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**RESEARCH*ARTICLE***
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# Effect of soil Amelioration on Fungal and Bacteria Communities in Kurdistan Calcareous Land.

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## ABSTRACT

Calcareous soils in Kurdistan pose significant challenges to agricultural productivity due to low fertility and poor structure. This study investigates the effect of soil amelioration practices, specifically organic matter (OM) and ash applications, on fungal and bacterial communities to improve soil health and functionality. The objective was to evaluate the diversity, phylogeny, and functional roles of soil microbes under varying levels of OM (0–6 t/ha) and ash (0–4 t/ha). Using 16S and 28S rRNA gene sequencing and phylogenetic analysis, microbial diversity and evolutionary relationships were assessed. The results demonstrated that high OM levels (6 t/ha) significantly enhanced microbial diversity, supporting nitrogen-fixing bacteria (*Azotobacter chroococcum*) and beneficial fungi (*Trichoderma reesei*). Ash applications influenced microbial composition, promoting resilient species such as *Xanthobacter sp.* and *Sphingomonas sanguinis*. Phylogenetic analysis confirmed close genetic relationships within microbial genera, reflecting their adaptability to calcareous soil conditions. Beneficial microbes played key roles in nutrient cycling, plant growth promotion, and soil resilience. In conclusion, integrating OM and microbial-based approaches can improve the fertility and biological health of calcareous soils. While ash can condition soil, its long-term impacts on microbial communities should be monitored. It is recommended to adopt regular OM applications and bio-inoculants containing *Pseudomonas* and *Trichoderma* to sustainably enhance soil productivity. These findings provide a foundation for tailored soil management strategies in the agricultural landscapes of Kurdistan.

***Keywords*:** Soil Amelioration**,** Fungi, Bacteria, Calcareous soil.



## INTRODUCTION

Calcareous soils are characterized by high pH and significant calcium carbonate content, which pose challenges for microbial activity due to their alkaline nature and limited nutrient availability. Despite these constraints, bacterial and fungal communities play critical roles in maintaining soil health and fertility. Bacteria in these soils are vital for processes such as nitrogen fixation, organic matter decomposition, and phosphorus solubilization, which are essential for nutrient cycling [1]. Fungi, particularly mycorrhizal species, contribute to nutrient uptake by plants, enhance soil aggregation, and promote OM decomposition [2]. However, the high pH of calcareous soils often limits the diversity and activity of these microbial communities, requiring specific adaptations or amendments to enhance their functionality. OM inputs, such as compost or biochar, can stimulate microbial activity, leading to improved nutrient availability and soil structure [3]. Understanding the dynamics of fungal and bacterial interactions in calcareous soils is vital for developing effective management practices aimed at improving agricultural productivity and soil sustainability.

The calcareous soils in the Kurdistan region pose significant challenges to agriculture due to their poor structure, low fertility, and limited water retention capacity [4,5]. These conditions adversely affect plant growth and productivity, necessitating effective soil amelioration strategies to enhance soil and agricultural output. Soil amelioration practices, such as the incorporation of organic amendments, have been shown to influence soil microbial communities, including bacteria and fungi, which play important roles in nutrient cycling, organic matter decomposition, and overall soil fertility. For instance, the application of carbon-rich organic materials like wheat straw and compost has been observed to alter the structure of bacterial and fungal communities, potentially leading to improved soil and plant growth [6]. In calcareous soils, the response of microbial communities to amelioration practices can be complex. Studies have indicated that certain bacterial and archaeal taxa serve as reliable indicators of soil restoration progress, reflecting changes in soil properties and [7, 8&9]. Additionally, the succession of plant communities in these soils can significantly influence the composition and diversity of soil fungi and bacteria, further affecting soil quality and ecosystem functioning [10]. Until now, there is no study available in this regard particularly in Kurdistan's calcareous lands. It is essential to comprehend

how soil amelioration affects the microbial communities in Kurdistan's calcareous fields to create strategies to improve soil fertility and agricultural output. Hence, the objective of this study was to evaluate the diversity, phylogeny, and functional roles of soil microbes under varying levels of OM (0–6 t/ha) and ash (0–4 t/ha).

**Materials and Methods**

We relied on data on the areas of land cultivated in the Bazian region with wheat, cucumbers and field tomatoes. This crop was chosen for several reasons, such as because it is the oldest and most accurately documented compared to other crops. We were unable to use the production amount of these lands due to the inaccuracy of the production data and its change. Using wheat cultivated areas as a proxy for Predictive economic growth and is an interpreted, object-oriented, high-level programming language with dynamic semantics in Python used pandas and stats models. The Temperature data was taken from the weather station in Bazian area.

Data Processing and Computational Workflow Two Independent Variable choosing for the research temperature and thermal shocks; for the study area (Bazian Plain) From the Meteorological Department, monthly temperature rates were obtained for the years 1973-2023 Then, thermal shocks were found for each monthly rate for each year.

To analyze the effect of thermal shocks on Predicted economic growth in the Bazian Plain computational tools were employed for data preprocessing statistical modeling and regression analysis. During these processes program outputs and error handling were managed systematically using standard output (STDOUT) and standard error (STDERR) streams.

Standard Output (STDOUT) This stream was used to monitor the results of key computations, including the regression coefficients, summary statistics, and thermal shock metrics. Intermediate results, such as yearly averages of thermal shocks, were displayed and validated through this output stream to ensure consistency and accuracy.

Standard Error (STDERR) Any issues encountered during the computational process, such as missing temperature data, unexpected input formats, or errors in regression model convergence, were captured through the standard error stream. These diagnostic messages facilitated the identification and resolution of errors before proceeding to the next stage of analysis. For instance, missing temperature readings for certain months were detected via STDERR and addressed by imputing values based on adjacent data [10].

**Multiple Linear Regression**

Using a formula known as a linear regression model. It is a statistical method used to examine the relationship between a dependent variable (in this case, Economic Growth) and one or more independent variables (Thermal Shock, Cultivated Area). The equation includes:

Predicted Economic Growth=10,849.31+1,438.58×Thermal Shock+1.01625×Cultivated Area [11, 12]

$\beta_0$: The intercept, representing the baseline level of economic growth when thermal shocks are zero = 10,849.31 (calculated from the regression)

$\beta_1$: The coefficient that measures the impact of thermal shock on economic growth, indicating the direction and magnitude of the relationship = 1,438.58 (calculated from the regression)

β2: The coefficient that measures the impact of thermal shock on economic growth, indicating the direction and magnitude of the relationship = 1.016

ϵt: standard component of regression models, representing the variability not captured by the explanatory variable (Thermal Shock, Cultivated Area).

**Results and Discussions**

**Materials and Methods**

The geographical of this study was Grdarash site, which is located at an elevation of 418 meters above sea level (Table 1), indicating moderately elevated terrain that may influence local climate, vegetation, and soil formation processes. The GPS coordinates for this site were X=411359X = 411359X=411359 and Y=3997002Y = 3997002Y=3997002, specifying its precise position on the Earth's surface.

(Table1) he location of Grdarash (G.P.S) reading and elevation

| Location | Elevation (m) | (G.P.S) Reading (X) | (G.P.S) Reading (y) |
|---|---|---|---|
| Grdarash | 418 | 411359 | 3997002 |

The field was planted with Bellary, which manure (OM) and ash was applied on January 1, 2024. The samples were collected on April 17, 2024, to assess microbial activity.

**Isolation of Fungi and Bacteria from Soil**

Soil samples from 12 subcultures are used to isolate fungal and bacterial colonies on Petri dishes containing culture media. The media contains 40 g/L dextrose, 10 g/L peptone, and agar as a solidifying agent, incubated at 30°C in a humidified environment for fungal growth. For the bacteria culture, the media contains 15 g/L agar,

peptone, and beef extract, with suitable nutrients including amino acids and glucose at pH of 7. A total of 54 fungal isolates and 62 bacterial isolates were obtained from different locations within the Erbil governorate at Grdarash filed.

**DNA Extraction**

Genomic DNA was extracted from both fungal and bacterial isolates using specialized kits. For fungi, the Beta Bayern Tissue DNA Preparation Kit (Beta Bayern GmbH, Germany) was used, while the PGA Bacterial DNA Extraction Kit (PF230-050, Iran) was utilized for bacterial isolates. The extracted DNA served as a template for downstream polymerase chain reaction (PCR) analysis.

**PCR Amplification of Ribosomal RNA Genes**

For Fungi (28S rRNA), PCR amplification targeted the partial 28S rRNA gene using a 50 µL reaction mixture containing 2x Taq DNA Polymerase Master Mix (AMPLIQON), 10 pmol of forward primer LSU (5'-ACCCGCTGAACTTAAGC-3'), 10 pmol of reverse primer LSU (5'-CGCCAGTTCTGCTTACC-3'), DNase-free water, and template DNA.

For Bacteria (16S rRNA), PCR amplification targeted the partial 16S rRNA gene using a similar reaction mixture (Table 2) with 10 pmol of forward primer UN-16S (5'-AGAGTTTGATCCTGGCCTCAG-3') and reverse primer UN-16S (5'-GGCTACCTTGTTACGACTT-3'). The thermocycler conditions were identical to those used for fungal PCR.

Table 2. 16S rRNA PCR Amplification Reagents

| No. | PCR components | Concentration | Volume (µl) |
|---|---|---|---|
| 1 | Master Mix | 2x | 25 |
| 2 | Forward Primer | 20 Pmol | 3 |
| 3 | Reverse Primer | 20 Pmol | 3 |
| 4 | DNase free Water | - | 15 |
| 5 | Template DNA | 50ng/µl | 4 |
| Total | | | 50 |

**Visualization of DNA Fragments**

The PCR products were visualized using gel electrophoresis. A 1.5% agarose gel prepared with 1X TBA buffer was stained with ethidium bromide. DNA fragments were separated under an electric field for 30 minutes and examined under a UV trans-illuminator to identify the presence and size of PCR amplicons.

**DNA Sequencing**

PCR products from both fungi (28S rRNA gene) and bacteria (16S rRNA gene) were sequenced. For fungi, the ABI 3130X Genetic Analyzer (Applied Biosystems) was used, and chromatograms of the 28S rRNA gene were processed using Finch TV software. For bacteria, the sequencing of 16S rRNA gene products was carried out using the ABI Prism Terminator Sequencing Kit (Applied Biosystem). Chromatograms were analysed and edited for base calls using Finch TV software.

**Sequence Alignment and Submission**

The obtained sequences were analysed using the Basic Local Alignment Search Tool (BLAST)available on the NCBI website(https://blast.ncbi.nlm.nih.gov/Blast.cgi). This tool was used to compare the query sequences with existing biological sequences in the database to identify similarities and potential matches. Both fungal (28S rRNA) and bacterial (16S rRNA) sequences were aligned and compared for taxonomic and phylogenetic studies.

**RESULTS AND DISCUSSION**

**Soil Properties and Microbial Communities**

The study examined the chemical and physical properties of Grdarash soil (Table 3), revealing a silty clay loam texture with 99.9 g $Kg^{-1}$ sand, 519.6 g $Kg^{-1}$ silt, and 380.5 g $Kg^{-1}$ clay. This texture suggests good water retention and nutrient-holding capacity but may pose drainage and aeration challenges due to the higher clay content. The soil's ion composition was dominated by calcium ($Ca^{2+}$, 4 meq $L^{-1}$), indicating high carbonate content, with moderate to low levels of magnesium ($Mg^{2+}$, 1.9 meq $L^{-1}$), sodium ($Na^{+}$, 1.06 meq $L^{-1}$), and potassium ($K^{+}$, 0.35 meq $L^{-1}$). Among anions, bicarbonate ($HCO_3^{-}$, 3.75 meq $L^{-1}$) was the most abundant, suggesting carbonate buffering, while sulphate ($SO_4^{2-}$, 1.62 meq $L^{-1}$) and chloride ($Cl^{-}$, 2.1 meq $L^{-1}$) were present in traceable amounts. The absence of carbonate ($CO_3^{2-}$) aligns with the soil toward slight alkaline pH [5].

The soil's slightly alkaline pH (7.75) and low salinity (EC 0.55 dS $m^{-1}$) make it suitable for most crops, but the low OM content (9.1 g $Kg^{-1}$) indicates a need for amendments to enhance soil structure and microbial activity. The high total (240 g $Kg^{-1}$) and active (44 g $Kg^{-1}$) calcium carbonate levels confirm its calcareous nature, impacting pH buffering and phosphorus availability. Nutrient levels were suboptimal, with low total nitrogen (0.31 g $Kg^{-1}$) and available phosphorus (3.2 mg $Kg^{-1}$), suggesting the need for fertilization. The moderate-to-high cation exchange capacity (CEC 23.8 Cmolc $Kg^{-1}$) indicates good nutrient-holding potential, largely influenced by its clay content and organic matter [4]

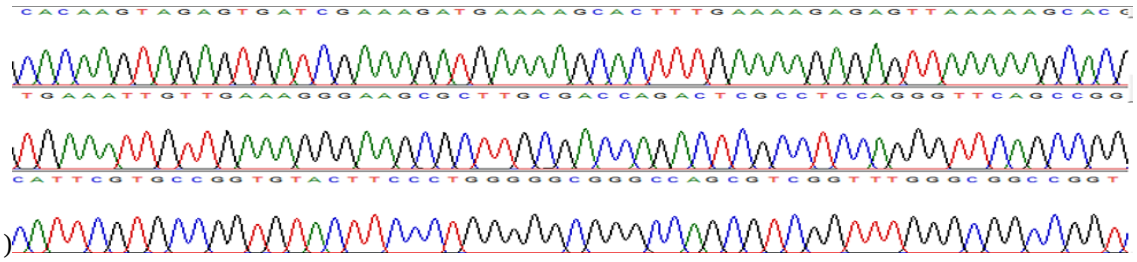
(Table 3) The results of key chemical and physical properties of the Grdarash soil.

| | |
|---|---|
| Physical properties | |
| Particle Size Distribution | |
| Sand | 99.9 g $Kg^{-1}$ |
| Silt | 519.6 g $Kg^{-1}$ |
| Clay | 380.5 g $Kg^{-1}$ |
| Textural name | Silty clay loam (SiCL) |
| Chemical properties | |
| pH | 7.75 g $Kg^{-1}$ |
| EC | 0.55 dS $m^{-1}$ |
| Organic matter | 9.1 g $Kg^{-1}$ |
| Carbonate mineral $CaCO_3$ | 240 g $Kg^{-1}$ |
| Active $CaCO_3$ | 44 g $Kg^{-1}$ |
| Total Nitrogen | 0.31 g $Kg^{-1}$ |
| Available Phosphorus | 3.2 mg $Kg^{-1}$ |
| CEC | 23.8 Cmolc K $g^{-1}$ |
| Cation | |
| $Ca^{2+}$ | 4 meq $L^{-1}$ |
| $Mg^{2+}$ | 1.9 meq $L^{-1}$ |
| $Na^{+}$ | 1.06 meq $L^{-1}$ |
| $K^{+}$ | 0.35 meq $L^{-1}$ |
| Anion | |
| Chloride $CL^{-1}$ | 2.1 meq $L^{-1}$ |
| Bicarbonate $HCO_3^{-}$ | 3.75 meq $L^{-1}$ |
| Carbonate CO3^2− | 6.12 meq $L^{-1}$ |
| Sulfate $SO4^{-2}$ | 1.62 meq $L^{-1}$ |

**PCR amplification of partial 16S rRNA gene**

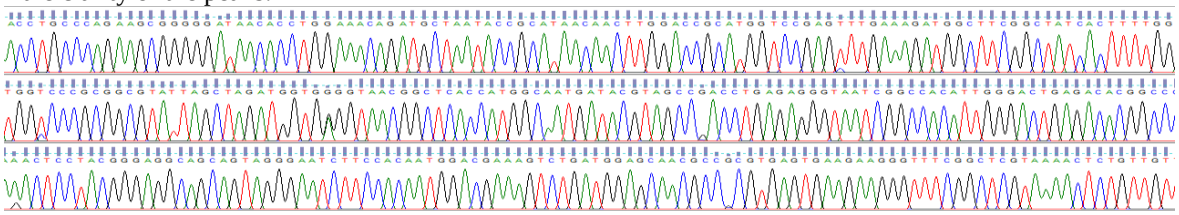
(Figure1) displays result of chromatogram analysis. The chromatogram displays the raw sequencing output where each peak corresponds to a specific nucleotide (adenine, thymine, cytosine, or guanine). The colours (green for A, red for T, blue for C and black for G) indicated the identity of the nucleotides and the sequence was aligned below the peaks. The peaks were sharp and evenly spaced, suggesting a high-quality sequence with minimal noise or overlap in the readable regions. This indicates that the sequencing process was successful. The sequence data corresponds to a partial 28S rRNA gene, as identified in the description.

The PCR amplification of the partial 16S rRNA gene and subsequent sequencing yielded high-quality results. The chromatogram analysis displayed sharp and evenly spaced peaks, indicating a high-quality sequence with minimal noise or overlap. The DNA template used was derived from the source material, and ribosomal gene-specific primers were designed for PCR amplification. The sequencing data corresponded to a partial 28S rRNA gene, and the chromatogram confirmed successful sequencing of the 16S rRNA gene partial region. The high-quality, distinct peaks suggest an accurate read of the nucleotide sequence [7,9].



(Figure 1) The chromatogram sequences result of partial gene of 28S rRNA sequence (PCR amplification)

(Figure2) displays the chromatogram sequences result of partial gene of 16s rRNA sequences. The chromatogram confirmed successful sequencing of the 16S rRNA gene's partial region. The high-quality, distinct peaks suggest an accurate read of the nucleotide sequence. The DNA template for sequencing was derived from PCR amplification of the 16S rRNA gene. The primers designed for this gene yielded approximately 1200 bp in size. This indicated successful amplification before sequencing. DNA sequencing was carried out using only the forward primer and the results were analysed using chromatographic software. The quality of sequencing depends on the clarity of the peaks.



(Figure2) .The chromatogram sequences result of partial gene of 16s rRNA sequences.

**Molecular Identification of Genus and Species of Fungus**

(Table4) displays the result of molecular identification and partial gene of 16S rRNA of 54 query sequences. The identified species include common genera such as *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, *Basidiomycota* and *Hypocrea*. These fungi were often associated with soil and organic decomposition. *Aspergillus flavus*, *A. oryzae*, *A. sergii*and *Penicillium javanicum* were the most frequently identified species. Other notable species include *Trichoderma longibrachiatum*, *Trichoderma reesei* and *Fusarium sp.*

The presence and diversity of fungal species vary with the levels of OM and ash in the soil (Table 4). High OM (6 t/ha) with varying ash levels identified species including *Aspergillus flavus*, *A. sergii* and *Penicillium javanicum*. Low OM or absence of ash revealed species such as *Fusarium sp.* and *Basidiomycota sp.* The distribution of fungi indicates the influence of soil treatments (OM and ash) on fungal communities. These species play roles in soil nutrient cycling, OM decomposition and maybe as plant pathogens (*Fusarium sp.*, *Aspergillus flavus*) or biocontrol agents (*Trichoderma*).

The molecular identification of 54 fungal query sequences revealed common genera such as *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, *Basidiomycota*, and *Hypocrea*. These fungi are often associated with soil and organic decomposition. *Aspergillus flavus*, *A. oryzae*, *A. sergii*, and *Penicillium javanicum* were the most frequently identified species. The presence and diversity of fungal species varied with the levels of OM and ash in the soil. High OM (6 t/ha) with varying ash levels identified species including *Aspergillus flavus*, *A. sergii*, and *Penicillium javanicum*. Low OM or absence of ash revealed species such as *Fusarium sp.* and *Basidiomycota sp.* The distribution of fungi indicates the influence of soil treatments (OM and ash) on fungal communities [8].

(Table 4). Molecular Identification and Partial Gene of 16S rRNA of 54 query sequences.

| Isolation sources | Fungi Identified | Accession Numbers |
|---|---|---|
| 2 organic matter and 2 ash t/ha | *Aspergillus flavus* | PQ424611 |
| | *Fusarium sp.* | PQ424612 |
| | *Basidiomycota sp.* | PQ424613 |
| | *Penicillium javanicum* | PQ424614 |
| 6 organic matter and 0 ash t/ha | *Aspergillus oryzae* | PQ424615 |
| | *Aspergillus flavus* | PQ424616 |
| | *Aspergillus oryzae* | PQ424617 |
| | *Aspergillus sergii* | PQ424618 |
| | *Penicillium javanicum* | PQ424719 |
| 6 organic matter and 4 ash t/ha | *Aspergillus flavus* | PQ432401 |
| | *Aspergillus oryzae* | PQ432402 |
| | *Aspergillus arachidicola* | PQ432403 |
| | *Penicillium javanicum* | PQ432404 |
| 4 organic matter and 2 ash t/ ha | *Trichoderma longibrachiatum* | PQ425463 |
| | *Aspergillus sergii* | PQ425464 |
| | *Rhizoctonia solani* | PQ425465 |
| 0 organic matter and 4 ash t/ha | *Hypocrea schweinitzii* | PQ425472 |
| | *Hypocrea jecorina* | PQ425473 |
| | *Penicillium javanicum* | PQ425474 |

| | | |
|---|---|---|
| | *Aspergillus oryzae* | PQ425475 |
| | *Trichoderma longibrachiatum* | PQ425477 |
| | *Trichoderma reesei* | PQ425478 |
| 2 organic matter and 0 ash t/ ha | *Aspergillus oryzae* | PQ425479 |
| | *Aspergillus sergii* | PQ425480 |
| | *Penicillium javanicum* | PQ425481 |
| | *Trichoderma reesei* | PQ432405 |
| | *Hypocrea schweinitzii* | PQ432406 |
| 0 organic matter and 2 ash t/ha | *Penicillium javanicum* | PQ432407 |
| | *AseqAspergillus arachidicola* | PQ432408 |
| | *Aspergillus minisclerotigenes* | PQ432409 |
| | *Fusarium sp.* | PQ432410 |
| | *Basidiomycota sp.* | PQ432411 |
| 0 organic matter and 0 ash t/ha | *Penicillium javanicum* | PQ432412 |
| | *Aspergillus oryzae* | PQ432413 |
| | *Trichoderma reesei* | PQ432414 |
| | *Aspergillus flavus* | PQ432415 |
| | *Fusarium sp.* | PQ432416 |
| 2 organic matter and 4 ash t/ha | *Basidiomycota sp.* | PQ432417 |
| | *Penicillium javanicum* | PQ432418 |
| | *Aspergillus oryzae* | PQ432419 |
| 6 organic matter and 2 ash t/ha | *Trichoderma avellaneum* | PQ425484 |
| | *Verticillium cf. fungicola* | PQ425485 |
| | *Aspergillus sergii* | PQ425486 |
| | *Penicillium javanicum* | PQ425487 |
| 4 organic matter and 0 ash t/ha | *Trichoderma citrinoviride* | PQ425489 |
| | *Aspergillus flavus* | PQ425490 |
| | *Fusarium sp.* | PQ425491 |
| | *Basidiomycota sp.* | PQ425492 |
| | *Penicillium javanicum* | PQ425493 |
| | *Aspergillus sergii* | PQ432420 |
| 4 organic matter and 4 ash t/ha | *Aspergillus oryzae* | PQ432421 |
| | *Penicillium javanicum* | PQ432422 |
| | *Aspergillus flavus* | PQ432423 |

(Table 5) exhibits the result of molecular identification and partial gene of 16S rRNA of 62 query sequences. The study identified 10 bacterial genera with diverse ecological roles, including nitrogen-fixing bacteria (*Azospirillum sp.*, *Azotobacter chroococcum*, *Xanthobacter sp.*), soil decomposers (*Bacillus sp.*, *Bacillus pumilus*, *Bacillus safensis*), plant-growth promoters (*Pseudomonas putida*, *Enterobacter sp.*) and opportunistic bacteria (*Klebsiella sp.*, *Staphylococcus sp.*). These bacteria contributed to soil fertility, nutrient cycling and plant health, indicating their importance in agricultural and ecological systems.

The study showed that varying levels of OM and ash significantly influence bacterial diversity and composition (Table 5). High OM (6 t/ha) supported rich microbial diversity, including nitrogen-fixing bacteria (*Azotobacter chroococcum*, *Azospirillum sp.*) and beneficial species such as *Bacillus pumilus* and *Pseudomonas japonica*. In contrast, low OM (0-2 t/ha) was dominated by resilient bacteria such as *Bacillus sp.*, *Staphylococcus sp.* and *Enterobacter sp.* Increased ash (0-4 t/ha) promotes the presence of adaptable genera such as *Xanthobacter sp.* and *Pseudomonas putida*, likely due to their tolerance to alkalinity or heavy metals, while moderate ash (2 t/ha) supports a balanced community of nitrogen fixers and soil decomposers.

The study also identified 10 bacterial genera with diverse ecological roles, including nitrogen-fixing bacteria (*Azospirillum sp.*, *Azotobacter chroococcum*, *Xanthobacter sp.*), soil decomposers (*Bacillus sp.*, *Bacillus* [6,10]

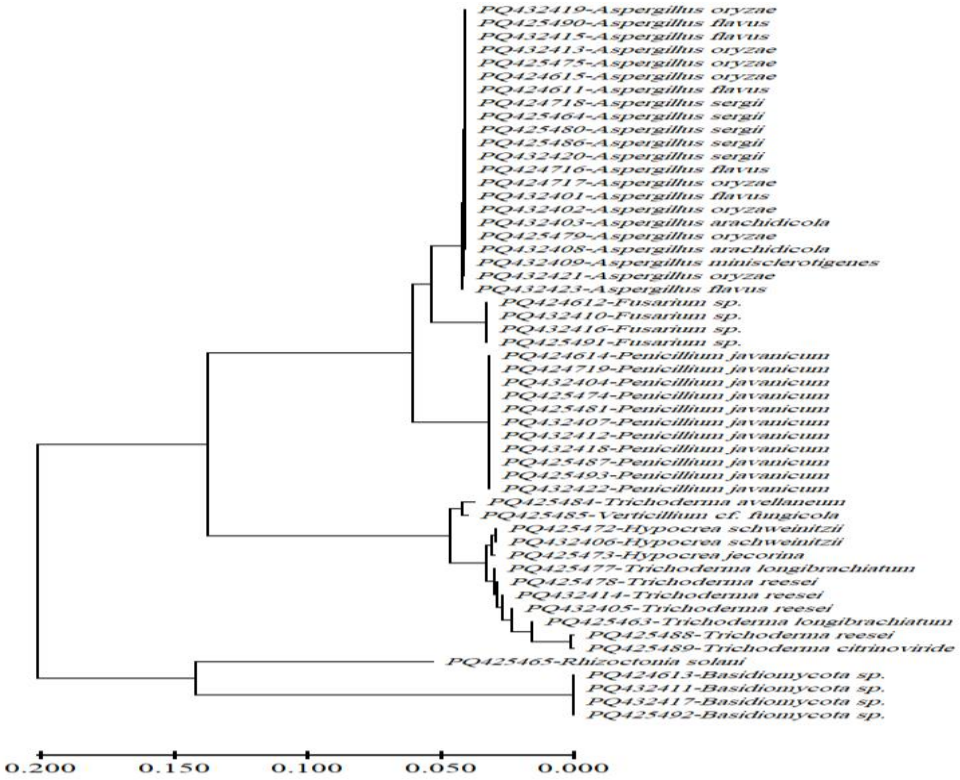
(Table5). Molecular identification and partial gene of 16S rRNA of 62 query sequences.

| Isolation sources | Bacterial Identified | Accession Numbers |
|---|---|---|
| 2 organic matter and 2 ash t/ha | *Bacillus safensis* | PQ209619 |
| | *Bacillus pumilus* | PQ209620 |
| | *Vibrio sp.* | PQ209621 |
| | *Staphylococcus sp.* | PQ209622 |
| | *Sphingomonas paucimobilis* | PQ209623 |
| | *Sphingomonas sanguinis* | PQ209624 |
| 6 organic matter and 0 ash t/ha | *Staphylococcus sp* | PQ209625 |
| | *Azospirillum sp.* | PQ209626 |
| | *Klebsiella sp.* | PQ209627 |
| | *Azotobacter chroococcum* | PQ209628 |
| | *Stenotrophomonas chelatiphaga* | PQ213483 |
| | *Stenotrophomonas maltophilia* | PQ213484 |
| 6 organic matter and 4 ash t/ha | *Azotobacter chroococcum* | PQ213485 |
| | *Bacillus sp.* | PQ213486 |
| | *Enterobacter sp.* | PQ213487 |
| | *Sphingomonas paucimobilis* | PQ214189 |
| | *Sphingomonas sanguinis* | PQ214190 |
| | *Bacillus sp.* | PQ214191 |
| 4 organic matter and 2 ash t/ha | *Xanthobacter sp.* | PQ214192 |
| | *Klebsiella sp.* | PQ214193 |
| | *Enterobacter sp.* | PQ214194 |
| | *Pseudomonas japonica* | PQ214195 |
| | *Sphingomonas sanguinis* | PQ216436 |
| | *Bacillus sp.* | PQ216437 |
| | *Xanthobacter sp.* | PQ216438 |
| 0 organic matter and 4 ash t/ha | *Enterobacter sp.* | PQ216439 |
| | *Azotobacter chroococcum* | PQ216440 |
| | *Vibrio sp.* | PQ216441 |
| | *Staphylococcus sp.* | PQ216442 |
| | *Azospirillum sp.* | PQ216443 |
| | *Bacillus sp.* | PQ216444 |
| 2 organic matter and 0 ash t/ha | *Xanthobacter sp.* | PQ216445 |
| | *Enterobacter sp.* | PQ216446 |
| | *Azotobacter chroococcum* | PQ216447 |
| | *Sphingomonas zeae* | PQ215533 |
| | *Alphaproteobacteria bacterium* | PQ215534 |
| 0 organic matter and 2 ash t/ha | *Staphylococcus sp.* | PQ215535 |
| | *Enterobacter sp.* | PQ215536 |
| | *Azotobacter chroococcum* | PQ215537 |
| | *Xanthobacter sp.* | PQ215657 |
| 0 organic matter and 0 ash t/ha | *Enterobacter sp.* | PQ215658 |
| | *Azotobacter chroococcum* | PQ215659 |
| | *Bacillus thuringiensis* | PQ219764 |
| 0 organic matter and 4 ash t/ha | *Klebsiella sp.* | PQ219765 |
| | *Pseudomonas japonica* | PQ219766 |
| | *Pseudomonas putida* | PQ219767 |
| | *Bacillus sp.* | PQ218985 |
| 6 organic matter and 2 ash t/ha | *Xanthobacter sp.* | PQ218986 |
| | *Enterobacter sp.* | PQ218987 |
| | *Bacillus pumilus* | PQ219602 |
| | *Xanthobacter sp.* | PQ219603 |
| 4 organic matter and 0 ash t/ha | *Enterobacter sp.* | PQ219604 |
| | *Azotobacter chroococcum* | PQ219605 |
| | *Staphylococcus sp.* | PQ219606 |

| | | |
|---|---|---|
| 4 organic matter and 4 ash t/ha | *Azospirillum sp.* | PQ219607 |
| | *Klebsiella sp.* | PQ219608 |
| | *Xanthobacter sp.* | PQ218975 |
| | *Azotobacter chroococcum* | PQ218976 |
| | *Staphylococcus sp.* | PQ218977 |
| | *Azospirillum sp.* | PQ218978 |
| | *Klebsiella sp.* | PQ218977 |
| | *Pseudomonas putida* | PQ218980 |

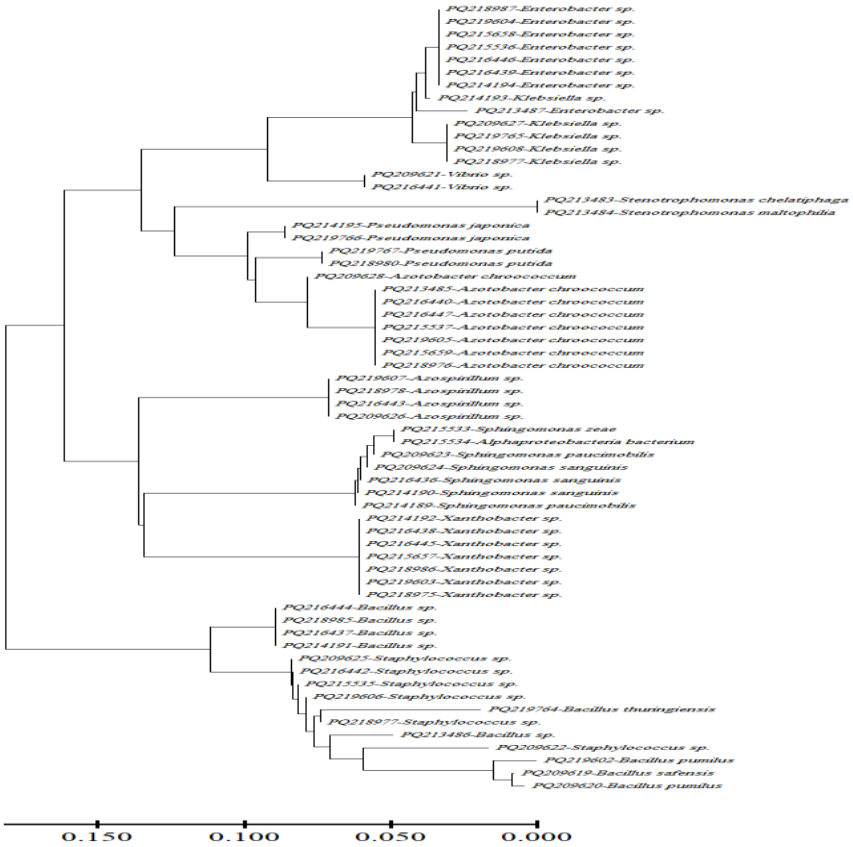
**Phylogenetic inferences**

The phylogenetic tree shown in the (Figure3) illustrates the evolutionary relationships among 54 fungal species identified using partial 28S rRNA gene sequences. The species clustered into distinct branches corresponding to their respective genera, reflecting their genetic similarity and evolutionary closeness. The phylogenetic analysis highlighted the clustering of fungal species by genus, such as *Aspergillus*, *Penicillium* and *Trichoderma*, indicating close genetic relationships within these groups. High similarity within branches confirmed the alignment of isolates with known GenBank species, while divergence across genera, such as *Aspergillus*, *Fusarium* and *Basidiomycota*, showed evolutionary differences and distinct ecological roles [6,7]. The tree also showed the diversity and adaptability of fungal genera to various environmental conditions, providing strong evidence for the accurate identification and classification of soil fungal species and their evolutionary relationships.




(Figure3). Phylogenic tree of soil fungus species. The analysis was conducted using the Maximum Likelihood method in MEGA11 software, applying the Tamura-Nei model based on partial DNA sequences of 28S rRNA gene.

The phylogenetic tree constructed using partial 16S rRNA gene sequences highlighted the genetic relationships among 62 soil bacterial species. The analysis revealed clear clustering by genus, with closely related species grouped together, such as *Enterobacter sp.*, *Klebsiella sp.* and *Xanthobacter sp.* High similarity within branches confirmed that the isolates align with known species in GenBank, while the separation of branches reflected evolutionary divergence among genera. The tree underscores the genetic diversity of soil bacteria and their classification based on nucleotide sequence data, providing robust evidence for accurate identification and evolutionary relationships in soil ecosystems [10].



(Figure4). Phylogenic tree soil bacterial species. The analysis was conducted using the Maximum Likelihood method in MEGA11 software, applying the Tamura-Nei model based on partial DNA sequences of 16S rRNA gene.

**Soil Amelioration and Microbial Communities**

The present study highlights the effects of soil amelioration on fungal and bacterial communities in this region, focusing on diversity, ecological functions, and phylogenetic relationships. The findings showed that the soil was slightly alkaline (pH 7.75), typical of calcareous soils, which can limit the availability of micronutrients such as iron, zinc, and phosphorus. Its low salinity (EC 0.55 dS $m^{-1}$) makes it suitable for most crops, but the OM content (9.1 g $Kg^{-1}$) is low, indicating a need for amendments to enhance soil structure and microbial activity. The high total (240 g $Kg^{-1}$) and active (44 g $Kg^{-1}$) calcium carbonate levels confirmed its calcareous nature, which impacted pH buffering and phosphorus availability. Nutrient levels were suboptimal, with low total nitrogen (0.31 g $Kg^{-1}$) and available phosphorus (3.2 mg $Kg^{-1}$), suggesting the need for fertilization. The moderate-to-high cation exchange capacity (CEC 23.8 Cmolc $Kg^{-1}$) indicated good nutrient-holding potential, largely influenced by its clay content and organic matter. These findings agreed with previous results [11], which indicated that amendments influence soil microbial communities by improving soil structure and nutrient content. Fungi and bacteria are essential for nutrient cycling and soil health, and they can respond positively to increased OM and ash applications [12]. These practices enhance microbial diversity and functions, such as nitrogen fixation and phosphorus solubilization.

Fungal species displayed notable shifts in diversity and composition with soil amendments. Genera such as *Aspergillus* and *Trichoderma* were dominant under higher OM levels, indicating their ability to decompose organic substrates and support nutrient cycling. *Trichoderma* also plays a key role in biocontrol and plant root development [13].

Species such as *Rhizoctonia solani* and *Verticillium sp.* were prevalent in ash-amended soils, showing their adaptation to high pH and altered nutrient profiles. This supports findings that ash can act as a soil conditioner, improving physical properties and challenging microbial adaptability [14]. Moreover, the phylogenetic tree based on 28S rRNA sequences highlights genera including *Aspergillus*, *Penicillium*, and *Fusarium* exhibited tight clustering, indicating their evolutionary closeness and shared ecological roles in decomposition and nutrient cycling. The divergence of *Trichoderma* and *Rhizoctonia* indicates evolutionary and functional differences, with *Trichoderma* contributing to biocontrol and *Rhizoctonia* representing potential pathogenic species.

The bacterial phylogenetic analysis demonstrated a high diversity of soil bacteria influenced by varying organic matter and ash levels. Adding OM enriched the soil with carbon sources, promoting the proliferation of nitrogen-fixing bacteria such as *Azotobacter chroococcum* and plant-growth-promoting species such as *Pseudomonas putida*. These bacteria enhance soil fertility and plant health, aligning with findings that OM improves microbial metabolic activity and diversity [15]. Resilient species such as *Bacillus sp.* And *Staphylococcus sp.* dominated, indicating their adaptability to nutrient-poor soils. These

bacteria probably play a vital role in maintaining minimal decomposition and nutrient cycling processes. The presence of ash-tolerant species, including Xanthobacter sp. and Sphingomonas sp., suggests adaptation to alkaline and metal-rich conditions, consistent with studies showing ash can influence microbial selection due to ph and heavy metal content [16]. Based on partial 16S rRNA sequences, revealed species including *Enterobacter sp.*, *Klebsiella spp*, and *Azospirillum sp.* grouped within their respective genera, indicating close genetic relationships. This clustering supports the reliability of 16S rRNA as a marker for bacterial taxonomy [17]. Nitrogen fixers such as *Azotobacter* formed distinct branches, separated from decomposers such as *Bacillus sp.*, reflecting their specialized ecological roles. The prevalence of genera including *Pseudomonas* and *Xanthobacter* in amended soils demonstrates their ecological plasticity, enabling them to thrive under altered environmental conditions.

The introduction of OM supports the proliferation of both bacterial and fungal decomposers, enhancing nutrient cycling and organic carbon availability. Nitrogen-fixing bacteria such as *Azotobacter* and fungi including *Aspergillus* improve soil fertility, reducing the dependency on synthetic fertilizers [18]. Calcareous soils are characterized by high calcium carbonate content and alkaline pH, which often challenging for microbial survival. The dominance of resilient microbes including *Xanthobacter sp.* and *Trichoderma sp.* in ash-amended soils underscores their adaptability and potential to stabilize soil under such conditions. The presence of plant-growth-promoting bacteria (e.g., *Pseudomonas putida*) and biocontrol fungi (e.g., *Trichoderma reesei*) suggests a dual benefit of soil amelioration: improving crop productivity and suppressing pathogens. This aligns with sustainable agriculture goals [20].

Soil management in Kurdistan's calcareous soils can benefit significantly from targeted practices. Regular application of OM enhances microbial diversity and soil functionality, particularly in nutrient-deficient areas. Moreover, ash can improve soil physical properties, its effects on microbial diversity require careful monitoring to ensure long-term health. Additionally, integrating beneficial microbes such as *Trichoderma* and *Pseudomonas* through bio-inoculants can complement amelioration efforts, bolstering soil resilience and improving crop productivity. These strategies collectively support sustainable agriculture in the region.

**Conclusion**

This study reported the significant role of soil amelioration practices, including OM and ash applications, in shaping microbial communities within Kurdistan's calcareous soils. The diversity and functionality of both bacterial and fungal species were significantly influenced by these treatments, with OM fostering nutrient-rich environments and microbial diversity. At the same time, ash contributed to soil conditioning but required careful monitoring for microbial health. Phylogenetic analyses revealed distinct evolutionary relationships and clustering patterns within microbial genera, underscoring their adaptability to environmental conditions and functional roles in soil ecosystems. Beneficial microbes such as *Trichoderma*, *Pseudomonas*, and nitrogen-fixing bacteria (*Azotobacter* and *Azospirillum*) emerged as key contributors to soil fertility and plant growth promotion. These findings provide a framework for sustainable soil management practices, emphasizing the integration of organic inputs and microbial-based interventions to enhance soil resilience, and agricultural productivity in the region.

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# تأثير تحسين التربة على مجتمعات الفطريات والبكتريا في الأراضي الجيرية في كردستان

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الخلاصة
تشكل التربة الجيرية في كردستان تحديات كبيرة للإنتاجية الزراعية بسبب انخفاض الخصوبة وضعف البناء. تبحث هذه الدراسة في تأثير ممارسات تحسين التربة، وخاصة تطبيقات المادة العضوية والرماد، على المجتمعات الفطرية والبكتيرية لتحسين صحة التربة وفعاليتها. كان الهدف هو تقييم تنوع وتطور وأدوار الميكروبات في التربة تحت مستويات متفاوتة من المادة العضوية (0-6 طن / هكتار) والرماد (0-4 طن / هكتار). باستخدام تسلسل جينات ***S***16 و ***S rRNA***28 ، تم تقييم التنوع الميكروبي والعلاقات التطورية. أظهرت النتائج أن مستويات المادة العضوية العالية (6 طن / هكتار) عززت بشكل كبير التنوع الميكروبي، ودعمت البكتيريا المثبتة للنيتروجين (***Azotobacter chroococcum***) والفطريات المفيدة (***Trichoderma reesei***). أثرت تطبيقات الرماد على التركيب الميكروبي، مما عزز الأنواع المرنة مثل ***Xanthobacter sp***. و ***Sphingomonas sanguinis***. وقد أكد التحليل الوراثي وجود علاقات وراثية وثيقة بين الأجناس الميكروبية، مما يعكس قدرتها على التكيف مع ظروف التربة الجيرية. ولعبت الميكروبات المفيدة أدوارًا رئيسية في دورة المغذيات، وتعزيز نمو النباتات، ومرونة التربة. وفي الختام، يمكن أن يؤدي دمج ***OM*** والنهج القائمة على الميكروبات إلى تحسين خصوبة التربة الجيرية وصحتها البيولوجية. وفي حين يمكن للرماد أن يكيف التربة، يجب مراقبة تأثيراته طويلة المدى على المجتمعات الميكروبية. ويوصى بتبني تطبيقات ***OM*** المنتظمة والملقحات الحيوية التي تحتوي على ***Pseudomonas*** و***Trichoderma*** لتعزيز إنتاجية التربة بشكل مستدام. توفر هذه النتائج الأساس لاستراتيجيات إدارة التربة المصممة خصيصًا في المناظر الطبيعية الزراعية في كردستان

الكلمات المفتاحية: تحسين الترب، الفطريات، البكتيريا، الأراضي الجيرية.