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Soil bacterial communities in a 10-year fallow rotational shifting cultivation field and an 85-year-old terraced paddy field in Northern Thailand

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Abstract

Rotational shifting cultivation (RSC) and terraced paddy farming are the primary rice cultivation systems in the upland areas of Northern Thailand. Despite their prevalence, few studies have compared the microbial communities in these systems, particularly in rhizosphere and bulk soils. This study investigated microbial diversity and abundance in a 10-year fallow RSC field and an 85-year-old terraced paddy field. Results showed that microbial richness and evenness were significantly higher in terraced soils compared to RSC soils. In contrast, RSC soils, lacking contour walls and ridges, are more vulnerable to erosion and leaching, resulting in a loss of essential nutrients like soil carbon and nitrogen. The microbial community in RSC soils is characterized by a greater relative abundance of Firmicutes and Actinobacteria, whereas Proteobacteria and Acidobacteria predominate in terraced soils. Although terraced rhizosphere soils differed significantly from terraced bulk soils, they exhibited lower richness and diversity than the bulk soils. Moreover, no significant differences were observed between RSC bulk and RSC rhizosphere samples. *Bacillus* was the dominant genus in RSC bulk soil. *Geobacter* and *Sideroxydans* were more abundant in the terraced rhizosphere, while *Paenibacillus* and *Effusibacillus* dominated the RSC rhizosphere samples. Predicted enzyme abundances indicated that RSC soils were enriched in cellulase, alkaline phosphatase, amidase, and chitinase, reflecting a microbial emphasis on carbon, nitrogen, and phosphorus cycling processes. Meanwhile, terraced soils exhibited higher abundances of nitrite reductase, nitrate reductase, and methane monooxygenase, suggesting enhanced microbial capacities for nitrogen transformation and methane metabolism. Long-term monitoring is recommended to better understand microbial community dynamics and their role in nutrient cycling in these distinct agricultural systems.

Keywords Rotational shifting cultivation, Terraced paddy fields, Microbial diversity, Rhizosphere and bulk soils, Soil nutrient cycling

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Introduction

Microbial communities play a crucial role in sustaining soil health, enhancing crop productivity, and regulating greenhouse gas (GHG) emissions. These microorganisms drive key soil processes such as nutrient cycling [1–3], organic matter decomposition [4, 5], plant–microbe interactions [6, 7], and soil structure formation [8]. The interactions between microbial populations and their environment significantly influence the fertility and sustainability of agricultural systems. With increasing concerns about climate change and the demand for sustainable agricultural practices, understanding the dynamics of soil microbial communities is vital. By investigating these communities, we can uncover the mechanisms that maintain soil health and crop productivity while identifying strategies to mitigate GHG emissions.

Rotational shifting cultivation (RSC) and terraced paddy practices, two distinct approaches to rice cultivation in upland areas [9–11], create different environmental conditions that influence microbial communities in unique ways. Terracing, with its long-standing history, is well known for preventing soil erosion and efficiently managing water [12–14]. Terraced paddy fields are characterized by controlled water management, with fields remaining flooded throughout the cultivation period, minimal soil disturbance, and the application of chemical fertilizers. The flooding during the cultivation period and drying after harvest in terraced rice paddies create alternating reduced and oxidized conditions, fostering diverse microbial habitats. This dynamic environment facilitates the synergistic breakdown of complex organic matter by various microbial groups, such as fermenters, denitrifiers, sulfate and Fe(III) reducers, methanogens, and methanotrophs [15–17]. On the other hand, practices such as burning in RSC can cause episodic shifts in soil conditions, significantly impacting microbial communities. Although burning releases available nutrients like phosphorus, calcium, magnesium and potassium, it can also temporarily reduce microbial biomass and diversity due to the high temperatures involved [18]. Ash after burning alters soil pH, favoring microbes that can tolerate more alkaline conditions [19]. Moreover, upland rice cultivation in RSC occurs under aerobic conditions, in contrast to the flooded conditions typical of terraced paddy fields, leading to differences in root-associated microbial communities [20]. Transplanting is typically practiced in terraced rice paddies, while direct seeding is used for upland rice cultivation in RSC fields. These differences in planting methods can significantly influence the abundance and diversity of bacterial communities in the rhizosphere soil [21], as plant roots actively select specific microbial communities from the soil [22, 23]. However, no study in Thailand has investigated or compared the microbial communities in RSC and terraced paddy fields.

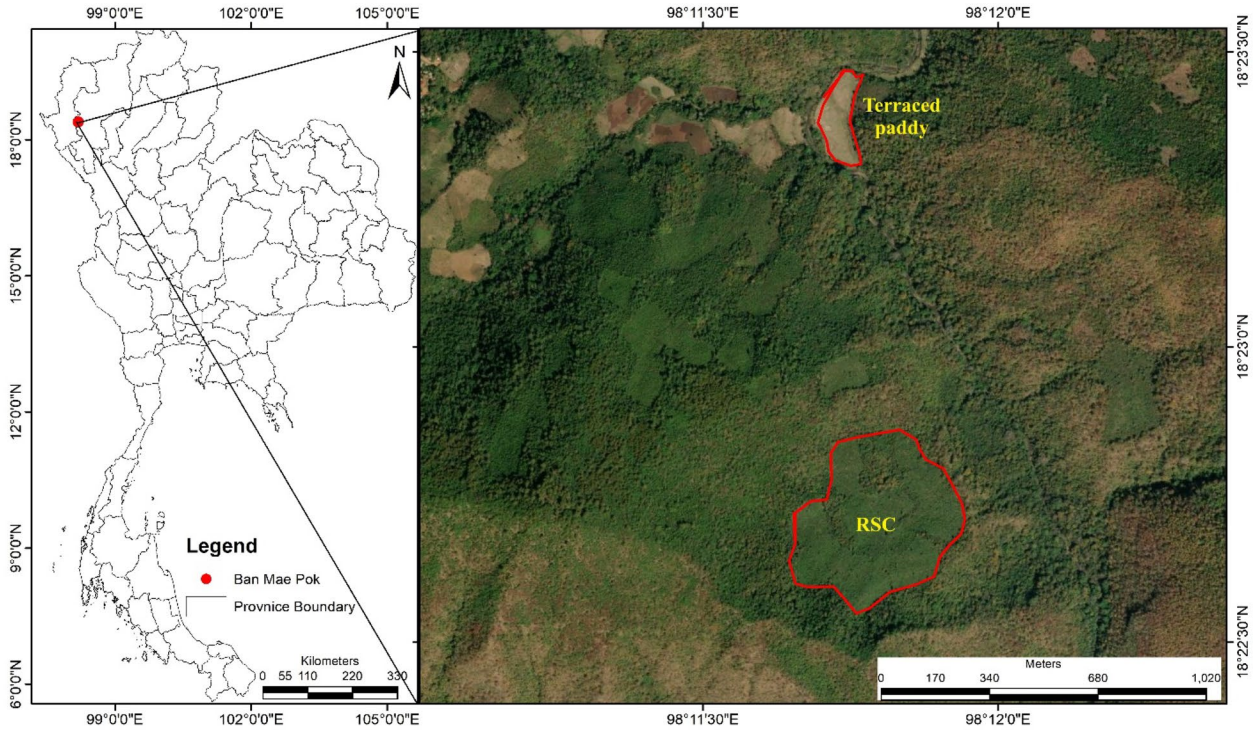
Understanding how different cropping systems in highland areas influence microbial communities is crucial, as their functional potential is highly sensitive to environmental changes. While significant research has been conducted on soil microbial communities, gaps persist, particularly regarding the effects of varied agricultural practices in upland regions. Most studies have either focused on bulk soil or root-associated soil, with few making direct comparisons. Bulk soil, which lacks direct root influence, generally has lower microbial diversity and activity due to limited nutrient availability. Its microbial community structure and diversity are primarily shaped by changes in soil physicochemical properties [24]. In contrast, root-associated soil, or the rhizosphere, benefits from higher concentrations of organic matter and nutrients from root exudates, supporting greater microbial diversity and activity [1, 25, 26]. This distinction is critical because root-associated microbes are key players in nutrient cycling, plant growth promotion, and soil health. However, the specific effects of terraced paddy versus RSC systems on microbial diversity and abundance remain underexplored.

This study aims to address these knowledge gaps by comparing microbial communities in bulk and rhizosphere soils across two agricultural systems: RSC and terraced paddy fields. The research has two primary objectives: (1) to evaluate differences in microbial diversity between these practices and how they shape community composition and functionality, and (2) to assess and compare soil bacterial communities between bulk and rhizosphere soils, as well as their functional potential. We hypothesize that: terraced paddy fields would exhibit higher microbial relative abundance and diversity compared to RSC fields (H1), and rhizosphere soil would show greater microbial relative abundance and diversity than bulk soil (H2). By addressing these objectives, this study offers practical management strategies centered on microbial diversity, ultimately contributing to sustainable agriculture and enhanced soil health management in upland regions.

Materials and methods

Study areas

The study area is located in Ban Mae Pok, Ban Thab Subdistrict, Mae Chaem District, Chiang Mai Province, Northern Thailand (Fig. 1). The rainy season lasted from May to October, with annual rainfall ranging from 1105 to 2688 mm. The winter season extended from October to February, with temperatures ranging from 3.2 to 22.1 °C, while the summer season occurred from



RSC field

Terraced paddy field

Fig. 1 Study area

February to April, with temperatures between 35 and 40 °C during 2021–2022 [18].

A field with a 10-year fallow period in RSC was investigated (Fig. 1). The RSC field (18°22′44.5′′N, 98°11′45.3′′E) was cut and burned in May 2023 for upland rice cultivation. No-tillage practices were followed, with the drilling method typically used. A hoe was employed to dig the soil to a depth of 10 cm, and upland rice seeds were then dropped by hand. No chemical fertilizers, herbicides, or insecticides used, and weeds were removed by hands. These practices represent the traditional methods used for RSC farming in this area. The upland rice relied solely on rainfall, and the harvest took place in October 2023. After harvesting, the field was left fallow to allow for natural recovery.

For a comparative study with similar geographic origin, soil formation, and microclimate conditions, a terraced paddy field (18°23′23.0′′N, 98°11′43.0′′E) was selected

(Fig. 1). According to the recorded history of the Ban Mae Pok community, this terraced paddy field has been under continuous cultivation since early 1939, spanning over 85 years. The transplanting method was used for terraced rice cultivation. The nursery plot (5 × 5 m) was prepared in a separate area of the terraced paddy field in May. Rice seeds were placed in bags and soaked in water for 24 h, then drained and dried in a shaded area for another 24 h. Pre-germinated rice seeds were sown by hand in the nursery plot. In mid-June, following puddling preparation under minimum tillage to a depth of 30 cm, rice seedlings were removed from the nursery plot and manually transplanted into the terraced paddy fields. Once all the rice seedlings were removed, the nursery plot was also prepared for transplanting. Chemical fertilizers, including 46-0-0 (156.25 kg ha^{-1}) and 16-16-8 (156.25 kg ha^{-1}) were applied. Harvesting took place in October by hand, with all rice residues left in the field without burning.

Soil sampling and soil physicochemical properties analysis

Soil samples were collected from both RSC and terraced paddy fields for comparative analysis. Samples were taken from a depth of 0–20 cm, representing the root zone in this study area. The soil samples were collected one month after harvest in November 2023. For the rhizosphere soil, 20–30 individual rice plants were randomly selected from each plot. After gently shaking off the loosely adhered soil, the remaining soil (approximately 1–2 cm thick around the roots) was homogenized to form one composite sample. For the bulk soil, samples were taken from outside the root zone of the rice plants using a mini shovel. Approximately 30 g of rhizosphere and bulk soils were placed into zip-lock plastic bags. In each field, 10 composite samples (five replicates for rhizosphere and five for bulk soils) were collected, and all samples were stored at − 20 °C for DNA extraction.

To determine the soil physicochemical properties of both RSC and terraced paddy fields, soil samples were collected from a depth of 0–20 cm from both rhizosphere and bulk soils. The samples were mixed, and 10 composite samples were taken from each field. Approximately 1 kg of soil from each composite sample was placed into a plastic bag. All soil samples were air-dried at room temperature, sieved through a 2 mm mesh, and analyzed for various soil physicochemical properties.

The soil bulk density (BD) was determined using a steel soil core (5.0 cm width × 5.5 cm length) and dried at 105 °C for 24 h [27]. The particle size distribution of sand, silt, and clay was analyzed using the hydrometer method [27]. Soil pH was measured with a pH meter in a 1:1 soil-to-water ratio [28], and electrical conductivity (ECe) was assessed with an EC meter [28]. Total nitrogen (Total N) was analyzed using the micro-Kjeldahl method [28]. Exchangeable calcium (Exch. Ca), magnesium (Exch. Mg), and potassium (Exch. K) were measured via atomic absorption spectrometry following extraction with NH_4OAc at pH 7.0 [29, 30]. Available phosphorus (Avail. P) was quantified using the Bray II extraction method [29, 31], and soil organic carbon (SOC) was determined through the potassium dichromate ($K_2Cr_2O_7$) oxidation method in sulfuric acid [29, 32].

DNA extraction and 16S rRNA gene sequencing

Soil DNA was isolated using the DNeasy PowerSoil Pro DNA Kit (Qiagen). The V3-V4 hypervariable region of the 16S rRNA gene was amplified with modified degenerate primers 341F (5′-CCTAYGGGDBGCWSCAG-3′) and 805R (5′-GGACTACNVGGGTHTCTAAT-3′), based on the design by Klindworth et al. [33]. The amplified products were sequenced on an Illumina Miseq platform in paired-end mode at the Center for Omics Sciences and Bioinformatics, Chulalongkorn University, Bangkok, Thailand. The resulting sequence data are available in the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA1152301.

Sequence processing and amplicon sequence variant (ASV) identification

Raw paired-end sequences underwent quality filtering and adapter trimming using FASTP [34], with subsequent quality checks performed using FASTQC [35] and MultiQC [36]. The cleaned paired-end reads were subsequently merged into single sequences using FLASH (version 1.2.7) [37] and processed in QIIME2 (version 2020.06) [38] to generate an ASV table via the DADA2 pipeline (version 1.14) [39]. This process included filtering, trimming, dereplication, error correction, ASV generation, and chimera removal. Taxonomic classification of ASVs was achieved using the consensus BLAST method within the 'qiime feature-classifier' plugin with the SILVA SSU rRNA database (v138) [40].

Diversity analysis

Diversity metrics were calculated using the "qiime diversity" plugin in QIIME2. Sequence reads were rarefied to the smallest sample size for consistent sampling depth across samples. Alpha diversity indices, including Shannon's index, Chao1, and Simpson's index, were computed and compared using the Kruskal–Wallis test. Beta diversity was assessed with Bray–Curtis dissimilarity and visualized through Principal Coordinate Analysis (PCoA). Statistical significance in beta diversity was evaluated using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations.

Enzyme prediction and functional composition analysis

The functional composition of microbial communities, predicted based on enzyme abundances, was inferred using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) method [41].

Statistical analysis

Statistical analyses were carried out using SPSS (Version 20.0). The *t*-test and least significant difference (LSD) tests ($p < 0.05$) were used to analyze the differences in soil physical and chemical properties between RSC and terraced paddy fields. Redundancy Analysis (RDA) was applied to evaluate the influence of soil properties on the composition of soil bacterial communities, with the significance of these relationships confirmed by the Mantel test. To identify differentially abundant bacterial taxa, Statistical Analysis of Metagenomic Profiles (STAMP) software was used [42]. Pairwise comparisons of bacterial genera were conducted using Welch's t-test with Benjamini–Hochberg false discovery rate (FDR) correction, and results were visualized as extended bar plots showing mean proportions and 95% confidence intervals. This analysis was used to evaluate compositional differences between management systems (RSC vs. Terraced) and soil compartments (bulk vs. rhizosphere). Additionally, STAMP was also used to detect predicted enzymes with significant changes in relative abundance between treatment groups, using default settings.

Results

Soil physicochemical properties

Significant differences were observed in certain soil physicochemical properties between terraced rice and RSC fields (Table 1). The pH of terraced rice was notably lower (4.93) compared to RSC (5.52), indicating a more acidic condition in terraced rice. The ECe was higher in RSC (0.13 dS/m) than in terraced rice (0.06 dS/m), suggesting higher salinity levels. Both soil types had similar organic matter content, total nitrogen, and avail.P levels, with no significant differences observed. This may be because slash-and-burn practices temporarily boost nutrient levels, and short cultivation followed by fallow periods helps retain them, whereas terraced fields maintain nutrients through fertilization but may also experience losses due to continuous cropping. Exch. K was higher in terraced rice (70.70 mg/kg) than in RSC (46.30 mg/kg). Exch. Ca was also greater in terraced rice (2017.20 mg/kg) compared to RSC (1663.90 mg/kg), whereas exch. Mg was higher in RSC (406.70 mg/kg) than in terraced rice (327.30 mg/kg). Moreover, RSC had a higher BD (1.29 mg/m^3) compared to terraced rice (1.08 mg/m^3). The textural composition of the soils differed as well: RSC contained more sand (21.30%) and less silt (26.60%) compared to terraced rice (13.40% sand and 30.60% silt), while the clay content was higher in terraced rice (56.00%) than in RSC (52.10%) (Table 1). However, no significant differences in soil physicochemical properties were found when comparing bulk and rhizosphere soils within terraced rice and RSC fields (Table 1).

The redundancy analysis (RDA) revealed that soil physical and chemical properties accounted for 68.3% of the total variation of the bacterial community in both the

Table 1 Physicochemical properties of bulk and rhizosphere soils in terraced rice and RSC fields

Soil properties	Bulk soil		Rhizosphere soil	
	Terraced rice	RSC	Terraced rice	RSC
pH (1:1)	4.93 ± 0.32^{aA}	5.52 ± 0.08^{bB}	5.01 ± 0.24^{aA}	5.60 ± 0.11^{bB}
ECe (dS/m)	0.06 ± 0.01^{aA}	0.13 ± 0.02^{bB}	0.05 ± 0.01^{aA}	0.11 ± 0.02^{bB}
OM (%)	3.55 ± 0.23^{aA}	3.37 ± 0.10^{aB}	3.51 ± 0.21^{aA}	3.40 ± 0.12^{aB}
Total N (%)	0.21 ± 0.01^{aA}	0.20 ± 0.01^{aB}	0.26 ± 0.02^{aA}	0.23 ± 0.01^{aB}
Avail.P (mg/kg)	33.10 ± 1.52^{aA}	45.40 ± 3.17^{aB}	39.15 ± 1.33^{aA}	43.87 ± 2.09^{aB}
Exch.K (mg/kg)	70.70 ± 2.83^{aA}	46.30 ± 8.41^{bB}	75.65 ± 2.05^{aA}	51.05 ± 5.33^{bB}
Exch.Ca (mg/kg)	2017.20 ± 100.10^{aA}	1663.90 ± 442.49^{bB}	2215.11 ± 201.22^{aA}	1769.13 ± 311.55^{bB}
Exch.Mg (mg/kg)	327.30 ± 14.10^{aA}	406.70 ± 8.54^{bB}	368.02 ± 15.18^{aA}	426.99 ± 11.23^{bB}
BD (Mg/m^3)	1.08 ± 0.04^{aA}	1.29 ± 0.22^{bB}	-	-
Sand (%)	13.40 ± 1.17^{aA}	21.30 ± 1.16^{bB}	10.60 ± 1.20^{aA}	18.80 ± 1.23^{bB}
Silt (%)	30.60 ± 1.26^{aA}	26.60 ± 1.43^{bB}	35.50 ± 1.11^{aA}	31.20 ± 1.15^{bB}
Clay (%)	56.00 ± 1.33^{aA}	52.10 ± 1.66^{bB}	53.90 ± 1.02^{aA}	50.00 ± 1.20^{bB}

$^{a-b}$ indicates significant differences ($p < 0.05$) between terraced rice and RSC fields for bulk and rhizosphere soils

$^{A-B}$ indicates significant differences ($p < 0.05$) between bulk and rhizosphere soils within terraced rice and RSC fields

terraced rice and RSC fields. The results indicated that total N and BD were strongly associated with the terraced rice field. Conversely, avail. P was positively associated with bulk soil in the RSC field, whereas OM, Exch. Ca, ECe, and Exch. Mg were positively associated with rhizosphere soils in the RSC field (Fig. 2). The remaining variation of the bacterial community could be due to factors like plant root exudates, microbial interactions, and unmeasured soil properties.

Diversity, taxonomic distribution and community composition

To characterize bacterial communities in the soil samples, 16S rRNA gene amplicon sequencing was conducted on all 20 samples collected from both RSC and terraced paddy fields. Sequencing depth prior to rarefaction ranged from 24,373 to 76,198 reads per sample. To ensure consistent sampling effort and minimize bias in diversity estimation, sequence reads were rarefied to a depth of 24,000 reads per sample, allowing all samples to be retained for downstream analyses. After quality control, denoising, and rarefaction, a total of 11,665 amplicon sequence variants (ASVs) were identified across all samples.

Alpha diversity indices, including Chao1, Shannon, and Simpson, were used to assess bacterial richness and diversity across the four treatments: RSC bulk, RSC rhizosphere, Terraced bulk, and Terraced rhizosphere (Fig. 3). The Chao1 index, which estimates species richness, indicated that both terraced treatments had significantly higher richness compared to the RSC samples ($p < 0.05$). Similar trends were observed for the Shannon and Simpson indices, which account for both richness and evenness, with the latter placing more emphasis on evenness. All three indices revealed that terraced soils exhibited significantly greater microbial diversity and evenness compared to RSC soils ($p < 0.05$). While terraced rhizosphere samples were significantly different from terraced bulk soils ($p < 0.05$), they showed lower richness and diversity than the bulk soils. No significant differences were observed between RSC bulk and RSC rhizosphere samples ($p > 0.05$). These findings suggest that land use management plays a dominant role in shaping bacterial diversity, while root compartment effects may become more evident under more favorable or stable conditions, such as those found in terraced fields.

Following the alpha diversity analysis, which revealed significant differences between RSC and terraced

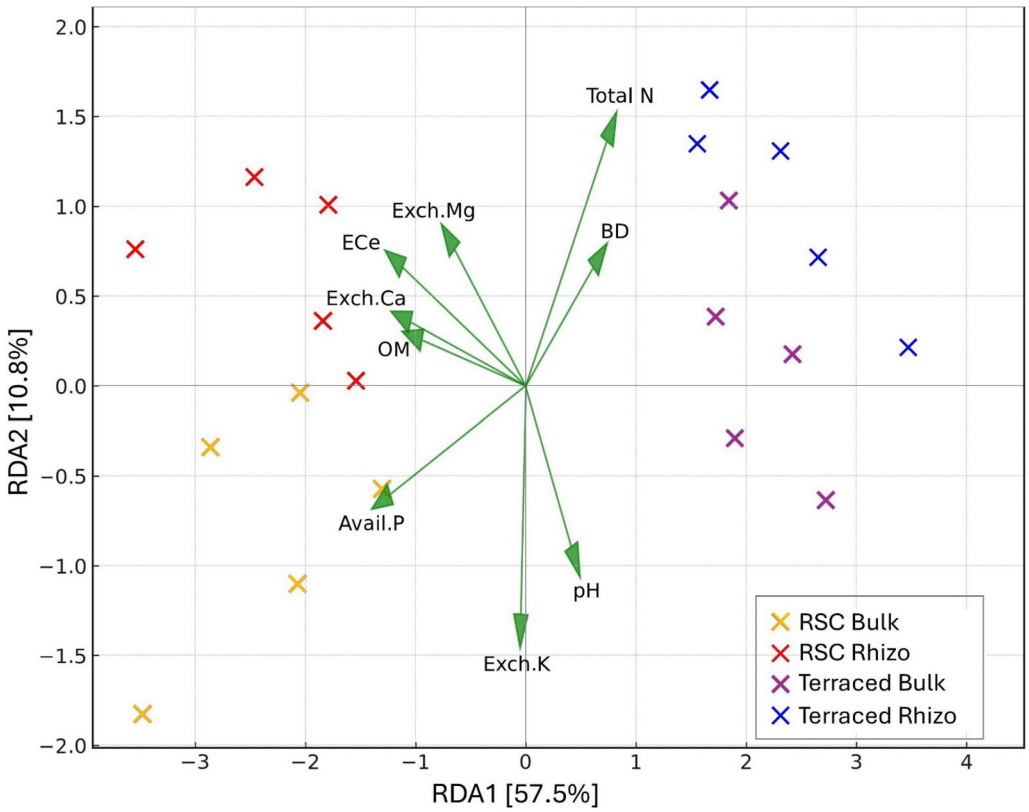


Fig. 2 Redundancy analysis (RDA) showing the soil physical and chemical properties significantly correlated with the variation of the bacterial community in terraced rice and RSC fields

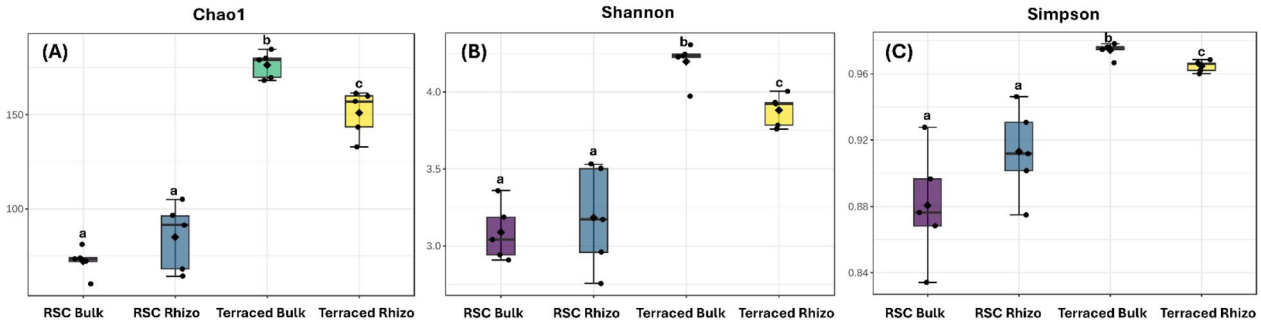


Fig. 3 Alpha diversity indexes of soil bacteria from different locations. **A** Chao1, **B** Shannon index and **C** Simpson index. Different lowercase letters indicate statistically significant differences among treatments ($p < 0.05$)

soils, the beta diversity of bacterial communities was assessed using principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity (Fig. 4). The PCoA plot displays all four treatment groups, enabling simultaneous evaluation of the effects of soil management and root compartment. Samples clustered primarily according to management system, with all pairwise comparisons between RSC and Terraced groups showing statistically significant differences ($p < 0.01$). In contrast, differences between rhizosphere and bulk soils within the same management type were not statistically significant (RSC: $p = 0.59$; Terraced: $p = 0.30$), although partial separation was observed in the ordination space. These findings support the hypothesis that

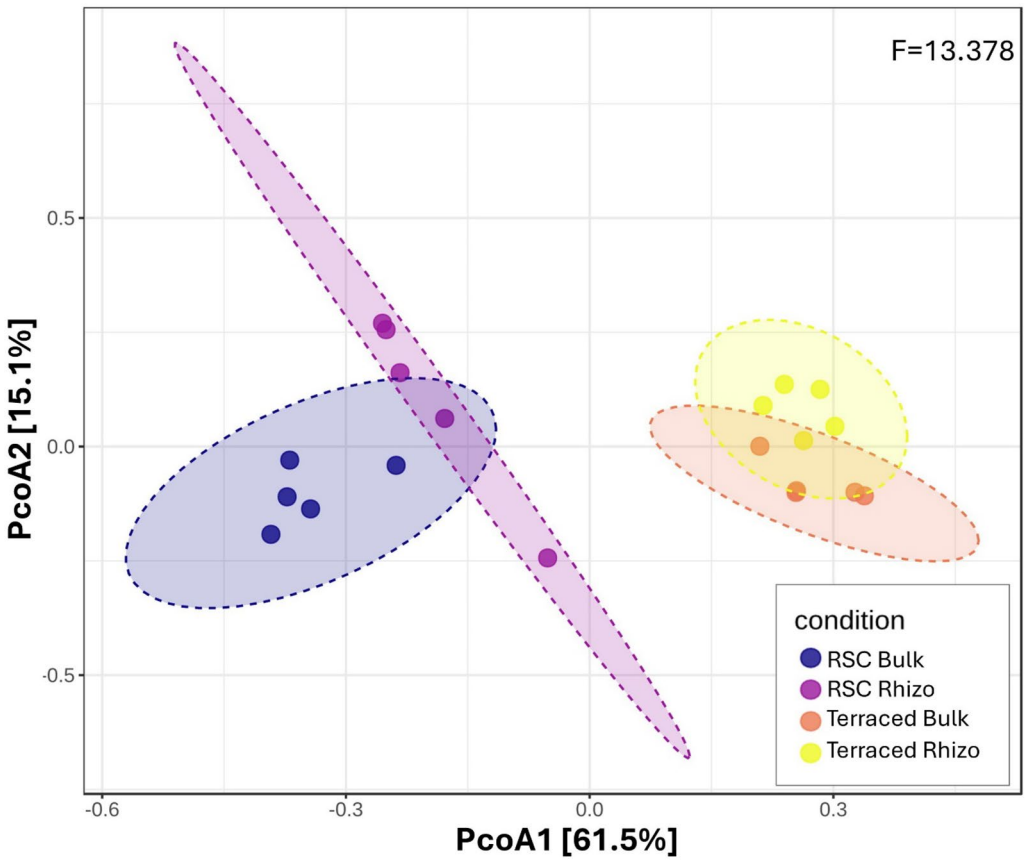


Fig. 4 PCoA analysis of bacterial communities. Each point corresponds to an individual community, with closer points indicating greater similarity in community composition

the management system is the primary driver of bacterial community composition, with minor contributions from rhizosphere–bulk compartmentalization under certain conditions.

The relative abundance of bacterial phyla across soil samples from RSC and terraced fields is presented in Fig. 5. A total of 25 phyla were identified in this study. The five most dominant phyla across both field types were Proteobacteria, Acidobacteria, Verrucomicrobia, Actinobacteria, and Firmicutes. RSC soils exhibited a notably higher relative abundance of Firmicutes and Actinobacteria, which appeared as the most visually dominant phylum in these samples. In contrast, Proteobacteria and Acidobacteria were more prominent in the terraced soils. Additionally, terraced soils displayed greater diversity among the less abundant phyla, whereas RSC soils were more strongly dominated by a few major phyla.

To investigate compositional differences in bacterial communities at the genus level, pairwise comparisons were performed using STAMP extended bar plots based on Welch's t-test with false discovery rate (FDR) correction (Fig. 6). In the comparison between Terraced-bulk and RSC-bulk soils (Fig. 6A), several genera, including *Nitrospira*, *Gaiella*, and *Geobacter*, were significantly more abundant in terraced soils. Although other genera such as *Gemmatimonas*, *Ammoniphilus*, and *Flavisolibacter* were more prevalent in RSC bulk soils, the genus *Bacillus* was particularly dominant, accounting for a markedly higher proportion in this group compared to all others. In the rhizosphere comparison (Fig. 6B), genera such as *Geobacter*, *Sideroxydans*, and *Nitrospira* were more abundant in the terraced rhizosphere, whereas *Paenibacillus* and *Effusibacillus* were dominant in RSC rhizosphere samples.

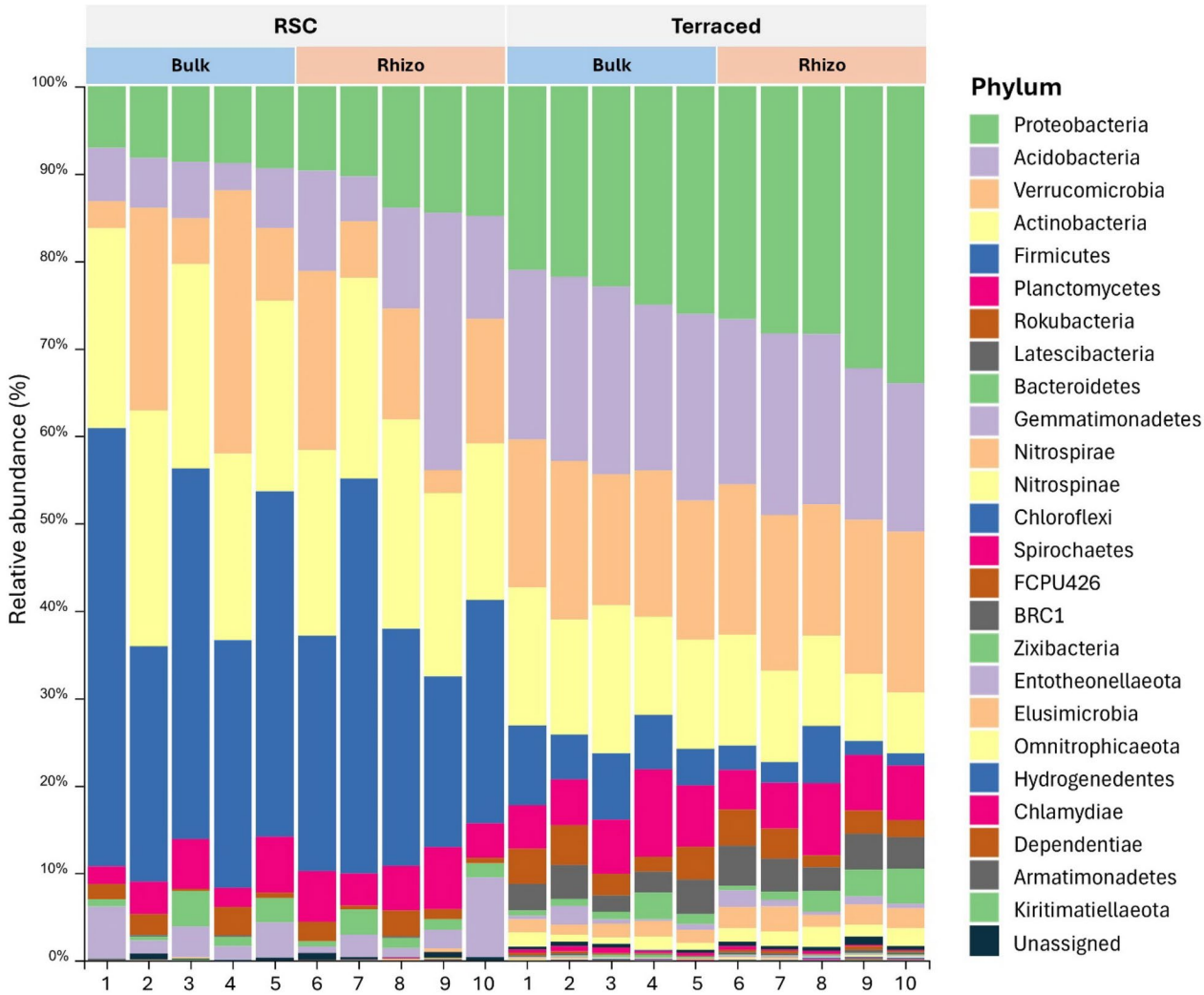


Fig. 5 Bacterial phyla composition in RSC and terraced soils

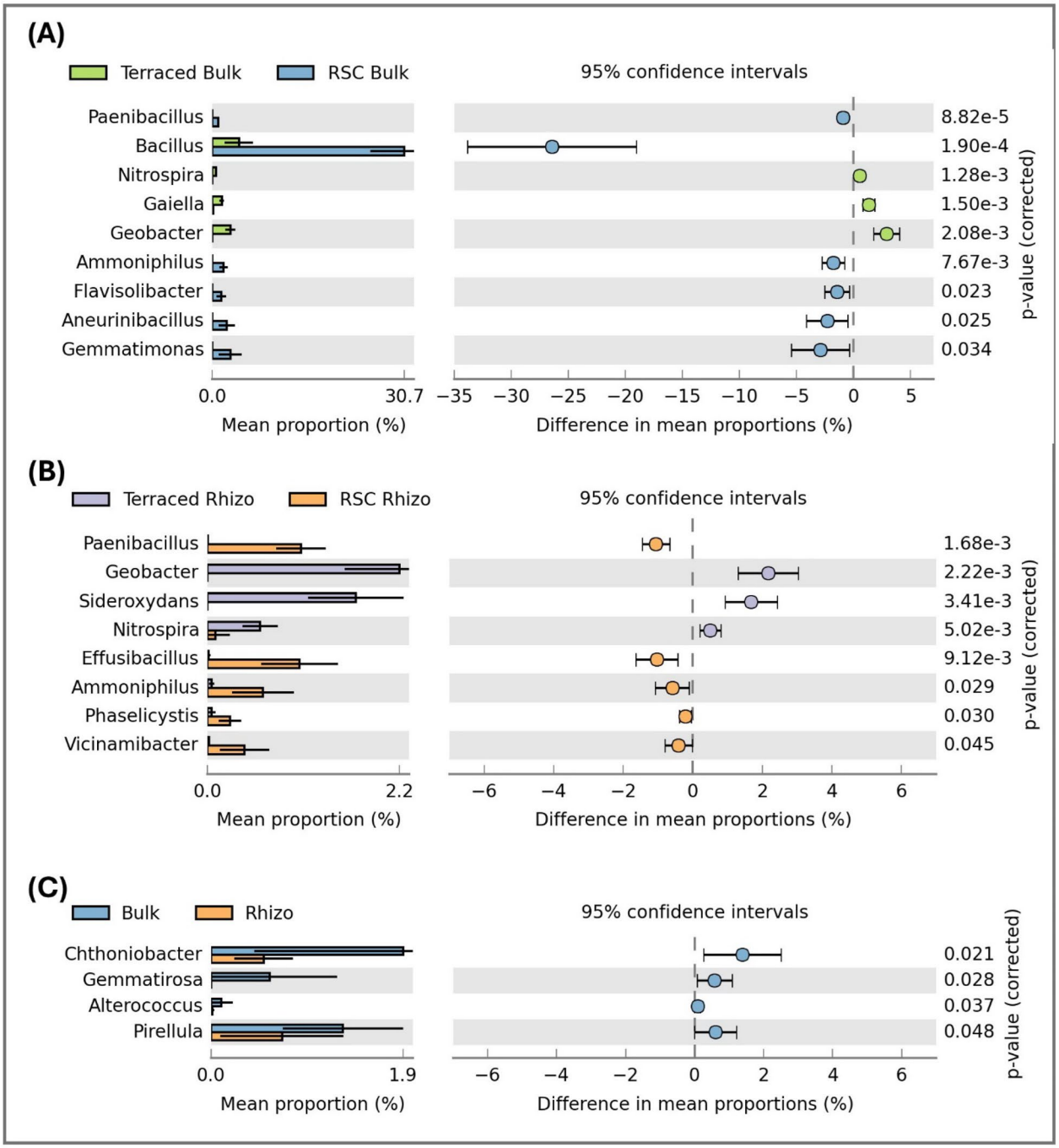


Fig. 6 Differential abundance of bacterial genera across treatment groups based on STAMP analysis. Extended bar plots show genera with significantly different relative abundances in (**A**) Terraced bulk vs RSC bulk, (**B**) Terraced rhizosphere vs RSC rhizosphere, and (**C**) Bulk vs Rhizosphere (pooled across systems). Statistical significance was determined using Welch's *t*-test with FDR correction ($p < 0.05$)

When comparing bulk and rhizosphere compartments across all samples (Fig. 6C), *Chthoniobacter*, *Gemmatirosa*, *Alterococcus*, and *Pirellula* exhibited significantly higher relative abundances in bulk soils. These findings offer a more nuanced understanding of bacterial community composition, revealing distinct taxonomic signatures associated with both land management practices and root compartmentalization.

Predictive function

In addition to analyzing bacterial community diversity, the functional composition of microbial communities was assessed by examining predicted enzyme abundances inferred using the PICRUSt2 method. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity was employed to visualize differences in predicted enzyme profiles across the four treatment groups (Fig. 7). The ordination revealed clear separation among treatments, particularly between RSC and terraced soils, with all pairwise comparisons between management systems showing statistically significant differences ($p < 0.05$). Notably, while predicted enzyme profiles in terraced bulk and rhizosphere soils did not differ significantly ($p = 0.12$), a significant difference was observed between RSC bulk and RSC rhizosphere samples ($p < 0.05$). This contrasts with the bacterial community composition, where no significant differences were detected between compartments within either system. These findings suggest that although land management is the primary driver of both taxonomic and functional shifts, functional profiles may be more responsive to compartmental variation, particularly under the dynamic and stress-prone conditions characteristic of RSC fields.

The heatmap (Fig. 8) illustrates the relative abundances of 20 predicted enzymes involved in the cycling of carbon, nitrogen, phosphorus, and methane across RSC and terraced soils. A clear distinction in predicted enzyme activity is evident between the two soil types. Terraced soils exhibited higher relative abundances of predicted enzymes such as nitrite reductase, nitrate reductase, and particulate methane monooxygenase, suggesting an enhanced microbial potential for nitrogen and methane cycling. In contrast, RSC soils showed elevated levels of predicted enzyme including cellulase, alkaline phosphatase, amidase, and chitinase, which are primarily associated with carbon, nitrogen, and phosphorus cycling. These predicted enzyme activity profiles indicate that microbial communities in RSC and terraced soils are functionally adapted to their respective environments, reflecting distinct emphases in biogeochemical processes.

The differential abundance of predicted microbial enzyme functions was assessed across treatment groups

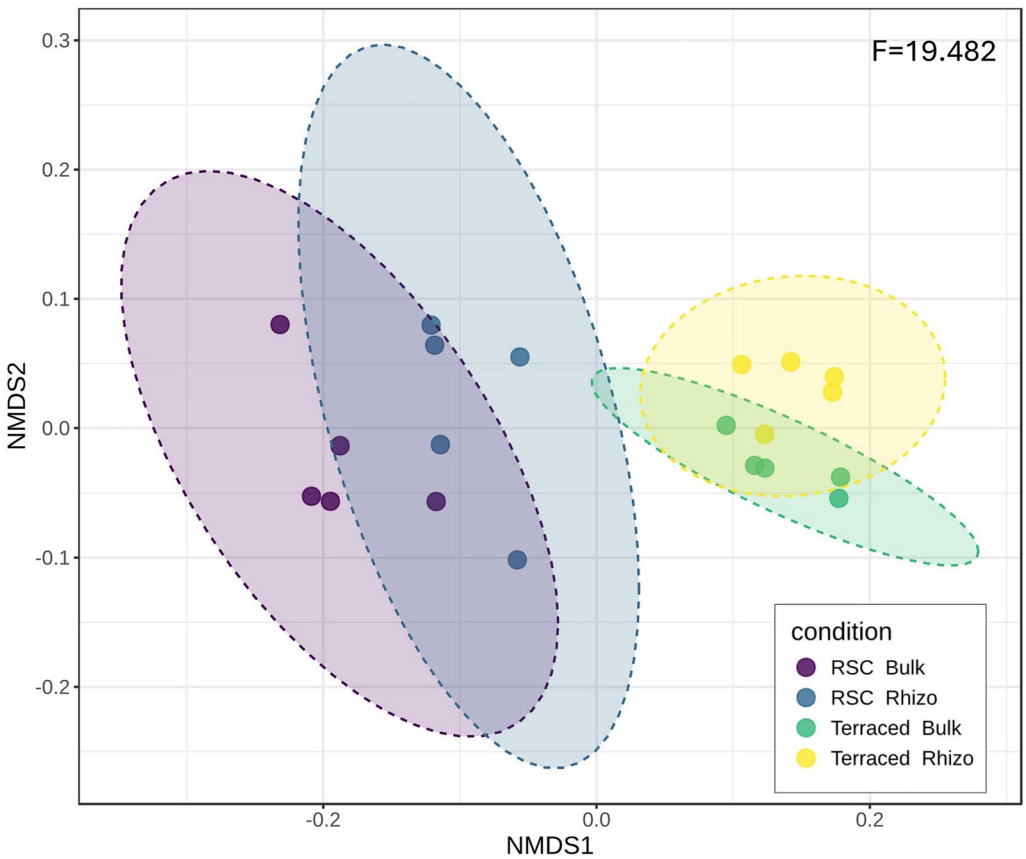


Fig. 7 Non-metric multidimensional scaling (NMDS) plot based on Bray–Curtis dissimilarity showing differences in predicted enzymes profiles across four treatment groups

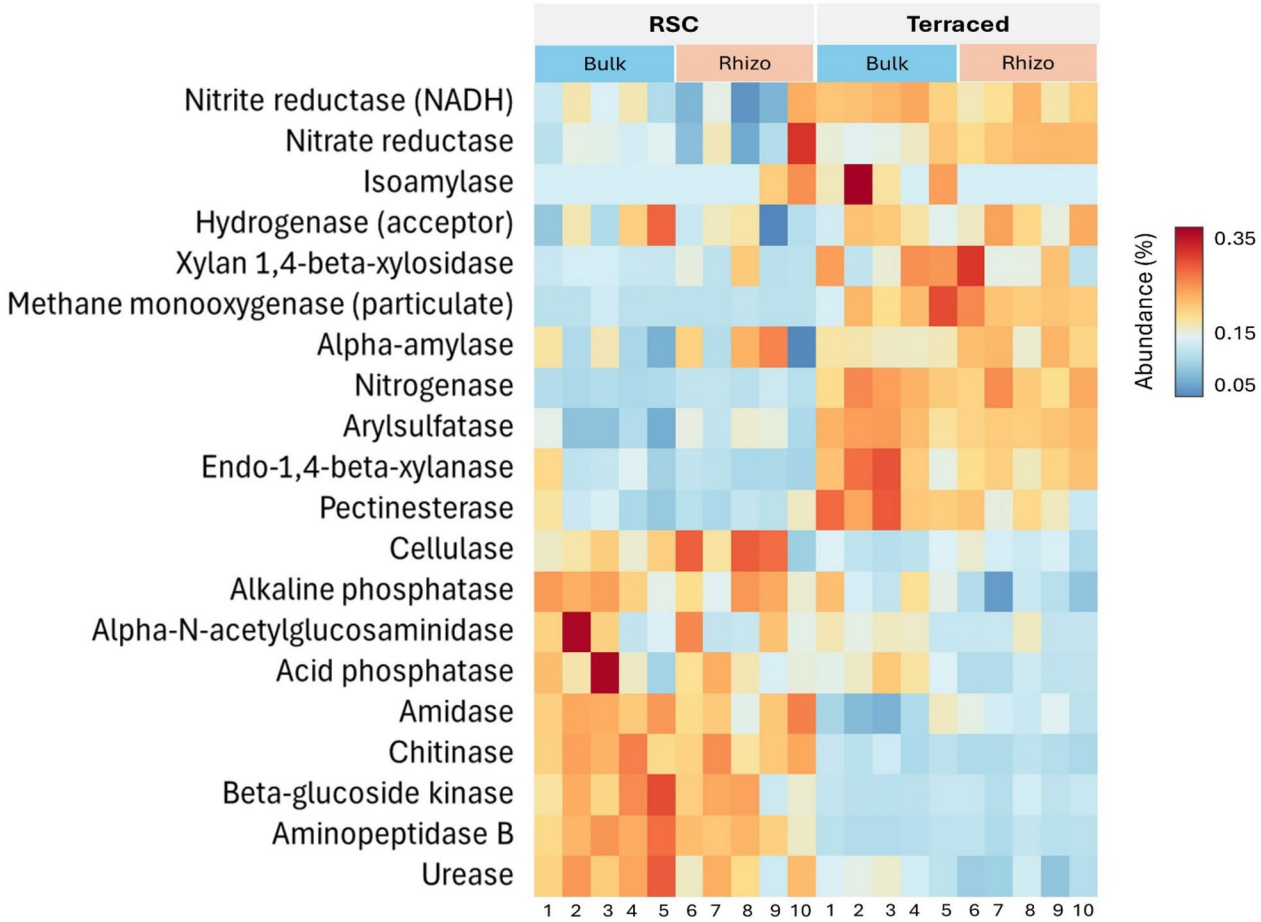


Fig. 8 Heatmap of enzymes potentially produced by bacteria in RSC and terraced soils

using STAMP analysis (Fig. 9). Significant differences in predicted enzyme profiles were observed between RSC and terraced soils under both bulk and rhizosphere conditions. In the bulk soil comparison (Fig. 9A), predicted enzymes such as arylsulfatase, nitrogenase, and nitrite reductase (NADH) were more abundant in terraced soils, whereas aminopeptidase B, amidase, urease, and beta-glucoside kinase were enriched in RSC bulk soils. Similarly, in the rhizosphere comparison (Fig. 9B), several predicted enzymes—including aminopeptidase B, alkaline phosphatase, amidase, urease, and chitinase—were significantly more abundant in RSC rhizosphere soils, while nitrogenase exhibited higher abundance in the terraced rhizosphere.

In contrast, no statistically significant differences were detected when comparing bulk versus rhizosphere compartments across both systems, indicating that the land management system (RSC vs. terraced) has a stronger influence on microbial functional potential than root proximity alone. These findings underscore the role of field management and environmental conditions in shaping microbial functional capacity, particularly with respect to nutrient cycling enzymes.

Discussion

Terraced soil supports a more diverse bacterial community than RSC soil

Both alpha diversity and beta diversity are significantly higher in the terraced soil compared to the RSC soil (Figs. 3 and 4), supporting the acceptance of hypothesis H1. This is attributed to soil flooding, the use of chemical fertilizers, and disturbance from minimum tillage in terraced soils. Terracing represents a critical engineering intervention for soil and water conservation, performing numerous essential functions such as nutrient preservation and erosion control [14, 43]. These systems benefit from diverse nutrient inputs, including rice straw and stubble, weeds, other plant residues, and chemical fertilizers. Furthermore, the periodic inundation of rice terraces enhances the concentration of elements like iron

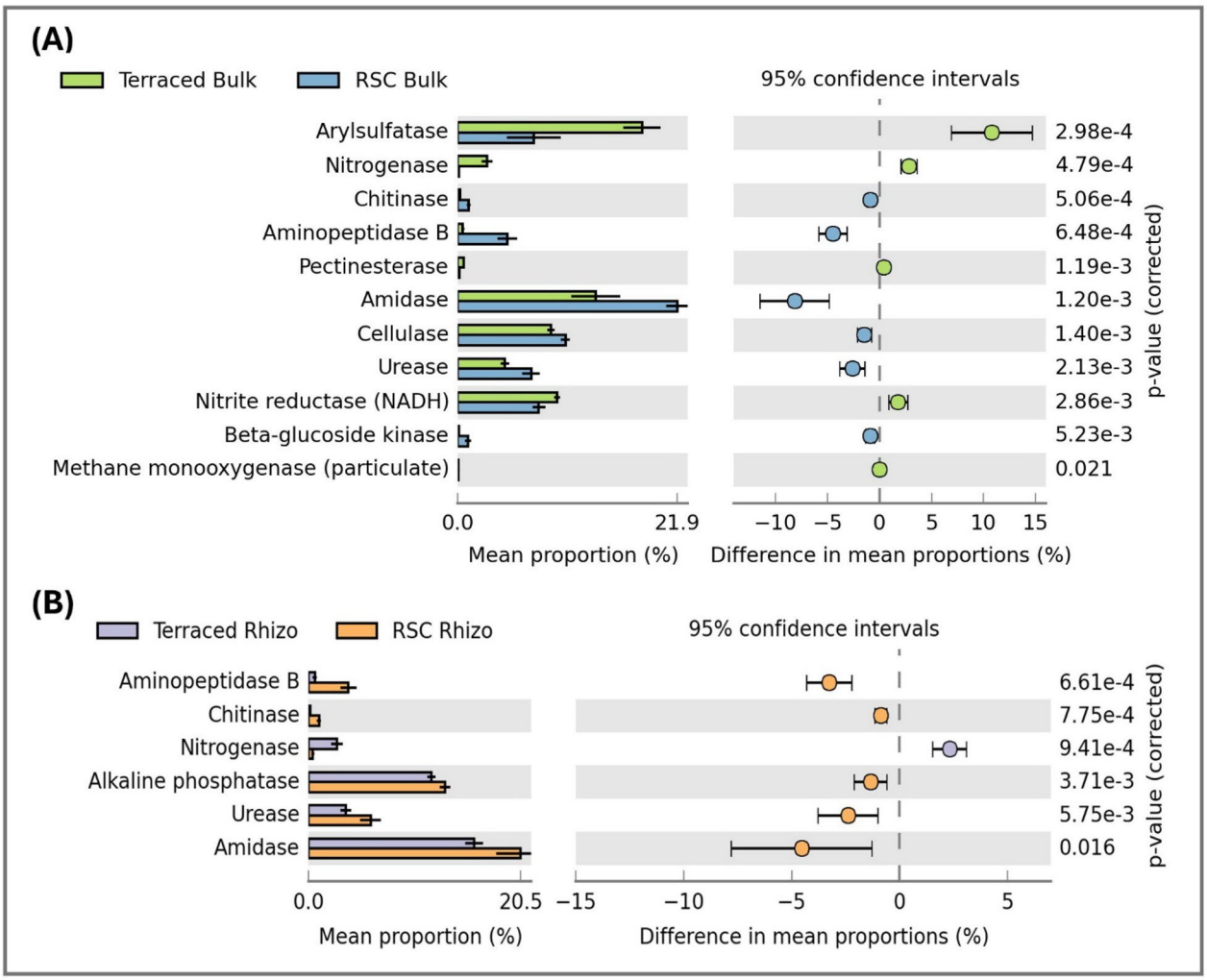


Fig. 9 Differential abundance of predicted enzyme profiles across treatment groups based on STAMP analysis. Extended bar plots show significantly different predicted enzymes in (**A**) Terraced Bulk vs RSC Bulk, and (**B**) Terraced Rhizosphere vs RSC Rhizosphere. Statistical significance was determined using Welch's *t*-test with FDR correction ($p < 0.05$)

and manganese, which play a pivotal role in stabilizing and safeguarding soil organic matter [44]. The submerged conditions also suppress microbial activity, thereby diminishing the mineralization of organic matter and facilitating the retention of more recalcitrant components from plant residues [45]. Sun et al. [46] observed multiple mutualistic interactions among sulfate-reducing bacteria, Fe(III)-reducing bacteria, and methane-oxidizing bacteria in the Gaoyao paddy terraces. Moreover, the use of chemical fertilizers, particularly nitrogen-based variants, in terraced paddy fields significantly influences soil microbial communities, introducing another dynamic to the alteration of soil biota [47]. A meta-analysis by Geisseler et al. [47] demonstrated that nitrogen generally promotes an increase in microbial biomass within paddy soils, particularly when carbon availability is sufficiently high [48]. Zhang et al. [49, 50] observed that nitrogen fertilizer can stimulate the proliferation of gram-positive bacteria in specific rice soils, while organic amendments contribute to a general increase in bacterial abundance. Minimum tillage in terraced paddy fields involves soil disturbance to a depth of 30 cm, compared to no-tillage in RSC fields. This increases pore space for air and water infiltration, facilitating the transport of nutrients from the surface soil to deeper layers, thereby creating a more favorable environment for soil microorganisms. Sun et al. [51] found that minimum tillage combined with organic farming enhances soil organic matter and microbial biomass, while also promoting a more diverse and abundant microbial community. Meanwhile,

ash, charcoal, and plant litter are the primary nutrient sources in RSC fields, decomposing and accumulating in the surface soil layer [18, 52]. Without contour walls and ridges, soils in hilly areas like RSC fields are more vulnerable to erosion from rain runoff and leaching, leading to the loss of soil carbon, nitrogen, and other nutrients [14, 53]. Moreover, no-tillage prevents rice roots from penetrating deeper soil layers due to poor nutrient infiltration and limited nutrient availability, causing the roots to spread primarily within the surface soil. This results in reduced root carbon inputs, such as root cell sloughing, root debris, mucilage, and exudates, into deeper soil layers. The above explanations suggest that lower nutrient levels in RSC soil, compared to terraced soil, lead to a reduced diversity in the bacterial community.

RSC soil notably exhibits a significantly higher relative abundance of Firmicutes and Actinobacteria (Fig. 5), which aligns with previous research. Nelson et al. [54] reported a marked increase in the abundance of Firmicutes and Actinobacteria in soils impacted by wildfires. Similarly, Arunrat et al. [18, 27] observed a rise in Firmicutes abundance following fire in RSC fields in Northern Thailand during the summer, with levels declining throughout the rainy and winter seasons. Furthermore, Zhu et al. [55] found that Firmicutes were most abundant in the 0–10 cm soil layer affected by smoke deposition, with a decline observed after 180 days. This persistence of Firmicutes and Actinobacteria can be attributed to their resistance to high temperatures, desiccation, and radiation [56, 57], making them more likely to survive soil heating events [58]. Meanwhile, Proteobacteria and Acidobacteria are more dominant in terraced soils (Fig. 5). Although some nutrients, such as organic matter, total nitrogen, and available phosphorus, showed no significant differences, most exchangeable ions (K, Ca, and Mg) were significantly different between terraced and RSC soils. Additionally, terraced soils exhibited significantly lower pH levels compared to RSC fields (Table 1). These factors may explain the predominance of Proteobacteria and Acidobacteria in terraced soils. Proteobacteria, as r-strategists, thrive in nutrient-rich environments and exhibit rapid growth [59, 60]. The persistent redox conditions in rice terraces likely enhance carbon and nutrient accumulation in the topsoil, creating favorable conditions for r-strategist microbes [49, 59]. Despite flooding and low oxygen levels, Proteobacteria can flourish in terraced soils due to their adaptability and ability to quickly exploit nutrients released from root exudates and decomposing organic matter [61]. Minimum tillage in terraced fields facilitates root distribution not only in the surface layer but also in deeper soil layers, resulting in a greater spread of rice roots compared to RSC fields. This is consistent with the findings of Pini et al. [62], who observed that Proteobacteria often form symbiotic relationships with plant roots. Acidobacteria may be highly correlated with low soil pH, as observed by Fierer and Jackson [63] and Li et al. [52], who found that the relative abundance of Acidobacteria is higher in acidic soils, typically within a pH range of 3.5–6.0. Interestingly, both Proteobacteria (r-strategists) and Acidobacteria (k-strategists) were observed in the terraced fields, likely because flooded soils create diverse microenvironments that allow fast- and slow-growing microbes to coexist, each occupying distinct niches.

The presence of *Bacillus* species in RSC bulk soil (Fig. 6A) aligns with findings from our previous study conducted in RSC fields in Northern Thailand [27] and in shifting cultivation fields in Bangladesh [64]. *Geobacter* and *Sideroxydans* have been widely recognized as predominant taxa in paddy soils globally [65–67], consistent with their high abundance observed in the terraced rhizosphere soils of this study (Fig. 6A). Nercessian et al. [68] demonstrated that *Geobacter* species play a major role in Fe(III) reduction, coupled with the oxidation of acetate or ammonium, in paddy soils. The genus *Sideroxydans* comprises microaerophilic bacteria capable of oxidizing Fe(II) and is commonly found rhizosphere soils [69]. As *Paenibacillus* was dominant in the RSC rhizosphere samples (Fig. 6B), it is noteworthy that *Paenibacillus* is often associated with plant roots; these rhizobacteria are known to promote plant growth and can fix nitrogen in or around the root zone [70]. Although *Effusibacillus* is typically considered a low-abundance taxon with a limited known ecological role in most soils, it was found to be dominant in the RSC rhizosphere samples in the present study (Fig. 6B). This environment, characterized by being one year post-fire and nutrient-depleted due to runoff in a high-slope area, suggests that *Effusibacillus* may exhibit resilience or act as an opportunistic colonizer following disturbances such as fire.

Rhizosphere and bulk soils show no significant differences in microbial abundance and diversity in RSC soil, but differ in terraced soil

Although terraced rhizosphere soils differed significantly from terraced bulk soils, they exhibited lower richness and diversity than their bulk counterparts (Fig. 3), thereby leading to the rejection of hypothesis H2. Moreover, no significant differences in richness or diversity were observed between RSC bulk and rhizosphere samples (Fig. 3). Our findings contrast with previous studies, which may be explained by similar nutrient availability, balanced root-soil interactions, and functional redundancy across rhizosphere and bulk soils. Meanwhile, Lopes and Schachtman [71] suggested that changes in soil properties—primarily soil nitrogen and moisture—drive

variations in bacterial communities between rhizosphere and bulk soils. Soil organic matter content [24] and nitrogen content [72] predominantly influence the structure and diversity of bulk soil microbial communities. Chen et al. [23] and Lopes et al. [73] also highlighted the significant impact of nitrogen concentration and pH on the composition of root-associated microbial communities. Rhizosphere zones in terraced fields were significantly different from bulk soils (Fig. 3) because the continuous cultivation practices in terraced systems enhance root activity, soil structure, and nutrient levels, creating a distinct microenvironment around the roots. In these fields, plant roots release root exudates—such as sugars, amino acids, and organic acids—that serve as energy sources for microbes and selectively enrich specific microbial groups [74]. This leads to higher microbial abundance, activity, and community shifts in the rhizosphere compared to the surrounding bulk soil, which is not directly influenced by root processes. Simply put, the root-soil interactions in terraced fields are stronger and more sustained, promoting clear microbial and chemical differences between the rhizosphere and bulk zones.

Dry soil conditions in the RSC field likely influence root zone microbial communities due to low moisture availability. Limited soil moisture restricts microbial activity and growth, as most soil bacteria depend on adequate moisture for metabolism and reproduction. Reduced diffusion of root exudates under dry conditions results in fewer nutrients reaching rhizosphere microbes, thereby weakening root–microbe interactions. In addition, water scarcity may hinder root development, leading to a smaller or less active rhizosphere. Thus, in RSC soils, the combination of dry conditions and frequent disturbance likely contributes to the absence of significant differences between rhizosphere and bulk microbial communities (Fig. 3).

Terraced and RSC soils display distinct dominant relative abundances of predicted enzymes

Soil enzymes connect microbial community dynamics with nutrient availability, acting as indicators of microbial function within the soil system [75]. These enzymes, which facilitate the transformation of carbon, nitrogen, and phosphorus, are essential for various soil biochemical processes [76]. RSC soils exhibit higher abundances of predicted enzymes such as cellulase, alkaline phosphatase, amidase, and chitinase (Fig. 8). These enzymes are commonly found across various soil systems [77–81] and play a crucial role in plant litter decomposition and maintaining soil quality. They play a crucial role in decomposing major litter components, thereby facilitating the cycling of carbon, nitrogen, and phosphorus [82]. Terraced soils, on the other hand, show higher relative abundances of predicted enzymes such as nitrite reductase (NADH), nitrate reductase, and particulate methane monooxygenase, suggesting a greater capacity for nitrogen and methane cycling (Fig. 8). Nitrite reductase facilitates the conversion of nitrite into ammonia [83], while nitrate reductase is essential for utilizing nitrate, the most readily available form of nitrogen in soil [84]. Methanotrophic microorganisms are classified into two groups. Aerobic methanotrophs utilize the enzyme methane monooxygenase to activate inert methane molecules [85], while anaerobic methanotrophs rely on external electron acceptors other than oxygen [86, 87]. In this study, however, the predicted enzyme abundances were derived using PICRUSt2 and should ideally be validated through direct measurements of enzyme activities.

Conclusion

This study compared soil bacterial communities and metabolic activities in RSC and terraced paddy fields. The results indicate that both the richness and evenness of microbial communities are significantly higher in terraced soils compared to RSC soils. This difference can be attributed to factors such as soil flooding, the application of chemical fertilizers, and disturbances from minimum tillage practices in terraced fields. Conversely, the absence of contour walls and ridges in hilly areas like RSC fields renders these soils more susceptible to erosion from rain runoff and leaching, resulting in the loss of soil carbon, nitrogen, and other essential nutrients. Additionally, the no-tillage practice in RSC fields restricts rice roots from penetrating deeper soil layers, leading to a decrease in root carbon inputs. Notably, RSC soils exhibited a significantly higher relative abundance of Firmicutes and Actinobacteria, whereas Proteobacteria and Acidobacteria were the dominant phyla in terraced soils. While terraced rhizosphere samples differed significantly from terraced bulk soils, they exhibited lower richness and diversity compared to the bulk soils. In contrast, no significant differences were observed between RSC bulk and RSC rhizosphere soils. *Bacillus* was the dominant genus in RSC bulk soil. *Geobacter* and *Sideroxydans* were more abundant in the terraced rhizosphere, while *Paenibacillus* and *Effusibacillus* dominated the RSC rhizosphere samples. Furthermore, RSC soils demonstrate higher abundances of predicted enzymes such as cellulase, alkaline phosphatase, amidase, and chitinase, which play crucial roles in the cycling of carbon, nitrogen, and phosphorus. Conversely, terraced soils exhibit higher relative abundances of predicted enzymes such as nitrite reductase (NADH), nitrate reductase, and particulate methane monooxygenase, indicating a greater potential

for nitrogen and methane cycling. Long-term monitoring of microbial community dynamics and enzyme activities in both RSC and terraced paddy fields is crucial for evaluating changes over time.

Author contributions

Noppol Arunrat: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding acquisition, Writing—Original Draft, Writing—Review & Editing. Toungporn Uttarotai: Methodology, Formal analysis, Writing—Original Draft. Wuttichai Mhuantong: Methodology, Formal analysis. Praeploy Kongsurakan: Methodology, Investigation, Writing—Original Draft. Sukanya Sereenonchai: Conceptualization, Methodology, Investigation, Writing—Original Draft. Ryusuke Hatano: Supervision, Writing—Original Draft.

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Data availability

The sequencing data relevant to this research are available at the National Center for Biotechnology Information (NCBI), recorded under the BioProject accession number PRJNA1152301.

Declarations

Conflict of interests

The authors declare no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

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