

Microbial diversity, functional potential, and antimicrobial resistance across soil depth in fire-affected rotational shifting cultivation soils

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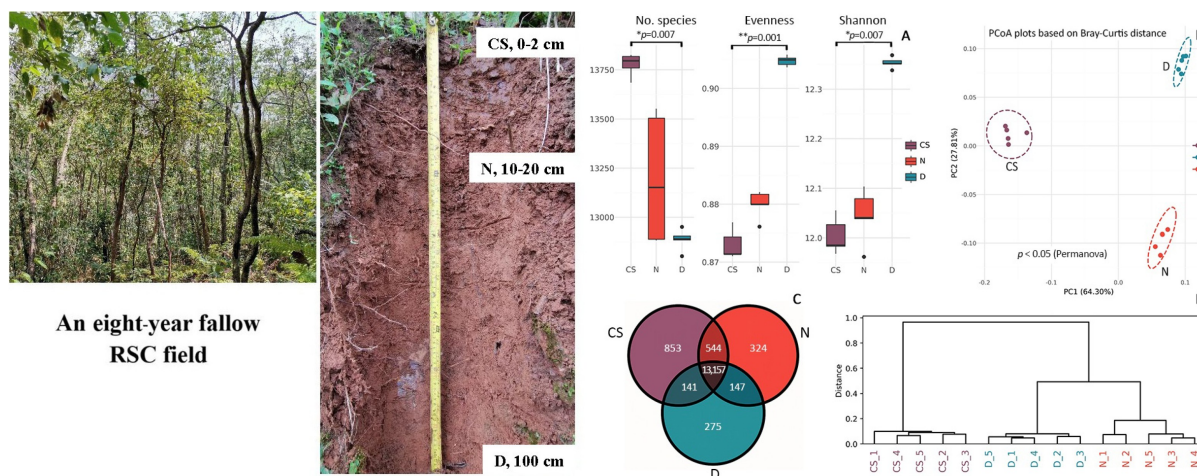
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ABSTRACT



- Charcoal-rich surface hosts distinct, low-evenness microbiome.
- Nitrogen-fixing taxa peak in the nutrient-leaching layer.
- Sharp vertical shift from copiotrophs to oligotrophs with depth.
- Surface soils act as hotspots of methane and nitrogen transformations.
- AMR genes persist across depths without anthropogenic inputs.

Fire is a recurring disturbance in rotational shifting cultivation (RSC) system, yet the depth-related organization of soil microbial communities in these systems remains poorly understood. This study investigated microbial diversity, functional potential, and antimicrobial resistance (AMR) in relation to soil depth and fire legacy in fire-affected RSC soils of northern Thailand using metagenomic sequencing. Soil samples were collected from three depth layers: the charcoal-mixed surface (CS, 0–2 cm), nutrient-leaching (N, 10–20 cm), and deep (D, 100 cm) horizons. Results revealed significant depth-dependent variation in soil physicochemical properties, microbial diversity, and taxonomic composition. The CS layer exhibited higher nutrient content and metabolic activity but lower Shannon diversity and evenness compared with deeper soils. Actinomycetota and Bacillota dominated surface soils, while Acidobacteriota and Pseudomonadota were enriched in subsoils, indicating a transition from copiotrophic to oligotrophic communities with increasing depth. Functional gene profiles (COG and KEGG) demonstrated strong vertical differentiation: surface soils were enriched in genes for amino acid metabolism, nutrient transport, and energy production, whereas deeper layers showed higher abundances of genes associated with DNA repair, replication, and stress tolerance. Functional genes linked to carbon, nitrogen, sulfur, and phosphorus cycles displayed clear stratification, with surface layers supporting greater biogeochemical activity. AMR genes, particularly those conferring resistance to *Rifamycin*, *Macrolide*, and *Glycopeptide* antibiotics, were most abundant in the D horizon. These patterns were observed in fire-affected RSC soils and are associated with both soil depth and fire legacy, and may reflect microbial responses to environmental stress conditions.

Keywords rotational shifting cultivation, metagenomics, soil microbial diversity, functional genes, antimicrobial resistance

1 Introduction

Soils are among the most biologically diverse ecosystems on Earth, hosting a vast array of microorganisms that regulate nutrient cycling, organic matter decomposition, and ecosystem productivity (Khaziev, 2011; Anthony et al., 2023). The vertical distribution of soil microbial communities is shaped by a combination of physical, chemical, and biological processes that vary with depth. Surface layers generally receive direct inputs of organic residues and are strongly influenced by climatic fluctuations and human activities, while deeper layers provide relatively stable microenvironments characterized by limited carbon availability, lower oxygen diffusion, and slower nutrient turnover (He et al., 2023; Philipp et al., 2025). Understanding how microbial diversity and functional potential vary with soil depth is essential for elucidating the mechanisms that underpin soil fertility, resilience, and ecosystem functioning (Fierer, 2017; Brewer et al., 2019).

In tropical upland ecosystems, fire is a pervasive disturbance that plays a dual role in shaping soil microbial communities (Ludwig et al., 2018). Fire combustion can drastically alter soil physicochemical properties by increasing pH, modifying nutrient availability, and generating pyrogenic organic matter (biochar), which can persist for decades and influence soil carbon sequestration (Rafie et al., 2024; Arunrat et al., 2024a). However, repeated burning may also lead to nutrient loss, soil erosion, and a temporary reduction in microbial biomass and activity (Fonturbel et al., 2021; Arunrat et al., 2023a). The extent to which microbial communities recover from such disturbances depends on fire intensity, post-fire vegetation succession, and soil depth (Certini, 2005; Buscardo et al., 2015; Pausas and Keeley, 2017). While the surface layer is directly affected by thermal shock and ash deposition, deeper horizons are typically insulated, allowing certain microbial taxa to survive and recolonize burned soils. These vertical differences create a depth-dependent pattern of microbial succession and functional reorganization, which remains underexplored in tropical agricultural systems.

One traditional land-use practice that exemplifies the interplay between fire, soil, and microbial dynamics is rotational shifting cultivation (RSC), widely practiced in the uplands of Southeast Asia, including Northern Thailand (Arunrat et al., 2023b; 2024b; 2025a; 2025b). RSC involves the cyclical clearing and burning of vegetation followed by short-term cultivation and long fallow periods that allow natural rege-

neration of vegetation and soil recovery. This system has historically sustained rural livelihoods and biodiversity in mountainous regions (Berkasem et al., 2009). However, increasing population pressure, shortened fallow cycles, and policy-driven land-use changes have raised concerns about its environmental sustainability. Despite debates surrounding its ecological impacts, evidence suggests that when practiced with adequate fallow periods, RSC can maintain or even enhance soil organic matter and microbial functions relative to continuous cultivation (Arunrat et al., 2022; Williams et al., 2022).

The microbial component of soil recovery under RSC remains poorly characterized, particularly regarding its vertical structure and functional potential. Most previous studies have focused on surface soils (0–20 cm), overlooking deeper horizons where long-term organic matter stabilization and nutrient transformation occur. Yet, the subsoil can contain over half of the total soil organic carbon and nitrogen pools, playing a pivotal role in regulating greenhouse gas fluxes and ecosystem resilience (Chabbi et al., 2009; Rumpel and Kögel-Knabner, 2011; Kautz et al., 2013). Deep soil environments are characterized by lower nutrient availability, reduced oxygen, and greater physicochemical stability, which can support distinct microbial communities and functional traits compared to surface soils. Therefore, including deeper layers (e.g., 100 cm) is essential for capturing the full vertical stratification of microbial diversity and functional potential, particularly in complex systems such as RSC.

Advancements in metagenomic sequencing now allow comprehensive assessment of microbial community composition, functional genes, and metabolic pathways across soil depths (Ejaz et al., 2024). Through high-throughput sequencing and bioinformatics analyses such as Clusters of Orthologous Genes (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway mapping, it is possible to identify microbial functions related to nutrient cycling, stress tolerance, and resistance traits. These approaches offer unprecedented insights into how microbial communities adapt and reorganize following disturbance, highlighting the mechanisms of functional redundancy and resilience that underpin soil ecosystem recovery (Tatusov, 2000; Kanehisa and Goto, 2000; Nam et al., 2023). In fire-affected soils, particular attention has been given to genes involved in carbon (C), nitrogen (N), sulfur (S), and phosphorus (P) cycling—key pathways that regulate soil fertility and greenhouse gas dynamics (Arunrat et al., 2025b; Zaman et al., 2025). The abundance and diversity of these functional genes across depths can thus reveal how fire legacy influences biogeo-

chemical cycling and microbial ecosystem functions over time (Revillini et al., 2025). However, the presence of antimicrobial resistance (AMR) genes in post-fire soils represents an emerging concern, as selective pressures from fire-induced stress and heavy metal enrichment may enhance horizontal gene transfer and promote the persistence of resistance even in natural ecosystems—a phenomenon that has not yet been thoroughly investigated.

Despite growing recognition of the ecological significance of soil microbes under RSC, comprehensive metagenomic analyses that integrate taxonomic, functional, and resistance gene profiles across soil depths are still lacking. Addressing this knowledge gap is crucial for understanding the ecological sustainability of RSC systems and their role in maintaining soil functional integrity under changing land-use pressures. Therefore, this study aimed to (i) assess the vertical stratification of microbial diversity, community composition, and functional potential across soil depths, (ii) determine depth-related functional specialization of microbial assemblages, and (iii) evaluate the distribution of key biogeochemical and antimicrobial resistance genes in relation to soil resilience and ecosystem functioning. We hypothesized that microbial diversity and community composition vary significantly with soil depth. Specifically, surface layers enriched in charcoal from fire inputs are expected to support lower diversity and specialized taxa, whereas deeper horizons harbor more diverse and evenly distributed microbial communities. We further hypothesized that microbial functional potential exhibits vertical stratification, with surface soils enriched in genes related to nutrient metabolism, while subsoils are dominated by genes associated with stress response and adaptation. Finally, we anticipated that genes involved in nutrient cycling and AMR would also display depth-dependent patterns, reflecting distinct ecological roles that shape soil resilience, fertility, and overall ecosystem functioning in shifting cultivation systems.

2 Materials and methods

2.1 Study area

The study site was situated in Ban Mae Pok, Ban Thab Subdistrict, Mae Chaem District, Chiang Mai Province, Northern Thailand (18°23'30.8" N, 98°103'56.1" E). The region receives an annual rainfall ranging from 1105 to 2688 mm, most of which occurs during the rainy season (May–October). During the cool season (October–February), minimum temperatures range between 3.2 °C and 22.1 °C, while during the hot season (February–April), maximum temperatures reach 35–40 °C (Arunrat et al., 2024b). The soils in these highland areas, characterized by slopes greater than 35%, are classified as Slope Complex

series (LDD, 1992).

The RSC field (18°23'02.8" N, 98°11'49.3" E; elevation 660 m a.s.l.) was previously used for upland rice cultivation and has remained abandoned since the harvest in 2017, resulting in an eight-year fallow period by 2025. At 0–5 cm depth, the soil was slightly acidic (pH 5.14) with low electrical conductivity (0.16 dS m⁻¹). It contained relatively high soil organic matter (5.62%) and total nitrogen (0.17%), along with available phosphorus of 12.53 mg kg⁻¹. The concentrations of exchangeable K, Ca, and Mg were 219.46, 461.01, and 184.54 mg kg⁻¹, respectively. The soil texture was classified as silty loam, comprising 30.53% sand, 53.65% silt, and 15.82% clay (Arunrat et al., 2025b).

2.2 Experimental design and soil sampling

Soil samples were collected in October 2025. The RSC field was divided into five transects located along the upper, middle, and lower slopes. Within each transect, three plots (1 m × 1 m) were established at the upper, middle, and lower slope positions for sampling. In each plot, soil samples were collected from three distinct layers along the soil profile, each characterized by varying charcoal distributions extending to deeper horizons. The vertical distribution of charcoal was identified based on observable physical characteristics, including soil color and charcoal particle content, which guided layer classification. Stones, grasses, roots, and other residues were manually removed before sampling.

Steel knives were used to collect soil samples. The charcoal-mixed surface layer (0–2 cm) was designated as the CS layer, where fine charcoal particles were visible in the surface soil. The nutrient leaching layer (10–20 cm) was designated as the N layer, and the deep, undisturbed soil layer (100 cm) was designated as the D layer. Samples from the upper, middle, and lower slope positions were composited to obtain one representative sample per layer within each transect. Approximately 1 kg of soil per layer was collected in plastic bags for physicochemical analyses, while about 100 g was placed in sterile zip-lock bags and stored at -20 °C for DNA extraction.

2.3 Soil physical and chemical properties analysis

Soil pH was measured using a pH meter in a 1:1 soil-to-water suspension (Soil Survey Staff, 2014). Electrical conductivity (ECe) was determined from the saturation paste extract using an EC meter (Soil Survey Staff, 2014). Total nitrogen (TN) was analyzed by the micro-Kjeldahl method (Soil Survey Staff, 2014). Exchangeable calcium (Ca), magnesium (Mg), and potassium (K) were extracted with 1 M ammonium acetate (NH₄OAc) at pH 7.0 and quantified using atomic absorption spectrometry (Thomas, 1982;

Jones, 2001). Available phosphorus (P) was determined using the Bray II extraction method followed by molybdate blue colorimetry (Bray and Kurtz, 1945; Jones, 2001). Organic carbon (OC) was measured by potassium dichromate ($K_2Cr_2O_7$) oxidation in sulfuric acid following the Walkley and Black method (Walkley and Black, 1934; Jones, 2001), and soil organic matter (SOM) was estimated by multiplying OC by 1.724. Soil texture was analyzed using the hydrometer method (Beretta et al., 2014).

2.4 DNA extraction and metagenome shotgun sequencing

Genomic DNA was extracted from 250 mg of soil per sample. Environmental DNA was isolated using the DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. DNA quality was verified by 1% agarose gel electrophoresis, and DNA concentrations were determined using a NanoDrop Eight spectrophotometer (Thermo Scientific, Wilmington, DE, USA) (Arunrat et al., 2025b).

Shotgun metagenomic sequencing was performed using 200 ng of DNA per sample for library preparation. Sequencing libraries were constructed with the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, USA), with unique index codes assigned to each sample according to the manufacturer's instructions. DNA fragments were sheared to approximately 350 bp by sonication, followed by end-repair and purification using the AMPure XP system (Beckman Coulter Life Sciences, USA). Library size distribution was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, USA), and library concentrations were quantified by real-time PCR. Indexed libraries were clustered on a cBot Cluster Generation System and sequenced in paired-end mode (2×150 bp, 300 cycles) on the NovaSeq 6000 platform (Illumina Inc., USA) after dilution to 0.6 nM (Arunrat et al., 2025b).

2.5 Diversity analysis and taxonomic classification

Raw sequencing data were processed for quality control to remove low-quality reads and adapter sequences. Reads with an average Phred quality score below 30 were discarded, and adapters were trimmed using FASTP (Chen et al., 2018). High-quality reads were taxonomically classified with Kraken 2 (Wood et al., 2019) against the NCBI nt database (release November 2023; available at the website of benlangmead.github.io/aws-indexes/k2), and relative abundances at the species level were refined using Bracken (Lu et al., 2017). Only taxa supported by ≥ 100 assigned reads in at least three replicates per layer were retained for downstream analysis. Taxonomic profiles were analyzed in QIIME2 (Bolyen et al., 2019) (version 2024.10). Alpha diversity (species richness and Shannon index) was used to

assess within-sample diversity, while beta diversity was evaluated using Bray–Curtis distances. Statistical differences in microbial communities were tested via PERMANOVA with 999 permutations.

To examine genes associated with key biogeochemical processes, including nitrogen, phosphorus, sulfur, and methane cycles, high-quality reads were aligned to the NCycDB (Tu et al., 2019), PCycDB (Zeng et al., 2022), SCycDB (Yu et al., 2021), and MCycDB (Qian et al., 2022) databases using DIAMOND (Buchfink et al., 2015) with parameters set to $\geq 80\%$ sequence identity and alignment length $>50\%$. Raw read counts were normalized to enable robust quantification and comparison of gene abundances across pathways using DESeq (Love et al., 2014).

In parallel, quality-filtered reads were assembled into contigs using metaSPAdes (Nurk et al., 2017) with default k-mer settings. Contigs shorter than 500 bp were removed prior to gene prediction with metaProdigal (Hyatt et al., 2012). Predicted genes were functionally annotated using EggNOG-mapper v2 (Cantalapiedra et al., 2021) against the EggNOG v5.0 database. Functional classifications, including Clusters of Orthologous Groups (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, were extracted, and the abundance of each gene category was expressed as counts per million (CPM), calculated as the relative proportion multiplied by 1 000 000.

2.6 Statistics analysis and visualization

All statistical analyses were conducted using IBM SPSS Statistics software (version 20; IBM Corporation, USA). Differences in soil physicochemical parameters among the three layers were examined using one-way ANOVA followed by Tukey's HSD post hoc test. Variations in taxonomic composition, alpha diversity indices, and the relative abundances of KEGG pathways and COG categories among soil metagenomes were evaluated using Welch's *t*-test implemented in STAMP (Parks et al., 2014). Associations between microbial community structure and soil physicochemical characteristics were assessed through Spearman's rank correlation using the SciPy package (Virtanen et al., 2020). Data visualization, including bar charts, heatmaps, and ordination plots, was carried out using ggplot2 (Wilkinson, 2011) in R and plotnine in Python.

3 Results

3.1 Soil physicochemical properties across soil layers

Significant variations in soil physicochemical properties were observed among the three layers (CS, N, and D) (Table 1). The surface charcoal-rich layer (CS) exhibited the highest

Table 1 Soil physicochemical properties across soil layers.

Properties	Layers		
	CS	N	D
pH	6.17±0.20 ^a	5.16±0.10 ^b	4.60±0.10 ^c
ECe (dS m ⁻¹)	0.45±0.10 ^a	0.14±0.00 ^b	0.07±0.00 ^c
Total N (%)	0.31±0.04 ^a	0.10±0.01 ^b	0.04±0.010 ^c
SOM (%)	6.60±0.30 ^a	2.95±0.16 ^b	0.77±0.094 ^c
Avail.P (mg kg ⁻¹)	137.95±13.60 ^a	49.96±2.80 ^b	17.42±2.20 ^c
Exch.K (mg kg ⁻¹)	2155.68±141.80 ^a	127.57±7.80 ^b	24.32±2.30 ^c
Exch.Ca (mg kg ⁻¹)	9126.16±336.80 ^a	2078.41±149.20 ^b	805.33±15.90 ^c
Exch.Mg (mg kg ⁻¹)	2065.87±105.50 ^a	628.88±66.90 ^b	184.73±65.90 ^c
Sand (%)	20.55±0.70 ^a	18.36±1.90 ^b	18.68±0.82 ^b
Silt (%)	46.60±1.40 ^a	39.11±1.07 ^b	35.41±0.55 ^c
Clay (%)	32.85±1.30 ^a	42.54±2.48 ^b	45.91±1.09 ^c

^{a-c} Denotes significant differences ($p \leq 0.05$) determined by one-way ANOVA followed by Tukey's HSD post hoc test.

soil fertility and nutrient availability, followed by the nutrient leaching layer (N), while the deep layer (D) showed the lowest values for most parameters. Soil pH decreased significantly with depth, from 6.17 in the CS layer to 5.16 in the N layer and 4.60 in the D layer. Similarly, ECe declined markedly from 0.45 dS m⁻¹ in the CS layer to 0.14 dS m⁻¹ and 0.07 dS m⁻¹ in the N and D layers, respectively. Total nitrogen (0.31%), soil organic matter (6.60%), available phosphorus (137.95 mg kg⁻¹), and exchangeable cations (K, Ca, and Mg) were all significantly higher in the CS layer than in the N and D layers, indicating strong nutrient enrichment in the surface horizon. In contrast, the D layer exhibited substantial depletion in these parameters, reflecting limited organic input and nutrient leaching to deeper horizons. Soil texture also varied with depth: the CS layer contained the highest proportion of silt (46.60%) and the lowest clay content (32.85%), while the D layer was relatively clay-rich (45.91%) and silt-poor (35.41%). These textural differences suggest that surface layers are more porous and well-aerated, whereas the deeper layers are more compact and less favorable for microbial activity.

3.2 Richness and diversity

Comparisons of species numbers across the three layers (CS, N, and D) revealed significant differences among certain pairs. Specifically, the CS layer contained a significantly higher number of species than the D layer, indicating greater microbial richness. Shannon diversity and evenness indices also differed significantly among soil depths in the rotational shifting cultivation system (Fig. 1A). Pairwise comparisons showed that the CS layer exhibited significantly lower microbial diversity and evenness compared with the D layer.

Beta diversity patterns are presented in Fig. 1B. The first

two principal coordinates explained 64.3% and 27.8% of the total variation, respectively, accounting for more than 92% of the community dissimilarity. The ordination revealed a clear separation of the CS layer from the D layer along the first axis, while partial overlap was observed between the CS and N layers. In contrast, the D and N layers clustered more closely together, indicating greater similarity in their microbial community composition. Differences in community structure among the three layers were further supported by PERMANOVA, which revealed statistically significant differences between groups ($p < 0.05$). These patterns suggest that the CS layer harbors a distinct microbial assemblage compared with the D and N layers.

The Venn diagram in Fig. 1C shows that the three layers share a substantial core microbiome comprising 13 157 species. The CS layer harbored the highest number of unique species (853), supporting a more diverse and evenly distributed microbial community compared with the D (275 species) and N (324 species) layers. This pattern is consistent with Fig. 1D, which highlights the distinct clustering of the CS layer relative to the D and N layers.

3.3 Taxonomic profiles and diversity

The relative abundance of microbial taxa showed distinct differences across soil depths. Bacteria were dominant in all layers, with Actinomycetota, Acidobacteriota, Bacillota, and Pseudomonadota comprising the majority of sequences. In the CS layer, Bacillota (36%–41%) and Actinomycetota (38%–43%) were most abundant, while Acidobacteriota and Pseudomonadota occurred at lower levels (0.48%–0.54% and 33%–41%, respectively). In the N, layer, Bacillota (42%–45%) and Actinomycetota (33%–38%) remained dominant, with a slightly higher proportion of Acidobacteriota (1.0%–1.5%) compared to CS soil. In the D soil, Actino-

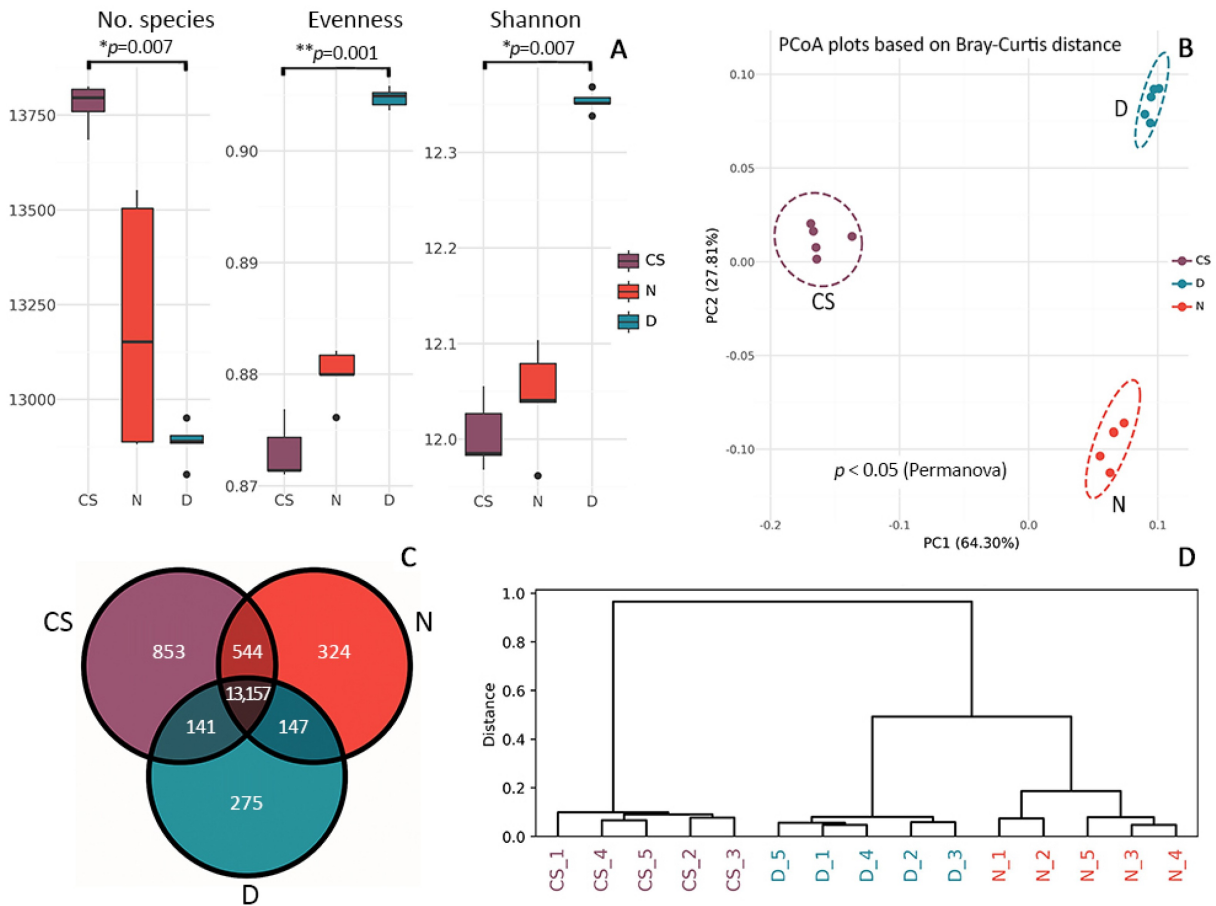


Fig. 1 Comparison of alpha and beta diversity. (A) Species richness, evenness, and Shannon diversity index. (B) Principal Coordinates Analysis (PCoA) based on Bray–Curtis distances. (C) Number of shared and unique species in three layers (CS, D, and N). (D) Hierarchical clustering constructed using Ward's linkage method.

mycetota abundance increased (39%–44%), while Bacillota decreased markedly (30%–34%), indicating a shift toward taxa adapted to more oligotrophic conditions. Acidobacteriota were also more prevalent in D (1.8%–2.3%) than in upper layers (Fig. 2A).

Eukaryotic taxa, including Fungi, Metazoa, and Viridiplantae, were consistently detected across all depths but at much lower relative abundance (<12%). Fungal sequences ranged from ~1.0% in CS layer to 1.4%–1.5% in D layer. Metazoa and Viridiplantae showed higher relative abundance in deeper soils (11.5%–11.7% and 1.9%–2.0%, respectively) compared with the surface (7.6%–8.6% and 1.4%–1.7%). Archaea were consistently low in abundance (<1%) across all depths (Fig. 2A).

Relative abundance analysis revealed clear depth-related patterns among archaeal taxa. In the CS layer, halophilic taxa such as *Halobaculum*, *Halorubrum*, and *Nitrososphaera* were notably enriched. *Nitrososphaera* exhibited particularly high relative abundance in CS samples, whereas its abundance sharply decreased in deeper soils (D and N layers). Similarly, *Halobaculum* maintained high abundance in CS compared to reduced levels in deeper layers (Fig. 2B). In

contrast, the deep soil layer was characterized by enrichment of methanogenic archaea. *Methanosarcina* displayed a striking increase and remaining elevated across D soil samples, compared to CS layer. Likewise, *Methanothrix* was more abundant in D layer than in CS layer (Fig. 2B). The nutrient-leaching layer (N layer) exhibited intermediate patterns. Some halophilic taxa such as *Halobacterium* and *Halorussus* maintained relatively stable abundances across CS, N, and D layers, while ammonia-oxidizing archaea (*Nitrosocosmicus* and *Nitrososphaera*) were significantly reduced in N layer relative to CS layer (Fig. 2B).

The bacterial community composition varied notably across the CS, N, and D layers (Fig. 2C). Among the Actinomycetota, *Streptomyces* was the most dominant taxon overall, with the highest relative abundance observed in the D layer (15%–17%), followed by N (13%–14%) and CS (11%–12%) layers. In contrast, *Nocardioides* and *Arthrobacter* were more abundant in the CS layer (3%–4%) but markedly lower in D (0.6%–1.4%) layer. Additionally, *Mycobacterium* and *Mycolicibacterium* were more abundant in N (1.8%–2.3%) layer, suggesting adaptation to stable soil conditions. Among the Pseudomonadota, nitrogen-fixing

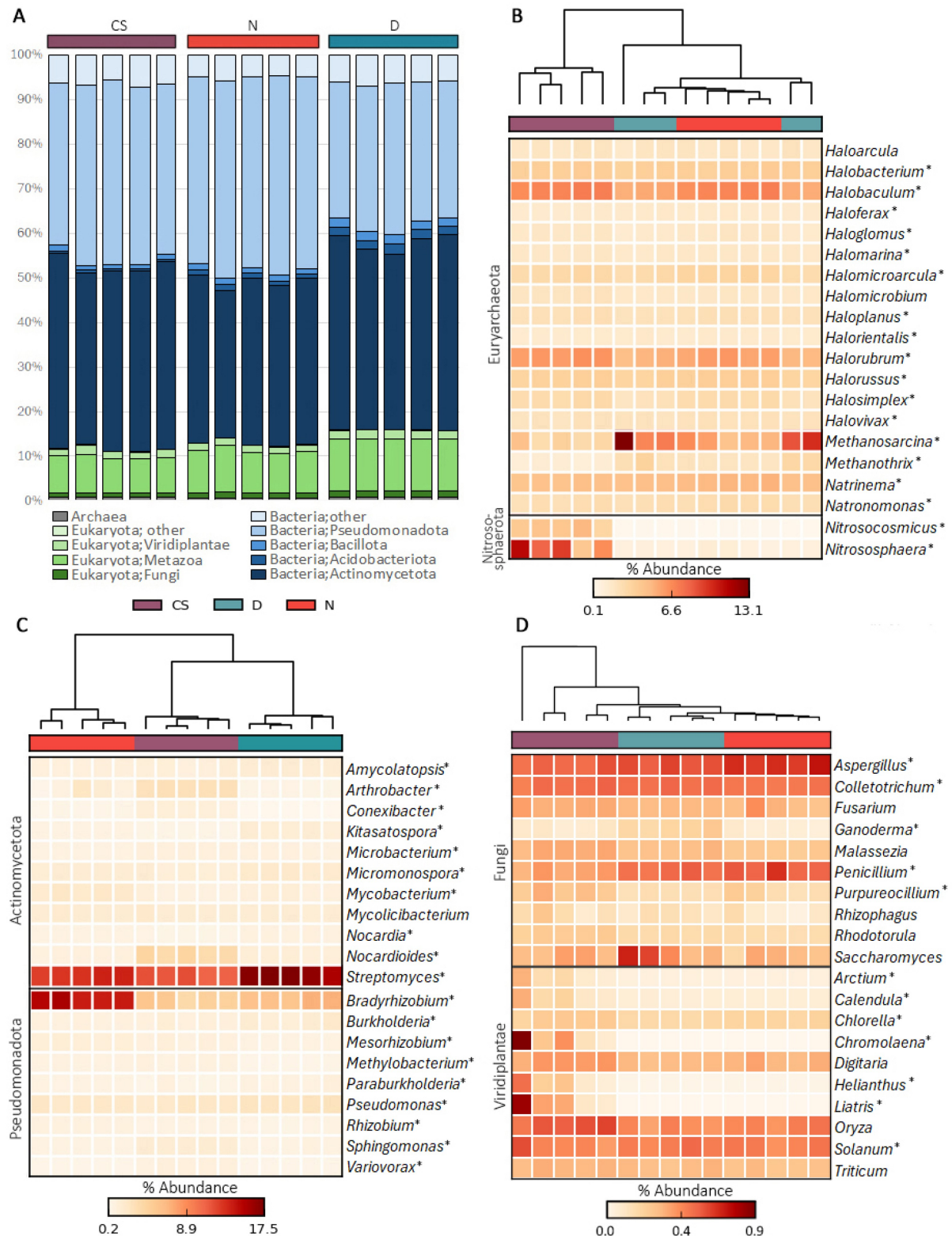


Fig. 2 Comparative taxonomic profiles of layer CS, D, and N soil samples. (A) The relative abundance of Archaeal, Bacterial, and Eukaryotic domains. Dendrogram heatmaps showing the relative abundance (%) of the top 20 taxa within the archaea (B), bacteria (C), and eukaryote (D), respectively.

bacteria, including *Bradyrhizobium*, *Rhizobium*, and *Mesorhizobium*, were strongly enriched in the N layer, where *Bradyrhizobium* reached 14%–15%, compared with only

6%–7% in D layer and 4%–5% in CS layer. *Pseudomonas* maintained a relatively stable abundance across layers (~2%–3%), while *Burkholderia* was more enriched in D

(~1.4%–2.1%) layer and *Sphingomonas* was more abundant in CS (~1.6%–1.7%) layer compared with N and D (~0.7%–0.8%) layers (Fig. 2C).

The relative abundances of fungal and Viridiplantae taxa varied among the CS, N, and D soil layers (Fig. 2D). Among fungi, *Aspergillus* and *Penicillium* were consistently abundant across all layers, with higher relative abundance in the D and N layers (~0.58%–0.65%) compared with CS (~0.50%–0.52%). *Colletotrichum* remained stable across layers (~0.48%–0.52%), while *Fusarium* showed moderate values with a peak. In contrast, *Saccharomyces* displayed sharp fluctuations, being low in CS (~0.29%–0.38%) layer (Fig. 2D). For Viridiplantae taxa, *Oryza* was the most abundant taxon overall, with the highest mean relative abundance in the CS layer, followed by the N layer and the D layer. *Solanum* displayed a relatively stable abundance across all layers. *Digitaria* and *Triticum* were moderately abundant, both showing a slight decline with increasing depth. *Chromolaena* and *Liatris* were detected exclusively in the CS layer with high variability among replicates, and were completely absent in both D and N layers (Fig. 2D).

3.4 Functional potential of microbial communities (COG categories)

The distribution of COG functional categories revealed distinct differences across the CS, N, and D soil layers (Fig. 3A). For cellular processes and signaling, functions of cell cycle control, cell division, and chromosome partitioning (COG D) showed the highest relative abundance in the CS layer but declined in the D and N layers. Cell wall/membrane/envelope biogenesis (COG M) was most enriched in the D layer, followed by CS and N layers. Cell motility (COG N) was higher in the CS layer compared with the D and N layers. Signal transduction mechanisms (COG T) were also more abundant in CS and N layers compared with D layer (Fig. 3B).

In term of information storage and processing, translation, ribosomal structure, and biogenesis (COG J) functions were consistently higher in CS layer, while transcription (COG K) and replication, recombination, and repair (COG L) were strongly enriched in the D layer (Fig. 3B).

Within metabolism, energy production and conversion (COG C) and amino acid transport and metabolism (COG E) functions were highest in the CS layer and lowest in D layer. Carbohydrate (COG G), lipid (COG I), and inorganic ion transport and metabolism (COG P) functions showed relatively balanced levels across layers, although CS generally exhibited slightly higher values. In contrast, secondary metabolites biosynthesis, transport, and catabolism (COG Q) functions were lower in D layer compared with CS and N layers (Fig. 3B).

Overall, the CS layer was characterized by higher abun-

dances in translation, energy production, amino acid metabolism, and motility, while the D layer was enriched in transcription, replication, repair, and cell wall biogenesis. The N layer generally displayed intermediate values but tended to resemble CS in several categories such as signal transduction and lipid metabolism.

3.5 Kyoto encyclopedia of genes and genomes (KEGG) pathway-based functional profiling

The KEGG pathway analysis revealed distinct functional profiles among the CS, N, and D layers (Fig. 3C). The D layer exhibited the highest relative abundance in core functions such as metabolic pathways, carbon metabolism, oxidative phosphorylation, and biosynthesis of secondary metabolites, suggesting a strong capacity for energy production and secondary metabolism in subsoil microbes. In contrast, the CS layer showed enrichment in ABC transporters, biosynthesis of amino acids, purine and pyrimidine metabolism, and glyoxylate and dicarboxylate metabolism, indicating enhanced nutrient acquisition, amino acid synthesis, and nucleotide metabolism near the surface (Fig. 3D). Meanwhile, the N layer was characterized by higher abundances of microbial metabolism in diverse environments, pyruvate metabolism, quorum sensing, and the two-component system, reflecting greater microbial communication and environmental adaptability (Fig. 3D). Collectively, these findings suggest functional specialization across soil depths, with the D layer emphasizing energy generation, the CS layer nutrient biosynthesis, and the N layer adaptation and signaling functions.

3.6 Gene abundance of nitrogen, phosphorus, sulfur and methane cycles

Differential abundance analysis revealed clear stratification of functional genes associated with biogeochemical cycles across soil depths in the RSC system (CS: 0–2 cm; N: 10–20 cm; D: 100 cm) (Fig. 4A). The methane-related genes were found in relatively low proportions compared with the other nutrient cycles (Fig. 4B). The CS layer exhibited the highest overall gene abundance across all functional categories, indicating enhanced microbial potential for methane oxidation, nitrogen transformation, and nutrient cycling near the soil surface. In contrast, the D layer consistently showed the lowest gene abundance, reflecting reduced microbial activity and substrate availability with depth, while the N layer displayed intermediate values, suggesting a transitional microbial community between surface and deeper horizons.

For the methane cycle, genes associated with methane oxidation and carbon metabolism—such as *GLYA* (glycine hydroxymethyltransferase), *ACDA* (acetate–CoA ligase subunit α), and *ACS* (acetyl–CoA synthetase)—were domi-

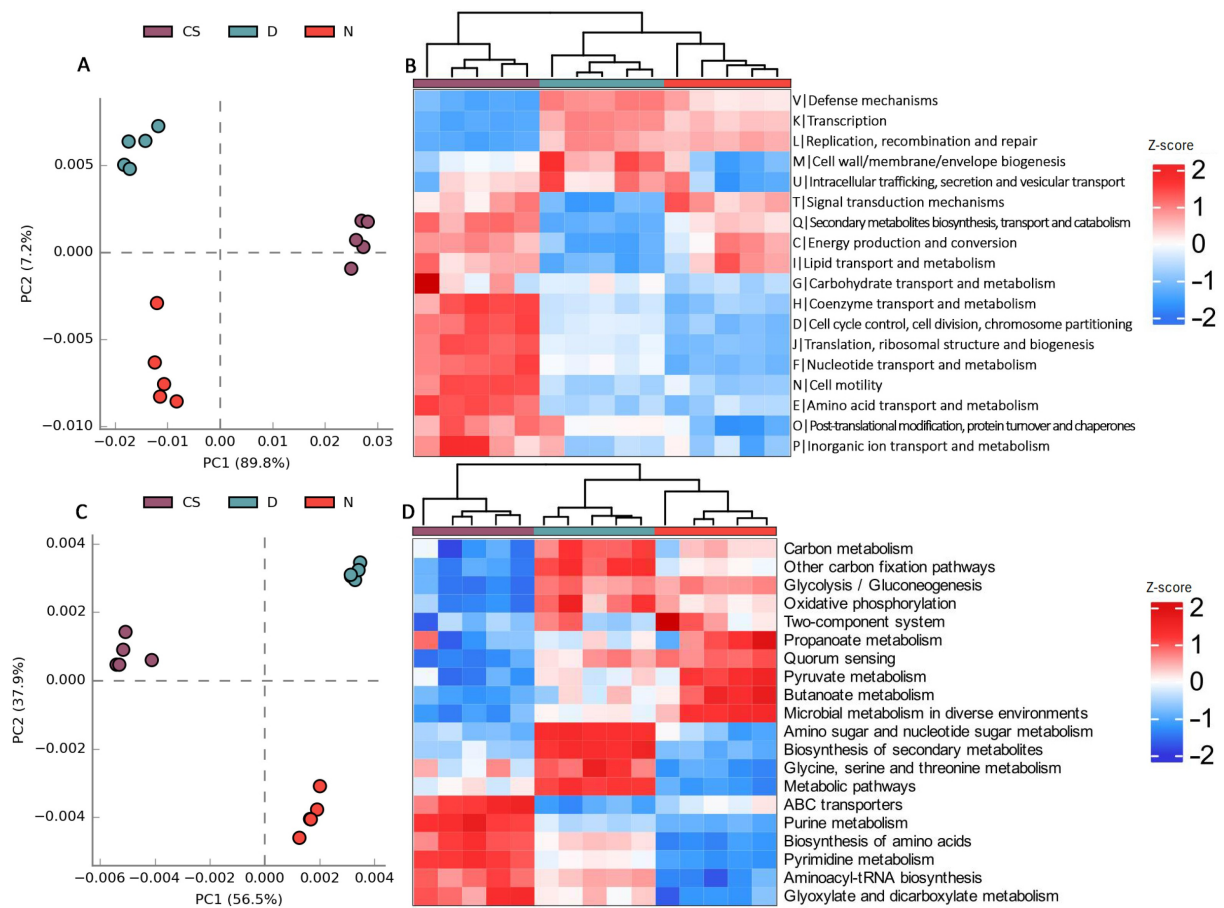


Fig. 3 Functional annotation of metagenomic genes. (A) Principal Component Analysis (PCA) of COG profiles, (B) Z-score heatmap of each COG category, (C) Principal Component Analysis (PCA) of KEGG pathway profiles, (D) Z-score heatmap of the top 20 KEGG pathways.

nant in the CS layer. Conversely, genes including *ACKA* (Acetate kinase), *FDOG* (Formate dehydrogenase major subunit), and *MDH-K00024* were enriched in the D and N layers, indicating enhanced energy metabolism and redox processes under suboxic conditions. Genes associated with glycolysis and carbon flow—such as *GPMI* (2,3-bisphosphoglycerate-independent phosphoglycerate mutase), *ENO* (enoyl-acyl carrier protein reductase), and *PPC* (phosphoenolpyruvate carboxylase)—showed a gradual decline with increasing soil depth, indicating that soil stratification strongly influences microbial carbon-processing functions. Overall, methane cycle genes displayed a clear depth-dependent pattern, with the CS layer favoring aerobic methane oxidation and carbon fixation, while deeper layers supported anaerobic energy metabolism (Fig. 4B).

Similarly, the nitrogen cycle genes exhibited distinct stratification (Fig. 4B). The CS layer exhibited the highest abundance of key nitrogen-cycle genes—*NOSZ* (nitrous oxide reductase), *NIRK* and *NIRS* (nitrite reductases involved in NO formation), and *GLNA* (glutamine synthetase)—indicating enhanced microbial potential for denitrification and ammonium assimilation in surface soils. This suggests that the

upper soil horizon supports more active nitrogen transformation processes, likely driven by higher organic matter and oxygen availability. The N layer displayed intermediate abundance, while the D layer showed the lowest levels, consistent with limited nitrogen turnover at depth. Genes involved in nitrous oxide and nitrite reduction (*NOSZ*, *NIRK*, *NIRS*) were markedly reduced in deeper horizons, indicating declining denitrification potential. The enrichment of *GLNA* and *NMO* (2-naphthoate monooxygenase) in the surface layer further underscores the predominance of active nitrogen assimilation and oxidative processes near the soil surface.

In the sulfur cycle, most sulfur-associated genes—*CYSA* (sulfate/thiosulfate transport system ATP-binding protein), *SSUB* (sulfonate transport system ATP-binding protein), *BETB* (betaine-aldehyde dehydrogenase), and *CYSC* (adenylylsulfate kinase)—were significantly more abundant in the CS layer, indicating enhanced microbial sulfur oxidation and assimilation under aerobic surface conditions. The D layer showed the lowest gene abundance, indicating suppressed sulfur metabolism under limited oxygen and nutrient conditions. The N layer demonstrated a moderate recovery of certain genes, suggesting residual sulfur trans-

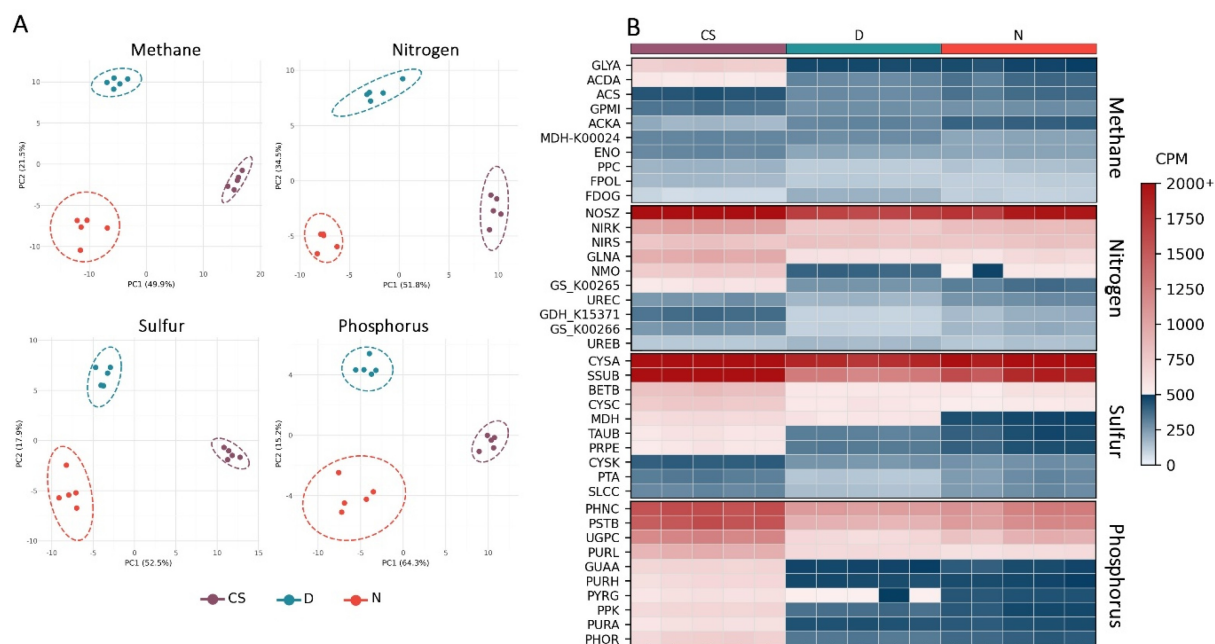


Fig. 4 Abundances of functional genes. (A) Principal Component Analysis (PCA) illustrating the distribution of functional genes across soil depths (CS, D, and N layers), (B) Relative abundances of functional genes associated with the methane, nitrogen, sulfur, and phosphorus cycles. CPM = Counts Per Million values (CPM).

formation potential in subsoils. Overall, sulfur-related gene abundance decreased with depth, confirming that surface layers support the highest sulfur metabolic activity (Fig. 4B).

In the phosphorus cycle, most phosphorus-associated genes—*PHNC* (phosphonate transport system ATP-binding protein), *PSTB* (phosphate transport system ATP-binding protein), and *UGPC* (sn-glycerol 3-phosphate transport system ATP-binding protein)—were predominantly enriched in the CS layer and declined markedly with depth, reflecting a reduction in microbial phosphate uptake and utilization in deeper soil horizons. Genes such as *PURL* (phosphoribosyl-formylglycinamide synthase), *GUAA* (GMP synthase (glutamine-hydrolyzing)), and *PURH* (phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase) exhibited similar overall depth-related trends but showed slight enrichment in the N layer, suggesting that microbial phosphorus metabolism and nucleotide biosynthesis remain active even in subsurface horizons. Overall, phosphorus gene abundance followed a clear vertical gradient, with strong cycling potential in the surface layer and diminished yet sustained activity in deeper soils (Fig. 4B).

Collectively, these findings reveal a pronounced depth-dependent pattern in functional gene abundance across methane, nitrogen, sulfur, and phosphorus cycles. The soil mixed with charcoal (CS layer) supports a metabolically diverse and active microbial community driven by oxygen availability and organic inputs, whereas deeper layers (N and D) host microorganisms adapted to energy conservation and redox balance under more reduced conditions.

3.7 Abundance of antimicrobial resistance genes

The analysis revealed clear differences in the abundance of antimicrobial resistance (AMR) genes across soil depths (CS, N, and D layers) (Fig. 5). *Rifamycin* resistance genes were by far the most abundant across all samples, with average values exceeding 55% in CS and N layers, and slightly higher levels in the deeper D layer (~64%–68%). *Macrolide* resistance genes ranked second in abundance, consistently ranging from 15%–20% across all depths. Moderate abundances were observed for *aminocoumarin* (~5%–7%) and *glycopeptide* resistance genes. *Glycopeptide* resistance was particularly enriched in the D layer (~7%–10%) compared to CS and N (~2%–5%) layers. Similarly, *aminocoumarin* showed elevated abundance in the D and N layers relative to CS layer. Other AMR gene families, including *mupirocin-like*, *penicillin beta-lactam*, *phenicol*, and *tetracycline*, occurred at lower levels (~1%–4%), with *phenicol* resistance showing sporadic but high peaks in the N layer (~6%–7%). *Aminoglycoside* resistance genes exhibited the lowest overall abundance (<3%), though slightly higher values were detected in the CS layer compared to D and N layers.

4 Discussion

4.1 Influence of fire legacy and soil depth on microbial diversity and community structure

The results clearly demonstrate that both fire legacy and soil

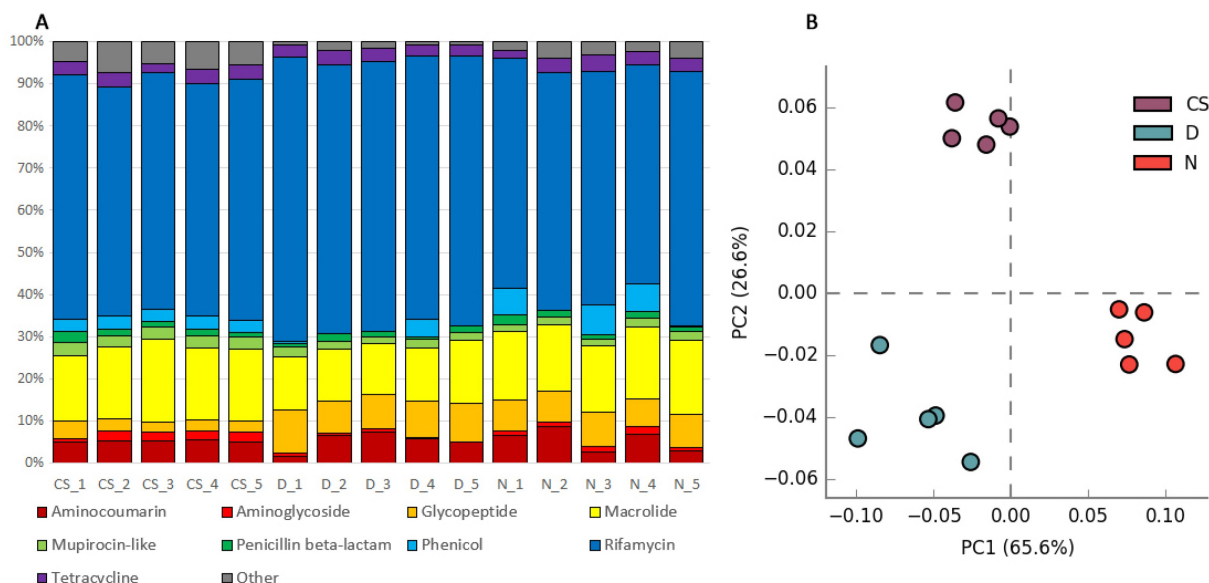


Fig. 5 The distribution of antimicrobial resistance (AMR) genes. (A) Percent relative abundance of AMR genes, (B) Principal Component Analysis (PCA) of AMR genes.

depth exerted a strong influence on microbial diversity and community assembly in RSC soils. The charcoal-rich surface layer (CS) harbored the highest number of observed features but exhibited significantly lower Shannon diversity and evenness compared with deeper layers (Fig. 1). This pattern indicates that although surface soils contain many taxa, community structure is unevenly distributed and dominated by a few specialized groups. The reduced diversity in the CS layer may be attributed to the selective pressure of fire-altered soil properties, including increased pH, the presence of recalcitrant pyrogenic carbon, and altered nutrient stoichiometry. Such conditions often favor the proliferation of stress-tolerant or opportunistic microorganisms while suppressing more sensitive taxa, as observed in other fire-affected ecosystems (Dooley and Treseder, 2012; Whitman et al., 2019). Charcoal residues can also adsorb labile organic compounds, reducing substrate availability and creating microsites that favor specialized decomposers and spore-forming taxa such as *Bacillus* and *Actinomyces* (Fig. 2).

In contrast, the deeper mineral layers (D and N) supported more diverse and evenly distributed microbial communities, suggesting a gradual recovery of community balance with increasing depth. These layers experience more stable moisture and temperature regimes and are less influenced by surface fire disturbance, allowing for the persistence of oligotrophic and slow-growing taxa. The increased evenness in the D layer likely reflects niche diversification and reduced competition for easily decomposable substrates. Beta diversity analysis further highlighted the vertical stratification of microbial communities, with a clear separation between CS and D layers and partial overlap between CS and N layers. This distinct clustering indicates that fire-induced changes at

the surface extend beyond immediate impacts on abundance, altering long-term microbial assembly processes and niche partitioning. The shared core microbiome among layers suggests a degree of vertical continuity, yet the large number of unique taxa in the D layer underscores depth-specific ecological specialization (Fig. 1). The distinct microbial assemblage in the CS layer can also be linked to the physical and chemical legacy of fire-derived charcoal, which increases soil porosity and creates microsites conducive to specific taxa capable of utilizing aromatic carbon compounds. Members of Actinomycetota and Bacillota, which were abundant in the CS layer (Fig. 2C), are known to degrade complex organic materials and tolerate harsh post-fire conditions (Wolińska et al., 2017; Yaradoddi and Kontro, 2022). Their dominance suggests that these taxa play pivotal roles in the early stages of post-fire soil recovery by contributing to organic matter decomposition and nutrient turnover. Conversely, the higher relative abundance of Acidobacteriota and Pseudomonadota in deeper layers indicates adaptation to oligotrophic environments with lower carbon and oxygen availability.

Collectively, these results suggest that the legacy of fire and the resulting vertical gradients in soil properties create a hierarchical structure of microbial diversity and composition. The CS layer represents a specialized niche dominated by copiotrophic and stress-resistant microorganisms adapted to fluctuating conditions and complex organic inputs. On the other hand, deeper horizons harbor more functionally stable and diverse communities that underpin long-term soil ecosystem recovery. This vertical partitioning underscores the resilience of RSC soils, where microbial communities reorganize along depth gradients to maintain essential ecological functions despite periodic fire disturbance.

While this study demonstrates clear depth-dependent stratification in microbial diversity and functional potential, the absence of an unburned reference soil limits our ability to fully separate fire legacy effects from inherent vertical gradients. Depth-related variations in microbial communities are well established and driven by changes in substrate availability, oxygen, and soil properties. Therefore, some observed patterns may reflect general soil processes rather than fire effects alone.

Nevertheless, the marked differences in the charcoal-rich surface layer—such as higher nutrient levels, distinct taxa, and enriched metabolic and stress-related genes—indicate that fire-derived inputs play an important role in shaping microbial communities. Thus, our findings should be interpreted as fire-associated patterns within a burned system rather than definitive causal effects. Future studies including paired burned and unburned soils are needed to confirm fire-specific impacts.

4.2 Depth-dependent taxonomic shifts reflect niche specialization and resource availability

The taxonomic composition of microbial communities revealed pronounced depth-dependent differentiation, reflecting the combined influence of fire legacy, nutrient gradients, and oxygen availability on microbial niche specialization. The surface charcoal-rich layer (CS) was dominated by Bacillota and Actinomycetota, while deeper layers exhibited increasing proportions of Acidobacteriota and Pseudomonadota (Fig. 2C). This vertical shift in dominant phyla indicates a transition from copiotrophic to oligotrophic lifestyles with increasing depth, consistent with decreasing labile carbon and oxygen availability. Bacillota and Actinomycetota are well-known for their metabolic versatility, spore-forming ability, and resilience to environmental stresses such as desiccation, temperature fluctuation, and fire-derived compounds (Nicholson et al., 2000; Subramani and Sippkema, 2019). Their enrichment in the CS layer suggests an adaptive advantage in fire-affected soils where periodic disturbance creates dynamic redox conditions and intermittent nutrient pulses.

In contrast, Acidobacteriota, which increased markedly in the D layer (Fig. 2C), are typically slow-growing, acid-tolerant organisms adapted to nutrient-poor and stable environments (Männistö et al., 2013). Their abundance in deeper horizons suggests an ecological strategy oriented toward energy conservation and efficient substrate utilization under oligotrophic conditions. Similarly, the enrichment of Pseudomonadota, particularly nitrogen-fixing taxa (Tao et al., 2024) such as *Bradyrhizobium*, *Rhizobium*, and *Mesorhizobium*, in the N layer highlights the role of these bacteria in maintaining nitrogen availability during post-fire recovery (Fig. 2C). The predominance of *Bradyrhizobium* in subsoils

(up to 15% relative abundance) suggests strong adaptation to microaerophilic conditions and potential symbiotic association with plant roots penetrating lower horizons (Gitonga et al., 2021). Such taxa are crucial for sustaining nitrogen inputs in RSC systems, particularly after burning events that lead to N volatilization and loss.

At the genus level, distinct depth-associated patterns further emphasize microbial niche differentiation. *Streptomyces*, abundant in the D layer (Fig. 2C), are filamentous Actinomycetes known for decomposing complex organic matter such as lignin and cellulose under limited nutrient conditions (Javed et al., 2021). Their prevalence at depth indicates active participation in the slow turnover of recalcitrant soil organic carbon. In contrast, *Nocardioides* and *Arthrobacter*, which were more abundant in the CS layer (Fig. 2C), are opportunistic decomposers that thrive on labile carbon compounds derived from charred plant residues. These taxa are also associated with polyaromatic hydrocarbon degradation (Plotnikova et al., 2011; Ma et al., 2023), suggesting that fire-derived organic compounds serve as selective substrates in the surface horizon.

Archaeal communities also exhibited clear vertical stratification corresponding to soil redox and energy gradients. The CS layer was enriched with halophilic and ammonia-oxidizing taxa, including *Halobaculum*, *Halorubrum*, and *Nitrososphaera* (Fig. 2B), reflecting the presence of oxidized microsites and active nitrification potential near the surface (Podell et al., 2014; Nakagawa et al., 2025). In contrast, methanogenic archaea such as *Methanosarcina* and *Methanotherix* dominated the D layer (Fig. 2B), where reduced oxygen conditions favor methanogenesis (Enzmann et al., 2018). This pattern indicates a functional shift from aerobic ammonia oxidation in surface soils to anaerobic methane production at depth—a hallmark of stratified redox environments.

Eukaryotic taxa, though less abundant overall, also followed consistent depth-dependent trends. Fungal taxa such as *Aspergillus* and *Penicillium* were more prevalent in the D and N layers (Fig. 2D), consistent with their roles as efficient decomposers under nutrient-limited conditions (Jabinski et al., 2024). Their ability to degrade complex carbon compounds likely contributes to the maintenance of soil organic matter turnover in subsoils. Conversely, the lower abundance of *Saccharomyces* in surface soils reflects the sensitivity of yeasts to fluctuating temperature and moisture conditions post-fire. Plant-derived sequences, including *Oryza* and *Digitaria*, detected in the upper layers (Fig. 2D), likely originate from root-associated microbiomes or rhizodeposits, indicating residual root-microbe interactions that contribute to surface nutrient cycling (Hanzawa et al., 2013; Oreja et al., 2025).

The observed taxonomic transitions along the soil profile highlight the interplay between resource availability, oxygen

gradients, and microhabitat stability in shaping microbial community composition. The surface layer favors fast-growing, stress-tolerant, and metabolically flexible microorganisms capable of exploiting transient nutrient inputs from fire residues. In contrast, deeper layers host more specialized and slow-growing taxa adapted to energy-limited, microaerophilic conditions. This vertical differentiation underscores a key ecological principle in soil microbiomes: stratified resource and redox environments foster distinct microbial guilds that collectively sustain ecosystem functions across depths. Overall, the observed depth-dependent taxonomic patterns provide strong evidence for niche partitioning and functional complementarity within the RSC soil profile. Fire-induced heterogeneity at the surface selects for specialized taxa capable of rapid nutrient cycling, while the stability and reduced energy flux at depth promote microbial groups that maintain long-term carbon and nitrogen turnover.

4.3 Functional specialization across soil depths

The vertical differentiation of microbial functional profiles revealed clear evidence of depth-dependent specialization, indicating that microbial communities have adapted distinct metabolic strategies to the changing physicochemical environments with soil depth. The surface charcoal-rich layer (CS) exhibited pronounced enrichment of genes related to nutrient acquisition, energy metabolism, and stress tolerance (Fig. 3A), reflecting the legacy of fire disturbance and the higher availability of labile organic inputs (VanderRoest et al., 2024). In contrast, the deeper mineral layers (N and D) were dominated by genes associated with cell replication, repair, carbon degradation, and nitrogen transformation (Fig. 3A), consistent with the more stable and oligotrophic conditions found below the surface (Xie et al., 2025).

The enrichment of carbohydrate metabolism and ABC transporter pathways in the CS layer (Fig. 3B) suggests active utilization of easily available carbon sources derived from decomposing plant residues and root exudates following cultivation and fire events. ABC transporters facilitate the uptake of sugars, amino acids, and secondary metabolites (Yazaki, 2006), providing an adaptive advantage in environments with fluctuating substrate availability. Similarly, the dominance of genes involved in amino acid and lipid metabolism in the CS layer indicates microbial strategies to rapidly exploit nutrient pulses associated with post-fire organic matter mineralization. These metabolic patterns align with the copiotrophic lifestyle of surface-dwelling taxa such as Bacillota and Actinomycetota, which are well-known for their ability to metabolize a wide spectrum of carbon compounds and produce extracellular enzymes involved in organic matter decomposition (Li et al., 2021).

At intermediate depths (N layer), functional profiles shifted toward genes related to oxidative phosphorylation, cofactor

and vitamin metabolism, and signal transduction (Fig. 3B). This suggests a transitional zone where microbial communities maintain moderate metabolic activity while adapting to reduced carbon and oxygen availability (Dolferus et al., 1994). Such intermediate horizons often serve as zones of microbial turnover, balancing inputs from the surface with diffusion-driven nutrient fluxes from below. The presence of genes associated with membrane transport and stress response further implies adaptation to fluctuating redox conditions typical of mid-depth soils.

In the deeper layer (D), the enrichment of genes for DNA replication, repair, and secondary metabolite biosynthesis highlights microbial strategies for survival under energy-limited and nutrient-poor conditions (Fig. 3B). These functions reflect a shift toward oligotrophy and dormancy, where microbes invest in genome maintenance and stress adaptation rather than growth. The higher relative abundance of nitrogen and sulfur cycle genes in the D layer—such as those involved in denitrification, ammonification, and sulfur oxidation—suggests that deeper microbial communities play a critical role in sustaining essential nutrient transformations despite limited organic carbon inputs (Dong et al., 2024).

These findings emphasize that microbial functional diversity in RSC soils is not randomly distributed but reflects a finely tuned depth-related specialization shaped by both fire legacy and resource availability. This vertical functional stratification represents an essential mechanism supporting the resilience and nutrient recovery of RSC ecosystems, allowing soils to maintain ecological functionality even under recurrent disturbance cycles. It is important to note that metagenomic analyses provide insights into the functional potential of microbial communities based on gene presence, rather than direct evidence of active metabolic processes. The detection of functional genes does not necessarily indicate their expression or activity under field conditions. Therefore, the functional patterns observed in this study should be interpreted as potential capabilities of the microbial community. Future studies integrating transcriptomic approaches or enzyme activity assays would be valuable to verify the extent to which these pathways are actively expressed *in situ*.

4.4 Linkages between functional genes and biogeochemical cycling

The distribution of functional genes across soil depths revealed clear linkages between microbial community structure and the regulation of methane, nitrogen, sulfur, and phosphorus cycling processes (Fig. 4). These depth-dependent variations reflect both the legacy effects of fire-derived organic inputs and the progressive decline in substrate quality and nutrient availability with depth. Collectively, the observed patterns indicate that fire-altered surface environ-

ments favor rapid turnover of labile nutrients, whereas deeper layers sustain slower but continuous biogeochemical activity essential for long-term soil fertility.

Dominant genes such as *GLYA*, *ACDA*, and *ACS* were highly abundant in the surface (CS) layer, reflecting strong microbial potential for methane oxidation and carbon assimilation under aerobic conditions. *GLYA* is linked to one-carbon metabolism and the serine pathway for methane-derived carbon assimilation (Ogawa et al., 2000). *ACDA* and *ACS* are key enzymes in the conversion of acetate to acetyl-CoA, an essential step in both methanotrophic and acetogenic pathways (Ferry, 2015; Ouboter et al., 2023). The enrichment of *ACKA*, *FDOG*, and *MDH-K00024* in deeper layers suggests a metabolic shift toward anaerobic oxidation of methane (AOM) and alternative electron-accepting processes, consistent with suboxic or anoxic conditions at depth (Smemo and Yavitt, 2011; Su et al., 2022). These patterns indicate that the CS layer supports active aerobic methane oxidation, while the D and N layers harbor facultative or obligate anaerobes capable of methane-linked redox metabolism.

The N cycle was dominated by *NOSZ*, *NIRK*, *NIRS*, and *GLNA* genes in the CS layer, which are involved in denitrification and ammonium assimilation (Kandeler et al., 2006). *NOSZ* encodes nitrous oxide reductase, the key enzyme responsible for reducing N_2O to N_2 , thereby completing the denitrification pathway (Müller et al., 2022). *NIRK* and *NIRS* catalyze the reduction of nitrite to nitric oxide, pivotal steps under oxygen-limited yet nitrate-rich conditions (Irisa et al., 2014; Zhu et al., 2025). The high abundance of *GLNA* suggests enhanced ammonium assimilation and nitrogen retention in surface soils (Lin et al., 2025). In contrast, the decline of these genes in the D layer reflects restricted nitrogen turnover, likely due to lower organic inputs and limited electron acceptors at depth. These results align with studies showing that surface soils, rich in oxygen and labile organic matter, sustain greater denitrification and nitrification gene abundance than subsoils (Hernandez and Mitsch, 2007).

Sulfur-associated genes, particularly *CYSA*, *SSUB*, *BETB*, and *CYSC*, dominated in the CS layer, indicating active sulfur oxidation and assimilation near the surface. *CYSA* and *CYSC* encode enzymes in the assimilatory sulfate reduction pathway (Iwanicka-Nowicka et al., 2007). *SSUB* is involved in dissimilatory sulfite reduction (Marietou et al., 2018), while *BETB* participates in osmoprotection and sulfur-containing amino acid synthesis (Niazian et al., 2021). The sharp decrease in these genes with depth suggests that limited oxygen and reduced carbon inputs constrain sulfur oxidation processes in deeper horizons. The moderate presence of sulfur genes in the N layer implies partial retention of redox flexibility, enabling microbial adaptation to fluctuating oxygen and moisture regimes typical of transitional zones.

Phosphorus-associated genes such as *PHNC*, *PSTB*, and *UGPC* were dominant in surface soils, consistent with active phosphate uptake and regulation under nutrient-rich and aerobic conditions. *PSTB* encodes a component of the phosphate-specific transporter (Pst) system responsible for high-affinity phosphate uptake (Hudek et al., 2016), while *PHNC* and *UGPC* participate in phosphonate metabolism and glycerophosphodiester degradation, respectively (Schweizer and Boos, 1985; Martín and Liras, 2021). The enrichment of *PURL*, *GUAA*, and *PURH* in both CS and N layers indicates sustained phosphorus metabolism related to purine biosynthesis, even under reduced substrate availability at depth (Flannigan et al., 1990). The decline of these genes in the D layer reflects diminished microbial phosphate mobilization potential, corresponding to the lower organic matter and microbial biomass typically observed in subsoils.

4.5 Antimicrobial resistance (AMR) gene distribution

The dominance of *Rifamycin* and *Macrolide* resistance genes across all soil depths highlights the widespread distribution and ecological resilience of these resistance mechanisms in natural and agricultural soils. *Rifamycin* resistance is primarily mediated by mutations or methyltransferases that modify the RNA polymerase β -subunit encoded by *rpoB*, conferring high-level resistance (Andre et al., 2017). Its strong presence in both surface and deeper layers suggests that rifamycin-resistant taxa (e.g., *Actinobacteria*, *Streptomyces*) (Parra et al., 2023) are integral components of the soil microbiome, reflecting their ecological role in antibiotic biosynthesis and natural resistance. *Macrolide* resistance genes, second in abundance, are typically associated with *erm* (rRNA methyltransferase) and *mef* (efflux pump) gene families (Gomes et al., 2017). Their relatively consistent abundance across depths suggests stable maintenance of these genes in both aerobic and anaerobic conditions, potentially due to horizontal gene transfer (HGT) and the widespread occurrence of mobile genetic elements in soil (Suzuki et al., 2022).

The enrichment of *Glycopeptide* resistance genes in the D layer indicates the proliferation of microbial taxa capable of producing or resisting vancomycin-like compounds (Arthur and Courvalin, 1993). These genes, often associated with *vanA*, *vanB*, and *vanHAX* operons (Arthur et al., 1992; Arthur and Quintiliani, 2001), confer resistance via alteration of the peptidoglycan D-Ala-D-Ala target to D-Ala-D-Lac or D-Ala-D-Ser (Arthur et al., 1996; Hugonnet et al., 2014). The increased abundance in deeper layers aligns with reduced competition and slower nutrient turnover, conditions favoring resistant spore-forming genera.

Aminocoumarin resistance, also more pronounced in subsoils, reflects adaptation to natural antibiotics produced

by soil actinomycetes (e.g., novobiocin, clorobiocin) (Heide, 2014). These compounds target DNA gyrase, and resistance genes typically involve gyrase-protective mutations or efflux mechanisms (Schmutz et al., 2003). Elevated levels in the deeper layers suggest a legacy effect of microbial secondary metabolite production from earlier successional stages, or a residual selection pressure driven by slow organic matter turnover (Li and Heide, 2006), favoring specialized decomposers such as *Streptomyces*.

Lower but detectable levels of *Phenicol*, *Tetracycline*, and *Beta-lactam* resistance genes point to background resistance reservoirs commonly found in agricultural soils, likely maintained through co-selection with metal or biocide resistance (Roberts and Schwarz, 2016; Amarasekara et al., 2023). The sporadic peaks of *Phenicol* resistance in the N layer may reflect transient enrichment of specific microbial groups capable of chloramphenicol degradation under intermediate redox conditions (Zhang et al., 2020).

While fire-induced stress and shifts in microbial community composition likely contribute to the observed distribution of AMR genes, it is important to recognize that multiple environmental factors may act as co-selective pressures. Previous studies have shown that heavy metals (Baker-Austin et al., 2006; Guo et al., 2025), animal manure (Biggel et al., 2026) and microplastics (Balta et al., 2025) can promote the enrichment and persistence of resistance genes in soils through co-selection and horizontal gene transfer (Murray et al., 2024). In the present study, these factors were not directly measured and therefore cannot be excluded as potential contributors to the observed AMR patterns.

Consequently, the role of fire should be interpreted as one of several plausible mechanisms influencing AMR gene distribution in RSC soils, rather than a single dominant driver. This perspective aligns with the concept that soil resistomes are shaped by complex interactions among environmental stressors and microbial ecological processes (Wright, 2007; Forsberg et al., 2014). Future studies integrating measurements of heavy metals, antibiotic residues, and mobile genetic elements would provide a more comprehensive understanding of the processes governing AMR dynamics in fire-affected ecosystems.

5 Conclusion

Microbial diversity, taxonomic composition, and functional potential vary significantly with soil depth. The surface charcoal-rich layer exhibited lower microbial diversity but higher metabolic activity, with communities dominated by copiotrophic and stress-tolerant taxa. In contrast, deeper layers (N and D) supported higher richness and evenness, comprising oligotrophic taxa associated with nutrient conservation and redox balance. These patterns indicate clear

depth-dependent niche differentiation and are consistent with potential legacy effects of past burning interacting with vertical resource gradients. Functional gene analyses based on COG and KEGG annotations revealed strong vertical differentiation in microbial metabolism. Surface soils were enriched in genes related to amino acid biosynthesis, nutrient acquisition, and energy metabolism, whereas subsoil layers showed higher relative abundances of genes associated with DNA repair, replication, and stress adaptation. These findings suggest functional stratification of microbial communities across soil depths in fire-affected RSC soils. The distribution of biogeochemical functional genes indicated that methane, nitrogen, sulfur, and phosphorus cycling processes follow distinct vertical patterns, with surface layers supporting greater relative functional potential. These depth-dependent trends likely reflect differences in oxygen availability, organic matter inputs, and environmental conditions, and are consistent with patterns observed in fire-affected RSC soils. Similarly, the presence of diverse AMR genes across depths—particularly those conferring resistance to *Rifamycin*, *Macrolide*, and *Glycopeptide* antibiotics—suggests that microbial communities maintain genetic capacities for stress tolerance under varying soil conditions. However, given the absence of unburned control sites and pre-fire baseline data, these findings should be interpreted as associations rather than direct causal effects of fire. The observed patterns are consistent with potential legacy effects of past burning but may also reflect broader soil environmental gradients. Maintaining appropriate fallow cycles and avoiding excessive intensification may help sustain microbial-mediated nutrient cycling and long-term soil functionality in tropical upland agroecosystems.

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Author contributions

Noppol Arunrat: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Wuttichai Mhuan-tong: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. Sukanya Sereenonchai: Writing – original draft, Investigation, Conceptualization.

Data availability

The raw sequence data have been deposited in the NCBI

BioProject database under accession number PRJNA1401473.

Competing interests

The authors declare no competing interests.

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