrive in low-nutrient conditions makes them essential for maintaining soil fertility in stressed or depleted ecosystems. While members within *Gaiella* play an important role in the nitrogen cycle, specifically in the reduction of nitrate to nitrite (Albuquerque et al., 2011), and are an indicator of soil condition (Severino et al., 2019). *Gemmatimonas* are crucial for phosphorus metabolism and are resilient to moisture fluctuations and are often dominant in arid or semi-arid soils (Mujakić et al., 2022; He et al., 2020). *Rubrobacter*, being extremophilic, which resists high temperature, desiccation, and drying out, is considered a "pioneer species" in harsh environments, and its presence often indicates soils that experience high UV exposure or periodic drought (Chen et al., 2021; Sghaier et al., 2016). *Chthoniobacter* could potentially play a role in the degradation of complex carbohydrates derived from plant cell walls and fungal biomass, which is vital for the global carbon cycle (Kant et al., 2011). It is also worth mentioning that the genus *Bradyrhizobium* (with 11,473 counts), *Rhizobium* (4950 reads), known to fix nitrogen with legumes (Bellabarba et al., 2019), endophytic *Azoarcus* (6997 reads) commonly in inside rice/grass, a free-living nitrogen fixing *Azonexus* (3504 reads), *Microvirga* (8731 reads), and *Ensifer/Sinorhizobium*, with a total sequence read of 391, were identified. Furthermore, *Bacillus*, P*seudomonas, and Streptomyces* were identified in the community, which are known for their ability to solubilize phosphate (Rawat et al., 2021). *Sphingomonas* (23025 reads), known to promote plant growth under environmental stress like drought or salinity and produce plant hormones.

Free-living nitrogen fixers such as *Azoarcu*s and *Azonexus* (Gaby and Buckley 2020) were also present. Overall, the bacterial landscape of Kiambu and Makueni is defined by a high degree of taxonomic diversity and functional versatility. The distinct dominance of oligotrophic and stress-tolerant genera—such as *Brevitalea*, *Gaiella*, and *Rubrobacter*—indicates a microbial community specifically adapted to the potentially acidic and semi-arid conditions of the study regions. Furthermore, the presence of a robust suite of plant growth-promoting bacteria (PGPB), including key nitrogen fixers like *Bradyrhizobium* and *Rhizobium* alongside efficient phosphate solubilizers like *Bacillus* and *Pseudomonas*, underscores a high biological potential for supporting soil fertility. While Makueni exhibits a higher total ZOTU richness, both counties harbor a core microbiome capable of maintaining essential biogeochemical cycles—carbon degradation, nitrogen transformation, and phosphorus mobilization—which are vital for sustained ecosystem health and agricultural productivity.

16S rRNA sequence analyses for the archaeal domain

Bioinformatic analysis yielded a total of 147,996 Zero-radius Operational Taxonomic Units (ZOTUs) for the archaeal domain, comprising 57,723 from Kiambu and 90,273 from Makueni, implying that Makueni is more diverse in terms of archaeal community composition. The archaeal community across the 74 samples (30 from Kiambu and 44 from Makueni) is highly specialized, with two ammonia-oxidizing genera, *Candidatus Nitrososphaera* (52.5%) and *Nitrososphaera* (40.95%), dominating the composition of the archaeal domains. The remaining (6.5%) of the archaeal population belongs to different genera comprising *Candidatus Methanomethylophilus, Methanomassiliicoccus, Methanobrevibacter,*

Methanogenium, Aciduliprofundum, and other minor groups. It is worth noting that *Candidatus Nitrososphaera* is a genus of ammonia-oxidizing archaea (AOA) belonging to the phylum Thaumarchaeota. Its members play a fundamental role in the global nitrogen cycle, particularly in terrestrial and soil environments (Stieglmeier et al. 2014). They are exceptionally well-adapted to low-nutrient (oligotrophic) environments because they have a much higher affinity for ammonia than their bacterial counterparts, giving them a comparative advantage to thrive where nitrogen is scarce. Furthermore, functional redundancy is well implicated in the community as shown by the high percentages of two closely related groups (*Candidatus* and *Nitrososphaeria*), suggesting a robust capability for ammonia oxidation, which is critical for ecosystem health and nitrogen availability for plants or other microbes. In summary, the archaeal community structure in Kiambu and Makueni is characterized by a high degree of specialization and a distinct dominance of ammonia-oxidizing taxa. The overwhelming prevalence of *Candidatus Nitrososphaera* and *Nitrososphaera*—accounting for over 93% of the total archaeal composition—highlights the critical role of the phylum Thaumarchaeota in these soil ecosystems. The greater ZOTU richness observed in Makueni, coupled with the functional redundancy between these two dominant genera, suggests a highly stable and efficient nitrogen cycling mechanism. This specialized architecture ensures sustained ammonia oxidation even in nutrient-limited environments, ultimately supporting soil fertility and ecosystem resilience across both regions.

ITS region sequence analyses for fungi

Across the entire dataset, a total of 2,234,424 sequence reads were analyzed, leading to the identification of 964 unique fungal genera (938 from Makueni and 932 from Kiambu), of which 184 genera represent more than 90% of the total sequence reads, suggesting a high degree of functional redundancy within a rich and complex fungal ecosystem. Regional distribution analysis showed that Makueni County accounted for 1,334,630 sequence reads, while Kiambu County yielded 899,794, indicating that Makueni possesses marginally higher fungal diversity and abundance.

The community was dominated by five primary genera: *Fusarium* (199,969 counts), *Cladosporium* (96,512 counts), *Mortierella* (91,850 counts), *Lectera* (65,625 counts), and *Humicola* (52,409 counts). These genera represent a mix of ecological roles. For example, *Mortierella* (Ozimek & Hanaka, 2021) and *Humicola* (Ibrahim et al., 2021) are recognized for their beneficial roles in soil health, specifically in phosphorus dissolution and the decomposition of complex organic matter like cellulose. Conversely, *Fusarium* (Kůdela, 2001), *Cladosporium* (Bensch et al., 2012), and *Lectera* (Cannon et al., 2012) are frequently identified as plant pathogens, suggesting a potential risk for crop health alongside beneficial nutrient cycling processes. Generally, the fungal communities of Kiambu and Makueni are characterized by high taxonomic richness and a distinct bimodal functional profile (both beneficial and harmful in this case). The significant presence of saprotrophic fungi like *Mortierella* and *Humicola* highlights a robust capacity for organic matter decomposition and nutrient mobilization, which are essential for soil fertility. However, the high prevalence of pathogenic genera, such as *Fusarium* and *Cladosporium,* suggests that the fungal architecture in these regions also harbors a significant phyto-pathological burden. The greater abundance and diversity observed in Makueni County indicate a more complex fungal network. However, the

functional redundancy across both sites implies that core ecosystem processes, such as carbon and phosphorus cycling, are likely maintained in the systems.

Conclusions

From County diversity gradient perspectives**,** across all three domains (Archaea, Bacteria, and Fungi), Makueni County consistently exhibits higher microbial diversity and sequence abundance than Kiambu County, where 56% more ZOTUs, 52% more ZOTUs, and 60% more for archaea, bacteria, and fungi, respectively. This is because high diversity is expected in agroforestry and intercropping systems. Farms integrated fruit-crop-livestock and maize + beans + fruit trees are likely to harbor the highest microbial diversity. The reason is that the variety of root exudates from different species (maize, pigeon pea, avocado, mango) is expected to support diverse functional groups of microbes, which is the case in Makueni. This suggests that Makueni's soil environment may support a more complex and varied microbial network, potentially due to differences in soil type, land use, or environmental stressors. From functional redundancy and ecosystem stability perspectives, a significant finding across the Archaeal, bacterial, and fungal domains is that a relatively small number of genera dominate the community (e.g., 154 bacterial genera represent >80% of reads; 184 fungal genera represent >90% of reads). This indicates high functional redundancy, meaning multiple species perform similar ecological functions. This redundancy is vital for ecosystem resilience, ensuring that critical processes, such as ammonia oxidation, carbon degradation, and phosphorus cycling, continue even if specific species are lost due to environmental changes.

From adaptation to stress and low nutrients perspectives, the microbial "fingerprint" of these regions suggests adaptation to oligotrophic (nutrient-poor) and semi-arid conditions, as implicated by members in Archaea, such as *Nitrososphaera*, which thrives on low levels of ammonia where bacteria cannot. Furthermore, prevalent members in bacteria such as *Brevitalea* and *Rubrobacter* are specialized for acidic, nutrient-poor, and high-UV/drought environments. Regarding fungi, the presence of *Gemmatimonas* and *Humicola* points to a community resilient to moisture fluctuations and capable of breaking down rough organic matter. From an integrated biogeochemical cycling viewpoint, **t**he data reveal a complete "microbial engine" capable of supporting plant life through three key cycles, such as the nitrogen cycle, which is driven by archaeal ammonia oxidizers (*Candidatus Nitrososphaera*) and Bacterial nitrogen fixers (*Bradyrhizobium*, *Rhizobium*). In addition, the carbon cycle is expected to be facilitated by primary degraders of complex plant polysaccharides (*Brevitalea*, *Chthoniobacter*, and saprotrophic fungi like *Humicola*). For the phosphorus cycle, the presence of specialized bacteria (*Bacillus*, *Pseudomonas*) and fungi (*Mortierella*) can guarantee the functions to continue in the systems.

From the bimodal fungal profile (risk vs. benefit) standpoint, **t**he fungal analysis highlights a critical management insight. While the soil is rich in beneficial decomposers that improve fertility, it also carries a significant phyto-pathological burden. The dominance of *Fusarium* and *Cladosporium* suggests that there is a high natural pressure from plant pathogens that could impact agricultural productivity. Overall, the soil microbiome of Kiambu and Makueni is a highly specialized, stress-tolerant system with a robust capacity for nutrient mobilization. While Makueni serves as a hotspot for diversity, both regions harbor a core microbiome that can sustain the ecological functions. For agricultural planning, the high presence of

beneficial growth-promoting bacteria is a major asset, though it is tempered by a prevalent fungal pathogenic load that may require integrated pest and soil management.

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Annexes

Table 1. Summary of study sites' characteristics. MK=Makueni, KM=Kiambu (NB: the agroecological/organic treatment plots are highlighted in blue)

Farm code	Cropping systems	Soil T (°C)	Plot type	Remark
MK-107	Maize intercropped with pigeon pea	24	Control	
MK-107 (Txt)	Maize intercropped with pigeon pea	25	4tons/ha manure	Mixed crop-livestock systems
MK-106	Maize intercropped with pigeon pea	25	control	
MK-106 (Txt)		26	Manure	Mixed crop-livestock systems
MK-109	Maize intercropped with pigeon pea	25.7	control	Mixed crop-livestock systems
MK-109 (Txt)	Maize intercropped with pigeon pea	24.5	Biopesticides (neem tree)	
MK-117	Maize intercropped with pigeon pea	25		
MK-111	Maize intercropped with pigeon pea	24		
MK-111 (Txt)		24.5	Manure treatment	
MK-004	Maize intercropped with pigeon pea, integrated with fruit trees such as mangoes and oranges	22	control	
MK-004 (Txt)		20	Manure (4t/ha)	Mixed crop-livestock systems; Water conservation practice
MK-073	Maize intercropped with pigeon pea integrated with fruit (avocado, orange, banana, mango)	23		Integrated Fruit Trees-Crop-Livestock (Agroforestry)
MK-073			Manure	
MK-077	Maize + Mangoes	24		Drip irrigation using water harvesting holes with plastic layers
MK-081	Maize intercropped with pigeon pea integrated with various fruit trees	30		Moisture conservation
MK-081 (Txt)		28	Manure (6.7t/ha)	
MK-010	Maize intercropped with pigeon pea	28.7		The soil is deeper and better
MK-010 (Txt)			Neem biopesticide	
MK-014	Only maize as the sole crop with FYM application. Pigeon pea at the hedge	29		
MK-023	Maize intercropped with pigeon pea	28.5		
MK-022	Maize intercropped with pigeon pea	32.5	Manure (2t/ha)	
MK-022 (Txt)		35	IPM (Neem biopesticide txt, manure 2t/ha)	
MK-045	Maize + common beans	29.7		
MK-045 (Txt)	Maize intercropped with common bean	30		Moisture conservation
MK-128	Maize intercropped with pigeon pea, mangoes on the hedge, and poultry production	19.5		
MK-128 (Txt)	Maize intercropped with common bean	19	Manure (4t/ha)	
MK-129	Maize with common bean	20		

MK-129 (Txt)	Maize with common bean	20	Neem tree as a biopesticide	
MK-036	Maize + common bean + orange + mangoes + pigeon pea	24		
MK-030	Maize + pigeon pea + mango tree	27		
MK-030 (Txt)		28	Neem tree as a biopesticide	
MK-026	Maize + beans + fruit trees, manure applied	28		
Mk-026 (Txt)	Maize + beans + fruit trees (mangoes, oranges, papaya)	27	Biopesticide + manure	Zai pits (2 ft by 2 ft for moisture conservation)
MK-136	Maize intercropped with pigeon pea, mango trees in the field	30		
MK-051	Maize intercropped with pigeon pea	31		
MK-051 (Txt)	Maize + common bean	27.5	Manure (4t/ha)	
MK-053	Maize +pigeon pea + cassava intercropped. Citrus fruits on the hedge	30		
MK-056	Maize intercropped with pigeon pea	30		
MK-061	Maize intercropped with pigeon pea. Mango trees on the hedge	30		
MK-044	Maize intercropped with pigeon pea and mango trees on the hedge	19		
MK-227	Maize intercropped with pigeon pea, mixed with citrus fruits	22.7		
MK-182	Maize intercropped with pigeon pea	26.5		
MK-158	Maize intercropped with pigeon pea. Manure was applied	26.3		
MK-147	Maize intercropped with pigeon pea, maize with common bean, and orange fruit. Farmers used FYM	31		Zai pits for water conservation
MK-194	Maize intercropped with pigeon pea and mango trees.	31		Irrigated, manure is applied
KM-091	Vegetables, such as spinach and kale	17.5		
KM-016	Maize intercropped with field pea	17.5		Fertilized with FYM
KM-015	Horticultural crops (potato and cabbage)	17.5		
KM-064	Maize intercropped with beans and potatoes	18		Fertilized with FYM
KM-094	Avocado + different vegetables	21.7		Fishpond and water harvesting pond
KM-053	Vegetables (spinach, kale) + avocado	19		Mulching
KM-065	Maize intercropped with common bean	31		
KM-011	Maize only	16		
KM-001	Maize intercropped with common bean	18.5		FYM applied to organic farming systems
KM-003	Horticulture (spinach and cabbage)	20		FYM and mulching
KM-113	Maize only for silage	17		Organic farming
KM-079	Maize only	19		Compost applied, organic farming
KM-049	Maize intercropped with beans	21		Compost applied

KM-010	Maize intercropped with beans	22	Fertilized with FYM
KM-009	Maize intercropped with beans	23	Fertilized with biogas slurry
KM-032	Maize intercropped with beans	17	FYM applied
KM-036	Maize intercropped with beans, relay cropping with potato	18	FYM applied
KM-031	Maize intercropped with beans	19.5	FYM applied
KM-033	Maize intercropped with beans. Avocado trees and vegetables are also components of the system	17	Fertilized with compost
KM-024	Cabbage with Lucerne as agroforestry	20.5	FYM applied
KM-045	Maize intercropped with beans	20	Compost applied
KM-013	Cabbage mulched	18.5	Fertilized with FYM and compost
KM-069	Maize	22	
KM-056	Peas, maize, and Napier grass	24	
KM-038	Maize intercropped with beans	16	Compost applied
KM-089	Maize intercropped with beans, with an agroforestry practice	16.7	Both compost and FYM were applied. Agroecological principle
KM-037	Maize intercropped with field peas	17	Compost applied
KM-067	Maize intercropped with beans	17.5	Fertilized with FYM
KM-057	Maize intercropped with beans and pumpkins	16,5	Fertilized with FYM
KM-055	Maize intercropped with field pea	17	Fertilized with FYM

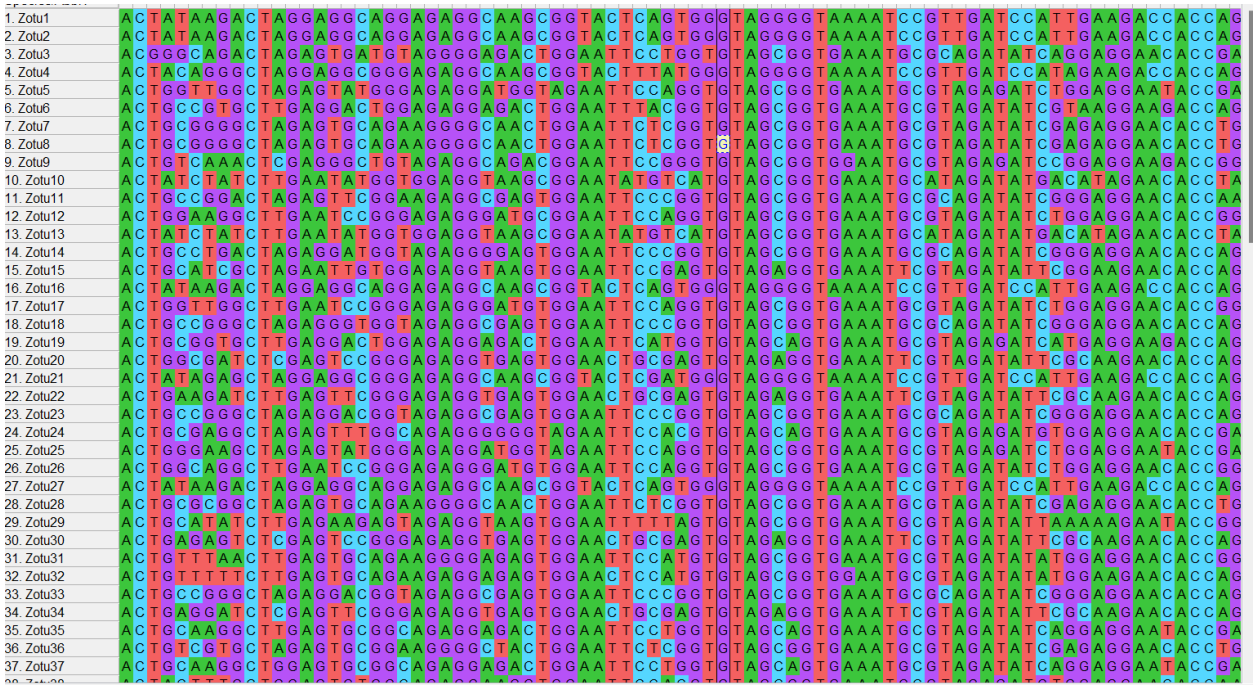

Fig. 1. Some snapshot Alignments for 16S rRNA for some ZOTUs, which highlights that the 16S rRNA sequence is highly conserved

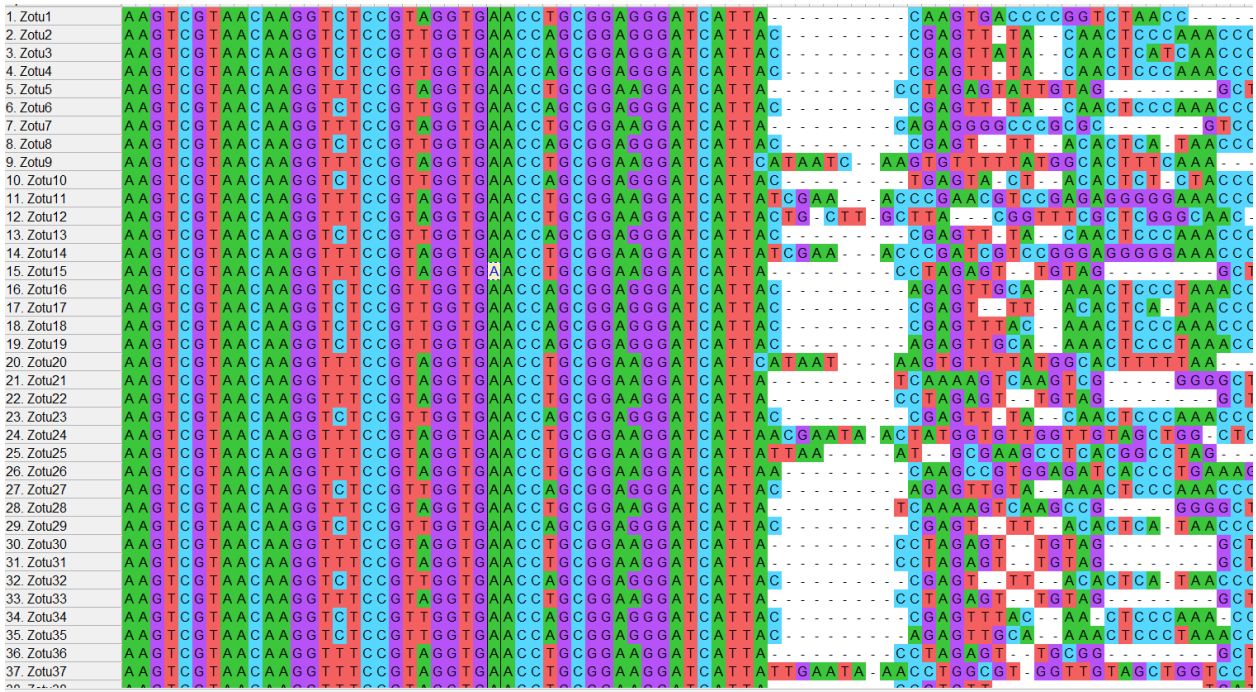

Fig. 2. Snapshot alignment for ITS regions for some ZOTUs, which highlights the variable nature of the ITS region

Table 2. Summary of key findings: Soil microbiome architecture

Domain	Total diversity/abundance	Key dominant genera	Primary ecological role identified	Key County-wise findings
Bacteria	1,729,881 ZOTUs (1,156 genera)	*Brevitalea, Gaiella, Gemmatimonas, Rubrobacter,* Chthoniobacter	Carbon sequestration, Nitrogen cycling, Phosphorus metabolism, Stress tolerance (UV/Drought).	**Makueni** is 52% more diverse than Kiambu
Archaea	147,996 ZOTUs	*Candidatus Nitrososphaera* (52.5%), *Nitrososphaera* (40.95%)	Specialized ammonia oxidation (AOA) in nutrient-poor (oligotrophic) soils.	**Makueni** is 56% more diverse than Kiambu.
Fungi	2,234,424 sequence reads (964 genera)	*Fusarium, Cladosporium, Mortierella, Lectera, Humicola*	**Bimodal profile:** Beneficial decomposition/P-dissolution vs. high phyto-pathological burden (pathogens).	**Makueni** has 60% higher sequence abundance.

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