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Integrated Microdosing Selected Basal Fertilizers and Lime Modulates Fungal and Bacterial Populations in Acidic Soils under Maize Production in the Meru Highland, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. The successful completion of this study involved the collective efforts of authors MKF, HON, GOO-A, MEM, JKK, and ERM. The authors contributed to field soil sampling, laboratory investigations, processing and analysis of the data, interpretation of the findings, and preparation of the manuscript. They also participated in the critical assessment and revision of the manuscript to ensure scientific accuracy and clarity. All authors reviewed the final manuscript, agreed with its contents, and approved the final version for publication.

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Abstract

The biological component of soil health, particularly bacteria and fungi, plays a central role in organic matter decomposition, nutrient cycling and mineralization, thereby contributing to the maintenance of soil fertility and overall soil functioning. However, continuous cultivation, inadequate nutrient replenishment and inappropriate fertilizer management can lead to soil degradation. Soil acidification can further constrain biological functioning by reducing microbial activity, increasing aluminium toxicity and limiting the availability of essential nutrients. Addressing these challenges requires integrated, resource-efficient soil management approaches that enhance soil fertility, support biological functioning and promote environmental sustainability. The combined use of mineral fertilizers, lime and farmyard manure (FYM), together with efficient nutrient placement through microdosing, may provide an effective approach for improving soil chemical and biological conditions. Information on the effects of fertilizer and lime microdosing on soil microbial populations, particularly bacteria and fungi, in strongly acidic maize-growing soils of the Kenyan highlands remains limited. A two-season field experiment was conducted from October 2024-January 2025 and March-July 2025 using a randomized complete block design arranged in a split-plot layout with three replications. The main-plot factor comprised microdosing (MD; 75% of recommended fertilizer and lime rates) and no microdosing (NMD; 100%), while the subplots comprised eighteen (18) fertilizer and lime treatments involving DAP, NPK 23:23:0, calcite lime, dolomitic lime, FYM, their combinations and a control, replicated three times, giving 108 experimental units. Under no microdosing (NMD), all inputs were applied once at 100% of the recommended rates: 10 t FYM ha^{-1}, 141.3 kg DAP ha^{-1}, 282.6 kg NPK ha^{-1} and 0.3 t ha^{-1} of either calcite or dolomitic lime. Under microdosing (MD), inputs were applied at 75% of the recommended rates: 7.5 t FYM ha^{-1}, 106.0 kg DAP ha^{-1}, 212.0 kg NPK ha^{-1} and 0.225 t ha^{-1} of either calcite or dolomitic lime. Soil sampling and analysis were conducted before the experiment and after each cropping season. Culturable fungal and bacterial populations were determined using the serial dilution and spread-plate method and expressed as colony-forming units (CFU) g^{-1} soil. Data were log_{10}-transformed before pooled split-plot ANOVA, and significant means were separated using Tukey's Honestly Significant Difference (HSD) test at 5%. Pooled analysis of variance showed that microdosing significantly affected both fungal and bacterial populations ($P < 0.01$), while the microdosing × treatment interaction was also highly significant for both fungal and bacterial populations ($P < 0.001$). The highest fungal population was recorded under MD with calcite + NPK 23:23:0 + FYM, with 1.36×10^7 CFU g^{-1} soil ($Log_{10} = 7.134$) in Season 1 and 1.41×10^7 CFU g^{-1} soil ($Log_{10} = 7.149$) in Season 2. These values were significantly higher ($P < 0.05$) than the corresponding NMD treatment, which recorded 1.28×10^7 CFU g^{-1} soil ($Log_{10} = 7.107$) and 1.33×10^7 CFU g^{-1} soil ($Log_{10} = 7.124$) in Seasons 1 and 2, respectively. The highest bacterial populations were similarly recorded under MD with calcite + NPK 23:23:0 + FYM, reaching 1.89×10^8 CFU g^{-1} soil ($Log_{10} = 8.276$) in Season 1 and 1.95×10^8 CFU g^{-1} soil ($Log_{10} = 8.289$) in Season 2. These were significantly higher ($P < 0.05$) than the corresponding NMD values of 1.81×10^8 CFU g^{-1} soil ($Log_{10} = 8.258$) and 1.84×10^8 CFU g^{-1} soil ($Log_{10} = 8.264$), respectively. The controls consistently recorded the lowest microbial populations. For fungi, the MD and NMD controls recorded 3.97×10^6 and 4.00×10^6 CFU g^{-1} soil ($Log_{10} = 6.598$ and 6.602) in Season 1 and 4.00×10^6 and 4.07×10^6 CFU g^{-1} soil ($Log_{10} = 6.602$ and 6.609) in Season 2 with the two control treatments reported as statistically similar ($P < 0.05$). The results demonstrate that combining microdosing with calcite, NPK 23:23:0 and FYM enhanced culturable fungal and bacterial populations compared with both the corresponding NMD treatment and the untreated controls. The improved microbial populations were likely associated with localized nutrient supply, organic carbon addition and amelioration of soil acidity. Microdosing therefore has potential to improve nutrient use efficiency while creating favourable conditions for soil microbial proliferation in acidic maize growing soils.

Keywords: *Soil health; culturable microorganisms; fungal populations; bacterial populations; microdosing; lime; fertilizer; soil acidity.*

1. Introduction

Soil health is a fundamental component of sustainable agricultural production because it determines the capacity of soils to sustain plant growth and productivity while maintaining essential ecosystem functions (Handayani & Hale, 2022). Globally, soil degradation, nutrient depletion, acidification, loss of soil organic matter and disruption of biological processes are increasingly recognized as major threats to agricultural sustainability and food security (Aliyeva, 2026; Naik *et al*., 2025). Soil microorganisms are particularly important because they regulate organic matter decomposition and contribute to carbon, nitrogen and phosphorus cycling, thereby influencing nutrient availability and soil fertility (Dlamini *et al*., 2026; Romero *et al*., 2026). Changes in soil chemical conditions, organic matter inputs and nutrient management can consequently alter microbial abundance and community composition, with implications for soil biological functioning and crop productivity (Lori *et al*., 2017; Cui *et al*., 2023). The challenge of maintaining soil health is particularly important in sub-Saharan Africa, where declining soil fertility, nutrient depletion, low organic matter and soil acidity constrain agricultural productivity and the livelihoods of smallholder farmers (Amede *et al*., 2023). Limited access to fertilizers, organic resources and soil amendments further constrains farmers' capacity to restore degraded soils (Amulpandi *et al*., 2026). These challenges contribute to low and unstable crop yields and increase pressure on agricultural land. Consequently, improving soil health in African farming systems requires research-driven and economically feasible innovations that improve input-use efficiency, restore soil fertility and maintain the environment (Ghosh, 2026). Such approaches are important for economic sustainability because they can potentially reduce the inefficient use of costly agricultural inputs while maintaining or improving crop productivity.

In East Africa, soil acidity is among the major chemical constraints affecting agricultural productivity, particularly in highly weathered soils and intensively cultivated highland areas (Scherwietes *et al*., 2024; Luna & Larrea, 2024). Acidification is associated with declining soil pH, increased exchangeable acidity and, under strongly acidic conditions, increased availability of aluminium (Al) to potentially toxic levels (Zhang *et al*., 2026; Kopittke *et al*., 2019). Soil acidity is a major constraint to agricultural productivity in highly weathered tropical soils because low pH, exchangeable acidity and aluminium (Al) toxicity can restrict root development, reduce nutrient availability and impair nutrient-use efficiency (Regasa *et al*., 2025a, 2025b; Penn & Camberato, 2019). In Kenya, soil acidity is a widespread constraint, particularly in the highland areas where maize production is

intensive (Muindi *et al.,* 2015; Muindi *et al*., 2017; Kibet *et al*., 2023; Golicha *et al.,* 2026). Studies of Kenyan maize-growing soils have reported pH values as low as 4.07-4.85, exchangeable aluminium concentrations exceeding 2 cmol kg^{-1} and aluminium saturation above 20%, demonstrating the severity of acidity in some production environments (Muindi *et al*., 2016; Esilaba *et al*., 2023; Mwakidoshi *et al.,* 2026; Mbaka *et al.,* 2026). Such conditions can restrict maize root development, reduce phosphorus availability and impair nutrient acquisition. In addition, continued use of nitrogen-containing fertilizers without adequate replacement of soil bases can contribute to further acidification over time (Chojnacka & Baltrusaitis, 2025; Goulding, 2016). Recent evidence similarly demonstrates that sustained mineral fertilization can reduce soil pH and alter soil microbial communities, highlighting the close relationship between fertilizer management, soil acidification and soil biological functioning (Xu *et al*., 2026). Addressing these interconnected constraints requires multidisciplinary research that links soil chemical assessment, nutrient management and biological indicators to develop practical innovations for sustainable maize production.

Soil microorganisms provide an important biological dimension for assessing the response of acid soils to management (Kuryntseva *et al*., 2026; Suanu *et al*., 2026). Fungi and bacteria participate in organic matter decomposition, nutrient mineralization and transformation and, therefore, influence the availability of nutrients required for plant growth (Delgado-Baquerizo *et al.,* 2018). Microbial populations are sensitive to soil pH, nutrient availability, organic carbon and other changes arising from agricultural management (Li *et al*., 2019; Cui *et al*., 2023; Wang & Kuzyakov, 2024; Bahram *et al*., 2018). Bacterial communities generally show strong associations with soil pH, whereas many fungal communities are relatively more tolerant of acidic conditions (Rousk *et al*., 2010; Bahram *et al*., 2018). Therefore, changes in fungal and bacterial populations can provide useful biological indicators of how soil acidity and fertility-management practices influence soil functioning. Integrating these biological responses with soil chemical and agronomic measurements provides a stronger multidisciplinary basis for evaluating soil-management innovations and their effects on soil health and crop productivity. The close interactions among soil acidity, microbial populations, soil fertility and crop performance highlight the need for integrated management approaches that combine soil chemistry, plant nutrition and soil biology to improve the sustainability of agricultural production. These interconnected relationships are summarized in Fig. 1.

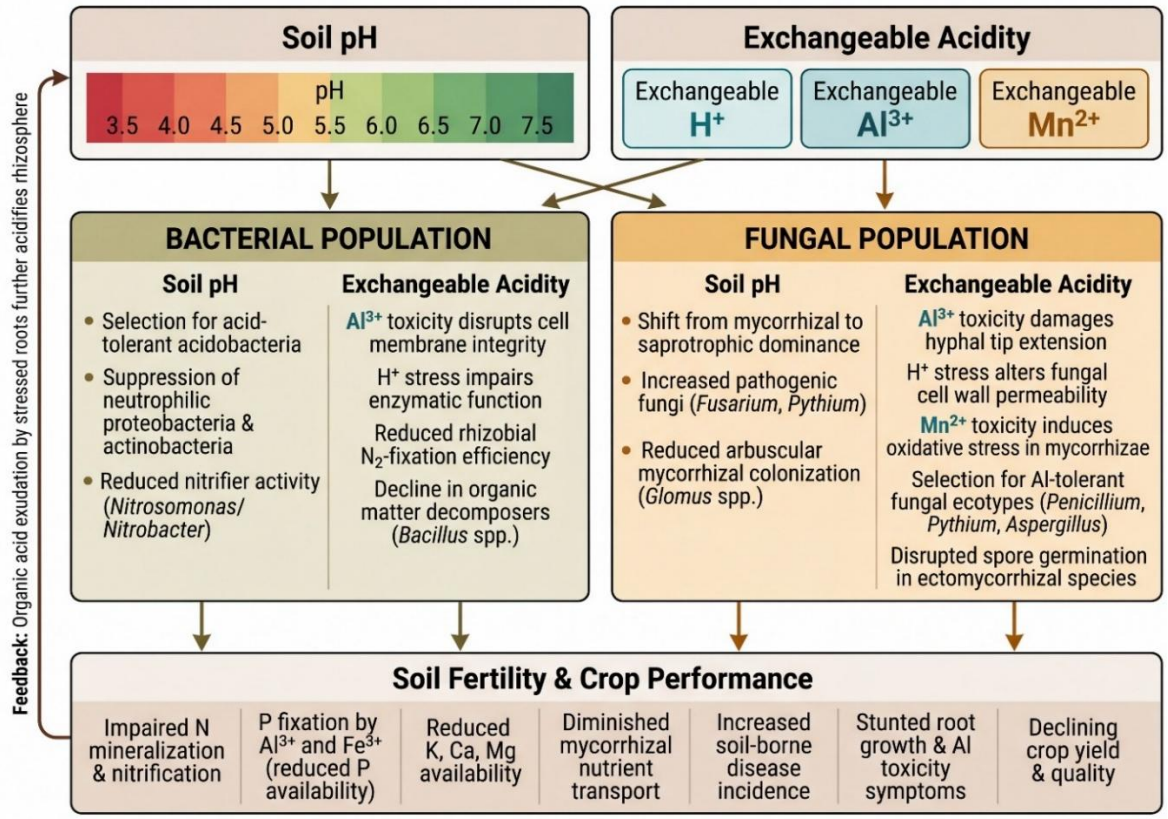


Fig. 1. Conceptual effects of soil acidity on fungal and bacterial populations, soil fertility and crop performance

The management of acidic soils commonly involves liming to neutralize acidity and reduce the effects of exchangeable Al, together with mineral fertilizers to correct nutrient deficiencies. Liming increases soil pH and can improve phosphorus availability by reducing Al-related phosphorus fixation, while mineral fertilizers supply readily available nutrients required for crop growth (Goulding, 2016; Kisinyo *et al*., 2013; Muindi, 2019; Muindi, 2020). However, neither intervention alone fully addresses the multiple constraints affecting degraded acid soils. Farmyard manure (FYM) can complement lime and mineral fertilizers by supplying organic carbon and nutrients and providing substrates that support microbial growth and activity (Lori *et al*., 2017; Bai *et al*., 2023; Cui *et al*., 2023; Shu *et al*., 2022; Shu *et al*., 2023). Recent evidence indicates that organic amendments can improve soil chemical properties and enhance soil microbial functioning, while combinations of organic and inorganic amendments can provide complementary benefits for soil fertility and biological activity (Yang *et al*., 2023). The integration of lime, mineral fertilizer and

FYM therefore represents a multidisciplinary soil management approach that combines soil acidity amelioration, plant nutrition and biological restoration, with potential benefits for both agricultural productivity and environmental management. Field evidence from the central highlands of Kenya also supports the combined use of lime, manure and inorganic fertilizer for improving soil chemical conditions and maize performance in acidic soils (Kimiti *et al*., 2021). Recent research further indicates that liming can modify microbial carbon-use efficiency and microbial biomass, with responses shaped by initial soil pH, supporting the assessment of biological responses alongside chemical indicators (Cha *et al*., 2021; Schroeder *et al*., 2024).

Despite these potential benefits, the cost of mineral fertilizers and liming materials can constrain their use among smallholder farmers in sub-Saharan Africa (Vanlauwe *et al*., 2015; Sheahan & Barrett, 2017). Fertilizer and lime microdosing provides an opportunity to improve input-use efficiency by applying reduced quantities of inputs in targeted locations and at appropriate times relative to crop requirements (Hayashi *et al*., 2008; Tovihoudji *et al*., 2017). Fertilizer microdosing has demonstrated potential to improve crop productivity and fertilizer-use efficiency under African production conditions, although responses vary according to soil fertility, climate and management conditions (Tovihoudji *et al*., 2017; Tovihoudji *et al*., 2022). Integrating microdosed fertilizers and lime with FYM could potentially reduce input requirements while simultaneously supplying nutrients, ameliorating soil acidity and improving the biological environment for soil microorganisms. This approach represents the innovation dimension of multidisciplinary research by bringing together soil science, plant nutrition, soil microbiology and resource-use efficiency to address a complex agricultural constraint. By reducing the quantity of costly inputs while targeting their application to crop requirements, microdosing also has potential to contribute to economic sustainability among smallholder farmers. Recent multi-site work in acid soils showed that an adequate lime microdose could maintain crop and nutrient-recovery responses comparable with conventional full-rate liming while using less lime material (Agyin-Birikorang *et al*., 2024). Maize field evidence from southern Ethiopia likewise indicates that microdosed nitrogen and phosphorus can improve crop responses, although effectiveness depends on fertilizer rate and placement method (Mohammed & Abera, 2025). Under semi-arid conditions in Zimbabwe, nitrogen microdosing combined with cattle manure and in situ rainwater harvesting improved maize yield and nutrient-use efficiency, illustrating the potential value of integrated resource management in smallholder systems (Kugedera & Kokerai, 2024).

The potential of microdosing in acidic soils therefore extends beyond fertilizer-use efficiency. Microdosing selected fertilizers and lime at reduced rates, when integrated with FYM, may simultaneously improve nutrient availability, ameliorate exchangeable acidity and enhance the biological environment for soil microorganisms. Improved soil fertility and biological functioning can contribute to more productive and resilient maize production, while efficient use of fertilizer and lime can reduce input wastage and support environmentally responsible management of agricultural soils. Thus, the approach directly links research and innovation with economic sustainability by seeking to achieve effective soil management using reduced and strategically targeted external inputs. Most microdosing studies have focused on crop yield, nutrient uptake and fertilizer use efficiency, with comparatively limited attention to microbial responses in strongly acidic maize soils (Tovihoudji *et al*., 2017; Tovihoudji *et al*., 2022). In particular, limited information is available on whether reduced rates of DAP and NPK 23:23:0 combined with calcite or dolomitic lime and FYM can maintain or enhance culturable fungal and bacterial populations relative to corresponding full-rate applications. This knowledge gap is important because microbial responses provide a biological perspective on whether soil-management interventions improve soil functioning in addition to supplying nutrients to crops. Generating evidence on these responses can support research-based soil fertility recommendations and the development of policies that promote efficient input use, sustainable soil management, food security and environmental conservation.

Therefore, this study evaluated the effects of fertilizer and lime microdosing, alone and in combination with FYM, on culturable fungal and bacterial populations in an acidic maize soil at Kariene, Kenya. Specifically, the study determined the effects of microdosing selected fertilizers and lime on fungal and bacterial populations across two cropping seasons and compared microbial responses between microdosed and non-microdosed management regimes. By integrating soil acidity amelioration, nutrient management, organic resource use and soil biological assessment, the study provides multidisciplinary research evidence on a resource efficient soil management innovation. The findings are relevant to the conference subtheme of research and policy for food security and environmental management because improved soil biological functioning and efficient nutrient management can contribute to sustainable maize production, while reduced and targeted input use has potential to improve input-use efficiency and economic sustainability for smallholder farmers in acid-affected agricultural systems.

2. Materials and Methods

2.1 Site Description

The study was conducted on a farmer-managed field in Kariene, Abothuguchi Central Ward, Meru Central Sub-County, Meru County, Kenya, during two consecutive cropping seasons: October 2024-February 2025 and March-July 2025. The experimental site is located along the Nairobi-Meru Highway at approximately 0.038471°S and 37.660618°E, at an elevation of about 1,400 m above sea level. The area receives approximately 1,250 mm of mean annual rainfall, distributed mainly between the long rainy season from March to May and the short rainy season from October to December (Jaetzold *et al*., 2007). Mean annual temperatures range from 14°C - 24°C, providing generally favourable conditions for the cultivation of maize and other food and cash crops. Agriculture is the main economic activity in the area, with farmers practising predominantly smallholder mixed crop-livestock production. Maize is widely cultivated as a major food crop, often in rotation or association with crops such as beans, while other crops including bananas, potatoes, vegetables and various horticultural crops are also grown. Livestock keeping, particularly dairy cattle, is commonly integrated with crop production, providing farmyard manure that is used as an organic soil amendment and source of nutrients. Farming activities in the area therefore include the use of mineral fertilizers, organic manure and other soil fertility management practices to sustain crop production. Soils at the study site are predominantly classified as Humic Nitisols, which are deep, well-drained and generally suitable for intensive agricultural production (Jaetzold *et al*., 2007).

2.2 Experimental Design, Treatment Combinations and Layout

The study employed a randomized complete block design (RCBD) in a split-plot configuration, with three replications. The fertilizer and lime application strategy was considered the main-plot factor and had two levels. Under microdosing (MD), 75% of the recommended fertilizer and lime rates were applied, whereas the no-microdosing (NMD) treatment received the full recommended rates (100%). The 18 soil fertility treatments were assigned to the subplots and independently randomized within each replication. The subplot treatments comprised a control and combinations involving DAP, NPK (23:23:0), calcite lime, dolomitic lime, and farmyard manure (FYM). Specifically, the treatments were: control, DAP, NPK (23:23:0), calcite lime, calcite lime + DAP, calcite lime + NPK (23:23:0), dolomitic lime, dolomitic lime + DAP, dolomitic lime + NPK (23:23:0), FYM, FYM + DAP, FYM + NPK (23:23:0), calcite lime + FYM, dolomitic lime + FYM, calcite lime + NPK (23:23:0) + FYM, calcite lime + DAP + FYM, dolomitic lime + DAP + FYM, and dolomitic lime + NPK (23:23:0) + FYM. All eighteen treatments were represented under each fertilizer application strategy in every replication. Consequently, the experiment consisted of 108 experimental plots, obtained from two main-plot treatments × 18 subplot treatments × three replications, as illustrated in Fig. 2. The maize was established using a spacing of 75 cm between rows and 25 cm between plants within rows. Each experimental plot contained four rows, with six plants established in each row, resulting in 24 plants per experimental unit. The resulting plot dimensions were 2.25 m × 1.80 m, giving a gross area of 4.05 m² per plot. A 0.5 m pathway was left between neighbouring plots and a 1.0 m separation between replications to minimize treatment interference and provide adequate access for field operations, including planting, input application, weed management, and data collection. The selected planting configuration provided an estimated plant density of 53,333 plants per hectare.

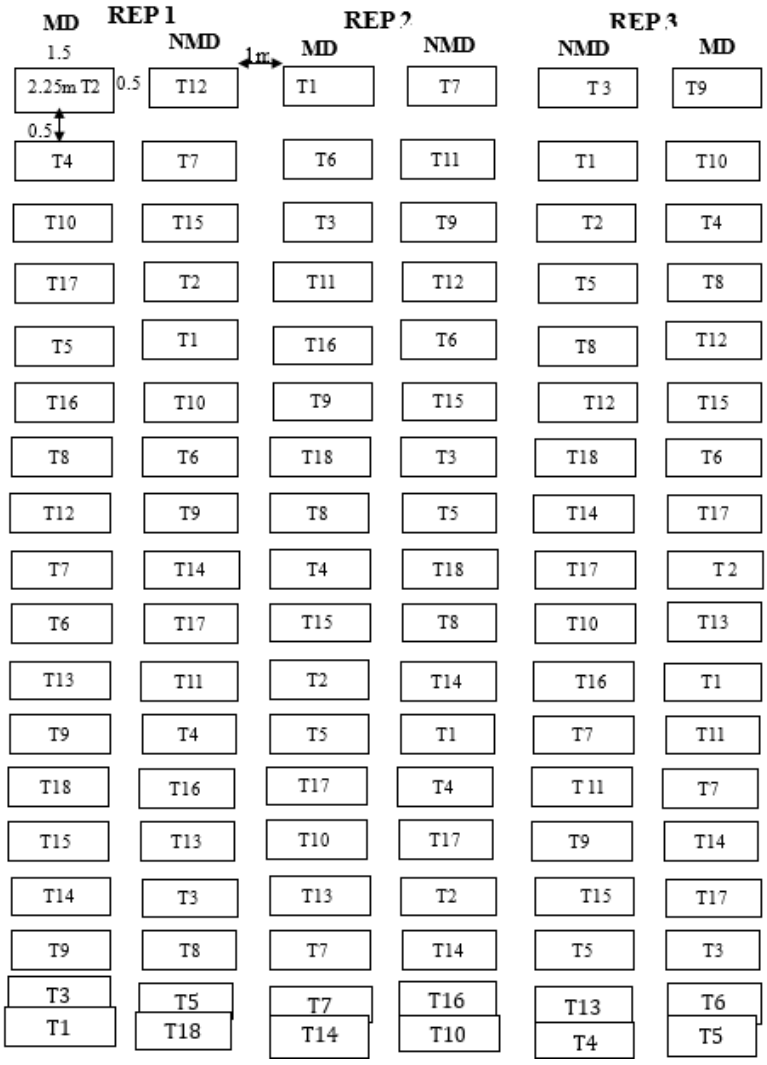


Fig. 2. Field experimental layout

Key: Two main plots; microdosing (MD); no microdosing (NMD). T1 (Control), T2 (Dolomite lime), T3 (Calcite lime), T4 (Farmyard manure (FYM]), T5 (NPK 23:23:0), T6 (Diammonium phosphate (DAP), T7 (FYM + Calcite lime), T8 (FYM + Dolomite lime), T9 (FYM + NPK 23:23:0), T10 (FYM + DAP), T11 (Calcite lime + NPK 23:23:0), T12 (Calcite lime + DAP), T13 (Dolomite lime + DAP), T14 (Dolomite lime + NPK 23:23:0), T15 (Dolomite lime + DAP + FYM), T16 (Dolomite lime + NPK 23:23:0 + FYM), T17 (Calcite lime + DAP + FYM), and T18 (Calcite lime + NPK 23:23:0 + FYM)

2.3 Soil Sampling, Analysis and Land Preparation

Initial soil sampling was carried out before applying fertilizers, lime, and farmyard manure to establish the baseline chemical characteristics of the experimental field. Four soil cores were randomly collected from the 0–30 cm depth in each of the 108 experimental plots, avoiding the plot margins. The four cores from each plot were thoroughly mixed to obtain one composite sample, giving a total of 108 composite soil samples for the initial soil chemical analysis. Soil samples for bacterial and fungal analysis were collected separately from the same plots at the tasselling stage of maize during each of the two cropping seasons. Tasselling was selected because maize is actively growing at this stage, with high nutrient demand and active root development, making it an appropriate stage for assessing treatment effects on soil microbial populations. Four soil cores were collected from the 0–30 cm depth at randomly selected points within each plot, avoiding the plot margins, and mixed thoroughly to obtain one composite sample per plot. The fresh samples were placed in labelled bags, transported to the laboratory as soon as possible, and stored at approximately 4°C until analysis to minimize changes in microbial populations. Bacterial and fungal populations were determined using the serial dilution and spread-plate technique, following Küster and Williams (1964) and Thatcher and Clark (1968). The populations were expressed as colony-forming units per gram of soil (CFU g^{-1}). Collecting microbial samples at tasselling in both seasons allowed the effects of the different soil fertility treatments on bacterial and fungal populations to be assessed under the conditions prevailing in each cropping season. For chemical analysis, the initial composite soil samples were air-dried at room temperature, gently crushed using a wooden mortar and pestle, and passed through a 2 mm sieve to remove stones, roots, and other coarse materials. Soil pH was determined according to Okalebo *et al.* (2002), total nitrogen was determined using the Kjeldahl digestion method (Bremner, 1996), total organic carbon was determined using the Walkley-Black wet oxidation method (Walkley & Black, 1934), and exchangeable acidity was determined by extraction with 1 M KCl followed by titration (Thomas, 1982).

2.4 Treatment Application and Agronomic Practices

All experimental inputs were sourced from reputable and authorized agro dealers to ensure the quality, authenticity and uniformity of the materials used throughout the study. To supply the recommended phosphorus requirement, 141.3 kg DAP ha^{-1} (18:46:0) or 282.6 kg NPK (23:23:0) ha^{-1} was applied, depending on the assigned treatment. Under the no-microdosing (NMD) treatment, all fertilizers and lime were applied at 100% of the laboratory-recommended rates in a single application. Accordingly, plots receiving FYM were supplied with 10 t ha^{-1}, DAP-treated plots received 141.3 kg DAP ha^{-1}, NPK 23:23:0-treated plots received 282.6 kg ha^{-1}, while both calcite and dolomitic lime were applied at 0.3 t ha^{-1}. In the combined treatments, each fertilizer or soil amendment component was applied once at its full recommended rate according to the treatment specifications.

Under the microdosing (MD) treatment, all fertilizers and soil amendments were applied at 75% of their respective recommended rates. Consequently, FYM was applied at 7.5 t ha^{-1}, DAP at 106.0 kg ha^{-1}, NPK (23:23:0) at 212.0 kg ha^{-1}, while both calcite and dolomitic lime were each applied at 0.225 t ha^{-1}. In the combined treatments, each fertilizer and lime component was similarly applied at 75% of its respective recommended rate. To enhance nutrient use efficiency and synchronize nutrient availability with crop demand, the inputs were applied in two equal splits according to the type of amendment. Lime was applied in two equal splits, with the first application three months before planting and the second one month before planting. Farmyard manure was also applied in two equal splits, with the first application two weeks before planting and the second at planting. The mineral fertilizers DAP and NPK 23:23:0 were applied by microdosing in two equal splits, with the first application at planting and the second two weeks after planting. Planting was carried out using certified PAN 3M-05 maize seed. One seed was sown per planting hole at a depth of approximately 3 cm, maintaining a spacing of 75 cm between rows and 25 cm between plants. The soil around each seed was lightly compacted to ensure good seed-to-soil contact and promote uniform germination. Throughout the experimental period, all plots received uniform agronomic management to minimize experimental variability. Hand weeding was carried out as required to control weed competition, while pests and diseases were managed using recommended control practices. In addition, calcium ammonium nitrate (CAN) was applied as a top-dressing fertilizer at a rate of 92.3 kg ha^{-1} to balance the required nitrogen.

2.5 Data Collection Procedures

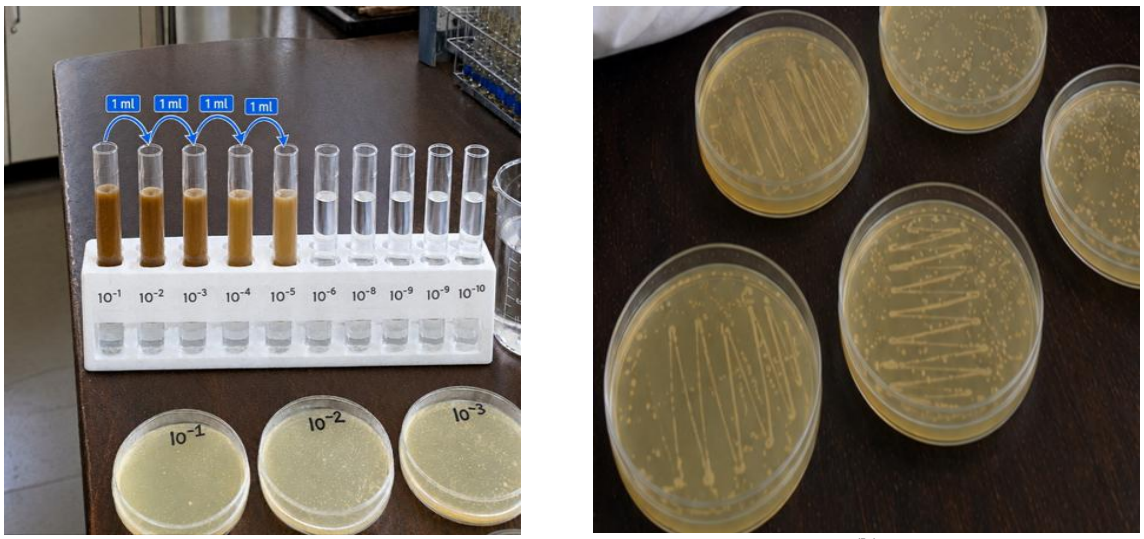
2.5.1 Effects of Microdosing Selected Fertilizers and Lime on Fungal and Bacterial Populations in Acidic Soils of the Meru Highland

Soil samples for bacterial and fungal analysis were collected at the tasselling stage of maize in each of the two cropping seasons. Four soil cores were randomly collected from the 0–30 cm soil depth from each experimental plot using a clean, sterilized soil auger, while avoiding the plot margins. The four cores from each plot were thoroughly mixed to form one composite sample representing the respective treatment and replicate. Approximately 100 g of fresh composite soil was placed in a clean, labelled sample bag and transported to the laboratory immediately after collection. The samples were maintained in a fresh condition and stored at approximately 4°C until microbial analysis to minimize changes in microbial populations before enumeration. Soil bacterial and fungal populations were determined using the serial dilution and spread-plate technique following standard microbiological procedures (Küster & Williams, 1964; Thatcher & Clark, 1968). For each treatment and replicate, 1 g of fresh soil was aseptically transferred into a sterile test tube containing 9 mL of sterile distilled water and thoroughly mixed to obtain the initial 10^{-1} soil suspension. A series of tenfold serial dilutions was then prepared by transferring 1 mL of the suspension into successive tubes containing 9 mL of sterile distilled water (Fig. 3). Dilutions were prepared up to 10^{-5} for bacterial enumeration and 10^{-4} for fungal enumeration. From the appropriate dilution, 0.1 mL was aseptically transferred and evenly spread onto sterile culture plates (Fig. 3) containing Nutrient Agar (NA) for bacterial enumeration and Potato Dextrose Agar (PDA) supplemented with tetracycline for fungal enumeration. Tetracycline was added to the PDA medium to suppress bacterial growth and facilitate fungal

isolation. The culture media were prepared according to the manufacturer's instructions and sterilized by autoclaving at 120°C for 20 minutes before use. The PDA plates were incubated at 25°C for five days to allow adequate fungal growth and development of distinct colonies, while the Nutrient Agar plates were incubated at 37°C for 24 hours to allow bacterial colonies to develop. Following incubation, visible colonies were counted using a digital colony counter. Plates containing 30-300 colonies were considered suitable for enumeration to improve the reliability of the microbial counts. Microbial populations were expressed as colony-forming units per gram (CFU g^{-1}) of oven dry soil. The microbial population was calculated using the equation:

$CFU\ g^{-1} = N \times D/V$

where; CFU g^{-1} = colony-forming units per gram of soil, N = average number of colonies counted on the plate, D = dilution factor, V = volume of diluted sample plated (mL).



(a) (b)

Fig. 3. (a) Laboratory serial dilution; (b) Planting procedures

2.5.2 Data Analysis

Data on soil bacterial and fungal populations were subjected to analysis of variance (ANOVA) using a split-plot model consistent with the experimental design. Statistical analyses were performed using R software version 4.5.1. Prior to analysis, microbial population counts expressed as CFU g^{-1} were $\log_{10}$-transformed to improve data distribution, stabilize variance, and satisfy the assumptions of normality and homogeneity of variances required for ANOVA. The transformed data were used for statistical testing, while microbial population values were presented in their original units where appropriate for ease of interpretation. The effects of season, microdosing strategy, soil fertility management treatments, and their interactions were evaluated using the appropriate error terms for the split-plot arrangement. Where significant treatment effects were detected, means were separated using Tukey's Honestly Significant Difference (HSD) test at the 5% significance level**.** Results were summarized using treatment means, coefficient of variation (CV%), and HSD values. Seasonal means and pooled means across the two seasons were presented to facilitate assessment of both seasonal variation and treatment effects. The statistical model used for analysis of the split-plot experiment is expressed as:

$$Yijkl=\mu + \alpha i + \beta j + \gamma j\ (ij) + \delta k + (\alpha\delta)ik + \epsilon ijkl$$

Where:

Yijkl: overall response (from replications, microdosing fertilizers and lime)
μ: overall mean
αi: effects of main plot factor microdosing vs. no microdosing (1, 2)
βj: Effects of the blocks/replicates (1,2,3)
γj(ij): Random error for the main plot (replication by microdosing)
δk: Effects of subplot (fertilizers and lime combinations 1.2.3…18)
(αδ)ik: Interaction effect between microdosing (main plot) and fertilizer-lime combinations(subplots)
ϵijkl: Random (residual) error associated with subplot treatments (fertilizer and lime combinations) assumed to be independently and normally distributed with mean zero and constant variance

Fungi and Bacteria population data were $\log_{10}$-transformed using the formula $\log_{10}\ \text{CFU}\ g^{-1}\ \text{soil} = \log_{10}(X)$, where X represents the microbial population expressed as CFU g^{-1} soil

3. Results and Discussions

3.1 Selected Initial Soil Characteristics

The initial soil was strongly acidic, with a pH of 4.8 and exchangeable acidity of 2.82 cmol (+) kg^{-1} as shown in Table 1. It also had relatively low total nitrogen (0.13%), available phosphorus (11.7 mg kg^{-1}) and organic carbon (1.2%), suggesting conditions that could constrain microbial growth. The initial bacterial population was 2.31×10^4 CFU g^{-1} soil**,** whereas the fungal population was 3.20×10^5 CFU g^{-1} soil, representing a fungal population approximately 14-fold greater than the bacterial population. The greater fungal than bacterial population is consistent with findings that bacteria are generally more sensitive to soil acidity than fungi. These findings were consistent with Rousk *et al.* (2010), who, using an arable soil pH gradient of 4.0-8.3, reported that bacterial abundance and diversity increased with increasing pH, whereas fungal abundance was largely unaffected by pH and fungal diversity was only weakly related to pH. Similarly, Wang *et al*. (2022) found that increasing soil acidification significantly reduced bacterial α-diversity, while fungal diversity showed little response, indicating greater sensitivity of bacterial communities to soil acidification.

Table 1. Initial soil Characteristics

Soil properties	Value
Soil pH	4.8
Total nitrogen %	0.13
Available phosphorous ppm	11.7
Exchangeable acidity (cmol (+)/kg	2.82
Total organic carbon %	1.2
CEC (cmol(+)/kg)	20.03
Bacteria density CFU of soil	2.31 x 104
Fungi CFU of soil	3.20 x 105

The present results are also supported by the recent meta-analysis of Li *et al*. (2025)**,** which synthesized 821 observations from 121 studies and found that soil acidification significantly reduced bacterial diversity, while fungal diversity was comparatively stable. These findings provide a plausible explanation for the substantially higher fungal population observed in the present soil at pH 4.8. However, contrary findings indicate that fungi are not universally insensitive to soil acidity. Carrino-Kyker *et al.* (2020) reported that reversal of soil acidification and phosphorus limitation produced significant and distinct responses in both bacterial and fungal communities, demonstrating that fungal communities can also respond to changes in soil pH and nutrient availability. Thus, the higher fungal population observed in the present study should not be attributed to pH alone. Differences in soil type, nutrient availability, organic carbon, microbial taxa and management history can modify microbial responses to acidity.

3.2 Effects of Microdosing Selected Fertilizers and Lime on Fungal Populations in Acidic Soils of the Meru Highland

The pooled ANOVA in Table 2 shows that fungal populations were significantly influenced by microdosing and the different fertilizer and lime treatments, while season did not have a significant effect. Microdosing had a significant effect on fungal populations ($P < 0.01$), and the fertilizer and lime treatments also differed significantly ($P < 0.001$). The significant microdosing × treatment interaction ($P < 0.001$) indicates that fungal populations responded differently to the various fertilizer and lime combinations depending on whether microdosing was applied. In contrast, the interactions involving season were not significant at the 5% level, suggesting that the effects of microdosing and the different treatments were generally consistent across the two seasons.

The fungal population varied considerably among fertilizer, lime and organic amendment treatments, with microdosing (MD) generally recording higher fungal populations than the corresponding non-microdosed (NMD) treatments. The highest fungal populations were recorded where lime, mineral fertilizer and FYM were combined**.** In Season 1, MD CALCITE + NPK 23:23:0 + FYM produced the highest value, 1.36×10^7 CFU g^{-1} (7.134 Log_{10} CFU g^{-1}), which was significantly ($P < 0.05$) higher than the corresponding no-microdosing treatment, NMD CALCITE + NPK 23:23:0 + FYM, which recorded 1.28×10^7 CFU g^{-1} (7.107 Log_{10} CFU g^{-1}). The same trend was maintained in Season 2, where MD CALCITE + NPK 23:23:0 + FYM recorded fungal populations of 1.41×10^7 CFU g^{-1} **(**7.149 Log_{10} CFU g^{-1}) and was significantly ($P < 0.05$) higher than NMD CALCITE + NPK 23:23:0 + FYM, which recorded 1.33×10^7 CFU g^{-1} (7.124 Log_{10} CFU g^{-1}). The control treatments consistently recorded the lowest fungal populations **i**n both seasons. In Season 1, the MD control had 3.97×10^6 CFU g^{-1} soil (6.598 Log_{10} CFU g^{-1})**,** while the corresponding NMD control recorded 4.00×10^6 CFU g^{-1} (6.602 Log_{10} CFU g^{-1})**.** The two controls were statistically similar ($P < 0.05$)in seasons. A similar pattern was observed in Season 2, where MD and NMD controls recorded statistically similar ($P < 0.05$) fungal populations of 4.00×10^6 and 4.07×10^6 CFU g^{-1} soil, corresponding to 6.602 and 6.609 Log_{10} CFU g^{-1}**,** respectively. The higher fungal population under MD may be attributed to the complementary effects of localized nutrient supply, organic carbon addition and amelioration of soil acidity**,** creating a favourable microsite for fungal growth. Both the MD control and NMD control recorded significantly lower fungal populations compared with the amended treatments, indicating that the absence of fertilizer, FYM and lime limited the conditions required for greater fungal proliferation. The initial soil conditions provide an important explanation for this response. The soil had a pH of 4.8 and exchangeable acidity of 2.82 cmol (+) kg^{-1}**,** indicating substantial acidity, while total N **(**0.13%**)** and total organic carbon **(**1.2%**)** were relatively low. Although fungi are generally more tolerant of acidic conditions than many bacteria and can remain active under low soil pH, severe acidity combined with limited nutrient and carbon availability can constrain fungal growth. Thus, the low fungal populations observed in both controls were likely associated

not simply with soil acidity but with the combined effect of acidic conditions and low supplies of N and organic carbon**,** which restricted the substrates and nutrients available for fungal proliferation. This finding is consistent with Wang *et al.* (2018)**,** who reported that fertilizer application increased fungal abundance compared with unfertilized soil and that the response was particularly pronounced where organic manure was included. The authors attributed the response partly to increases in soil organic C, total N and available P, which are important resources for soil microorganisms. The present study therefore agrees with these findings, since the unfertilized controls had the lowest fungal populations under conditions of relatively low soil N and organic C, whereas treatments receiving FYM and mineral fertilizers provided additional substrates and nutrients that could support fungal proliferation. The organic amendment likely supplied carbon substrates while the mineral fertilizer supplied readily available nutrients, resulting in a more favourable resource environment for microbial growth.

Table 2. Effects of microdosing selected fertilizers and lime on fungal populations across the two maize cropping seasons

Season	MD/NMD	Treatment	Fungal population (CFU g^{-1} soil)	Fungal population (Log_{10} CFU g^{-1} soil)
1	MD	CONTROL	3.97×10^{06y}	6.598^{w}
	NMD	CONTROL	3.9700×10^{06y}	6.598^{w}
	MD	DOLOMITELIME	4.77×10^{06vw}	6.678^{tu}
	NMD	DOLOMITELIME	4.43×10^{06x}	6.647^{v}
	MD	CALCITELIME	5.37×10^{06t}	6.730^{rs}
	NMD	CALCITELIME	4.70×10^{06vw}	6.672^{u}
	MD	FYM	8.67×10^{06l}	6.938^{jk}
	NMD	FYM	7.73×10^{06n}	6.888^{l}
	MD	NPK23:23:0	5.87×10^{06rs}	6.768^{pq}
	NMD	NPK23:23:0	5.00×10^{06uv}	6.699^{t}
	MD	DAP	5.53×10^{06t}	6.743^{rs}
	NMD	DAP	4.53×10^{06w}	6.656^{uv}
	MD	CALCITELIME+FYM	1.20×10^{07fg}	7.079^{ef}
	NMD	CALCITELIME+FYM	1.11×10^{07h}	7.047^{g}
	MD	DOLOMITELIME+FYM	1.10×10^{07h}	7.041^{g}
	NMD	DOLOMITELIME+FYM	1.04×10^{07i}	7.017^{h}
	MD	FYM+NPK23:23:0	9.60×10^{06j}	6.982^{t}
	NMD	FYM+NPK23:23:0	8.80×10^{06kl}	6.944^{j}
	MD	FYM+DAP	9.10×10^{06k}	6.959^{tj}
	NMD	FYM+DAP	8.30×10^{06m}	6.919^{k}
	MD	CALCITE+NPK23:23:0	7.10×10^{06o}	6.851^{m}
	NMD	CALCITE+NPK23:23:0	6.30×10^{06pq}	6.799^{no}
	MD	CALCITE+DAP	6.80×10^{06o}	6.832^{m}
	NMD	CALCITE+DAP	6.00×10^{qr}	6.778^{op}
	MD	DOLOMITE+DAP	6.10×10^{06o}	6.785^{nop}
	NMD	DOLOMITE+DAP	5.30×10^{06tu}	6.724^{s}
	MD	DOLOMITE+NPK23:23:0	6.40×10^{06p}	6.806^{n}
	NMD	DOLOMITE+NPK23:23:0	5.60×10^{06st}	6.748^{qr}
	MD	DOLOMITE+DAP+FYM	1.26×10^{07d}	7.100^{cde}
	NMD	DOLOMITE+DAP+FYM	1.18×10^{07g}	7.072^{f}
	MD	DOLOMITE+NPK23:23:0+FYM	1.33×10^{07ab}	7.124^{ab}
	NMD	DOLOMITE+NPK23:23:0+FYM	1.25×10^{07de}	7.097^{cde}
	MD	CALCITE+DAP+FYM	1.30×10^{07bc}	7.114^{abc}
	NMD	CALCITE+DAP+FYM	1.22×10^{07ef}	7.086^{def}
	MD	CALCITE+NPK23:23:0+FYM	1.36×10^{07a}	7.134^{a}
	NMD	CALCITE+NPK23:23:0+FYM	1.28×10^{07cd}	7.107^{bcd}
2	MD	CONTROL	4.00×10^{06z}	6.602^{x}
	NMD	CONTROL	4.07×10^{06z}	6.609^{wx}
	MD	DOLOMITELIME	5.00×10^{06x}	6.699^{u}
	NMD	DOLOMITELIME	4.20×10^{06yz}	6.623^{w}
	MD	CALCITELIME	5.30×10^{06vw}	6.724^{st}
	NMD	CALCITELIME	4.43×10^{06y}	6.647^{v}
	MD	FYM	9.10×10^{06l}	6.959^{jk}
	NMD	FYM	8.30×10^{06n}	6.919^{l}
	MD	NPK23:23:0	6.30×10^{06t}	6.799^{pq}
	NMD	NPK23:23:0	5.50×10^{06v}	6.740^{s}
	MD	DAP	5.90×10^{06u}	6.771^{r}
	NMD	DAP	5.10×10^{06w}	6.707^{tu}
	MD	CALCITELIME+FYM	1.25×10^{07fg}	7.097^{ef}
	NMD	CALCITELIME+FYM	1.17×10^{07h}	7.068^{g}
	MD	DOLOMITELIME+FYM	1.17×10^{07h}	7.068^{g}
	NMD	DOLOMITELIME+FYM	1.09×10^{07i}	7.037^{h}
	MD	FYM+NPK23:23:0	1.02×10^{07j}	7.007^{i}

Season	MD/NMD	Treatment	Fungal population (CFU g^{-1} soil)	Fungal population (Log_{10} CFU g^{-1} soil)
	NMD	FYM+NPK23:23:0	9.30×10^{06kl}	6.968^{j}
	MD	FYM+DAP	9.53×10^{06k}	6.979^{j}
	NMD	FYM+DAP	8.80×10^{06m}	6.944^{k}
	MD	CALCITE+NPK23:23:0	7.60×10^{06o}	6.881^{m}
	NMD	CALCITE+NPK23:23:0	6.80×10^{06qr}	6.832^{no}
	MD	CALCITE+DAP	7.30×10^{06p}	6.863^{m}
	NMD	CALCITE+DAP	6.50×10^{06st}	6.813^{op}
	MD	DOLOMITE+DAP	6.60×10^{06rs}	6.819^{nop}
	NMD	DOLOMITE+DAP	5.80×10^{06u}	6.763^{r}
	MD	DOLOMITE+NPK23:23:0	6.90×10^{06q}	6.839^{n}
	NMD	DOLOMITE+NPK23:23:0	6.03×10^{06u}	6.781^{qr}
	MD	DOLOMITE+DAP+FYM	1.31×10^{07d}	7.117^{cde}
	NMD	DOLOMITE+DAP+FYM	1.23×10^{07g}	7.090^{f}
	MD	DOLOMITE+NPK23:23:0+FYM	1.38×10^{07b}	7.140^{ab}
	NMD	DOLOMITE+NPK23:23:0+FYM	1.30×10^{075e}	7.114^{cde}
	MD	CALCITE+DAP+FYM	1.35×10^{07c}	7.130^{abc}
	NMD	CALCITE+DAP+FYM	1.27×10^{07f}	7.104^{def}
	MD	CALCITE+NPK23:23:0+FYM	1.41×10^{07a}	7.149^{a}
	NMD	CALCITE+NPK23:23:0+FYM	1.33×10^{07cd}	7.124^{bcd}
		Mean	8.44×10^{06}	6.893
		CV (%)	6.22	3.98
		HSD ($P < 0.05$)	1.61×10^{05}	0.015

Key: Values are means within each season and response variables. Means followed by the same superscript letters $^{(a, b, c...)}$ are not significantly different according to Tukey's HSD test at P <0.05. CFU = colony-forming units; Log_{10} = base-10 logarithm

Table 3. Pooled split-plot analysis of variance for fungal populations

Source	DF	Fungal population (CFU g^{-1} soil) F	Fungal population (CFU g^{-1} soil) P	Fungal population (Log_{10} CFU g^{-1} soil) F	Fungal population (Log_{10} CFU g^{-1} soil) P
Season (S)	1	65.081	0.107	11.084	0.0621
Replication within Season	4	21.901	0.2116	9.893	0.081
Microdosing (M)	1	43.98	<0.01	231.67	<0.01
S × M	1	116.178	0.324	150.081	0.916
Error A	4	—	—	—	—
Treatment (T)	17	31.345	<0.001	59.341	<0.001
S × T	17	209.300	0.347	82.904	0.0808
M × T	17	69.022	<0.001	182.501	<0.001
S × M × T	17	143.081	0.108	0.432	0.891
Error B	136	—	—	—	—

DF = degrees of freedom; S = season; M = microdosing; T = Fertilizers and lime amendments. P-values below 0.05 indicate statistically significant effects

The significantly greater fungal populations under MD than the corresponding NMD treatments (Table 2; Fig. 4) may further be attributed to the localized application and split timing of fertilizer under microdosing. Concentrating the inputs near the maize root zone may have created nutrient-rich microsites where nutrients and organic substrates were more readily available to fungi associated with the rhizosphere. The lower total fertilizer quantity under MD may also have reduced the intensity of fertilizer-induced chemical changes in the wider soil volume, while split application provided nutrients over a longer period and reduced the likelihood of a large instantaneous nutrient concentration. The Kenyan study by Kisinyo *et al*. (2015) similarly demonstrated the potential of microdosing as a strategy for applying smaller quantities of fertilizer and lime to acid soils while improving nutrient availability and maize performance. Thus, the present finding extends the usefulness of microdosing from crop nutrient management to the creation of localized soil conditions that may also favour microbial proliferation. The beneficial response to lime in the present study should, however, be interpreted cautiously because the response of fungi to liming is not consistently positive across all soils and fungal groups. Yin *et al.* (2021) found that liming did not significantly alter overall fungal diversity or richness in wheat soils, although particular fungal taxa increased or decreased following lime application. Similarly, a recent review concluded that liming can increase, decrease or have no effect on fungal abundance, depending on soil conditions and fungal taxa. These findings contrast with the present study to some extent, where lime-containing treatments generally supported greater fungal populations than the controls. The difference may be related to the initially strongly acidic conditions of the present soil (pH 4.8), where amelioration of acidity may have provided a stronger benefit than in soils where fungi were already adapted to less restrictive chemical conditions. Further support for this interpretation comes from research conducted in strongly acidic Ultisols. Ye *et al.* (2020) reported that soil pH and organic C were among the most important factors determining fungal community structure and found that NPK 23:23:0 combined with lime or manure altered fungal community composition compared with the unfertilized control. This is broadly consistent with the present findings, particularly the greater fungal populations recorded where mineral fertilizer was combined with lime and FYM. However, the same study found that long-term fertilization did not necessarily increase

overall fungal diversity in strongly acidic soil, demonstrating that improvements in soil chemical conditions may alter fungal community composition without necessarily increasing all measures of fungal diversity.

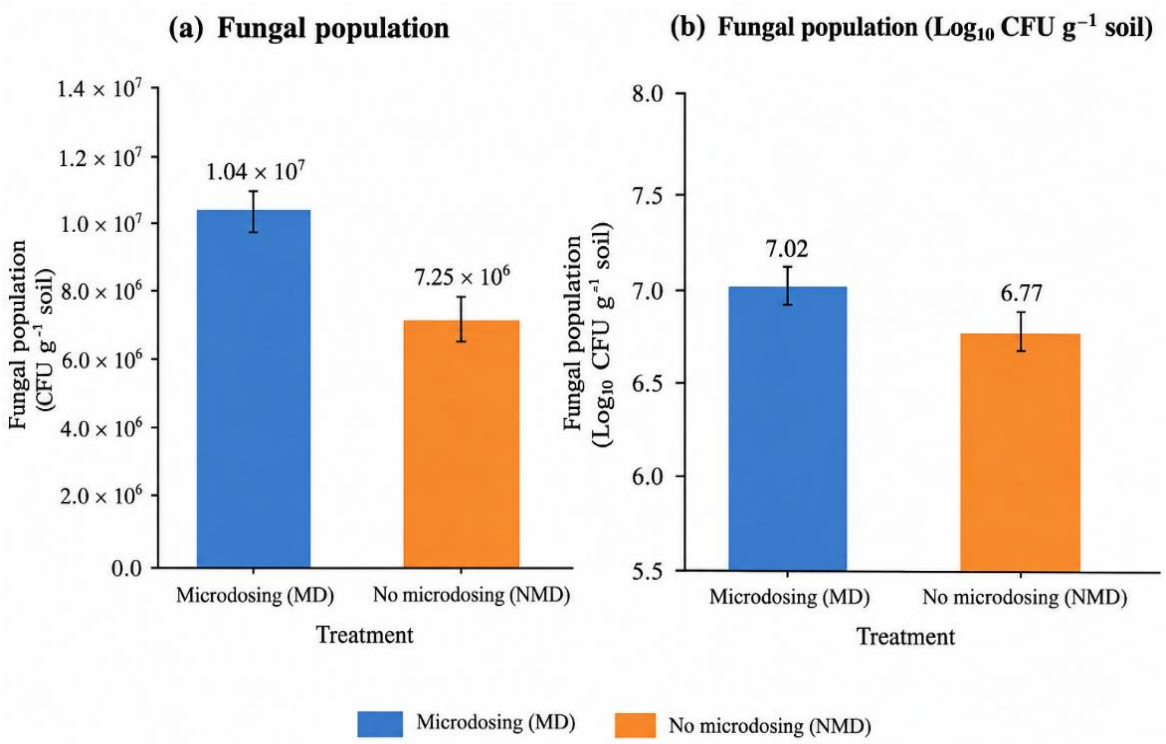


Fig. 4. Effects of microdosing and no microdosing on fungal populations expressed as a) CFU g^{-1} soil; b) corresponding log_{10}-transformed values

The comparatively higher fungal populations in treatments receiving FYM, lime and mineral fertilizer**,** particularly under MD, can therefore be interpreted as a consequence of the complementary effects of the three inputs. FYM provided organic C and additional nutrients, mineral fertilizer supplied readily available N and P, while lime reduced acidity and potentially alleviated acidity related constraints on microbial activity. The localized placement of these inputs under MD may have further concentrated these resources in the maize rhizosphere. This interpretation is supported by Wang *et al.* (2018**)**, who found stronger fungal responses under manure and manure mineral fertilizer combinations than under mineral fertilizer alone, and by studies showing that organic amendments can counteract some of the effects of mineral fertilization on fungal communities. The comparatively higher fungal populations observed under the NPK23:23:0 treatments compared with the corresponding DAP treatments may partly be attributed to differences in the forms of nitrogen supplied and their subsequent transformation in the soil. The NPK23:23:0 fertilizer formulation contained both ammonium- and nitrate-N, such that a proportion of the applied N was supplied directly as nitrate and did not require nitrification before plant uptake**.** This may have reduced the amount of ammonium-N undergoing nitrification and, consequently, the associated release of H^+ ions into the soil. In contrast, DAP supplied nitrogen predominantly in the ammonium form, which is subject to nitrification following application. The nitrification of NH_4^+ releases H^+ ions and can therefore contribute to increased soil acidity. Thus, the greater proportion of N supplied in a form that does not require nitrification under NPK 23:23:0 may have resulted in lower nitrification-related acidification**,** creating relatively more favourable soil chemical conditions for fungal proliferation than under the corresponding DAP treatments.

The present results agree with studies showing that nutrient and organic carbon enrichment can increase fungal abundance, particularly where organic and mineral fertilizers are combined. They also partly contrast with studies reporting weak or inconsistent fungal responses to liming, emphasizing that the effect of lime is context-dependent. In the present acidic Nitisol, the combination of improved nutrient and carbon availability, reduced acidity and localized nutrient supply under microdosing appears to have created more favourable conditions for fungal proliferation than those prevailing in the untreated MD and NMD controls. The results, however, differ from studies in which fungal communities showed limited response to liming. Yin *et al*. (2021) reported that liming did not significantly alter overall fungal richness and diversity in agricultural soils, while Li *et al.* (2021) found that fungal communities were less responsive to liming than prokaryotic communities. These apparently contrary findings may reflect differences in the microbial parameters measured. The present study quantified culturable fungal populations using CFU, whereas the cited studies primarily evaluated fungal diversity and community composition. Lime may therefore increase the number of culturable fungi without necessarily producing a comparable increase in overall fungal diversity. Differences in soil type, lime rate, organic matter availability and fungal taxa may also account for variation among studies. These findings are consistent with Barcelos *et al.* (2024**)**, who found that lime favoured several fungal and N cycling groups in a tropical soil, with soil pH being the strongest factor determining microbial structure. They are also consistent with Li *et al.* (2021**)**, who found that pH and soil organic matter jointly explained fungal community variation under lime application.

3.3 Effects of Microdosing Selected Fertilizers and Lime on Bacterial Populations in Acidic Soils of Kariene Highland

Season had no significant effect on bacterial populations ($P > 0.05$), indicating that bacterial populations were relatively consistent across the two seasons in Table 5. However**,** microdosing significantly affected bacterial populations ($p < 0.01$), while fertilizer and lime treatments had a highly significant effect ($p < 0.001$). The microdosing × treatment interaction was also highly significant ($p < 0.001$), indicating that the response of bacterial populations to fertilizer and lime amendments depended on whether microdosing was applied. In contrast, season × microdosing, season × treatment, and season × microdosing × treatment interactions were not significant ($P > 0.05$), indicating that treatment responses were generally consistent across seasons as shown in Table 5. The treatment means in Table 4 show a consistent pattern across the two maize cropping seasons. In both seasons, the highest bacterial population was recorded under MD, with MD CALCITE + NPK 23:23:0 + FYM recording bacterial populations of 1.89×10^8 CFU g^{-1} soil ($Log_{10} = 8.276$) and 1.95×10^8 CFU g^{-1} soil ($Log_{10} = 8.289$) in Seasons 1 and 2, respectively. These values were significantly ($p<0.05$) higher than the corresponding NMD CALCITE + NPK 23:23:0 + FYM treatment, which recorded 1.81×10^8 CFU g^{-1} soil ($Log_{10} = 8.258$) and 1.84×10^8 CFU g^{-1} soil ($Log_{10} = 8.264$) in Seasons 1 and 2, respectively. In Season 1, MD and NMD controls recorded 3.29×10^7 and 3.30×10^7 CFU g^{-1} soil, corresponding to Log_{10} values of 7.514 and 7.519, respectively. In Season 2, both MD and NMD controls recorded 3.23×10^7 CFU g^{-1} soil ($Log_{10} = 7.510$). MD and NMD controls were not significantly different from each other ($P > 0.05$). From these results, MD CALCITE + NPK 23:23:0 + FYM recorded approximately 5.7–6.0 times more culturable bacteria than the controls, with Log_{10} differences of approximately 0.76–0.78 log units. The superscript separation between the control and the best treatment confirms that this difference was statistically significant at $P < 0.05$. Thus, microdosing increased the bacterial population in the best-performing treatment by approximately 4.4% in Season 1 and 6.0% in Season 2 relative to NMD. The higher bacterial population under MD may be associated with the more targeted and temporally distributed supply of nutrients. In the present experiment, MD involved applying 75% of the recommended fertilizer rate in two applications, whereas NMD received the full recommended amount at planting. The smaller, split applications may have reduced the likelihood of excessive nutrient concentrations immediately after application and maintained nutrient availability closer to periods of active crop and microbial demand.

Table 4. Effects of microdosing selected fertilizers and lime on bacterial populations across the two maize cropping seasons

Season	MD/NMD	Treatment	Bacterial population (CFU g^{-1} soil)	Bacterial population (Log_{10} CFU g^{-1} soil)
1	MD	CONTROL	3.29×10^{07x}	7.514^{w}
	NMD	CONTROL	3.30×10^{07x}	7.519^{w}
	MD	DOLOMITELIME	6.10×10^{07u}	7.785^{t}
	NMD	DOLOMITELIME	5.30×10^{07w}	7.724^{v}
	MD	CALCITELIME	6.57×10^{07u}	7.817^{s}
	NMD	CALCITELIME	5.57×10^{07v}	7.746^{u}
	MD	FYM	1.22×10^{08k}	8.086^{j}
	NMD	FYM	1.14×10^{08l}	8.057^{k}
	MD	NPK23:23:0	8.20×0^{07qr}	7.914^{pq}
	NMD	NPK23:23:0	7.40×10^{07s}	7.869^{r}
	MD	DAP	7.60×10^{07s}	7.881^{r}
	NMD	DAP	6.80×10^{07t}	7.832^{s}
	MD	CALCITELIME+FYM	1.68×10^{08f}	8.225^{e}
	NMD	CALCITELIME+FYM	1.60×10^{08g}	8.204^{f}
	MD	DOLOMITELIME+FYM	1.58×10^{08g}	8.200^{f}
	NMD	DOLOMITELIME+FYM	1.50×10^{08h}	8.176^{g}
	MD	FYM+NPK23:23:0	1.36×10^{08i}	8.134^{h}
	NMD	FYM+NPK23:23:0	1.28×10^{08j}	8.107^{i}
	MD	FYM+DAP	1.29×10^{08j}	8.111^{i}
	NMD	FYM+DAP	1.21×10^{08k}	8.083^{j}
	MD	CALCITE+NPK23:23:0	1.01×10^{08m}	8.004^{l}
	NMD	CALCITE+NPK23:23:0	9.30×10^{07no}	7.968^{m}
	MD	CALCITE+DAP	9.50×10^{07n}	7.978^{m}
	NMD	CALCITE+DAP	8.70×10^{07p}	7.939^{no}
	MD	DOLOMITE+DAP	8.80×10^{07p}	7.944^{n}
	NMD	DOLOMITE+DAP	8.00×10^{07r}	7.903^{q}
	MD	DOLOMITE+NPK23:23:0	9.20×10^{07o}	7.964^{m}
	NMD	DOLOMITE+NPK23:23:0	8.40×10^{07q}	7.924^{op}
	MD	DOLOMITE+DAP+FYM	1.76×10^{08d}	8.245^{cd}
	NMD	DOLOMITE+DAP+FYM	1.68×10^{08f}	8.225^{e}
	MD	DOLOMITE+NPK23:23:0+FYM	1.84×10^{08b}	8.265^{ab}
	NMD	DOLOMITE+NPK23:23:0+FYM	1.76×10^{08d}	8.245^{cd}
	MD	CALCITE+DAP+FYM	1.81×10^{08c}	8.258^{bc}
	NMD	CALCITE+DAP+FYM	1.73×10^{08e}	8.238^{de}
	MD	CALCITE+NPK23:23:0+FYM	1.89×10^{08a}	8.276^{a}
	NMD	CALCITE+NPK23:23:0+FYM	1.81×10^{08c}	8.258^{bc}

Season	MD/NMD	Treatment	Bacterial population (CFU g^{-1} soil)	Bacterial population (Log_{10} CFU g^{-1} soil)
2	MD	CONTROL	3.23×10^{07x}	7.510^{v}
	NMD	CONTROL	3.23×10^{07x}	7.510^{v}
	MD	DOLOMITELIME	6.60×10^{07u}	7.819^{s}
	NMD	DOLOMITELIME	5.80×10^{07w}	7.763^{u}
	MD	CALCITELIME	7.00×10^{07t}	7.845^{r}
	NMD	CALCITELIME	6.20×10^{07v}	7.792^{t}
	MD	FYM	1.27×10^{08k}	8.104^{j}
	NMD	FYM	1.19×10^{08l}	8.076^{k}
	MD	NPK23:23:0	8.57×10^{07r}	7.933^{p}
	NMD	NPK23:23:0	7.13×10^{07t}	7.853^{r}
	MD	DAP	7.50×10^{07s}	7.875^{q}
	NMD	DAP	6.67×10^{07u}	7.824^{s}
	MD	CALCITELIME+FYM	1.73×10^{08f}	8.238^{e}
	NMD	CALCITELIME+FYM	1.65×10^{08g}	8.217^{f}
	MD	DOLOMITELIME+FYM	1.63×10^{08g}	8.212^{f}
	NMD	DOLOMITELIME+FYM	1.55×10^{08h}	8.190^{g}
	MD	FYM+NPK23:23:0	1.41×10^{08i}	8.149^{h}
	NMD	FYM+NPK23:23:0	1.33×10^{08j}	8.124^{i}
	MD	FYM+DAP	1.34×10^{08j}	8.127^{i}
	NMD	FYM+DAP	1.26×10^{08k}	8.100^{j}
	MD	CALCITE+NPK23:23:0	1.06×10^{08m}	8.025^{l}
	NMD	CALCITE+NPK23:23:0	9.83×10^{07no}	7.993^{m}
	MD	CALCITE+DAP	1.00×10^{08n}	8.001^{m}
	NMD	CALCITE+DAP	9.20×10^{07p}	7.964^{no}
	MD	DOLOMITE+DAP	9.30×10^{07p}	7.968^{n}
	NMD	DOLOMITE+DAP	8.50×10^{07r}	7.929^{p}
	MD	DOLOMITE+NPK23:23:0	9.70×10^{07o}	7.987^{m}
	NMD	DOLOMITE+NPK23:23:0	8.90×10^{07q}	7.949^{o}
	MD	DOLOMITE+DAP+FYM	1.81×10^{08d}	8.258^{cd}
	NMD	DOLOMITE+DAP+FYM	1.74×10^{08f}	8.240^{e}
	MD	DOLOMITE+NPK23:23:0+FYM	1.90×10^{08b}	8.279^{ab}
	NMD	DOLOMITE+NPK23:23:0+FYM	1.81×10^{08d}	8.258^{cd}
	MD	CALCITE+DAP+FYM	1.87×10^{08c}	8.271^{bc}
	NMD	CALCITE+DAP+FYM	1.78×10^{08e}	8.250^{de}
	MD	CALCITE+NPK23:23:0+FYM	1.95×10^{08a}	8.289^{a}
	NMD	CALCITE+NPK23:23:0+FYM	1.84×10^{08d}	8.264^{bcd}
		Mean	1.17×10^{08}	8.025
		CV (%)	4.72	3.986
		HSD ($P < 0.05$)	5.00×10^{5}	0.011

Key: Values are means. Within each season and response variable, superscript letters (a, b, c…) are not significantly different according to Tukey's HSD test at P <0.05. CFU = colony-forming units; Log_{10} = base-10 logarithm

Table 5. Pooled split-plot analysis of variance for bacterial populations

Source	DF	Bacterial population (CFU g^{-1} soil) F	Bacterial population (CFU g^{-1} soil) P	Bacterial population (Log_{10} CFU g^{-1} soil) F	Bacterial population (Log_{10} CFU g^{-1} soil) P
Season (S)	1	6.732	0.9315	164.892	0.0611
Replication within Season	4	33.654	0.3123	19.721	0.651
Microdosing (M)	1	117.081	<0.01	87.451	<0.01
S × M	1	11.085	0.071	126.905	0.922
Error A	4	—	—	—	—
Treatment (T)	17	27.082	<0.001	182.33	<0.001
S × T	17	52.098	0.817	278.013	0.911
M × T	17	135.061	<0.001	46.3231	<0.001
S × M × T	17	13.083	9.874	4.905	0.721
Error B	136	—	—	—	—

DF = degrees of freedom; S = season; M = microdosing; T = Fertilizers and lime amendments. P-values below 0.05 indicate statistically significant effects

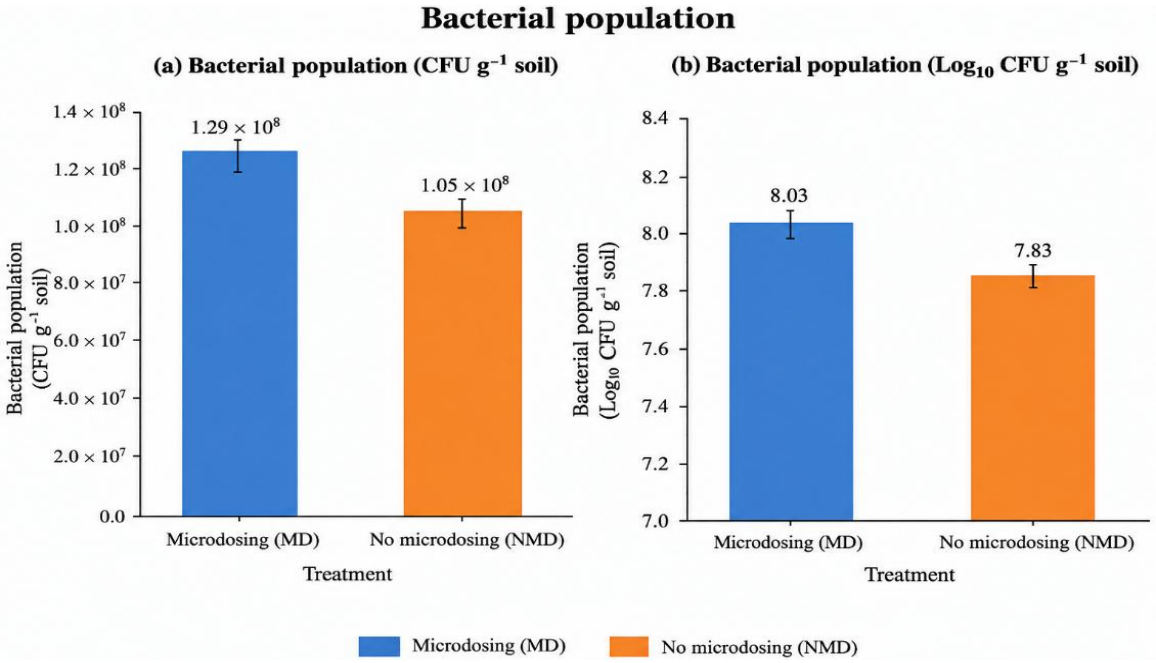


Fig. 5. Effects of microdosing and no microdosing on bacteria populations expressed as a) CFU g⁻¹ soil; b) corresponding log₁₀-transformed values

Farmyard manure provides readily decomposable organic substrates and nutrients that can stimulate heterotrophic bacterial growth, while calcite supplies Ca and neutralizes soil acidity. The mineral fertilizer provides readily available N and P to support microbial growth and plant development. Evidence from manure meta-analyses indicates that manure application can substantially increase microbial biomass and accelerate C and N cycling, while integrated applications of mineral fertilizer and organic inputs can improve microbial functioning. The results are also consistent with observations from acidic soils in Kenya. Gitari (2014) reported substantially greater bacterial and fungal populations where manure and agricultural lime were combined compared with the control, demonstrating the potential of simultaneously improving soil chemical conditions and supplying organic substrates to stimulate soil microorganisms. Similarly, long-term studies in acidic soils have shown that combining NPK with FYM and/or lime improves soil biological properties. In particular, NPK + lime increased bacterial populations by 145% compared with NPK alone, while NPK + FYM significantly improved microbial functions and soil fertility (Vishwanath *et al.,* 2022).

The higher bacterial populations under MD than NMD (Table 4; Fig. 5), despite the lower total quantity of inputs, can be scientifically explained by improved nutrient use efficiency and localized nutrient concentration. Microdosing places fertilizer and lime in a relatively small volume of soil close to the crop root zone. Consequently, the nutrients are less widely dispersed and can produce localized nutrient-rich microsites. These microsites can simultaneously serve the plant and associated microbial community. This interpretation is particularly relevant to the initial soil condition shown in Table 1. The initial pH of 4.8 and exchangeable acidity of 2.82 cmol (+) kg^{-1} indicate an acidic environment in which microbial activity, particularly that of many bacterial groups, can be constrained. At the same time, available P was only 11.7 mg kg^{-1}, total N was 0.13%, and TOC was 1.2%. Therefore, the initial bacterial population of 2.31 × 10^4 CFU g^{-1} was likely constrained by a combination of acidity, nutrient deficiency and limited readily available carbon. Localized application of lime and fertilizer under MD could have alleviated these constraints immediately around the application zone rather than attempting to modify the entire soil volume.

The low bacterial population in the controls can therefore be related to the initially poor soil fertility conditions, particularly the acidic soil reaction and relatively low concentrations of organic C and N. Bacteria are generally sensitive to soil chemical conditions, and soil pH is an important regulator of bacterial abundance and community composition. Long-term fertilizer research has shown that chemical fertilization can alter bacterial communities through changes in soil pH, with bacterial richness and diversity often reduced under acidifying fertilizer regimes compared with manure or manure-fertilizer combinations. The beneficial effect of liming observed in the present study is supported by recent evidence showing that liming can alleviate soil acidity and improve microbial abundance and activity. Mitsuta *et al*. (2025) reported that liming increased the abundance of several microbial groups, while also buffering microbial populations against seasonal variation. More recent work similarly identified soil pH as an important driver of microbial community structure and showed that lime application can alleviate acid stress and favour bacterial groups involved in nutrient cycling. Previous work in Kenya supports the efficiency of microdosing in acidic soils. Kisinyo *et al*. (2015) demonstrated that relatively small quantities of N, P and lime applied through microdosing improved nutrient recovery and maize performance in an acidic Kenyan soil. Their findings showed that combining fertilizer and lime improved fertilizer recovery, supporting the concept that localized amendment can increase input-use efficiency. More recently, Rotich and Kisinyo (2025) reported that integrating lime with nutrient microdosing in acidic soils of Western Kenya improved pH, reduced exchangeable acidity and enhanced nutrient availability, further supporting the effectiveness of combining acidity amelioration with targeted nutrient supply. Other studies showed that lime and nitrogen status were important controls of bacterial community structure,

indicating the importance of soil chemical conditions in regulating microbial populations. More recent work similarly identified soil pH as the most important driver of soil microbial community structure and showed that lime application alleviated acidity stress and favoured microbial groups involved in nitrogen cycling, including beneficial bacterial groups (Barcelos *et al*., 2024). Similarly, the Kenyan microdosing study by Kisinyo *et al*. (2015) demonstrated that localized application of N, P and lime improved nutrient recovery and maize productivity on acidic soil. Although that study did not directly evaluate bacterial populations, its findings support the underlying principle that concentrating limited inputs in the crop root zone can improve their effectiveness under acidic, nutrient-deficient conditions.

The superiority of calcite over dolomite combinations can also be related to the initial chemical condition of the soil. The initial soil contained relatively low exchangeable Ca (1.4 cmol (+) kg^{-1}) and Mg (0.60 cmol (+) kg^{-1}), while exchangeable acidity was relatively high at 2.82 cmol (+) kg^{-1} (Table 1). Calcite primarily supplies Ca and neutralizes soil acidity, whereas dolomite supplies both Ca and Mg. The slightly better performance of calcite combinations may therefore indicate that rapid amelioration of acidity and replenishment of Ca were particularly important under the experimental conditions. Ca is also important in maintaining soil aggregation and the stability of microbial habitats. Furthermore, because the soil already contained some Mg, the additional Mg supplied by dolomite may have offered less biological advantage than the acid-neutralizing and Ca-supplying effect of calcite. The difference was most apparent in the highest performing integrated treatments. Nevertheless, the difference between calcite and dolomite was relatively small compared with the much larger difference between integrated and unamended treatments. Therefore, the results suggest that both liming materials improved the microbial environment, with calcite providing a slightly more favourable response under the particular chemical conditions of Kariene. This conclusion should not be generalized to all acidic soils because the relative performance of calcite and dolomite depends on soil Mg status, lime quality, particle size, neutralizing value and rate of application. An important finding is that the initial soil in Table 1 had a fungal population of 3.20×10^5 CFU g^{-1}, approximately an order of magnitude greater than the bacterial population of 2.31×10^4 CFU g^{-1}. However, following amendment application, bacterial populations became much greater than the fungal populations reported in the subsequent microbial results. Increased pH and reduced acidity can stimulate bacterial activity and diversity, while the addition of readily available mineral nutrients can produce rapid bacterial multiplication. Thus, the reversal from fungi-dominated initial abundance to substantially greater bacterial abundance after amendment is consistent with a shift in microbial community conditions following acidity amelioration and nutrient enrichment.

4. Conclusion

The study demonstrated that microdosing (MD) and the type of fertilizer and lime amendments significantly influenced bacterial populations in the maize soil, while seasonal variation had no significant effect. Microdosing generally resulted in higher bacterial populations than the corresponding non-microdosing (NMD) treatments, indicating that the split application of 75% of the recommended fertilizer rate provided favourable conditions for fungal and bacterial proliferation. The significant microdosing × treatment interaction further demonstrated that the fungal and bacterial response to microdosing depended on the fertilizer and lime combination applied. Among the treatments, calcite + NPK 23:23:0 + FYM under MD consistently recorded the highest bacterial population in both cropping seasons, indicating a beneficial interaction between acidity amelioration by calcite, nutrient supply from NPK 23:23:0 and organic substrates supplied by FYM. In contrast, the controls recorded the lowest bacterial populations and were significantly lower than the amended treatments, highlighting the limitations imposed by the initially acidic and relatively nutrient-deficient soil conditions. The findings indicate that integrating microdosing with lime, mineral fertilizer and FYM can improve soil fungal and bacterial populations in acidic maize soils. The superior performance of MD compared with the corresponding NMD treatments suggests that applying nutrients in smaller, split doses can support microbial populations while using less mineral fertilizer. Thus, MD combined with appropriate organic and inorganic amendments, particularly calcite + NPK 23:23:0 + FYM, offers a promising soil fertility management strategy for improving biological soil health in acidic maize-growing soils.

5. Limitation

The study was conducted at one field site over two consecutive cropping seasons and quantified culturable fungal and bacterial populations using plate-based enumeration. Accordingly, the findings represent the culturable fraction of the soil microbial community under the conditions evaluated and do not describe total microbial diversity, community composition or functional activity. The observed treatment responses should therefore, be interpreted within the site-specific acidic soil and management conditions. Longer-term and multi-site studies using complementary microbial approaches would be useful for assessing the spatial and temporal consistency of these responses.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results and scholarly content. QuillBot was used to assist with grammatical refinement, while Oreate was used to support the preparation and formatting of figures. The authors take full responsibility for the accuracy, integrity, originality and final content of the manuscript**.** The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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